X-Ray Footprinting Overview and Progress at the ALS

Corie Ralston, Sayan Gupta

A Brief History of Footprinting

1970 | 1980 | 1990 | 2000

Chemical modification methods developed

1972
Gilbert, CIBA Found. Symp. 7.
Demonstrates that protein binding protects DNA from enzymatic digestion

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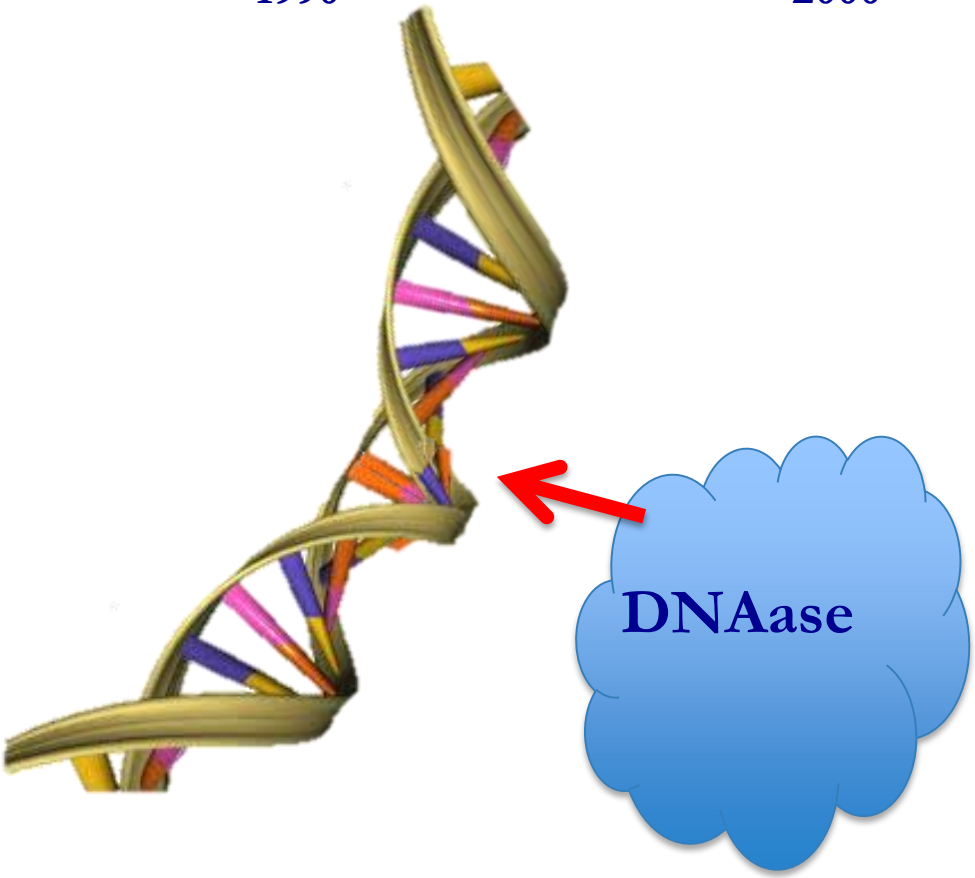
Galas and Schmitz, Demonstration of enzymatic footprinting (using DNAase). Nucleic Acids Research 1978, v5, 3157.



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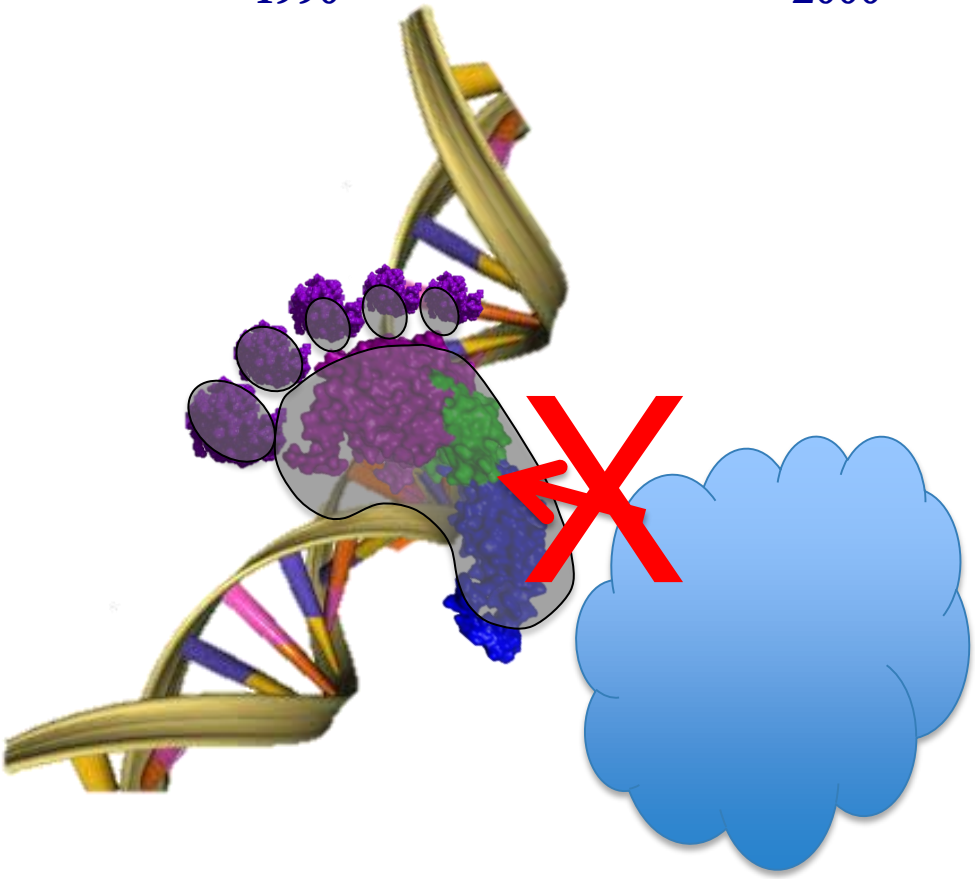

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More chemical and enzymatic modification FP methods developed

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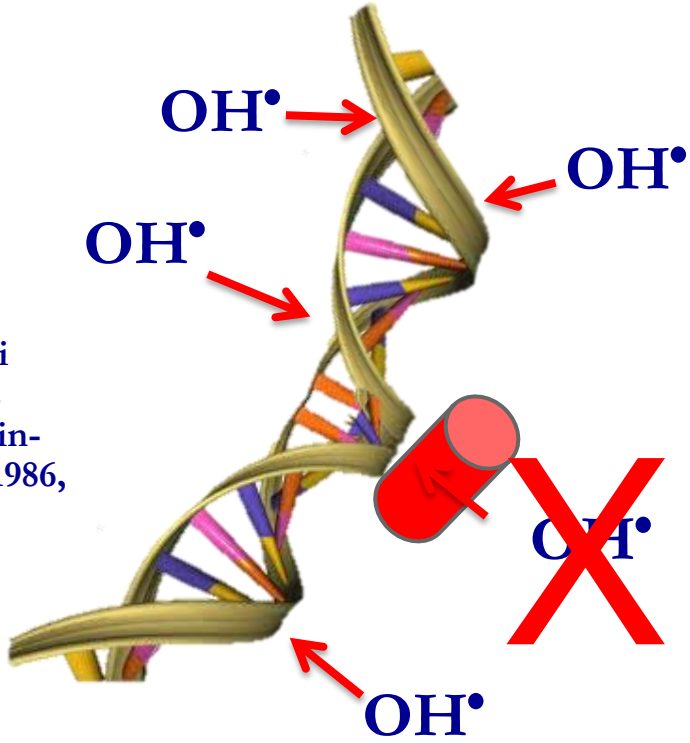

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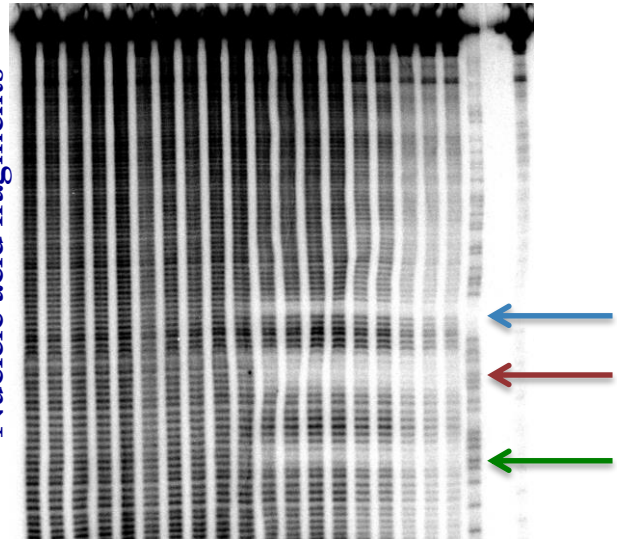
A Brief History of Footprinting

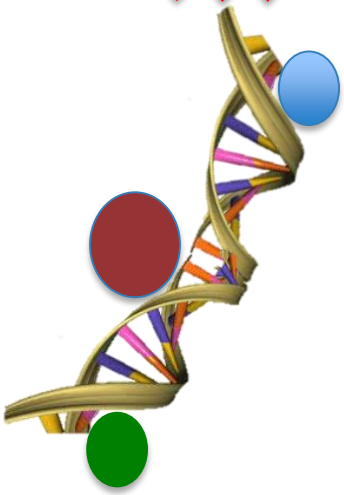
1970 1980 1990 2000

Time (msec)

Nucleic acid fragments



X-rays



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A Brief History of Footprinting

1970 1980 1990 2000

- Covalent modifications
- Residue-specific
- Time resolution capability
- Bound water location

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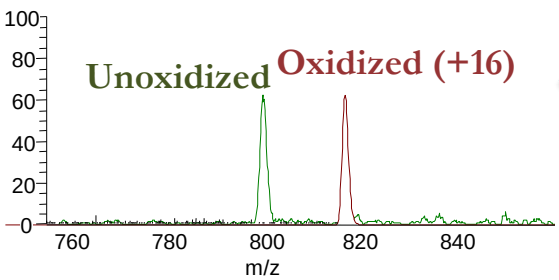
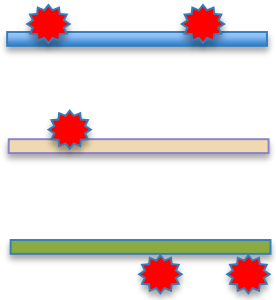
Maleknia et al, Millisecond radiolytic modification of peptides by synchrotron X-rays identified by mass spectrometry, Analytic Chem. 1999, 71, 8965.

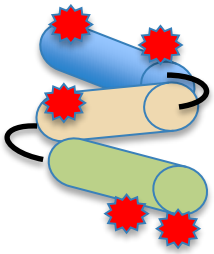
LC/MS



Protease Digestion



X-rays



Footprinting Methods Comparison

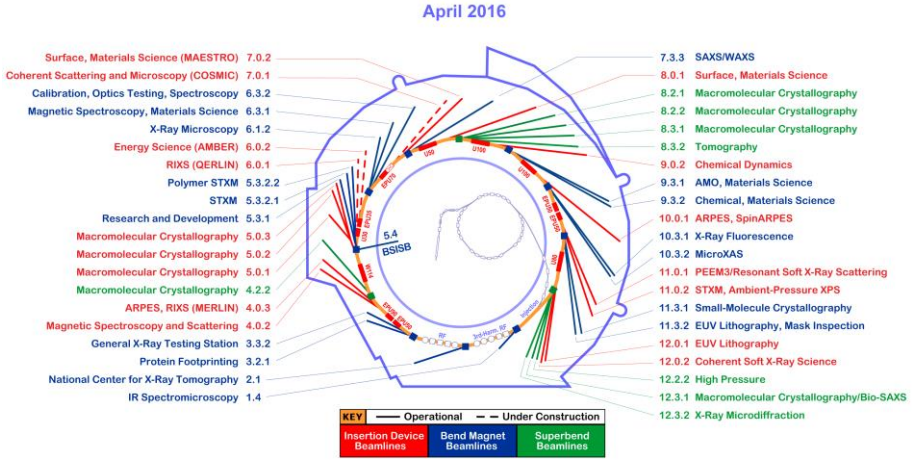
- Protease mapping and cross-linking
- Electron beam radiolysis
- Hydrogen-Deuterium Exchange
- UV photolysis of H_2O_2: "FPOP"
- Fenton Chemistry

"Fast Photochemical Oxidation of Proteins (FPOP) and Mass Spectrometry Follow Sub-millisecond Protein Folding at the Amino-Acid Level," J. Chen… M. L. Gross, *J. Am. Chem. Soc.*, *134*, 18724–18731 (2012).

"Protein footprinting by pyrite shrink-wrap laminate," M.Leser… M. Brenowitz, *Lab Chip.*, *15*, 1646–1650 (2015).

Advantages of Synchrotron

- No need for H_2O_2
- Low concentrations of protein
- Permanent modifications
- Dynamics experiments possible
- National User Program

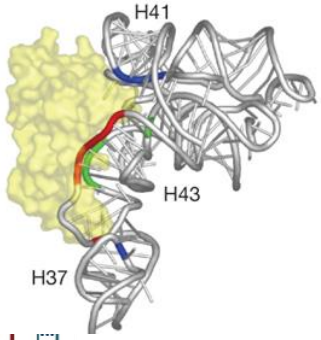


XFP Tackles Progressively More Challenging Projects

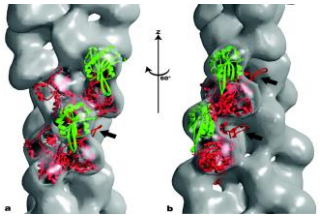
2000

2003
Kiselar et al, Ca dependent changes in Gelsolin, PNAS 2003.

2006
Adilakshmi et al, In-vivo footprinting NAR 2006, 104, 7910.



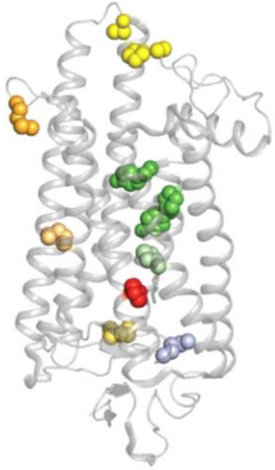
2007
Kamal et al, Actin-cofilin interaction (cell motility, division, morphology) PNAS 2007, 104, 7910.



2008
Bohon et al, ATP-dependent structural changes in a protease, Structure 2008, 16, 1157.

2008
Adilakshmi et al, Time-resolved XFP on ribosome assembly Nature 2008, 455, 1268.

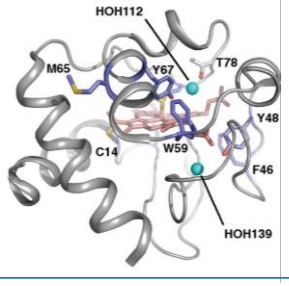
2009
Angel et al, Photoactivation of Rhodopsin PNAS 2009, 106 14367.



2010
Wang et al, Glycosylated GP120 Biochem 2010, 49 9032.

2014

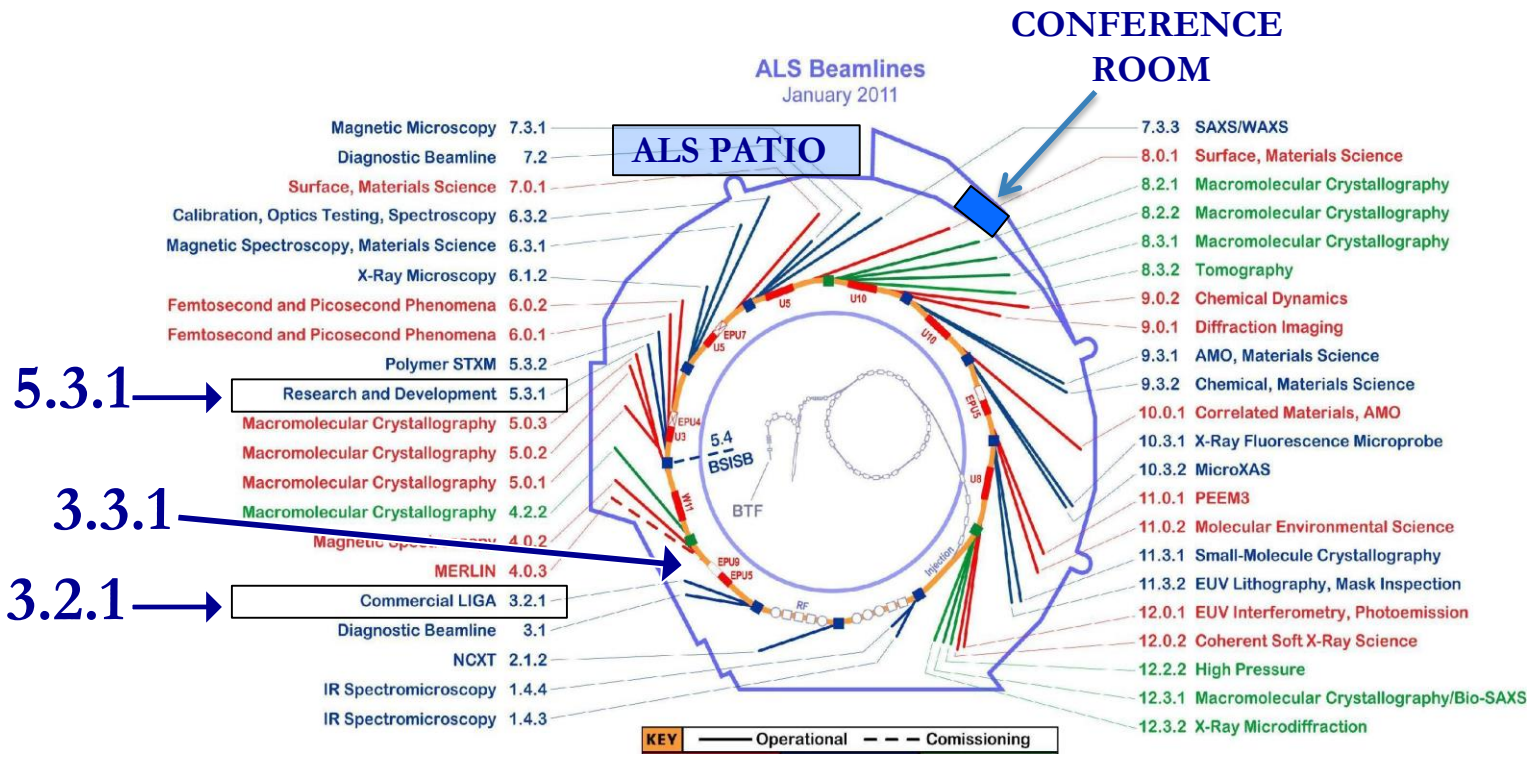
2012
Gupta et al, Location and dynamics of protein waters PNAS 2012, 109 14882.



2013
Clatterbuck et al, Advances in in-vivo XFP Mol Cell 2013, 52, 506.

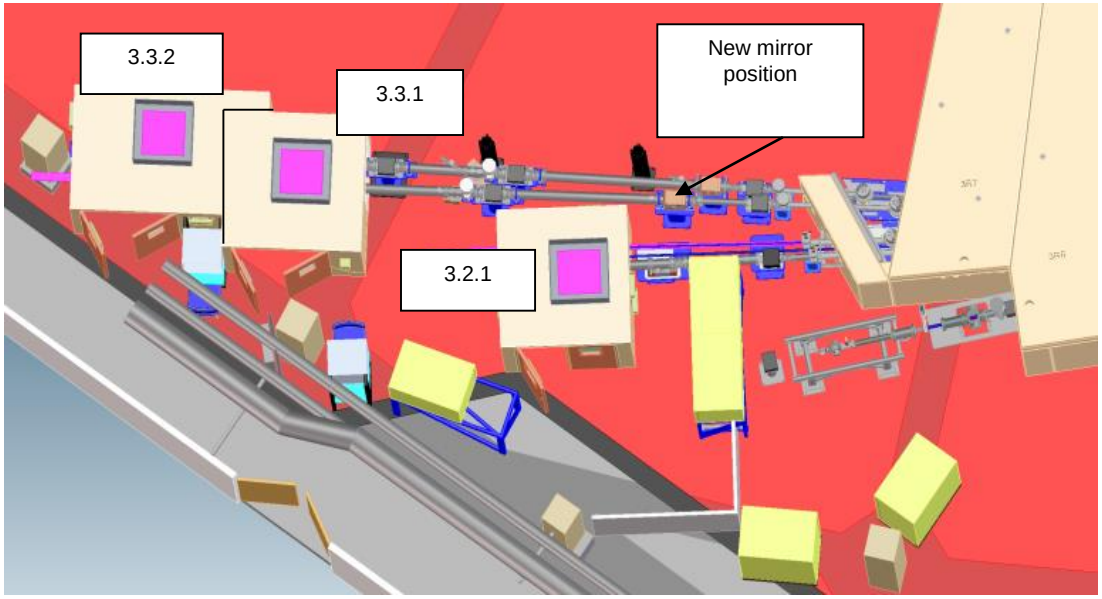
2014
Gupta et al, Transporter gating mechanism Nature 2014, 512(7512), 101.

FOOTPRINTING AT THE ALS



A DEDICATED XFP BEAMLINE AT THE ALS

- 3.3.1 is currently decommissioned
- Radiation safe for top-off mode
- Beamline Readiness Review needed
- An old NSLS mirror is available



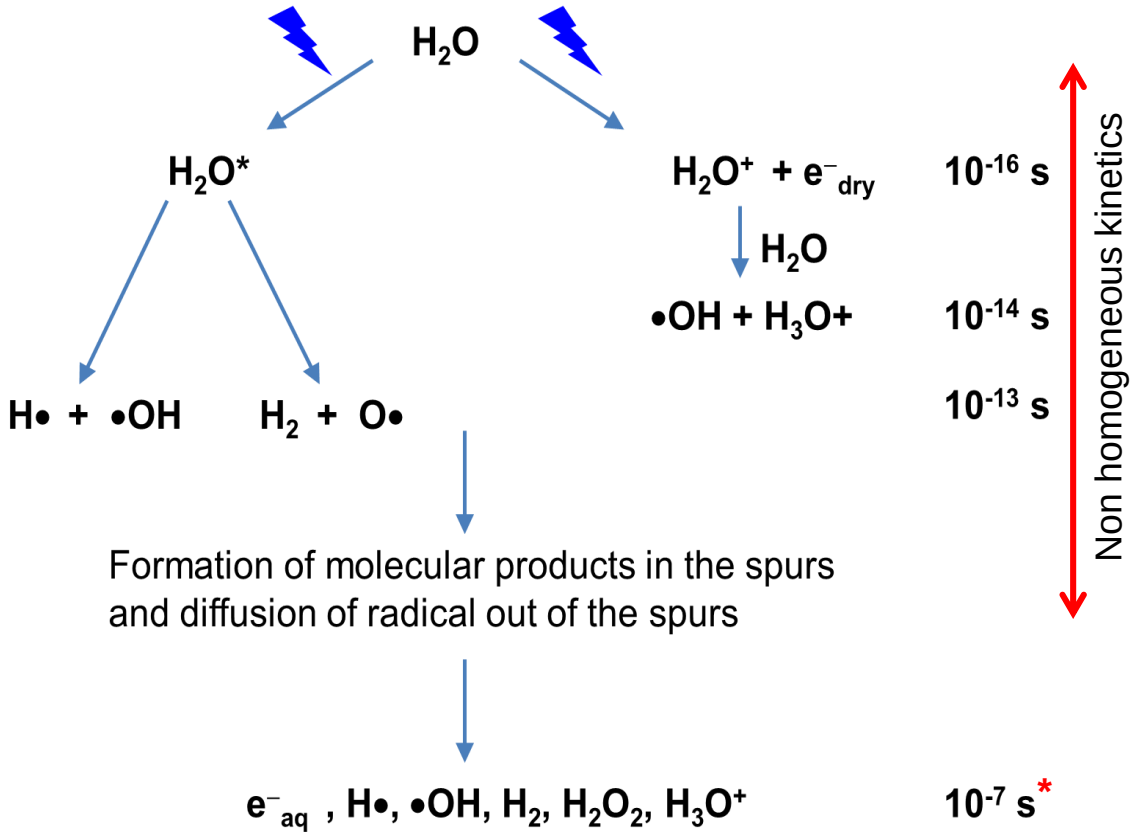
Other Applications for XFP

- H_2O^{18} vs H_2O^{16} for investigating water dynamics
- XFP on protein crystals to determine packing interactions
- In-vivo XFP on proteins

TIME	
9:30 – 11:30	**Welcome and Overview** Corie Ralston **Using Footprinting to Characterize Gating Events in Zinc Transporter Proteins** Dax Fu **Characterizing Conformation Changes in Chloride Transporters** Tanmay Chavan **The Orange Carotenoid Protein: Mechanism of a Photoswitch** Corie Ralston **Designing and Characterizing Organic-IronOxide Interfaces and Applications for Biohybrid Engineering** Behzad Rad
11:30 – 1:00	**Lunch**
1:00 – 2:00	**Application of MS-based Footprinting in Drug Discovery and Development** Janna Kiselar **In-situ X-ray Footprinting of Intact, Functional Mitochondria** Awuri Asuru **The New X-Ray Footprinting Beamline at the NSLS-II** Mark Chance
2:00–4:00	**Hands-on Tutorial**
4:00-4:30	**Coffee Break and Discussion**
4:30-5:00	**Tour of the Beamlines**

X-Ray Radiolysis of Water

- **Water radiolysis & primary radical products**



- **Secondary radical product**

$$e_{aq}^- + e_{aq}^- = H_2 + 2OH^-$$

$$e_{aq}^- + \bullet OH = OH^-$$

$$e_{aq}^- + H_3O^+ = H\bullet + H_2O$$

$$e_{aq}^- + O_2 = \bullet O_2^-$$

$$H\bullet + H\bullet = H_2$$

$$H\bullet + O_2 = \bullet HO_2$$

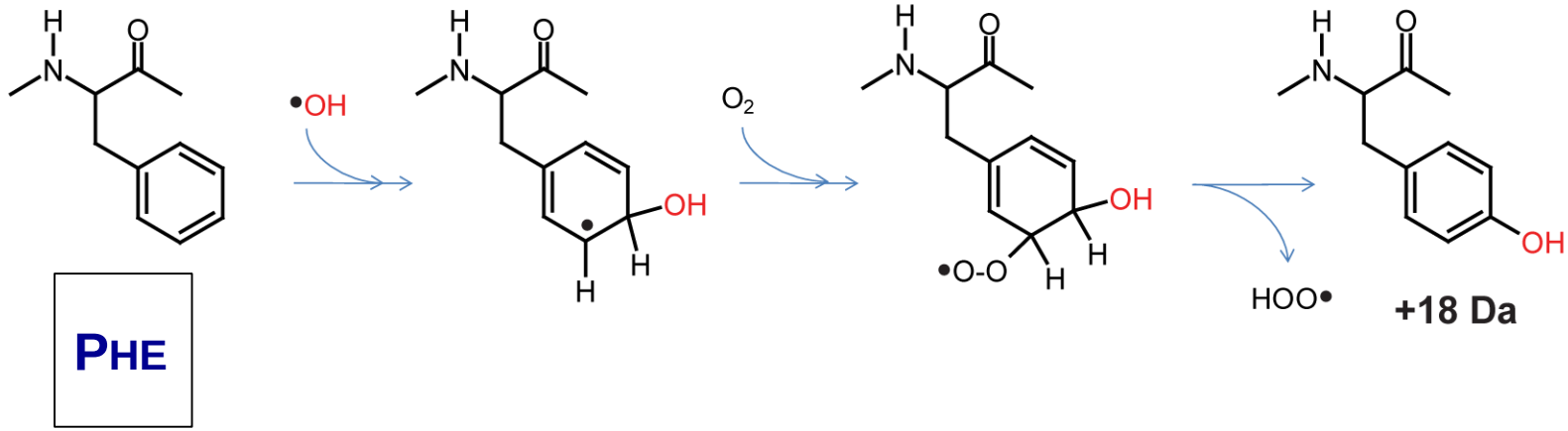
$$\bullet OH + \bullet OH = H_2O_2$$

$$\bullet OH + H\bullet = H_2O$$

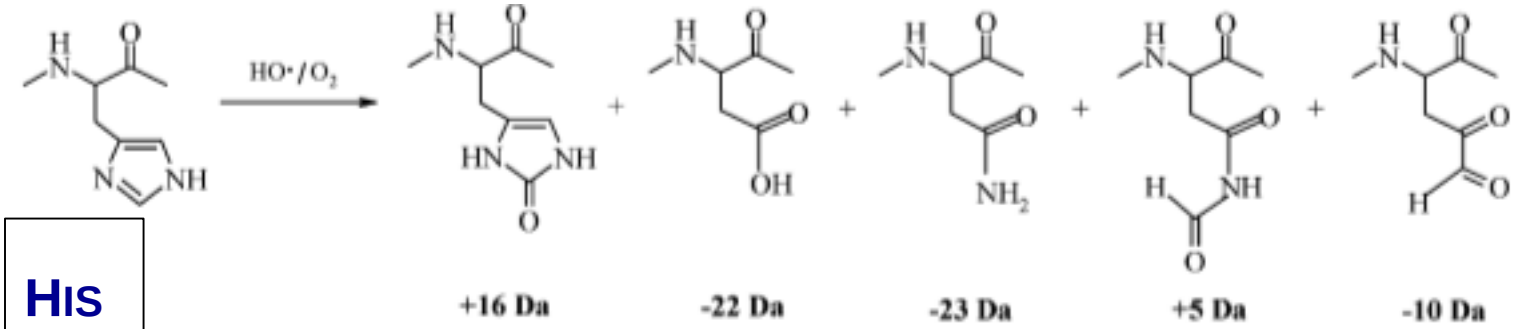
$$H_3O^+ + OH^- = H_2O$$

Gupta et. al. JSR. 2014. 21(Pt 4):690-9 / Pryor WA. A. R. Physiol. 1988. 48, 657-667 / Buxton et al. JPC Ref. D. 1988. 17-34

Variation in Reactions by Residue



Phe



His

Gupta et. al. PNAS 2012. 109(37):14882-7

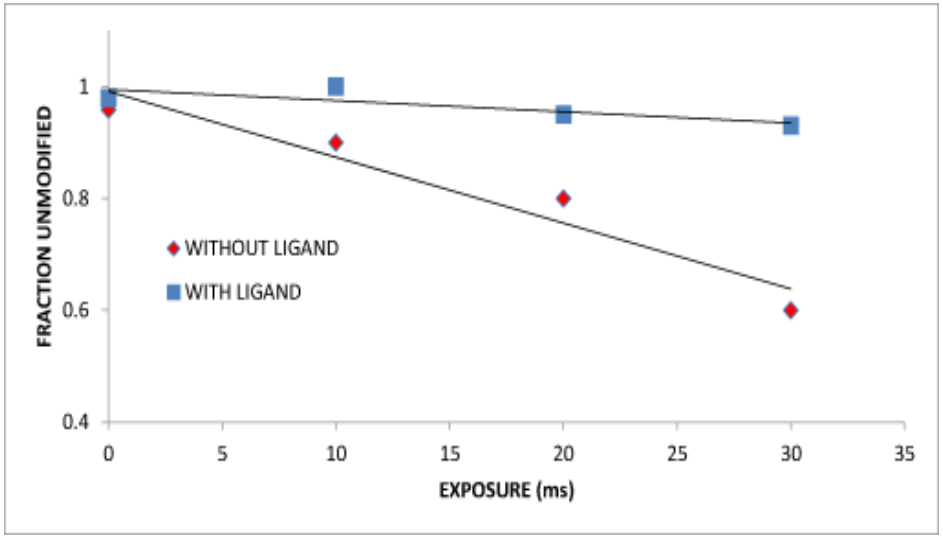
Dealing with Residue-Specific Reactivity

Chemical Reviews, 2007, Vol. 107, No. 8 3519

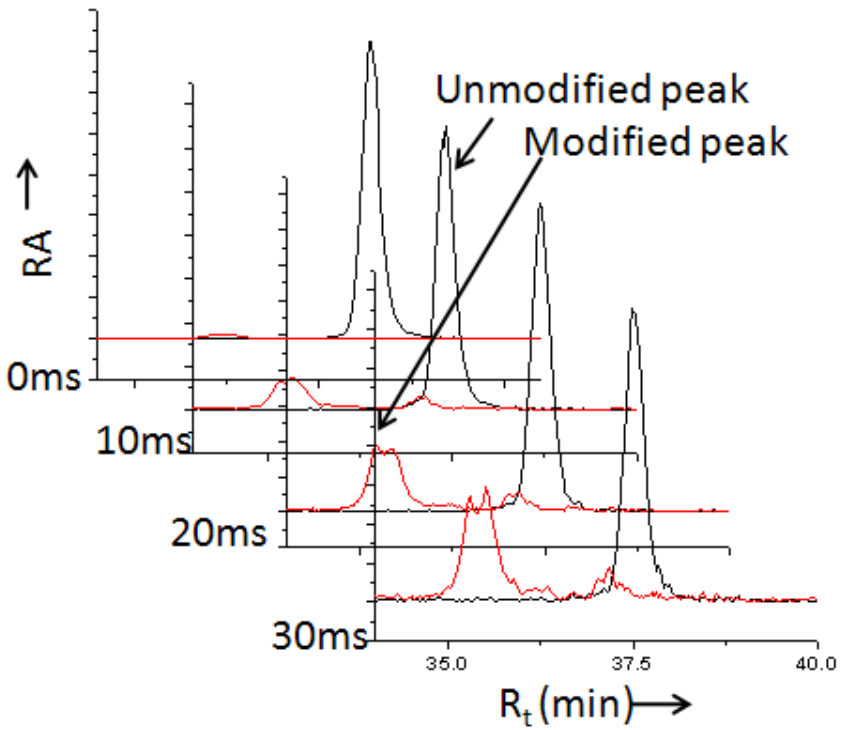
Table 1. Rate Constants for Reaction of Amino Acids with Hydroxyl Radical and Hydrated Electrons[a]

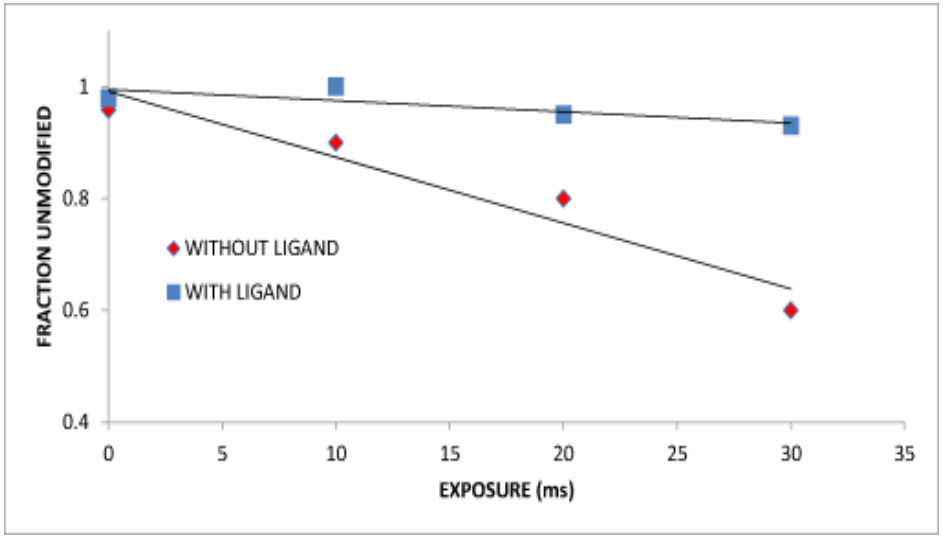
	HO^-		e_{aq}^{-1}	
substrate	rate (M^{-1} s^{-1})	pH	rate (M^{-1} s^{-1})[b]	pH
Cys	3.5×10^{10}	7.0	1.0×10^{10}	−7
Trp	1.3×10^{10}	6.5−8.5	3.0×10^{8}	7.8
Tyr	1.3×10^{10}	7.0	2.8×10^{8}	6.6
Met	8.5×10^{9}	6−7	4.5×10^{7}	7.3
Phe	6.9×10^{9}	7−8	1.6×10^{7}	6.9
His	4.8×10^{9}	7.5	6.0×10^{7}	−7
Arg	3.5×10^{9}	6.5−7.5	1.5×10^{8}	6.1
cystine	2.1×10^{9}	6.5	1.5×10^{10}	6.2
Ile	1.8×10^{9}	6.6	N/A	N/A
Leu	1.7×10^{9}	~6	$<1 \times 10^{7}$	6.5
Val	8.5×10^{8}	6.9	$<5 \times 10^{6}$	6.4
Pro	6.5×10^{8}	6.8	2.0×10^{7}	6.7
Gln	5.4×10^{8}	6.0	N/A	N/A
Thr	5.1×10^{8}	6.6	2.0×10^{7}	7.0
Lys	3.5×10^{8}	6.6	2.0×10^{7}	7.4
Ser	3.2×10^{8}	~6	$<3 \times 10^{7}$	6.1
Glu	2.3×10^{8}	6.5	$1\text{--}2 \times 10^{7}$	5.7−7
Ala	7.7×10^{7}	5.8	1.2×10^{7}	7.4
Asp	7.5×10^{7}	6.9	1.8×10^{7}	7.0
Asn	4.9×10^{7}	6.6	1.5×10^{8}	7.3
Gly	1.7×10^{7}	5.9	8.0×10^{8}	6.4

[a] http://allen.rad.nd.edu/browse compil.html. [b] Davies, M. J.; Dean, R. T. *Radical-mediated protein oxidation: from chemistry to medicine*; Oxford University Press: 1997; pp 44−45.



LIMITING EXPOSURE





- **Fraction Unmodified = 1-[modified/(total of mod+unmod)]**
- **Limit exposure to stay in linear region**