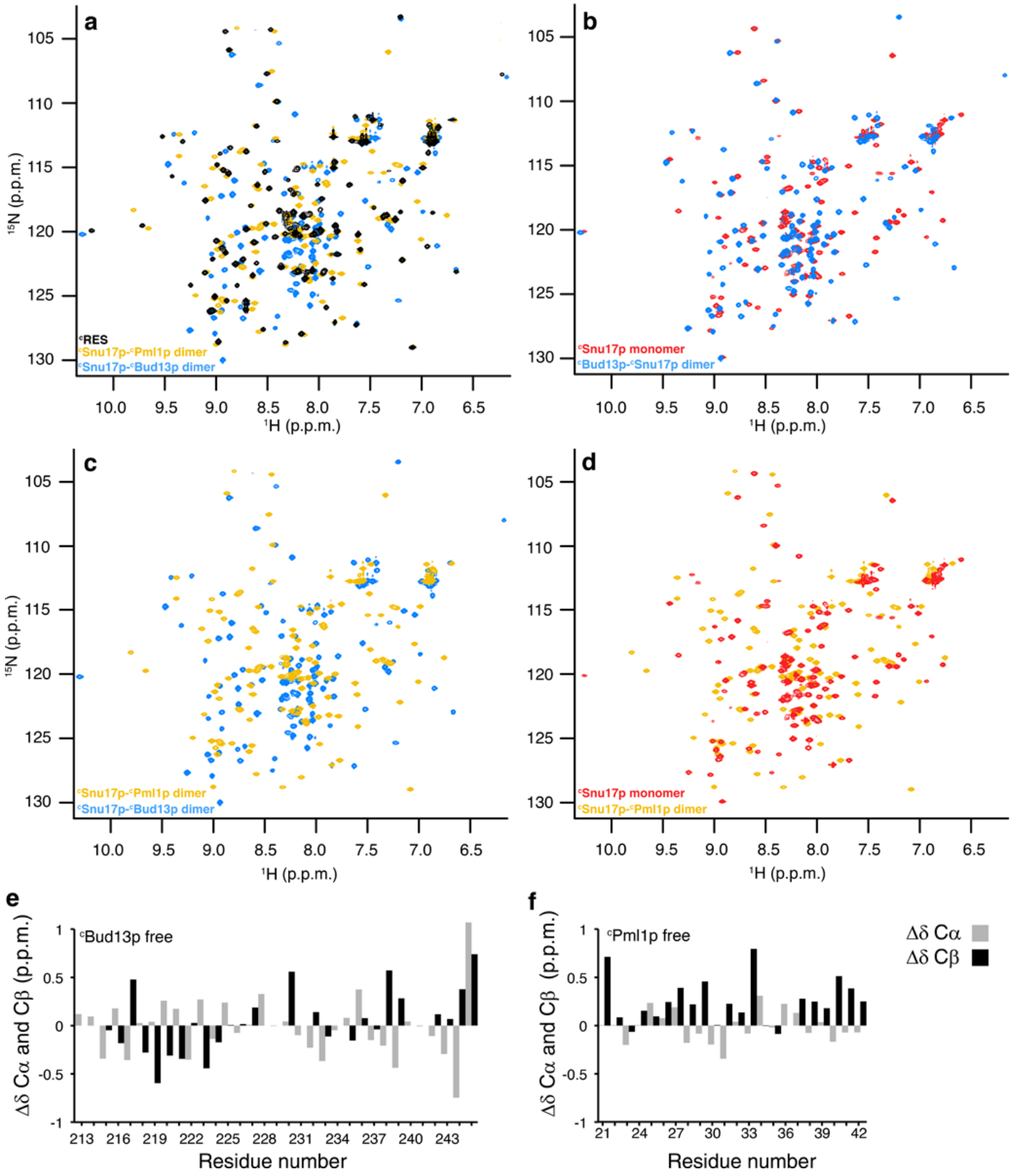
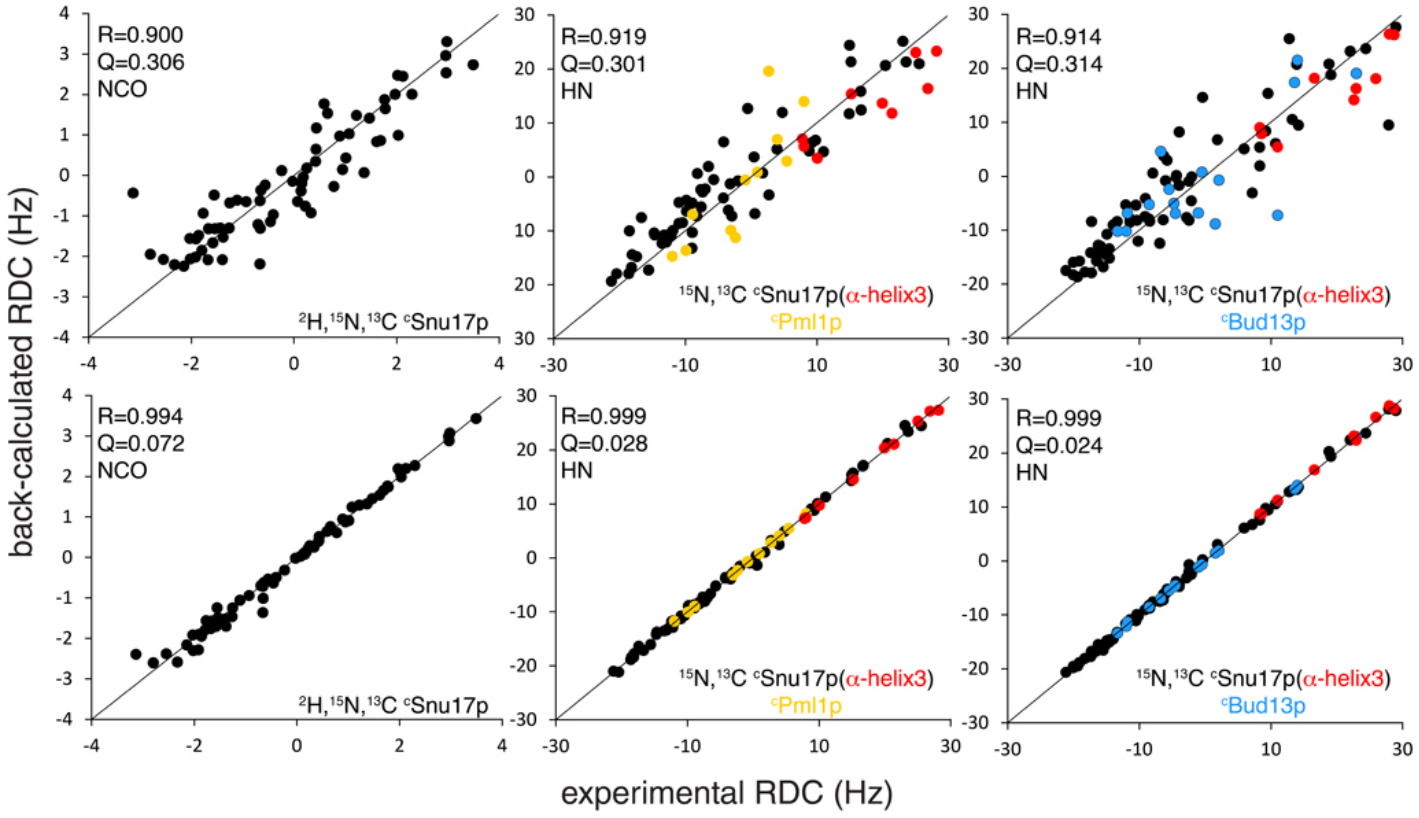

a
b
c
d
e
f
105
110
115
120
125
130
^{15}N (p.p.m.)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
^{1}H (p.p.m.)
^{c}RES
^{c}Snu17p-^{c}Pml1p dimer
^{c}Snu17p-^{c}Bud13p dimer
^{c}Snu17p monomer
^{c}Bud13p-^{c}Snu17p dimer
^{c}Snu17p monomer
^{c}Snu17p-^{c}Pml1p dimer
^{c}Bud13p free
^{c}Pml1p free
Δδ Cα
Δδ Cβ
Δδ Cα and Cβ (p.p.m.)
1
0.5
0
-0.5
-1
213
216
219
222
225
228
231
234
237
240
243
21
24
27
30
33
36
39
42
Residue number

Supplementary Figure 1

Comparison of ^{1}H-^{15}N HSQC of ^{c}Snu17p in various complexes and secondary chemical shift of free ^{c}Pml1p and ^{c}Bud13p.

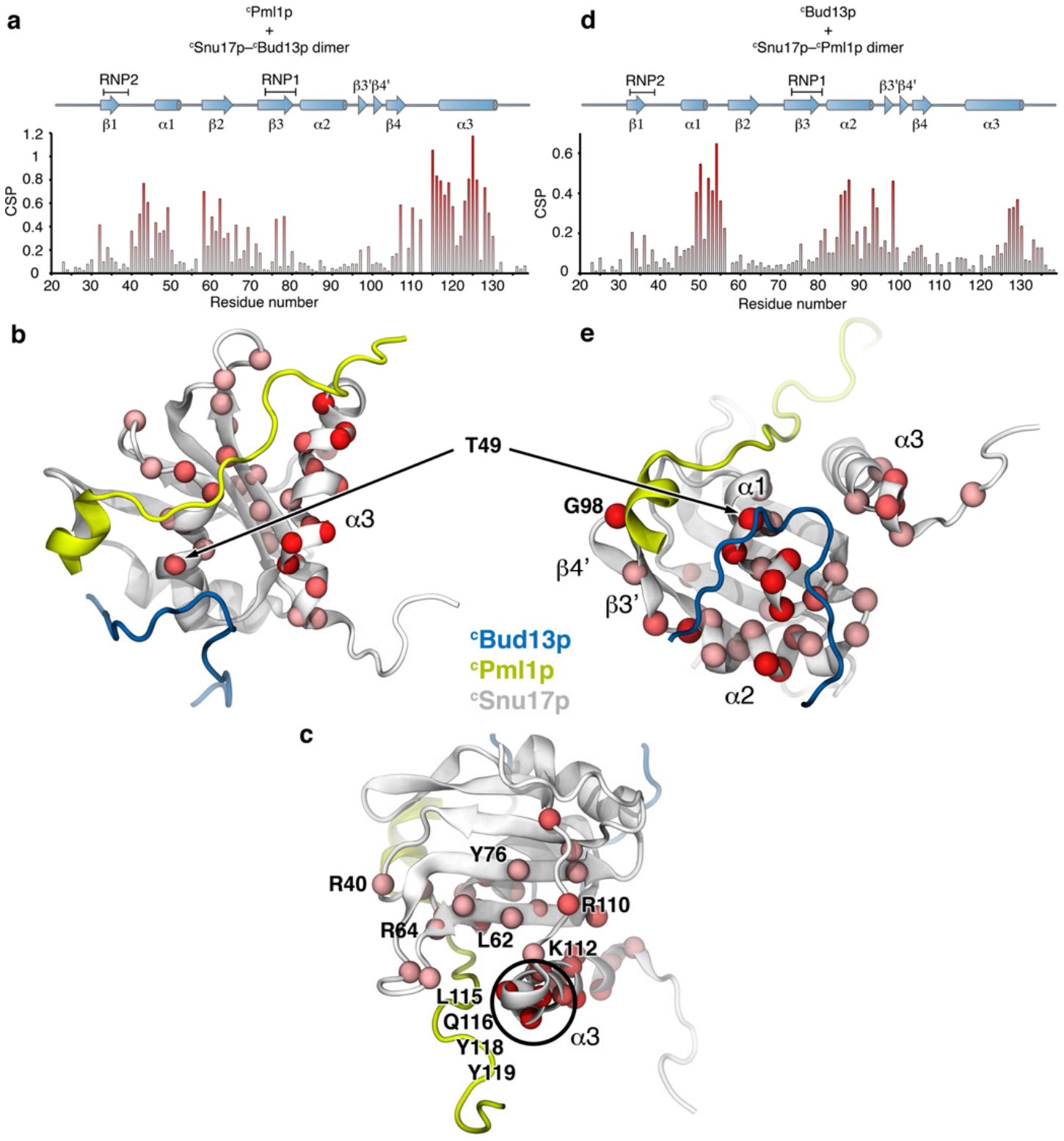
(**a**) ^{1}H, ^{15}N HSQC of ^{c}Snu17p in ^{c}Pml1p–^{c}Snu17p dimer, ^{c}Bud13p–^{c}Snu17p dimer, and ^{c}RES. (**b**) ^{1}H, ^{15}N HSQC of ^{c}Snu17p in ^{c}Pml1p–^{c}Snu17p dimer and ^{c}Snu17p monomer. (**c**) ^{1}H, ^{15}N HSQC of ^{c}Snu17p in ^{c}Pml1p–^{c}Snu17p dimer and ^{c}Bud13p–^{c}Snu17p dimer. (d) ^{1}H, ^{15}N HSQC of ^{c}Snu17p in ^{c}Pml1p–^{c}Snu17p dimer and ^{c}Snu17p monomer. (**e**) Secondary chemical shift ($\Delta\delta$) of free ^{c}Bud13p. (**f**) Secondary chemical shift ($\Delta\delta$) of free ^{c}Pml1p.



Supplementary Figure 2

Validation of the ^{c}RES structure with residual dipolar couplings (RDCs).

Plots of experimental vs back-calculated RDCs before (upper panel) and after (lower panel) structure refinement with RDCs. RDCs from the C-terminal α-helix (117-126) backbone amides are indicated in red, those of ^{c}Pml1p in yellow and ^{c}Bud13p in blue.

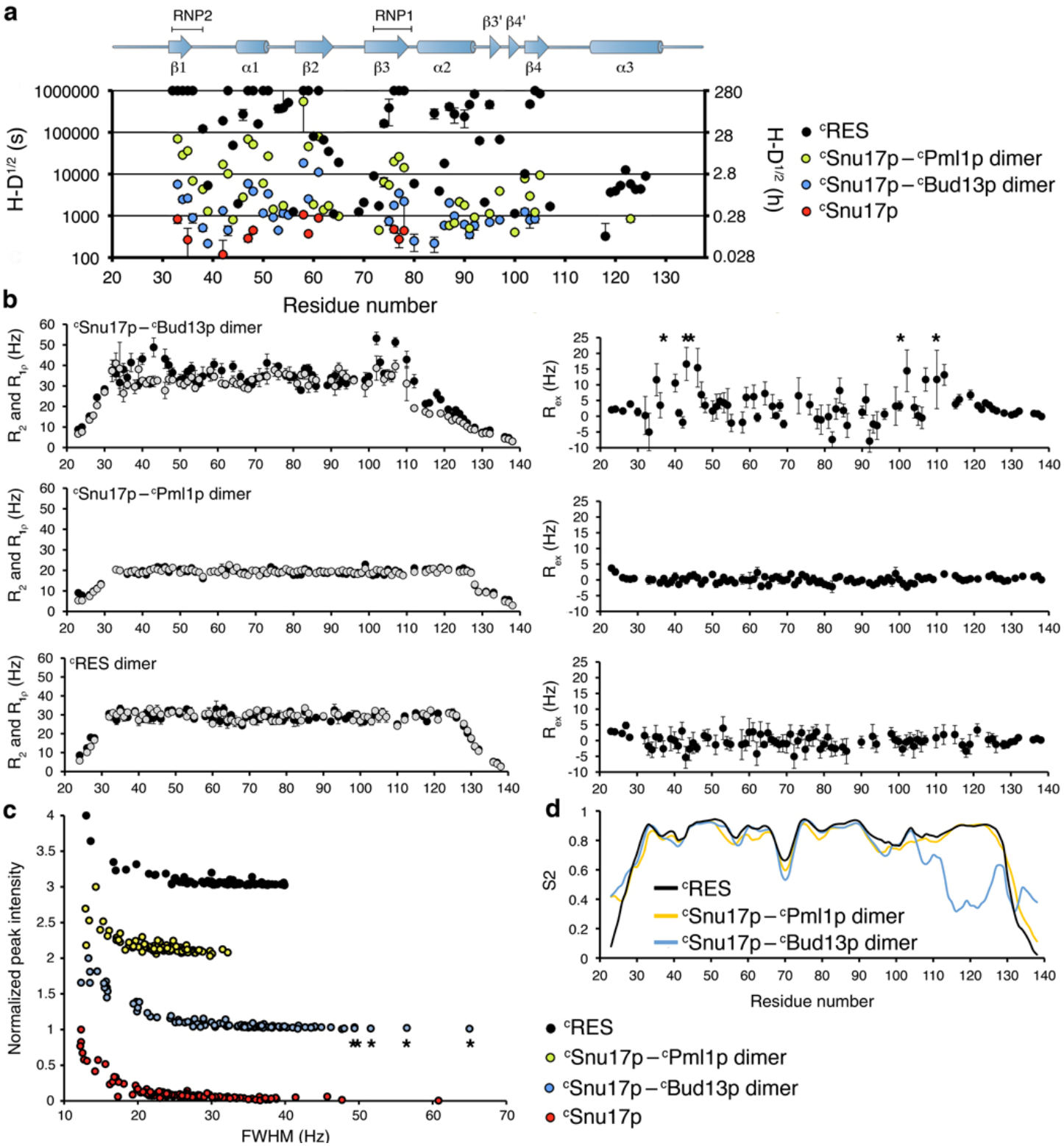


Supplementary Figure 3

^{1}H-^{15}N chemical-shift perturbation (CSP) of ^{c}Snu17p–^{c}Bud13p or ^{c}Snu17p–^{c}Pml1p when titrated with ^{c}Pml1p or ^{c}Bud13p, respectively.

(**a**) Plot of CSP imposed on ^{c}Snu17p–^{c}Bud13p dimer when titrated with ^{c}Pml1p. (**b**) The aforementioned plot mapped onto the structure

of ^{c}RES with spheres colored as described above; T49 is indicated (**c**) Residues that are common between this CSP experiment (when ^{c}Snu17p-^{c}Bud13 dimer is titrated with ^{c}Pml1p) and when ^{c}RES is titrated with RNA (CUUCAUCUUUUUG) are labeled. (**d**) Plot of CSP imposed on ^{c}Snu17p–^{c}Pml1p dimer when titrated with ^{c}Bud13p. (**e**–**f**) The aforementioned plot mapped onto the structure of ^{c}RES with spheres colored as described above; T49 is indicated. Only 224 to 238 residues of ^{c}Bud13p are shown for clarity. Significant CSPs were grouped and color-coded into three categories according to: medium (light pink) if 2σ>CSP>1σ, strong (pink) if 3σ>CSP>2σ, very strong (red) if CSP>3σ, were σ is the standard deviation of the mean.

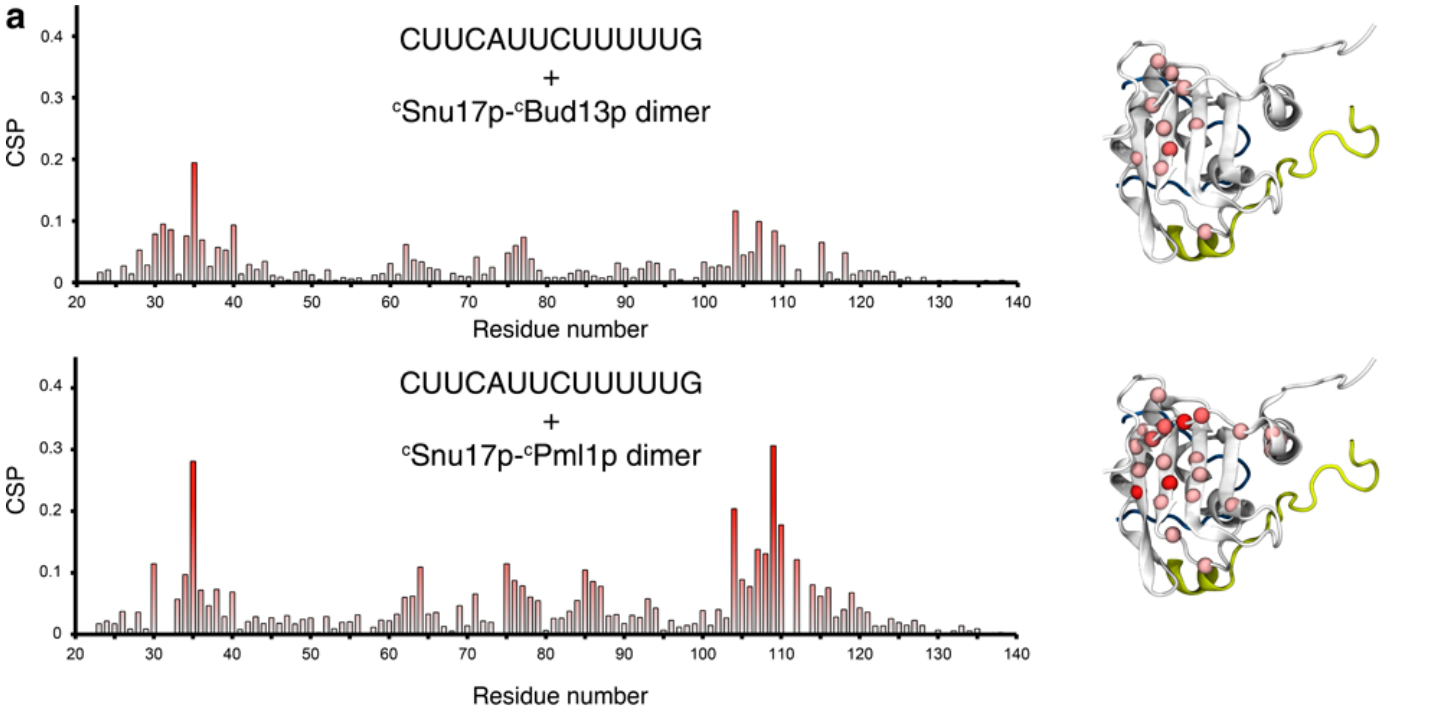


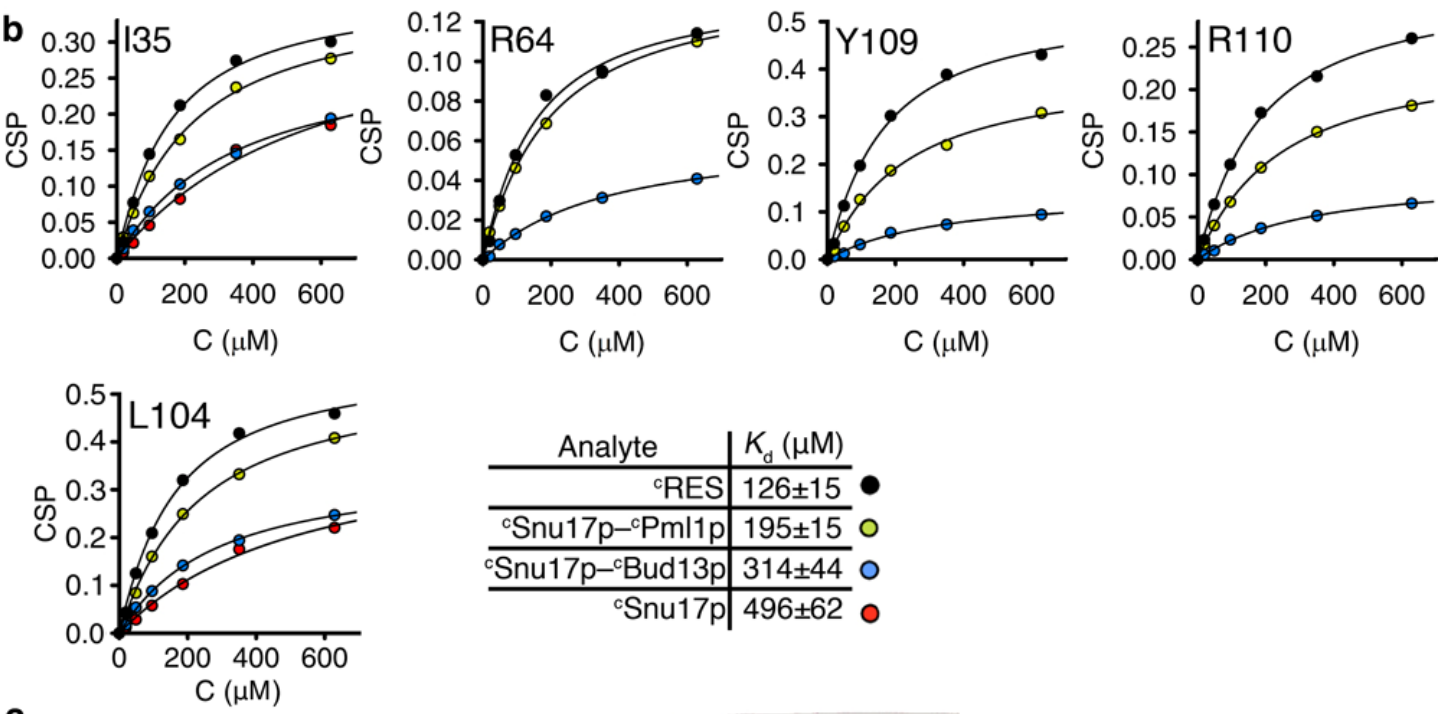
Supplementary Figure 4

Analysis of ^{c}Snu17p monomer, ^{c}Snu17p–^{c}Pml1p dimer, ^{c}Snu17p–^{c}Bud13p dimer and ^{c}RES dynamics.

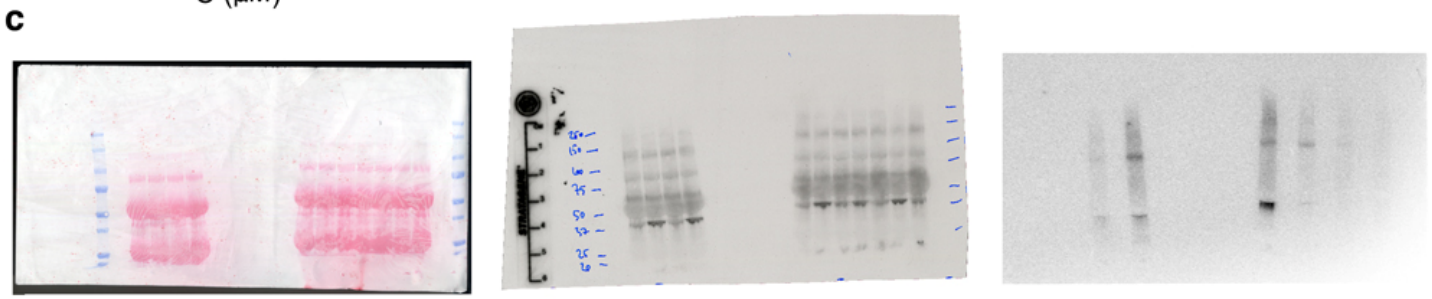
(**a**) Plot of residue specific hydrogen-deuterium exchange half-life H-D$^{1/2}$ for ^{c}RES (black), ^{c}Snu17p–^{c}Pml1p dimer (yellow), ^{c}Snu17p–

^{c}Bud13p dimer (blue), ^{c}Snu17p monomer (red). (**b**) R_2 (black) and $R_{1\rho}$ (grey) plots (left panel) for the aforementioned complexes. R_{ex} estimates derived from R_2 and $R_{1\rho}$ difference (right panel). Positions marked with an asterisk correspond to the five most broadened peaks in **c**). (**c**) Normalized intensity vs ^{1}H full width at half maximum (FWHM) of ^{1}H, ^{15}N HSQC peaks for the aforementioned complexes and ^{c}Snu17p monomer. Intensity values are offset between each group by 1. Please note that both ^{c}RES and ^{c}Snu17p–^{c}Bud13p dimer experience a decrease in the overall tumbling due to apparent increase in size related to peptide binding and/or the presence of C-terminal α-helix. This effect is small in ^{c}Snu17p–^{c}Pml1p dimer since ^{c}Pml1p is shorter and C-terminal α-helix is folded. (**d**) S2 order parameter derived from the chemical shift for the aforementioned complexes.





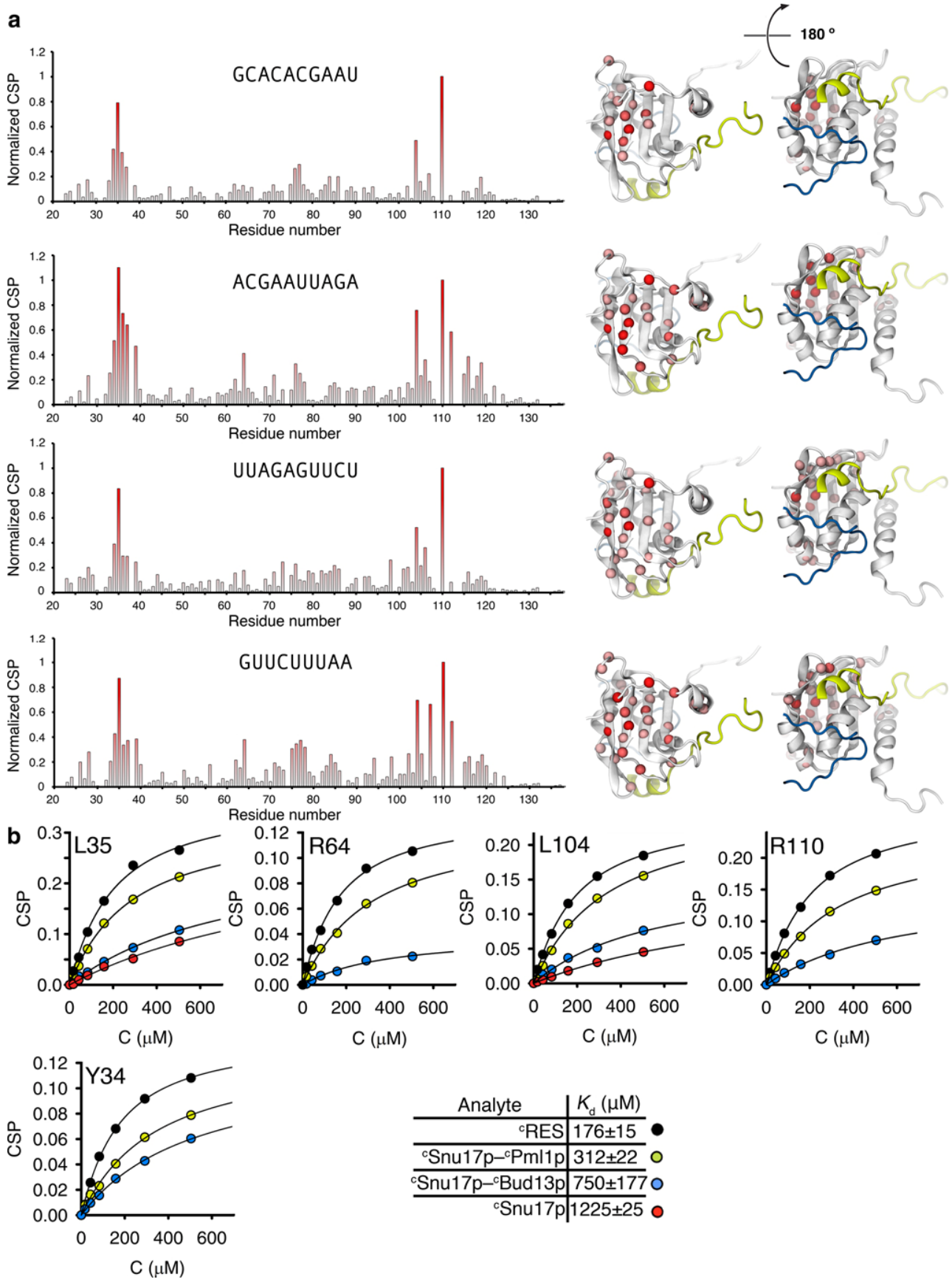
Analyte	K_d (μM)	
cRES	126±15	●
cSnu17p–cPml1p	195±15	●
cSnu17p–cBud13p	314±44	●
cSnu17p	496±62	●



Supplementary Figure 5

RES-RNA interaction.

(**a**) ^{1}H, ^{15}N chemical shift perturbation (CSP) imposed on ^{c}Snu17p in ^{c}Snu17p–^{c}Pml1p dimer and in ^{c}Snu17p–^{c}Bud13p dimer upon titration with CUUCAUCUUUUUG RNA. CSP mapped on the structure of ^{c}RES (left of each graph). Significant CSPs were grouped and color-coded into three categories according to: medium (light pink) if $2\sigma>CSP>1\sigma$, strong (pink) if $3\sigma>CSP>2\sigma$, very strong (red) if $CSP>3\sigma$, were σ is the standard deviation of the mean for the CUUCAUCUUUUUG to ^{c}RES titration. Only 224 to 238 residues of ^{c}Bud13p are shown for clarity. (**b**) CSP binding curves derived from a representative set of residues experiencing high CSP and residue-averaged dissociation constants (K_d) of CUUCAUUCUUUUUG and all four members of the ^{c}RES assembly pathway. (**c**) Uncropped western blots from main text Fig. 5a. From left to right, Ponceau-stained membrane, peroxidase-anti-peroxidase detection of RES-TAP and autoradiography.


a
GCACACGAAU
ACGAAUUAGA
UUAGAGUUCU
GUUCUUUAA
Normalized CSP
Residue number
180 °
b
L35
R64
L104
R110
Y34
CSP
C (μM)
Analyte
K_d (μM)
cRES 176±15
cSnu17p–cPml1p 312±22
cSnu17p–cBud13p 750±177
cSnu17p 1225±25

Supplementary Figure 6

^{1}H-^{15}N normalized chemical-shift perturbation (CSP) imposed on ^{c}Snu17p in ^{c}RES upon titration with various RNAs.

(**a**) NCSP mapped on the structure of ^{c}RES (left of each graph). Significant NCSPs were grouped and color-coded into three categories according to: medium (light pink) if 2σ>NCSP>1σ, strong (pink) if 3σ>NCSP>2σ, very strong (red) if NCS >3σ, were σ is the standard deviation of the mean. Only 224 to 238 residues of ^{c}Bud13p are shown for clarity. The given RNA sequence is indicated above each graph. (**b**) CSP binding curves derived from a representative set of residues experiencing high CSP upon ACGAAUUAGA titration and average binding affinity (below). * value derived from CSP of two residues.