**Supplemental Material for "Viewing Single λ Site-Specific Recombination Events from Start to Finish"**

## DNA Ligation in the Presence of SDS

We hypothesized that covalent Int/DNA cleavage-generated complexes are not trapped in ensemble reactions because SDS fails to quench Int ligation activity fast enough to prevent reformation of the initial phosphodiester bonds. This hypothesis was confirmed using an adaptation of a ligation assay previously described by Pan and Sadowski (see Fig. S1; (Pan and Sadowski, 1992). In this assay, we first trapped covalent λ Int-DNA cleavage adducts using a radiolabeled "suicide" substrate with an appropriately positioned Int cleavage site (see diagram in Fig. S1). To trap these intermediates, DNA ligation is prevented through a rapid loss of the 5'-OH nucleophile which is carried away by diffusion of the DNA trinucleotide. Following this, high concentrations of a 5'-OH hexanucleotide were added to the reaction in the presence of SDS and the products were separated by SDS-PAGE. In agreement with our hypothesis, about 70% of covalently-bound Int was released by ligation to the 5'OH hexanucleotide in the presence of 1% SDS (see Fig. S1). We expect that this ligation reaction is even more efficient in a normal recombination reaction since the attacking 5'-OH nucleophile is present within, or immediately adjacent to, the active site and does not depend upon diffusion, as is the case in this artificial assay. In other experiments (not shown), no Int-dependent DNA cleavage in the presence of SDS was observed.

We conclude that the failure to observe in solution the levels of covalent Int-DNA intermediates predicted by the single molecule experiments is due to the reversal of these intermediates upon addition of the traditional SDS quenching solution. It should be noted that given sufficient time, SDS will denature Int and prevent ligation. When the 5'-OH hexanucleotide is added subsequently to SDS, the amount of ligation depends on the time interval between the two additions. By 8s SDS denatures Int to the extent that no ligation activity is detectable (data not shown). It should also be noted that somewhat similar conclusions have been reached with two other Tyrosine recombinase family members, Cre and XerC/D. In this case, when high concentrations of ethidium bromide are used to quench the recombination reactions, Holiday junction reaction intermediates are trapped that are not seen when SDS is used to quench (Kilbride et al., 2006; Barre et al., 2000). However, thus far we have not (yet) found conditions where ethidium bromide traps unique intermediates in the λ excision reaction.

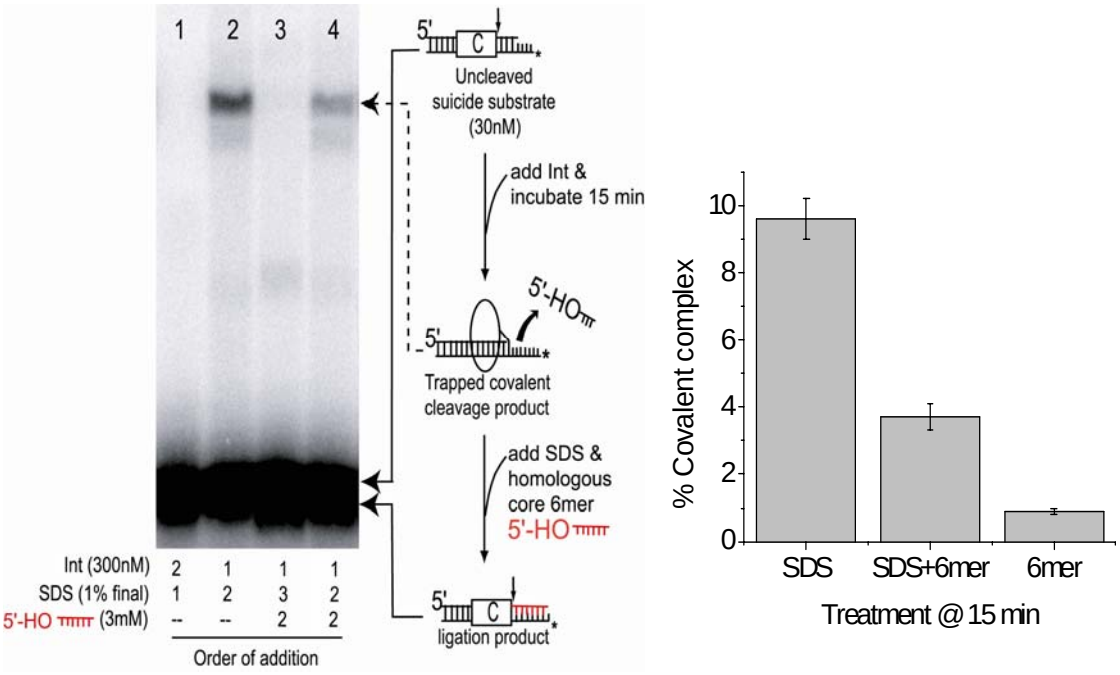


**Figure S1. Int-mediated DNA ligation can occur in the presence of SDS.**

When preparing the Int core-site suicide substrate the bottom strand oligonucleotide was radiolabed with $^{32}$P-γATP (Perkin Elmer, Wellesly, MA) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA) before annealing it to the top strand oligonucleotide. SDS-PAGE purified oligonucleotides (Operon, Huntsville, AL) used in the ligation assay are (top strand) 5'-gatatttatatcattttacgtttctcgttcagctttttt, (bottom strand) 5'-gtataaaaaagctgaacgagaaacgtaaaatgatataaatatc, and (homologous core 6mer) 5'-tttata. Ligation assays were carried out in 50 mM Tris(hydroxymethyl)-aminomethane at pH 8.0, 75 mM NaCl, 11.3 mM $CaCl_2$, 1 mM EDTA, 1 mM DTT, 0.005 mg/ml fish sperm DNA (Sigma-Aldrich, St. Louis, MO), and 0.5 mg/ml molecular biology grade BSA (Roche, Indianapolis, IN). After the addition of purified Int, reactions were incubated at 25°C for 15 minutes before the addition of SDS and core 6mer and (concentrations are indicated in figure). Reactions were incubated an additional 1 min before loading on a 5% polyacrylamide gel containing 1% SDS. The gel was dried and the percentage of covalent Int cleavage product converted from radiolabeled suicide substrate was determined using a Fuji BAS-2500 phosphorimaging scanner. Results from three separate experimental trials were averaged (standard deviation indicated by error bars) and plotted on the bar graph.

**Additional Materials and Methods: DNA Substrate Preparation**

All DNAs used to tether the beads were 1943 bp and were made using polymerase chain reaction (PCR) with *pfu* DNA polymerase (Stratagene, La Jolla, CA). pBR322, pJT23 (Thompson et al., 1986) and pSN55 (Nunes-Düby et al., 1997) were used as templates for non-*att*, *attL*, and *attR* substrates, respectively (Supplemental Table S1). In the case of the *attL+attR* and *attLinvattR* substrates, a two step PCR protocol was used in which individual PCR products, each containing an *attL* or *attR*, were purified on a 1.2% agarose gel and annealed to template a single DNA in a second round of PCR. All DNAs used in these experiments were 1943 bp in length. Where noted, PCR primers were commercially 5' labeled (Operon, Huntsville, AL) with either biotin or digoxigenin for attachment to the flow chamber surface and a polystyrene bead. In all cases, the *att* sites were ≥250 bp from the DNA ends.

A 1943 bp radiolabeled biotinated DNA, made by PCR incorporating [$\alpha$-$^{32}$P] deoxycytidine triphosphate and a primer with biotin linked to the 5′ end, was used to measure the density of DNA attachment on the flow chamber surface as described (Finzi and Gelles, 1995). Attachment density was proportional to the amount of DNA incubated in the flow chamber at concentrations < $4.0 \times 10^{-9}$ M. Pre-treating the streptavidin-coated surface with biotin before DNA incubation reduced the number of counts retained on the surface by 99%, demonstrating specific attachment. In single-molecule experiments, the incubation solution contained $1.0 \times 10^{-12}$ M DNA. Assuming random attachment, this surface density ensured that ≤7% of DNA molecules have a neighboring DNA near enough to attach to the same bead.

**Table S1. Oligonucleotides used in the ligation assay and the PCR construction of 1943 bp substrates.**

| PCR PRODU | TEMPLATE | PRIMER | PRIMER SEQUENCE |
|---|---|---|---|
| non *att* | pBR322 | BR322For2 | 5'[C6Bio]gaggaggaggtaccggcgccg gacgcatccgtc |
| | | BR322rev6 | 5'[C6dig]gtgcactctcagtacaatctgctc |
| *attL* | pSN55 | SN55for12 | 5'[C6dig]agccacgaacgcggtaccaaa gggtaatcggggaaggattcc |
| | | SN55rev6 | 5'[C6Bio]ggcgggaccagagaaaaat c |
| *attR* | pSN66 | SN66for4 | 5'[C6dig]gacgacgacgacgacgacga cgtagttcgccagttaatagtttgcgc |
| | | SN66rev12 | 5'[C6Bio]aaaactgcagccaatgcaccg gaacgatggcgcagcaccgccg |
| ½ *attL* | pSN55 | SN55for12 | 5'[C6dig]agccacgaacgcggtaccaaa gggtaatcggggaaggattcc |
| | | SN55rev3 | 5'gtcgtcgtcgtcgtcgtcggcgtagag gatccgcctacc |
| ½ *attR* | pSN66 | SN66for4 | 5'gacgacgacgacgacgacgtagtt cgccagttaatagtttgcgc |
| | | SN66rev12 | 5'[C6Bio]aaaactgcagccaatgcaccg gaacgatggcgcagcaccgccg |
| *attL+attR* | ½ *attL* plus | SN55for12 | 5'[C6dig]agccacgaacgcggtaccaaa gggtaatcggggaaggattcc |
| | ½ *attR* | SN66rev12 | 5'[C6Bio]aaaactgcagccaatgcaccg gaacgatggcgcagcaccgccg |
| ½ *invattR* | pSN66 | inv66rev1 | 5'gacgacgacgacgacgacgaccaagg agatggcgcccaacagtc |
| | | inv66for1 | 5'-[C6Bio]taaacaaataggggttccgcg cac |
| *attLinvattR* | ½ *attL* plus | SN55for12 | 5'[C6dig]agccacgaacgcggtaccaaa gggtaatcggggaaggattcc |
| | ½ *invattR* | inv66for1 | 5'-[C6Bio]taaacaaataggggttccgcg cac |

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