Structure Determination of PR772 Virus from Single-particle XFEL Data

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1. Outline
- In the absence of stochastic artifacts, XFEL single-particle snapshots can differ in particle orientations and conformations.
- Using experimental data from PR772 virus (SPI-AMO86615), we show that manifold embedding is able to determine the 3D structure of this virus to the resolution corresponding to the detector edge.

2. XFEL Snapshots of PR772 Virus
- Large viruses scatter millions of photons per shot
- This is much larger than needed for 3D reconstruction in the absence of strong stochastic effects
- However, diffraction patterns are strongly affected by extraneous effects such as shot-to-shot variations in intensity, aperture scattering, and background stochastic noise, etc.

3. Manifold Embedding Approach
- Manifold Embedding = nonlinear PCA
- All we have is ensemble of diffracted intensities.
- Ensemble of snapshots produces a cloud of points
- Manifold embedding by Diffusion Map extracts (nonlinear) manifold describing the signal.
- Diffusion Map provides mathematical link to “cloud of points” via Laplace-Beltrami operator.

4. Single-particle Hit Finding
- Manifold of raw data projected onto the first two Laplace-Beltrami eigenfunctions \( \psi_1 \) and \( \psi_2 \) reveals a parabolic characteristic.
- Parabola reveals shot-to-shot intensity variations of XFEL pulses.
- Diffusion Map identifies \( \sim 38,000 \) single-particle hits located at the lower edge of the manifold
- The single-particle data set is used for orientation recovery after appropriate preprocessing steps

5. Diffraction Volume from Manifold
- After removing imaging artifacts (background noise, intensity variations, etc.), manifold of single-particle snapshots from PR772 virus is consistent with Icosahedral Wigner D-functions.
- The object orientation is determined for each snapshot.
- 3D diffraction volume from \( \sim 38,000 \) 2D snapshots is recovered.
- Resolution of the recovered 3D volume extends to edge of detector (11nm determined by FSC).

6. 3D Structure from Manifold
- 3D electron density obtained from diffraction volume via iterative phasing.

7. Validation of Orientation Recovery
- Fourier-Shell correlation (FSC) is computed between electron densities of two random subsets.
- Reveals statistically significant information out to detector edge (11nm).

8. Summary
- Demonstrated single-particle 3D structure recovery to detector edge
- Manifold embedding a powerful end-to-end platform for single-particle structure recovery

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