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Supplemental Information

KRAS Prenylation Is Required for Bivalent Binding with Calmodulin in a Nucleotide-Independent Manner

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Table S1: Characterization of components studied by sedimentation velocity

Table S2: Summary of binding parameters obtained of CaM binding to GDP-bound KRAS4b variants via SV-AUC, SPR and ITC

Table S3. Summary of binding affinities obtained for CaM binding to various KRAS4b constructs via ITC

Figure S1. *Unprenylated GDP and GNP-bound KRAS4b do not interact with CaM* **(A)** Sedimentation velocity absorbance c(*s*) profiles obtained for individual CaM and KRAS4b variants at 70 μM CaM (cyan) and ~10 μM GDP and GNP-bound KRAS4b-FMe, KRAS4b 2-185 and KRAS4b 2-169. **(B)** Sedimentation velocity absorbance c(*s*) profiles for mixtures of 30 μM CaM and 20 μM GDP and GNP-bound KRAS4b constructs as indicated. Faster sedimenting species was only observed for CaM and GDP and GNP-bound KRAS4b-FMe. (**C**) ITC heat of dilution data acquired upon injection of buffer into 30 μM KRAS4b-FMe and 1 mM CaM into buffer. **(**D) ITC data obtained for CaM (1 mM) titrated into GDP and GNP-bound KRAS4b 2-185 (30 μM).

Figure S2. *Prenylated GDP and GNP-bound KRAS4b-FMe forms a 2:1 complexes with CaM.* Absorbance (top panel) and interference (bottom panel) sedimentation velocity c(*s*) profiles for the titration of **(A)** CaM into 6 μM GDP-bound KRAS4b-FMe, CaM into 3 μM GDP-biund KRAS4b-FMe, and GDP-bound KRAS4b-FMe into 6 μM CaM, **(B)** CaM-C into 3 μM GDP-bound KRAS4b-FMe and **(C)** CaM-N into 3 μM GDP-bound KRAS4b-FMe at the concentrations indicated. The weighted-average sedimentation coefficient S_w used for analysis in Figure 2 is obtained by integration of the c(*s*) profiles shown.

Figure S3. *NMR data further supports that unprenylated GDP and GNP-bound KRAS4b do not interact with CaM.* 2D ^{1}H-^{15}N HSQC of ^{15}N-labeled GDP and GNP-bound KRAS4b2-185 at 50 μM titrated with 500 μM CaM. Again, no CSP or line broadening was observed between unprenylated KRAS4b and CaM.

Figure S4. *NMR data of GDP-bound KRAS4b-FMe binding to CaM-C and CaM-N.* **(A)**2D ^{1}H-^{15}N HSQC of ^{15}N-labeled CaM-N titrated with GDP-bound KRAS4b-FMe at a 1.5:1 ratio. (**B)** 2D ^{1}H-^{15}N HSQC of ^{15}N-labeled CaM-C titrated with GDP- bound KRAS4b-FMe at a 1.5:1 ratio. (**C-D)** A histogram of normalized ^{1}H-^{15}N chemical shift changes vs. residue number calculated from the HSQC spectra for CaM-N and CaM-C upon addition of GDP-bound KRAS4b-FMe. (**E-F)** Cartoon representation of CaM structure (PDB ID: 3CLN) highlighting residues that exhibited substantial (>0.05 ppm) chemical shift changes for CaM-N(red) or CaM-C(magenta).

Figure S5. *NMR data further supports that unprenylated GDP and GNP-bound KRAS4b do not interact with CaM.* 2D ^{1}H-^{15}N HSQC of ^{15}N-labeled CaM at 50 μM titrated with 500 μM upon titration of unprenylated KRAS4b into CaM.

Figure S6. *KSKTKC-FMe does not induce secondary structural rearrangements in CaM.* FarUV circular dichroism spectra obtained for CaM (magenta) and the KSKTKC-FMe: CaM complex(green).

Table S1: Characterization of components studied by sedimentation velocity.

Component	Concentration studied (μM)	$S_{20,w}$ (S)	M (kDa)
CaM	70	2.0	18
CaM-N	110	1.4	10
CaM-C	25	1.5	13
GDP KRAS4b-FMe	10	2.2	23
GNP KRAS4b-FMe	10	2.2	22
GDP KRAS4b 2-185	10	2.2	23
GNP KRAS4b 2-185	10	2.2	23
GDP KRAS4b 2-169	10	2.2	21
GNP KRAS4b 2-169	10	2.3	21
MSP1D1 POPC:POPS 70:30 Nanodisc	4.5	3.1	84

Table S2: Summary of binding parameters obtained of CaM binding to GDP-bound KRAS4b variants via SV-AUC, SPR and ITC

Technique	Sample 1	Sample 2	Kd (μM)	ΔH (kcal/mol)	-TΔS (kcal/mol)	ΔG (kcal/mol)
SV-AUC	CaM	KRAS4b-FMe	Co-Op*			
SV-AUC	CaM-C	KRAS4b-FMe	0.4 ± 0.1			
SV-AUC	CaM-N	KRAS4b-FMe	4 ± 1			
SPR	CaM	KRAS4b-FMe	0.4 ± 0.1			
SPR	CaM-C	KRAS4b-FMe	0.6 ± 0.1			
SPR	CaM-N	KRAS4b-FMe	3 ± 0.2			
ITC	CaM	KRAS4b-FMe	0.3 ± 0.1			
ITC	CaM-C	KRAS4b-FMe	0.5 ±0.1	-5.1 ± 0.3	-3.5	-8.6
ITC	CaM-N	KRAS4b-FMe	4 ± 1	-7.3 ± 0.5	-0.2	-7.5

Co-op* Data were modeled in terms of two non-symmetric sites with microscopic binding constants. The microscopic binding constants observed for binding to CaM-N and CaM-C were applied and fixed to obtain a favorable co-operative term.

Table S3: Summary of binding affinities obtained for CaM binding to various KRAS4b constructs via ITC

Cell	Syringe	K_d
KRAS4b-FMe (44 μM)	CaM (120 μM)	0.3 μM ± 0.08 μM
KRAS4b-FMe (30 μM)	CaM-C (300 μM)	0.5 μM ± 0.07 μM
KRAS4b-FMe (30 μM)	CaM-N (500 μM)	4 μM ± 1 μM
KRAS4b-Farnesyl (57 μM)	CaM (300 μM)	2 μM ± 0.2 μM
KRAS4b-2-180-AAAAC-Farnesyl (48 μM)	CaM (440 μM)	10 μM ± 1 μM
HVR-FMe (50 μM)	CaM (150 μM)	0.3 μM ± 0.06 μM
KSKTKC-FMe (50 μM)	CaM (130 μM)	0.4 μM ± 0.1 μM
KSKTKC-GMe (30 μM)	CaM (200 μM)	3 μM ± 0.01 μM
KSKTKC-PMe (30 μM)	CaM (800 μM)	30 μM ± 3 μM
GDP KRAS4b 2-185 (30 μM)	CaM (1 mM)	ND *
GNP KRAS4b 2-185 (30 μM)	CaM (1 mM)	ND *

ND* No binding detected.

Figure S1

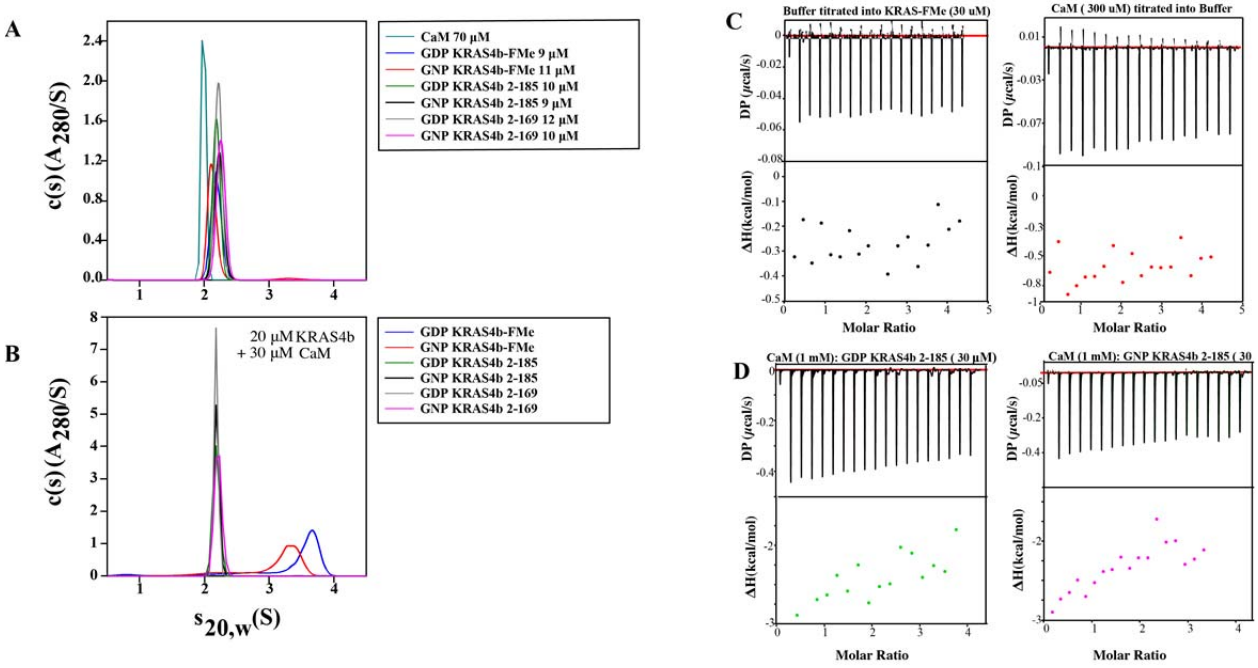

A
c(s)(A280/S)
CaM 70 µM
GDP KRAS4b-FMe 9 µM
GNP KRAS4b-FMe 11 µM
GDP KRAS4b 2-185 10 µM
GNP KRAS4b 2-185 9 µM
GDP KRAS4b 2-169 12 µM
GNP KRAS4b 2-169 10 µM
B
20 µM KRAS4b
+ 30 µM CaM
GDP KRAS4b-FMe
GNP KRAS4b-FMe
GDP KRAS4b 2-185
GNP KRAS4b 2-185
GDP KRAS4b 2-169
GNP KRAS4b 2-169
s20,w(S)
C
Buffer titrated into KRAS-FMe (30 uM)
CaM (300 uM) titrated into Buffer
DP (µcal/s)
ΔH(kcal/mol)
Molar Ratio
D
CaM (1 mM): GDP KRAS4b 2-185 (30 µM)
CaM (1 mM): GNP KRAS4b 2-185 (30
DP (µcal/s)
ΔH(kcal/mol)
Molar Ratio

Figure S2

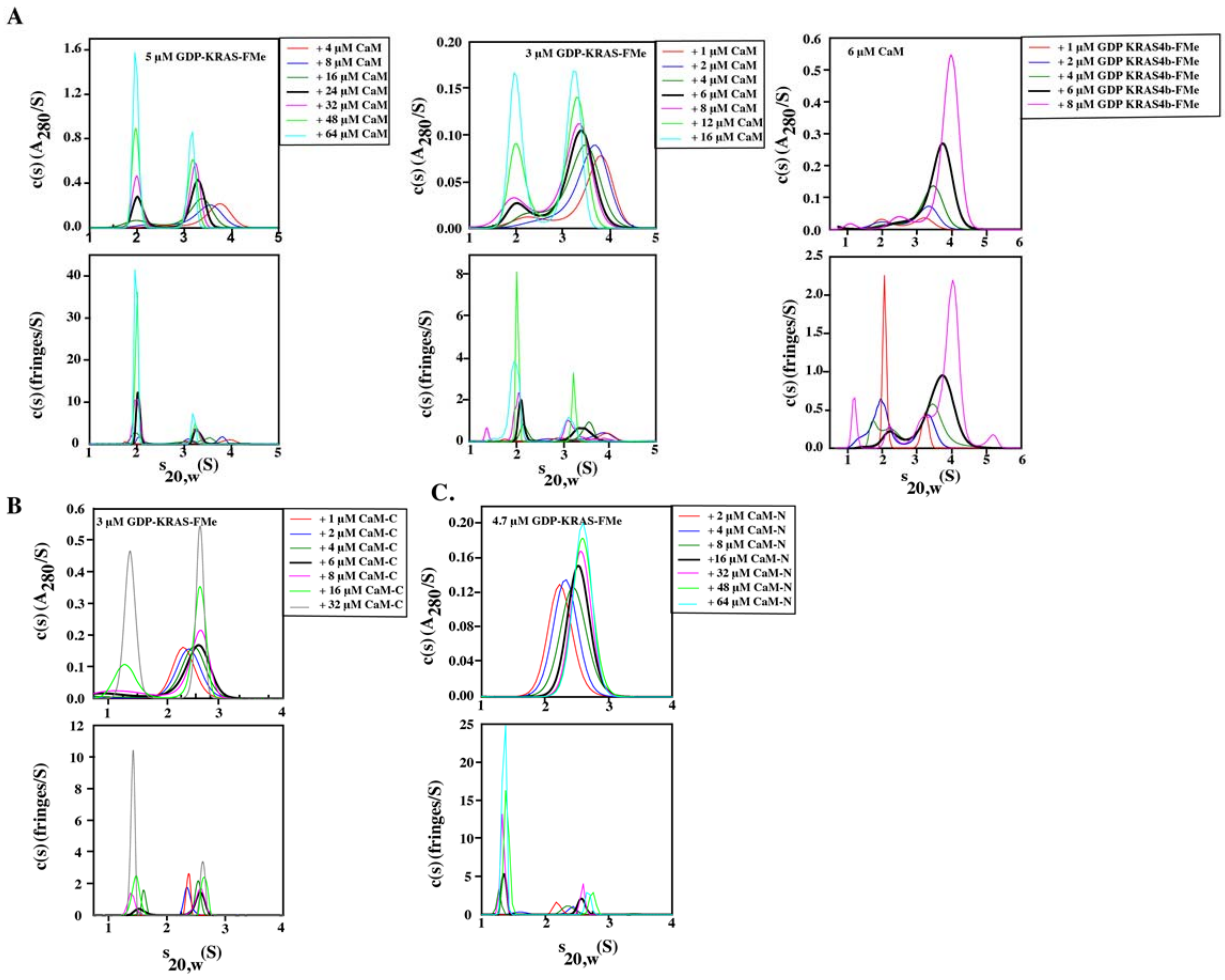

A
5 µM GDP-KRAS-FMe
+ 4 µM CaM
+ 8 µM CaM
+ 16 µM CaM
+ 24 µM CaM
+ 32 µM CaM
+ 48 µM CaM
+ 64 µM CaM
3 µM GDP-KRAS-FMe
+ 1 µM CaM
+ 2 µM CaM
+ 4 µM CaM
+ 6 µM CaM
+ 8 µM CaM
+ 12 µM CaM
+ 16 µM CaM
6 µM CaM
+ 1 µM GDP KRAS4b-FMe
+ 2 µM GDP KRAS4b-FMe
+ 4 µM GDP KRAS4b-FMe
+ 6 µM GDP KRAS4b-FMe
+ 8 µM GDP KRAS4b-FMe
c(s) (A280/S)
c(s) (fringes/S)
s20,w (S)
B
3 µM GDP-KRAS-FMe
+ 1 µM CaM-C
+ 2 µM CaM-C
+ 4 µM CaM-C
+ 6 µM CaM-C
+ 8 µM CaM-C
+ 16 µM CaM-C
+ 32 µM CaM-C
C.
4.7 µM GDP-KRAS-FMe
+ 2 µM CaM-N
+ 4 µM CaM-N
+ 8 µM CaM-N
+16 µM CaM-N
+ 32 µM CaM-N
+ 48 µM CaM-N
+ 64 µM CaM-N

Figure S3

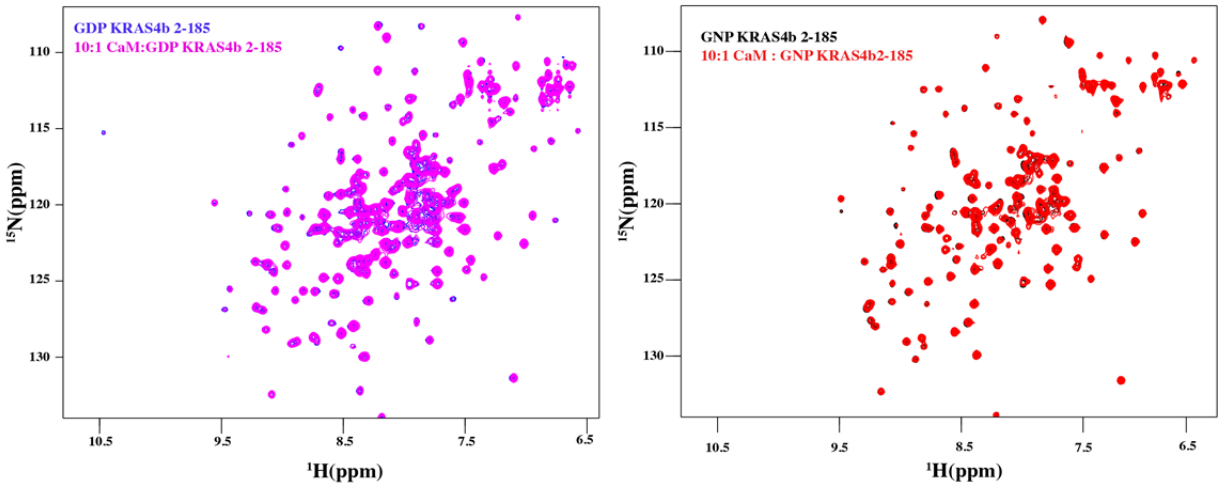

GDP KRAS4b 2-185
10:1 CaM:GDP KRAS4b 2-185
^{15}N(ppm)
^{1}H(ppm)
GNP KRAS4b 2-185
10:1 CaM : GNP KRAS4b2-185
^{15}N(ppm)
^{1}H(ppm)

Figure S4

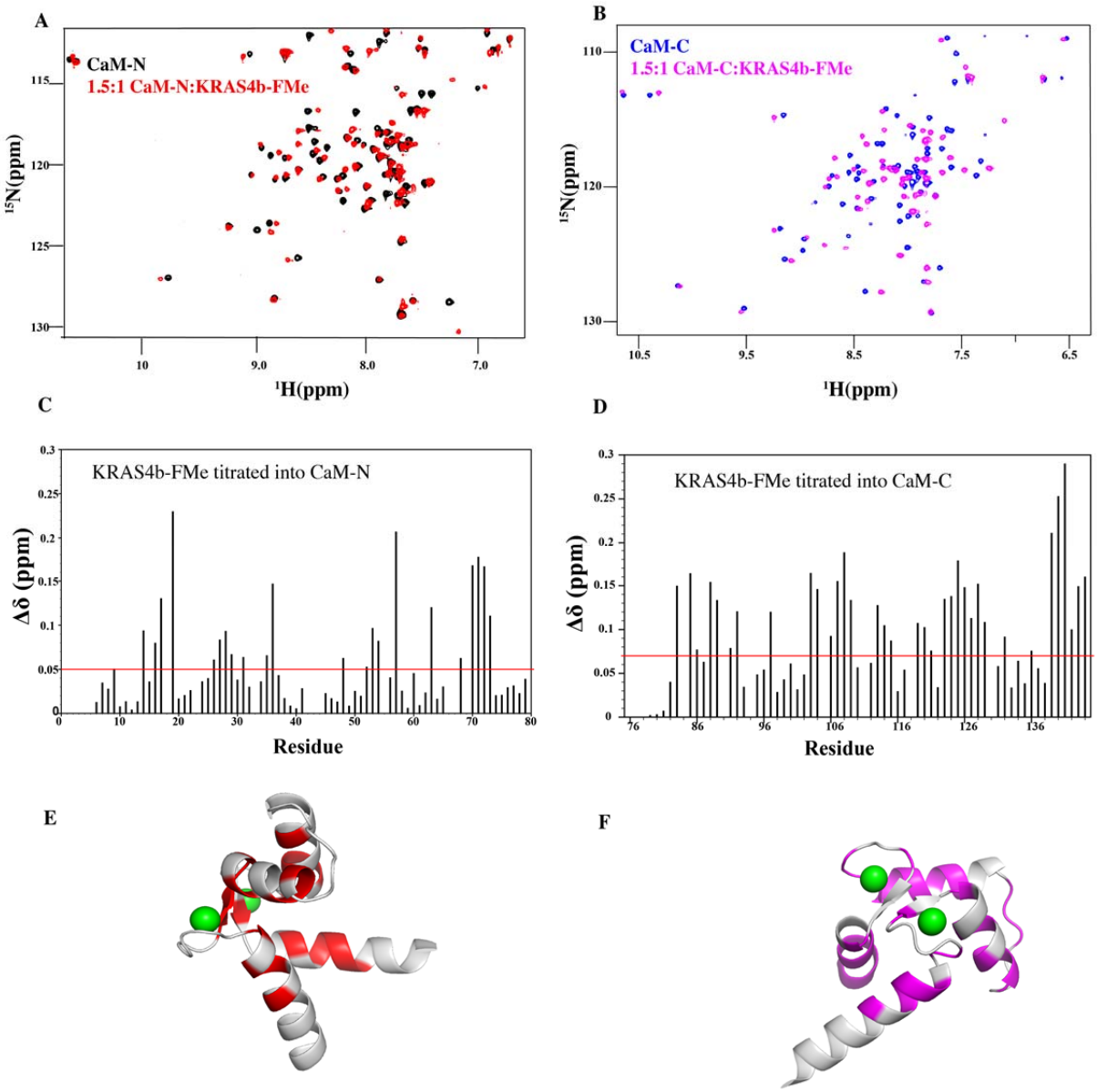

A
CaM-N
1.5:1 CaM-N:KRAS4b-FMe
^{15}N(ppm)
^{1}H(ppm)
B
CaM-C
1.5:1 CaM-C:KRAS4b-FMe
^{15}N(ppm)
^{1}H(ppm)
C
KRAS4b-FMe titrated into CaM-N
Δδ (ppm)
Residue
D
KRAS4b-FMe titrated into CaM-C
Δδ (ppm)
Residue
E
F

Figure S5

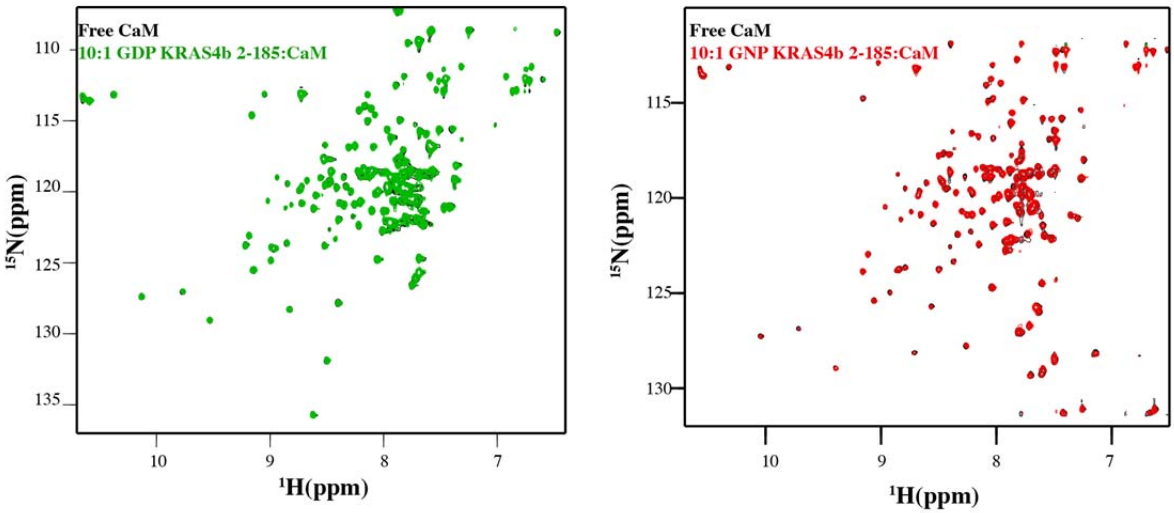
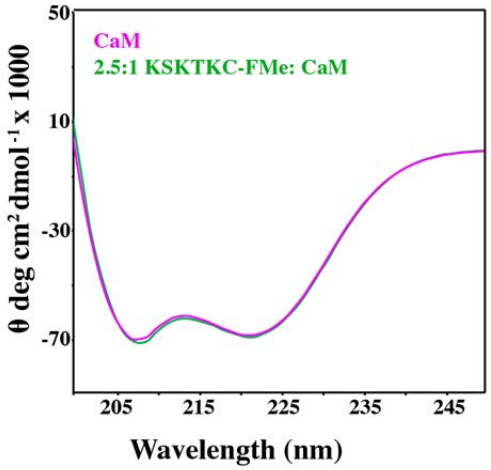

Free CaM
10:1 GDP KRAS4b 2-185:CaM
^{15}N(ppm)
^{1}H(ppm)
Free CaM
10:1 GNP KRAS4b 2-185:CaM
^{15}N(ppm)
^{1}H(ppm)

Figure S6


CaM
2.5:1 KSKTKC-FMe: CaM
θ deg cm² dmol⁻¹ x 1000
50
10
-30
-70
205
215
225
235
245
Wavelength (nm)