Characterization of structure, dynamics and detergent interactions of the anti-HIV chemokine variant 5P12-RANTES

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Supporting Material

RANTES-E66S

5’-gga tcc gac gac gac gac aag tcc cca tat tcc tcg gac acc aca ccc tgc tgc ttt gcc tac att gcc-3’

G S D D D D K S P Y S S D T T P C C F A Y I A

-7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

G S D D D D K Q G P P L M A T Q S C C F A Y I A

5’-gga tcc gac gac gac gac aag cag ggc cca cct tta atg gcc acc caa tcc tgc tgc ttt gcc tac att gcc-3’

5P12-RANTES-E66S

0Q-S1G-RANTES-E66S primer pair

5’- ga tcc gac gac gac gac aag cag ggc cca tat tcc tcg gac acc -3’

5’- ggt gtc cga gga ata tgg gcc ctg ctt gtc gtc gtc gtc gga tc -3’

T8Q-P9S-RANTES-E66S primer pair

5’- c cca tat tcc tcg gac acc caa tcc tgc tgc ttt gcc tac att g -3’

5’- c aat gta ggc aaa gca gca gga ttg ggt gtc cga gga ata tgg g -3’

Y3P-S4L-S5M-D6A-RANTES-E66S primer pair

5’- gac gac aag cag ggc cca cct tta atg gcc acc caa tcc tgc tgc -3’

5’- gca gca gga ttg ggt ggc cat taa agg tgg gcc ctg ctt gtc gtc -3’

Fig. S1 Generation of 5P12-RANTES-E66S DNA. Fragment of pGEV2 plasmid with the N-terminus of RANTES-E66S (top) and three primer pairs designed for site-directed mutagenesis PCR (bottom). Differing nucleotides and amino acids were marked with green (RANTES-E66S) and red (5P12-RANTES-E66S).

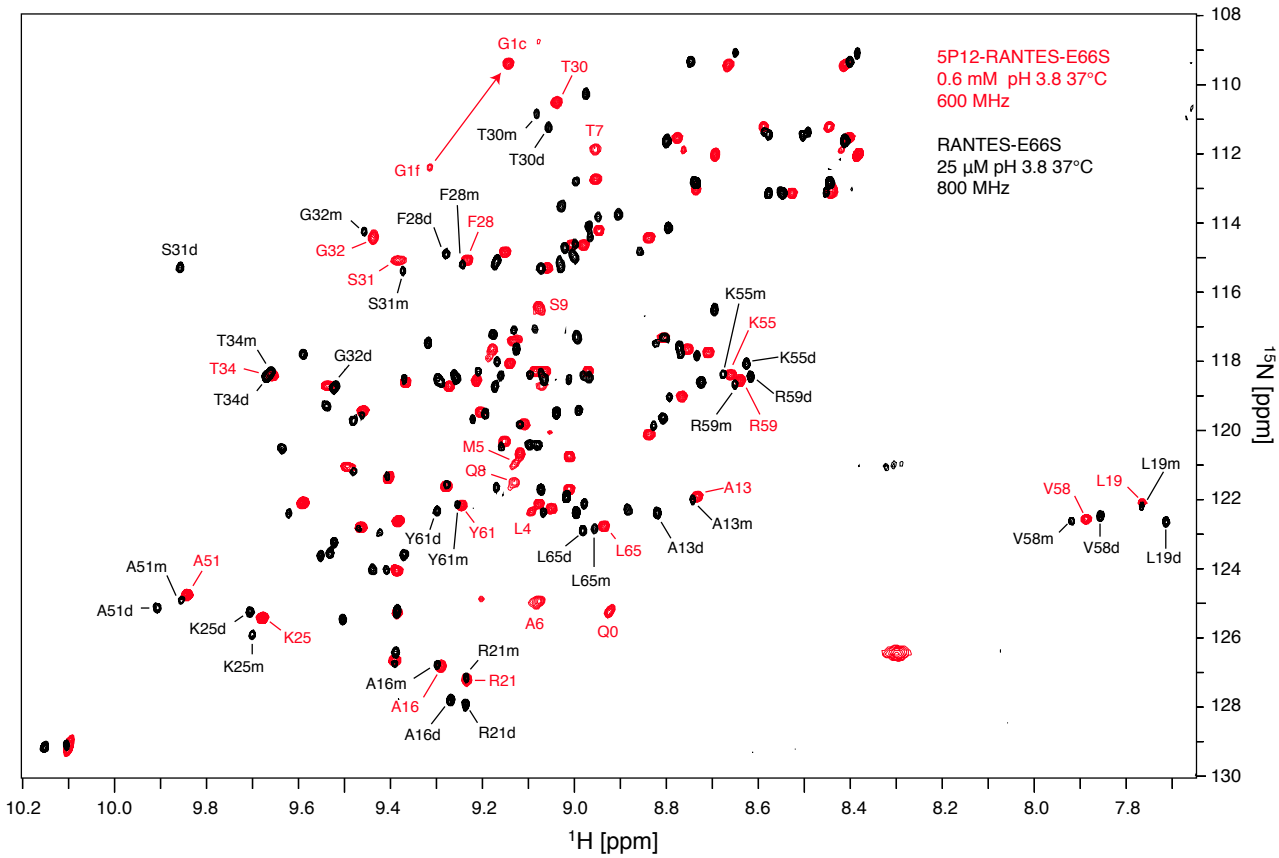


Fig. S2 Overlay of ^{1}H-^{15}N HSQC spectrum of 25 μM RANTES-E66S recorded intentionally at low concentration to increase monomer/dimer ratio (black) onto the spectrum of 0.6 mM 5P12-RANTES-E66S (red). Exemplary resonances are labeled with assignment information and with letters m for monomers and d for dimers. Chemical shifts of 5P12-RANTES-E66S resonances (A13, R21, S31, A51, Y61, etc.) are more similar to RANTES-E66S monomer (A13m, R21m, S31m, A51m, Y61m, etc.) than to RANTES-E66S dimer (A13, R21d, S31d, A51d, Y61d, etc.) resonances. As a result of the N-terminal Q0 cyclization of 5P12-RANTES-E66S a shift from the “free” G1f to the “cyclic” G1c is observed (red arrow).

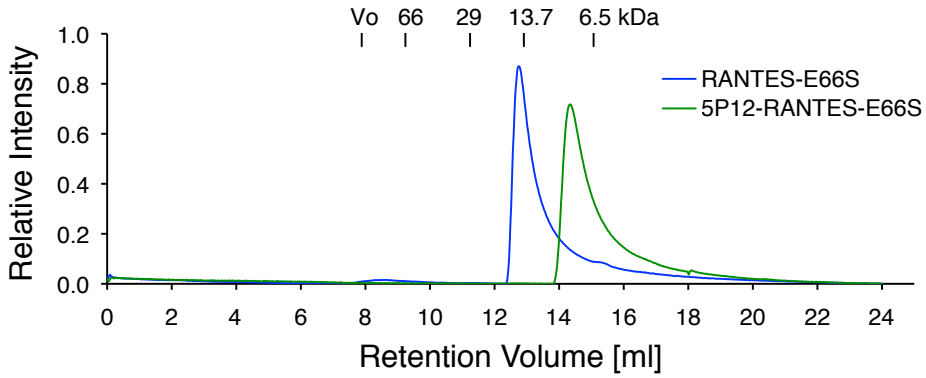


Fig. S3 Size exclusion chromatography of RANTES-E66S (blue) and 5P12-RANTES-E66S (green). RANTES-E66S elutes at ~12.8 ml which corresponds to 15.7 ± 0.1 (SD) kDa and matches the size of RANTES-E66S dimer (15.6 kDa). 5P12-RANTES-E66S elutes at ~14.3 mL which corresponds to 8.5 ± 0.4 kDa (N=2) and is a good agreement with the mass of 5P12-RANTES-E66S monomer (7.9 kDa). The experiment was performed with 40 μg of each RANTES variant on a Superdex 75 10/300 GL column (GE Healthcare, Little Chalfont, UK) in 20 mM Na_2HPO_4 pH 7.4, 180 mM NaCl at RT and 0.5 mL/min flow rate. The column was calibrated using blue dextran, albumin, carbonic anhydrase, aprotinin (Sigma-Aldrich, St. Louis, USA) and ribonuclease A (Invitrogen, Carlsbad, USA). Vo, void volume (7.9 mL).

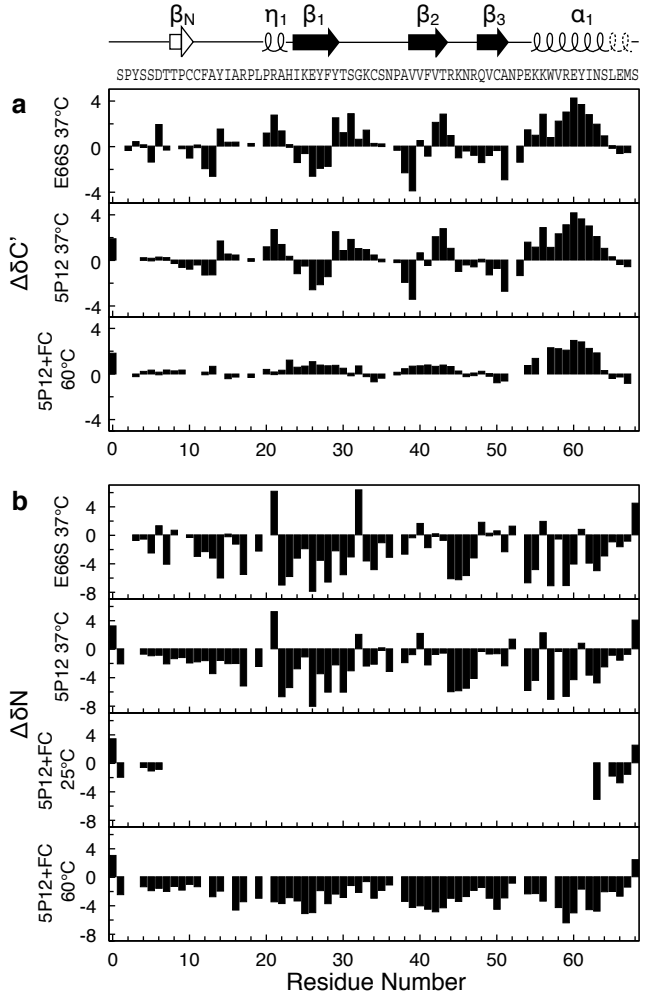


Fig. S4 Secondary $^{13}C'$ (a) and ^{15}N (b) chemical shifts analysis of RANTES-E66S dimer (37 °C), 5P12-RANTES-E66S (37 °C) and 5P12-RANTES-E66S in the presence of 1 % FC-12 (at 25 °C and 60 °C). Secondary structure elements according to the crystal structure 1EQT and the amino acid sequence of the wild type RANTES are drawn at the top.

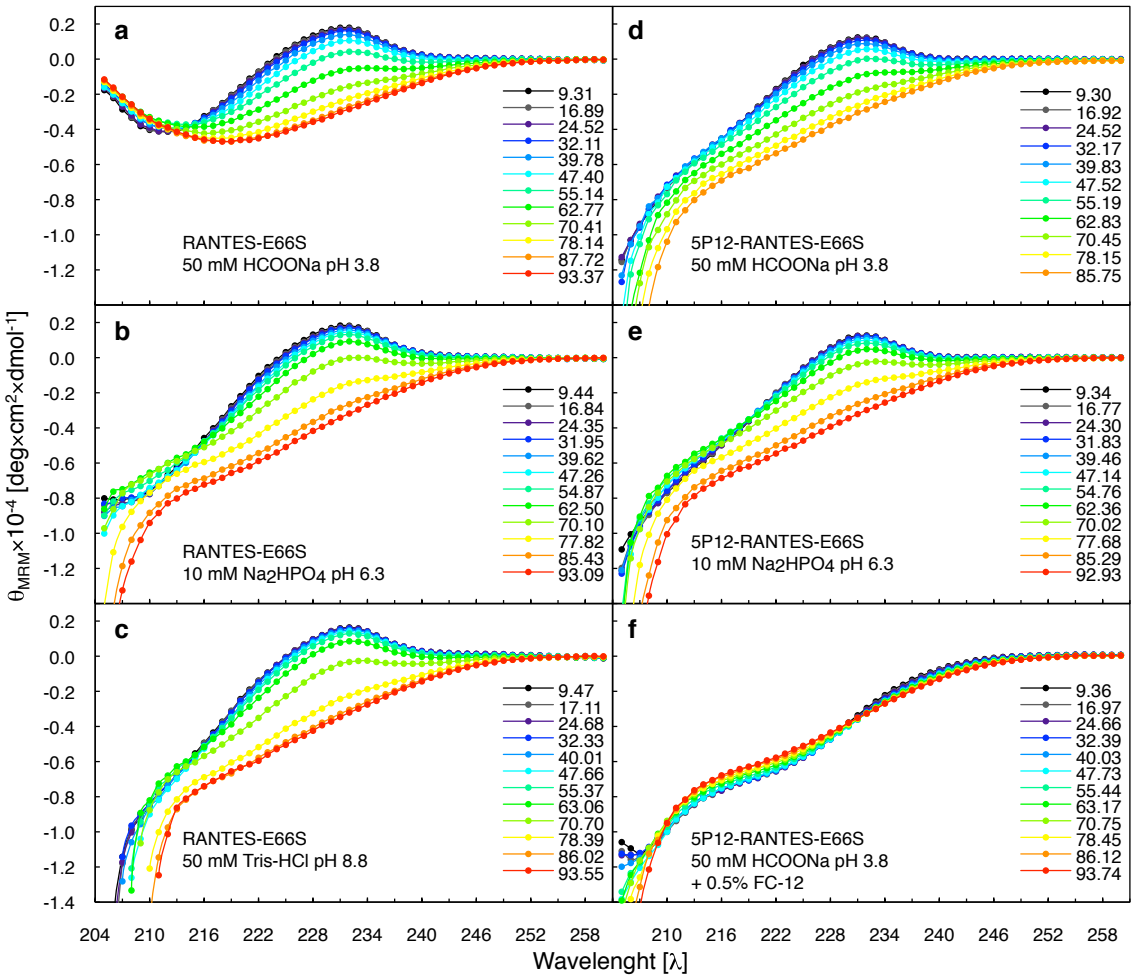
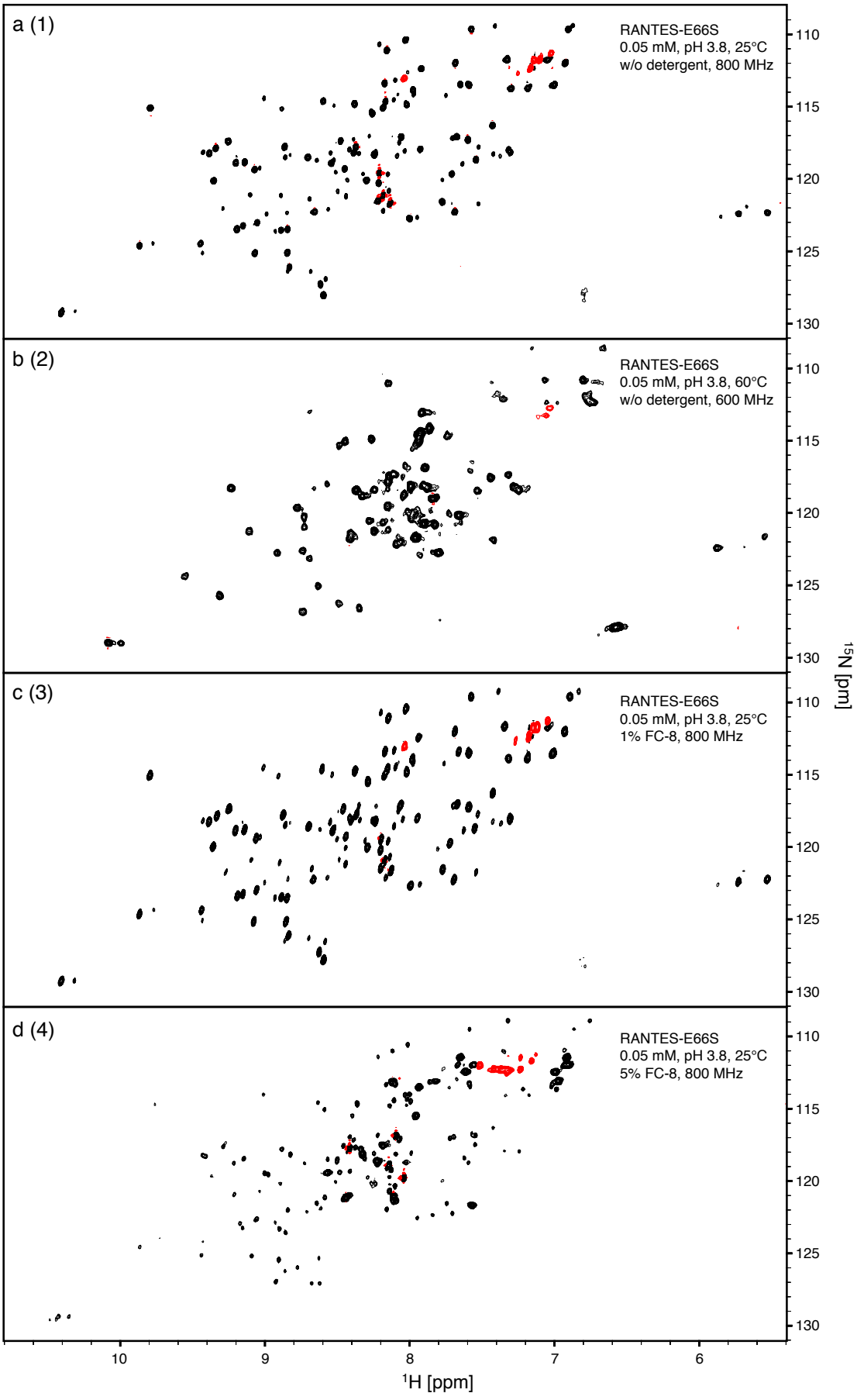
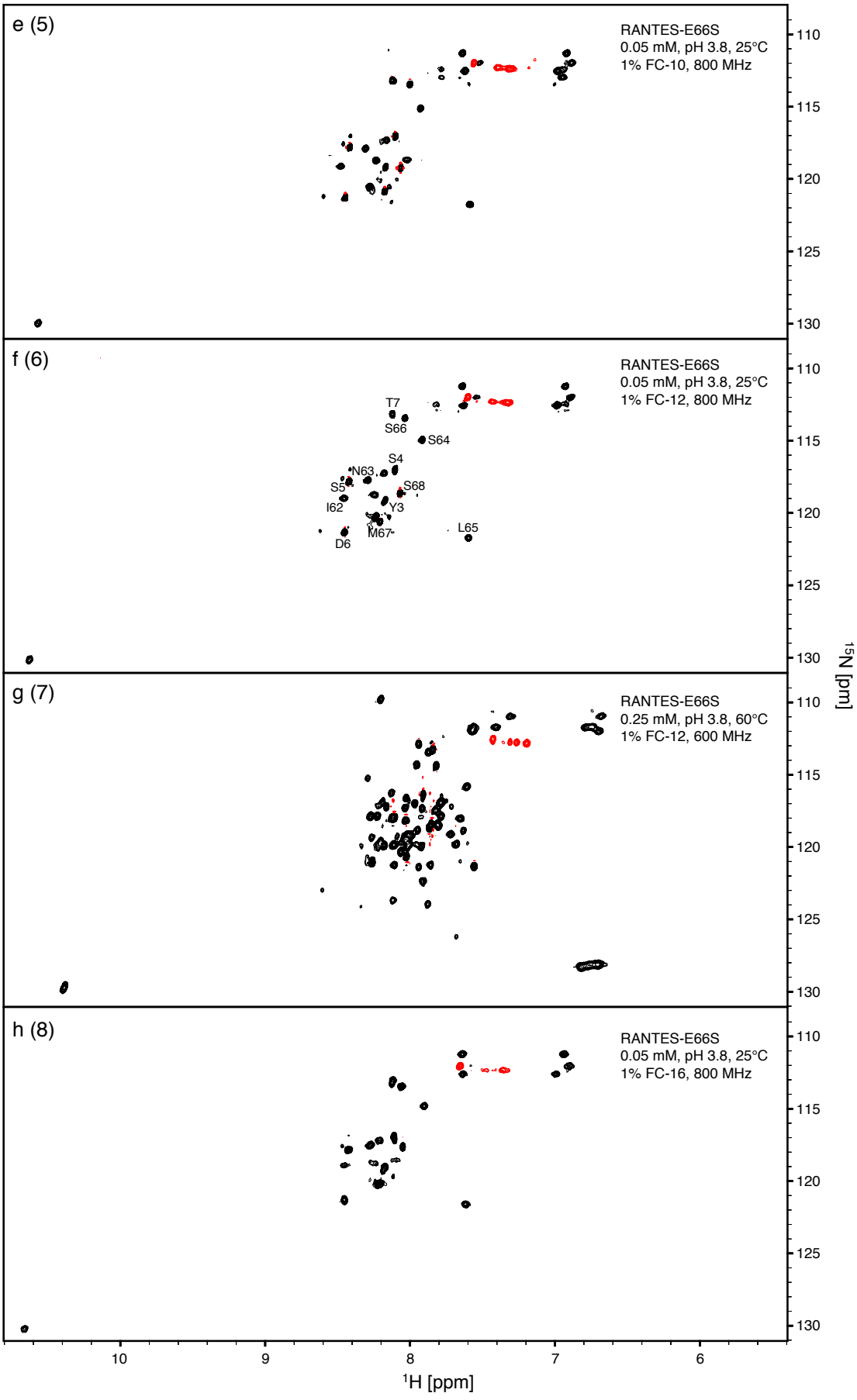
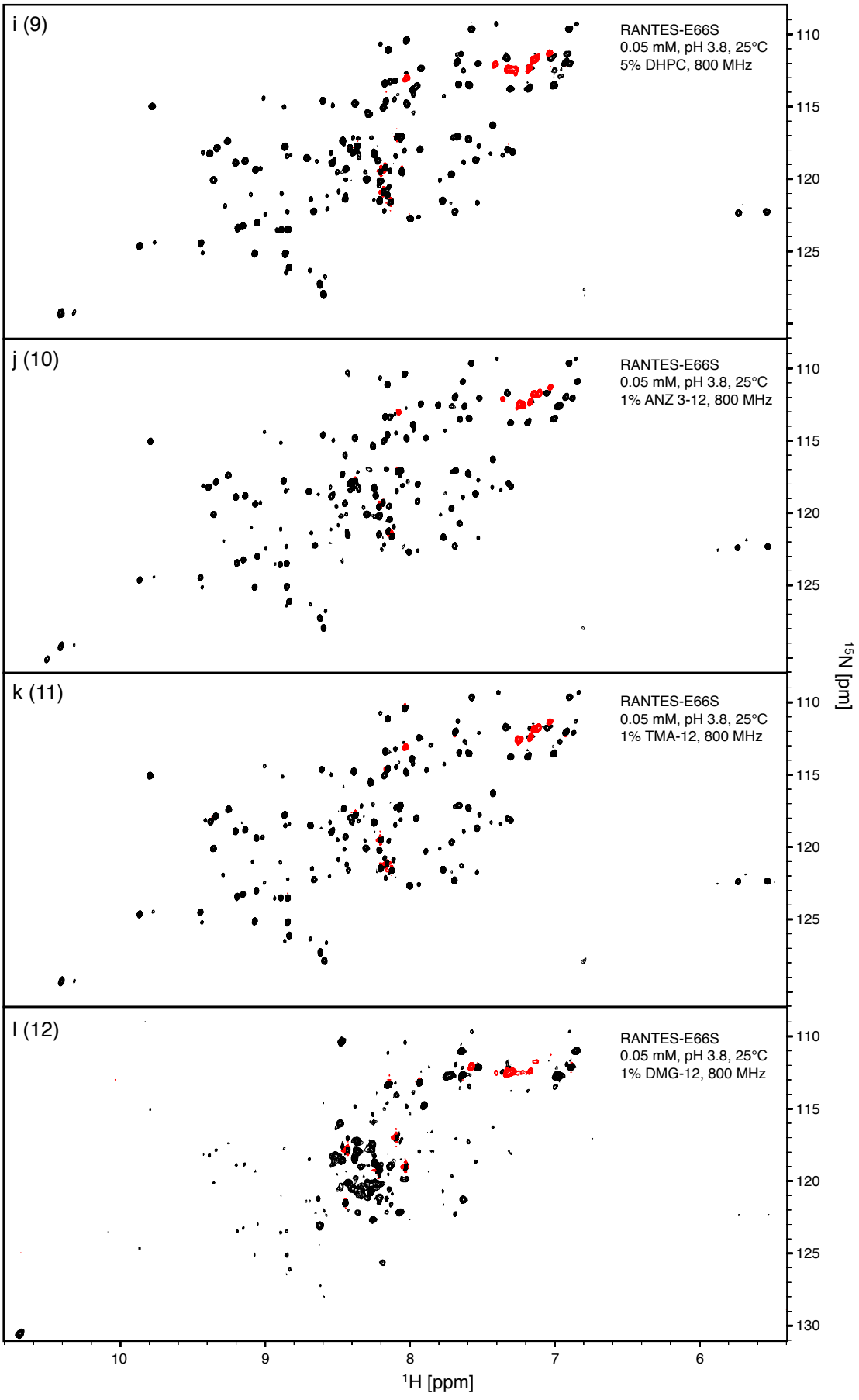
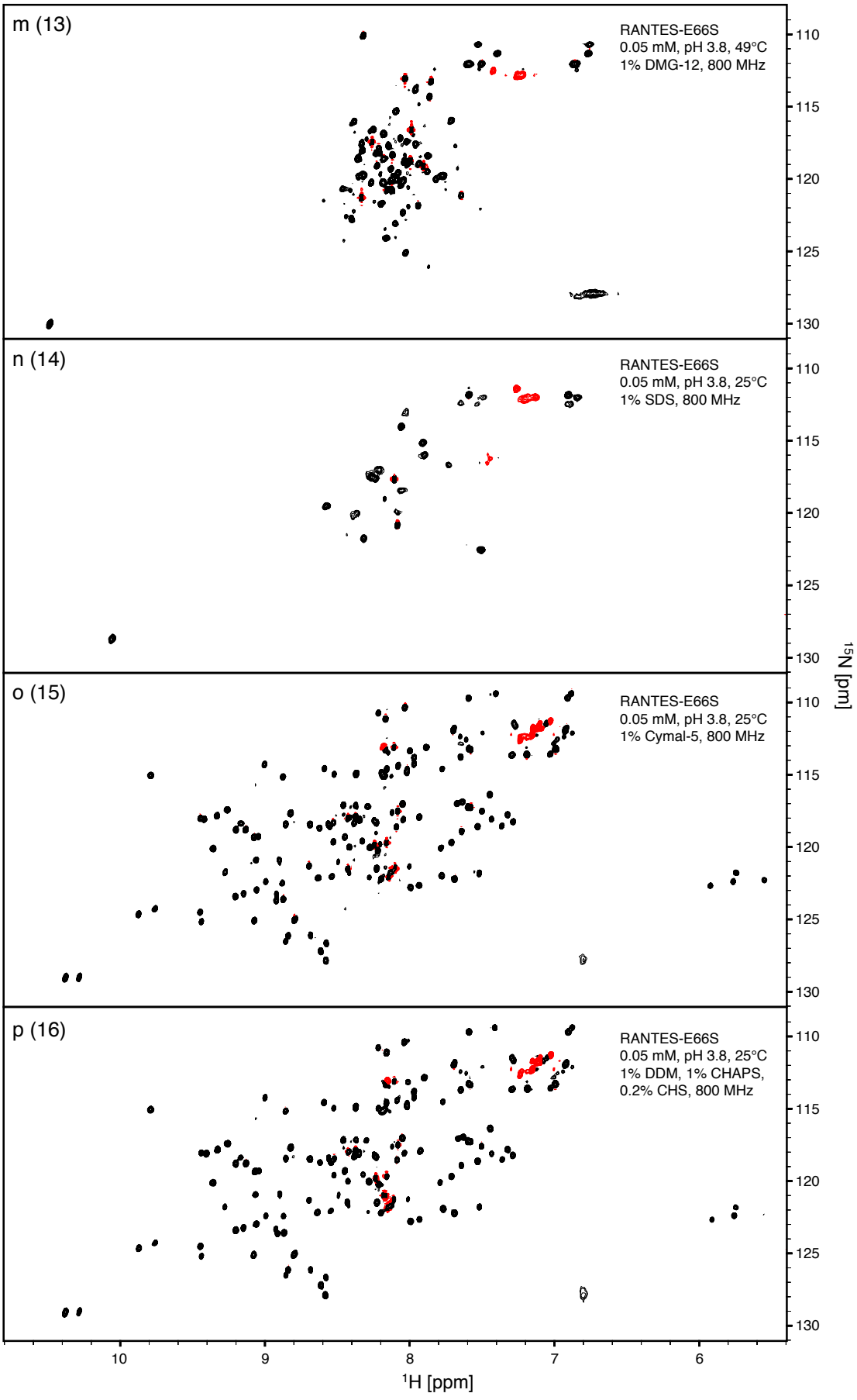


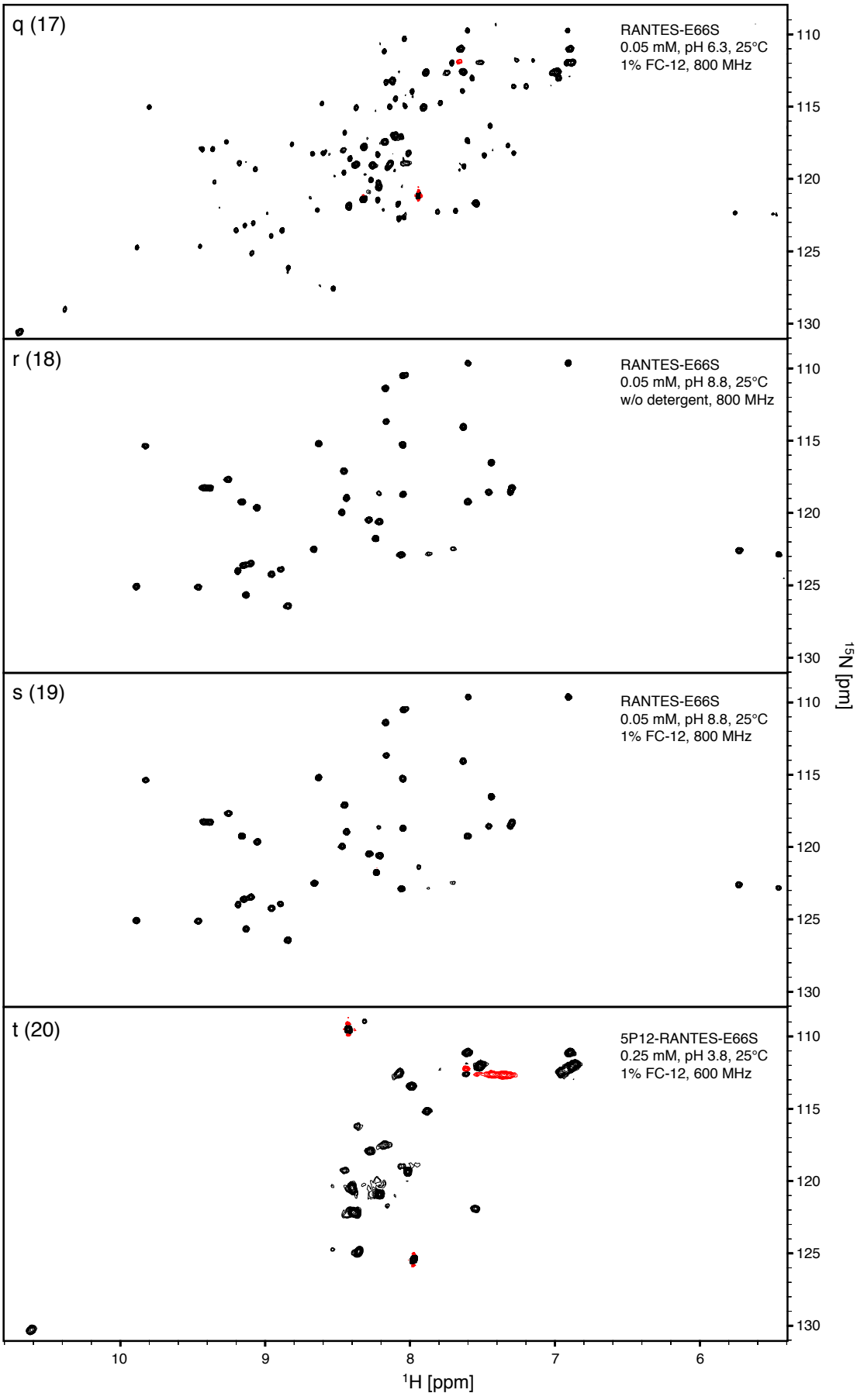
Fig. S5 CD thermal denaturation experiments on RANTES-E66S (a-c) and 5P12-RANTES-E66S (d-f) at various conditions monitored as θ_{MRM} in the 204-260 nm range. Spectra were collected every 2 degrees from 10 °C to 98 °C (set values). The labels correspond to the real temperature monitored by an independent sensor placed in the sample cuvette. For clarity of the presentation every forth measurement is shown.

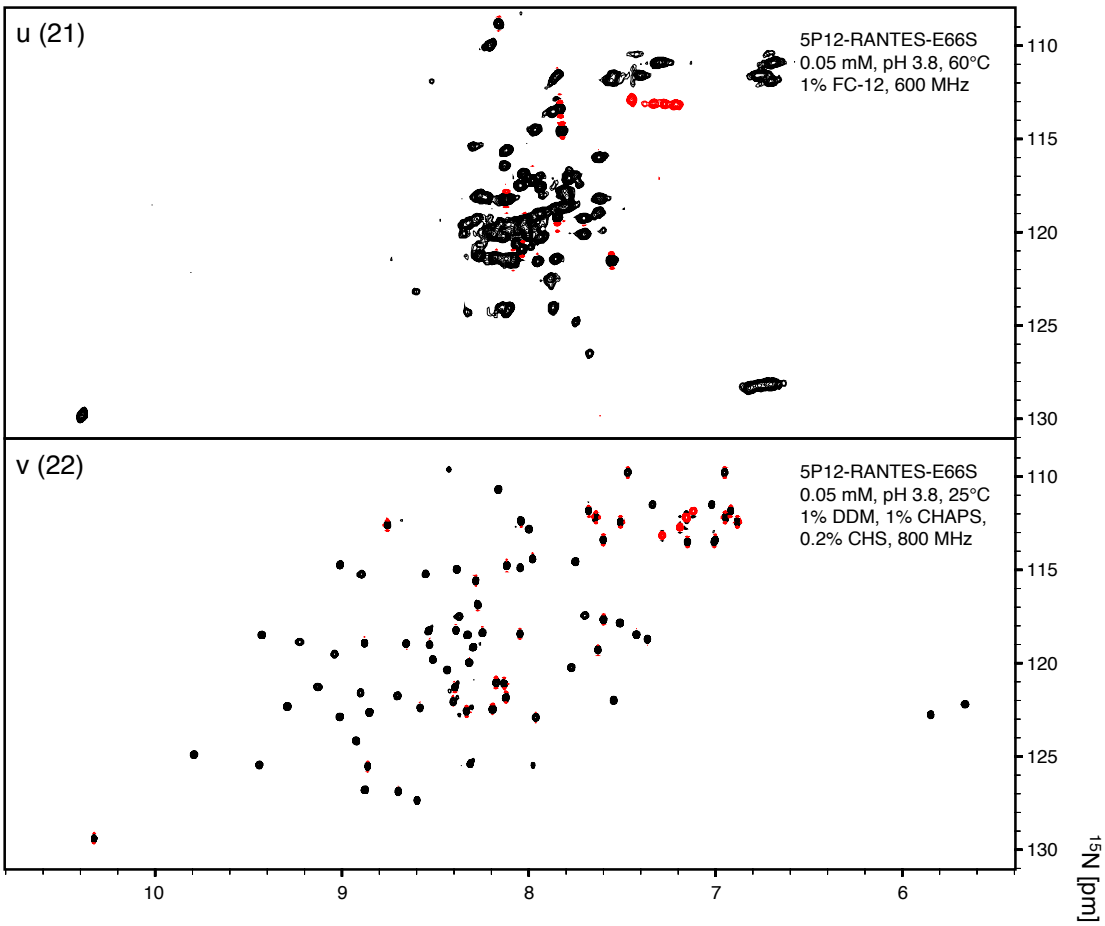

a (1)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
w/o detergent, 800 MHz
b (2)
RANTES-E66S
0.05 mM, pH 3.8, 60°C
w/o detergent, 600 MHz
c (3)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% FC-8, 800 MHz
d (4)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
5% FC-8, 800 MHz
110
115
120
125
130
10
9
8
7
6
^{1}H [ppm]
^{15}N [ppm]

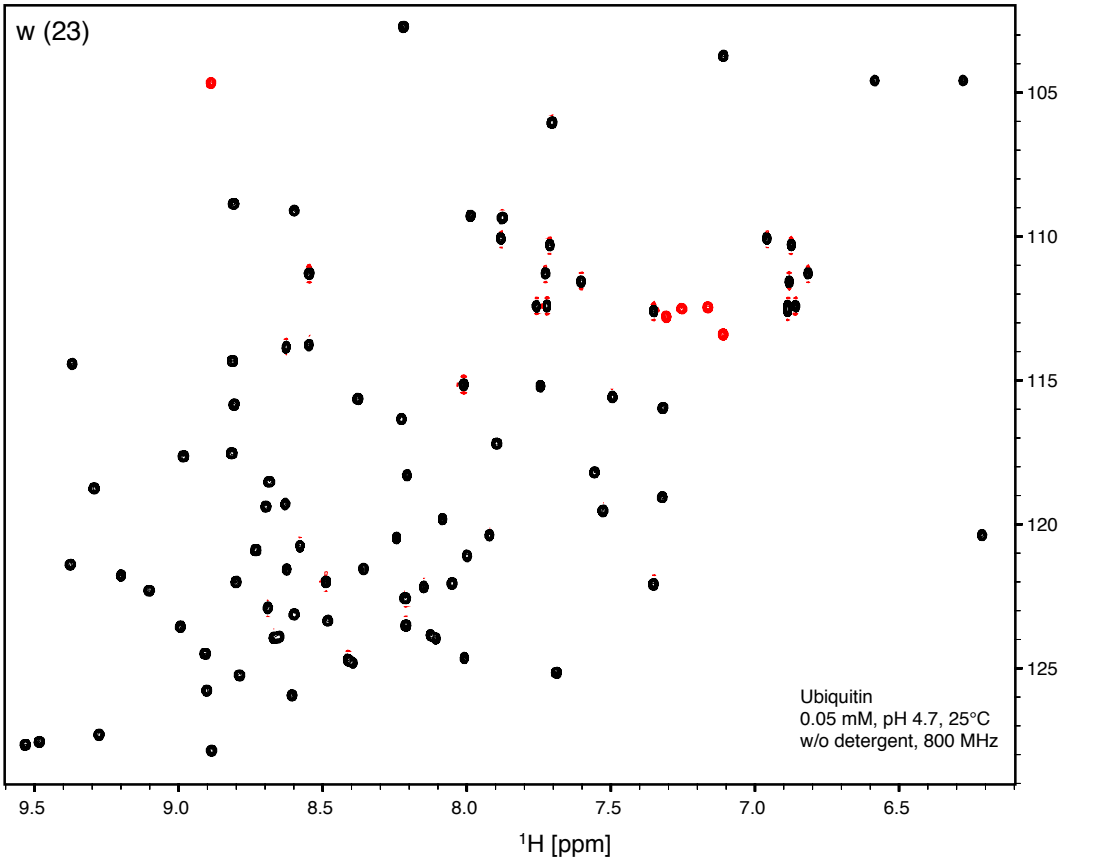

e (5)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% FC-10, 800 MHz
f (6)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% FC-12, 800 MHz
T7
S66
S64
S4
N63
S5
S68
I62
Y3
M67
L65
D6
g (7)
RANTES-E66S
0.25 mM, pH 3.8, 60°C
1% FC-12, 600 MHz
h (8)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% FC-16, 800 MHz
110
115
120
125
130
10
9
8
7
6
^{1}H [ppm]
^{15}N [ppm]


i (9)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
5% DHPC, 800 MHz
j (10)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% ANZ 3-12, 800 MHz
k (11)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% TMA-12, 800 MHz
l (12)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% DMG-12, 800 MHz
110
115
120
125
130
10
9
8
7
6
^{1}H [ppm]
^{15}N [ppm]


m (13)
RANTES-E66S
0.05 mM, pH 3.8, 49°C
1% DMG-12, 800 MHz
n (14)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% SDS, 800 MHz
o (15)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% Cymal-5, 800 MHz
p (16)
RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% DDM, 1% CHAPS,
0.2% CHS, 800 MHz
110
115
120
125
130
10
9
8
7
6
^{1}H [ppm]
^{15}N [ppm]


q (17)
RANTES-E66S
0.05 mM, pH 6.3, 25°C
1% FC-12, 800 MHz
r (18)
RANTES-E66S
0.05 mM, pH 8.8, 25°C
w/o detergent, 800 MHz
s (19)
RANTES-E66S
0.05 mM, pH 8.8, 25°C
1% FC-12, 800 MHz
t (20)
5P12-RANTES-E66S
0.25 mM, pH 3.8, 25°C
1% FC-12, 600 MHz
110
115
120
125
130
10
9
8
7
6
^{1}H [ppm]
^{15}N [ppm]


u (21)
5P12-RANTES-E66S
0.05 mM, pH 3.8, 60°C
1% FC-12, 600 MHz
v (22)
5P12-RANTES-E66S
0.05 mM, pH 3.8, 25°C
1% DDM, 1% CHAPS,
0.2% CHS, 800 MHz
110
115
120
125
130
10
9
8
7
6
^{15}N [ppm]


w (23)
Ubiquitin
0.05 mM, pH 4.7, 25°C
w/o detergent, 800 MHz
105
110
115
120
125
9.5
9.0
8.5
8.0
7.5
7.0
6.5
^{1}H [ppm]

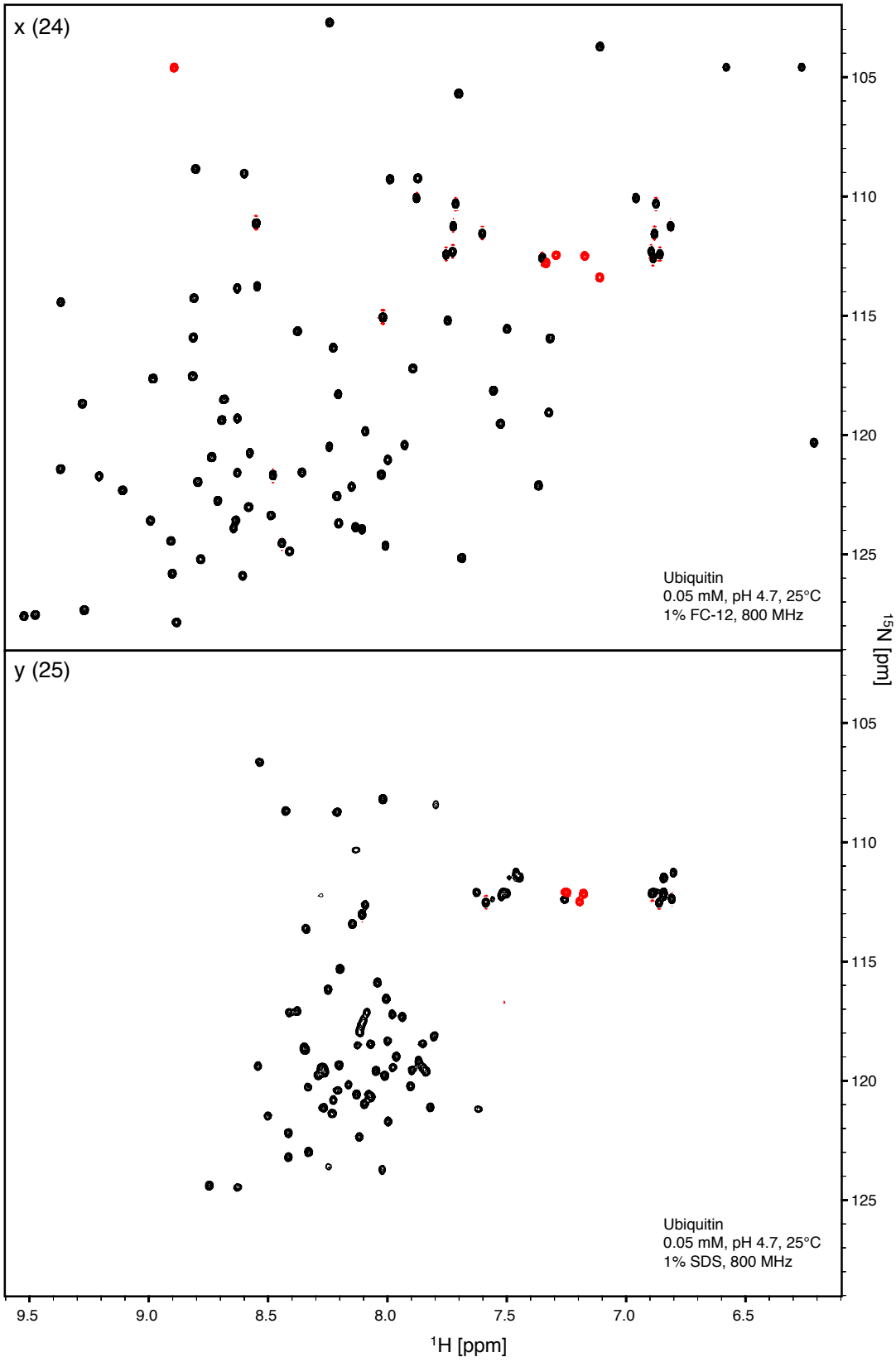


Fig. S6 ^{1}H-^{15}N HSQC spectra (black/red, positive/negative signal) of RANTES-E66S (a-s), 5P12-RANTES-E66S (t-v) and ubiquitin (w-y) at various conditions specified in the top/bottom right corners. Numbers in the brackets correspond to the sample numbers in Table 1.

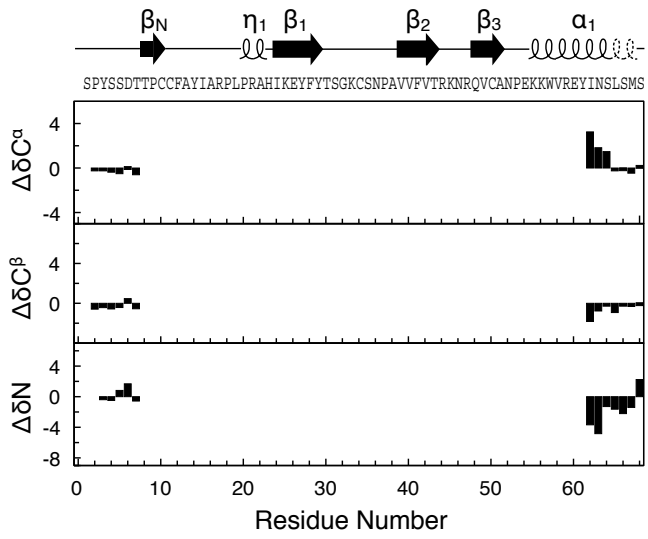


Fig. S7 Secondary $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{15}N chemical shifts analysis of the visible terminal resonances of RANTES-E66S in the presence of detergent at 25 °C (50 μM ^{2}H,^{15}N,^{13}C-labeled protein, 50 mM DCOONa pH 3.8, 1% FC-12, 5% D_2O) shows strong helical propensity for residues 62-64. Secondary structure elements according to the crystal structure 1EQT and the amino acid sequence of RANTES-E66S are drawn at the top.

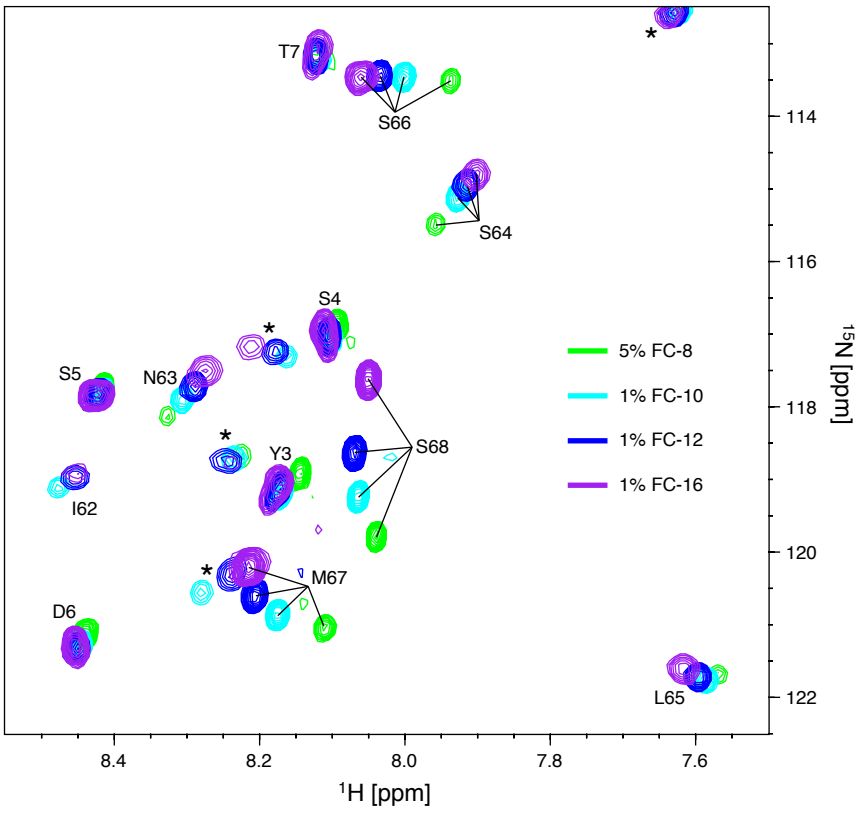
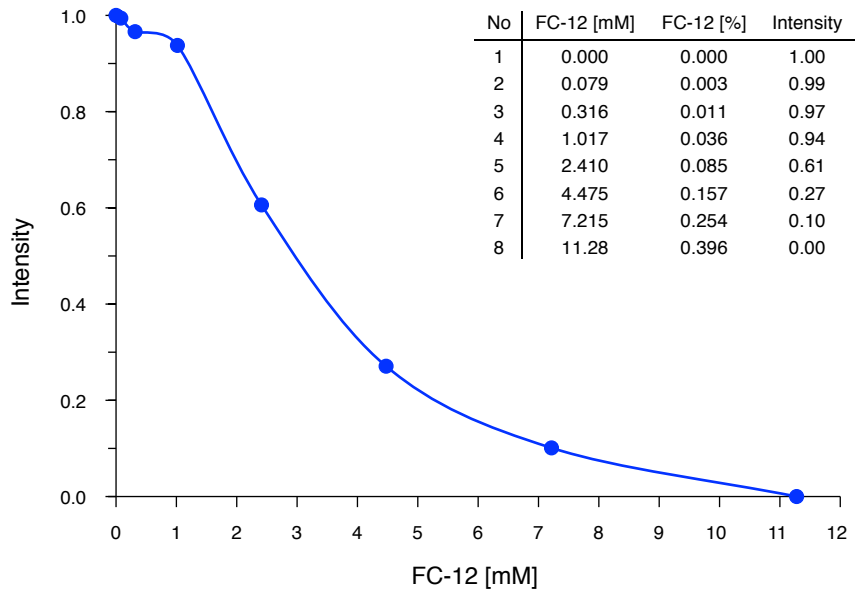


Fig. S8 ^{1}H-^{15}N HSQC spectra of RANTES-E66S (50 μM ^{2}H,^{15}N,^{13}C-labeled protein, 50 mM DCOONa pH 3.8, 5% D_2O) at 25 °C in the presence of 5 % FC-8 (green, higher threshold than in Fig. S7d), 1 % FC-10 (cyan), 1 % FC-12 (blue) and 1 % FC-16 (purple). The C-terminal (N63-S68), but not the N-terminal (Y3-T7) resonances shift significantly with the increasing length of the detergent hydrocarbon tail. Unassigned resonances were marked with an asterisk.



No	FC-12 [mM]	FC-12 [%]	Intensity
1	0.000	0.000	1.00
2	0.079	0.003	0.99
3	0.316	0.011	0.97
4	1.017	0.036	0.94
5	2.410	0.085	0.61
6	4.475	0.157	0.27
7	7.215	0.254	0.10
8	11.28	0.396	0.00

Fig. S9 Intensity of native-state 5P12-RANTES-E66S backbone ^{1}H-^{15}N resonances (average of residues L4-S68) as a function of FC-12 concentration. With the exception of G1, the intensity of the resonances decreases uniformly throughout the amino acid sequence (Fig. S7b).

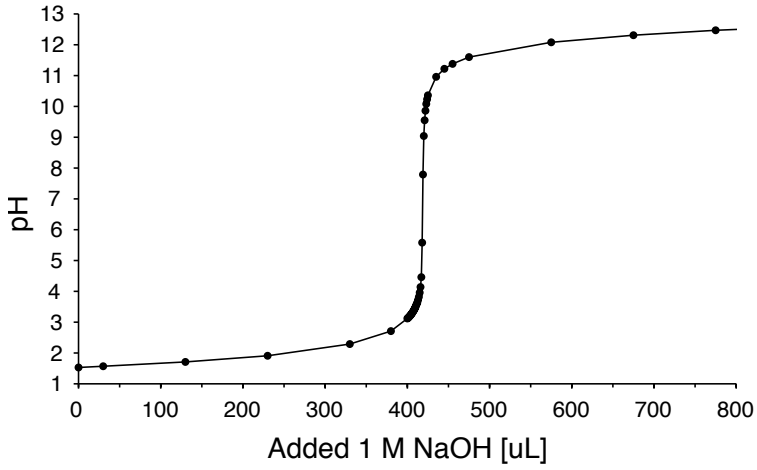


Fig. S10 Titration of 20 mM FC-12 (10 mL). The solution was acidified to pH 1.5 with HCl and subsequently titrated with 1 M NaOH.

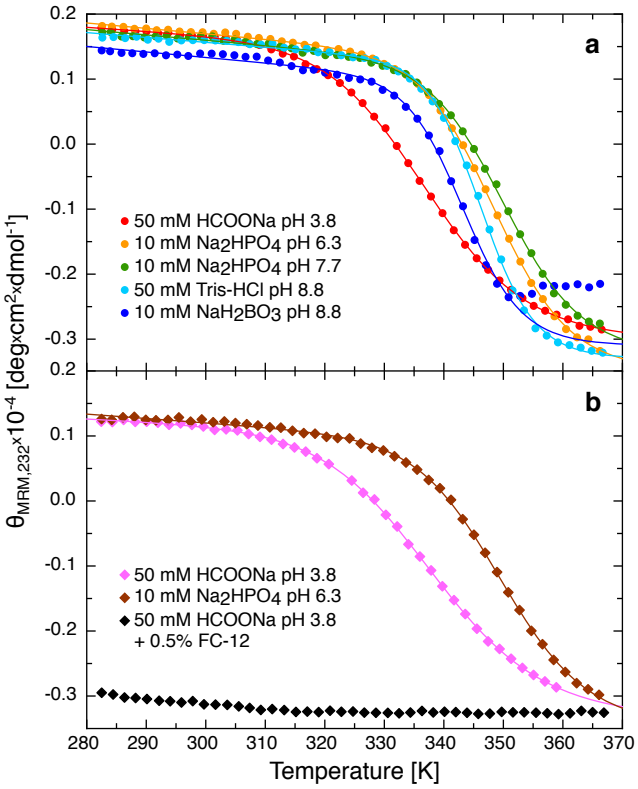
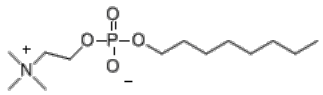
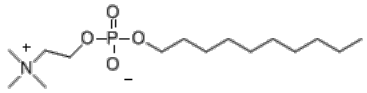
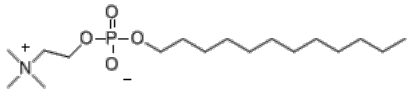
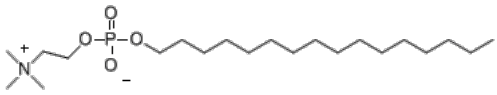
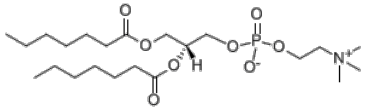
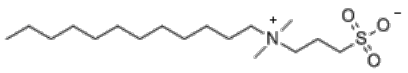
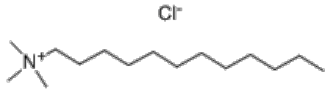
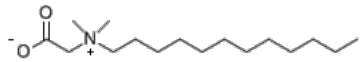
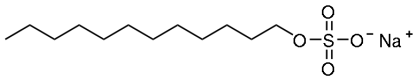
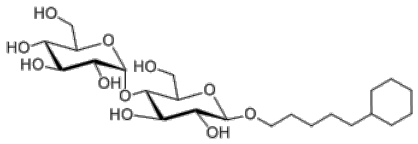
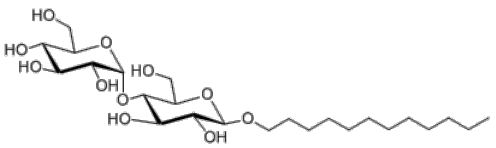
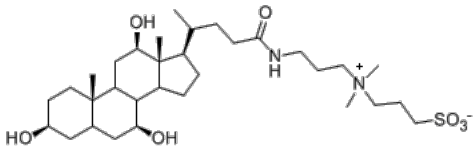
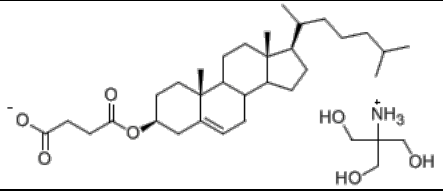


Fig. S11 CD thermal denaturation experiments on RANTES-E66S (a) and 5P12-RANTES-E66S (b) at various conditions monitored as θ_{MRM} at 232 nm. For the measurement in 10 mM NaH_2BO_3 pH 8.8 (blue) data points beyond 352 K were omitted from fitting (protein precipitation). The pH was adjusted at RT. For the measurement in 50 mM Tris-HCl pH 8.8 (cyan), the real pH values at a given temperature deviates from the value at RT due to the large temperature coefficient of the Tris protonation ($d(pK_a)/dT = -0.028$).

Table S1 List of used detergents including their acronyms, chemical names, molecular weight (MW), approximate critical micelle concentration (CMC), approximate aggregation number (AN) and chemical structure.

Detergent	MW	CMC [mM]	CMC [%]	AN	Chemical Structure
FC-8 (Fos-Choline-8) n-Octylphosphocholine	295.4	114[1]	3.4	N/A	
FC-10 (Fos-Choline-10) n-Decylphosphocholine	323.4	11[1]	0.35	24[1]	
FC-12 (Fos-Choline-12) n-Dodecylphosphocholine	351.5	1.5[1]	0.047	54[1]	
FC-16 (Fos-Choline-16) n-Hexadecylphosphocholine	407.5	0.013[1]	0.00053	178[1]	
DHPC 1,2-Diheptanoyl-*sn*-Glycero-3-Phosphocholine	481.5	1.4[2]	0.067	N/A	
ANZ-3-12 (Anzergent-3-12) n-Dodecyl-N,N-Dimethyl-3-Ammonio-1-Propanesulfonate	335.5	2.8[1]	0.094	55-87[1]	
TMA-12 N-Dodecyl Trimethylammonium Chloride	263.9	1-20[3]	0.03–0.53	N/A	
DMG-12 n-Dodecyl-N,N-Dimethylglycine	271.4	1.5[1]	0.041	N/A	
SDS Sodium Dodecyl Sulfate	288.4	8[2]	0.23	62[4]	
Cymal-5 5-Cyclohexyl-1-Pentyl-β-D-Maltoside	494.5	2.4[1]	0.12	47[1]	
DDM n-Dodecyl-β-D-Maltopyranoside	510.6	0.17[1]	0.0087	78-149[1]	
CHAPS 3-[(3-Cholamidopropyl)-Dimethylammonio]-1-Propane Sulfonate	614.9	8[1]	0.49	10[1]	
CHS Cholesteryl Hemisuccinate Tris Salt	607.9	N/A	N/A	N/A	

[1] According to Anatrace product information
[2] According to Avanti product information
[3] Depending on salt according to Sarac and Bester-Rogac (1)
[4] According to Turro and Yekta (2)

References

1. Sarac, B., and M. Bester-Rogac. 2009. Temperature and salt-induced micellization of dodecyltrimethylammonium chloride in aqueous solution: a thermodynamic study. J Colloid Interface Sci 338:216-221.
2. Turro, N. J., and A. Yekta. 1978. Luminescent probes for detergent solutions. A simple procedure for determination of the mean aggregation number of micelles. J Am Chem Soc 100:5951–5952.