Characterization of knots and links arising from site-specific recombination on twist knots

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Abstract
We develop a model characterizing all possible knots and links arising from recombination starting with a twist knot substrate, extending the previous work of Buck and Flapan. We show that all knot or link products fall into three well-understood families of knots and links, and prove that given a positive integer $n$, the number of product knots and links with minimal crossing number equal to $n$ grows proportionally to $n^5$. In the (common) case of twist knot substrates whose products have minimal crossing number one more than the substrate, we prove that the types of products are tightly prescribed. Finally, we give two simple examples to illustrate how this model can help determine previously uncharacterized experimental data.

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1. Introduction
The central axis of the famous DNA double helix can become knotted or linked as a result of numerous biochemical processes, most notably site-specific recombination [1–3]. A wide variety of DNA knots and links have been observed [4–16]. Characterizing the precise knot or link type can often help understand structural or mechanistic features of the biochemical reaction [16–27]. Experimentally, such a characterization is typically achieved via gel electrophoresis (which stratify DNA products according to their minimal crossing number) [28] and electron microscopy (which allows us to visualize the over- and under-crossings of the DNA molecule) [29, 30] together with knot invariants such as the Jones polynomial (amongst many others) [31]. However, gel electrophoresis is not straightforward and often the precise over- or under-crossing cannot be categorically determined. Partial information

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can be gleaned by using gel electrophoresis but as there are 1,701,936 prime knots with minimal crossing number less than 17 this information is not sufficient [32]. Furthermore, gel electrophoresis does not distinguish between handedness of chiral knots, so this does not give the full picture. Thus topological techniques, such as those presented here, can aid experimentalists in characterizing DNA knotted and linked molecules by restricting the types of knots or links that can arise in a particular context. Here we focus on the most common biochemical reaction that yields DNA knots and links: site-specific recombination. Site-specific recombination is an important cellular reaction that has been studied extensively since the 1960s. It involves a reciprocal exchange between defined DNA segments. Biologically, this results in a variety of processes (see [33] and references therein). Apart from their fundamental functions in the cell, site-specific recombinases give scientists an elegant, precise and efficient way to insert, delete and invert segments. Thus they are rapidly becoming of pharmaceutical and agricultural interest as well as being used in the development of biotechnological tools [34, 35]. Twist knots are one of the most common DNA conformations. This is not surprising as in the cell most DNA is (plectonemically) supercoiled (like an over-used phone cord) and in the lab most experiments done with site-specific recombinases use small (plectonemically) supercoiled circular DNA molecules, so an unknot can be transformed to a twist knot by a single crossing change (see figure 1). Unlike (2, n)-torus knots, twist knots occur as knots (not links) for both odd and even minimal crossing numbers, MCN(K). Thus ubiquitous DNA twist knots arise as a result of a variety of site-specific recombination reactions [3, 5–11]. Despite the biological importance of this twist knot family, there has 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 knots [36–38].) Here we rectify this by presenting a model, extending the work of [37], classifying all possible knots and links that can arise from site-specific recombination on a twist knot. Our model is built on three assumptions for which biological evidence is provided in [38, 39]. We construct a model that predicts all possible knots and links that can arise as products of a single round of recombination, multiple rounds of (processive) recombination and of distributive recombination, given a twist knot substrate \( C(2, v) \) and our three assumptions. We predict that products arising from site-specific recombination on a twist knot substrate \( C(2, v) \) must be members of the three families of products illustrated in figure 2. Members of these families of knots and links include prime and composite knots and links with up to three components (see section 2.3). Our model can also distinguish between the chirality of the product molecules of site-specific recombination (see section 5). Our model is independent of site orientation, and we make no assumption on the number of base pairs of the molecule(s).

1.1. Structure of our paper

This paper is organized as follows: in section 2 we give a concise introduction to site-specific recombination and introduce notation. In section 3 we state and explain the three assumptions about the recombinase complex, the substrate and the mechanisms of recombination. (Biological justifications for these assumptions can be found in [38, 39].) In section 4 we determine the pre-recombinant and post-recombinant conformations of the recombination sites and all possible conformations of the DNA-protein complex; we also prove the necessary background lemmas for section 5. In section 5 we prove theorems 1 and 2 which determine all the putative DNA knot and link products of (non-distributive) site-specific recombination on a twist knot substrate. We show that these products belong to one of the three families of knots and links illustrated in figure 2. (These families of knots and links are defined in section 2.3.) We also identify all knots and links that arise as products of distributive
site-specific recombination. In section 6, we prove theorem 4 which shows that all the possible DNA knot and link products of site-specific recombination on a twist knot substrate are a very small fraction of all knots and links. We also further restrict the knot and link types of products that have minimal crossing number one more that of the substrate. Finally, in section 7 we consider two simple uses of our model. For a detailed biological discussion of applications of our model, and how to use this model as a tool in a variety of site-specific recombination systems, we refer the reader to [39].

2. Biological systems and terminology

In this section we give a concise introduction to site-specific recombination, introduce notation and describe the families of knots and links that arise as products of site-specific recombination on twist knot substrates.
2.1. Site-specific recombination

Site-specific recombination reshuffles DNA sequences by inserting, deleting or inverting DNA segments of arbitrary length. As such, it mediates a variety of important cellular processes including chromosome segregation and viral infections. (See the review [33] for more details.) Minimally, site-specific recombination requires both particular proteins (site-specific recombinases) and two short (30–50bp) DNA segments (the crossover sites) within one or two DNA molecules (the substrate). (More complex site-specific recombination systems may also require additional proteins (called accessory proteins) and DNA sites (called enhancer sequences).) Site-specific recombinases can be broadly divided into two subfamilies: serine site-specific recombinases and tyrosine site-specific recombinases, based on their catalytic residues. Site-specific recombination roughly has three stages (see figure 3). First, two recombinase molecules bind to each of two crossover sites and bring them close together. (The sites together with the four bound recombinases is called the synaptic complex.) Second, the crossover sites are cleaved, exchanged and resealed. (The precise nature of this intermediary step is determined by the recombinase subfamily, see assumption 3 and figures 4(a) and (b).) And finally, the rearranged DNA (the product) is released. Multiple rounds of strand exchange can occur before releasing the DNA: this process is known as processive recombination. (See assumption 3 and figure 5.) This is in contrast to distributive recombination, where multiple rounds of the entire process of recombination (including releasing and rebinding) occur. Only serine recombinases can mediate processive site-specific recombination, but both types of recombinases can mediate distributive recombination. In this work we use the term substrate to refer specifically to the DNA prior to the first cleavage. We treat processive recombination as one extended process (with several intermediate exiting points for the reaction).

2.2. Mathematical terminology

A twist knot is a knot that admits a projection with a row of \( v \neq 0 \) vertical crossings and a hook, as in figure 6(b) and is denoted by \( C(\pm 2, v) \). If \( r = -2 \), by flipping the top loop we get \( r = +2 \) and add a positive crossing to the row of \( v \) crossings (see this isotopy illustrated in figure 6(c)). Thus from now on we assume that our substrate is the twist knot \( C(2, v), v \neq 0 \).
Figure 4. (a). Biological assumption 3: serine recombinases. Serine recombinases perform simultaneous double-stranded breaks, rotate one half of the recombinase complex relative to the other by 180° and rebind the DNA (b). Biological assumption 3: tyrosine recombinases. Tyrosine recombinases cleave one strand from each duplex, exchange the cleaved strands and ligates them to form a Holliday junction (rightmost two panels). Isomerization of this junction alternates the catalytic activity and the same process happens with the other two DNA strands. These images are modifications of figures 3 and 11 in [33].

Figure 5. Mathematical assumption 3: serine recombinases. Begin with all possible projections of the pre-recombinant conformation of the recombinase complex, with zero or one crossings. Follow with projections of the post-recombinant conformations of the productive synapse at each round of processive recombination.

(See [40–44] for a detailed discussion on twist knots.) Note that twist knots can be generalized to clasp knots. A clasp knot $C(r, v)$ is a knot that has two non-adjacent rows of crossings, one with $r \neq 0, \pm 1$ crossings and the other with $v \neq 0$ crossings (figure 6(a)). (By adjacent rows of $r$ and $v$ crossings we mean that the two rows cannot be considered as a single row of $r + v$ crossings as they can in the case of the torus knots and links $T(2, r + v)$. A clasp knot $C(r, v)$ with $r = \pm 2$ is a twist knot.) We use the following terminology and notation. We consider the central axis of the DNA double helix and therefore, when we illustrate DNA molecules we draw this axis (and not the two DNA backbones that make up the double helix). Let $J$ denote the twist knot substrate molecule. Once the synaptic complex has been formed, the recombinase complex, $B$, denotes the convex hull of the four bound recombinase molecules together with the two crossover sites. (Note that $B$ is a topological ball.) The recombinase-DNA complex, $J \cup B$, denotes the union of the substrate $J$ with the recombinase complex $B$. Let $C = \text{cl}(\mathbb{R}^3 - B)$ and let $C \cap J$ denote the complement of the recombinase complex. If the
recombinase complex meets the substrate in precisely the two crossover sites then we say the recombinase complex is a **productive synapse**, see figure 7. In particular, for recombinases that utilize an enhancer sequence or accessory proteins, the recombinase complex is a productive synapse if the accessory sites and proteins are sequestered from the crossover sites\(^2\).

2.3. **Notation for families of knots and links that arise from site-specific recombination on a twist knot**

We now discuss the three families of knots and links that we encounter in the main results of this paper. The families of knots and links illustrated in figures 2(a), (b) and (c) are

\(^2\) Note that if \(B\) is a productive synapse, then it can be thought of as a (2-string) tangle. However, unlike in the traditional tangle model, the complement of \(B\) may take a variety of forms (not necessarily that of a tangle), so we avoid this potentially confusing terminology.
Figure 8. (a) A Montesinos knot or link has a projection as illustrated here. The $R_i$ are rational tangles. (b) A rational tangle with alternating crossings. (c) The partial sum of two tangles refers to the tangle diagram resulting from the insertion of two tangle diagrams into the shaded discs. (d) Numerator closure of a tangle.

referred to as $F(p, q, r, s, t, u)$, $G_1$ and $G_2$ respectively. We note that $F(p, q, r, s, t, u)$ is a special family of knots and links. In [45] it is shown that a standard rational tangle diagram corresponds to any expansion of a rational number $\frac{p}{q}$ as a continued fraction; in this paper we choose the convention that a choice of the expansion in which all terms have alternating sign gives an alternating diagram (see figure 6(d) for a convention on crossings and figure 8(b) for an example of a rational tangle diagram). A Montesinos link is a link $L$ that admits a diagram $D$ composed of $m \geq 3$ rational tangle diagrams $R_1, \ldots, R_m$ and $k \geq 0$ half-twists glued together as in figure 8(a) (and see e.g. [50]). Members of $F(p, q, r, s, t, u)$ are obtained by the numerator closure of Montesinos tangles of the form $(\frac{r}{p+1}, \frac{r}{q+1}, \frac{r}{t+1})$. That is, for three standard rational tangle diagrams with fractions $\frac{r}{p+1}$, $\frac{r}{q+1}$ and $\frac{r}{t+1}$, take their partial sum as in figure 8(c) and then the closure of the diagram as in figure 8(d).

Denote the tangle with corresponding rational number $\frac{r}{p+1}$, $\frac{r}{q+1}$ and $\frac{r}{t+1}$ by $R_1$, $R_2$ and $R_3$ respectively. As in [37], we define the family of small Montesinos knots and links to be the family of Montesinos links for $i = 3$, $R_k$ as above and $k = 0$. Thus, our family of knots and links $F(p, q, r, s, t, u)$ illustrated in figure 2(a) is a subfamily of small Montesinos knots and links.

In the family $F(p, q, r, s, t, u)$ of knots and links, the variables $p, q, r, s, t, u$ describe the number of crossings between two strands in that particular row of crossings. Note that knots that are members of this family can be prime or composite and links belonging to this family can have up to three components. In this family, the variables $p, q, r, s, t, u$ can be positive, negative or zero. By letting the variables equal 0 or $\pm 1$ as appropriate, we obtain the subfamilies illustrated in figure 9. Subfamily 1 is denoted by $F_S(0, q, r, s, t, u)$ with $|r| > 0$, $|t| > 1$. Subfamily 2 is denoted $F_S(\pm 1, q, r, s, t, u)$ with $|r| > 1$. Subfamily 3 is denoted $F_S(\pm 1, q, r, s, t, u)$ with $|r|, |t| > 1$. Subfamily 4 is denoted $F_S(p, q, r, s, t, u)$ when we forbid $p, t, r = [0, 1]$. Subfamily 5 is composite knots or links $T(2, u)\# C(p, q)$ formed from a torus knot and a twist knot. Subfamily 6 is a subfamily of $F(p, q, r, s, t, u)$ with $p + q = 0$. Subfamily 7 is a family of clasp knots and links, $C(r, s)$. (Recall that it is a generalization of the family of twist knots, which we consider in this paper as the substrate molecule for site-specific recombination.) Subfamily 8 is the family of torus knots and links, $T(2, r)$. Finally, subfamily 9 is the family of pretzel knots $K(p, q, s, u)$. (Note that some of the subfamilies in figure 9 are special cases of other subfamilies, for example in subfamily 2; if we let $q = 0$, then we get subfamily 5. Similarly for subfamilies 1, 7, 3 and 6.) In the families $G_1(k)$ and $G_2(k)$ of knots and links, the variable $k$ describes the number of crossings between...
Figure 9. Subfamilies of the family illustrated in figure 2(a). The nine subfamilies obtained by setting $p, q, r, s, t$, and/or $u$ equal to 0 or ±1 in the family of knots and links $F(p, q, r, s, t, u)$.

Top: product subfamily $F_1(0, q, r, s, t, u)$ with $|r|, |t| > 1$, product subfamily $F_2(±1, q, r, s, ±1, u)$ with $|r| > 1$, product subfamily $F_3(±1, q, r, s, t, u)$ with $|r|, |t| > 1$, product family $F_4( p, q, r, s, t, u)$ with $|t|, |r|, |p| > 1$, product subfamily of composite knots $T(2, u) ⊪ \mathcal{C}(p, q)$.

Bottom: product subfamily $F(−1, 1, r, s, t, u)$ with $|t|, |r| > 1$, product subfamily of clasp knots and links $\mathcal{C}(r, s)$, product subfamily of torus knots and links $T(2, r)$, product subfamily of pretzel knots $K(p, s, u)$.

3. Assumptions

In this section we state and explain the three assumptions about the recombinase complex, the substrate and the mechanisms of recombination. (Biological justifications for these assumptions can be found in [38, 39].) We make the following three assumptions about the recombinase-DNA complex, which we state in both biological and mathematical terms. These assumptions are similar in [37, 38]. However, for assumption 2 in particular, we introduce new terminology and prove a necessary result in order to re-state this biological assumption in precise mathematical terms. In [38, 39] we provide experimental evidence showing that each of these assumptions is biologically reasonable.

**Biological assumption 1.** The synaptic complex is a productive synapse, and there is a projection of the crossover sites which has at most one crossing between the sites and no crossings within a single site.

**Mathematical assumption 1.** $B \cap J$ consists of two arcs and there is a projection of $B \cap J$ which has at most one crossing between the two arcs, and no crossings within a single arc.

Fix a projection of $J$ such that $B \cap J$ has one of the forms illustrated in figure 10. Observe that form $B1$ can be rotated by $90°$ to obtain form $B2$. However, we list forms $B1$ and $B2$ as two different forms to make subsequent figures easier to follow (similarly for forms $B3$ and $B4$). Note that hooked productive synapses, illustrated in figure 6(d), are biologically possible because there exist many recombinases whose productive synapse is not characterized, and

the two strands. Depending on the value of $k$, we obtain either a knot or a link: for $G_i(k)$ for $i = 1, 2$, if $k$ is odd, the members of these families are knots. If $k$ is even, then the members of this family are two component links. These families are illustrated in figures 2(b) and (c). Note that there are a few knots and links that belong to both $F(p, q, r, s, t, u)$ and either $G_1(k)$ 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 $k = 2$. 
Figure 10. Assumption 1: projections of the pre-recombinant productive synapse. Assumption 1 states that there is a projection of the pre-recombinant productive synapse with at most one crossing. Note that it does allow productive synapses like the hook, where there is a projection with one crossing but no projections with zero crossings. Assumption 3 for tyrosine recombinases: after recombination with a tyrosine recombinase, the productive synapse has a projection with at most one crossing.

Figure 11. By continuously deforming $B \cap J$ within a neighbourhood of the hook, we can obtain a projection of the hook with exactly one crossing. This affects the projection of the rest of the substrate only by adding one positive crossing to the row of $v$ crossings.

for these systems $B \cap J$ could be hooked. However, this does not contradict assumption 1, since a hook has no projections with no crossings, but has projections where there is only one crossing. There is an isotopy of the substrate molecule taking a hook from a projection with two crossings to a projection with one crossing, without affecting the projection of the substrate molecule outside a neighbourhood of the hook (figure 11 illustrates this).

Biological assumption 2. The synaptic complex does not pierce through a supercoil or a branch point in a non-trivial way and the supercoiled segments are closely juxtaposed. Also, no persistent knots or links are trapped in the branches of the DNA on the outside of the synaptic complex.

Here persistent knots or links are those that remain after a continuous deformation of the DNA molecule, keeping $B$ fixed.

Before we can state assumption 2 mathematically, we need to introduce some terminology. We define a planar surface with twists as in [37]. Consider a surface lying in a plane together with a finite number of arcs in the surface whose endpoints are on the boundary of the surface (see figure 12(a)). We can use this planar surface with arcs to obtain a non-planar surface by replacing a neighbourhood of each arc in the original surface by a half-twisted band and removing the top and bottom ends of the band (see figure 12(b)). Figure 12 illustrates how such a surface can be obtained from a doubly-punctured planar disc together with a collection of arcs defining the twists. A planar surface with twists is defined to be any surface which can be obtained from a planar surface in this way. Define a surface $D$ with boundary $J$ to be a spanning surface for $J$ if $D$ is topologically equivalent to a doubly-punctured planar
Figure 12. Obtain a planar surface with twists by replacing a neighbourhood of each arc by a half-twisted band. Here our planar surface is a doubly-punctured disc and our non-planar surface with twists is a surface whose boundary is the twist knot $C(2,v)$.

Figure 13. A surface $D$ with boundary $J$ is a spanning surface for $J$ if $D$ is topologically equivalent to a doubly-punctured planar disc with twists when $J$ is a twist knot $C(2,v)$. (We can think of a spanning surface for $J$ as a soap film surface with boundary $J$.) In the construction of this spanning surface, in figure 12(a), we choose the twisted band that replaces the arc connecting the boundary of the planar disc and the right-most puncture and the twisted band that replaces the arc connecting the two punctures, such that the corresponding crossings defined on the non-planar surface with twists make a set of $+2$ horizontal crossings that we call a clasp, illustrated in figures 12 and 13. Figure 14 shows examples of the relationship between the spanning surface $D$ and the recombinase complex. Observe that in illustrations (i) and (ii) $D \cap \partial B$ consists exactly two arcs. In illustration (iii), no matter how the spanning surface $D$ is chosen, $D \cap \partial B$ contains at least one circle as well as two arcs, whereas in illustration (iv) there is an isotopy that removes the circle in $D \cap \partial B$. Mathematically, a spanning surface $D$ is pierced non-trivially by $B$ if and only if $D \cap \partial B$ contains at least one circle in addition to the required two arcs, and there is no ambient isotopy of $D$ that removes this additional circle.

Claim. The intersection of any spanning surface for $J$ and $\partial B$ contains exactly two arcs.

Proof. By assumption 1, $B$ contains exactly two arcs of $J = \partial D$; thus, $\partial B \cap J$ is precisely four points. It follows that the intersection of any spanning surface for $J$ with $\partial B$ contains exactly two arcs, whose endpoints are the four points $\partial B \cap J$. By biological assumption 2, $B$ does not pierce the interior of any spanning surface $D$ in a non-trivial way. Thus $D \cap \partial B$ consists of exactly two arcs and no circles that cannot be removed by an ambient isotopy of $D$. □
Figure 14. (i) A knot is trapped in the DNA branches outside of $B$. (ii) An unknotted substrate with the synaptic complex formed. (iii) The recombinase complex pierces a supercoil in a non-trivial way, $D \cap \partial B$ contains at least one circle as well as two arcs. (iv) The productive synapse trivially pierces through a supercoil and the circle contained in $D \cap B$ can be removed via an isotopy of $C$. Scenarios (i) and (iv) are allowed by our assumptions, the other two scenarios are not.

Suppose that $D$ is a spanning surface for $J$. We know by biological assumption 2 that the supercoiled segments of the DNA molecule $J$ are closely juxtaposed; this means that we can visualize the spanning surface $D$ as a narrow soap film surface. In particular, this means that the two arcs in $D \cap \partial B$ are each very short, so we can assume that they are co-planar. (Note that this does not mean that the crossover sites themselves ($\partial D \cap B$) are co-planar.) We can now define a surface $D \cap C$ to be unknotted relative to $\partial B$ if there is an ambient isotopy of $C$, point-wise fixing $\partial B$, that takes $D \cap C$ to a doubly-punctured planar disc with twists, where the end points of the arcs defining the twists are disjoint from $\partial B$. Illustration (ii) of figure 14 shows an example of $D \cap C$ unknotted relative to $\partial B$. Illustration (i) shows a knot trapped in the substrate molecule outside of $B$. We are now ready to state assumption 2 mathematically.

**Mathematical assumption 2.** $J$ has a spanning surface $D$ such that $D \cap \partial B$ consists of exactly two arcs; the two arcs are co-planar and $D \cap C$ is unknotted relative to $\partial B$.

The fact that $J$ has a spanning surface $D$ satisfying assumption 2 means our model is independent of the projection of the substrate $J$, so we now fix a projection of $J$ as in figure 6(b) and from now on we work with this particular projection $J$. Note that here we are referring specifically to the substrate $J$ before the synaptic complex is formed. The conformations of the pre-recombinant recombinase-DNA complex are dealt with in section 4. Recall that site-specific recombinases fall into two subfamilies, the serine recombinases and the tyrosine recombinases. The details of the mechanism differ depending on which subfamily the recombinase belongs to. Assumption 3 addresses the mechanism for each subfamily of recombinases.

**Biological assumption 3 for serine recombinases.** Serine recombinases perform recombination via the ‘subunit exchange mechanism.’ This mechanism involves making two simultaneous double-stranded breaks in the sites, rotating two recombinase molecules in opposite sites by $180^\circ$ within the productive synapse and resealing the new DNA partners (figure 4(a)). In each subsequent round of processive recombination, the same set of subunits is exchanged and the sense of rotation remains constant.
Biological assumption 3 for tyrosine recombinases. After recombination mediated by a tyrosine recombinase, there is a projection of the crossover sites which has at most one crossing (figure 4(b)).

The mathematical statement is as follows:

Mathematical assumption 3 for serine recombinases. After (each round of processive) recombination mediated by a serine recombinase, there is precisely one additional crossing between the crossover sites (see figure 5).

Mathematical assumption 3 for tyrosine recombinases. After recombination mediated by a tyrosine recombinase, there is a projection of the crossover sites which has at most one crossing (figure 10).

4. Possible forms of the productive synapse and its complement

In this section we determine the pre-recombinant and post-recombinant conformations of the recombination sites and all possible conformations of the DNA-protein complex. We also prove the necessary background lemmas for section 5.

4.1. Possible forms of the productive synapse $B \cap J$

As a result of assumption 2, we have fixed a projection of $J$ prior to cleavage such that $B \cap J$ has form $B_1$, $B_2$, $B_3$ or $B_4$, illustrated in figure 10. It follows from assumption 3 that after $n$ recombination events with serine recombinases, we have added a row of either $n - 1$, $n$ or $n + 1$ identical crossings that can be positive, negative or zero. Without loss of generality, we assume that after $n$ recombination events with serine recombinases, we add a row of $n$ identical crossings that can be positive, negative or zero. Thus after $n$ recombination events our fixed projection of $B \cap J$ is isotopic to one of the forms $n_1$ or $n_2$ illustrated in figure 5. (Note that from $n_1$ we can obtain $n_2$ by rotating by $90^\circ$. However, we list them as separate forms in order to make it easier to follow the use of figure 15 in the proof of theorem 2.) For tyrosine recombinases, without loss of generality we assume that the post-recombinant projection of $B \cap J$ has one of the eight forms in figure 16. Note that conformations $B_5$, $B_6$, $B_7$ and $B_8$ are hooks. Hooks have no projections with no crossings but do have projections with one crossing, so we allow these conformations. Forms $B_1$, $B_3$, $B_5$ and $B_7$ are equivalent by a $90^\circ$ rotation, to forms $B_2$, $B_4$, $B_6$ and $B_8$ respectively. (We list them separately to make it easier to follow the use of figure 17 in the proof of theorem 1.)

4.2. Possible forms of the complement of the productive synapse $C \cap J$

In this section we determine all the possible conformations of $C \cap J$, and determine the respective pre-recombinant conformations of $B \cap J$ for each form of $C \cap J$. For simplicity, we will use the phrase ‘$C \cap J$ has a particular form’ when we mean that ‘$C \cap J$ is ambient isotopic, pointwise fixing $\partial B$, to that form’. The forms of $C \cap J$ referred to in the lemma are illustrated in figure 18.

Lemma 1. Suppose that assumptions 1, 2 and 3 hold for a particular recombinase-DNA complex with substrate $J$. Let $J$ be a twist knot $C(2, v)$. Then $C \cap J$ has one of possible five forms listed below. For each of these, $B$ has corresponding forms.

If $C \cap J$ has the form
Figure 15. Products of recombination with serine recombinases, theorem 2: all possible projections of the post-recombinant conformation of the recombinase-DNA complex $J \cup B$ and the productive synapse $B$ after $n$ rounds of processive recombination with a serine recombinase. The images inside the circles denote forms $n_1$ and $n_2$ of $B$ after $n$ rounds of processive recombination.

Figure 16. Mathematical assumption 3: tyrosine recombinases. Projections of the possible post-recombinant conformations of the recombinase complex. Hooks are allowed because they have projections with at most one crossing between the sites.

- $C_1$, then $B \cap J$ has the form $B_1$
- $C_2$, then $B \cap J$ has the form $B_1$, $B_3$ or $B_4$
- $C_3$, then $B \cap J$ has the form $B_2$, $B_3$ or $B_4$
- $C_4$, then $B \cap J$ has the form $B_1$
- $C_5$, then $B \cap J$ has the form $B_3$ or $B_4$.

**Proof.** By assumption 2, we can choose a spanning surface $D$ to be a doubly-punctured planar disc with twists as in figure 12, such that $D \cap \partial B$ is two co-planar arcs and $D \cap C$ is unknotted rel $\partial B$. Consider the doubly-punctured disc that generates $D$ in figure 12(a). We can consider this punctured planar disc with arcs as a thrice-punctured $S^2$ with a collection of arcs connecting the punctures (the three punctures are numbered 1, 2 and 3 in figure 12(a)). A thrice-punctured $S^2$ in $S^3$ with arcs connecting the three punctures can be regarded as a graph with three points and a collection of arcs connecting them, as illustrated in figure 19. We determine all possible conformations of $C \cap J$ in three steps as follows: First we consider all different possible locations of the specific sites $D \cap \partial B$ on the thrice-punctured $S^2$. The two specific sites can be located either both on the boundary of one puncture of $S^2$ or on a
Figure 17. Products of recombination with tyrosine recombinases. Theorem 1: projections of all possible conformations of the post-recombinant recombinase-DNA complex \( J \cup B \) and the productive synapse \( B \) after a reaction with a twist knot substrate \( C(2, v), v \neq 0 \) mediated by a tyrosine recombinase.

Figure 18. Summary of lemma 1. All the possible distinct forms that the substrate molecule \( C \cap J \) can take, up to isotopy, along with the corresponding forms of \( B \) for each form of \( C \cap J \).

Figure 19. A thrice-punctured \( S^2 \) in \( S^3 \), with arcs connecting the three punctures can be regarded as a graph with three points and a collection of edges connecting them.

Combination of these, so we consider each case. Note, however, that from the symmetry of the graph described above it is enough to consider only the cases where the sites are located either.
Figure 20. Characterization of the recombinase-DNA complex. Specific sites situated either case (11): both on the boundary of puncture 1 of $S^2$, or case (23): one on the boundary of puncture 2 of $S^2$ and the other on the boundary of puncture 3 of $S^2$. In cases where the right-most column says ‘not allowed’, we mean that we cannot allow such a conformation because when bringing the two specific sites together inside $B$, we get $C \cap D$ non-planar, which is not allowed by assumption 2.

- **Case (11):** both on the boundary of puncture 1.
- **Case (22) or (33):** both on the boundary of puncture 2 (equivalent to both on puncture 3).
- **Case (12) or (13):** one site on the boundary of puncture 1 and the other on the boundary of puncture 2 (equivalent to one site on puncture 1 and the other on puncture 3).
- **Case (23):** one site on the boundary of puncture 2 and the other on puncture 3.

Next, on the corresponding spanning surface $D$ (generated by the thrice-punctured $S^2$) we consider all possibilities for $D \cap B$, which can either be two discs or a (possibly twisted) band. Finally, we perform an appropriate isotopy of this spanning surface which maps it to a spanning surface having one of the conformations of $C \cap J$ illustrated in figure 18 as boundary. We do this for every case. In figures 20, 21 and 22 we illustrate the isotopy of $C \cap J$ to one of the standard forms $C_1, C_2, C_3, C_4$ or $C_5$ (or we show that such a case is not allowed by assumption) for each case. Even though cases (22) and (33) (and (12) and (13)) are equivalent, we illustrate all of them since it may be more straightforward to visualize the isotopy in one case or the other. In figures 20, 21 and 22, inside each box we have three sets of illustrations.

Left illustration: thrice punctured $S^2$ with (thin, long) arcs which define the twists on a non-planar surface and (thick, short) arcs on the boundary of one of the punctures (or a combination of these punctures) defining the arcs $D \cap \partial B$. 


Figure 21. Characterization of the recombinase-DNA complex. Specific sites situated either case (22): both on the boundary of puncture 2 of $S^2$, case (33): both on the boundary of puncture 3 of $S^2$. Note that cases (22) and (33) are equivalent, but we consider both here because it may be more straightforward to visualize the isotopy of $C \cap J$ to one of the standard forms in one case or the other.

Middle illustration: the spanning surface $D$ of our substrate $J = C(2, v)$ with the arcs $D \cap \partial B$ illustrated by a pair of thick, short arcs.

Right illustration: corresponding conformation of $C \cap J$. Since there are many cases and some of whose isotopies are not very complicated, we have illustrated all the cases in figures 20, 21 and 22. Here we describe two of the most involved in detail.

Case (11c): Assume both arcs of $D \cap \partial B$ lie on the boundary of puncture 1 of the thrice-punctured $S^2 \subset S^3$. Consider case (11c) as illustrated in figure 20. The thrice-punctured $S^2$ generates the spanning surface $D$ illustrated. Figure 23 illustrates a continuous deformation taking this conformation of $D$ to a conformation whose boundary is of the form $C_1$.

Case (12a): In figure 22 consider case (12a). The thrice-punctured $S^2$ generates the spanning surface $D$ illustrated. Figure 24 illustrates a continuous deformation taking this conformation of $D$ to a conformation whose boundary is of the form $C_1$. Here $C \cap D$ is unknotted rel $\partial B$ so the left and middle forms illustrated in figures 20, 21 and 22 yield (up to isotopy, fixing $\partial B$) the corresponding forms of $C \cap J$ illustrated on the right images. Thus, we can also specify the pre-recombinant form of $B \cap J$ for each conformation of $C \cap J$ as shown in figure 18. □

Observations. Since $B \cap J$ contains at most one crossing, the component of $D$ with almost all of the twists of $C(2, s)$ must be contained in $C$. In form $C_2$, while there may be twists to the right of $B$, they are topologically insignificant, since they can be removed by rotating
Figure 22. Characterization of the recombinase-DNA complex. Specific sites situated either case (12): one on the boundary of puncture 1 of $S^2$ and the other on the boundary of puncture 2 of $S^2$. case (13): one on the boundary of puncture 1 of $S^2$ and the other on the boundary of puncture 3 of $S^2$. In cases where the right-most column says ‘not allowed’, we mean that we cannot allow such a conformation because when bringing the two specific sites together inside $B$, we get $C \cap D$ non-planar, which is not allowed by assumption 2. Note that cases (12) and (13) are equivalent, but we consider both here because it may be more straightforward to visualize the isotopy of $C \cap J$ to one of the standard forms in one case or the other.

Figure 23. Isotopy for case (11c): assume that both arcs of $D \cap \partial B$ lie on boundary 1 of the thrice-punctured $S^2 \subset S^3$. The thrice-punctured $S^2$ generates the spanning surface $D$ that has boundary illustrated on the second image from the left. Next, a continuous deformation taking this conformation of $J$ to form $C_1$.

$C \cap D$ by some multiple of $\pi$. In form $C_1$, any twists which had occurred above $B$ can be removed and added to the row of twists below $B$ by rotating $C \cap D$ by some multiple of $\pi$. These rotations can occur while pointwise fixing $B$. Thus the five forms of $C \cap J$ illustrated in figure 18 are the only ones possible.
5. Characterization of knots and links arising as products of site-specific recombination on a twist knot

In this section we prove theorems 1 and 2 which determine all the putative DNA knot and link products of (non-distributive) site-specific recombination on a twist knot substrate. We
show that these products belong to one of the three families of knots and links illustrated in figure 2. (These families of knots and links are defined in section 2.3.) We also identify all knots and links that arise as products of distributive site-specific recombination. Here we use our preliminary work from section 2.3 to prove our main results. In this section, we suppose that the substrate is a twist knot $C(2,v)$ and that all three of our assumptions hold for a particular recombinase-DNA complex. We prove theorems 1 and 2 which characterize all possible knotted or linked products brought about by a non-distributive reaction with a tyrosine recombinase and a serine recombinase, respectively. Most knotted and linked products are as products of different scenarios of site-specific recombination on twist knots.

**Theorem 1.** Suppose that assumptions 1, 2 and 3 hold for a particular tyrosine recombinase-DNA complex with substrate $J$. If $J$ is a twist knot $C(2,v)$, then the only possible products (of a non-distributive reaction) are the unknot, the Hopf link, $C(r,s)$ for $r = \{1,2,3,4\}$, $T(2,m)$, a connected sum $T(2,m) \# C(2,s)$, a member of the family $F(p,q,r,s,t,u)$ in figure 2(a) with $|r| \geq 2, |l| = 1 \text{ or } 2, |p| \leq 1$, or a member of the family of knots and links $G_1$ or of the family of links and links $G_2$.

The possible products are illustrated in figure 17.

**Proof.** By assumption 3, after recombination with a tyrosine recombinase $B \cap J$ has one of the eight post-recombinant forms illustrated in figure 16. By lemma 1, $C \cap J$ has one of the five forms illustrated in figure 18. The products of recombination mediated by a tyrosine recombinase are obtained by replacing the pre-recombinant forms of $C \cap J$ in each of the forms of $C \cap J$ (in figure 18) with each of the eight post-recombinant forms of $B \cap J$ (in figure 16). The resulting products are illustrated in figure 17. More specifically, suppose that $J$ is $C(2,v)$. Then by lemma 1, $C \cap J$ can have form $C_1, C_2, C_3, C_4$ or $C_5$. Hence by figure 17, the possible products are the unknot, $C(r,s)$ for $r = \{1,2,3,4\}$, $T(2,m)$, a Hopf link, a connected sum $T(2,m) \# C(2,s)$, a member of the family $F(p,q,r,s,t,u)$ in figure 2(a) with $|r| \geq 2, |l| = 1 \text{ or } 2, |p| \leq 1$ and a knot or a link that has a projection in either $G_1$ or $G_2$. Note that from figure 17 we can see that all the possible products of site-specific recombination mediated by a tyrosine recombinase on a twist knot substrate belong to one of the subfamilies of $F(p,q,r,s,t,u)$ as illustrated in figure 9, with one exception. For the image on column 7, row 5 of figure 17, depending on the value of $v$, we get different knots or links.

- If $v$ is an odd number, then the product is a knot.
- If $v$ is a negative odd number, the product is a knot belonging to family $G_1$ with $k = |v|$. 
- If $v$ is a positive odd number, the product is a knot belonging to family $G_2$ with $k = |v| - 1$.
- If $v$ is an even number, the product is a two-component link.
- If $v$ is a negative even number, the product is a link belonging to family $G_1$ with $c = |v|$.

If $v$ is a positive even number, the product is a link belonging to family $G_2$ with $c = |v| - 1$. Thus, with the exception of one sequence of products, all products of recombination with a tyrosine recombinase belong to the family of small Montesinos knots and links illustrated in figure 2(a).

It follows from theorem 1 that every product of recombination with tyrosine recombinases is a member of the family in figure 2(a) with $|l| = 1, 2$ and $|p| \leq 1$, or a member of the families...
51 and G2. Also, it follows from figure 9 that C(2, s) (possibly with an additional trivial component) T(2, m) and T(2, m)\(\cap\)C(2, s) can be obtained as knots or links in F(p, q, r, s, t, u) with |t| \(=\) 1, 2 and |p| \(\leq\) 1.

**Theorem 2.** Suppose that assumptions 1, 2 and 3 hold for a particular serine recombinase-DNA complex with substrate J. If J is C(2, v), then the only possible products (of a non-distributive reaction) are the C(r, s), T(2, m), a connected sum T(2, m)\(\cap\)C(2, s) and any member of the family in figure 2(a) with |r| \(\geq\) 2, t \(\neq\) 0 and |p| \(\leq\) 1.

The possible products are illustrated in figure 15.

**Proof.** By assumption 3, after recombination with a serine recombinase, \(B \cap J\) has one of the two post-recombinant forms \(n1\) and \(n2\) illustrated in figure 5. Also, by lemma 1, \(C \cap J\) has one of the five forms illustrated in figure 18. For assumption 3 for serine recombinases, for each of the forms of \(C \cap J\), the products of recombination with serine recombinases are obtained by replacing each of the pre-recombinant forms of \(B \cap J\) with their corresponding post-recombinant form of \(B \cap J\) after n rounds of processive recombination according to figure 5. The resulting products are illustrated in figure 15. More specifically, suppose that J is C(2, v). Then according to lemma 1, \(C \cap J\) can have forms C1, C2, C3, C4 or C5. When \(C \cap J\) has form C1, \(B \cap J\) must have form B1. It follows from figure 5 that the post-recombinant form of \(B \cap J\) must be of form \(n2\). Thus, by replacing \(B \cap J\) with \(B1\) in C1, we obtain that the products can be any knot or link in subfamily 3 illustrated in figure 9. When \(C \cap J\) has form C2, \(B \cap J\) must have form B2, B3 or B4. In this case by figure 5, the post-recombinant form of \(B \cap J\) must be of form \(n1\) or \(n2\). We see from form C2 in figure 18 that the products can be any knots or links in subfamily 5 or subfamily 7 illustrated in figure 9. A similar analysis is made on the other possible forms of \(C \cap J\) to arrive to the conclusion that the products can be any knots or links in subfamily 1, 3, 5, 7 or 8 illustrated in figure 9 and thus, are members of the family F(p, q, r, s, t, u) (see figure 15).

Table 1 summarizes the results of theorems 1 and 2. Note that theorems 1 and 2 distinguish between the chirality 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 right-handed (or (+)) trefoil), according to our model, site-specific recombination mediated by a tyrosine recombinase yields T(2, 5), which is a (+) \(S1\) (among other products) and can never yield T(2, \(-5\)), which is a (–) \(S1\). For an explicit strategy see our paper [39].

5.1. Knots and links that cannot arise as products

There are a number of simple knots and links that cannot arise as products of non-distributive site-specific recombination.

**Corollary 3.** Suppose that assumptions 1, 2 and 3 hold for a particular site-specific recombinase-DNA complex with substrate a twist knot C(2, v). Any product arising that falls outside of families F(p, q, r, s, t, u), G1 or G2 must arise from distributive recombination.

For example, the knot \(8_{18}\) is a knot that is not Montesinos; thus, it does not belong to our family of small Montesinos knots and links. It also does not belong to either G1 or G2, so \(8_{18}\) is an example of a knot that cannot arise as a product of non-distributive recombination on a twist knot substrate. Knots and links in F(p, q, r, s, t, u) that cannot arise
Table 1. Products of non-distributive recombination predicted by our model.

| Recombinase type | Substrate | Product |
|------------------|-----------|---------|
| Tyrosine         | C(2, v)   | C(r, s) for r = 1, 2, 3, 4, T(2, m), Hopf link, T(2, m)♯C(2, s), F(p, q, r, s, t, u) with | |
|                  |           | | t| = 1 or 2, |
|                  |           | | p| ≤ 1, knots or links in families G1 or G2 |
| Serine           | C(2, v)   | C(r, s), T(2, m), T(2, m)♯C(2, s), F(p, q, r, s, t, u) with |
|                  |           | | p| ≤ 1 and t ≠ 0 |

from recombination mediated neither by a serine recombinase, nor a tyrosine recombinase.
The knot 10_{141} cannot be expressed in F(p, q, r, s, t, u) with t ≠ 0 and |p| ≤ 1. Recall that all products from recombination with a tyrosine recombinase or a serine recombinase belonging to F(p, q, r, s, t) can be expressed with r > 2, t ≠ 0 and |p| ≤ 1. Thus 10_{141} cannot arise as a product. Knots that cannot arise from recombination mediated by a tyrosine recombinase. There are knots and links in F(p, q, r, s, t, u) which do not have a projection with |t| = {±1, ±2} and |p| ≤ 1, for example, the knot 8_{11} = F(2, 2, 2, −1, −3, 0). By inspection we can see that there is no way to express 8_{11} as a member of F(p, q, r, s, t, u) with t = {±1, ±2} and |p| ≤ 1; hence, 8_{11} is not a product of recombination with a tyrosine recombinase. The knot 10_{64} is another example of this. 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 illustrated in figure 2(a) with t ≠ 0 (not just t = {±1, ±2}) and |p| ≤ 1, can occur as a consequence of theorem 2. The knot 8_{11} mentioned above is an example of this; this knot is a possible product of recombination with a serine recombinase, but not with a tyrosine recombinase.

6. Minimal crossing number of our model

In this section we prove theorem 4 which shows that all the possible DNA knot and link products of site-specific recombination on a twist knot substrate are a very small fraction of all knots and links. We also further restrict the knot and link types of products that have minimal crossing number one more than that of the substrate.

6.1. The growth of product knots and links is proportional to n^5

To prove the main theorem of this section, theorem 4, we will split the family F(p, q, r, s, t, u) of knots and links into seven smaller subfamilies illustrated in figure 25. Theorem 4 is independent of how family F(p, q, r, s, t, u) is split up since we are using these subfamilies to count all the possible knots and links belonging to F(p, q, r, s, t, u).

Definition. For a knot or link K the minimal crossing number MCN(K) is the smallest number of crossings over all possible projections. For a knot or link K, denote its minimal crossing number by MCN(K).

The number of prime knots and links (links with up to two components and counting chiral pairs separately) with minimal crossing number n grows exponentially as a function of n [46]. By contrast, we now prove that the total number of knots and links with MCN(K) = n that are putative products of site-specific recombination on a twist knot substrate grows linearly as a function of n^5. Our families include prime and composite knots and links with up to three components. For the purposes of this section, we do not distinguish handedness of chiral...
knots; however, even including both versions of chiral knots, our family grows slower than the function of $n^5$ multiplied by 2. This actually means that all the possible prime knot and (two-component) link products of site-specific recombination on a twist knot substrate are a very small fraction of all knots and links. First, we consider knots and links belonging to $F(p, q, r, t, s, u)$. Note that, while the knots and links in this family have at most six non-adjacent rows containing $p, q, r, s, t$ and $u$ signed crossings respectively, it does not follow that the minimal crossing number of such a knot or link is $|p| + |q| + |r| + |s| + |t| + |u|$. If the knot or link is not alternating, it is quite possible that the number of crossings can be significantly reduced. Thus, \textit{a priori}, there is no reason to believe that the number of knots and links in this product family should grow linearly with $n^5$.

**Definition.** A link diagram is called reduced if it does not contain any ‘removable’ or ‘nugatory’ crossings. A reduced alternating link diagram is a link diagram that is reduced and also alternating.

Murasugi [47] and Thistlethwaite [48] proved that any reduced alternating diagram has a minimal number of crossings. Buck and Flapan [37] used this to show that for a twist knot $C(r, s)$ if $r$ and $s$ have the same sign, then $MCN(C(r, s)) = |r| + |s| - 1$, and if $r$ and $s$ have opposite sign, then $MCN(C(r, s)) = |r| + |s|$. To prove our result, we consider a \textit{Hara–Yamamoto projection}: a projection of a knot or a link in which there is a row of at least two crossings and which has the property that if this row is cut off from the rest of the projection and the endpoints are resealed in the two natural ways, then both resulting projections are reduced alternating (see figure 26). Hara and Yamamoto showed that any Hara–Yamamoto projection has a minimum number of crossings [49]. We make use of the following theorem proved by Lickorish and Thistlethwaite, in [50]:

**Theorem** (Lickorish–Thistlethwaite). If a link $L$ admits an $n$-crossing projection of the form as in figure 8(a) with $k = 0$ and each $R_i$ a reduced alternating rational tangle diagram with one crossing between the two arcs at the bottom of each $R_i$ and at least one more crossing, then $L$ cannot be projected with fewer than $n$ crossings.

We refer to such a projection as a \textit{reduced Montesinos diagram}. We can deduce from the theorem that any projection of a knot or link that is a reduced Montesinos diagram has a minimal number of crossings. We begin with two lemmas.

**Lemma 2.** The number of distinct knots and links in the product family illustrated in figure 2(a) with $MCN = n$ grows linearly with $n^5$. 

![Figure 26. Hara–Yamamoto: a projection of a knot or link is Hara–Yamamoto if when we cut off the row of $p$ crossings on the left and reseal the strands in the two natural ways, both resulting projections are reduced alternating.](image)
Figure 27. Example of strand movement: by moving the two strands, we reduce from $|r|+|u|+|q|$ crossings originally to $|r|+|u|+|q| - 2$ crossings in the alternating diagram.

**Proof.** Fix $n$ and suppose $K$ is a knot or a link projection in the family of figure 2(a) with minimal crossing number $n$. Then this projection has $|p| + |q| + |r| + |s| + |t| + |u|$ crossings. We divide the proof into three cases.

**Case 1. $K$ reduced alternating or reduced Montesinos.** If projection $K$ is reduced alternating or a reduced Montesinos diagram, then $|p| + |q| + |r| + |s| + |t| + |u| = n$.

We now show that if $K$ is not reduced alternating or a reduced Montesinos diagram, then it is ambient isotopic to one of 121 possible projections which have minimal number of crossings.

**Case 2. $K$ can be isotoped to a reduced alternating or reduced Montesinos diagram.** Figure 27 illustrates an example of how to reduce the number of crossings in a projection $K$ that is not reduced alternating or reduced Montesinos diagram. Observe that for the link in figure 27, the part containing the rows of $r$ and $q$ crossings is alternating if and only if $r$ and $q$ have opposite signs. Similarly for the section containing the rows of $r$ and $u$ crossings. If $r$ and $q$ have the same sign, then by moving a single strand, this part of the knot or link becomes alternating. This isotopy removes a crossing from both the $r$ row and the $q$ row and adds a single new crossing. Thus we reduce this part of the diagram from having $|r| + |q|$ crossings in a non-alternating form to having $(|r| - 1) + (|q| - 1) + 1$ crossings in an alternating form. Similarly, for the middle and left-hand side of the diagram, a non-alternating diagram having $|r| + |q| + |u|$ crossings is reduced to an alternating for having $(|r| - 1) + (|u| - 1) + 1$ crossings. So overall, our original non-alternating diagram having $|r| + |q| + |u|$ crossings is reduced by an isotopy of two strand movements to a reduced alternating diagram having $(|r| - 1) + (|q| - 1) + (|u| - 1) + 2 = n$ crossings. Note that we can also change non-alternating diagrams to reduced Montesinos diagrams using strand movements like these.

**Case 3. $K$ cannot be isotoped to either a reduced alternating or reduced Montesinos diagram.** There are also cases where we cannot obtain a reduced alternating or reduced Montesinos diagram via strand movements of $K$. We describe a specific example illustrated in figure 26. Let $K$ be a knot or link diagram in our family $F(p, q, r, s, t, u)$ with $t, p = 1, r > 1, s = 1, q, u < -1$. In its original form, the projection has $(r - 1) + (|u| + 1) + (|q| + 1)$ crossings. The projection on the left of figure 26 is Hara–Yamamoto because the projections (on the right) obtained by resealing the endpoints are both reduced alternating. Thus, this projection has a minimum number of crossings.

We consider 121 cases according to the values of $p, q, r, s, t$ and $u$, and show that in all but the Hara–Yamamoto case $K$, the initial diagram is isotopic to a diagram that is either reduced alternating or reduced Montesinos and hence has minimal crossing number. Since
there are so many cases, we display the results in tables 2–5 rather than discussing each case individually. We make the following notes and observations with respect to the tables.

- To compute tables 2–5, the family of knots and links $F(p, q, r, s, t, u)$ is broken down into seven smaller subfamilies, shown in figure 25. We count knots and links belonging to subfamilies denoted by $F_S(0, q, r, s, t, u)$ with $|r| > 0$, $|t| > 1$, $F_S(\pm 1, q, r, s, t, u)$ with $|r| > 1$, $F_S(p, q, r, s, t, u)$ with $|t| > 1$. $F_S(p, q, r, s, t, u)$ with $|t|, |r|, |p| > 1$, $T(2, r, s, t, u)$ and the unlink (subfamilies illustrated with a double arrow in between indicate that they give the same knots and links, so we count only one of them).

Observe that in subfamily $F_S(\pm 1, q, r, s, t, u)$, the rows of crossings containing $u$ and $q$ crossings are interchangeable, so we treat the variables $u$ and $q$ as interchangeable. Similarly, in subfamily $F_S(\pm 1, q, r, s, t, u)$, the tangles $R_1$ and $R_2$ are interchangeable, so we treat the variables $r$ and $t$ as interchangeable and $s$ and $u$ as interchangeable. A similar consideration is given to subfamilies $F_S(0, q, r, s, t, u)$ and $F_S(p, q, r, s, t, u)$. For certain specific values of $p, q, r, s, t$ and $u$, we may obtain a trivial knot or link. However, we do not specifically exclude these cases from our tables.

- For all tables column 2 lists the form of the knot or link which has a minimal number of crossings (e.g. reduced alternating). If the knot or link is isotopic to a clasp, pretzel, or

| Values of $p, q, r, s, t, u$ for $r \geq 0$ | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|--------------------------------------------|-----------------------|--------------|-------------------------------------|-------------------------------|
| $p = 0, r \geq r \geq 2, u, s \leq -1$     | Reduced               | 0            | $t + |u| + r + |s|$                     | $n^3$                        |
| $p = 0, t, r \geq 2, u, s \geq 1$         | Reduced alternating   | 2            | $(t - 1) + (u - 1) + (r - 1) + (s - 1) + 2$ | $n^3$                        |
| $p = 0, t \leq -2, r \geq 2, u, s \geq 1$ | Reduced alternating   | 1            | $(r - 1) + (s - 1)$ + $|t| + u + 1$ | $2n^3$                      |
| $p = 0, s \geq 1, u \leq -1, \text{ord} |u| < |s|$                     | Reduced alternating | $|u| + 1?$ | $|t| + r + (s - |u|) - 1?$ | $4n^2$                      |
| $p = 0, s = -u$                            | $T(2, t + r)$         | $|s|$        | $|t + r|$                          | 1                            |
| $p = 0, s, u = 0$                          | $T(2, t + r)$         | 0            | $|t + r|$                          | 1                            |
| $p = 0, r = 1, t = \pm 1$                 | $T(2, (u \pm 1) + (s + 1))$ | 0        | $(u \pm 1) + (s + 1)$ | 1                            |
| $p = 0, r = 1$                             | $C(t, u + s + 1)$     | 1?           | $|t| + |u + s + 1| - 1$ | $4n$                        |
| $p = 0, u = 0, t, r \geq 2, s \geq 1$     | Reduced alternating   | 2            | $(t - 1) + (r - 1) + (s - 2) + 2$   | $n^2$                      |
| $p = 0, u = 0, t \leq -2, r \geq 2, s \leq -1$ | Reduced alternating | 1            | $(|t| - 1) + r + (|s| - 1) + 1$ | $n^2$                      |
| $p = 0, u = 0, t \leq -2, r \geq 2, s \geq 1$ | Reduced alternating | 1            | $|t| + (r - 1) + (s - 1) + 1$ | $n^2$                      |
| $p = 0, u = 0, t, r \geq 2, s \leq -1$    | Reduced alternating   | 0            | $t + r + |s|$                      | $n^2$                      |
| $p = 0, u = 0, t$                          | $T(2, r)$             | 0            | $|t|$                              | 1                            |
| $p, r, t = 0$                              | Unlink                | 0            | 0                                  | 0                            |
| $p, r, t = \pm 1$                          | $K(u \pm 1, s \pm 1, q \pm 1)$ | 0        | $(|u \pm 1|) + (|s \pm 1|) + (|q \pm 1|)$ | $8n^2$                      |
Values of \( p, q, r, s, t, u \) for \( r \geq 0 \)

| Values of \( p, q, r, s, t, u \) for \( r \geq 0 \) | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|---|---|---|---|---|
| \( p, t = \pm 1, u, q = 0 \) | \( C(r, s) + O \) | 1? | \(|r| + |s| - 1?\) | 4n |
| \( p, t = \pm 1, r = 1 \) | \( K(u, s + 1, q) \) | 0 | \(|u| + |s + 1| + |q|\) | 4n^2 |
| \( p, t = \pm 1, r = 1, s = 1 \) | \( T(2, u) \) | 0 | \(|u| + |q|\) | 2n |
| \( p, t = \pm 1, r > 1, q = 0 \) | \( T(2, u) \) | 1? | \(|u| + |r + |s| - 1?\) | 4n^2 |
| \( p, t = \pm 1, r > 1, uq = -1 \) | \( T(2, r) \) | 0 | \( r\) | 1 |
| \( p, t = \pm 1, r > 1, uq = \) | \( C(\pm 2, r) \) | 0 | \( 2 + r - 1?\) | 2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \( u + (r - 1) + (s - 1) + 2 n^2\) | |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \( r + (-s - 1) + 2\) | n |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 2 | \(-u + (r - 1) + (s - 2) + 2\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 0 | \(-u - q + r - s\) | n^3 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 2 | \((u - 1) + (q - 1) + (r - 2) + 2\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(-u + (q - 1) + (r - 1) + 1\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(-u + (q + s)\) | |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(|r + u + 1|\) | 1 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(u + r + (-s - 1) + 1\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \((-u - 2) + r + (-s - 2) + 1\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(u + q + (r - 1) + 1\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \((-u - 1) + q + (r - 1)\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 2 | \((-u-1)+(r-1)+(s+1)+2\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \((u - 1) + r - s + 1\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Trivial | 2 | \(0 \neq n\) | 0 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 3 | \((u - 2)+(r-1)+(s+3)+2\) | n^2 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 0 | \(|u| + |q| + r - s\) | 4n^3 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(|u| + |q| + (r - 1) + (s - 1) + 1\) | 4n^3 |
| \( p, t = \pm 1, r > 1, uq = \) | Reduced alternating | 1 | \(-u + 2 + (r - 1)\) | n |
| \( p, t = \pm 1, r > 1, uq = \) | Hara- Yamamoto | 1 | \(-u + (-q - 1) + (r - 1)\) | n^2 |

Table 3. Proof of theorem 4: the minimal crossing forms of knots and links in subfamily \( F_S(\pm 1, q, r, s, \pm 1, u) \) illustrated in figure 25.
Table 4. Proof of theorem 4: the minimal crossing forms of knots and links in subfamily $F_{S_3}(\pm 1, q, r, s, t, u)$ illustrated in figure 25.

| Values of $p, q, r, s, t, u$ for $r \geq 0$ | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|-------------------------------------------|-----------------------|---------------|-------------------------------------|-------------------------------|
| $p = 1, t, r \geq 2, q = -1, -1 \leq u, s \leq 0$, not both $u, s = 0$ | Reduced alternating | 0 | $|r| + |s| + |t| + |u| + (q)(\pm 1)$ | $n^4$ |
| $p = 1, t, r \geq 2, u, s, q \leq -1$ | Reduced Montesinos | 0 | $|r| + |s| + |t| + |u| + (q)(\pm 1)$ | $n^4$ |
| $p = 1, t, r \geq 2, u, s, q = 1$ | Reduced alternating | 2 | $(r - 1) + (t - 1) + 3$ | $n$ |
| $p = 1, t, r \geq 2, u = 0, s, q = 1$ | Reduced alternating | 1 | $(r - 1) + (s - 1) + t + 1$ | $2n^2$ |
| $p = 1, t, r \geq 2, u, s, q > 1$ | Reduced alternating | 2 | $(r - 1) + (t - 1) + (s - 1) + (u - 1) + 2$ | $n^4$ |
| $p = 1, t, r \geq 2, -1 \leq u, s \leq 0, q = 1$, not both $u, s = 0$ | Reduced alternating | 2 | $(r - 1) + (t - 1) + 1$ | $n$ |
| $p = 1, t, r \geq 2, u, s \leq -2, q \geq 2$ | Reduced alternating | 1 | $r + |t| + u + |s| + q$ | $n^4$ |
| $p = 1, t \leq -2, r \geq 2, 0 \leq u, s \leq 1, -1 \leq s \leq -1, q = 0$ | Reduced alternating | 1 | $r + |t| + u + |s|$ | $4n^3$ |
| $p = 1, t, s, q \leq -2, r, u \geq 2$ | Reduced Montesinos | 0 | $r + |t| + u + |s| + |q|$ | $4n^4$ |
| $p = 1, r \geq 2, t \leq -2, -1 \leq u, s \leq 0, q = 1$ | Reduced Montesinos | 1 | $r + |t| + (|u| - 1) + |s| + 1$ | $2n^3$ |
| $p = 1, r \geq 2, t \leq -2, u, s \leq -2, q \geq 2$ | Reduced Montesinos | 1 | $r + (|t| - 1) + (|u| - 1) + |s| + q + 1$ | $2n^4$ |
| $p = 1, t, r \geq 2, u, s = 0, q = -1$ | Reduced alternating | 1 | $(r - 1) + (t - 1) + 1$ | $2n$ |
| $p = 1, t, r \geq 2, u = 1, s, q = -1$ | Reduced alternating | 2 | $(r - 1) + (t - 2) + 1$ | $2n$ |
| $p = 1, t, r, u \geq 2, q, s \leq -2$ | Reduced Montesinos | 1 | $r + (t - 1) + (u - 1) + |s| + |q|$ | $2n^3$ |
| $p = 1, r \geq 2, t \leq -2, u = 0, s = 1, q = 1$ | Reduced alternating | 1 | $|t| + r$ | $2n$ |
| $p = 1, r \geq 2, t \leq -2, u, s = 1, q = -1$ | Reduced alternating | 2 | $|t| + r - 1$ | $n$ |
Table 4. (Continued.)

| Values of $p, q, r, s, t, u$ for $r \geq 0$ | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|---------------------------------------------|----------------------|--------------|--------------------------------------|-------------------------------|
| $p = 1, r, u, s \geq 2, t, q \leq -1$      | Reduced Montesinos   | 1            | $(r - 1) + (s - 1) + |t| + u + |q| + 1$ | $n^4$                        |
| $p = 1, t, r \geq 2, u = 0, q = 1, s = -1$ | Reduced alternating  | 0            | $t + r + 2$                      | $2n$                         |
| $p = 1, t, r \geq 2, u, q = 1, s = -1$    | Reduced alternating  | 2            | $(t - 1) + r + 2$               | $2n$                         |
| $p = 1, t, r, u, q \geq 2, s \leq -1$     | Reduced Montesinos   | 1            | $(t - 1) + (u - 1) + r + |s| + |q| + 1$ | $2n^3$                       |
| $p = 1, r \geq 2, t \leq -2, u = 0, q \leq 1, s = 1, q = 1$ | Reduced alternating | 0            | $(|t| + r + 2$               | $2n$                         |
| $p = 1, r \geq 2, t \leq -2, u, s, q \leq -1$ | Reduced Montesinos   | 2            | $(|t| - 1) + (u - 1) + |s| + |q| + 1$ | $2n^4$                       |
| $p = 1, t, r \geq 2, u = 1, s = 0, q = -1$ | Reduced alternating  | 1            | $(t - 1) + r$                      | $2n$                         |
| $p = 1, t, r \geq 2, u, s = 1, q = -1$    | Reduced alternating  | 2            | $(t - 1) + (r - 1) + 1$                  | $n$                           |
| $p = 1, t, r, s, u \geq 2, q \leq -1$     | Reduced Montesinos   | 2            | $(t - 1)+(r - 1)+(u - 1) + (s - 1) + |q| + 1$ | $n^4$                        |
| $p = 1, r \geq 2, t \leq -2, 0 \leq u \leq 1, s = -1, q = 1$ | Reduced alternating | 2            | $(t - 2) + (r - 1) + 1$               | $2n$                         |
| $p = 1, r, u \geq 2, t \leq -2, q \geq 1, s = -1$ | Reduced Montesinos   | 0            | $r + u + q + |t| + |s|$                   | $2n^4$                       |
| $p = 1, r = s = 1$                         | $T(2, q)\#C(t, u)$ | 1?           | $|q| + 1 + (|t| - 1? + (u| - 1?) + (|s| - 1?) + (|t| - 1?) + (s - 1) + |q| + 1$ | $4n^2$                       |
| $p = 1, q = 0$                             | $C(r, s)\#C(t, u)$ | 2?           | $(|r| - 1?) + (|s| - 1?) + (|t| - 1?) + (u - 1) + |q| + 1$ | $8n^3$                       |
| $p = 1, q = 0, u s = -1$                   | $T(2, r)\#T(2, t)$ | 2            | $|t| + |r| + 1$                      | $2n$                         |
| $p = 1, q, s = 0$                          | $T(2, r)\#C(t, u)$ | 1?           | $(|t| - 1?) + (|u| - 1?) + (|s| - 1?) + (|t| - 1?) + (|s| - 1?) + (|r| + 1) + 1$ | $4n^2$                       |
| $p = 1, u, s = 0$                          | $C(t + r, q)$        | 1?           | $(|t + r| - 1?) + (|u| - 1?) + (|s| - 1?) + (|q| + 1 - 1?) + 1$ | $4n$                         |
| $p = 1, u, s, q = 0$                       | $T(2, t + r)$        | 0            | $|t + r| + 1$                      | $1$                           |
| $p = 1, t, r = 0$                          | unknot              | $|q|$        | 0                                   | 0                             |
Table 5.  Proof of theorem 4: the minimal crossing forms of knots and links in subfamily $F_{1n}$ $(p,q,r,s,t,u)$ illustrated in figure 25.

| Values of $p,q,r,s,t,u$ for $r \geq 0$ | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|----------------------------------------|------------------------|---------------|--------------------------------------|-------------------------------|
| $r,t,p \geq 2, s \leq 1 \leq u,s,q \leq u, t,p \geq 0,$ no two of $u,s,q =$ 0 | Reduced alternating 0 | 0 | $t+|u|+|r+s|+p+|q|$ | $2n^2$ |
| $r,t,p \geq 2, s \leq -1 | Reduced Montesinos 0 | 0 | $t+|u|+|r+s|+p+|q|$ | $2n^5$ |
| $r,t,p \geq 2, s \leq 0 \leq u, t,p \geq 0,$ no two of $u,s,q =$ 0 | Reduced alternating 3 | 3 | $(t-1)+(|u|-1)+(|r|) \leq 1, -1 \leq s \leq 0 no two of $u,s,q =$ 0 | Reduced alternating 0 | 0 | $|t|+|p|+|q|+r+u+q$ | $2n^2$ |
| Reduced alternating 0 | 0 | $|u|+|p|+|q|+r+t+q$ | $4n^3$ |
| Reduced alternating 0 | 0 | $|u|+|p|+|q|+r+t+q$ | $4n^2$ |
| Reduced alternating 1 | 1 | $t+(r-1)+(s-1)+|p|+3+q$ | $4n^5$ |
| Reduced alternating 2 | 2 | $(t-1)+(u-1)+(r-1)+(s-1)+|p|+2+q$ | $4n^5$ |
| Reduced alternating 2 | 2 | $(|r|-1)+(u-1)+(|s|-1)+(|p|-1)+(|q|-1)+s+2+r$ | $2n^3$ |
| Reduced alternating 1 | 1 | $|t|+|u|+(|p|-1)+(|q|-1)+s+2+r$ | $2n^5$ |
| Reduced alternating 1 | 1 | $(r-1)+(s-1)+|p|+q+|t|+u+1$ | $2n^3$ |
| Reduced alternating 0 | 0 | $r+|p|+q+|t|+u$ | $2n^5$ |
| Values of $p, q, r, s, t, u$ for $r \geq 0$ | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|-------------------------------------------|----------------------|--------------|-------------------------------------|-------------------------------|
| $r \geq 2, t, p \leq 0$                  | Reduced alternating  | 1            | $(|p| - 1) + (|q| - 1) + |s| + r + |t| + 1$                          | $4n^5$                        |
| $-2, q \leq 1, -1 \leq s, u \leq 0$     | Reduced Montesinos 2 | 2            | $(t - 1) + (u - 1) + (r - 1) + (s - 1) + p + |q| + 2$                         | $3n^5$                        |
| $p, r, t \geq 2, u, s \geq 0, q \leq 0$ | Reduced Montesinos 1 | 1            | $(t - 1) + (u - 1) + |r| + |s| + p + |q| + 1$                          | $2n^5$                        |
| $0, no two of $u, s, q = 0$              | Reduced Montesinos 3 | 3            | $(|p| - 1) + (|u| - 1) + (p - 1) + (q - 1) + (r - 1) + (s - 1) + 3$ | $2n^5$                        |
| $t, r \geq 2, p \leq 0, u, q \leq 0$   | Reduced Montesinos 2 | 2            | $t + u + (|p| - 1) + (|q| - 1) + (r - 1) + (s - 1) + 2$ | $2n^5$                        |
| $0, no two of $u, s, q = 0$              | Reduced Montesinos 3 | 3            | $r + s + (|p| - 1) + (|q| - 1) + (t - 1) + (u - 1) + 2$ | $2n^5$                        |
| $r \geq 2, t, p \leq 0$                 | Reduced Montesinos 1 | 1            | $|p| + |q| + (|t| - 1) + (|u| - 1) + r + |s| + 1$ | $2n^5$                        |
| $-2, u, s \geq 0, q \leq 0$             | Reduced Montesinos 3 | 3            | $(|p| - 1) + (|u| - 1) + (|p| - 1) + (|q| - 1) + (r - 1) + (s - 1) + 3$ | $2n^5$                        |
Table 5. (Continued.)

| Values of \( p, q, r, s, t, u \) for \( r \geq 0 \) | Minimal crossing form | Strands moved | MCN as a sum of non-negative integers | Upper bound on number of links |
|---------------------------------------------------|------------------------|---------------|--------------------------------------|------------------------------|
| \( r \geq 2, t, p \leq -2, s, u, q \leq 0, \text{no two of } u, s, q = 0 \) | Reduced Montesinos | 2 | \((|r| - 1) + (|u| - 1) + (|p| - 1) + (|q| - 1) + r + s + 2\) | \(n^3\) |
| \( r = 1, s = -1 \) | \(C(t, u)\#C(p, q)\) | 2? | \((|r| - 1)? + (|u| - 1)? + (|p| - 1)? + (|q| - 1)? + \text{?}\) | \(8n^2\) |
| \( r = 1, s = -1, p = \pm 1 \) | \(T(2, q)\#C(t, u)\) | 1? | \((|r| - 1)? + (|u| - 1)? + (|q|\pm 1)? + \text{?}\) | \(8n^2\) |
| \( r = 1, s = -1, p = \pm 1, q = \mp 2 \) | \(T(2, u)\#T(2, q)\) | 0 | \((|u| \pm 1) + (|q| \pm 1)\) | \(2n\) |

torus knot or link or a composition of any of these, we list the specific form. Also, if one of the knots or links contains a trivial component, we use the shorthand \(+O\) to indicate this. Column 3 shows the number of strand movements needed to achieve a diagram with minimal number of crossings. We write an expression with \((\pm \#)\) at the end to indicate that there may be \# more or less number of strand movements, depending on the values of the relevant variables. Column 4 shows the MCN of the corresponding reduced alternating or reduced Montesinos conformation. The MCN is listed as an unsimplified function of \( p, q, r, s, t, u \) and \( v \) to help the reader recreate the isotopy taking the original form to the minimal crossing form. As a consequence, in column 4 we write an expression with \((\pm \#)\) at the end to indicate that the MCN may be \# smaller or bigger. For example, when the minimal crossing form of the knot is a clasp knot \( C(r, s) \), if we do not know the signs of \( r \) and \( s \), on column 2, we write an expression with \(+1?\) and in column 3 we write an expression with \(-1?\), see figure 27. In column 5 we obtain the upper bounds for the number of links in each case by expressing MCN\(=n\) as a sum of non-negative integers. This enables us to find an upper bound for the number of knots and links with MCN\(=n\) in each case. Note that the upper bounds given are intended to be simple rather than as small as possible. In particular, a number of our cases overlap, and thus some knots and links are counted more than once.

- For all tables we consider a knot or link and its mirror image to be of the same link type, and hence we do not count both. Thus without loss of generality, we assume that \( r \geq 0 \).

There are 118 non-trivial cases in tables 2–5. Any knots and links appearing more than once in the tables are counted only once. Thus there are at most 111 distinct families of knots and links listed in the tables. The number of knots and links in each of these families is bounded above by \(4n^5\) (in fact, for most of the cases there are significantly fewer than \(4n^5\) knot and link types). It follows that for a given \( n \), the number of distinct knots and links in the product family \( F(p, q, r, s, t, u) \) which have MCN\(=n\) is bounded above by \(4n^5 \times 111 = 444n^5\). In particular, the number of distinct knots and links with the form of figure 2(a) which have MCN\(=n\) grows linearly with \(n^5\). \(\square\)
We now consider product knots and links belonging to $G_1$ or $G_2$. These come about as products of recombination with a tyrosine recombinase on a recombinase-DNA complex with conformation $C$ illustrated in figure 18 and the post-recombinant conformation $B = B_6$.

**Lemma 3.** For a fixed $n$ there exists at most one knot type in $G$ with MCN equal to $n$. Similarly, for $G_2$.

**Proof.** $G_1$ is reduced alternating, and hence has minimal number of crossings. Thus, it is clear that $MCN(G_1) = 4 + |v| = n$. Similarly, $G_2$ is reduced alternating and thus has minimal crossing number, so $MCN(G_2) = 3 + v = n$. □

We are now ready to prove theorem 4.

**Theorem 4.** The number of putative knots and links resulting from site-specific recombination on a substrate that is the twist knot $C(2, v)$ with MCN equal to $n$ grows linearly with $n^5$.

**Proof.** There are at most $111 + 2 = 113$ non-trivial, distinct families of knots and links that are putative products of site-specific recombination on a substrate that is $C(2, v)$; 111 belong to the family of small Montesinos knots and links illustrated in figure 2(a) (the number of such knots and links is bounded above by $4n^5$) and 2 belong to the families $G_1$ and $G_2$ illustrated in figures 2(b) and (c). It follows that for a given $n$, after recombination on a twist substrate, the number of distinct knots and links which have $MCN = n$ is bounded above by $4n^5 \times 113 = 452n^5$. In particular, the number of distinct knots and links that belong to the families $G_1$, $G_2$ and/or $F(p, q, r, s, t, u)$ that have $MCN = n$ grows linearly with $n^5$. □

It follows from theorem 4 that the proportion of all knots and (two-component) links which are contained in the families $F(p, q, r, s, t, u)$, $G_1$ and $G_2$ decreases exponentially as $n$ increases. Thus, for a knotted or linked product, knowing its MCN and that it belongs to one of these families allows us to significantly narrow the possibilities for its precise knot or link type. The model described herein thus provides an important step in characterizing DNA knots and links which arise as products of site-specific recombination.

6.2. Products whose MCN is one more than the substrate

We now prove a more directly applicable theorem. Site-specific recombination often increases the MCN of a knotted or linked substrate by 1, see for example table 1 in [38]. If the substrate is $C(2, v)$, with minimal crossing number $m$ and the product of a single recombination event has $MCN = m + 1$, then we can further restrict the resulting knot or link type. Recall that $MCN(C(2, v)) = 2 + |v|$ for $v < 0$ and $MCN(C(2, v)) = 1 + v$ for $v > 0$. We remark that site-specific recombination that increases the minimal crossing number of the product by 1 could result in a change in the number of components. For example, if the substrate is $C(2, 2)$ which is a one-component link (a knot) one of the possible products according to theorem 5 is $T(2, 4)$, a two-component link.

**Theorem 5.** Suppose that assumptions 1, 2, and 3 hold for a particular recombinase-DNA complex with substrate $J = C(2, v)$, $v \neq 0$ and denote $MCN(J) = n > 0$. Let $L$ be the product of a single recombination event and suppose $MCN(L) = n + 1$. Then: If $v > 0$, $L$ is either $C(2, v + 1)$, $C(2, -v)$, $C(-2, v)$, $C(-2, -(1 + v))$, $C(3, v)$, $T(2, \pm(2 + v))$, $F_{S_0}(0, q, 2, s, 2, u)$ where $u + s = v$, $F_{S_0}(\pm1, \pm1, 2, s, \pm1, u)$ where $u + s = v$ or $s \neq 0$ or $F_{S_0}(\pm1, 0, 2, s, 2, u)$ where $u + s = v$ and $s, u \neq 0$. If $v < 0$, $L$ is
either \( C(2, 2 + |v|) \), \( C(2, -(1 + |v|)) \), \( C(-2, 1 + |v|) \), \( C(-2, -(2 + |v|)) \), \( C(3, v) \), \( C(-4, v) \), \( T(2, \pm(3 + |v|)) \) or \( F_{S_5}(\pm 1, \pm 1, 2, s, \pm 1, u) \) for \( u + s = v \).

**Proof.** First note that \( n \geq 2 \). Note also that if \( v = 1 \), then \( n = 2 \), but there are no non-trivial knots with minimal crossing number equal to 2, so the substrate must be the unknot, which is considered in [37] and [38]. We exclude the case when \( v = 1 \). For \( n = 3 \), \( C(2, v) \) is the trefoil knot \( 3_1 \) (i.e. \( v = -1 \)) so \( L \) must be the figure of eight knot \( 4_1 = C(2, -2) \) or the torus link \( T(2, \pm 4) \), since these are the only knots and links with minimal crossing number equal to 4. Now assume that \( n \geq 4 \), that is \( v \leq -2 \) or \( v \geq 3 \). By assumption 1, there is a projection of \( J \) such that \( B \cap J \) has at most one crossing. Since \( J = C(2, v) \), the proof of lemma 1 shows that \( C \cap J \) has the form \( C1, C2, C3, C4 \) or \( C5 \) (figure 18). When \( C \cap J \) has form \( C1, u + s = v \). By assumption 3 and figures 5 and 16, the post-recombinant form of \( B \cap J \) is one of those illustrated in figure 16. Thus any knotted or linked product \( L \) has one of the forms illustrated in figure 17. Now, suppose that \( L \) has one of the forms illustrated when \( C \cap J \) has form \( C2, C3, C4 \) or \( C5 \). \( L \) cannot be either \( T(2, 2) \cap C(2, v) \) or \( T(2, -2) \cap C(2, v) \) because \( MCN(L) = n + 2 \). \( L \) can certainly not be a Hopf link, an unknot or \( C(2, v) \) with a trivial component, since \( MCN(L) \neq n + 1 \). Finally, \( L \) cannot be \( C(4, v) \) because if \( v > 0 \), \( MCN(L) = 3 + v \) and if \( v < 0 \), \( MCN(L) = 4 + |v| \). If \( L = C(2, n) \), then \( n = 1 + v \) or \( -v \) when \( v > 0 \) or \( n = 2 + |v| \) or \( v - 1 \) when \( v < 0 \). If \( L = C(k, v) \), then \( k = 3 \forall v, k = -2 \) for \( v > 0 \) and \( k = -4 \) for \( v < 0 \). If \( L = T(2, n) \), then for \( v > 0 \) \( n = \pm(1 + |v|) \) and for \( v < 0 \) \( n = \pm(3 + |v|) \). If \( L = F_{S_5}(0, q, 2, s, t, u) \) for some value of \( t \), then \( L \) has a projection in this product subfamily with \( t = \pm 2 \). So we can assume that \( L \) has a projection of the form \( F_{S_5}(0, q, 2, s, u) \) with \( u + s = v \). If \( t = -2 \), for \( v < 0 \) \( MCN(L) = 1 + [u + |s| + 1 = 3 + |v| = n + 1 \) so this case is possible and for \( v > 0 \) \( MCN(L) = 3 + u + 1 + s = 3 + v \neq n + 1 \) so this case is not possible. If \( t = +2 \), for \( v < 0 \) \( MCN(L) = 2 + |u| + 2 + |s| = 4 + |v| \neq n + 1 \) so this case is not possible and for \( v > 0 \) \( MCN(L) = 1 + u + 1 + s = 2 + v = n + 1 \) so this case is possible. So \( L = F_{S_5}(0, q, 2, s, -2, u) \) only for \( v < 0 \) and \( L = F(2, s, 2, u) \) for \( v > 0 \). Now suppose that \( L \) has one of the forms illustrated when \( C \cap J \) has form \( C1 \). Suppose \( L \) has a projection of the form \( F_{S_5}(\pm 1, q, 2, s, \pm 1, u) \) with \( u + s = v \). For \( v > 0 \) and \( s = 0 \), \( q \) must be 0; however,
this is isotopic to $T(2, u)$, which has $MCN = v$; thus, this is not allowed. For $v > 0$ and $s \neq 0$, $q = \pm 1$ and for $v < 0$, $q = \pm 1$. That is, for $v > 0$, $L = F_{s_1}(\pm 1, \pm 1, 2, s, \pm 1, u)$ for $s \neq 0$ and $s + u = v$, and for $v < 0$ $L = F_{s_2}(\pm 1, \pm 1, 2, s, \pm 1, u)$ for $u + s = v$. If $L$ has a projection of the form $F_{s_1}(\pm 1, q, 2, s, t, u)$ for some value of $t$, then $L$ has a projection in this product subfamily with $t = \pm 2$. Thus we now assume that $L$ has a projection of the form $F_{s_2}(\pm 1, q, 2, s, \pm 2, u)$ with $u + s = v$. If $t = 2$, for $v < 0$, $MCN(L) = 2 + |u| + 2 + |s| + |q| = 4 + |v| + |q| > n + 1$ for any value of $q$, so this case is not possible, for $v > 0$ $MCN(L) = 1 + u + 1 + s + |q| = 2 + v + |q|$, so this case is possible for $q = 0$. In this particular case, if one of $u$ or $s$ equals 0, then $MCN(L) = 3 + v + |q| > n + 1$ for any value of $q$, so $L = F(q, 2, s, u)$ only for $v > 0$ and $s, u \neq 0$. If $t = -2$, for $v > 0$ $MCN(L) = |−2| + u + 1 + s + |q| = 3 + v + |q| > n + 1$ for any value of $q$, so this case is not allowed and for $v < 0$ $MCN(L) = |−1| + |u| + 2 + |s| + |q| = 3 + |v| + |q|$ so this case is possible for $q = 0$. In this particular case, if one of $u$ or $s$ equals 0, then $MCN(L) = 4 + |v| + |q| > n + 1$ for any value of $q$, so $L = F(q, 2, s, −2, u)$ only for $v < 0$ and $s, u \neq 0$. In summary, $L = F_{s_1}(\pm 1, 0, 2, s, −2, u)$ for $v < 0$ and $L = F(0, 2, s, 2, u)$ for $v > 0$, both with $u, s \neq 0$ are the only possibilities allowed. Finally, suppose $L$ has a projection of the form $G1$. Then $v < 0$ and $MCN(L) = 4 + |v| > 3 + |v| = n + 1$; thus, $L$ cannot have this conformation. Suppose $L$ has a projection of the form $G2$; then $v > 0$ and $MCN(L) = 3 + v > 2 + v = m + 1$ and so $L$ cannot have this projection either. This completes the proof. □

7. Applications of our model

We discuss how the model developed here can be a useful tool to analyse previously uncharacterized data in a variety of settings in [39]. These applications fall into four broad categories.

- **Application 1**: our model can help determine the order of products of processive recombination.
- **Application 2**: in the common situations where the products of site-specific recombination have $MCN$ one more than the $MCN$ of the substrate, our model can help reduce the number of possibilities for these products.
- **Application 3**: our model can help predict products of processive and distributive recombination.
- **Application 4**: our model can help distinguish between products of processive and distributive recombination.

To give a flavour of how to use this model, we conclude with a simple example of applications 1 and 2.

**Application 1**. Our model can be used to help understand processive recombination mediated by a serine recombinase. Using figure 15 and table 1 which summarize the conclusions of theorem 2, we can narrow the possibilities for the sequence of products in multiple rounds of processive recombination. Suppose that for a twist knot substrate of the form $C(−2, v)$ with $v \neq 0$, experimental conditions minimize distributive recombination and the products of multiple rounds of processive recombination are twist knots, unknots (or $C(−2, s) + O$) and the connected sum of a torus knot and a twist knot $C(−2, s)T(2, m)$. Then from figure 15(g) we can determine that recombination happens from the twist knot substrate to the clap knot with a trivial component $C(−2, v) + O$, product of the first round of recombination, to the connected sum of torus knots and clap knots, product of the second round of recombination.
Moreover, any products of further rounds of recombinations are connected sums of the form $C(−2, v)\#T(2, m)$ with increasing minimal crossing number.

**Application 2.** We now demonstrate an application of theorem 5. Suppose the twist knots $C(2, 5)$ and $C(2, 7)$ (which have MCN equal to 6 and 8 respectively) are used as substrates for a site-specific recombination reaction with a tyrosine recombinase, where experimental conditions eliminate distributive recombination and products are knots and links with minimal crossing number 7 and 9. In this case the minimal crossing number is not sufficient to determine the knot type, since there are 7 knots, 8 two-component links and 1 three-component link with MCN = 7 and 49 knots, 61 two-component links and 22 three-component links with MCN = 9. However, we can use theorem 6 and table 7 to significantly reduce the number of possibilities for these products. It follows from theorem 6 that the possible seven-crossing products are $7_1$, $7_2$, $7_3$, $7_6$, $7_2^2$, $7_1^2$ or $3_1\#4_1$; and the possible nine-crossing products are $9_1$, $9_2$, $9_3$, $9_8$, $9_7$, $9_1^2$, $9_8^2$, $6_1\#3_3$ or $4_1\#5_2$. In table 7 we show how to do this. We have reduced from 16 choices for 7-noded knots to just 7, from 132 possibilities for 9-noded knots and links to just

| Products with 7 crossings | Products with 9 crossings |
|---------------------------|---------------------------|
| $C(2, 6) = 7_2^*$         | $C(2, 8) = 9_1^*$         |
| $C(2, −5) = 7_2^*$        | $C(2, −7) = 9_2^*$        |
| $C(−2, 5) = 7_2^*$        | $C(−2, 7) = 9_3^*$        |
| $C(−2, −6) = 7_2^*$       | $C(−2, −8) = 9_8^*$       |
| $C(3, 5) = 5_1^2$         | $C(3, 7) = 9_1^2$         |
| $T(2, ±7) = 7_1^*$        | $T(2, ±9) = 9_1^*$        |
| $F_5(0, q, 2, 1, 2, 4) = 7_3^*$ | $F_5(0, q, 2, 1, 2, 6) = 9_1^2$ * |
| $F_5(0, q, 2, 2, 2, 3) = 7_3^*$ | $F_5(0, q, 2, 2, 2, 5) = 9_1^2$ * |
| $F_5(±1, 1, 2, ±1, 1, 4) = 7_3^*$ | $F_5(0, q, 2, 3, 4) = 9_1^2$ * |
| $F_5(±1, −1, 2, ±1, ±1, 4) = 5_1$ | $F_5(±1, 1, 2, ±1, 1, 6) = 9_1^*$ |
| $F_5(±1, 1, 2, ±1, ±1, 3) = 7_2^*$ | $F_5(±1, −1, 2, ±1, 1, 6) = 7_1$ |
| $F_5(±1, −1, 2, ±1, ±1, 3) = \text{unlink}$ | $F_5(±1, 1, 2, ±1, 1, 5) = 7_2^*$ |
| $F_5(±1, 1, 2, ±1, ±1, 2) = \text{unknot}$ | $F_5(±1, −1, 2, ±1, ±1, 4) = 5_2$ |
| $F_5(±1, −1, 2, ±1, ±1, 1) = 7_2^*$ | $F_5(±1, −1, 2, ±1, ±1, 4) = 5_2$ |
| $F_5(±1, 1, 2, ±1, ±1, 1) = \text{Hopf link}$ | $F_5(±1, 1, 2, ±1, ±1, 3) = 7_2^*$ |
| $F_5(±1, −1, 2, ±1, ±1, 3) = 5_1^2$ | $F_5(±1, 1, 2, ±1, ±1, 2) = 9_8^*$ |
| $F_5(±1, 1, 2, ±1, ±1, 2) = 4_1$ | $F_5(±1, −1, 2, ±1, ±1, 2) = 4_1$ |
| $F_5(±1, 1, 2, ±1, ±1, 1) = 9_1^2$ * | $F_5(±1, −1, 2, ±1, ±1, 1) = \text{Hopf link}$ |
| $F_5(±1, 1, 2, ±1, ±1, 1) = \text{Hopf link}$ | $F_5(±1, 0, ±1, 2, 2, 5) = 6_1\#3_3^*$ |
| $F_5(±1, 0, 2, 2, 2, 3) = 3_1\#4_1^*$ | $F_5(±1, 0, 2, 2, 2, 4) = 4_1\#5_2^*$ |

| Products with 7 crossings | Products with 9 crossings |
|---------------------------|---------------------------|
| $F_5(±1, 0, 2, 1, 2, 4) = 5_2$ | $F_5(±1, 0, 2, 2, 2, 5) = 6_1\#3_3^*$ |
| $F_5(±1, 0, 2, 2, 2, 3) = 3_1\#4_1^*$ | $F_5(±1, 0, 2, 3, 2, 4) = 4_1\#5_2^*$ |
9 possibilities. Thus, theorem 6 can help us to significantly reduce the knot and link type of products of site-specific recombination that add one crossing to the substrate.

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