



Predicting Knot and Catenane Type of Products of Site-Specific Recombination on Twist Knot Substrates

Karin Valencia^{1*} and Dorothy Buck²

¹Department of Mathematics, Imperial College London, South Kensington Campus, Office: 640, London SW7 2AZ, UK

²Department of Mathematics, Imperial College London, South Kensington Campus, Office: 623, London SW7 2AZ, UK

Received 22 March 2011;
received in revised form
30 May 2011;
accepted 31 May 2011
Available online
7 June 2011

Edited by M. Gottesman

Keywords:

site-specific recombination;
DNA knots;
serine recombinase;
tyrosine recombinase;
DNA topology

Site-specific recombination on supercoiled circular DNA molecules can yield a variety of knots and catenanes. Twist knots are some of the most common conformations of these products, and they can act as substrates for further rounds of site-specific recombination. They are also one of the simplest families of knots and catenanes. Yet, our systematic understanding of their implication in DNA and important cellular processes such as site-specific recombination is very limited. Here, we present a topological model of site-specific recombination characterizing all possible products of this reaction on twist knot substrates, extending the previous work of Buck and Flapan. We illustrate how to use our model to examine previously uncharacterized experimental data. We also show how our model can help determine the sequence of products in multiple rounds of processive recombination and distinguish between products of processive and distributive recombinations.

This model studies generic site-specific recombination on arbitrary twist knot substrates, a subject for which there is limited global understanding. We also provide a systematic method of applying our model to a variety of different recombination systems.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

Site-specific recombination

Site-specific recombination is a cellular process that involves reciprocal exchange between defined DNA sites. Prototypes of site-specific recombination include the integration of bacteriophage λ into the *Escherichia coli* chromosome and the DNA inversions responsible for flagellar phase variation in *Salmonella*.¹ Apart from their fundamental functions in the cell, site-specific recombinases give scientists an elegant, precise and efficient way to insert, delete and invert DNA segments. This means that they are rapidly becoming of pharmaceutical

and agricultural interest and are being used in the development of biotechnological tools.^{2–5}

Minimally, site-specific recombination requires one or two duplex DNA molecules (linear, relaxed and plectonemically supercoiled, covalently closed circular DNAs are all good substrates for many site-specific recombination reactions; however, plectonemically supercoiled, covalently closed circular DNA molecules are the most prevalent in topological enzymology studies, and thus, we focus on these substrates here) containing two short (30–50 bp) sequence-specific DNA segments, the *crossover sites* and specialized proteins and *site-specific recombinases*, responsible for recognizing the sites and breaking and rejoining the DNA with conservation of the phosphodiester bond energy (Fig. 1). The sites are usually nonpalindromic; thus, each can be assigned an orientation, and if the sites are on a single DNA molecule, they can be either in *direct* orientation (head to tail) or in *inverted* orientation (head to head). Depending on the initial arrangement of the

*Corresponding author. E-mail address:

karin.valencia06@imperial.ac.uk.

Abbreviation used: MCN, minimal crossing number.

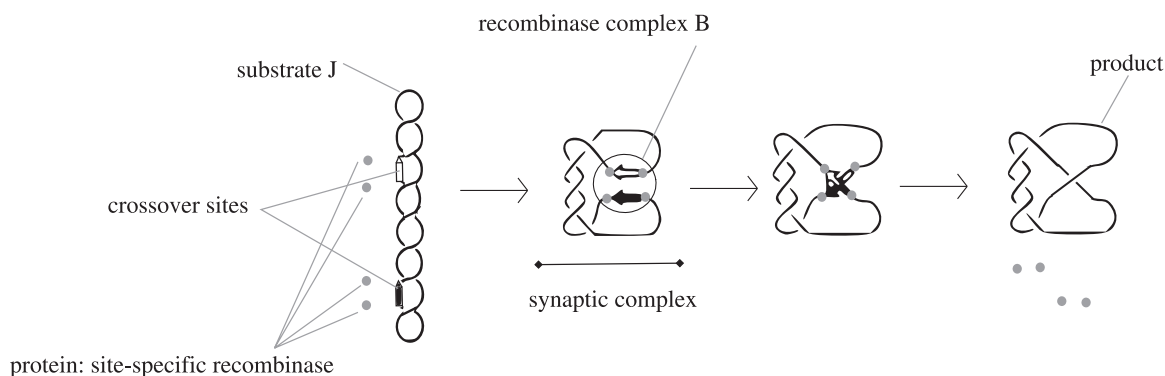


Fig. 1. Illustration of a small site-specific recombination reaction. The black line represents the central axis of the DNA molecule. Left: four dimer site-specific recombinases. The substrate molecule: an unknotted, duplex, covalently closed circular and plectonemically supercoiled DNA molecule with the crossover sites (open and filled arrows). Left middle: juxtaposition of the crossover sites, creating the synaptic complex. Right middle: recombination of the crossover sites. Right: knotted product molecule and detached proteins.

parental recombination sites and recombinase used, site-specific recombination has one of three possible outcomes: integration, excision or inversion. Larger site-specific recombination systems may also require additional proteins (e.g., accessory proteins) and sites (e.g., accessory sequences).

The reaction starts when a recombinase dimer binds at each of the two recombination sites (referred to as sites hereafter). The sites are then brought together to form the *synaptic complex* with the crossover sites juxtaposed, possibly trapping a fixed number of (interdomainal) supercoils. The sites are cleaved, exchanged and resealed. Finally, the proteins dissociate, releasing the product molecule and completing the reaction (Fig. 1).

We refer to the region of space containing the two juxtaposed sites and the recombinase molecules as the *recombina-se complex*. The synaptic complex is called a *productive synapse* if the recombinase complex meets the substrate in precisely the two crossover sites. In this model (as opposed to the tangle model), we assume that any enhancer sequences and/or accessory proteins are sequestered from the recombinase complex and that the

recombinase complex meets the substrates at precisely the two crossover sites. That is, we assume that the synaptic complex is a productive synapse (Fig. 2). During the intermediate step, once the crossover sites have been cleaved, multiple rounds of strand exchange can occur before resealing the DNA; this is called *processive recombination*. The entire process of recombination (including releasing and rebinding) can also occur multiple times, either at the same site or at different sites; this process is called *distributive recombination*. In this work, we use the term *substrate* to refer specifically to the DNA prior to the first cleavage. Processive recombination is treated as one extended process, given an initial substrate with several intermediate exiting points for the reaction.

Site-specific recombinases can be broadly divided into two subfamilies: serine recombinases and tyrosine recombinases, based on sequence homology, catalytic residues and their mechanisms of cutting and rejoining the DNA. Only serine recombinases can mediate processive recombination. See Ref. 1 (and references therein) for a detailed exposition of site-specific recombination.

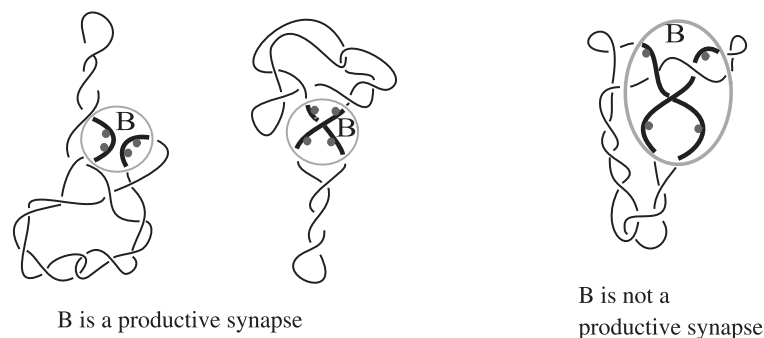


Fig. 2. Productive synapse. We assume that the synaptic complex is a productive synapse. *B* (light-gray circle) denotes the smallest region containing the four bound recombinase molecules (small gray discs) and the two crossover sites (highlighted in black). Left and middle: the synaptic complex is a productive synapse. Right: the synaptic complex is not a productive synapse. In this case, we cannot draw *B* such that only the two crossover sites are inside it without also including the third (horizontal, non-highlighted) strand.

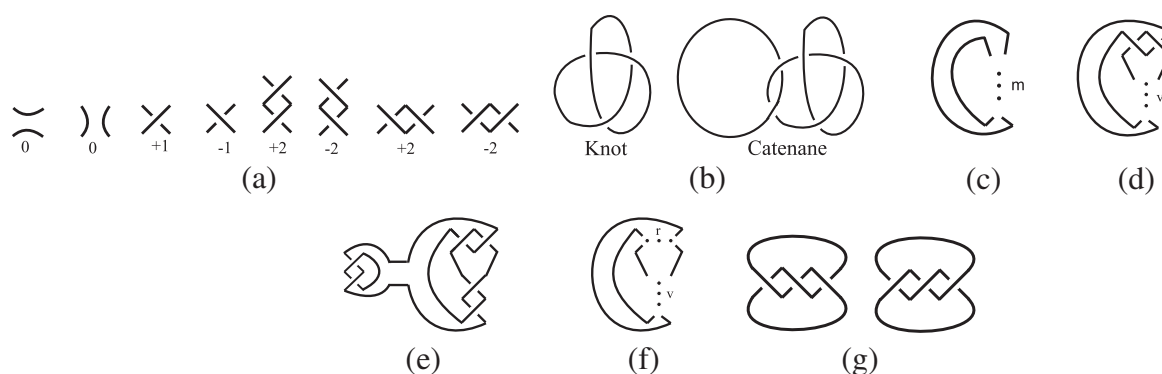


Fig. 3. Background terminology. (a) Crossing sign convention used in this paper: 1, -1; 2 and -2 vertical; 2 and -2 horizontal, also called hooked junctions. (b) An example of a knot and a catenane. (c) Generalized structures of torus knots (m is odd) and catenanes (m is even) denoted $T(2, m)$. (d) The substrate we consider, the twist knot $C(2, v)$. (e) A clasp knot $C(r, s)$. (f) An example of a composite catenane. This particular example, denoted $T(2, 2) \# C(-2, 2)$, consists of the catenane $T(2, 2) = 2_1^2$ and the twist knot $C(-2, 2) = 4_1$. (g) The (+) trefoil and (-) trefoil, respectively, from left to right.

DNA knots and catenanes

A variety of DNA knots and catenanes have been observed since their discovery in the 1960s.^{6–8,12} (Experimentally, two techniques have been widely used to resolve DNA knots and catenanes: electron microscopy and electrophoretic migration.^{9–11}) However, they arise more commonly as products of topological enzymology experiments on artificially constructed small (3–5 kb) DNA plasmids. Knots and catenanes are generated and then used as experimental tools to investigate the mechanisms of enzymes acting on DNA. They are used as substrates for these reactions, and careful analysis of the topology of the product DNA molecules allows inferences to be drawn about the detailed mechanism of the reaction.^{9,13–41}

DNA twist knots as substrates for site-specific recombination

A *twist knot* $C(2, v)$ is a knot that admits a projection, as illustrated in Fig. 3d. Mathematically,

twist knots are the simplest family of knots [after the torus knots and catenanes $T(2, m)$, which admit a projection, as in Fig. 3c] and appear more prevalently for small *minimal crossing number* (MCN) (see [Mathematical terminology](#) for a definition).

Twist knots are ubiquitous DNA knot molecules *in vivo* and *in vitro*. Most DNA inside prokaryotic cells is plectonemically supercoiled, and in the laboratory, most experiments performed with site-specific recombinases use small plectonemically supercoiled circular DNA molecules. This supercoiling promotes strand collision and DNA entanglement. A simple crossing change in such a molecule can result in knotting of the DNA into twist knots (Fig. 4).

Together with torus knots and catenanes, twist knots are the most common products of site-specific recombination both *in vivo*⁴¹ and *in vitro*,^{13–16,41,42} mediated by serine recombinases and tyrosine recombinases on unknotted, unlinked and torus knot and torus catenane substrates (see Table 1 in Ref. 43 and references therein). For example, recombination mediated by λ Int on the torus

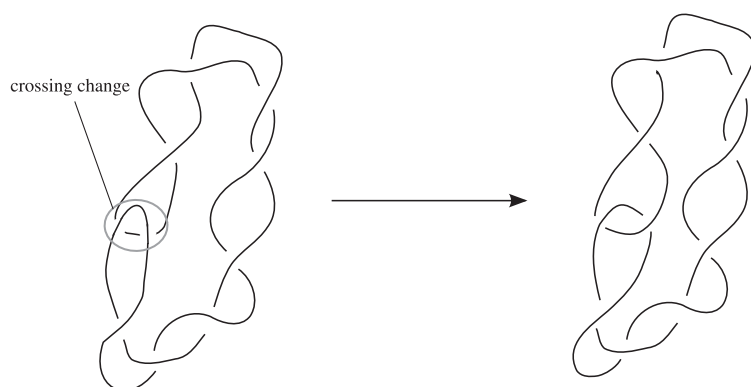


Fig. 4. Twist knots are ubiquitous DNA knots. DNA *in vivo* and *in vitro* is plectonemically supercoiled; thus, an unknot can be transformed into a twist knot by a single crossing change.

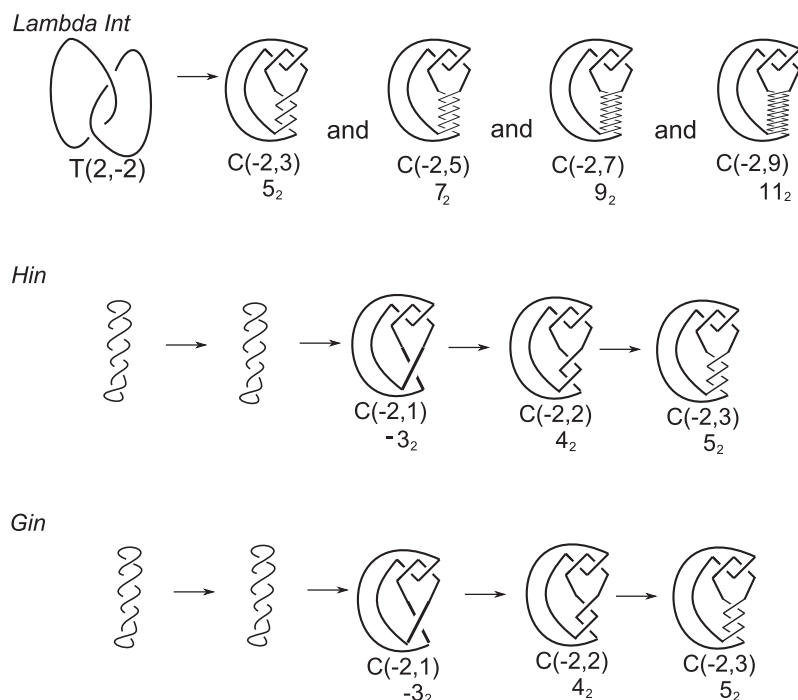


Fig. 5. Site-specific recombination mediated by both serine recombinases and tyrosine recombinases often yields twist knot products.

catenane $T(2, -2)$ with *attP* and *attB* bacteriophage λ attachment sites, one on each of the components of the catenane substrate, yields the twist knot products $C(-2, 3)$, $C(-2, 5)$, $C(-2, 7)$ and $C(-2, 9)$ (Table 7 in Ref. 16; see also the top image of Fig. 5). Site-specific recombination mediated by *Hin* recombinase on an unknot substrate with inverted sites has the following sequence of processive recombination: $C(-2, -1)$ (substrate) $\rightarrow C(-2, -1) \rightarrow C(-2, 1) \rightarrow C(-2, 2) \rightarrow C(-2, 3)$ ⁴¹ (see also the middle image of Fig. 5). The twist knots $C(-2, 1)$, $C(-2, 2)$ and $C(-2, 3)$ are products of the second, third and fourth rounds of processive recombination on an unknotted substrate, respectively.

Experimental conditions do not always preclude distributive rounds of recombination, and both can occur[†]. In multiple rounds of processive and distributive recombinations on unknot, unlink and torus knot and catenane substrates, twist knots can become substrates of new recombination reactions. For example, in experiments of Kanaar *et al.*, site-specific recombination on an unknot substrate with inverted sites, mediated by *Gin*, yields the following products of processive recombination: $C(-2, -1)$ (substrate) $\rightarrow C(-2, -1) \rightarrow C(-2, 1) \rightarrow C(-2, 2) \rightarrow C(-2, 3)$ ^{20,41} (see also the middle image of Fig. 5). The twist knots $C(-2, 1)$, $C(-2, 2)$ and $C(-2, 3)$ are products of the second, third and fourth rounds of

processive recombination, respectively. Also, the composite knot on six crossings, the granny knot $C(-2, 1) \neq C(-2, 1)$, was analyzed to be a product of distributive recombination on two trefoils, each a product from the first round of recombination.

Thus, a better understanding of DNA twist knots and their role in site-specific recombination reactions may contribute to the understanding of the mechanisms of this cellular process.

Our model

Given the variety of DNA knots and catenanes that arise from site-specific recombination, it is clear that stratification of these products is necessary. Topological techniques such as those presented here can aid experimentalists in characterizing DNA knot and catenane products. Our model predicts the exact topology and chirality of possible products, thus restricting the knot or catenane type of the products observed.

Despite the ubiquity and biological importance of these knots, previous systematic study of twist knots involved in DNA-protein interactions has been limited, and there has yet to be a systematic model incorporating these as substrates for a generic site-specific recombinase. Earlier predictions of knots arising from site-specific recombination did not consider twist knot substrates.^{19,43,44} Here, we rectify this by presenting a model, extending the work of Buck and Flapan,⁴⁴ classifying all possible knots and links that can arise from site-specific recombination on twist knot substrates.

[†] Distributive recombination can be minimized, for example, by stereostructural impediments or diluted protein concentration (see Refs. 14 and 46).

Our model is built on three biological assumptions, stated in [Assumptions of Our Model](#). From these, we construct a model that predicts all possible knots and catenanes that can arise as products of a single round of recombination, multiple rounds of (processive) recombination or distributive recombination on a plectonemically supercoiled twist knot substrate $C(2, v)$. We predict that products arising from site-specific recombination on a twist knot substrate $C(2, v)$ must be members of one family of products (illustrated in [Fig. 6a](#)). Our model is independent of site orientation. We make no assumption on the size (number of base pairs) of the molecule(s). In [Ref. 45](#), we provide detailed topological proofs for the model presented here.

Note that although the three assumptions are not for a generic recombinase but, rather, for general site-specific recombination, our model can restrict the topology of products in a specific system. If, for example, the site orientation is taken into account, the model further restricts the possible products of such a reaction. We illustrate this in [Discussion and Applications](#) with many examples.

Structure of this paper

This article is organized as follows. In [Mathematics Terminology and Notation](#), we explain mathematical terminology and notation. In [Assumptions of Our Model](#), we state the three assumptions of our model. In [Results](#), we explain how, given a twist knot $C(2, v)$, all possible knotted or catenated products fall into one characterized family with two special subfamilies. We also consider the (common) case of products that have MCN one more than the substrate and show that the product knot or catenane type is even more tightly prescribed. Finally, in [Discussion and Applications](#), we discuss how the model can help predict all possible products of (non-distributive) recombination mediated by a

serine recombinase and a tyrosine recombinase, determine the order of products of processive recombination, distinguish products of distributive recombination and narrow the possible knot or catenane type for previously uncharacterized experimental data.

Mathematics Terminology and Notation

In this section, we define a few mathematical terms and introduce notation. [Figures 2 and 3](#) present diagrams for each one of the terms defined. (We note that all line segments in these images represent the central axis of the double helix of a duplex DNA molecule.)

Throughout this article, we adopt the convention for crossings illustrated in [Fig. 3a](#).

Mathematical terminology

A *catenane* L is a collection of separate rings that may or may not be knotted, called *components*. ([Fig. 3b](#)) A *knot* K is considered to be a catenane of one component ([Fig. 3b](#)) Roughly, two knots or catenanes K, J are *equivalent* if there is a continuous deformation from K to J (without cutting them). A *torus knot or catenane*, $T(2, m)$, is a knot or catenane formed by closing a row of m plectonemic twists (such a closing is achieved by identifying the top and bottom endpoints of the arcs representing twists without introducing or removing further crossings; see [Fig. 3c](#)). A *twist knot* $C(2, v)$ is a knot that admits a projection with two nonadjacent rows of crossings: a row of $v \neq 0, 1$ vertical crossings and a *hook*, of the form shown in [Fig. 3d](#). Note that $C(-2, v-1) = C(2, v)$. The equivalence of these two forms can be seen via a continuous deformation by flipping the hook. Note that twist knots can be generalized to *clasp knots* ([Fig. 3f](#)). A clasp knot $C(r, v)$

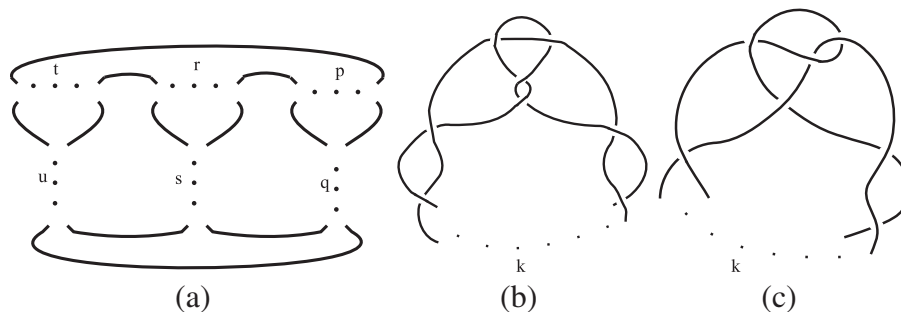


Fig. 6. The family of knots and catenanes: $F(p, q, r, s, t, u)$, containing the subfamilies $G_1(k)$ and $G_2(k)$ (from left to right respectively). Given the three assumptions in [Assumptions of Our Model](#), we predict that all product knots and catenanes of (non-distributive) site-specific recombination on twist knots with a tyrosine recombinase or a serine recombinase fall within family $F(p, q, r, s, t, u)$ of knots and links. In (a), the letters p, q, r, s, t and u denote the number of crossings in that particular row of crossings. In (b) and (c), k describes the number of crossings between the two DNA duplexes. Note that, depending on the value of k , a member of $G_1(k)$ or $G_2(k)$ is either a knot or a catenane. Families $G_1(k)$ and $G_2(k)$ are important subfamilies of $F(p, q, r, s, t, u)$.

is a knot that has two nonadjacent rows of crossings, one with $r \neq 0, \pm 1$ crossings and the other with $v \neq 0$ crossings. A clasp knot $C(r, v)$ with $r = \pm 2$ is a twist knot.

In this work, we may use the standard Rolfsen notation and the $T(2, m)$, $C(2, r)$ notation interchangeably. We give examples of both notations for knots and catenanes with the smallest MCN (the standard Rolfsen notation is on the left): $0_1 = C(2, 1)$, $(+)_2^2 = T(2, 2) = C(2, 0)$, $(-)_3^1 = C(-2, 1)$, $(+)_3^1 = C(2, -1)$, $4_1 = C(2, -2)$, $(-)_4^2 = T(2, -4)$, $(+)_5^1 = T(2, 5)$, $5_2 = C(2, -3)$, $6_1 = C(2, -4)$. For more examples, see Ref. 47. Note that $T(2, m)$ is a catenane if m is even and a knot if m is odd (Fig. 3c).

Given two knots or catenanes K_1 and K_2 , their composite knot or catenane, written $K_1 \# K_2$, is obtained by removing an unknotted arc from each and gluing the resulting two endpoints of K_1 to the two endpoints of K_2 without introducing (or removing) any additional knotting (Fig. 3e). A prime knot is one that can only be decomposed into two sub-knots $K_1 \# K_2$ if one is trivial (i.e., equivalent to the unknot). The MCN of a knot or catenane K , denoted $\text{MCN}(K)$, is the fewest number of crossings with which it can be drawn. For example, $\text{MCN}(\text{unknot}) = 0$ and $\text{MCN}(C(2, -2)) = 4$. Similarly, $\text{MCN}(C(2, v)) = |v| + 2$ if $v < 0$ or $\text{MCN}(C(2, v)) = v + 1$ if $v > 0$. See Refs. 47–49 for a mathematical study of knots and catenanes.

Let J denote the substrate $C(2, v)$ (Fig. 1). Recall that, in this model, we assume that the synaptic complex is a productive synapse (defined previously; see also Fig. 2); thus, let B denote the smallest region containing the four bound recombinase molecules and the two crossover sites, that is, the recombinase complex. (B is a topological ball; i.e., it can be continuously deformed to a round ball; see Fig. 1) The recombinase–DNA complex $B \cup J$ is the recombinase complex B along with the rest of the substrate molecule J .

Notation for product families

In Results, we show that all knots and catenanes arising from site-specific recombination on a twist knot substrate must fall the family of knots and catenanes: $F(p, q, r, s, t, u)$, illustrated in Fig. 6a.

In the family $F(p, q, r, s, t, u)$ of knots and catenanes, the variables p, q, r, s, t, u describe the number of crossings between two DNA duplexes in that particular row of crossings. In this family, the variables p, q, r, s, t, u can be positive, negative or zero. t, r and p can take horizontal and vertical zero crossings and only horizontal nonzero crossings. u, s and q can only take both zero and nonzero vertical crossings. In the subfamilies $G_1(k)$ and $G_2(k)$ of knots and catenanes, the variable k describes the number of crossings between the two DNA duplexes. Depending on the value of k , we obtain either a knot or a catenane: if k is odd, the members of these

families are knots, and if k is even, then the members of these families are two-component catenanes. (See Algorithm 3, point (3) in Discussions and Applications.) Note that there are knots and catenanes that have projections in both $F(p, q, r, s, t, u)$ and one of $G_1(k)$ or $G_2(k)$. For example, the trefoil knot has a projection as a member of $F(p, q, r, s, t, u)$ with $p=0, t, u=1, r=2, s=-1$ and a projection as a member of $G_2(k)$ with $k=2$.

Families $G_1(k)$ and $G_2(k)$ fall within family $F(p, q, r, s, t, u)$. We present these explicitly as they are a natural way to visualise some products of recombination mediated by a tyrosine recombinase.

Note that not all knots and catenanes in family $F(p, q, r, s, t, u)$ are predicted to arise as products of recombination. In particular, $F(p, q, r, s, t, u)$ contains catenanes with up three components. However, it is impossible to yield a three component catenane from recombination on a knot substrate. Figure 11 show the exact topology of the products predicted. Notice that they are all knots or catenanes with up to two components.

Assumptions of Our Model

Given a twist knot substrate and a given recombinase, we now state assumptions about the recombinase–DNA complex. Evidence that these assumptions are biologically reasonable is given in Section 2 of Ref. 43.

Assumption 1. The recombinase complex is a productive synapse, and there is a projection of the crossover sites with zero or one crossing between the sites and no crossings within a single site.

Figure 7 illustrates projections of B before recombination.

Assumption 2. The productive synapse does not pierce through a supercoil or a branch point in a nontrivial way, and the supercoiled segments are closely juxtaposed. Also, no persistent knots or catenanes are trapped in the branches of the DNA on the outside of the productive synapse.

Figure 2 illustrates examples of recombinase complexes that are either productive or not productive synapses. Figure 8 illustrates different examples of DNA molecules that are allowed and

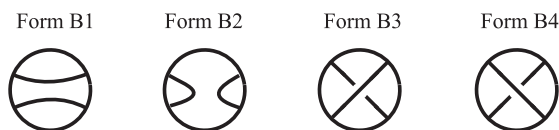


Fig. 7. Assumption 1: projections of the pre-recombinant B . Assumption 1 states that there is a projection of the pre-recombinant recombinase complex with at most one crossing.

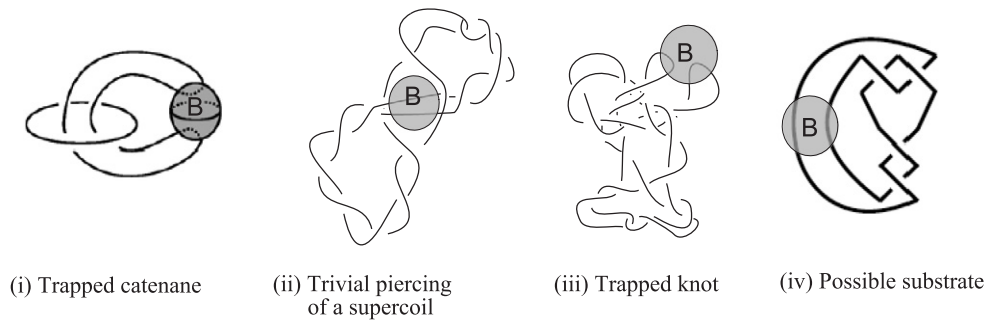


Fig. 8. Different scenarios for Assumption 2. (i) A catenane is trapped in the DNA branches outside of B . (ii) The productive synapse pierces a supercoil in a nontrivial way. (iii) A knot is trapped in the DNA branches outside of B . (iv) An unknotted substrate with the synaptic complex already formed. Scenarios (i) and (iii) are not allowed by the assumption; (ii) and (iv) are.

not allowed according to Assumption 2. Note that we allow hooked junctions (see Fig. 3a) because these have projections where there is only one crossing between the sites but no projections with no crossings between the sites.

Assumption 3 for serine recombinases. Serine recombinases perform recombination via the **subunit exchange mechanism**.¹ This mechanism involves making two simultaneous double-stranded breaks in the sites, rotating two recombinase monomers in opposite sites by 180° within the productive synapse and resealing the new DNA partners. In each subsequent round of processive recombination, the same set of subunits is exchanged, and the sense of rotation remains constant.

Figure 9 illustrates Assumption 3 for serine recombinases. It illustrates projections of B at each round of processive recombination mediated by a serine recombinase. Recall that, in processive recombination, the term substrate refers specifically to the DNA prior to the first cleavage.

Assumption 3 for tyrosine recombinases. After recombination mediated by a tyrosine recombinase, there is a projection of the crossover sites that has zero or one crossing.

Figure 10 illustrates Assumption 3 for tyrosine recombinases. It illustrates all possible projections of B after recombination mediated by a tyrosine recombinase. For the post-recombinant synapse (illustrated in Fig. 7), note that we allow hooked junctions because these have projections where there is only one crossing between the sites but no projections with no crossings between the sites. Note also that tyrosine recombinases can sometimes give the appearance of “processive recombination” in the circumstances involving multiple rounds of recombine and reset, without loss of accessory factor binding. In this work, we regard

this action mediated by tyrosine recombinases as distributive recombination.

Results

Given the three assumptions in the previous section, we predict that all product knots and catenanes of (non-distributive) site-specific recombination on twist knots with a tyrosine recombinase (Theorem 1) or with a serine recombinase (Theorem 2) fall within family $F(p, q, r, s, t, u)$ of knots and catenanes illustrated in Fig. 6a. We also predict the exact knot and catenane type of possible products of one round of recombination on a twist knot substrate that have MCN one more than the substrate molecule. The technical proofs of these results can be found in Ref. 45.

Products of non-distributive site-specific recombination belong to three families of knots and catenanes

Theorem 1 (Tyrosine recombinases). Suppose that Assumptions 1, 2 and 3 hold for a particular tyrosine recombinase–DNA complex. Then the only possible products of (non-distributive) recombination on a twist knot $C(2, v)$ are those illustrated in the left half of Fig. 11.

That is, if the substrate knot is a twist knot $C(2, v)$, then the only possible products (of a non-distributive reaction) are the unknot; the two-node catenane 2_1^2 ; $T(2, m)$ for $m=v, v\pm 1, v\pm 2$; $C(2, s)$ for $s=v\pm 1, v\pm 2$; $C(r, v)$ for $r=\{\pm 2, \pm 3, 4\}$; a connected sum $T(2, \pm 2)\#C(2, v)$; a member of the family $F(p, q, r, s, t, u)$ with $r=2, p=0$ (vertical), $|t|\leq 2$ or a member of the family of knot and links $G_1(k)$ or of the family of knots and links $G_2(k)$.

Theorem 2 (Serine recombinases). Suppose that Assumptions 1, 2 and 3 hold for a particular serine

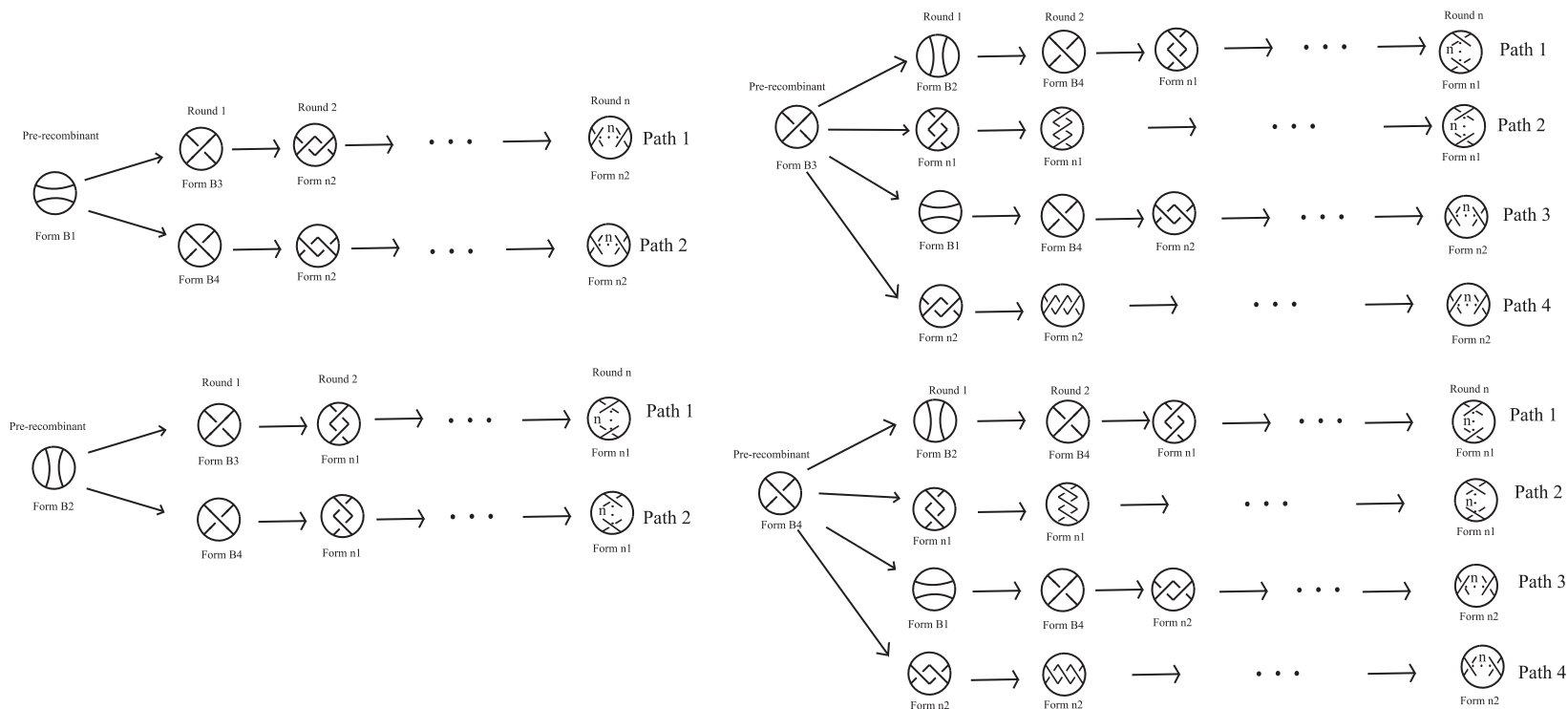


Fig. 9. Assumption 3 for serine recombinases. Starting with a projection of pre-recombinant B with zero or one crossings, we illustrate projections of the post-recombinant conformations of B at each round of processive recombination. Processive recombination can result in a row of n vertical crossings, which we denote $n1$, or in a row of n horizontal crossings, which we denote $n2$.

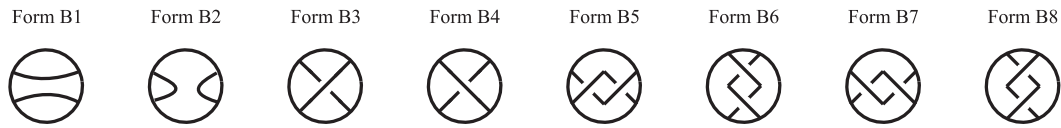


Fig. 10. Assumption 3 for tyrosine recombinases. All possible projections of B after recombination mediated by a tyrosine recombinase.

recombinase–DNA complex. Then the only possible products of n rounds of processive (non-distributive) recombination on a twist knot $C(2, v)$ are those illustrated in the right half of Fig. 11.

That is, if the substrate knot is $C(2, v)$, then the only possible products of n rounds of (non-distributive) processive recombination are the $C(r, v)$ for $r = \pm n, \pm n + 2$; $C(2, s)$ for $s = v, v \pm n$; $T(2, v \pm n)$; a connected sum $T(2, \pm n) \# C(2, v)$; and any member of the family $F(p, q, r, s, t, u)$ with $t = \pm n, r = 2, p = 0$.

Note: Theorems 1 and 2 distinguish between the chiralities of the product DNA molecules, since using our model, we can work out the *exact conformation* of all possible products of site-specific recombination starting with a particular twist knot substrate and site-specific recombinase. For example, starting with the twist knot substrate $C(2, -1)$ [a (–) trefoil, see Fig. 3g for an illustration of a (–) trefoil and a (+) trefoil], then, according to our model, site-specific recombination mediated by a tyrosine recombinase yields $T(2, -5)$, which is a (–) 5_1 (among other products) and can never yield $T(2, 5)$, which is a (+) 5_1 .

Characterization of products of distributive recombination

Theorem 3. Any products whose knot or catenane type is not listed in Theorems 1 and 2 must arise from distributive recombination.

Knots and links in $F(p, q, r, s, t, u)$ that cannot arise from recombination mediated by a serine recombinase or by a tyrosine recombinase. Recall that all products from recombination with a tyrosine recombinase or a serine recombinase belonging to $F(p, q, r, s, t, u)$ can be expressed with $t = \pm n, p = 0, r = 2$. Thus, any knots that cannot be expressed in this form cannot arise as products of recombination with either a serine recombinase or a tyrosine recombinase. 8_{18} and 10_{141} are examples of such knots.

Knots that can arise as products of recombination mediated by a serine recombinase but not by a tyrosine recombinase. In contrast with Theorem 1, any knot or link in the family $F(p, q, r, s, t, u)$ with $|t| > 2, p = 0, r = 2$ can occur as a consequence of Theorem 2. The knot $F(0, -1, 2, -1, 3, -1) = 8_{13}$ is an example of this; this knot is a possible product of recombination with a serine recombinase but not with a tyrosine recombinase.

Products whose MCN is one more than the substrate

Often, recombination increases the MCN of a knotted or catenated substrate by one (see Ref. 50). In this case, we can further restrict the knot and catenane type of the possible products of recombination.

Theorem 4. Suppose that Assumptions 1, 2 and 3 (for serine recombinases and for tyrosine recombinases) hold for a particular recombinase–DNA complex with substrate $J = C(2, v)$ and suppose that site-specific recombination increases the MCN by one. Let L be the product of a single recombination event. Then, for $v > 0$, L can be any of the knots and catenanes illustrated in the left half of Fig. 12, and for $v < 0$, L can be any of the knots and catenanes illustrated in the right half of Fig. 12. These are the only possibilities for L .

That is, if $v > 0$, L is one of the following: $C(2, v+1)$, $C(2, -v)$, $C(-2, v)$, $C(-2, -(1+v))$, $C(3, v)$, $T(2, \pm(2+v))$, $F(0, q, 2, s, 2, u)$ where $q = v, F(0, \pm 1, 2, s, 0, u)$ where $q = v$ and $s \neq 0$ or $F(0, 0, 2, s, 2, u)$ where $q = v$.

If $v < 0$, L is one of the following: $C(2, 2+|v|)$, $C(2, -(1+|v|))$, $C(-2, 1+|v|)$, $C(-2, -(2+|v|))$, $C(3, v)$, $C(-4, v)$, $T(2, \pm(3+|v|))$ or $F(0, \pm 1, 2, s, 0, u)$ for $q = v$.

Discussion and Applications

Our model predicts products of processive and distributive recombinations in a number of ways: We outline three algorithms that help us predict all possible products of (non-distributive) processive site-specific recombination on twist knot substrates mediated by a serine recombinase, determine the sequence of products of processive recombination given a substrate and a list of experimentally characterized products and determine all the products of (non-distributive) recombination of a twist knot substrate mediated by a tyrosine recombinase. We illustrate how to use these algorithms in Applications 1, 2 and 3. In Application 3, we employ Algorithm 3 to analyze previously uncharacterized products of distributive recombination mediated by a tyrosine recombinase. In Application 4, we discuss how our model can reduce the number of possibilities of products in situations where they have MCN one more than the

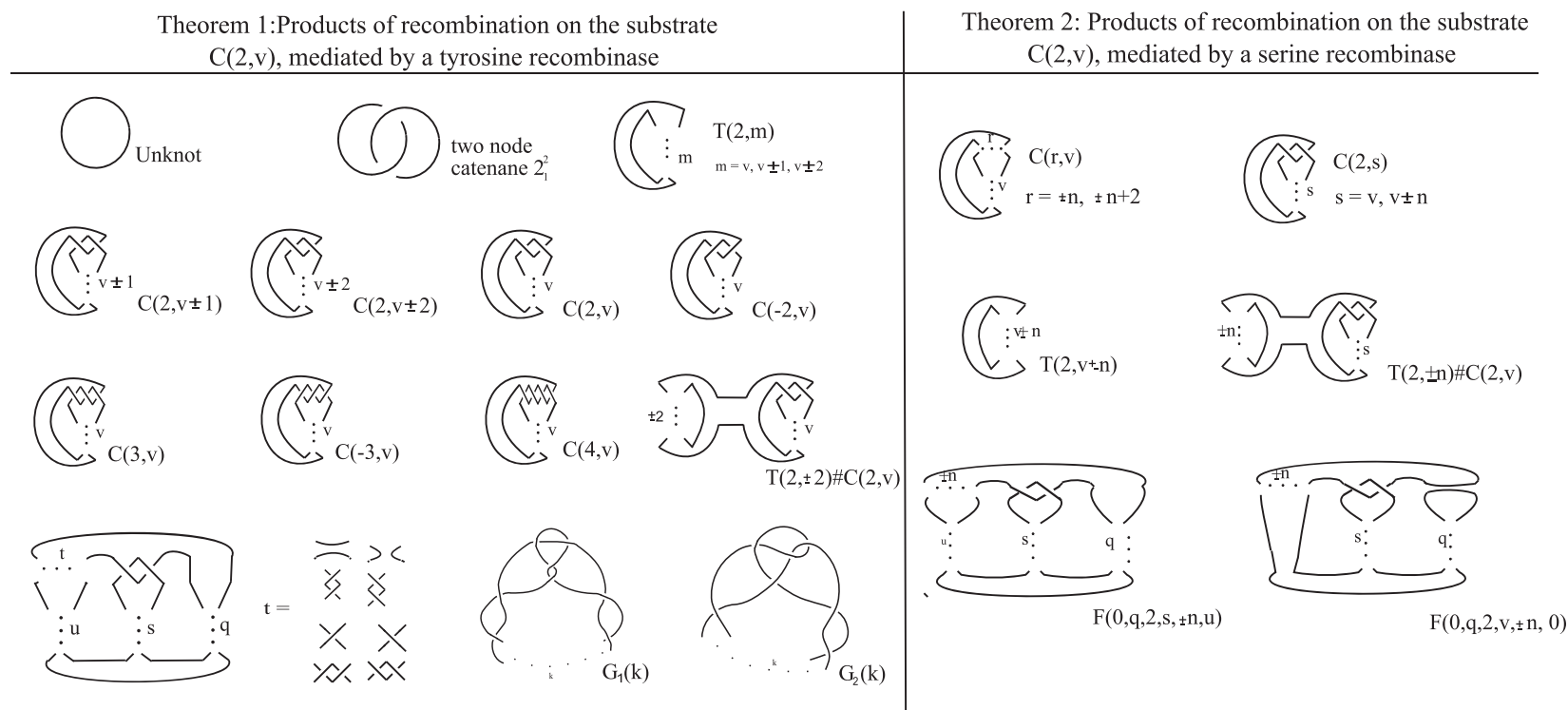


Fig. 11. Left: summary of [Theorem 1](#). These are all the possible products of a reaction mediated by a tyrosine recombinase on a twist knot substrate $C(2, v)$, predicted by the model. Right: summary of [Theorem 2](#). Similarly, these are all the possible products of a reaction mediated by a serine recombinase on a twist knot substrate $C(2, v)$, predicted by the model. For all, $q + s = v$. These products are listed in the same order as in the theorems (left to right, top to bottom). Note that all products predicted are knots or catenanes with up to two components. On the right half of the image, n is an integer.

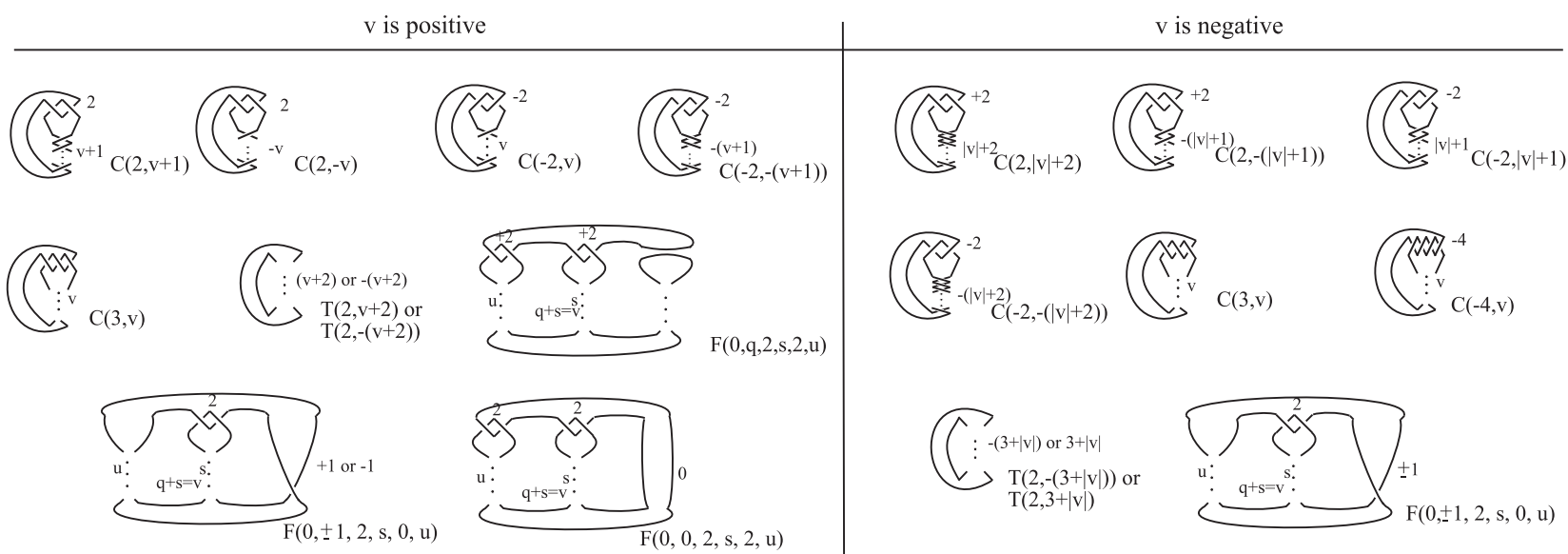


Fig. 12. Summary of [Theorem 4](#). Products of a recombination reaction with a twist knot substrate that have MCN one more than that of the substrate. If the substrate is a twist knot $C(2, v)$ with $\text{MCN}(C(2, v)) = m$ and the product has MCN equal to $m + 1$, then the knots and catenanes illustrated here are the only possible such products. Depending on whether v is positive or negative (see [Fig. 3a](#) for the convention on crossings), then we obtain different possible products. For all, $q + s = v$. These products are listed in the same order as in the theorems (left to right, top to bottom).

substrate. In Application 5, we explain how our model can be used to distinguish between products of processive and distributive recombinations.

Algorithm 1: Determining all products of recombination mediated by a serine recombinase

- (1) Choose a twist knot substrate. Determine if the substrate is of the form $C(-2, v)$ or $C(2, v)$. If necessary, employ a deformation of the substrate molecule by flipping the hook to make the substrate molecule the twist knot $C(2, v)$ [instead of $C(-2, v-1)$]. Determine v .
- (2) The right half of Fig. 11 illustrates all the possible conformations of products of site-specific recombination on the twist knot $C(2, v)$ mediated by a serine recombinase. For each, replace v with the value of v (i.e., v should be replaced for the value of v of the substrate. For instance, if the substrate is the trefoil knot $C(2, -1)$, then the number of crossings in the row of crossings v in these figures should be replaced by one negative crossing) and recall that $qs=v$; thus, consider each combination of q and s such that $qs=v$.
- (3) For a substrate that does not have mismatched sites: for obtainment of all possible products of the k th round of processive recombination, for each image of the right half of Fig. 11, replace n by k positive or negative crossing if the handedness of recombination of the enzyme is known or both positive and negative crossings if the handedness is not known.
- (4) For a substrate that has mismatched sites: for obtainment of all products of the k th round of processive recombination, replace n by $2k$ crossings.
- (5) Exclude any predicted products that are not possible due to the relative orientation and alignment of the specific sites or any other special properties of the particular system in question. [Depending on the relative orientation (direct or inverted repeat) of the sites, catenanes may or may not be possible products of the reaction. Also, the alignment (parallel or antiparallel) of the sites may produce mismatched crossover sites. In this case, an even number of site exchanges is necessary for the recombinase to be able to reseal the DNA sites, leaving the parental genomic sequence intact but introducing two or more crossings.]

Application 1. Predicting all products of recombination on a twist knot substrate mediated by a serine recombinase. Suppose that we choose the substrate $C(2, -2)$ with wild-type sites. Then, $v=-2$; thus,

replace $v=-2$ in each image of the right-hand side of Fig. 11. Suppose also that recombination proceeds through one round of strand exchange during each round of processive recombination and that we do not know the direction of rotation of one-half of the recombinase complex relative to the other during strand exchange. We want to know all possible products of the first three rounds of processive recombination.

The products of the first round of recombination [obtained by replacing n with one (positive and negative) crossing] are as follows: $C(1, -2), C(-1, -2), C(1\ 2, -2), C(-1\ 2, -2), C(2, -2), C(2, -2\ 1), C(2, -2-1), T(2, -2\ 1), T(2, -2-1), T(2, 1)\#C(2, -2), T(2, -1)\#C(2, -2), F(0, 0, 2, -2, 1, u), F(0, 0, 2, -2, -1, u), F(0, -1, 2, -1, 1, u), F(0, -1, 2, -1, -1, u), F(0, -2, 2, 0, 1, u), F(0, -2, 2, 0, -1, u), F(0, q, 2, -2, 1, u), F(0, q, 2, -2, -1, u)$.

The products of the second round of recombination [obtained by replacing n with two (positive and negative) crossings] are as follows: $C(2, -2), C(-2, -2), C(2\ 2, -2), C(-2\ 2, -2), C(2, -2\ 2), C(2, -2-2), T(2, -2\ 2), T(2, -2-2), T(2, 2)\#C(2, -2), T(2, -2)\#C(2, -2), F(0, 0, 2, -2, 2, u), F(0, 0, 2, -2, -2, u), F(0, -1, 2, -1, 2, u), F(0, -1, 2, -1, -2, u), F(0, -2, 2, 0, 2, u), F(0, -2, 2, 0, -2, u), F(0, q, 2, -2, 2, u), F(0, q, 2, -2, -2, u)$.

The products of the third round of recombination [obtained by replacing n with three (positive and negative) crossings] are as follows: $C(3, -2), C(-3, -2), C(3\ 2, -2), C(-3\ 2, -2), C(2, -2\ 3), C(2, -2-3), T(2, -2\ 3), T(2, -2-3), T(2, 3)\#C(2, -2), T(2, -3)\#C(2, -2), F(0, 0, 2, -2, 3, u), F(0, 0, 2, -2, -3, u), F(0, -1, 2, -1, 3, u), F(0, -1, 2, -1, -3, u), F(0, -2, 2, 0, 3, u), F(0, -2, 2, 0, -3, u), F(0, q, 2, -2, 3, u), F(0, q, 2, -2, -3, u)$.

These correspond to (knots) $0_1, (-)3_1, 4_1, 5_2, (-)6_1, 7_2$, (catenanes) $(+)2_1^2(-)4_1^2$, (composite knots and catenanes) $(\pm 3_1)\#4_1, (\pm 2_1^2)\#4_1$ and any products belonging to the infinite families $F(0, 0, 2, -2, \pm n, u), F(0, -1, 2, -1, \pm n, u), F(0, -2, 2, 0, \pm, u), F(0, q, 2, -2, \pm n, u)$ for any positive integer u . Two important observations: the products not in the infinite families are very tightly prescribed, and the handedness of all the chiral products (except 2_1^2) is negative.

Algorithm 2: Determining the sequence of products of processive recombination (given a specific substrate and experimentally characterized products)

When the products of processive recombination are known but not the ordering, the algorithm below can determine the sequence of products.

- (1) Determine if the substrate is of the form $C(-2, v)$ or $C(2, v)$. If necessary, employ a deformation of the substrate molecule by

- flipping the hook to make the substrate molecule the twist knot $C(2, v)$ [instead of $C(-2, v-1)$]. Determine v .
- (2) [Figure 13](#) lists all the possible conformations of the synaptic complex and the possible products that particular conformations yield. Use the characterized products from the experiment to determine which image in [Fig. 13](#) is the conformation of the synaptic complex. Perform the rest of the algorithm only for that conformation.
 - (3) For [Figs. 13b–h](#), replace v by its value (i.e., v should be replaced by the value of v of the substrate). In [Fig. 13a](#), recall that the number of crossings q and s should add up to the number of crossings v . Thus, consider each combination of q and s such that $q + s = v$.
 - (4) [Figure 13](#) also gives the possible pre-recombinant forms of B for each conformation of the synaptic complex, and [Fig. 9](#) gives the post-recombinant forms of B at each stage of processive recombination. Use the characterized products and the images in [Fig. 13](#) to determine the pre-recombinant form of B and for that form, use [Fig. 9](#) to work out which path yields those products.
 - (5) In the synaptic complex form chosen, replace B with each of its post-recombinant forms at

each stage of processive recombination to obtain a sequence of products.

- (6) Exclude any predicted products that are not possible due to specific properties of the particular system in question. [See step (5) of the algorithm in [Algorithm 1: Determining all products of recombination mediated by a serine recombinase](#).]

This algorithm gives the order of products of processive recombination, and any products arising from the experiment that are not in the list of predicted products are assumed to arise from distributive recombination.

Application 2. Determining the possible sequences of products of processive recombination.

Example. Suppose that, for the twist knot substrate $C(-2, 3)$, experimental conditions minimize distributive recombination, and analysis of the products reveals unknots, (unknown) torus knots and (unknown) clasp knots $C(r, s)$.

We can determine the order of products of recombination by using [Figs. 9 and 13](#) as follows:

- We perform a deformation of the substrate molecule by flipping the hook to make the substrate molecule the twist knot $C(2, 4)$ [instead of $C(-2, 3)$].

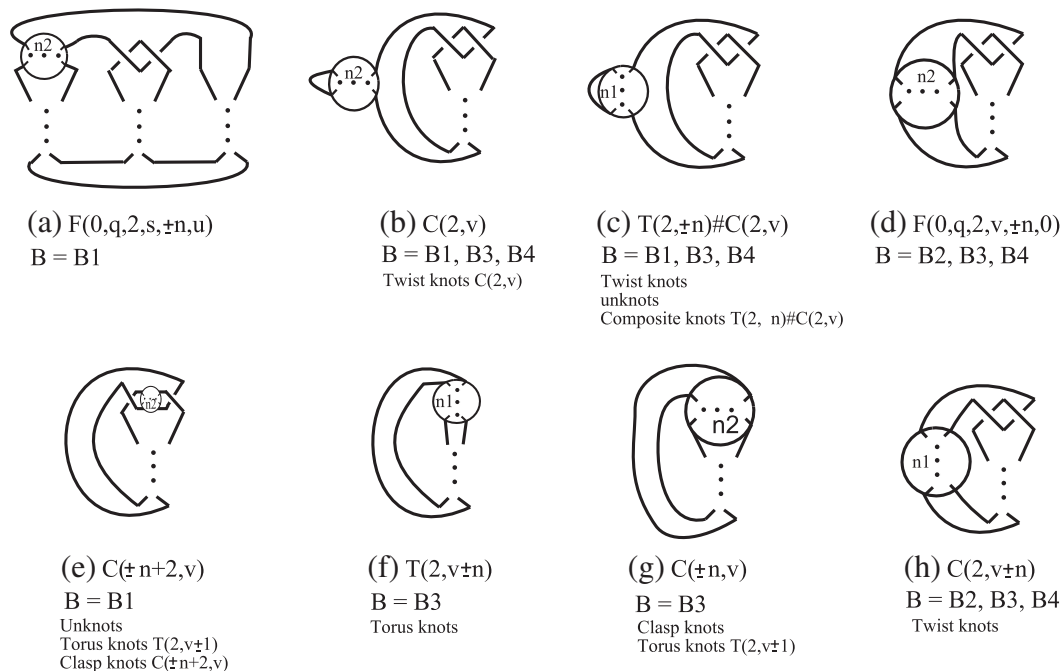


Fig. 13. Summary of [Theorem 2](#). After n rounds of processive recombination with a serine recombinase on a twist knot substrate, the product DNA molecule must have one of the forms illustrated here. The images inside the circles denote B after n rounds of processive recombination. There are two possible conformations, a vertical row of n crossings ($n1$) and a horizontal row of n crossings ($n2$). Under each conformation, we list some of the possible products that can arise from that particular conformation after n rounds of processive recombination.

- From Fig. 13, we see that the conformation of the synaptic complex must be that illustrated in Fig. 13e. This is the only conformation of the synaptic complex that yields unknots, torus knots (and catenanes) and clasp knots.
- Also from Fig. 13, the pre-recombinant conformation of B must be of the form $B1$.
- From Fig. 9, we see that path 1 is the only path that gives an unknot as a product (of the second round of recombination).

Thus, in Fig. 13e, replace the B by the post-recombinant form after each round of processive recombination. We deduce that the sequence of products is $C(2, 4)$ (substrate) $\rightarrow T(2, -14) \rightarrow C(0, 4)$ (unknot) $\rightarrow T(2, 14) \rightarrow C(-2, 4) \rightarrow C(-3, 4)$. Any products of further rounds of processive recombination are clasp knots $C(r, 4)$ with increasing MCN.

Example (substrate with mismatched sites). In cases where there is a mismatch in the crossover sites, two subunit exchanges are necessary in order for the recombinase to be able to reseal the DNA sites (i.e., processive recombination performs two 180° rotations of one-half of the productive synapse relative to the other before ligating the sites.)

Assume that, for the twist knot substrate $C(-2, 2)$, experimental conditions minimize distributive recombination and that processive recombination encounters mismatched sites after one round of exchange; thus, it proceeds through two exchanges of the crossover sites per round of recombination. Suppose that the products of multiple rounds of processive recombination are twist knots and connected sums of a torus knot and a twist knot. Then, we employ a similar method to that explained in the example above:

- We perform a deformation of the substrate molecule by flipping the hook to make the substrate molecule the twist knot $C(2, 3)$ [instead of $C(-2, 2)$].
- Figure 13c gives the conformation of the synaptic complex because this is the only conformation that gives connected sums of torus knots or catenanes and twist knots.
- B is either $B3$ or $B4$ because, from Fig. 13c, $B=B1, B3$ or $B4$, but all paths of $B1$ give only the twist knot $C(2, 3)$.
- The path must be path 1 for both $B3$ and $B4$ because path 2 does not give any twist knots and paths 3 and 4 give only the twist knot $C(-2, v)$.

Thus, using $B=B3$, path 1, we obtain the following sequence of products: $C(2, 3)$ (substrate) $\rightarrow C(2, 3) \rightarrow T(2, -3) \# C(2, 3) \rightarrow T(2, -5) \# C(2, 3)$. Moreover, any products of further rounds of recombination are

connected sums of the form $T(2, m) \# C(2, 3)$ for an odd negative integer m , with increasing MCN.

Using $B=B4$, path 1, we obtain the following sequence of products: $C(2, 3)$ (substrate) $\rightarrow C(2, 3) \rightarrow T(2, 3) \# C(2, 3) \rightarrow T(2, 5) \# C(2, 3)$. Moreover, any products of further rounds of recombination are connected sums of the form $T(2, m) \# C(2, 3)$ for an odd positive integer m , with increasing MCN.

Algorithm 3: Determining all products of (non-distributive) recombination mediated by a tyrosine recombinase

- (1). Use the characterized products from the experiment to determine if the substrate is a twist knot of the form $C(2, v)$ or $C(-2, v)$. If necessary, employ a deformation of the substrate molecule by flipping the hook to make the substrate molecule the twist knot $C(2, v)$ [instead of $C(-2, v-1)$]. Determine v .
- (2). The left half of Fig. 11 illustrates all the possible conformations of a product of site-specific recombination on the twist knot $C(2, v)$ mediated by a tyrosine recombinase. For products other than $G_1(k)$ or $G_2(k)$, replace v (and/or q and s ; recall $q=s=v$, and thus, consider all combinations) with the value of v (i.e., v should be replaced for the value of v of the substrate).
- (3). For products of the form $G_1(k)$ or $G_2(k)$:
 - If v is a negative odd number, the product is a knot belonging to family $G_1(k)$ with $k=|v|$.
 - If v is a positive odd number, the product is a knot belonging to family $G_2(k)$ with $k=|v|-1$.
 - If v is a negative even number, the product is a link belonging to family $G_1(k)$ with $k=|v|$.
 - If v is a positive even number, the product is a link belonging to family $G_2(k)$ with $k=|v|-1$.
- (4). Exclude any predicted products that are not possible due to specific properties of the particular system in question. [See step (5) of the algorithm in Algorithm 1: Determining all products of recombination mediated by a serine recombinase.]

For both serine and tyrosine site-specific recombinases, any product molecules that arise from the experiment that are not in the list of knots and catenanes predicted by the algorithms are assumed to have arisen from distributive recombination reactions.

Application 3. Narrowing possible knot and catenane type for previously uncharacterized experimental data: products of distributive recombination of a tyrosine

recombinase. We now consider products of multiple rounds of distributive recombination mediated by a tyrosine recombinase. Crisna *et al* performed experiments using the Flp recombinase of the yeast 2- μ m plasmid on unknotted substrates.¹⁶ They studied Flp inversion reactions by carrying out the experiments on plasmids containing two inverted Flp recognition target sites. Note that Flp recognition target sites in inverted repeats cannot yield catenanes as products of one round of recombination with an Flp recombinase. Flp can catalyze multiple rounds of distributive recombination; thus, it forms both even- and odd-noded knots. In their paper, they were only interested in the odd-noded knots, as they are products of the first round of distributive recombination. They found that trefoil knots $C(2, -1) = 3_1$ were among these products and did not identify product of further rounds of distributive reactions.

An interesting question is: what are the possible products of further rounds of distributive recombination? This can be answered using Theorem 1 and Fig. 11 (left-hand side). We assume that the product of the first round of distributive recombination (and thus our substrate) is the trefoil knot $C(2, -1)$. In Fig. 11 (left-hand side), we set $v = -1$ in each image [since the substrate is $C(2, -1)$]. From this, we can see that the possible products of a second round of distributive recombination by Flp recombinase on the unknot are $0_1, 3_1, 4_1, 5_1, 5_2, 0_1^2, 2_1^2, 4_1^2, 2_1^2 \# 3_1$ and any non-catenane products belonging to the family $F(p, q, 2, s, t, u)$ with $p=0, q+s=-1, |t| \leq 2$, as illustrated in Fig. 11 (left-hand side). (Recall that the catenanes predicted are not biologically possible.) [Note that, here, we did not take into account handedness, but it can be revealed by writing the products in $C(r, s)$ and $T(2, m)$ notation, as in the left-hand side of Fig. 11.] Recall that, for these images, the vertical row of u crossings can be any number of crossings. This means that, topologically, there are infinitely many possibilities for these product knots. However, biologically, due to physical and other constraints of the DNA molecule, conformations with a small value of u would probably be the most abundant.

Other applications of our model

Application 4. *Products of recombination reactions that increase the MCN by one.* Very commonly, site-specific recombination adds one crossing to the substrate, resulting in an increase by one of the MCN of the substrate. For example Bath *et al.* used the catenanes $T(2, 6)$ and $T(2, 8)$ as substrates for XerCD recombination, yielding product knots with MCN equals to 7 and 9.⁵¹ They did not characterize these products beyond their MCN (although they did make strong biological predictions as to their exact topology). Buck and Flapan significantly

reduced the possibilities for each of these products,⁴³ Darcy used the tangle model to reduce the number of mathematical solutions to the tangle equations involving the 4-noded catenane 4_1^2 (product of one round of recombination on an unknot substrate) and a 7-noded knot (products of one round of recombination on the torus catenane 6_1^2)²³ and Vazquez *et al.* designed a three-dimensional model for Xer recombination.²⁴

As DNA twist knots are common recombination substrates, considering a scenario similar to that of the Xer example above is relevant. To do this, we apply Theorem 4. Figure 12 summarizes this theorem. Suppose that the twist knots $C(2, 5)$ and $C(2, 7)$ (MCNs 6 and 8, respectively) are used as substrates for a site-specific recombination reaction with Xer recombinase, where experimental conditions minimize distributive recombination and products are knots and catenanes with MCNs 7 and 9. (Note that Xer has been shown to recombine on both directly and inversely repeated sites; thus, knots or catenanes are expected depending on site orientation.) In this case, the MCN is not sufficient to determine the knot type, since there are 7 knots and 8 two-component catenanes with MCN=7 and 49 knots and 61 two-component catenanes with MCN=9. However, we can use Theorem 4 (and Fig. 12) to significantly reduce the number of possibilities for these products. Through replacement of v for five crossings in each illustration of Fig. 12, it follows that the possible seven-crossing products are $7_1, 7_2, 7_3, 7_6, 7_2^2, 7_3^2$ or $3_1 \# 4_1$. Similarly, through replacement of v for seven crossings in each illustration of Fig. 12, it follows that the possible nine-crossing products are $9_1, 9_2, 9_3, 9_8, 9_{11}, 9_1^2, 9_{10}^2, 6_1 \# 3_1$ or $4_1 \# 5_2$. We have reduced 15 choices for 7-noded knots to just 7 choices and 110 possibilities for 9-noded knots and catenanes to just 9 possibilities. Thus, Theorem 4 can help to significantly reduce the knot and catenane type of products of site-specific recombination that add one crossing to the substrate.

Application 5. *Processive versus distributive recombination.* In some cases, processive recombination does not preclude distributive rounds of recombination, and both occur in a recombination reaction. Our model can be helpful in distinguishing between products of distributive recombination and products of processive recombination.

Example. Suppose that a trefoil knot $C(-2, 1)$ is used as a substrate for a reaction with a serine recombinase and that electron microscopy and gel electrophoresis reveal the figure of eight knot $C(-2, 2)$ as the primary product and $T(2, 2) \# C(-2, 1)$, $T(2, 2) \# C(-2, 2)$ and a three-component catenane as secondary products. It follows from Theorem 2 that recombination proceeds from the trefoil knot to $C(-2, 2)$, product of the first round of processive

recombination. The original $C(-2, 1)$ and the product $C(-2, 2)$ are then substrates yielding the composite catenanes $T(2, 2) \# C(-2, 1)$ and $T(2, 2) \# C(-2, 2)$, products of the first round of distributive recombination. The product knots and these composite catenanes are then used as substrates to yield the three-component catenanes, products of the second round of distributive recombination (however, this composite is not one of the substrates that we consider). Overall, this would be akin to the serine recombinase performing multiple rounds of processive and distributive recombinations.

Conclusions and Directions for Further Research

We have developed a model to predict and characterize the topology and chirality of DNA knots and catenanes that can arise as a result of a site-specific recombinase acting on a twist knot substrate. Our model is based on three biological assumptions about site-specific recombination. Our model predicts that all knotted or catenated products of such enzyme actions are in one of the three families of Fig. 6, as described in Theorems 1 and 2.

In Ref. 45, we have also shown that the total number of knots and catenanes in our product families with $MCN=n$ grows linearly with n^5 , whereas the total number of all knots and catenanes increases exponentially with the MCN .⁵³ Hence, the calculation n^5/e^n gives the proportion of all knots and catenanes that are putative recombination products, and as n increases, n^5/e^n decreases exponentially rapidly to zero. Knowing the MCN of a product and knowing that the product is in one of our families allow us to significantly narrow the possibilities for its knot or catenane type. The model described herein thus provides an important step in characterizing DNA knots and catenanes, which arise as products of site-specific recombination.

We have shown how our model can be helpful in determining the sequences of products of processive recombination on twist knot substrates and how it can help distinguish between products of processive and distributive recombinations. The algorithms presented allow the interested reader to apply our results to a specific site-specific recombination system.

We plan to expand this project in three main ways. First, we are further testing the model with experimental data from our experimental collaborators. Second, we are developing a computer program based on the model presented on this paper and in Ref. 45. This will allow the automatic computation of products of site-specific recombi-

nation on any twist knot substrate. Finally, although we have assumed that the productive synapse has only two crossover sites and that any accessory sites are sequestered from the synaptic complex, electron micrographs of recombinase complexes such as those of Gin and Hin^{13–15,41,52} reveal three DNA duplexes looping out of the enzyme complex. This suggests that our model could be developed by making biologically reasonable assumptions of a synaptic complex with three crossover sites (see Ref. 25) and predicting the putative products that could arise.

Acknowledgements

We wish to thank Erica Flapan, Mauro Mauricio, Julian Gibbons, Kai Ishihara and Ken Baker for insightful discussions and the referees for their valuable suggestions. D.B. was supported in part by Engineering and Physical Sciences Research Council Grants EP/H0313671, EP/G0395851 and EP/J1075308 and thanks the London Mathematical Society for their Scheme 2 Grant. K.V. was also supported by EP/G0395851.

References

- Grindley, N. D. F., Whiteson, K. L. & Rice, P. A. (2006). Mechanisms of site-specific recombination. *Annu. Rev. Biochem.* **75**, 567–605.
- Kilby, N. J., Snaith, M. R. & Murray, J. A. H. (1993). Site-specific recombinases: tools for genome engineering. *Trends Genet.* **120**, 413–421.
- Jia, F., Wilson, K. D., Sun, N., Gupta, D. M., Huang, M., Li, Z. *et al.* (2010). A nonviral minicircle vector for deriving human iPS cells. *Nat. Methods*, **7**, 197–199.
- Zhao, N., Fogg, J. M., Zechiedrich, L. & Zu, Y. (2010). Transfection of shRNA-encoding Minivector DNA of a few hundred base pairs to regulate gene expression in lymphoma cells. *Gene Ther.* **18**, 220–224.
- Feil, R. (2007). *Conditional Somatic Mutagenesis in the Mouse Using Site-Specific Recombinases*, 178, pp. 3–28, Springer-Verlag, Berlin, Germany.
- Dulbecco, R. & Vogt, M. (1963). Evidence for a ring structure of pyloma viral DNA. *Proc. Natl. Acad. Sci.* **50**, 236–243.
- Weil, R. & Vinograd, J. (1963). The cyclic helix and cyclic coil forms of pyloma viral DNA. *Proc. Natl. Acad. Sci.* **50**, 730–738.
- Yan, J., Magnasco, M. O. & Marko, J. F. (1999). A kinetic proofreading mechanism for disentanglement of DNA by topoisomerases. *Nature*, **401**, 932–935.
- Krasnow, M. A., Stasiak, A., Spengler, A. J., Dean, F., Koller, T. & Cozzarelli, N. R. (1983). Determination of the absolute handedness of knots and catenanes of DNA. *Nature*, **304**, 559–560.

10. Trigueros, S., Arsuaga, J., Vazquez, M. E., Sumners, D. W. & Roca, J. (2001). Novel display of knotted DNA molecules by two-dimensional gel electrophoresis. *Nucleic Acids Res.* **29**, E67.
11. Zechiedrich, E. L. & Crisona, N. J. (1999). Coating DNA with RecA protein to distinguish DNA path by electron microscopy. *Methods Mol. Biol.* **94**, 99–107.
12. Fiers, W. & Sinsheimer, R. L. (1962). The structure of the DNA of bacteriophage Phi - X174. I, II, III. *J. Mol. Biol.* **5**, 424–434.
13. Crisona, N. J., Kanaar, R., Gonzalez, T. N., Zechiedrich, E. L., Klippel, A. & Cozzarelli, N. R. (1994). Processive recombination by wild-type Gin and an enhancer-independent mutant. Insight into the mechanisms of recombination selectivity and strand exchange. *J. Mol. Biol.* **243**, 437–457.
14. Heichman, K. A., Moskowitz, I. P. & Johnson, R. C. (1991). Configuration of DNA strands and mechanism of strand exchange in the Hin invertasome as revealed by analysis of recombinant knots. *Genes Dev.* **5**, 1622–1634.
15. Kanaar, R., Klippel, A., Shekhtman, E., Dungan, J. M., Kahmann, R. & Cozzarelli, N. R. (1990). Processive recombination by the phage Mu Gin system: implications for the mechanisms of DNA strand exchange, DNA site alignment, and enhancer action. *Cell*, **62**, 353–366.
16. Crisona, N. J., Weinberg, R. L., Peter, B. J., Sumners, D. W. & Cozzarelli, N. R. (1999). The topological mechanism of phage lambda integrase. *J. Mol. Biol.* **289**, 747–775.
17. Spengler, S. J., Stasiak, A. & Cozzarelli, N. R. (1985). The stereostructure of knots and catenanes produced by phage λ integrative recombination: Implications for mechanism and DNA structure. *Cell*, **42**, 325–334.
18. Colloms, S. D., Bath, J. & Sherratt, D. J. (1997). Topological selectivity in Xer site-specific recombination. *Cell*, **88**, 855–864.
19. Sumners, D. W., Ernst, C., Spengler, A. & Cozzarelli, N. (1995). Analysis of the mechanism of DNA recombination using tangles. *Q. Rev. Biophys.* **28**, 253–313.
20. Kanaar, R., Klippel, A., Shekhtman, E., Dungan, J. M., Kahmann, R. & Cozzarelli, N. R. (1990). Processive recombination by the phage Mu Gin system: implications for the mechanisms of the DNA strand exchange, DNA site alignment and enhancer action. *Cell*, **62**, 353–366.
21. Ernst, C. & Sumners, D. W. (1990). A calculus for rational tangles: applications to DNA recombination. *Math. Proc. Cambridge Philos. Soc.* **108**, 489–515.
22. Vazquez, M. & Sumners, D. W. (2004). Tangle analysis of Gin site-specific recombination. *Math. Proc. Cambridge Phil. Soc.* **136**, 565–582.
23. Darcy, I. (2011). Biological distances on DNA knots and links: applications to Xer recombination. *J. Knot Theory Ramif.* **10**, 269–294.
24. Vazquez, M., Colloms, S. D. & Sumners, D. W. (2005). Tangle analysis of Xer recombination reveals only three solutions, all consistent with a single three-dimensional topological pathway. *J. Mol. Biol.* **346**, 493–504.
25. Ibarraa, H. C. & Lizrraga Navarrob, D. A. (2010). An algorithm based on 3-braids to solve tangle equations arising in the action of Gin DNA invertase. *Appl. Math. Comput.* **216**, 95–106.
26. Darcy, I. K., Luecke, J. & Vazquez, M. (2009). Tangle analysis of difference topology experiments: applications to a Mu protein–DNA complex. *Algebraic and Geometric Topology*, **9**, 2247–2309.
27. Kim, S. & Darcy, I. K. (2009). Topological analysis of DNA–protein complexes. In *Mathematics of DNA Structure, Function, and Interactions*. (Benham, C. J., Harvey, S., Olson, W. K., Sumners, D. W. L. & Swigon, D., eds), Springer Science + Business Media, LLC, New York, NY.
28. Darcy, I. K. (2008). Modeling protein–DNA complexes with tangles. *Comput. Math. Appl.* **55**, 924–937.
29. Vetcher, A. A., Lushnikov, A. Y., Navarra-Madsen, J., Scharein, R. G., Lyubchenko, Y. L., Darcy, I. K. & Levene, S. D. (2006). DNA topology and geometry in Flp and Cre recombination. *J. Mol. Biol.* **4**, 1089–1104.
30. Darcy, I. K. & Sumners, D. W. (2000). Rational tangle distances on knots and links. *Math. Proc. Cambridge Phil. Soc.* **128**, 497–510.
31. Saka, Y. & Vazquez, M. (2002). TangleSolve: topological analysis of site-specific recombination. *Bioinformatics*, **18**, 1011–1012.
32. Darcy, I. K., Ishihara, K., Medikonduri, R. & Shimokawa, K. (2009) Rational tangle surgery and Xer recombination on catenanes. Preprint.
33. Shimokawa, K., Ishihara, K. & Vazquez, M.. Tangle analysis of DNA unlinking by the Xer/FtsK system. To appear in Bussei Kenkyu (2009), Proceedings of the International Conference "Knots and soft-matter physics: topology of polymers and related topics in physics, mathematics and biology."
34. Ernst, C. (1996). Tangle equations. *J. Knot Theory Ramif.* **5**, 145–159.
35. Ernst, C. (1996). Tangle equations II. *J. Knot Theory Ramif.* **6**, 1–11.
36. Darcy, I. K. & Scharein, R. G. (2006). TopoICE-R: 3D visualization modeling the topology of DNA recombination. *Bioinformatics*, **22**, 1790–1791.
37. Buck, D. & Verjovsky Marcotte, C. (2005). Tangle solutions for a family of DNA-rearranging proteins. *Math. Proc. Cambridge Phil. Soc.* **139**, 59.
38. Buck, D. & Verjovsky Marcotte, C. (2007). Classification of tangle solutions for integrases, a protein family that changes DNA topology. *J. Knot Theory Ramif.* **16**, 969–995.
39. Buck, D. & Mauricio, M. M. (2010) Tangle solutions for composite knots: applications to Hin recombination arXiv:1007.0948v1.
40. Grainge, I., Buck, D. & Jayaram, M. (2000). Geometry of site alignment during Int family recombination: antiparallel synapsis by the Flp recombinase. *J. Mol. Biol.* **298**, 749–764.
41. Merickel, S. K. & Johnson, R. C. (2004). Topological analysis of Hin-catalysed DNA recombination *in vivo* and *in vitro*. *Mol. Microbiol.* **51**, 1143–1154.
42. Wasserman, S. A., Dungan, J. M. & Cozzarelli, N. R. (1985). Discovery of a predicted DNA knot substantiates a model for site-specific recombination. *Science*, **229**, 171–174.
43. Buck, D. & Flapan, E. (2007). Predicting knot or catenane type of site-specific recombination products. *J. Mol. Biol.* **374**, 1186–1199.
44. Buck, D. & Flapan, E. (2007). A topological characterization of knots and links arising from site-specific recombination. *J. Phys. A: Math. Theor.* **40**, 12377–12395.

45. Valencia, K. & Buck, D. (2011). Characterization of knots and links arising from site-specific recombination on twist knots. *J. Phys. A: Math. Theor.* **44**, 045002.
46. Johnson, R. C. & Bruist, M. F. (1989). Intermediates in Hin-mediated DNA inversion: a role for Fis and the recombinational enhancer in the strand exchange reaction. *EMBO J.* **8**, 1581–1590.
47. Rolfsen, D. (2003). *Knots and Links*. AMS, Providence, RI.
48. Cromwell, P. (2005). *Knots and Links*. Cambridge University Press, Cambridge, MA.
49. Kawauchi, A. (1996). *A Survey of Knot Theory*. Birkhauser, Boston, MA.
50. Cox, M. M. (1989). DNA inversion in the 2 μ m plasmid of *Saccharomyces cerevisiae*. In *Mobile DNA*, pp. 661–670, American Society for Microbiology, Washington, DC.
51. Bath, J., Sherratt, D. J. & Colloms, S. D. (1999). Topology of Xer recombination on catenanes produced by λ integrase. *J. Mol. Biol.* **289**, 873–883.
52. Pathania, S., Jayaram, M. & Harshey, R. M. (2002). Path of DNA within the Mu transpososome: transposase interactions bridging two Mu ends and the enhancer trap five DNA supercoils. *Cell*, **109**, 425–436.
53. Ernst, C. & Sumners, D. W. (1987). The growth of the number of prime knots. *Math. Proc. Cambridge Phil. Soc.* **102**, 303.