Using DNA as a fiducial marker to study SMC complex interactions with the Atomic Force Microscope

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SUPPLEMENTARY MATERIALS AND METHODS

Analytical gel filtration

Gel filtration was performed at room temperature using a Superdex 200 HR 10/30 column (GE Healthcare) connected to an AKTA FPLC instrument (GE Healthcare). The column was equilibrated with a buffer of 50 mM Tris-Cl, pH 7.5, 1 mM EDTA, 150 mM NaCl and 0.1 mM DTT. In each case, SMC was injected at a concentration of 3 µM and ScpA and ScpB were at concentrations of 15 µM. All proteins were run individually and in combination with either one or both of the other proteins in such a way that every possible combination of the three subunits was investigated. Proteins were mixed in a 600 µl volume and left on ice for 10 minutes before injection into a 500 µl loop on the FPLC injection port. All experiments were performed using an identical method in order that a valid comparison of traces from different experiments could be made. The column was run at 0.5 ml/min, with 0.5 ml fractions collected over a total volume of 1.5 column volumes. Fractions were subjected to acetone precipitation, with 1.2 ml of cold acetone added per 300 µl of each fraction, and left at -20°C overnight. Fractions were then spun for 8 minutes at 13500 rpm using a benchtop microfuge and the acetone was removed. Finally, the protein pellet was resuspended in SDS loading buffer and boiled for 5 minutes prior to SDS PAGE analysis.

Protein expression and purification

For expression of the SMC complex subunits, *E. coli* BL21 (DE3) cells were transformed with the pET22b-SMC, pET28a-ScpA or pET22b-ScpB plasmids. Cells were grown in LB supplemented with ampicillin at 37°C until mid -log phase. Protein expression was induced by the addition of IPTG (1 mM), followed by shaking at 37°C for a further three hours (for ScpB), or at 27°C for approximately 14 hours (SMC and ScpA). Cells were harvested by centrifugation, resuspended in 20 ml resuspension buffer (50 mM TrisCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM DTT and 10% sucrose) and then lysed by sonication in the presence of 0.1 mM PMSF.

For purification of SMC (or SMC^{E1118Q} mutant) protein, the cell lysate was bound to a HiTrap Blue column (GE Healthcare, Chalfont St Giles, UK) and SMC was eluted with a gradient of 50 – 2000 mM NaCl in buffer A (50 mM Tris, pH 7.5, 1 mM EDTA, 0.1 mM DTT). Peak fractions were dialysed against buffer A + 50 mM NaCl before loading onto a HiTrap Heparin column (GE Healthcare, Chalfont St Giles, UK) followed by elution with a gradient from 50 – 300 mM NaCl in buffer A. Peak fractions were dialysed against buffer A + 50 mM NaCl and subsequently

applied to an MonoQ column (GE Healthcare, Chalfont St Giles, UK). SMC protein was eluted using a gradient of 50 – 400 mM NaCl in buffer A. Peak fractions were pooled, dialysed against buffer A + 100 mM NaCl + 20% Glycerol, snap frozen and stored at -80^{o}C. Protein concentration was determined using a theoretical extinction coefficient of 51230 M^{-1} cm^{-1}. For purification of ScpA protein, cell lysate was passed over a HiTrap QFF column (GE Healthcare, Chalfont St Giles, UK) and ScpA eluted with a gradient of 50 – 1000 mM NaCl in buffer A. Peak fractions were pooled, dialysed against buffer A + 3 M NaCl and then run on a HiTrap Butyl FF column (GE Healthcare, Chalfont St Giles, UK). ScpA was eluted from the column using a gradient of 3000 – 50 mM NaCl in buffer A. Peak fractions were loaded directly onto a MonoQ column and ScpA was eluted with a gradient of 200 – 600 mM NaCl in buffer A. Peak fractions were collected and loaded onto a Superdex200 gel filtration column equilibrated in buffer A + 150 mM NaCl. Peak fractions were pooled, dialysed against buffer A + 100 mM NaCl + 20% Glycerol, snap frozen and stored at -80^{o}C. Protein concentration was determined using a theoretical extinction coefficient of 16390 M^{-1} cm^{-1}. ScpB was purified using the same chromatographic steps as for ScpA. Following gel filtration, peak fractions were pooled, dialysed against buffer A + 100 mM NaCl + 20% Glycerol, snap frozen and stored at -80^{o}C. Protein concentration was determined using a theoretical extinction coefficient of 15930 M^{-1} cm^{-1}.

Table S1 Proteins used in this work

Protein	MW (kDa)	V_{AFM}/V_{REF}	K (kDa^{-1})
ScpB	21.89	0.30 ± 0.03	$11.0 \pm 0.1 \times 10^{-3}$
ScpA	29.46	0.41 ± 0.04	$11.9 \pm 0.1 \times 10^{-3}$
$ScpB_2$	43.78	0.58 ± 0.04	$11.9 \pm 0.1 \times 10^{-3}$
SSB	74.40	0.90 ± 0.15	$11.3 \pm 0.1 \times 10^{-3}$
SMC	135.39	1.6 ± 0.1	$11.37 \pm 0.07 \times 10^{-3}$
SMC_2	270.78	3.1 ± 0.2	$11.22 \pm 0.07 \times 10^{-3}$
AddAB	275.67	3.3 ± 0.7	$11.8 \pm 0.2 \times 10^{-3}$

Proteins used in this study and their molecular weights (columns 1 and 2). Experimental relative volumes, and constant K as defined in **Eq. 4** (columns 3 and 4).

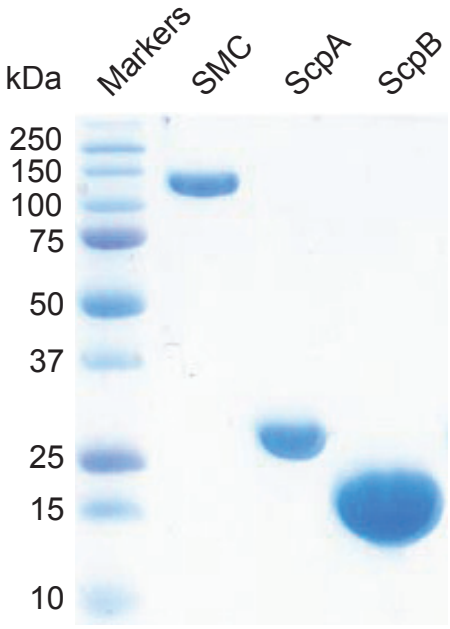

Figure S1 SDS-PAGE gel showing the three component polypeptides of the SMC complex; SMC, ScpA and ScpB. The proteins were purified to homogeneity without the use of tags as described in the Materials and Methods section.

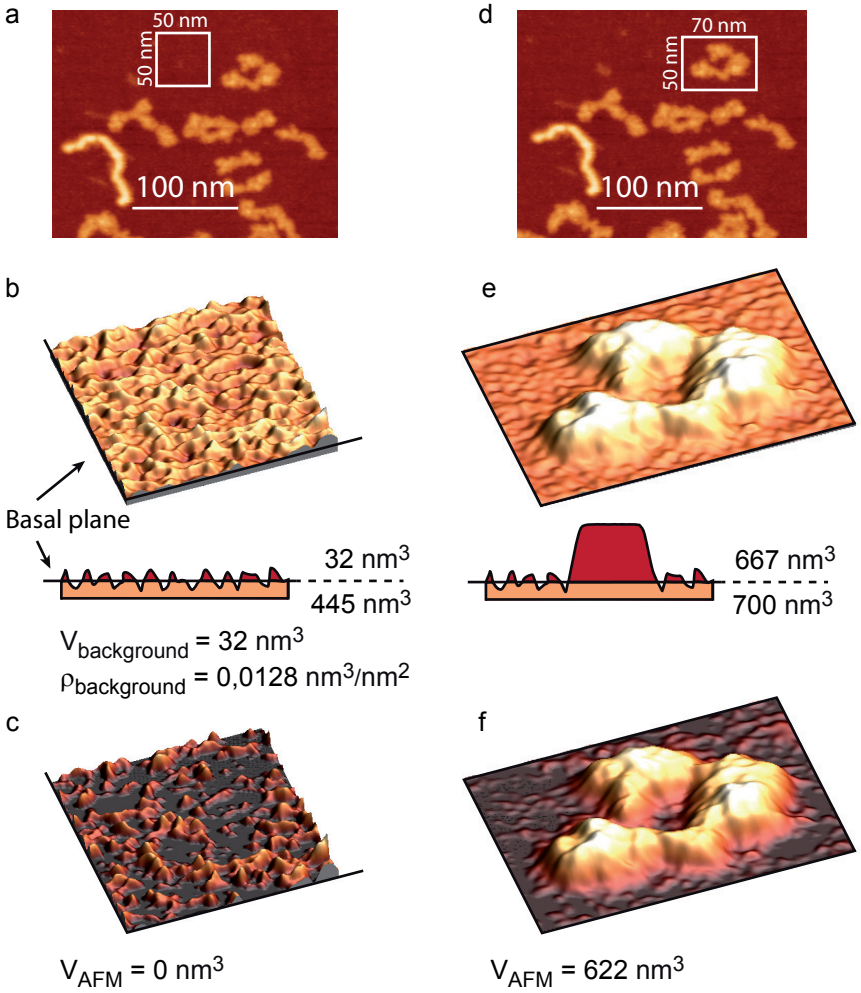

Figure S2 AFM method to measure volumes of proteins or DNA. (**a**) To calculate the basal plane and the background density, a bare area was selected close to the protein of interest (white square in the AFM picture). (**b**) 3D representation of the area chosen in (**a**). At this magnification the background noise is obvious. Firstly, the basal plane was determined by fitting a plane to the image. The volume above this plane is the background noise volume ($V_{Background}$) and in this example is 32 nm^3. Next, the background noise volume density ($\rho_{background}$) was calculated by dividing $V_{Background}$ by the area considered. In this example $\rho_{background}$ = 0.0128 nm^3/nm^2. (**c**) Same image as (**b**) after subtraction of the basal plane. Application of **Eq. 3** to this image gives V_{AFM} = 0 nm^3, as expected. (**d**) Once $\rho_{background}$ is determined the volume of a protein can be calculated. In this example we chose an SMC dimer contained in a 50 nm x 70 nm window. This window was chosen to fit to the shape of the protein. (**e**) Detail of the SMC dimer in a 3D representation. The volume above the basal plane calculated previously was 667 nm^3. $V_{Background}$ for this example is 44.8 nm^3. Application of **Eq. 3**, gives a volume for a SMC dimer of V_{AFM} = 622 nm^3. (**f**) 3D representation of the SMC dimer after subtraction of the basal plane.

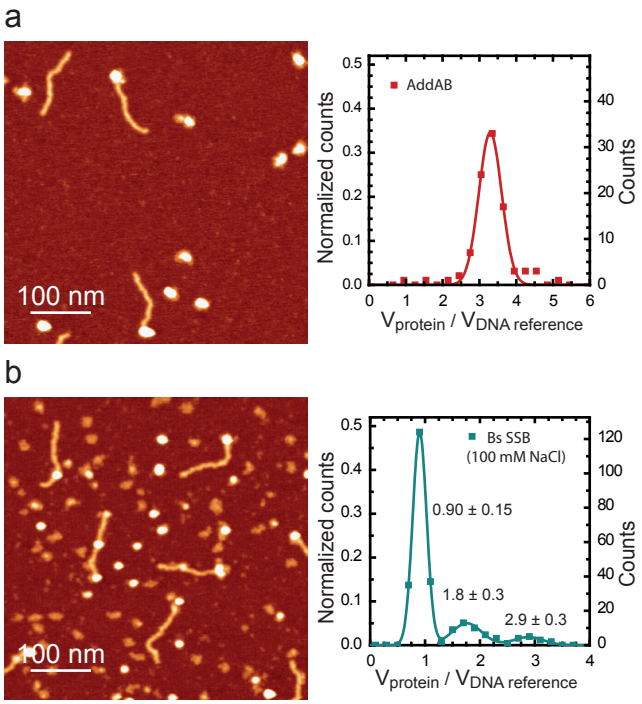

Figure S3 AFM volumetric analysis of AddAB and SSB. (**a**) AFM image (left) and histogram of normalized volumes (right) of AddAB. The AddAB histogram had a single peak at 3.3 ± 0.4. AddAB is a helicase-nuclease from *B. subtilis* that binds at DNA ends and this can also be seen in the image. (**b**) AFM image (left) and histogram of normalized volumes (right) of SSB protein. We included 100 mM NaCl in the buffer to prevent aggregation of this protein. The SSB histogram displayed a main peak at 0.90 ± 0.15 which corresponds to the expected SSB homotetramer as well as two smaller peaks suggesting the formation of larger oligomers. Color scale from dark to bright is 0-2 nm in all AFM images.

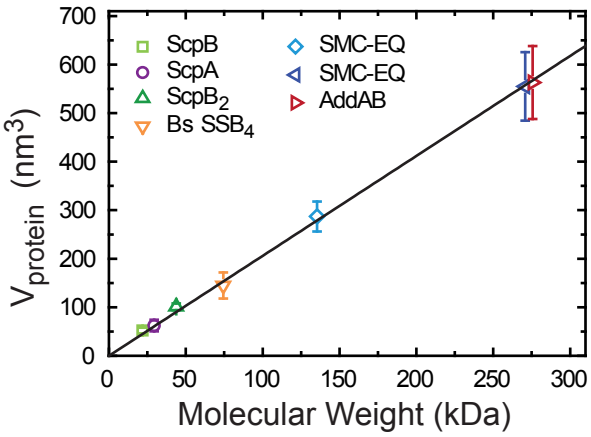

Figure S4 Absolute protein volumes determined by AFM. The molecular weights of the proteins shown in this graph are described in **Table 1** and span from 22 to 300 kDa. Data was fit to Equation S1 (1-2)

(S1) $$V_h = \frac{MW}{NA}(\bar{V}_2 + \delta\bar{V}_1)$$

Where MW is the molecular weight of the protein, NA is the Avogadro´s number, $\bar{V}_2$ is the partial specific volume of the protein (0.741 cm^3 / g), $\bar{V}_1$ is the partial specific volume of water (1 cm^3 / g), and δ is the hydration of the protein (in g H_2O / g protein). Interestingly, data fit nicely to Equation S1 with δ=0.5 g H_2O / g protein, a bit larger than the values reported for globular proteins (about 0.3-0.4 g H_2O / g protein). This fact may reflect the non-globular nature of some of the proteins considered here. On the other hand, it is surprising that data correlate nicely with this fit given the known effect of tip convolution present in all AFM images. Other geometric models (2-3) were not considered here due to the non-globular shape of our proteins.

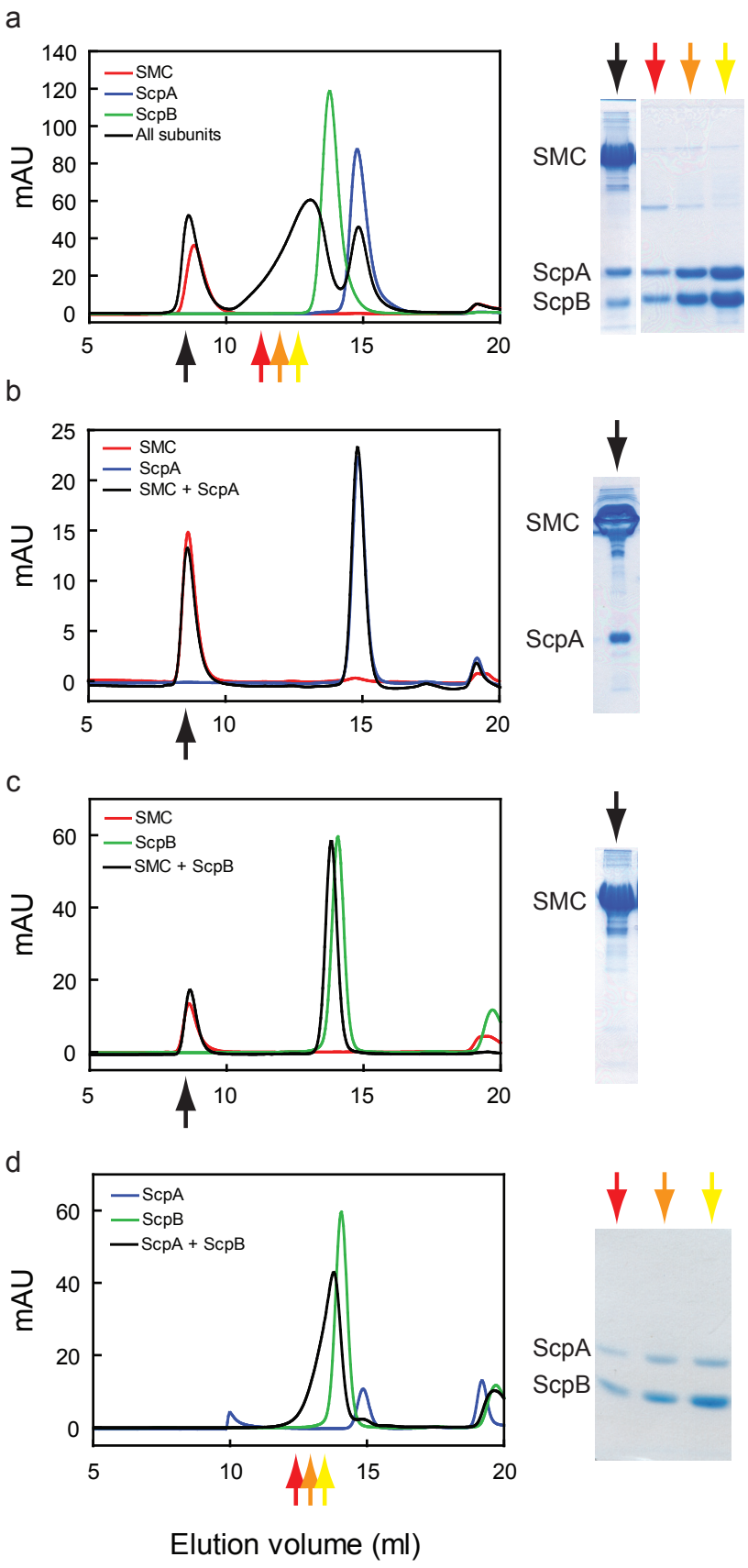

Figure S5 Gel filtration analysis of interaction between the components of the SMC complex. (**a**) Gel filtration of SMC complex components individually and in different combinations. Upon mixing of all subunits, there was a small decrease in the elution volume of the earliest peak, compared to that of SMC alone, indicating a larger species had been formed (black arrow) and this was shown to contain both the small ScpA and ScpB subunits, indicating that all three proteins interact to form a complex. ScpA and ScpB also bound to each other independently of SMC (red arrow). (**b**) ScpA binds to SMC independently of ScpB. (**c**) No association was detected between ScpB and SMC. (**d**) ScpA binds to ScpB as both proteins were found in the volumes marked with red, orange and yellow arrows.

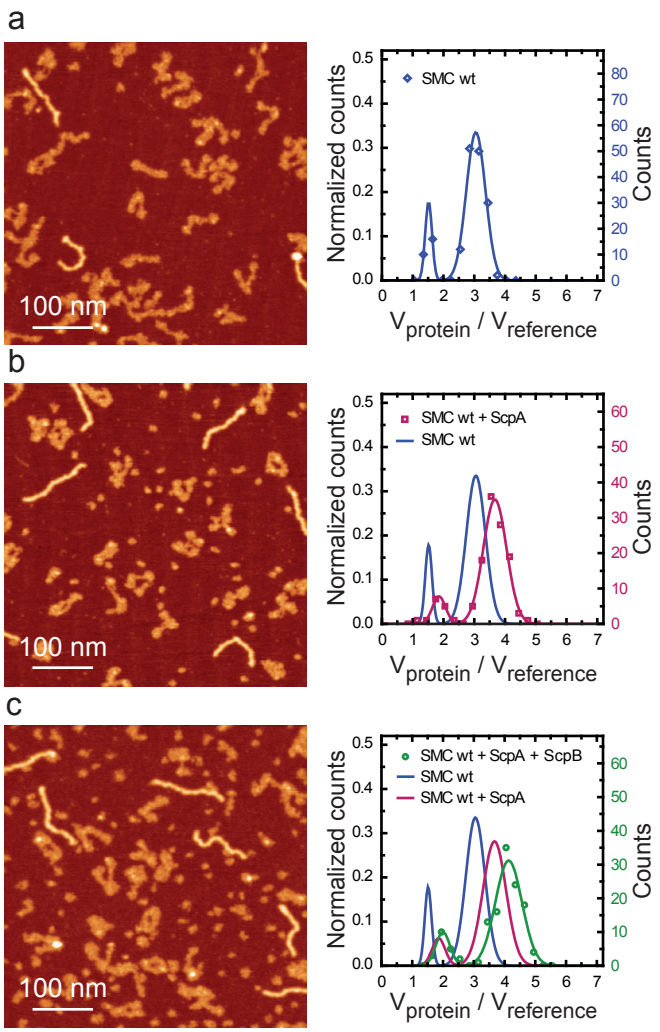

Figure S6. The wild type SMC protein behaves identically to the ATP-mutant SMC^{E1118Q}. Key experiments were repeated with the wild type SMC protein. (**a**) SMC behaves as a dimer; (**b**) SMC binds to ScpA; and (**c**) SMC binds to ScpA and ScpB.

SUPPORTING REFERENCES

1. Cantor, C. R., and P. R. Schimmel. 1980. Biophysical Chemistry. Part II. Techniques for the study of biological structure and function. W.H. Freeman and company, New York.
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3. Neaves, K. J., L. P. Cooper, J. H. White, S. M. Carnally, D. T. Dryden, J. M. Edwardson, and R. M. Henderson. 2009. Atomic force microscopy of the EcoKI Type I DNA restriction enzyme bound to DNA shows enzyme dimerization and DNA looping. Nucleic Acids Res 37:2053-2063.