A Novel Secondary DNA Binding Site in Human Topoisomerase I Unravelled by using a 2D DNA Origami Platform

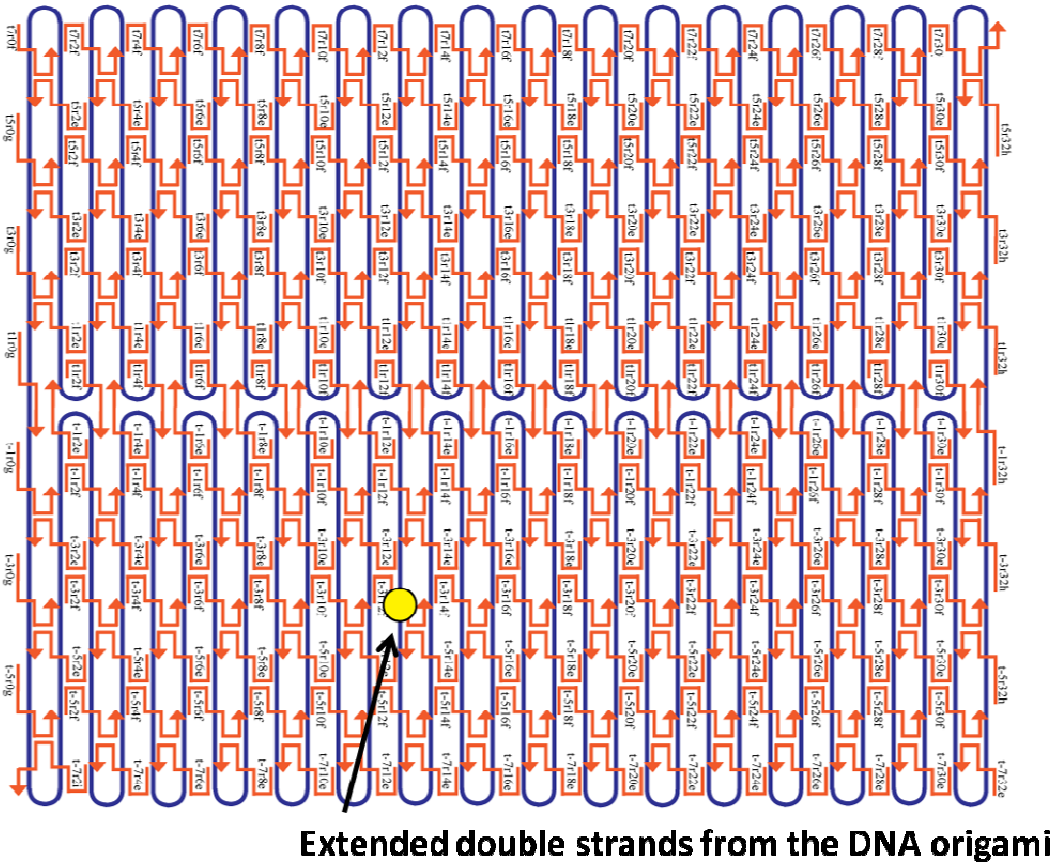

Figure S1: Design of DNA origami template with the protruding baitDNA highlighted with a yellow circle. The DNA origami is assembled by mixing single-stranded M13mp18 DNA with 225 staple strands and the two oligonucleotides constituting the protruding baitDNA, as described in the Methods section.

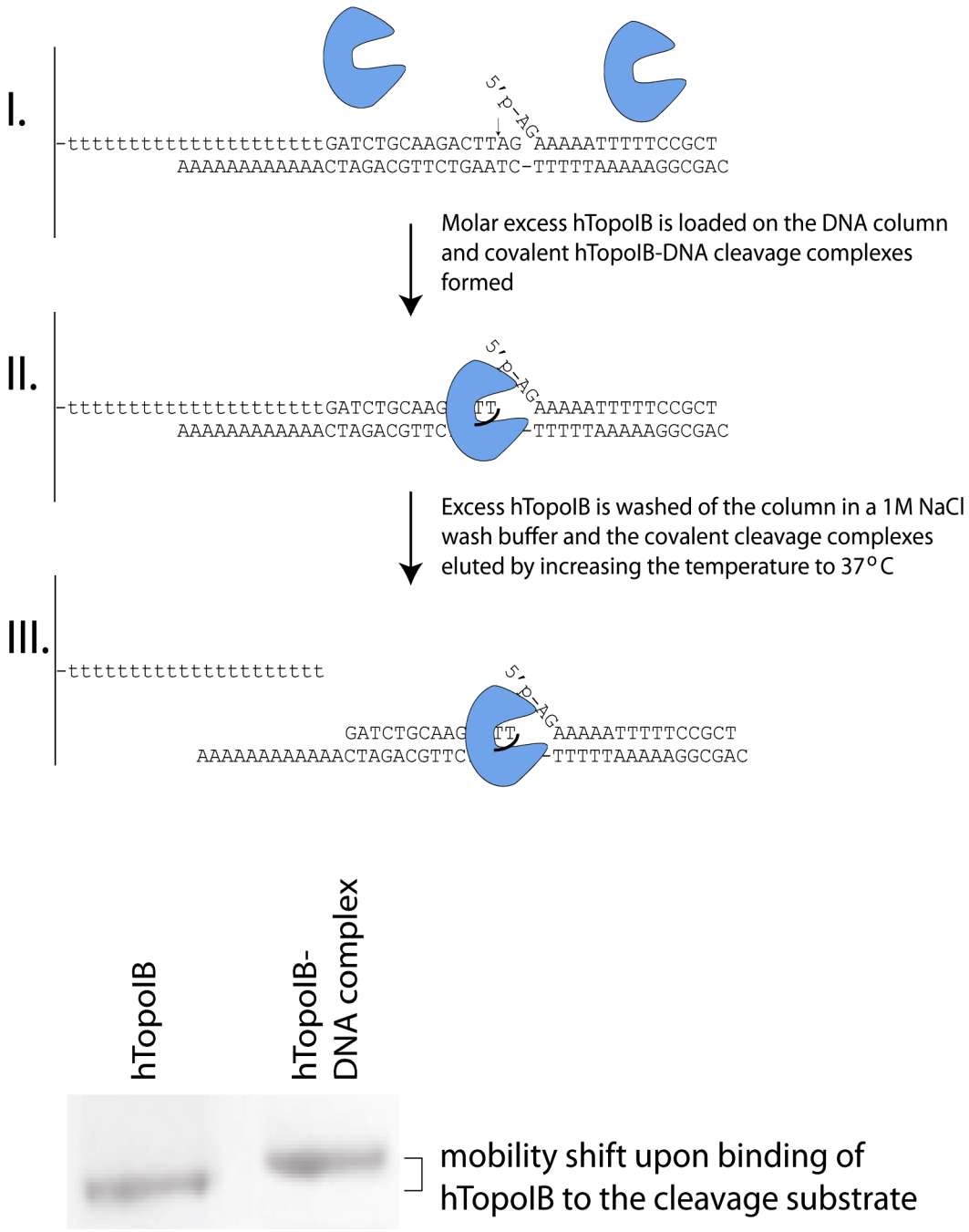

Figure S2: The top panel shows a flow chart schematically illustrating preparation and purification of hTopoIB-DNA cleavage complexes. The lower panel shows the result of analysing the purified cleavage complexes in a 10% SDS polyacrylamide gel in comparison to DNA-free hTopoIB. The protein was visualized by Coomassie staining. As evident from the gel picture covalent attachment to the cleavage substrate caused a slight retardation of hTopoIB in the gel. No DNA-free hTopoIB could be observed in the fraction eluted from the DNA column, which is consistent with the lack of relaxation activity in this fraction (data not shown).

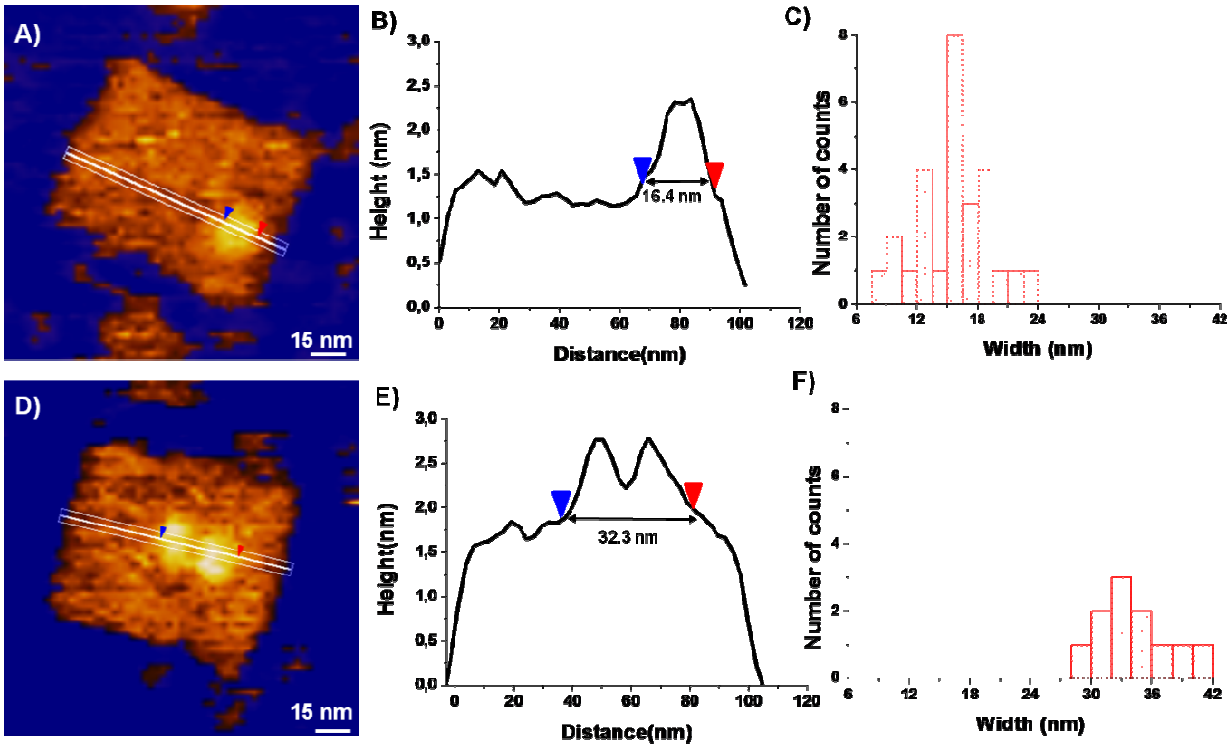

Figure S3: Discrimination between the T_1 and T_2 interaction modes. (A) AFM image of a hTopoIB-DNA cleavage complex binding a naked baitDNA exposed on the DNA origami (T_1 interaction mode). (B) Line profile measurement to calculate the width of a hTopoIB-DNA cleavage complex bound to the baitDNA. (C) Histogram of the widths of baitDNA-bound hTopoIB-DNA cleavage complexes measured from images like the one shown in (A). (D) AFM image of a hTopoIB-DNA cleavage complex binding a hTopoIB-bound baitDNA exposed from the DNA origami (T_2 interaction mode). (E) Line profile measurement to calculate the width of the interaction complex shown in (D). (F) Histogram of the widths of interaction complexes measured from images like the one shown in (E).

The average width of interaction complexes as the one shown in (A) of 15.6 ± 3.5 nm (C), which corresponds to the calculated average width (17.0 ± 3.5 nm) of individual hTopoIB enzymes (estimated in Fig 2D), suggests these complexes to be the result of interaction mode T_1. The average width of interaction complexes like the one shown in (D) of 34.2 ± 3.7 nm (F), strongly suggests that these complexes represent the interaction of two DNA-bound TopoIB enzyme and, hence, represent interaction mode T_2.

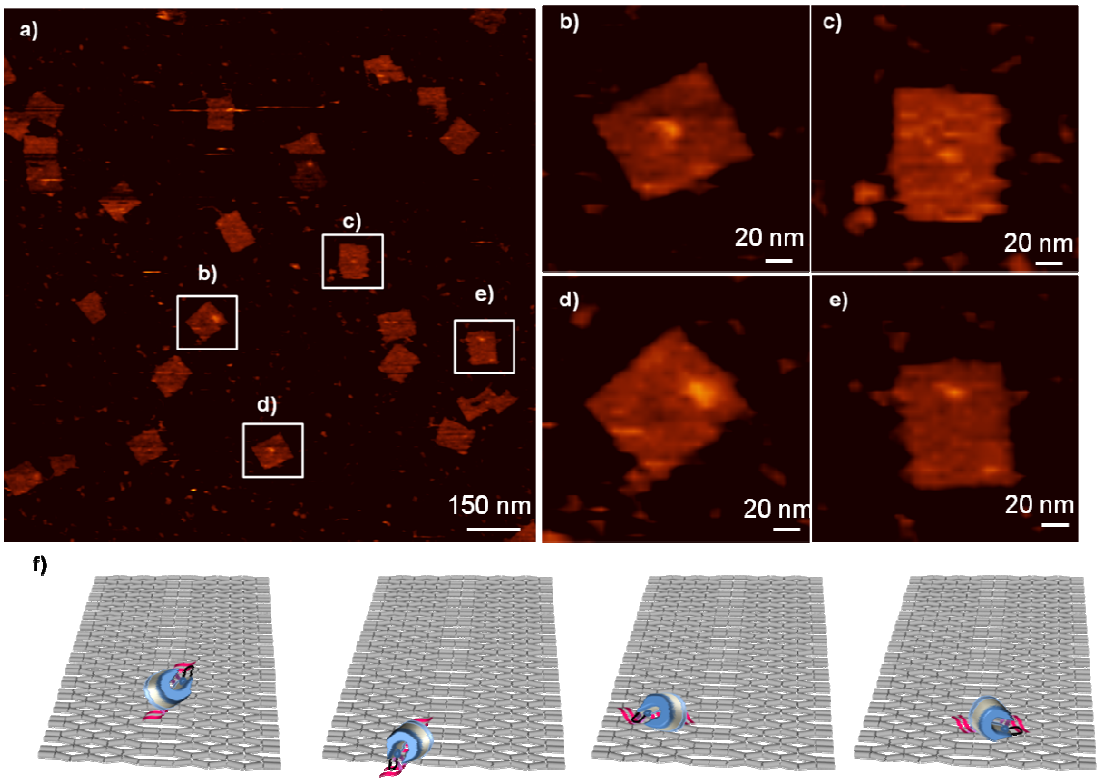

Figure S4: (A) Large-scale AFM image of free hTopoIB enzymes binding on the DNA origami template. (B,C,D and E) Zoom-in AFM images from (A), (marked in (A) with white squares) showing different possible positions of hTopoIB on the origami template due to the flexibility of the protruding baitDNA. (F) Schematic representation of the different positions of hTopoIB on the DNA origami. The DNA origami with protruding baitDNA and hTopoIB are not drawn to scale.

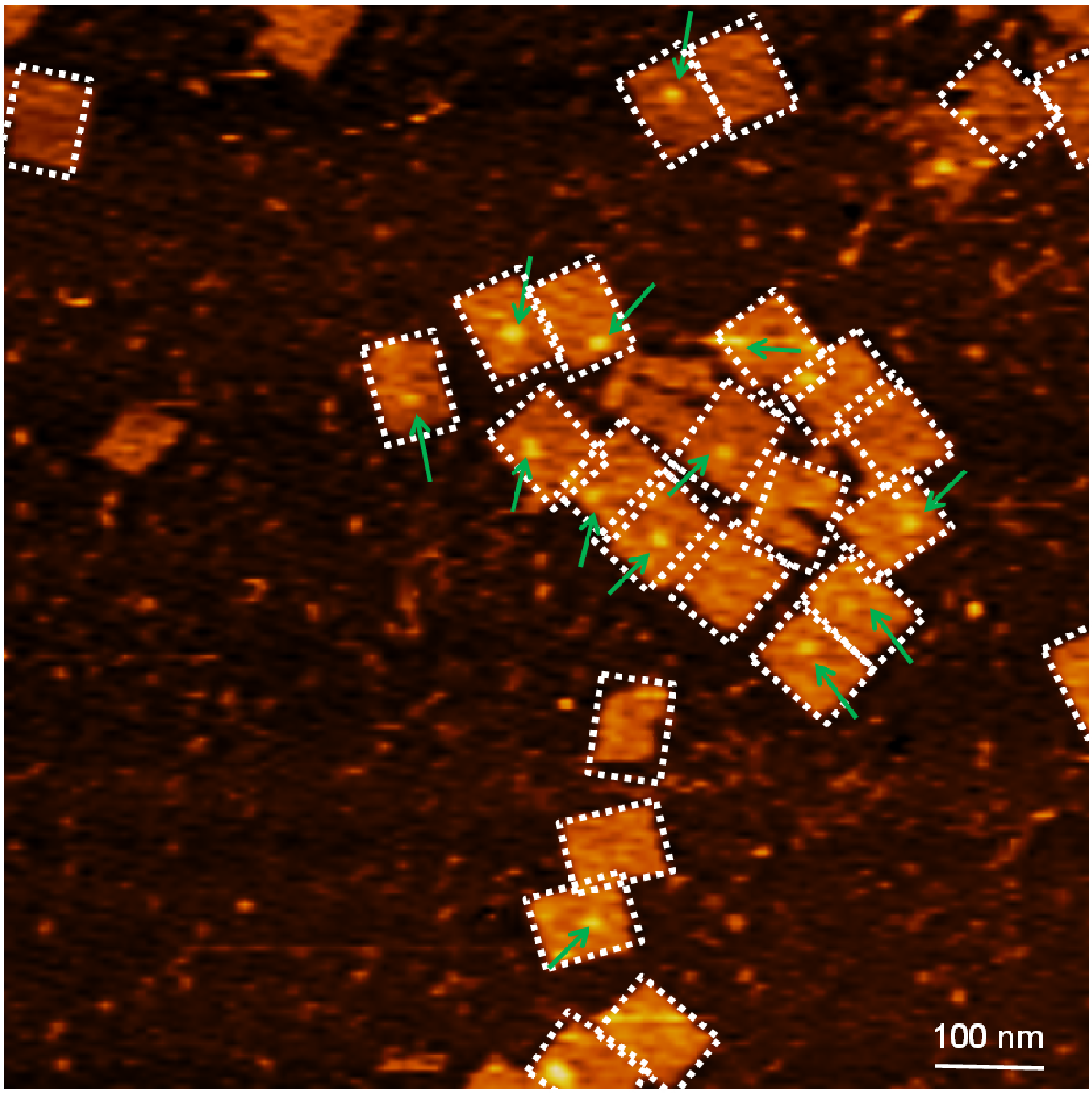

Figure S5: Large-scale AFM image of origamis incubated with purified hTopoIB-DNA cleavage complexes, illustrating the different positions of the hTopoIB-DNA complexes on the DNA origami templates. The DNA origamis are marked by white squares and baitDNA-bound hTopo-DNA cleavage complexes are indicated by green arrows.

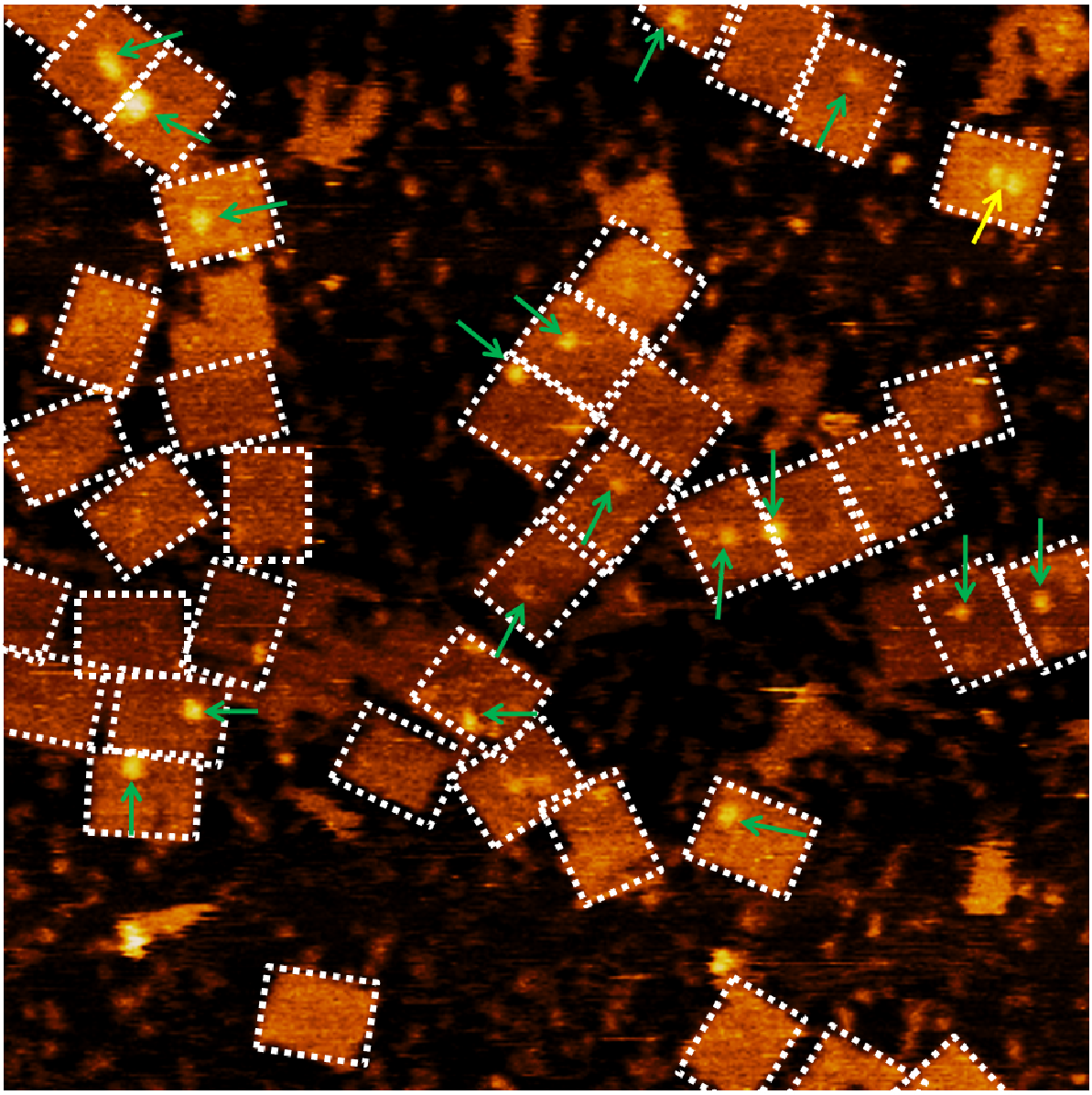

Figure S6: Large-scale AFM image showing hTopoIB-DNA cleavage complexes interacting with baitDNA-bound hTopoIB on the DNA origami templates, illustrating the different positions of the hTopoIB-DNA complexes on the DNA origamis. The DNA origamis are marked with white squares and baitDNA-bound hTopoIB indicated by green arrows. On one origami an hTopoIB-DNA cleavage complex interacting to an baitDNA bound hTopoIB (indicated by a yellow arrow) is shown.