CLASSIFICATION OF TANGLE SOLUTIONS FOR INTEGRASES, 
A PROTEIN FAMILY THAT CHANGES DNA TOPOLOGY

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Abstract

Integrase proteins acting on circular double-stranded DNA often change its topology by transforming unknotted circles into torus knots and links. Two systems of tangle equations—corresponding to the two initial DNA sequences—arise when modelling this transformation: direct and inverted.

With no a priori assumptions on the constituent tangles, we utilize Dehn surgery arguments to completely classify the tangle solutions for each of the two systems. A key step is to combine work of our previous paper [7] with recent results of Kronheimer, Mrowka, Ozsváth and Szabó [31] and Ernst [18] to show a certain prime tangle must in fact be a generalized Montesinos tangle.

These tangle solutions are divided into three classes, common to both systems, plus a fourth class for the inverted system that contains the sole generalized Montesinos tangle. We discuss the possible biological implications of our classification, and of this novel solution.

1. Introduction

DNA is often circular, and so can be knotted or linked [34]. To aid in essential cellular tasks, many proteins have thus evolved to manipulate the geometry and topology of DNA. Important examples of these tasks include: replication (creating 2 copies from one double strand of DNA), inversion (inverting a subsequence of DNA), and integration and deletion (inserting and deleting DNA sequences into/from other DNA segments). For instance, certain proteins invert crossings of two double strands of DNA to unknot or unlink DNA [3].

It is important for biologists to understand how these proteins operate. So a model is often generated based on data obtained by experimentally probing a particular system. For example, if a particular protein can knot or link circular DNA, then by running DNA through a gel, one can determine the crossing number of the DNA knots or links. One can then use electron microscopy to identify the exact knot or link type [32].

By modelling different regions of the (possibly knotted) DNA molecule as tangles, we can describe the protein’s action as a change in one of the constituent tangles. Mathematicians can help biologists by finding all tangle combinations that may explain the protein’s action. Some of these mathematically possible solutions can then be eliminated through biological considerations.

Based on the biological work of Wasserman and Cozzarelli [50, 51], Ernst and Summers developed the tangle model to describe and make predictions—later experimentally verified—about how the protein Th3 interacts with DNA [20]. The tangle model has since been used to determine specific features of a wide variety of protein-DNA interactions (see for example, [7, 11, 13, 15, 17, 19, 21, 24, 42, 44, 48, 52]).
1.1. The integrase family of recombinases. We will focus on one particular family of proteins that affects DNA topology: the integrase family of site-specific recombinases, also known as the tyrosine recombinases. For an overview of this family, see the review of Grainge and Jayaram [25]. The integrase family includes Flp, λ Int, and Cre. Integrases are involved in a wide variety of biological activities, including integrating (i.e., inserting) a virus’ DNA into a host cell’s DNA. Integrase proteins have a common biochemical mechanism and share a subsequences of amino acids. Given varying numbers of DNA axis self-crossings (supercoils) these proteins can transform unknotted circular DNA into a variety of torus knots or links [25].

Members of the other family of site-specific recombinases, the serine recombinases, act very differently. These proteins, such as Tn3, require a fixed number of supercoils. The protein then cuts and rearranges the DNA (occasionally multiple times) before releasing it. For several serine recombinases, it has been possible to completely solve the tangle model equations (see [20, 21, 48]). In contrast, for integrase proteins, the unbound DNA’s configuration varies from molecule to molecule, depending on the degree of supercoiling. Further, these proteins act only once before releasing the DNA. So, to model the varying configurations, and its effects on the resulting DNA products after a single round of recombination, we must use a larger number of tangles than needed for a serine recombinase. Rather than four fixed tangles, a solution to the (direct or inverted) system of equations consists of three fixed tangles, $P$, $R$ and $O_c$, and an indexed family of tangles, $O_{kf}$. In general, there are an infinite number of solutions to such a system. The increased complexity of the tangle model for the integrase family has thus far prevented a full solution to the tangle model. Previous work found solutions by making several assumptions which are thought to be biologically reasonable and which lead to considerable mathematical simplification [11, 24, 44].

We previously considered the tangle model for a generic member of the integrase family [7] without any a priori assumptions on the constituent tangles. We gave and exemplified three (topologically motivated) classes of solutions for $O_{kf}$, $O_c$ and $P$, all of which are valid for both systems. We then developed Dehn Surgery arguments to eliminate all (in the direct system) or all but one (in the inverted system) other possibilities.

In this paper, we now complete the classification of all possible solutions to the systems of equations arising from both the direct and inverted cases. In particular, we utilize recent work of Kronheimer, Mrowka, Ozsváth and Szabó [31], and Ernst [18, 19] to determine the fourth solution in the inverted case. In addition, we determine all possible solutions for $R$ in both the direct and inverted settings. Combining the results derived below with our previous work [7], we obtain a complete classification of all solutions to the equations arising from either of the direct or inverted system:

**Theorem 1.** There are three classes of solutions to the equations that model integrase-mediated DNA recombination

\[
N(O_{kf}^k + O_c + P) = b(1, 1) = \text{unknot for } k \in \{0, 1, 2, 3\}
\]

\[
N(O_{kf}^k + O_c + R) = b(2k, 1) = T_{2k, 2} \text{ torus link for direct sites}
\]

or

\[
N(O_{kf}^k + O_c + R) = b(2k + 1, 1) = T_{2k+1, 2} \text{ torus knot for inverted sites}
\]

1. $P = (\infty)$, $O_c$, $O_{kf}^k$ and $R$ are integral tangles.

2. $P$ and $O_c$ are integral, $O_{kf}^k = (\infty)$ for at most one value of $k$, integral for at most two values of $k$ and otherwise is vertical or the sum of a vertical plus an integral tangle. $R = (\infty)$, integral or vertical plus integral. However if
$O_k^f = (\infty)$ for some $k$, then $R$ cannot be $(\infty)$, nor can it be integral unless $k = 0$.

3. $P$ and $R$ are strictly rational, $O_c$ is integral, and $O_k^f$ is integral for at most value of $k$ and otherwise strictly rational.

For the direct system, there are no other classes of solutions.

For the inverted system, there is precisely one additional class of solutions:

4. $P = \pm(p)$, $R = \pm(1+p)$, $O_c$ is integral, $O_k^f$ is the prime Montesinos tangle $\mp(\frac{1}{2}, \frac{2}{3}, p-1)$ and $O_j^{k\neq 2} = \pm(-p + \frac{1}{2k})$ or $\mp(p + \frac{1}{2k+2})$, where $p \in \{0, 1\}$.

Theorem 1 is a consequence of Theorem 1 in [7] and Theorem 4.8, as well as Theorems 5.2, 5.3 and 5.4. Note that $O_c$ is always integral. Also note that the only thing that keeps the solutions in Class 4 from being solutions in Class 2 is tangle $O_k^f$.

Note that solutions are unique up to compensating negative and positive vertical sums of vertical tangles or horizontal sums of horizontal tangles. See Section 2 for a discussion of these equivalence classes.

The paper is organized as follows: In Section 2 we review some basic facts about tangles, four-plats and their corresponding double branch covers. In Section 3 we provide the biological motivation and background for our work: the action of an integrase on DNA with either inverted or direct repeats. We also present the generalization of Ernst and Sumners’ mathematical model in terms of tangles and four-plats [20]. In Section 4 we focus on the inverted system. Here we harness recent results of Kronheimer, Mrowka, Ozsváth and Szabó [31] and Ernst [18, 19] to limit the possibilities for $O_k^f$, and then determine the complete fourth solution.

In Section 5 we present examples of all classes of solutions to these tangle equations, including the novel inverted solution. We conclude with some remarks on the biological relevance of our model and solutions, as well as possible directions for future (biological and mathematical) work.

2. TANGLES, FOUR-PLATS AND THEIR DOUBLE BRANCH COVERS

We begin by recalling a few elementary facts about tangles. (For a more comprehensive introduction to tangles, see [7] and [22]). A tangle $T$ is a pair $(B^3, t)$, where $B^3$ is a 3-ball with a given boundary parametrization with four distinguished boundary points labelled NW, NE, SW, SE, and $t$ consists of a pair of properly embedded unoriented arcs with endpoints NW, NE, SW and SE. We say two tangles $A$ and $B$ are equivalent if there exists an isotopy taking $A$ to $B$, which remains the identity on $\partial A$.

Tangles can be divided into three mutually exclusive families: locally knotted, rational and prime.

A tangle is locally knotted if there exists a sphere in $B^3$ meeting $t$ transversely in 2 points such that the 2-ball bounded by the sphere intersects $t$ in a knotted spanning arc. Locally knotted tangles have reducible double branch covers (branching over $t$).

We note that locally knotted tangles do not occur in our context since the DNA molecule starts off unknotted, $P$, $O_c$ and $O_k^f$ cannot be locally knotted. Suppose $R$ were locally knotted, with $S \subset R$ be a sphere bounding a ball $B$ containing the locally knotted arc. Then, since every product is a prime knot, $N(O_k^f + O_c + R) - B$ must be an unknotted arc, for all $k$. But this implies that $N(O_j^f + O_c + R) = N(O_f^j + O_c + R)$ for $j \neq k$, a contradiction. So none of the constituent tangles are locally knotted.

Rational tangles are the second family; they are so called because their equivalence classes are in one-to-one correspondence with the extended rational numbers...
A tangle whose corresponding rational number is \( \frac{p}{q} \) will be denoted by \( \left( \frac{p}{q} \right) \). Rational tangles are formed by an alternating series of horizontal and vertical half-twists of two (initially untwisted) parallel arcs (and hence are freely isotopic to them). Any continued fraction decomposition of \( \frac{p}{q} = a_n + \frac{1}{a_{n-1} + \ldots + (1/a_1)} \) yields a finite list of integers \( [a_1, \ldots, a_n] \) which tell us how to twist the strands around each other to get a diagram of the tangle. The \((0)\) tangle corresponds to two untwisted horizontal arcs (one joining NE to NW and one joining SE to SW), whereas the \((\infty)\) tangle corresponds to two untwisted vertical arcs. The double branch cover of a rational tangle is a solid torus.

All locally unknotted, non-rational tangles are prime. The double branch cover branched over \( t \) of a prime tangle is irreducible and has incompressible boundary \([33]\). Bleiler demonstrated that the minimal prime tangle has a minimal projection with five crossings \([4]\).

There are several operations one can perform on tangles. We concentrate on three. The first operation forms a knot or 2-component link from a given tangle \( A \): the numerator closure, \( N(A) \). This adds an unknotted arc joining the northern endpoints, and another unknotted arc joining the southern endpoints, or equivalently, the boundary of \( A \) and \((0)\) are identified so that \( E_A \) is identified with \( E_{(0)} \) for \( E \in \{NE, NW, SE, SW\} \).

The second operation, tangle sum, takes a pair of tangles \( A \) and \( B \), and, under certain restrictions, yields a third tangle, \( A + B \), by identifying the eastern hemispheric boundary disk of \( A \) with the western one of \( B \) in such a way that \( NE_A \) is identified with \( NW_B \) and \( SE_A \) is identified with \( SW_B \). Note that the \((0)\) tangle is the identity under this operation: \( A + (0) = A \). Beware that under tangle addition, we cannot distinguish between \( A + (p) \) added to \( B + (-p) \) and \( A \) added to \( B \). Thus although tangle summands are written in their simplest form, they are unique only up to an arbitrary number of compensating positive and negative horizontal twists.

The last operation is the vertical sum \( \ast \), which takes two tangles \( A \) and \( B \) and, under certain restrictions, yields a third tangle, \( A \ast B \), by identifying the southern hemispheric boundary disk of \( A \) with the northern hemispheric boundary disk of \( B \) in such a way that \( SE_A \) is identified with \( NE_B \) and \( SW_A \) is identified with \( NW_B \). Note that under the numerator closure operation, given two tangles \( A \) and \( B \), although \( (A \ast (1/n)) + B \neq A + (B \ast (1/n)) \) in fact \( N((A \ast (1/n)) + B) \simeq N((A \ast (1/n)) + B) \). Also, \( N((A \ast (1/n)) + (B \ast (-1/n))) \simeq N(A + B) \). As with horizontal tangle sums, we cannot distinguish between these two cases. Thus although tangle solutions are written in the simplest form for a given equivalence class, they are unique only up to an arbitrary number of compensating positive and negative vertical twists.

A particular class of prime tangles is obtained by tangle sum of rational tangles and will be of interest to us: Montesinos tangles \([36]\). We will use the notation \( \left( \frac{a_1}{b_1}, \frac{a_2}{b_2}, \ldots, \frac{a_n}{b_n} \right) \) to denote the Montesinos tangle obtained by the tangle sum of the rational (possibly integral) tangles \( \left( \frac{a_1}{b_1} \right), \left( \frac{a_2}{b_2} \right), \ldots, \left( \frac{a_n}{b_n} \right) \). A tangle that is ambient isotopic to a sum of rational tangles called a generalized Montesinos tangle. Since the sum of a rational tangle and an integral tangle yields a rational tangle \([39]\), a Montesinos (non-rational) tangle must have at least two non-integral summands.

The numerator closure of a rational tangle yields a four-plat, a knot or 2-component link that admits a projection consisting of a braid on 4 strings, with one strand free of crossings \([1]\). Given two rational tangles \( \left( \frac{p}{q} \right) \) and \( \left( \frac{p'}{q'} \right) \) then \( N \left( \frac{p}{q} \right) = N \left( \frac{p'}{q'} \right) \) iff \( p = p' \) and \( q \equiv q' \mod p \). Thus given a 4-plat, we can write...
it as the numerator closure of a rational tangle that is unique up to the relationship above. (See [29] for a classification of rational knots.) Schubert showed that all four-plats are prime knots [43]. A four-plat obtained by the numerator closure of \((\frac{p}{q})\) is written as \(b(p, q)\). For example, we can write the unknot as \(b(1, 1)\), and the trefoil as \(b(3, 1)\).

2.1. Double Branch Covers. If \(T\) is a tangle, then \(\tilde{T}\) will mean the double cover of \(B^3\), branched over \(t\). In general, we will write \(dbc(K)\) to denote the three-manifold that is the double cover of \(S^3\) branched over the set \(K\). We now turn our attention to the (compact, connected and orientable) three-manifolds that arise as double branch covers of tangles or four-plats.

If \(P\) is a rational tangle, then \(\tilde{P}\) is a solid torus, which we will denote by \(V_P\). Schubert showed that \(dbc(b(p, q))\) is the lens space \(L(p, q)\). Two four-plats \(b(p, q)\) and \(b(p', q')\) are equivalent if and only if their corresponding double branch covers, the lens spaces \(L(p, q)\) and \(L(p', q')\), are homeomorphic [43], so \(b(p, q) = b(p', q')\) if and only if \(p = p'\) and \(q \equiv q' \mod p\). (See Rolfsen [40], for a good introduction to lens spaces.)

A summing disk of a tangle (either the western or eastern summing one) lifts to an annulus on the boundary of the double branch cover. This annulus can be meridional (its core bounds a meridional disk of the solid torus), longitudinal (its core intersects a meridian once) or neither (intersects a meridian more than once). When two tangles are summed, this corresponds to gluing two solid tori along the annuli that are lifts of the summing disks. Hence, a natural subdivision of rational tangles arises: We say a rational tangle is integral, and write it as \((n)\), if it consists of a series of \(n\) horizontal half-twists, where \(n \in \mathbb{Z}\). We denote this class as \(\mathbb{Z}\).

Integral tangles have summing disks that lift to longitudinal annuli. Similarly, a tangle is the infinity tangle, denoted by \((\infty)\), if it consists of two vertical parallel strands with no twists. The infinity tangle has a summing disk that lifts to a meridional annulus. We say a tangle is strictly rational, and denote this class as \(\mathbb{Q}\) if it is neither integral nor the infinity tangle, and so \(\mathbb{Q} = \mathbb{Q}! \cup \mathbb{Z} \cup \{\infty\}\). Strictly rational tangles have summing disks that lift to annuli that are neither meridional nor longitudinal. Within the class of strictly rational tangles we distinguish vertical tangles, written as \((\frac{1}{n})\), which consists of a series of \(n\) vertical half-twists (\(|n| > 1\)).

3. Biological Motivation and Model

We can now describe in full detail a generalization [44] of the original tangle model of Ernst and Summers [20].

We illustrate our model with a member the integrase family of recombinases, the protein \(Flp\) (pronounced ‘flip’). Flp has served as the paradigm for site-specific recombination [53], and there are a number of proteins (including Cre, and \(\lambda\) Int acting on LR sites) whose products are, topologically speaking, identical to those of Flp.

Roughly speaking, Flp recognizes two copies of a specific DNA sequence, binds at these sites, cuts the DNA at the sites, moves the strands in space, reseals the break, and releases the DNA. When acting on circular DNA, Flp can change the underlying knot type of the DNA, for example turning the unknot into the trefoil knot. The distribution of knot/link products should reflect the supercoiling density [38]. We call a DNA molecule that has not been acted on by Flp a substrate, and a molecule that has been acted on a product. In these terms, the substrate is always an unknot and the products are torus knots or links.

We model each of the substrates and products as the numerator closure of the sum of three tangles. Each tangle arc represents a segment of double-stranded DNA.
In the tangle model pioneered by Ernst and Sumners [20], the cutting and joining of DNA is assumed to be completely localized: two of the tangles are unchanged by the action of the protein. In the substrate, the first tangle, \( P \) (Parental), represents two short identical sites that Flp recognizes and to which it chemically binds and then cuts, rearranges and re-seals. This action can be thought of as removing \( P \) and replacing it with a new tangle, \( R \) (Recombinant), in the product. The second tangle, \( O_c \), represents the part of the DNA that is physically constrained, but unchanged, by the protein (\( O \) stands for Outside and \( c \) for constrained). The last tangle, \( O_f \), represents the part of the DNA that is free (hence the subscript \( f \)) from protein binding constraints. \( O_f^k \) can vary depending on the amount of DNA supercoiling present at the time Flp acts. The superscript \( k \) indexes these different possibilities.

In terms of tangles, this amounts to saying that our substrate and products can be modelled as:

\[
N(O_f^k + O_c + P) = \text{substrate (before recombination)}
\]
\[
N(O_f^k + O_c + R) = \text{product (after recombination)}
\]

where \( k \in \{0, 1, 2, 3\} \). \( O_f^k \) varies as \( k \) varies, so we obtain different products, as described below. We use \( O^k \) to mean the part unchanged by Flp, that is, \( O^k = O_f^k + O_c \). When there is no mathematical distinction between \( O_f^1 \) and \( O_c \), we use \( O_1 \) and \( O_2 \) to represent them interchangeably, i.e., \( \{O_1, O_2\} = \{O_f^1, O_c\} \).

Recall that, in contrast to integrases, proteins in the serine family of recombinases, such as Tn3, require a fixed number of supercoils before they begin cutting and rejoining DNA. Once this requirement is met, they rearrange the DNA, occasionally multiple times, before releasing it. The corresponding tangle equations: from substrate \( N(O_f + O_c + P) = K_0 \) to products \( N(O_f + O_c + nR) = K_n \), were first solved (i.e., all constituent tangles have been characterized, given the 4-plats) by Ernst and Sumners [20]. Note that the free part, \( O_f \), does not vary. This single, fixed \( O_f \) is what has thus far made the serine recombinase tangle equations more tractable than the integrases, whose equations involve a family of tangles \( O_f^k \), indexed by \( k \).

### 3.1. Two Systems: Inverted and Direct

Flp identifies two short identical sequences, called repeats, on a molecule of DNA. These sites are non-palindromic sequences, and can thus be given an orientation, and hence on circular DNA, the strings can be in head to head (inverted repeats) or head to tail (direct repeats) orientation. Action on inverted repeats on a circular molecule of DNA yields a knot, and action on direct repeats yields a two-component link. When Flp acts on DNA it yields a variety of torus knots (inverted repeats) and links (direct repeats) that depend on \( O_f^k \).

When Flp acts on a DNA molecule with inverted sites, experiments have shown that the resulting DNA can be an unknot (with a different DNA sequence), or a knot with up to 11 crossings [25]. Crisona et al. have obtained images (using electron microscopy) of the simplest products, and has shown that they are the torus knots \( b(\pm 1, 1) \) (the unknot), primarily positive \( b(3, 1) \) and exclusively positive \( b(5, 1) \) [11]. This experimental evidence indicates that Flp begins with an unkotted DNA substrate with inverted repeats, \( b(\pm 1, 1) \) and converts it via tangle surgery into a torus knot \( b(\pm (2k + 1), 1) \), where \( k \in \{0, 1, 2, 3\} \). (The chirality of the products for \( k = 1 \) and \( k = 2 \) has not been determined for all members of the integrase family, so we remain in the general situation. Corollary 4.9 considers the specific setting for Flp (and \( \lambda \) Int acting on LR sites), whose products’ handedness are known.)
We thus model the action of Flp on DNA with inverted repeats as:

Before: \( N(O^k_f + O_c + P) = b(\pm 1, 1) = \text{unknot}, \text{ for } k \in \{0, 1, 2, 3\} \)

After: \( N(O^0_f + O_c + R) = b(\pm 1, 1) = \text{unknot} \)

\[ N(O^k_f + O_c + R) = b(\pm (2k + 1), 1) = \text{torus knot for } k \in \{0, 1, 2, 3\} \]

3.2. Strategy. Given the set of tangle equations above, whose products (4-plats) are known, the goal is to determine the constituent tangles. The interplay of tangles and four-plats with their corresponding double branch covers is the key to many of our results in tangle calculus. For instance, if \( C \) and \( D \) are tangles, and \( D \) is a rational tangle, then the \( \text{dbc}(C + D) \) is obtained by gluing \( \bar{C} \) and \( \bar{D} = V_D \) along annuli that are the lifts of their corresponding gluing disks. If \( D \) is integral, then the gluing annulus is boundary reducible, and \( \text{dbc}(C + D) \approx \bar{C} \).

The sum and subsequent numerator closure of two tangles \( C \) and \( D \) induces a gluing of the boundaries of their respective double branch covers \( \bar{C} \) and \( \bar{D} \). If \( N(C + D) \) yields a four-plat \( b(p, q) \), then \( \bar{C} \cup_h \bar{D} \) must be the lens space \( L(p, q) \), where \( h \) is the map that takes \( \mu_{\bar{C}} \) to \( p\lambda_{\bar{A}C} + q\mu_{\bar{A}D} \). In particular, when \( C \) and \( D \) are both rational, \( \bar{C} = V_C \) and \( \bar{D} = V_D \) are solid tori, and they form a Heegaard splitting \( V_C \cup_h V_D \) of \( L(p, q) \).

Replacing tangle \( P \) in \( N(O + P) \) by tangle \( R \) to obtain \( N(O + R) \) is called tangle surgery. If \( P \) and \( R \) are rational tangles, then tangle surgery corresponds to replacing \( V_P \) with \( V_R \) in the double branch cover, and thus corresponds to different Dehn fillings of \( \partial^k \). In the case of \( N(O^k + P) = b(1, 0) \), the unknot, then since \( \text{dbc}(b(1, 0)) = S^3 \), and the tangle surgery corresponds to Dehn surgery on a knot complement \( (O^k) \) in \( S^3 \). If \( O^k \) is not rational, then the knot is non-trivial.

Previous work (Ernst and Sumners [20], and the authors [7] via different techniques for \( R \)) proved that \( P \) and \( R \) are rational for both direct and inverted repeats. So the tangle surgery of replacing \( P \) with \( R \) corresponds to Dehn surgery in the double branch covers.

Thus the strategy is to use restrictions on the type of Dehn surgeries of \( S^3 = \text{dbc}(b(1, 1)) \) that yield lens spaces. This in turn restricts the possible tangle solutions.

4. The Fourth Case for Inverted Repeats

In [7], we asked whether there were any solutions in the single remaining open inverted case: \( P \) rational, \( O_1 \) integral, \( O_2 \) prime (and hence \( O^k = O^k_f + O_c \) prime). We now can give a positive answer for \( k = 2 \) and eliminate this case for all other \( k \).

In this section, we consider only the inverted system, as we previously completely classified the direct system [7].
4.1. Double branch cover of $O_f^2$ is a trefoil knot complement. We begin by restricting the possibilities for $O^k$, in part by harnessing a powerful recent result of Kronheimer, Mrowka, Oszváth and Szabó:

**Theorem 4.1** ([31], Corollary 8.4). If $K$ is a knot in $S^3$, such that for some $r \in \mathbb{Z}$, $M_K(r) = L(p,q)$ is a lens space where $|p| < 9$, then $K$ must either be the unknot or the trefoil knot.

**Theorem 4.2.** In the inverted repeats system, $O^k$ is rational for $k \in \{0, 1, 3\}$. Further, if $O^2$ is not rational, then $\tilde{O}^2$ must be the complement of a trefoil knot.

**Proof:** Recall that since both $P$ and $R$ are rational tangles, then the tangle equations $N(O^k + P) = b(1, 1)$ and $N(O^k + R) = b(2k + 1, 1)$ correspond to a Dehn surgery along core$(V_P)$ in the (possibly trivial) knot complement $\tilde{O}^k := S^3 \setminus V_P$ that yields $L(2k + 1, 1)$.

The possibilities of $\tilde{O}^k$ being satellite (for all $k$) or torus (for $k \neq 2$) have been eliminated in Theorem 7.1 of [7]. Gordon [23] and Moser [37] have classified all surgeries on a generic torus knot $T_{a,b}$ complement that yield lens spaces: $L(p,qb^2)$ iff $p = qab \pm 1$. A straightforward calculation shows that only $L(5, 4)$ can be obtained from a torus knot—the trefoil knot $T_{3,2}$.

It now remains only to rule out the possibility of $\tilde{O}^k$ being hyperbolic. Assume $\tilde{O}^k$ is a hyperbolic knot ($K$) complement. Then by the Cyclic Surgery Theorem [12], the surgery slope must be integral, and in fact must be $2k + 1$, since $H_1(M_K(s/t)) = \mathbb{Z}_{[s]}$. In our setting, the integral surgery slope $2k + 1$ is strictly less than 9, since $k \in \{0, 1, 2, 3\}$. Hence, we can apply Corollary 8.4 of [31], and obtain that $\tilde{O}^k$ must be a solid torus or a trefoil knot complement, neither of which is hyperbolic. Therefore the hyperbolic case is impossible.

We have thus shown that $\tilde{O}^k \neq 2$ must be the complement of the unknot (a solid torus), and therefore $O^k$ is a rational tangle. Further, $O^2$ is either a solid torus or trefoil knot complement, and therefore $O^2$ is either a rational tangle or a tangle whose double branch cover is a trefoil knot complement.

4.2. $O_c$ is integral and $O_f^2$ is prime. We can now use the rationality of $P$ and $O^k := O_c + O_f^k$ for $k \neq 2$ to place restrictions on the summand tangles.

**Theorem 4.3.** In the inverted system, assume $O^2 = O_1 + O_2$ has a trefoil knot complement double branch cover. Then $O_c$ must be integral and $O_f^2$ must be prime with $O_f^2$ is a trefoil knot complement.

**Proof:** We first show that our only option in this setting is $O_1$ is integral and $O_2$ is prime.

From Section 6 (as summarized in Table 1) of our previous paper [7], the only cases not eliminated are (i) $P$ rational, $O_1$ prime and $O_2$ integral, and (ii) $P$ integral, $O_1 = (\infty)$ and $O_2$ prime. We now show possibility (ii) cannot occur.

Theorem 6.8(v) of [7] eliminates the case when $O_c = O_1 = (\infty)$ and $O_f^2 = O_2$ is prime. Alternately, the case with $O_c$ prime and $O_f^k = (\infty)$ can occur for at most 1 value of $k$, since for $k \neq j$, $N(O_f^k + O_c + R) \neq N(O_f^j + O_c + R)$. By Theorem 6.8(viii) in [7], $O_f^2$ must be integral for all other values of $k$. But then for these 3 (or 4) values of $k$, $O^k := O_f^k + O_c = prime + integral$, is prime [39], a contradiction to Theorem 4.2.

Thus the only possibility that can occur is $O_1$ is integral and $O_2$ is prime. Suppose that $O_c = O_2$ is prime and $O_f^2 = O_1$ is integral. Then since $O_c + O_f^2$ is rational
Proof. From Corollary 4.5, we know that if \( O^k_j = (\infty) \) for \( k = 0, 1 \) and 3 by Cam Van [39]. But for \( k \neq j \), \( O^k \neq O^j \) as these are different recombination products. Thus \( O_e \) must be integral, and \( O^3_j \) prime.

Since \( O_e \) is integral, \( dbc(O_e + O^3_j) \simeq \widetilde{O}_j \simeq O^2 \), the trefoil knot complement from the preceding theorem.

4.3. \( O^3_j \) is a Montesinos tangle, and \( P \) and \( R \) are integral. Montesinos links were first considered by Bonahon [6] and Montesinos [36], by using work of Tollefson [47] who determined that every involution of a Seifert fiber space with non-empty boundary must respect a Seifert fibration. Therefore if the double branch cover of a link in \( S^3 \) admits a Seifert fibration which is invariant under the covering involution the link is either a torus link or what is now called a Montesinos link. Ernst [18], with a later clarification by Darcy [13], used related techniques to determine that any tangle whose double branch covers is Seifert fiber space must be a rational or a generalized Montesinos tangle:

**Theorem 4.4** (Ernst [18], and Darcy [13]). If \( M \) is a SFS with orbit surface a disk and \( n \geq 0 \) exceptional fibers and if \( M \) is the 2-fold branch cover of a tangle \((B,t)\), then \( B \) is a generalized Montesinos tangle.

**Corollary 4.5.** In the inverted system, the case where \( P \) is a rational tangle, \( O_e \) is an integral tangle, and \( O^3_j \) is a prime tangle, then \( O^3_j \) is a generalized Montesinos tangle with two non-integral rational tangents.

**Proof.** From Theorem 4.3 we have that \( \widetilde{O}_j \) is a trefoil knot complement. By Theorem 4.4, \( O^3_j \) then must be a generalized Montesinos tangle with two non-integral summands.

The following result was first used without proof by Darcy in [13]. We give a short self-contained proof below, since we will also need this for the fourth solution.

**Corollary 4.6.** If \( N(O^3_j + 0) \) is a four-plat, then \( O^3_j \) is of the form \( (a/b, c/d) \times (m/e) \) with \( m \in \mathbb{Z} \).

**Proof.** From Corollary 4.5, we know that \( O^3_j \) is a generalized Montesinos tangle with two non-integral rational tangents. Note that \( (a/b, c/d) \times (m/e) = (a/b, c/d + em/d) \), a Montesinos tangle with two non-integral summands.

As a consequence of the above and [14], such a generalized Montesinos tangle is of the form

\[
\left( \left( \left( \frac{a}{b} \cdot \frac{c}{d} \right) * \left( \frac{1}{m_n} \right) \right) + \left( m_{n-1} \right) \right) * \ldots + \left( m_1 \right)
\]

if \( n \) is even, or

\[
\left( \left( \frac{a}{b} \cdot \frac{c}{d} \right) * \left( \frac{1}{m_n} \right) \right) + \left( m_{n-1} \right) \right) * \ldots * \left( \frac{1}{m_1} \right)
\]

if \( n \) is odd.

We will show that if \( n > 1 \) then \( N(O^3_j + 0) \simeq N(O^3_j) \) cannot be a four-plat. Note first that if \( n \) is odd, then \( N \left( \left( \frac{a}{b} \cdot \frac{c}{d} \right) * \left( \frac{1}{m_n} \right) \right) * \ldots * \left( \frac{1}{m_1} \right) \) \( \simeq \)

\[
N \left( \left( \frac{a}{b} \cdot \frac{c}{d} \right) * \left( \frac{1}{m_n} \right) \right) + \left( m_{n-1} \right) * \ldots * \left( m_2 \right).
\]

Hence we need only examine the case when \( n > 1 \) is even.

Recall that \( N (A \star (1/n)) + B \simeq N (A + (B \star (1/n))). \) Hence by a simple inductive argument we can show that \( N \left( \left( \frac{a}{b} \cdot \frac{c}{d} \right) \star \left( \frac{1}{m_n} \right) \right) + \left( m_{n-1} \right) \right) * \ldots * \left( m_1 \right) \) \( \simeq \)

\[
N \left( \frac{a}{b} \cdot \frac{c}{d} \right) + \left( \left( m_{n-1} \right) + \ldots + \left( m_3 \right) + \left( m_1 \right) * \left( \frac{1}{m_n} \right) \right) * \left( \frac{1}{m_1} \right) \) * \ldots * \left( \frac{1}{m_1} \right) \). The
Case 1: O

4.4. The Final Inverted Solution. From the previous section, this fourth case reduces to \( P, R \) and \( O_c \) are integral and \( O^*_f \) is Montesinos tangles. We now determine all solutions to the tangle equations in this case, by applying an algorithm of Ernst whose completeness is guaranteed [19, Theorem 3.1].

**Theorem 4.7** (Ernst [19]). In the inverted system, if \( O^2 \) a Montesinos tangle with two non-integral rational summands, then \( P \) and \( R \) are integral tangles.

**Proof.** Recall that \( N(O^2 + P) = b(1, 1) \), and \( N(O^2 + R) = b(5, 1) \), whose double branch covers are \( S^3 \) and \( L(5, 1) \) respectively. Now \( O^2 \) is a Montesinos tangle with two non-integral summands. If \( P \) were not integral, then the double branch cover of \( N(O^2 + P) \) would be a Seifert fibered space with three exceptional fibers, a contradiction, as it is a lens space. Similarly with \( R \).

**Theorem 4.8.** In the fourth solution of the inverted case, the tangle solutions are \( P = \pm(p), O_c \) integral, \( R = \pm(1 + p) \), \( O^*_f = \mp\left(\frac{2}{3}, \frac{2}{3}, p - 1\right) \) and \( O^k f_2 = \pm(-p + \frac{1}{2k}) \) or \( \mp(p + \frac{1}{2k+2}) \), where \( p \in \{0, 1\} \).

**Proof:** First note that if \( P = (p) \), where \( p \neq 0 \), we can assume that \( P = (0) \) by moving the \( P \) horizontal twists into \( O^k \). So given a solution \( P = (0), R = (r), \) and \( O^k \), then the solution corresponding to \( P = (p) \) would be \( R = (r + p) \) and \( O^k = O^k + (−p) \).

**Case 1: \( k = 2 \).** From Theorems 4.2 and 4.7, \( O^2 \) is a Montesinos tangle of two non-integral summands, and \( P \) and \( R \) are integral tangles. In this section we will use the same notation as in the algorithm in [19]: \( O^2 = (u/v, x/y), P = (m_0) \) and \( R = (m) \). By moving the \( m_0 \) horizontal twists of \( P \) into \( O^2 \), we can set \( P = (0), R = (m) \), and \( O^2 = (u/v, x/y, m_0) \). We can now apply the algorithm to determine \( m_0, m, u, v, x \) and \( y \).

For \( k = 2 \), we begin with \( b(\alpha_1, \beta_1) = b(1, 0) \) and obtain \( b(\alpha_2, \beta_2) = b(5, 1) \). Ernst’s algorithm yields \( m_0 = m = -1, u = 1, v = x = 2 \) and \( y = 3 \). So the solution is either \( R = (−1), \) and \( O^2 = (u/v, x/y, m_0) = (\frac{2}{3}, \frac{2}{3}, -1) = (\frac{1}{2}, \frac{1}{3}) \) or their mirror images: \( R = (1), \) and \( O^2 = (−1, \frac{2}{3}, \frac{2}{3}, 1) \).

**Case 2: \( k \neq 2 \).** We will show that if \( P = (0) \), then \( O^k = \mp\left(\frac{1}{q}\right) \) or \( \pm\left(\frac{1}{2k+1}\right) \).

We have that \( N(O^k + P) = N(O^k + (0)) = N(O^k) = N\left(\frac{1}{q}\right) \) and \( N(O^k + R) = N\left(\frac{1}{q}\right) = N\left(\pm\frac{2k+1}{q}\right) \).

By the correspondence between numerator closure of rational tangles and 4-plats, \( N\left(\frac{1}{q}\right) = N\left(\frac{p}{q}\right) \) if and only if \( p = q \) and \( q \equiv q' \mod p \) [10], [43]. This tells us that \( O^k \) is a vertical tangle \( \left(\frac{1}{q}\right) \). The tangle \( O^k + R = \left(\frac{1}{q} \pm 1\right) = \left(\frac{1+q}{q}\right) \), where the sign is the sign of \( R \). Hence \( 1 \pm q = \pm 2k + 1 \) and so \( q = 2k \) or \( -2k - 2 \) when \( R = (1) \), and \( q = -2k \) or \( 2k + 2 \) when \( R = (-1) \).

We thus obtain the general solution by converting as described above. □

Note that as \( O_c \) is integral, then \( O_c \) is either zero or the integral part of \( O^*_f \).
We reiterate that, although solutions are given in their simplest form, they are unique only up to compensating positive and negative vertical twists. So for example, as noted by Darcy, \( P = (0), \ R = (1) \) and \( O^2 = (\frac{1}{2}, \frac{3}{2}) \) is also a solution for \( k = 2 \) [16].

For both Flp and \( \lambda \) Int (acting on LR sites), Crisona et al. have numerous electron microscope images of two of the products [11]. Most of trefoils \((k = 1)\) and all of the pentafoils \((k = 2)\) examined are positive. This chirality further constrains the tangle possibilities for Flp and \( \lambda \) Int (on LR sites).

**Corollary 4.9.** For both Flp and \( \lambda \) Int (acting on LR sites), if \( P = (p) \), then \( p \in \{0, -1\} \) and \( R = (p - 1) \), \( O^2 = (\frac{2}{3}, -p - 1) \), \( O^1 = (-p - \frac{1}{2}) \), and \( O^k = (-p + \frac{1}{2k}) \) or \((-p + \frac{1}{2k+2})\) for \( k = 0 \) or 3.

**Proof:** Note that the right-handed products, when \( P = (0) \), are given when \( O^2_f = (\frac{1}{2}, \frac{3}{2}, -1) \) and \( O^2 = (\frac{1}{2}, \frac{3}{2}, -p - 1) \). Therefore, the handedness of the product \( b(\pm 5, 1) \), as determined through electron microscopy by Crisona et al. [11] means that \( p = (-p) \), \( R = (-1 - p) \) and \( O^2 = (\frac{1}{2}, \frac{3}{2}, -p - 1) = (\frac{1}{2}, -\frac{1}{3}) \) for \( p \in \{0, 1\} \). By including the negative sign in \( p \) we get the resulting \( P, R \) and \( O^2_f \).

In the case \( k = 1 \), further electron micrographs of Crisona et al. show the majority of the product is \( b(\pm 3, 1) \). Since \( R = (-p - 1) \), the corresponding right-handed solution is \( O^1 = (-p - \frac{1}{2}) \).

No chirality information exists for \( k = 0 \) or 3. Therefore there are still two possibilities for \( k = 0 \) or 3. \( \square \)

### 5. Solutions

In this section, we present all tangle solutions for an integrase acting on DNA with direct or inverted sites. In particular, we determine what \( R \) must be in each case.

We should note that when \( O^k = O_c + O^k_f \) is known to be rational then the following theorem allows us to find a values of \( O^k \) and \( R \) that will satisfy tangle equations arising from an unknotted substrate and four-plat products.

**Theorem 5.1** ([13] Lemma 14, [20]). With \( c'd - cd' = 1 \):

\[
N \left( \frac{a}{b} + \frac{c}{d} \right) = N \left( \frac{ad + bc}{ad' + bc'} \right)
\]

For instance, in our case we have the unknot as substrate, and torus knots and links (that are the numerator closures of integral tangles) as products.

**Theorem 5.2.** Given \( O^n \), \( P = \frac{2}{q} \) and \( R \) are rational tangles such that:

\[
N (O^n + P) = N \left( \frac{1}{n} \right), \text{ the unknot}
\]

\[
N (O^n + R) = N \left( \frac{1}{n-1} \right), \text{ the torus knot or link } T_{\frac{n}{n-1}}
\]

Then \( O^n = \frac{r+pt}{s+qt} \) \& \( R = \frac{r+pt}{s+qt} \) for \( rq + ps = 1 \), for any constant \( t \).

**Proof.** We begin by noting:

Claim. Suppose \( p \) and \( q \) are relatively prime, and \( rq + ps = 1 \). Then \( r'q + p's' = 1 \) if and only if \( r' = r \equiv \pm pt \) and \( s' = s \equiv qt \).

**Proof of Claim.** If \( r' = r \equiv \pm pt \) and \( s' = s \equiv qt \) then checking that \( r'q + p's' = 1 \) is a simple calculation. Conversely, \( r'q + p's' = rq + ps \), so \( r'(r' - r)q = p(s - s') \). Since \( p \) and \( q \) are relatively prime, then \( q | (s - s') \), so \( s - s' \equiv qt \) for some \( t \). Therefore \( r'q = rq + p(s - s') = rq + pqt \) so \( r' = r + pt \). Therefore \( rq + pqt + p's' = 1 \), i.e., \( rq + p(qt + s') = 1 \), and so \( s = s' + qt \), and hence \( s' = s - qt \).
Applying Theorem 5.1 to $N\left(\frac{r \pm pn}{s \mp qn} + \frac{t}{q}\right)$ and $N\left(\frac{r \pm pn}{s \mp qn} + \frac{r \pm pt}{s \mp qt}\right)$ now gives the result: In the first case, $N\left(\frac{r \pm pn}{s \mp qn} + \frac{t}{q}\right) = N\left(\frac{1}{1+\frac{t}{q}}\right)$. In the second case, note that $-p(-s \pm qt) - (-q)(r \mp pt) = 1$, so $N\left(\frac{r \pm pn}{s \mp qn} + \frac{r \pm pt}{s \mp qt}\right) = N\left(\pm t\frac{q}{t}\right)$. □

Since for inverted repeats $N(O^k + R) = T(\pm(2k+1), 2)$, setting $n = 2k + 1 + t$ gives a solution for the inverted system. For direct repeats $N(O^k + R) = T(\pm2k, 2)$, so setting $n = 2k + t$ gives a solution for the direct system.

Let us illustrate this by picking an arbitrary fraction for $P$. For $P = (\frac{1}{7})$ we get $T = 1 + \frac{1}{1 + \frac{1}{1 + \frac{1}{1 + \frac{1}{1 + \frac{1}{1}}}}}$ so $P = [3, 1, 1, 1]$. Note 7(−3) + 11(2) = 1, so let $r = -3$ and $s = 2$. Then we get:

$$O^k = \frac{r \pm p(2k + t + 1)}{s \mp q(2k + t + 1)} = \frac{-22k \pm 11t \pm 11 - 3}{14k \pm 7t \pm 7 + 2}$$

and

$$R = \frac{-3 \pm 11t}{-2 \mp 7t} = \frac{3 \mp 11t}{2 \pm 7t}$$

are solutions to the system for the given $P$, for any constant $t$.

**Corollary 5.3.** If $P$ is an integral tangle $(n)$ then $R$ is either the infinity tangle, an integral tangle, or the sum of a vertical and integral tangle.

**Proof.** Theorem 5.2 tells us that, since if $r = 1 - n$ and $s = 1$ then $r + ns = 1$, $R$ must be

$$\left(\frac{1 - n \pm nt}{-1 \pm t}\right) = (n) + \left(\frac{1}{t \mp 1}\right).$$

If $t = 0$ or $\pm 2$ then $R$ is an integral tangle. If $t = \pm 1$ then $R$ is the infinity tangle. Otherwise, it is the sum of a vertical and integral tangle. □

In [7] we showed there are three classes of solutions for $P$, $O^k_1$ and $O_c$ to both systems. The example illustrated above lies in Class 3, described below.

**Class 1.** $P = (\infty)$ and $O_c$ and $O^k_1$ are integral. In this case Theorem 5.1 tells us that $R$ must be integral: $\frac{R}{q} = \frac{1}{1}$, so for $s = 1$ and any $r$, say $r = 0$ we get $R = \left(\frac{\text{nt}}{1}\right)$. In addition, simply knowing that $O^k$ is rational, then Theorem 5.1 gives us that $O^k$ must be integral: $O^k = \left(\frac{\pm(2k+1)}{1}\right)$ for the inverted case.

The simplest example is when $R = (0)$. In that case $O^k_1 + O_c + O^k = 2(2k+1)$ for inverted, or $O^k = (\pm 2k)$ for direct [24]. We can, however, choose $R$ to be any integral tangle, which corresponds to varying $t$, and $O^k$ changes accordingly.

**Class 2.** $P$ and $O_c$ are integral, and $O^k_1$ (and hence $O^k$) is $(\infty)$ for at most value of $k$, integral for at most 2 values of $k$—for all $k$ in both the directed and inverted systems—and strictly rational otherwise. When $O^k_1$ is strictly rational, it must be either vertical or the sum of a vertical and an integral tangle [7], possibly of different signs. (Note that if the vertical and the horizontal twists have opposite sign the tangle is said to be in “non-canonical” form.) As shown in Theorem 5.3, $R$ must be the infinity tangle, integral, or the sum of a vertical and integral tangle. However, $R$ and $O^k_1$ for some $k$ cannot both be $(\infty)$.

The simplest example in this class is when $P = (0)$, $R = (\infty)$ and $O^k = (\pm 1/(2k + 1))$ for inverted, and $O^0 = (\infty)$ and $O^k = (\pm 1/2k)$ ($k > 0$) for direct, as first considered in [11]. This is biologically equivalent to the first example, in that the tangle surgery consists in interchanging the two tangles with no crossings. In general two solutions are biologically equivalent if their three-dimensional arrangement is the same, but have different projections. See our previous paper [7] and [49] for further discussion of biological equivalence.
Another example, which is biologically non-equivalent, is when \( P = (\pm 2) \). In this case \( R = (\pm 1) \) and \( O^0 \) is infinity for inverted repeats and \( O^0 \) and \( O^1 \) are integral for direct repeats; all other \( O^k \)'s are strictly rational. For direct repeats \( O^0 = (\mp 1) \), \( O^1 = (\pm 3) \) or \( (\mp \frac{5}{3}) \), \( O^2 = (\mp \frac{9}{5}) \), and \( O^3 = (\mp \frac{13}{9}) \). For inverted repeats \( O^0 = (\infty) \) or \( (\mp \frac{3}{2}) \), \( O^1 = (\pm \frac{3}{2}) \), \( O^2 = (\mp \frac{5}{3}) \) and \( O^3 = (\mp \frac{11}{5}) \).

If a solution has \( O^k = (\infty) \), for some \( k \), as in the second example, then in fact it must satisfy several conditions:

**Theorem 5.4.** Given a solution from Class 2 then:

(i) If \( \exists i \) s.t. \( O^i_f = (\infty) \), then \( R \) must be vertical or \((\pm 1)\).

(ii) If \( \exists i \) and \( j \) s.t. \( O^i_f = (\infty) \) and \( O^j_f \) is integral, then \( P \in \{0, \pm 2\} \).

(iii) If \( \exists i \) s.t. \( O^i_f = (\infty) \) and \( P \neq (0) \), then \( i = 0 \). If in addition \( O^j_f \) is integral, then \( j = 1 \).

**Proof.** The first two items were shown in [7]. The third item can be proved by looking at \( O^k \) and \( R \), which can be re-written as \( R = \left( \frac{1 - p + (\pm 1)}{1 + (\pm 1)} \right) \) and \( O^k = \left( -p + \frac{1}{1 + (\pm 1)} \right) \) for inverted, and \( O^k = \left( -p + \frac{1}{1 + (\pm 1)} \right) \) for direct, where \( P = (p) \neq (0) \). As \( R \) is vertical, this means that either \( s \mp t = 0 \) or \( p(s \mp t) = 2 \).

If \( s \mp t = 0 \), then, if \( O^i = (\infty) \), that \( 2i + 1 = 0 \) for inverted, or \( 2i = 0 \) for direct. The first is impossible, so \( i = 0 \) for direct. If further, \( O^1 \) is integral, then \( \pm (2i + 1) = \pm 1 \) for inverted, or \( \pm 2j = \pm 1 \) for direct. The first case gives \( j = 0 \), the second is impossible. The second case is similar: Since in this case \( p = \pm 2 \), this means \( s \mp t = \pm 1 \). So \( 2i = \mp 1 \) for direct, which is impossible, and \( 2i + 1 = \mp 1 \) for inverted, so \( i = 0 \). If in addition \( O^j \) is integral, then \( \pm 1 \mp (2j + 1) = \pm 1 \) for inverted, which is impossible, and \( \pm 1 \mp (2j) = \pm 1 \) so \( j = 1 \), or \( j = 0 \) which cannot occur since \( i = 0 \), so \( j = 1 \).

**Class 3.** \( P \) and \( O^k_f \) are strictly rational and and \( O_c \) is integral. In this case \( R \) is also strictly rational: given \( O^k = \left( \frac{2}{b} \right) \), with \( b \neq \pm 1 \), if \( R = (c) \) then \( N(O^k + R) = N\left( \frac{a + bc}{k} \right) = N\left( \frac{2k+1}{k} \right) \), which implies \( b = \pm 1 \), a contradiction.

An illustration of the last class of solutions was given above, following Theorem 5.2. This class includes many different (biologically non-equivalent) possible actions.

**Class 4 (inverted case only).** In the preceding section we demonstrated that there is an additional solution for inverted sites: \( R \), \( O_c \) and \( P = (p) \) are integral, and \( O^j_f \) is Montesinos with two non-integral tangles, and in fact is the minimal prime tangle [4]. In particular, \( P = (p) \), \( R = (1 + p) \) and \( O_c \) are integral, \( O^j_f \) is the prime Montesinos tangle \( \mp (\frac{1}{2}, \frac{2}{5}, p - 1) \) and \( O^{k\neq2}_f = \mp (p + \frac{1}{2k}) \) or \( \mp (p + \frac{1}{2k+2}) \), where \( p \in \{0, 1\} \). This is the only case in which one of the constituent tangles is not rational, and in this case it is the smallest prime tangle (plus possibly a single horizontal twist).

### 6. Conclusion

We have completely classified all possible tangle solutions corresponding to a generic member of the integrase family of recombinases acting on DNA with either direct or inverted sites. We proved there are exactly three classes of solutions in the direct system, and exactly four in the inverted.

The biological assumption is that the protein mechanism is consistent for a given protein across a variety of substrates. The integrase family of recombinases is forgiving of substrates—these members will perform recombination between two sites on the same (circular, as discussed here, or linear) or different (circular and/or
linear) molecules. However, if an integrase requires a particular alignment of the two sites when they are brought together, then a substrate with inverted sites may have a different number of crossings introduced into $O$ than a substrate with direct sites. (For example, it was shown that Flp aligns the sites in antiparallel, and so in the simplest case, $O_c = (1)$ for inverted and $(0)$ for direct [24].) Biologically, the unequal number of solutions between the inverted and direct cases means that the fourth case is not likely, as this would mean that $O_k$ differs not only in the number of crossings but in the actual type of tangle (prime versus rational). This would in turn imply a different mechanism for bringing the sites together depending whether they are in direct or inverted repeats (only for one product, corresponding to $k = 2$). A small protein being able to “sense” the global orientation (inverted versus direct) of the two sites would be unusual. However, there are examples of proteins which act in response to global characteristics, notably Topoisomerase II, which preferentially changes crossings to unknot DNA [41].

6.1. Future Directions. We conclude with several observations, and avenues for future biological and mathematical research.

Electron microscopy images by Crisona and colleagues determine that for Flp in the cases $k = 1$ and $k = 2$, the products are in fact torus knots [11]. For the related recombinase, $\lambda$ integrase acting on LR or PB sites, electron microscopy has shown that the products are almost exclusively positive torus knots (inverted) and negative torus links (direct) [11], [45]. Biologically, one would expect a fixed, precise mechanism for this family of recombinases, which would predict that for Flp and other integrases, every product should be a torus knot or link. However, although one can experimentally demonstrate that higher-crossing products exist and determine their respective crossing number, their precise knot/link type has not been experimentally verified for any other integrase family member. As the crossing number increases, resolving the precise knot type becomes more crucial (and difficult) since the number of knots with a given crossing number increases dramatically—for instance, there are $1,701,936$ knots with $\leq 16$ crossings [27].

**Question 1:** For Flp and other integrases (not $\lambda$ Int), can we obtain experimental confirmation that every product is of the form $b(2k, 1)$ (direct) or $b(2k + 1, 1)$ (inverted)?

If they are, one might be able develop similar Dehn surgery arguments such as the result from [31] used here to restrict $O_k$. For example, for $k \leq 10$, we can eliminate the possibility of $O_k$ having as double branch cover a satellite knot complement by work of Bleiler and Litherland [5], and, in many cases, a torus knot complement by Moser and Gordon [23, 37]. So in many cases, the first step would be to consider $O_k$ such that $\tilde{O}_k$ is a hyperbolic knot complement.
Second, we consider the chirality of the resulting products. Crisona et al. have characterized the first two nontrivial products of Flp-mediated inversion as almost exclusively positive torus knots [11].

**Question 2**: Can we determine the chirality of any of the Flp deletion products, or the higher crossing inversion products?

If so, the classes of solutions could be tightened significantly by removing the possibility of mirror images, as in Corollary 4.9.

Third, although the third class of solutions is mathematically possible, biological considerations such as DNA’s stiffness impeding a high number of crossings, make them biologically unlikely. For this reason, biologists often assume in the tangle model that $P$ and $R$ each have 0 or 1 crossings.

Other work, notably [11] and [44], have incorporated a number of biologically reasonable assumptions into the tangle model which reduce the number of putative tangle solutions. For example the Generalized Random Collision assumes that the original DNA is exclusively negatively supercoiled (i.e., $O^f_n = (n)$ or $(-\frac{1}{2})$ for $n \geq 0$), which then in turn restricts $O_c$ and $P$ [11], [44]. These assumptions are biologically reasonable, and preclude tangle solutions such as those of Class 3. But topologically, the more exotic cases cannot be excluded.

**Question 3**: Can the third class of solutions be eliminated or restricted experimentally, or computationally using models (e.g., [35]) of DNA flexibility?

If the number of crossings of the constituent tangles can be bounded with any degree of certainty, this reduces the solutions from an infinite number to a mere handful. For example, we consider the serine recombinase Xer acting on circular DNA with direct psi sites, whose corresponding tangle equations are $N(O + P) = b(1, 1)$ and $N(O + R) = n(\frac{1}{2}) = b(4, 1)$ [9]. Assuming all tangles are rational and $P = (0)$, and so $O = (\frac{1}{2})$, then Darcy showed that $R \in \left\{\frac{1}{2}, \frac{3}{5+7}, \frac{5}{5+7}, \frac{46-1}{4+3(4k-1)}\right\}$ [13].

Additionally, Vazquez et al. by carefully analyzing the biological data, and assuming any nontrivial topology is in $O_c$, show $P$ is trivial (and hence in their setting can be chosen to be $(0)$), and show $O$ is rational using techniques similar to [7]. Further they assume $R$ is integral or $\infty$, and show there exist 3 solutions: $O = (\frac{1}{2}), R = (-1)$ or $O = (\frac{-1}{2}), R = (+1)$ or $O = (\frac{-1}{2}), R = (\infty)$, which can be biologically equivalent [49].

Additionally given torus link substrates constructed by $\lambda$ Int, Xer-mediated recombination results in more complex knots: $N(O_2 + P) = N(\frac{1}{2})$, and $N(O_2 + R)$ = seven-crossing product (knot), and $N(O_3 + P) = N(\frac{1}{2})$ $N(O_3 + R)$ = nine-crossing product [2]. Darcy also uses this information to analyze how differing seven-crossing products reduce the number of putative solutions [13].

**Question 4**: Can the precise type of knot resulting in the action of Xer on $N(\frac{1}{2})$ be determined? Can similar techniques to those used here be used to examine the Xer system in their full generality, without simplifying assumptions?

Finally, we reiterate that, although we have motivated our work by considering Flp, our results can be applied to any protein in the integrase family, such as Cre or $\lambda$ Int (acting on LR sites), whose products are the above torus knots or links.

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