Supporting Information for:

Liquid crystal formation of RecA-DNA filamentous complexes

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Substrates. RecA protein was purified by the procedure as described before,[1] or purchased from Epicentre Biotechnologies (Wisconsin, USA). Purification of the M13mp18 single-stranded DNA (*ss*DNA) was carried out according to the reported method.[2] *Escherichia coli* strain XL-1 Blue MRF´ was grown in 50 mL of 2× YT broth containing 5 mM $MgCl_2$ and infected with the M3mp18 phage. After 5 h the cells were centrifuged, and the supernatant solution was treated with 3.6% polyethylene glycol 8000 and 2.7% NaCl. The precipitated bacteriophage particles were recovered by centrifugation, and resuspended in TE buffer (10 mM Tris-Cl, pH 7.5, and 1 mM EDTA). The solution was extracted with TE equilibrated phenol and the circular *ss*DNA was precipitated in 2-propanol. The purified DNA was dissolved in TE buffer. Purification of the pUC118 *ss*DNA was carried out as described above, except that *Escherichia coli* strain MV1184 transformed with pUC118 was grown in 50 mL of 2× YT broth containing 150 μg mL^{-1} ampicillin and 70 μg mL^{-1} kanamycin, and infected with M13KO7 helper phage. For the preparation of the M13mp18 linear *ss*DNA, the

single-stranded M13mp18 DNA was first annealed with an oligonucleotide d(GGATCCCCGGGTACCG), which has a complementary sequence to an M13mp18 multi-cloning site, and then digested with the *Sma* I endonuclease. The reaction product was loaded onto agarose-gel electrophoresis containing ethidium bromide, and the DNA fragment was recovered using a dialysis membrane (RECOCHIP; TaKaRa, Shiga, Japan). The DNA solution was purified by extraction with phenol/chloroform/isoamyl alcohol and precipitation in 2-propanol. The purified DNA was dissolved in TE buffer. Small oligonucleotide fragments bound to the single-stranded DNA were removed by small-scale gel filtration (Chroma SPIN 200; Clontech, Mountain View, CA, USA).

Formation of Liquid Crystals.

Liquid crystal of RecA protein: About 10 mL of the purified RecA protein was dialyzed against 20 mM Tris-Cl, pH 7.5, 1 mM dithiothreitol, 0.1 mM EDTA and 10% (v/v) glycerol. The protein solution was concentrated by ultrafiltration (centriprep YM-30; Millipore, Billerica, MA, USA) until a final volume of about 500 μL was reached. The concentrated RecA solution was found to form a liquid crystal as judged by a polarizing microscope. The final protein concentration was 2.1 mM as determined by UV absorption.[3]

Liquid crystal of RecA protein complexed with linear *ss*DNA: The filament formation was carried out at room temperature for 60 min in 2.5 mL of the reaction solution containing 20 mM Tris-acetate, pH 7.5, 25% (v/v) glycerol, 400 μM linear *ss*DNA and 132 μM RecA protein. The protein solution was concentrated by ultrafiltration (Amicon Ultra 30,000 MWCO, Millipore) until a final volume of about 50 μL was reached. The final concentration of the protein and the *ss*DNA were determined by the Bradford method (Bio-Rad protein assay) using a low concentration of RecA protein as a standard and the UV difference spectra,

and estimated as 1.0 mM and 2.6 mM, respectively. The concentration of DNA is expressed as moles of nucleotide residues.

Liquid crystal of RecA protein complexed with circular *ss*DNA: The RecA protein and pUC118 circular *ss*DNA were dissolved in 25 mM Tris-acetate, pH 7.5 and 25% (v/v) glycerol, and the protein solution was concentrated by ultrafiltration (Amicon Ultra 30,000 MWCO, Millipore) at room temperature until a final volume of about 100 μL was reached. The final concentrations of the protein and the *ss*DNA were estimated as 2.2 mM and 4.4 mM, respectively. The concentration of DNA is expressed as moles of nucleotide residues.

Optical Microscopic Observations. Polarizing optical microscopic observations were carried out with an E600POL polarizing optical microscope (Nikon, Tokyo, Japan) equipped with a DS-5M CCD camera (Nikon) connected to a DS-L1 control unit (Nikon). The sample solution was placed on a glass plate without a cover glass to develop the planar structure before observations at ambient temperature (20– 25 °C).

AFM Observations. For AFM observations, the liquid crystal of RecA-*ss*DNA complex was diluted 500 times with the sample buffer (20 mM Tris-acetate, pH 7.5, 25% (v/v) glycerol), and immediately loaded onto the gel filtration column (Sepharose 2B, Amersham Biosciences, Uppsala, Sweden) equilibrated with the same buffer. The sample was eluted within 80 sec, and immediately spread over aminopropyltriethoxysilane-mica (AP-mica), positively-charged mica that had been functionalized with aminopropyltriethoxysilane. AFM images were acquired with a NanoScope IV MultiMode system (Veeco Instruments Inc., Santa Barbara, USA) in air at ambient temperature with standard silicon cantilevers (NCH, NanoWorld, Neuchâtel, Switzerland) in the tapping mode.

Supporting References

1 T. Nishinaka, A. Takano, Y. Doi, M. Hashimoto, A. Nakamura, Y. Matsushita, J. Kumaki., E. Yashima, *J. Am. Chem. Soc.*, 2005, **127**, 8120-8125.

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3 S. Kuramitsu, K. Hamaguchi, H. Tachibana, T. Horii, T. Ogawa, H. Ogawa, *Biochemistry*, 1984, **23**, 2363-2367.

Fig. S1

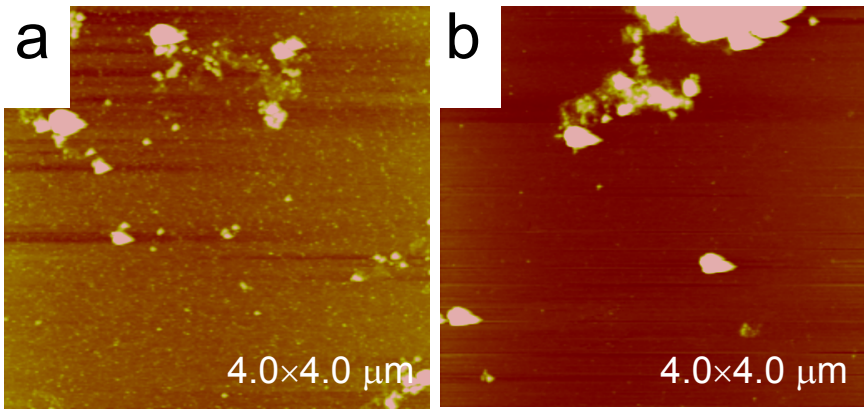

Fig. S1 AFM images of large aggregations observed in the solution of RecA containing ATPγS. RecA protein was diluted to 0.18 μM with 20 mM Tris-acetate, pH 7.5, 10 mM $Mg(CH_3COO)_2$ and 10 mM ATPγS (a) or 20 mM Tris-acetate, pH 7.5 and 10 mM ATPγS (b), and then spread over AP-mica for AFM observations.