

Radiation damage effects on protein conformation at room temperature and 100K

Silvia Russi¹, Shawn Kann¹, Henry van den Bedem^{1,2}, Lillian Kenner³ & Ana M. González¹

¹ Structural Molecular Biology, SSRL, Menlo Park CA, USA

² Joint Center for Structural Genomics, SSRL, Menlo Park CA, USA

³ Department of Bioengineering and Therapeutic Sciences, UCSF, San Francisco CA, USA

Abstract: To increase the lifetime of protein crystals, X-ray diffraction data collection at synchrotrons is customarily carried out at cryogenic temperatures to reduce radiation damage to the crystal. Yet for a host of crystallographic experiments, e.g. the unsuitability of cryo-protectants, the induced lattice changes or promotion of internal disorders during the cooling process, and the convenience of collecting data directly from the crystallization plates, data collections are increasingly carried out at room temperature. Several recent investigations have shed light on the effects that flash-freezing has on the conformational ensemble of crystal structures [1], and that these can hide from observation important functional mechanisms [2]. Though radiation damage at cryo-temperatures has been studied in depth, its effects at room temperature remain poorly understood. We investigated the effects of data collection temperature on secondary local damage to the side chain and main chain from different proteins. Data were collected from crystals of thaumatin, lysozyme and cyclophilin A at 100 K and room temperature. To carefully control the total absorbed dose, full data sets at room temperature were assembled from a few diffraction images per crystal. Several data sets were collected at increasing levels of absorbed dose. While a mild trend of increased conformational variability with radiation damage was observed at cryogenic temperatures, at room temperature was not the case. The break-up of the disulfide bonds also occur at different relative rate, perhaps because of a more active repair mechanism at room temperature.

[1] J.S. Fraser, H. van den Bedem, A.J. Samelson, P.T. Lang, J.M. Holton, N. Echols & T. Alber. *PNAS* (2011), vol. 108, no. 39, 16247-16252

[2] H. van den Bedem, G. Bhabha, K. Yang, P.E Wright & J.S. Fraser. *Nat Meth* (2013), vol. 10, no. 9, 896-902