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Comment

Thermodynamics and kinetics of TopoII action: A consensus on T-segment curvature selection? Comment on "Disentangling DNA Molecules" by Alexander Vologodskii

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In his *Physics of Life* review, Vologodskii [1] advocates for a "merging" of the hooked-juxtaposition [2] and bend-angle (bent G segment) [3,4] models of disentangling by type II topoisomerases (topoIIs). His review is significant because it signals an emerging consensus, at least among theoreticians, that topoIIs perform T-segment selection [5]. As a researcher of DNA topology who has made important contributions, and particularly as a long-time proponent of active bending of the G-segment by topoII with *no* curvature selection of the T-segment [4], Vologodskii's change of opinion is compelling.

Although structural data exist only for a topoII-bound curved G-segment [6], indirect evidence that narrows theoretical choices to a hooked-juxtaposition-like T-segment selection mechanism has been accumulating. For instance, a recent single-molecule measurement ruled out an alternative kinetic proofreading model [7]. Moreover, an atomic force microscopy study showed that G-segment bending is not the sole determinant of a topoII's disentangling power [8]. Perhaps most notably, direct evidence has been obtained for T-segment selection, though the property being selected remains to be elucidated [9].

In fact, theoretical support for T-segment selection, especially with regard to topoII's Lk narrowing action, is more preponderant than that presented by Vologodskii, who stated that analysis of $\langle(\Delta Lk)^2\rangle$ reduction "has been performed for only one model of the phenomenon" [1]. But there are two other theoretical studies of Lk narrowing [5,10]. In particular, using a wormlike DNA model [11] together with an analytical formulation, we have obtained $\langle(\Delta Lk)^2\rangle$ reduction factors $R_{Lk} \approx 1.7$—1.8 for DNA circle size $\gtrsim$3.5 kb by segment passage at hook-like juxtapositions. The computed R_{Lk} values are in good agreement with experiment [5,9,12]. A broader view of the predicted disentangling

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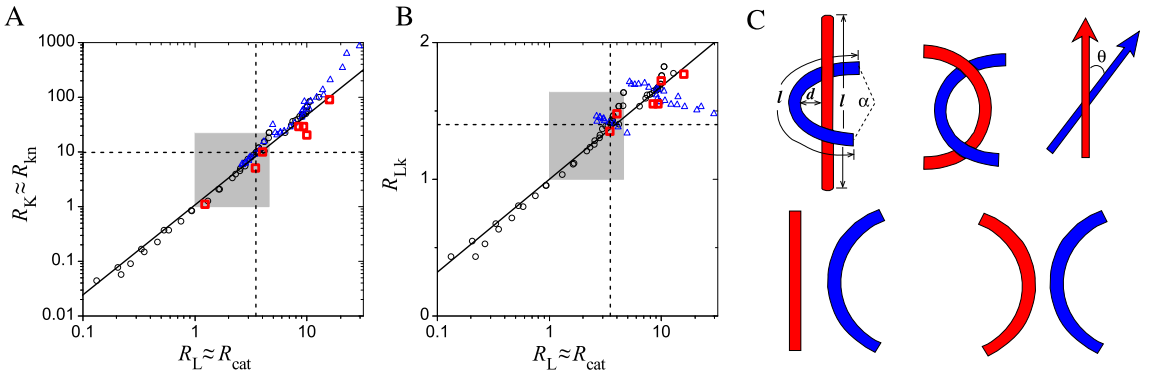

Fig. 1. Extensive Agreement between Theoretical and Experimental TopoII Disentanling Effects. The simulated knot and link (catenane) reduction R_K and R_L (A) and supercoil narrowing R_{Lk} (B) factors were computed in our wormlike chain model [11] for different half-hooked and hooked juxtapositions (C) with $l = 10$ nm (d, α, and θ varied) for 3.5 kb DNA circles in [NaCl] = 0.154 M (black circles). The results satisfy $(R_{Lk} - 1) \approx 0.42 \log_{10} R_K \approx 0.68 \log_{10} R_L$ and thus $R_K \approx (R_L)^{1.6}$. This predicted scaling [5] agrees with that among the corresponding [13] experimental R_{kn}, R_{cat}, and R_{Lk} of Rybenkov et al. (red squares) [14]. The grey boxes (A, B) indicate possible disentangling effects via various half-hooks (first juxtaposition in (C)). The dashed lines (A, B) are for an example half-hooked juxtaposition with $d = 5$ nm, $\alpha = 150^\circ$, and $\theta = 90°$ [15]. The blue triangles were computed by varying DNA circle size. R_K, $R_L < 1$ values were computed using variations of the "free" juxtapositions in the bottom row of (C) [15]. Part of this figure was adapted from [5]; simulation data were from [5,15].

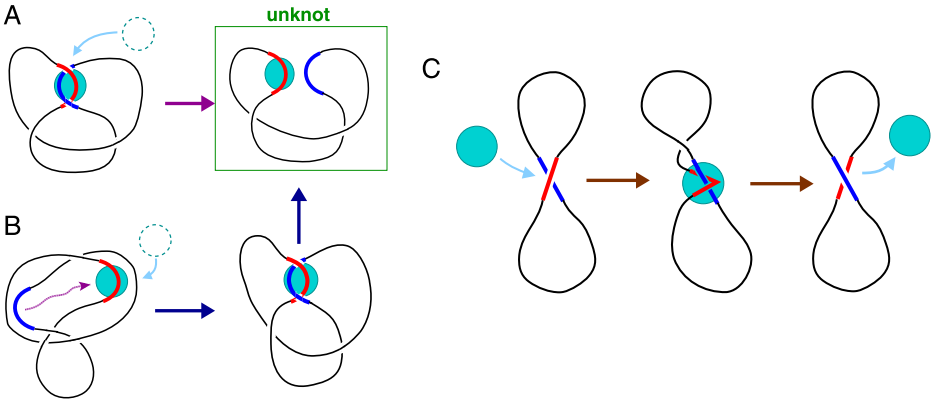

Fig. 2. Possible Two-Stage TopoII Action and Kinetic Noise Introduced by Active Bending of the G-Segment. For any given juxtaposition geometry, (A) one-step (magenta arrow) and (B) two-step (blue arrows) recognition of the juxtaposition by topoII (cyan circle) can produce essentially the same topological steady state [13,5]. (C) Schematics of hypothetical active bending of a straight segment to a V-shape hairpin by topoII.

powers of segment passage via various half-hooked and hooked juxtaposition geometries is provided in Fig. 1, in which topoII's experimental diminishing effect on Lk narrowing with decreasing DNA circle size versus a corresponding enhancing effect on knot and catenane reduction [12] is computationally reproduced and thus rationalized (blue triangles). Segment passages via half-hooked juxtapositions (grey boxes in Fig. 1A and B) produce essentially the same disentangling as the bent G segment model [13]. As has always been clear and re-emphasized by Vologodskii [1], curvature selection of the G-segment alone is insufficient to account for the high disentangling powers of certain real topoIIs [14] (red squares in Fig. 1).

The realization that the disentangling effect of segment passage at a hooked juxtaposition recognized by a topoII in two steps is essentially equal to that at a hooked juxtaposition recognized in one step (Fig. 2A, B) is a major change in perspective on the part of Vologodskii, since he has stated previously that "if the protein binds the G segment first and keeps/creates the bent conformation of the segment until it binds a T segment, the model becomes nearly identical to the hairpin-like G segment model" [4]. However, it is not a new idea that "the hooked-juxtaposition hypothesis is agnostic as to whether the two segments are recognized at the same time or not" [5]. In fact, we have provided a formula relating the two-step knot reduction factor $R_K^{[j/2]}$ with the one-step knot reduction factor R_K, viz.,

$R_K^{[j/2]} = R_K\{[(P_U^{[j/2]})_{eq}/(P_K^{[j/2]})_{eq}][(P_K)_{eq}/(P_U)_{eq}]\}$, where the quantity $\{\ldots\} \approx 1$, hence $R_K^{[j/2]} \approx R_K$ (p. 280 of [13] and Eq. (14) of [5]). In view of this general mathematical relationship, it is not surprising that Vologodskii's latest simulation results in Fig. 9a and Fig. 10 of [1] are very similar to those in Figs. 2 and 4 of [15] and Fig. 5C of [5].

Note that $R_K^{[j/2]} \approx R_K$ applies only if the recognition by topoII is essentially passive, or if active bending of the G-segment as envisioned by Vologodskii is involved [3,4], there is sufficient lag time before T-segment passage so that thermodynamic equilibrium among DNA conformations within each topological state can be re-established after the bending disruption. Otherwise, if T-segment passage occurs immediately after G-segment deformation (Fig. 2C), the topological outcome would be that of the juxtaposition before but not after active bending [5]. Therefore, active bending diminishes topological discrimination. It is equivalent to allowing passage at multiple juxtaposition geometries in equilibrated DNA ensembles. According to the formulas for knot and catenane reduction via multiple juxtapositions (Eqs. (38,39) of [10]), this would result in less disentangling. In other words, active bending allows leakage back in the direction of topological equilibrium. This is a kinetic shortcoming of Vologodskii's active bending model that perhaps he could have addressed. Active bending has a presumed advantage over passive recognition in that it could speed up disentangling kinetics by creating a hairpin from a straight DNA segment [3]. This advantage is lost, however, if topoII has to wait for a naturally curved T-segment after all. Vologodskii's Monte Carlo simulations could have tackled these kinetic questions. It would be instructive if quantitative aspects of simulation kinetics, such as initial positions of the curved DNA segments, were provided in his review.

The above critique notwithstanding, Vologodskii is right in focusing steadfastly on the kinetic viability of proposed topoII mechanisms. Supercoiling [16] can facilitate DNA disentangling [17,18] in the cellular environment. A valid model of topoII action, nonetheless, has to ultimately account kinetically for experiments on relatively relaxed DNA circles as well. Now that the essential thermodynamics of G,T-segment curvature selection has been agreed upon, efforts can be directed toward kinetic issues, and the fundamental question as to whether, and if so, how T-segment selection is effectuated by topoII.

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