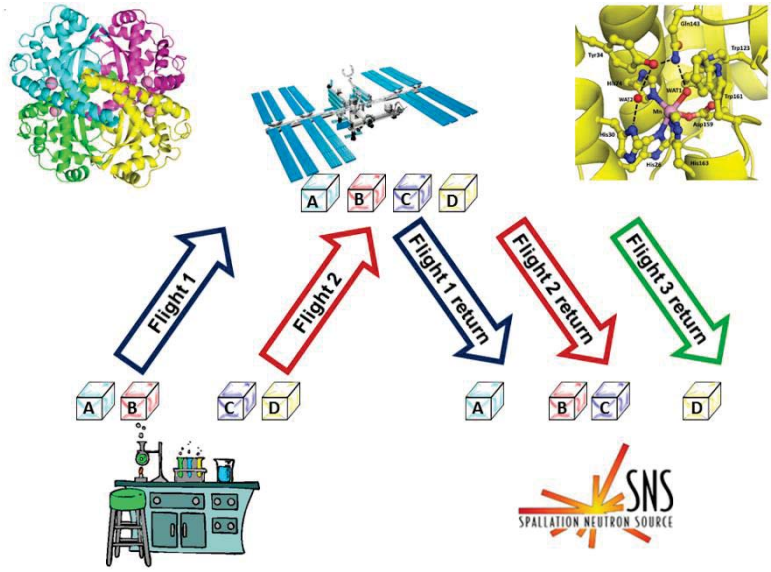
Growth of Large, Perfect Crystals of Human MnSOD for Neutron Crystallography

Gloria E. O. Borgstahl

Eppley Institute for Cancer and Allied Diseases, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, USA

Mitochondrial manganese-containing superoxide dismutase (MnSOD) is an important antioxidant enzyme that protects all living cells against toxic oxygen metabolites, also known as reactive oxygen species. SODs are the first defense against propagation of damaging oxidative reactions through elimination of superoxide. Normal metabolism and/or ionizing radiation generates superoxide. Each catalytic cycle dismutes two molecules of superoxide to oxygen and hydrogen peroxide via cyclic reduction and oxidation half reactions using the active site metal ion. Mutations in MnSOD lead to aging and degenerative diseases such as amyotrophic lateral sclerosis, diabetes, and cancer.

Our flights to International Space Station (ISS) will provide the critical crystal samples needed for a detailed study of human MnSOD. *Despite the biological and medical importance of MnSOD, the complete enzymatic mechanism is still unknown.* We need precise structural data to understand the binding sites of the diatomic substrate and product as well as the source of the protons in the reaction. Only neutron crystallography provides information at this level of detail. Perdeuterated human MnSOD will be the target for large volume crystal ($\geq$ 1mm^3) growth for structure determination by neutron crystallography. Our previous research during the space shuttle era showed that in the quiescent environment afforded by microgravity crystals grow not only large enough for neutron studies but also of a quality that approaches perfection. In 2001, the Borgstahl laboratory successfully grew large crystals of MnSOD using microgravity conditions on ISS. With NASA's renewed interest in implementing the microgravity environment on the ISS for protein crystal growth we look forward to crystallizing MnSOD on ISS. Capillary counterdiffusion crystallization protocols will achieve these goals. The ISS microgravity environment is essential to form a stable supersaturation gradient to obtain the required large, high quality crystals. We collect our neutron diffraction data at the MaNDi beamline at Oak Ridge National Laboratory (ORNL).



The principal outcomes will be to identify the role of hydrogen atoms in enzymatic activity, discern superoxide from peroxide, and water from hydroxide ion by their protonation states and to decipher a structure-based mechanism for human MnSOD more precisely than from previous X-ray crystallographic models determined from Earth-grown crystals.