Methods

Expression and purification of GCAP1 – The full-length cDNA clone for chicken GCAP1 used here was previously described (Semple-Rowland et al., 1996). A Gly→Ser mutation at position 6 was introduced to promote high affinity interaction with yeast N-myristoyl transferase (NMT) (Duronio et al., 1991). GCAP1 cDNA was inserted into pFastBac HT vector (Invitrogen, Carlsbad, CA) to generate pFBcGCAP1 with GCAP1 flanked by the following sequences:"...[ATG]TCGTACTAC[CATCACCATCACCATCAC]GATTACGATATCC CAACGACC[GAAAACCTGTATTTTCAG**GGC]AACATGGAT~GAGGCTGCCCA G**[TAA][GCGGCCGC]..." where the initiation codon, the 6×His-tag coding sequence, the tobacco etch virus (TEV) recognition site coding sequence, the stop codon and the *NotI* recognition site are sequentially shown in brackets. The GCAP1 coding sequence is shown in bold letters. The expressed recombinant GCAP1 sequence (Supplementary Material, Fig. S5A) shows the 6×His-tag in orange and the blue arrow indicates the TEV cleavage site. pFBcGCAP1 was used to generate GCAP1 encoding bacmid via transposition to a bacmid acceptor in the DH10Bac bacterial strain following instructions in Invitrogen's Bac-to-Bac manual. Bacmid, isolated from 250 ml of overnight culture with the PrepEase Bac purification kit (USB Corp., Cleveland, OH), was used to generate baculovirus in Sf9 cells as described in Invitrogen's Bac-to-Bac manual. GCAP1 was expressed in High-Five insect cells grown in suspension in Express Five SFM media supplemented with 90 mL/L of 200 mM L-glutamine. The culture was propagated at 27 °C in 2 L glass baffled flasks shaken at 120 rotations/min.

Just before the addition of baculovirus, High-Five cells were diluted about twofold with fresh media to a density of 2×10^6 cells/mL. Expression was initiated by the addition of 1 mL of baculovirus (passage 2) per 100 mL of culture. Infected cells were collected 50 h later by a 10 min centrifugation in 50 mL conical tubes at 300*g*. Pellets were resuspended in 3 mL of 50 mM HEPES, pH 7.0, 40 mM NaCl, and 360 mM KCl. One tablet of EDTA-free protease inhibitors (Roche, Mannheim, Germany) was added to each 50 mL of buffer. Samples were frozen in liquid nitrogen and stored at -80 °C. To prepare clarified cell lysates for column purification, cell pellets from 250 mL of culture were thawed and sonicated three times for 20 s by a Sonifier Model 150D (Branson, Danbury, CT) with the power-level set to 5, and each sonication was followed by 1 min incubation on ice. The homogenate was centrifuged at 25,000*g* for 30 min at 4 °C to remove crude cell debris. Next, the supernatant was ultracentrifuged at 100,000*g* for 30 min followed by another centrifugation for 1 h at 4 °C to remove cell membranes. The column (7.5 mm diameter) packed with 2.5 mL of TALON metal affinity resin (Clontech, Mountain View, CA) was equilibrated with 10 resin volumes of 50 mM HEPES, pH 7.0, 40 mM NaCl, and 360 mM KCl. After sample loading, it was washed with 20 resin volumes of the same buffer containing 10 mM imidazole. Elution of purified GCAP1 was achieved with 50 mM HEPES, pH 7.4, 360 mM KCl, 40 mM NaCl, and 200 mM imidazole. Protein-containing 1 mL fractions, identified with the Bio-Rad protein assay (Bio-Rad, Hercules, CA), were combined. TEV protease was added at a molar ratio of 1:50 (TEV:GCAP1) to cleave off the 6×His-tag and the mixture was incubated for 24-48 h at 4 °C. SDS-PAGE was used to verify complete removal of the tag. Finally, the buffer was changed to 50 mM HEPES, pH 7.4, 90 mM KCl, and 10 mM NaCl, with 10 ml Zeba

Desalt Spin Columns (Thermo Scientific, Rockford, IL) following manufacturer's instructions. GCAP1 was snap frozen in liquid nitrogen and stored at -80 °C until further use. A typical yield from 250 mL of insect culture was 10 mg at >95% purity. Activity of GCAP1 was assayed at high and low Ca^{2+} concentrations (Fig. 6A). Mass spectrometry of the intact protein was employed to assess its purity (Fig. 6B). Charged states, i.e. $(GCAP1+12H)^{12+}$ to $(GCAP1+12H)^{28+}$, were used to calculate the deconvoluted mass (Fig. 6C) as previously described (Zhang and Marshall, 1998).

Expression and purification of Saccharomyces cerevisiae N-myristoyl-transferase (NMT) – The 6×His-tag-NMT cDNA clone in pBB131 plasmid was a kind gift of Dr. Jeffrey I. Gordon (Washington University, St. Louis, MO). Because of our preference for kanamycin selection in bacteria we have transferred the 6×His-tag-NMT coding sequence to the pET30B (Novagen, Madison, WI). The resulting pET30b-NMT expression plasmid was transformed into the Rosetta 2(DE3)pLysS bacteria (Novagen, Madison, WI) and the plasmid-bearing colonies were selected on LB-agar plates with 50 μg/mL kanamycin and 34 μg/mL chloramphenicol. A single colony was used to inoculate 250 mL liquid culture, which was carried out in Luria-Bertani (LB) medium (USB Corp., Cleveland, OH) with 50 μg/mL kanamycin and 34 μg/mL chloramphenicol, and was incubated overnight at 37 °C in a shaking incubator. The next day the starter culture was diluted to 6 L with the same media and antibiotics as above and grown under the same conditions until optical density at 600 nm (OD_{600}) reached 0.25. At that point the incubation temperature was lowered to 30 °C. After the OD_{600} reached the value of 0.5, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM. The culture

was incubated in a shaker at 30 °C for another 6 h, after which the cells were harvested by 20 min centrifugation at 7,000*g*. These expression conditions resulted in soluble NMT. All buffers and centrifuges used in the following steps were chilled to 4 °C. To prepare clarified cell lysate suitable for column purification the combined pellets were resuspended in 60 mL of 50 mM HEPES pH 7.0, 360 mM KCl, 40 mM NaCl. The suspension was divided between two tubes and sonicated four times for 20 s with a sonifier model 150D (Branson, Danbury, CT) set at a power-level of 7. To remove crude cellular debris the samples were centrifuged at 25,000*g* for 30 min and the supernatant collected was first centrifuged at 100,000*g* for 30 min followed by a second centrifugation at 100,000*g* for 1 h to remove cell membranes. In the meantime a column (15 mm diameter) was packed with 4 mL (~8 mL of slurry) of TALON metal affinity resin (Clontech, Mountain View, CA) and equilibrated with 40 mL of 50 mM HEPES, pH 7.0, 360 mM KCl, 40 mM NaCl. The cleared cell lysate was loaded at a flow rate of ~1 mL/min and the column was washed with 80 mL of 50 mM HEPES, pH 7.0, 360 mM KCl, 40 mM NaCl, 10 mM imidazole at a flow rate of ~2 mL/min. Purified NMT was eluted at a flow rate of 1 mL/min with 20 mL of 50 mM HEPES, pH 7.4, 360 mM KCl, 40 mM NaCl, 200 mM imidazole in 1 mL fractions. Fractions containing protein as identified with the Bio-Rad protein assay (Bio-Rad, Hercules, CA) were combined. Next, the buffer was exchanged with 50 mM HEPES, pH 7.4, 90 mM KCl, 10 mM NaCl using 10 mL Zeba Desalt Spin Columns (Pierce Biotechnology, Rockford, IL) following the producer's instructions. The sample was then concentrated to ~1.75 mL (50 mg/mL) by Centriprep YM-10 (Millipore, Billerica, MA) following the producer's instructions at 4

°C. The purity of NMT was assessed by SDS-PAGE (Fig. S5, panel B). The purified NMT was aliquoted, frozen by immersion in liquid nitrogen and stored at -80 °C.

REFERENCES

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Table S1. Deuterium uptake rates of Ca^{2+}-bound GCAP1. Deuterium uptake was calculated as described in the methods section "*Amide hydrogen-deuterium exchange analysis and representation*". The raw data were fitted to equation 2**.**

ID	m/z	z	Sequence	A_1(Da)	k_1(min^{-1})	A_2(Da)	k_2(min^{-1})
1	722.3	1+	^{2}GNMDSKA.V	2.6	3.2	0.5	0.10
2	966.7	2+	^{20}WYKKFMTECPSGQLTL	5.3	2.0	1.7	0.10
3	1179.4	1+	^{25}MTECPSGQLTL	4.8	3.0	1.5	0.10
4	861.3	1+	^{36}YEFKQF	0.8	1.0	0.6	0.01
5	963.8	2+	^{42}FGLKNLSPSANKYVEQM	5.5	1.5	1.5	0.01
6	879.6	2+	^{59}FETFDFNKDGYIDF	0.8	8.0	2.2	0.03
7	805.3	1+	^{59}FETFDF	0.5	10.0	0.5	0.01
8	617.5	2+	^{63}DFNKDGYIDF	0.9	1.5	0.6	0.02
9	971.4	1+	^{65}NKDGYIDF	1.6	5.8	1.6	0.01
10	796.3	1+	^{73}MEYVAAL	0.3	10.0	0.6	0.01
11	544.3	1+	^{79}LSLVL	0.7	1.5	0.01	0.01
12	763.6	2+	^{98}YDVDGNGCIDRGEL	1.5	6.0	1.0	0.60
13	671.4	1+	^{112}LNIIKA	0.5	6.0	1.2	0.02
14	871.3	2+	^{112}LNIIKAIRAINRCNE	5.4	2.2	1.4	0.01
15	545.0	2+	^{118}IRAINRCNE	4.9	3.4	0.2	0.00
16	818.1	2+	^{137}VFDKIDINGDGELSL	6.0	4.0	1.0	0.10
17	648.0	2+	^{154}FMEGVQKDEVL	2.0	1.3	1.7	0.04
18	925.9	2+	^{165}LDILTRSLDLTHIVKL	5.5	4.0	2.0	0.05
19	523.5	2+	^{165}LDILTRSLD	2.0	6.0	2.0	0.04
20	526.6	2+	^{172}LDLTHIVKL	3.4	5.2	0.5	0.01
21	823.5	1+	^{174}LTHIVKL	3.6	3.8	0.1	0.10
22	710.5	1+	^{175}THIVKL	1.1	4.0	0.6	0.11
23	889.7	2+	^{181}IQNDGKNPHAPEEAEE	7.5	10.0	1.5	0.01
24	725.1	2+	^{181}IQNDGKNPHAPEE	4.0	5.0	1.0	0.10

Table S2. GCAP1 sequence coverage based on peptic fragments generated in the presence of EDTA. Deuterium uptake was calculated as described in the methods section "*Amide hydrogen-deuterium exchange analysis and representation*". The raw data were fitted to equation 2**.**

ID	m/z	z	Sequence	A_1(Da)	k_1(min^{-1})	A_2(Da)	k_2(min^{-1})
1	722.3	1+	^{2}GNMDSKA	3.0	7.0	0.3	0.05
2	966.7	2+	^{20}WYKKFMTECPSGQLTL	5.4	3.0	0.6	0.01
3	873.6	2+	^{21}YKKFMTECPSGQLTL	3.8	6.0	1.9	0.04
4	818.3	1+	^{28}CPSGQLTL	0.8	6.0	2.2	0.10
5	715.4	1+	^{29}PSGQLTL	2.8	2.5	0.6	0.01
6	861.4	1+	^{36}YEFKQF	0.7	3.0	2.0	0.01
7	995.3	2+	^{38}FKQFFGLKNLSPSANKY	5.5	8.0	1.5	0.01
8	963.7	2+	^{42}FGLKNLSPSANKYVEQM	5.6	5.0	2.2	0.04
9	805.2	1+	^{59}FETFDF	1.0	3.0	0.2	0.05
10	879.6	2+	^{59}FETFDFNKDGYIDF	2.2	3.0	2.0	0.03
11	1233.4	1+	^{63}DFNKDGYIDF	1.7	1.1	1.14	0.01
12	971.4	1+	^{65}NKDGYIDF	1.9	3.4	0.1	0.10
13	796.3	1+	^{73}MEYVAAL	1.3	0.8	1.1	0.02
14	544.3	1+	^{79}LSLVL	0.3	0.9	0.5	0.10
15	735.7	2+	^{82}VLKGKVDQKLRW	3.6	4.5	0.01	0.01
16	1525.5	1+	^{98}YDVDGNGCIDRGEL	3.3	10.0	0.1	0.10
17	671.4	1+	^{112}LNIIKA	0.7	4.0	1.4	0.04
18	545.3	2+	^{118}IRAINRCNE	4.7	8.9	0.8	0.10
19	718.0	2+	^{137}VFDKIDINGDGEL	2.8	6.0	0.8	0.04
20	818.0	2+	^{137}VFDKIDINGDGELSL	4.6	4.5	1.4	0.01
21	1294.5	1+	^{154}MEGVQKDEVL	1.2	1.2	1.6	0.01
22	1147.4	1+	^{155}FMEGVQKDEVL	2.9	5.3	1.0	0.01
23	925.8	2+	^{165}LDILTRSLDLTHIVKL	4.2	3.0	0.8	0.10
24	1055.5	1+	^{169}TRSLDLTHI	3.6	5.0	0.1	0.10
25	698.7	2+	^{169}TRSLDLTHIVKL	2.8	8.5	0.4	0.26
26	823.5	1+	^{174}LTHIVKL	2.6	3.8	0.2	0.10
27	710.4	1+	^{175}THIVKL	1.4	4.0	0.3	0.01
28	889.6	2+	^{181}IQNDGKNPHAPEEAEE	6.7	8.0	0.1	0.01

Table S3. myr-GCAP1 sequence coverage based on peptic fragments generated in the presence of Ca^{2+}. Deuterium uptake was calculated as described in the methods section "*Amide hydrogen-deuterium exchange analysis and representation*". The raw data were fitted to equation 2.

ID	m/z	z	Sequence	A_1(Da)	k_1(min^{-1})	A_2(Da)	k_2(min^{-1})
1	932.5	1+	myr-^{2}GNMDSKA	1.6	10.0	0.1	0.02
2	966.7	2+	^{20}WYKKFMTECPSGQLTL	2.5	8.0	2.0	0.04
3	861.3	1+	^{36}YEFKQF	0.6	7.0	0.4	0.04
4	963.8	2+	^{42}FGLKNLSPSANKYVEQM	3.3	7.0	2.0	0.05
5	879.6	2+	^{59}FETFDFNKDGYIDF	0.5	7.0	0.4	0.03
6	805.3	1+	^{59}FETFDF	0.5	10.0	0.01	0.01
7	971.4	1+	^{65}NKDGYIDF	0.6	10.0	0.1	0.01
8	796.3	1+	^{73}MEYVAAL	0.3	10.0	0.01	0.01
9	544.3	1+	^{79}LSLVL	0.1	10.0	0.01	0.20
10	735.7	2+	^{82}VLKGKVDQKLRW	2.4	12.0	0.40	0.01
11	763.6	2+	^{98}YDVDGNGCIDRGEL	1.4	13.0	0.10	0.10
12	671.4	1+	^{112}LNIIKA	0.6	10.0	0.01	0.01
13	871.3	2+	^{112}LNIIKAIRAINRCNE	3.1	6.0	0.50	0.05
14	545.0	2+	^{118}IRAINRCNE	4.0	9.0	0.10	0.01
15	718.1	2+	^{137}VFDKIDINGDGEL	1.6	8.0	0.50	0.10
16	648.0	2+	^{154}FMEGVQKDEVL	1.9	10.0	0.80	0.01
17	523.5	2+	^{165}LDILTRSLD	1.4	5.0	0.70	0.03
18	823.5	1+	^{174}LTHIVKL	1.2	8.0	1.20	0.03
19	710.5	1+	^{175}THIVKL	0.9	3.0	0.40	0.01
20	889.7	2+	^{181}IQNDGKNPHAPEEAEE	6.0	15.0	0.01	0.10

Table S4. myr-cGCAP1 sequence coverage based on peptic fragments generated in the presence of EDTA. Deuterium uptake was calculated as described in the methods section "*Amide hydrogen-deuterium exchange analysis and representation*". The raw data were fitted to equation 2**.**

ID	m/z	z	Sequence	A1(Da)	k1(min^{-1})	A2(Da)	k2(min^{-1})
1	932.5	1+	myr-^{2}GNMDSKA	2.4	14.0	0.1	0.05
2	1179.4	1+	^{25}MTECPSGL	3.9	11.0	2.0	0.08
3	861.3	1+	^{36}YEFKQF	2.8	6.0	0.1	0.01
4	963.8	2+	^{42}FGLKNLSPSANKYVEQM	5.0	12.0	3.5	0.08
5	879.6	2+	^{59}FETFDFNKDGYIDF	4.4	6.0	0.5	0.01
6	805.4	1+	^{59}FETFDF	1.4	6.0	0.2	0.04
7	617.5	2+	^{63}DFNKDGYIDF	2.3	6.0	0.3	0.01
8	971.4	1+	^{65}NKDGYIDF	1.9	6.0	0.2	0.09
9	796.3	1+	^{73}MEYVAAL	1.4	3.0	1.8	0.10
10	544.3	1+	^{79}LSLVL	1.0	3.0	0.6	0.08
11	763.6	2+	^{98}YDVDGNGCIDRGEL	4.2	8.0	1.0	0.05
12	671.4	1+	^{112}LNIIKA	1.0	4.0	1.8	0.03
13	871.3	2+	^{112}LNIIKAIRAINRCNE	5.6	3.5	0.2	0.01
14	545.0	2+	^{118}IRAINRCNE	5.8	10.0	0.8	0.10
15	718.1	2+	^{137}VFDKIDINGDGEL	4.2	8.0	1.0	0.20
16	648.0	2+	^{154}FMEGVQKDEVL	3.8	8.0	1.6	0.04
17	523.5	2+	^{165}LDILTRSLD	2.8	8.0	1.2	0.30
18	823.5	1+	^{174}LTHIVKL	2.8	11.0	0.5	0.05
19	710.5	1+	^{175}THIVKL	1.6	10.0	0.7	0.06
20	1024.8	2+	^{181}IQNDGKNPHAPEEAEEAAQ	10.0	10.0	0.1	0.10
21	889.7	2+	^{181}IQNDGKNPHAPEEAEE	8.2	13.0	1.0	0.01

Table S5. Peptides studied in using footprinting experiments. The results show the identified peptides used for the footprinting analysis following cleavage of GCAP1 and myr-GCAP1 in the Ca^{2+}-free and Ca^{2+}-bound forms using trypsin. Modified amino acid residues are shows in red. Solvent accessible surface area was calculated from the X-ray structure of Ca^{2+}-bound myr-GCAP1 using Chimera version 1.3. Modification rates for all four experimental setup are also indicated were calculated by fitting data presented in Fig S3 to equation 4.

ID	Sequence	m/z	m/z ox	z	Å^2	GCAP1-[Ca^{2+}]-bound	GCAP1-[Ca^{2+}]-free	myr-GCAP1-[Ca^{2+}]-bound	myr-GCAP1-[Ca^{2+}]-free
						k(s^{-1})			
1	^{24}FMTECPSGQLTLYEFK	947.4	956.4	2+	74.6	3.57	4.00	16.66	14.28
2	^{54}YVEQMFETFDFNK	849.3	857.3	2+	3.2	19.23	12.04	66.66	25.00
3	^{141}IDINGDGELSLEEFMEGVQK	1112.2	1120.0	2+	39.6	3.22	2.04	3.57	3.33
4	^{171}SLDLTHIVK	513.2	521.2	2+	46.6	0.04	0.03	0.22	0.25
5	^{187}NPHAPEEAEEAAQ	696.8	704.8	2+	N/A	0.40	0.52	1.72	1.85

Figure legends – Supplementary material

Figure S1. Proteolysis of GCAP1 and myr-GCAP1. GCAP1 and myr-GCAP1 were digested with pepsin (*panel A*) and trypsin (*panel B*). The sequence of myr-GCAP1 is indicated by the blue star above the Gly1 residue.

Figure S2. Deuterium uptake profile of Ca^{2+}-bound and Ca^{2+}-free forms of GCAP1 and myr-GCAP1. Panels A-K show deuterium uptake profiles of common peptides found in the four experimental setups: (■) GCAP1-Ca^{2+}-bound form, (●) GCAP1-Ca^{2+}-free form, (▲) myr-GCAP1-Ca^{2+}-bound form, and (▼) myr-GCAP1-Ca^{2+}-free form. Primary sequences of the peptides are indicated at the top right of each plot.

Figure S3. Footprinting of GCAP1. *Panels A, B, C,* and *D* show footprinting results of the four different experimental setups. The representation of the four experimental setups is identical to the one described for Fig. S2. The modified amino acid identified by MS/MS shown in red. Footprinting was performed as described in "Experimental procedures".

Figure S4. Molecular dynamics simulations of GCAP1 and myr-GCAP1 in the Ca^{2+}-free and Ca^{2+}-bound forms. *Panel A* shows the X-ray structure of the myr-GCAP1-Ca^{2+}-bound form in red (Ca^{2+} as red spheres) whereas the snapshot extracted at 5 ns of molecular dynamics is shown in cyan (Ca^{2+} as cyan spheres). The N-terminus and the first α-helix, namely Gly^{2} to Glu^{17}, also are indicated in the figure by blue arrows. *Panel B* shows the X-ray structure of the myr-GCAP1-Ca^{2+}-bound form superposed with the structure of the GCAP1-Ca^{2+}-free form at 5 ns. *Panels C* and *D* show superposed

structures of myr-GCAP1 in the Ca^{2+}-free and Ca^{2+}-bound form with the X-ray structure, respectively. *Panel E* shows the RMSD of the α-Carbon atoms as a function of the simulation time whereas *panel F* shows the RMSD of each residue as a function of the simulation time.

Figure S5. Expression and purification of GCAP1, yeast NMT, and myr-GCAP1. *Panel A* shows the primary sequence of GCAP1. The 6×His tag is colored orange and Gly1 is indicated by the blue arrow at the TEV cleavage site. *Panel B* shows the SDS-PAGE patterns of: *M*, the molecular ladder; *lane 1* is the soluble fraction of the bacterial lysate *lanes 2-7* are the flow through in the purification of NMT; and *lanes 8* and *9* are purified NMT (0.1µl and 0.025 µl, respectively). *Panel C* illustrates the flowchart for the *in vitro* myristoylation reaction of GCAP1. NMT with its 6×His tag was bound first to myristoyl CoA (MCoA) (C1) followed by incubation with GCAP1 for 1 h at 4 ^{o}C (C2). Finally, NMT was removed by binding to the IMAC resin, leaving purified myristoylated GCAP1 in solution (panel C3). Panel D shows the SDS-PAGE migration pattern of purified GCAP1 and myr-GCAP1 under different conditions (i.e., with and without Ca^{2+} and EDTA).

Figure S6. Flowchart of the footprinting procedure. Following exposure to X-rays GCAP1-selected residues exposed to OH· radicals become oxidized (red spheres). Following digestion with trypsin the peptides are analyzed by HPLC coupled to mass spectrometry (Experimental procedures) and the unmodified fraction is calculated as the ratio described in equation 3.

Figure S7. Myristoylated fragments of myr-cGCAP1. *Panel A* exhibits the ion m/z =932 corresponding to the myr-GNMDSKA peptide obtained after pepsin cleavage (left) together with its MS/MS profile (right) showing the *b* and *y* ions. *Panel B* displays the ion m/z=861.5 corresponding to the myr-GNMDSK peptide obtained after trypsin cleavage (left) together with its MS/MS profile (right).

Figure S1

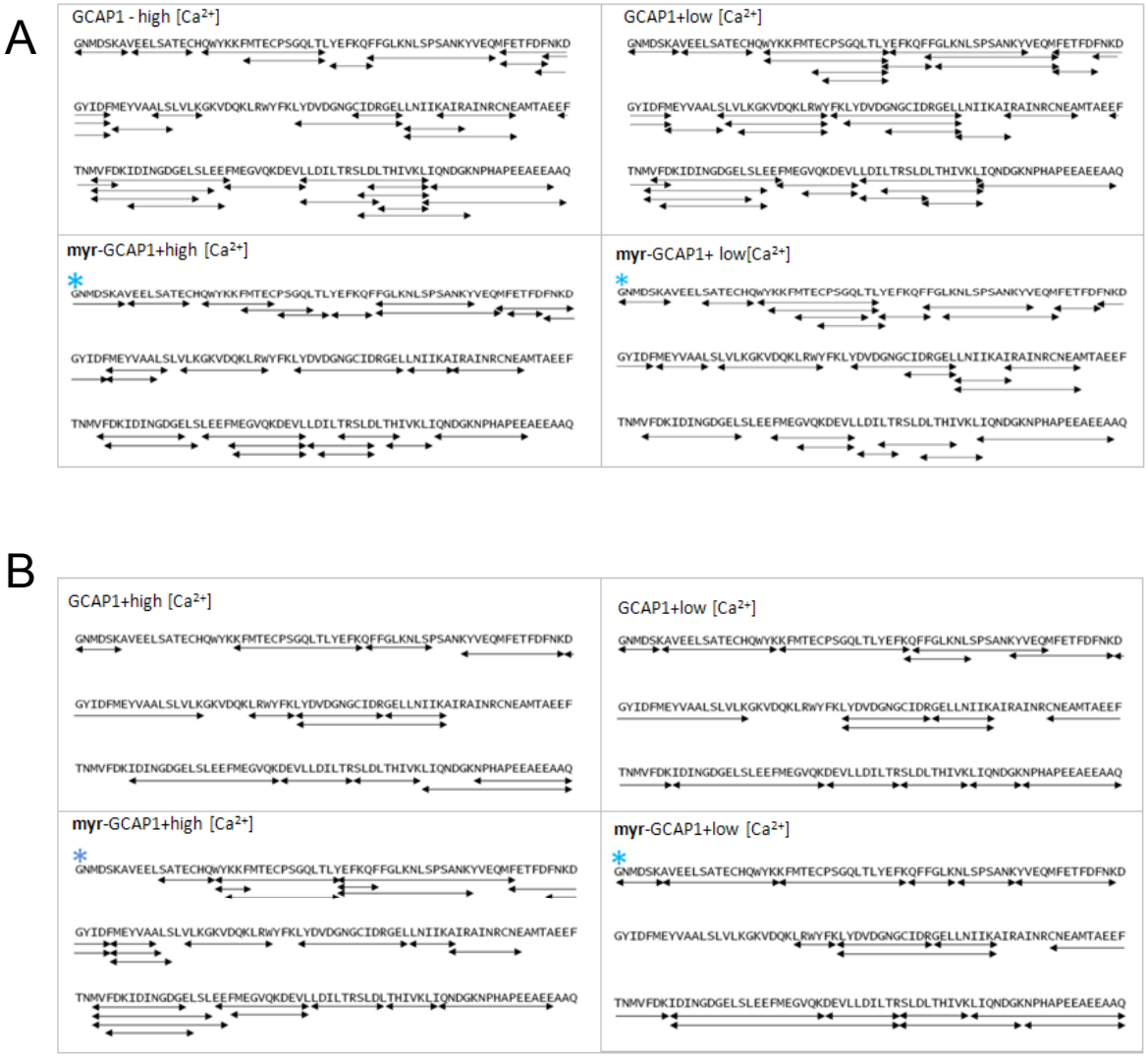

A
GCAP1 - high [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
GCAP1+low [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
myr-GCAP1+high [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
myr-GCAP1+ low[Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
B
GCAP1+high [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
GCAP1+low [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
myr-GCAP1+high [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ
myr-GCAP1+low [Ca2+]
GNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEFKQFFGLKNLSPSANKYVEQMFETFDFNKD
GYIDFMEYVAALSLVLKGKVDQKLRWYFKLYDVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEF
TNMVFDKIDINGDGELSLEEFMEGVQKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ

Figure S2

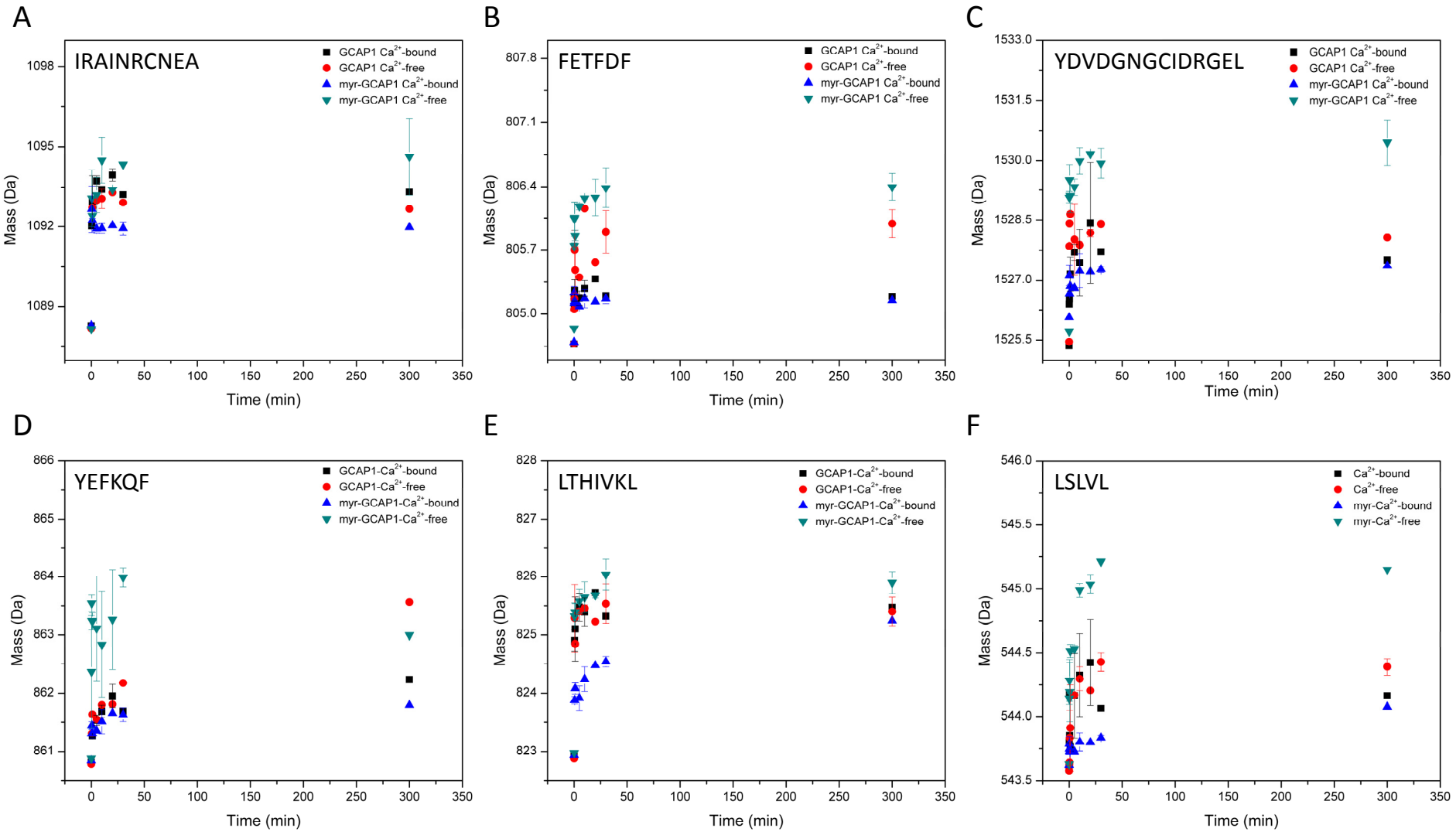

A
IRAINRCNEA
GCAP1 Ca2+-bound
GCAP1 Ca2+-free
myr-GCAP1 Ca2+-bound
myr-GCAP1 Ca2+-free
Mass (Da)
Time (min)
B
FETFDF
C
YDVDGNGCIDRGEL
D
YEFKQF
GCAP1-Ca2+-bound
GCAP1-Ca2+-free
myr-GCAP1-Ca2+-bound
myr-GCAP1-Ca2+-free
E
LTHIVKL
F
LSLVL
Ca2+-bound
Ca2+-free
myr-Ca2+-bound
myr-Ca2+-free

Figure S2 continuation

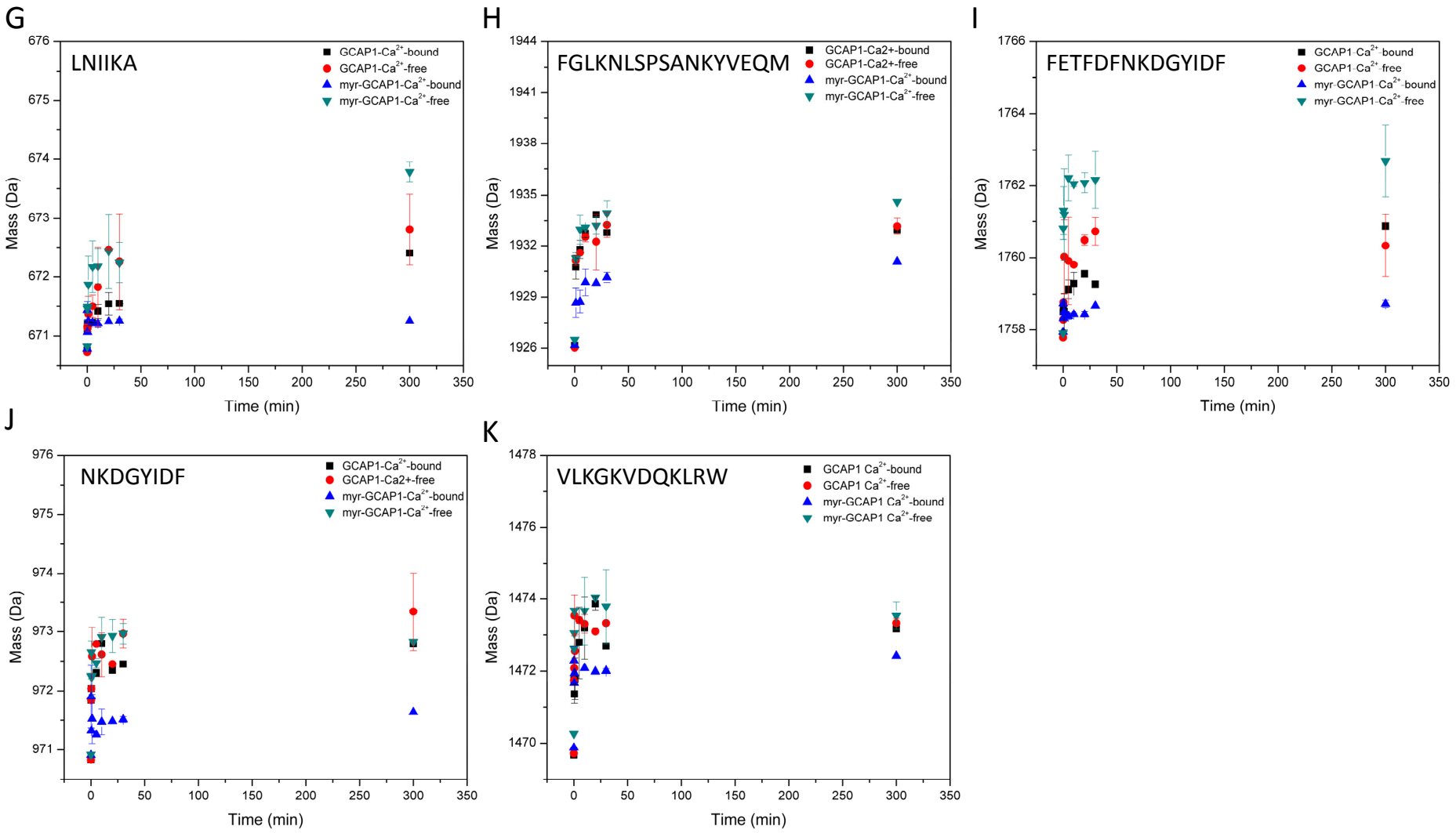


Figure S3

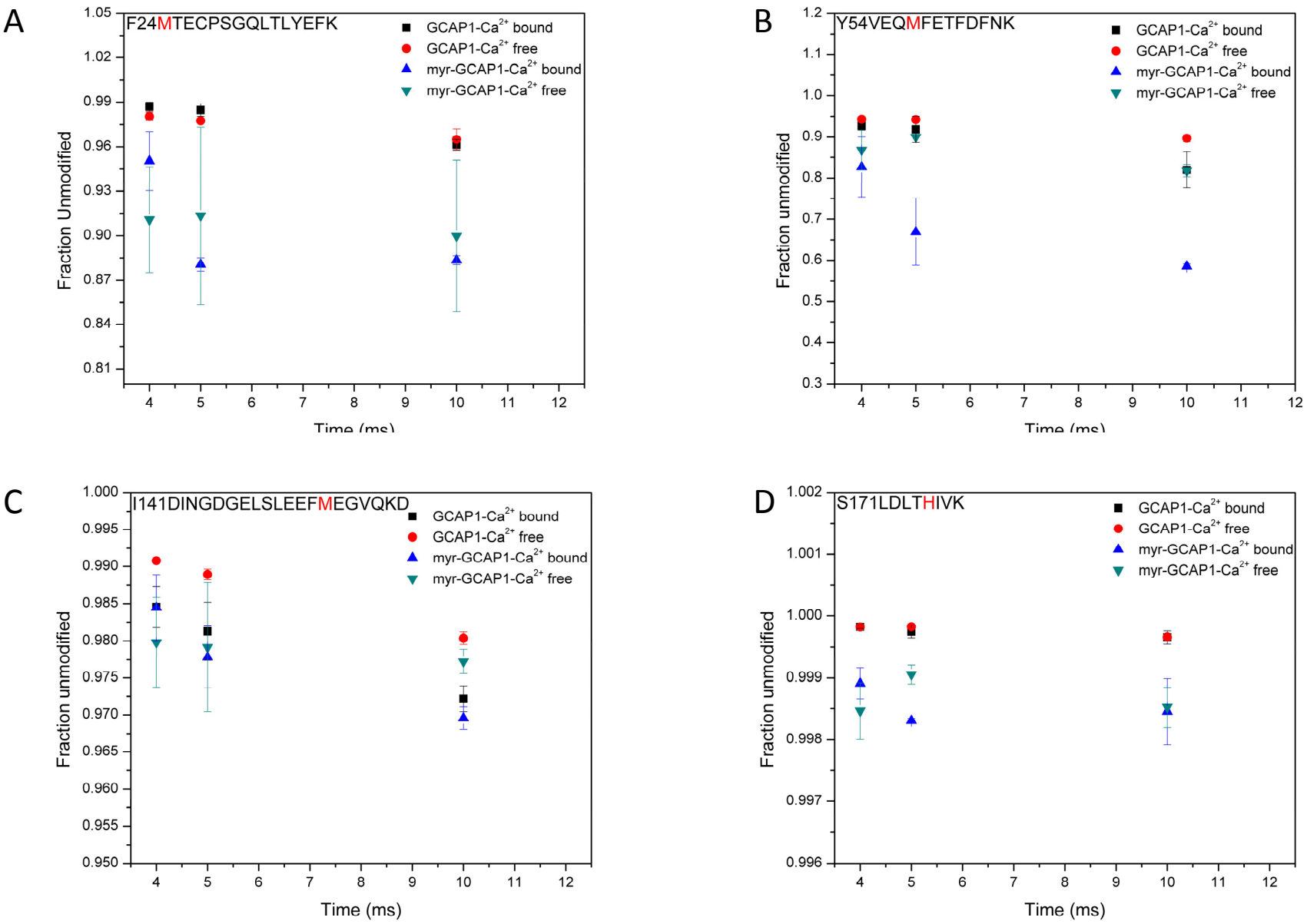

A
F24MTECPSGQLTLYEFK
GCAP1-Ca2+ bound
GCAP1-Ca2+ free
myr-GCAP1-Ca2+ bound
myr-GCAP1-Ca2+ free
Fraction Unmodified
Time (ms)
B
Y54VEQMFETFDFNK
GCAP1-Ca2+ bound
GCAP1-Ca2+ free
myr-GCAP1-Ca2+ bound
myr-GCAP1-Ca2+ free
Fraction unmodified
Time (ms)
C
I141DINGDGELSLEEFMEGVQKD
GCAP1-Ca2+ bound
GCAP1-Ca2+ free
myr-GCAP1-Ca2+ bound
myr-GCAP1-Ca2+ free
Fraction unmodified
Time (ms)
D
S171LDLTHIVK
GCAP1-Ca2+ bound
GCAP1-Ca2+ free
myr-GCAP1-Ca2+ bound
myr-GCAP1-Ca2+ free
Fraction unmodified
Time (ms)

Figure S4

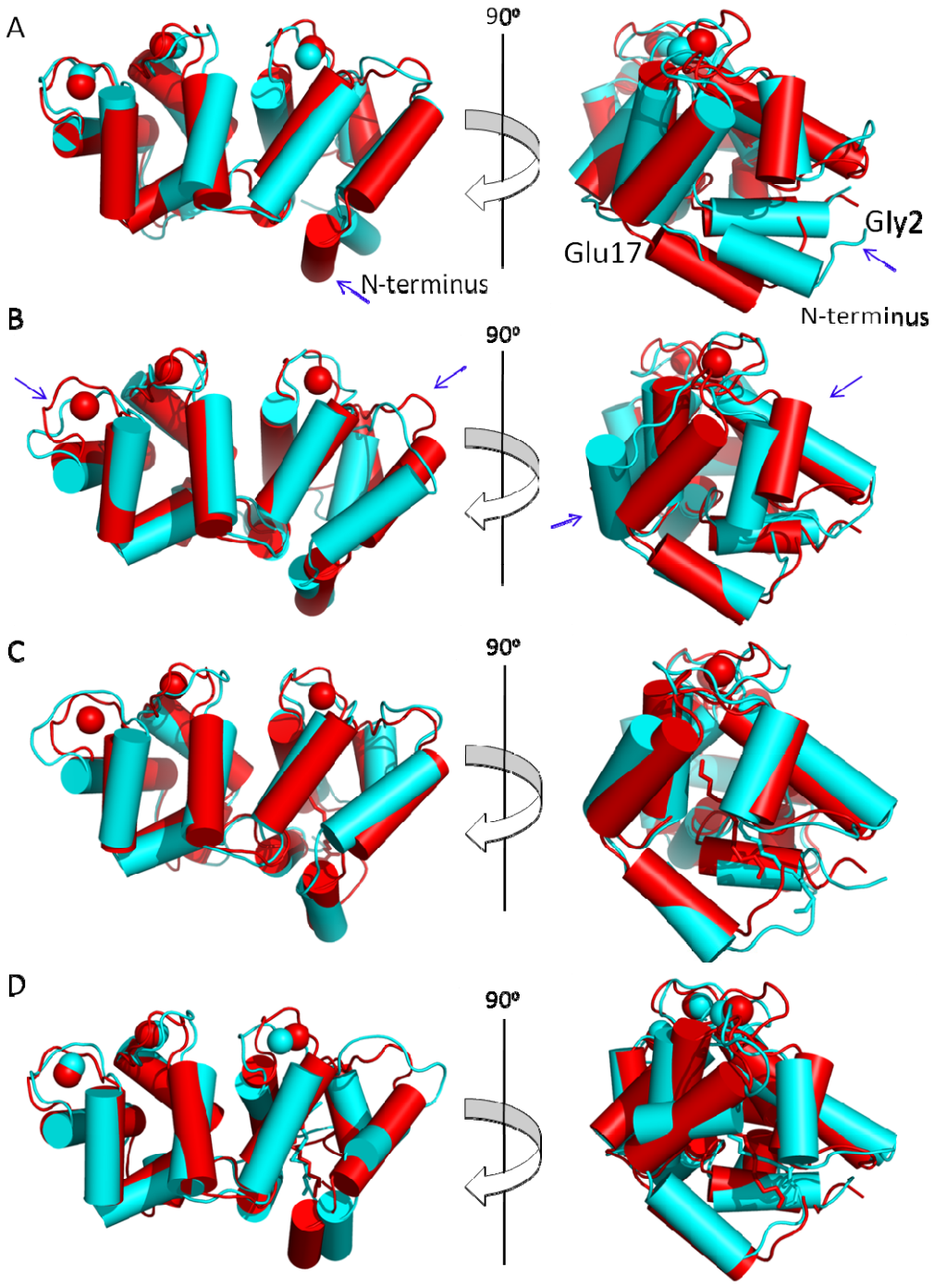
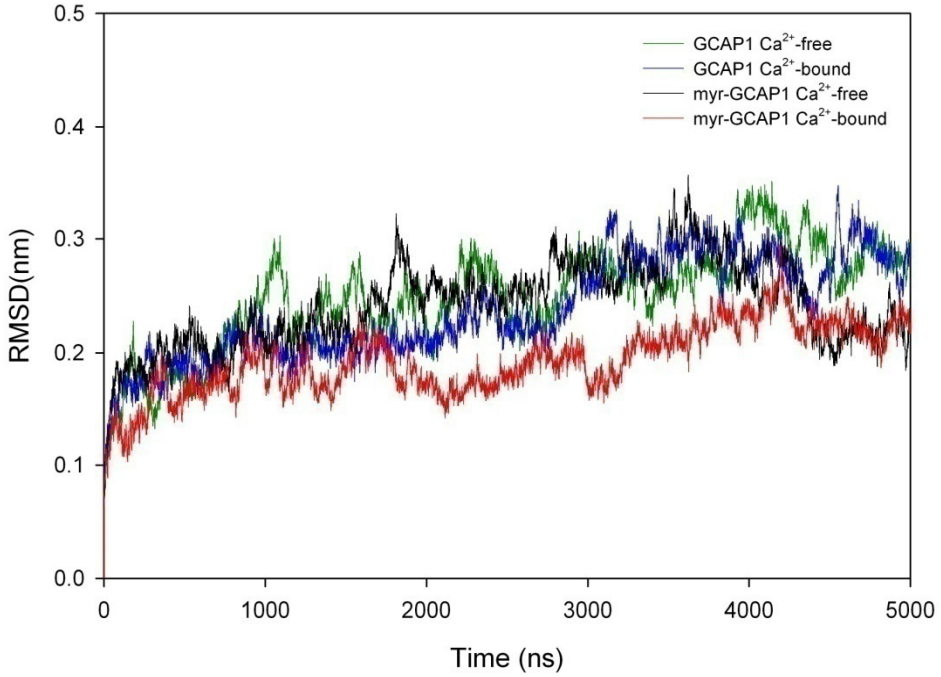


Figure S4

E


GCAP1 Ca2+-free
GCAP1 Ca2+-bound
myr-GCAP1 Ca2+-free
myr-GCAP1 Ca2+-bound
RMSD(nm)
Time (ns)
0.0
0.1
0.2
0.3
0.4
0.5
0
1000
2000
3000
4000
5000

F

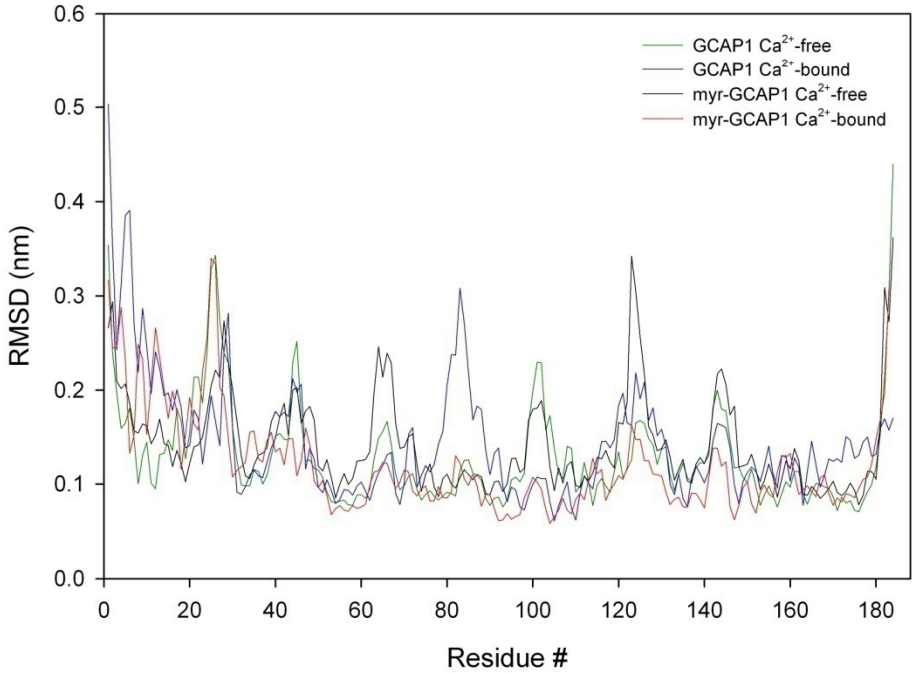

GCAP1 Ca2+-free
GCAP1 Ca2+-bound
myr-GCAP1 Ca2+-free
myr-GCAP1 Ca2+-bound
RMSD (nm)
Residue #
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
20
40
60
80
100
120
140
160
180

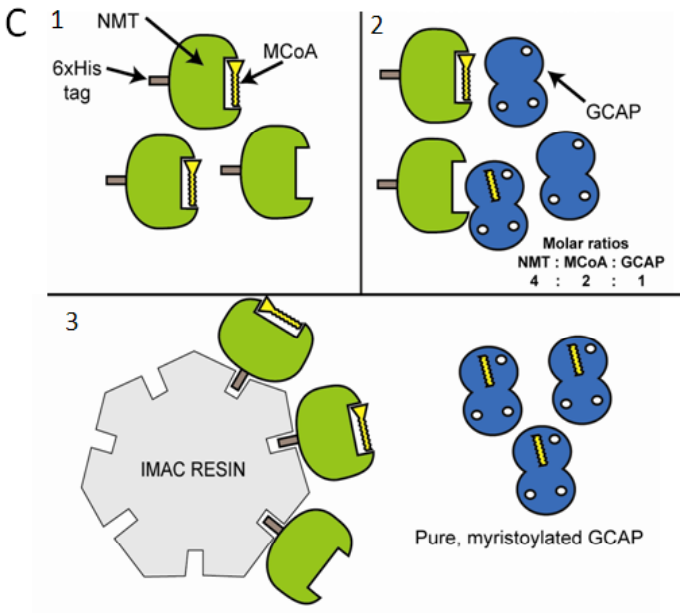
Figure S5

A

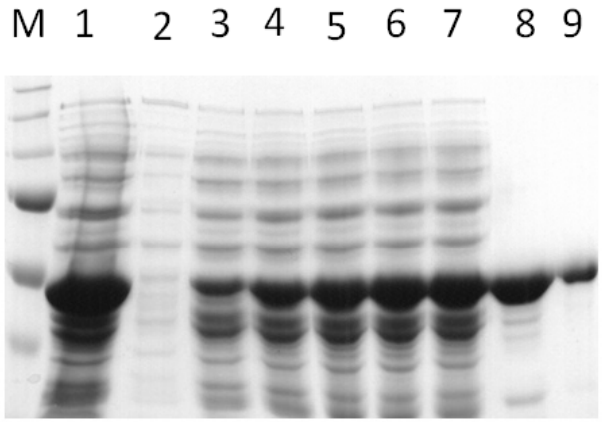
cGCAP-G6S

TEV cleavage site

MSYYHHHHHHDYDIPTTENLYFQGNMDSKAVEELSATECHQWYKKFMTECPSGQLTLYEF
KQFFGLKNLSPSANKYVEQMFETFDFNKDGYIDFMEYVAALSLVLKGKVDQKLRWYFKLY
DVDGNGCIDRGELLNIIKAIRAINRCNEAMTAEEFTNMVFDKIDINGDGELSLEEFMEGV
QKDEVLLDILTRSLDLTHIVKLIQNDGKNPHAPEEAEEAAQ



B



D

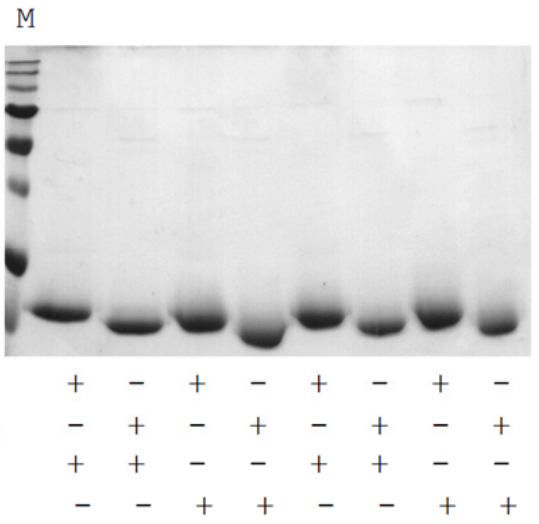
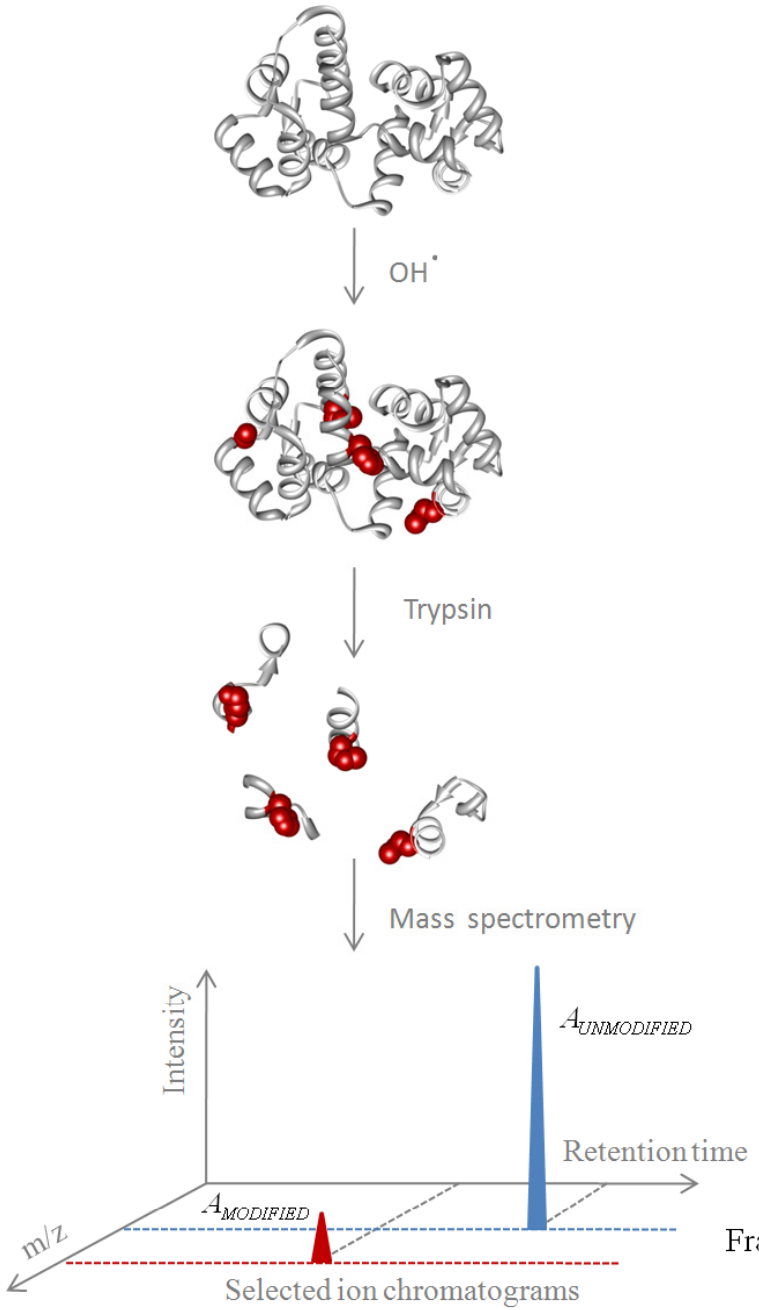
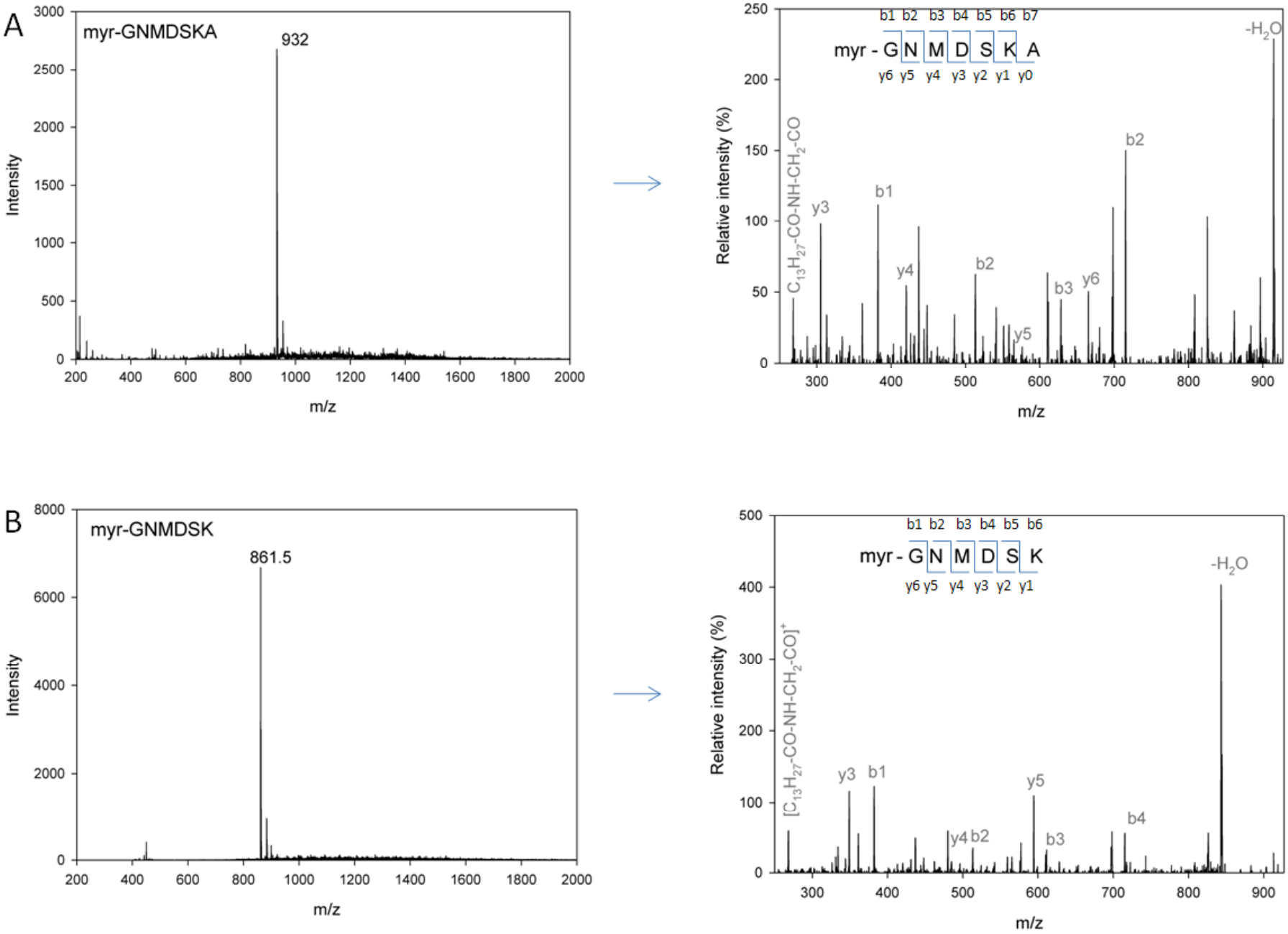


Figure S6



$$\text{Fraction unmodified} = \frac{A_{UNMODIFIED}}{A_{UNMODIFIED} + A_{MODIFIED}}$$

Figure S7


A
myr-GNMDSKA
932
Intensity
m/z
b1 b2 b3 b4 b5 b6 b7
myr - G N M D S K A
y6 y5 y4 y3 y2 y1 y0
-H2O
Relative intensity (%)
C13H27-CO-NH-CH2-CO
B
myr-GNMDSK
861.5
Intensity
m/z
b1 b2 b3 b4 b5 b6
myr - G N M D S K
y6 y5 y4 y3 y2 y1
-H2O
Relative intensity (%)
[C13H27-CO-NH-CH2-CO]+