MODELLING THE UNKNOTTING FUNCTION OF TOPOISOMERASES AND KNOT ADJACENCY

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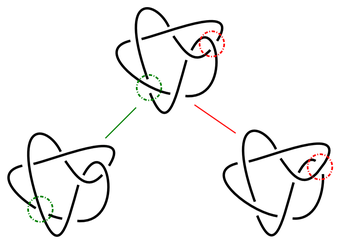
Abstract

The action of **type II topoisomerases** on covalently closed DNA molecules can change their topology, resulting in a range of different **knot** types [1]. Here we model the configuration space of a knotted DNA molecule as a graph. The vertices are planar projections (*i.e.* shadows) of the molecule, which can be thought as a closed polymer in space, and these are connected by edges representing inter-segmental passages. This diagram focused approach is applied to investigate **knot adjacency** and the **unknotting function** of topo II. We complement and synthesise earlier works [2] [3] by looking at neighbouring subspaces in the graph of the configurations, modeled as a network of grid diagrams with increasing complexity. We suggest a **grid-based** calculation as a new and computationally convenient method for investigating unbiased knotting probability of biopolymers. Furthermore, in this setting we are able to emulate previous simulations [4], [5] in the lattice model to investigate the role of **local juxtaposition geometry** [6] for Topoisomerases action.

Our Model

Double stranded, covalently closed DNA molecules are often modeled as twisted ribbons [1]. In this setting, the core of the ribbon (*i.e.* the central axes of the double helix) is a closed curve in space, that is, a **mathematical knot**. Projections of a closed curve on a plane in which the only intersections allowed involve two arcs are called **knot diagrams** [7].

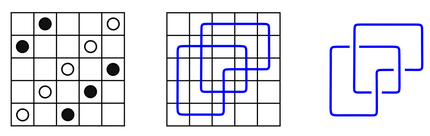
Such intersections, together with the information of which arc is the over-passing one,are called **crossings**. A single action of **type II topoisomerases** corresponds to **changing a crossing** in the diagram (that is, exchanging the over and under passing arcs).



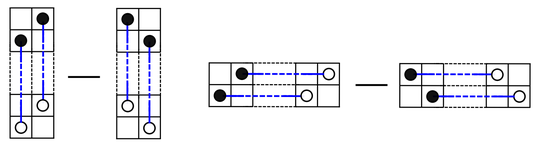
The **configuration space** of a **closed DNA molecule** undergoing the action of **type II Topoisomerases** can be modeled as a **network** in which the vertices are the **projections** of the configurations, connected through edges representing **crossing changes**.

Grid Diagrams

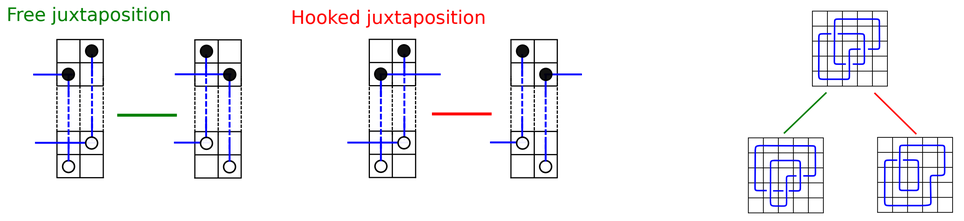
Grid diagrams are a special kind of knot diagrams, first introduced in [8]; they are composed by a square planar grid of dimension n, where n is called the **grid number GN**, in which 2n markings are placed. Each line/column must contain exactly two markings.



A diagram can be created from a grid as follows: connect any two markings on the same line and column, so thath each vertical strand is an over-pass.



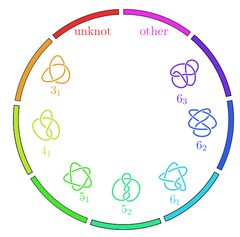
Crossing changes are achieved on grids by a process called **interleaving commutation**: it swaps the positions of two adjacent and interleaved rows or columns.



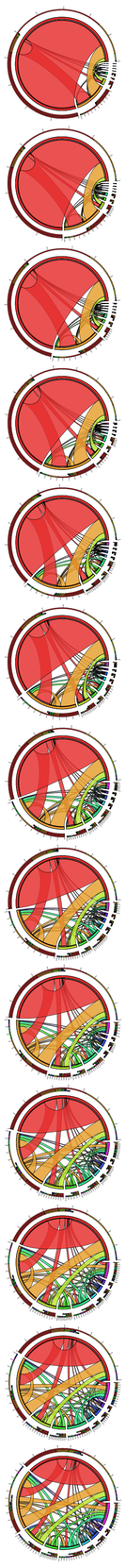
By imposing that the crossing changes happen only at specific local configurations resembling the **hooked geometries** [6], we can test the Buck and Zechiedrich **hooked-juxtaposition hypothesis** [9]: topo II achieves disentaglement by performing strand passages only at hooked juxtapositions. Each grid diagram (and the crossing changes applicable to it) can be described by a **pair of permutations** on n = GN elements, defining the placement of the markings. This allows for a easily **computable theory**, in which theoretically it is feasible to work with grids of arbitrary dimensions.

Methods

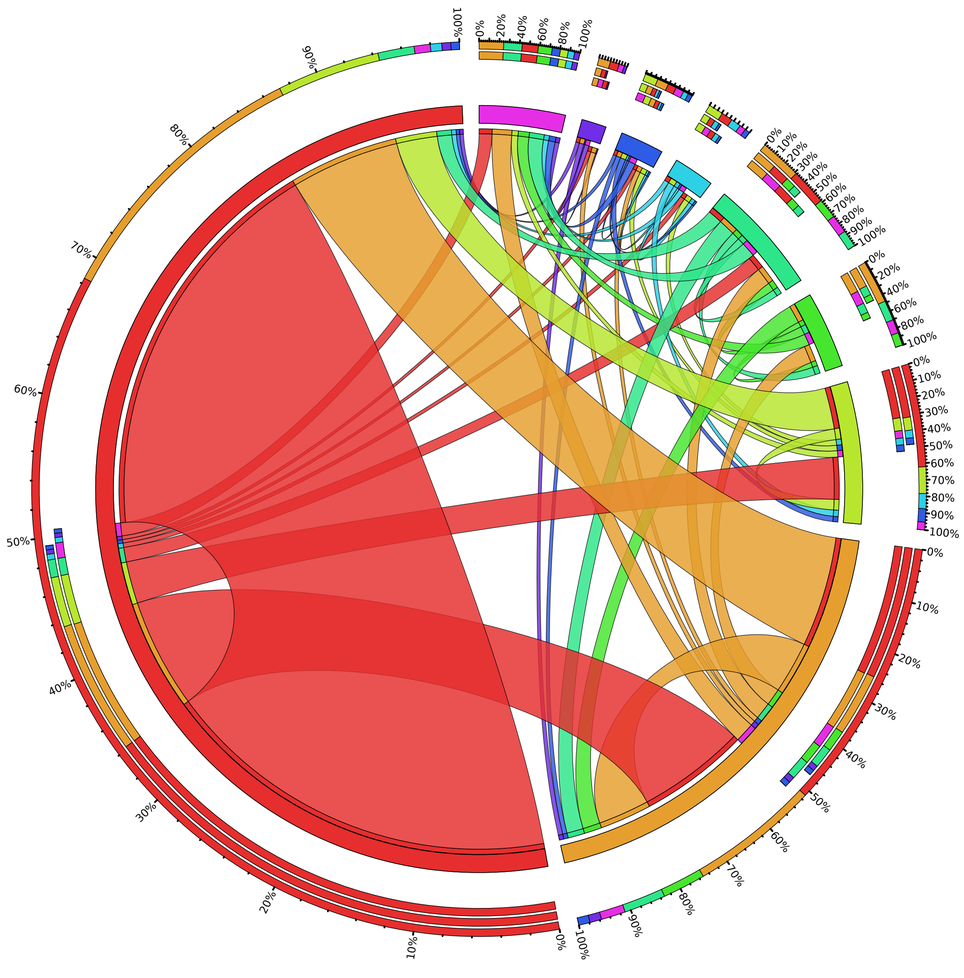
We begin our analysis by **enumerating all grid diagrams** with grid number **GN < 8**, and we detect their corresponding knot types using a combination of knot invariants (knot polynomials, determinant, signature). We then keep track of every possible crossing change from each configuration. The number of configurations grows super-factorially with GN, and an exhaustive computation for higher values of GN becomes quickly unfeasible; the investigation on diagrams with higher grid number is then performed through **random sampling**. Since a grid diagram is completely determined by a pair of permutations defining the positions of the markings, **uniformity of sampling is automatically built** in our model. We restrict our analisys to knots with **minimal crossing number up to 6**. For every GN the obtained data is visualised using **circos plots** [10], in whch different knot types (labeled following Alexander-Briggs' notation) are identified using a colour code, shown on the right. These results provide a **control group** that we can subsequently compare with the data obtained by counting only the crossing changes happening exclusevely at local configurations that resemble the **hooked geometries** [6].



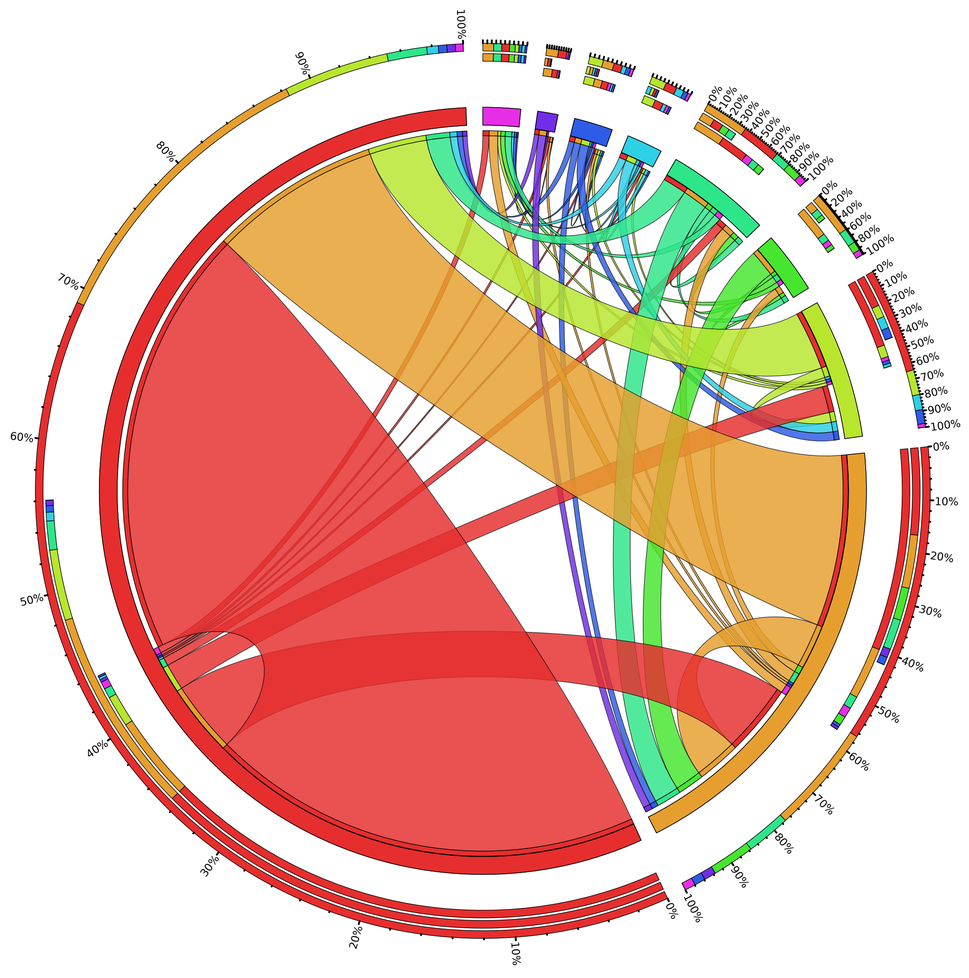
Results and Discussion



Each plot on the left summarises the data on random **unbiased random strand passages** between configurations with a specific GN. Grid numbers are considered in the range [8,20]. The three external segments above each knot type represent (from the innermost) the percentages of outgoing and incoming exchanges between the knot and its neighbours, following the colour code. The external segments represent the sum of the previous two. The internal segments' sizes encode the **occurence values** of each knot type, and ribbons (whose sizes depend on the values of the **transition probabilities**) are coloured according to the starting knot types. In the lower GN, we observe a prevalance of configurations representing the trivial knot, decreasing as the GN increases. This agrees with the notion that, for closed polymers, the probability that a polymer is unknotted decreases as the length of the polymer increases [11] [12]. Indeed, diagrams with GN = g may be tought as closed polymers of length ~2g [13]. The total data is then summarised in a single plot, and compared with with the circos plot representing the **transition probabilities** of crossing changes happening at **hooked juxtapositions**.

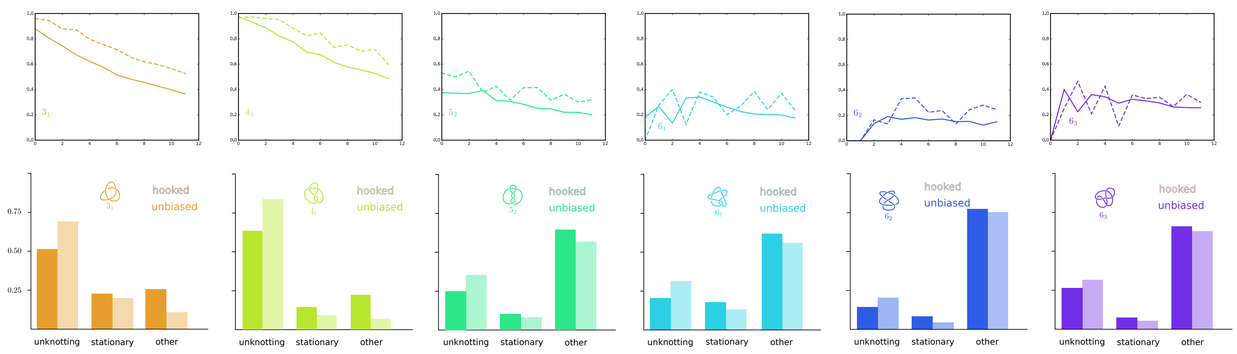


Total data for unbiased strand passages, with GN in the range [8,20].



Total data for strand passages happening only at hooked juxtapositions with GN in the range [8,20].

The right-most circos plot shows a clear **increment in the unknotting probability** (*i.e.* the the probability with which a specific knot type gets converted into the trivial knot) of each knot type; futhermore, in the case of the knot type 5_1 (It is known that it cannot be transformed into the trivial knot by a single crossing change), we note an **increased transition probability** towards knots with **higher unknotting probabilities**. Unknotting probabilities are plotted on the right, as a function of GN. Since the occurence probabilities of six-crossing knots is significantly lower than for the other knot types, the diagrams on the right are affected by statistical noise. To remedy this, we ran a random sampling restricted the each knot type. Results (N = 1000 for each knot type) are shown in the bar diagram. Note that the **stationary probability** (*i.e.* the the probability with which a specific knot type remains the same after a strand passage) of the 6_1 knot (see the bar diagram) is remarkably high with respect to the other six-crossings knots. This is in disagrement with a conjecture from [3], claiming that the probability of remaining in the same knot type after a single random intersegmental passage decreases with the L/D ratio of the corresponding ideal knots [14].



Previous simulations [4], [5] show that strand passages happening at specific hooked geometries present an unknotting preference consistent with experimental data [15]. Besides providing further evidence confirming the **hooked-juxtaposition hypothesis** [9] using a different knot representation, our data provides a map of the i**ntensities of exchanges** between configurations representing a closed DNA molecule, undergoing the action of a **hypothetical unbiased topo II**. We obtain a picture for one-passage connectivity between knots with up to 6 crossings, that can be compared with previous simulations. For instance, we observe a clear difference with results in [3] in the stationary probabilities of each knot type (ours being sensibly lower, with the exception of the 6_1 knot), in the relevant range of GN. Despite these discrepancies, we observe a good agreement in the unknotting probabilities for knots represented by grid diagrams with GN between 12-16. Although these discrepancies may be explained with the significant differences between the models, another reason might be in the different methods of sampling used. While uniformity of sampling is built in in our model, in [3] the equilateral polymers are obtained by applying random moves on the **ideal configurations** [14] of each knot type, possibly creating non-negligible biases.

References

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