

# SUPREX & SPROX: Where Do They Fit In The H/D Exchange & Covalent Labeling Landscape?

Graham M. West  
Fitzgerald Lab  
Duke University

## Labeling Methods

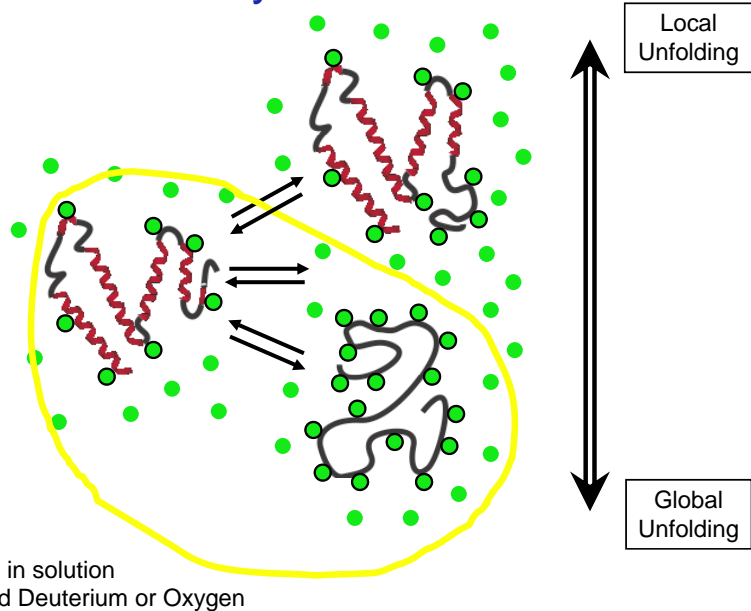
- H/D Exchange **SUPREX**
- H<sub>2</sub>O<sub>2</sub> labeling (Met) **SPROX**
- OH radical labeling (Cys, Met, Trp, Tyr, etc)
- Cross-linking

## Basic Strategies

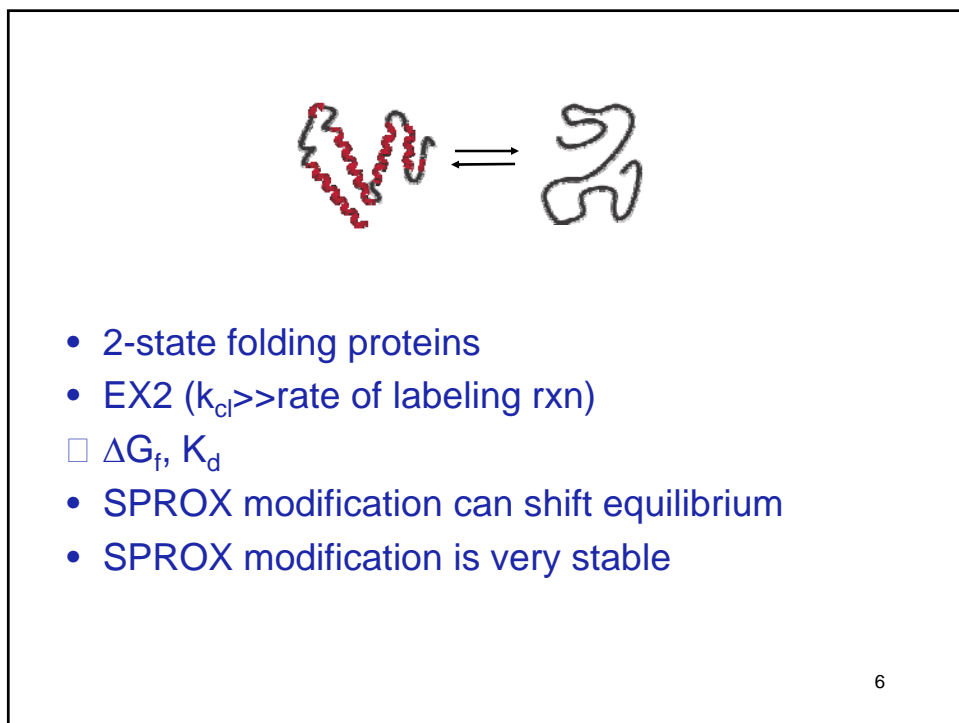
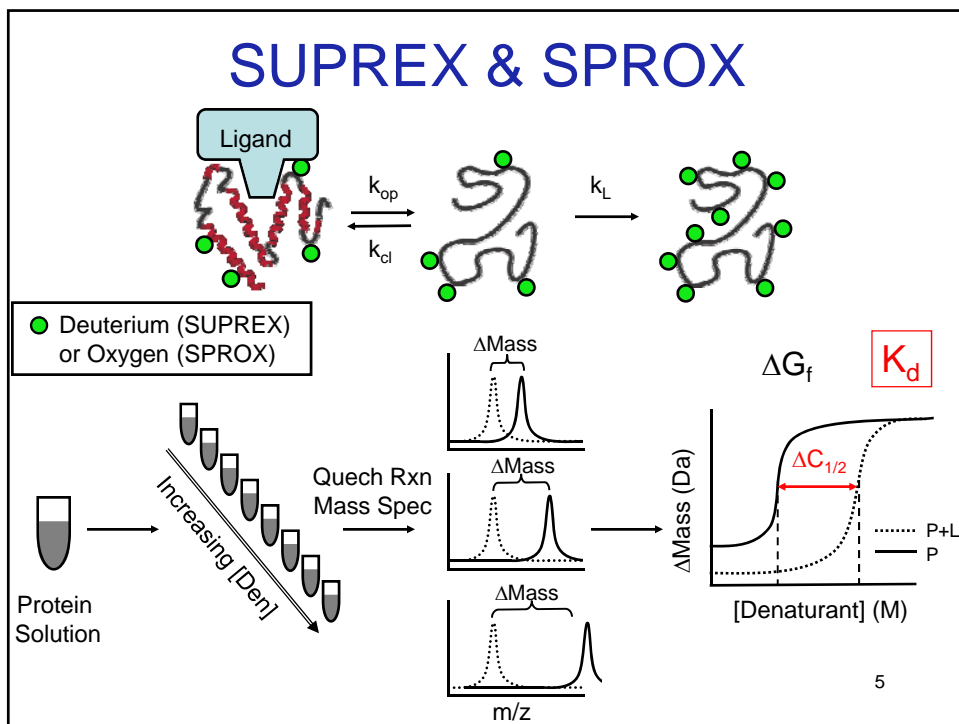
- Modification vs. Time
- Modification vs. [Ligand] PLIMSTEX
- Modification vs. [Denaturant] SUPREX & SPROX

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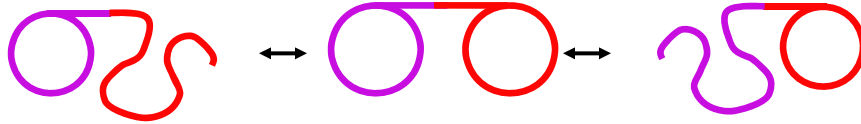
## SUPREX & SPROX: Why Denaturant?



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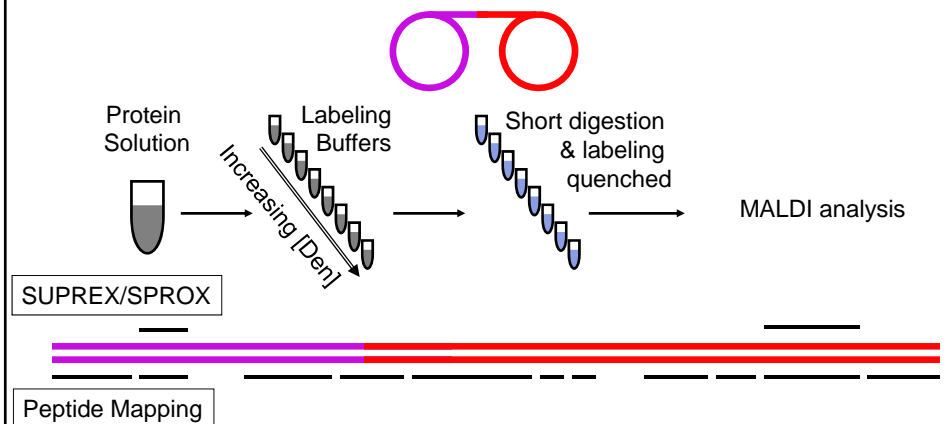
## Protease Digestion



- For non 2-state proteins
- Applicable to SUPREX & SPROX
- Protocol is similar to H/D mapping
- Used to follow sub global thermodynamic stability

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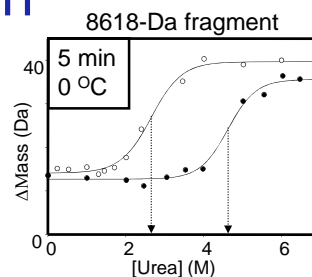
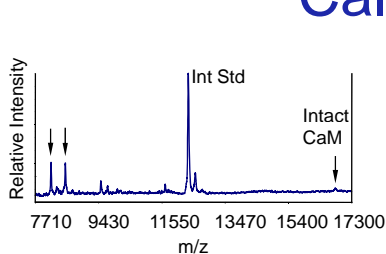
## Protease Digestion Protocol



- Conventional H/D Ex & Peptide Mapping - need coverage
- SUPREX/SPROX - only need one peptide/domain
- Need globally protected peptide
- SPROX - peptide must have a Met

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## Protease Digestion Protocol: Calmodulin



$\Delta\Delta G_f$  (kcal mol<sup>-1</sup>)

	SUPREX	Spectroscopic
N (1-77)	0	0
C (78-148)	-2.4 ± 0.3	-2.1

Ca<sup>2+</sup> binding affinity

	$\Delta\Delta G^{\text{SUPREX}}$ (kcal mol <sup>-1</sup> )	Titration $K_d$ ( $\mu\text{M}$ )
N (1-77)	-0.6 ± 0.5	39.8 & 2.5
C (78-148)	-2.2 ± 0.5	12.6 & 0.3

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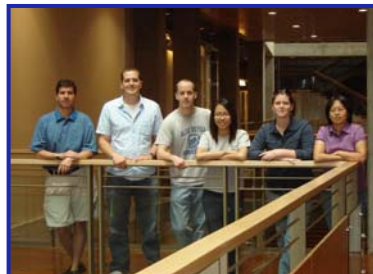
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## Conclusions

- Denaturant - probe global & sub-global unfolding rxns
- Lower resolution than time course studies
- Higher Throughput
  - 1 protein, many ligands
  - many proteins, one ligand (SPROX)
- Ligand Discovery Technique

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## Acknowledgements



- Professor Michael Fitzgerald
- Ying Xu - Tues 10am HsP chaperone binding
- Patrick DeArmond - Poster #661 (Thurs am) HTS
- Erin Hopper
- Graham West - Poster #483 (Thurs am) SPROX

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