Figure S-1: (A) ESI-MS spectrum of porcine CaM. 10 pmol of CaM loaded onto a C_8 column, pre-equilibrated with 100 μL of 0.2% formic acid in water. The column was then washed with 300 μL of 0.2% formic acid in water and CaM was eluted with an isocratic flow 40% Solvent B. The positive ion at *m/z* 1120.3 corresponds to a +15 charge state. (B) MaxEnt1 decharged spectrum of A. The MW of CaM is 16789 ± 1 Da.

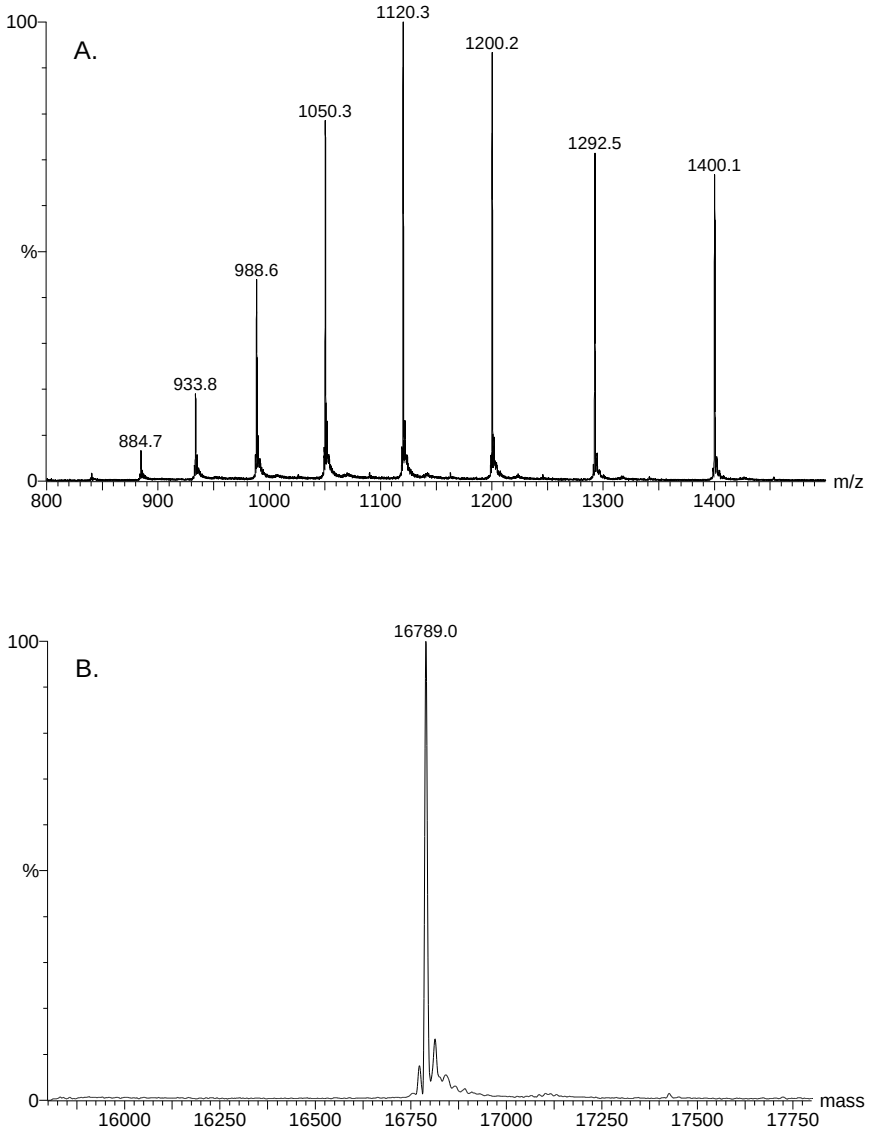
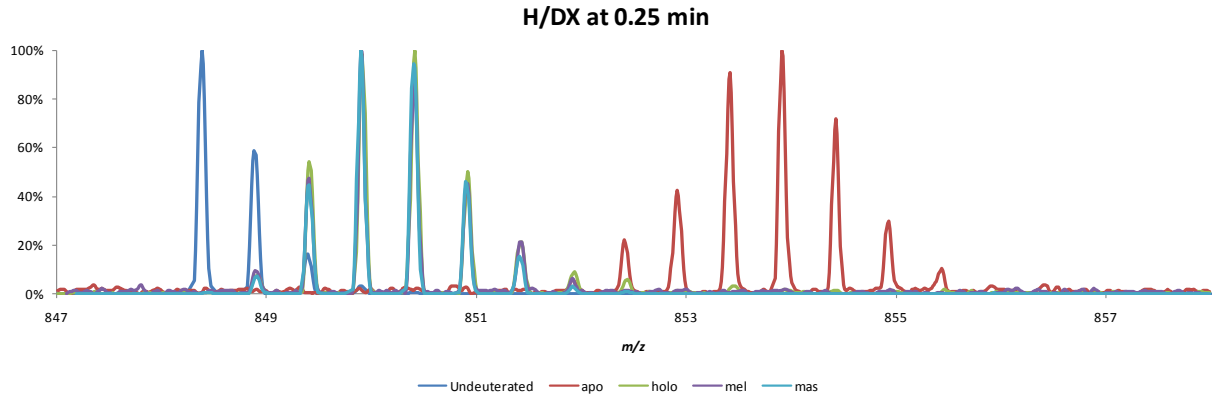
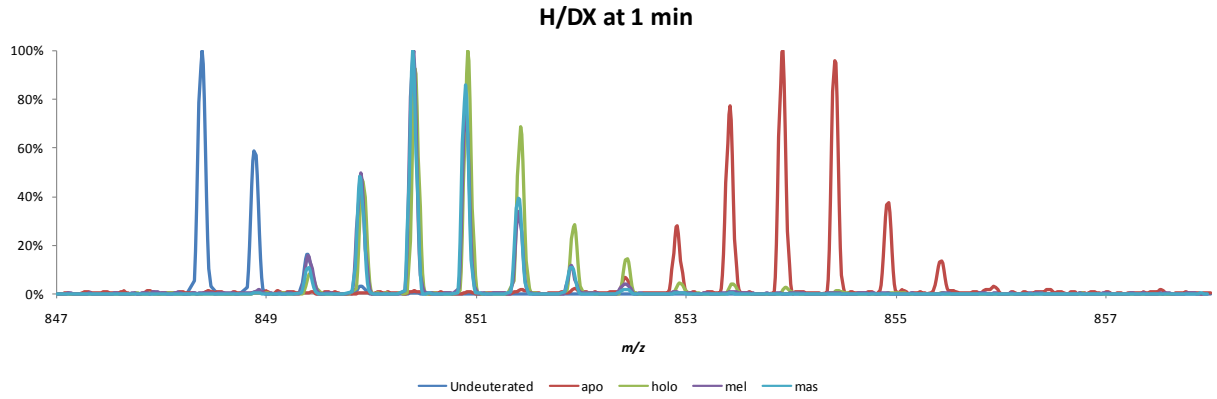
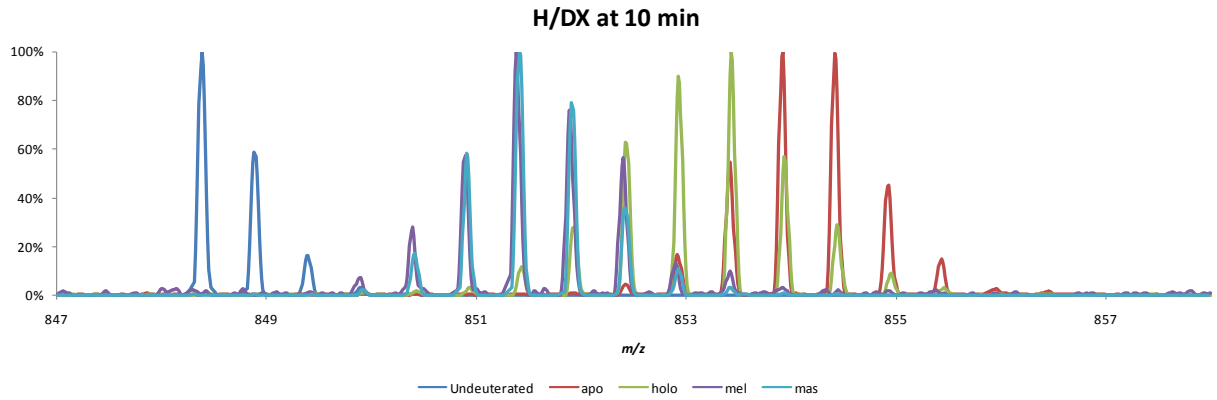


Figure S-2: Local H/DX kinetics at four exchange times for residues 124-138 representing EF hand 4 in calmodulin. The undeuterated mass spectrum is provided as a reference for each exchange time. The apo state shows nearly complete exchange at 0.25 min. When calmodulin is bound by Ca^{2+} and either melittin or mastoparan, the extent of exchange decreases.


H/DX at 0.25 min
100%
80%
60%
40%
20%
0%
847
849
851
853
855
857
m/z
Undeuterated
apo
holo
mel
mas


H/DX at 1 min
100%
80%
60%
40%
20%
0%
847
849
851
853
855
857
m/z
Undeuterated
apo
holo
mel
mas


H/DX at 10 min
100%
80%
60%
40%
20%
0%
847
849
851
853
855
857
m/z
Undeuterated
apo
holo
mel
mas

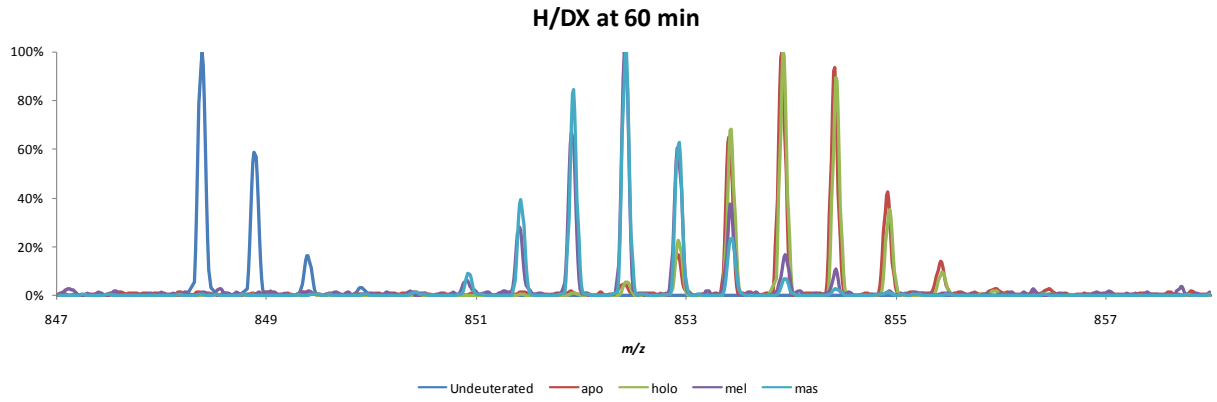

H/DX at 60 min
100%
80%
60%
40%
20%
0%
847
849
851
853
855
857
m/z
Undeuterated
apo
holo
mel
mas

Figure S-3: Local H/DX kinetics experiments in the regions with little change in D uptake. Peptide 12-18 represents part of the N-terminal tail, 69-72 the beginning of the central α-helix, 117-128 is part of EF hand 4, and 141-148 is the C-terminal tail. Four states of CaM are shown: CaM with no Ca^{2+} (squares), CaM with 2 mM Ca^{2+} (diamonds), 2.9:1 melittin:CaM with 2 mM Ca^{2+} (triangles), 2.9:1 mastoparan:CaM with 2 mM Ca^{2+} (circles) and 2.9:1 MLCK:CaM with 2mM Ca^{2+} (open squares).

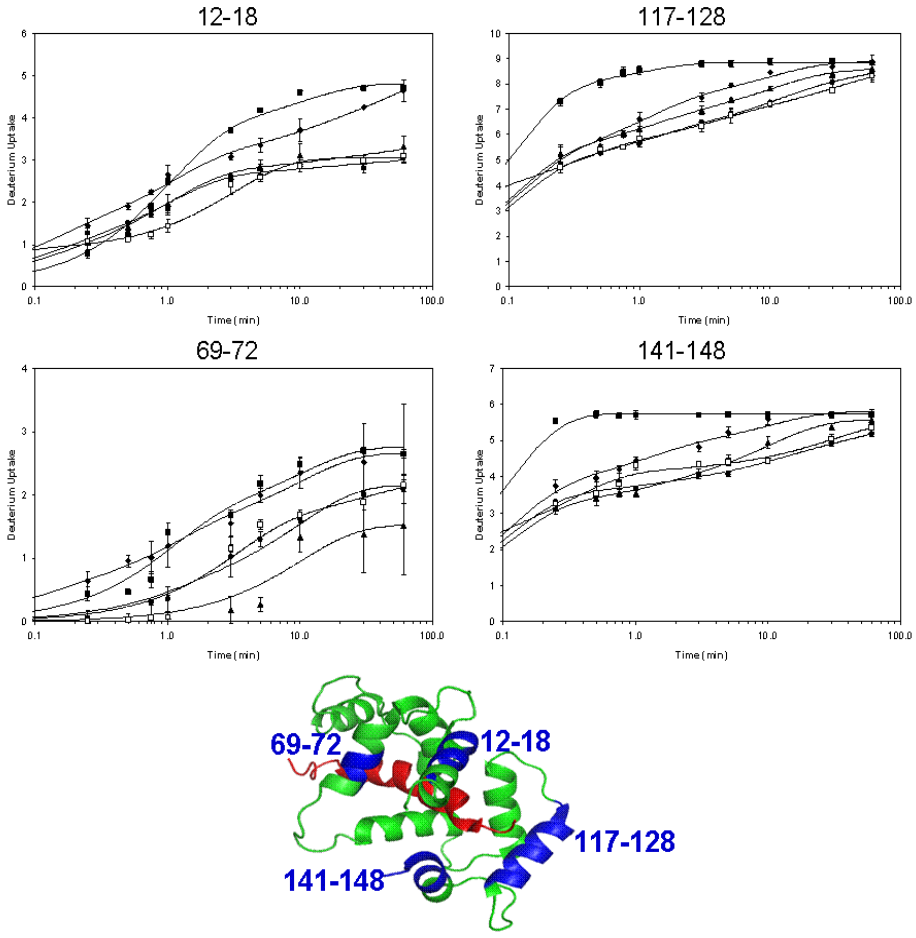


Figure S-4: CaM fractional species output from the PLIMSTEX modeling. A. Titration of CaM with Ca^{2+}, B. 2.9:1 MEL:CaM titrated with Ca^{2+}, and C. 2.9:1 MAS:CaM titrated with Ca^{2+}. The CaM:3Ca^{2+} and 4Ca^{2+} states are the dominant species in A. The CaM:2Ca^{2+} and 4Ca^{2+} states are the dominant species in B and C.

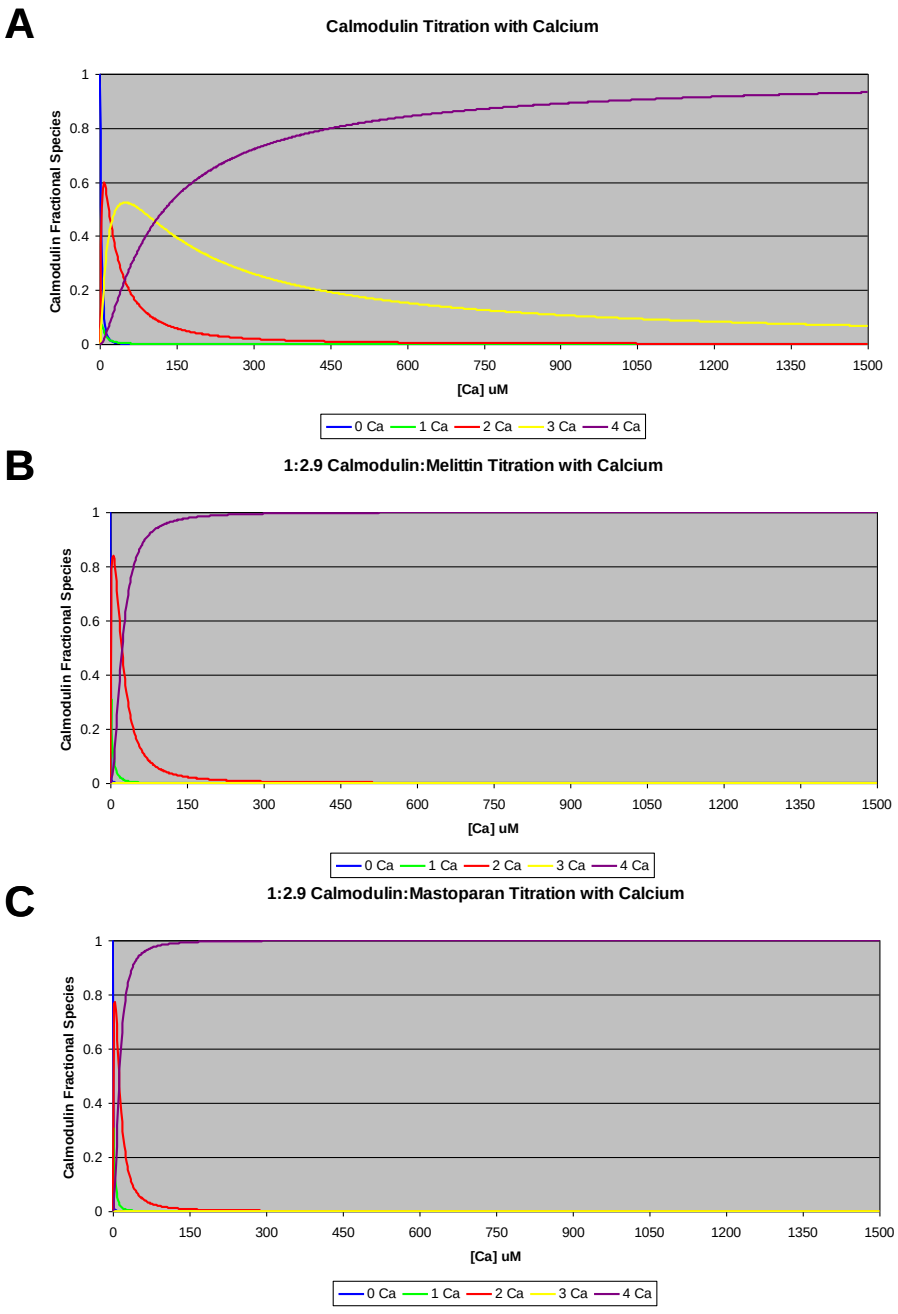


Figure S-5: High resolution titration experiments of EF hands 1, 3, and 4: calmodulin titrated with Ca^{2+} (squares), 2.9:1 melittin:calmodulin titrated with Ca^{2+} (diamonds), and 2.9:1 mastoparan:calmodulin titrated with Ca^{2+} (circles). H/D exchange was conducted at a constant 10 min with 97% D_2O, 10 mM HEPES (pH 7.4), 150 mM KCl, and 2.9 μM calmodulin. Peptide 19-36 represents EF hand 1, 85-102 EF hand 3, and 125-138 EF hand 4.

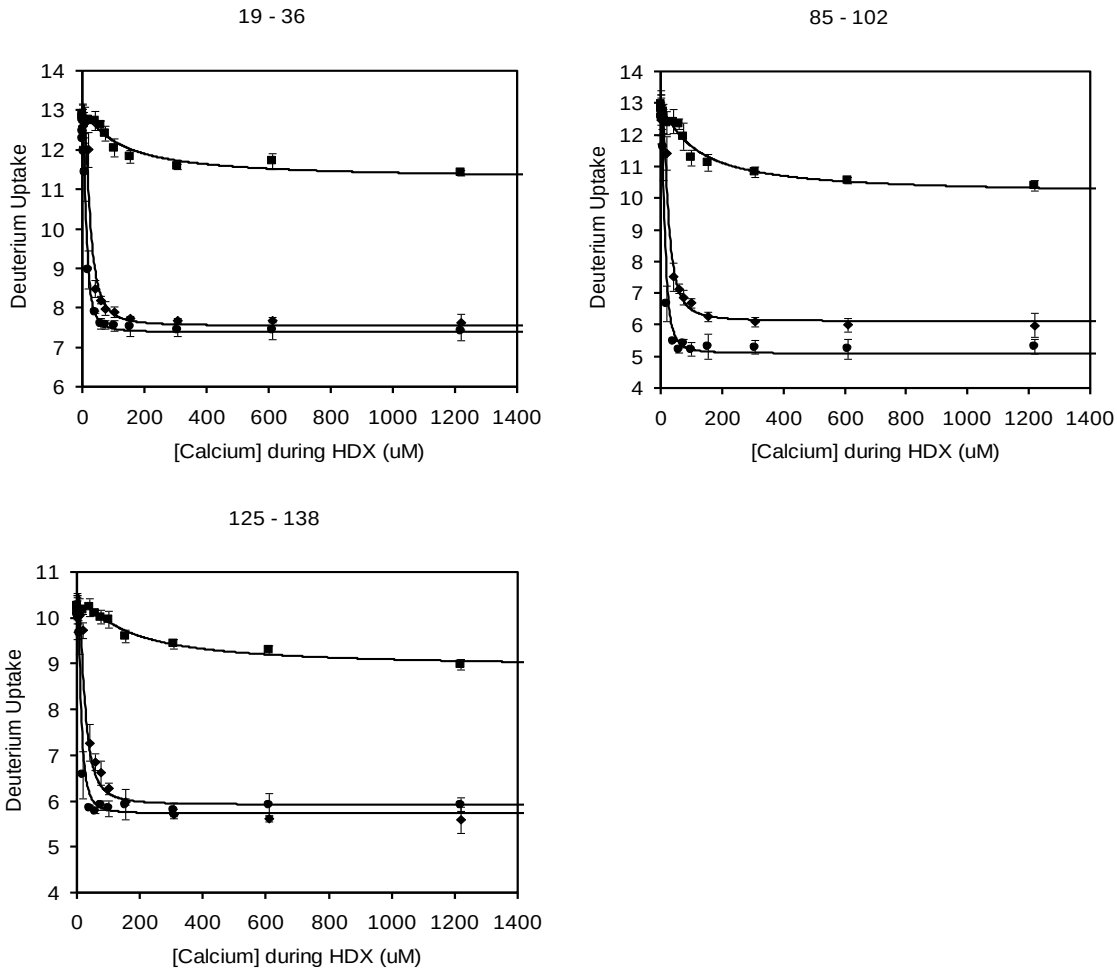


Figure S-6. High resolution titration experiments of the linker regions between EF hands 1 and 2 (peptide 37-48) and 3 and 4 (peptides 103-112, 103-119, and 103-120): calmodulin titrated with Ca^{2+} (squares), 2.9:1 melittin:calmodulin titrated with Ca^{2+} (diamonds), and 2.9:1 mastoparan:calmodulin titrated with Ca^{2+} (circles). H/D exchange was conducted at a constant 10 min with 97% D_2O, 10 mM HEPES (pH 7.4), 150 mM KCl, and 2.9 μM calmodulin.