BSR16

Jointly organized by RCSB PDB, NSLS-II, APS, SSRL, LCLS, ALS, CHESS and CLS

August 21-24, 2016

12th International Conference

BIOLOGY and SYNCHROTRON RADIATION

Abstract Booklet

SSRL LCLS SLAC

The 12th International Conference on Biology and Synchrotron Radiation (BSR 2016) - August 21, 24, 2016
Panofsky Auditorium (SUSB - Buildling 53) at SLAC National Accelerator Laboratory, Menlo Park, CA, USA

Sunday, August 21, 2016		
15:00-17:00	Arrival and Registration	
17:00-19:00	Reception	
Monday, August 22, 2016		
08:15-08:30	Soichi Wakatsuki, Stephen Burley, Mike Dunne, Kelly Gaffney, and Henry van den Bedem	Welcome
Membrane Proteins (Chair: Poul Nissen)		
08:30-08:35	Poul Nissen, Aarhus University	Introduction
08:35-08:55	Zhi-Jie Liu, iHuman Institute	Structural and functional studies of human GPCRs
08:55-09:15	Brian Kobilka, Stanford University	The dynamic process of G protein activation
09:15-09:35	Irmgard Sinning, Heidelberg University	Regulation of protein targeting: when SRP meets the ribosome
09:35-09:55	Jian-Ren Shen, Okayama University	High resolution structure of photosystem II and the mechanism of photosynthetic water-splitting
09:55-10:15	Liz Carpenter, University of Oxford	Structure and function of K2P ion channels from synchrotron data from Diamond and serial femtosecond crystallography at LCLS
10:15-10:30	Alexander Kintzer, UCSF	Structure, inhibition, and regulation of a two-pore channel TPC1
10:30-11:00	*Break / Group Photo*	
Macromolecular Complexes (Chair: Bill Weis)		
11:00-11:05	Bill Weis, Stanford University	Introduction
11:05-11:25	Osamu Nureki, University of Tokyo	Structure-based development of genome-editing tool, CRISPR-Cas9 towards medical applications
11:25-11:45	Richard Garratt, University of São Paulo	Septins, a molecular jigsaw
11:45-12:05	Yvonne Jones, University of Oxford	Cell surface signaling systems, structural insights in developmental biology
12:05-12:25	Jim Hurley, UC Berkeley	
12:25-12:45	Natalie Strynadka, University of British Columbia	Structure-based piecing together of the Type III secretion system puzzle
12:45-13:00	Karthik Sathiyamoorthy, Stanford University	Structural basis for Epstein-Barr virus host cell tropism mediated by gp42 and gHgL entry glycoproteins

13:00-14:30	*Lunch Break/Poster Setup*	*For those that pre-paid for lunch, it is available in Trinity Room across from Panofsky Auditorium. For those that did not pre-pay for lunch, food is available for purchase at SLAC Café or Starbucks by Stanford Guest House.*
14:30-16:00	Poster Session 1	
Hybrid Methods (Chair: Robert McKenna)		
16:00-16:10	Robert McKenna, University of Florida	Introduction
16:10-16:30	Gabriel Waksman, University College London/Birkbeck	Structural and molecular biology of bacterial secretion systems
16:30-16:50	Jill Trewhella, University of Sydney	Small-angle scattering: an effective constraint in modelling complex biomolecular structures
16:50-17:10	Mike Hough, University of Essex	Fingerprinting redox and ligand states in protein crystals: applications for validation and determining structural movies
17:10-17:30	Flora Meilleur, Oak Ridge National Laboratory	Radiation damage free structural studies of cellulolytic redox enzymes using neutron scattering and diffraction
17:30-17:50	Martin Beck, EMBL Heidelberg	*In situ* structural analysis of the human nuclear pore complex
17:50-18:10	Hélène Déméné, CNRS	Towards a mechanistic understanding of the opioid mu receptor activation
18:10-18:30	Orion Shih, NSRRC	Linear oligomerization process of BAX revealed from coexisting intermediates in solution
Tuesday, August 23, 2016		
Bioinformatics and Computing (Chair: Helen Berman)		
08:30-08:35	Helen Berman, Rutgers University - Introduction	Introduction
08:35-08:55	Michael Levitt, Stanford University School of Medicine	Combinatorial methods solve a difficult structural problem to reveal how chaperonins work in eukaryotes
08:55-09:15	Christine Orengo, University College London	CATH-FunFams: New domain families to explore protein structure and function space
09:15-09:35	Paul Adams, Lawrence Berkeley National Laboratory	New methods for low resolution structure refinement
09:35-09:55	Dan Rigden, University of Liverpool	Exploiting structural bioinformatics for unconventional molecular replacement
09:55-10:15	Wladek Minor, University of Virginia	High throughput computing on high output synchrotron facilities
10:15-10:30	Daniel Franke, EMBL-Hamburg	Rapid shape classification of biological macromolecules from small angle x-ray scattering data
10:30-11:00	*Break*	
Science with Upgraded SRS (Chair: Stefan Vogt)		
11:00-11:10	Stefan Vogt, Argonne National Laboratory	Introduction
11:10-11:30	Pieter Glatzel, ESRF	Hard X-ray photon-in/photon-out spectroscopy at ESRF-EBS
11:30-11:50	Marjolein Thunnissen, MAX IV Laboratory	Possibilities for the life sciences at the first diffraction limited light-source MAX IV
11:50-12:10	Gayle Woloschak, Northwestern University	Upgrading X-ray fluorescence imaging
12:10-12:30	Lin Yang, Brookhaven National Laboratory	The life science x-ray scattering (LiX) beamline at NSLS-II
12:30-14:00	*Lunch Break/Poster Setup*	*For those that pre-paid for lunch, it is available in Trinity Room across from Panofsky Auditorium. For those that did not pre-pay for lunch, food is available for purchase at SLAC Café or Starbucks by Stanford Guest House.*
14:00-15:30	Poster Session 2	
Industrial or Pharmaceutical Applications (Chair: Nigel Walker)		

16:00-16:10	Nigel Walker, Molecular Consulting - Introduction	The application of structural biology to pharmaceutical drug discovery
16:10-16:30	Benjamin Bax, GlaxoSmithKline, UK	How is movement coupled to catalysis in DNA gyrase?
16:30-16:50	Chun-Wa Chung, GlaxoSmithKline R&D, UK	How crystallography can (and can't) help us find drugs
16:50-17:10	Avni Bhatt, University of Florida	Crystallographic insight into enhanced catalytic activity of carbonic anhydrase II using "activating" ligands
17:10-19:00	SLAC Tour - Pre-registration required	
19:00-22:00	BBQ Dinner - Pre-registration required	
Wednesday, August 24, 2016		
X-ray/IR Imaging/SR-CDI (Chair: Chris Jacobsen)		
08:30-08:35	Chris Jacobsen, Argonne National Laboratory	Introduction
08:35-08:55	Tanja Dučić, CELLS-ALBA	Multimodal synchrotron spectro-microscopy for elucidation cellular disorders in neuro-degenerative diseases
08:55-09:15	Liz Duke, Diamond Light Source	Cryo soft x-ray microscopy: new opportunities for structural biology
09:15-09:35	Patrick La Riviere, University of Chicago	Development of "color" x-ray histology using multiple metal stains and multi-energy synchrotron CT
09:35-09:55	Andreas Menzel, Paul Scherrer Institute	Bio-imaging using x-ray ptychography - the method, recent advances, and applications
09:55-10:15	Carol Hirschmugl, University of Wisconsin - Milwaukee	Rapid 2D and 3D IR imaging applied to biologically and chemically complex systems
10:15-10:30	Michael Martin, LBNL	Biological SINS: Broadband synchrotron infrared nano-spectroscopy of biological materials
10:30-11:00	*Break*	
Dynamics (Chair: Keith Moffat)		
11:00-11:05	Keith Moffat, University of Chicago	Introduction
11:05-11:25	Marius Schmidt, University of Wisconsin - Milwaukee	Ultrafast dynamics in proteins investigated by time-resolved serial femtosecond crystallography
11:25-11:45	Lois Pollack, Cornell University	Time-resolved studies: SAXS of protein-DNA complexes and mixing jets for XFELs
11:45-12:05	Rajmund Mokso, MAX IV Laboratory	Fast tomographic microscopy to capture the dynamics of life
12:05-12:25	Doeke Hekstra, UT Southwestern	Probing the mechanics of molecular machines with electric fields and X-rays
12:25-12:45	James Fraser, UC San Francisco	Birth of the cool: multitemperature multiconformer x-ray crystallography
12:45-13:00	Daniel Keedy, UCSF	Multitemperature synchrotron crystallography and ligand scanning reveal novel allosteric modulators of the therapeutic target PTP1B
13:00-14:30	*Lunch Break/Poster Setup*	*For those that pre-paid for lunch, it is available in Trinity Room across from Panofsky Auditorium. For those that did not pre-pay for lunch, food is available for purchase at SLAC Café or Starbucks by Stanford Guest House.*
14:30-16:00	Poster Session 3	
7 Years of XFEL in Structural Biology (Chair: Janet Smith)		
16:00-16:05	Chi-Chang Kao, SLAC National Accelerator Laboratory	
16:05-16:10	Janet Smith, University of Michigan	Introduction
16:10-16:30	Richard Neutze, University of Gothenburg	Time-resolved serial femtosecond crystallography studies of bacteriorhodopsin - a light-driven proton pump.

16:30-16:50	Ilme Schlichting, Max Planck Institute for Medical Research	Phasing serial femtosecond crystallography data
16:50-17:10	Henry Chapman, DESY	Macromolecular diffractive imaging using disordered crystals
17:10-17:30	Axel Brunger, Stanford University	XFEL crystal structure of the Synaptotagmin-1 : SNARE complex
17:30-17:50	So Iwata, RIKEN SPring-8 Center/Kyoto University	Macromolecular crystallography at SACLA
17:50-18:10	Andy Aquila, SLAC National Accelerator Laboratory	Single particle imaging at the Linac Coherent Light Source
18:10-18:30	Jan Kern, LBNL	Mechanism of water oxidation in photosystem II studied by room temperature fs x-ray crystallography and spectroscopy
18:30-19:00	Soichi Wakatsuki and Stephen Burley	Closing Remarks

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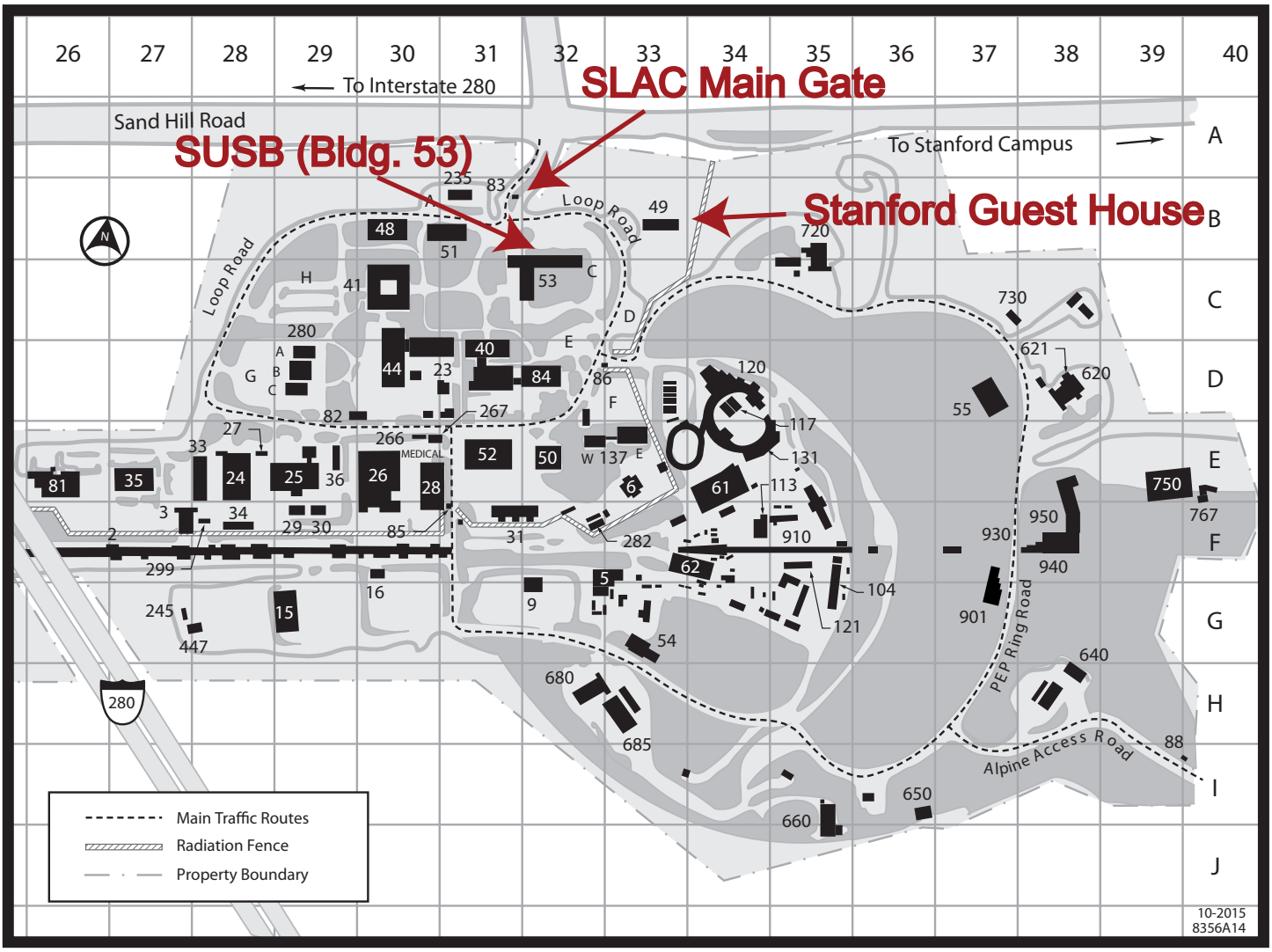

SLAC Area Map

	Building Number	Grid Number
Administration and Engineering Building (A&E)	41	30-C
Alpine Gate Entrance (automated gate badge required)	88	40-I
Arrillaga Recreation Center	55	37-D
Auxiliary Control Building	3	27-F
Beam Switch Yard Access	9	32-G
Central Hazardous Waste Management Area	245	27-G
Central Laboratory	40	31-D
Central Laboratory Annex	84	32-D
Central Utility Building	23	31-D
Chemical Storage Building	36	29-E
Cleaning Facility Building	30	29-F
Collider Experimental Hall (CEH)	750	39-E
Communications Office	53	32-C
Computer Building (SCS)	50	32-E
Controls Building	34	28-F
Cryogenics Laboratory	6	33-E
End Station A (ESA)	61	34-E
End Station B (ESB)	62	34-F
Environmental Protection Restoration	299	28-F
Environmental Safety and Health (ES&H)	41	30-C
Exercise Room/Shops Dining Room	27	28-E
Experimental Facilities Department Shops (EFD)	104	35-F
Gate 17 (automated gate badge required)	86	33-D
General Services Building (Shipping & Receiving)	81	26-E
Hazardous Waste Storage Area	447	28-G
Heavy Fabrication Building	26	30-E
International Services Office	53	32-C
Kavli Building	51	31-B
Klystron Gallery (Visitors Alcove, Sector 27)	2	27-F
Laboratory Offices and Shops (LOS)	137	33-E
LCLS Beam Transport Hall	910	33/34/35-F
LCLS Far Hall Tunnel Entrance	767	40-E
LCLS Near Hall	930/940/950	37/38-F
LCLS Office Building	901	37-G
Light Assembly Building	33	28-E
Light Fabrication Building	25	29-E

	Building Number	Grid Number
Main Control Center (MCC)	52	31-E
Main Gate (Information Booth)	83	31-B
Master Substation	16	30-F
Medical (room 11)	28	30-E
Metal Stores Shelter	29	29-F
Orientation Theater	53	32-C
Operations Support Building	28	30-E
Panofsky Auditorium	53	32-C
Parking Lots	A and C to H	
PEP Beam Facility/SSRL	650	36-I
PEP Beam Facility/SSRL	730	37-C
PEP Control Room	685	33-H
PEP Interaction Region 2 (IR-2)	620	38-D
PEP Interaction Region 4 (IR-4)	640	38-H
PEP Interaction Region 6 (IR-6)	660	35-I
PEP Interaction Region 8 (IR-8)	680	32-H
PEP Interaction Region 12 (IR-12)	720	35-B
Physics and Engineering Building	280	29-D
Plant Maintenance and Utilities	35	27-E
Power Conversion	15	29-G
Research Office Building (ROB)	48	30-B
Research Support Building	52	31-E
Science User Support Building (SUSB)	53	32-C
Sector 30 Guard House (automated gate badge required)	85	31-F
Security	53	32-C
SLAC Café	53	32-C
SLC Engr. Trailer South (Fort Apache)	282	32-F
SPEAR Control Room	117	34-D
Stanford Guest House	49	33-B
Stanford Research Computing Facility	54	33-G
Stanford Synchrotron Radiation Lab (SSRL)	120	34-D
Stanford Synchrotron Radiation Lab (SSRL)	131	34-E
Test Beam Facility	121	35-F
Test Laboratory	44	30-D
User Support	53	32-C
Vacuum Assembly Building	31	31-F
Visitor, User, Employee Center (VUE)	53	32-C

BSR 2016 Poster Assignment List - Presentation Order

Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
M01	Hybrid methods	Avni	Bhatt	University of Florida	Structure activity relationships of benzenesulfonamide-based inhibitors towards carbonic anhydrase isoform specificity
M02	Hybrid methods	Pawel	Grochulski	Canadian Light Source	Future of biological and life-science facilities at the CLS
M03	Hybrid methods	Nelly	Hajizadeh	EMBL Hamburg Outstation	Intelligent Agents for Improving Data Collection Efficiency at the EMBL P12 BioSAXS Beamline, Hamburg
M04	Hybrid methods	Seung Joong	Kim	UCSF (University of California, San Francisco)	Molecular Architecture and Function of the SEA Complex, a Modulator of the TORC1 Pathway
M05	Hybrid methods	Thomas	Kroll	SLAC National Accelerator Laboratory	Extracting Electronic Structure and Bond Strength Information from 1s2p RIXS: Electron Transfer and Apoptosis in the Cytochrome c protein
M06	Hybrid methods	Michael	Mara	Stanford University	Characterization of the Cytochrome C Iron-Thioether Bond and Its Regulation by the Protein
M07	Hybrid methods	Ivan	Rajkovic	SLAC/SSRL	Recent Developments at the Beamline for Biological Small Angle X-ray Scattering BL4-2 at SSRL
M08	Hybrid methods	Ursula	Schulze-Gahmen	UC Berkeley	Insights into HIV-1 proviral transcription from an integrative structure of the Tat:AFF4:P-TEFb:TAR complex
M09	Hybrid methods	Farzaneh	Tondnevis	University of Florida	Solution Structure of an “open” E. coli Pol III Clamp Loader
M10	Hybrid methods	Yasufumi	Umena	Okayama University	Estimation of valences and radiation damage of four Mn atoms in photosystem II crystals using anomalous diffraction
M11	Hybrid methods	Matthias	Wilmanns	European Molecular Biology Laboratory	Molecular mechanism of reversible elasticity in the muscle filament bridge protein myomesin
M12	Hybrid methods	Petrus	Zwart	LBNL	Advances in Fluctuation Scattering
M13	Macromolecular complexes	Asha	Bhushan	All India Institute of Medical Sciences	Crystal Structure of the Complex of Lactoperoxidase with an Anti-thyroid drug Propylthiouracil
M14	Macromolecular complexes	Teck Khiang	Chua	MacCHESS, Cornell University	Innovative Application of Pressure Cryo-cooling
M15	Macromolecular complexes	Jan	Dohnalek	Institute of Biotechnology CAS	Protein-nucleic acids interactions studied by synchrotron radiation and complementary techniques

BSR 2016 Poster Assignment List - Presentation Order

Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
M16	Macromolecular complexes	Jonathan	Herrmann	Stanford University	Calcium Mediates Structural Dynamics of RsaA, the S-Layer Protein from Caulobacter Crescentus
M17	Macromolecular complexes	Tatiana	Isabet	Synchrotron SOLEIL	PROXIMA-1 : macromolecular crystallography beamline @ synchrotron SOLEIL
M18	Macromolecular complexes	Tatiana	Isabet	Synchrotron SOLEIL	Automated devices for BIOSAXS at synchrotron SOLEIL
M19	Macromolecular complexes	Sheng-Jun	Liu	Stanford University / Xiamen City University	Data filtering method for Correlated X-ray Scattering
M20	Macromolecular complexes	Suzanne	Norwood	University of Queensland	Structural characterisation of the retromer complex
M21	Macromolecular complexes	Natalia	Orlova	The City College of New York	A plasmid that became a chromosome
M22	Macromolecular	Karthik	Sathiyamoorthy	Stanford University	Structural Basis for Epstein-Barr Virus Host Cell Tropism
M23	Macromolecular complexes	Orion	Shih	NSRRC	Linear Oligomerization Process of BAX Revealed from Coexisting Intermediates in Solution
M24	Macromolecular complexes	Christopher	Warner	University of California, Santa Cruz	Small angle X-ray scattering analysis reveals that introduction of D-glutamate at a critical residue of Aβ42
M25	Macromolecular complexes	Jui-Hung	Weng	IBC, Academia Sinica	Uncovering the mechanism of FHA domain-mediated TIFA oligomerization that plays a central role in immune
M26	Macromolecular complexes	Hsiang-Yi	Wu	Institute of Biological Chemistry, Academia Sinica	Structural Basis of the Antizyme-Mediated Inhibition and Degradation of Ornithine Decarboxylase
M27	Membrane proteins	Bobby	Baravati	Arizona State University	Structural Biology of Coronavirus Envelope (CoV E) Proteins
M28	Membrane proteins	Isabel	De Moraes	Diamond Light Source / Imperial College London	Tailoring Synchrotrons to Membrane Protein Structure Determination
M29	Membrane proteins	Patrick	Frank	SLAC/Stanford University	Spin-Polarization and Spectroscopic Validation of the Through-Bond Electron Transfer Mechanism of Redox
M30	Membrane proteins	Alexander	Johs	Oak Ridge National Laboratory	Small-angle Neutron Scattering as a probe for leaflet asymmetry in biomembranes
M31	Membrane proteins	Alexander	Kintzer	University of California at San Francisco	Structure, inhibition, and regulation of a two-pore channel TPC1

BSR 2016 Poster Assignment List - Presentation Order

Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
M32	Hybrid methods	Yang	Ha	Stanford University	XAS studies on Fe2S2 ferredoxin binding with Δ9 desaturatase
M33	Hybrid methods	Hyeongtaek	Lim	Stanford University	Cu K-beta X-ray Emission Spectroscopy as a Probe of Coordination Environments of Cu(I) Sites
M34	Hybrid methods	James	Yan	Stanford University	L-Edge Spectroscopic Investigation of {FeNO}6: Factors Determining Delocalization vs Antiferromagnetic Coupling
T01	Bioinformatics and computing	Daniel	Franke	EMBL Hamburg	Assessing Goodness of Fit with the Correlation Map Test
T02	Bioinformatics and computing	Eugene	Krissinel	CCP4, STFC, Research Complex at Harwell, Rutherford-Appleton	CCP4 Web-Services and Cloud Computing Developments
T03	Bioinformatics and computing	Sergey	Stepanov	Argonne National Laboratory	Integration of fast detectors into beamline controls at GM/CA@APS: Pilatus3 6M and Eiger 16M
T04	Bioinformatics and computing	Ville	Uski	STFC/CCP4	CCP4: a resource for macromolecular crystallography
T05	Science with upgraded SRS	Stephanie	Bachas-Daunert	Stanford University	Arsenic and the Gut Microbiome: A Case Study for Application of Synchrotron Radiation in Microbiome Research
T06	Science with upgraded SRS	Florian	Dworkowski	Swiss Light Source @ Paul Scherrer Institut	Kinoform diffractive lens based micro focusing upgrade of the macromolecular crystallography beamline X10SA at the
T07	Science with upgraded SRS	Robert	Fischetti	Advanced Photon Source, Argonne National	Serial Millisecond Crystallography of Microcrystals at the Advanced Photon Source
T08	Science with upgraded SRS	Andreas	Förster	DECTRIS Ltd.	High-speed detectors enable synchrotron serial crystallography
T09	Science with upgraded SRS	Gavin	Fox	Synchrotron SOLEIL	Crystallographic data collection using microbeams with a photon-counting detector at PROXIMA2-A
T10	Science with upgraded SRS	Mutairu Bolaji	Olatinwo	Louisiana State University	Quantitative X-ray Grating-based Interferometry Brown Adipose Tissue in Mice
T11	Science with upgraded SRS	Thomas	Sorensen	Diamond Light Source, UK	New MX beamline dedicated to in situ diffraction experiments

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Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
T12	Science with upgraded SRS	Doletha	Szebenyi	MacCHESS, Cornell University	MacCHESS, a Synchrotron Source with Unique Opportunities for structural Biology
T13	Science with upgraded SRS	Charles	Titus	Stanford	Probing symmetry, spin, and valency of metal centers via ultra-sensitive soft X-ray detectors
T14	Science with upgraded SRS	Yusuke	Yamada	High Energy Accelerator Research Organization	Upgrade of a macromolecular crystallography beamline, BL-17A, at the Photon Factory
T15	Science with upgraded SRS	Fumiaki	Yumoto	KEK/High Energy Accelerator Research	Photon Factory as a Research Hub in the Platform of Drug Discovery, Informatics, and Structural Life Science
T16		Masahiko	Hiraki	Mechanical Engineering Center, KEK	Upgrade of automated protein crystallization and imaging system
T17		Tsutomu	Matsui	SLAC National Accelerator Laboratory	Time-resolved SAXS with low sample consumption: a way to pursue conformational changes of biomolecules
T18		Hideo	Okumura	Japan Synchrotron Radiation Research Institute (JASRI)	Present status of SPring-8 macromolecular crystallography beamlines
T19		John	Rose	SER-CAT/ University of Georgia	SER-CAT/UGA Native-SAD Highlights
T20		Chien-chang	Tseng	NSRRC	TPS-05A Protein Microcrystallography Beamline at the National Synchrotron Radiation Research Center
T21	Industrial or pharmaceutical applications	Ximena	Barros-Alvarez	University of Washington	Structure guided approach in the design of inhibitors against pathogenic protozoa targeting aminoacyl-tRNA synthetases
T22	Industrial or pharmaceutical	Anja	Burkhardt	Deutsches Elektronen-Synchrotron DESY	Macromolecular crystallography at beamline P11 at PETRA III
T23	Industrial or pharmaceutical	Martin	Gifford	Lyncean Technologies, Inc	The Lyncean Compact Light Source: X-ray Synchrotron Radiation for Analytical and Imaging Applications
T24	Industrial or pharmaceutical	Vitul	Jain	ICGEB	Structure of prolyl-tRNA synthetase-Halofuginone complex provides basis for development of novel drugs against
T25	Industrial or pharmaceutical	Anne	Mulichak	IMCA-CAT/Hauptman Woodward Research	IMCA-CAT Advanced Photon Source Facility for Pharmaceutical Drug Discovery
T26	Industrial or pharmaceutical applications	Weifeng	Shang	Illinois Institute of Technology	Recent SAXS developments dedicated for solution scattering of biological macromolecules at the BioCAT beamline 18-ID at the Advanced Photon Source

BSR 2016 Poster Assignment List - Presentation Order

Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
T27	Industrial or pharmaceutical applications	Nina	Wurzler	BAM	Chemical and electrochemical interaction mechanisms of metal reducing bacteria on steel surfaces
T28		Chun-Jung	Chen	NSRRC	
W01	X-ray/IR imaging/SR-CDI	Michael	Becker	GM/CA@APS, Argonne National Laboratory	Towards user operations of the SONICC system at GM/CA@APS beamline 23ID-B at the Advanced Photon Source
W02	X-ray/IR imaging/SR-CDI	Suzana	Car	Dartmouth College	Imaging the plant ionome: Synchrotron X-ray fluorescence and its applications in a study of zinc homeostasis in
W03	X-ray/IR imaging/SR-CDI	Kenneth	Fahy	SiriusXT	3D Cell Structure Imaging with Laboratory Scale Cryo Soft X-ray Tomography
W04	X-ray/IR imaging/SR-CDI	Chi-Feng	Huang	Department of Applied Chemistry, National Chiao Tung University	Imaging Individual Drug-Carrying Liposome Particles by Free-Electron-Laser Coherent Diffraction
W05	X-ray/IR imaging/SR-CDI	Courtney	Krest (Roach)	Stanford Synchrotron Radiation Lightsource	Hard X-ray Fluorescence Imaging and μ-X-ray Absorption Spectroscopy
W06	X-ray/IR imaging/SR-CDI	Ting-kuo	Lee	Academia Sinica, Taiwan	Recovery of missing central diffraction intensities by using template method
W07	X-ray/IR imaging/SR-CDI	Po-Nan	Li	Stanford University	Resolution enhancement of transmission x-ray microscopy using coherent diffraction
W08	X-ray/IR imaging/SR-CDI	Keng	Liang	Institute of Physics, Academia Sinica, Taiwan	Measurement and simulation of interference enhancement in coherent X-ray diffraction imaging of gold nano particles
W09	X-ray/IR imaging/SR-CDI	Michael	Martin	Advanced Light Source, LBNL	Biological SINS: Broadband synchrotron infrared nano-spectroscopy of biological materials
W10	X-ray/IR imaging/SR-CDI	Peng	Qi	University of Saskatchewan	A Bent Laue Energy Dispersive Monochromator: An Example Application of Speciation Imaging at the Selenium
W11	X-ray/IR imaging/SR-CDI	Shenglan	Qiao	Stanford University	Angular correlations of photons from solution diffraction at a free electron laser encode molecular structure
W12	X-ray/IR imaging/SR-CDI				

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W13	Dynamics	Daniel	Keedy	University of California, San Francisco	Multitemperature synchrotron crystallography and ligand scanning reveal novel allosteric modulators of the
W14	Dynamics	Michael	Thompson	University of California, San Francisco	Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Time-Resolved Crystallographic
W15	7 years of XFEL in structural biology	Jeffrey	Donatelli	Lawrence Berkeley National Lab	Multi-Tiered Iterative Phasing for Fluctuation X-ray Scattering and Single-Particle Diffraction
W16	7 years of XFEL in structural biology	Jan	Kern	Lawrence Berkeley National Lab	Mechanism of water oxidation in photosystem II studied by room temperature fs x-ray crystallography and spectroscopy
W17	7 years of XFEL in structural biology	Elena	Kovaleva	Stanford Synchrotron Radiation Lightsource	Characterization of Ferryl Intermediate in DypB Peroxidase Using Femtosecond Crystallography, Optical and X-Ray
W18	7 years of XFEL in structural biology	Anna	Munke	Uppsala University	Coherent diffraction of single Rice Dwarf Virus particles using hard X-rays at the Linac Coherent Light Source
W19	7 years of XFEL in structural biology	Yoshinori	Nishino	Research Institute for Electronic Science, Hokkaido University	Controlled Environment Nano-Imaging Free From Radiation Damage by X-ray Laser Diffraction
W20	7 years of XFEL in	Kensuke	Tono	Japan Synchrotron	Platforms for biological researches at SACLA
W21	7 years of XFEL in structural biology	Rahel	Woldeyes	UCSF	Using XFELs to visualize solvent in the Flu M2 Proton Channel

BSR 2016 Poster Assignment List - Alphabetical Order by Presenter's Last Name

Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
T5	Science with upgraded SRS	Stephanie	Bachas-Daunert	Stanford University	Arsenic and the Gut Microbiome: A Case Study for Application of Synchrotron Radiation in Microbiome Research
M27	Membrane proteins	Bobby	Baravati	Arizona State University	Structural Biology of Coronavirus Envelope (CoV E) Proteins
T21	Industrial or pharmaceutical applications	Ximena	Barros-Alvarez	University of Washington	Structure guided approach in the design of inhibitors against pathogenic protozoa targeting aminoacyl-tRNA synthetases
W1	X-ray/IR imaging/SR-CDI	Michael	Becker	GM/CA@APS, Argonne National Laboratory	Towards user operations of the SONICC system at GM/CA@APS beamline 23ID-B at the Advanced Photon Source
M1	Hybrid methods	Avni	Bhatt	University of Florida	Structure activity relationships of benzenesulfonamide-based inhibitors towards carbonic anhydrase isoform specificity
M13	Macromolecular complexes	Asha	Bhushan	All India Institute of Medical Sciences	Crystal Structure of the Complex of Lactoperoxidase with an Anti-thyroid drug Propylthiouracil
T22	Industrial or pharmaceutical applications	Anja	Burkhardt	Deutsches Elektronen-Synchrotron DESY	Macromolecular crystallography at beamline P11 at PETRA III
W2	X-ray/IR imaging/SR-CDI	Suzana	Car	Dartmouth College	Imaging the plant ionome: Synchrotron X-ray fluorescence and its applications in a study of zinc homeostasis in Arabidopsis thaliana
T28		Chun-Jung	Chen	NSRRC	
M14	Macromolecular complexes	Teck Khiang	Chua	MacCHESS, Cornell University	Innovative Application of Pressure Cryo-cooling
M28	Membrane proteins	Isabel	De Moraes	Diamond Light Source / Imperial College London	Tailoring Synchrotrons to Membrane Protein Structure Determination
M15	Macromolecular complexes	Jan	Dohnalek	Institute of Biotechnology CAS	Protein-nucleic acids interactions studied by synchrotron radiation and complementary techniques
W15	7 years of XFEL in structural biology	Jeffrey	Donatelli	Lawrence Berkeley National Lab	Multi-Tiered Iterative Phasing for Fluctuation X-ray Scattering and Single-Particle Diffraction
T6	Science with upgraded SRS	Florian	Dworkowski	Swiss Light Source @ Paul Scherrer Institut	Kinoform diffractive lens based micro focusing upgrade of the macromolecular crystallography beamline X10SA at the SLS
W3	X-ray/IR imaging/SR-CDI	Kenneth	Fahy	SiriusXT	3D Cell Structure Imaging with Laboratory Scale Cryo Soft X-ray Tomography

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Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
T7	Science with upgraded SRS	Robert	Fischetti	Advanced Photon Source, Argonne National Laboratory	Serial Millisecond Crystallography of Microcrystals at the Advanced Photon Source
T8	Science with upgraded SRS	Andreas	Förster	DECTRIS Ltd.	High-speed detectors enable synchrotron serial crystallography
T9	Science with upgraded SRS	Gavin	Fox	Synchrotron SOLEIL	Crystallographic data collection using microbeams with a photon-counting detector at PROXIMA2-A
M29	Membrane proteins	Patrick	Frank	SLAC/Stanford University	Spin-Polarization and Spectroscopic Validation of the Through-Bond Electron Transfer Mechanism of Redox Metalloproteins
T1	Bioinformatics and computing	Daniel	Franke	EMBL Hamburg	Assessing Goodness of Fit with the Correlation Map Test
T23	Industrial or pharmaceutical applications	Martin	Gifford	Lyncean Technologies, Inc	The Lyncean Compact Light Source: X-ray Synchrotron Radiation for Analytical and Imaging Applications
M2	Hybrid methods	Pawel	Grochulski	Canadian Light Source	Future of biological and life-science facilities at the CLS
M32	Hybrid methods	Yang	Ha	Stanford University	XAS studies on Fe2S2 ferredoxin binding with Δ9 desaturatase
M3	Hybrid methods	Nelly	Hajizadeh	EMBL Hamburg Outstation	Intelligent Agents for Improving Data Collection Efficiency at the EMBL P12 BioSAXS Beamline, Hamburg
M16	Macromolecular complexes	Jonathan	Herrmann	Stanford University	Calcium Mediates Structural Dynamics of RsaA, the S-Layer Protein from Caulobacter Crescentus
T16		Masahiko	Hiraki	Mechanical Engineering Center, KEK	Upgrade of automated protein crystallization and imaging system
W4	X-ray/IR imaging/SR-CDI	Chi-Feng	Huang	Department of Applied Chemistry, National Chiao Tung University	Imaging Individual Drug-Carrying Liposome Particles by Free-Electron-Laser Coherent Diffraction
M17	Macromolecular complexes	Tatiana	Isabet	Synchrotron SOLEIL	PROXIMA-1 : macromolecular crystallography beamline @ synchrotron SOLEIL
M18	Macromolecular complexes	Tatiana	Isabet	Synchrotron SOLEIL	Automated devices for BIOSAXS at synchrotron SOLEIL
T24	Industrial or pharmaceutical applications	Vitul	Jain	ICGEB	Structure of prolyl-tRNA synthetase-Halofuginone complex provides basis for development of novel drugs against Malaria and Toxoplasmosis
M30	Membrane proteins	Alexander	Johs	Oak Ridge National Laboratory	Small-angle Neutron Scattering as a probe for leaflet asymmetry in biomembranes

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Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
W13	Dynamics	Daniel	Keedy	University of California, San Francisco	Multitemperature synchrotron crystallography and ligand scanning reveal novel allosteric modulators of the therapeutic target PTP1B
W16	7 years of XFEL in structural biology	Jan	Kern	Lawrence Berkeley National Lab	Mechanism of water oxidation in photosystem II studied by room temperature fs x-ray crystallography and spectroscopy
M4	Hybrid methods	Seung Joong	Kim	UCSF (University of California, San Francisco)	Molecular Architecture and Function of the SEA Complex, a Modulator of the TORC1 Pathway
M31	Membrane proteins	Alexander	Kintzer	University of California at San Francisco	Structure, inhibition, and regulation of a two-pore channel TPC1
W17	7 years of XFEL in structural biology	Elena	Kovaleva	Stanford Synchrotron Radiation Lightsource	Characterization of Ferryl Intermediate in DypB Peroxidase Using Femtosecond Crystallography, Optical and X-Ray Absorption Spectroscopies
W5	X-ray/IR imaging/SR-CDI	Courtney	Krest (Roach)	Stanford Synchrotron Radiation Lightsource	Hard X-ray Fluorescence Imaging and μ-X-ray Absorption Spectroscopy
T2	Bioinformatics and computing	Eugene	Krissinel	CCP4, STFC, Research Complex at Harwell, Rutherford-Appleton Laboratory, UK	CCP4 Web-Services and Cloud Computing Developments
M5	Hybrid methods	Thomas	Kroll	SLAC National Accelerator Laboratory	Extracting Electronic Structure and Bond Strength Information from 1s2p RIXS: Electron Transfer and Apoptosis in the Cytochrome c protein
W6	X-ray/IR imaging/SR-CDI	Ting-kuo	Lee	Academia Sinica, Taiwan	Recovery of missing central diffraction intensities by using template method
W7	X-ray/IR imaging/SR-CDI	Po-Nan	Li	Stanford University	Resolution enhancement of transmission x-ray microscopy using coherent diffraction
W8	X-ray/IR imaging/SR-CDI	Keng	Liang	Institute of Physics, Academia Sinica, Taiwan	Measurement and simulation of interference enhancement in coherent X-ray diffraction imaging of gold nano particles and influenza virus in water at SACLA
M33	Hybrid methods	Hyeongtaek	Lim	Stanford University	Cu K-beta X-ray Emission Spectroscopy as a Probe of Coordination Environments of Cu(I) Sites
M19	Macromolecular complexes	Sheng-Jun	Liu	Stanford University / Xiamen City University	Data filtering method for Correlated X-ray Scattering
M6	Hybrid methods	Michael	Mara	Stanford University	Characterization of the Cytochrome C Iron-Thioether Bond and Its Regulation by the Protein

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Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
W9	X-ray/IR imaging/SR-CDI	Michael	Martin	Advanced Light Source, LBNL	Biological SINS: Broadband synchrotron infrared nano-spectroscopy of biological materials
T17		Tsutomu	Matsui	SLAC National Accelerator Laboratory	Time-resolved SAXS with low sample consumption: a way to pursue conformational changes of biomolecules
T25	Industrial or pharmaceutical applications	Anne	Mulichak	IMCA-CAT/Hauptman Woodward Research Institute	IMCA-CAT Advanced Photon Source Facility for Pharmaceutical Drug Discovery
W18	7 years of XFEL in structural biology	Anna	Munke	Uppsala University	Coherent diffraction of single Rice Dwarf Virus particles using hard X-rays at the Linac Coherent Light Source
W19	7 years of XFEL in structural biology	Yoshinori	Nishino	Research Institute for Electronic Science, Hokkaido University	Controlled Environment Nano-Imaging Free From Radiation Damage by X-ray Laser Diffraction
M20	Macromolecular complexes	Suzanne	Norwood	University of Queensland	Structural characterisation of the retromer complex
T18		Hideo	Okumura	Japan Synchrotron Radiation Research Institute (JASRI)	Present status of SPring-8 macromolecular crystallography beamlines
T10	Science with upgraded SRS	Mutairu Bolaji	Olatinwo	Louisiana State University	Quantitative X-ray Grating-based Interferometry Brown Adipose Tissue in Mice
M21	Macromolecular complexes	Natalia	Orlova	The City College of New York	A plasmid that became a chromosome
W10	X-ray/IR imaging/SR-CDI	Peng	Qi	University of Saskatchewan	A Bent Laue Energy Dispersive Monochromator: An Example Application of Speciation Imaging at the Selenium K-edge
W11	X-ray/IR imaging/SR-CDI	Shenglan	Qiao	Stanford University	Angular correlations of photons from solution diffraction at a free electron laser encode molecular structure
M7	Hybrid methods	Ivan	Rajkovic	SLAC/SSRL	Recent Developments at the Beamline for Biological Small Angle X-ray Scattering BL4-2 at SSRL
T19		John	Rose	SER-CAT/ University of Georgia	SER-CAT/UGA Native-SAD Highlights
M22	Macromolecular complexes	Karthik	Sathiyamoorthy	Stanford University	Structural Basis for Epstein-Barr Virus Host Cell Tropism mediated by gp42 and gHgL Entry Glycoproteins
M8	Hybrid methods	Ursula	Schulze-Gahmen	UC Berkeley	Insights into HIV-1 proviral transcription from an integrative structure of the Tat:AFF4:P-TEFb:TAR complex

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Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
T26	Industrial or pharmaceutical applications	Weifeng	Shang	Illinois Institute of Technology	Recent SAXS developments dedicated for solution scattering of biological macromolecules at the BioCAT beamline 18-ID at the Advanced Photon Source
M23	Macromolecular complexes	Orion	Shih	NSRRC	Linear Oligomerization Process of BAX Revealed from Coexisting Intermediates in Solution
T11	Science with upgraded SRS	Thomas	Sorensen	Diamond Light Source, UK	New MX beamline dedicated to in situ diffraction experiments
T3	Bioinformatics and computing	Sergey	Stepanov	Argonne National Laboratory	Integration of fast detectors into beamline controls at GM/CA@APS: Pilatus3 6M and Eiger 16M
T12	Science with upgraded SRS	Doletha	Szebenyi	MacCHESS, Cornell University	MacCHESS, a Synchrotron Source with Unique Opportunities for structural Biology
W14	Dynamics	Michael	Thompson	University of California, San Francisco	Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Time-Resolved Crystallographic Studies of Protein Dynamics
T13	Science with upgraded SRS	Charles	Titus	Stanford	Probing symmetry, spin, and valency of metal centers via ultra-sensitive soft X-ray detectors
M9	Hybrid methods	Farzaneh	Tondnevis	University of Florida	Solution Structure of an “open” E. coli Pol III Clamp Loader Sliding Clamp Complex
W20	7 years of XFEL in structural biology	Kensuke	Tono	Japan Synchrotron Radiation Research Institute	Platforms for biological researches at SACLA
T20		Chien-chang	Tseng	NSRRC	TPS-05A Protein Microcrystallography Beamline at the National Synchrotron Radiation Research Center
M10	Hybrid methods	Yasufumi	Umena	Okayama University	Estimation of valences and radiation damage of four Mn atoms in photosystem II crystals using anomalous diffraction analysis
T4	Bioinformatics and computing	Ville	Uski	STFC/CCP4	CCP4: a resource for macromolecular crystallography
M24	Macromolecular complexes	Christopher	Warner	University of California, Santa Cruz	Small angle X-ray scattering analysis reveals that introduction of D-glutamate at a critical residue of Aβ42 stabilizes a pre-fibrillary aggregate with enhanced toxicity.
M25	Macromolecular complexes	Jui-Hung	Weng	IBC, Academia Sinica	Uncovering the mechanism of FHA domain-mediated TIFA oligomerization that plays a central role in immune responses
M11	Hybrid methods	Matthias	Wilmanns	European Molecular Biology Laboratory	Molecular mechanism of reversible elasticity in the muscle filament bridge protein myomesin

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Assignment (M=Monday; T=Tuesday; W=Wednesday)	Category	First Name	Last Name	Institution	Abstract Title
W21	7 years of XFEL in structural biology	Rahel	Woldeyes	UCSF	Using XFELs to visualize solvent in the Flu M2 Proton Channel
M26	Macromolecular complexes	Hsiang-Yi	Wu	Institute of Biological Chemistry, Academia Sinica	Structural Basis of the Antizyme-Mediated Inhibition and Degradation of Ornithine Decarboxylase
T27	Industrial or pharmaceutical applications	Nina	Wurzler	BAM	Chemical and electrochemical interaction mechanisms of metal reducing bacteria on steel surfaces
T14	Science with upgraded SRS	Yusuke	Yamada	High Energy Accelerator Research Organization	Upgrade of a macromolecular crystallography beamline, BL-17A, at the Photon Factory
M34	Hybrid methods	James	Yan	Stanford University	L-Edge Spectroscopic Investigation of {FeNO}6: Factors Determining Delocalization vs Antiferromagnetic Coupling
T15	Science with upgraded SRS	Fumiaki	Yumoto	KEK/High Energy Accelerator Research Organization	Photon Factory as a Research Hub in the Platform of Drug Discovery, Informatics, and Structural Life Science
M12	Hybrid methods	Petrus	Zwart	LBNL	Advances in Fluctuation Scattering
W12	X-ray/IR imaging/SR-CDI				

New Methods for Low Resolution Structure Refinement

P.D. Adams[1], P.V. Afonine[1], D. Baker[2], F. DiMiao[2], N. Echols[1], J. Headd[1], O.V. Sobolev[1], T.C. Terwilliger[3]

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[3] Los Alamos National Laboratory, Los Alamos, USA

X-ray crystallography is a well-established technique that is now routinely used to determine the structures of macromolecules. It is able to provide atomic resolution information that has been a prerequisite to the understanding the fundamentals of life, from the structure of the double helix to the structures of intact ribosomes. It is also a method that is central to the development of new therapeutics for human disease. Increasingly, researchers are studying larger complexes and/or systems will inherent flexibility, often leading to low resolution diffraction data. This lack of experimental observations presents challenges for obtaining high quality atomic models. Therefore, we have developed methods for low-resolution crystallographic refinement method, including the combination of the Rosetta energy function with reciprocal-space X-ray refinement in Phenix. These methods will be described along with new real space approaches for refinement of models against cryo-electron microscopy maps.

Single Particle Imaging at the Linac Coherent Light Source

A. Aquila[1]

[1] SLAC National Accelerator Laboratory, Menlo Park, USA

The Single Particle Imaging (SPI) Initiative is a community-involved initiative tasked with the goal of overcoming the technical challenges for reaching better and ultimately atomic resolution in X-ray imaging of single-particles. It has more than 100 participating members from 21 institutions spanning 8 countries. Since its inception, just over one year ago, the SPI Initiative has completed seven experiment at the Linac Coherent Light Source (LCLS) using two different beamtimes; this talk will review the results/technical discoveries from each of the SPI beamtimes and discuss how the field has progressed.

Arsenic and the Gut Microbiome: A Case Study for Application of Synchrotron Radiation in Microbiome Research

S. Bachas-Daunert[1], J. Lezama Pacheco[2], C. Criddle[1], D.A. Relman[3]

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[2] Department of Earth System Science, Stanford University, Stanford, CA, USA
[3] Departments of Medicine and Microbiology & Immunology, Stanford, CA, USA

Synchrotron radiation is rarely employed in microbiome research, despite its potential utility for elucidating important features of intermolecular dynamics, reaction geometries, and speciation in these complex biological systems. Our work serves as a case study for the application of synchrotron science to microbiome research. The human gut microbiota and its genes and genomes (or, the "microbiome") play a role in nutrition, immunity, and detoxification of ingested substances. Conventional risk assessment fails to emphasize possible microbiome interactions with ingested environmental contaminants, instead relying upon universal dose-response curves. We focus on characterizing the effects of chronically ingested arsenic on the gut microbiome, laying groundwork for the novel field of microbiome-based risk assessment and informing future interventions in affected communities worldwide. In the US, 13 million Americans are exposed to arsenic levels exceeding US Water Quality Standards, and over 25 million people in Bangladesh are chronically exposed. Synchrotron analyses at the SLAC Synchrotron Radiation Lightsource (SSRL) play a vital role in tracking the potential arsenic biotransformative capability of the microbiome, as XAS analyses provide an extremely accurate and sensitive tool for detecting arsenic speciation changes at environmental concentrations in mixed solid and liquid phase samples, as well as provide insights into reaction geometries via EXAFS. At SSRL, we analyzed a time series of samples from anaerobic bioreactors inoculated with stool from one healthy human subject under two arsenic regimes: a 1 mM arsenate (As(V)) spike at inoculation and a 0.1 mM As(V) spike at inoculation. Our preliminary feasibility study illustrated that within a 45.5-hour time series, biotransformation of arsenic occurred at significant rates in the 1 mM bioreactor; by hour 29.5, 25% of the total arsenic had been converted from As(V) to As(III), and by hour 45.5, 40% of the total arsenic was As(III). EXAFS data showed suspected arsenic-arsenic interactions. In follow-up analyses of a 54.5-hour time series from one individual's inoculum, we found that (1) the 1 mM As(V) reactor illustrated ~50% conversion from As(V) to As(III) and (2) there were concentration effects, with the 0.1 mM reactor converting As(V) to As(III) at slower rates than the 1 mM regime. We also analyzed the unaltered stool, and detected no arsenic. The results are encouraging, illustrating the potential biotransformative capability of the gut microbiome, along with the viability and utility of this type of sample analysis on synchrotron radiation sources. Synchrotron analyses allow for simultaneous assay of extracellular and intracellular matter, as well as finer insights at the molecular level via EXAFS—both advantages over conventional instrumentation. Future work includes analyses of two US healthy subjects and six Bangladeshi subjects (chronically/minimally exposed, with/without disease) under the same reactor regimes.

Structural Biology of Coronavirus Envelope (CoV E) proteins

B. Baravati[1], J. Carillo[1], B.R. Cherry[1], S.M. Daskalova[1], P. Fromme[1], M. Goryll[1], D.T. Hansen[1], B.G. Hogue[1], H.H. Hogue[1], W. Liu[1], P. Rath[1], W.D. Van Horn[1], M.D. Vaughn[1], J. Yang[1], J. Zook[1]

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The Coronavirus small envelope (CoV E) proteins have been implicated in various stages of the viral life cycle, and are therefore promising drug targets. To this end, structural studies are necessary in fully elucidating the roles of the CoV E proteins in pathogenesis and developing intervention strategies, as well as contributing to the fundamental understanding of viroporins. To date, there is no full length structure reported for any of the Coronavirus small envelope (CoV E) proteins. Here, the recent progress towards solving the structure of CoV Mouse Hepatitis Virus Envelope (MHV-E) protein will be outlined. Development of a single step on-column method provided high purity MHV-E at high yield which overcame a major barrier that has prevented previous structural work, and allowed for rapid screening of different conditions. Ion channel activity of the purified proteins in DPC micelles was confirmed by BLM electrophysiology measurements. The secondary and quaternary structures of CoV E in various micelles were further analyzed using CD spectroscopy and Native PAGE, respectively. TROSY NMR spectra of CoV E were acquired in different micellar and mixed micellar systems to screen for a suitable membrane mimetic and pave the way for resonance assignment and structure determination using solution NMR spectroscopy.

Structure guided approach in the design of inhibitors against pathogenic protozoa targeting aminoacyl-tRNA synthetases

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Human African Trypanosomiasis (HAT), also called Sleeping Sickness, and Visceral Leishmaniasis (VL), also called kala-azar, are devastating and neglected infectious diseases that threaten millions of the poorest people in tropical and subtropical areas. There is an urgent need for better drugs against the causative agents, the protozoan pathogens *Trypanosoma brucei* and two species of *Leishmania* (L. *donovani and L. infantum*), respectively. We target parasitic aminoacyl-tRNA synthetases (aaRS) since they are essential for protein synthesis and therefore potential targets for the development of anti-typanosomatid drugs. Significant sequence differences between the human and parasitic methionyl-tRNA synthetase (MetRS) and tyrosyl-tRNA synthetase (TyrRS) warrant a structure-guided design effort of high affinity and selective inhibitors.

T. brucei MetRS•Met crystals were soaked with multiple different compounds providing more than 50 crystal structures of *T. brucei* MetRS•inhibitor complexes that feed the iterative optimization of inhibitors including compounds obtained initially by high-throughput screening against *T. brucei* MetRS. Our best compounds so far, are able to cure *T. brucei* infected mice in an acute disease model with no signs of toxicity.

For *L. donovani* TyrRS, we used nanobodies as crystallization chaperones in combination with a potent TyrRS inhibitor to solve a 2.4 Å resolution structure that differs importantly from the human counterpart. Interestingly, while the human TyrRS is a homodimer with chains containing each an active site domain and an anti-codon binding domain, the parasitic enzyme is a pseudodimer which likely contains only one functional active site (in the N-terminal half) and one functional anticodon recognition site (in the C-terminal half). In the *L. donovani* TyrRS structure, the inhibitor binds to the N-terminal active site and knowledge of its binding mode may be useful in the process of structure-guided design of new anti-leishmania compounds.

How is movement coupled to catalysis in DNA gyrase?

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Type IIA topoisomerases (topo2As) are essential enzymes that regulate DNA topology by: (i) creating a four base-pair staggered break in one DNA duplex (ii) passing another DNA duplex through the break and (iii) religating the double-stranded DNA break. The bacterial type IIA topoisomerase, DNA gyrase, functions as a B_2A_2 heterotetramer, but BA fusions (equivalent to the dimeric eukaryotic typeIIA topoisomerases) retain activity. We have used fusion proteins of the C-terminal Toprim domain of GyrB, with the N-terminal domains of GyrA to solve multiple crystal structures of DNA complexes of DNA gyrase with both antibacterial (Bax *et al*., 2010, Nature **466**:935-940) and anti-cancer drugs (Chan *et al*., 2015, Nat. Commun. **6**:10048), including structures with two new classes of antibacterial agents currently in phase II clinical trials (gepotidacin, a NBTI related to GSK299423 and AZD0914, a derivative of QPT-1). Gepotidacin (Biedenbach et al., 2016, Antimicrob. Agents Chemother. 60:1918-1923) was developed within GlaxoSmithKline with support of funding from outside agencies - reflecting the growing role of public/private partnerships within the changing landscape of antibacterial drug discovery. In Europe GlaxoSmithKline is part of the New Drugs for Bad Bugs IMI (http://www.imi.europa.eu/content/nd4bb).

This talk will focus on the catalytic mechanism of DNA Gyrase and other topo2As. A fully dissociative mechanism for ATP hydrolysis (and synthesis) at the N-terminal ATP-gate of DNA gyrase will be described (Agrawal *et al*., 2013 Biochem. J. 456:263-273). Our multiple structures of DNA-cleavage complexes with different compounds show how the central DNA-cleavage and religation gate of the enzyme can ‘wriggle’, suggesting that, just as a chemist may use heat, DNA gyrase can use directional movement to protonate specific oxygens on phosphates to catalyse phosphotranferase reactions.

Title: *In situ* structural analysis of the human nuclear pore complex

Abstract

Nuclear pore complexes (NPCs) are fundamental components of all eukaryotic cells. They mediate nucleocytoplasmic exchange, regulate gene expression and are of high human health relevance. Elucidating their 110 MDa structure imposes a formidable challenge and requires *in situ* structural biology approaches. Fifteen out of about thirty nucleoporins (Nups) are structured and form the Y- and inner ring complexes. These two major scaffolding modules assemble in multiple copies into an eight-fold symmetric structure that fuses the inner and outer nuclear membranes to form a central channel of ~60 nm in diameter. The scaffold is decorated with transport channel Nups that often contain phenylalanine (FG)-repeat sequences and mediate the interaction with cargo complexes. Until very recently, it was unclear how the Y- and inner ring complexes oligomerizes *in situ*. We combined cryo-electron tomography with mass spectrometry, biochemical analysis, perturbation experiments and structural modeling to investigate nuclear pore architecture *in situ*. We obtained the most comprehensive architectural model of the NPC to date. We conclude that, similarly to coated vesicles, multiple copies of the same structural building block - although compositionally identical - engage in different local sets of interactions and conformations.

Towards user operations of the SONICC system at GM/CA@APS beamline 23ID-B at the Advanced Photon Source

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Second order nonlinear optical imaging of chiral crystals (SONICC), based on femtosecond laser scanning microscopy, has been implemented at GM/CA@APS beamline 23ID-B for rapid protein crystal localization and centering. The technique is based on infrared laser light impinging on non-centrosymmetric crystals of proteins, which may selectively yield a frequency-doubled, visible signal generated by the anharmonic response of the electron cloud of the protein in response to the laser field. One aim of this method is to locate small crystals grown in opaque crystallization media for centering in X-ray beams of only a few microns or less in cross-section, such as for membrane-protein crystals grown in mesophase. An initial optical system implemented for generation and detection of Second Harmonic Generation (SHG) signals at beamline 23IDB has been described [1]. The system also provides the capability to scan visible laser light across the sample and to detect signals in other imaging modes, and recent work with synchronous digitization provides for user-selectable acquisition of 3 modes of video-rate images simultaneously [2]. For incident green laser light, the 3 modes are (a) fluorescence (FLUO), (b) two-photon-excited UV fluorescence (TPE-UVF), and (c) laser infrared (IR) brightfield (BR). For incident IR laser light, the 3 modes are (d) second harmonic generation (SHG), (e) two-photon excited fluorescence (TPEF), and (f) laser IR brightfield (BR). The upgraded system has increased speed of image acquisition to 15 Hz, improved signal-to-noise by a factor of ~ 15.6, and resolution of crystals down to ~ 1 μm. The SONICCscan GUI for user control of the system at the beamline has been adapted to incorporate the advanced imaging modes, as well as to incorporate user-friendly features for intuitive operation. An update will be provided on these features and on the initial release of the system to the beamline user program.

[1] J.T. Madden, S. Toth, C. Dettmar, J. Newman, R. Oglesbee, H. Hedderich, R. Everly, M. Becker, J. Ronau, S. Buchanan, V. Cherezov, M. Morrow, S. Xu, D. Ferguson, O. Makarov, C. Das, R. Fischetti, G. Simpson, J Synchrotron Rad., 2013, 20, 531-540.

[2] J.A. Newman, S. Zhang, S.Z. Sullivan, X.Y. Dow, M. Becker, M.J. Sheedlo, S. Stepanov, M.S. Carlsen, R.M. Everly, C. Das, R.F. Fischetti, G.J. Simpson, J Synchrotron Rad., 2016 – *in press*

Crystallographic insight into enhanced catalytic activity of carbonic anhydrase II using "activating" ligands

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Carbonic anhydrase is a zinc metalloenzyme whose structure and function plays an important role in therapeutic design. In particular, carbonic anhydrase II (CA II) is the fastest isoform of the enzyme, rapidly catalyzing the interconversion of carbon dioxide to bicarbonate and a proton. This reaction is essential for pH regulation and is instrumental in the function of various neurotransmitters, such as glutamate, GABA and dopamine. In the hydration direction, the enzyme functions by utilizing a catalytic zinc-bound hydroxide for the formation of bicarbonate, leaving a zinc-bound water. To convert the enzyme back to its active form, a proton bound to the zinc must be released via a proton wire and expelled from the active site. A class of synthesized small-molecule ligands has shown to significantly increase CA II's catalytic conversion rate, and have previously demonstrated a strong nootropic effect in rat models, improving memory, learning, and cognition. Since this is the rate-limiting step of the reaction, it is believed that these activating ligands function by assisting in the proton-transfer step, serving as a secondary site for proton release. However, the precise mechanism of this activation was previously unknown. We present the crystal structure of CA II in complex with one such activating ligand, depicting the precise binding motif in the active site to a resolution of 1.6A. This structure validates the postulated method of activation through an enhanced proton shuttling mechanism and provides insight into the endogenous catalytic function of the enzyme. Furthermore, the network of active site water molecules can be closely studied using neutron diffraction to understand the differences in solvent organization with the addition of the activating ligand. This work will vastly impact the design of CA activating ligands for future therapeutic applications.

Structure activity relationships of benzenesulfonamide-based inhibitors towards carbonic anhydrase isoform specificity

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Carbonic anhydrases (CAs) are implicated in a wide range of diseases, including the upregulation of CA IX and XII in many aggressive cancers. However, effective inhibition of disease-implicated CAs should minimally affect the ubiquitously expressed CA I and II to reduce side effects and improve directed distribution of the inhibitors to tumor sites. Four benzene sulfonamide inhibitors are presented here, synthesized using the “tail approach” that display nM affinity for CAs. The crystal structures of these inhibitors bound to a CA IX-mimic and CA II are presented, and their binding interactions analyzed. The crystallographic results demonstrate that the CA IX and II active site residue differences along the hydrophobic pocket- particularly at positions 92 and 131- dictate the positional binding and affinity of inhibitors, and that specific functional groups on the inhibitor tail group modulate CA isoform specificity. Modeling was also performed with each inhibitor docked into CA XII and CA I, identifying important residues that contributed to steric hindrance and will play key roles for drug design. These studies will further our understanding of obtaining high isoform specificity when designing small molecule CA inhibitors.

Crystal structure of the complex of GLPO with an anti-thyroid drug PTU

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Lactoperoxidase is a member of mammalian heme peroxidase superfamily that consists of lactoperoxidase (LPO), myloperoxidase (MPO), thyroid peroxidase (TPO), eosinophil peroxidase (EPO). So far crystal structures of only two mammalian peroxidases LPO and MPO have been determined. The structures of LPO have been determined from the samples obtained from bovine, buffalo, goat and sheep. Their complexes have also been determined with inhibitors and substrate analogues. However, the structures of EPO and TPO have not been obtained so far. Due to significant sequence identity between LPO and TPO, the structures of these proteins are expected to be similar. Similarly their substrates will also have similarity as well as the substrate and enzyme interactions will be similar. Therefore, a complex of LPO was prepared with propylthiouracil (PTU), a compound used as a drug in thyroid ailments. The complex was crystallized using co-crystallization. The crystals of native LPO were also soaked in the solution containing PTU. The crystals belonged to monoclinic space group with cell dimensions a = 80.2 Å, b = 82.5, c = 95.0, β = 73.7º There were four molecules of LPO in the asymmetric unit. The structure determination of the complex revealed that PTU binds to the LPO at the distal heme site. It is held at this site through several hydrogen bonds and van der Waals contacts. The mode of binding and number of interactions suggest that a similar mode of binding may occur with TPO. Based on the information of interactions and the missing potential interactions, the modifications in the structure of PTU are suggested so that the improved design of the TPO inhibitor is obtained.

XFEL Crystal Structure of the Synaptotagmin-1 : SNARE Complex

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Synaptotagmin-1 and neuronal SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein receptor) proteins play key roles in evoked synchronous neurotransmitter release. However, it has been unknown how they cooperate to trigger synaptic vesicle fusion. We recently reported crystal structures of Ca^{2+} and Mg^{2+}-bound complexes between synaptotagmin-1 and the neuronal SNARE complex, one of which was determined with diffraction data from an X-ray free electron laser (XFEL) at the Linac Coherent Light Source (LCLS), leading to a near-atomic resolution structure with accurate rotamer assignments for many sidechains. We developed methods that allowed us to determine the structure on relatively few diffraction images. The crystal structure of the synaptotagmin-1 : SNARE complex revealed a large, specific, Ca^{2+}-independent, and evolutionarily conserved interface. This interface is essential for Ca^{2+}-triggered neurotransmitter release in neuronal synapses and for Ca^{2+}-triggered vesicle fusion in a reconstituted system.

Reference: Zhou, et al. *Nature* **525**, 62-67 (2015).

Macromolecular crystallography at beamline P11 at PETRA III

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The "Bio-Imaging and Diffraction Beamline" P11 at PETRA III currently provides two state-of-the-art endstations for structural investigations of biological samples at different length scales: an X-ray microscope utilizing tender X-rays between 2.4 and 10 keV [1] and a crystallography experiment operated between 5.5 and 30 keV [2]. In future, a third experimental endstation especially dedicated to serial crystallography and pump-probe experiments will be available (see Fig. 1).

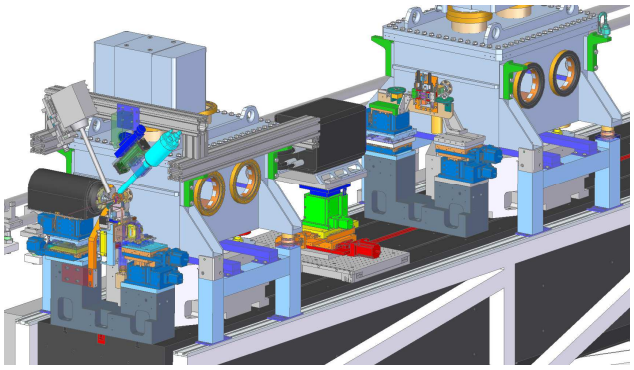

Figure 1: MX at P11. Standard crystallography experiment (left) and future endstation for serial crystallography and pump-probe experiments (right).

Basis of beamline design was to make full use of the excellent source properties of PETRA III and to deliver most of the photons from the source into a very small focal spot at the sample position. The optics concept involves the generation of a secondary source at 65.5 m using three dynamically bendable KB mirrors located in the optics hutch. A second KB system which is installed at 72.8 m can be used for refocusing the X-ray beam down to 4 × 9 μm^2 (v × h, FWHM) with full flux from the source (2 × 10^{13} ph/s at 12 keV). Smaller beam sizes down to 1 × 1 μm^2 with more than 2 × 10^{11} ph/s in the focus can be obtained by slitting down the secondary source at the cost of flux. The flexible X-ray optics allow for tailoring the beam properties to the needs of the experiment: A large parallel beam is available for structure determinations from large unit cell systems, such as large molecular complexes [3]. A highly intense microbeam allows for serial crystallography experiments on microcrystals using liquid delivery systems [4] or Silicon microchips [5, 6].

In addition, P11 is ideally suited for high-throughput crystallography and fast crystal screening. Crystals can be mounted within 10 s using an automatic sample changer. A large storage Dewar provides space for 368 samples.

[1] A. Meents *et al.*, Proc. SPIE 8851, 88510K (2014); [2] A. Burkhardt *et al.*, EPJ Plus 131:56 (2016); [3] K. Schulte *et al.*, BMC Biology 14:14 (2016); [4] F. Stellato *et al.*, IUCrJ 1, 204 (2014); [5] P. Roedig *et al.*, Sci. Rep. 5, 10451 (2015); [6] P. Roedig *et al.*, J. Appl. Cryst. 49, 968 (2016).

Imaging the plant ionome: Synchrotron X-ray fluorescence and its applications in a study of zinc homeostasis in *Arabidopsis thaliana*

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Every organism has a unique elemental composition, or ionome. This elemental profile is shaped by an interplay of various genetic and environmental factors. The ionome varies between individuals and fluctuates as an organism develops and is exposed to different environmental stimuli (e.g., climate, nutrient supply, stress). Mutants lacking functional copies of particular genes may also have an altered elemental profile. In order to quantitatively track changes in the ionome, volume-averaged techniques like inductively coupled plasma mass spectroscopy (ICP-MS) can be applied. Such techniques yield numerical information about the average concentration of different elements, but they do not provide a visual of those elements' spatial distribution. To map the actual localization of elements in tissues and organs, we utilize a spatially-resolved elemental analysis technique, synchrotron X-ray fluorescence (SXRF).

Here, we demonstrate how two-dimensional SXRF imaging can produce an ionomic snapshot of a plant. We show the distinct localization of four macro- and micronutrients in the shoots and roots of an *Arabidopsis thaliana* plant, and provide examples of how SXRF can be used to measure changes in the plant ionome caused by genetic, developmental, and environmental factors.

Additionally, we utilize this technique to help answer one of the ongoing questions in our lab—how do plants acquire, sequester, and distribute zinc? Zinc is an essential micronutrient that serves as a critical cofactor for a myriad of proteins. In humans, zinc deficiency compromises the immune system, gastrointestinal function, neurobehavioral development, and reproductive outcome. Unfortunately, it is estimated that more than two billion people worldwide suffer from zinc deficiency. Because plants are the major source of human dietary zinc, one strategy to reduce the incidence of zinc deficiency is the biofortification of food crops. Of course, the success of this strategy hinges on our understanding of zinc homeostasis in plants.

We commonly use a reverse genetic approach where we analyze mutant plants in order to decipher the function of a particular protein. Because we are interested in the transport of zinc, knowing where the zinc localizes in wild type plants, and how disrupting particular genes alters its localization, is extremely valuable and makes SXRF particularly well-suited to our studies. Using SXRF, we have identified two proteins responsible for the acquisition of zinc from the soil. This knowledge will aid future efforts to engineer crops that are richer in zinc.

Structure and function of K2P ion channels from synchrotron data from Diamond and serial femtosecond crystallography at LCLS.

Liz Carpenter, University of Oxford

Structural biology of membrane proteins is going through a revolution with the advent of new technologies which will allow us to solve structures of ever the most challenging of targets. Standard X-ray crystallography continues to be the workhorse of the field, but new methods such as serial femtosecond crystallography, using free electron lasers, now brings a new dimension to solving protein structures. At the SGC we have developed a pipeline for producing human membrane proteins and solving their structures. These methods have allowed us to begin to understand how the polymodal TREK-1 and TREK-2 K2P potassium channels are regulated by a range of stimuli (Dong et al., Science, 2015). We have solved structures of TREK-2 in several conformations, including as a complex with the inhibitor norfluoxetine, the breakdown product of Prozac. These structures of TREK-2 have suggested mechanisms for inhibition by small molecules and have revealed how mechanical stretch could activate the channel. When studying the structure of the canonical K2P channel, TREK-1, we initially obtained a structure from crystals in high magnesium, which produced an interested, but very unusual conformation of the channel. Crystallisation of TREK-1 in low magnesium concentrations gave nanocrystals which proved to be impossible to optimize for standard crystallography, but worked well for data collection using serial femtosecond crystallography at LCLS. The xFEL dataset give a structure for TREK-1 at 3.0A, which showed a more conventional conformation for TREK-1 and suggests new gating mechanisms for the TREK channels by lipids.

Macromolecular Diffractive Imaging using Disordered Crystals

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X-ray crystallography suffers from the well-known phase problem. This means that it is not possible to reconstruct an image of a molecule from its crystal diffraction pattern without additional measurements or assumptions about the structure. Single-molecule diffraction overcomes this restriction since the continuous diffraction of a non-periodic object gives access to more information than available only at the Bragg peaks produced by crystals. The challenge has been that diffraction from single molecules is exceedingly weak, even when using powerful pulses from an X-ray free-electron laser. We have demonstrated [1] a new method to record "single molecule" diffraction and obtain molecular images. The trick is to place many oriented molecules into the focused X-ray beam, which we do with a crystal! However, we require the crystal to be disordered—the loss of translational order, usually assumed to be bad for crystallography, gives access to the continuous diffraction. We demonstrate this technique by reconstructing 3D images of photosystem II complexes at 3.5 Å resolution, improving upon the 4.5 Å resolution of a structure refinement obtained by conventional crystallographic analysis of the Bragg intensities. Diffraction patterns were recorded at the CXI beamline of the Linac Coherent Light Source of the SLAC National Accelerator Laboratory.

1. K. Ayyer, O. M. Yefanov, D. Oberthür, S. Roy-Chowdhury, L. Galli, V. Mariani, S. Basu, J. Coe, C. E. Conrad, R. Fromme, A. Schaffer, K. Dörner, D. James, C. Kupitz, M. Metz, G. Nelson, P. L. Xavier, K. R. Beyerlein, M. Schmidt, I. Sarrou, J. C. H. Spence, U. Weierstall, T. A. White, J.-H. Yang, Y. Zhao, M. Liang, A. Aquila, M. S. Hunter, J. S. Robinson, J. E. Koglin, S. Boutet, P. Fromme, A. Barty, and H. N. Chapman. Macromolecular diffractive imaging using imperfect crystals. Nature **530**, 202–206 (2016).

Innovative Application of Pressure Cryo-cooling

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High-pressure cryo-cooling is a technique that was developed by Kim et. al to eliminate the need for penetrative cryoprotectants in protein crystals. To reduce damage during cryo-cooling, high pressure up to 200 Mpa, using helium gas was applied prior to rapidly cooling the protein crystal in liquid nitrogen. The development of pressure cryo-cooling opened up new possibilities in structural biology research. One application is to trap reactants or reaction intermediates in protein crystals using biologically active gases in pressurized form. Here, we show that applying pressurized carbon dioxide gas (CO2) on bacterial β-class carbonic anhydrase (bCA) protein crystals could successfully trap its substrate CO2 (Aggarwal et al. 2015).

How crystallography can (and can't) help us find drugs

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Crystallography has become an integral part of the drug discovery process. Classically, it has been used both in structure based drug design during lead optimisation, and to enable lead identification by structure-based virtual screening. Todays high throughput approaches allow crystallography to be used as an assay, both in validating hits from biochemical and biophysical screens, and as a primary assay to detect weak "fragment" compounds.

This presentation highlights the synergistic use of multiple drug discovery approaches within GSK to deliver chemical tools and clinical candidates for epi-enzymes[1-3] and epi-reader[4-6] proteins. These include phenotypic assays, structure/fragment guided methodologies and GSK's proprietary encoded library technology (ELT)[7]. The examples given will illustrate the power of crystallography to influence the direction of drug discovery programs, as well as key challenges where it is powerless to assist.

1 Kruidenier, Laurens, et al. "A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response." *Nature* **488** (2012): 404-408.

2 Lewis, Huw D., et al. "Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation." Nature Chemical Biology **11** (2015): 189-191.

3 S. M. Westaway, et. al "Cell penetrant inhibitors of the JMJD2 (KDM4) and JARID1 (KDM5) families of histone lysine demethylases. Part 1 &2 " J. Med. Chem. **59** (2016): 1357-1369, 1370-1387

4 Nicodeme, Edwige, et al. "Suppression of inflammation by a synthetic histone mimic." *Nature* 468.7327 (2010): 1119-1123.

5 Dawson, Mark A., et al. "Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia." *Nature* 478 (2011): 529-533.

6 P. Bamborough & C. Chung "Fragments in bromodomain drug discovery. " MedChemComm **6**, 1587-1604(2015).

7 Clark, Matthew A., et al. "Design, synthesis and selection of DNA-encoded small-molecule libraries." *Nature chemical biology* 5 (2009): 647-654.

Tailoring Synchrotrons to Membrane Protein Structure Determination

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Over the years membrane proteins have fascinated scientists for playing a fundamental role in many critical biological processes. Located across the native cell membrane or mitochondria wall, integral membrane proteins perform a large diversity of vital functions. Mutations or improper folding of these proteins are associated with many known diseases such Alzheimer's, Parkinson's, cancer and many others. It is estimated that more than one quarter of the human genome codes integral membrane proteins and therefore imperative to investigate the role of these proteins in human health and diseases.

For more than 50 years, synchrotron radiation (SR) has been fundamental in many areas of science including physics, material sciences, chemistry, biology and medicine. In the field of membrane protein structural biology, advances in SR have also revolutionized the field. Despite the advent of many innovative crystallization approaches, the growth of well-ordered membrane protein crystals is still a major problem. Crystals are often very small, extremely fragile, poorly ordered and very sensitive to radiation damage. Collecting data from such crystals is not a straightforward task and the resolution of the X-ray diffraction data not only depends on the crystal quality but also on the characteristics of the data collection apparatus.

A major breakthrough in addressing these difficulties was the arrival of 3rd generation synchrotrons. However, was only with dedicated micro/nano focus beamlines, combined with modern developments in sample handling, sample visualization, automatic crystal centering, cryo-cooling systems, raster scanning systems, fast readout detectors, new data collection strategies and the appearance of new algorithms for merging data collected from different crystals that recently yielded many novel high-resolution membrane protein structures. In addition, the use of *in situ* diffraction screening/data collection of membrane protein crystals has also proved to be enormously valuable. Dealing with large number of crystals that are small in size and extremely fragile to loop mounting (due to mechanical shocks) can be challenging. This technique allows crystals to be tested directly in their crystallization plates at room temperature without any physical manipulation of crystals. Finally, developments in SAXS and CD beamlines are also now playing an important role in Membrane Protein Structure Determination.

TOWARDS A MECHANISTIC UNDERSTANDING OF THE OPIOID MU RECEPTOR ACTIVATION

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ABSTRACT

Opioid receptors (OR), members of the G protein-coupled receptor (GPCR) superfamily, constitute the major and the most effective target for the treatment of pain[1]. The use of opioid drugs acting at these receptors is however a leading cause of death by overdose in Europe and North America. Both beneficial and adverse effects of illicit opioid drugs (opium, heroin) as well as approved therapeutics (morphine and codeine) are mediated by the activation of the mu-opioid receptor (μOR).

We recently described the structure of an inactive and active conformation of the μOR[2],[3] bound to a G protein mimetic nanobody. It provided important information regarding the binding site of small morphinan antagonists and agonists, and demonstrated the key molecular determinants for ligand binding and activation process common to other GPCRs. However, much remains to be learned about the mechanisms by which different agonists can induce distinct levels of Gi protein activation and/or arrestin recruitment upon activation of μOR. Pharmacological and biophysical studies suggest that this versatility can be achieved through the structural plasticity of GPCRs[4].

In this work, we analyze the conformational landscape of the μOR in distinct pharmacological conditions using liquid-state NMR spectroscopy by monitoring signals from methyl-labelled lysines. We also investigate the structure and dynamics changes upon binding to different ligands ranging from agonist to antagonists, as well as upon binding the effector Gs protein and a mimetic nanobody thereof. Our results show that there is very weak allosteric coupling between the agonist binding pocket and G protein coupling interface (transmembrane TM 5 and 6). Furthermore, the analysis provides clues on the successive structural events leading to the full active conformation of μoR [5]. We know extend this approach to biased ligands, that are able to elicit G-protein activation without arrestin activation. A better knowledge of the structural basis of all activation pathways for opioid drug efficacy may lead to new therapeutic approaches with limited side effects.

REFERENCES:

[1] *Melnikova I (2010) Pain market.* ***Nat. Rev. Drug Discov.*** *9(8):589-590.*

[2] *Manglik A, et al. (2012) Crystal structure of the micro-opioid receptor bound to a morphinan antagonist.* ***Nature*** *485(7398):321-326.*

[3] Huang, W, et al. Nature. 2015,524(7565):315-2

[4] *Ghanouni P, et al. (2001) Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta 2 adrenergic receptor.* ***J. Biol. Chem.*** *276(27):24433-24436.*

[5] Sounier, R., et al. (2015) ***Nature*** *524, 375-378.*

Protein-nucleic acids interactions studied by synchrotron radiation and complementary techniques

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Interactions between proteins and nucleic acids (NAs) are essential in many key biological processes. Our projects comprise studies of proteins responsible for RNA synthesis (RNA polymerase) and NAs degradation (nucleases). DNA transcription in gram-positive bacteria performed by RNA polymerase utilizes specific subunits and protein partners. Protein HelD, is a protein partner of *Bacillus subitlis* RNAp, which, in cooperation with the delta subunit, enhances transcription in an ATP-dependent manner and is likely involved in DNA template recycling [1]. Approximate localization of HelD on RNAp was determined, however details of function, structure and interaction with NAs remain elusive. We have cloned and characterized close and distant homologs of *B. subitlis* HelD to characterize their properties and NA binding by a combination of techniques.
Protein-NA interactions in nucleases determine their specificity. In S1-P1 Zn-dependent 3'-nucleases the interactions rely on varied nucleotide binding sites [2-4]. The enzymes with various roles in plants, fungi and bacteria (senescence, securing nutrients, pathogen-host interactions) have modified specificity for the given purpose. TBN1 cleaves ss and dsDNA, RNA and structured RNA, which is reflected by the enzyme surface [2,4]. Fungal S1 nuclease shows strict ssDNA preference. We have determined X-ray structures of a fungal enzyme showing a range of protein-ligand interactions. The required enzyme specificity is connected with arrangement of surface sites. S1-P1 nuclease is also present in gram negative bacteria and protozoan parasites pathogenic to humans [4]. We have first specificity details of an enzyme from an opportunistic pathogen.
The work is supported by CSF (no. 15-05228S) and by MEYS CR (LG14009).

[1] Wiedermannova J, Sudzinova P, Koval T, *et al.* Nucleic Acids Res. 2014;42:5151-63. [2] Matousek J, Podzimek T, Pouckova P, *et al. Neoplasma* 2010;57;339–48. [3] Romier C, Dominguez R, Lahm A, *et al. Proteins* 1998;32;414–24. [4] Koval' T, Lipovová P, Podzimek T, *et al. Acta Crystallogr.* 2013;D69;213-26. [5] Guimarães-Costa AB, DeSouza-Vieira TS, Paletta-Silva R, *et al. Infect Immun.* 2014;82;1732-40.

Multi-Tiered Iterative Phasing for Fluctuation X-ray Scattering and Single-Particle Diffraction

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With recent advances in imaging technology, we are now able to overcome the limitations of traditional imaging techniques by performing new imaging experiments that were previously impossible. One such emerging experimental technique is fluctuation X-ray scattering (FXS), where one collects a series of diffraction patterns from multiple particles in solution using an ultrashort X-ray pulse, which is able to take snapshots below rotational diffusion times of the particles. The resulting images contain angularly varying information from which angular correlations can be computed, yielding several orders of magnitude more information than traditional solution scattering methods. However, determining molecular structure from FXS data introduces several challenges, since, in addition to the classical phase problem, one must also solve a hyper-phase problem to determine the 3D intensity function from the correlation data. In another technique known as single-particle diffraction (SPD), several diffraction patterns from individual particles are collected using an ultrabright X-ray beam. However, the samples are delivered to the beam at unknown orientations and may also be present in several different conformational states. In order to reconstruct structural information from SPD, one must determine the orientation and state for each image, extract an accurate 3D model of the intensity function from the images, and solve for the missing complex phases, which are not measured in diffraction images.

In this talk, we present the multi-tiered iterative phasing (M-TIP) algorithm for determining molecular structure from FXS and SPD data. This algorithm breaks up the associated reconstruction problems into a set of simpler subproblems that can be efficiently solved by applying a series of projection operators. These operators are combined in an iterative framework which is able to simultaneously determine missing parameters, the 3D intensity function, the complex phases, and the underlying structure from the data. In particular, this approach is able to leverage prior knowledge about the structural model, such as finite size or symmetry, to obtain a reconstruction from very limited data with excellent global convergence properties and high computational efficiency. We show results from applying M-TIP to determine molecular structure from both simulated data and experimental data collected at the Linac Coherent Light Source (LCLS).

Multimodal synchrotron spectro-microscopy for elucidation cellular disorders in neuro-degenerative diseases

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Molecular neuroscience is in need of the new techniques that would broaden the set of tools available for elucidation and investigation of known and to be discovered disease features [1]. Current advances in X-ray imaging techniques, and the establishment of dedicated facilities, have made X-ray microscopy an important novel tool for cellular and tissue analysis.

Here, two examples of X-ray imaging aspects in neuroscience will be presented: *i)* the 3D-insight into sub-cellular level changes in cortical astrocytes isolated from a transgenic rat model for Amyotrophic lateral sclerosis, by using soft X-ray full field tomography, and *ii)* hard X-ray fluorescence imaging and spectroscopy on primary dopaminergic midbrain rat neurons relevant for Parkinson's disease.

X-ray cryo-microscopy is a rather novel microscopic approach in life sciences in general, complementary to other conventional microscopy. It can provide information on the organization of cellular organelles and subcellular features in whole intact and unstained cells, at resolution intermediate between that of visible light- and electron- microscopy (around 30-40 nm). The typical single cell intrinsic sample thickness does not limit X-ray microscopy, and thus it can collect data without cell sectioning, or chemical fixation, which in classical microscopy introduces artifacts in cellular elemental and structural compartmentalization. In addition, the correlation of information with visible light-, electron- or infrared-microscopy has been an important source of data in study of biological events at different levels. Combining complementary imaging techniques, including X-ray microscopies, will allow us to generate deeper insight into structural and molecular modification on the cellular/organelles level.

This approach allows us to draw conclusions about pathophysiological role of trace metals and other chemical compounds in different diseases. The final goal is to unify the efforts in different synchrotron based microscopy imaging modalities to understand cellular disorders in neurodegenerative diseases.

[1] T. Salditt and T. Dučić, in *Super-Resolution Microscopy Techniques in the Neurosciences Series*, eds. E. Fornasiero and R. Silvio, 2014, pp. 257–290.

Kinoform diffractive lens based micro focusing upgrade of the macromolecular crystallography beamline X10SA at the SLS

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Beamline X10SA (PXII) is the second undulator beamline dedicated to macromolecular crystallography (MX) at the Swiss Light Source. It is funded by the three beamline partners Hoffman-La Roche, Novartis and the Max Planck Society, and has been in user operation since 2005. Based on the design of the first SLS MX beamline X06SA, it focuses light from the U19 in vacuum undulator to a 50 (h) × 10 (v) μm^2 monochromatic (0.02 % bandwidth) X-ray beam at the sample position, 23.75 m downstream of the source, via a sagittal focusing crystal in the double-crystal monochromator and a vertical focusing mirror, resulting in a flux density of 6.0×10^9 ph sec^{-1} μm^{-2} at 12.4 keV. The increasing amount of micrometer sized crystals as well as progress in dynamic sample delivery systems for serial crystallography makes it necessary to focus to smaller beamspots without sacrificing flux density.

Here we present our current plans to introduce discrete micro-focusing (<5 (h) × 1 (v) μm^2) in an on-demand arrangement using the recently demonstrated kinoform diffractive lenses developed at the Paul Scherrer Institut[1]. Due to the high efficiency of these focusing devices, we expect a flux density of 6.0×10^{10} ph sec^{-1} μm^{-2} at the sample position. Together with the emerging high throughput sample delivery systems, like the HVE injector [2], this system will be invaluable for serial crystallography experiments, as well as for challenging micro-crystal samples.

References

[1] Karvinen, P., et al. (2014). "Kinoform diffractive lenses for efficient nano-focusing of hard X-rays." Optics Express 22(14): 16676-16685.
[2] Botha, S., et al. (2015). "Room-temperature serial crystallography at synchrotron X-ray sources using slowly flowing free-standing high-viscosity microstreams." Acta Crystallogr D Biol Crystallogr 71(Pt 2): 387-397.

3D Cell Structure Imaging with Laboratory Scale Cryo Soft X-ray Tomography

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Cryo-soft X-ray tomography (cryo-SXT) is an extremely powerful technique that allows the imaging of an entire cell in its fully hydrated state with natural contrast. Whole cells up to 10-15 microns thick can be imaged at a 3D resolution approaching 30 nm. Cryo-SXT preserves volatile structures, and since the cell is fully hydrated, avoids artefacts associated with sample shrinkage during dehydration. Cryo-SXT can also image the thickest parts of the cell, including the perinuclear region that contains many of the cell's organelles, which cannot be imaged in 3D by other techniques. Great progress has been made over the last decade in developing cryo-SXT as an imaging technique on synchrotron hosted microscopes [1-4]. Workflows have improved which allow non-synchrotron researchers to access the technique, and significant expertise has been developed in correlating SXT and cryo fluorescence data [5-7]. This amalgamation of techniques integrates 3D molecular localisation data with a high-resolution, 3D reconstruction of the cell. Here we report on the development of a compact lab based microscope that aims to deliver synchrotron performance in a system that will turn cryo-SXT into an affordable, efficient laboratory tool, thus increasing the scope and throughput of possible research projects. The key to this is the development of a sufficiently bright and compact source of soft X-rays.

The technology at the core of the SiriusXT instrument is a high-performance soft X-ray light source based on laser-produced plasma emission with the appropriate size, wavelength and brightness, combined with smart optics whose optical quality is not degraded by the debris generated by the plasma. This unique combination enables the deployment of a lab-scale stable and robust light source suitable for cryo-SXT. We show data on light source performance and first images from our microscope.

[1] C.A. Larabell & K.A. Nugent, Curr. Opin. Struct. Bio. **20** (2010), p623.
[2] G. Schneider et al, Nature Methods **7** (2010), p985.
[3] R. Carzaniga et al, Protoplasma **251** (2014), p449.
[4] J.L. Carrascosa et al, Journal of Structural Biology **168** (2009), p234.
[5] B.P. Cinquin et al, Journal of Cellular Biochemistry **115** (2014), p209.
[6] R. Carzaniga et al, Methods in Cell Biology **124** (2014), p151.
[7] K.C. Dent et al, Methods in Cell Biology **124** (2014), p179.

Serial Millisecond Crystallography of Microcrystals at the Advanced Photon Source

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The scarcity of XFEL facilities severely limits the use of serial femtosecond crystallography (SFX)[1] while synchrotron sources are becoming viable options as a real alternative for serial millisecond crystallography (SMX) experiments. The number of SMX experiments is rapidly growing and, so far, six experiments[2-7] have been reported. Here, we present the first injector-based SMX experiments carried out at a U.S. synchrotron source, the Advanced Photon Source (APS). These experiments were conducted at the GM/CA 23-ID-D beamline. Micro-crystals between 1 and 10 μm from six proteins (lysozyme, thaumatin, PSII, phycocyanin, human adenosine A2A receptor (A2AAR) and beta-2 adrenergic receptor (β2AR)) were delivered to the beam suspended in lipidic cubic phase or agarose, using a high viscosity injector[8]. For each protein target, hundreds of thousands of diffraction patterns were collected in shutterless mode at a repetition rate of 10 Hz with a photon energy of 12 keV, 10 μm beam size, using a Pilatus detector. In-house hit-finding software developed at APS and SFX data-reduction and analysis software suites, Cheetah[9] and CrystFEL[10], enabled efficient SMX data monitoring, reduction and processing. Although, hits were found for almost all proteins tested, the best diffracting crystals were from lysozyme, thaumatin and A2AAR with hit rates of 38.1%, 15.1% and 4.7%, respectively, and corresponding indexing rates ranging from 14.0% to 1.8%. The lysozyme data enabled the determination of the structure from the 2.5 Å data set, demonstrating the feasibility of serial data collection at the APS using 1 – 10 μm crystals of small proteins. Only 11 μL of lysozyme/LCP material was used for this study, which is far below the volumes used in other serial experiments at LCLS. Our results clearly demonstrate that SMX is feasible at the APS with microcrystals. The planned APS-Upgrade will increase the intensity in microfocused beams by at least two orders of magnitude enabling SMX for larger macromolecules.

References:
1- Chapman HN, Fromme P, Barty A, et al. Femtosecond X-ray protein nanocrystallography. Nature, 470 (7332):73-

77 (2011) 2- Gati C, Bourenkov G, Klinge M, et al. Serial crystallography on in vivo grown microcrystals using synchrotron radiation. IUCrJ; 1 (Pt 2):87-94 (2014)
3-Stellato F, Oberthür D, Liang M, et al. Room-temperature macromolecular serial crystallography using synchrotron radiation. IUCrJ; 1 (Pt 4):204-212 (2014)
4- Botha S, Nass K, Barends TR et al. Room-temperature serial crystallography at synchrotron X-ray sources using slowly flowing free-standing high-viscosity micro-streams. Acta Crystallographica Section D, D71, 387-397 (2015)
5- Nogly P, James D, Wang D, et al. Lipidic cubic phase serial millisecond crystallography using synchrotron radiation. IUCrJ, 2 (Pt 2):168-176 (2015)
6- Coquelle N, Brewster AS, Kapp U, et al. Raster-scanning serial protein crystallography using micro- and nano-focused synchrotron beams. Acta Crystallographica Section D: Biological Crystallography; 71 (Pt 5):1184-1196 (2015)
7- Murray, T, Lyubimov, AY, Ogata,CM, et. al. A high-transparency, micro-patternable chip for X-ray diffraction analysis of microcrystals under native growth conditions, Acta Crystallographica Section D, D71, 1987-1997 (2015)
8- Weierstall, U., James, D., Wang, C., White, T. A., Wang, D., Liu, W., et al. Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography. Nature Communications, 5:3309 (2014)
9. Barty A., Kirian R.A., Maia F.R.N.C., Hanke M., et al. Cheetah: software for high-throughput reduction and analysis of serial femtosecond X-ray diffraction data.Journal of Applied Crystallography 47, 1118-1131 (2014)
10. White T.A., Kirian R.A., Martin A.V., et al. CrystFEL: A software suite for snapshot serial crystallography. J Appl Cryst 45, 335- 341 (2012)

High-speed detectors enable synchrotron serial crystallography

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With the recently introduced EIGER, hybrid photon counting enters a new dimension of spatial and temporal resolution and expands the field of x-ray experimentation. The absence of detector noise in combination with an image bit depth of 32 bit and high spatial resolution ensure uncompromised data quality. Continuous read-out with frame rates in the hundreds of hertz and a pixel size of 75 μm open EIGER detectors to various time-resolved and high-throughput experiments.

A short outline of the differences between EIGER and PILATUS3 will highlight key aspects of the new detector technology. The main focus of the presentation will be on synchrotron serial crystallography (SSX). This novel approach to data collection brings strategies developed at X-ray free-electron lasers (XFELs) to synchrotron beamlines. Appropriate sample supports (e.g. mesh loops [1], mesophase sandwiches [2] or micro-patterned Si chips [3]) can be automatically screened for the presence of crystals by their diffraction [1] or by X-ray microscopy [4]. Identified crystals are targeted for the acquisition of partial datasets that are then merged until sufficient data has been acquired for structure solution. Alternatively, crystals can be injected in the beam into the beam in glass capillaries, grease matrices [5] or lipidic cubic phase jets [6]. Individual diffraction images from non-oriented crystals can be merged to yield complete datasets.

SSX allows the study of crystals of sizes formerly thought to require heavily oversubscribed XFELs for their structural study. The crystals can be prepared in aqueous solutions or in lipidic mesophases and at room temperature or at cryogenic temperatures, and can be presented to the beam in a variety of ways. Despite their differences, all kinds of SSX have one thing in common - a critical dependence of their success on noise-free detectors operating at high speed, like EIGER.

1. U. Zander *et al.* (2015) *Acta Cryst.* D**71**, 2328-2343
2. C.-Y. Huang *et al.* (2016) *Acta Cryst.* D**72**, 93-112
3. P. Roedig *et al.* (2015) *Sci. Rep.* **5**:10451
4. J.A. Wojdyla *et al.* (2016) *J. Appl. Cryst.* **49**, 944-952
5. S. Muniyappan *et al.* (2015) *Bio Design* **3**, 98-110
6. P. Nogly *et al.* (2015) *IUCrJ.* **2**, 168-176

Crystallographic data collection using microbeams with a photon-counting detector at PROXIMA2-A

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PROXIMA2-A (PX2-A) is a tuneable microfocus beamline for X-ray crystallography[1], and the latest addition to the world-class facilities for Structural Biology at Synchrotron SOLEIL. The beamline is highly automated, and the sample environment incorporates a high performance goniometer, a large capacity robotic sample-changing system, and an EIGER X 9M photon-counting detector. PX2-A has been designed to handle crystals down to a few microns in size with versatile sample characterisation and data collection methods including; X-ray centring, translation scanning, helical collections, and grid scans, integrated into a user-friendly workflow in the MXCuBE[2] interface.

The sensitivity and relatively small pixel size (75 microns) of the EIGER X 9M coupled with an intense and very stable microbeam offers the possibility to collect excellent quality X-ray diffraction data from small or non-homogenous biological crystals. The current state-of-the-art in beamline technology enables PX2-A users to make informed choices on how to extract optimal crystallographic data from the most challenging and fragile samples. The challenges and opportunities for optimized and ultra-fast data collection strategies exploiting a photon-counting detector with small X-ray beams at PX2-A will be presented.

[1] Denis Duran *et al., J. Physics: Conf Series,* 03/2013

[2] Gabadinho *et al., J. Synchrotron Rad,* **2010**, *17*, 700-707

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Spin-Polarization and Spectroscopic Validation of the Through-Bond Electron Transfer Mechanism of Redox Metalloproteins

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Abstract: Sulfur K-edge XAS spectra of the monodentate sulfate complexes [M^{II}(itao)(SO_4)(H_2O)$_{0,1}$] and [Cu(Me_6tren)(SO_4)] exhibit well-defined pre-edge transitions at 2479.4 eV, 2479.9 eV, 2478.4 eV, and 2477.7 eV, respectively (M = Co, Ni, Cu), despite having no direct metal-sulfur bond. The sulfur K-edge XAS of [Cu(itao)(SO_4)] uniquely exhibits a weak transition at 2472.1 eV, an extraordinary 8.7 eV below the first inflection of the rising K-edge. Ground state and time-dependent Density Functional Theory (TDDFT) calculations indicate electron transfer from coordinated sulfate to paramagnetic late transition metals, which produces spin polarization that differentially mixes the spin-up (α) and spin-down (β) spin-orbitals, producing negative spin density at sulfate sulfur. Sulfur 3p character then mixes into ligand anti-bonding sigma orbitals. This process produced the 2471.2 eV transition. This evidence of spin-radical mixing from Cu(II) through sulfur and across an H-bond bridge into distant sigma-bond molecular orbitals of the itao ligand, provides the first direct spectroscopic confirmation of the through-bond electron transfer mechanism of redox-active metalloproteins.

Rapid Shape Classification of Biological Macromolecules from Small Angle X-ray Scattering Data

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Small Angle X-Ray Scattering (SAXS) is routinely used for examining the low resolution structures and shapes of biological macromolecules in solution. An issue with modeling SAXS data is that the inherently low-information content may result in ambiguous shape assignments. Furthermore, the application of *ab initio* or rigid body modeling to data that has been considered monodisperse, but is in fact measured from a polydisperse sample, results will be deceiving. Therefore we have developed a systematic approach to automatically classify SAXS patterns into defined object classes prior to modeling providing investigators with a qualifier that acts as an independent and unbiased parameter for subsequent data interpretation and model refinement.

Based on 450.000 scattering patterns predicted from geometrical objects with uniform density (BODIES; Konarev et al., 2003) and 550.000 scattering patterns from random chains (EOM; G. Tria et al., 2015) that were randomly split into training and cross-validation data sets, a number of Support Vector Machines has been trained to reliably distinguish between compact objects with or without cavities, extended and flat objects, random chains, as well as “unrecognizable data”, i.e. anything not suitable for the other categories. With almost 95% classification accuracy on the cross-validation dataset, the machine learner system greatly exceeded expectations. To validate the plausibility of the class boundaries we mapped the positions of more than 100.000 high-resolution models deposited in the Protein Data Bank (H. M. Berman et al. 2000) within the classification space. Here, approximately 93% of all structures are classified as compact or compact with cavities, 3% as extended, 4% as flat, 168 as random chain and only 83 are considered not classifiable by the software. This result not only indicates a realistic separation between classes and an excellent coverage of the SAXS space of biological macromolecules, but also highlights the utility of SAXS as a rapid tool for screening high-potential crystallization targets.

In summary, the presented classifier provides a unique and nearly instantaneous method to assess shape from SAXS scattering patterns and provides an independent guide for subsequent modeling procedures. It may thus act as a building block in SAXS expert software systems and automated data analysis pipelines for high-throughput structural screening and modeling.

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Assessing Goodness of Fit with the Correlation Map Test

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Small Angle Scattering (SAS) of biological macromolecules in solution is a universal technique to obtain structural information in low resolution. SAS data collection and analysis at synchotrons are generally highly automated, which often results in large amounts of scattering data and structural models. A vital component of subsequent data interpretation are adequate answers to questions as “Are these data sets identical?” or “Does this model fit the data?”. Traditionally, quantitative assessments of similarity between independent data sets, or between models and data, have been performed using the reduced $|^2$ test, which in addition to the provision of experimental data and fit, also requires that the experimental errors have been correctly estimated. However, accurate error estimates may be difficult to obtain, potentially rendering the classical statistical method invalid to use.

Due to the previous lack of viable alternatives to the reduced $|^2$ test, investigators may disregard test outcomes they consider unlikely, e.g. due to incorrect error estimates, and apply empirical decision rules instead. Such decision rules may lack statistical validity, resulting in incorrect data interpretation and conclusions. Here we present the Correlation Map (CorMap), an approach that sidesteps the problem of explicit error estimation. The pair-wise CorMap test only takes into account the distribution of signs of the residuals, but nonetheless maintains a statistical power to detect differences comparable to that of the reduced $|^2$ test with correct error estimates. Based on the design of the CorMap test, exact probabilities of difference in data-data and data-model fits may be obtained (Franke et al., Nature Methods, 2015).

We conducted extensive simulation studies to compare the statistical validity and power of the CorMap with the reduced $|^2$ test and the $|^2_{free}$ resampling variation test (Rambo & Tainer, Nature, 2013). Here, CorMap shows a statistical power to detect systematic deviations comparable to that of a valid reduced $|^2$ test. Notably, when correct error estimates are provided, the outcomes of the reduced $|^2$ and $|^2_{free}$ tests are identical, i.e., $|^2_{free}$ affords no advantage over the $|^2$ test. In turn, if no, or incorrect, error estimates are available, both the reduced $|^2$ and $|^2_{free}$ tests are invalid whereas the CorMap always maintains its power to detect differences.

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Birth of the Cool: Multitemperature Multiconformer X-Ray Crystallography

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Protein conformational landscapes are complex and predicting the conformational response to physiologically relevant perturbations like mutation or small molecule binding is a major challenge. Often, functionally-relevant states are nearly isoenergetic (separated in energy by a few kT, or less), meaning that at physiological temperatures, multiple conformational states populate the ensemble. Using newly developed multiconformer models of X-ray data, we have shown how population shifts can result from temperature perturbation. Our experience over multiple systems has demonstrated that temperature sensitive conformational states are the same ones used by evolution to create new functions, by small molecules in creating new binding sites, and by enzymes to transit through a catalytic cycle. Using an easily controllable physical perturbation (temperature) to predict the conformational response to physiological perturbations suggests the specific conformations to enforce at allosteric sites to achieve long-range control over protein activity.

Septins, a molecular jigsaw

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Septins are GTP-binding proteins which polymerize into membrane associating hetero-filaments involved in membrane remodelling events and barrier formation. By combining in different ways, septin paralogues can potentially generate hundreds of different hetero-filaments which are based on the polymerization of hexameric or octameric core complexes. In order to form physiologically competent filaments, individual monomers must interact specifically via two inter-subunit interfaces (G and NC) and then associate with neighbouring filaments in order to form higher-order complexes. We are attempting to unravel the structural basis of correct filament assembly and bundling using both crystallographic approaches and a series of complementary biophysical techniques. We demonstrate that a C-terminal coiled-coil domain is important for the recognition of partner septins at one of the NC interfaces as well as contributing to the formation of higher-order assemblies. Furthermore the NC interface at the extremity of the core complex (which is essential for polymerization) presents unusual properties and may be sensitive to GTP binding and hydrolysis. Comparison of the GDP and GTP-bound complexes reveals that consecutive interfaces along a filament are coupled by a sliding strand mechanism which is predicted to affect membrane binding. In summary, our data suggest mechanisms for self-assembly, filament bundling and the importance of GTP binding and hydrolysis for filament assembly and membrane association.

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The Lyncean Compact Light Source: X-ray Synchrotron Radiation for Analytical and Imaging Applications

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The Lyncean Compact Light Source (CLS) is a true miniature synchrotron x-ray source with undulator output x-ray characteristics (inherently monochromatic, tunable, high flux). The compact size (8m x 4m) is accomplished by employing a low energy (45 MeV) electron beam storage ring combined with a sub-micrometer period "laser undulator" replacing the permanent magnets of traditional undulators (see Fig.1).

The output beam of the Lyncean CLS is axially symmetric with 4 mrad beam divergence, a 4% bandwidth and tunable from 8 to 35 keV by changing the energy of the stored electron beam. It delivers 10^{10} photons per second to experimental end stations located outside of the CLS shielded enclosure.

The first commercial installation of a Lyncean CLS is at the Technical University Munich (TUM) in Germany. Applications pursued there are primarily Talbot grating based multi-modal imaging and tomography (quantitative absorption/phase contrast, dark field) and high-resolution x-ray tomography. The Lyncean CLS is very well matched to these measurements due to the inherent coherence property, Monochromaticity (no beam hardening + quantitation) and the high flux.

Analytical applications using specifically developed multilayer focusing optics have been demonstrated at the Lyncean factory in the USA. Protein crystallography with freely selectable x-ray energy to enable advanced phasing techniques such as single wavelength anomalous dispersion (SAD) are possible. Other examples include powder diffraction and small angle scattering to name a few.

Operating the Lyncean CLS has been made extremely simple for users. The complexity of the system is packaged into easy to use interfaces enabling non-experts to run the machine after one week of training.

The special characteristic of the Lyncean CLS of producing a truly symmetric and monochromatic beam without contamination by higher x-ray energies (compared to traditional synchrotrons) allows very simple beam transport systems and experimental stations with relaxed shielding requirements to be utilized.

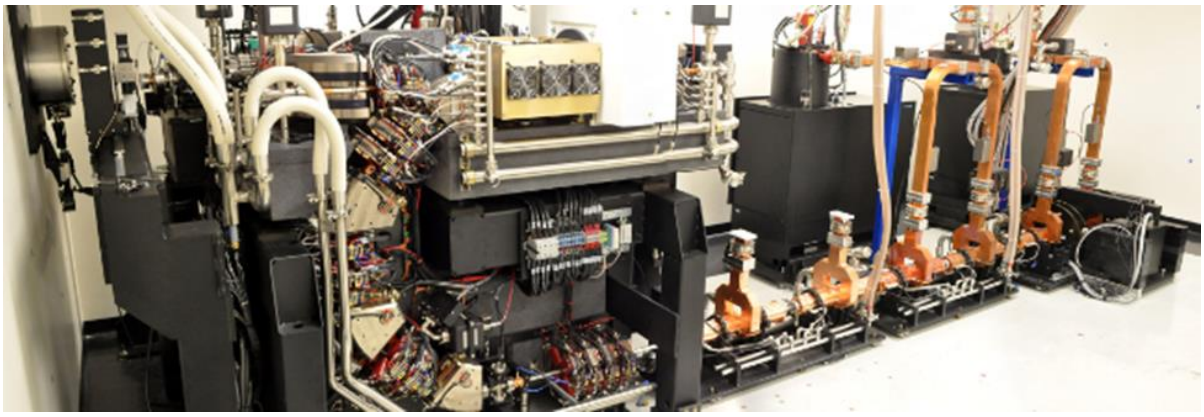

Figure 1. Photograph of the Lyncean Compact Light Source miniature synchrotron.

Hard X-ray photon-in/photon-out spectroscopy at ESRF-EBS

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The second phase of the ESRF upgrade (EBS - **E**xtremely **B**rilliant **S**ource) will improve the horizontal emittance from currently 4000 pm·rad to 147 pm·rad. This will provide an enormous increase of the coherent fraction of the photon flux and allow for significantly reduced spot sizes on the sample. Spectroscopic techniques rarely make direct use of the coherence properties of the incoming X-ray beam and high photon densities aggravate the problem of sample degradation under the beam. Many applications of X-ray spectroscopy in biology will therefore not benefit directly from the higher brilliance.

The smaller horizontal emittance results in a cleaner undulator spectrum with sharper undulator peaks. Spectroscopic techniques that can use the pink beam such as non-resonant X-ray emission (photon-out) spectroscopy (XES) can take advantage of that. A single pink beam pulse may contain more than 10^{11} photons in the undulator fundamental energy around the K-edges of 3d transition metals. Inspired by the research performed at XFELs the ESRF is now planning experimental stations for single shot XES experiments.

Biological samples often exhibit low absorber concentrations and X-ray spectroscopy must be performed in fluorescence detection mode. Experiments on dilute and radiation sensitive samples thus greatly benefit from improvements of the X-ray detection instrumentation. Photon-out spectroscopy in the hard X-ray range employs perfect crystal Bragg optics to achieve an energy bandwidth that is below the core hole lifetime broadening which is on the order of 1eV. The technique provides information on the sample well beyond what can be obtained with standard x-ray absorption spectroscopy (XAS) alone. Photon-out spectroscopy has experienced an enormous increase in interest in recent years mainly for two reasons: 1) User-friendly theoretical codes have been developed that provide a direct link between spectral features and the electronic structure around the absorber atom and 2) free-electron lasers have embraced the technique because of its suitability for single-shot experiments.

The quality of spherically bent Bragg crystals has improved considerably over the past years. Smaller bending radii allow increasing the luminosity of the spectrometer without broadening the energy bandwidth. At the same time, spectrometers were developed that capture large solid angles around the sample. The ESRF is currently designing a new, high luminosity instrument for the energy range 1.5 - 5 keV that operates in Johansson geometry. This instrument will be ideally suited for measurements with absorber concentrations of tens of ppm and thus open up new possibilities in biology research.

Future of biological and life-science facilities at the CLS

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The Canadian Light Source Inc. (CLS) is a mid-size 3rd generation 2.9 GeV synchrotron located on the campus of the University of Saskatchewan in Saskatoon. The newly created Bio/Life Sciences department is composed of the following facilities: Canadian Macromolecular Crystallography Facility (CMCF), Mid Infrared Spectromicroscopy (Mid-IR), Biomedical Imaging and Therapy (BMIT) and, recently built, Biological X-ray Absorption Spectroscopy (BioXAS) which is currently being commissioned. The department is well equipped to study biological objects ranging from atomic resolution (CMCF and BioXAS) to cells and tissues (BioXAS and Mid-IR) through to larger samples such as organs, live animals and plants (BMIT). Since 2006, researches acquired data at the Bio/Life Sciences beamlines to produce a total of 540 peer-reviewed articles, 59 doctoral theses and 49 masters theses used data acquired at the associated beamlines. The CMCF is composed of two beamlines and it serves more than 65 Canadian and some international labs. The techniques available at the CMCF are as follows; high resolution macromolecular X-ray crystallography, Multi/Single wavelength Anomalous Dispersion (MAD/SAD), small molecule crystallography and EXAFS on crystals. BMIT is composed of two beamlines. The following techniques are available at BMIT; conventional absorption imaging, Diffraction Enhanced Imaging (DEI), K-edge Subtraction (KES), Multiple Image Radiography (MIR) and Phase Contrast Imaging both in planar and Computed Tomography (CT) mode. BioXAS is composed of 3 beamlines. Two of them are dedicated to X-ray absorptrion spectroscopy and are being commissioned, while one will be a multi-mode X-ray fluorescence imaging line. Techniques include X-ray Fluorescence (XRF), X-ray Absorption Spectroscopy (XAS) Imaging, multi-mode X-ray fluorescence imaging & micro-XAS. The beamlines are tailored for biological and health-related studies of metals in living systems using X-ray absorption spectroscopy (XAS) and XAS-imaging. At the Mid-IR beamline, the following experiments are performed; full field spectromicroscopy at diffraction-limited spatial resolutions (single point mapping and large area mapping utilizing a Focal Plane Array detector), Photoacoustic Spectroscopy, Polarization Modulation IR Spectromicroscopy and Time-Resolved measurements. At the Mid-IR beamline, the following experiments are performed; full field spectromicroscopy at diffraction-limited spatial resolutions (single point mapping and large area mapping utilizing a Focal Plane Array detector), Photoacoustic Spectroscopy, Polarization Modulation IR Spectromicroscopy and Time-Resolved measurements. Future developments at each facility will be discussed.

XAS studies on Fe2S2 ferredoxin binding with Δ9 desaturatase

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Fe2S2 ferredoxins can bind with Δ9 desaturatase to facilitate the electron transfer from the latter to its substrate. Previous studies suggest that this may be due to the change of bond covalencies of iron-sulfur clusters, which are very sensitive to their local environment. In this study, we tried to use S K-edge XAS, coupled with protein crystallography, docking models and DFT calculations to model the environmental change and evaluate the impact. we show that the covalency change of the dry protein is due to the reorientation of the H-bond at the surface of ferredoxin, and the covalency change of the bond form is because of the lost of solvent water upon binding.

Intelligent Agents for Improving Data Collection Efficiency at the EMBL P12 BioSAXS Beamline, Hamburg

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Small Angle X-ray Scattering (SAXS) is becoming the method of choice for sample characterization and low resolution shape determination of biological macromolecules in solution (Graewert & Svergun, 2013). Among other advantages, SAXS has the ability to directly control and rapidly screen diverse sample environments, probing structural changes caused by altering sample conditions. Previous advances in the automation of SAXS data collection and processing (Franke et al., 2012; Graewert et al., 2015) have already significantly reduced the workload for scientists using the EMBL P12 BioSAXS beamline at PETRA-III (DESY, Hamburg) with respect to data collection and analysis (Blanchet et al., 2015). Nonetheless, the local staff has the responsibility to equip the users with a knowledge base of the beamline and its inner workings to hundreds scientists visiting P12 each year, providing them with basic troubleshooting capabilities.

To strengthen this effort, we developed intelligent agents to help supervising the instrument and to provide users with meaningful information and support at all times. Here, an intelligent agent is a software system that, based on sensory inputs may (autonomously) act in the beamline environment to optimize the SAXS data collection. Currently two such agents are available at the beamline. First, a recommender system continuously monitors the stochastic environment for critical events like beam drift or loss, capillary fouling, but also for the availability of consumables and resources, notifying users of actions required. In addition, the recommender is able to provide the user with explicit instructions on how to resolve common hardware issues, suggesting the appropriate step-by-step actions to transform the system into a state where data collection is possible again. Once this is achieved, the second (scheduling) agent computes the optimal data collection strategy for the specimens currently marked for measurement. For this, the scheduler assesses the beamline status as well as the requirements of the sample, for instance any changes in temperature. The agent also considers the different sample environments (SEC-SAXS, regular batch mode) and adapts the measurement approach accordingly. This strategy is dynamically updated and recomputed as the beamline state changes, supporting and enabling the visiting scientist to make the most efficient use of the allocated beamtime slot.

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Graewert MA, Svergun DI. (2013) *Curr. Opin. Struct. Biol.* 23(5)
Graewert MA et al. *Sci Rep* 5
Blanchet CE, et. al. (2015). J Appl Crystallogr 48(Pt 2):
Franke, D.,et. al. (2012). Nuc Inst Meth A.

Probing the mechanics of molecular machines with electric fields and X-rays

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The 3D structure of proteins guides their motions. These restricted motions are important for each aspect of protein function but are hard to study experimentally. I will describe a new method combining strong electric field pulses to exert precise forces on proteins, and time-resolved crystallography to see the resulting motions in detail. These experiments lay the basis for a systematic mapping of the mechanical properties of proteins and their role in protein function.

Calcium Mediates Structural Dynamics of RsaA, the S-Layer Protein from *Caulobacter Crescentus*

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Surface layers are paracrystalline, proteinaceous cell wall structures found in archaea and many bacteria. This evolutionarily conserved macromolecular structure has been implicated in diverse functions including pathogenicity, adhesion, protection, maintaining cell shape, and is thought to be one of the oldest forms of a cell wall. Due to their stability and paracrystalline properties, surface layer proteins have been utilized for a variety of biotechnological applications including nanopatterning and antigen display. While it is generally accepted that S-layers cover the entirety of the cell, the mechanisms that allow them to form a network of 2D crystalline patches are predominantly unknown. In *Caulobacter crescentus*, a ubiquitous alphaproteobacterium found in freshwater environments, the S-layer consists of one 98 kDa protein, RsaA, that self-associates into a hexagonal crystal lattice in the presence of calcium ions. Previous research indicates that although the S-layer covers the entire surface of the cell, not all of the protein is crystalline. Here, we explore the various structural states of RsaA using a variety of structural and biochemical techniques including electron microscopy, protein stability assays, and Small Angle X-ray Scattering/Diffraction. We have found that calcium mediates the structural dynamics of RsaA in multiple ways. The biophysical mechanisms by which S-layer proteins adapt their structure to the extracellular environment promise to hold insights into cellular physiology as well as greater manipulation and control of these distinct nanomaterials.

Upgrade of automated protein crystallization and imaging system

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Synchrotron X-ray crystallography is one of the most powerful techniques to determine protein crystal structures. However, crystallization process remains one of the bottlenecks in crystallographic analysis. In order to screen thousands of crystallization conditions automatically, we have developed fully-automated large-scale protein crystallization and monitoring system named PXS [1]. The PXS consists of sitting drop method dispensing system, plate handling robot, incubators, imaging system and storage server. We have operated the PXS for more ten years and we are now upgrading to realize more efficient crystallization, especially a dispensing system and an imaging system. Although the dispensing system of PXS was special ordered for high-throughput crystallization, new dispensing system was developed using commercially available devices for reduction in development period and easier maintenance. Liquid handling device Mosquito LCP (ttplabtech), dispensing device BIOTEQUE (MS-Technos), microplate sealer PS (micronix) and a special designed consumables supplying device are placed around plate handling robot MOTOMAN (Yasukawa). We replaced imaging system to RockImager2 (Formulatrix) for frequently imaging and SONICC (Formulatrix) for special UV and SHG imaging. The new dispensing and imaging systems are connected to the plate handling robot of PXS and the crystallization plates are carried to the incubators of PXS. In addition, we developed incubator that includes the imaging device RockImager2. This new incubator is also connected to the plate handling robot of PXS and mainly used for low temperature crystallization.

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[1] M. Hiraki, R. Kato, M. Nagai, T. Satoh, S. Hirano, K. Ihara, N. Kudo, M. Nagae, M. Kobayashi, M. Inoue, T. Uejima, S. Oda, L. M. G. Chavas, M. Akutsu, Y. Yamada, M. Kawasaki, N. Matsugaki, N. Igarashi, M. Suzuki and S. Wakatsuki., Acta Cryst. D62, 1058-1065, 2006.

Rapid 2D and 3D IR imaging Applied to Biologically and Chemically Complex Systems

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The holy grail of chemical imaging is to provide spatially and temporally resolved information about heterogeneous samples on relevant scales. Synchrotron-based Fourier Transform infrared imaging[1] combines rapid, non-destructive chemical detection with morphology at the micrometer scale, to provide value added results to standard analytical methods. Hyperspectral cubes of spatially and spectrally resolved data (x,y,z,Abs(λ))) are obtained employing spectromicrotomography[2], which is a label free approach. This method inherently evaluates a broad array of wide organic materials, with minimal sample preparation and modification. Examples presented here (polymer composites, single cells and colonies of cells) demonstrate the broad applicability of this approach to detect complex chemical information of intact samples.

References

1 Nasse, M. J., Walsh, M. J., Mattson, E. C., Reininger, R., Kajdacsy-Balla, A., Macias, V., Bhargava, R., and Hirschmugl, C. J. (2011) *Nat.Methods* **8,** 413-416

2 Martin, M. C., Dabat-Blondeau, C., Unger, M., Sedlmair, J., Parkinson, D. Y., Bechtel, H. A., Illman, B., Castro, J. M., Keiluweit, M., Buschke, D., Ogle, B., Nasse, M. J., and Hirschmugl, C. J. (2013) *Nat.Methods* **10,** 861-864

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Fingerprinting Redox and Ligand States in Protein Crystals: Applications for Validation and Determining Structural Movies

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It is crucial to assign the correct redox and ligand states to crystal structures of proteins in order to gain valid functional information and to prevent misinterpretation. Single-crystal spectroscopies, particularly when applied *in situ* at MX beamlines during the collection of crystallographic data, allow spectroscopic validation of redox and ligand states, comparison with solution data and the identification of reaction intermediates. I will describe recent developments in methodology [1] and data analysis [2], together with applications to mechanistic and ligand binding studies of Cu and heme proteins. The combination of *in situ* spectroscopies with X-ray-induced redox reactions allows characterisation of multiple intermediate states accessible by electron transfer, and the determination of X-ray driven 'structural movies' of enzyme reactions.

Our approach provides a powerful integrated methodology relevant to a wide range of protein systems.

References

[1] Kekilli, D., Dworkowski, F.S.N, Pompidor, G., Fuchs, M.R., Andrew, C.R, Antonyuk, S., Strange, R.W., Eady, R.R., Hasnain, S.S. & Hough, M.A. (2014) Fingerprinting redox and ligand states in haemprotein crystal structures using resonance Raman spectroscopy. Acta crystallographica. Section D, Biological crystallography, 70(5), 1289-1296.

[2] Dworkowski, F.S.N., Hough, M.A., Pompidor, G. & Fuchs, M.F. (2015) Challenges and solutions for the analysis of in situ, in crystallomicro-spectrophotometric data. Acta crystallographica Section D, Biological crystallography 71(1), 27-35

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[2] Dworkowski, F.S.N., Hough, M.A., Pompidor, G. & Fuchs, M.F. (2015) Challenges and solutions for the analysis of in situ, in crystallomicro-spectrophotometric data. Acta crystallographica Section D, Biological crystallography 71(1), 27-35

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Imaging Individual Drug-Carrying Liposome Particles by Free-Electron-Laser Coherent Diffraction

Chi-Feng Huang[1,2], Po-Nan Li[1],Tsung-Tse Lee[1], Tsui-Ling Hsu[3], Yi-Yun Chen[1], Shun-Min Yang1, Yoshitaka Bessho[1], Shih-Hsin Huang[4], Wei-Hau Chang[4], Yasumasa Joti5, Takashi Kimura[6], Yoshinori Nishino[6], Ting-Kuo Lee[2], Peilin Chen[7], Cheng-Zhi Shi[8], Wei-Hsiang Wang [8,] Yu-Fang Hu[8], Chi-Huey Wong[3] , Keng S. Liang[1] and Y. Hwu [1]

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We used the SACLA (RIKEN/HARIMA, Japan) X-ray free electron laser (X-FEL) to implement coherent diffraction imaging (CDI) of individual liposome particles in water, with or without inserted doxorubicin nanorods. In spite of the low cross section, the diffracted intensity of blank (drug-free) liposomes was sufficient for spatial reconstruction yielding quantitative structural information. When the particles contained doxorubicin, we could measure the structural parameters of the nanorods. In both cases, the information went well beyond what can be obtained by small-angle X-ray scattering (SAXS) and electron microscopy. This is important for the potential drug efficiency optimization and, in general, for X-FEL analysis of individual low-cross-section nanoparticles.

PROXIMA-1: macromolecular crystallography beamline @ synchrotron SOLEIL

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The beamline PROXIMA-1 at the synchrotron SOLEIL classically operates at 450 mA stored current with world-class stability and flux (1.4 x 1E12 ph/s @ 8keV). The beamline is equipped with a full kappa goniometry, a pixel detector (Pilatus-6M, 25Hz) to speed up data collection, and operates at a monochromatic beam with a tunable energy in a range between 5 - 15 KeV, which covers most of the commonly exploited edges for anomalous experiments. The sample exchange robot CATS is fully implemented on the beamline, configured to interface with sample cassettes of the UniPuck-1 format. We recently improved the quality of the data collected through the installation of a capillary / pinhole that allows reducing air diffusion and scattering to unreached levels. Coupled with a newly designed direct beam-stopper, exceptionally low background noise diffraction images can be recorded.

The sample environment on the beamline has been designed for accepting the maximum flexibility for allowing performing various types of experiments. The latest developments include the implementation of removable devices to permit *in situ* data measurements. In this context and as an example of the potentials of the beamline, concrete results on *in house* produced microchips will be presented.

AUTOMATED DEVICES FOR BIOSAXS AT SYNCHROTRON SOLEIL

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SWING is the SAXS/WAXS beamline at Synchrotron SOLEIL, dedicated to structural biology and soft condensed matter. For solution biology, Agilent HPLC modules, including two independent circuits of purification directly coupled to the SAXS measurement cell, are implemented for Size-Exclusion online purification (SEC) [1], allowing to record data under two different buffer conditions without waiting for column equilibration between two shots. Using columns with small volumes (2.5mL), complete elution profiles can be obtained in as short time as 10 minutes, with enough resolution to separate macromolecules from aggregates. In the course of the elution, a SAXS acquisition of buffer frames is automatically performed before the dead volume and another set of frames are collected during the elution of the sample. The SEC-SAXS procedure naturally results in an optimized buffer subtraction and a range of concentration with a single injection.
Very simple to use Graphical User Interfaces were generated using the Passerelle graphical language, to let the user parameterize and launch the sequences of SEC elutions. The user can program a series of injections and parameterize each of them independently. A sequence of multiple HPLC injections and their related SAXS acquisitions can then be launched with as a single click. For an easier understanding of all the acquired data, both the SAXS data and the UV-Vis absorption reports from the Agilent device are automatically named according to the sample acronym and stored in the same folder.
An efficient and compact home-made autosampler is also available for direct injection. The time between two successive injections is close to 4 minutes for 40μL of sample, including automated cleaning and drying of the cell. The sample followed by an air bubble is pushed by a pumping set-up filled with water within a previously dried capillary, resulting in the absence of dilution effects or spurious air bubble generation. A dedicated interface, very similar to that used for the SEC experiments, allows users to switch in less than 2 minutes between direct and SEC experiments without any intervention on the instruments.

[1] David, G.; Perez, J. J. Appl. Crystallogr. 2009, 42, 892– 900

Macromolecular Crystallography at SACLA

So Iwata
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Radiation damage of crystals is one of the most hampering problems in the current macromolecular crystallography. The X-ray beams at the latest beamlines, including microfocus beamlines, are so intense that the crystals suffer serious radiation damage during even very-short exposure-time. The problem is particularly severe for crystals of membrane proteins or macromolecular assemblies, which are extremely radiation sensitive. X-ray Free Electron Laser could provide a solution to this problem. Very high dose rates delivered by the intense femtosecond pulses of XFELs reduce the amount of damage suffered by a crystal during its irradiation. Single shot diffraction patterns are collected from a series of small crystals and by combining them, we could swiftly complete the dataset without any serious radiation damage. At the Japanese XFEL facility, SACLA, we are currently developing a data collection system focusing on drug-target protein crystals including those from membrane proteins and flexible multi-modular proteins. The system is composed of a diffraction chamber with a sample injector and a fast readout multiport CCD (mpCCD) detector. The sample injector is optimized for the data collection from crystals in the lipidic cubic phase (LCP), which are common for membrane proteins. The injector is also capable to handle the crystals obtained from solutions by making the solution viscous using additives including gels and grease. The system requires only several 100 micrograms of proteins to complete the dataset. The system can dramatically accelerate the structure determination of membrane proteins.

The SFX system is also suitable for time-resolved crystallography including visualization of ultrafast protein structural dynamics on the femtosecond to picosecond time-scale, as well as time-resolved diffraction studies of non-cyclic reactions. I am convinced that this will lead to a revolution of time-resolved protein crystallography. In my talk, I will present some recent results of pump and probe experiments at SACLA.

＜CV＞

He was awarded a PhD at University of Tokyo in 1991. He joined Imperial College London in 2000 (- 2015) as the Chair of Membrane Protein Crystallography. He also served as a Diamond Fellow at Diamond Light Source, Oxford. Since 2007, he has undertaken a position of Professor at Graduate School of Medicine, Kyoto University. Since 2012, he has been serving as the group director of SACLA Science Research Group, RIKEN SPring-8 Center. His current research interest includes X-ray crystallography of membrane proteins, macromolecular assemblies, G-protein-coupled receptors (GPCR) and protein crystallography using free electron laser.

Structure of prolyl-tRNA synthetase-Halofuginone complex provides basis for development of novel drugs against Malaria and Toxoplasmosis

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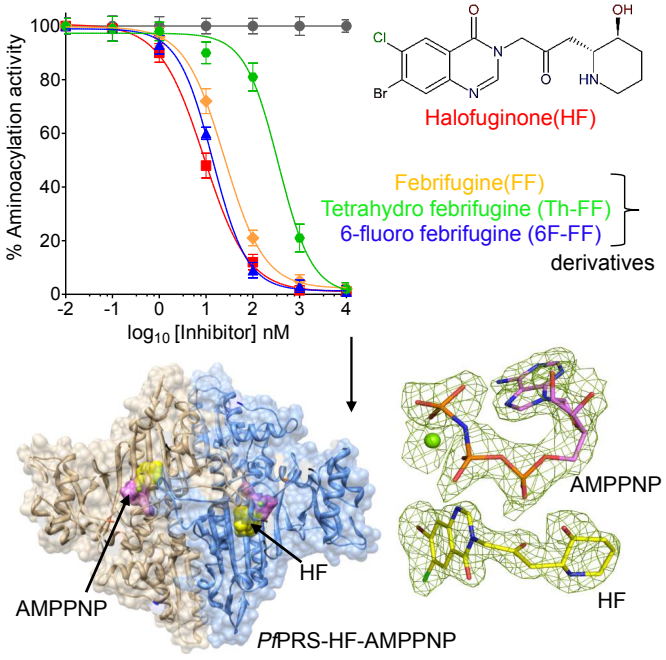
The natural herb *Dichroa febrifuga* has treated malaria-associated fever traditionally. Its active component febrifugine (FF) and derivatives like halofuginone (HF) are potent anti-malarials. Here, we show that FF-based derivatives arrest parasite growth by direct interaction with and inhibition of protein translation enzyme prolyl-tRNA synthetase (PRS). Dual administration of inhibitors that target different tRNA synthetases suggests high utility of these drug targets. We reveal ternary complex structure of PRS-HF and AMPPNP where the latter facilitates HF integration into PRS active site. Structural analyses also highlight spaces within PRS architecture for HF derivatization of its quinazolinone but not piperidine moiety. We also show remarkable ability of HF to kill the related human parasite *Toxoplasma gondii*, suggesting wider HF efficacy against parasitic prolyl-tRNA synthetases. Hence, our cell, enzyme and structure-based data on FF-based inhibitors strengthen case for their inclusion in anti-malarial and anti-toxoplasmosis drug development efforts.



Small-angle Neutron Scattering as a probe for leaflet asymmetry in biomembranes

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The distribution of phospholipids in cellular membranes is almost universally asymmetric with individual leaflets containing different lipid species at varying concentrations. This asymmetry is essential for many biological functions such as protein-membrane interactions, membrane trafficking, and cellular signaling. Membrane asymmetry in living cells is maintained by the activity of translocases, which actively transport specific lipid species across membrane bilayers. We have purified and reconstituting the well-characterized *E. coli* phospholipid ABC transporter MsbA into proteoliposomes that mimic the phospholipid composition of a physiological bacterial membrane. The translocation of phospholipids by MsbA resulting in membrane asymmetry was followed using small-angle neutron scattering (SANS). SANS is exceptionally well suited for studying the phospholipid distribution perpendicular to the bilayer plane due to its nondestructive nature and sub-nanometer resolution. Furthermore, the hydrogen/deuterium (H/D) neutron scattering contrast from isotopic phospholipid labeling enables us to track the translocation of lipid membrane components without introducing bulky fluorescence or spin labels. In this study, we describe SANS experiments to study the MsbA-mediated translocation of the phospholipid phosphatidylethanolamine (PE) between proteoliposome leaflets and the formation of asymmetric bilayers. This new approach for probing membrane asymmetry in active proteoliposomes is a key step towards the development of model systems with sustained asymmetry and will help elucidate role of asymmetric membranes for biological processes at cellular interfaces.

Cell surface signalling systems, structural insights in developmental biology.

E. Yvonne Jones

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Cell-cell signalling is central to many developmental processes and, conversely, is implicated in a number of cancers. The interaction of an extracellular ligand with a cell surface receptor is central to the signalling process, however, for many systems regulation of receptor activation and functional output involves additional interactions and multi-component cell surface assemblies. Multi-disciplinary analyses, combining a range of structural methods with functional studies, are providing insight into the ligand-receptor, receptor-receptor and receptor-membrane interactions which contribute to the mechanisms of signalling and regulation of these systems. I will illustrate this integrated approach with recent results from my laboratory.

Multitemperature synchrotron crystallography and ligand scanning reveal novel allosteric modulators of the therapeutic target PTP1B

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Predicting conformational coupling within proteins is a current challenge with significant implications for basic biology and therapeutic development. Recently, X-ray crystallography has been used to reveal temperature-dependent multiple conformations for coupled residues; this approach provides a new avenue for connecting protein conformational heterogeneity to important biological functions like allosteric regulation. We have combined novel structural and chemical biology techniques to comprehensively map the inherent allosteric capability and small-molecule bindability of the phosphatase PTP1B, a highly validated but "undruggable" target. First, we use multiconformer modeling and direct comparison of electron density maps to reveal differences in conformational heterogeneity as a function of temperature throughout PTP1B, including >8 Å opening/closing of the dynamic active-site loop and dramatic disordering/ordering of the C-terminal helix. Second, we scan the entire surface of PTP1B with drug-like small molecules using the site-directed tethering method to identify binding hotspots. Strikingly, our higher-temperature multiconformer structures for apo PTP1B recapitulate the same conformations seen in the previously solved cryogenic-temperature structure of PTP1B in complex with a moderate-affinity allosteric inhibitor, revealing that the free enzyme pre-samples both the active state and the allosterically inhibited state. Moreover, our newly resolved multiple conformations and binding hotspots converge to a previously hidden, putatively allosteric binding site 30 Å from the active site. A more focused tethering screen at this site identified small molecules that significantly inhibit or activate enzymatic function. We hypothesize that these molecules operate by differentially modulating conformational heterogeneity along the predicted allosteric pathway. The new site is distinct from other previously reported allosteric sites in PTP1B, and has several sequence differences compared to the closest homolog, TCPTP, offering the possibility of specifically regulating PTP1B activity. Our hybrid structural/chemical approach is entirely general, and may yield insights into allostery and druggability for other systems.

Mechanism of Water Oxidation in Photosystem II Studied by Room Temperature fs X-ray Crystallography and Spectroscopy

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Photo-induced oxidation of water in the dimeric membrane protein photosystem II (PSII) is responsible for most of the dioxygen in the atmosphere. We obtained the *room temperature* structures of cyanobacterial PSII in the dark and various illuminated states at ~2.5 Å resolution using fs pulses of the X-ray free electron laser LCLS. Distinct differences in the overall structure compared to the reported *cryogenic temperature* structures are observed, which include higher side-chain mobility with multiple conformers, expansion of the dimer in the membrane plane with changes in the helix orientations, and longer cofactor-cofactor distances. On the other hand, in the S_1-state, the Mn_4CaO_5 cluster is similar to the structure at cryogenic temperature. Major structural changes are not observed either in the peptide backbone or the Mn_4CaO_5 cluster between the dark and illuminated states, precluding mechanisms that require large changes in the S_3 state. In order to establish the O-O bond formation mechanism in PSII the binding sites of the substrate waters on the catalytic Mn_4CaO_5 cluster in the OEC must be determined. NH_3, a water analog, known to bind to the Mn_4CaO_5 cluster in the S_2 and S_3 states can be used to distinguish substrate from non-substrate waters. We used a combination of EPR and EXAFS data together with our room temperature X-ray diffraction data to investigate the potential binding sites for NH_3 at the Mn_4CaO_5 cluster. In addition, Mn L-edge spectra and time resolved *in-situ* X-ray emission spectra from PSII were collected at LCLS to follow changes in the electronic structure of the Mn-cluster over the reaction cycle. The emission spectra from time-points between the S_3 to S_0 transition demonstrate that the kinetics of oxidation/reduction of the Mn_4CaO_5 cluster are complex. The different classes of suggested water oxidation mechanisms are discussed in light of our results.

Molecular Architecture and Function of the SEA Complex, a Modulator of the TORC1 Pathway

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The TORC1 signaling pathway plays a major role in the control of cell growth and response to stress. Here we demonstrate that the SEA complex physically interacts with TORC1 and is an important regulator of its activity. During nitrogen starvation, deletions of SEA complex components lead to Tor1 kinase delocalization, defects in autophagy, and vacuolar fragmentation. TORC1 inactivation, via nitrogen deprivation or rapamycin treatment, changes cellular levels of SEA complex members. We used affinity purification and chemical cross-linking to generate the data for an integrative structure modeling approach, which produced a well-defined molecular architecture of the SEA complex and showed that the SEA complex comprises two regions that are structurally and functionally distinct. The SEA complex emerges as a platform that can coordinate both structural and enzymatic activities necessary for the effective functioning of the TORC1 pathway.

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Structure, inhibition, and regulation of a two-pore channel TPC1

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Two-pore channels (TPCs) comprise a subfamily of eukaryotic voltage- and ligand-gated cation channels that contain two non-equivalent tandem pore-forming subunits that then dimerize to form quasi-tetramers. Found in vacuolar or endolysosomal membranes, they regulate the conductance of Na^+ and Ca^{2+} ions, intravesicular pH, trafficking of filoviruses, excitability, and cellular amino acid homeostasis. Membrane potential and cytosolic Ca^{2+}-ions activate TPCs, whereas luminal low pH and Ca^{2+}, phosphorylation, and binding of pharmacophores are inhibitory. We report the crystal structure of TPC1 from Arabidopsis thaliana at 2.87Å resolution as a basis for understanding ion permeation, channel activation, the location of voltage-sensing domains, and regulatory ion-binding sites. We determined sites of phosphorylation in the N-terminal and C-terminal domains that are positioned to allosterically modulate cytoplasmic Ca^{2+}-activation. One of the two voltage sensing domains (VSD2) encodes voltage sensitivity and inhibition by luminal Ca^{2+} locks VSD2 in a resting conformation, distinct from the activated VSDs observed in structures of other voltage-gated ion channels. The structure shows how potent pharmacophore trans-Ned-19 allosterically acts to inhibit channel opening. In animals, trans-Ned-19 prevents infection by Ebola virus and Filoviruses by blocking fusion of the viral and endolysosomal membranes, thereby preventing delivery of their RNA into the host cytoplasm. The structure of TPC1 paves the way for understanding the complex function of these channels and may aid the development of antiviral compounds.

Characterization of Ferryl Intermediate in DypB Peroxidase Using Femtosecond Crystallography, Optical and X-Ray Absorption Spectroscopies

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Spectroscopic techniques, in conjunction with X-ray diffraction studies, can provide a more complete picture of both the geometric and electronic structures of metallo-enzyme active sites. This multidisciplinary approach is of particular significance to investigation of the reaction mechanisms of metalloenzymes, where spectroscopy serves not only as a source of additional mechanistic insight but as an indicator of radiation-induced chemistry as well. Our study with dye-decolorizing peroxidase (DypB), focused on spectroscopic (Vis and XAS) and structural (XFEL) characterization of ferryl intermediate trapped in crystallo, has successfully demonstrated the applicability of this combined approach. The 1.45 Å crystal structure of high-valent intermediate also illustrates the currently under-utilized potential of femtosecond crystallography for the field of structural mechanistic enzymology.

Hard X-ray Fluorescence Imaging and μ-X-ray Absorption Spectroscopy

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The X-ray fluorescence (XRF) imaging facility at the Stanford Synchrotron Radiation Lightsource (SSRL) consists of three beamlines, which cover a wide range of x-ray energies and beam sizes, allowing for the ability to conduct an extensive variety of experiments. As a facility, we need to cater to a broad range of users including those from the Biological, Environmental, and Material Science communities, which often require different beam line conditions based on sample composition. Needs can include varying sample sizes (5uM to 0.5m), varying resolution (<2uM to >200uM), and varying energy ranges (2100eV-20,000eV). These needs have been achieved with the utilization of three beam lines for microfocus experiments:

1) Beamline 2-3: High spatial resolution (beam size ~2uM), good for spectroscopy/ diffraction, medium to small sample sizes, ~5,000eV to ~20,000 eV, tomography
2) Beamline 10-2: Moderate resolution, (10uM – 200uM), high flux, rapid scanning, medium to large sample sizes, OK spectroscopy, ideal diffraction, ~5,000eV to ~20,000eV, tomography, confocal microscopy
3) Beamline 14-3: Lower energy (~2,000 to ~4,500eV), high spatial resolution (~5uM beam size), good spectroscopy, small to medium sample sizes, Helium sample environment

These three beamlines are tied together via a cohesive suite of software developed by Sam Webb. All imaging beamlines at SSRL share the same suite of software to control the beamline as well as analyze the data. These programs also share the same look and many features as the bulk XAS lines at SSRL for ease of transition between many beamlines. The capabilities of the imaging facility are always expanding at SSRL, future directions include the addition of zone plates at beamline 10-2 allowing beam sizes < 100nM as well as a hexapod stage to allow for easy changes between optics and thus beam sizes.

CCP4 Web-Services and Cloud Computing Developments

E. Krissinel, V. Uski, C. Ballard

CCP4, Research Complex at Harwell, Rutherford Appleton Laboratory, Harwell Campus, UK

The Collaborative Computational Project Number 4 in Protein Crystallography (CCP4) exists to maintain, develop and provide world-class software that allows researchers to determine macromolecular structures by X-ray crystallography and other biophysical techniques. Over 37 years, the CCP4 Software was assembled and distributed as an integrated Suite of programs, installable on either user's personal PCs or centralized facilities. The Suite is traditionally operated via CCP4i(2) Graphical User Interface and is available for all major Linux, Mac OSX and Windows platforms.

Modern trends in computing suggest a fast-growing interest to mobile platforms and cloud solutions for data storage and operations in practically all areas. These trends are observed in both hardware (such as the appearance of Chromebooks-like and tablet devices) and operating systems, which now routinely include a number of cloud services from their vendors, changing the pattern of ordinary computing. In context of crystallographic computing, cloud solutions become increasingly appealing also in view of recent advances in automated structure solution methods, which are demanding for both computing power and various databases, making them less convenient for offline setups. Yet another reason for mobile trend to persist is that the cloud model of operations simplifies software and data management for both software provider and end users. Although CCP4 invested a considerable effort into development and maintenance of its dynamic update system, keeping both software and data resources in sync proves to be a burden for many users with limited computer or Internet resources.

CCP4 steps into the area of mobile computing and cloud services in 3 different ways. Firstly, it provides a set of free web services for automated structure solution. In many cases, an upload of reflection data and sequence is all what a user needs to do in order to solve their structure. Secondly, a system of cloud-based virtual machines with per-user persistent storage, access to collected data at DLS synchrotron and pre-installed CCP4 software is under development to serve computing needs of MX community in the UK. Thirdly, RESTful API is being developed, which will provide access to remote CCP4 computing on script level. Further plans include the development of native HTML5 interface for CCP4 Software, which would be useable on all mobile devices. We will discuss these developments, achieved results and future directions in middle-term perspective.

Extracting Electronic Structure and Bond Strength Information from 1s2p RIXS: Electron Transfer and Apoptosis in the Cytochrome c protein

T.Kroll[1,2], R.G. Hadt[1], S.A. Wilson[1], M. Lundberg,[1,3] J.J. Yan[1], T.-C. Weng[2], D. Sokaras[2], R. Alonso-Mori[4], D. Casa[5], M.H. Upton[5], M. Mara[1], H. Lim[1], M.E. Reinhart[6], R. Hartsock[6], D. Zhu[4], M. Chollet[4], K. Gaffney[2,4], B. Hedman[2], K.O. Hodgson[1,2], and E.I. Solomon[1,2]

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The protein cytochrome c plays an active role in biological processes in humans and animals. The knowledge of the electronic structure of this protein including bonding and reaction properties related to the valence state of its central iron ion is crucial, but difficult to address directly. In this study, the hard X-ray based method of resonant inelastic X-ray scattering (1s2p RIXS) will be introduced together with its application to investigate the protein's bonding properties. 1s2p RIXS is a powerful method that allows the study of systems in difficult conditions with the resolution of soft X-ray absorption spectroscopy [1]. New insights were obtained by correlating spectral differences of cyt *c* to a bis-imidazole porphyrin model complex to define the differential orbital covalency of the iron site in the highly covalent porphyrin environment through charge transfer multiplet calculations that are correlated to DFT calculations. [2]
Here, this presentation serves two goals: The introduction of 1s2p RIXS in terms of bonding property extraction through experiment and theory, and the use of this information for the electron transfer and apoptosis mechanisms using SSRL and LCLS.

[1] Lundberg et al., JACS 135, 17121 (2013)
[2] Kroll et al., JACS 136, 18087 (2014)

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The protein cytochrome c plays an active role in biological processes in humans and animals. The knowledge of the electronic structure of this protein including bonding and reaction properties related to the valence state of its central iron ion is crucial, but difficult to address directly. In this study, the hard X-ray based method of resonant inelastic X-ray scattering (1s2p RIXS) will be introduced together with its application to investigate the protein's bonding properties. 1s2p RIXS is a powerful method that allows the study of systems in difficult conditions with the resolution of soft X-ray absorption spectroscopy [1]. New insights were obtained by correlating spectral differences of cyt *c* to a bis-imidazole porphyrin model complex to define the differential orbital covalency of the iron site in the highly covalent porphyrin environment through charge transfer multiplet calculations that are correlated to DFT calculations. [2]
Here, this presentation serves two goals: The introduction of 1s2p RIXS in terms of bonding property extraction through experiment and theory, and the use of this information for the electron transfer and apoptosis mechanisms using SSRL and LCLS.

[1] Lundberg et al., JACS 135, 17121 (2013)
[2] Kroll et al., JACS 136, 18087 (2014)

Development of “color” x-ray histology using multiple metal stains and multi-energy synchrotron CT

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In this talk, we will discuss a technique for performing three-dimensional, “color” x-ray histology using multiple heavy-metal stains and a multi-energy computed tomography (CT) acquisition technique. The technique employs biologically specific heavy-metal stains such as osmium tetroxide and uranyl acetate that have been widely used in electron microscopy. While such stains have previously been used in high-resolution x-ray imaging of biological samples, previous studies have focused on single-stained specimens. Using monochromatic synchrotron radiation, we demonstrate that it is possible to produce quantitatively accurate maps of the distribution of two distinct stains. We validate the quantification using both L-edge subtraction and single stain controls. We demonstrate using a juvenile zebrafish that the differential distribution of uranyl acetate and osmium tetroxide can be used to differentiate tissues in much the same way as hematoxylin and eosin are used in a conventional optical histology image. While the technique is demonstrated using zebrafish, it is expected to be applicable to other biological specimens, potentially including human pathology tissue, allowing for three-dimensional virtual histology without physical sectioning. We conclude with a brief discussion of the potential for translating the technique into lab-based benchtop CT systems.

Recovery of missing central diffraction intensities by using template method

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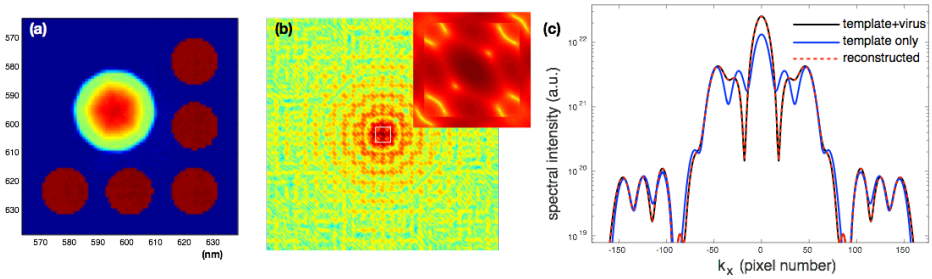
Third-generation synchrotron radiation and free-electron laser (FEL) light sources have enabled the determination of structure of biological molecules in higher resolution. However, several technical or physical limitations still prevent scientists from accessing images of weakly scattering biological samples. In particular, X-ray coherent diffraction imaging (CDI), which has been widely employed at synchrotron and FEL beamlines for imaging noncrystalline samples [1], relies on robust phase retrieval and is vulnerable to quantum noise and missing waves in the center of diffraction pattern.

Previously we have presented an innovative approach, namely template method, for CDI experiments [2]. Such approach allows significant image fidelity enhancement by placing a template made of heavy atoms with the weakly scattering biological sample. This result is counter-intuitive as it greatly boosts the number of scattering photons without increasing the noise level which would have buried the signal of weak samples.

Here we further report that the template method is also capable of mitigating the missing center problem, whereby the diffraction intensities in the low-*k* region which are critical for structure determination are blocked by the beamstop [3]. To circumvent, we simulate the diffraction pattern of a Mimivirus with metallic template (panel (a)) and perform the phase retrieval process GHIO with the Fourier transform of the template *per se* as *a priori*. Both amplitude and phase are allowed to be updated in the iterative process. Our simulation result shows the Mimivirus can be reconstructed in the presence of a large missing center, which would be otherwise inaccessible if without using the template. Panels (b) and (c) show the missing waves have been recover with acceptable errors (ER=0.0043).



[1] J. Miao et al., IEEE J. Select. Topics Quantum Electron. **18**, 399 (2012).
[2] T.-Y. Lan, P.-N. Li and T.-K. Lee, New J. Phys. **16**, 033016 (2014).
[3] J. Miao et al., Phys. Rev. Lett. **95**, 085503 (2005).

COMBINATORIAL METHODS SOLVE A DIFFICULT STRUCTURAL PROBLEM TO REVEAL HOW CHAPERONINS WORK IN EUKARYOTES

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Eukaryotic group II Chaperonin TRiC or CCT is a 0.95 megadalton protein complex that is essential for the correct and efficient folding of cytosolic polypeptides. The closed form is a 16 nm sphere made of two hemi-spherical rings of 8 subunits (~550 residues/subunit) that rotate to open a central folding chamber. In eukaryotes, 8 different genes encode the subunits of this ATP-powered nanomachine. The high sequence identity of subunits made the 40,320 (8-factorial) possible arrangements indistinguishable in previous cryo-electron microscopy and crystallographic analysis.

First we use cross-linking, mass spectrometry and combinatorial homology modeling. We react bovine TRiC under native conditions with a lysine-specific cross-linker, follow up with trypsin digestion, and use mass spectrometry to identify 63 cross-linked pairs providing distance restraints. Independently of the cross-link set, we construct all 40,320 homology models of the TRiC particle. When we compared each model with the cross-link set, we discovered that one model is significantly more compatible than any other model. Bootstrapping analysis confirms that this model is 10 times more likely to result from this cross-link set than the next best-fitting model.

Second, we re-examine the 3.8 Å resolution X-ray data of yeast TRiC. Our method of R-Value Exploration (RVX) exhaustively tests all 2,580,460 possible models. This unbiased analysis singles out with overwhelming significance one model, which is fully consistent with our previous biochemical data and refines to a much lower Rfree value than reported previously with the same X-ray data. With four-fold averaging, our structure reveals remarkably resolved details of the unique conformation of each subunit, and suggests a mechanism for the initiation of transition to the open state. More generally, we expect RVX to resolve ambiguity in low-resolution crystallographic studies.

Resolution enhancement of transmission x-ray microscopy using coherent diffraction

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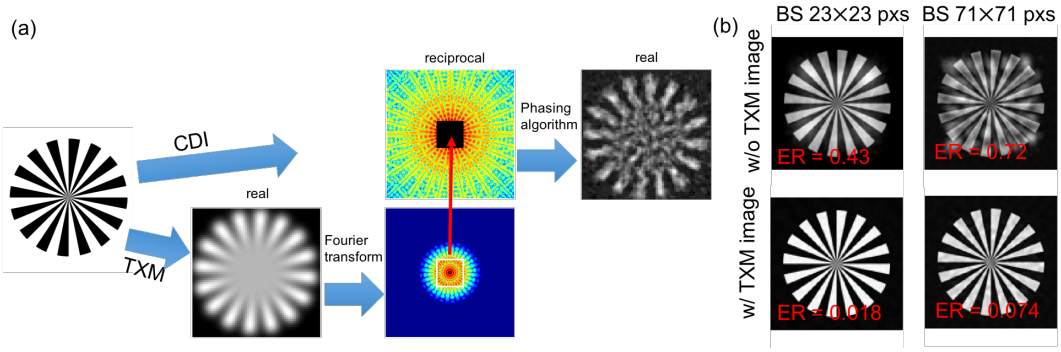
Coherent diffraction imaging (CDI) has been proposed for investigating the non-periodic structure of biological systems such as viruses and cells. In principle, the spatial resolution of CDI is only limited by the geometry of the detector and quantum noise. However, there are many practical factors (such as the missing data due to the beam stop) that complicate the image reconstruction and, subsequently, impair the image fidelity. Transmission X-ray microscopy (TXM) employing Fresnel zone plates has been used for such studies, however, the resolution is limited by the zone plate fabrication technique to 10-30 nm.

Here we describe and numerically demonstrate a new approach for X-ray microscopy that combines the strengths of coherent diffraction and zone plate based imaging techniques in a complimentary fashion: the TXM image helps solve the missing data problem of CDI, while the CDI provides high resolution data. Therefore this approach may be capable of providing high fidelity biological images.



In our simulation, the object is imaged by CDI and TXM simultaneously, and the central part of the Fourier transform (FT) is incorporated into the CDI diffraction pattern as *a priori* information, as the red arrow in figure panel (a) indicates. The hybrid input-output (HIO)[1] algorithm is then employed to reconstruct the image, giving the results shown in figure panel (b). Our method can significantly enhance the image fidelity even when the beam stop (BS) area is large, i.e. when several low-*q* components are missing, and is still valid in the scenario where the FT of the TXM image has low resolution and doesn't fully cover the beam stop region (not shown here). The present method can be readily realized experimentally through the addition of a detector in a TXM that captures the required high-*q* information.

[1]Fienup, Opt. Lett. **3**, 27 (1978).

Abstract for BSR16 (June 19, 2016)

Measurement and simulation of interference enhancement in coherent X-ray diffraction imaging of gold nano particles and influenza virus in water at SACLA

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Abstract

At SACLA, BL3 currently delivers a coherent X-ray beam at 4 keV with the pulse duration approximately 10 fs, the flux about 6.225×10^{24} photons $\mu m^{-2} sec^{-1}$ with FWHM beam spot diameter being 1.5 μm. We have performed coherent diffraction measurements using SACLA beam on both samples of Au nano particles of sizes for 10 to 40 nm and influenza virus of ~100 nm in unmixed and mixed conditions. Prepared solution was dipped and sealed in a micro-cell of 20μm x 20μm with thickness 1.5μm. The concentration of our liquid samples was carefully adjusted to maximize the probability of hitting only one single or few nano particles in the cell. Single-shot speckle patterns were recorded with a 2399×2399 CCD detector. The speckle patterns collected from single Au particles serve as a calibration of our measurements. A consistency between measured X-ray intensities and Au nano particles was demonstrated. In mixed samples, the phase interference in the speckle patterns is studied for enhancement of phase retrieval and resolution of CDI images. Such effect, which is of special interest for the study of weak scatters of biological objects, will be addressed in detail.

Cu Kβ X-ray Emission Spectroscopy as a Probe of Coordination Environments of Cu(I) Sites

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Cu(I) sites are considered to be spectroscopically silent because they have d^{10} closed subshell configuration and do not show spectroscopic signatures in many conventional spectroscopic methods. Kβ X-ray emission spectroscopy (XES) probes transitions from the occupied states to the 1s core hole and thus is particularly useful for Cu(I) sites. The Kβ XES spectra of Cu(I) model complexes have been investigated to establish background theory and to identify analytically useful features. The density functional theory calculations which well reproduced the experimental spectra allowed for quantitative analysis. These results play an important role in analyzing Kβ XES applied to Cu(I) active sites in Cu enzymes.

Data filtering method for Correlated X-ray Scattering

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More and more experiments indicate that there will be great potential to use the correlated X-ray scattering (CXS) to study the system of macromolecules solution and the three-dimension structure parameters can even be obtained. Derek showed that the CXS technique is capable of extracting detailed atomic-scale structures from a random ensemble of particles in solution [1].

Compared with other reported models of particles solution system, the difficulty for the biological macromolecules solution lies in the nonunion of electron density and there is no obvious boundary with the medium. So the low rate of signal and noise was obtained for the X-ray scattering patterns. And there are many invalid data in the X-ray scattering patterns from random orientations of the sample.

We explored the data filtering method for billions of scattering patterns. After the effective data filtering, we can use the CXS to study the biological macromolecules in solution better. Three kinds of solution sample were scattered by the x-ray Free Electron Laser in Spring8 in Japan. The first kind of sample is the Peg solution, the second kind of sample is the linear DNA from salmon sperm, and the third kind of sample is plasmid DNA. Millions of scattering patterns were obtained from the x-ray Free Electron Laser experiment. One of the most challenge for the analysis of the scattering patterns is the artefacts which reduce the ration of single and noise. Because there are many reasons for the experimental artefacts such as whether the shutter is on or off, whether there is the beam line or not for the shot, the swaying of the nozzle and the shadow of the detector. It is very challenge for the analysis of the scattering patterns.

Based on the median and variance of the scattering intensity, two obvious clusters were obtained for the plasmid DNA in peg, one obvious cluster were obtained for peg and more clusters were obtained for the linear DNA from salmon sperm. Based on the density based scan, some of the scattering patterns with high artifacts were removed and different clusters were clarified. We will compute and analyse the angular auto-correlation of different clusters in future.

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Reference

[1] Wide-angle correlated X-ray scattering from gold nanoparticles demonstrated precise agreement with an atomic twinning model, Derek Mendez, Herschel Watkins, Shenglan Qiao, Kevin S. Raines, Thomas J. Lane, Gundolf Schenk, Kensuke Tono, Yasumasa Joti, Makina Yabashi, Daniel Ratner and Sebastian Doniach (submitted for publication).

Structural and functional studies of Human GPCRs

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Cell surface receptors and their related intracellular proteins are responsible for human cellular communications with each other and their environment, and are involved in a wide range of physiological activities. Such a central role in human biology makes cell signaling the target for intervention for tuning physiological responses and fighting numerous conditions and diseases. G protein coupled receptors (GPCRs) are involved in a wide range of physiological systems where they are responsible for around 80% of transmitting extracellular signals into cells.

In humans, GPCRs signal in response to a diverse array of stimuli including light molecules, hormones, and lipids, where these signals affect downstream cascades to impact both health and disease states. Yet, despite their importance as therapeutic targets, detailed molecular structures of only ~30 unique GPCRs have been determined to date. A key challenge to their structure determination is adequate protein expression and crystallization. Here we report the quantification of protein expression in an insect cell expression system for all 826 human GPCRs using two different fusion constructs. Expression characteristics are analyzed in aggregate and among each of the five distinct subfamilies. These data can be used to identify trends related to GPCR expression between different fusion constructs and between different GPCR families, and to identify and prioritize lead candidates for future structure determination efforts.

Characterization of the Cytochrome C Iron-Thioether Bond and Its Regulation by the Protein

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Cytochrome *c* (cyt *c*) is a paradigm protein for probing structure-function relationships in bioinorganic chemistry. While cyt *c* is an electron transfer protein, utilizing an Fe-S(Met) bond to lower E^0, it also functions as a peroxidase in programmed cell death (e.g., apoptosis), which requires cleaving the Fe-S(Met) bond. Although the Fe-S(Met) bond is rather weak,[1] it remains intact at room temperature despite being entropically unfavorable, implying that the protein bulk plays a role in stabilizing this bond under physiological conditions. The Fe-S(Met) bond is broken photochemically by optical excitation at the heme π-π* bands in ferrous cyt c,[2-3] providing a convenient method for studying the mechanism of bond dissociation and rebinding in this protein. In this study, we interrogate the ferrous cyt c photoproduct by laser pump/X-ray probe measurements, utilizing the ultrafast X-ray pulses generated at LCLS; we use K-edge XAS measurements to probe the local structure of the iron, and Kβ XES to probe the iron spin state. We relate the ligand recombination with heat dissipation from the heme following photoexcitation to determine the strength of the Fe-S(Met) bond in the protein, revealing the protein entatic contribution to stabilizing this bond and regulating protein function.

[1] Kroll et al., JACS, 136, 18087 (2014)
[2] Negrerie et al., JPC B 110, 12766 (2006)
[3] Wang et al., JPC B, 104, 10789 (2000)

Biological SINS: Broadband synchrotron infrared nano-spectroscopy of biological materials

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By probing intrinsic vibrational and phonon modes, infrared (IR) spectroscopy continues to be a powerful analytical technique for chemical identification, but the spatial resolution of this technique has been traditionally diffraction-limited to several microns, even with synchrotron sources. Here, we describe synchrotron infrared nano-spectroscopy (SINS), in which spectrally bright and broad IR light from a synchrotron source is coupled to a scattering-type-scanning near-field optical microscope (s-SNOM), enabling sensitive vibrational spectroscopy spanning the entire mid- and far-infrared regions with nanometer spatial resolution [1]. This highly powerful combination provides access to a qualitatively new form of nano-chemometric analysis with the investigation of nanoscale, mesoscale, and surface phenomena that were previously impossible to study with IR techniques. The Beamline 5.4 SINS end-station at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory is open to users, such that it can be broadly applied to biological, surface chemistry, materials, environmental science, and other scientific problems. In this talk I will show the performance and results of SINS on a variety of natural and engineered biological systems, including cells, biominerals, proteins, and peptoid nanosheets.

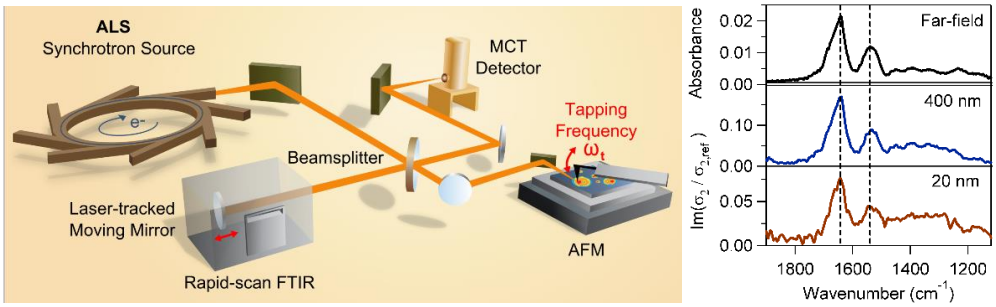

Fig. 1. Overview of the SINS setup at the ALS (left). Example of how IR spectra obtained with SINS from only 20 nm of a protein sample compared to the far-field FTIR spectrum (right).

References. 1. H.A. Bechtel, E.A. Muller, R.L. Olmon, M.C. Martin, M.B. Raschke, *PNAS*, 2014, **111**, 7191–7196.

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Time-resolved SAXS with low sample consumption: a way to pursue conformational changes of biomolecules

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Time-Resolved Small Angle X-ray Scattering (TR-SAXS) is an extremely powerful tool to investigate *in-situ* the conformational changes that biological systems are undergoing during the course of their biological function. The time regime of such changes is generally depending on the scale of the event. A TR-SAXS experiment using a stopped-flow mixer typically can cover the time regime greater than millisecond time scale. Therefore this is an ideal tool to detect large scale transitions such as tertiary or quaternary structural changes of biological macromolecules. However the use of stopped-flow mixers for TR-SAXS experiments on biological samples has often been difficult due to the large amount of material necessary.

Here we will update the latest status of fast TR-SAXS setup at SSRL using a customized stopped-flow mixer in order to reduce the sample consumption. This new setup allows us to obtain a TR-SAXS data set from as little as 30ul of sample volume at the present moment. It also eliminates the sample consuming priming of the tubing inside the stopped flow (no dead volume between shots) and thus substantially reduces the sample amount required for such experiments. Semi-automatic sample injection and wash cycle for the sample cell are employed at every single data collection. Recent scientific results using this setup are also being discussed.

Radiation damage free structural studies of cellulolytic redox enzymes using neutron scattering and diffraction

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Fungal polysaccharide monooxygenases (PMOs) are a recently discovered family of extracellular enzymes that break glycosidic bonds at the surfaces of crystalline carbohydrates, and enhance the susceptibility of the substrate to further enzymatic degradation by "classical" glycoside hydrolases. PMOs are copper containing metallo-enzymes which require input of two electrons from their redox partners, the cellobiose dehydrogenase enzymes (CDHs), and one oxygen molecule to achieve hydroxylation of one carbon in the glycosidic bond.

X-ray crystallographic studies to date have failed to unambiguously determine the chemical nature of the oxygen species bound to the catalytic center. This is due in part to metal photo-reduction by exposure to the X-ray beam leading to a mix Cu(I)/Cu(II) catalytic center.

In marked contrast to X-rays, neutrons do not cause radiation damage and are therefore well suited to study the enzymatic mechanism of redox enzymes. We will discuss our recent X-ray and neutron crystallographic studies that provide new insight into the PMO mechanism.

Redox complexes are often transient and their structural characterization can be challenging. We are using small angle X-ray and neutron scattering to investigate the intramolecular electron transfer between the dehydrogenase and cytochrome domain of CDH and the intermolecular electron transfer between the CDH cytochrome domain and PMO. We will present the structural knowledge we gained of CDH and of the PMO-CDH complex from these low resolution techniques.

Bio-Imaging using X-Ray Ptychography
The Method, Recent Advances, and Applications

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Ptychography is a scanning variant of coherent diffractive imaging, or “lens-less” imaging, that is rapidly maturing into a versatile and user-friendly approach to high-resolution microscopy. In combination with tomographic methods, isotropic 3D resolving power better than 20 nm has been demonstrated using X-rays with 2 Å wavelength [1], which are capable of penetrating samples tens of microns thick. Image contrast can reliably be related to quantitative density information on an absolute scale [2], facilitating reliable image segmentation and allowing for images from different samples or different instruments to be reliably compared. We will discuss recent developments relevant to imaging of biological specimens. These include experimental design and instrumentation that allows for imaging of frozen-hydrated, and include as well advances in image reconstruction. Applications range from characterizing hard materials, e.g. from endo- or exoskeletons, i.e., bone, chitin, etc., to single-cell and tissue imaging.

References

[1] M. Holler *et al.*, “X-ray ptychographic computed tomography at 16 nm isotropic 3D resolution” *Sci. Rep.* **4** (2014) 3857. DOI: 10.1038/srep03857.
[2] A. Diaz *et al.*, “Three-dimensional mass density mapping of cellular ultrastructure by ptychographic X-ray nanotomography” *J. Struct. Biol.* **192**(3) (2015) 461. DOI: 10.1016/j.jsb.2015.10.008; A. Diaz *et al.*, “Addendum to ‘Three-dimensional mass density mapping of cellular ultrastructure by ptychographic X-ray nanotomography’ [J. Struct. Biol. 192 (2015) 461–469]” *J. Struct. Biol.* **193**(1) (2016) 83. DOI: 10.1016/j.jsb.2015.12.003.

High throughput computing on high output synchrotron facilities

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Abstract

X-ray crystallography is one of the most detailed “microscopes” available today for examining macromolecular structures. However, structures are only simplified models of target proteins and/or nucleic acids, and should be seen as a framework for generating hypotheses to be explored. Numerous biochemical and biophysical experiments, including new diffraction experiments, can and should be performed to verify or disprove these hypotheses. Reconciling structural information with functional, experimental, and sequential data in the context of pathways or interaction networks with other bio-macromolecules and/or bioactive chemical compounds increasingly requires the use of Big Data paradigms for effective data management and for checking data integrity and accuracy. This is easy to say, but extremely difficult to implement. A combination of advancements in high-quality data harvesting, validation, mining, and data management tools would make it possible to convert high-throughput pipelines into high-output pipelines in target-based drug discovery and academic biomedical research.

Fast tomographic microscopy to capture the dynamics of life.

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In-vivo X-ray imaging of small animals is routinely performed down to a spatial resolution of several tens of micrometers. Breaking the 10 µm barrier is very difficult due to the high radiation dose deposited in the tissue and the motion of the sample during tomographic acquisition [1]. To toggle these challenges we propose new strategies to visualize fast micrometer-scale internal movements in small animals. We optimize the radiation dose by retrieving the information encoded in the refraction of X-rays by the tissue [2]. To eliminate the motion artifacts new strategies were successfully applied to various biological systems [1,3]. For periodic motions such as breathing, heart-beat or wing-beat the optical flow guided retrospective gating of tomographic projections is a promising new method [4]. In more complex biological systems with multiple motion patterns one will benefit of the high brilliance of the new synchrotron sources [5]. We can conclude that a true one micrometer resolution for in vivo X-ray imaging is within reach.

[1] G. Lovric, S. F. Barré, J. C. Schittny, M. Roth-Kleiner, M. Stampanoni, R. Mokso, J. Appl. Crystallogr. 46(4), 856 (2013).
[2] R. Mokso, F. Marone, S. C. Irvine, M. Nyvlt, D. Schwyn, K. Mader, G. K. Taylor, H. G. Krapp, M. Skeren, M. Stampanoni, J. Phys. D. Appl. Phys. 46, 494004 (2013).
[3] S. M. Walker, D. A. Schwyn, R. Mokso, M. Wicklein, T. Müller, M. Doube, M. Stampanoni, H. G. Krapp, G. K. Taylor, PLoS Biol. 12, e1001823 (2014).
[4] R. Mokso, D. A. Schwyn, S. M. Walker, M. Wicklein, T. Müller, M. Doube, M. Stampanoni, G. K. Taylor, H. G. Krapp, Scientific Reports 5, 8727 (2015).
[5] M. Eriksson, J. F. van der Veen, C. Quitmann, J. Synchrotron Radiation 21, 837-842 (2014)

IMCA-CAT Advanced Photon Source Facility for Pharmaceutical Drug Discovery

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The Industrial Macromolecular Crystallography Association Collaborative Access Team operates a data-collection facility located at Sector 17 of the Advanced Photon Source that was founded to meet the demands of IMCA member pharmaceutical companies for reliable, high-throughput, high-quality data collection, while ensuring a secure environment for proprietary research. The 17ID high-flux beamline, equipped with a Pilatus 6M pixel array detector, allows for very fast data collection times. The beam size can be easily optimized for each sample using a mini-beam quad collimator, with user-selectable beam sizes down to 5 micron. Automated sample mounting is performed by a Rigaku ACTOR robot compatible with both Rigaku and ALS/Unipuck style magazines and all common pin types. Mail-in is now the predominant beamtime access mode for IMCA members, utilizing fully unattended data collection for routine samples with typical throughput rates of 19 data sets per hour, and manual collection by beamline staff for more challenging projects. IMCA-CAT is currently working in collaboration with MiTeGen on a modular crystallization plate design, to enable *in situ* data collection at the beamline without major modification of experimental components. Access to IMCA-CAT is available, on-site and via remote access, to other industrial users needing regular and guaranteed proprietary beamtime through subscription memberships, and to academic researchers through the APS General User program.

Coherent diffraction of single Rice Dwarf Virus particles using hard X-rays at the Linac Coherent Light Source

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This work was performed as part of the Single Particle Imaging Initiative, a collaboration between many institutions. See ref. 1 for a complete affiliation list.

X-ray Free Electron Lasers (XFELs) have enabled “diffraction before destruction” experiments in structural biology, both in single particle imaging (SPI) and crystallography. The added value of XFELs as oppose to conventional crystallography at synchrotrons is their high brilliance, short femtosecond pulses and the ability to collect data at room temperature. Consequently, diffraction from micro- and nanocrystals[2] and even single particles can be detected before radiation damage occurs[3], in a time resolved fashion under physiological conditions. Isolated objects, such as viruses[4] and cells[5], have previously been imaged to nanometer resolution.

The SPI initiative[6] was formed to identify and solve the challenges posed by high resolution imaging with XFELs and the Rice Dwarf Virus (RDV) was selected for the first experiment. RDV is an icosahedral RNA virus and the causative agent of rice dwarf disease, which creates severe economic damage in Asia. The virus is c. 70 nm in diameter and has seven structural proteins making up the virus particles’ two shells, consisting of an inner and an outer capsid[7].

RDV was aerosolized and delivered via an aerodynamic lens injector into hard X-ray pulses at the Coherent X-ray Imaging (CXI) instrument of the Linac Coherent Light Source (LCLS). Coherent diffraction images were recorded in two planes on Cornell-SLAC Pixel Array Detectors. Measurable photons were recorded from the sample up to a scattering angle that is commensurate with 5.9 Å resolution[1].

1. Munke, A. et al Coherent diffraction of single Rice Dwarf Virus particles using hard X-rays at the Linac Coherent Light Source, Sci. Data, in press
2. Chapman, H.N., et al. Femtosecond X-ray protein nanocrystallography. Nature, 470, 73-77 (2011)
2. Neutze, R., et al. Potential for biomolecular imaging with femtosecond X-ray pulses. Nature, 406, 752-757 (2000)
3. Seibert, M.M., et al. Single mimivirus particles intercepted and imaged with an X-ray laser. Nature, 470 (2011)
4. van der Schot, G., et al. Imaging single cells in a beam of live cyanobacteria with an X-ray laser. Nat. Commun. 6, 5704 (2015)
5. Aquila, A. et al. The linac coherent light source single particle imaging road map. Struct. Dyn. 2, 041701 (2015)
6. Nakagawa, A. et al. The atomic structure of Rice dwarf virus reveals the self-assembly mechanism of component proteins. Structure, 11, 1227-1238 (2003)

Time-Resolved serial femtosecond crystallography studies of bacteriorhodopsin - a light-driven proton pump

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XFEL radiation has revolutionized experimental approaches to structural biology. One area where XFEL radiation is having a large impact is time-resolved structural studies of protein conformational changes. Bacteriorhodopsin is a light-driven proton pump which has long been used as a model system in biophysics. The mechanism by which light-driven isomerization of a retinal chromophore is coupled to the transport of protons “up-hill” against a transmembrane proton concentration gradient involves protein structural changes. I will describe collaborative studies performed at SACLA that have investigated the nature and time-scale of these structural changes at high resolution. We used Time-Resolved Serial Femtosecond Crystallography (TR-SFX) to probe structural changes in microcrystals on a time-scale from nanoseconds to milliseconds. Structural results from these studies enabled a complete picture of structural changes occurring during proton pumping by bacteriorhodopsin to be recovered. These results provide new chemical insight into one mechanism by which the energy of sunlight is directed into the biosphere.

Controlled Environment Nano-Imaging Free From Radiation Damage by X-ray Laser Diffraction

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Coherent diffractive imaging (CDI) is a growing technique in photon science. CDI has been demonstrated to be a powerful tool in visualizing cells and organelles using synchrotron radiation. X-ray free-electron lasers (XFELs) with femtosecond pulse durations further extends the ability of CDI to achieve spatial resolution beyond the conventional radiation-damage limitation. We performed live cell nano-imaging using a Japanese XFEL facility, SACLA. We employed pulsed coherent X-ray solution scattering (PCXSS), a form of X-ray CDI, developed by our group [1,2]. A unique feature of PCXSS is to keep solution sample under a controlled environment in micro-liquid enclosure array (MLEA) chips. We succeeded in reconstructing a live cell image from a coherent diffraction pattern recorded with a single XFEL shot. The reconstructed image quantitatively revealed the internal structures, *e.g.* high image intensity structure indicative of dense DNA [2]. PCXSS can also be effectively applied to nano-imaging of materials functional in solution. For example, we successfully imaged gold nanoparticle self-assemblies, developed as drug delivery carriers, in solution [3,4]. We also started industrial application of PCXSS in collaboration with Toyota Motor Corp [5].

[1] J. Pérez and Y. Nishino, “Advances in X-ray scattering: from solution SAXS to achievements with coherent beams”, Curr. Opin. Struct. Biol. 22, 670 (2012).
[2] T. Kimura *et al.*, “Imaging live cell in micro-liquid enclosure by X-ray laser diffraction”, Nature Commum. 5, 3052 (2014).
[3] R. Iida *et al.*, “Synthesis of Janus-Like Gold Nanoparticles with Hydrophilic/Hydrophobic Faces by Surface Ligand Exchange and Their Self-Assemblies in Water”, Langmuir 31, 4054 (2015).
[4] J. Wei *et al.*, “Yolk/Shell Assembly of Gold Nanoparticles by Size Segregation in Solution”, J. Am. Chem. Soc. 138, 3274 (2016).
[5] R. Yoshida *et al.*, “Extending the potential of x-ray free-electron lasers to industrial applications – an initiatory attempt at coherent diffractive imaging on car-related nanomaterials”, J. Phys. B 48, 244008 (2015).

Application of small angle X-ray scattering to protein drug development

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Development of new pharmaceutical proteins and peptides require thorough characterizations of the structural/biophysical properties for understanding mode-of-action and rational design of stable pharmaceutical formulations. Small angle X-ray scattering (SAXS) offers important information about the behaviour of protein and peptides at various sample conditions that provides information on both a structural and biophysical level. This information can be utilized for selecting the right analogue or finding optimum formulation conditions. The implementation of SAXS as a tool in our daily research and development at Novo Nordisk will be discussed.

Structural characterisation of the retromer complex

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Retromer is a multi-functional protein assembly that plays a central role in endosomal trafficking, forming an interaction hub for a wide array of regulatory proteins, lipids and cargo molecules. Involved in a broad range of physiological, developmental and pathological processes, retromer is vital for normal cell homeostasis in all eukaryotic organisms and an emerging target for the treatment of human neurodegenerative diseases and HCV infection.

The classical mammalian retromer complex consists of a core heterotrimeric cargo recognition sub-complex (VPS26, VPS29 and VPS35) associated with a dimer of proteins from the SNX–BAR sorting nexin family that drives membrane deformation and tubulation. By recruiting the cargo-selective sub- complex to the forming tubules, the SNX–BAR coat complex mediates the retrograde transport of proteins from endosomes to the trans-Golgi network. Recent studies, however, have highlighted the molecular and functional diversity of retromer and the identification of new interacting proteins has revealed that the role of this protein complex extends to aspects of endosome-to-plasma membrane sorting and regulation of signalling events. Emerging evidence indicates that cargo specificity is mediated by specific sorting nexins. These include SNX3, involved in the trafficking of the Wntless/MIG-14 protein, and SNX27, a PX-FERM protein that mediates the retrieval of the β2-adrenergic receptor.

Using the MX and SAXS/WAXS beamlines at the Australian Synchrotron, we have acquired crystallographic and small angle scattering data to determine how the core cargo recognition sub-complex assembles and to characterise retromer-associated sorting nexins and regulatory proteins. We have also recently started cryo-EM studies to probe the structure of retromer alone, and in complex with interacting proteins, allowing us to study the conformational changes and oligomeric states of retromer that are triggered by ligand and membrane binding. This structural information is being used in combination with biochemical and biological studies to understand retromer-mediated endosomal protein sorting and how this fascinating protein complex contributes to a diverse set of cellular processes in health and disease.

Structure-based development of genome-editing tool, CRISPR-Cas9 towards medical applications

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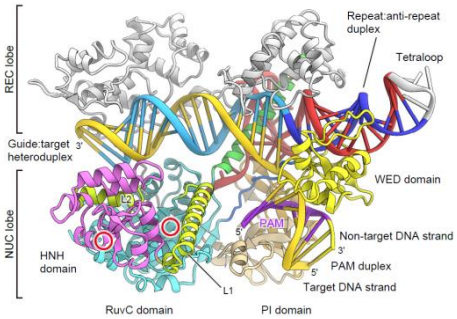
Abstract

The CRISPR-associated endonuclease Cas9 can be targeted to specific genomic loci by single guide RNAs (sgRNAs). Here, we solved the crystal structure of *Streptococcus pyrogenes* Cas9 (SpCas9) in complex with sgRNA and its target DNA at 2.5 A resolution. The structure revealed a bilobed architecture consisting of target recognition and nuclease lobes (Rec and Nuc lobes, respectively), accommodating the sgRNA:DNA heteroduplex in a positively-charged groove at their interface. While Rec lobe is essential for binding sgRNA and DNA, Nuc lobe contains the HNH and RuvC nuclease domains, which are properly located for cleavage of the complementary and noncomplemantary strands of the target DNA, respectively. Nuc lobe also contains a C-terminal domain responsible for the recognition of the protospacer adjacent motif (PAM). We further solved the crystal structure of more compact *Staphyrococcus* aureus Cas9 (SaCas9) and large *Francisella novicida* Cas9 (FnCas9) complexed with their guide RNAs and double-stranded target DNAs at 2.6 and 1.8 A resolutions, respectively. These high-resolution structures combined with functional analyses revealed the molecular mechanism of RNA-guided DNA targeting by Cas9, and uncovered the distinct mechanisms of PAM recognition. On the basis of the structures, we succeeded in changing the specificity of PAM recognition, which paves the way for rational design of new, versatile genome-editing technologies. Recently, we solved the crystal structure of type-V CRISPR, Cpf1 in complex with crRNA and target dsDNA. The structure explains striking similarity and major differences between Cas9 and Cpf1.



<References>

1. "Crystal Structure of Cas9 in Complex with Guide RNA and Target DNA" H. Nishimasu, F. A. Ran, P. D. Hsu, S. Konermann, S. I. Shehata, N. Dohmae, R. Ishitani, F. Zhang F and O. Nureki. ***Cell*** **156**, 935-949 (2014). doi: 10.1016/j.cell.2014.02.001.
2. "Crystal structure of *Staphylococcus aureus* Cas9" H. Nishimasu, L. Cong, W. X. Yan, F. A. Ran, B. Zetsche, Y. Li, A. Kurabayashi, R. Ishitani, F. Zhang and O. Nureki ***Cell*** **162**, 1113-1126 (2015). doi: 10.1016/j.cell.2015.08.007.
3. "Structure and Engineering of Francisella novicida Cas9" H. Hirano, J. S. Gootenberg, T. Horii, O. O. Abudayyeh, M. Kimura, P. D. Hsu, T. Nakane, R. Ishitani, I. Hatada, F. Zhang, H. Nishimasu and O. Nureki. ***Cell*** **164**, 950-961 (2016) doi: 10.1016/j.cell.
4. "Structural basis for the altered Pam specificities of engineered CRISPR-Cas9" S. Hirano, H. Nishimasu, R. Ishitani and O. Nureki. ***Mol. Cell*** **61**, 886-894 (2016) doi 10.1016/j.molcel.2016.02.018.
5. "Crystal structure of Cpf1 in complex with guide RNA and target DNA" T. Yamano, H. Nishimasu, B. Zetsche, H. Hirano, I. M. Slaymaker, Y Li, I. Fedorova, T. Nakane, K. S. Makarova, E. V. Koonin, R. Ishitani, F. Zhang and O. Nureki. ***Cell*** **165**, 949-962 (2016) doi: 10.1016/j.cell.2016.04.003.

Present status of SPring-8 macromolecular crystallography beamlines

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At SPring-8, JASRI and RIKEN are collaboratively developing five beamlines dedicated to macromolecular crystallography. Each beamline shares to serve broad requests from beamline users and liaises to develop new applications for enhancing each characteristic property.

Undulator beamlines, BL41XU[1] and BL32XU[2], focus on cutting edge analyses exploiting high flux microbeam produced by high-magnification focusing optics. In BL41XU, the two step focusing achieved beam size of 2 μm × 2 μm - 35 μm (H) × 50 μm (V). The wide beam size range allows both micro-crystallography and high-resolution data collection that makes use of crystal volume. In addition for high resolution analysis, ultra-high resolution (~0.4 Å) data could be collected by using higher energy beam in range of 20 keV to 35 keV focused by using compound refractive lenses. Meanwhile, BL32XU can provide finest beam with typical horizontal size of 1 μm. This micro-beam is very suited to data collection using small crystals, especially membrane protein crystals grown in LCP that are important targets on the beamline. For such crystals, an original raster diffraction scan system using low-dose X-rays is installed in both beamlines. It is performed in advance of multicrystal data collection for microcrystals; the crystal alignment tool, SHIKA, provides 2D spot population map of raster scan, and KUMA is a tool suggesting data collection strategy with predicted radiation damage.

On the other hand, the bending magnet beamlines BL26B1/B2 and BL38B1 are focused for automation and routine data collection exploiting stable and easily tunable beam. Recently, humidifiers have been installed for the HAG (Humid Air and Glue-coating) mounting method[3], which involves a combination of controlled humid air and water-soluble polymer glue for crystal coating. By this technique, most protein crystals are stable at room temperature and is able to be cryo-cooled under optimized humidity. Micro-spectroscopic measurement system has also been developing. This system has coaxial optics for absorption and Raman spectroscopic measurement against x-ray beam for precise evaluation of radiation damage, detailed validation of cofactor state, and detection of entrapped reaction intermediates on protein crystals.

References:

[1] K. Hasegawa, *et al.*, ***J. Synchrotron Rad.***, 20, 910-913 (2013)
[2] K. Hirata, *et al.*, ***J. Phys.: Conf. Ser.***, 425, 1-4 (2013)
[3] S. Baba, *et al.*, ***Acta Cryst.***, D69, 1839-1849 (2013)

Quantitative X-ray Grating-based Interferometry Brown Adipose Tissue in Mice

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A rapid, non-invasive assessment imaging method is needed to assess the presence of metabolically-active brown adipose tissue (BAT) and compare both concentration and activity to white adipose tissue (WAT). Pfeiffer and co-workers showed detection of BAT in infant mice carcass with stepped-grating X-ray interferometry/tomography.[1] BAT is observable in the differential phase contrast and dark-field (X-ray scattering) image modalities, but not in the conventional X-ray absorption images.

Fat activation is a highly promising target for long-term weight loss maintenance. Fat activating agents have emerged as a powerful tool to increase adipose tissue energy expenditure, either by increasing classical non-shivering thermogenic activity within the more metabolically-active brown adipose tissue (BAT), defined by the presence of uncoupling protein 1 (UCP1), or to induce de novo UCP1 expression and thus thermogenic activity within adipocytes (”beige” or ”brite” cells) in the white adipose tissue (WAT).

Twenty-four C57BL6 mice on low-fat diet were cold exposed for 7 days and sacrificed. Both cold exposure and 3-adrenergic agonists (CL-316, 243) stimulate thermogenesis in rodent models. Mice were divided among thermoneutral (30 ^{0}C, number of mice, N = 6), room temperature (24 ^{0}C, N = 6), and cold (10 ^{0}C, N = 12) groups. Fat samples from mice were extracted, submerged in formalin, and imaged in test tubes using a Talbot-Lau stepped grating X-ray interferometer. Tomography data sets were analyzed based on the ratio of dark-field to absorption signals.

Reference:

1. Wang Z, Hauser N, Singer G, Trippel M, Kubik-Huch RA, Schneider CW*, et al.* Non-invasive classification of microcalcifications with phase-contrast X-ray mammography. *Nat Commun.* **5**, (2014).

CATH-FunFams: New domain families to explore protein structure and function space

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There are vast amounts of protein structure and especially protein sequence data in public repositories ? more than 100,000 proteins structures in the PDB and over 60 million sequences in UniProt and ENSEMBL. Applying an evolutionary lens we have developed new approaches to identify families of homologous protein domains that share highly similar structural and functional characteristics. These are mostly orthologues and those paralogues which have retained similar functional characteristics. In some cases they are extremely distant relatives allowing us to capture structural and sequence characteristics conserved over very large evolutionary timescales and which can inform on functional constraints on the domain. These functional families (called FunFams) are therefore useful for capturing functional sites and we have applied them to understand how mutations of residues at or close to these sites cause functional shifts. We have also used them to build accurate structural models for uncharacterised proteins. Using our FunMod pipeline nearly 70% of human proteins can be assigned known structural domains or good quality models and these can be used to build good models of 5000 human protein complexes. Our data is being used to rationalise the impacts of genetic mutations linked to cancer and other diseases.

A plasmid that became a chromosome

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The majority of bacteria possess a single chromosome where replication is mediated by the conserved replication system consisting of the replication origin *OriC* and initiator protein DnaA. However, a small number of bacterial species scatter their genome across multiple chromosomes. For example, *Vibrio cholerae* possesses two chromosomes, and the replication of the secondary chromosome is mediated by a separate replication system consisting of replication origin *OriCII* – and a unique replication initiator protein RctB. RctB initiator does not bear any sequence similarity to other proteins. To date, no structural information was available for the RctB initiator, and this has hindered our understanding of how DNA replication is initiated on the secondary bacterial chromosome.

Here, we report that *Vibrio cholerae* RctB consists from four domains, and we have determined the crystal structures of three of these four RctB domains. The structures reveal that RctB possesses three winged-helix motifs involved in binding of the replication origin. RctB crystallized as a head-to-head dimer that is likely to be the dimer present in solution. The head-to-head RctB dimer is not compatible with a head-to-tail arrangement of the binding sites within *OriCII*, therefore a structural rearrangement has to take place before RctB can bind the replication origin and initiate DNA replication. The obtained structural information allowed for a development of an *OriCII*-RctB complex model accounting for the presence of three DNA-binding domains in each RctB molecule, and it explains the possible regulation mechanism that is achieved by different functionality of RctB head-to-head dimer and RctB monomer. Interestingly, the middle fragment of RctB possesses a high degree of structural similarity with plasmid replication initiators, which supports a theory that secondary chromosomes evolved from megaplasmids.

A Bent Laue Energy Dispersive Monochromator: An Example Application of Speciation Imaging at the Selenium K-edge

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Obtaining a 3D image of chemical forms of an element within a living system would be insightful for many health research areas. XAS can derive chemical form information, but is challenging to apply to 3D objects. Meanwhile, K-Edge Subtraction imaging (KES) can reveal the 3D distribution of an element, but traditionally with no chemical information. This project aims to study the feasibility of 3D speciation imaging with KES by developing our monochromator and analysis algorithms using Se in a living plant system. Future studies will apply our methods to biomedical systems.

We have developed a class of bent Laue monochromators which have very good energy resolving capabilities which we have used for iodine, barium and xenon imaging in biomedical systems at the CLS biomedical beamline. These reflection planes are asymmetric which significantly improves the efficiency and by proper choice of asymmetry angle, give excellent focal and energy dispersion properties. For this work around the Se K-edge at 12.658keV, we purchased 0.80mm thick silicon wafers which were thinned to 0.3mm for optimal transmission or Laue geometry diffraction performance. The wafers had a [2,2,4] surface normal and we used for diffraction [1,1,-1] reflection. The [1,1,-1] lattice planes were inclined 5 degrees from being perpendicular to the surface in the diffraction plan which was chosen to provide nearly optimal energy dispersive properties. The monochromator was bent to a 2m radius with a ~1m focus downstream. The energy range in the beam was over 200eV. The detector is located approximately 2m downstream.

Using the CLS biomedical bend magnet beamline we measured with an air filled ionization chamber a photon rate of 7.9 x 1013 ph/s. As a comparison, a calculation of the vertically integrated photon rate for the same conditions using a double-crystal Si (1,1,1) Bragg monochromator is 6.5 x 1011 ph/s; a factor of over 100 times lower photon rate.

The energy resolution was blurred by about 1.5eV Gaussian width compared to measurements using a dedicated XAS beamline using a Si (2,2,0) Bragg type monochromator for three Se compounds.

Samples were both projection and computed tomography (CT) imaged using the monochromator and these results will also be presented. Initial tests of the system are quite encouraging with sufficient energy resolution to give good results for CT. Crystal polishing of the crystal was done and new results obtained and the new results will be presented.

Angular correlations of photons from solution diffraction at a free electron laser encode molecular structure

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During exposure of a molecular solution, photons scattered from the same molecule are correlated. If molecular motion is insignificant during exposure, then differences in momentum transfer between correlated photons are direct measurements of the molecular structure. In conventional small- and wide-angle solution scattering, photon correlations are ignored. We present advances in a new bio-molecular structural analysis technique, correlated X-ray scattering (CXS), which uses angular intensity correlations to recover hidden structural details from molecules in solution [1]. Due to its intense, rapid pulses, an X-ray Free Electron Laser (XFEL) is an excellent tool for CXS experiments. A protocol is outlined for analysis of a CXS dataset comprising a total of half a million X-ray exposures of solutions of small gold nanoparticles recorded at the Spring-8 Angstrom Compact XFEL facility (SACLA). From the scattered intensities and their correlations, two populations of nanoparticle domains within the solution are distinguished: small, twinned and large, probably non-twinned domains. It is analytically shown how, in a solution measurement, twinning information is only accessible via intensity correlations, demonstrating how CXS reveals atomic-level information from a disordered solution of like-molecules.

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[1] Mendez, D., Watkins, H., Qiao, S., Raines, K. S., Lane, T.J., Schenk, G., Nelson, G., Tono, K., Joti, Y., Yabashi, M., Ratner D., & Doniach, S. (2016). *Angular correlations of photons from solution diffraction at a free electron laser encode molecular structure. Submitted.*

Recent Developments at the Beamline for Biological Small Angle X-ray Scattering BL4-2 at SSRL

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The small-angle x-ray scattering station BL4-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) is a permanent experimental station dedicated to structural biology and biophysics, providing state-of-the-art experimental facilities for structural studies on nucleic acids, proteins, protein assemblies, virus particles, biological fibers, lipid membranes and their complexes. A range of specialized sample environments for variety of different SAXS experiments is available at the beamline, including a fully automated high-throughput sample delivery robot for static solution scattering on biological macromolecules. This robot automatically performs all the steps necessary for high quality data collection. A software pipeline can be run simultaneously that will automatically reduce the data and carry out initial analysis in real time, summarizing the results for the user in an easy to view html table. In case of aggregation prone samples or weakly bound complexes a size-exclusion chromatography (SEC) setup can be directly connected to the instrument allowing in-line SEC-SAXS experiments to provide the highest sample quality for the SAXS data collection. In addition to the static solution scattering measurements we also provide state-of-the-art instrumentation for time resolved experiments on biological systems on the millisecond time scale. Our stopped-flow device has been optimized to substantially reduce the required sample volume in order to allow time-resolved measurements on systems that are difficult to produce in larger amounts. Here we will further discuss recent technological developments and present some of the recent scientific results obtained at the beamline.

Exploiting structural bioinformatics for unconventional molecular replacement

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Molecular Replacement (MR) is the most popular route to protein crystal structure solution but is limited by the availability of suitable structures in the PDB to act as search models. Where such structures are simply unavailable, as with new folds, computational production of suitable search models is desirable. Even when PDB structures are recognisably similar to the target, they may still be too divergent to succeed: in such cases processing of the available homologues may tip the balance towards success. AMPLE is a pipeline for generating and processing search models in each of these scenarios. Using local Rosetta or the Quark server, small globular domains are frequently modelled ab initio with sufficient accuracy for successful MR. The ab initio structure predictions are clustered and rationally truncated to produce sets of search models to trial. Coiled-coil proteins also work extremely well by this approach, while incorporation of additional restraints - derived from evolutionary covariance - extends success to larger targets. Where multiple structures distantly homologous to the target are detectable in the PDB, AMPLE's truncation approach can effectively generate core ensembles which may succeed where conventional treatments fail. Finally, methods for the identification of structurally conserved cores even in single distant homologues are presented, enhancing their value for MR.

SER-CAT/UGA Native-SAD Highlights

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Native-SAD phasing uses the anomalous scattering signal of light atoms in the crystalline, native sample of macromolecules collected from single-wavelength X-ray diffraction experiments. These atoms include sodium, magnesium, phosphorus, sulfur, chlorine, potassium and calcium. The technique is challenging and critically dependent on the collection of accurate data and mitigation of all sources of noise (error) in the data collection chain from source ➔ crystal ➔ detector and other endstation components.

Using a generous grant of General User beam time from the APS, SER-CAT/UGA has initiated a pilot program aimed at enabling routine Native-SAD data collection on 22BM. The granted beam time will be used (1) to enhance beamline stability at longer wavelengths on 22BM (2) to carry out commissioning studies on selected Native-SAD targets and (3) to collect Native-SAD data from samples provided by SER-CAT members and the Community and (4) to advance through collaborative efforts with particicipating researchers to make Native-SAD phasing a routine process for macromolecular structure determination.

A web site (UGA-APS.Pilot.uga.edu) is being developed to serve as a clearing house for information related to the Native-SAD experiment and serve as a portal for requesting Native-SAD beam time on 22BM.

Details of the Native-SAD pilot project, hardware upgrades to 22BM to support routine Native-SAD data collection and information about how to participate in the SER-CAT/UGA Native-SAD program will be presented.

Structural Basis for Epstein-Barr Virus Host Cell Tropism mediated by gp42 and gHgL Entry Glycoproteins

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Herpesvirus entry into host cells is mediated by multiple virally-encoded receptor binding and membrane fusion glycoproteins. Despite their importance in host cell tropism and disease pathology, the essential interactions between these viral glycoproteins remain poorly understood. Epstein-Barr Virus (EBV) is an important pathogen and the prototypical member of the subfamily *gammaherpesvirinae.* EBV is etiologic of acute infectious mononucleosis (IM) in children. EBV is an oncogenic virus, causally associated with malignancies of lymphoid system such as Burkitt's and Hodgkin's lymphoma, and epithelial cell disorders like nasopharyngeal and gastric carcinomas in immunocompromised patients. EBV requires a viral glycoprotein complex of gH, gL and gp42 for its entry into B cells. Binding of gHgL/gp42 complexes to host receptor (HLA class II) activates the viral gB protein to drive membrane fusion. Both gp42 and gp42-derived peptides bound to gHgL inhibit fusion with epithelial cells, regulating target cell infection. Here, we describe the crystal structure of the gHgL/gp42 complex bound to an anti-gHgL monoclonal antibody (E1D1). The structure reveals the gp42 N-domain, which forms a high affinity interaction with gHgL by wrapping around the exterior of gH domains D-II to D-IV. This interaction positions the transmembrane domains of gH and gp42 at the same end of the complex, consistent with their predicted orientation in the viral lipid bilayer. The gp42 N-domain, disordered in the absence of gH, adopts a well-defined, extended conformation over 40 amino acids forming extensive contacts with 5 gH subsite pockets. The gp42 C-terminal domain also interacts with gH, through a previously identified hydrophobic pocket (HP). Two adjacent histidines located centrally within the gp42 HP contact glutamic acids in gH D-II, which could play a role in modulating open-closed states of the gHgL/gp42 complex in a pH-dependent manner. The E1D1 Fab binds unexpectedly to the tip of the gHgL domain I (D-I), exclusively engaging gL amino acids and not gH residues previously implicated in its binding. E1D1 has a substantial inhibition of epithelial cell fusion but no inhibition of B cell fusion. Mutants of gL that disrupt E1D1 binding mimic the effects of antibody, with little impact on B cell fusion and partial inhibition of epithelial cell fusion, highlighting specific differences in their respective host receptor-mediated activation steps. These studies provide insights into the structural determinants of gp42-mediated specificity of EBV infection of host cells and highlight regions of gHgL that are functionally divergent in cell-specific virus entry.

Phasing Serial Femtosecond Crystallography data

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Serial Femtosecond Crystallography (SFX) at X-ray free-electron lasers (XFELs) offers unprecedented possibilities for time-resolved crystallography and for macromolecular structure determination of systems prone to radiation damage. However, phasing XFEL data *de novo* is complicated by the inherent inaccuracy of SFX data. So far, only a few successful examples have been reported, mostly based on exceedingly strong anomalous or isomorphous signals. Recently, a number of model systems that diffract to high resolution were phased using the weak anomalous scattering from the endogenous sulphur atoms. We present an overview of successes and challenges of SAD phasing of SFX data.

Ultrafast Dynamics in Proteins Investigated by Time-Resolved Serial Femtosecond Crystallography

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Fundamental events in chemical reactions such as bond braking, bond formation and isomerization happen on the femtosecond time scale. Isomerizations are of particular importance to both chemistry and biology. Cis-trans isomerizations lie at the base of light perception and numerous highly important reactions such as proton pumping and energy generation by light. These reactions sparked the interest of theorists and experimentalists alike. Although computational approaches to characterize these reactions “in-silico” as well as spectroscopic approaches to determine the dynamics were extremely successful, the three dimensional structures remained experimentally elusive. The reason for this was that structure determination could not be performed fast enough to follow the evolution of the electron density distribution during these ultrafast events. With the advent of the free electron lasers such as the Linac Coherent Light Source (LCLS) this changed. By exploiting the 40 femtosecond X-ray pulse duration at these machines, femtosecond processes can now be time-resolved. We established that time-resolved serial femtosecond crystallography (TR-SFX) works at the LCLS [1] and followed the trans to cis isomerization of the central para-cinnamic acid (pCA) chromophore in the photoactive yellow protein in real time [2]. Excited state (ES) structural dynamics of the pCA initiates the trans to cis isomerization. Relaxation from the ES potential energy surface (PES) to the ground state (GS) PES occurs at about 600 fs, after which the configuration of the chromophore is cis. By comparing X-ray structures on both the ES-PES and the GS-PES on time scales from 100 fs to 3 ps we structurally characterize the long sought after transition through a conical intersection for the first time.

1. Tenboer J, Basu S, Zatsepin N, Pande K, Milathianaki D, Frank M, Hunter M, Boutet S, Williams GJ, Koglin JE, Oberthuer D, Heymann M, Kupitz C, Conrad C, Coe J, Roy-Chowdhury S, Weierstall U, James D, Wang D, Grant T, Barty A, Yefanov O, Scales J, Gati C, Seuring C, Srajer V, Henning R, Schwander P, Fromme R, Ourmazd A, Moffat K, Van Thor JJ, Spence JC, Fromme P, Chapman HN, Schmidt M (2014) Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein. Science 346 (6214):1242-1246. doi:10.1126/science.1259357

2. Pande K, Hutchison CDM, Groenhof G, Aquila A, Robinson JS, Tenboer J, Basu S, Boutet S, Deponte D, Liang M, White T, Zatsepin N, Yefanov O, Morozov D, Oberthuer D, Gati C, Subramanian G, James D, Zhao Y, Koralek J, Brayshaw J, Kupitz C, Conrad C, Roy-Chowdhury S, Coe JD, Metz M, Lourdu Xavier P, Grant TD, Koglin J, G. K, Fromme R, Srajer V, Henning R, Spence JHC, Ourmazd A, Schwander P, Weierstall U, Frank M, Fromme P, Barty A, Chapman HN, Moffat K, Van Thor JJ, Schmidt M (2016) Femtosecond Structural Dynamics Drives the Trans/Cis Isomerization in Photoactive Yellow Protein. Science 352 (6286):725-729

Insights into HIV-1 proviral transcription from an integrative structure of the Tat:AFF4:P-TEFb:TAR complex

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HIV-1 Tat hijacks the human superelongation complex (SEC) to promote proviral transcription. HIV-1 Tat forms a physical complex with P-TEFb (Cyclin T1 and CDK9), AFF1/4, and HIV-1 TAR RNA. Solution structures of peptide:TAR complexes and a crystal structure of Tat:AFF4:P-TEFb are known, but the structure of the active Tat:AFF4:P-TEFb:TAR complex has been elusive. We used hydrogen-deuterium exchange, small angle x-ray scattering, and selective 2'-hydroxyl acylation analyzed by primer extension to determine the integrative structure of the complex. The structure reveals direct contacts between helix α2 of AFF4 and TAR, along with contributions of Cyc T1 TRM and Tat ARM to TAR major groove binding. Point mutations in helix α2 of AFF4 reduced TAR binding to Tat-AFF4-P-TEFb in solution, and the corresponding mutations in AFF1 reduce Tat transactivation in cell-based reporter assays. These findings provide a structural framework for a critical complex in the regulation of HIV-1 latency.

Recent SAXS developments dedicated for solution scattering of biological macromolecules at the BioCAT beamline 18-ID at the Advanced Photon Source

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Abstract

The 18ID undulator beamline of Biophysics Collabortive Access Team (BioCAT) at the Advanced Photon Source (APS), Argonne National Laboratory was designed and commissioned in 1997 for the study of partially ordered and disordered biological materials with a strong focus in fiber diffraction and X-ray absorption spectroscopy. In the past decade, the increasing demand of small angle X-ray scattering in the structural biology community motivates us to develop incrementally the SAXS capability dedicated for biological macromolecules in solution. A modular SAXS camera has been designed and optimized to cover the momentum transfer Q from 0.004 to 0.4 $Å^{-1}$ with a 3.5 m flight tube and a 2.5 mm beamstop. In recent years, data quality are significantly improved after the deployment of a Pilatus 3 1M detector and the implementation of coupling a size-exclusion chromatography system directly to the quartz flow cell for X-ray exposure. In order to utilize the continuous flow systems for time resolved studies, a Compound Refractive Lens (CRL) was commissioned to reduce the focused beam size down to 5 x 20 μm which perfectly matches the channel width of microfluidics chaotic and laminar flow mixers. The instrumentation of equilibrium SAXS and time resolved SAXS using a laminar flow mixer is described in detail. The system performance is demonstrated in the high quality data obtained in recently published user experiments.

High resolution structure of photosystem II and the mechanism of photosynthetic water-splitting

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Photosystem II (PSII) is a membrane-protein complex consisting of 20 subunits with a molecular mass of 350 kDa for a monomer, and catalyzes light-induced water oxidation in photosynthesis, resulting in the production of molecular oxygen indispensible for aerobic life on the earth. The structure of dimeric PSII has been solved at 1.9 Å resolution using synchrotron radiation X-rays [1], which provided the first atomic structure of the oxygen-evolving complex (OEC), the catalytic center for water-oxidation. The structure of the OEC was revealed to be a Mn_4CaO_5-cluster organized in a distorted chair form. The inter-atomic distances within the cluster, however, were suggested to be slightly longer than those obtained from EXAFS and theoretical (QM/MM) calculations; which were taken as evidence suggesting partial reduction of the Mn ions by X-ray radiation damage during data collection. In order to remove such radiation damage, we employed femtosecond X-ray free electron lasers (XFEL) at SACLA, Japan, to determine the damage-free structure of PSII. By using more than 100 large PSII crystals with an approach of fixed-target crystallography at cryo-temperature, we were able to determine the damage-free structure of PSII at 1.95 Å resolution [2], which showed slightly shorter inter-atomic distances for most of the Mn-Mn and some of the Mn-ligand bonds within the Mn_4CaO_5-cluster. We further determined the structure of a reaction intermediate produced by flash-illumination of small PSII crystals at room temperature, using the serial femtosecond X-ray crystallography (SFX) at SACLA. Difference Fourier-map between the light-induced intermediate and the dark-stable structure showed clear structural changes, which provides important clues to the reaction mechanism of water-splitting. Based on these results, I will discuss the mechanism of photosynthetic water oxidation and O=O bond formation catalyzed by the Mn_4CaO_4-cluster.

References

1. Umena Y., Kawakami K., Shen J.-R., Kamiya N., *Nature*, 2011, **473**, 55-60.
2. Suga M., Akita F., Hirata K., Ueno G., Murakami H., Nakajima Y., Shimizu T., Yamashita K., Yamamoto M., Ago H. and Shen J.-R., *Nature*, 2015, **517**, 99-103.

Linear Oligomerization Process of BAX Revealed from Coexisting Intermediates in Solution

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Upon receipt of apoptotic stimuli, the proapoptotic B-cell lymphoma 2 (Bcl-2) associated X protein (BAX) changes conformation and oligomerize to permeabilize the mitochondrial outer membrane. The critical oligomerization process and intermediate structures of BAX from monomer to membrane-associated complex along the apoptotic signaling pathway are of interest but remain elusive over years. In this study, the co-existing intermediate structures of BAX including dimer, tetramer, and octamer in solution are revealed using scanning small-angle X-ray scattering (SAXS) with online size exclusion chromatography. These intermediate structures are reconstructed on the basis of integrated SAXS-envelops structure and the skeleton structures determined by electron spin resonance (ESR). The hence revealed structural features of the intermediates and the corresponding population evolutions observed during the reaction suggest a linear oligomerization process of BAX, and project higher order of BAX oligomers to linear segments, arcs, and ring-like structures reported very recently.

Regulation of protein targeting: when SRP meets the ribosome

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Most membrane proteins are delivered to the membrane by the signal recognition particle (SRP) pathway which relies on the recognition of an N-terminal signal sequence. SRP is a universally conserved RNA-protein complex in which the RNA is much more than a scaffold. The SRP RNA plays a central role for the function of both, the Alu domain and the S domain. For the Alu domain we show how it tunes translation by a “dock and block” mechanism. We showed how SRP68 remodels the RNA in eukaryotic SRP. SRP68 shapes a part of the S domain RNA which is important for ribosome binding and efficient protein translocation. Our data visualize the structural evolution of SRP and show how the SRP system is regulated, and how functions of the SRP RNA have been transferred to proteins.

New MX beamline dedicated to *in situ* diffraction experiments

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Crystallization is an iterative process where conditions are optimized until diffraction quality crystals are obtained. But, crystallization is currently separated from the diffraction experiment. Diamond has pioneered microfocus experiments [1] and explored the feasibility of in situ diffraction experiments [2]. This has highlighted the potential value of a microfocus beamline dedicated to automated in situ diffraction experiments: (1) no manipulation of individual crystals, thus preserving the crystal integrity, (2) immediate feedback on the diffraction, crystal quality and, in many cases, unit-cell parameters, space group, (3) full automation with high reliability, (4) a route for data collection from crystals that consistently lose diffraction capability when conventionally harvested, or else are hazardous and may not be harvested for safety reasons.

With this in mind, a new fully integrated, highly automated and remotely operated microfocus beamline is currently under construction at DLS. This new beamline will replace the existing MX beamline I02 by the end of 2016. This undulator beamline have two monochromator options: a Si(111) DCM ($\Delta E/E \sim 10^{-4}$, $>10^{12}$ photon/s) and a Ru/B_4C DMM ($\Delta E/E \sim 10^{-2}$, $>10^{14}$ photon/s), and a KB mirror pair for focusing. The endstation is equipped with high-resolution sample viewing, SBS-format vertical goniometry operating at 4°C and 20°C, and a high-performance detector. Plate storage and imaging for >1000 plates at 4°C and 20°C will be an integrated part of the beamline and directly linked to the endstation with fully automated plate delivery. A web-based user interface provides combined database access to sample/crystallization information and diffraction/analysis information in ISPyB.

We will present our latest results on multilayer datacollection/processing, image-based sample alignment, and the mechanical performance of the specialized endstation setup.

References

[1] Evans G, Axford D, Waterman D, Owen R. Macromolecular microcrystallography. Crystallography Reviews, (2011) 17:105-142

[2] Axford D, Owen RL, Aishima J, Foadi J, Morgan AW, Robinson JI, Nettleship JE, Owens RJ, Moraes I, Fry EE, Grimes JM, Harlos K, Kotecha A, Ren J, Sutton G, Walter TS, Stuart DI, Evans G In situ macromolecular crystallography using microbeams.. Acta Crystallogr D Biol Crystallogr. (2012) 68:592-600

Integration of fast detectors into beamline controls at GM/CA@APS: Pilatus3 6M and Eiger 16M

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Fast detectors revolutionize many operations at MX beamlines, from introducing shutterless data collection and on-the-fly rastering scans to new standards for beamline stability, detector-goniometer synchronization at 100Hz data collection, and new ways to display data (e.g. plotting spots count in addition to visual inspection of individual frames). Fast crystal screening and data collection with such detectors also create new requirements for the computing environment, including fast automatic data processing and analysis pipelines on clusters, high-speed storage, distribution and transfer of data to remote institutions, and software to handle new data formats such as HDF5. The General Medical Sciences and Cancer Institutes Structural Biology Facility at the Advanced Photon Source (GM/CA @ APS) has operated the Pilatus3 6M detector at the 23ID-D beamline for 2.5 years and just started operating the Eiger 16M at 23ID-B. In this presentation we report how we incorporated controls for these detectors into our EPICS-based JBluIce control system, how we collect, display and automatically process data on computing clusters at speeds up to 100 frames per second, how we re-arranged our network and distributed storage to accommodate greater data speeds and volumes, and how we are optimizing our ScienceDMZ network to speed up remote data backups.

Abstract

Structure-based piecing together of the Type III Secretion System Puzzle

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Bacteria have evolved several dedicated and sophisticated assemblies to transport proteins across their biological membranes. Recent advances in our understanding of the molecular details governing the specific actions of these protein secretion systems has benefited from an integrated x-ray crystallography, NMR, mass spectroscopy, electron microscopy, and Rosetta-based molecular modeling toolbox. Highlights of recent advances in our piece wise structure/function analysis of the Type III Secretion system "injectisome" will be presented. A molecular understanding of the Type III systems being garnered from these studies provides the foundation for the development of new classes of vaccines and antimicrobials to combat infection in the clinic and community.

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MacCHESS, a Synchrotron Source with Unique Opportunities for Structural Biology

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MacCHESS ("**Mac**romolecular diffraction at **CHESS**") is an NIH-funded facility at the Cornell High Energy Synchrotron Source; we provide an excellent user facility with an exceptional level of staff support, as well as pursuing research to benefit the entire structural biology community.

Crystallography – We provide a high-flux monochromatic beamline equipped with a Pilatus3 6M detector, single-axis goniometer with automounter, excellent crystal visualization and centering capability, and extras such as on-line crystal annealing, operated by a reliable data collection interface which supports remote data collection. BSL-2 biohazards can be accommodated.

BioSAXS – A dual SAXS/WAXS setup, using two Pilatus 100K detectors, is standard. An integrated computer-controlled flow system supports either robotic sample loading from a 96-well tray or in-line SEC-SAXS, with monitoring by DLS/MALS. Custom sample cells use thin flat glass windows to minimize scattering artifacts. User-friendly software controls both collection and analysis of data. Periodic workshops educate potential users in the intricacies of BioSAXS.

High pressure cryocooling (HPC) – Cryocooling under pressure can reduce cooling-induced degradation and the need for penetrating cryoprotectants. It can also stabilize mobile ligands, and reduce some kinds of lattice disorder. We offer HPC as a service to CHESS users, while continuing to develop the method.

Research highlights – In-house research projects include aspects of serial microcrystallography (microbeams, background reduction, crystal transport, detectors, software), BioSAXS (time-resolved experiments, microfluidics, sample cell optimization, CryoSAXS), and analysis of diffuse scattering from crystals.

More information – To request beamtime, submit an on-line proposal at https://userdb.chess.cornell.edu. Mail-in service is available for crystallography (standard or using HPC) and BioSAXS. We welcome a chance to collaborate on "non-standard" experiments. For more information, contact User Administrator Kathy Dedrick (kd73@cornell.edu).

Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Time-Resolved Crystallographic Studies of Protein Dynamics

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Time-resolved X-ray crystallography is among the most information-rich experimental techniques in structural biology. To date, systems that have been successfully studied are those in which a protein conformational change is coupled to excitation of a photoactive ligand molecule, because the conformational change can be initiated with an ultrafast laser pulse. Unfortunately, the number of proteins that undergo photochemistry as part of their functional cycle is small, and there is a fundamental need to develop generalized methods that can be used to synchronously excite conformational transitions in *any* protein molecule, even in the absence of specific photochemistry. A recent "multi-temperature" crystallographic study of a model enzyme, cyclophilin A (CypA) demonstrated that temperature perturbation is an effective way to experimentally manipulate the conformational ensemble of a crystalline protein. Our current goal is to develop time-resolved crystallographic experiments that utilize laser-induced temperature-jump (T-jump) excitation methods to synchronize conformational dynamics. Initial SAXS/WAXS experiments demonstrate that even modest T-jumps produce a measurable change in X-ray scattering by the protein, and allow us to develop a kinetic model for how the X-ray scattering signal changes following the IR laser pulse. We are now developing crystallographic T-jump experiments using the serial femtosecond crystallography technique at the LCLS. Because laser T-jump methods exploit photochemistry of the solvent, and not the protein molecules, we hope they will be universally applicable as a tool for studying protein dynamics.

Possibilities for the Life Sciences at the first diffraction limited light-source MAX IV.

Marjolein Thunnissen
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MAX IV is the next generation synchrotron radiation facility in Sweden. It replaces the old MAX-lab that has served a wide scientific community for nearly 30 years. The new synchrotron consists of two new storage rings (1.5 GeV and 3 GeV) using a 7-bend achromat design that leads to exceptionally low emittance and an unprecedented brilliance for a synchrotron source. MAX IV can be considered as the first in a new generation of diffraction limited synchrotron light-sources.

Not only will the 3 GeV storage ring provide scientist with high brilliant X-rays, but the coherence fraction of these X-rays will also be enhanced. Both this brilliance and the higher coherence will supply life scientists with unique opportunities. MAX IV is working with a suite of beamlines that will cover a wide range of length scales and also offers possibilities for time resolved experiments.

Progress of MAX IV commissioning both the machine and beamlines will be presented.

Probing symmetry, spin, and valency of metal centers via ultra-sensitive soft X-ray detectors

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Nature has found molecules to efficiently store, transport, and utilize energy, and research at the intersection of biology and physics aims to model and mimic these molecular systems. Energy transformations often feature an active metal site surrounded by a large matrix of light elements. One of the grand challenges in this field is to understand the unique local electronic structure that gives rise to the efficiency and selectivity of natural systems.

Core-level spectroscopy is a unique probe of local structure that offers a window into the electronic configuration of an active metal site. L-edge spectrosocpy of 3d transition metals in the soft x-ray regime can yield element, site, symmetry, and spin selective spectral information, but the application of these measurements has been hampered by the inability of current technology to detect low concentrations of metal embedded in a larger matrix, especially in radiation sensitive samples.

Superconducting transition edge sensor (TES) technology has been used to build novel detectors with greatly increased sensitivity in the x-ray regime at intermediate energy resolution (~1 eV, with a future goal of 0.5 eV). This spring we commissioned a new soft x-ray TES spectrometer at SSRL, with a scientific agenda driven in part by ultra-low concentration active site measurements in biology. We will present the most recent demonstrations from this new detector and scientific prospects for the TES at synchrotrons and free electron lasers. Recent measurements include model iron compounds and preliminary results on dry hemoglobin samples.

Solution Structure of an "open" *E. coli* Pol III Clamp Loader Sliding Clamp Complex

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ABSTRACT

Sliding clamps are opened and loaded onto primer template junctions by clamp loaders, and once loaded on DNA, confer processivity to replicative polymerases. Previously determined crystal structures of eukaryotic and T4 clamp loader-clamp complexes have captured the sliding clamps in either closed or only partially open interface conformations. In these solution structure studies, we have captured for the first time the clamp loader-sliding clamp complex from *Escherichia coli* using size exclusion chromatography coupled to small angle X-ray scattering (SEC-SAXS). The data suggests the sliding clamp is in an open conformation which is wide enough to permit duplex DNA binding. The data also provides information about spatial arrangement of the sliding clamp with respect to the clamp loader subunits and is compared to complex crystal structures determined from other organisms.

Platforms for biological researches at SACLA

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Among a variety of applications of an X-ray free electron laser (XFEL), biology is one of the most important targets. About 30% of the beamtime at SPring-8 Angstrom Compact Free Electron Laser (SACLA) is allocated to coherent diffraction imaging (CDI) and crystallography for biological samples, of which diffraction patterns are recorded in a “diffraction-before-destruction” scheme. A duration of X-ray pulse at SACLA is short enough (<10 fs) to outrun radiation damage of samples [1]. This feature helps to realize damage-free structure analysis [2,3] and to investigate fast structural dynamics.

To offer a wide variety of opportunities for advanced biological researches, we have developed experimental platforms for CDI and serial femtosecond crystallography (SFX); Multiple Application X-ray Imaging Chamber (MAXIC) [4] and Diverse Application Platform for Hard X-ray Diffraction in SACLA (DAPHNIS) [5], respectively. These instruments are characterized by the use of sample delivery systems for the “diffraction-before-destruction” experiment. A fast raster-scanning system is usually employed in MAXIC. X-ray pulses are applied to sample particles on a thin membrane with an enough separation between adjacent shot positions. This system can be applied to a custom-made membrane array such as a micro-liquid enclosure array [6]. In SFX using DAPHNIS, micrometer-size crystals are dispersed in a fluid and delivered with a fluid injector. Three types of injectors are available [7,8]; a liquid jet injector with the gas dynamic virtual nozzle, viscous fluid injector using a carrier such as a lipidic-cubic-phase matrix or grease, and pulsed droplet injector.

In this presentation, we will give an overview of the imaging and crystallography platforms at SACLA. Upgrade plans of MAXIC and DAPHNIS to facilitate pump-probe measurement will also be shown.

References

[1] I. Inoue et al., Proc. Natl. Acad. Sci. USA 113, 1492 (2016).
[2] K. Hirata et al., Nature Methods 11, 734 (2014).
[3] M. Suga et al., Nature 517, 99 (2015).
[4] C. Song et al., J. Appl. Cryst. 47, 188 (2014).
[5] K. Tono et al., J. Synchrotron Rad. 22, 532 (2015).
[6] T. Kimura et al., Nature Communications. 6, 3052 (2014).
[7] M. Sugahara et al., Nature Methods 12, 61, (2015).
[8] F. Mafune et al., Acta Cryst. D72, 520 (2016).

Small-Angle Scattering: an Effective Constraint in Modelling Complex Biomolecular Structures

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This talk will highlight studies of a key muscle protein where small-angle scattering, in combination with data from complementary methods, is advancing our understanding of its role in heart muscle action. Structural models of biomolecular complexes and assemblies are increasingly determined by integrative modeling that relies on varied types of experimental data and theoretical information. In this context, the small-angle scattering (SAS) of X-rays or neutrons from biological macromolecules in solution can provide unique structural insights. The rising interest in SAS in the structural biology community has multiple drivers, including advances in instrumentation and data analysis tools, but importantly because structural biology is concerned with larger, more complex and often partly flexible systems, such those that drive muscle action, that are well suited for study using SAS. For biomolecular complexes that can be reconstituted with specific components deuterated, neutron SAS with contrast variation provides reliable information on the shapes and dispositions of individual subunits within the complexes. This talk will focus on our work on the cardiac Myosin Binding Protein C (cMyBP-C) that plays both structural and regulatory roles in heart muscle. The broader interest in cMyBP-C arises from the fact that approximately half the cases of inherited hypertrophic cardiomyopathy (HCM) are associated with mutations in this protein. The incidence of HCM estimated to be as high as 1 in 200, and it is the most common cause of fatal heart attacks in the young. Thus, there is strong motivation to understand the relationship between the structural impacts of the clinically-linked mutations at the molecular level and the changes in the muscle sarcomere that lead to thickening of the heart wall, disease and potentially catastrophic heart failure.

TPS-05A Protein Microcrystallography Beamline at the National Synchrotron Radiation Research Center

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The routine use of synchrotron radiation for single crystal diffraction study in the past decades has revolutionized macromolecular structural biology. However, crystals of important macromolecules, such as membrane proteins and viruses, are usually small in sizes and have poor diffraction quality. Advances in synchrotron radiation sources, detectors, and software are necessary to tackle these challenging problems. This beamline aims for difficult structures as well as routine data collection. The X-ray source of TPS-05A is a three meters long in-vacuum undulator (IU22), producing a high-brilliant X-ray beam. The X-rays are monochromated by a liquid-nitrogen-cooling Si double-crystal monochromator, and focused by a pair of Kirkpatrick–Baez mirrors. The focused beam size at the sample is 65 μm (H) x 36 μm (V) with photon flux of 1.1×10^{13} photons/s at 12.4keV. Apertures are used to collimate the beam in the range of 50–5 μm. The beam divergence at the sample is less than 500 μrad (H) and 100 μrad (V), and the energy range is from 5.7 to 20 keV (wavelength 2.175-0.62 Å). Equipped with a high speed CCD area detector and a micro-diffractometer, TPS-05A is capable of not only shutterless data collection for much higher throughput and helical scan to mitigate the radiation damage but also grid scan to find the best diffraction areas or to locate micro crystals. The optional mini-κ goniometer of the high precision micro-diffractometer enables crystal reorientation. A robotic sample changer for automatically sample mounting and centering in installed, making the data acquisition more efficient. Substantial user-support, remote access and mail-in service are also provided.

Fig. 1: Experimental instrumentations of TPS-05A (left). The micro-diffractometer MD2 (middle). The high speed CCD area detector Rayonix MX300HS (right).

Estimation of valences and radiation damage of four Mn atoms in photosystem II crystals using anomalous diffraction analysis

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Photosystem II (PSII) is a membrane-protein complex consisting of 20 subunits, and catalyzes light-induced water oxidation in photosynthesis leading to the production of protons, electrons and molecular oxygen. We have analyzed the structure of PSII from cyanobacterium at 1.9 Å resolution (PDB code: 3WU2(3ARC) [1]) using synchrotron X-rays, which revealed the structure of a Mn_4CaO_5-cluster, the catalytic center of oxygen evolving complex (OEC), for the first time. However, the valences of each of the four Mn atoms in OEC are still under debates, and some reports[2][3] from quantum chemical calculation or X-ray absorption analysis suggested that the Mn atoms in the crystal structure may be partially reduced due to X-ray radiation damage, resulting in elongated inter-atomic distances in OEC.

In order to uncover the catalytic mechanism of water oxidation by OEC, it is important to determine the valence of each of these Mn atoms, and also evaluate the possible effects of X-ray radiation damage on OEC. In this study, we analysed the individual valence of these Mn atoms in the PSII crystals by crystallographic analysis using anomalous difference Fourier map (ADF map) collected at Mn absorption K-edge wavelength, where the anomalous scattering factor of Mn changes depending on the oxidation state of the Mn atom. The ADF maps obtained showed different intensities of the maps corresponding to each of the four Mn atoms, indicating the different oxidation sates of each of the Mn atoms. Using the same approach, we also evaluated the degree of reduction of these Mn atoms by collecting several datasets with different X-ray doses (from 14 kGy to 19 MGy). The results showed that the four Mn atoms behave differently in response to the X-ray dose exposed. We will discuss the valences of the four Mn atoms and their sensitivity toward X-ray radiation damage, and demonstrate the usability of this method for determination of the oxidation states of multi-metal clusters in protein crystals.

References

[1] Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya. *Nature*, 2011, **473**, 55-60
[2] A. Galstyan, A. Robertazzi, E.W. Knapp. *J. Am. Chem. Soc.*, 2012, **134**, 7442-7449
[3] C. Glöckner, J. Kern, V. Yachandra, J. Yano, et al. *J. Biol. Chem.*, 2013, **288**, 22607-22620

CCP4: a resource for macromolecular crystallography

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The Collaborative Computational Project Number 4 (CCP4) has been serving the software needs of the protein crystallography community for more than 30 years [1]. In this time the CCP4 Suite of software has been refined through contributions from some of the leading developers in the field of protein crystallographic software and the feedback of both expert and novice users. Today it is a highly comprehensive suite, providing tools and packages covering all aspects from data collection through to structure deposition. We present details of the latest release series of the Suite, version 7.0 (Gargrove).

Release 7.0 is hailed as a milestone for the Suite, bringing a number of essential components and features, as well as many updates. The famous SHELX [2] software programs are now included in CCP4, which greatly enhances the Experimental Phasing functionality of the Suite. The new graphical interface, ccp4i2, has an attractive design and a simplified interface with emphasis on greater automation. The new data processing software DIALS [3] and the *ab initio* phasing package ARCIMBOLDO [4] are included in CCP4 7.0.

CCP4 provides a number of computationally intensive pipelines as web services which makes use of a Linux cluster as a backend. We discuss the latest developments of these services and further plans to meet new challenges arising due to changing computing environments and trends towards automation, remote computations and hosting projects in the Cloud.

[1] M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin and K. S. Wilson (2011) Overview of the CCP4 suite and current developments, *Acta Cryst.* **D67** 235-242.
[2] G. M. Sheldrick (2008) A short history of SHELX, *Acta Cryst.* **D64** 112-122.
[3] D. G. Waterman, G. Winter, J. M. Parkhurst, L. Fuentes-Montero, J. Hattne, A. S. Brewster, N. K. Sauter and G. Evans (2013) CCP4 Newsletter on Protein Crystallography **49**, 16-19.
[4] D. D. Rodríguez Martínez, C. Grosse, S. Himmel, C. González, I. M. de llarduya, S. Becker, G. M. Sheldrick and Isabel Usón (2009) ARCIMBOLDO: Crystallographic Ab Initio protein solution below atomic resolution, *Nat. Methods* **6** 651-653.

Structural and Molecular Biology of Bacterial Secretion Systems

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Gram-negative pathogens commonly exhibit adhesive pili on their surface that mediate specific attachment to the host. A major class of pili is assembled via the chaperone/usher (CU) pathway. Type 1 and P pili have served as model systems for the elucidation of the CU biosynthetic pathway. Pilus assembly requires a periplasmic chaperone (FimC and PapD for type 1 and P pili, respectively) and an outer-membrane assembly platform termed “usher” (FiimD and PapC for type 1 and P pili, respectively). CU pilus subunits are produced in the cytoplasm, translocated to the periplasm by the Sec translocation machinery, and then taken up by a chaperone to cross the periplasmic space to reach the outer-membrane. At the outer-membrane, chaperone-subunit complexes are recruited to an outer-membrane assembly platform, the usher, which orchestrates recruitment and polymerization of subunits. Previous work has elucidated the molecular basis of chaperone function. Recent progress has shed light into the mechanism of pilus subunit assembly at the usher, leading to the elucidation of the entire cycle of pilus subunit incorporation.

References

1. M. Hospenthal, A. Redzej, K. Dodson, M. Ukleja, B. Frenz, C. Rodrigues, S. Hultgren, F. DiMaio, E. Egelman* and **G. Waksman** (2016). Structure of a Chaperone-Usher pilus reveals the molecular basis of rod uncoiling. **Cell**. 164:269-278.
2. S. Geibel, E. Procko, S.J. Hultgren, D. Baker*, and **G. Waksman** (2013). Structure and energetic basis of folded protein transport by the FimD usher. **Nature**. 496:243-246.
3. G. Phan, H. Remaut, T. Wang, W. Allen, K. Pirker, A. Lebedev, N. Henderson, S. Geibel, E. Volkan, J. Yan, M. Kunze, J. Pinkner, B. Ford, C. Kay, H. Li, S. Hultgren, D. Thanassi*, and G. Waksman* (2011). Crystal structure of the FimD usher bound to its cognate FimC:FimH substrate. **Nature**. 474:49-53.

Small angle X-ray scattering analysis reveals that introduction of D-glutamate at a critical residue of Aβ42 stabilizes a pre-fibrillary aggregate with enhanced toxicity

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Amyloid-beta 42 (Aβ42) is an aggregation-prone peptide that plays a pivotal role in Alzheimer's disease.[1] We report that a subtle perturbation to the peptide through a single chirality change at Glutamate 22 leads to the stabilization of a soluble, ordered macromolecular assembly as determined by small angle X-ray scattering (SAXS).[2] This subtle chiral edit was found to demonstrate an enhanced cytotoxicity to PC12 cells, pronounced delay in β-sheet adoption and an attenuated propensity of the peptide to form fibrils, which was correlated with changes at the level of fibrillary architecture. Furthermore, Aβ42 containing the chiral edit at position 22 forms amyloid structures that are immunologically distinct from those formed by wildtype Aβ42, as indicated by differential reactivity patterns against amyloid conformation-specific monoclonal antibodies.[3] Our results highlight the importance of advanced pre-fibrillary Aβ aggregates in neurotoxicity.

[1] Selkoe, D. J., *Behav. Brain Re*s. **2008**, *192*, 106-113. doi: 10.1016/j.bbr.2008.02.016
[2] Warner, C. J. A.; Dutta, S.; Rodriguez, A. R.; Raskatov, J. A., *Chem. Eur. J.*, **2016**, manuscript online. doi: 10.1002/chem.201601763
[3] Hatami, A.; Albay, R.; Monjazeb, S.; Milton, S.; Glabe, C., *J Biol. Chem.*, **2014**, 289(46):32131-43. doi: 10.1074/jbc.M114.594846.

Uncovering the mechanism of FHA domain-mediated TIFA oligomerization that plays a central role in immune responses

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Forkhead-associated (FHA) domain is the only signaling domain that recognizes phosphothreonine (pThr) specifically. TRAF-interacting protein with an FHA domain (TIFA) is involved in the activation of nuclear factor κB (NF-κB) by various stimulations including tumor necrosis factor α (TNF-α), interleukin-1, lipopolysaccharide, and hypoxia. Our previous study showed that TIFA is intrinsic dimer in solution and that upon stimulation by TNF-α, the Thr9 of TIFA is phosphorylated. This phosphorylation triggers TIFA oligomerization via pThr9-FHA domain binding and activates NF-κB. While the FHA domain-pThr binding is known to mediate protein dimerization, its role in oligomerization has not been demonstrated at the structural level. Our study of truncated TIFA (residues 1-150, with the unstructured C-terminal tail truncated) and its complex with TIFA N-terminal pThr9-peptide (residues 1-15) show unique features in the FHA structure (intrinsic dimer and extra β-strand) and in its interaction with the pThr-peptide (with residues preceding rather than following pThr). These structural features support our functional analyses. Furthermore, the structure of the complex suggests that the pThr9-FHA domain interaction can only occur between different sets of dimers rather than between the two protomers within a dimer, this provides the structural mechanism for TIFA oligomerization. Our results uncover the mechanism of FHA domain-mediated oligomerization in a key step of immune responses, and expand the paradigm of FHA domain structure and function.

Molecular mechanism of reversible elasticity in the muscle filament bridge protein myomesin

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Muscle cells comprise highly sophisticated protein architecture, as they generate substantial molecular forces by contraction/relaxation and simultaneously need to respond to them to maintain their overall structure. In striated skeletal and cardiac muscles, the sarcomeric M-band ensures packing regularity of thick myosin filaments and a uniform distribution of tension across these filaments during contraction. Myomesin presents a prominent filament-connecting protein across the M-band and is considered to act as strain absorber to keep thick filaments in register during muscle contraction. To unravel the mechanism of myomesin molecular elasticity required to excel its function, we previously determined the overall architecture of the C-terminal part of this filament by combining X-ray crystallography, small angle X-ray scattering data and electron microscopy data [1]. Our findings revealed a dimeric tail-to-tail filament structure My9-My10-My11-My12-$(My13)_2$-My12'-My11'-My10'-My9'. It is folded into an irregular superhelical coil of almost identical immunoglobulin domain modules My9, My10, My11, My12 and My13, separated by highly exposed α-helical linkers. Unfolding of these linkers at very low tension below 10 pN applied by atomic force microscopy (AFM) can reversibly stretch this myomesin segment to about 2.5 times, from 36 nm to about 85 nm in overall length.

To gain further insight into the overall molecular myomesin architecture and to identify the borders of molecular elasticity we extended our data by two additional domains, My7 and My8, resulting in a revised overall filament model that covers more than half of the myomesin filament dimer My7-My8-My9-My10-My11-My12-$(My13)_2$-My12'-My11'-My10'-My9'-My8'-My7' (**Figure 1).** Our new electron microscopy, high-resolution X-ray crystallography data and AFM data demonstrate how the C-terminal elastic part of myomesin is terminated by a compact domain/domain array, including fibronectin-like domains My7 and My8 and the first immunoglobulin-like domain My9. In parallel, recent preliminary insights by electron microscopy of the complete myomesin filament plus additional high resolution X-ray structures from the N-terminal part of myomesin allow the first time unraveling the overall molecular architecture of a complete muscle filament system. We are planning to apply our expertise generated to related larger muscle filament systems as well to unravel general molecular principles associated with muscle filament protein elasticity.

[1] Pinotsis *et al.* (2012) Superhelical architecture of the myosin filament-linking protein myomesin with unusual elastic properties. PLoS Biol. **10**(2):e1001261.

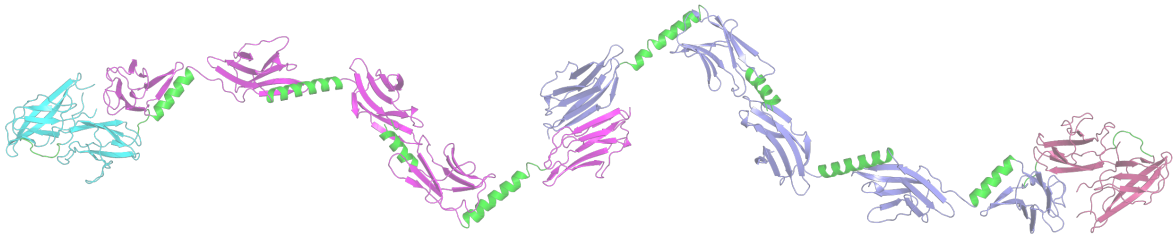

Figure 1: Composite structure of the C-terminal myomesin filament dimer: All individual domains are indicated; helical linkers are shown in green.

Using XFELs to visualize solvent in the Flu M2 Proton Channel

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The influenza A virus must acidify its interior to release its RNA. This important step in the viral life cycle relies on matrix protein 2 (M2), a pH-regulated proton channel embedded in the lipid envelope of the virus. M2 has been a subject of intense study both because it the target of adamantane compounds that can be used to treat flu and because it is an ideal model system to study proton conduction across membranes. The structural location of ordered water within the M2 channel plays a key role in both of these areas. Ordered water molecules are proposed to make key bridging interactions between adamantane compounds and the protein. Additionally, there is significant debate about the existence “water wires” of ordered solvent molecules that allow proton conduction by connecting the viral exterior and interior. It is therefore critical to determine the distribution of water molecules within the channel. Previous high resolution X-ray data of the M2 channel crystallized in the lipid sponge phase revealed interesting differences in the distributions of water as a function of pH. However, preliminary room temperature data called into question whether data collected from cryo-cooled crystals was informative for biologically relevant mechanisms of proton transport. Unfortunately, damage to the small M2 crystals from the radiation dose at room temperature precluded mechanistic interpretations of the solvent density. To circumvent these issues and to observe the functionally important structural dynamics of M2, we used X-ray Free Electron Lasers to collect radiation-damage free, 1.4 Å dataset of M2 at low pH. By avoiding the artifacts associated with cryo-cooling and eliminating the effects of radiation damage, the resulting room temperature data have allowed us to draw new conclusions about the solvent conformational heterogeneity. We will build on this work and use XFEL to monitor changes in the solvation of the pore of M2 as a function of both pH and time. Furthermore, these structures will help in the design of next-generation of antivirals address continuing problems with drug resistance.

Upgrading X-ray fluorescence imaging

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Present-day X-ray fluorescence microscopy was made possible by development of third generation synchrotrons (*1*). Nevertheless, less than 100 articles each in cell biology or neuroscience (according to Web of Science) refer to X-ray fluorescence as a "topic". At the same time, many thousands of publications on X-ray protein crystallography were produced over the same period. It is (still) impossible to make direst comparisons between structural biology and X-ray fluorescence imaging – biological information produced by these two techniques is distinctly different; nevertheless, new approaches for data acquisition and reconstruction have narrowed this gap. Thus, coherent diffraction imaging and hard X-ray fluorescence came to be mentioned as complementary techniques already in 2008 (*2*).

Upgrades that will make current synchrotrons into fourth generation instruments can be expected to improve photon flux and decrease imaging times (and/or enable imaging of samples inaccessible so far) in orders of magnitude. This will alleviate many challenges inherent in sample preparation and data acquisition; however, the possibility of sample degradation by radiation damage will increase as well as the difficulties of data processing. Fortunately, new sample preparation approaches are at hand as well as new computational developments.

Synchrotron upgrades will produce data rates that are orders of magnitude larger than at present. In order to enable users to extract more and new knowledge from their samples, to allow them to exploit the information their samples carry, new approaches are needed; such that can handle increased data volume, variety, and acquisition velocity. Thus, upgrading the X-ray fluorescence imaging will not depend only on new X-ray sources

References:

1. T. Paunesku, S. Vogt, J. Maser, B. Lai, G. Woloschak, X-ray fluorescence microprobe imaging in biology and medicine. *Journal of cellular biochemistry* **99**, 1489-1502 (2006).
2. G. J. Williams *et al.*, High-resolution X-ray imaging of Plasmodium falciparum-infected red blood cells. *Cytom. Part A* **73A**, 949-957 (2008).

Structural Basis of the Antizyme-Mediated Inhibition and Degradation of Ornithine Decarboxylase

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Polyamines are organic polycations essential for cell growth and differentiation; their aberrant accumulation is often associated with diseases, including many types of cancer. To maintain polyamine homeostasis, the catalytic activity and protein abundance of ornithine decarboxylase (ODC), the committed enzyme for polyamine biosynthesis, are reciprocally controlled by the regulatory proteins antizyme isoform 1 (Az_1) and antizyme inhibitor (AzIN). Az_1 suppresses polyamine production by inhibiting the assembly of the functional ODC homodimer and, most uniquely, by targeting ODC for ubiquitin-independent proteolytic destruction by the 26S proteasome. In contrast, AzIN positively regulates polyamine levels by competing with ODC for Az_1 binding. The structural basis of the Az_1-mediated regulation of polyamine homeostasis has remained elusive. Here we report crystal structures of human Az_1 complexed with either ODC or AzIN. Structural analysis revealed that Az_1 sterically blocks ODC homodimerization. Moreover, Az_1 binding triggers ODC degradation by inducing the exposure of a cryptic proteasome-interacting surface of ODC, which illustrates how a substrate protein may be primed upon association with Az_1 for ubiquitin-independent proteasome recognition. Dynamic and functional analyses further indicated that the Az_1-induced binding and degradation of ODC by proteasome can be decoupled, with the intrinsically disordered C-terminal tail fragment of ODC being required only for degradation but not binding. Finally, the AzIN–Az_1 structure suggests how AzIN may effectively compete with ODC for Az_1 to restore polyamine production. Taken together, our findings offer structural insights into the Az-mediated regulation of polyamine homeostasis and proteasomal degradation.

Chemical and electrochemical interaction mechanisms of metal reducing bacteria on steel surfaces

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Metal reducing bacteria (MRB) are able to utilize various materials such as iron, uranium and manganese as well as many organic compounds as electron acceptors. This process leads to the conversion of Fe(III) containing passive film species to soluble Fe(II) oxides and hydroxides. The reduction process triggers the acceleration of general and local corrosion processes. Electron transfer mechanisms are not yet fully understood. In literature it has been controversially discussed to which extend secreted electron shuttles contribute to the extracellular electron transfer (EET).

To understand the chemical and electrochemical interaction mechanisms of MRB with steel surfaces this project combines a variety of in-situ techniques. The changes in oxide chemistry on Fe/steel surfaces in the presence of biomolecules and MRB are under investigation using a newly designed electrochemical cell for in situ XANES (x-ray absorption near edge structure) spectroscopy. Electrochemical quartz crystal microbalance (eQCM) studies support the spectroscopic investigations to gain information about the kinetics of attachment processes and changes in biofilm viscosity. The biofilm structure and composition as well as cell viability are investigated by complementary ex situ spectroscopic and microscopic analysis.

Combining spectroscopic techniques and eQCM data with electrochemical measurements, biological processes and the resulting degradation of steel surfaces can be observed in a non-destructive manner. Selecting model systems and a defined biological medium allows the determination of individual effects of diverse surface and environmental parameters. The fundamental understanding of bacterial attachment mechanisms and initial steps of biofilm formation will contribute to the development of new antifouling strategies.

Upgrade of a macromolecular crystallography beamline, BL-17A, at the Photon Factory

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BL-17A is one of the macromolecular crystallography beamlines at the Photon Factory. The beamline was constructed in 2006, and due to its light source and dedicated optics, it was characterized as a beamline for diffraction experiments with small crystals, and it has contributed to a large number of significant achievements on important biological problems. However, recent trends in macromolecular crystallography moves to much more difficult targets, and demands from users to the beamline becomes much higher. In 2014 the beamline got an opportunity to be performed an extensive upgrade.

The upgrade includes mainly three subjects; (1) modification of optical layout, (2) installing a pixel array detector with a large active area and (3) installing a new diffractometer. Two existing focusing mirrors is moved to upstream and its focusing is adjusted to the upstream of end station to make a secondary source. The new KB mirror system is installed just upstream of the sample position to focus the X-ray beam from the secondary source more. The X-ray detector was replaced to Pilatus S6M in this autumn, which is a pixel array detector with a large active area (423.6 x 434.6 mm^2), short readout time (2.03 msec) and a relatively high frame rate (25 Hz in max.). The new diffractometer at BL17A is designed for the precise and efficient diffraction experiments with a smaller X-ray beam. The new diffractometer has an additional vertical goniometer head, which can handle a crystallization plate. This gives a new opportunity to users to perform in-situ data collection experiment, where crystals in a crystallization drop on the plate is directly irradiated to an X-ray beam.

The modification of optical layout and installing new apparatus were completed until March 2015, and the beamline commissioning was carried out from May to June 2015. After the commissioning, the beamline has been opened to general academic users and industrial users.

L-Edge Spectroscopic Investigation of {FeNO}6: Factors Determining Delocalization vs Antiferromagnetic Coupling

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NO is a classic non-innocent ligand and iron nitrosyls can have different electronic structure descriptions depending on their spin-state and coordination environment. These highly covalent systems are found in proteins and are also used as models for Fe-O_2 systems. This study utilizes iron L-edge X-ray absorption spectroscopy, interpreted using a valence bond configuration interaction multiplet model, to directly experimentally probe the electronic structure of the S=0 $\{FeNO\}^6$ compound $[Fe(PaPy_3)NO]^{2+}$ ($PaPy_3$ = N,N-bis(2-pyridylmethyl)amine-N-ethyl-2-pyridine-2-carboxamide) and the S=0 $[Fe(PaPy_3)CO]^+$ reference compound. This method allows separation of the σ-donation and π-acceptor interactions of the ligand through ligand-to-metal and metal-to-ligand charge transfer mixing pathways. The analysis shows that the $\{FeNO\}^6$ electronic structure is best described as Fe^{III}-NO(neutral), with no localized electron in an NO π* orbital or electron hole in an Fe dπ orbital. This delocalization comes from a large energy gap between the Fe-NO π-bonding and antibonding molecular orbitals relative to the exchange interaction between electrons in these orbitals.

The Life Science X-ray Scattering (LiX) Beamline at NSLS-II

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The LiX beamline is constructed as part of the Advanced Beamlines for Biological Investigations using X-rays (ABBIX) project funded by the National Institutes of Health (NIH). NIH is also supporting its continued development and operations through a P41 grant. This instrument is designed to operate in the x-ray energy range of 2.1-18 keV, provide variable beam sizes from 1 micron to ~0.5 mm, and support user experiments in three scientific areas: (1) high-throughput solution scattering, in-line size exclusion chromatography and flow mixers-based time-resolved solution scattering of biological macro-molecules, (2) diffraction from single- and multi-layered lipid membranes, and (3) scattering-based scanning probe imaging of biological tissues. In order to satisfy the beam stability required for these experiments and to switch rapidly between different types of experiments, we have adopted a secondary source with refractive lenses for secondary focusing, a detector system consisting of three Pilatus detectors, and specialized experimental modules that can be quickly exchanged and each dedicated to a defined set of experiments. The construction of the beamline has been completed in early 2016 and user operation is expected to start shortly. I will describe relevant details in the beamline design and innovations in the experimental station, as well as preliminary results from technical and scientific commissioning.

Photon Factory as a Research Hub in the Platform of Drug Discovery, Informatics, and Structural Life Science

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Platform of Drug Discovery, Informatics, and Structural Life Science (PDIS) is a project which has been open for all life scientists in Japan. It has three units such as “structural analysis” for protein production, structure determination, and bioinformatics, “regulation” for small molecule screening and the following medicinal chemistry, and “information” for integration of databases. Once a life scientist submitted a research proposal and got the approval, the researcher could be supported by experts in the units with a project manager who can guide the users to use structural information for designing a molecule in drug discovery after solving a protein-ligand structure at high resolution. Importantly, submission of a relatively short proposal to PDIS if it is approved, is only one requirement to obtain supports from the project without any cost. To accelerate the handling speed of peer reviewing process, a system in cloud has also been developed to facilitate communications among officers, administrators who have expertise in each field, and reviewers. At the structural analysis unit, the project in protein production, structural analysis with the biophysical techniques, and/or bioinformatics has supported more than 460 proposals, applied from principal investigators since end of FY2012. The researchers have published results and obtained new grants based on data obtained through the PDIS.

Photon Factory (PF) has played a pivotal role as a hub and also as a leading institute in the “structural analysis” unit which is consists of 46 groups in universities and national institutes. Life scientists have started collaborations with experts in biochemical and biophysical techniques such as protein crystallography, small angle X-ray scattering, NMR, cryo-electron microscopy, and structural bioinformatics. Infrastructure in the PF has also been upgraded, for instance, facility for native SAD experiment with a long wavelength X-ray has been developed. Many of new structures have been solved with the method. We will report on outcomes obtained through the project as a research hub to integrate structural biology, genomics, bioinformatics, and drug discovery efforts.

Advances in Fluctuation X-ray Scattering

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Fluctuation X-ray scattering (FXS)[1][2] is an X-ray scattering technique similar to small-angle X-ray scattering (SAXS), but is performed using X-ray exposures below sample rotational diffusion times. This technique, ideally performed with an ultra-bright X-ray light source, results in data containing significantly more information as compared to traditional scattering methods [3]. Data obtained from an FXS experiment comes in the form of intensity correlations, resulting in a double phase and amplitude determination problem that needs to be solved in order to obtain structural information. Recently, the general solution to structure determination from FXS data has been proposed [4], which has resulted in a number of structures determined from experimental data. Extension of this method to new sources and experimental modalities will be discussed.

1. Kam, Zvi (1977). "Determination of Macromolecular Structure in Solution by Spatial Correlation of Scattering Fluctuations". Macromolecules 10 (5): 927–934.

2. Kam, Z.; M. H. Koch, and J. Bordas (1981). "Fluctuation x-ray scattering from biological particles in frozen solution by using synchrotron radiation". Proceedings of the National Academy of Sciences of the United States of America 78 (6): 3559–3562.

3. Malmerberg, Erik; Cheryl A. Kerfeld and Petrus H. Zwart (2015). "Operational properties of fluctuation X-ray scattering data". IUCrJ 2 (3): 309–316.

4. Donatelli, Jeffrey J.; Peter H. Zwart, and James A. Sethian (2015). "Iterative phasing for fluctuation X-ray scattering" (PDF). Proceedings of the National Academy of Sciences of the United States of America 112: 10286–10291.