Center for Synchrotron Biosciences

X-ray Synchrotron Footprinting

NSLS Beamline X28C
Footprinting at the CSB: What We Do

- Provide access to world class facilities and expertise in x-ray synchrotron footprinting
- Provide a solution-state probe of protein and nucleic acid structure and dynamics at amino-acid side-chain or single-nucleotide level resolution
- Provide facilities to perform XF experiments in near-physiological to \textit{in vivo} conditions and/or on physiologically relevant timescales
Why Use X-ray Footprinting?

- Structural studies of macromolecules with samples in solution state in near-physiological to in vivo conditions
- Local probe of protein side chain or NA backbone solvent accessibility (amino-acid/single nucleotide level resolution)
- Reveals binding interfaces and conformational changes
- Macromolecular dynamics from msec to minutes
- Water dynamics
- Small amount of sample required (often pmoles to nmoles) for structural mass spectrometry

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History of Footprinting at the CSB

- 1995 – First x-ray footprinting experiments on X19C
- 1997 – Footprinting moved to X9A
- 1999 – Constructed/relocated X28B pinhole and received port X28C from NSLS (AECOM)
- 2000 – Built and commissioned dedicated XF beamline X28C
- 2007 - Installed toroidal focusing mirror, drastically expanding the scientific potential of the beamline
- 2012 – NSF MRI Award: Began design of NSLS-II XFP beamline
- 2013 – Established national benchmarks of BL performance
- Operate NSLS X28C until September 30, 2014
CSB Footprinting User Program 5-year Statistics

- Maintained and operated dedicated beamline X28C as the premier x-ray footprinting facility in the world
- 41 publications in peer reviewed journals (2 PhD dissertations focused on research at X28C)
- 32 separate User Groups (PIs), 213 experimental starts, ~25 users/yr maintain on-site BLOSA training (many Mail-in)
- 44 active projects – plus several multi-technique projects, mostly supported by NIH; support also from NSF, DOE, US and foreign institutions as well as industry
- Obtained NSF MRI funding for XFP beamline at NSLS-II, extended footprinting across US
- *Expanded scope of XF science!* Membrane proteins, water dynamics, live cell experiments...
X-ray Synchrotron Footprinting: Radiolysis

Radiolysis of water generates hydroxyl radicals

H₂O → H₂O⁺ + e⁻ (Fast)

H₂O⁺ + e⁻ → H₂O + OH⁻ (Slow)

H₂O⁺ → H⁺ + OH⁻

Rapid PERMANENT modification of AA side chains
Cleavage of NA phosphodiester backbone

General reactivity order of the 20 common amino acids

CYS > MET > TRP > TYR > PHE > HIS > LEU, ILE > ARG, LYS, VAL > THR, SER, PRO > GLU, GLN > ASN, ASP > ALA > GLY
Nucleic Acid Footprinting Technique

- Strand cleavage
- Protection
- Reaction Time
- Gel Electrophoresis
- Binding Isotherm
- Kinetic progress curve
- Site Specific $K_d$
- Site Specific Rate Constants, $k_{obs}$
The ‘Multi-Sample Holder’ Setup is used for steady-state experiments on small volume (5μl) samples — 10 ms minimum exposure time.
Quench Flow Experimental Setup

The KinTek® Quench-Flow Setup is used for steady-state experiments on larger volume (20 – 250 ml) samples and for time-resolved experiments requiring mixing.

- Can reach low ms exposure times
The Syringe Pump Setup is used for high volume samples (100s of μL to mL, including cell culture) and for exposures needing very high flux density. Easily transportable apparatus.

Adaptation to Live Cell Experiment: includes 2 pumps (1 for reaction initiation, 1 for exposure), incubator, fraction collector, capillary flow cell
**Scientific Highlight #1: Water Dynamics**

*Temperature dependent and H$_2^{18}$O exchange XF as novel probes of protein-associated water dynamics on microsecond to millisecond timescales*

XF distinguishes between residues reacting with bulk water from bound water (including surface-bound)

Progress curves for H$_2$O exchange for $^{18}$O labeled side chains; residues near bound waters exchange more slowly

Gupta et al. (2012) *PNAS* 109: 14882
Industrial partner (Genentech) used x-ray footprinting of a full IgG1 antibody to identify dimer interfaces.

Interface is head-to-head, but may be single (A) or double (B) arm-bound.

Leads the way for future mAb higher order structure characterization by XF.

Deperalta et al. (2013) MAbs 5: 86