In-vitro Enzyme Comparative Kinetics: Unwinding surface bound DNA Nanostructures by RecQ and RecQ1

Pietro Parisse[1], Alessandro Vindigni[1,2,3], Giacinto Scoles[1,4] and Loredana Casalis[1]

[1] Sincrotrone Trieste S.C.p.A., s.s.14 km163,5 in Area Science Park, Trieste, 34149, Italy

[2] International Centre for Genetic Engineering and Biotechnology, Padriciano 99, Trieste, 34149, Italy

[3] Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, MO, 63104, USA

[4] Department of Biological and Medical Science, University of Udine, Ospedale della Misericordia, Udine, 33100, Italy

MATERIALS AND METHODS

Atomic Force Microscopy: Equipment and settings

AFM Nanografting and imaging experiments have been performed on a XE-100 PARK/PSIA System and a MFP-3D- Stand Alone Asylum Research. The measurements were carried out in commercially available liquid cells using silicon cantilevers (nominal spring constants between 0.5-1.5 N m^{-1} for the nanografting process and 0.03 N m^{-1} for the imaging in contact mode). The grafting and imaging procedures have been performed according to the protocol reported in Ref. [30] with some slight changes. Nanografting of ss(ds)DNA on preformed self assembled monolayers of thiolated-Oligo-Ethylene Glycol (OEG) molecules on gold has been performed in a 5(2.5) μM thiolated ss(ds)DNA buffered solution (1M NaCl solution in TE (10mM Tris, 1mM EDTA, pH 7.1) buffer in MilliQ water (Millipore Simplicity, resistivity=18.2 MΩcm)). The sequences of the duplex are reported in Table S1. The

duplexes were prepared by incubating the two complementary strands in a 1:1 ratio at 37 °C in a 1M NaCl solution in TE buffer for 2 hours.

Table S1. Sequences used as forked substrate

30-bp duplex	5'AGTCTTCGTCCTCGTACCCGATGTTTTCGCTTTTTTTTTTTT 3'
with 12dTs	5'TTTTTTTTTTTTACGAAAACATCGGGTACGAGGACGAAGACT 3'-$(CH_2)_6$-SH

Image and data analysis

AFM images have been analyzed using XEI (Park Systems) and Igor Pro (Wavemetrics, Inc.) software. Equation 1 was fit using Igor Pro (WaveMetrics, Inc.) using a nonlinear least-squares (NLLS) fitting algorithm by holding the parameter *m* fixed. The numbers reported are the best fit value and the standard deviation for each parameter.

Protein purification

RecQ and RECQ1 were purified according to the procedure reported in ref. [13].

Helicase assays

Helicase assays were performed in 300 μl of a reaction mixture containing helicase buffer (20mM Tris, 2mM dithiothreitol DTT, 5mM $MgCl_2$, 10mM KCl, pH 7.5). RECQ (RECQ1) proteins were used at a concentration of 12.5 and 25 nM (50 and 150 nM). The reactions were started by the addition of 5mM ATP, after a 15 min preincubation at 37 °C, and the mixture was incubated at 37 °C as a function of time. The reactions were terminated by washing the sample with three different buffers (10mM Tris, 1mM EDTA, Tween 0.05%, 1M NaCl, pH 7.1), (10mM Tris, 1mM EDTA, 1M NaCl pH 7.1) and (100mM NaCl, 10mM

$MgCl_2$, 50mM Bis-Tris*HCl, pH 6.0) and the samples were imaged immediately after the washing procedure.

In figure S1 we report the topographic images of an array of DNA patches of different sizes, from 300 nm to 2 μm of lateral dimension. The height color bar is the same for all the three images (ssDNA, dsDNA and after the helicase reaction) and the simple visual inspection of the image already reveals that after the enzymatic reaction the unwinding has been effective for all the patches, independently of their size. In fact, after the helicase action the patches recover, within the experimental error, the initial ssDNA height.

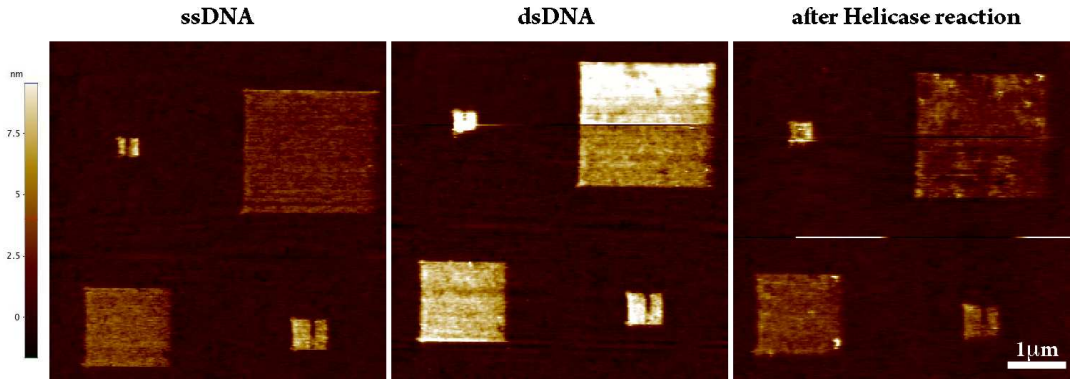

Figure S1. Atomic Force Microscopy topographic images and corresponding *average* line profiles of an array of patches. As grafted (ssDNA), after the hybridization (dsDNA) and after 3 hours of 50nM RecQ1 helicase incubation in the unwinding buffer (20 mM Tris, 2 mM DTT, 5 mM $MgCl_2$, 10 mM KCl, 5 mM ATP, pH 7.5).

Re-hybridization of DNA substrates on surface

After the helicase assay the DNA substrates were re-hybridized in order to check that the effective unwinding is not damaging the DNA substrate. Hybridization was performed by incubating surfaces in 1μM solutions of complementary DNA, in 1M NaCl TE-buffer. After hybridization, surfaces were washed with two buffers: (10mM Tris, 1mM EDTA, 1M NaCl pH 7.1) and (100mM NaCl, 10mM MgCl2, 50mM Bis-Tris*HCl, pH 6.0). In Figure S2 we

report the dispersion of the height of the rehybrized patches as a function of the initial height of the dsDNA matrices. The linear fit of the dispersion gives a slope of 0.95±0.05, perfectly consistent with a full recovery of the initial height. In the same figure we also report the values of the height of ssDNA grafted matrices as a function of the height of the dsDNA obtained by their hybridization. The linear fit of the dispersion gives a slope of 0.50±0.02 that will serve as a reference for the height of the patches after the action of the helicases.

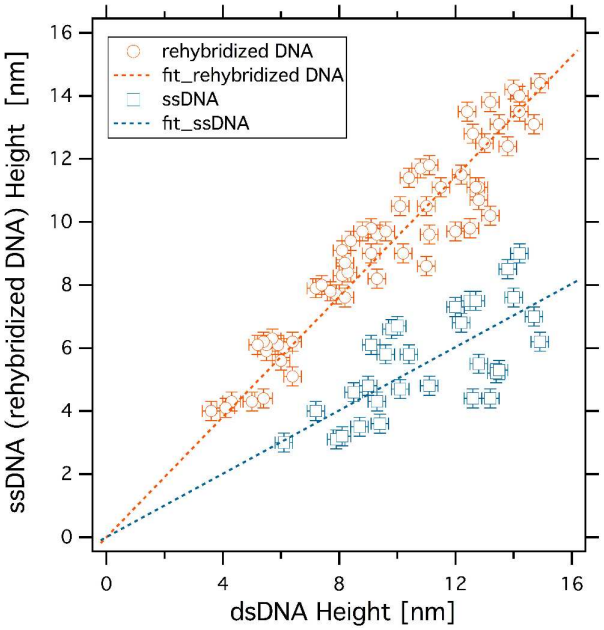

Figure S2. The plot shows the dispersion and relative fit of the height of the patches rehybridized after the helicase assays and for the starting ssDNA height versus the height of the dsDNA matrices before the helicase assays.

Pre-incubation effect

We checked our DNA patches after a 15 minutes incubation of RecQ without ATP washing only with high salt buffer (Tris-HCl 10mM, 1 mM EDTA, 1M NaCl, pH 7.1) and we observed the presence of a quite homogeneous layer on top of the patches, that resulted to be ~4 nm higher, as shown in Figure S3. This is consistent with the binding of a layer of the enzymes on the DNA and it is compatible with a one to one (or nearly one to one) ratio between DNA and enzyme.

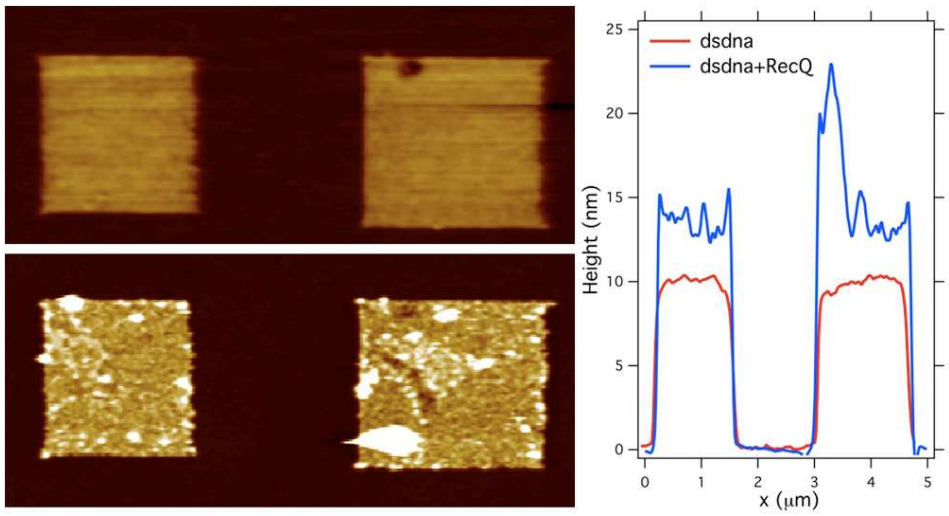

Figure S3. Atomic Force Microscopy topographic images and corresponding average line profiles of two patches (size of the images is 5 x 2 μm) of dsDNA before and after the 15 minutes incubation of RecQ without ATP.

Helicase assay in presence of a DNA trap

The experiments were also repeated with the introduction of a 1 μM DNA trap (a 40 oligonucleotides sequence 5'TAG CCG ATT ACC AAG CCA AGC CCA AGC CAA GTA GCC GAT T 3') for the helicases. In Fig. S4 we report the comparison between the assay performed with 25nM RecQ with and without the presence of the DNA trap. We cannot notice appreciable changes in the kinetic behavior, demonstrating that our assay is reasonably performed in single turnover conditions.

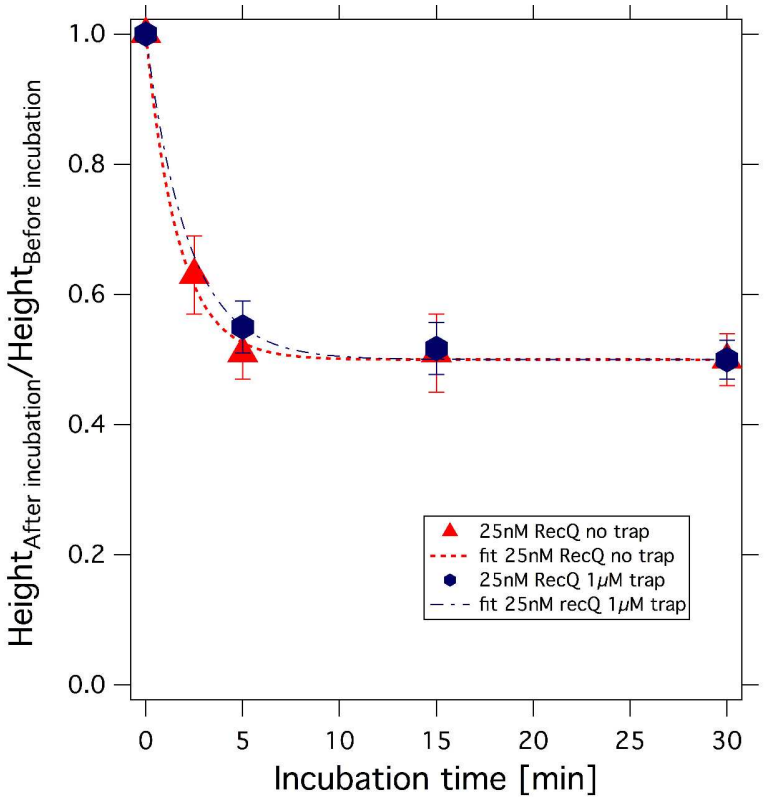

Fig. S4. Variation of the ratio between the height before and after the reaction as a function of the incubation time of RecQ for experiments performed with (blue exagons) and without (red triangles) a 1 μM DNA trap in the incubation solution.

The effect of dsDNA density on unwinding reactions inside dsDNA matrices.

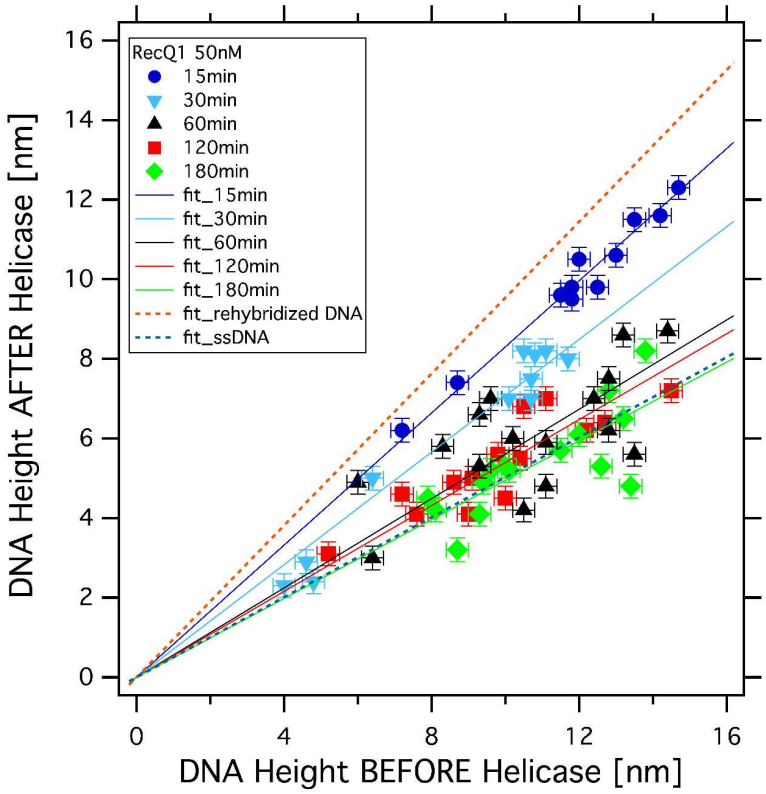

Fig. S5. The plot shows the dispersion and relative fit of the absolute height of the dsDNA sequences immobilized on the surface after reaction with RecQ1 helicase versus the initial height before the RecQ1 helicase assay for different incubation times (15, 30, 60, 120, 180 minutes). The dashed lines in orange and blue represent the linear fits for the rehybridized patches and the reference ssDNA height, respectively.