Control of Smc Coiled Coil Architecture by the ATPase Heads Facilitates Targeting to Chromosomal ParB/parS and Release onto Flanking DNA

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I.) Supplemental Data

**Figure S1** Expression, functionality and localization of ATPase mutant Smc proteins. Related to Figure 1.

(A) Colony formation assay using strains BSG1002, 1007, 1045, 1047, 1046, 1008 and 1083. Notably, Smc(EQ) mutant cells form colonies on minimal medium slightly more slowly than wild type or smc deletion mutants, suggesting that the mutant protein is mildly toxic when normal Smc function is lacking (Figure S1C, D) (Schwartz and Shapiro, 2011). The slow growth is likely due to a defect in replication origin segregation (Gruber et al., 2014; Schwartz and Shapiro, 2011; Wang et al., 2014), which provides a plausible explanation for the lower number of (replication origin-proximal) Smc foci in Smc(EQ) cells (see Figure S1H). (B) Protein extracts stained by Comassie Brilliant Blue. Immunoblotting of identical protein samples is shown in Figure 1B. (C) Same as in (A) with strains BSG1002, 1007, 1008, 1067, 1068 and 1855. (D) Immunoblotting of extracts from strains BSG1002, 1067, 1855, 1857, 1856, 1068, 1881, 1378, 1413, 1677, 1662, 1799 and 1798 with anti-GFP antiserum (top panel). SDS-PAGE of identical extracts stained by Comassie Brilliant Blue (bottom panel). (E) Immunoblotting against Smc protein using strains BSG1007, 1067, 1002, 1045, 1046, 1008, 2050, 2051. (F) Colony formation assay using strains BSG1002, 1007, 1045, 1046, 1008, 2050 and 2051. (G) ChiP-qPCR using anti-Smc antiserum on strains BSG1002, 1008, 1045, 1046, 2050 and 2051. (H) Quantification of Smc-GFP foci in strains shown in Figure 1C. Number of foci is displayed per unit cell length (µm). Standard deviation is derived from four different fields of view for each genotype. ‘n’ denotes the total number of individual cells counted.
Figure S1
Figure S2  Smc and ScpB ChIP-Seq in Smc and Smc(EQ) cells. Related to Figure 2.
(A) ChIP-Seq analysis of BSG1002 and 1008 using anti-Smc antiserum. Number of reads in 1 kb windows at 100bp intervals are shown for input (IN) and ChIP (IP) samples without prior normalization for input material. Normalized data of the same experiment is shown in Figure 2A and 2C. (B) ChIP-Seq analysis of strains BSG1470 and BSG1472 using anti-ScpB antiserum. Data analysis and display as in (A). (C) Whole-genome ChIP-Seq profile for anti-ScpB ChIP (on strain BSG1470), (also shown in Figure 7B; same experiment as in Figure S2B). Sequencing reads are put into 5 kb bins and normalized for input DNA. Please note the generally high degree of similarity between anti-Smc (Figure 2C) and anti-ScpB ChIP-Seq profiles with the ScpB profile possibly displaying a steeper gradient from the replication origin to the terminus (Kleine Borgmann et al., 2013). (D) Localization of Smc, Smc(S1090R) and Smc(E1118Q) to sites located on the chromosome arm analyzed by ChIP-qPCR using strains BSG1002, BSG1046 and BSG1008. Mean and standard deviation are calculated from three replicate experiments. Boxed insert displays results from the same experiment with “background” correction by subtraction of ChIP obtained with Smc(S1090R).
Figure S2
Figure S3  
Dimerization at the Smc hinge determines localization of Smc/ScpAB to parS. Related to Figure 3.
(A) Immunoblotting of cell extracts from strains BSG1007, 1067, 1002, 1051, 1406, 1052, 1387, 1890, 1893, 1889, 1891 and 1892 using anti-Smc antiserum. (B) Colony formation Bs strains BSG1007, 1008, 1889, 1892 and 1891 on minimal medium (SMG). (C) The hinge mutation (GGGG->AAAA) blocks dimerization of headless Smc protein (BsSmcH-CC300). 40 μg of purified proteins was injected onto a gel filtration column and analyzed by multi-angle light scattering (SEC-MALS). Absorbance (at A280) and light scattering is shown for wild-type and hinge mutant BsSmcH-CC300 (curves in red and blue colours, respectively). (D) Analysis of the major peak (in A280 absorbance) in SEC-MALS (as in C) of wild-type and hinge-mutant BsSmcH-CC300 indicates the existence of largely dimeric and monomeric protein species, respectively. (E) Immunoblotting of cell extracts from strains BSG1007, 1067, 1002, 1890, 1893, 1892, 1624, 1621 and 1620 using anti-Smc antiserum. (F) Colony formation of strains BSG1007, 1893, 1624, 1621, 1623 and 1620 on minimal medium (SMG). (G) Quantification of Smc-GFP foci in strains shown in Figure 3D. Number of foci is displayed per unit cell length (μm). Standard deviation is derived from four different fields of view for each genotype. ‘n’ denotes the total number of individual cells counted. (H) ChIP-qPCR analysis of strains BSG1893, 1892, 2144-2147 grown in SMG medium with anti-Smc antiserum.
Figure S3
Figure S4  Smc(K1151C) –the reporter for head engagement– is functional. Related to Figure 4. (A) Colony formation of strains BSG1002, 1007, 1360 and 1457 on minimal medium (SMG) and nutrient rich medium (NA). (B) Cross-linking of Smc(K1151C) in BSG1607, 1488, 1512 and 1513 with BMOE. Mean values and standard deviation from triplicate experiments are shown.
Figure S4
Figure S5  
Expression and functionality of hinge-less Smc protein. Related to Figure 5.

(A) Immunoblotting against the TAP tag on Smc in cell extracts from strains BSG1002, 1016, 1475, 1691, 1896, 1671, 1780, 1672, 1895, 1689 and 1779. Commassie staining of the same extracts is shown in the bottom panel. (B) Colony formation assay using strains BSG1007, 1008, 1626, 1619, 1896 and 1780. (C) Same as in (B) with strains BSG1002, 1007, 1008, 1520, 1689 and 1779. (D) Exemplary image of the SDS-PAGE analysis of disulfide cross-linked BsSmcH-CC300 samples harboring pairs of cysteines as annotated. (E) Quantification of intra- and inter-molecular disulfide formation (after 4 hr incubation) from Commassie stained SDS-PAGE gels for 16 pairs of cysteine mutants. (F) Schematic view of the folding of the Smc coiled coil. Anchor points setting the register of the Smc coiled coils – established by in vitro disulfide formation (see D and E)– are given as dashed lines connecting N- and C-terminal helix. Disruptions in the coiled coil register were detected by Marcoil prediction. The length of extra sequences in the C-terminal coiled coil as given by the experimentally determined coiled coil register are indicated at the corresponding positions. Regions relevant for the targeting of mini-Smc to parS are highlighted by labels in red colours.
Figure S5
Figure S6  
ChIP-Seq of Smc(EQ) to an ectopic parS site. Related to Figure 6.
Smc(EQ) is efficiently targeted to parS-amyE. ChIP-Seq analysis of BSG1471 (top panel) and BSG1008 (bottom panel) using anti-Smc antiserum. ChIP eluate sequence reads were mapped to 5 kb bins and normalized for input DNA. Please note that Smc(EQ) localization to endogenous parS sites is decreased by the presence of an extra parS site, being consistent with a titration effect. The bottom panel is identical to the bottom panel of Figure 2C.
Figure S6
II.) Supplemental Table

Supplemental Table 1: Genotypes

All strains are derivatives of *Bacillus subtilis* 1A700 provided by the BGSC (*Bacillus* Genetic Stock Center). All strains are auxotrophic for tryptophan (trpC2).

| Strain Code | Genotype Description |
|-------------|----------------------|
| BSG1002     | smcftsY::ermB         |
| BSG1007     | ΔsmcftsY::ermB        |
| BSG1008     | smc(E1118Q)ftsY::ermB |
| BSG1016     | smc-TAPftsY::ermB     |
| BSG1045     | smc(K37I)ftsY::ermB   |
| BSG1046     | smc(S1090R)ftsY::ermB |
| BSG1047     | smc(D1117A)ftsY::ermB |
| BSG1051     | smcftsY::ermB, parAB::kanR |
| BSG1052     | smcftsY::ermB, ΔparAB::kanR |
| BSG1067     | smc-mGFPmut1ftsY::ermB |
| BSG1068     | smc(E1118Q)-mGFPmut1ftsY::ermB |
| BSG1083     | smc(R57A)ftsY::ermB   |
| BSG1360     | smc(C1195, C4375, C6265, C11145)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB |
| BSG1378     | smc-mGFPmut1ftsY::ermB, specR::ΔscpA |
| BSG1387     | smc(E1118Q)ftsY::ermB, ΔparAB::kanR |
| BSG1406     | smc(E1118Q)ftsY::ermB, parAB::kanR |
| BSG1413     | smc(E1118Q)-mGFPmut1ftsY::ermB, specR::ΔscpA |
| BSG1457     | smc(C1195, C4375, C6265, C11145, K1151C)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB |
| BSG1469     | smcftsY::ermB, ΔamyE::parS-359::cat |
| BSG1470     | smcftsY::ermB, ΔamyE::mtparS-359::cat |
| BSG1471     | smc(E1118Q)ftsY::ermB, ΔamyE::parS-359::cat |
| BSG1472     | smc(E1118Q)ftsY::ermB, ΔamyE::mtparS-359::cat |
| BSG1475     | smc(E1118Q)-TAPftsY::ermB |
| BSG1488     | smc(C1195, C4375, C6265, C11145, K1151C, E1118Q)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB |
| BSG1509     | smc(C1195, C4375, C6265, C11145, K1151C)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB, specR::ΔscpA |
| BSG1512     | smc(C1195, C4375, C6265, C11145, K1151C, E1118Q)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB, specR::ΔscpA |
| BSG1513     | smc(C1195, C4375, C6265, C11145, K1151C, E1118Q)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB, specR::scpAΔscpB |
| BSG1520     | smc(E1118Q)-TAPftsY::ermB, specR::ΔscpA |
| BSG1547     | smc(G657A, G658A, G662A, G663A, E1118Q)-ftsY::ermB |
| BSG1597     | smc(C1195, C4375, G657A, G658A, G662A, G663A, C6265, C11145, K1151C)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB |
| BSG1598     | smc(C1195, C4375, G657A, G658A, G662A, G663A, C6265, C11145, E1118Q, K1151C)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB |
| BSG1607     | smc(K37I, C1195, C4375, C6265, C11145, K1151C)-TEV-His12-HaloTag(C61V, C262A)ftsY::ermB |
| BSG1619     | rncSmc[1-499]GGGGGSGSGGGGGG674-1186, E1118Q]ftsY::ermB |
| BSG1620     | smc(G657A, G658A, G662A, G663A, E1118Q)-ftsY::ermB, specR::ΔscpA |
| BSG1621     | smc(G657A, G658A, G662A, G663A, E1118Q)-ftsY::ermB, specR::scpA |
| BSG1624     | smc(G657A, G658A, G662A, G663A)ftsY::ermB, specR::scpA |
| BSG1626     | rncSmc[1-499]GGGGGSGSGGGGG674-1186]ftsY::ermB |
| BSG1662     | smc(G657A, G658A, G662A, G663A, E1118Q)-mGFP-ftsY::ermB |
| BSG1671     | smc(G657A, G658A, G662A, G663A)-TAP-ftsY::ermB, specR::scpA |
| BSG1672     | smc(G657A, G658A, G662A, G663A)-TAP-ftsY::ermB, specR::ΔscpA |
| BSG1677     | smc(G657A, G658A, G662A, G663A)-mGFP-ftsY::ermB |
| BSG1689 | smc(G657A, G658A, G662A, G663A, E1118Q)-TAP ftsY::ermB, specR::ΔscpA |
| BSG1691 | smc(G657A, G658A, G662A, G663A)-TAP ftsY::ermB, specR::scpAB |
| BSG1779 | smc(1-499 GGGSSGGSSGGG 674-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1780 | smc(1-499 GGGSSGGSSGGG 674-1186, E1118Q)-TAP:ermB, specR::scpAB |
| BSG1791 | smc(C119S, C437S, G657A, G658A, G662A, G663A, C826S, C1114S, E1118Q, K1151C)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB, specR::ΔscpA |
| BSG1798 | smc(G657A, G658A, G662A, G663A, E1118Q)-mGFP-ftsY::ermB, specR::ΔscpA |
| BSG1799 | smc(G657A, G658A, G662A, G663A)-mGFP-ftsY::ermB, specR::ΔscpA |
| BSG1800 | smc(C119S, C437S, G657A, G658A, G662A, G663A, C826S, C1114S, K1151C)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB, specR::ΔscpA |
| BSG1824 | smc(1-199 GGGSSGGSSGGG 999-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1825 | smc(1-219 GPG 983-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1826 | smc(1-243 GGGSSGGSSGGG 957-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1827 | smc(1-243 GGGSSGGSSGGG 943-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1828 | smc(1-261 GGGSSGGSSGGG 943-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1829 | smc(1-261 GGGSSGGSSGGG 912-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1830 | smc(1-277 GGGSSGGSSGGG 922-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1855 | smc(K37I)-mGFP1mut1 ftsY::ermB |
| BSG1856 | smc(S1090R)-mGFP1mut1 ftsY::ermB |
| BSG1857 | smc(D1171A)-mGFP1mut1 ftsY::ermB |
| BSG1871 | smc(1-468 GGGSSGGSSGGG 705-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1872 | smc(1-437 GGGSSGGSSGGG 736-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1873 | smc(1-315 GGGSSGGSSGGG 858-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1874 | smc(1-370 GGGSSGGSSGGG 803-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1875 | smc(1-414 GGGSSGGSSGGG 785-1186, E1118Q)-TAP:ermB, specR::ΔscpA |
| BSG1881 | smc(R57A)-mGFP1mut1 ftsY::ermB |
| BSG1889 | smc ftsY::ermB, specR::ΔscpA |
| BSG1890 | smc ftsY::ermB, specR::scpAB |
| BSG1891 | smc ftsY::ermB, specR::scpA ΔscpB |
| BSG1892 | smc(E1118Q) ftsY::ermB, specR::ΔscpA |
| BSG1893 | smc(E1118Q) ftsY::ermB, specR::scpAB |
| BSG1895 | smc(1-499 GGGSSGGSSGGG 674-1186)-TAP ftsY::ermB, specR::ΔscpA |
| BSG1896 | smc(1-499 GGGSSGGSSGGG 674-1186)-TAP ftsY::ermB, specR::scpAB |
| BSG1921 | smc(C119S, C437S, A715C, C826S, C1114S)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB |
| BSG1922 | smc(C119S, C437S, A715C, C826S, C1114S, E1118Q)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB |
| BSG1923 | smc(C119S, C437S, G657A, G658A, G662A, G663A, A715C, C826S, C1114S)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB |
| BSG1924 | smc(C119S, C437S, G657A, G658A, G662A, G663A, A715C, C826S, C1114S, E1118Q)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB |
| BSG1949 | smc(C119S, C437S, A715C, C826S, C1114S)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB, specR::ΔscpA |
| BSG1950 | smc(C119S, C437S, A715C, C826S, C1114S, E1118Q)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB, specR::ΔscpA |
| BSG1951 | smc(C119S, C437S, G657A, G658A, G662A, G663A, A715C, C826S, C1114S)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB, specR::ΔscpA |
| BSG2036 | smc(C119S, C437S, G657A, G658A, G662A, G663A, A715C, C826S, C1114S, E1118Q)-TEV-His12-HaloTag(C61V, C262A) ftsY::ermB, specR::ΔscpA |
| BSG2050 | smc(K37I, E1118Q) ftsY::ermB |
| BSG2051 | smc(S1090R, E1118Q) ftsY::ermB |
| BSG2144 | specR::scpAB, smc(G657A, G658A, G662A, G663A)-ftsY::ermB |
| BSG2145 | specR::ΔscpA, smc(G657A, G658A, G662A, G663A, E1118Q)-ftsY::ermB |
| BSG2146 | specR::scpA ΔscpB, smc(G657A, G658A, G662A, G663A, E1118Q)-ftsY::ermB |
| BSG2147 | specR::ΔscpAB, smc(G657A, G658A, G662A, G663A, E1118Q)-ftsY::ermB |
III.) Supplemental Experimental Procedures

In vivo expression of Smc proteins tested by immunoblotting
Cells were grown in SMG at 37°C to an OD_{600} of 0.02-0.03, harvested by centrifugation or filtrations and washed once in 2 ml PBSG (PBS + 0.1% glycerol). The OD_{600} was measured and equivalent amount of cells for all samples were taken (0.02 ml*OD_{600}). Cells were resuspended in PBSG, β-mercaptoethanol was added to a final concentration of 28.6 mM and kept on ice for 3 min. Lysozyme (12.8 U/µl final), Roche Complete protease inhibitor cocktail and Benzonase (0.4 U/µl; Sigma-Aldrich) were added and the samples were incubated at 37°C for 20 min. NuPage LDS loading dye (final 1x) and DTT (final conc. 100 mM) were added and the samples incubated at 70°C for 10 min. The extracts were loaded on a 4-12% NuPAGE Bis-Tris gel run in MOPS buffer for 50 min at 200 V. Proteins were transferred to a PVDF membrane which was treated with α-Smc, α-GFP (Life Technologies, A6455) or Peroxidase Anti-Peroxidase (PAP). α-Smc and α-GFP blots were treated with ECL Anti-rabbit IgG, HRP-linked whole antibody (from donkey) (GE healthcare). The blots were incubated with Supersignal West Femto (Thermo Scientific) and were imaged in a LAS4000 scanner.

Chromatin immuno-precipitation (ChIP) and qPCR
Cells were grown in SMG medium at 37°C overnight and diluted to OD_{600} 0.005 in SMG. At OD 0.02-0.03 40 ml of fixing solution (50mM Tris/HCl pH 8.0, 100mM NaCl, 1mM EDTA, 0.5mM EGTA, 11% formaldehyde) was added to 400 ml of culture and incubated at room temperature for 30 minutes. Cells were harvested by centrifugation or filtration and washed in 2 ml ice-cold PBS and OD_{600} was measured. Cells were resuspended in 1 ml TESS (50mM Tris/HCl 7.4, 10mM EDTA, 50mM NaCl, 500mM sucrose) and protoplasted by incubating in 1 ml TESS supplemented with 20mg/ml lysozyme (Sigma) and Roche Complete protease inhibitor cocktail for 30 min at 37°C shaking. Cells were washed once in 1 ml TESS, aliquoted according to the previously measured OD_{600} and stored at -80°C.

One aliquot of fixed cells was resuspended in 2 ml lysis buffer (50mM Hepes/KOH pH 7.5, 140mM NaCl, 1mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) sodium deoxycholate, 100mg/ml RNase, Roche Complete protease inhibitor cocktail) and transferred to a 5 ml round-bottom tube. The samples were sonicated 3 x 20 sec on a Bandelin Sonoplus with a MS-72 tip at 90% pulse and 35% power output. Lysates were transferred into 2 ml tubes and centrifuged 5 min at 21000g and the supernatant subsequently 10 min at 21000g at 4°C.

200 µl of the cleared lysates was kept separate as the input sample. 50 µl Protein G coupled dynabead (Invitrogen) were incubated with 50 µl antibody serum (α-Smc, α-ScpB or α-ParB generated in rabbit) for at least 1 hr rotating at 4°C. Beads were washed in lysis buffer and added to 800 µl of the cleared lysates. For experiments involving TAP-tagged strains, rabbit IgG coupled to 50 µl magnetic DynaBeads (Epoxy, M-270) (prepared according to the manufacturer’s protocol) was added to 800 µl cleared lysates. The beads with cleared lysates were incubated at 4°C rotating for 2-4 hours. Beads were washed once with each of the following buffers, lysis buffer, lysis buffer with high salt (500mM NaCl) and wash buffer (10mM Tris/HCl pH 8.0, 250mM LiCl, 1mM EDTA, 0.5% (w/v) NP-40, 0.5% (w/v) sodium deoxycholate). Beads were resuspended in 520 µl TES (50mM Tris/HCl pH 8.0, 10mM EDTA, 1% SDS), the input samples were combined with 300 µl TES and 20 µl 10% SDS solution and incubated overnight at 65°C shaking. DNA was purified by phenol chloroform extraction and ethanol precipitation. The DNA was dissolved in 100µl TE at 65°C for 20 min and purified on a Qiagen PCR purification column and eluted in 30 µl EB. For qPCR 4 µl of the input DNA (diluted 1:200) and IP samples (diluted 1:20) was used in a 10 µl reaction using 5 µl Takyon no ROX SYBR Mastermix blue dTTP (Eurogentec) and 1 µl primer pair stock solution (3 µM each primer) on a Qiagen Rotor-Gene Q in a 72 well rotor according to manufacturer’s instructions. Primer sequences are given in the table below. Curves were analyzed by determining the maximum of the 2^{nd} derivative using the Real-time PCR miner software (http://ewindup.info) (Zhao and Fernald, 2005). ChIP efficiencies were calculated as follows: (((IP/input)*100) for each primer pair.
List of primer pairs for qPCR:

| Primer | Forward  | Reverse  |
|--------|----------|----------|
| *pars*-356 | STG236: tgaagaagaatgcccatacaca | STG237: tgcacaagcaacaccctttac |
| *pars*-359 | STG097: aaaaagtgaattcggcagcag | STG098: aagaaccgccatctttcacagg |
| *dnaA* | STG199: gatcaatcggggaaagtgtg | STG200: gatggccttgtgatgttgg |
| *trnS* | STG404: ggttggacaccccttgta | STG405: aagcacaagttaatgctga |
| *cheC* | STG396: tttgcataagctggcaata | STG397: ttgaaacaatgcacactg |
| *yocGH* | STG099: ttcatatctctgcgttcatcg | STG100: attctgtatgtgcaatgg |

Protein purification and SEC-MALS

Wild-type and hinge mutant BsSmcH-CC300 protein (Smc residues 188-1011) were overexpressed from plasmid pNEA-th in *E. coli* with an N-terminal HIS6 tag (Diebold et al., 2011). The proteins were purified via a HisTrap column, concentrated by anion-exchange chromatography (MonoQ HiTrap) and eluted from a size exclusion chromatography column (Superdex 200 10/300) in 200mM NaCl, 25mM Tris/HCl pH 7.4 (4°C). Multi-angle light scattering coupled to size exclusion chromatography (SEC-MALS) was performed as described previously (Soh et al., 2015).

Viability spotting assay

Cells were grown in SMG medium overnight into stationary phase, diluted 81-fold and 59049-fold (in 9x steps) and spotted on nutrient agar plates (Oxoid) or SMG agar plates. Plates were incubated at 37°C for ~12 hr on NA or ~36 hr on SMG agar.

Mapping of the Bs Smc coiled coil register by disulfide formation

Intramolecular crosslinking reactions for the determination of the coiled coil register were performed essentially as described in (Waldman et al., 2015) using the BsSmcH-CC300 construct (Soh et al., 2015). The protein was expressed from the pNEA-th plasmid as a His-Tag fusion protein. For each double cysteine mutant, 50mL cultures were set-up. Cells were lysed by sonication and soluble extract was incubated for one hour with 300uL of Talon resin (Clontech). Beads were washed three times with the lysis buffer (200 mM NaCl, 50 mM NaPi pH 7.4, 5 mM Imidazole) and then resuspended in the lysis buffer supplemented with 1 mM magnesium chloride. 1uL of benzonase (Roche) was added to the beads that were shaked for 30min at room temperature to remove the bound DNAs. The beads were washed two more times with the lysis buffer and the proteins were eluted with elution buffer 200 mM NaCl, 500 mM Imidazole, 50 mM NaPi pH 7.4.

The proteins were then dialyzed against PBS buffer containing 4 mM DTT for 2 hours. For the crosslinking reaction, the protein was diluted to a concentration of 5 μM. The non-crosslinked sample was prepared by adding 1μM of iodoacetamide and heating at 70°C for 10 min. Disulfide formation was set-up by adding one volume of PBS supplemented with 5 mM NaAsO₂ (Fluka) to the protein and 100 μM dithio-bisnitrobenzoic acid DTNB (Merck) and 300 μM beta-mercaptoethanol. The reaction was incubated at 4°C under shaking. Samples were taken after 2, 4, 6 and 20h of reaction, and quenched by addition of 10 mM iodoacetamide at 70°C for 10 min.
After addition of non-reducing SDS-Page loading buffer the samples were boiled for 5 min at 95°C and run on a Bis-Tris 4-16% NuPage acrylamide gel (Novex) using MOPS buffer as running buffer for 50 min at 200 V.
Supplemental References

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