Poster Presentation

MS22.P25

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

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Protein crystallography data collection at synchrotrons today is routinely carried out at cryogenic temperatures to mitigate radiation damage to the crystal. Although damage still takes place, at 100 K and below, the immobilization of free radicals increases the lifetime of the crystals by orders of magnitude. Increasingly, experiments are carried out at room temperature. The lack of adequate cryo-protectants, the induced lattice changes or internal disorders during the cooling process, and the convenience of collecting data directly from the crystallization plates, are some of the reasons. Moreover, recent studies have shown that flash-freezing affects the conformational ensemble of crystal structures [1], and can hide important functional mechanisms from observation [2]. While there has been a considerable amount of effort in studying radiation damage at cryo-temperatures, its effects at room temperature are still not well 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 and lysozyme 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. Our analysis shows that while at cryogenic temperatures, radiation damage increases the conformational variability, at room temperature it has the opposite effect . We also observed that disulfide bonds appear to break up at a different relative rate at room temperature, perhaps because of a more active repair mechanism. Our analysis suggests that elevated conformational heterogeneity in crystal structures at room temperature is observed despite radiation damage, and not as a result thereof.

[1] *J.S. Fraser, H. van den Bedem, A.J. Samelson, et al. PNAS (2011), vol. 108, no. 39, 16247-16252,* ***[2]*** *H. van den Bedem, G. Bhabha, K. Yang, et al., Nat Meth (2013), vol. 10, no. 9, 896-902*

Keywords: radiation damage, room temperature, multiple conformation