X-ray Footprinting (XFP)

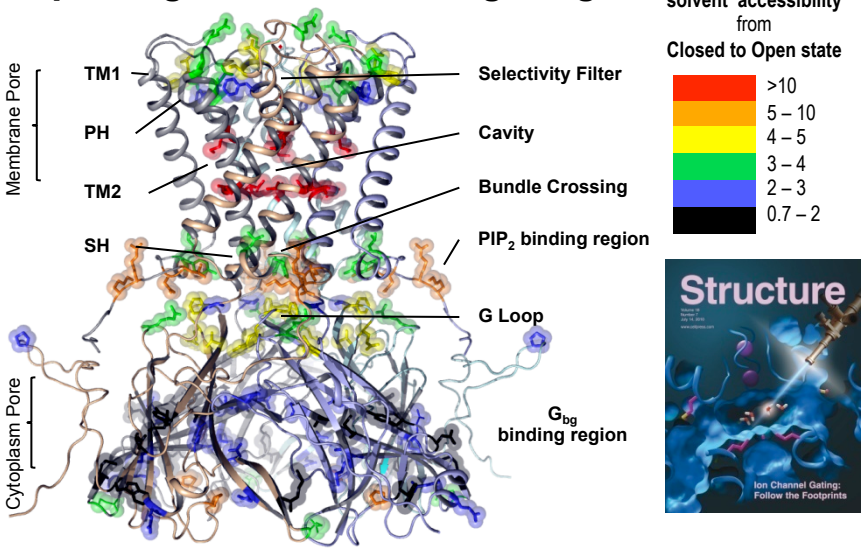
XFP at NSLS-II

- X-ray mediated hydroxyl-radical footprinting (XFP) will provide a local probe of solvent-accessibility for *in-vivo* and *in-vitro* structural studies of biomolecular complexes and their interactions.
- Time-resolved XFP studies to elucidate local structural dynamics from microsecond to millisecond time scales.
- The high flux density and beam energy range of NSLS-II DW will provide high quality data from microliter volumes of dilute solution samples in near physiological conditions.

Examples of Science Areas & Impact

- ***IN VIVO STUDIES***: Real time ribosomal biogenesis in living cell, cell surface receptor-ligand interactions (drug/protein, antibody/ antigen)
- ***MEMBRANE PROTEINS***: Understanding of structure and function at molecular level for ion channels, receptors (GPCR), gated pores, H^+-pumps, transporters, membrane enzymes, dynamics of bound waters in pores, channels and cavities
- ***MEGA DALTON COMPLEXES***: XFP provides structural information for intermediates and activated states of extremely large complexes (e.g. cell cytoskeleton, proteosome assemblies)
- ***HYBRID APPROACH***: XFP (local structural measures) along with SAXS (global) important in deciphering mechanism of biomolecular assemblies in "Biology Village" life sciences mode at NSLS II

Exploring the K^+ channel gating



Closed and open states of KirBac3.1 are irradiated with focused 'white beam' of beamline X28C of NSLS. The chemical modification mediated by the hydroxyl radical on the protein side chains are analyzed by high resolution mass spectrometry. The relative rate of modification between these two states are directly correlated to the changes solvent accessibility of residue undergoing modofications. This study has allowed the identification of **novel gating-sensitive residues** in the permeation pathways of the channel and also **residues that mediate the gating process through allosteric conformational rearrangements**.(Gupta et al. Structure 2010,18(7):839-46.)

Beamline Capabilities

TECHNIQUE(S): steady state and time-resolved x-ray hydroxyl-radical mediated protein and nucleic acid footprinting

SOURCE: damping wiggler

ENERGY RANGE / RESOLUTION: "white beam" (5-20 keV)

U.S. DEPARTMENT OF ENERGY

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