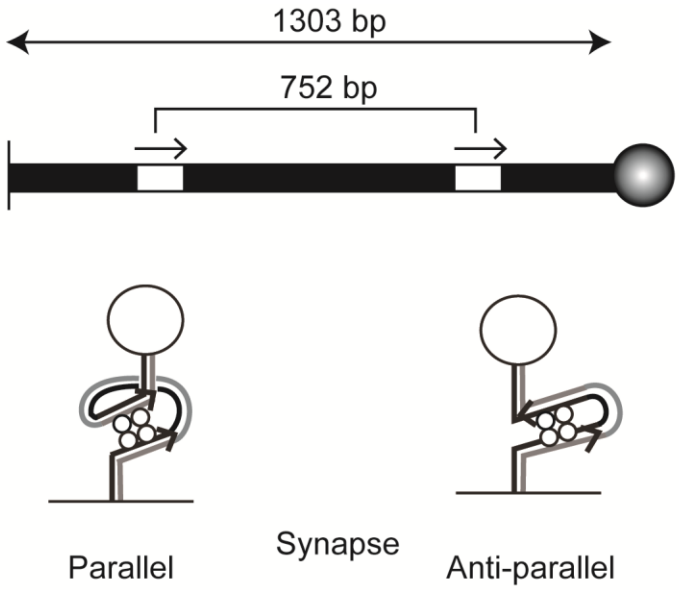
SUPPLEMENTARY DATA

A bipartite thermodynamic-kinetic contribution by an activating mutation to RDF-independent excision by a phage serine integrase

Hsiu-Fang Fan, Bo-Yu Su, Chien-Hui Ma, Paul A. Rowley and Makkuni Jayaram



DNA species	Characteristics	BM amplitude nm
S	Unbound substrate.	93-99
NP	Bound nonproductively. Reverts to S.	70-80
PS	Bound, pre-synaptic complex. Proceeds to WS or RS.	70-80
WS	Wayward synapse formed from PS. Dissociates to PS.	40-60
RS	Recombinogenic synapse formed from PS. Performs recombination.	54-60
BC	All bound complexs. NP + PS (WS + RS)	40-80
L	Linear recombinant.	40-45

Figure S1. DNA species identified by BM amplitude estimates during recombination reactions mediated by Int (or Int(E449K)) with or without gp3.

The schematic diagram at the top represents the substrates (each 1303 bp long) used for the present TPM studies, tethered at one end (shown by the vertical line) and attached to a polystyrene bead (shown by the sphere) at the other. The *att* sites (drawn as rectangular boxes) are spaced 752 bp apart. Except for one set of experiments each with Int and Int(E449K), the sites were in head-to-tail orientation, as shown here by the direction of the arrows. In synapsed molecules, the *att* sites bound by Int may come together in parallel geometry (functional in recombination) or anti-parallel geometry (nonfunctional in recombination). The distal disposition of the DNA entry and exit points with respect to the synapse is expected to exert less constraint on the tethered bead compared to their proximal disposition. The predicted higher BM amplitude of the recombination-competent parallel synapse than the abortive anti-parallel synapse (for head-to-tail oriented sites) is consistent with experimental results. The observed BM values signify distinct DNA species comprised of the substrate (S) and bound complexes (BC) in which the *att* sites are occupied by Int or Int plus gp3. Non-productively bound complexes (NP) as well as pre-synaptic complexes (PS) make up BC. Wayward synaptic complexes (WS); and recombinogenic synaptic complexes (RS) are derived from PS. The tethered linear excision product (L) is formed only within RS. The inactive WS synaptic complexes span a wide BM amplitude range, suggesting that they are heterogeneous in conformation.

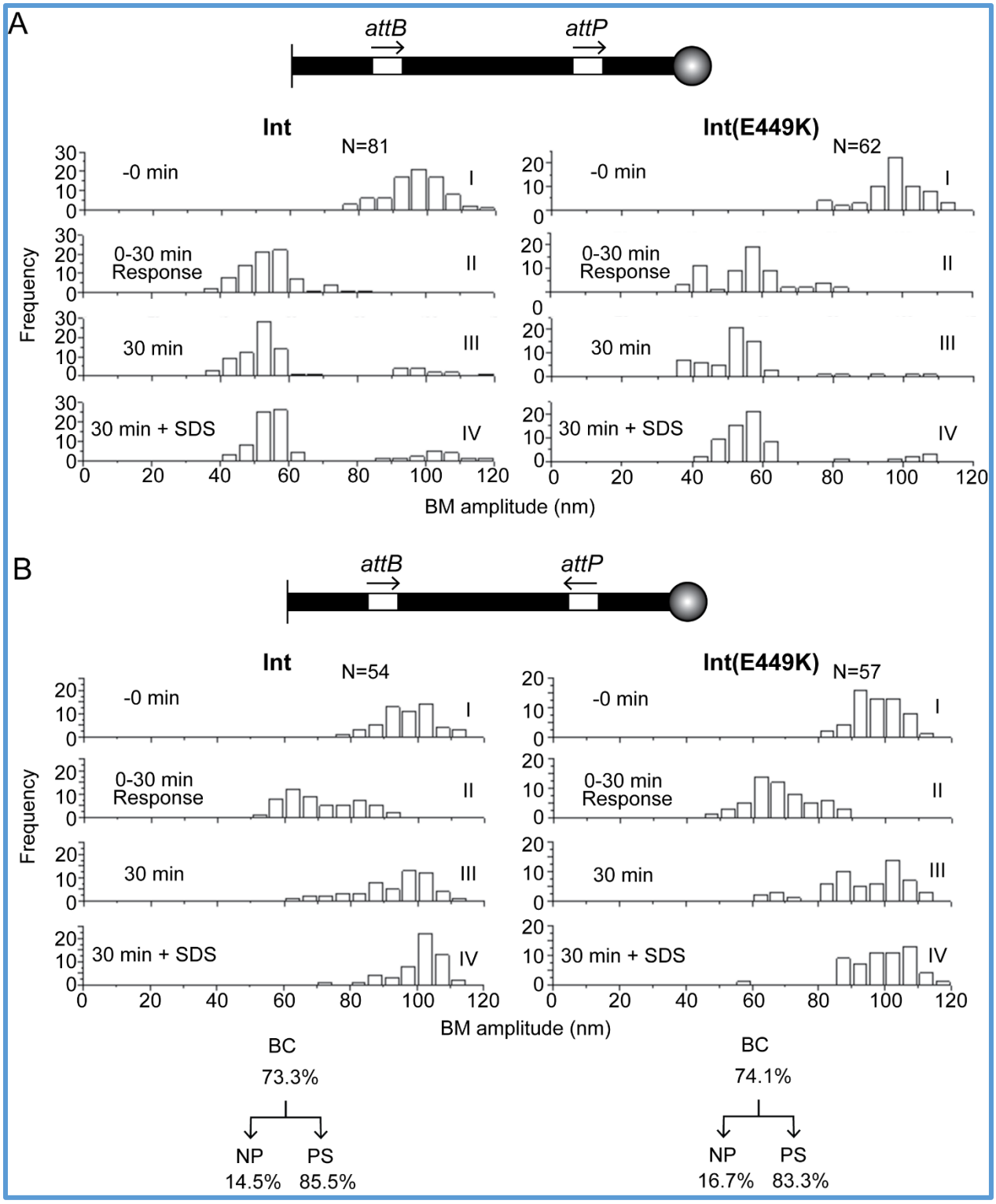


Figure S2. The BM amplitude data derived from cumulative single molecule time traces recorded for *attP* X *attB* recombination. The plots show the results for reactions of Int and Int(E449K) with the *att* sites in head-to-tail (**A**) and head-to-head (**B**) orientations. For each

assay set, the four panels arranged from top to bottom categorize the observations as follows: (**I**) molecules just prior to addition of Int or Int(E449K) (-0 min); (**II**) molecules shifted to lower BM amplitudes during the assay (0-30 min); (**III**) molecules at 30 min just before SDS addition; and (**IV**) molecules immediately after SDS addition. The BM amplitudes in (**II**) represent the first response of each individual molecule recorded in its time trace. These molecules could subsequently change BM amplitudes depending on the transitions they undergo. For the *attP* X *attB* (head-to-head) recombination reactions, the relative proportions of non-productive (NP) and PS (pre-synaptic) complexes formed from total bound complexes (BC) are shown at the bottom. The synaptic complexes formed from PS could not be separated into recombinogenic and wayward synapses (RS and WS, respectively). The BM amplitude change resulting from the dissociation of WS into unreacted substrate is not different from that caused by the conversion of RS into product, as there is no change in DNA length due to the inversion reaction.

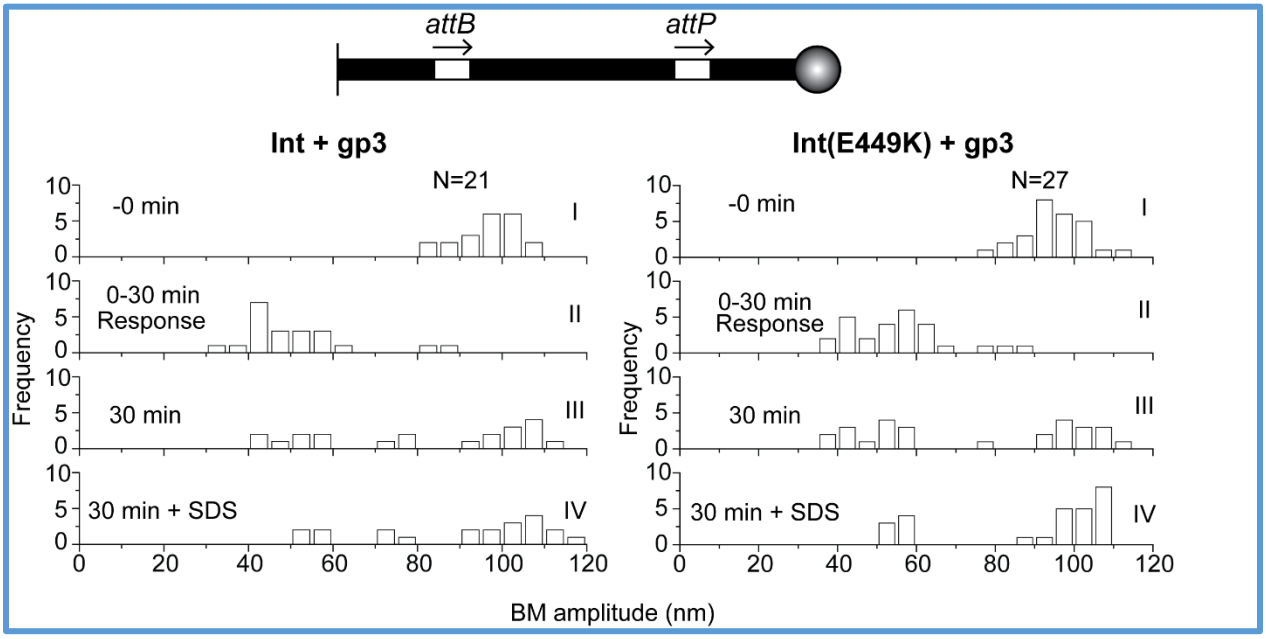


Figure S3. The effect of gp3 on *attP* X *attB* recombination by Int or Int(E449K). The assays were performed as described under Figure S2, except that Int or Int(E449K) together with gp3 was added to the reactions at time zero.

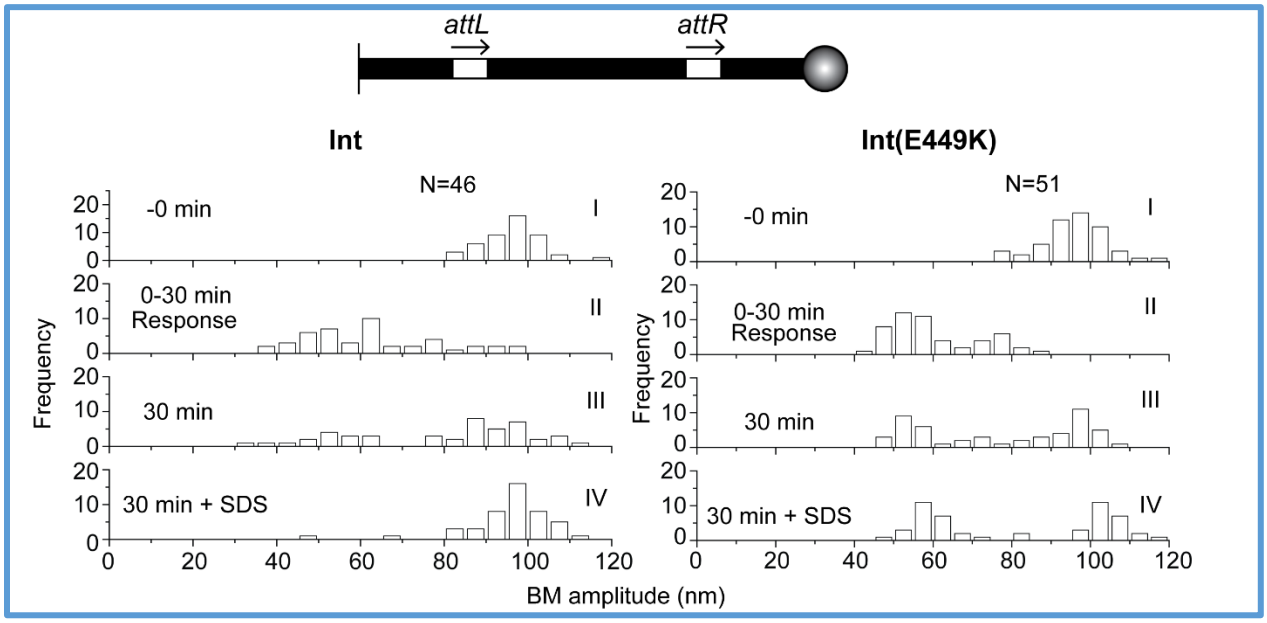


Figure S4. Int and Int(E449K) activities in *attL* X *attR* recombination. The analysis utilized the DNA substrate containing *attL-attR* in head-to-tail orientation. The data are assembled as in Figures S2 and S3.

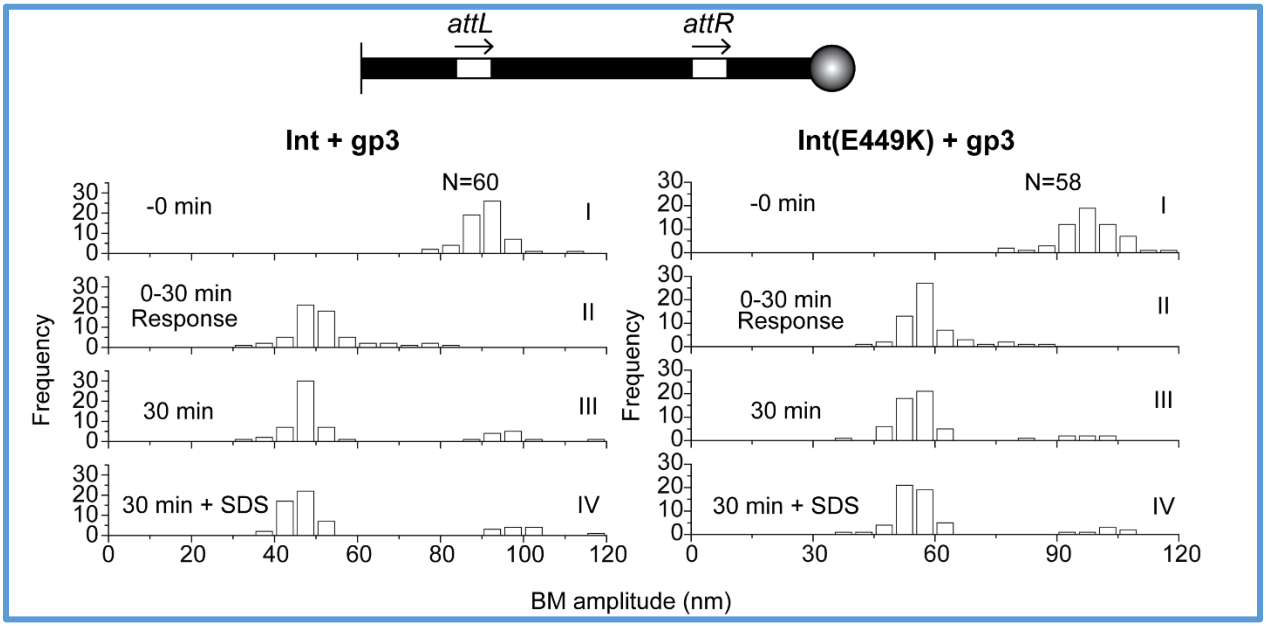


Figure S5. Activities of Int or Int(E449K) plus gp3 in *attL* X *attR* recombination. The assays were performed as described under Figure S4, except that Int or Int(E449K) together with gp3 was added at time zero.

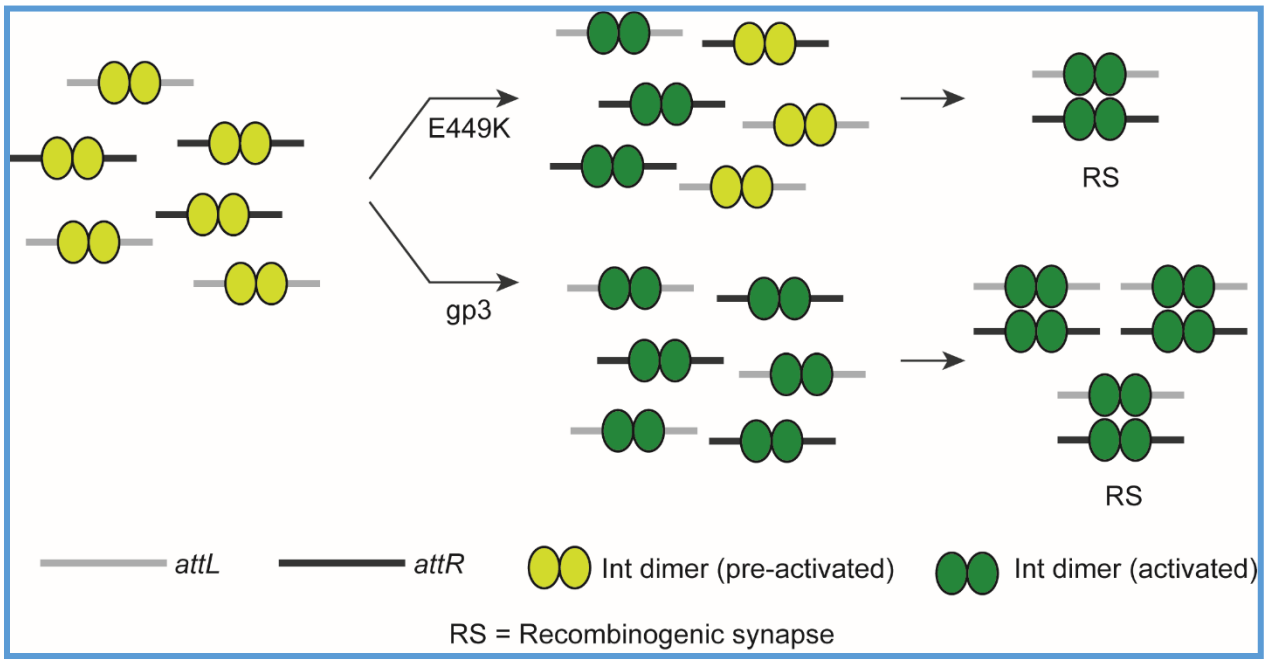


Figure S6. A model for the differences between E449K and gp3 in activating Int for *attL-attR* recombination. In the schematic diagrams, the activated and pre-activated Int dimers are shown as light and dark green twin ovals, respectively. For simplicity, only Int dimers bound to *attL* and *attR* sites are shown. The E449K substitution is less effective than gp3 in populating the active Int dimers. As a result, the functional synaptic structures assembled in the presence of gp3 are more abundant. In the TPM analysis, these recombination competent synapses constitute the RS complexes. The inactive synaptic structures assembled from pre-activated Int dimers or a paired combination of pre-activated and activated dimers fall into the WS category.

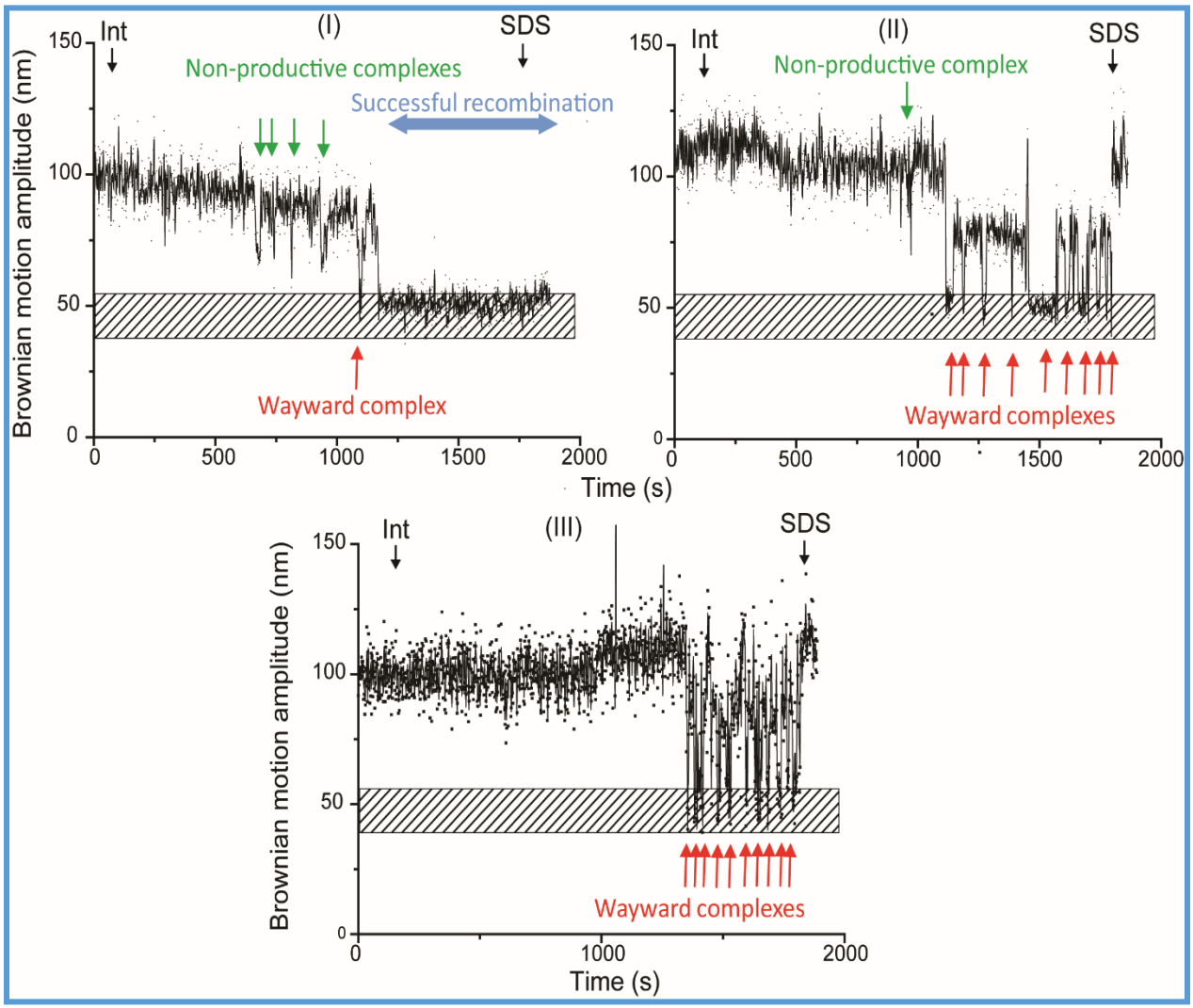


Figure S7. Dissociation of NP and WS complexes and their re-attempts to form productive synapsis. The time traces of three molecules observed by TPM over a 30 min time course from addition of Int to reaction quenching by SDS are shown, highlighting the dissociation and reassociation events that the non-productive (NP) and wayward synaptic (WS) complexes undergo. After multiple attempts, molecule (I) successfully assembled a recombinogenic synapse (RS complex). Similar attempts by molecules (I) and (II) resulted in the formation of WS complexes (failure of recombination).