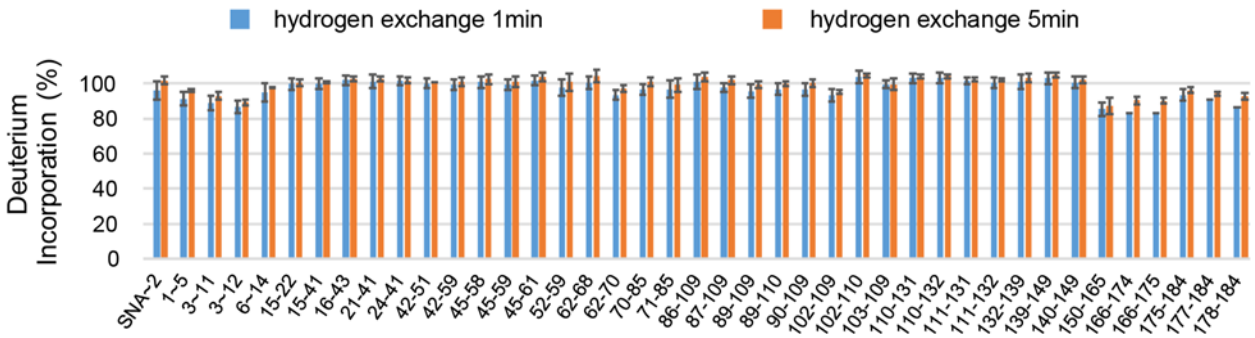

Figure S1. Related to Figure 1. Deuterium incorporation of peptic fragments in HX-MS within 1 min (blue) and 5 min (red) incubation in D_2O. Error bars indicate standard deviation (three independent experiments).

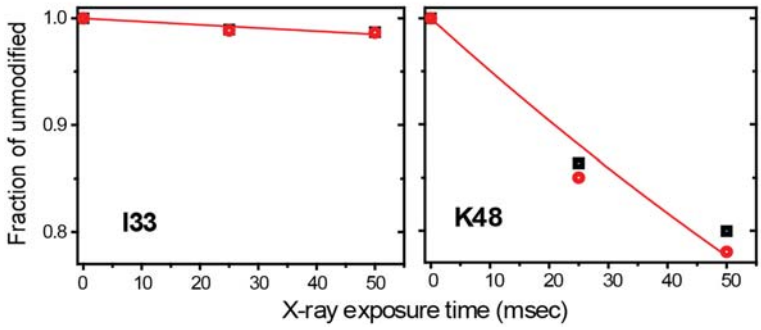

Figure S2. Related to Figure 4. Hydroxyl radical protein footprinting dose-response curves as a function of X-ray exposure time. Red lines are the least-squares fit to an exponential decay function (i.e., a normalized fraction of unmodified residues), each yielding a rate of footprinting (k_{fp}). The division by an intrinsic reactivity with hydroxyl radicals (k_R) provides a protection factor measure (PF = k_R/k_{fp}).

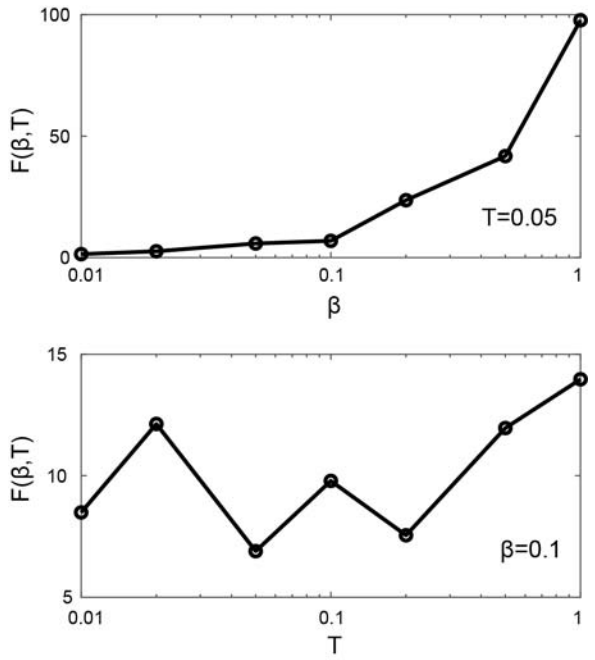

Figure S3. Related to Figure 4. The effective free energy F(β,T) as function of the parameters β and T. The values of β=0.1 and T=0.05 were chosen to give the local minimum of F(β,T) (see Eq. 4).

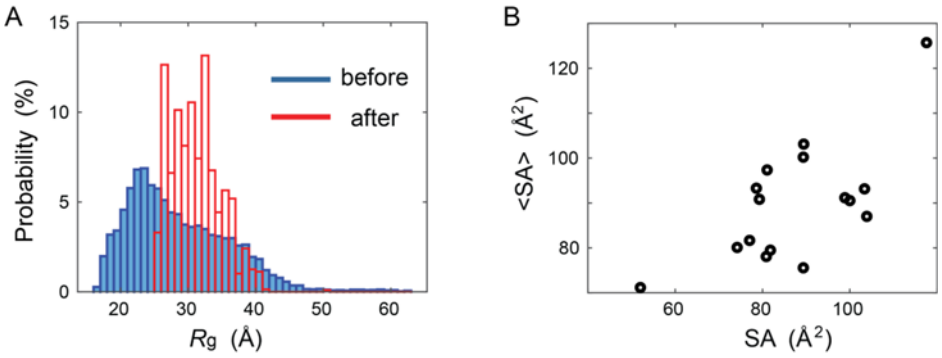

Figure S4. Related to Figure 4. Structure selection by the ensemble-fitting method against experimental scattering and footprinting data. (A) The histogram plot as a function of R_g before and after the ensemble fitting was applied. (B) The ensemble-averages of solvent-accessible surface area for the set of 16 footprinting-probed residues before (<SA>) and after (SA) the ensemble-fitting.

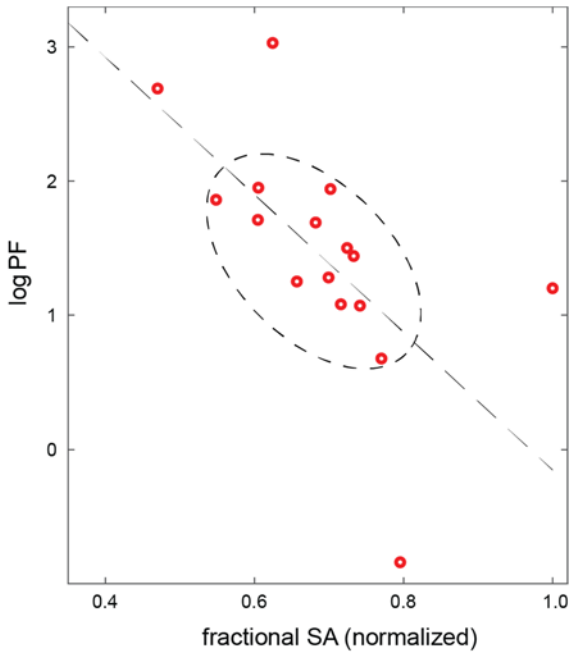

Figure S5. Related to Figure 4. Measured logPF values against the fractional SA values for the set of 16 probed residues. The factional SA value was the SA weighted by each residue's maximum possible accessible area.

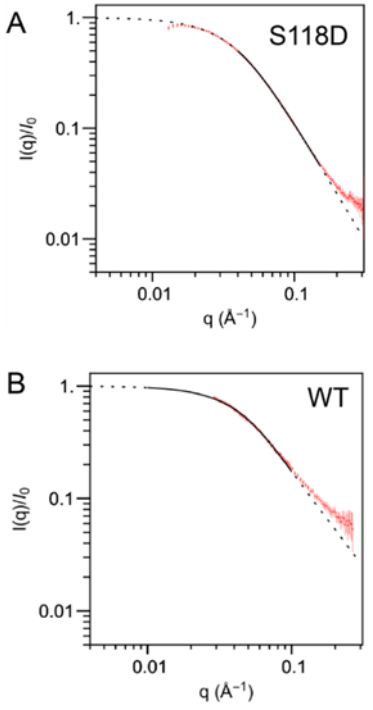

Figure S6. Related to Figure 5. SAXS data for mutant S118D (A), compared to the wild-type (B). Generated using the webserver at http://sosnick.uchicago.edu/SAXSonIDPs.

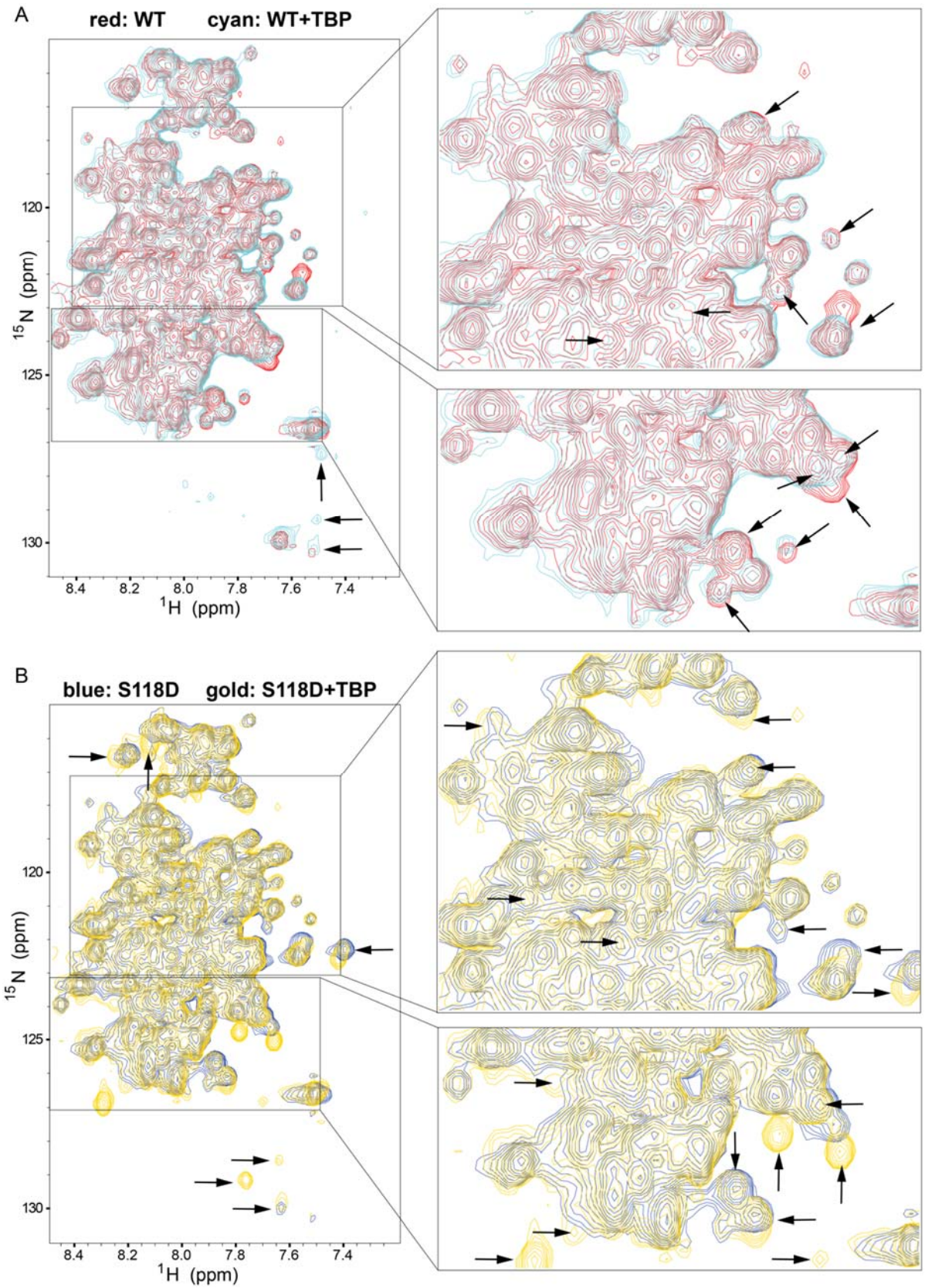

Figure S7. Related to Figure 5. Mutation-induced conformational changes and alteration of

coactivator TBP binding.

(A) Overlap of ^{1}H-^{15}N TROSY spectra of the WT in the absence (red) and presence (cyan) of TBP. Change/shift of peaks is marked by arrows.

(B) Overlap of ^{1}H-^{15}N TROSY spectra of S118D in the absence (blue) and presence (gold) of TBP.

Table S1. Related to Figure 4. SAXS data details and parameters.

Experiment date	June 12, 2017
SASBDB ID (https://www.sasbdb.org)	SASDEE2 (https://www.sasbdb.org/data/SASDEE2)
Beamline/Instrument	NSLS-II/16-ID-LiX, Pitaus3 S 1M/300k
Wavelength	0.9 Å
Sample detector distance	4.270 m / 0.856 m
Exposure time	1 second
Cell temperature	10 °C
protein concentration	2.5 mg/ml
Number of residues	187
Molecular weight	20.18 kDa
Protein buffer	20mM Hepes, pH7.4, 300mM NaCl, 5% glycerol, 1mM DTT, 0.5 mM PMSF, 1 mM EDTA

Table S2. Related to Figure 4. Radius of gyration (R_g) values derived from SAXS data using several fitting methods.

WT/S118D	Guinier	Debye	extended Guinier	Sosnick
R_g (Å)	30.0±1.6/35.8±0.5	32.9± 0.6/41.0±0.3	29.2±1.3/36.7±0.6	31.0±0.2/38.7±0.2
$(qR_g)_{max}$	1.3	3.0	2.0	3.0/5.2
reference	(Guinier, 1939)	(Debye, 1947;Calmettes et al., 1994)	(Zheng and Best, 2018)	(Riback et al., 2017)

Table S3. Related to Figure 4. Hydroxyl radical protein footprinting protection factors (FP-PFs) for the NTD residues. Listed are the probed residues and their corresponding values for footprinting rates (k_{fp}), logPF, and SA^{pred} predicted from the ensemble-structures.

Residue	k_{fp} (s^{-1})	*log(*PF)	SA^{pred} ($Å^2$)
L26	0.63±0.01	1.94±0.02	79.3
L31	1.26±0.14	1.25±0.11	74.2
I33	0.30±0.03	2.69±0.10	52.0
L35	1.50±0.13	1.08±0.09	80.9
L39	0.81±0.04	1.69±0.05	77.0
L44	0.98±0.20	1.50±0.20	81.8
K48	5.07±0.30	-0.84±0.06	117.6
Y54	1.70±0.15	1.95±0.09	89.4
Y73	2.17±0.09	1.71±0.04	89.3
Y80	1.87±0.092	1.86±0.05	81.0
F89	3.85±0.11	1.07±0.03	100.0
F97	5.68±0.19	0.68±0.03	103.9
F120	2.64±0.11	1.44±0.04	98.9
H124	0.45±0.029	3.03±0.07	78.6
Y130	3.32±0.13	1.28±0.04	103.4
P146	0.30±0.02	1.20±0.07	89.3