Mobility capillary electrophoresis-native mass spectrometry reveals dynamic conformational equilibrium of calmodulin and its complexes

Supporting information

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Calculation of protein hydrodynamic radius

The hydrodynamic radius of a protein or protein complexes could be determined by the Stokes equation:

$$R_{\mathrm{h}} = \frac{4k_B T\sigma^2}{\pi\eta t_R R_c^2} \quad (1)$$

in which k_B is the Boltzmann constant, T is the working temperature in the capillary, R_c is the capillary inner diameter, and η is the viscosity coefficient of the buffer solution, t_R and σ^2 are the peak arrival time and temporal standard deviation of the protein after Gauss fit.

In MCE experience, the R_h was corrected as:

$$R_{\mathrm{h}} = \frac{R_{hn}D_n}{D} = \frac{R_{hn}t_p^3\sigma_r^2}{t_r^3\sigma_p^2} \quad (2)$$

where R_{hn} is the hydrodynamic radius of phenol, t_p and σ_p are the peak arrival time and temporal standard deviation of phenol after Gauss fit, t_r and σ_r are the peak arrival time and temporal standard deviation of protein and protein complexes after Gauss fit.

Calculation of protein ellipsoid radii

The average charge state could be calculated

$$Z_{\mathrm{av}} = \frac{\sum_n nI_n}{\sum_n I_n} \quad (3)$$

I_n is the signal intensity of the charge state n

Then SASA could be computed by

$$\ln(\mathrm{SASA}) = \frac{\ln(Z_{av}) + 4.1902}{0.7045} \quad (4)$$

The semi-major axis (a) was obtained from (5)

$$\mathrm{SASA} = 4\pi a^2 \quad (5)$$

The following formulas were used to calculated the b and c:

$$R_{\mathrm{h}}\text{-}r_{\mathrm{s}} = \frac{8c_1}{3} \times \frac{1}{\frac{2\emptyset_1}{\emptyset_1^2 - 1} + \frac{2\emptyset_1 - \emptyset_1^3}{(1-\emptyset_1^2)^{1.5}} ln(\frac{1+\sqrt{1-\emptyset_1^2}}{1-\sqrt{1-\emptyset_1^2}})} \quad (6)$$

$$R_h\text{-}r_s = \frac{8a}{3} \times \frac{1}{\frac{-\emptyset_2}{1-\emptyset_2^2} - \frac{2\emptyset_2^2 - 3}{(1-\emptyset_2^2)^{1.5}} Arcsin\sqrt{1-\emptyset_2^2)}} \quad (7)$$

$$c = \frac{\emptyset_1 c_1 + \emptyset_2 c_2}{\emptyset_1 + \emptyset_2} \quad (8)$$

$$V = \frac{4}{3}\pi(R_h - r_s) = \frac{4}{3}\pi abc \quad (9)$$

in which $\emptyset_1 = c_1/a$, $\emptyset_2 = c_2/a$, r_s is the thickness of the hydration layer.

Manhattan Distance Method

```
import pandas as pd
import numpy as np
df = pd.read_excel('stackChartData2.xlsx',header=None)
graph_num = 3
x_num = 5
color_num = 5
total_graph1=[]
for i in range(0,graph_num):
    graph_item = {}
    graph_item['id']=i+1
    for j in range(0,color_num):
        x_axis = np.arange(0,x_num,1)
        y_axis = df.iloc[0:x_num,2+i*7+j]
        computeList = []
        computeList.extend(y_axis)
        color_name = 'color'+str(j+1)
        graph_item[color_name] = computeList
    total_graph1.append(graph_item)

for i in range(0,graph_num):
    graph1 = total_graph1[i]
    for j in range(1+i,graph_num):
        graph2 = total_graph1[j]
        scoreSum = 0
        for k in range(0,color_num):
            color_name = 'color'+str(k+1)
            a = graph1[color_name]
            b = graph2[color_name]
            c = [a[pp]-b[pp] for pp in range(0,x_num)]
            hh=0
            for cc in c:
                hh = hh +abs(cc)
            scoreSum = scoreSum +hh
        print('Graph'+str(i+1)+'-' +'Graph'+str(j+1)+' score: '+str(scoreSum))
        scoreSum = 0
```

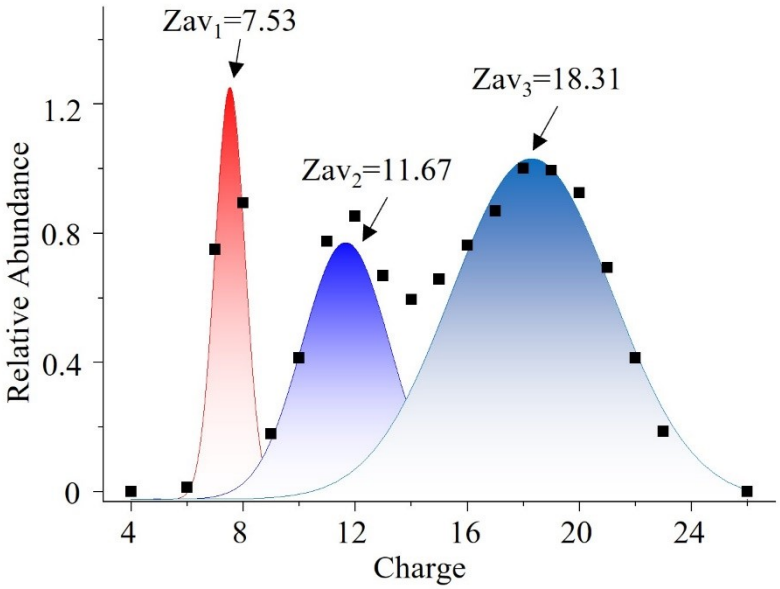

Figure S1. Gaussian-Fitting of apo-CaM mass spectrum. The black square represented the relative abundance of apo-CaM at different charge states, and the Gauss peaks from left to right corresponded to Group1, Group2, and Group3, respectively.

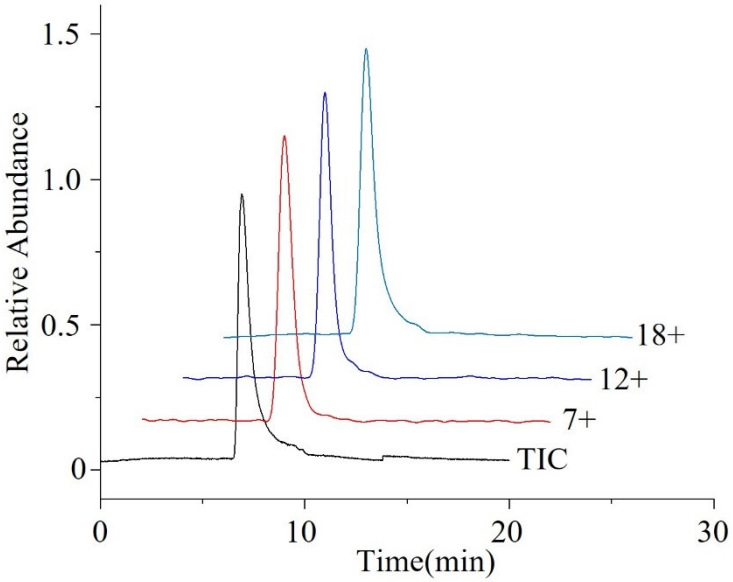

Figure S2. Ion flow diagram from size exclusion chromatography (SEC). (Bottom: Total ion chromatogram (TIC), followed by extracted ion chromatograms (EIC) for charge states 7+, 12+, and 18+, respectively.)

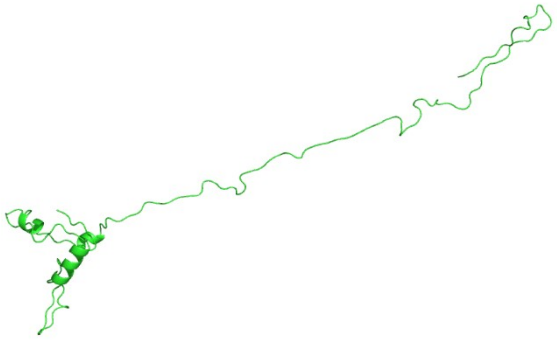

Figure S3. MD simulation of further unfolding structures of CaM during the ESI process [1].

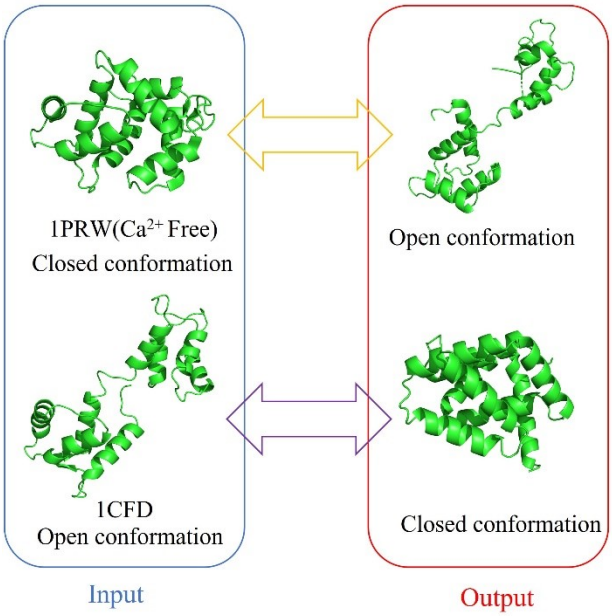

Figure S4. Str2str results of Apo-CaM interconverting between the closed and open conformations.

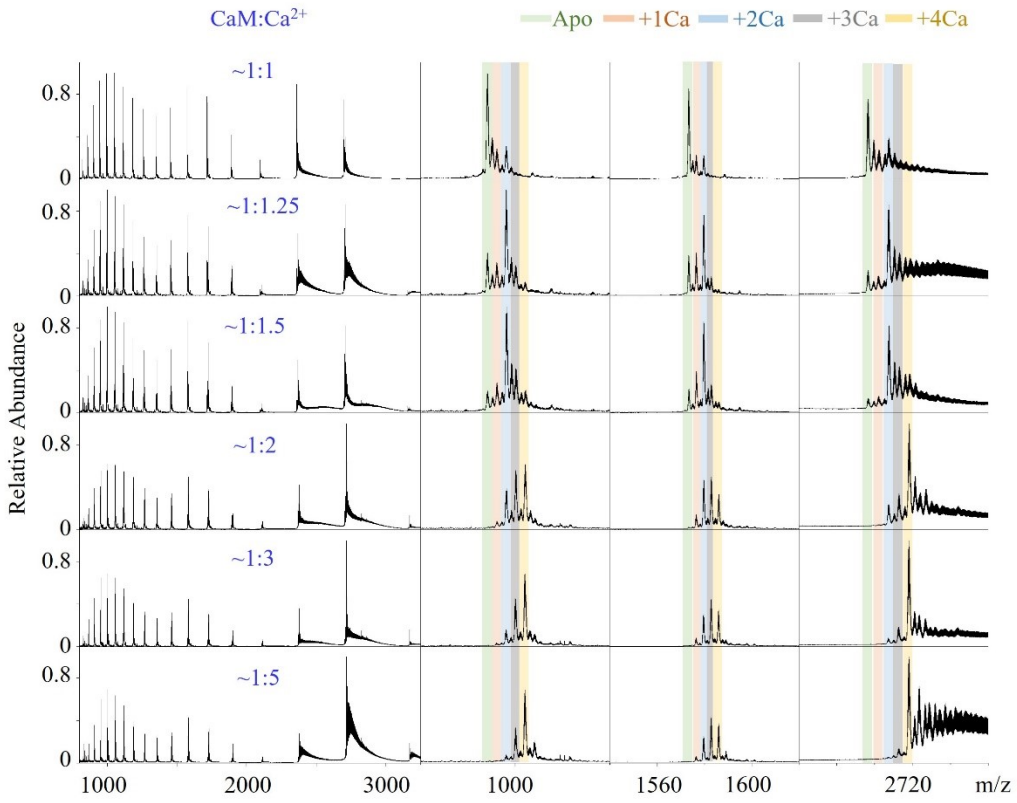

Figure S5. nMS spectrum of CaM at different CaM:Ca^{2+} ratios: 1:1, 1:1.25, 1:1.5, 1:2, 1:3 and 1:5.

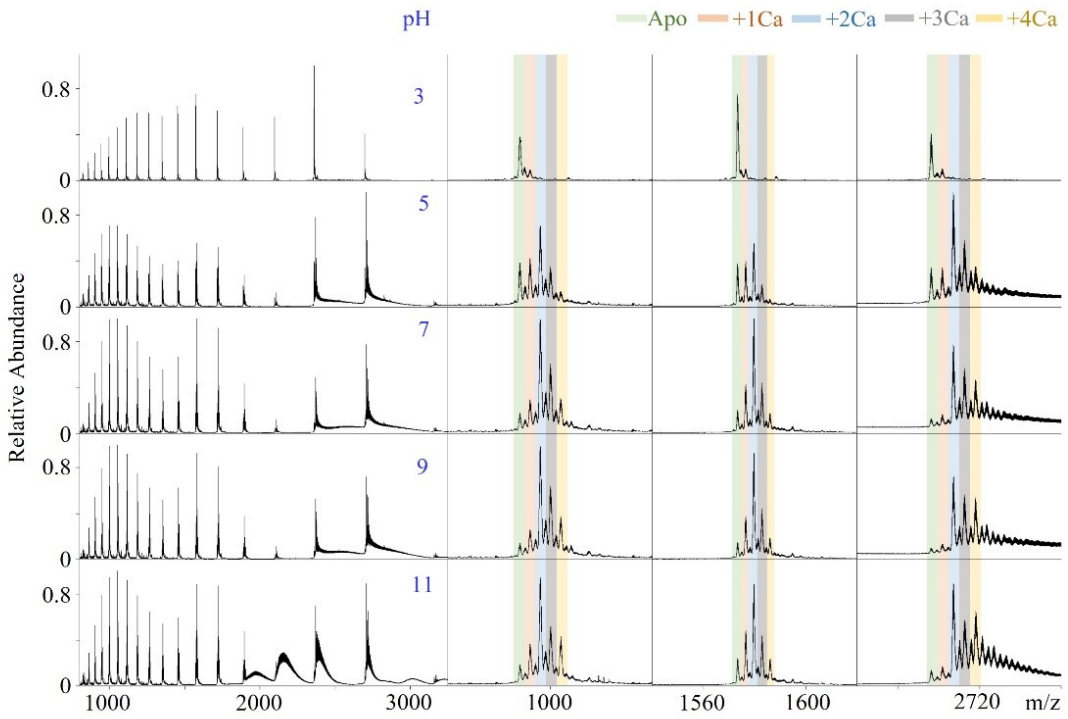

Figure S6. nMS spectrum of CaM at different pH(CaM:Ca^{2+} ratio was 1:1.5).

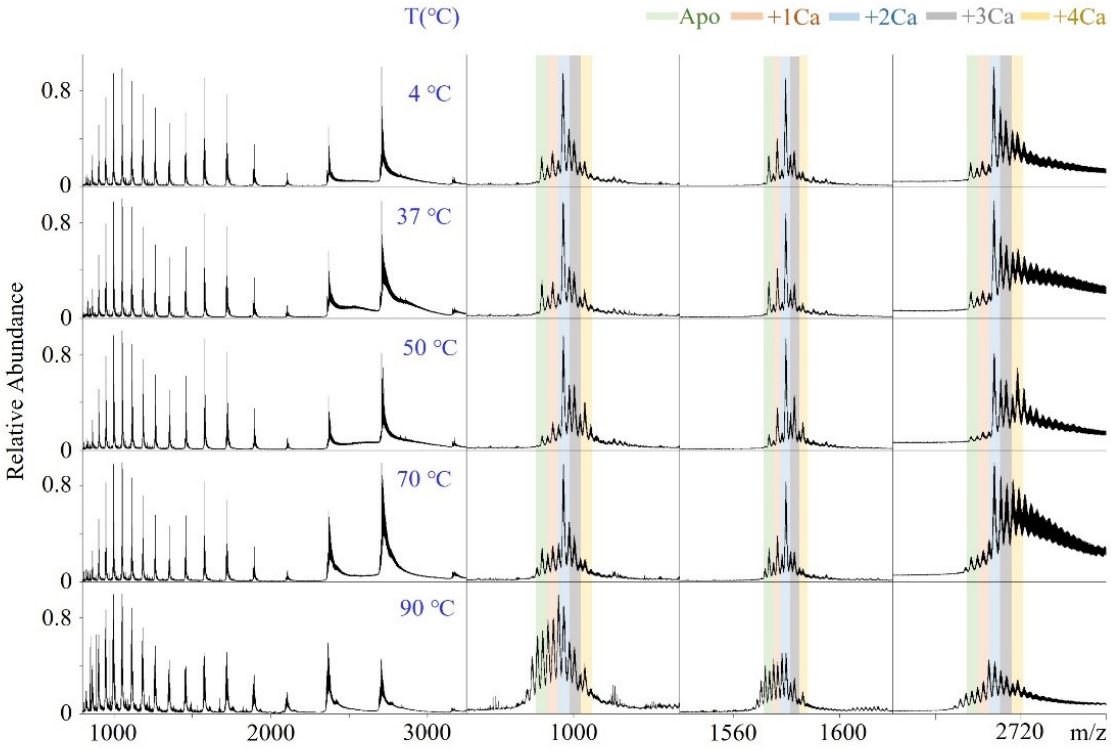

Figure S7. nMS spectrum of CaM at different temperature (CaM:Ca^{2+} ratio was 1:1.5).

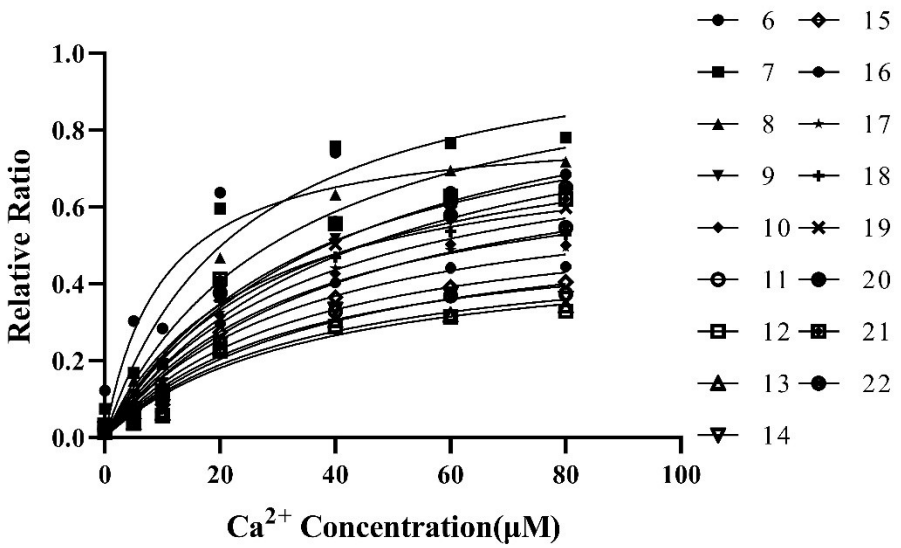

Figure S8. Cumulative K_d fitting curves of different charge states(The fitting equation is given by $y_m = \frac{B_{max} \times x}{K_d + x}$, where B_{max} denotes the maximum binding capacity, and K_d representes the dissociation constant. The term y_m is calculated as

$$y_m = \frac{S_{4Ca^{2+}-CaM}}{\sum_{l=0}^{4} S_{nCa^{2+}-CaM}}$$, where $S_{nCa^{2+}-CaM}$ signifies the absolute abundance of the nCa^{2+}-CaM at charge state m).

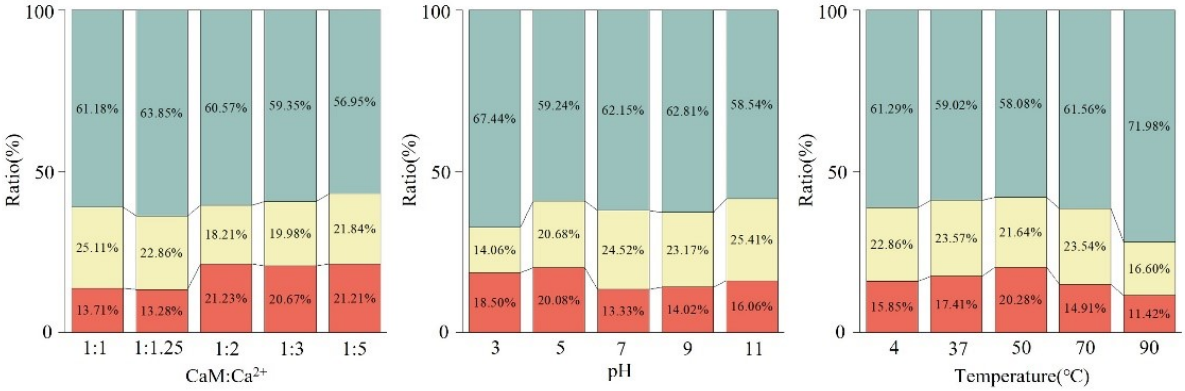

Figure S9. The proportion of three groups of CaM under different conditions. The red, yellow and blue sections represented group 1, group 2 and group 3, respectively.

Table S1 Ellipsoids radii of group 1 and group 2

Ellipsoids radii (Å)	*a*	*b*	*c*
Charge group 1	23	24	22
1CFD	24	21	19
Charge group 2	32	27	14
1PRW	35	22	16
$4Ca^{2+}$-CaM-Mel	28	23	19
8AHS	26	21	21

Table S2 Relative ratio(%) of nCa^{2+}-CaM at different Ca^{2+} concentration of charge group 1

	1:1	1:1.5	1:2	1:3	1:5
$0Ca^{2+}$-CaM	44.38	8.91	2.53	2.12	3.33
$1Ca^{2+}$-CaM	17.44	9.53	3.62	2.45	3.92
$2Ca^{2+}$-CaM	20.55	42.10	17.03	7.59	4.65
$3Ca^{2+}$-CaM	9.67	22.01	22.41	16.63	11.47
$4Ca^{2+}$-CaM	7.96	17.45	54.42	71.20	76.63

Table S3 Relative ratio(%) of nCa^{2+}-CaM at different Ca^{2+} concentration of charge group 2

	1:1	1:1.5	1:2	1:3	1:5
$0Ca^{2+}$-CaM	66.90	12.10	1.55	1.15	2.74
$1Ca^{2+}$-CaM	15.18	19.66	8.96	6.53	4.94
$2Ca^{2+}$-CaM	15.50	46.85	29.96	19.77	14.90
$3Ca^{2+}$-CaM	2.42	13.75	30.07	33.10	32.00
$4Ca^{2+}$-CaM	0.00	7.64	29.45	39.45	45.40

Table S4 Relative ratio(%) of nCa^{2+}-CaM at different Ca^{2+} concentration of charge

group 3

	1:1	1:1.5	1:2	1:3	1:5
$0Ca^{2+}$-CaM	58.19	11.14	2.66	1.94	2.59
$1Ca^{2+}$-CaM	16.99	13.62	5.68	3.01	4.44
$2Ca^{2+}$-CaM	17.32	45.71	25.39	15.61	11.94
$3Ca^{2+}$-CaM	4.46	19.37	33.37	34.50	31.04
$4Ca^{2+}$-CaM	3.05	10.17	32.91	44.95	49.99

Table S5 Relative ratio(%) of nCa^{2+}-CaM at different pH of charge group 1

	3	5	7	9	11
$0Ca^{2+}$-CaM	77.98	14.79	6..67	5.44	6.36
$1Ca^{2+}$-CaM	15.23	15.01	8.93	7.84	9.36
$2Ca^{2+}$-CaM	3.98	36.91	36.93	35.39	35.73
$3Ca^{2+}$-CaM	1.66	20.73	26.66	27.54	24.13
$4Ca^{2+}$-CaM	1.16	12.56	20.81	23.78	24.42

Table S6 Relative ratio(%) of nCa^{2+}-CaM at different pH of charge group 2

	3	5	7	9	11
$0Ca^{2+}$-CaM	70.03	25.21	9.57	6.91	10.99
$1Ca^{2+}$-CaM	13.50	23.21	17.83	16.28	18.02
$2Ca^{2+}$-CaM	14.76	34.97	44.36	44.90	37.60
$3Ca^{2+}$-CaM	1.20	11.77	19.23	20.48	18.54
$4Ca^{2+}$-CaM	0.51	4.84	9.01	11.44	14.84

Table S7 Relative ratio(%) of nCa^{2+}-CaM at different pH of charge group 3

	3	5	7	9	11
$0Ca^{2+}$-CaM	80.00	19.94	8.05	6.60	8.19
$1Ca^{2+}$-CaM	15.22	21.05	13.18	12.06	17.06
$2Ca^{2+}$-CaM	0.78	35.11	41.67	40.41	39.93

$3Ca^{2+}$-CaM	2.09	16.77	24.78	26.69	20.45
$4Ca^{2+}$-CaM	1.91	7.13	12.32	14.24	14.37

Table S8 Relative ratio(%) of nCa^{2+}-CaM at different temperature of charge group 1

	4	37	50	70	90
$0Ca^{2+}$-CaM	9.33	10.01	5.50	8.23	14.66
$1Ca^{2+}$-CaM	9.85	10.05	7.48	9.70	18.89
$2Ca^{2+}$-CaM	40.18	36.84	35.32	33.31	28.54
$3Ca^{2+}$-CaM	22.54	22.39	24.87	25.80	20.60
$4Ca^{2+}$-CaM	18.10	20.71	26.83	22.96	17.30

Table S9 Relative ratio(%) of nCa^{2+}-CaM at different temperature of charge group 2

	4	37	50	70	90
$0Ca^{2+}$-CaM	14.15	16.48	6.79	15.83	21.18
$1Ca^{2+}$-CaM	18.85	18.92	15.50	17.99	25.64
$2Ca^{2+}$-CaM	44.78	42.68	44.11	45.45	27.13
$3Ca^{2+}$-CaM	14.38	13.62	20.19	13.32	14.98
$4Ca^{2+}$-CaM	7.84	8.30	13.41	7.41	11.07

Table S10 Relative ratio(%) of nCa^{2+}-CaM at different temperature of charge group 3

	4	37	50	70	90
$0Ca^{2+}$-CaM	12.59	14.32	6.04	13.99	20.42
$1Ca^{2+}$-CaM	15.06	14.32	10.87	15.33	23.38
$2Ca^{2+}$-CaM	43.52	43.98	41.89	44.00	27.33
$3Ca^{2+}$-CaM	19.02	17.59	24.87	17.86	17.39
$4Ca^{2+}$-CaM	9.82	9.79	16.33	8.82	11.48

Table S11 Dissociation constants (K_d) of CaM at different charge states

Charge	6	7	8	9	10	11	12	13	14
K_d(μM)	9.88	23.62	31.76	25.08	40.83	37.80	35.46	34.35	32.20

Charge	15	16	17	18	19	20	21	22	
K_d(μM)	32.89	33.14	35.43	35.63	43.37	41.53	35.74	31.83	

Reference

1.Luan, M.; Hou, Z.; Zhang, B.; Ma, L.; Yuan, S.; Liu, Y.; Huang, G., Inter-Domain Repulsion of Dumbbell-Shaped Calmodulin during Electrospray Ionization Revealed by Molecular Dynamics Simulations. *Analytical chemistry* **2023,** *95* (23), 8798-8806.