# TOPOLOGICAL ANALYSIS OF A SITE-SPECIFIC RECOMBINATION REACTION

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Since Xer-mediated dimer resolution is efficient on torus knots with five or more crossings, and *psi* sites in inverted repeat, we provide a tangle analysis when the substrate has five crossings, and then we predict the outcome of Xer recombination for any substrate with 2n+1 crossings (n = 3, 4, 5). Specifically, the predictions are that an odd (2n+1)-noded knot with *psi* sites in direct repeat will undergo Xer recombination to yield an even-noded knot with one extra node i.e. containing 2(n+1) nodes.

## 1. SITE-SPECIFIC RECOMBINATION

Recombination refers to a process of DNA breakage and reunion, which results in the exchange of genetic material between DNA segments. In nature, a specialized type of recombination, called site-specific recombination, provides the means to rearrange large genomes. Such systems require specialized recombinase proteins to recognize specific sites, break and re-join the DNA[1]. These enzymes perform topological manipulations on cellular DNA in order to mediate a number of biological processes. For instance, λ Int catalyzes the integration of phage genomes[2], XerC and XerD function to resolve dimers in Escherichia Coli[3], while Hin serves to regulate the expression of flagellin genes in Salmonella[4]. In general, site-specific recombination involves the exchange of two pairs of DNA strands via a Holliday junction intermediate[5].

## 2. XER RECOMBINATION

In bacteria with circular chromosomes, an odd number of crossovers can unite two genomes to form chromosomal dimers[6]. Dimers cannot proceed with proper segregation, thus the cell needs some sort of machinery to convert these dimers to monomers. The Xer site-specific recombination system performs this cell cycle maintenance function by adding a crossover at *dif* in the replication terminus region of the E. coli chromosome. The Xer system also acts at sites found in naturally-occurring plasmids, such as *cer* in ColE1 and *psi* in pSC101, to resolve dimers, and guarantee stable plasmid inheritance[7]. Recent studies suggest the universality of the Xer system in the bacterial kingdom.

## 3. TOPOLOGICAL ANALYSIS

Plasmid dimer resolution and the related enzymatic mechanism constitute the focus of this study. Recombination at the plasmid sites *cer* and *psi* requires accessory proteins to assemble a synaptic complex with precise geometry. Recombination at the plasmid sites displays resolution selectivity by converting dimers to monomers, but not *vice versa*. Xer's topological features make it a good candidate for tangle analysis. Based on the observation that during a site-specific recombination reaction, an enzyme binds a DNA molecule to enable a crossover between two DNA strands, and that is precisely the definition of a 2-string tangle (see Fig. 1), we may apply the tangle method to model this two-strand interaction. In the 1990's, the tangle method was proposed by two topologists (Claus Ernst and DeWitt Sumners) to analyze site-specific recombination reactions.

In the tangle method, we assume the recombination event happens in $S^3$. So, we can consider the DNA substrate with its two recombination sites as an embedding of one or more circles in $S^3$. We can also consider the recombination sites as living inside a ball, with the ball representing the enzymes, and then the ball with the two strands of bound DNA represents the local synaptic complex. Inside the ball is where the two strands are cut and recombined. The exterior of the ball remains unchanged by this recombination. The ball divides the space in two regions, with each region defined by its biological role.

In the tangle method, we need to solve a system of tangle equations, where knots are written as the numerator closure of sums of unknown tangles. In the special case when the knots are 4-plats, we make use of knot theory and low-dimensional topology to solve a given system of tangle equations. The method models the pre-recombinant and post-recombinant stages with a system of tangle equations. Tangle analysis has been used extensively to study the topological mechanisms of several enzymes (e.g. Int, Cre, Tn3, Xer). Here we apply the tangle method to study how XerCD converts a torus knot with five crossings to a knot with six crossings. Our results suggest two possible mechanisms of action for the enzymes, one of which is consistent with experimental data.

To begin our investigation, we separate the DNA involved in the recombination event in three ways: we call $O_f$ the unbound or free DNA, which is not bound to the enzyme; we call $O_b$ the bound DNA, which does not change during recombination; and, we call P the bound DNA, which changes during recombination. If we let $O = O_f + O_b$ denote the outside tangle, which is unchanged during the recombination event, P the parental tangle, which is replaced with R the recombinant tangle during the recombination event, we may write down a system of tangle equations. The knot type of the substrate is controlled in the lab, so it is known. The products can be identified using gel electrophoresis. Thus, we need to solve this system for the unknown tangles $O_f$, $O_b$, R.

Figures/Illustrations

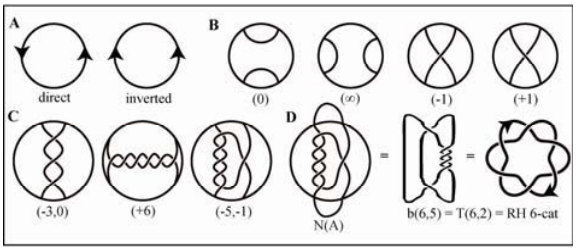


**Fig. 1.** Tangles. (A) Two free circles with recombination sites (arrows) in direct (head-to-tail) and inverted (head-to-head) repeats. (B) The four exceptional tangles. (C) Three rational tangles. (D) The numerator closure of a rational tangle produces a 4-plat knot. A right-handed 6 crossing torus knot is shown here.

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