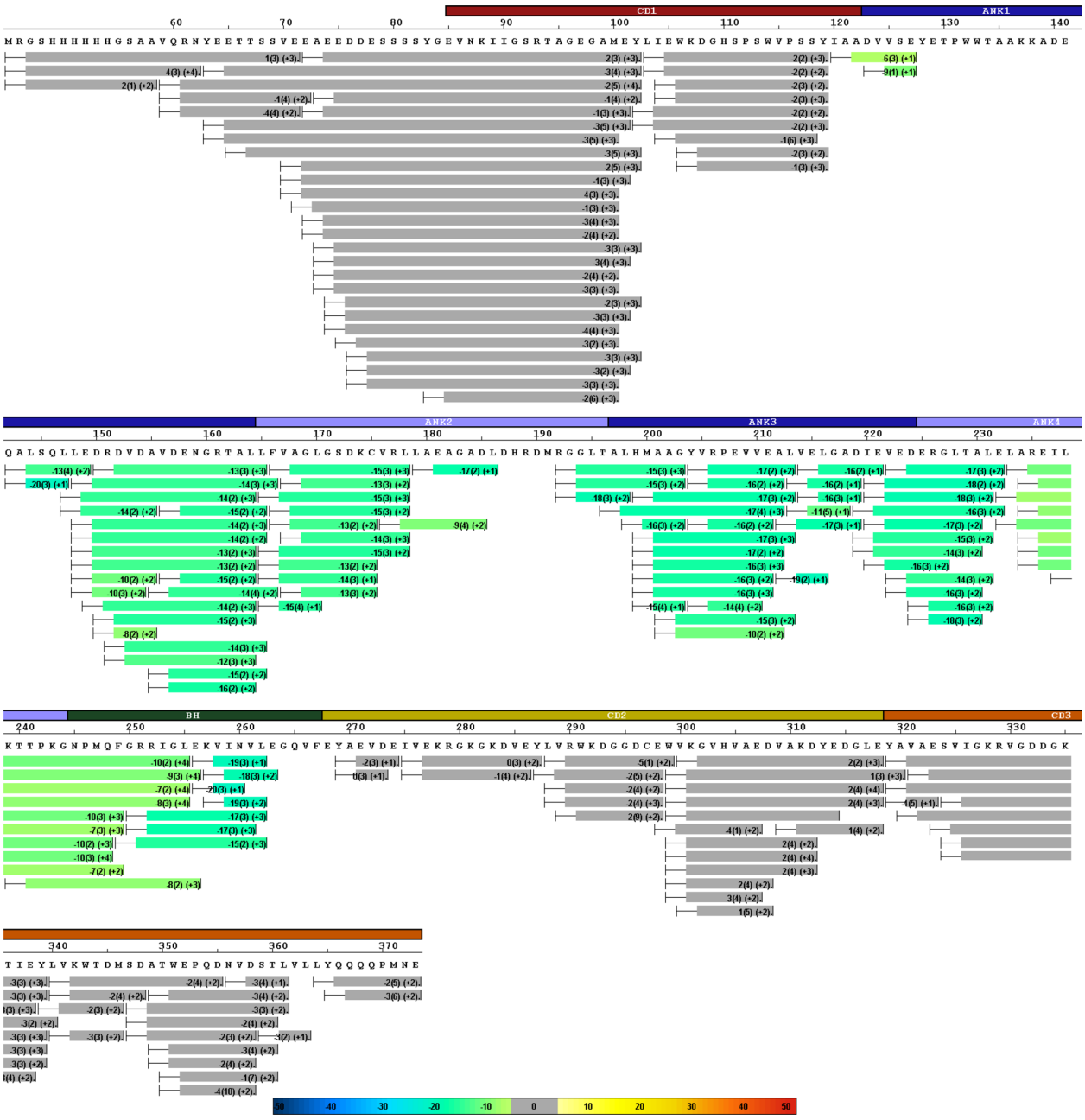
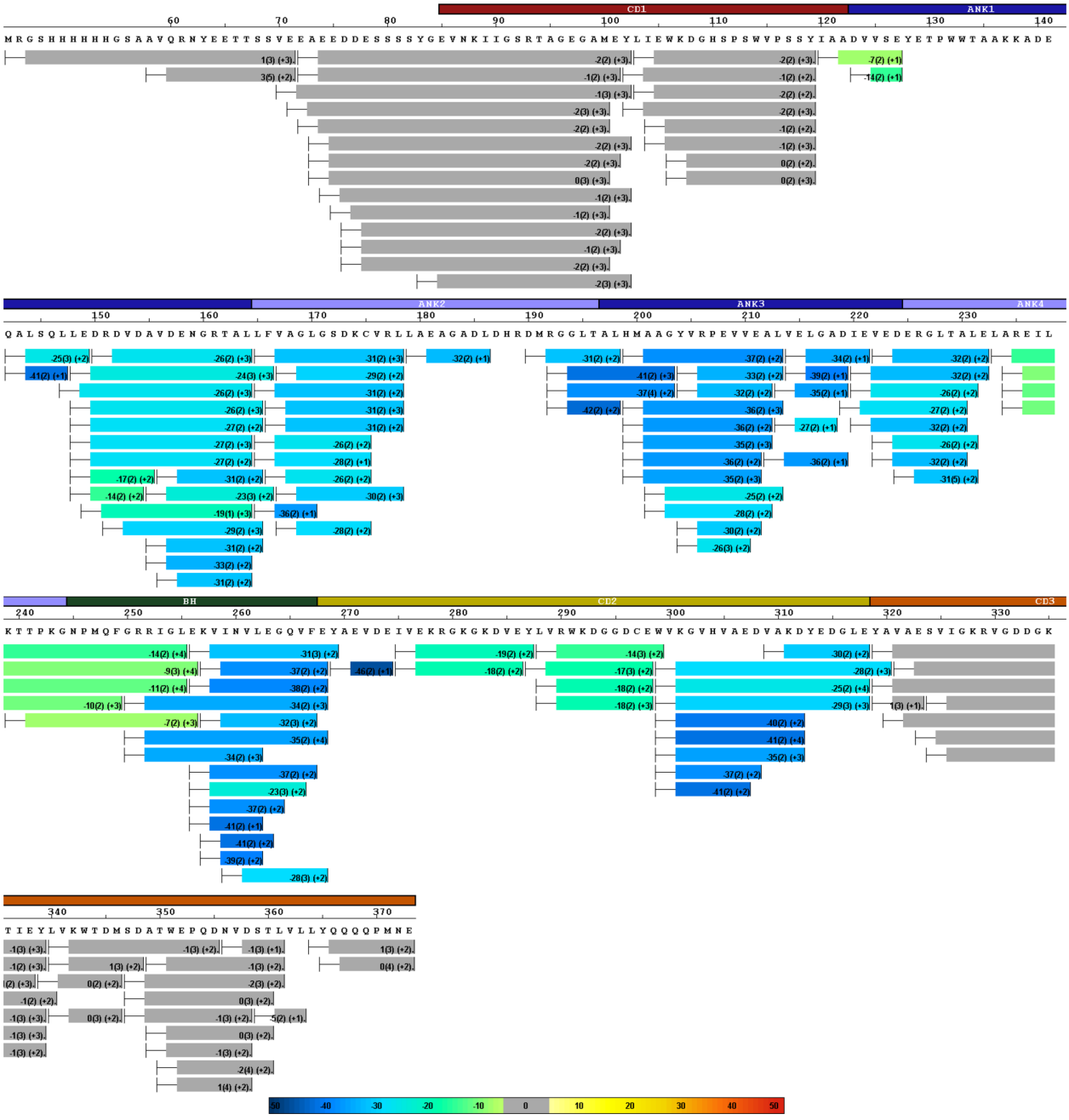
Supplementary Table 1. Summary of the apparent binding constants ($K_{1/2}$) of LHCP to individual cpSRP43 mutants, obtained from light scattering data with and without cpSRP54M peptide. All cpSRP43 mutants shown in this table are derived from cysless cpSRP43 (denoted as WT). 1 μM LHCP was used for all light scattering experiments, and a 1:1 molar ratio of cpSRP43:54M was used for +54M experiments.

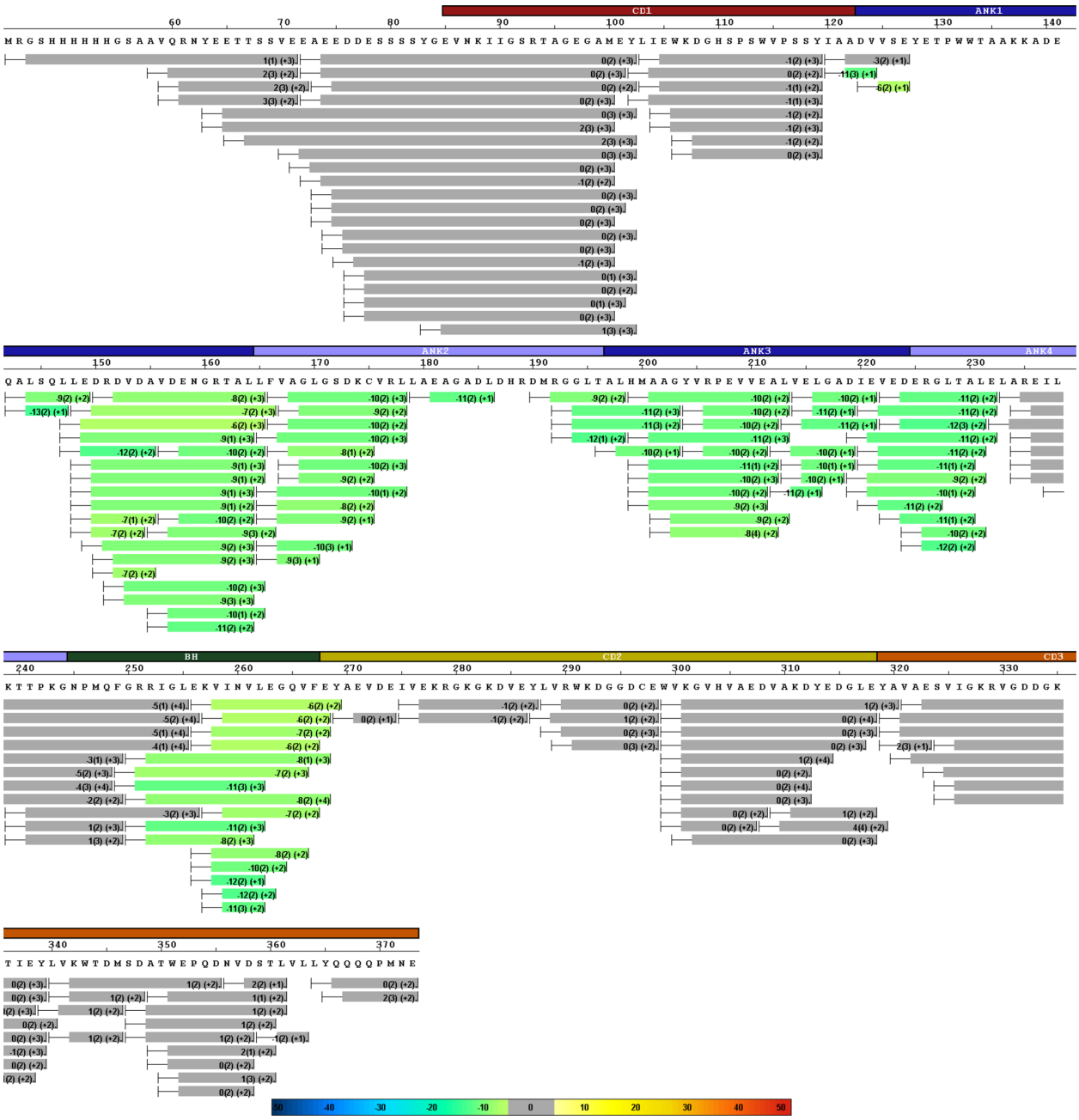
Construct	$K_{1/2}$, -54M (nM)	% Soluble at Saturation	Hill Coefficient	R value	$K_{1/2}$, +54M (nM)
WT	2100	85%	2.1	1.0	50
R252C	6800	88%	2.3	0.99	840
E256C	8400	80%	4.6	1.0	180
I259C	No binding	No binding	No binding	No binding	980
E263C	3500	100%	2.0	0.98	460



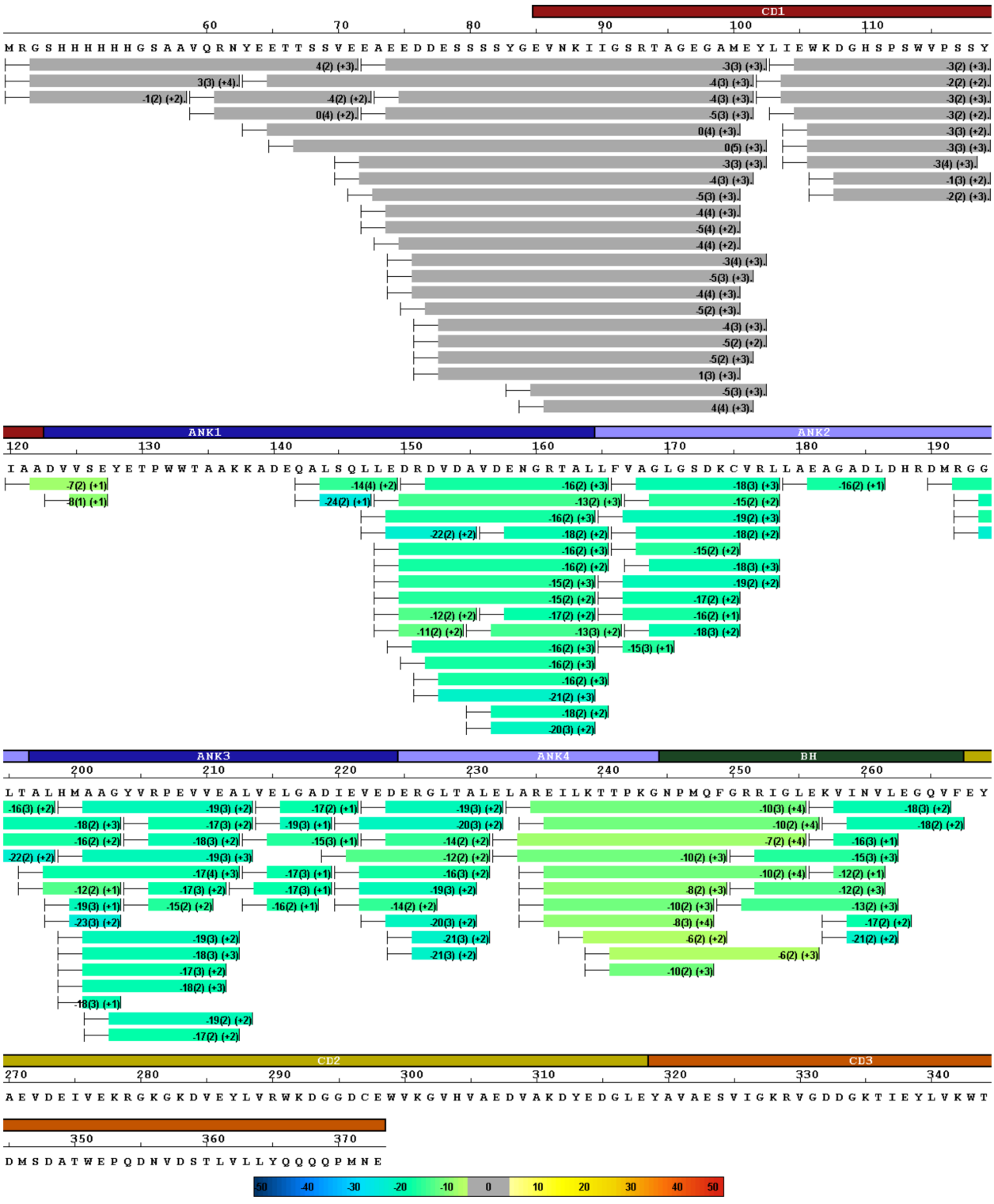
Supplementary Figure 1. Difference plot of deuterium incorporation into L18-bound cpSRP43 relative to apo-cpSRP43 are mapped onto the primary sequence of the chaperone. Colored bars denote quantified peptide fragments corresponding to different sequences in cpSRP43. Protection (reduced exchange) or deprotection (increased exchange) are denoted by pseudo-colors as indicated, and gray denotes no change.



Supplementary Figure 2. Difference plot of deuterium incorporation into 54M-bound cpSRP43 relative to apo-cpSRP43 are mapped onto the primary sequence of the chaperone. Colored bars denote quantified peptide fragments corresponding to different sequences in cpSRP43. Protection (reduced exchange) or deprotection (increased exchange) are denoted by pseudo-colors as indicated, and gray denotes no change.



Supplementary Figure 3. Difference plot of deuterium incorporation into cpSRP43(intein) relative to wildtype cpSRP43 are mapped onto the primary sequence of the chaperone. Colored bars denote quantified peptide fragments corresponding to different sequences in cpSRP43. Protection (reduced exchange) or deprotection (increased exchange) are denoted by pseudo-colors as indicated, and gray denotes no change.



Supplementary Figure 4. Difference plot of deuterium incorporation into cpSRP43-SBD relative to full-length cpSRP43 are mapped onto the primary sequence of the chaperone. Colored bars denote quantified peptide fragments corresponding to different sequences in cpSRP43. Protection

(reduced exchange) or deprotection (increased exchange) are denoted by pseudo-colors as indicated, and gray denotes no change.

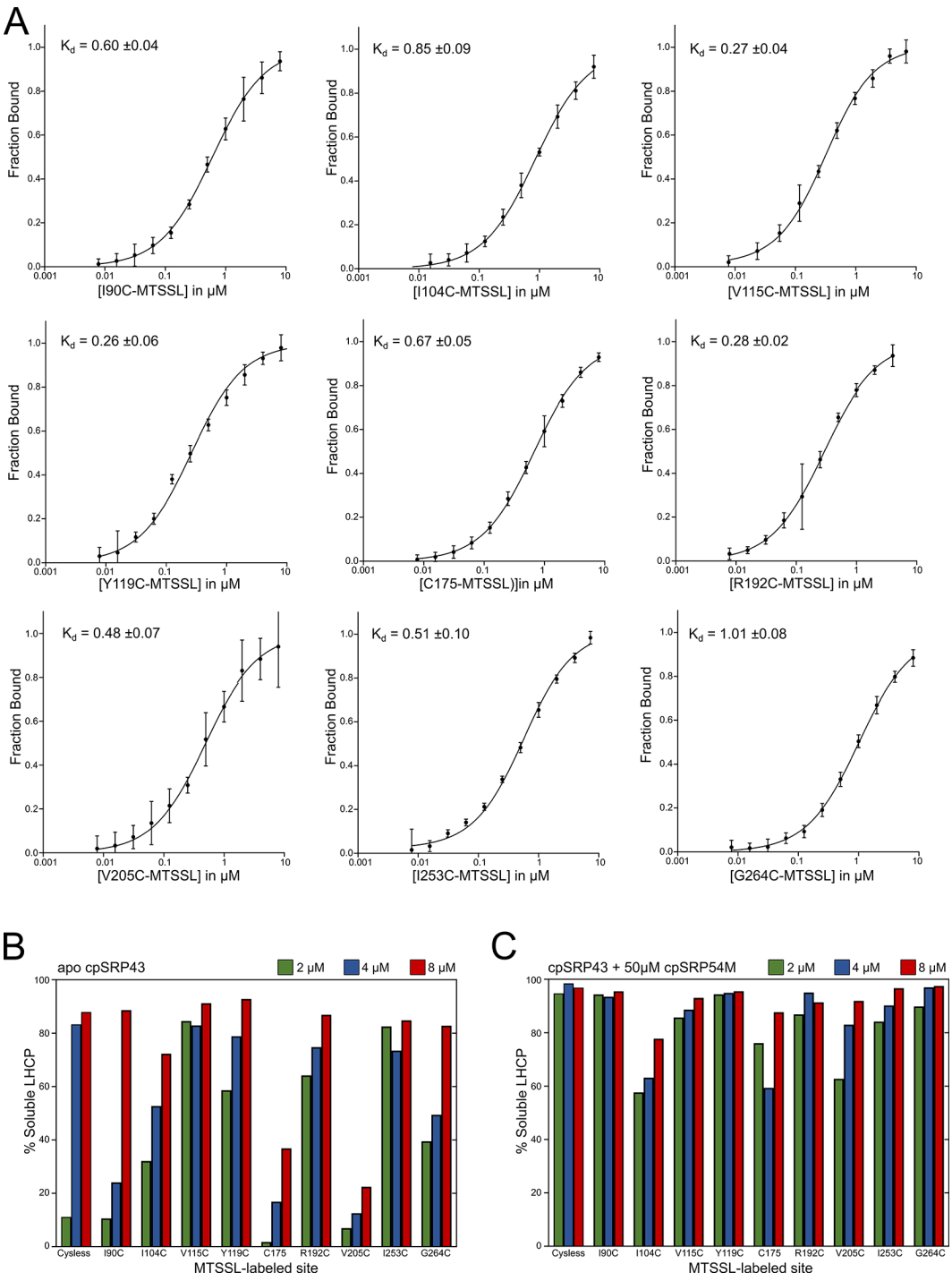


Figure S5. cpSRP54M binding and activation of MTSSL-labeled cpSRP43 single cysteine mutants. (A) Equilibrium titrations to measure the binding of MTSSL-labeled cpSRP43 to fluorescein-labeled 54M peptide, as described in the Methods. The data were fit to Eq. 1, and the obtained K_d values are indicated. **(B, C)** The % soluble LHCP calculated from the light scattering assays with either 2, 4, or 8 µM MTSSL-labeled cpSRP43 in the absence (B) or presence (C) of 50 µM cpSRP54M peptide.

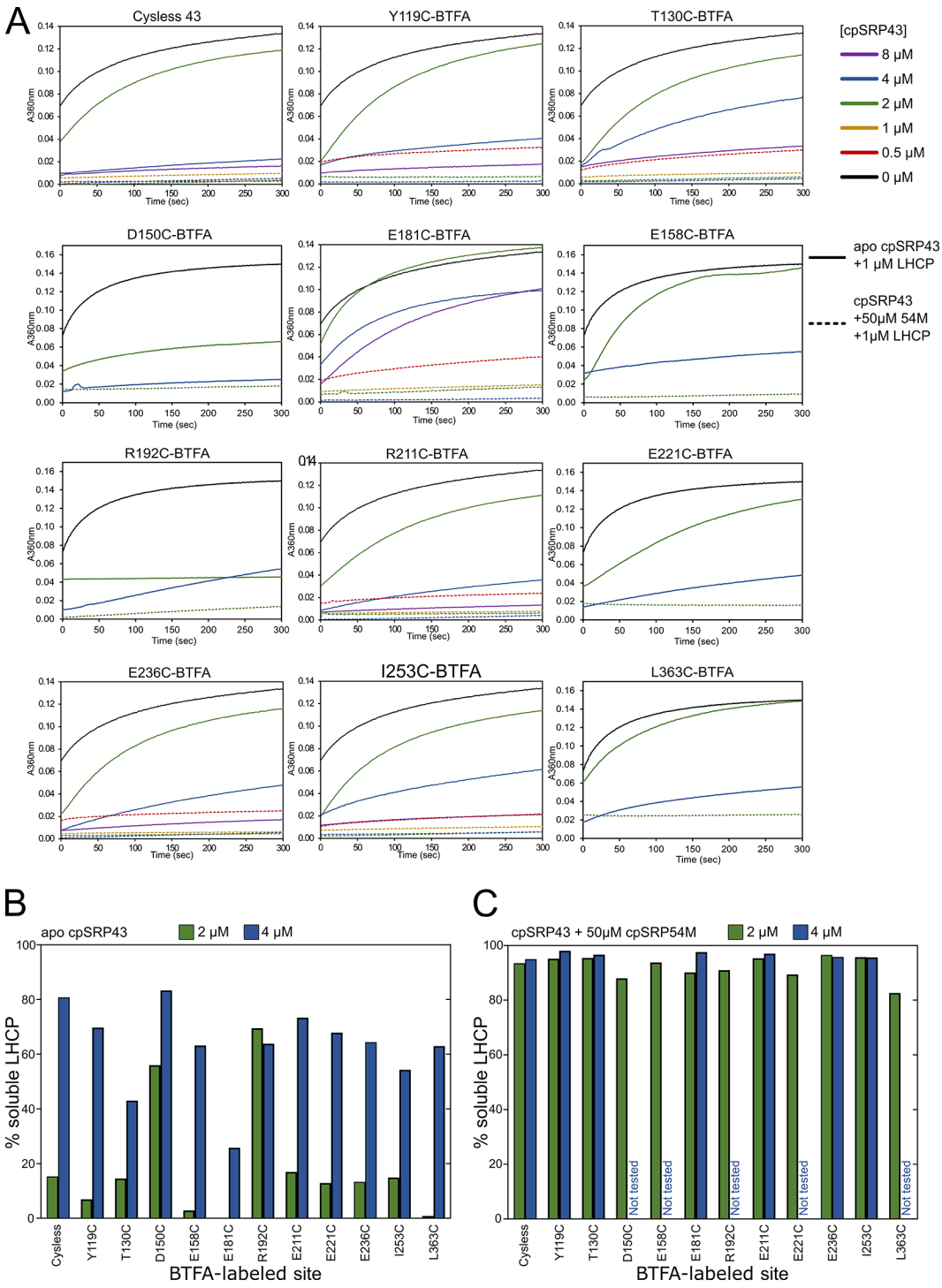


Figure S6. Light scattering assays showing chaperone activity of each BTFA-labeled cpSRP43. (A) Absorbances at A360nm measured over 5 minutes for 1 μM LHCP incubated with different concentrations of indicated concentrations of single cysteine cpSRP43 labeled with BTFA either in the absence or presence of the 54M peptide. **(B, C)** The % soluble LHCP calculated from the light scattering assays in A with either 2 or 4 μM cpSRP43-BTFA and in the absence (B) or presence (C) of 54M peptide.

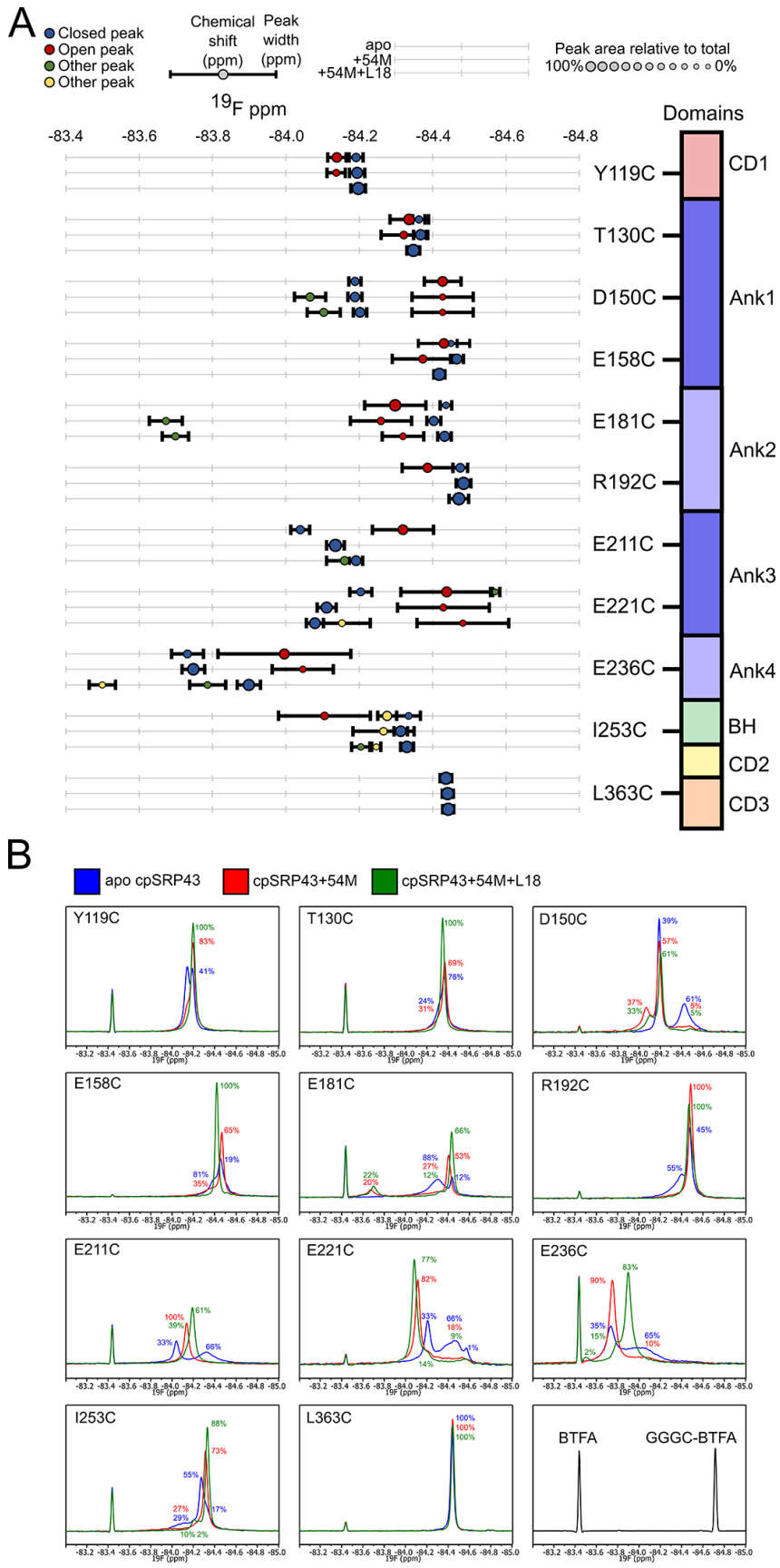

A
Closed peak
Open peak
Other peak
Other peak
Chemical shift (ppm)
Peak width (ppm)
apo
+54M
+54M+L18
Peak area relative to total
100%
0%
19F ppm
-83.4
-83.6
-83.8
-84.0
-84.2
-84.4
-84.6
-84.8
Domains
Y119C
T130C
D150C
E158C
E181C
R192C
E211C
E221C
E236C
I253C
L363C
CD1
Ank1
Ank2
Ank3
Ank4
BH
CD2
CD3
B
apo cpSRP43
cpSRP43+54M
cpSRP43+54M+L18
Y119C
T130C
D150C
E158C
E181C
R192C
E211C
E221C
E236C
I253C
L363C
BTFA
GGGC-BTFA
19F (ppm)

Figure S7. Quantification of peak characteristics from ^{19}F-NMR spectra of BTFA-labeled cpSRP43. (A) Diagram summarizing the parameters extracted from the NMR spectra of BTFA at the indicated site in apo cpSRP43 (top), cpSRP43 with 54M peptide (middle), and cpSRP43 with both 54M and L18 peptides (bottom). (B) NMR spectra for apo cpSRP43, cpSRP43+54M, and cpSRP43+54M+L18 conditions with BTFA labeled at each of the indicated positions. Each peak is labeled with the % of total area after deconvoluting the spectra. The BTFA labeled peptide (GGGC-BTFA) shows the chemical shift of BTFA labels at sites lacking any secondary or tertiary structure.

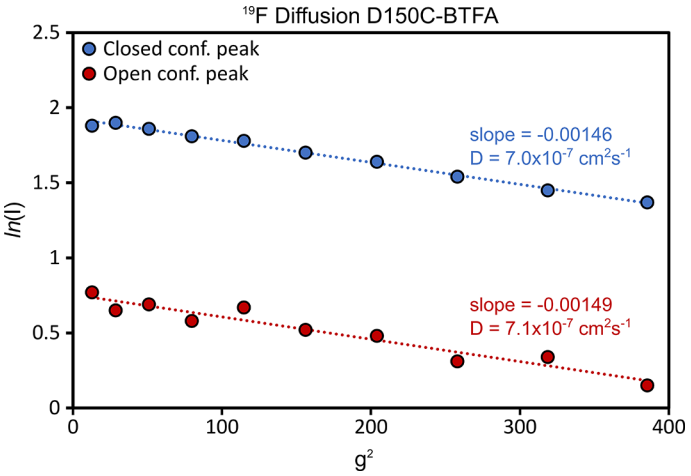


Figure S8. ^{19}F Diffusion Ordered Spectroscopy open and closed conformation peaks from apo cpSRP43 D150C-BTFA. ^{19}F diffusion spectra collected on D150C-BTFA for the closed and open conformation peaks. Intensity changes were fit to obtain diffusion coefficients for both peaks consistent with two monomeric species of identical size.

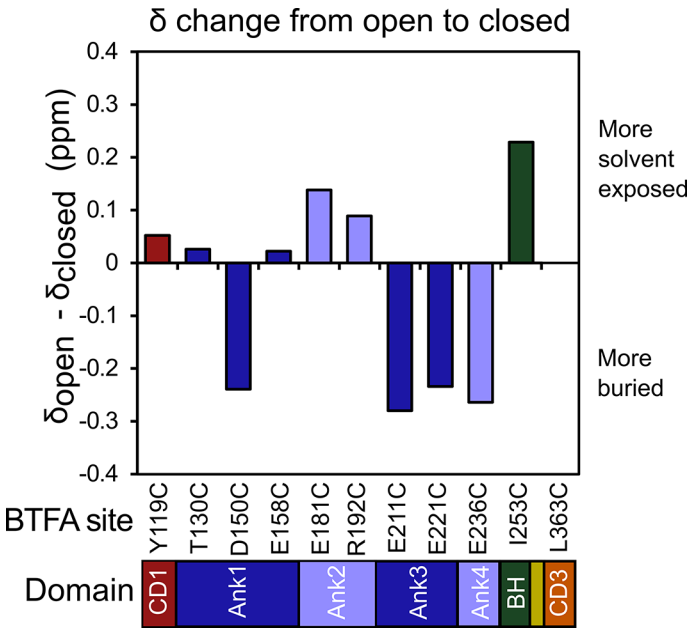


Figure S9. Solvent accessibility of the BTFA-labeled sites. Chemical shift perturbations calculated at each BTFA site by the difference between the closed and open conformation.

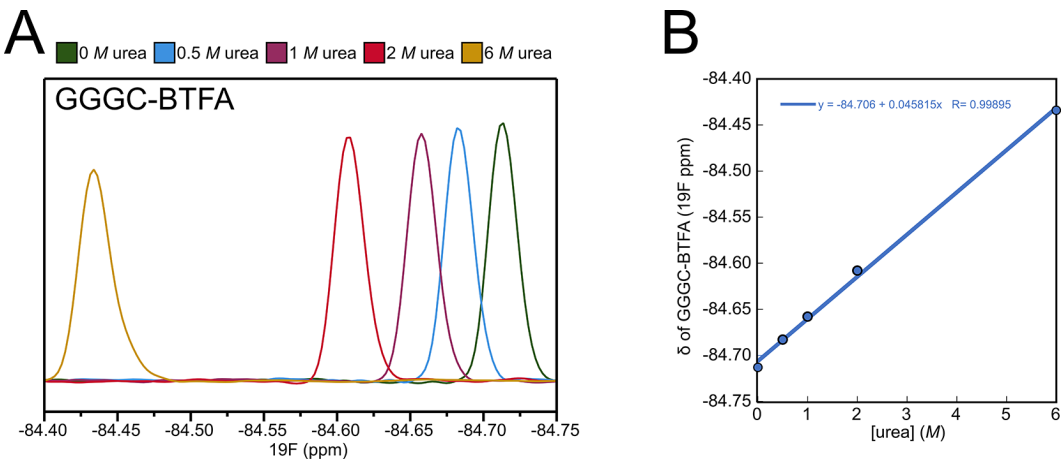


Figure S10. Solvent effect of urea on the ^{19}F chemical shift of BTFA-labeled peptide. (A) Increasing concentrations of urea induces a downfield shift of the ^{19}F peak of BTFA labeled on the GGGC peptide. All spectra were recorded with 200 μM GGGC-BTFA. (B) The ^{19}F chemical shift of GGGC-BTFA correlates with urea concentration.