

# Structure-specific recognition protein-1 (SSRP1) is an elongated homodimer that binds histones

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The histone chaperone complex facilitates chromatin transcription (FACT) plays important roles in DNA repair, replication, and transcription. In the formation of this complex, structure-specific recognition protein-1 (SSRP1) heterodimerizes with suppressor of Ty 16 (SPT16). SSRP1 also has SPT16-independent functions, but how SSRP1 functions alone remains elusive. Here, using analytical ultracentrifugation (AUC) and small-angle X-ray scattering (SAXS) techniques, we characterized human SSRP1 and that from the amoeba *Dictyostelium discoideum* and show that both orthologs form an elongated homodimer in solution. We found that substitutions in the SSRP1 pleckstrin homology domain known to bind SPT16 also disrupt SSRP1 homodimerization. Moreover, AUC and SAXS analyses revealed that SSRP1 homodimerization and heterodimerization with SPT16 (resulting in FACT) involve the same SSRP1 surface, namely the PH2 region, and that the FACT complex contains only one molecule of SSRP1. These observations suggest that SSRP1 homo- and heterodimerization might be mutually exclusive. Moreover, isothermal titration calorimetry analyses disclosed that SSRP1 binds both histones H2A–H2B and H3–H4 and that disruption of SSRP1 homodimerization decreases its histone-binding affinity. Together, our results provide evidence for regulation of SSRP1 by homodimerization and suggest a potential role for homodimerization in facilitating SPT16-independent functions of SSRP1.

In eukaryotes DNA is packaged into nucleosomes, which consist of 147-bp fragments of DNA wrapped around a histone octamer comprising two H2A–H2B heterodimers and one

H3–H4 heterotetramer (1). Nucleosomes represent a barrier for DNA replication, repair, and transcription machinery. Hence, they need to be reorganized to allow access to DNA. Nucleosome assembly is a process that occurs during transcription and DNA replication. It can be described as a two-step process where DNA is initially contacted by a histone H3–H4 tetramer and subsequently two histone H2A–H2B dimers (2–4). This two-step process is carried out by histone chaperones. There are two classes of histone chaperones grouped according to their mechanism of action. The first class uses ATP hydrolysis to move or modify histone structure along the DNA, and the second class reorganizes nucleosomes without ATP hydrolysis (5–7). Facilitates chromatin transcription (FACT)<sup>5</sup> belongs to the latter group (5–8).

FACT is a heterodimeric complex consisting of structure-specific recognition protein-1 (SSRP1) and suppressor of Ty 16 (SPT16) (9, 10). It plays important roles in DNA replication, transcription, and DNA repair by remodeling chromatin structure, although the mechanism remains elusive (11–14). Early *in vitro* studies demonstrated that FACT displaces the histone H2A–H2B dimer from the nucleosome (9, 15, 16), but Xin *et al.* (16) showed that FACT also increases nuclease access to DNA without H2A–H2B eviction. Based on these data, two models were proposed to explain FACT's mechanism of action, the “dimer eviction and insertion” model and the “accessibility and tethering” model. In the first model, FACT actively removes histones H2A–H2B from the nucleosome to enable DNA accessibility, and in the second model FACT tethers to the nucleosome components without H2A–H2B eviction and destabilizes the nucleosome conformation (17).

Several studies showed that FACT binds to all components of the nucleosome, including histones H2A–H2B and H3–H4, histone N-terminal tails, and DNA via domains within FACT subunits SPT16 and SSRP1 (9, 18–27). SPT16 consists of an N-terminal domain, a dimerizing domain, and a middle domain followed by an intrinsic disordered region at the C terminus (13, 24, 28). The SPT16 N-terminal domain adopts an amino-

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This article contains Figs. S1–S4.

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<sup>5</sup> The abbreviations used are: FACT, facilitates chromatin transcription; FL, full length; PH, pleckstrin homology; TEV, tobacco etch virus; ΔCTD, ΔC-terminal domain; ITC, isothermal titration calorimetry; AUC, analytical ultracentrifugation; SAXS, small-angle X-ray scattering; MD, middle domain; HMG, high-mobility group; SV, sedimentation velocity; SE, sedimentation equilibrium; Ni-NTA, nickel-nitrilotriacetic acid.

## SSRP1 homodimerization has a role in histone binding

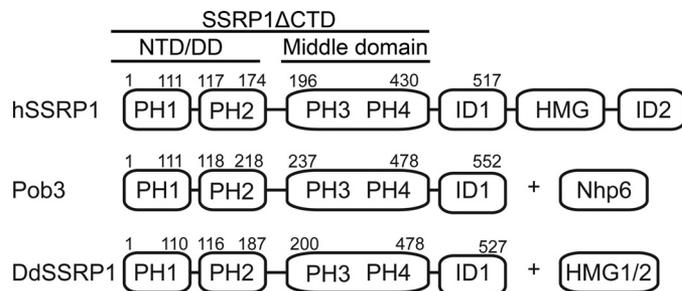
peptidase-like structure that binds histones H3–H4 (21, 23, 26). The dimerizing domain assumes a pleckstrin homology (PH)-like fold, important for SPT16–SSRP1 heterodimerization (20). The SPT16 middle domain adopts a double PH domain structure similar to that of Rtt106 and the SSRP1 middle domain and binds histone H2A–H2B and H3–H4 (20, 27). The C-terminal acidic region of SPT16 binds a hydrophobic pocket on H2B (29). SSRP1 consists of two N-terminal PH domains (PH1–PH2), a middle domain (MD) comprising a double PH domain (PH3–PH4), and a high-mobility group (HMG) domain flanked by two intrinsic disordered regions (ID1 and ID2) (Fig. 1). The homolog of SSRP1 in yeast is the Pob3–Nhp6 complex; Pob3 comprises two N-terminal PH domains and a double PH domain, whereas Nhp6 comprises a HMG-1 domain. Studies showed that SSRP1 binds histone H3–H4 (19); the PH2 domain interacts with the dimerizing domain of SPT16 (20); and SSRP1 MD and HMG domains interact with DNA (30–32). Interestingly, the C-terminal acidic regions of Pob3 and SPT16 bind overlapping sites on H2B suggesting that FACT might bind to the two symmetry-related H2A–H2B dimers in the nucleosome (29). In this manner, FACT would break up DNA–histone interactions to promote nucleosome reorganization.

In cells, the bulk of cellular SSRP1 is found in complex with SPT16 (9, 33). Thus, SSRP1 and SPT16 functions are tightly associated with the roles played by FACT in chromatin remodeling. However, several studies have shown that in addition to its FACT function, SSRP1 exhibits SPT16-independent functions. SSRP1 has been shown to associate with transcription factors such as the serum-response factor and p63 to regulate their activity (34, 35). Indeed, SSRP1 and SPT16 knockdown transcriptome analyses revealed a distinct set of genes that are regulated by SSRP1 (36). Furthermore, SSRP1 has been shown to facilitate microtubule growth in mitosis (37) and to regulate DNA demethylation in *Arabidopsis* (38). It remains unclear how SSRP1 behaves independently of SPT16. To further elucidate the function of SSRP1, we characterized its solution structure using analytical ultracentrifugation (AUC) and small-angle X-ray scattering (SAXS), and we showed that SSRP1 self-associates to generate an elongated homodimer. Mutational analyses revealed that SSRP1 PH2 and PH3 domains are essential for homodimerization. Moreover, SSRP1 homodimerization and SPT16 interaction utilize the same SSRP1 surface suggesting that both events might be mutually exclusive. Finally, we showed that optimal histone binding requires SSRP1 homodimerization. Collectively, our results provide a hint for how SSRP1 could function independently of SPT16.

## Results

### SSRP1 is a homodimer

To better characterize SSRP1 structure and function, we expressed and purified human SSRP1 lacking the C-terminal ID regions and the HMG domain (residues 1–433; hSSRP1 $\Delta$ CTD) for biochemical and biophysical analysis (Fig. 1). hSSRP1 $\Delta$ CTD, with a predicted molecular mass of 49.8 kDa, eluted between the 150- and 75-kDa molecular mass markers in size-exclusion chromatography, suggesting it might form an oligomer or adopt an elongated conformation (Fig. 2).



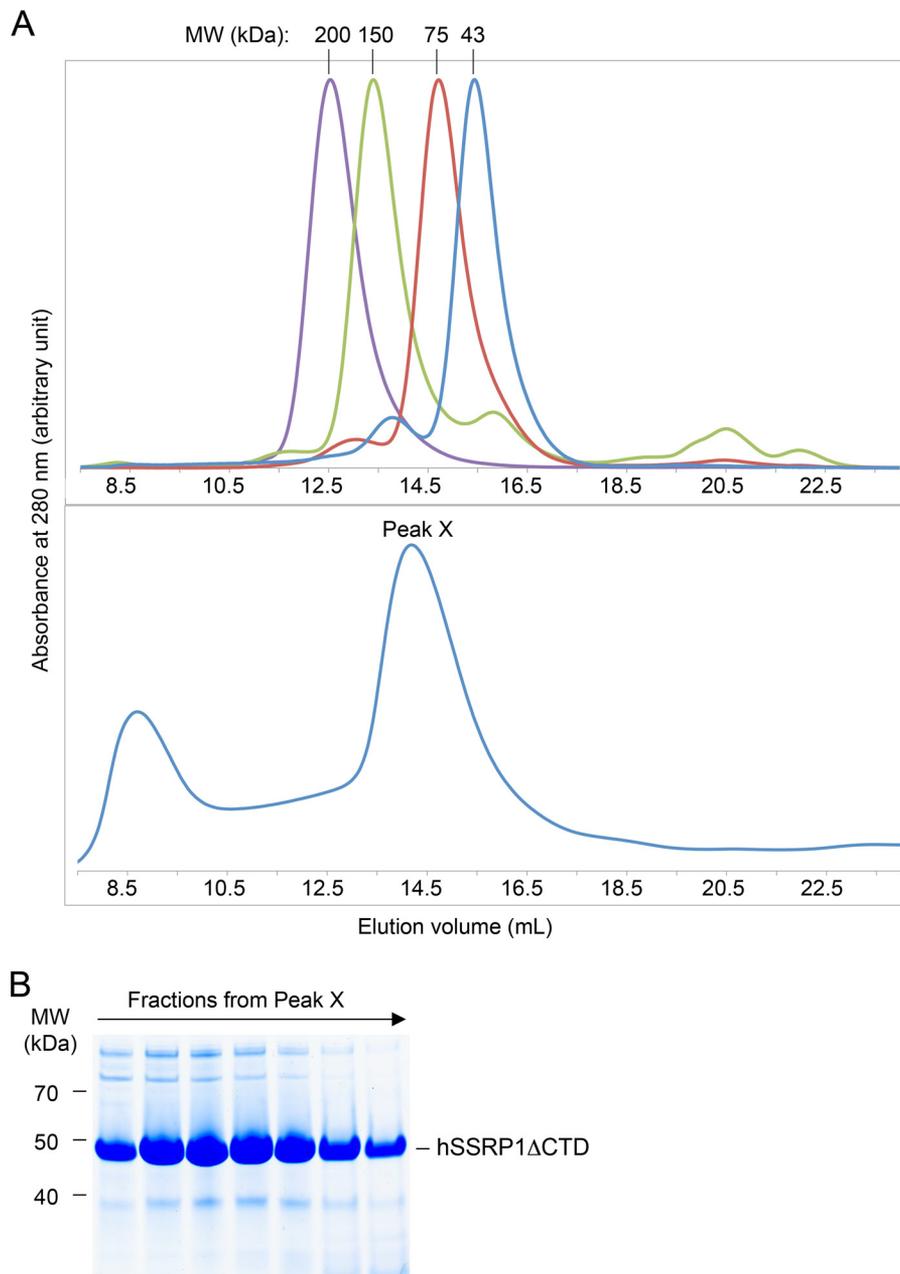
**Figure 1. Schematic representation of hSSRP1 (top), Pob3 (middle), and DdSSRP1 (bottom).** Nhp6, nonhistone protein 6.

To determine the oligomeric state and molecular mass of hSSRP1 $\Delta$ CTD in solution, AUC was performed. Sedimentation velocity (SV) data showed that hSSRP1 $\Delta$ CTD has an infinite dilution sedimentation coefficient  $s_{20,w}^0$  of  $3.32 \pm 0.21$  S. The frictional ratio ( $f/f_0$ ) provides information about the shape of the protein: globular proteins have a ratio between 1.2 and 1.3; elongated, asymmetric, or glycosylated proteins lie between 1.5 and 1.8, and unfolded or linear chains have a much higher ratio (39, 40). hSSRP1 $\Delta$ CTD gave a value of 1.63, suggesting that it is elongated in solution. Sedimentation equilibrium (SE) data globally fitted with a single species model yielded a molecular mass of  $91.8 \pm 3.7$  kDa, which is similar to the predicted molecular mass of a homodimer (99,615 Da; Fig. 3, A and B).

Next, we assessed whether SSRP1 homodimerization is conserved among different species. *Dictyostelium discoideum* SSRP1 (DdSSRP1) adopts a similar domain structure as Pob3 containing N-terminal PH domains followed by a C-terminal ID region (Fig. 1). We expressed and purified DdSSRP1 lacking the C-terminal ID region (residues 1–478; DdSSRP1 $\Delta$ CTD). Sedimentation velocity and equilibrium data confirmed that DdSSRP1 $\Delta$ CTD has an  $s_{20,w}^0$  ( $3.23 \pm 0.1$  S) similar to that of hSSRP1 $\Delta$ CTD and a molecular mass of  $104.8 \pm 22.7$  kDa (Fig. 3, C and D; predicted molecular mass of a dimer is 108,466 Da), demonstrating that homodimerization is conserved.

### Second PH domain and middle domain are required for homodimerization

SSRP1 consists of two N-terminal PH domains and a middle domain, which is a double PH domain. For simplicity, we designate these PH domains as PH1–4 (Fig. 1). The crystal structure of the SSRP1–SPT16 heterodimer reveals that SSRP1 PH2 is responsible for heterodimerization with the dimerizing domain of SPT16 (20). Because the dimerizing domain of SPT16 adopts a PH-like fold similar to those found in SSRP1, we hypothesized that homodimerization of SSRP1 may involve its PH2 domain. DdSSRP1 was used in all subsequent analyses as it was more stable when mutations were introduced. We introduced six DdSSRP1 mutations (6mut: C112A, N113A, W114A, F135R, M172R, and M175R) along the SSRP1 PH2–SPT16 dimerizing domain–binding interface and assessed whether this mutant has a defect in homodimerization. AUC analysis showed that DdSSRP1 $\Delta$ CTD\_6mut is a monomer in solution at all tested concentrations in the range of 0.2–60  $\mu$ M (Table 1), suggesting that SSRP1 PH2 is required for homodimerization. To further probe the mechanism of



**Figure 2. Analysis of hSSRP1 by gel-filtration chromatography.** A, elution profile from an SD200 10/30 size-exclusion chromatography column of protein standards:  $\beta$ -amylase (200 kDa), alcohol dehydrogenase (150 kDa), ovalbumin (43 kDa) and conalbumin (75 kDa) (top panel) and hSSRP1 $\Delta$ CTD. Peak X contains hSSRP1 $\Delta$ CTD (bottom panel). B, SDS-PAGE of fractions from peak X in A, bottom panel.

homodimerization, we generated DdSSRP1 containing only PH1 and PH2 domains (DdSSRP1<sub>NTD</sub>; Fig. 1). AUC analysis showed that DdSSRP1<sub>NTD</sub> is a monomer at all tested concentrations in the range of 2.4 to 304  $\mu$ M (Table 1), suggesting that the PH1 and PH2 domains are insufficient for homodimerization and require the presence of PH3–PH4.

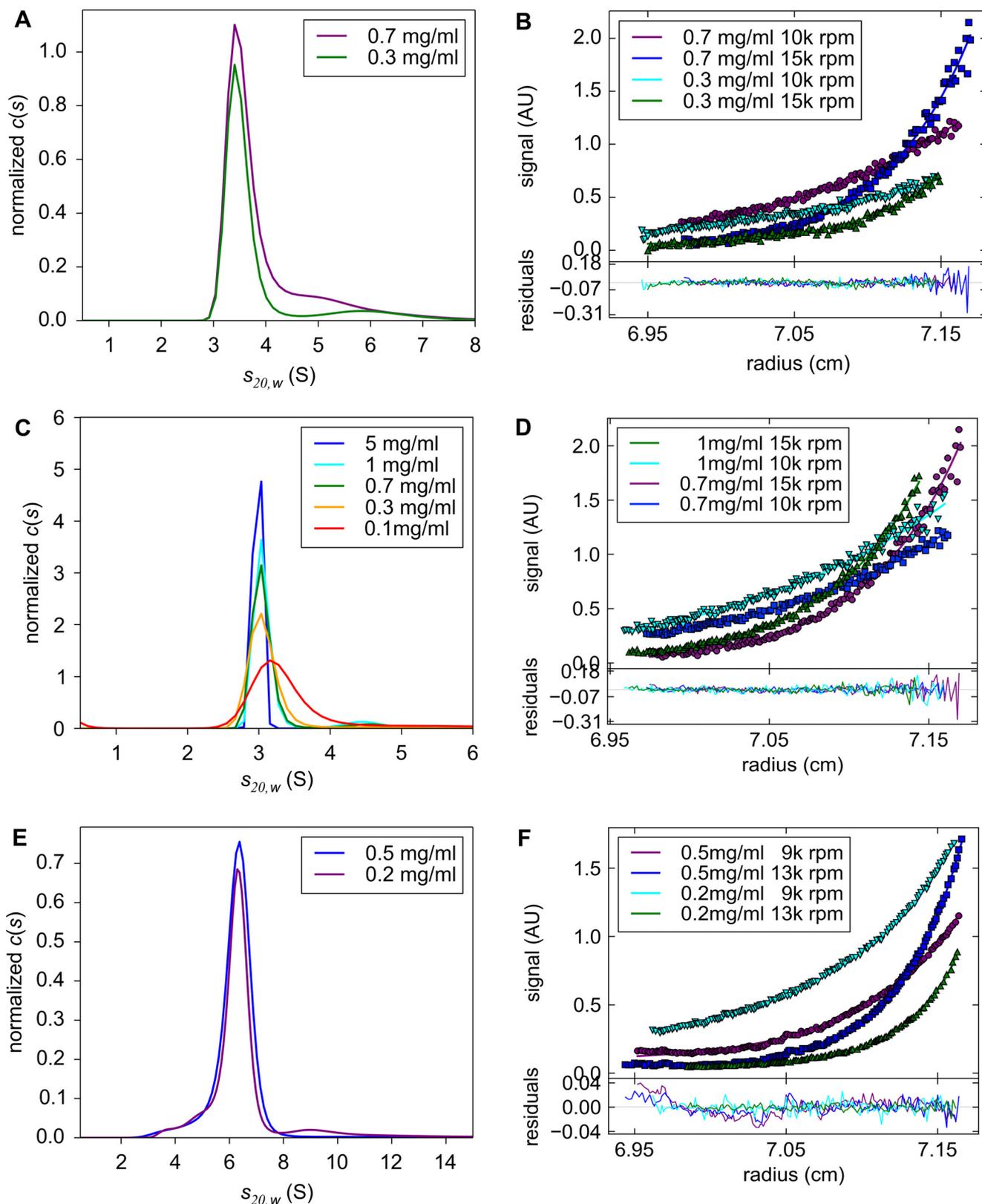
A single point mutation, Q308K, in yeast Pob3 causes defects in transcription and replication (24). This mutation has no effect on Pob3 MD structure but alters the surface charge in that region (24). We next assessed whether the corresponding substitution, Q306K, in DdSSRP1 $\Delta$ CTD has an effect on homodimerization. We performed AUC analysis on DdSSRP1 $\Delta$ CTD Q306K and found that it is a monomer at all tested concentrations in the range of 1–41

$\mu$ M (Table 1). Together, these results suggest that the SSRP1 homodimerization interface may involve both the PH2 and PH3 domains.

#### FACT is a heterodimer of SSRP1 and SPT16

Given that SSRP1 homodimerization and SSRP1–SPT16 heterodimerization require the same SSRP1 PH2 surface, we investigated the oligomeric state of FACT complex. To address this question, we performed AUC on the DdFACT complex. DdFACT was purified as described under “Experimental procedures.” SV data showed that DdFACT is homogeneous in solution with an  $s_{20,w}^0 = 6.09 \pm 0.52$  S, and a frictional ratio of 1.51, suggesting that the complex is elongated. SE data gave a molecular mass of  $162.6 \pm 18.8$  kDa, which agrees with the

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**Figure 3. AUC analyses of SSRP1.** A,  $c(s)$  distribution derived from SV of hSSRP1 $\Delta$ CTD at various protein concentrations. B, SE analyses of hSSRP1 $\Delta$ CTD. Data are globally fitted with a single species model. C,  $c(s)$  distributions derived from SV of DdSSRP1 $\Delta$ CTD revealing similar sedimentation coefficients as hSSRP1 $\Delta$ CTD. D, SE analyses of DdSSRP1 $\Delta$ CTD. Data are globally fitted with a single species model. E,  $c(s)$  distribution derived from SV of DdFACT showing that the protein is monodispersed with the same sedimentation coefficient at 0.5 and 0.2 mg/ml. F, SE analyses of DdFACT. Data are globally fitted with a single species model. Images were made using the software GUSI (65).

**Table 1**  
Sedimentation equilibrium analysis of DdSSRP1 constructs

Protein	Mass	$\sigma$	Mass expected
	Da		Da
DdSSRP1 $\Delta$ CTD_6mut	58,417.56	5346.65	54,102.16
DdSSRP1_NTD	20,249.15	2808.91	20,481.08
DdSSRP1 $\Delta$ CTD Q306K	54,827.28	4626.82	54,223.44

sequence molecular mass of 162,856 Da (Fig. 3, E and F), indicating that DdFACT does not oligomerize. Our results are consistent with the earlier finding showing that yeast FACT is an elongated heterodimer (33). Together, the data suggest that SSRP1 homodimerization and SSRP1–SPT16 heterodimerization cannot occur simultaneously.

### SAXS analysis

Solutions of DdSSRP1 $\Delta$ CTD, of the heterodimeric FACT complex, and of the DdSSRP1 $\Delta$ CTD mutants, 6mut and Q306K, were studied with synchrotron SAXS to gain insight into overall SSRP1 structure and oligomeric state. The results are presented in Fig. 4.

Guinier analysis (see “Experimental procedures”) was performed on the scattering profiles, yielding the radii of gyration ( $R_g$ ) and the molecular mass of the proteins, with the latter based on the scattering intensity extrapolated to zero angle ( $I(0)$ ). The  $R_g$  and mass values as functions of the protein concentration are shown in Figs. S1 and S2, respectively. These overall parameters suggest that the heterodimeric FACT complex and DdSSRP1 $\Delta$ CTD have similar sizes, with estimated  $R_g$  values between 5.5 and 6.0 nm. The estimated mass of the FACT complex (130–150 kDa) is relatively close to the mass calculated from its primary sequence (162.856 kDa). In contrast, the mass for DdSSRP1 $\Delta$ CTD (90–110 kDa), points to its homodimerization, as the mass calculated from the sequence is 54.233 kDa. These overall parameters thus further corroborate the results from AUC.

The SSRP1 $\Delta$ CTD mutants, 6mut and Q306K, are apparently smaller than DdSSRP1 $\Delta$ CTD, with the  $R_g$  around 4.5 nm, and their scattering profiles are similar to each other (Fig. 4A). The estimated mass values are in the range 50–80 kDa, which tend toward the sequence-calculated values (54.102 kDa for 6mut and 54.233 kDa for Q306K). Therefore, the overall parameters from SAXS suggest that the mutants are predominantly monomeric. However, the apparent mass and  $R_g$  values grew with concentration pointing to possible dimerization effects.

Table 2 reports the overall parameters obtained after appropriate merging of lower and higher concentrations of SAXS data, including  $R_g$ , the maximum particle size  $D_{\max}$ , the Porod (excluded) volumes, and the mass. The relative differences between the proteins measured are confirmed, as well as the indication of a dimeric assembly for the WT DdSSRP1 $\Delta$ CTD. The merged SAXS data for DdSSRP1 $\Delta$ CTD are further analyzed in Fig. 4, B–D. Both the pair distance distribution function ( $P(r)$ ) and the dimensionless Kratky plot suggest an elongated structure for the homodimer, deviating from the overall fold of typical globular proteins. The good linearity of the Guinier region confirms the successful removal of low- $s$  concentration dependences.

The merged profile for the FACT complex was further utilized to obtain the *ab initio* model of its low-resolution shape using the program DAMMIF (41) (see “Experimental procedures”). For the WT DdSSRP1 $\Delta$ CTD and its two mutants showing concentration-dependent dimerization, the program GasborMX was employed to generate the shapes of monomers and dimers while simultaneously fitting multiple scattering data sets at different concentrations (see “Experimental procedures”). The resulting *ab initio* models and fits to the experimental SAXS are displayed in Fig. 4, E and F, and the concentration dependence of the monomer and dimer fractions for DdSSRP1 $\Delta$ CTD WT, 6mut, and Q306K is given in Fig. S3.

From the above *ab initio* modeling, FACT heterodimer has an elongated asymmetric shape. Both DdSSRP1 $\Delta$ CTD and its mutants demonstrate a concentration-dependent homodimerization. However, only WT DdSSRP1 $\Delta$ CTD reveals significant amounts of dimers at low protein concentration. The mutants are mostly dissociated into monomers at concentrations below 1 mg/ml, with a slight tendency for increased dimerization at higher concentrations (Fig. S3). Given these results, at the (low) concentrations used for AUC, an essentially monomeric state is expected. All the DdSSRP1 $\Delta$ CTD homodimers are elongated (Fig. S4), with the length comparable with the FACT heterodimer but with a smaller cross-section. Interestingly, the dimers seem to adopt an extended, V-shaped conformation in solution.

### Homodimerization plays a role in histone binding

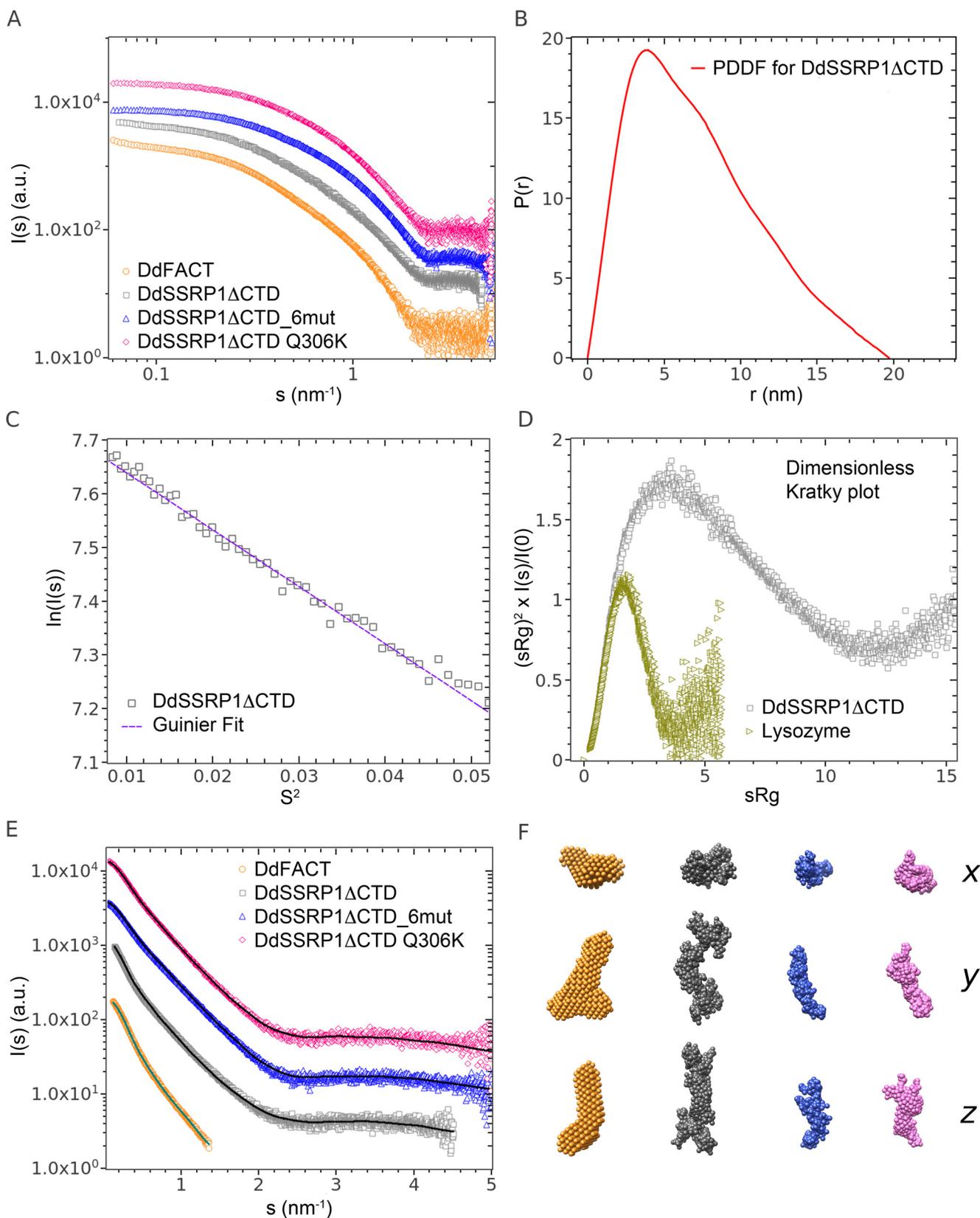
SSRP1 has been shown to have SPT16-independent function in gene transcription as knockdown of SPT16 and SSRP1 in human nonsmall cell lung carcinoma cells revealed a subset of genes that are regulated by SSRP1 independent of SPT16 (36). Previous studies showed that SSRP1 binds nucleosomes with high affinity and has a preference for binding histone H3–H4 (19). We hypothesized that SSRP1 homodimerization contributes to histone binding. Currently, we do not know how SSRP1 homodimerizes, and therefore it was difficult to generate an SSRP1 variant that solely affects homodimerization. Given that DdSSRP1 $\Delta$ CTD Q306K and DdSSRP1 $\Delta$ CTD\_6mut are monomeric in solution at low concentrations, we tested their effects on histone binding. We performed ITC analyses to assess how DdSSRP1 $\Delta$ CTD, DdSSRP1 $\Delta$ CTD Q306K, and DdSSRP1 $\Delta$ CTD\_6mut bind histones. DdSSRP1 $\Delta$ CTD bound histone H3–H4 with a  $K_d$  of  $0.57 \pm 0.05 \mu\text{M}$  (Fig. 5A and Table 3), which is similar to the binding affinity observed between human SSRP1 and *Xenopus laevis* histone H3–H4 (19). Interestingly, DdSSRP1 $\Delta$ CTD\_6mut and DdSSRP1 $\Delta$ CTD Q306K bound histone H3–H4 with a  $K_d$  of  $3.0 \pm 0.2 \mu\text{M}$  and  $1.37 \pm 0.07 \mu\text{M}$ , respectively (Fig. 5, B and C, and Table 3). Thus, disruption of homodimerization via Q306K substitution or mutations in the PH2 domain decreased histone H3–H4 binding affinity by 5- or 2.5-fold, respectively. We also tested SSRP1 binding affinity with histone H2A–H2B. DdSSRP1 $\Delta$ CTD bound histone H2A–H2B with a  $K_d$  of  $0.82 \pm 0.05 \mu\text{M}$  (Fig. 5D and Table 3), whereas DdSSRP1 $\Delta$ CTD\_6mut exhibited a slightly weaker binding affinity with a  $K_d$  of  $1.57 \pm 0.14 \mu\text{M}$  (Fig. 5E and Table 3). The ITC profile for DdSSRP1 $\Delta$ CTD Q306K binding is biphasic (Fig. 5F) suggesting that the Q306K substitution likely

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alters the surface charge thereby initiating a nonspecific H2A–H2B binding. Together, our ITC analyses show that SSRP1 homodimerization contributes to histone binding.

## DdSSRP1 ID1 region binds histone H2A–H2B

Recent studies on Pob3 showed that its acidic ID1 region binds directly to histone H2A–H2B (29, 42). To investigate



**Table 2**

Data collection and SAXS-derived parameters

Data collection parameters				
Radiation source	Petra III (DESY)			
Beamline	EMBL P12			
Detector	Pilatus 2M			
Beam geometry (mm)	0.2 x 0.12			
Wavelength (nm)	0.124			
Sample-detector distance (m)	3.1			
$s$ range (nm <sup>-1</sup> )	0.02-4.5			
Exposure time (s)	1 (=20 x 0.05 s)			
Temperature (K)	283.15 or 293.15			
Overall parameters (merged profiles)				
	DdFACT	DdSSRP1 ΔCTD	DdSSRP1 ΔCTD 6mut	DdSSRP1 ΔCTD Q306K
$R_g$ from Guinier approximation (nm)	5.89 ± 0.34	5.64 ± 0.12	4.42 ± 0.02	4.29 ± 0.13
$R_g$ from PDDF (nm)	6.06	5.68	4.48	4.38
$D_{MAX}$ (nm)	20 ± 1	20 ± 1	15 ± 1	15 ± 1
Porod Volume, $V_p$ (nm <sup>3</sup> )	368 ± 15	189 ± 15	92 ± 15	103 ± 15
DAMMIF excluded volume (nm <sup>3</sup> )	483 ± 20	n/a	n/a	n/a
Molecular weight from $V_p$ (kDa)	230	118	58	64
Molecular weight from excluded volume (kDa)	230	n/a	n/a	n/a
Molecular weight from sequence (kDa)	162.856	54.233	54.102	54.233
Software employed				
Primary data reduction	SASFLOW			
Data processing	PRIMUS			
<i>Ab initio</i> modelling	DAMMIF/DAMMIN, GASBORMX			
Validation and averaging	DAMMAVER			
Parameters of filtered <i>ab initio</i> DdFACT model				
	Normalized Spatial Discrepancy	Standard deviation of NSD	Number of models excluded	Final $\chi^2$ after DAMMIN
DdFACT	0.793	0.055	1	1.205

whether the ID1 region of DdSSRP1 also binds histone H2A–H2B, we performed ITC analyses using full-length DdSSRP1 (DdSSRP1\_FL) and DdSSRP1 containing only the ID1 region (residues 479–527; DdSSRP1\_ID1). Titration of DdSSRP1\_FL against histone H2A–H2B generated an exothermic thermogram with a  $K_d$  of  $0.31 \pm 0.03 \mu\text{M}$  (Table 3) that is different from the endothermic thermogram generated by titration of DdSSRP1ΔCTD against histone H2A–H2B (compare Figs. 5D and 6A), suggesting that the ID1 region likely binds histone H2A–H2B, and the interaction is exothermic. Indeed, DdSSRP1\_ID1 bound histone H2A–H2B with a  $K_d$  of  $0.19 \pm 0.03 \mu\text{M}$ , and the thermogram exhibited an exothermic reaction (Fig. 6B and Table 3). These results showed that the ID1 region of DdSSRP1 binds to histone H2A–H2B.

## Discussion

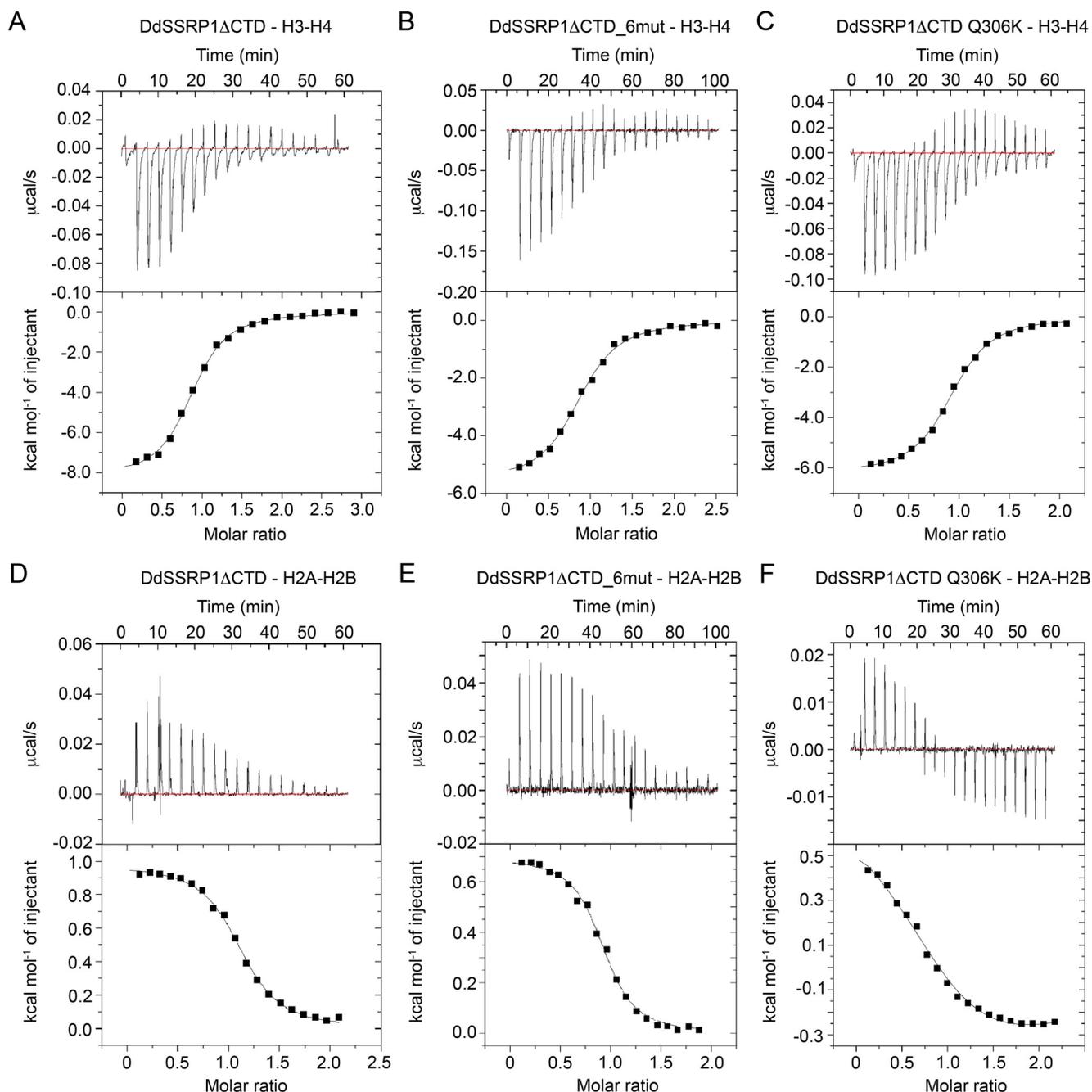
Little is known about how SSRP1 behaves independent of SPT16; our present work reveals a role of SSRP1 homodimerization. We show that both human and *D. discoideum* SSRP1 self-associate to form an elongated homodimer in solution using both AUC and SAXS analyses. Homodimerization

likely involves both PH2 and PH3 domains as mutations within these two domains yield monomeric protein. Notably, the PH2 surface that interacts with SPT16 also participates in homodimerization, suggesting that both binding events might be mutually exclusive. Indeed, we show that the DdFACT complex does not oligomerize, consistent with an earlier study on yeast FACT (33). Furthermore, we show that disruption of SSRP1 homodimerization decreases the binding affinity of SSRP1 for histones H2A–H2B and H3–H4, highlighting a role for homodimerization in the function of SSRP1.

Homodimerization has been observed in other histone chaperones such as Rtt106 (43, 44), yeast nucleosome assembly protein 1 (Nap1) (45), and vacuolar protein sorting 75 (Vps75) (46, 47). These histone chaperones harbor an N-terminal helical domain that is involved in dimerization. Homodimerization plays an important role in their functions as follows. Rtt106 homodimerization is essential for its association with histone H3–H4 tetramer and its function in transcriptional silencing (44); Nap1 homodimerization enables it to interact with two molecules of histones H2A–H2B or H3–H4 (48); and Vps75

**Figure 4. SAXS analyses of DdSSRP1ΔCTD.** A, double logarithmic plot showing the merged SAXS profiles used to extract overall parameters and for DAMMIF modeling of the FACT heterodimer, arbitrarily displaced in the vertical direction for display. B, DdSSRP1ΔCTD WT pair distance distribution function  $P(r)$  (PDDF). C, Guinier plot; D, dimensionless Kratky plot with a globular protein for comparison of DdSSRP1ΔCTD WT. E, comparison of the SAXS data used for structural modeling (symbols) with the calculated scattering curves from DAMMIF and GasborMX (solid lines). For the FACT complex, the DAMMIF modeling employed the merged curve up to  $1.4 \text{ nm}^{-1}$ . For DdSSRP1ΔCTD and mutants, the GasborMX modeling employed the SAXS profiles at the highest concentration (WT 9 mg/ml, 6mut 12.2 mg/ml, and Q306K 10.9 mg/ml). The pairs of fits are shifted by about 1 order of magnitude along the logarithmic axis for clarity. F, final averaged (“filtered”) *ab initio* model of FACT and three representative GasborMX models of DdSSRP1ΔCTD WT, 6mut, and Q306K in three orthogonal views along the  $x$ ,  $y$ , and  $z$  axes. From left to the right: DdFACT heterodimer (orange), DdSSRP1ΔCTD WT homodimer (gray), monomer DdSSRP1ΔCTD\_6mut (blue), and monomer DdSSRP1ΔCTD Q306K (pink).

## SSRP1 homodimerization has a role in histone binding

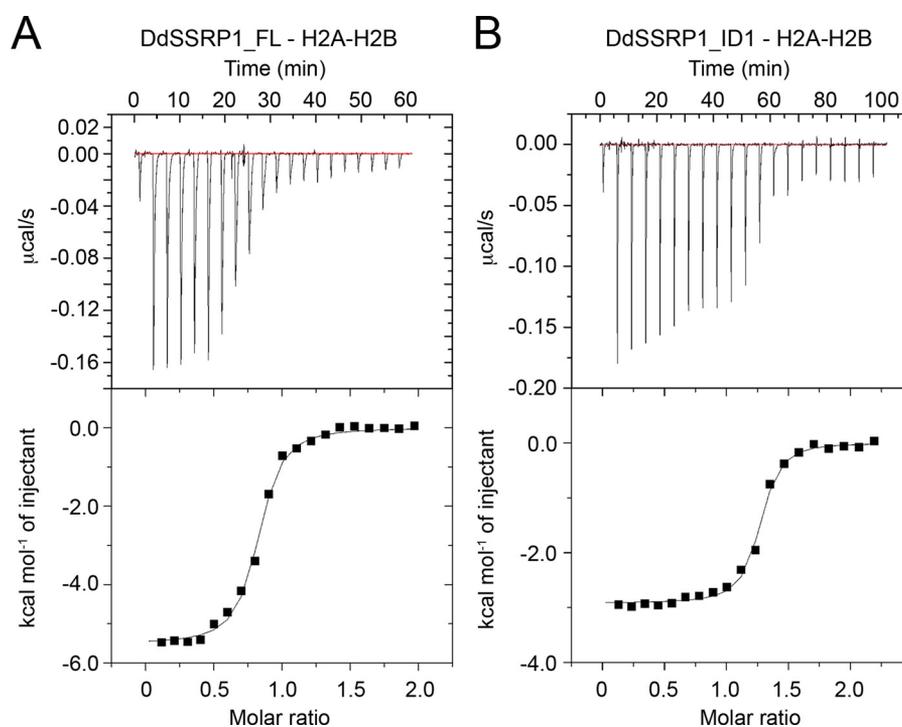


**Figure 5.** ITC profiles of the interaction between DdSSRP1 variants and histones. *A*, DdSSRP1 $\Delta$ CTD-H3-H4 binding profile. *B*, DdSSRP1 $\Delta$ CTD<sub>6mut</sub>-H3-H4 binding profile. *C*, DdSSRP1 $\Delta$ CTD Q306K-H3-H4 binding profile. *D*, DdSSRP1 $\Delta$ CTD-H2A-H2B binding profile. *E*, DdSSRP1 $\Delta$ CTD<sub>6mut</sub>-H2A-H2B binding profile. *F*, DdSSRP1 $\Delta$ CTD Q306K-H2A-H2B binding profile. Raw data (*top panel*) and integrated data (*bottom panel*) are shown. The data shown are representative of two independent experiments except *C*, *E*, and *F* that are representative of one experiment.

**Table 3**  
ITC analysis of DdSSRP1 constructs against histone H2A-H2B or H3-H4

SSRP1-histone binding	$K_d$
	$\mu\text{M}$
DdSSRP1 $\Delta$ CTD-H2A-H2B	$0.82 \pm 0.05$
DdSSRP1 $\Delta$ CTD <sub>6mut</sub> -H2A-H2B	$1.57 \pm 0.14$
DdSSRP1 $\Delta$ CTD-H3-H4	$0.57 \pm 0.05$
DdSSRP1 $\Delta$ CTD <sub>6mut</sub> -H3-H4	$3.0 \pm 0.2$
DdSSRP1 $\Delta$ CTD Q306K-H3-H4	$1.37 \pm 0.07$
DdSSRP1 <sub>FL</sub> -H2A-H2B	$0.31 \pm 0.03$
DdSSRP1 <sub>ID1</sub> -H2A-H2B	$0.19 \pm 0.03$

homodimerization is involved in binding and activating the catalytic activity of histone acetyltransferase Rtt109 (47, 49). SSRP1 lacks a defined homodimerization domain. We show that the PH1-PH2 domain is a monomer, and structural studies of MD from human and Pob3 also reveal a monomer (24, 30). Homodimerization seems to be achieved via multiple surfaces involving both PH2 and PH3 domains. The exact molecular details of homodimerization will require future studies. Here, we show that monomeric forms of SSRP1 mutants are able to bind both histones H2A-H2B and H3-H4 with low micromolar affinity, but the presence of a homodimer further



**Figure 6.** ITC profiles of the interaction between DdSSRP1 ID1 region and histone H2A–H2B. *A*, DdSSRP1\_FL–H2A–H2B binding profile. *B*, DdSSRP1\_ID1–H2A–H2B binding profile. Raw data (top panel) and integrated data (bottom panel) are shown. The data shown are representative of one independent experiment.

enhances the binding affinity. Given that SSRP1 interacts with other proteins independent of SPT16, future studies will be required to elucidate whether homodimerization has a role in other SSRP1 functions.

In our study, we show that both human and *D. discoideum* SSRP1 are homodimers, but whether homodimerization is conserved in other species remains unclear. Sequence analysis of SSRP1 from plants, fungi, and mammals shows that SSRP1 comprising N-terminal PH domains and the ID1 region is conserved. Closer inspection reveals that Gln-306 (in *D. discoideum*) is conserved from fungi to mammals, but the corresponding residue in plants is a lysine (Fig. 7). In our study, DdSSRP1 $\Delta$ CTD Q306K is predominantly a monomer at low protein concentrations, but it has the tendency to dimerize at high protein concentrations. Thus, this criterion alone is not sufficient to predict its oligomeric state. Future studies are required to assess the oligomeric state of SSRP1 in other species.

We showed that SSRP1 homodimerization and SPT16 heterodimerization utilize the same SSRP1's PH2 surface, and the FACT complex contains one molecule of SSRP1. These data suggest that both events could compete for the same pool of SSRP1. This raises the question of how SSRP1 oligomerization is regulated. In HeLa and yeast cells the FACT complex concentration is in the range of 0.1–0.5  $\mu$ M, suggesting that the bulk of SSRP1 is associated with SPT16 (9, 33). Our SAXS analyses showed that SSRP1 homodimerization is concentration-dependent where the monomeric fraction increases at lower protein concentrations (Fig. S3). At 1 mg/ml ( $\sim$ 18  $\mu$ M), DdSSRP1 $\Delta$ CTD is predominantly a dimer but with a small fraction in the monomeric state. Thus, it seems that at a low protein concentration, SSRP1 has a stronger preference to form a com-

plex with SPT16, and the SSRP1 homodimer would be present when SSRP1 is in excess of SPT16 and at higher protein concentration. Future studies are required to understand how SSRP1 homodimerization is regulated.

Studies have shown that human SSRP1 binds nucleosome, histone H3–H4, and DNA (19, 30), and Pob3 binds histone H3–H4 (50). In this study, we show that DdSSRP1 binds histones H2A–H2B and H3–H4 via different mechanisms. DdSSRP1 $\Delta$ CTD bind histone H3–H4 with a binding affinity similar to that of human SSRP1 (19). Interestingly, both PH1–4 domains (DdSSRP1 $\Delta$ CTD) and the C-terminal ID1 region (DdSSRP1\_ID1) bind histone H2A–H2B independently. A very recent study showed that the C-terminal acidic ID1 region of Pob3 containing a (D/E)XX $\Phi$  (where  $\Phi$  is Phe or Tyr, and X is any residue; DEDF in Pob3) motif binds H2B (29), and the reported binding affinity is similar to the binding affinity of DdSSRP1\_ID1 for histone H2A–H2B observed here. Furthermore, DdSSRP1 harbors a DDDY motif in the ID1 region, suggesting a similar mode of H2A–H2B binding. It is unclear how DdSSRP1 PH1–4 domains bind H2A–H2B, and whether the binding site is distinct or overlaps with the ID1 region requires future investigation. It is noteworthy that the C-terminal acid region of yeast Spt16 contains an EVSEY motif that binds to the same H2B pocket as the acidic ID1 region of Pob3, and their H2A–H2B binding mode is incompatible with H2A–H2B–DNA interaction in the nucleosome structure (29). Thus, it was proposed that the SPT16 and SSRP1 C-terminal acidic regions of FACT would bind to two symmetry-related H2A–H2B dimers to compete with DNA thereby promoting nucleosome reorganization. The distance between the two H2B-binding pockets in the nucleosome structure is about 60 Å, and the nucleosome has a dimension of 100  $\times$  100  $\times$  60 Å. With the



from the following: 1) extrapolation to zero scattering angle on absolute scale; 2) the excluded volume of the hydrated particle using the Porod invariant (59), and 3) the excluded volumes of *ab initio* bead models. The latter were generated from the low resolution data ( $s < 1.4 \text{ nm}^{-1}$ ) for the FACT complex by repeating 20 DAMMIF runs (61) without symmetry (P1) or anisotropy and refined with DAMMIN (41). Both algorithms construct bead models yielding a scattering profile with the lowest possible discrepancy ( $\chi$ ) from the experimental data while keeping beads interconnected and the model compact. Twenty independent *ab initio* reconstructions were performed and averaged using DAMAVER (62), which also provides a normalized spatial discrepancy as a measure of similarity among different reconstructions. SAXS methods and results are summarized in Table 2.

The homodimerization behavior of SSRP1 WT and mutants, as well as the structure of the dimers, were analyzed by GasborMX (59). The program simultaneously fits SAXS profiles of the concentration series with a linear combination of the scattering intensities of monomer and dimer. The monomer structure is represented as a collection of dummy residues, and the dimer is generated in a P2 symmetry. To better model the dimers, prolate anisotropy was imposed, with the dyadic axis of the symmetric dimer being transverse to the major axis of the model. For the concentration series treated, the modeling was repeated 10 times, and the best fitting models were selected. Alignment of the *ab initio* models for depiction and comparison purposes was performed using SUPCOMB (63). The *ab initio* models shown in Fig. 4 and in the supporting information were rendered using the UCSF Chimera package (64).

### Isothermal titration calorimetry

SSRP1 binding to histone H2A–H2B or H3–H4 was determined at 25 °C by using a MicroCal iTC200 microcalorimeter (Malvern Instruments Ltd., UK). All proteins were buffer-exchanged into 20 mM HEPES, pH 7.5, 200 mM NaCl, 1 mM DTT. Histone H2A–H2B or H3–H4 was loaded into the cell at a concentration of 20–30  $\mu\text{M}$ , and SSRP1 variants were loaded into the syringe at a concentration 10 times higher than that in the cell. 20 injections (2  $\mu\text{l}$  each) were added every 180 s to the cell. For control experiments, buffer or SSRP1 was injected into the cell containing histone or buffer, respectively. ITC data were generated by subtracting the raw data from the control experiment and were analyzed using Origin software (version 7, OriginLab).

**Author contributions**—G. M. data curation; G. M. and D. T. H. formal analysis; G. M. and D. T. H. supervision; G. M. and D. T. H. investigation; G. M. and D. T. H. writing-original draft; G. M. and D. T. H. project administration; G. M., S. D. V., D. I. S., and D. T. H. writing-review and editing; O. B. methodology; D. T. H. conceptualization; D. T. H. funding acquisition; S. D. V., G. T., and D. I. S. performed and analyzed SAXS experiment.

### References

- Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 [CrossRef Medline](#)
- Ransom, M., Dennehey, B. K., and Tyler, J. K. (2010) Chaperoning histones during DNA replication and repair. *Cell* **140**, 183–195 [CrossRef Medline](#)
- Groth, A., Rocha, W., Verreault, A., and Almouzni, G. (2007) Chromatin challenges during DNA replication and repair. *Cell* **128**, 721–733 [CrossRef Medline](#)
- Smith, S., and Stillman, B. (1991) Stepwise assembly of chromatin during DNA replication *in vitro*. *EMBO J.* **10**, 971–980 [Medline](#)
- Svejstrup, J. Q. (2002) Chromatin elongation factors. *Curr. Opin. Genet. Dev.* **12**, 156–161 [CrossRef Medline](#)
- Belotserkovskaya, R., and Reinberg, D. (2004) Facts about FACT and transcript elongation through chromatin. *Curr. Opin. Genet. Dev.* **14**, 139–146 [CrossRef Medline](#)
- Akey, C. W., and Luger, K. (2003) Histone chaperones and nucleosome assembly. *Curr. Opin. Struct. Biol.* **13**, 6–14 [CrossRef Medline](#)
- Orphanides, G., LeRoy, G., Chang, C. H., Luse, D. S., and Reinberg, D. (1998) FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell* **92**, 105–116 [CrossRef Medline](#)
- Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M., and Reinberg, D. (1999) The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature* **400**, 284–288 [CrossRef Medline](#)
- Brewster, N. K., Johnston, G. C., and Singer, R. A. (1998) Characterization of the CP complex, an abundant dimer of Cdc68 and Pob3 proteins that regulates yeast transcriptional activation and chromatin repression. *J. Biol. Chem.* **273**, 21972–21979 [CrossRef Medline](#)
- Wittmeyer, J., and Formosa, T. (1997) The *Saccharomyces cerevisiae* DNA polymerase  $\alpha$  catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. *Mol. Cell. Biol.* **17**, 4178–4190 [CrossRef Medline](#)
- Schlesinger, M. B., and Formosa, T. (2000) POB3 is required for both transcription and replication in the yeast *Saccharomyces cerevisiae*. *Genetics* **155**, 1593–1606 [Medline](#)
- Keller, D. M., and Lu, H. (2002) p53 serine 392 phosphorylation increases after UV through induction of the assembly of the CK2.hSPT16.SSRP1 complex. *J. Biol. Chem.* **277**, 50206–50213 [CrossRef Medline](#)
- Heo, K., Kim, H., Choi, S. H., Choi, J., Kim, K., Gu, J., Lieber, M. R., Yang, A. S., and An, W. (2008) FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. *Mol. Cell* **30**, 86–97 [CrossRef Medline](#)
- Belotserkovskaya, R., Oh, S., Bondarenko, V. A., Orphanides, G., Studitsky, V. M., and Reinberg, D. (2003) FACT facilitates transcription-dependent nucleosome alteration. *Science* **301**, 1090–1093 [CrossRef Medline](#)
- Xin, H., Takahata, S., Blanksma, M., McCullough, L., Stillman, D. J., and Formosa, T. (2009) yFACT induces global accessibility of nucleosomal DNA without H2A–H2B displacement. *Mol. Cell* **35**, 365–376 [CrossRef Medline](#)
- Winkler, D. D., and Luger, K. (2011) The histone chaperone Fact: Structural insights and mechanisms for nucleosome reorganization. *J. Biol. Chem.* **286**, 18369–18374 [CrossRef Medline](#)
- Formosa, T., Eriksson, P., Wittmeyer, J., Ginn, J., Yu, Y., and Stillman, D. J. (2001) Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. *EMBO J.* **20**, 3506–3517 [CrossRef Medline](#)
- Winkler, D. D., Muthurajan, U. M., Hieb, A. R., and Luger, K. (2011) Histone chaperone FACT coordinates nucleosome interaction through multiple synergistic binding events. *J. Biol. Chem.* **286**, 41883–41892 [CrossRef Medline](#)
- Hondele, M., Stuwe, T., Hassler, M., Halbach, F., Bowman, A., Zhang, E. T., Nijmeijer, B., Kotthoff, C., Rybin, V., Amlacher, S., Hurt, E., and Ladurner, A. G. (2013) Structural basis of histone H2A–H2B recognition by the essential chaperone FACT. *Nature* **499**, 111–114 [CrossRef Medline](#)
- Stuwe, T., Hothorn, M., Lejeune, E., Rybin, V., Bortfeld, M., Scheffzek, K., and Ladurner, A. G. (2008) The FACT Spt16 “peptidase” domain is a histone H3–H4 binding module. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 8884–8889 [CrossRef Medline](#)
- Kemble, D. J., Whitby, F. G., Robinson, H., McCullough, L. L., Formosa, T., and Hill, C. P. (2013) Structure of the Spt16 middle domain reveals func-

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- tional features of the histone chaperone FACT. *J. Biol. Chem.* **288**, 10188–10194 [CrossRef Medline](#)
23. VanDemark, A. P., Xin, H., McCullough, L., Rawlins, R., Bentley, S., Heroux, A., Stillman, D. J., Hill, C. P., and Formosa, T. (2008) Structural and functional analysis of the Spt16p N-terminal domain reveals overlapping roles of  $\gamma$ FACT subunits. *J. Biol. Chem.* **283**, 5058–5068 [CrossRef Medline](#)
24. VanDemark, A. P., Blanksma, M., Ferris, E., Heroux, A., Hill, C. P., and Formosa, T. (2006) The structure of the  $\gamma$ FACT Pob3-M domain, its interaction with the DNA replication factor RPA, and a potential role in nucleosome deposition. *Mol. Cell* **22**, 363–374 [CrossRef Medline](#)
25. Allain, F. H., Yen, Y. M., Masse, J. E., Schultze, P., Dieckmann, T., Johnson, R. C., and Feigon, J. (1999) Solution structure of the HMG protein NHP6A and its interaction with DNA reveals the structural determinants for non-sequence-specific binding. *EMBO J.* **18**, 2563–2579 [CrossRef Medline](#)
26. Marciànò, G., and Huang, D. T. (2016) Structure of the human histone chaperone FACT Spt16 N-terminal domain. *Acta Crystallogr. F Struct. Biol. Commun.* **72**, 121–128 [CrossRef Medline](#)
27. Tsunaka, Y., Fujiwara, Y., Oyama, T., Hirose, S., and Morikawa, K. (2016) Integrated molecular mechanism directing nucleosome reorganization by human FACT. *Genes Dev.* **30**, 673–686 [CrossRef Medline](#)
28. Tsunaka, Y., Toga, J., Yamaguchi, H., Tate, S., Hirose, S., and Morikawa, K. (2009) Phosphorylated intrinsically disordered region of FACT masks its nucleosomal DNA binding elements. *J. Biol. Chem.* **284**, 24610–24621 [CrossRef Medline](#)
29. Kemble, D. J., McCullough, L. L., Whitby, F. G., Formosa, T., and Hill, C. P. (2015) FACT disrupts nucleosome structure by binding H2A–H2B with conserved peptide motifs. *Mol. Cell* **60**, 294–306 [CrossRef Medline](#)
30. Zhang, W., Zeng, F., Liu, Y., Shao, C., Li, S., Lv, H., Shi, Y., Niu, L., Teng, M., and Li, X. (2015) Crystal structure of human SSRP1 middle domain reveals a role in DNA binding. *Sci. Rep.* **5**, 18688 [Medline](#)
31. Masse, J. E., Wong, B., Yen, Y. M., Allain, F. H., Johnson, R. C., and Feigon, J. (2002) The *S. cerevisiae* architectural HMG protein NHP6A complexed with DNA:DNA and protein conformational changes upon binding. *J. Mol. Biol.* **323**, 263–284 [CrossRef Medline](#)
32. Kasai, N., Tsunaka, Y., Ohki, I., Hirose, S., Morikawa, K., and Tate, S. (2005) Solution structure of the HMG-box domain in the SSRP1 subunit of FACT. *J. Biomol. NMR* **32**, 83–88 [CrossRef Medline](#)
33. Wittmeyer, J., Joss, L., and Formosa, T. (1999) Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase  $\alpha$ . *Biochemistry* **38**, 8961–8971 [CrossRef Medline](#)
34. Spencer, J. A., Baron, M. H., and Olson, E. N. (1999) Cooperative transcriptional activation by serum response factor and the high-mobility group protein SSRP1. *J. Biol. Chem.* **274**, 15686–15693 [CrossRef Medline](#)
35. Zeng, S. X., Dai, M. S., Keller, D. M., and Lu, H. (2002) SSRP1 functions as a co-activator of the transcriptional activator p63. *EMBO J.* **21**, 5487–5497 [CrossRef Medline](#)
36. Li, Y., Zeng, S. X., Landais, I., and Lu, H. (2007) Human SSRP1 has Spt16-dependent and -independent roles in gene transcription. *J. Biol. Chem.* **282**, 6936–6945 [CrossRef Medline](#)
37. Zeng, S. X., Li, Y., Jin, Y., Zhang, Q., Keller, D. M., McQuaw, C. M., Barklis, E., Stone, S., Hoatlin, M., Zhao, Y., and Lu, H. (2010) Structure-specific recognition protein 1 facilitates microtubule growth and bundling required for mitosis. *Mol. Cell Biol.* **30**, 935–947 [Medline](#)
38. Ikeda, Y., Kinoshita, Y., Susaki, D., Ikeda, Y., Iwano, M., Takayama, S., Higashiyama, T., Kakutani, T., and Kinoshita, T. (2011) HMG domain containing SSRP1 is required for DNA demethylation and genomic imprinting in *Arabidopsis*. *Dev. Cell* **21**, 589–596 [CrossRef Medline](#)
39. Smith, C. A. (1988) Estimation of sedimentation coefficients and frictional ratios of globular proteins. *Biomed. Education* **16**, 104–106
40. Dam, J., and Schuck, P. (2004) Calculating sedimentation coefficient distributions by direct modeling of sedimentation velocity concentration profiles. *Methods Enzymol.* **384**, 185–212 [CrossRef Medline](#)
41. Franke, D., and Svergun, D. I. (2009) DAMMIF, a program for rapid *ab-initio* shape determination in small-angle scattering. *J. Appl. Crystallogr.* **42**, 342–346 [CrossRef Medline](#)
42. Hoffmann, C., and Neumann, H. (2015) *In vivo* mapping of FACT-histone interactions identifies a role of Pob3 C erminus in H2A–H2B binding. *ACS Chem. Biol.* **10**, 2753–2763 [CrossRef Medline](#)
43. Su, D., Hu, Q., Li, Q., Thompson, J. R., Cui, G., Fazly, A., Davies, B. A., Botuyan, M. V., Zhang, Z., and Mer, G. (2012) Structural basis for recognition of H3K56-acetylated histone H3–H4 by the chaperone Rtt106. *Nature* **483**, 104–107 [CrossRef Medline](#)
44. Fazly, A., Li, Q., Hu, Q., Mer, G., Horazdovsky, B., and Zhang, Z. (2012) Histone chaperone Rtt106 promotes nucleosome formation using (H3–H4)<sub>2</sub> tetramers. *J. Biol. Chem.* **287**, 10753–10760 [CrossRef Medline](#)
45. Park, Y. J., and Luger, K. (2006) The structure of nucleosome assembly protein 1. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 1248–1253 [CrossRef Medline](#)
46. Tang, Y., Meeth, K., Jiang, E., Luo, C., and Marmorstein, R. (2008) Structure of Vps75 and implications for histone chaperone function. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 12206–12211 [CrossRef Medline](#)
47. Park, Y. J., Sudhoff, K. B., Andrews, A. J., Stargell, L. A., and Luger, K. (2008) Histone chaperone specificity in Rtt109 activation. *Nat. Struct. Mol. Biol.* **15**, 957–964 [CrossRef Medline](#)
48. Andrews, A. J., Downing, G., Brown, K., Park, Y. J., and Luger, K. (2008) A thermodynamic model for Nap1-histone interactions. *J. Biol. Chem.* **283**, 32412–32418 [CrossRef Medline](#)
49. Kolonko, E. M., Albaugh, B. N., Lindner, S. E., Chen, Y., Satyshur, K. A., Arnold, K. M., Kaufman, P. D., Keck, J. L., and Denu, J. M. (2010) Catalytic activation of histone acetyltransferase Rtt109 by a histone chaperone. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 20275–20280 [CrossRef Medline](#)
50. Zunder, R. M., Antczak, A. J., Berger, J. M., and Rine, J. (2012) Two surfaces on the histone chaperone Rtt106 mediate histone binding, replication, and silencing. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E144–E153 [CrossRef Medline](#)
51. Lambert, S. J., Nicholson, J. M., Chantalat, L., Reid, A. J., Donovan, M. J., and Baldwin, J. P. (1999) Purification of histone core octamers and 2.15 Å X-ray analysis of crystals in KCl/phosphate. *Acta Crystallogr. D Biol. Crystallogr.* **55**, 1048–1051 [CrossRef Medline](#)
52. Schuck, P. (2000) Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and Lamm equation modeling. *Biophys. J.* **78**, 1606–1619 [CrossRef Medline](#)
53. Laue, T. M., Shah, B. D., Ridgeway, T. M., and Peletier, S. L. (1992) *Analytical Ultracentrifugation in Biochemistry and Polymer Science* (Harding, S. E., Rowe, A. J., and Horton, J. C., eds) pp. 99–125, Society of Chemistry, Cambridge, UK
54. Vistica, J., Dam, J., Balbo, A., Yikilmaz, E., Mariuzza, R. A., Rouault, T. A., and Schuck, P. (2004) Sedimentation equilibrium analysis of protein interactions with global implicit mass conservation constraints and systematic noise decomposition. *Anal. Biochem.* **326**, 234–256 [CrossRef Medline](#)
55. Blanchet, C. E., Spilotros, A., Schwemmer, F., Graewert, M. A., Kikhney, A., Jeffries, C. M., Franke, D., Mark, D., Zengerle, R., Cipriani, F., Fiedler, S., Roessle, M., and Svergun, D. I. (2015) Versatile sample environments and automation for biological solution X-ray scattering experiments at the P12 beamline (PETRA III, DESY). *J. Appl. Crystallogr.* **48**, 431–443 [CrossRef Medline](#)
56. Round, A. R., Franke, D., Moritz, S., Huchler, R., Fritsche, M., Malthan, D., Klaering, R., Svergun, D. I., and Roessle, M. (2008) Automated sample-changing robot for solution scattering experiments at the EMBL Hamburg SAXS station X33. *J. Appl. Crystallogr.* **41**, 913–917 [CrossRef Medline](#)
57. Franke, D., Kikhney, A. G., and Svergun, D. I. (2012) Automated acquisition and analysis of small angle X-ray scattering data. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **689**, 52–59
58. Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H., and Svergun, D. I. (2003) PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* **36**, 1277–1282 [CrossRef](#)
59. Petoukhov, M. V., Franke, D., Shkumatov, A. V., Tria, G., Kikhney, A. G., Gajda, M., GORBA, C., Mertens, H. D., Konarev, P. V., and Svergun, D. I. (2012) New developments in the ATSAS program package for small-angle

- scattering data analysis. *J. Appl. Crystallogr.* **45**, 342–350 [CrossRef](#) [Medline](#)
60. Svergun, D. (1992) Determination of the regularization parameter in indirect-transform methods using perceptual criteria. *J. Appl. Crystallogr.* **25**, 495–503 [CrossRef](#)
61. Svergun, D. I. (1999) Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing. *Biophys. J.* **76**, 2879–2886 [CrossRef](#) [Medline](#)
62. Volkov, V. V., and Svergun, D. I. (2003) Uniqueness of *ab initio* shape determination in small-angle scattering. *J. Appl. Crystallogr.* **36**, 860–864 [CrossRef](#)
63. Kozin, M. B., and Svergun, D. I. (2001) Automated matching of high- and low-resolution structural models. *J. Appl. Crystallogr.* **34**, 33–41 [CrossRef](#) [Medline](#)
64. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605–1612 [CrossRef](#) [Medline](#)
65. Brautigam, C. A. (2015) Calculations and publication-quality illustrations for analytical ultracentrifugation data. *Methods Enzymol.* **562**, 109–133 [CrossRef](#) [Medline](#)

**Structure-specific recognition protein-1 (SSRP1) is an elongated homodimer that binds histones**

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