X-Ray Footprinting
Welcome and Overview

Corie Ralston, Sayan Gupta, Jen Bohon

ALS Users Meeting Workshop Oct 8, 2014

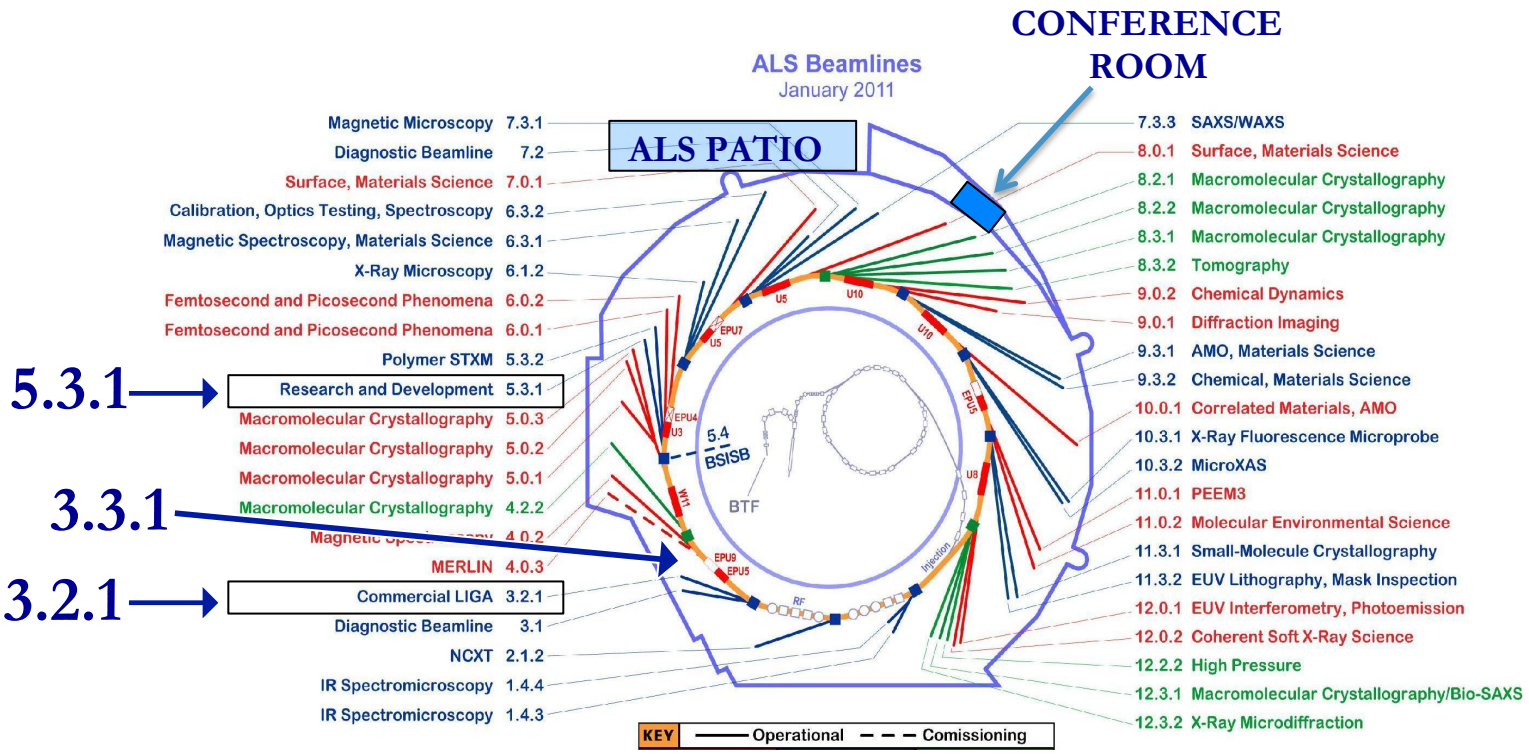
WORKSHOP AGENDA – MORNING SESSION

TIME	TALKS
9:30 – 10:30	Welcome and Overview Corie Ralston
	XF Mass Spectrometry and Advances in Data Analysis Approaches Janna Kiselar
10:30 – 10:45	Coffee Break
10:45 – 12:00	The Hybrid Approach: Combining XFP with SAXS Sichun Yang
	ProtMapMS: Software Solutions for High-Throughput Examination of Covalently Labeled Biomolecules by Structural Mass Spectrometry Parminder Kaur
12:00– 1:00	Lunch

WORKSHOP AGENDA – AFTERNOON SESSION

TIME	TALKS
1:00 – 2:00	Probing Ribosome Assembly in Live Cells Sarah Woodson
	A Synchrotron-based Hydroxyl Radical Footprinting Analysis of Amyloid Fibrils and Prefibrillar Intermediates with Reside-specific Resoultion Janna Kiselar
	Coffee Break
2:00 – 3:00	Using XFP to Probe Protein Conformational Changes Governing Photoprotection in Cyanobacteria Cheryl Kerfeld
	Visualizing Internal Water Interactions in Membrane Proteins by XFP Sayan Gupta
	Coffee Break
3:00 – 4:30	XFP Insights into IgG Galahad Deperalta
	Unmasking the initial Stages of HIV Env Glycoprotein Activation using H/D Exchange and X-Ray Footprinting Miklos Guttman
	The NSLS-II XFP Beamline and Beyond Jen Bohon
4:30 – 5:00	Beamline Tour

ALS BEAMLINES



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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DNAase

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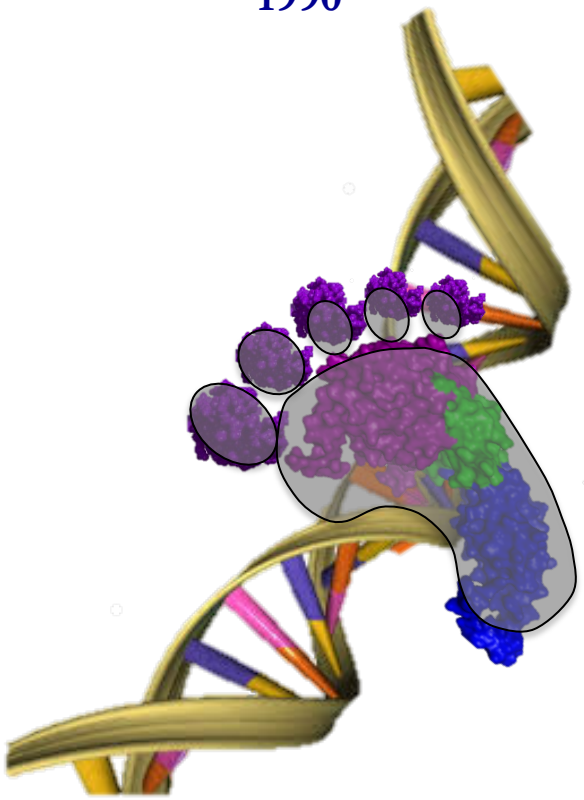

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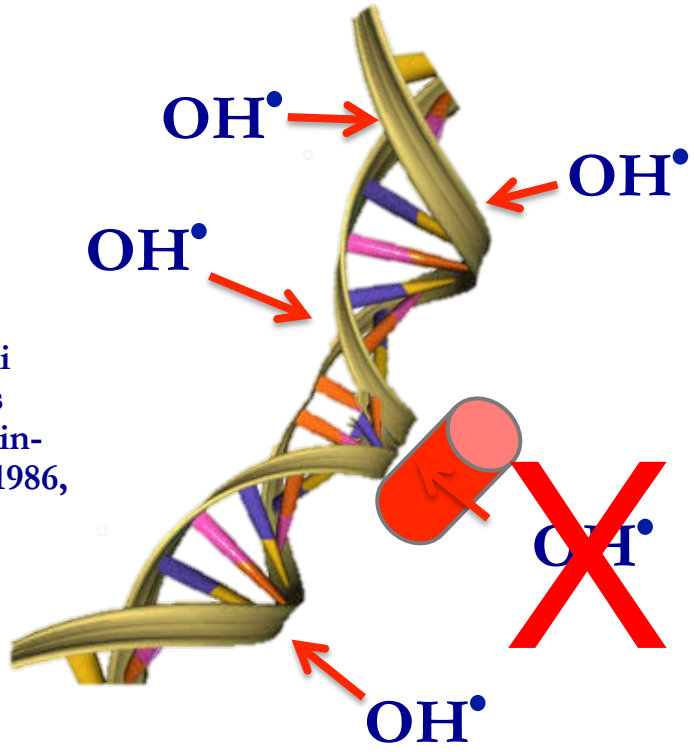

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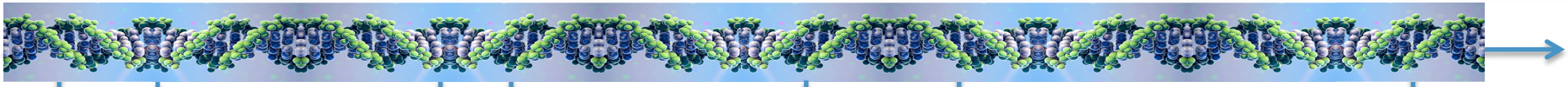

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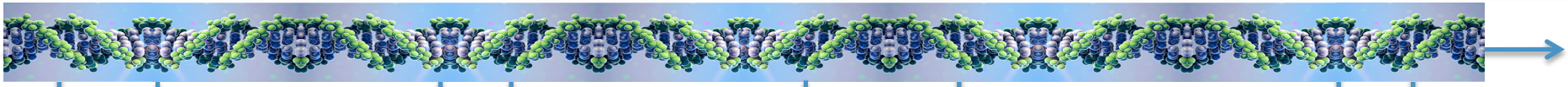

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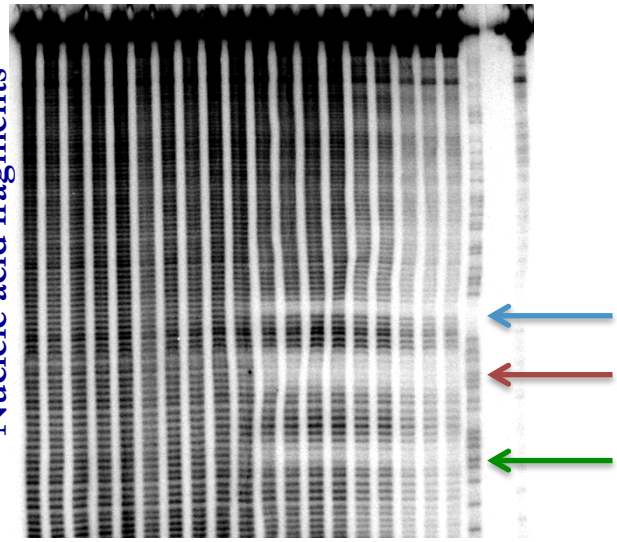
A Brief History of Footprinting

1970 1980 1990 2000

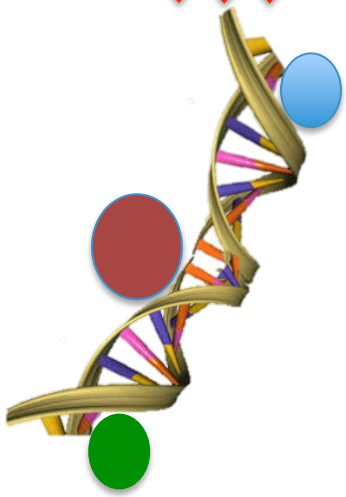
Concentration

Time (msec)

Nucleic acid fragments



X-rays



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Berkeley Lab

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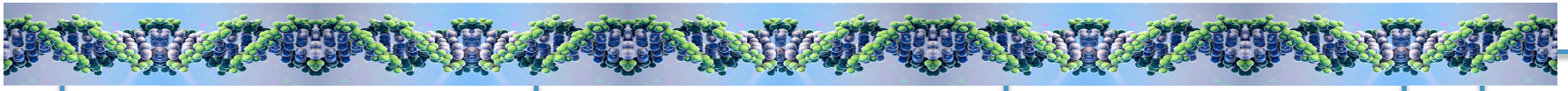

1970 1980 1990 2000

- Covalent modifications
- Residue-specific
- Time resolution capability

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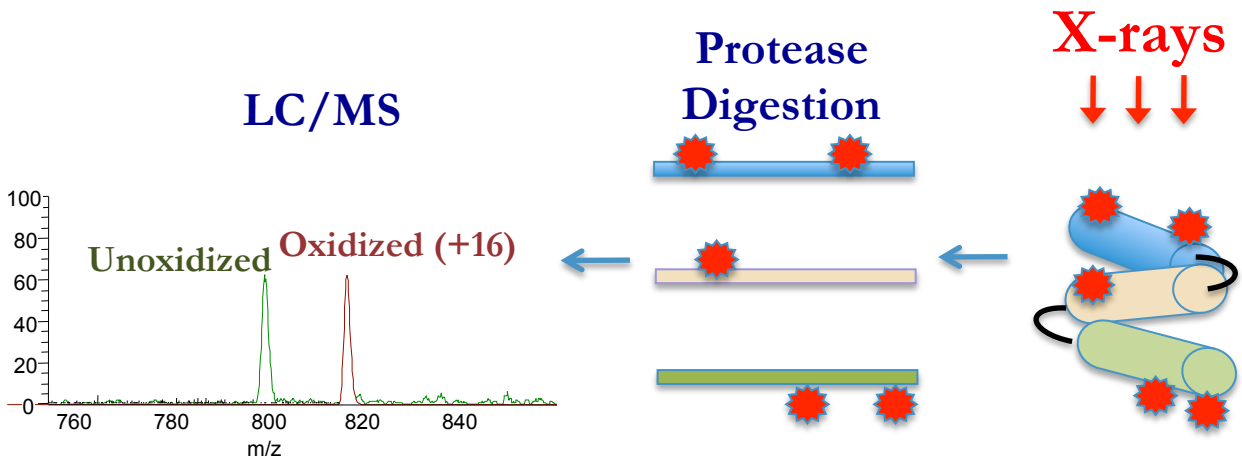
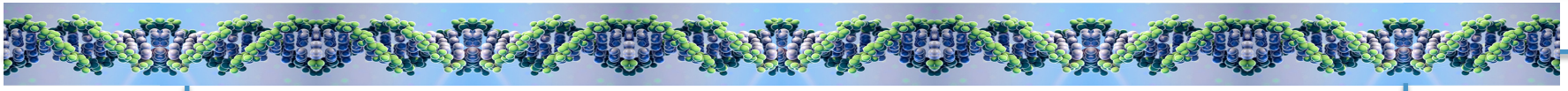


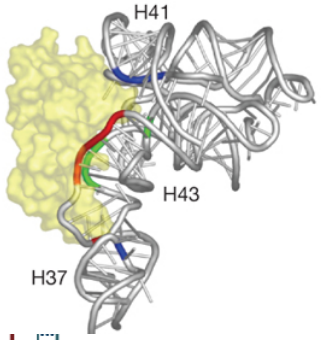
XFP Tackles Progressively More Challenging Projects



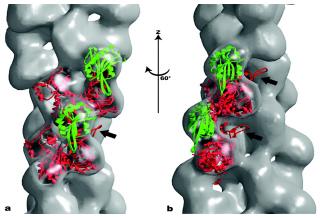
2000

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

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



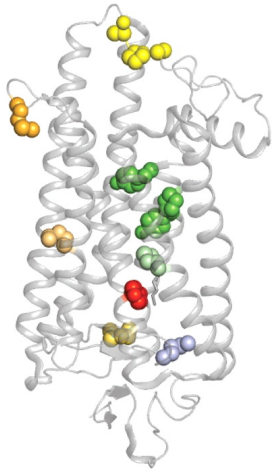
2007
Kamal et al, Actin-cofilin interaction (cell motility, division, morphology) PNAS 2007, 104, 7910.



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.

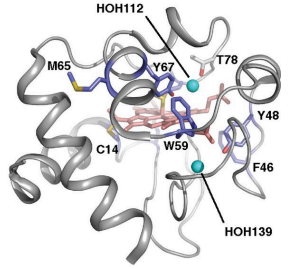
2009
Angel et al, Photoactivation of Rhodopsin PNAS 2009, 106 14367.



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.



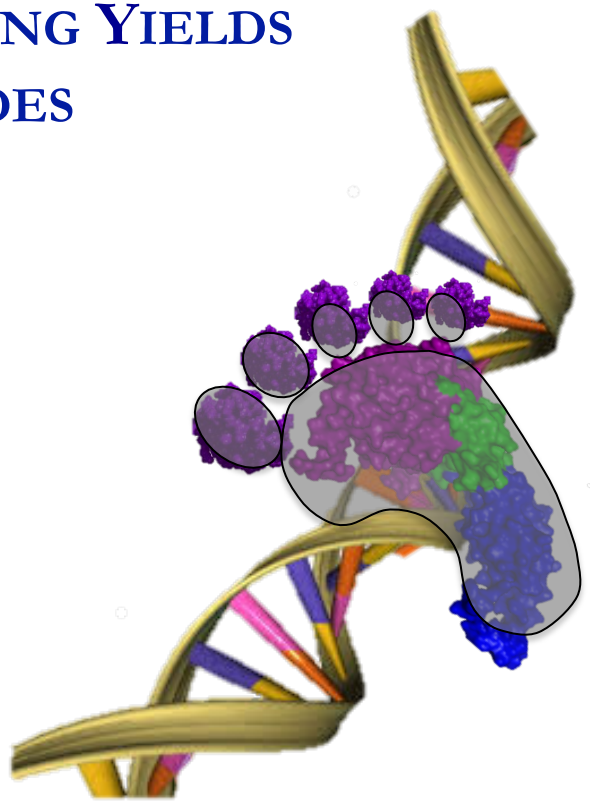
2013
Clatterbuck et al, Advances in in-vivo XFP Mol Cell 2013, 52, 506.

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

BERKELEY LAB

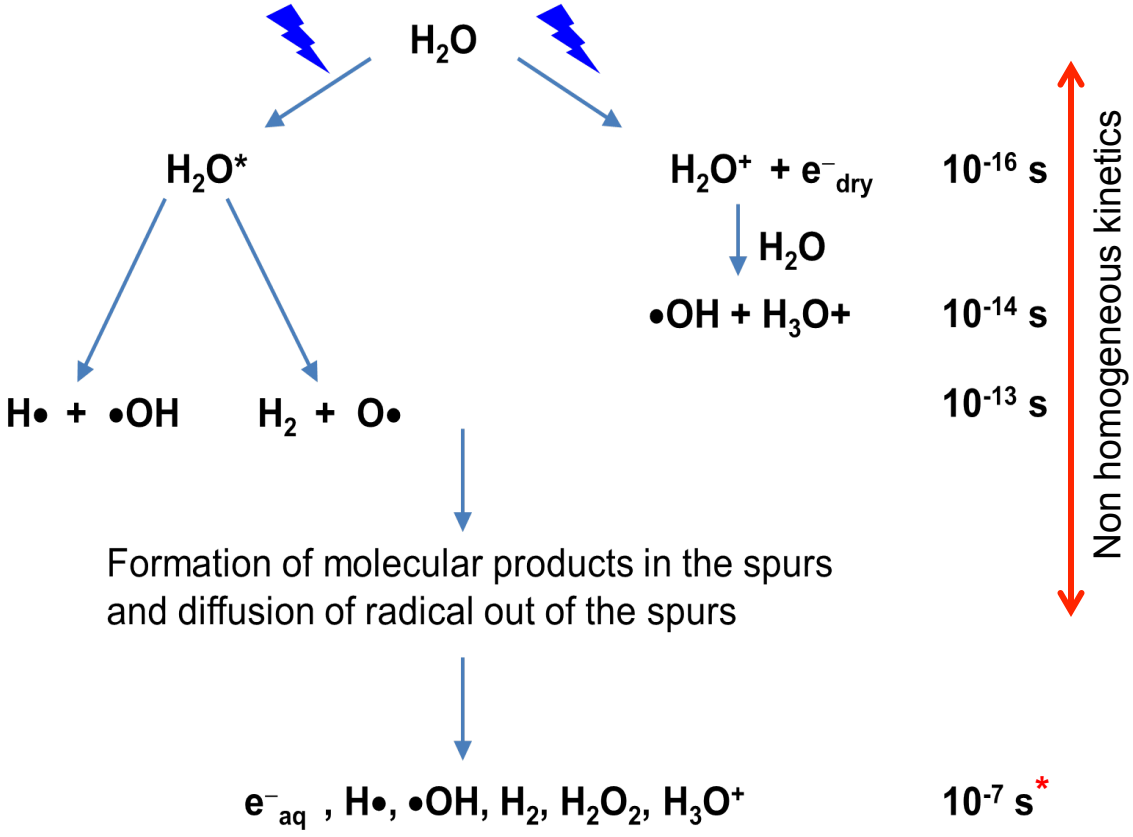
Other Considerations

- X-Ray Radiolysis of Water – What Happens?
- Factors That Reduce Footprinting Yields
- Variation in Reactivity of Peptides



X-RAY RADIOLYSIS OF WATER

- **Water radiolysis & primary radical products**



- **Secondary radical product**

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

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

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

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

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

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

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

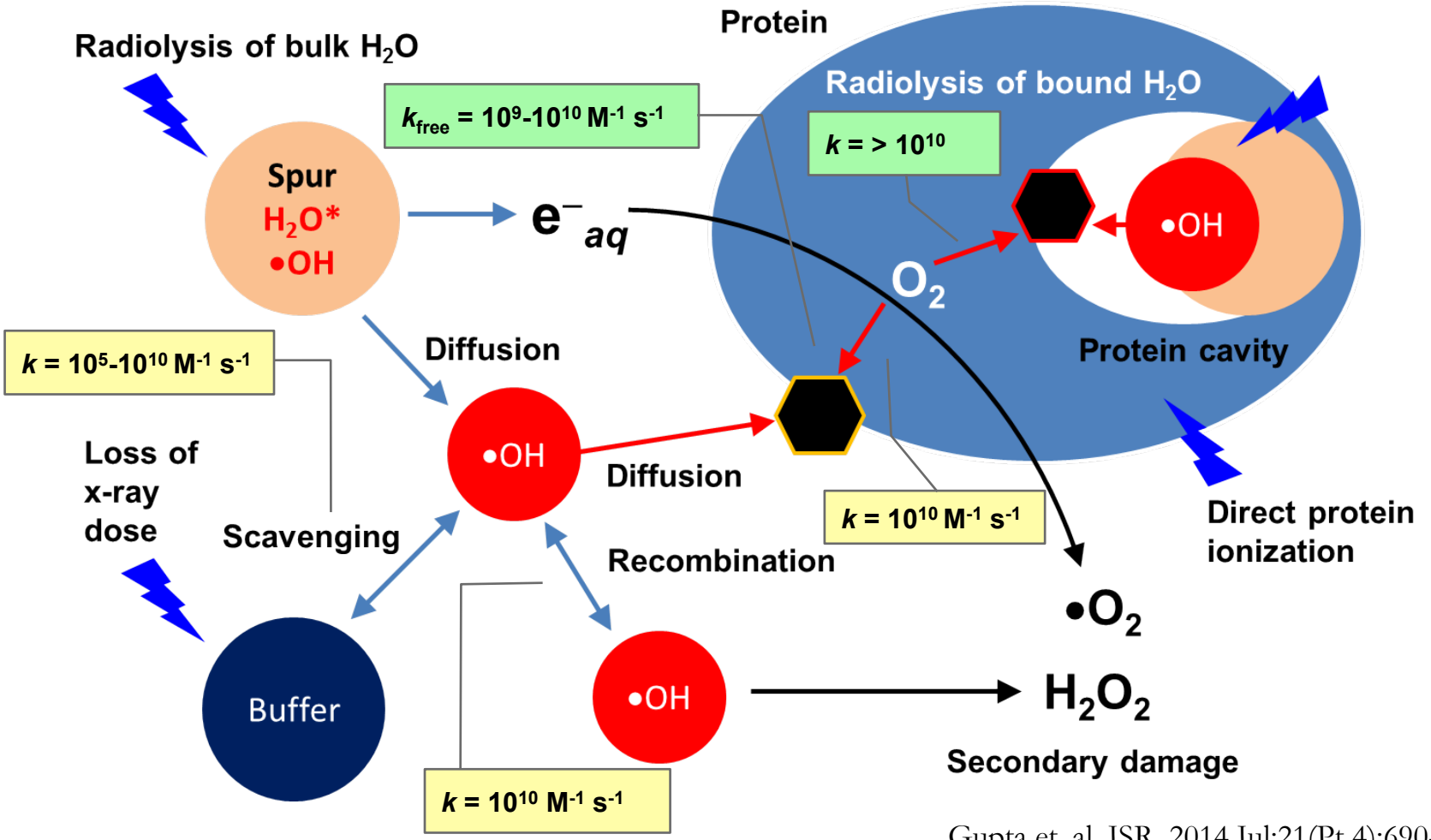
$$\bullet OH + H\bullet = H_2O$$

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

- **OH reacts within 1 to 5 molecular diameters of the site of formation***

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

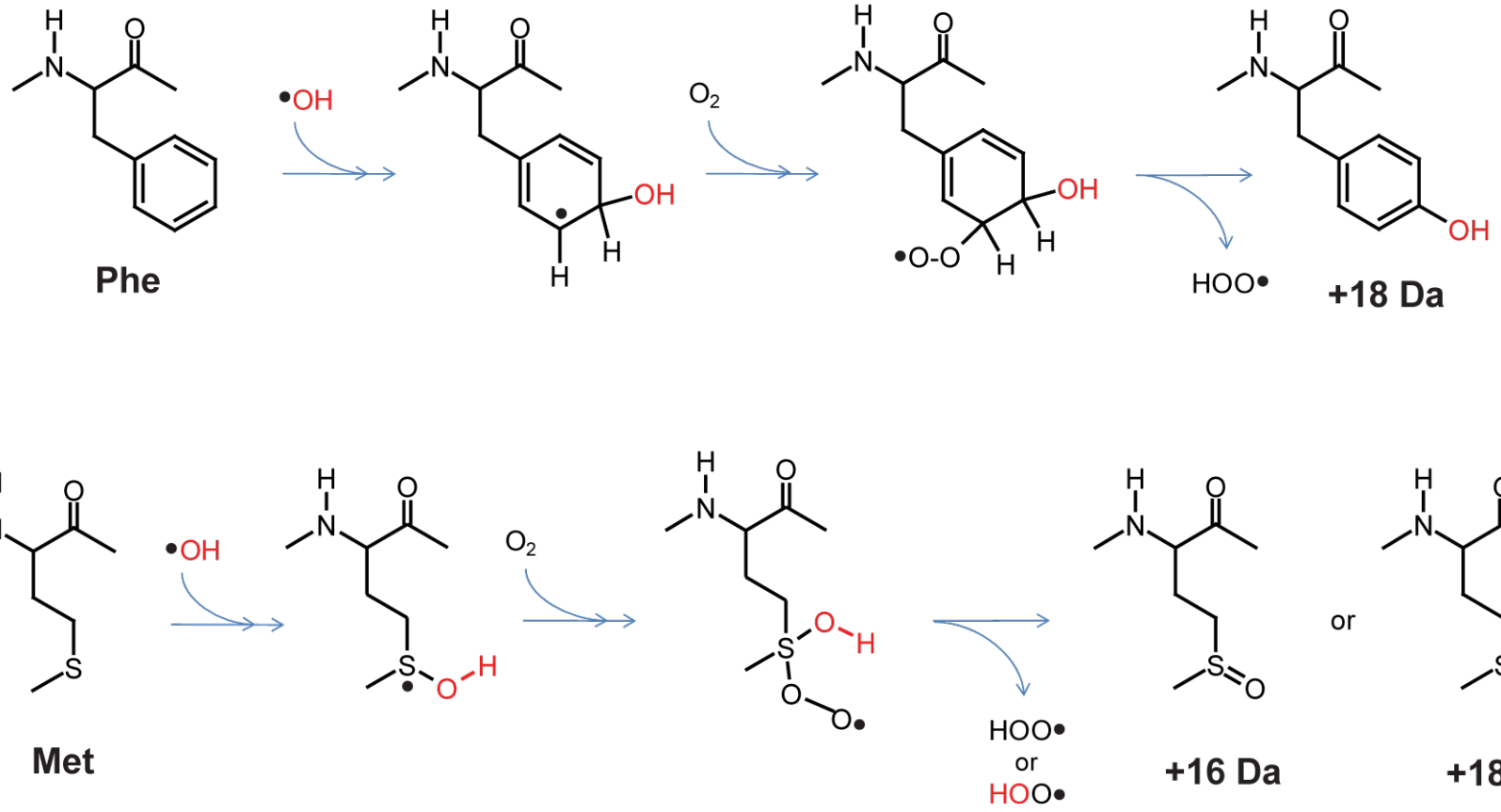
Reactions that Reduce Footprinting Yields



Gupta et. al. JSR. 2014 Jul;21(Pt 4):690-9

- Life time of OH in aqueous solution is < ms
- XF measures steady state reactivity, High flux density = High steady state [•OH]

VARIATION IN REACTIONS BY RESIDUE



- Reactivity varies by residue
- Dissolved oxygen is necessary for XF
- Experiment in $H_2{}^{18}O$ will include stoichiometric labeling

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

All the Things I didn't Cover

- Buffer "Calibration" – Optimizing the X-Ray Dose
- Considerations for Mixing Experiments
- Examination of Unfolding by Residue
- Process Development Possibilities in Industry
- Use in Drug Discovery Processes
- Complementary Techniques