Cyanylated cysteine reports site-specific changes at protein-protein interfaces without perturbation

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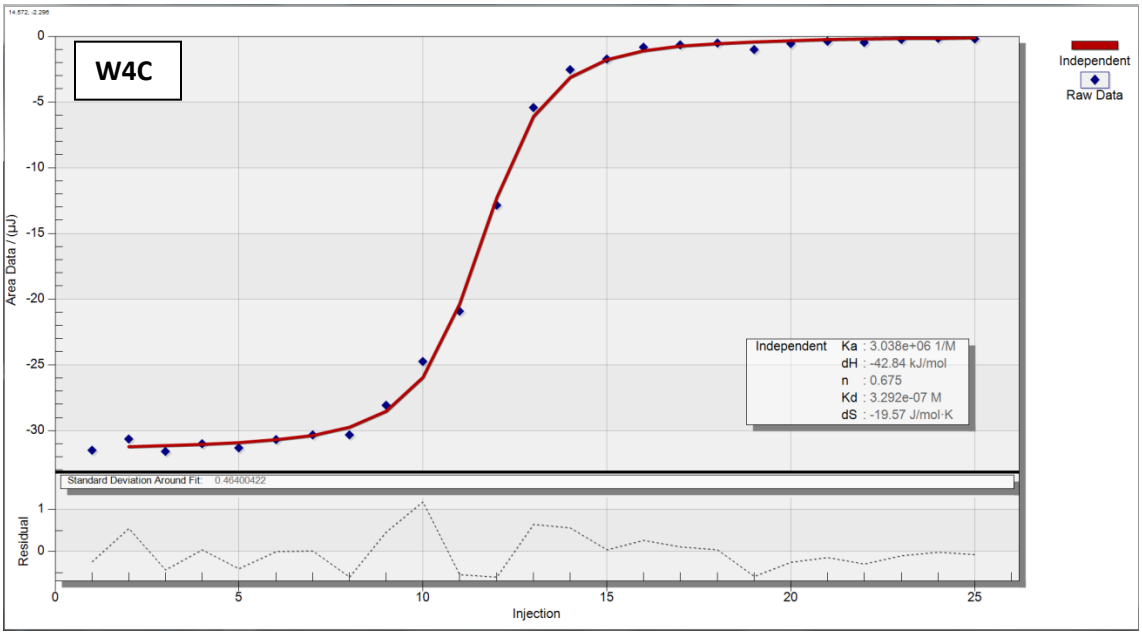
Supporting Information

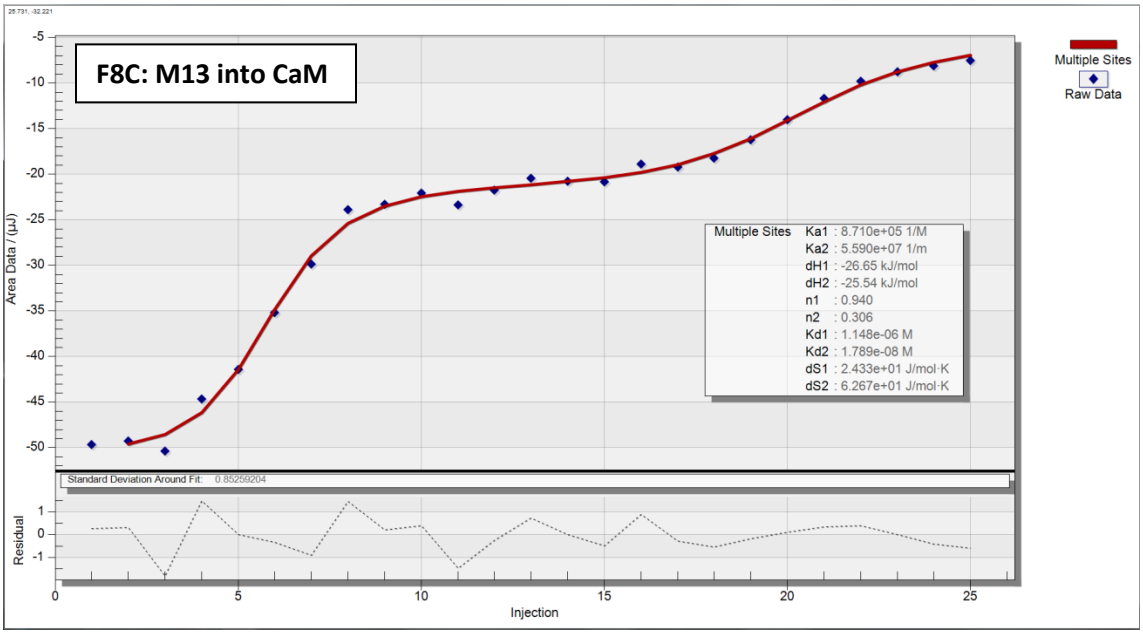
1. **Isothermal Titration Calorimetry**

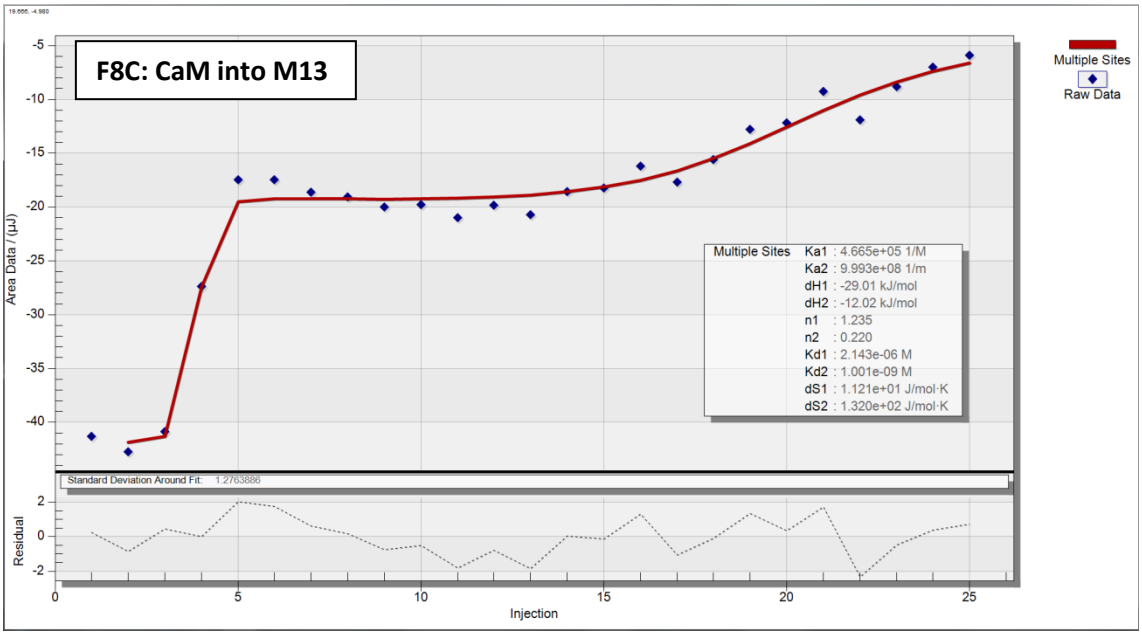
The following data were acquired for seven M13 peptides (see table 1 in the main manuscript for sequences, and "WT" is the unmodified M13 sequence) titrated with recombinant wild-type calmodulin. The data points shown below are peak areas determined following baseline correction of the raw titration data. The fits are all to either a single "independent" sigmoidal model for binding, or in some cases, to a "two-sites" double-sigmoidal model for binding for titrations where a second process was suggested by the data.

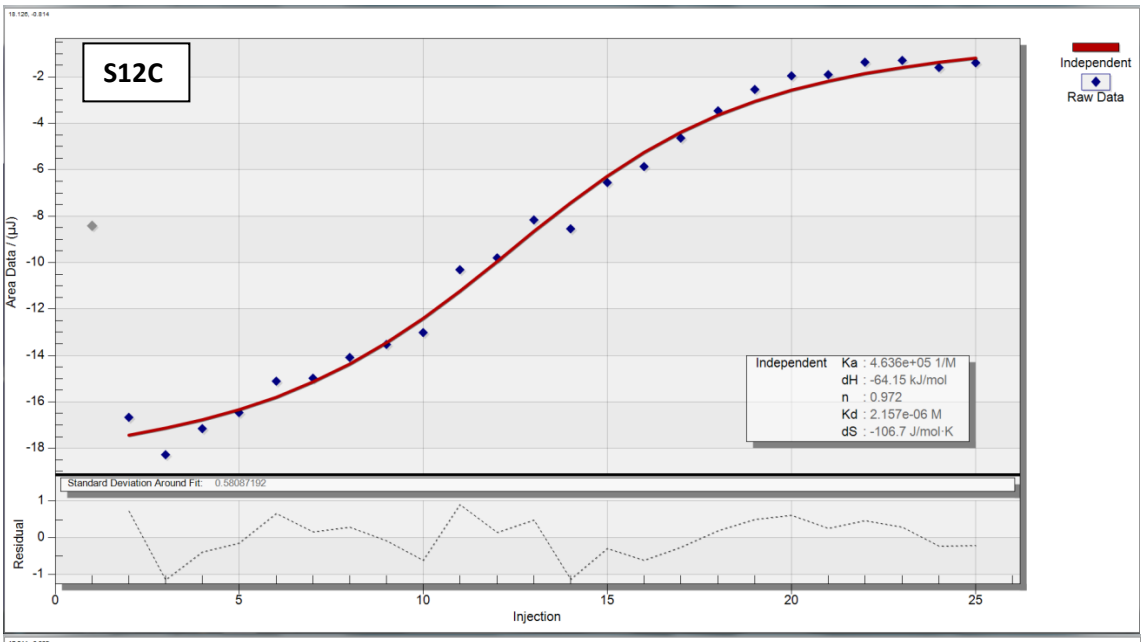
The signal:noise ratio for S23C* was not high enough to distinguish clearly between a one- and a two-sites fit. A two-sites fit is clearly implicated for F8C*, and this is discussed in the main manuscript; this led to our also performing the "backwards" titration experiment with an independent sample of F8C* which led to very similar fitting parameters.

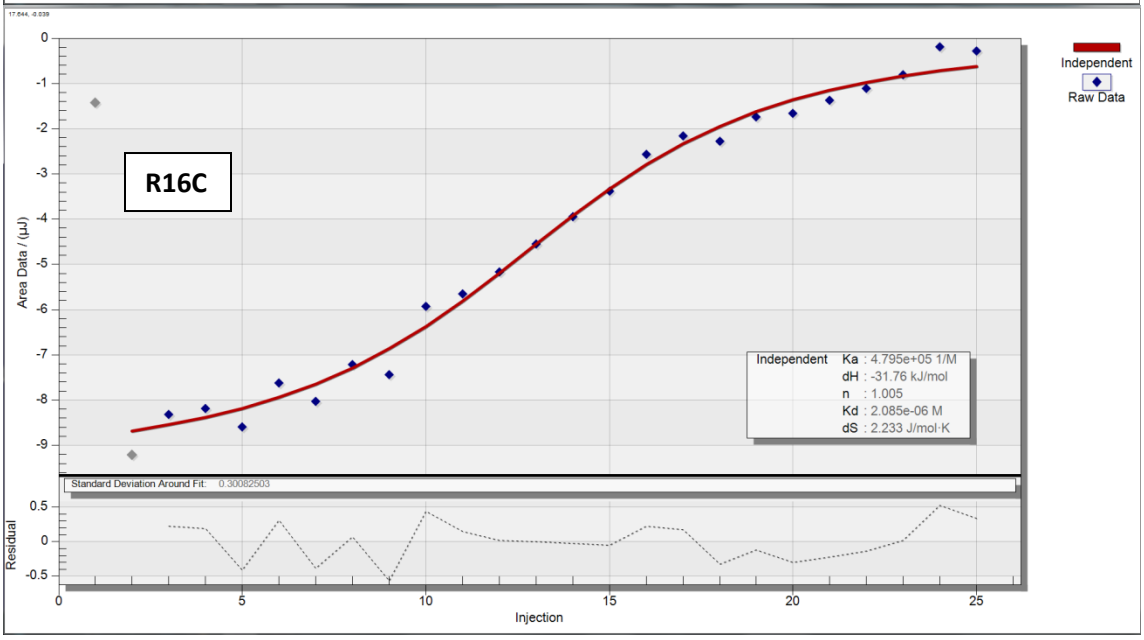
Figure S1 follows: integrated areas for ITC titrations of calmodulin into peptide solutions, with fits to independent (single-sigmoid) binding models. Full parameters calculated from these fits are then presented in Table S1.

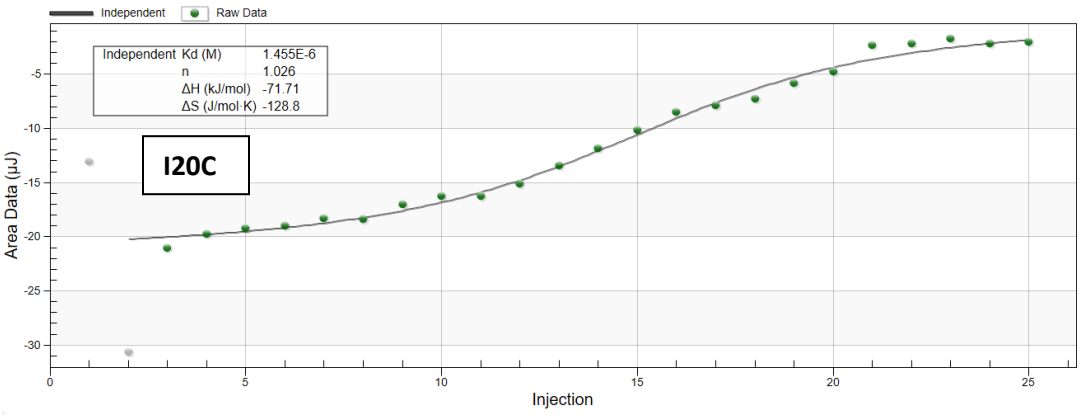

W4C
Independent
Raw Data
Area Data / (µJ)
Independent Ka : 3.038e+06 1/M
dH : -42.84 kJ/mol
n : 0.675
Kd : 3.292e-07 M
dS : -19.57 J/mol·K
Standard Deviation Around Fit: 0.46400422
Residual
Injection

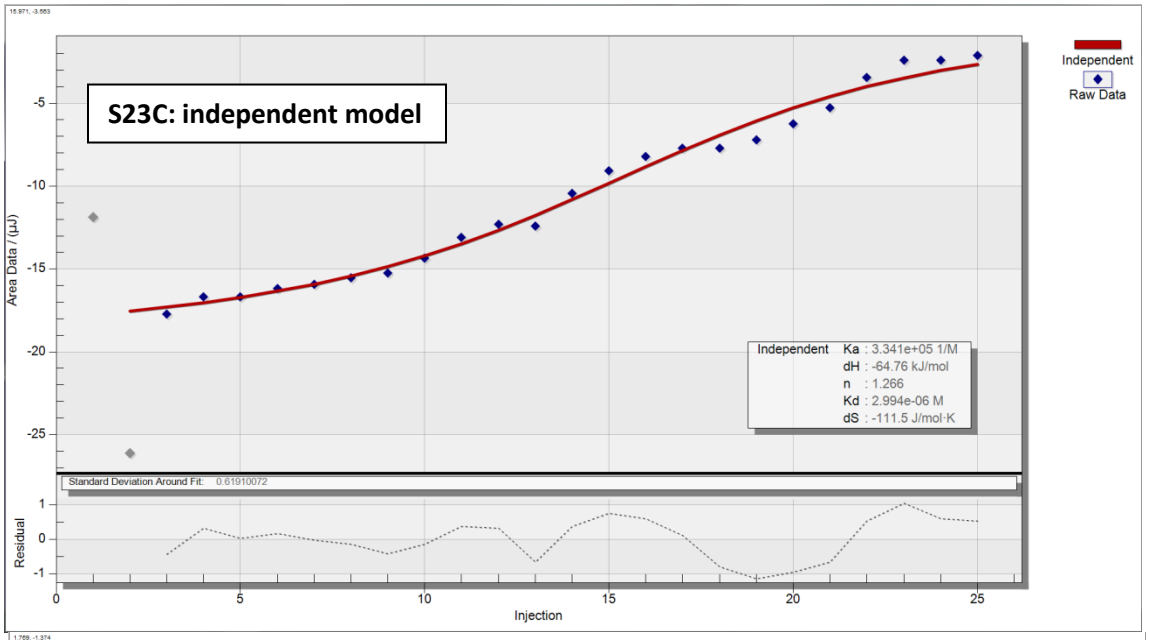

F8C: M13 into CaM
Multiple Sites
Raw Data
Area Data / (µJ)
Multiple Sites Ka1 : 8.710e+05 1/M
Ka2 : 5.590e+07 1/m
dH1 : -26.65 kJ/mol
dH2 : -25.54 kJ/mol
n1 : 0.940
n2 : 0.306
Kd1 : 1.148e-06 M
Kd2 : 1.789e-08 M
dS1 : 2.433e+01 J/mol·K
dS2 : 6.267e+01 J/mol·K
Standard Deviation Around Fit: 0.85259204
Residual
Injection

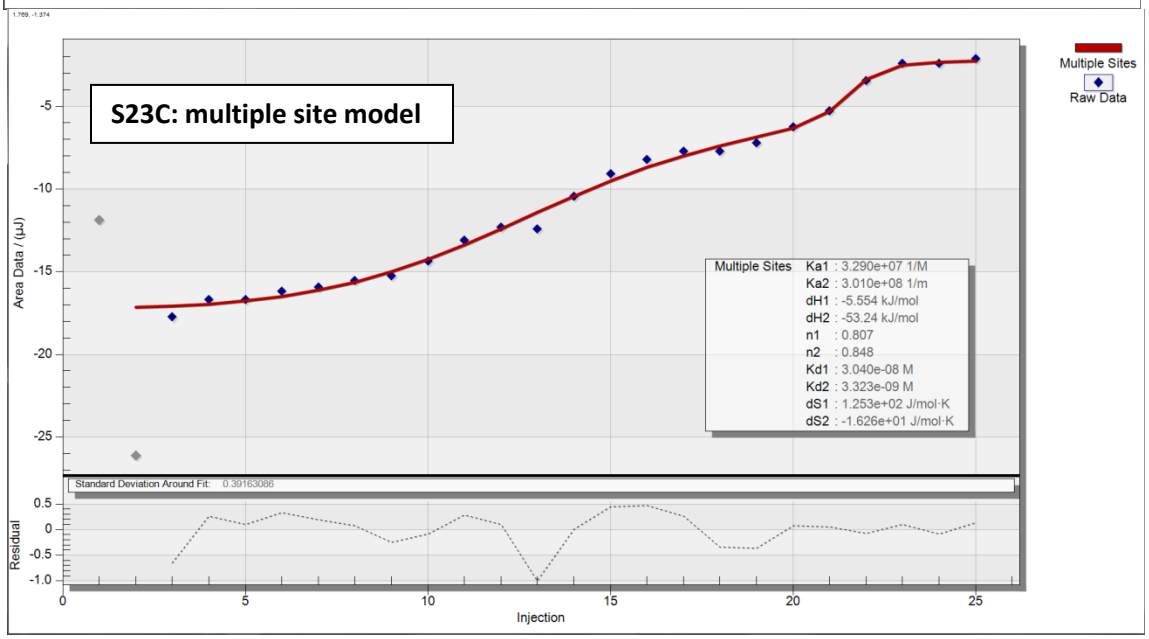

F8C: CaM into M13
Multiple Sites
Raw Data
Area Data / (µJ)
Multiple Sites Ka1 : 4.665e+05 1/M
Ka2 : 9.993e+08 1/m
dH1 : -29.01 kJ/mol
dH2 : -12.02 kJ/mol
n1 : 1.235
n2 : 0.220
Kd1 : 2.143e-06 M
Kd2 : 1.001e-09 M
dS1 : 1.121e+01 J/mol·K
dS2 : 1.320e+02 J/mol·K
Standard Deviation Around Fit: 1.2763886
Residual
Injection


S12C
Independent
Raw Data
Area Data / (µJ)
Independent Ka : 4.636e+05 1/M
dH : -64.15 kJ/mol
n : 0.972
Kd : 2.157e-06 M
dS : -106.7 J/mol·K
Standard Deviation Around Fit: 0.58087192
Residual
Injection


R16C
Independent
Raw Data
Area Data / (µJ)
Independent Ka : 4.795e+05 1/M
dH : -31.76 kJ/mol
n : 1.005
Kd : 2.085e-06 M
dS : 2.233 J/mol·K
Standard Deviation Around Fit: 0.30082503
Residual
Injection


Independent
Raw Data
Independent Kd (M) 1.455E-6
n 1.026
ΔH (kJ/mol) -71.71
ΔS (J/mol·K) -128.8
I20C
Area Data (µJ)
Injection


S23C: independent model
Independent
Raw Data
Area Data / (µJ)
Independent Ka : 3.341e+05 1/M
dH : -64.76 kJ/mol
n : 1.266
Kd : 2.994e-06 M
dS : -111.5 J/mol·K
Standard Deviation Around Fit: 0.61910072
Residual
Injection


S23C: multiple site model
Multiple Sites
Raw Data
Area Data / (µJ)
Multiple Sites Ka1 : 3.290e+07 1/M
Ka2 : 3.010e+08 1/m
dH1 : -5.554 kJ/mol
dH2 : -53.24 kJ/mol
n1 : 0.807
n2 : 0.848
Kd1 : 3.040e-08 M
Kd2 : 3.323e-09 M
dS1 : 1.253e+02 J/mol·K
dS2 : -1.626e+01 J/mol·K
Standard Deviation Around Fit: 0.39163086
Residual
Injection

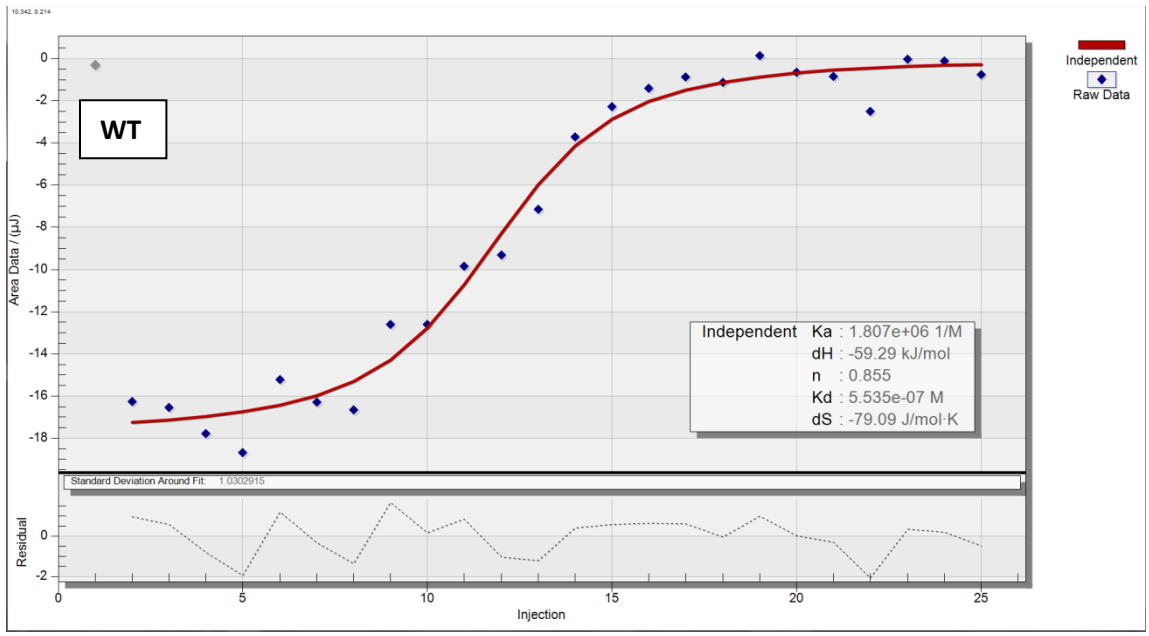

WT
Independent
Raw Data
Area Data / (µJ)
Independent Ka : 1.807e+06 1/M
dH : -59.29 kJ/mol
n : 0.855
Kd : 5.535e-07 M
dS : -79.09 J/mol·K
Standard Deviation Around Fit: 1.0302915
Residual
Injection

Table S1: binding parameters calculated from fits to the ITC titration curves.

variant	*n*	K_D	*ΔG* (kJ/mol)	*ΔH* (kJ/mol)	*ΔS* (J/mol K)	*ΔΔG* (kJ/mol)	*ΔΔH* (kJ/mol)	*ΔΔS* (J/mol K)
W4C*	0.9	3E-07	-37	-43	-20.	-1	16	59
F8C*	0.9	1E-06	-34	-27	24	2	33	100
S12C*	0.9	2E-06	-32	-65	-110	3	-5	-28
R16C*	1.0	2E-06	-32	-32	2.2	3	28	81
I20C*	1.1	1E-06	-33	-72	-130	2	-13	-51
S23C*	1.0	3E-06	-32	-65	-110	4	-5	-32
wt	0.9	5E-07	-36	-60.	-79			

2. **Infrared spectroscopy**

Table S2: the calculated lineshape parameters for each of the six C*-containing variants of M13, in buffer solution and with calmodulin. The standard deviation and FWHM were calculated as described in the experimental methods section.

Peptide	Buffer mode / cm^{-1}	CaM mode / cm^{-1}	Buffer mean / cm^{-1}	CaM mean / cm^{-1}	Buffer FWHM / cm^{-1}	CaM FWHM / cm^{-1}
W4C*	2164.6	2156.0	2163.2	2157.2	11.1	14.3
F8C*	2163.5	2157.7	2163.0	2155.6	12.2	13.3
S12C*	2163.5	2156.7	2162.8	2157.7	15.3	14.5
R16C*	2163.1	2161.2	2161.1	2159.8	14.8	19.2
I20C*	2163.5	2160.6	2161.6	2160.8	17.4	15.9
S23C*	2164.4	2163.5	2162.6	2161.6	14.6	15.3

3. **Supplementary data from molecular dynamics**

Angular distributions for orientation of probe side chains in MD simulations:

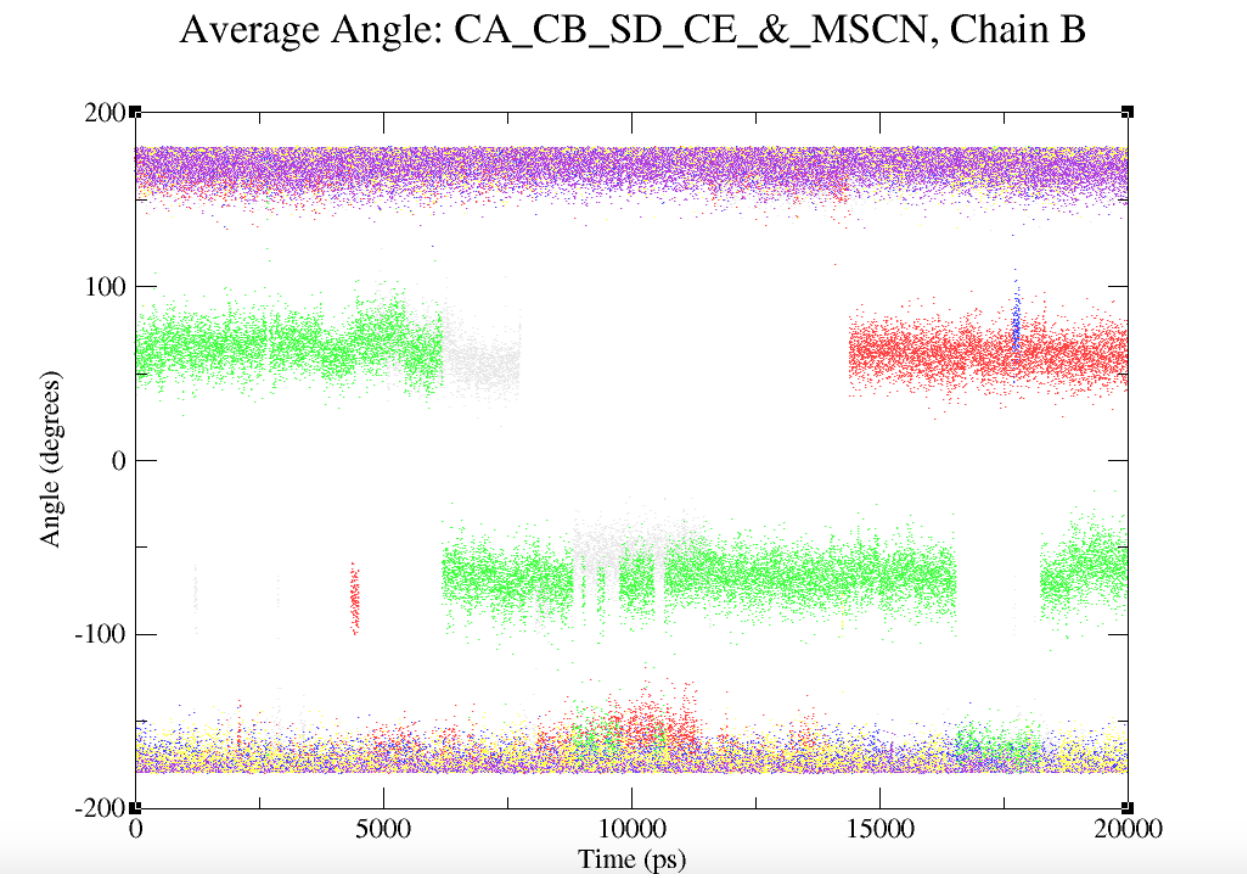


Figure S2. Dihedral angles (measured through Cα-Cβ-S-C for the cyanylated cysteine residue) throughout a 20 ns MD trajectory for labels at the six selected sites along the M13 peptide. (colors: yellow: W4C*; violet: F8C*; grey: S12C*; red: R16C*; green: I20C*; blue: S23C*) While the majority prefer a trans (180 degrees) configuration, a few sites in the middle region of M13 (S12C*, R16C*, I20C*) tend towards substantial sampling of gauche (60 or -60 degrees) configurations due to local steric restrictions. The dihedral angles, however, do not seem to correlate with either mean frequencies or linewidths. Fast (sub-ns) fluctuations of the SCN group orientation were in general *not* observed.

Distributions of solvent accessible surface area for each probe site:

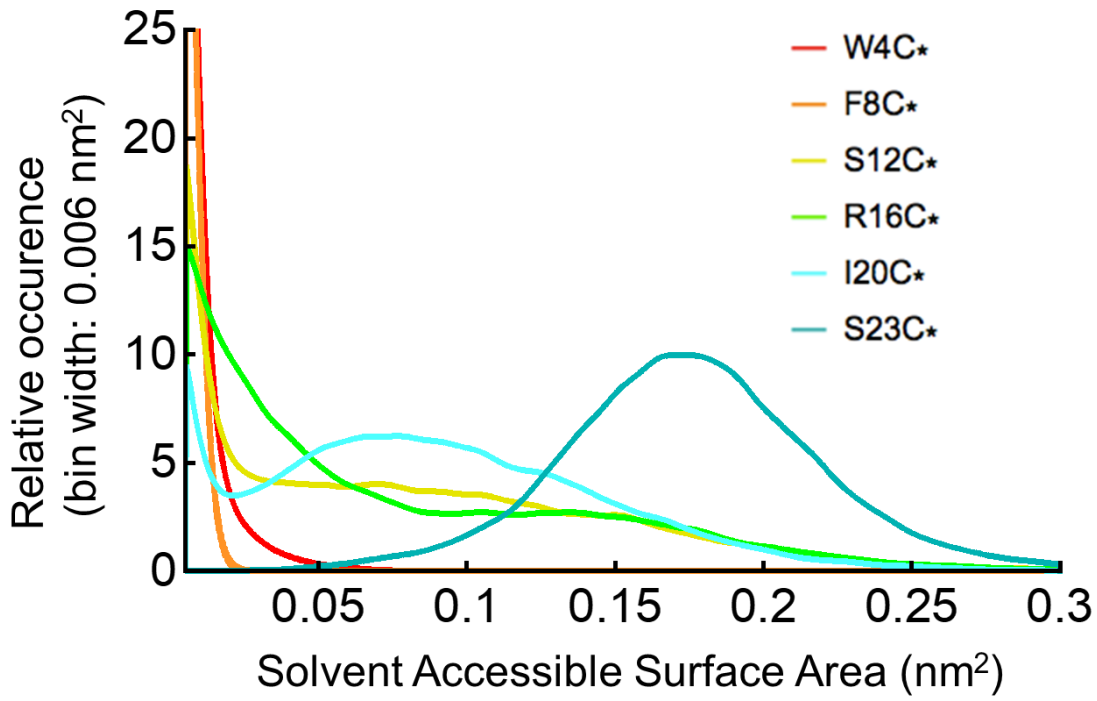


Figure S3. Histogram distributions for solvent accessible surface area for the SCN nitrogen atom calculated from the 20 ns MD trajectories, represented as lines.