Supplementary Figures

Figure S1. Monitoring CaM-Cx50$_{141-166}$ complex formation by mass spectroscopy. The MALDI-MS spectra of the free form of CaM (~17 kDa) and the complex form of CaM-Cx50$_{141-166}$ (~20 kDa). The inset showed the molecular weight of the Cx50$_{141-166}$ peptide.

Figure S2. Monitoring the interaction between Apo-CaM and Cx50 peptides by (^{1}H, ^{15}N)-HSQC spectroscopy. An overlay of HSQC spectra of apo-CaM (red) with the spectrum of the apo-CaM-Cx50$_{141-166}$ (green).

Figure S3. The dansyl fluorescence anisotropy. D-CaM (1 μM) was titrated with Cx50$_{141-166}$ in 100mM KCl, 5mM $CaCl_2$, 50mM Tris-HCl at pH 7.5. The fluorescence anisotropy were measured at λex = 335 nm and λem = 495 nm with an integration time of 20s. The inset shows the anisotropy change for D-CaM (free), and the complex of D-CaM-Cx50p (bound).

Figure S4. Comparison of the α family of connexins. An overlay of the HSQC spectra of holo-CaM (yellow), holo-CaM-Cx50p (green), and holo-CaM-Cx43p (violet). Arrows indicated that most signals shifted to the same direction due to identical binding mode, while rectangles highlighted some exceptions.

Figure S1

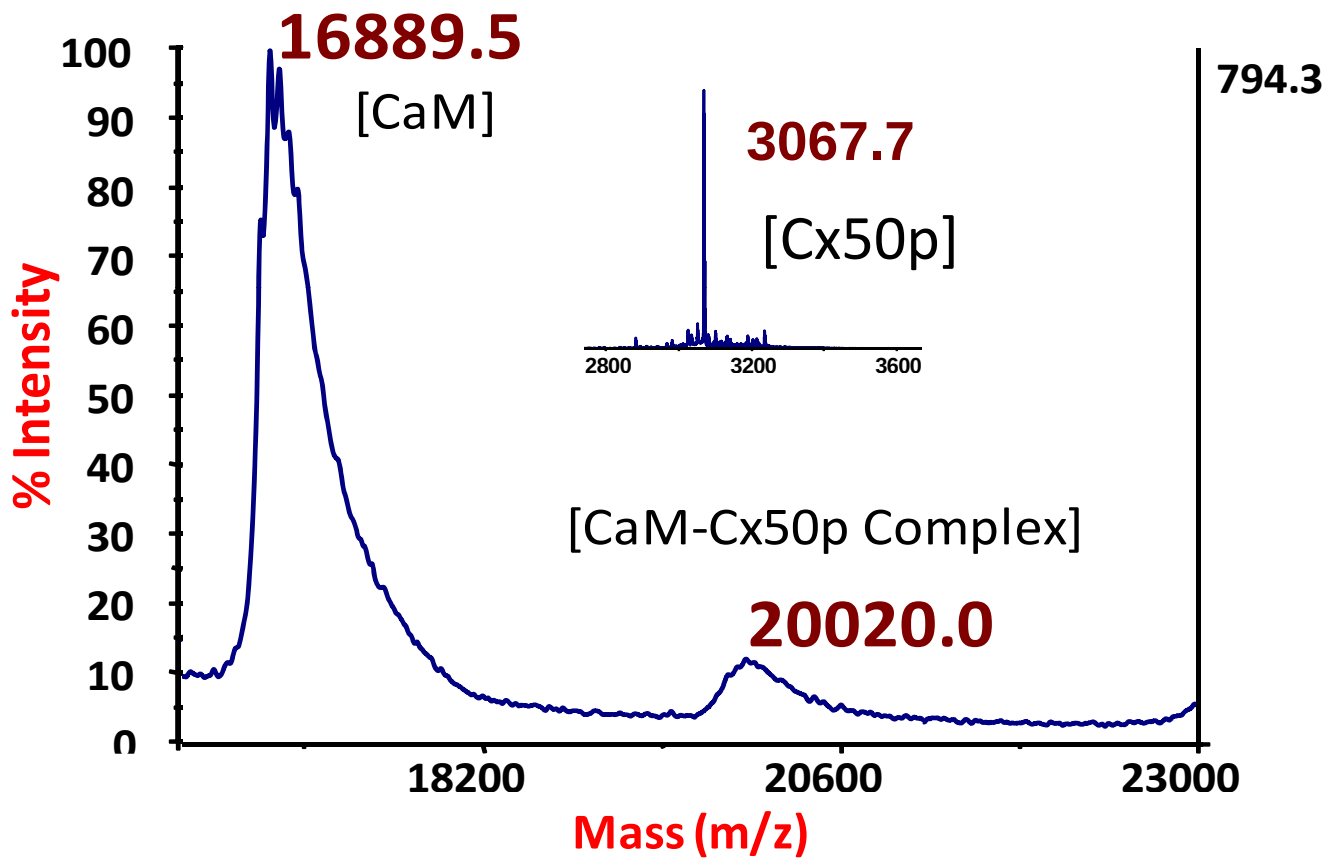

16889.5
[CaM]
3067.7
[Cx50p]
794.3
[CaM-Cx50p Complex]
20020.0
% Intensity
100
90
80
70
60
50
40
30
20
10
0
18200
20600
23000
Mass (m/z)
2800
3200
3600

Figure S2

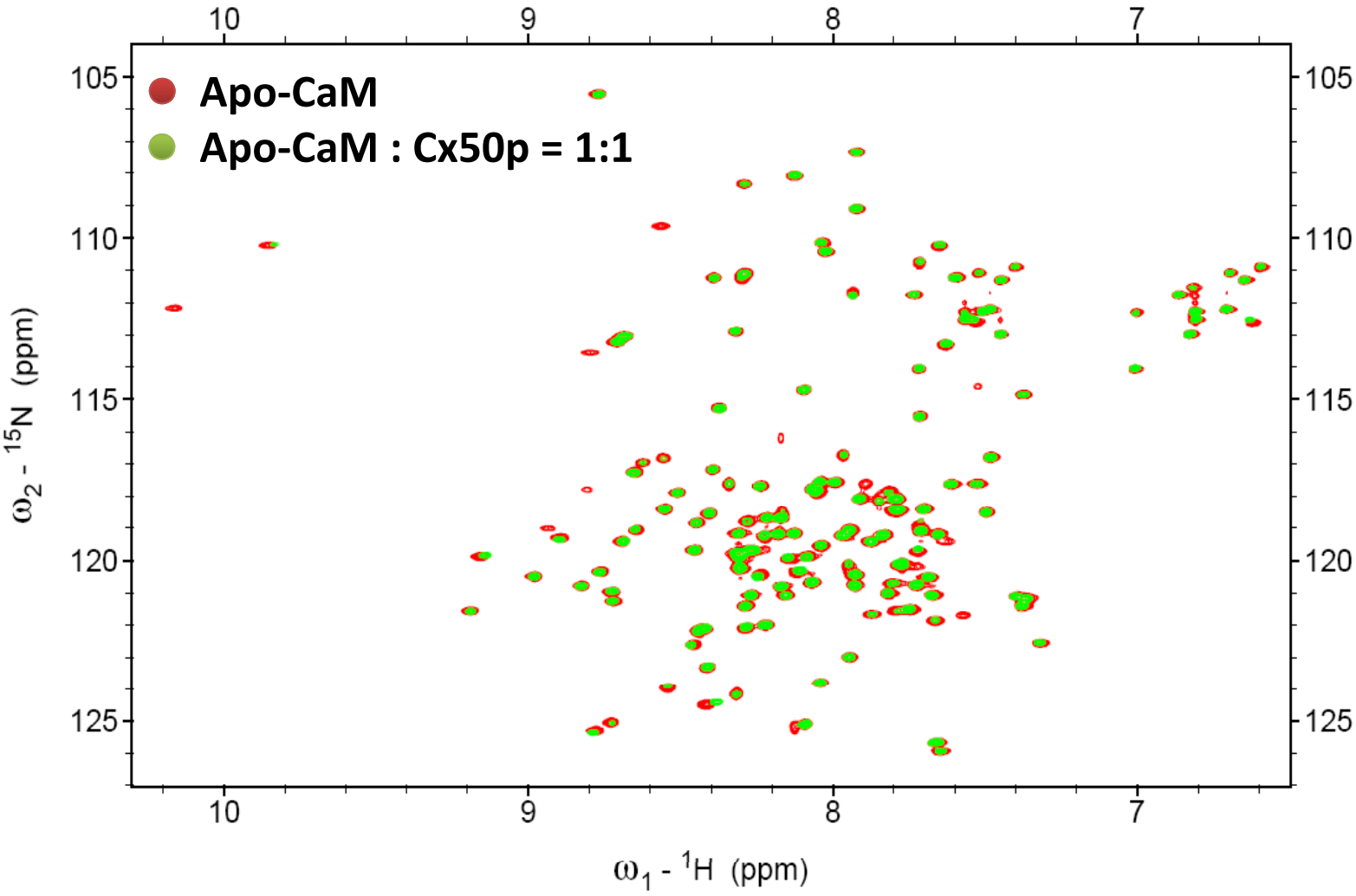

Apo-CaM
Apo-CaM : Cx50p = 1:1
10
9
8
7
105
110
115
120
125
ω2 - 15N (ppm)
ω1 - 1H (ppm)

Figure S3

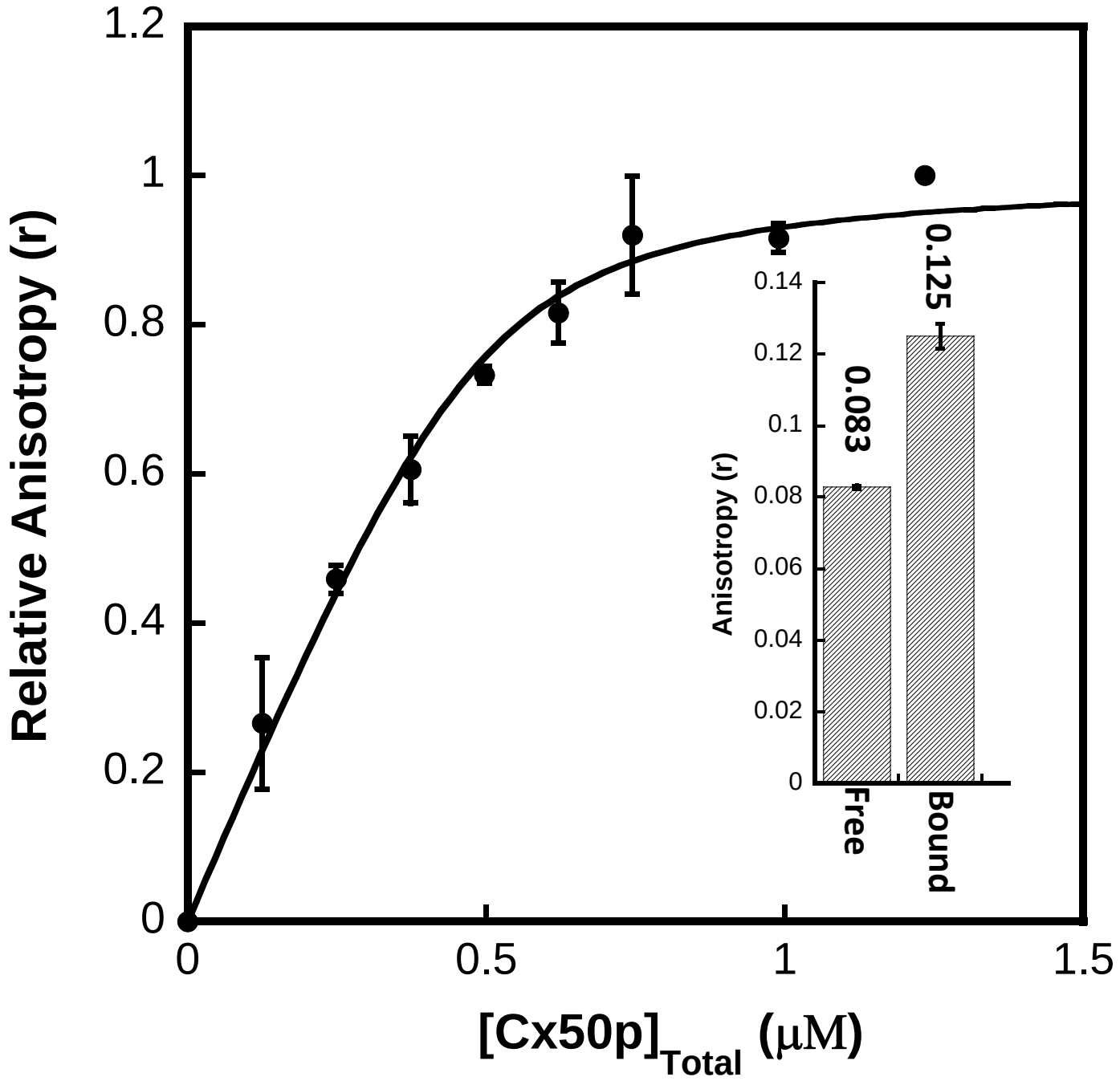
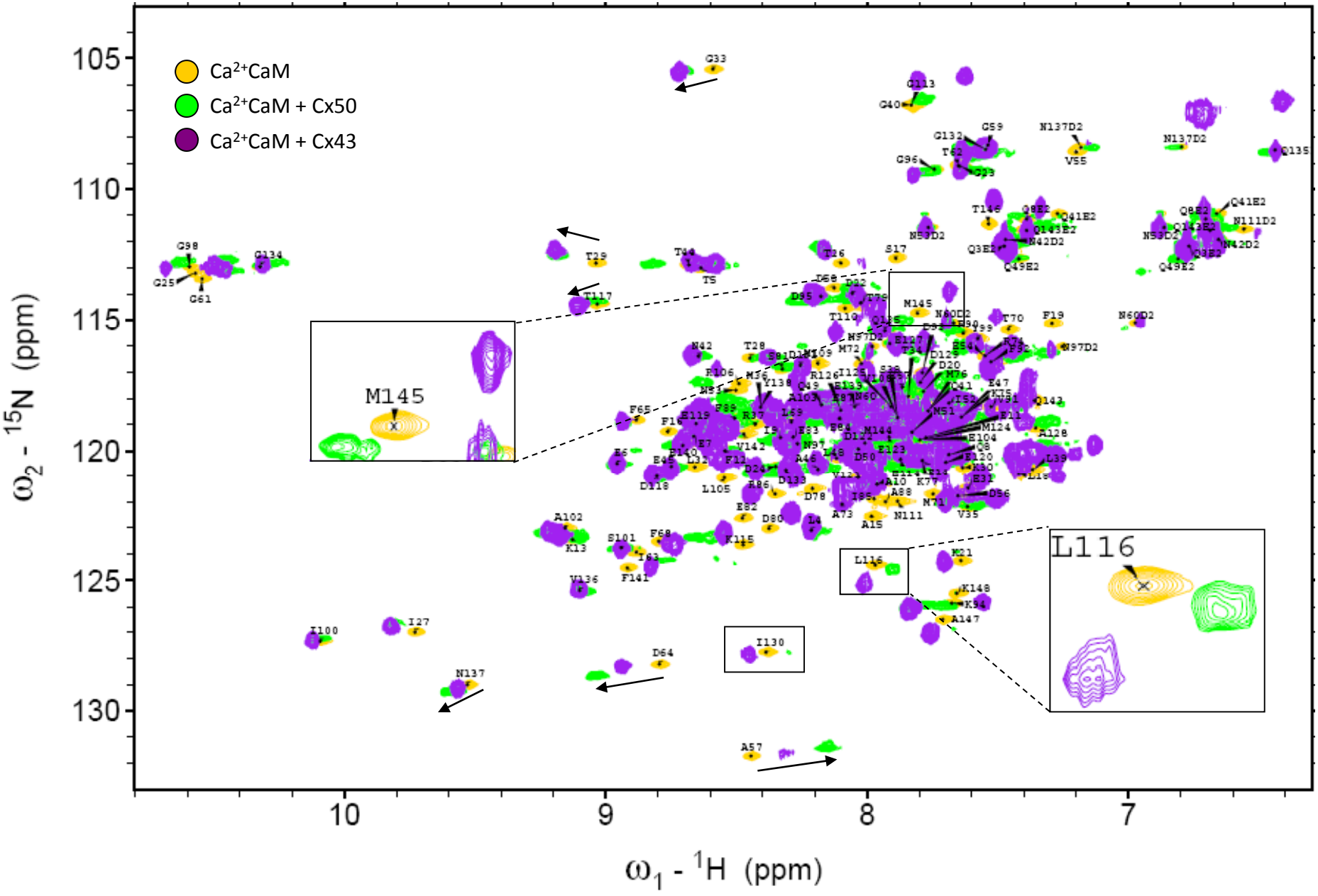

Relative Anisotropy (r)
1.2
1
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
[Cx50p]Total (μM)
Anisotropy (r)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0.083
0.125
Free
Bound

Figure S4


Ca2+CaM
Ca2+CaM + Cx50
Ca2+CaM + Cx43
M145
L116
105
110
115
120
125
130
10
9
8
7
ω2 - 15N (ppm)
ω1 - 1H (ppm)