Structural Studies of a Metalloenzyme Essential for Vision by X-ray Crystallography and X-ray Spectroscopy

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The Case Center for Synchrotron Biosciences (CSB), supported under a P30 grant mechanism by NIBIB, is participating in the development of world-class synchrotron resources at the NSLS-II in three areas including X-ray footprinting, X-ray absorption spectroscopy (XAS), and macromolecular crystallography (MX). We provide support for users who employ single approaches but have a special interest in comprehensive studies using multiple synchrotron-based techniques simultaneously. CSB personnel have a long-time collaboration with the Palczewski Laboratory on structural and functional studies of RPE65, an essential enzyme in the mammalian retinoid cycle. The protein was initially studied by MX and XAS, and the crystal structure of the active RPE65 in lipids was also determined (1, 2). The crystal structure of the detergent-solubilized form of RPE65 established the presence of a non-heme iron active site with 4 His ligands. A refined local active site structure and iron valence were determined by XAS. The mechanism by which RPE65 catalyzes retinoid isomerization remained elusive because of uncertainty about how retinoids bind in the active site. The crystal structures of RPE65 in complex with retinoid-mimetic compounds were published recently and show the retinoid-binding pocket located near the membrane-associating surface of the enzyme (3). A mechanism of catalysis which reconciles the existing biochemical and structural data has been proposed. This model of multi-technique biophysical approaches to complex problems in structural biology has the potential to engage collaborations across many P41 centers and across many technologies. Our center uses a concierge approach to engage investigators and help them assess what other technologies might be valuable for this research programs; we then arrange collaborations and beamtime, or provide computational approaches for data integration, to help complete the project.

References:

1. Kiser PD, Golczak M, Lodowski DT, Chance MR, Palczewski K (2009) PNAS 106:17325.
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3. Kiser PD, Zhang J, Badiee M, Li Q, Shi W, Sui X, Golczak M, Tochtrop GP, Palczewski K (2015) Nat Chem Biol 11:409.