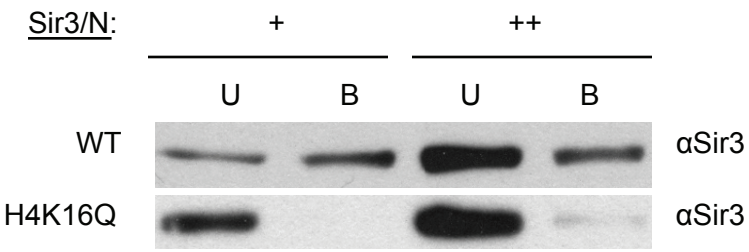
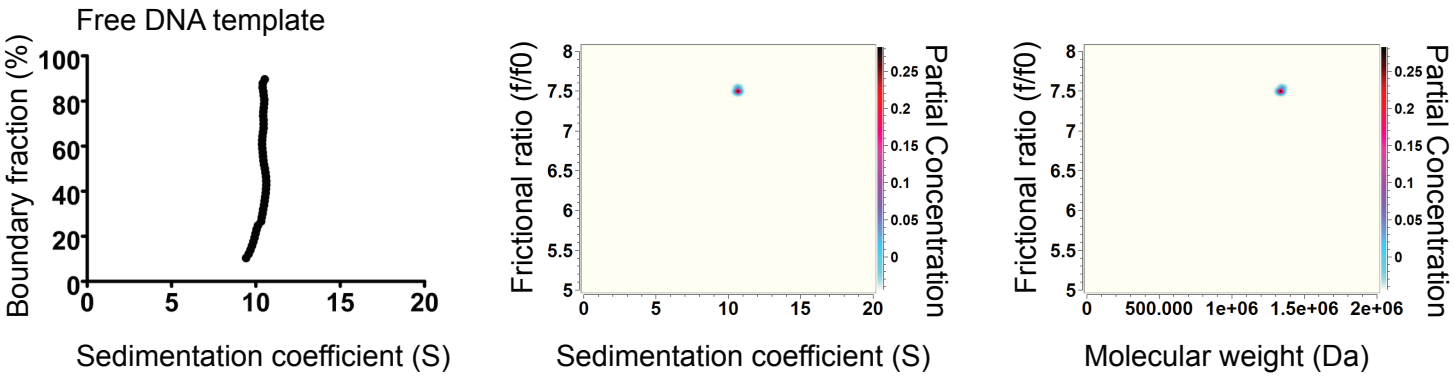
a

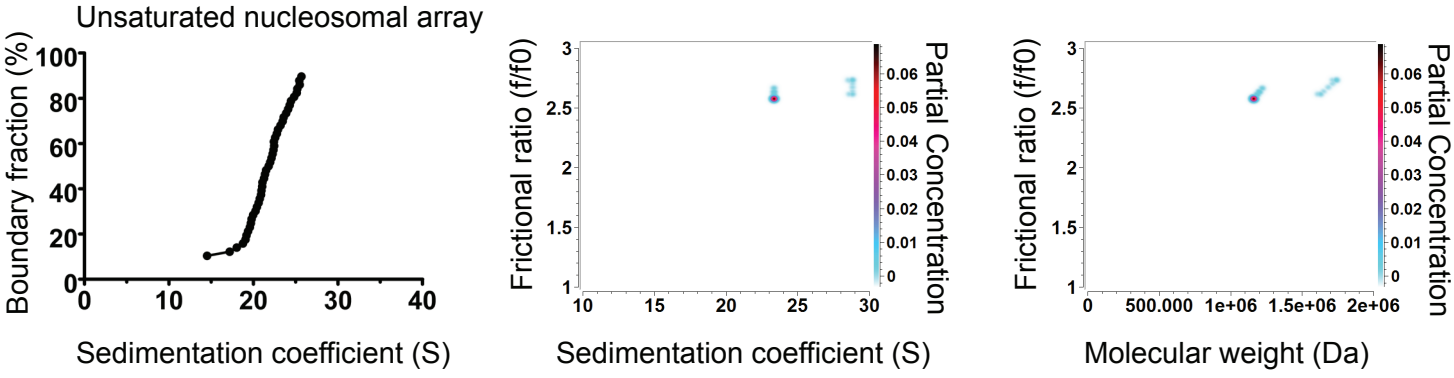


Supplementary Figure 1. Sir3 specifically binds to WT over H4-H16Q arrays in phosphate buffer containing ~40 mM Na+. (a) Nucleosomal array capture assay as in **Fig. 1a-b** showing a titration of Sir3 on WT and H4-K16Q arrays in 20 mM phosphate buffer.

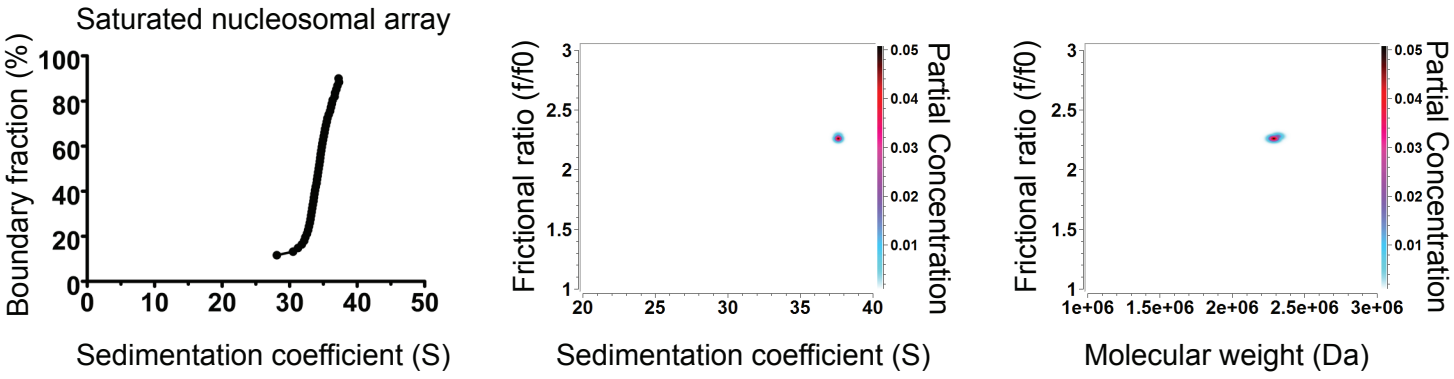
a



b



c

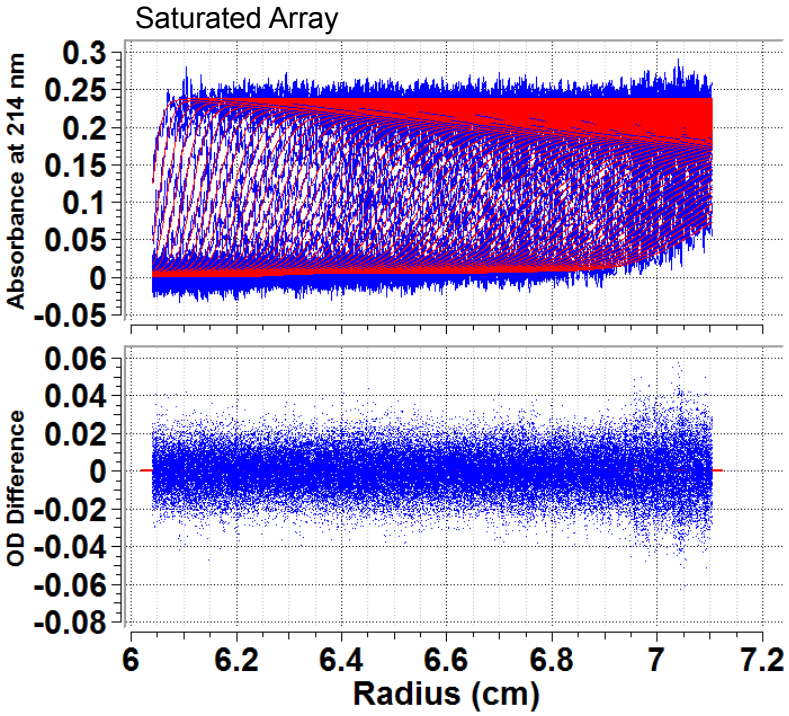


d

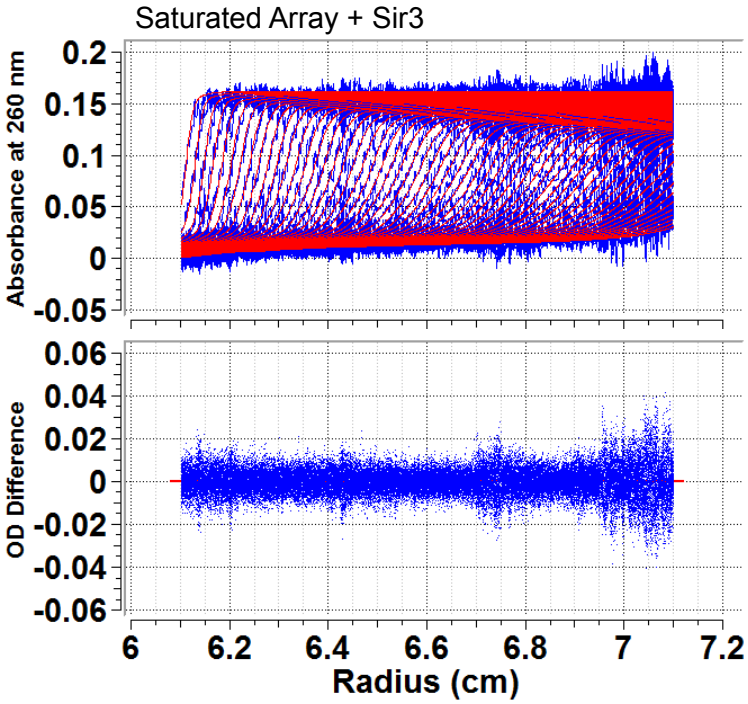
	Mol. Weight (MDa)	Sed. Coefficient (s^{-13})	Frictional Ratio
DNA template	1.335 [1.313] (1.333, 1.347)	10.654 (10.648, 10.668)	7.499 (7.486, 7.540)
Unsaturated array	1.167 [1.964] (1.140, 1.195)	23.341 (23.324, 23.358)	2.587 (2.546, 2.627)
Saturated array	2.296 [2.614] (2.272, 2.321)	37.594 (37.561, 37.627)	2.269 (2.252, 2.285)

Supplementary Figure 2. 2DSA/GA-MC modeling using predicted partial specific volumes does not accurately determine the molecular weights of complex chromatin macromolecules. (a-c) vHW and 2DSA/GA-MC plots of free 601-177-12 template DNA, an approximately half-saturated 12mer nucleosomal array, and a saturated 12mer nucleosomal array, respectively. **(d)** The molecular weight, sedimentation coefficient, and frictional ratio of samples in **(a-c)** as determined by 2DSA/GA-MC. Numbers in parentheses are 95% confidence intervals. Numbers in brackets represent theoretical molecular weights calculated by a sequence-based algorithm implemented in UltraScan3.

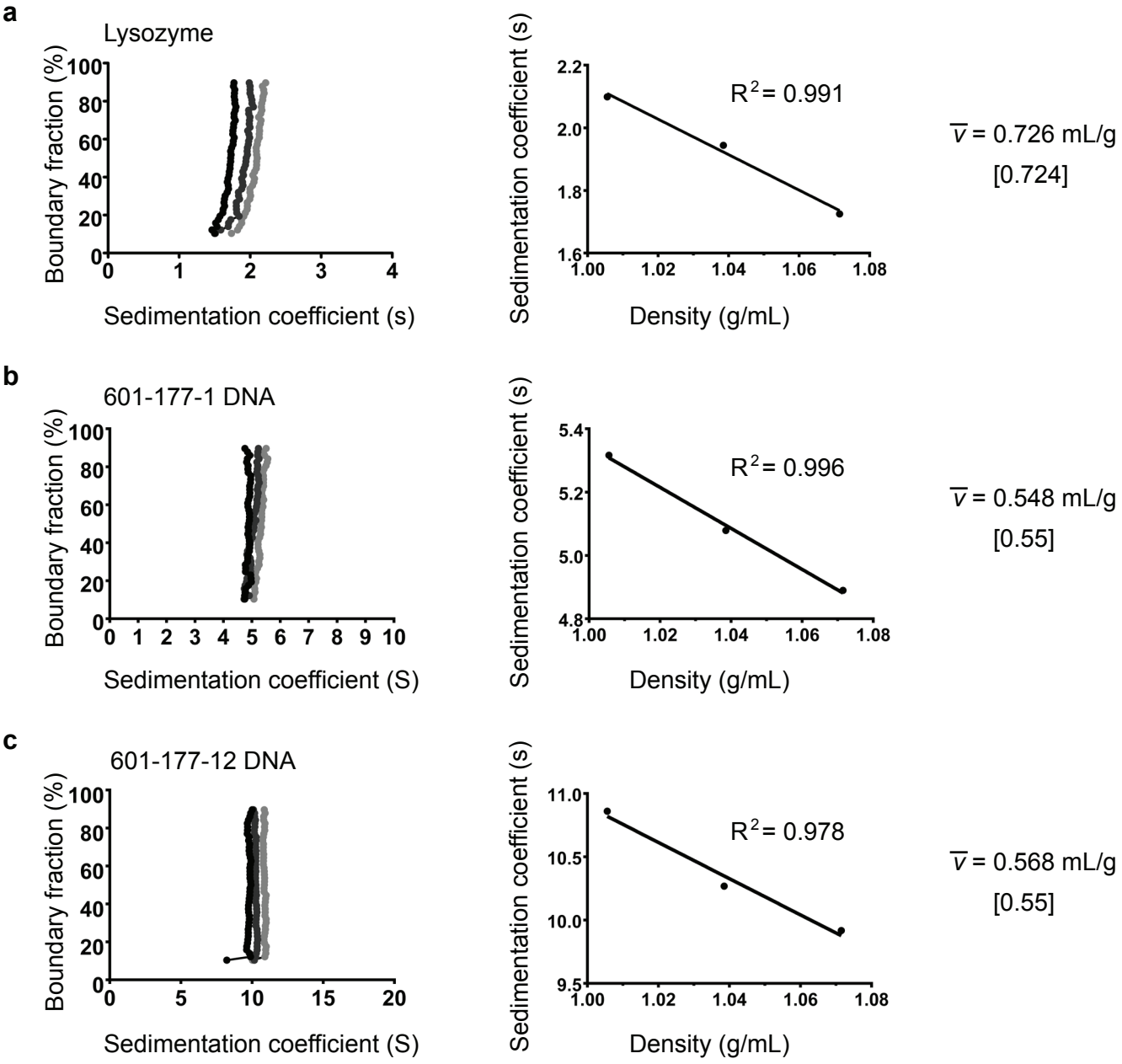
a



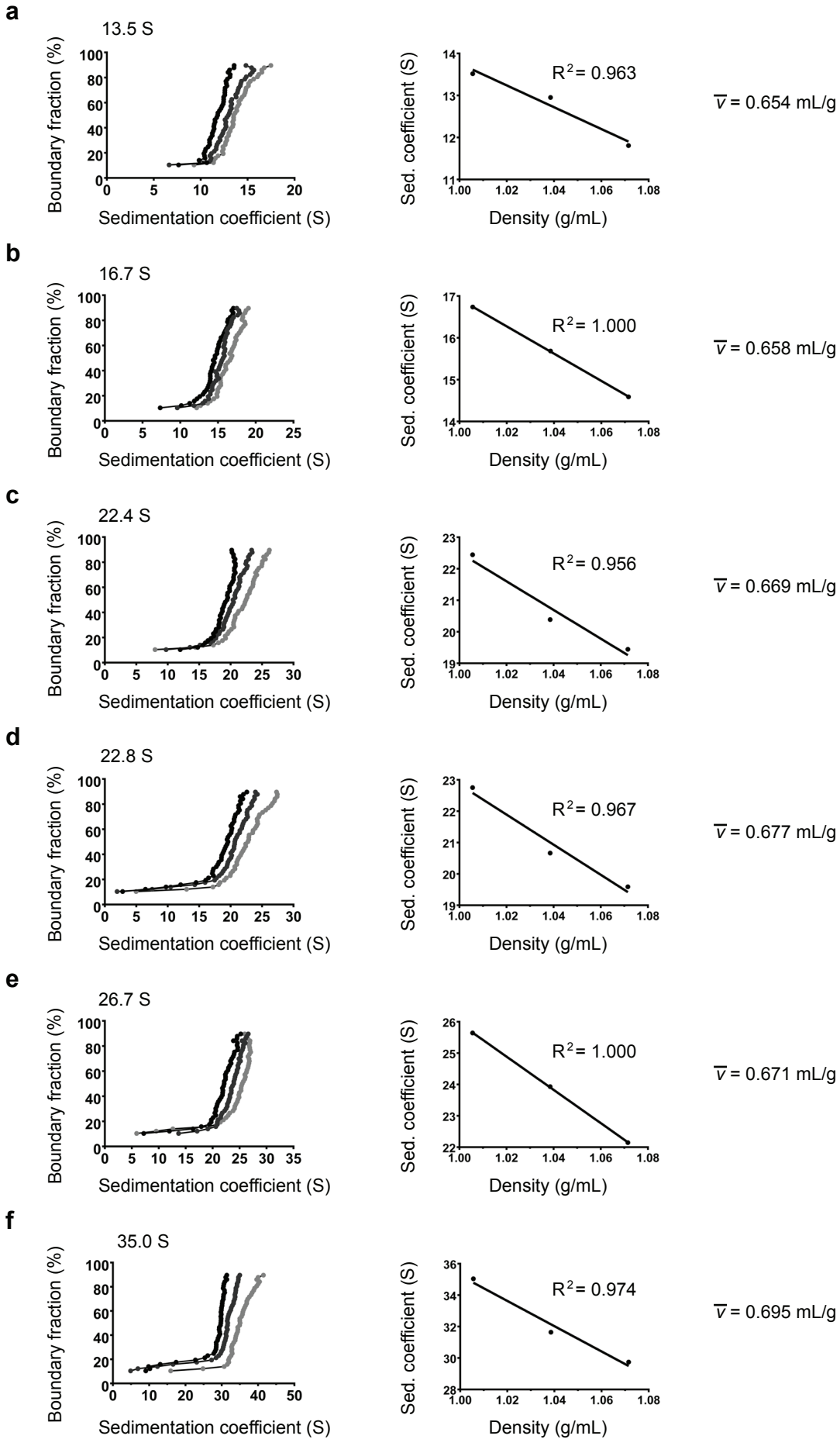
b



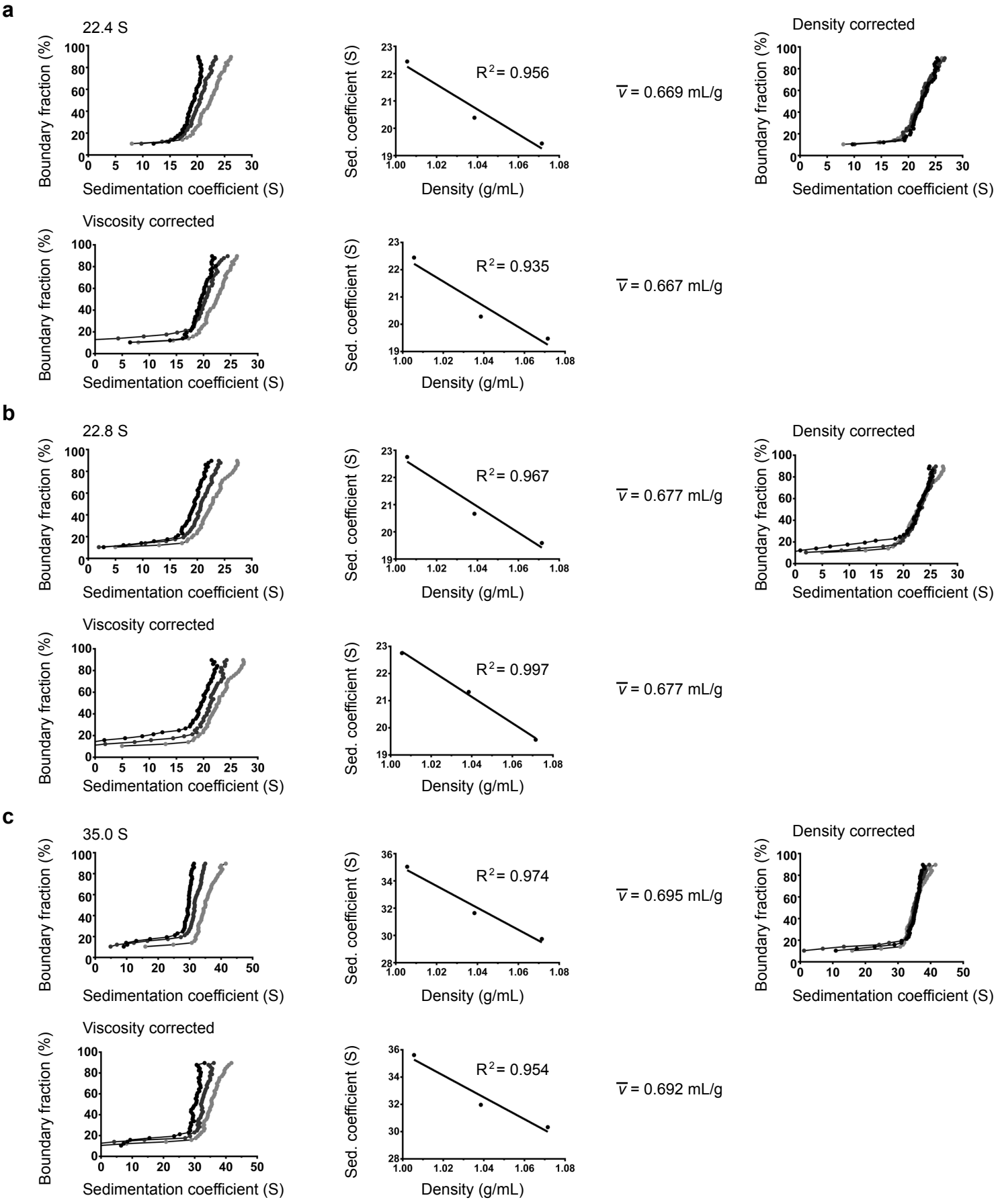
Supplementary Figure 3. 2DSA fitting is appropriate for chromatin samples. (a-b) 2DSA experimental vs. model scans (top, model is in red) and residuals (bottom) demonstrate good fits and random residuals for a WT array unbound **(a)** and bound by Sir3 **(b)**.



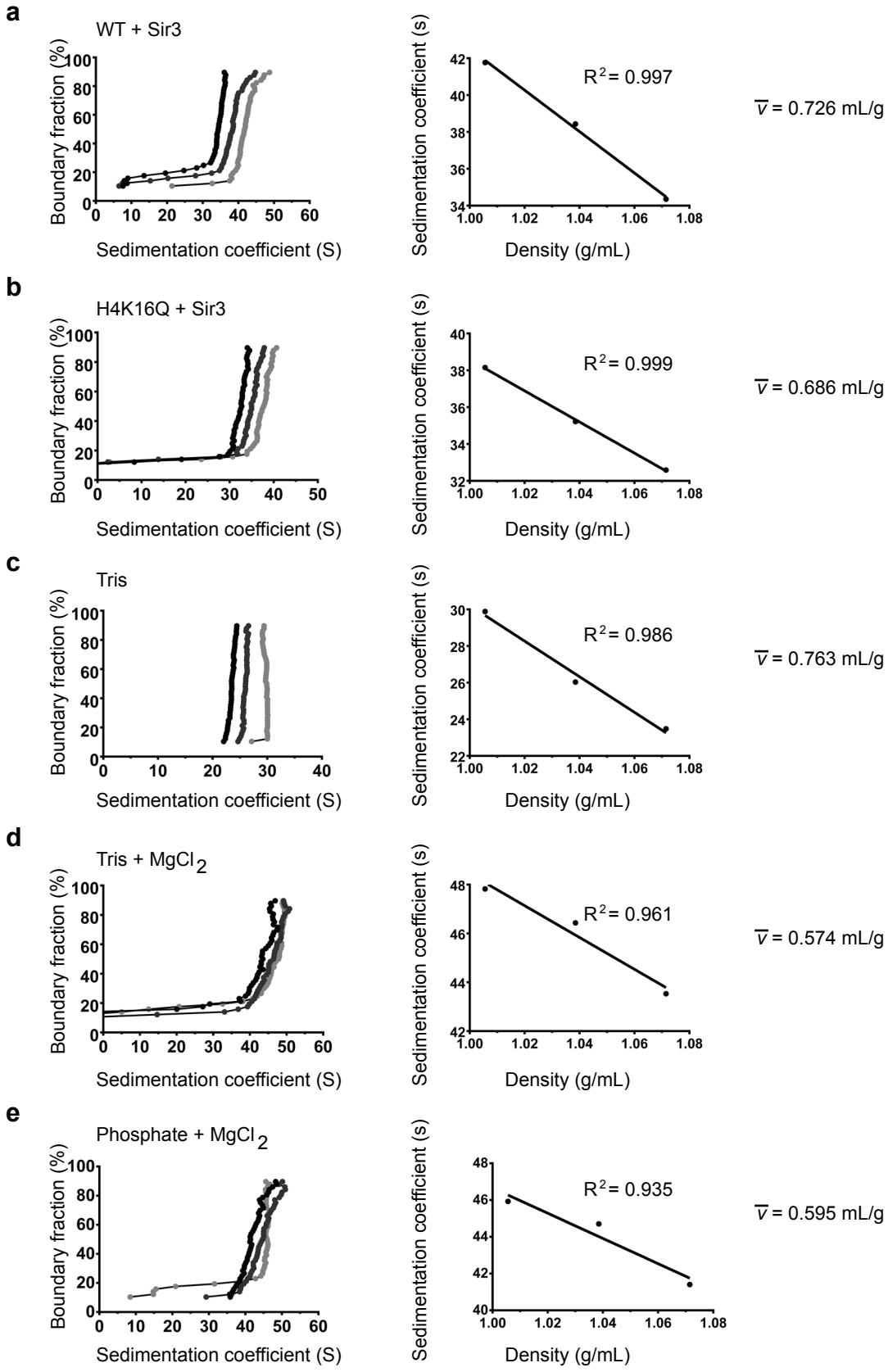
Supplementary Figure 4. The partial specific volume of molecules can be determined via sedimentation in solvents of known density. (a-c) vHW plots showing the sedimentation of molecules in 0% (light gray), 30% (dark gray), and 60% H2O18 (black) and plots of sedimentation coefficient vs. density for **(a)** lysozyme, **(b)** 601-177-1 template DNA, and **(c)** 601-177-12 template DNA. The $\overline{v}$ is calculated by dividing the slope of the fit line by the y-intercept. Numbers in brackets represent the $\overline{v}$ of the respective molecule as predicted by UltraScan3.



Supplementary Figure 5. The partial specific volume of nucleosomal arrays increases with histone octamer saturation and S. (a-f) Determination of the $\overline{v}$ of arrays in **Fig. 2** as in **Supplementary Fig. 4**.

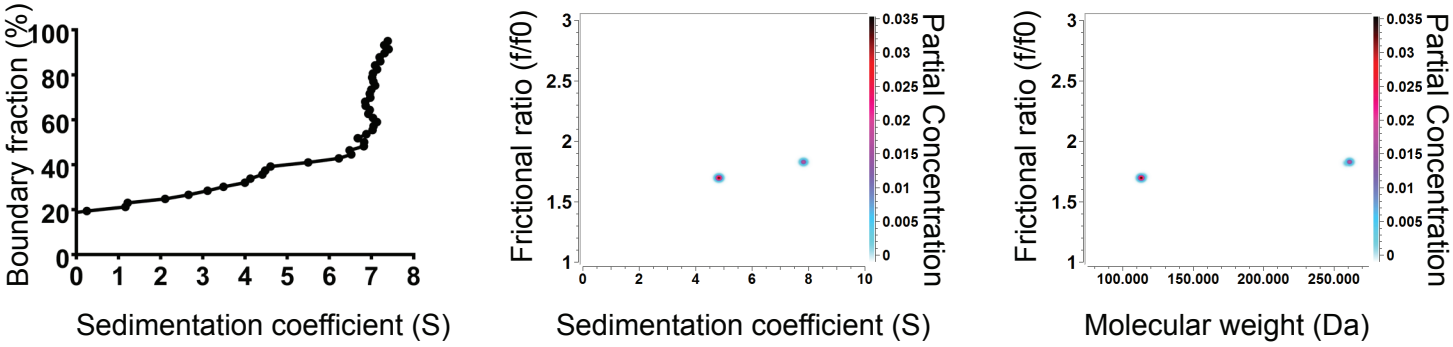


Supplementary Figure 6. The partial specific volume of nucleosomal arrays is independent of viscosity, and the sedimentation distribution of chromatin samples is highly reproducible. (a-c) The $\overline{v}$ determination of array samples in **Supplementary Fig. 5c,d,f** shown as used in **Fig. 2** (top panels) and corrected for viscosity (bottom panels). The vHW distributions corrected for density are in the top right panels.



Supplementary Figure 7. The partial specific volume of arrays decreases during Mg++-induced folding but increases upon Sir3 binding. (a-b) Example $\bar{v}$ determinations of WT and H4K16Q arrays with Sir3. Average $\bar{v}$'s from three experiments were used for 2DSA/GA-MC in **Fig. 3**. **(c-d)** Example $\bar{v}$ determinations of extended and folded arrays in Tris. Average $\bar{v}$'s from three experiments were used for 2DSA/GA-MC in **Fig. 4**. **(e)** Example $\bar{v}$ determinations of folded arrays in phosphate buffer.

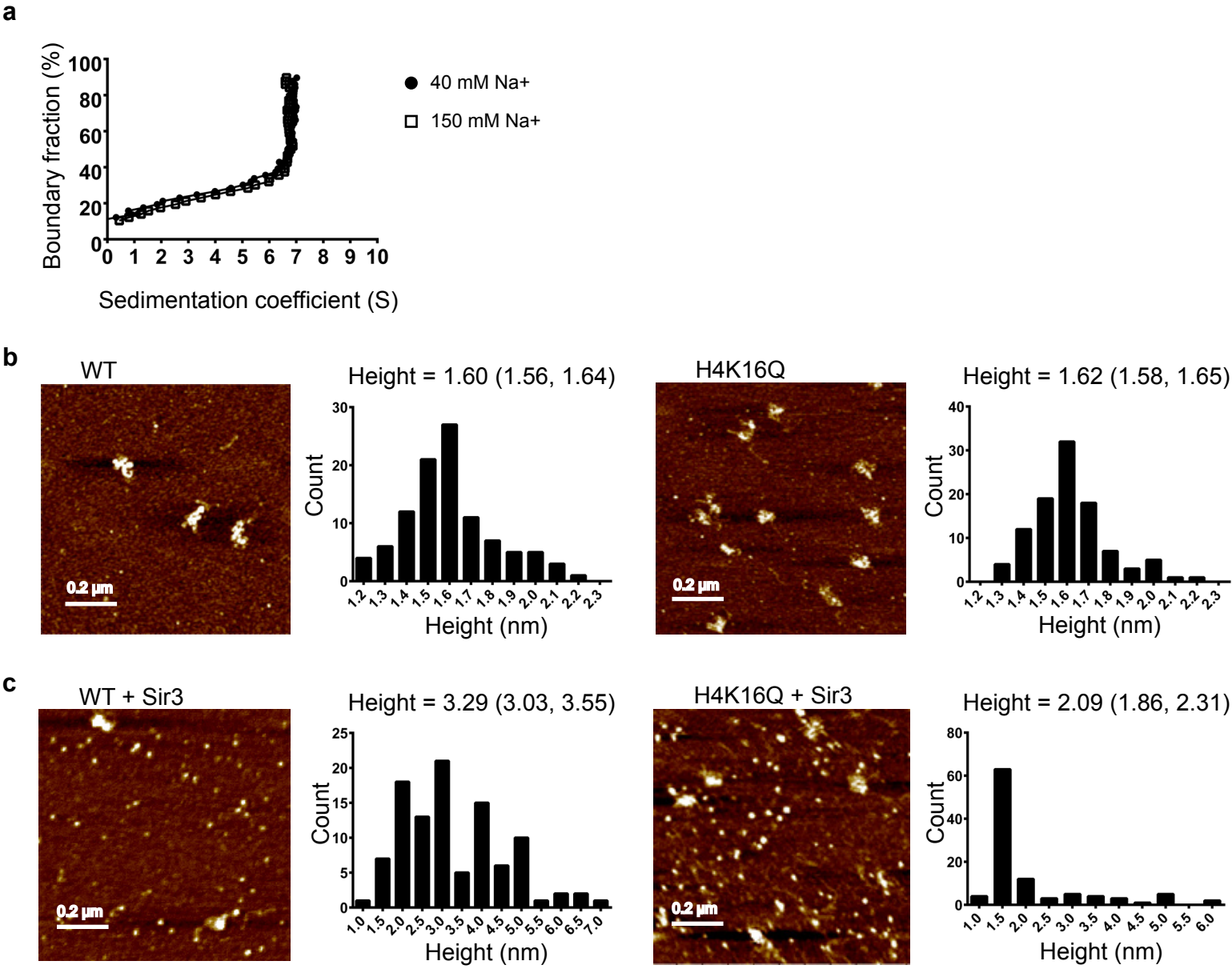
a



b

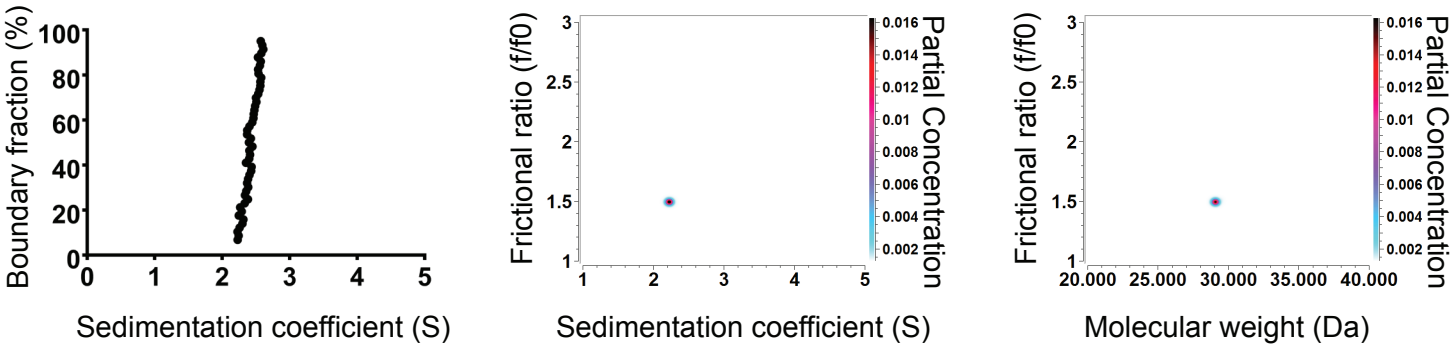
	Relative Percent	Sed. Coefficient (S)	Mol. Weight (kDa)	Frictional Ratio
Monomer	68.97 %	4.824 (4.815, 4.832)	113.43 (112.48, 114.38)	1.701 (1.693, 1.708)
Dimer	31.03 %	7.819 (7.806, 7.832)	260.53 (258.73, 262.33)	1.826 (1.819, 1.834)

Supplementary Figure 8. Sir3 exists as a mixture of monomers and dimers in solution. (a) Left panel, vHW analysis of Sir3 at 171 nM (corresponding to the concentration used for 2 monomers of Sir3 per nucleosome in **Fig. 1d-e** and **3**) in phosphate buffer. Middle and right panels, GA-MC plots of S vs. molecular weight and *f/f0* vs. molecular weight. **(b)** 2DSA/GA-MC statistics show 69% of Sir3 in solution is a monomer (113 kDa), and 31% exists as an oligomer with a molecular weight most closely corresponding to a dimer (theoretical molecular weight is 226 kDa).



Supplementary Figure 9. Sir3-array structure in 150 mM Na+ closely resembles Sir3-array structure in 40 mM Na+. (a) vHW analysis of 171 nM Sir3 in phosphate buffer containing ~40 mM Na+ or in phosphate buffer brought to 150 mM Na+. **(b)** WT and H4-K16Q arrays in phosphate buffer brought to 150 mM Na+ are equivalent in structure and height to arrays in phosphate buffer alone (compare to **Fig. 5b**). **(c)** WT and H4-K16Q arrays in phosphate buffer brought to 150 mM Na+ are similar in structure and height to arrays in phosphate buffer alone in the presence of 2 Sir3 monomers/nucleosome (compare to **Fig. 5c**).

a



b

	Sed. Coefficient (S)	Mol. Weight (kDa)	Frictional Ratio
Monomer	2.224 (2.222, 2.226)	29.119 [26.336] (28.965, 29.274)	1.498 (1.492, 1.503)

Supplementary Figure 10. Sir3 BAH exists as a monomer in solution. (a) Left panel, vHW analysis Sir3 BAH at 1.71 μM in phosphate buffer. Middle and right panels, GA-MC plots of S vs. molecular weight and *f/f0* vs. molecular weight. **(b)** 2DSA/GA-MC statistics show 100% of Sir3 BAH in solution is a monomer. Number in brackets is the expected molecular weight.