A Single Approach (LITPOMS) Reveals Composite Conformational Changes, Order of Binding, and Affinities for Calcium Binding to Calmodulin

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09/19/2019

Measuring P-L Binding Affinity

$$K_a = \frac{k_{on}}{k_{off}} = \frac{[PL]}{[P][L]} \qquad K_d = \frac{k_{off}}{k_{on}} = \frac{[P][L]}{[PL]}$$

- **Concentration Measurement**

  Fluorescence Polarization, Circular Dichroism, NMR, FT-IR

- **Rate Constant Measurement**

  Surface Plasmon Resonance

- **Thermodynamic Measurement**

  Isothermal Titration Calorimetry (Heat flow during titration)

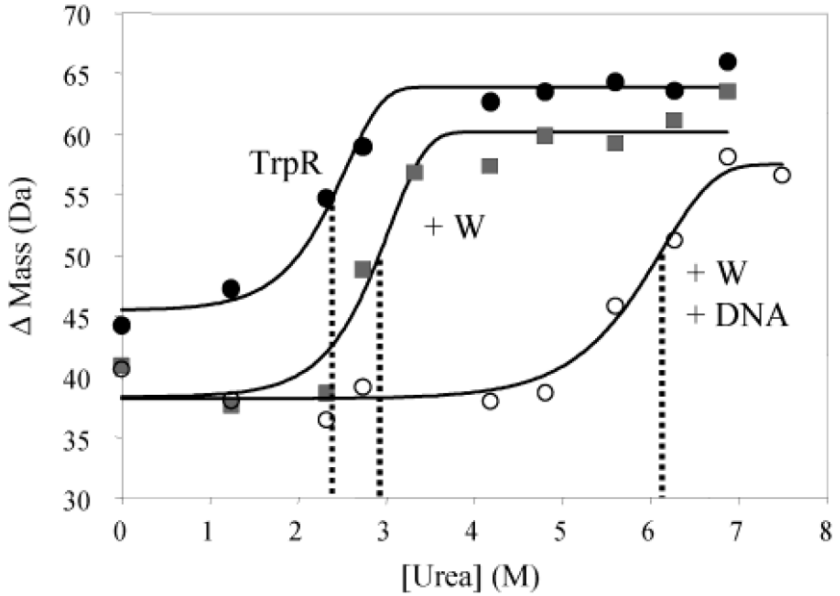
Specific sample preparation

Limited spatial resolution

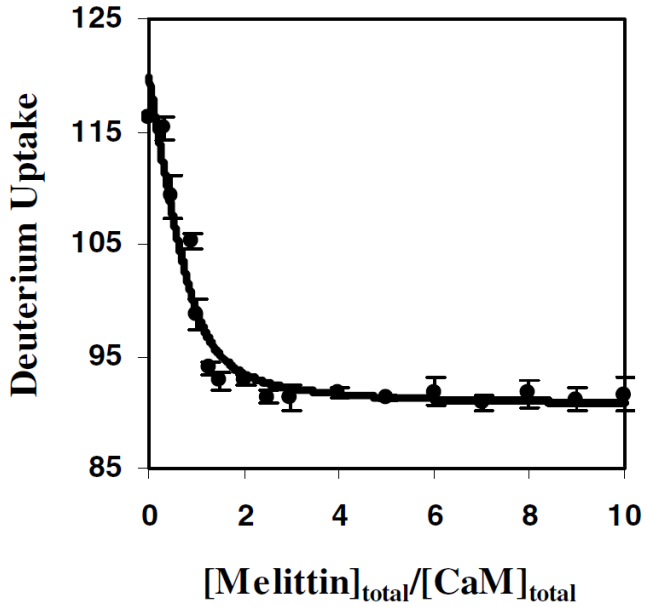
How could Mass Spectrometry contribute?

[1] Willams, M. A.; Daviter, T. *Protein-Ligand interactions, Methods and Applications*, 2nd ed.; Humana Press: New York, 2013
[2] Weis, D. D. *Hydrogen Exchange Mass Spectrometry of Proteins, Fundamentals, Methods, and Applications*; Wiley: Chichester, 2016

SUPREX and PLIMSTEX



Stability of Unpurified Proteins from Rates of H/D Exchange

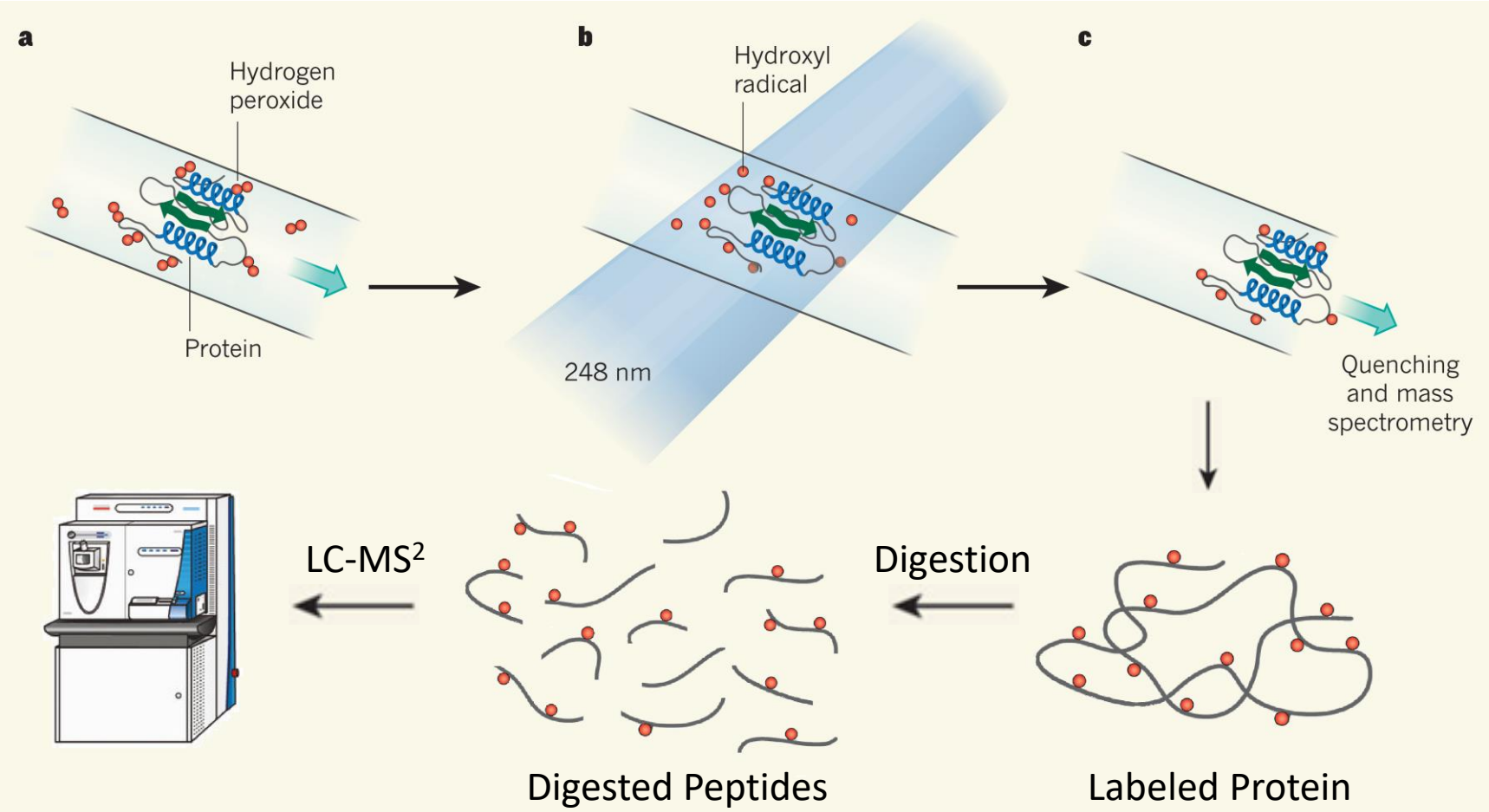


Protein-Ligand Interactions by Mass Spectrometry, Titration, and H/D Exchange

- Universal to various systems, low sample quantities, no special labeling, site-specific
- Backexchange, complicated picture by ligand off-rate and long H/D exchange time

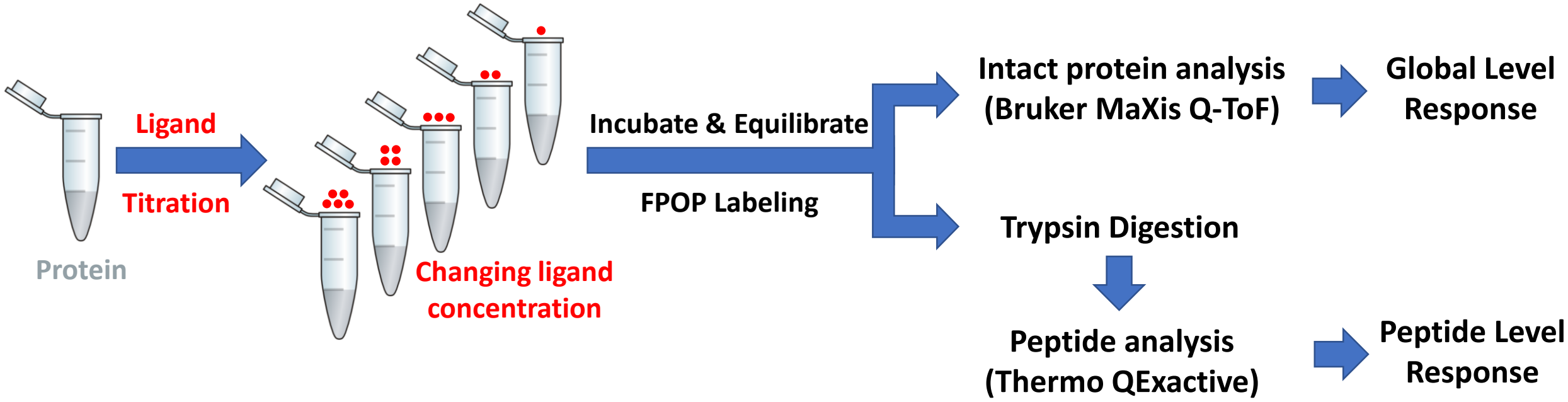
[1] Powell, K. D.; Ghaemmaghami, S.; Wang, M. Z.; Ma, L.; Oas, T. G.; Fitzgerald, M. C. *J. Am. Chem. Soc.* **2002**, *124*, 10256-10257
[2] Zhu, M. M.; Rempel, D. L.; Du, Z.; Gross, M. L. *J. Am. Chem. Soc.* **2003**, *125*, 5252-5253

Fast Photochemical Oxidation of Proteins (FPOP) & Mass Spectrometry



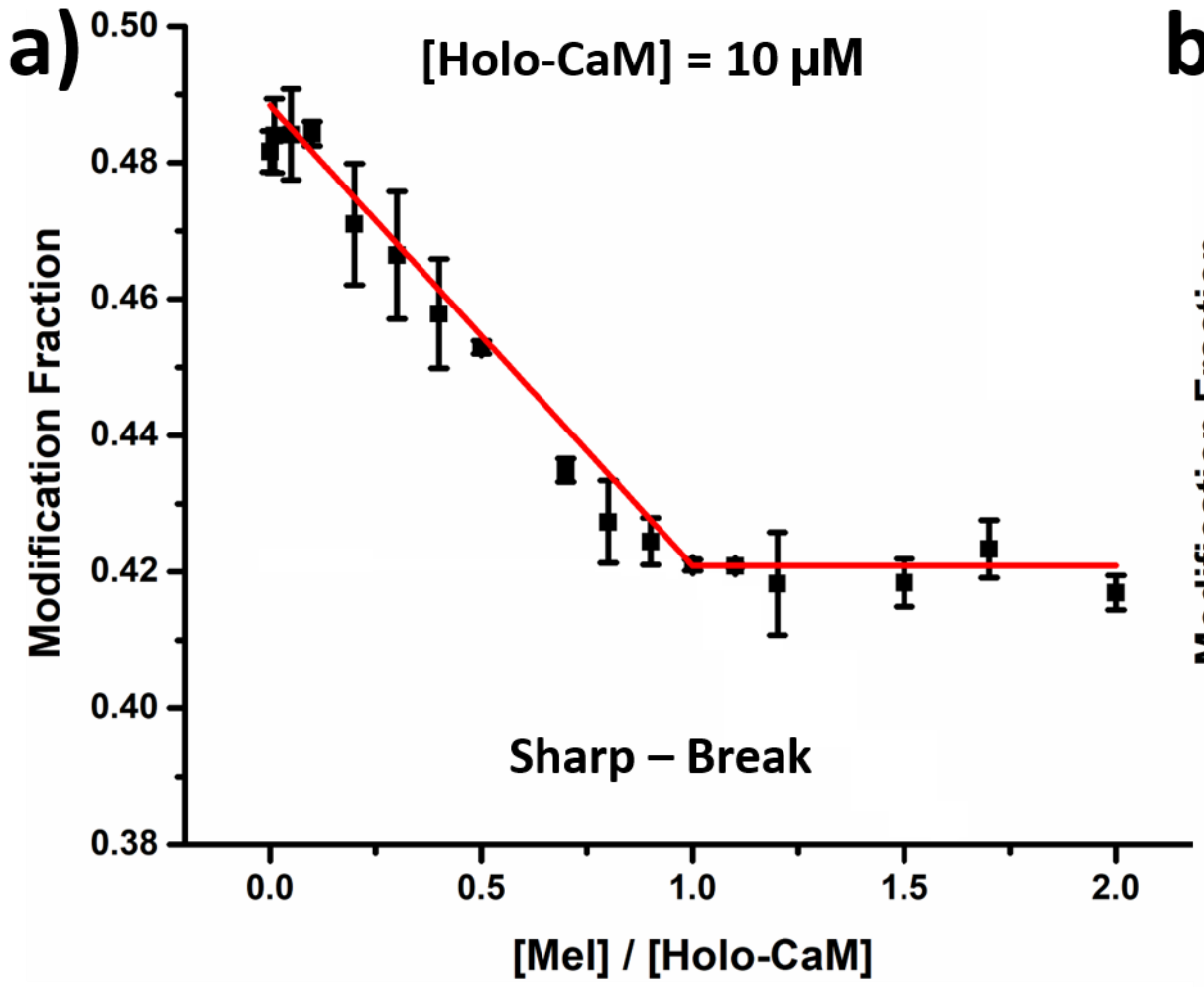
[1] Gruebele, M. *Nature* **2010**, *468*, 640-641
[2] Hambly, D. M.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 2057-2063
[3] Xu, G.; Chance, M. R. *Chem. Rev.* **2007**, *107*, 3514-3543

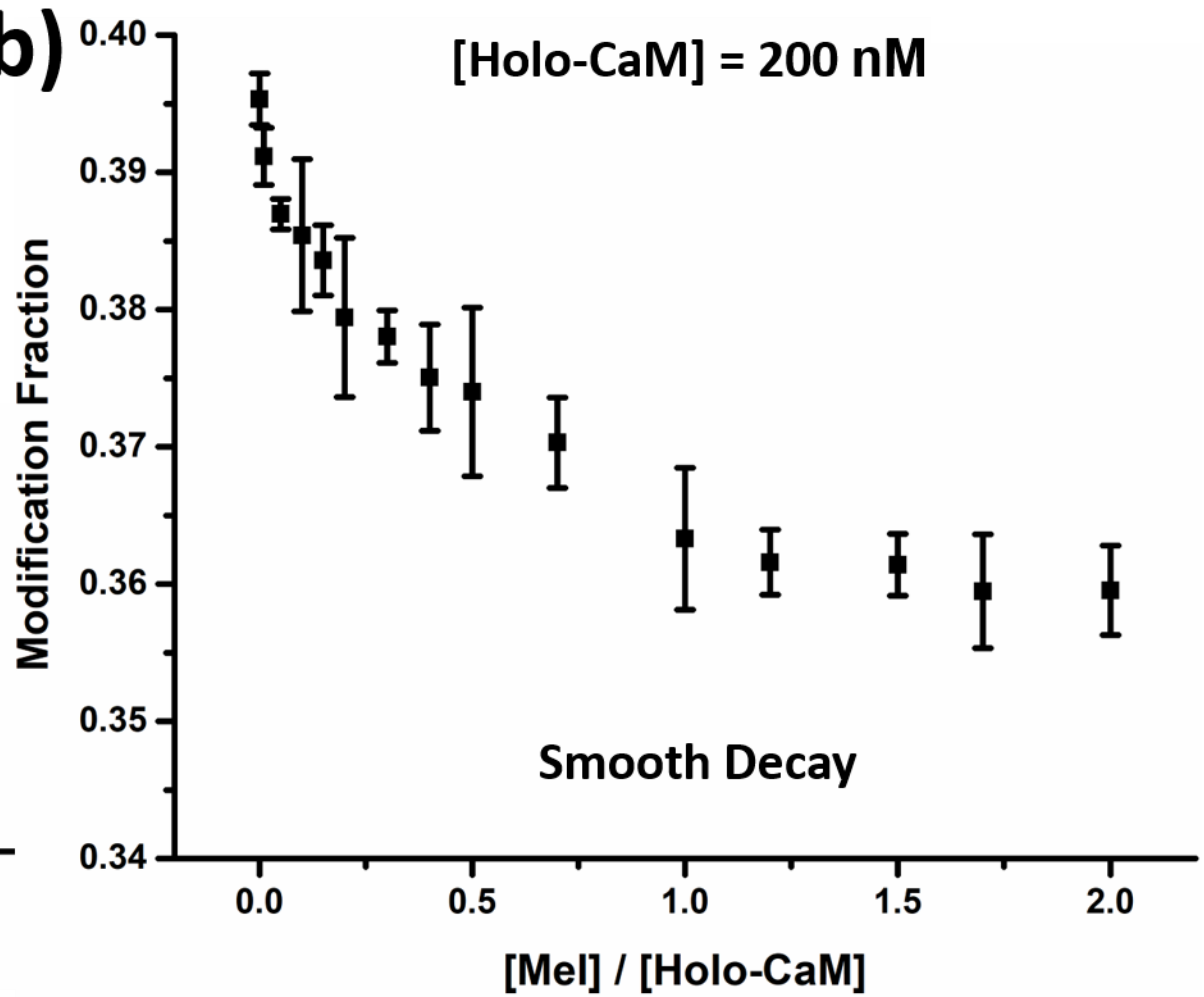
Protein-Ligand Interaction by Ligand Titration, Fast Photochemical Oxidation of Proteins and Mass Spectrometry: LITPOMS



[1] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 213-217
[2] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 5508-5512

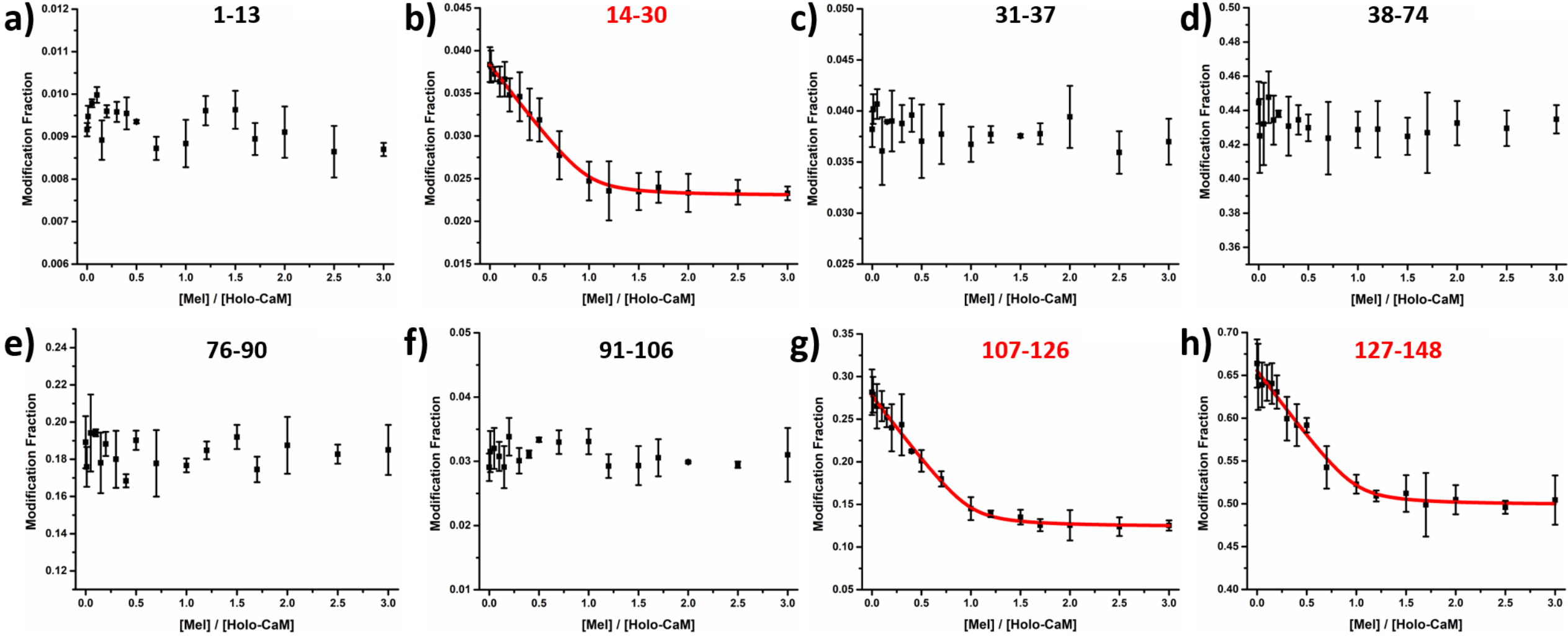
LITPOMS of Mel & Holo-CaM at Global Level





Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 213-217

LITPOMS of Mel & Holo-CaM at Peptide Level



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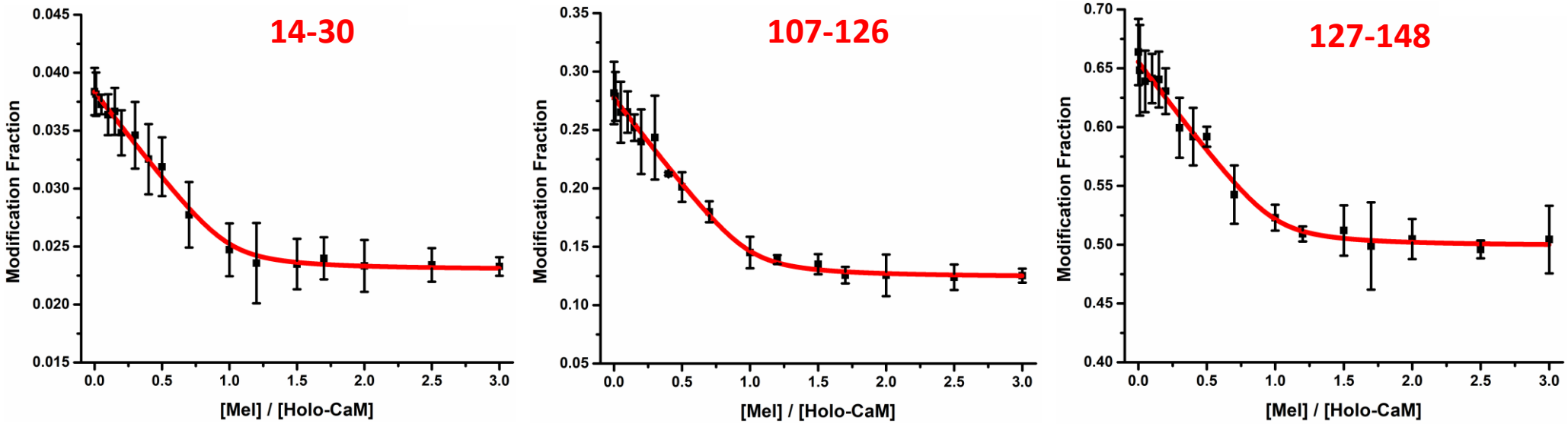
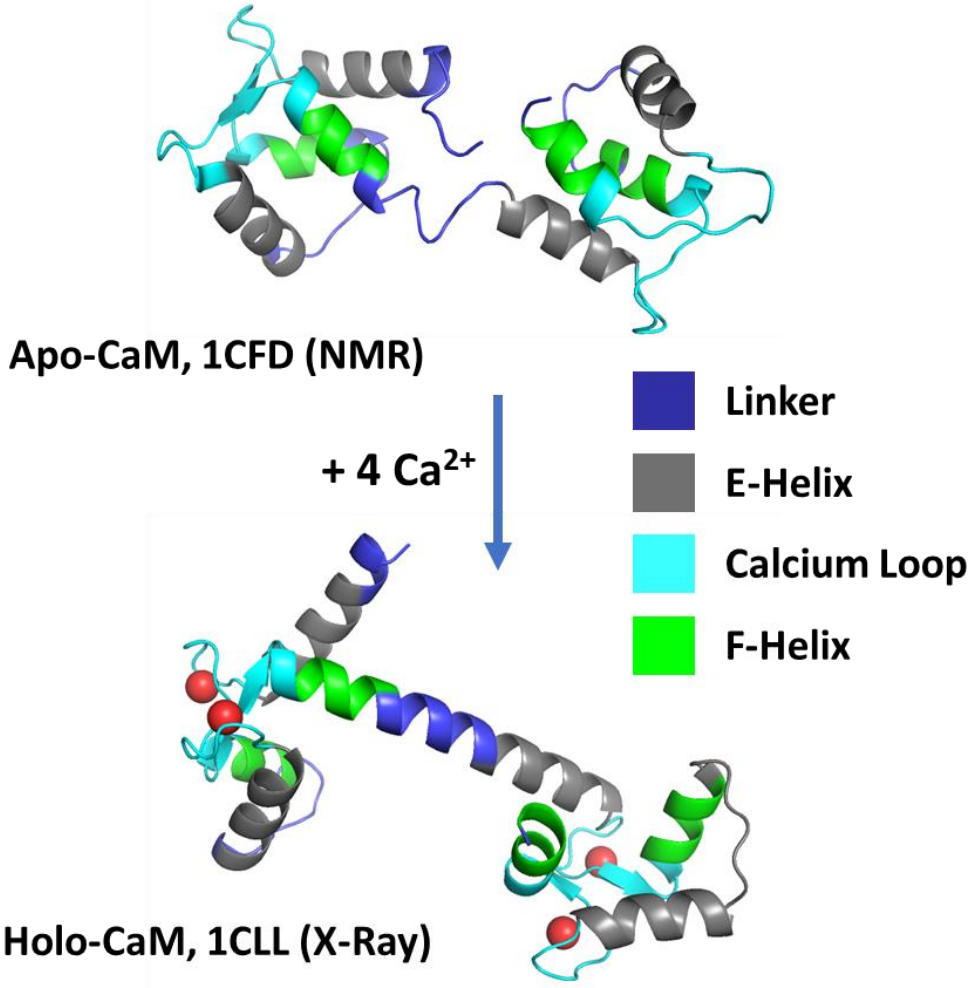
LITPOMS of Mel & Holo-CaM at Peptide Level



- Binding stoichiometry of 1 : 1
- Identified three binding sites: **14-30**, **107-126** & **127-148**
- Binding affinity (K_d) of 4.6 ± 2.7 nM (comparing with literature value of 3 nM)

[1] Comte, M.; Maulet, Y.; Cox, J. A. *Biochem. J.* **1983**, *209*, 269-272
[2] Schulz, D. M.; Ihling, C.; Clore, G. M.; Sinz, A. *Biochemistry* **2004**, *43*, 4703-4715
[3] Zhang, H.; Gau, B. C.; Jones, L. M.; Vidavsky, I.; Gross, M. L. *Anal. Chem.* **2011**, *83*, 311-318
[4] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 213-217

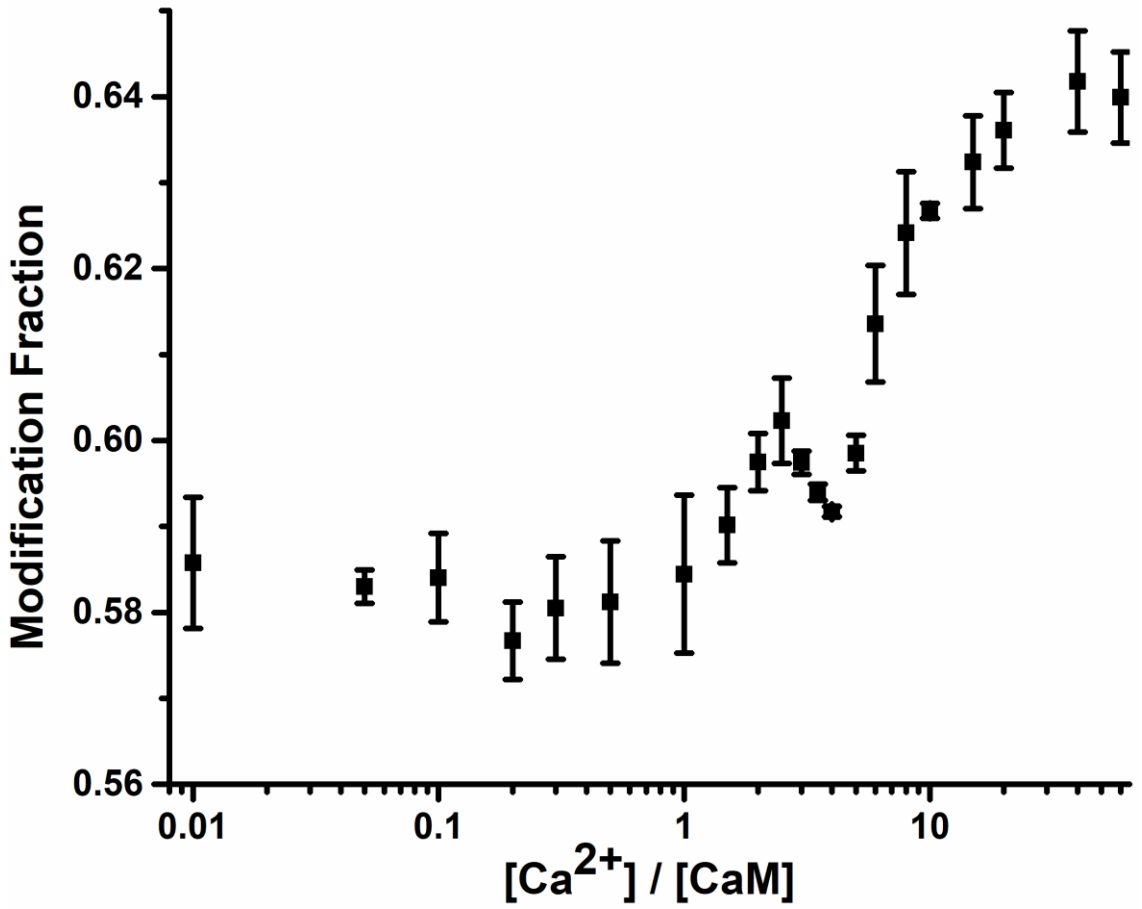
Calmodulin & Calcium in Tris – 1:4 System



- Found in all eukaryotic cells
- Major Ca^{2+} signaling pathway, regulating multiple intracellular process: cell growth, proliferation, apoptosis
- Four EF-hands with "helix-loop-helix" configuration
- Ca^{2+} binds to calcium loop through chelating with negatively charged residues
- Binding affinity of µM, sensitive to stimulations
- Hint of cooperativity / allosteric behavior during binding

[1] Koniwa, H.; Tjandra, N.; Grzesiek, S.; Ren, H.; Klee, C. B.; Bax, A. *Nat. Chem. Biol.* **1995**, *2*, 768-776
[2] Chattopadhyaya, R., Meador, W.E., Means, A.R., Quiocho, F.A. *J. Mol. Biol.* **1992**, *228*, 1177-1192
[3] Linse, S.; Helmersson, A.; Forsen, S. *J. Biol. Chem.* **1991**, *266*, 8050-8054
[4] Sorensen, R.; Shea, M. A. *Biochemistry* **1998**, *37*, 4244-4253

LITPOMS of Ca^{2+} & CaM at Global Level



- A more extended confirmation upon binding with calcium
- A decrease in modification fraction indicates a potential binding event
- A promising preliminary check before peptide & residue-level analysis

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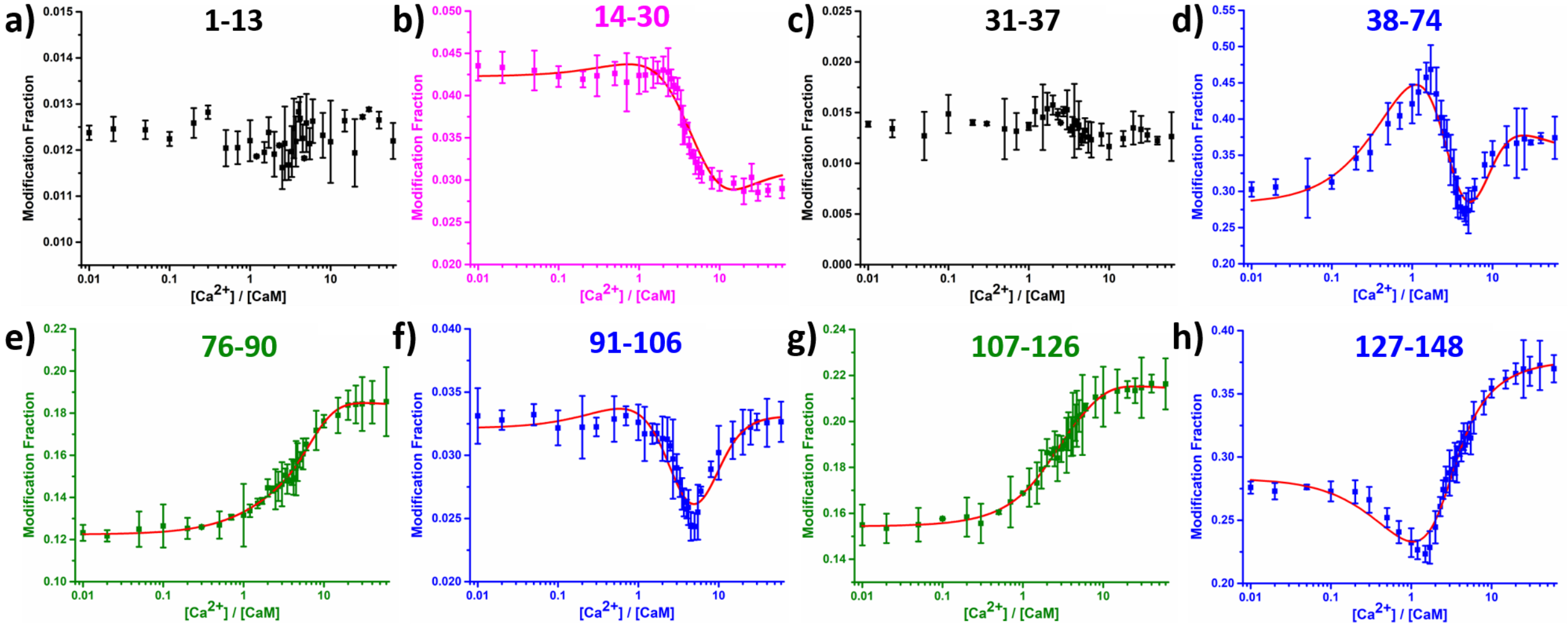
LITPOMS of Ca^{2+} & CaM at Peptide Level



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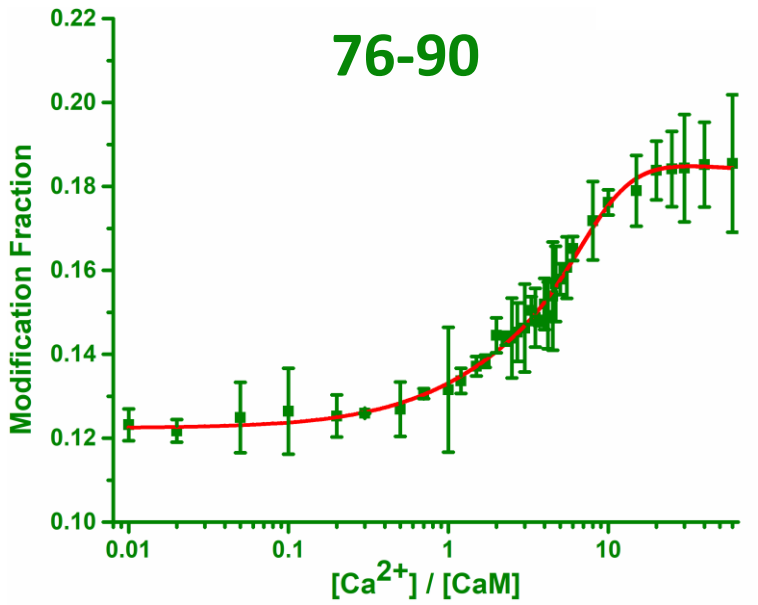
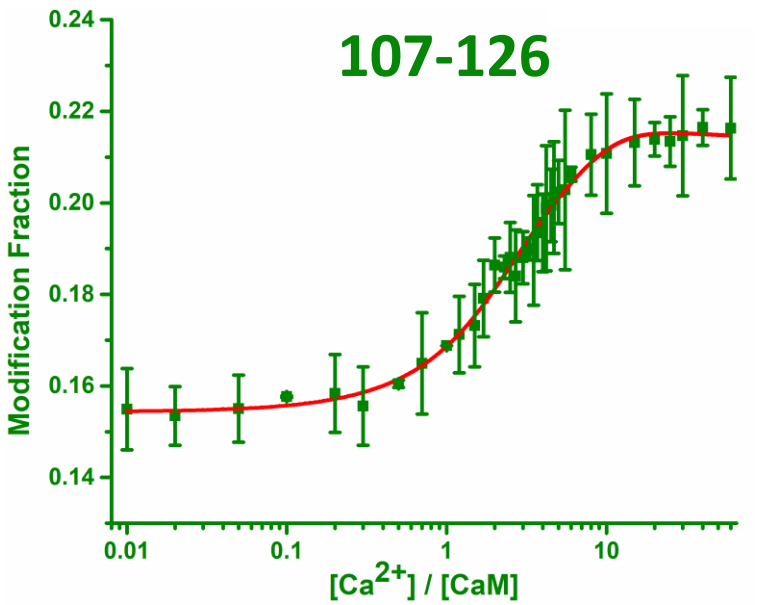
Class I Behavior – Loss of Protection





E-Helix

F-Helix

- Loss of protection upon binding with calcium, reporting the overall structural transition
- Linker region between EF-hands / more exposed helices

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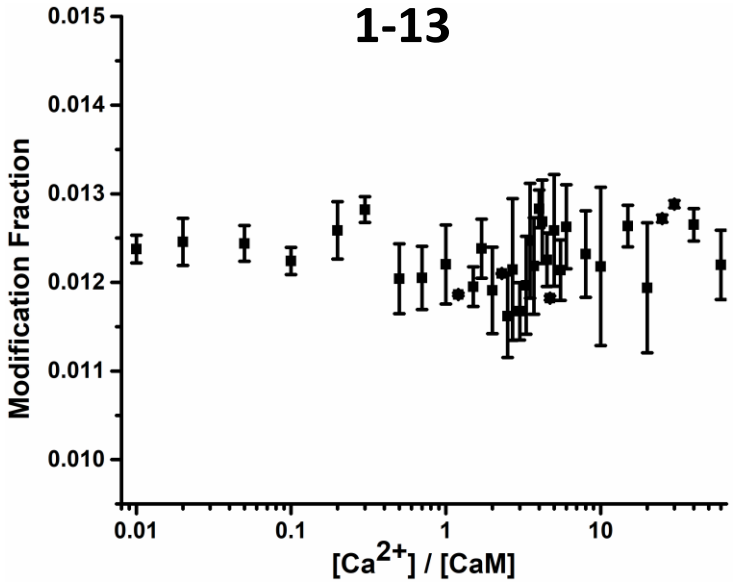
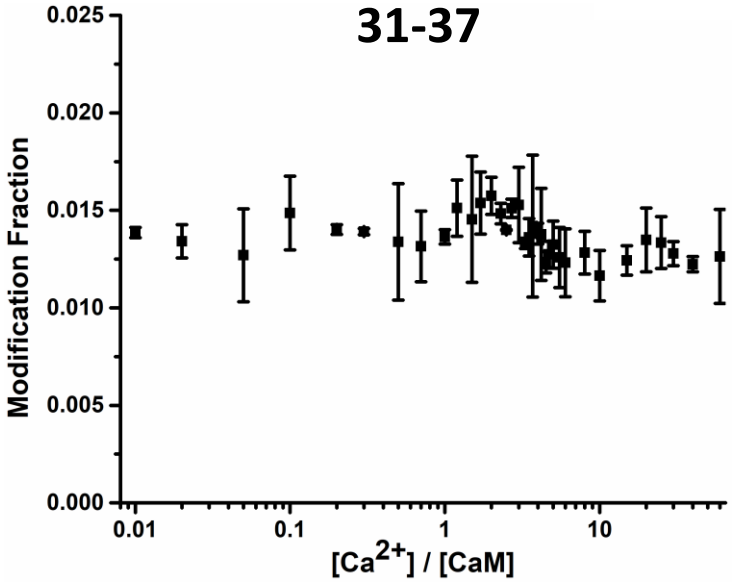
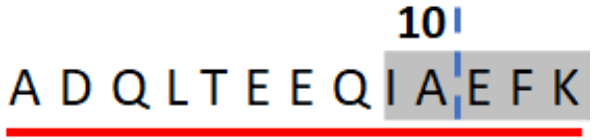
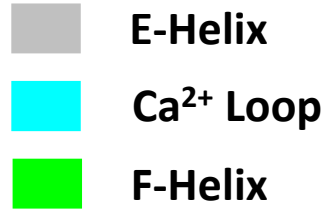
Class II Behavior – Constant Protection









- Constant protection upon binding with calcium
- Linker region between EF-hands / F-helix of EF-1

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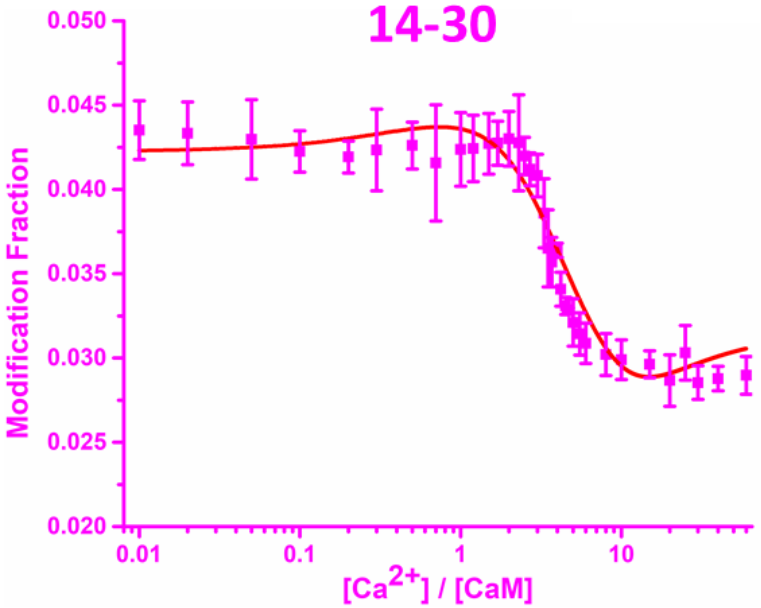
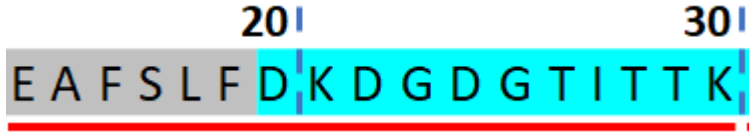
Class III Behavior – Classical Binding



- E-helix & calcium loop of EF-1



- More protected upon binding with calcium, a classical binding behavior

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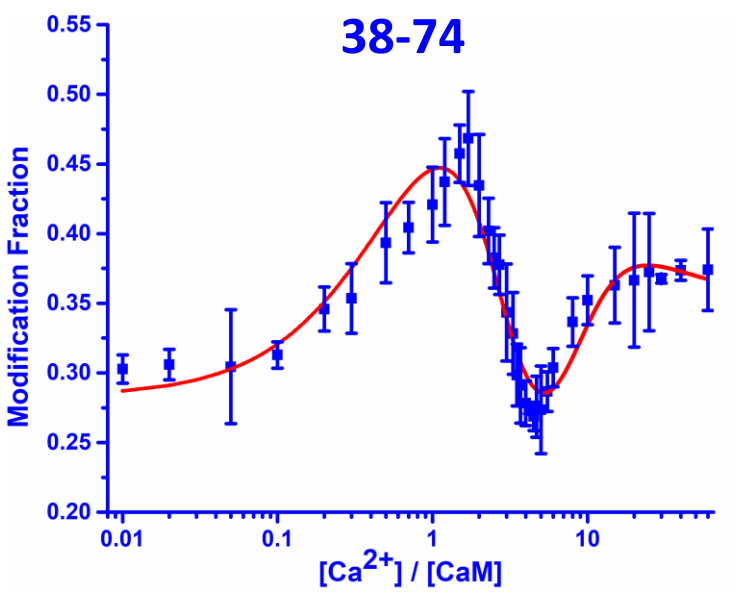
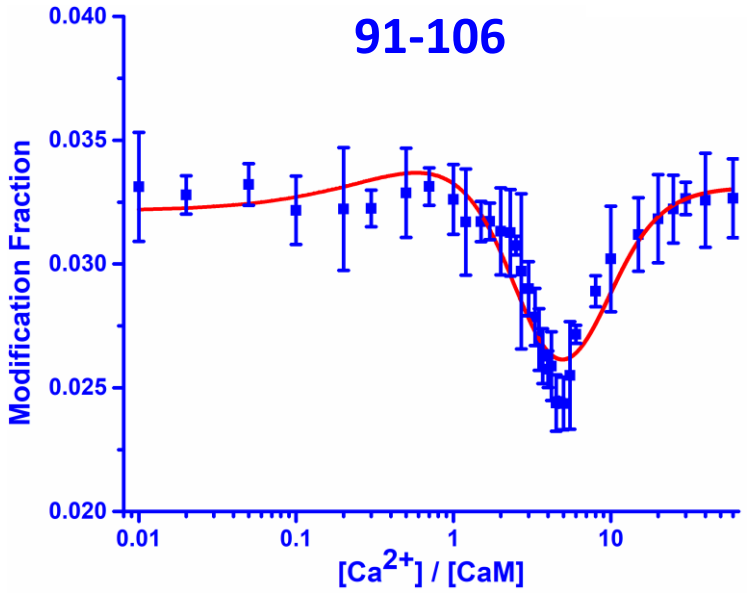
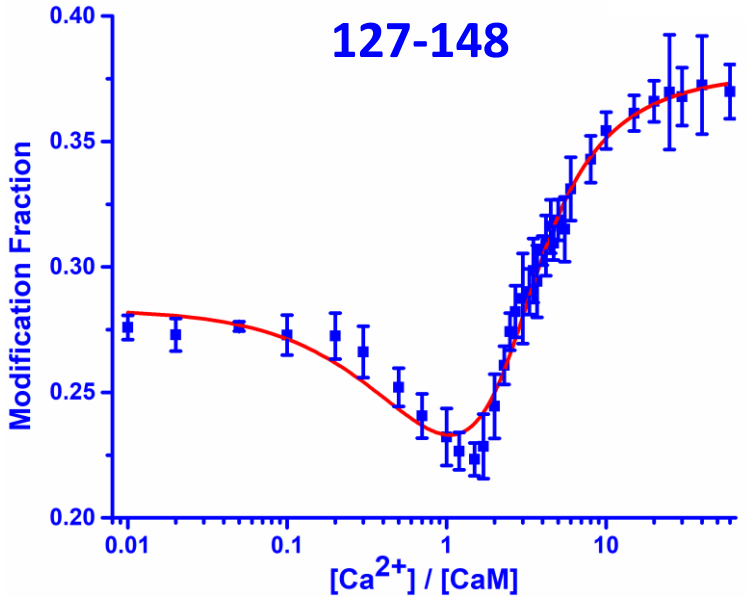
Class IV Behavior – Composite Behavior







E-Helix

Ca^{2+} Loop

F-Helix

- Covers EF-2 (38-74), EF-3 (91-106) and EF-4 (127-148)
- Binding & Allostery (Conf. change by a remote binding event)

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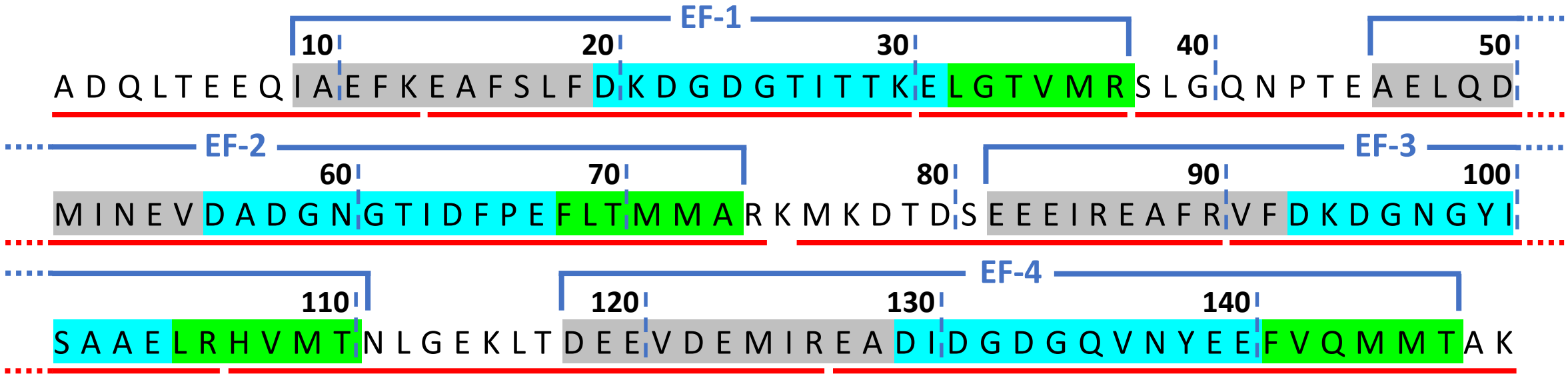
A Summarized Coverage Map



- **E-Helix**
- **Ca^{2+} Loop**
- **F-Helix**

- A 99% sequence coverage
- Four classes of behaviors
- Reveals composite behavior of calmodulin **during** binding with calcium

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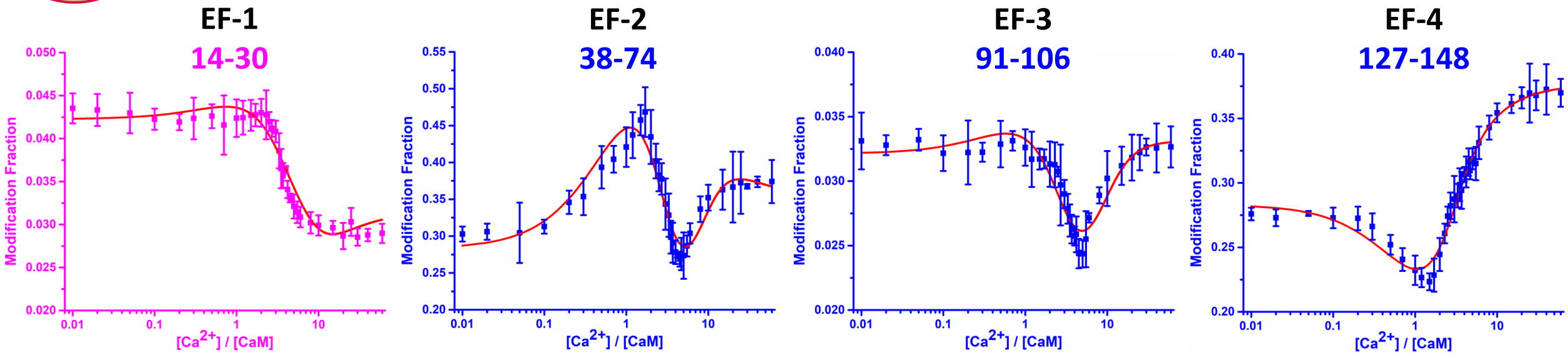
Allosteric Behavior / Binding Dynamics



- EF-4 (127-148) binds first, followed by a conformational change over helix F (more exposed)
- EF-2 (38-74) becomes more exposed owing to binding at EF-3 (91-106) and EF-4 (127-148), preparing them for Ca^{2+} binding
- EF-2, 3 and 4 become more exposed at high Ca^{2+} to facilitate binding at EF-1 (14-30)
- Calmodulin is saturated at $[Ca^{2+}]$ / [CaM] = 15

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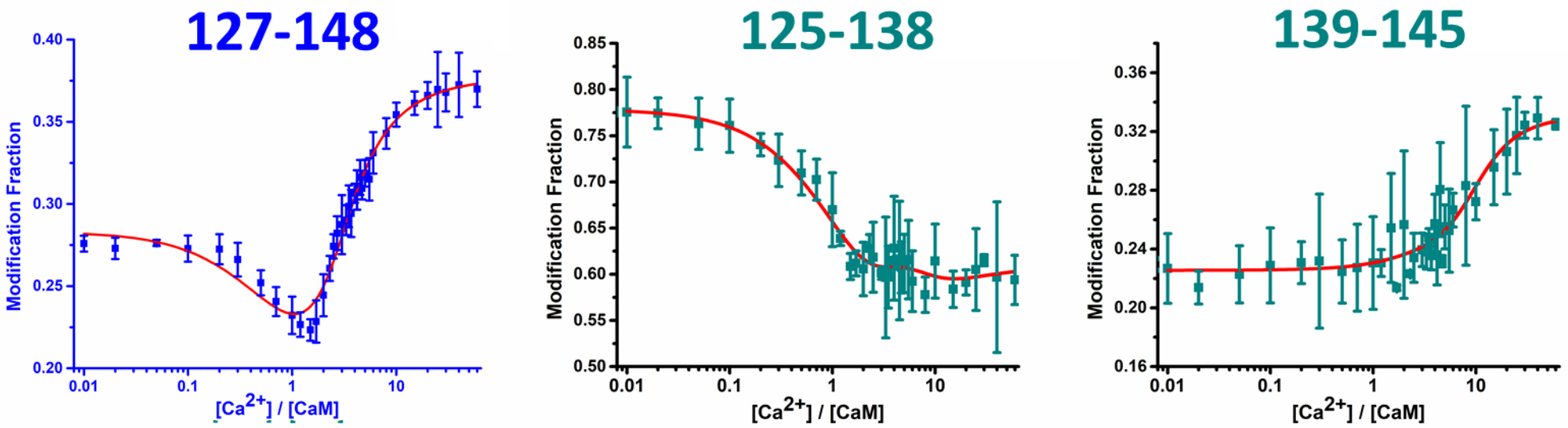
Dissect Composite LITPOMS by Chymotrypsin



E-Helix

Ca^{2+} Loop

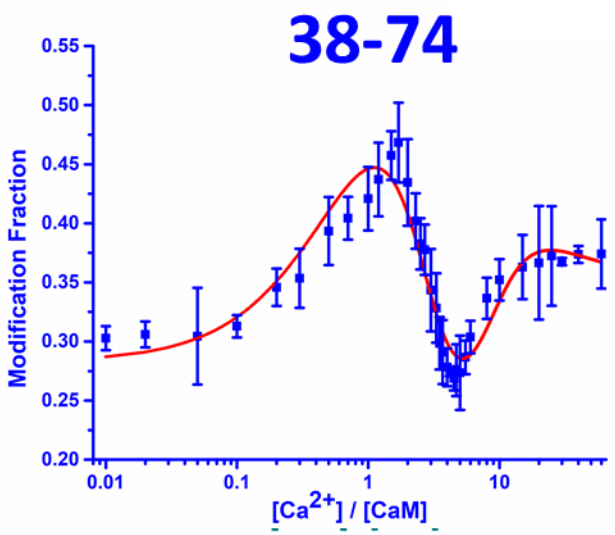
F-Helix

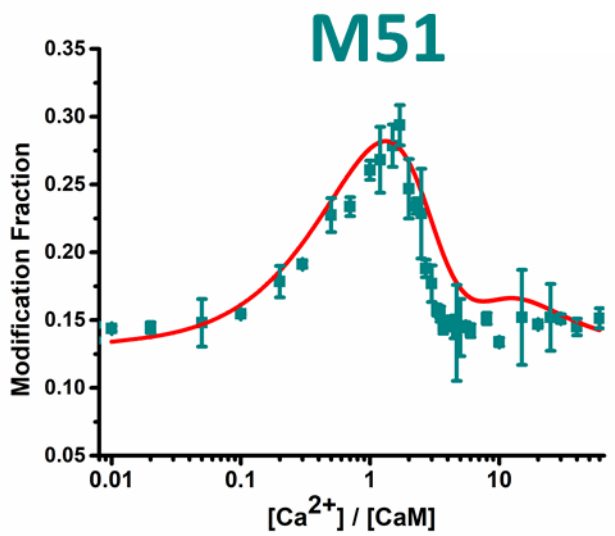
- Tryptic Peptide 127-148 covers EF-4 of calmodulin
- Chymotryptic peptides 125-138 and 139-145 show simple behavior of binding and opening

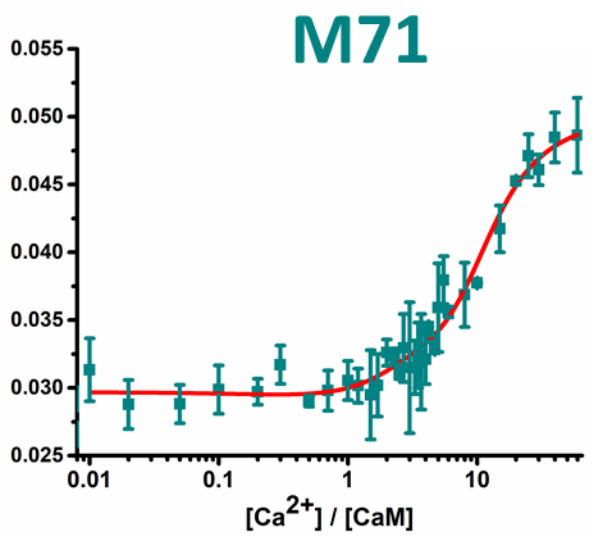
[1] Liu, X. R.; Rempel, D. L.; Gross, M. L. *Anal. Chem. Under Review*

[2] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 5508-5512

Dissect Composite LITPOMS by Residue-Level Analysis



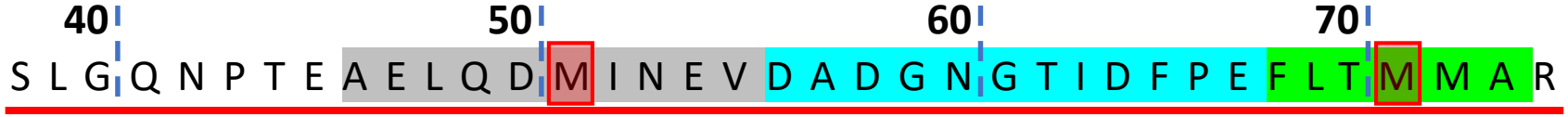




E-Helix

Ca^{2+} Loop

F-Helix



- Tryptic Peptide 38-74 covers EF-2 of calmodulin
- Residue M51 show deprotection initially, followed by binding induced protection and stay protected

[1] Liu, X. R.; Rempel, D. L.; Gross, M. L. *Anal. Chem. Under Review*

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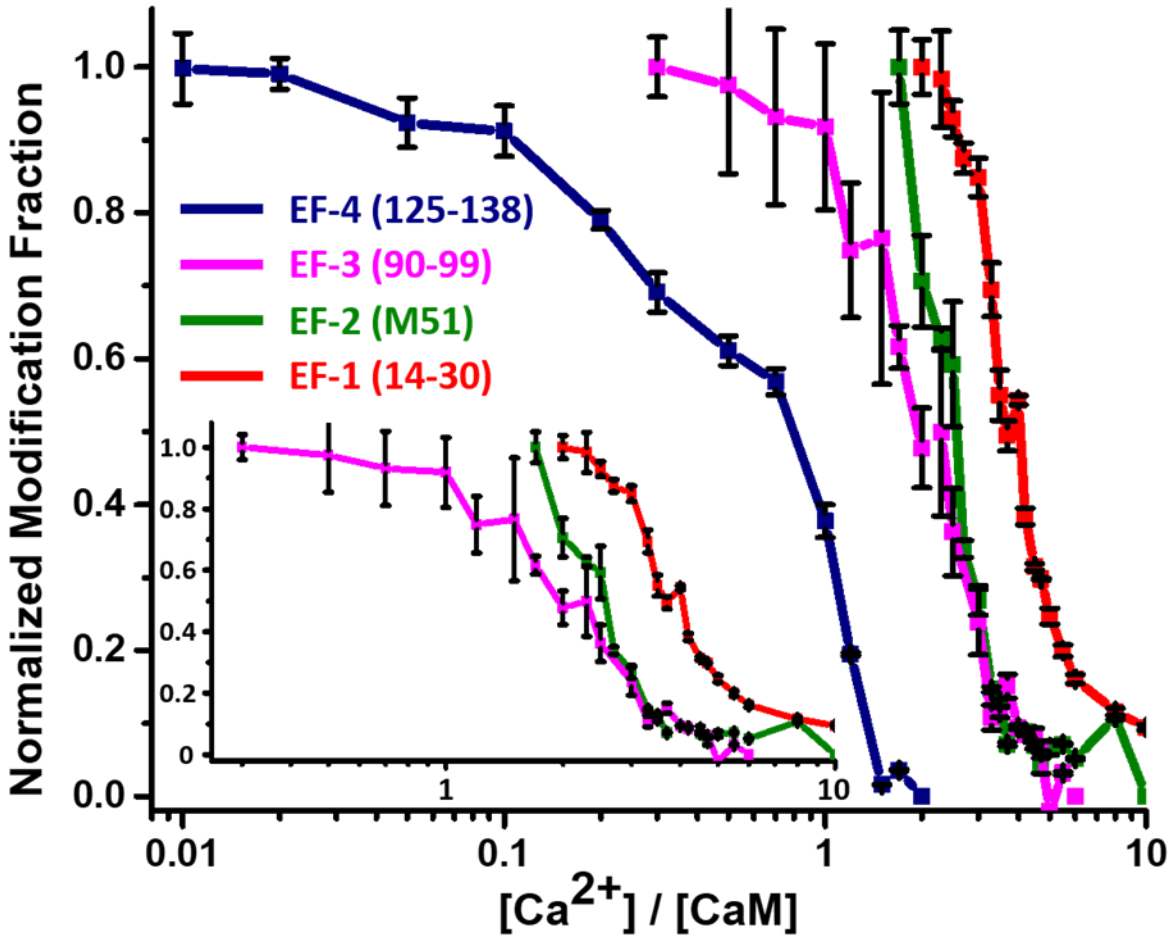
Binding Order by LITPOMS



EF-4 > EF-3 > EF-2 > EF-1

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[2] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 5508-5512

Site - Specific Binding Affinity

- Four distinct binding affinities through fitting

Binding Order	EF-hand	LITPOMS K_i (M^{-1})	Literature K_i (M^{-1})
1	EF-4 (127-148)	1.4×10^6	8.0×10^4
2	EF-3 (91-106)	6.2×10^6	4.0×10^6
	C-Term Lobe	8.6×10^{12}	3.2×10^{11}
3	EF-2 (38-74)	4.1×10^4	2.5×10^4
4	EF-1 (14-30)	2.9×10^6	4.0×10^5
	N-Term Lobe	1.2×10^{11}	1.0×10^{10}

- Agree with literature values within 20-fold

[1] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 5508-5512
[2] Weis, D. D. *Hydrogen Exchange Mass Spectrometry of Proteins, Fundamentals, Methods, and Applications*; Wiley: Chichester, 2016
[3] Linse, S.; Helmersson, A.; Forsen, S. *J. Biol. Chem.* **1991**, *266*, 8050-8054

Conclusion

- LITPOMS reveals the **composite behavior** and allostery of calmodulin upon binding with calcium
- March through the protein region-by-region, characterize binding at peptide & residue level
- Composite LITPOMS behavior can be dissected by a different protease or by analyzing residue-level LITPOMS curves
- Successfully demonstrate the capability of dealing with
    - Systems with various binding stoichiometries
    - Systems with binding affinities of μM to nM (both loose and tight binders)
    - Systems that bind with small peptides
    - Systems that bind with metal ions
- Readily applicable to other systems with complex binding schemes, e.g., signaling proteins

[1] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 213-217
[2] Liu, X. R.; Zhang, M. M.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 5508-5512
[3] Liu, X. R.; Rempel, D. L.; Gross, M. L. *Anal. Chem. Under Review*

Acknowledgement

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PROTEIN METRICS

Thank You!