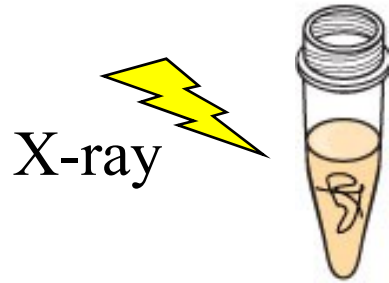




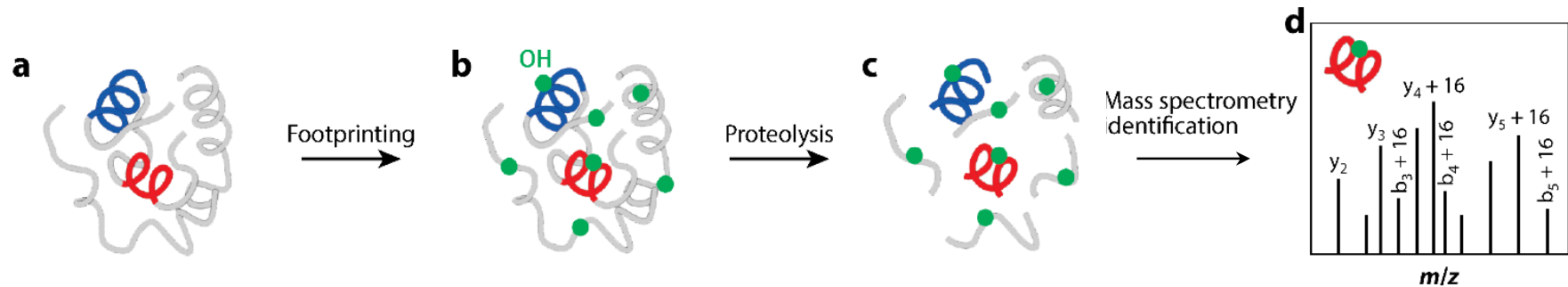
Probing protein structure by X-ray footprinting

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About X-ray footprinting



- Produced fast, (sub)ms
- Similar to water size, $\sim 3\text{\AA}$
- Highly reactive
- Fast reaction with 20 amino acids



Adapted from Fig.2. *Annu Rev Biophys.* 2018. 47:315-333

Solvent accessible surface at residue level

X-ray footprinting – about samples

- Picomoles / nanograms
- No size limit, peptide to large complex
- (membrane) protein, protein complex, *in vivo* (cells/tissues)
- disordered regions of proteins, disordered proteins
- Temperature, from -35 °C to +37 °C
- RNA/DNA

X-ray footprinting applications

Structure prediction

Biehn et al, Accurate protein structure prediction with hydroxyl radical protein footprinting data. Nat Commun. 2021; 12: 341.

Drug discovery

- **protein ligand interaction**
Stanford et al, Drug binding to LMPTP- A Nat Chem Bio. 2017, 13, 624.
- **antibody epitope mapping**
Sandercock, U Storz. Nature biotechnology 30 (7), 615-618, 2012

Protein protein interaction

Tetter et al, Evolution of a virus-like architecture and packaging mechanism in a repurposed bacterial protein. Science, 2021. 372(6547), 1220-1224

Membrane protein studies

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

Gupta S. Using X-ray Footprinting and Mass Spectrometry to Study the Structure and Function of Membrane Proteins. Protein Pept Lett. 2019;26(1):44-54

Time-resolved studies

Gupta et al. Structure and dynamics of protein waters revealed by radiolysis and mass spectrometry. (2012) PNAS 109: 14882

Hao et al, Time-resolved Hydroxyl Radical Footprinting of RNA with X-rays. Curr. Protoc. Nucleic Acid Chem. 2018, **73**(1), e52

X-ray footprinting applications

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Drug discovery Keys:

- protein ligand interaction
Stanford et al, Drug binding to LMP1. Nat Chem Biol. 2017, 13, 624.
 - antibody epitope mapping
Sandercock, U Storz, Nature biotechnology 30 (7), 610-618, 2012
- High and well-controlled X-ray dose
 - Mass spectrometry with high sensitivity
 - Computational structural biology

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