Coarse-grained modelling of DNA-RNA hybrids

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We introduce oxNA, a new model for the simulation of DNA-RNA hybrids which is based on two previously developed coarse-grained models—oxDNA and oxRNA. The model naturally reproduces the physical properties of hybrid duplexes including their structure, persistence length and force-extension characteristics. By parameterising the DNA-RNA hydrogen bonding interaction we fit the model's thermodynamic properties to experimental data using both average-sequence and sequence-dependent parameters. To demonstrate the model's applicability we provide three examples of its use—calculating the free energy profiles of hybrid strand displacement reactions, studying the resolution of a short R-loop and simulating RNA-scaffolded wireframe origami.

I. INTRODUCTION

DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are sufficiently similar that they can form stable DNA-RNA hybrids¹. In a biological context an important example of such hybrids is the R-loop, which forms when one of the strands in double-helical DNA is displaced by complementary RNA to create a hybrid duplex and an unpaired DNA strand². In vivo, short R-loops form during nuclear DNA replication by RNA primers, as well as during transcription when nascent RNA anneals to the DNA template inside an RNA polymerase³. The formation of an R-loop is also necessary for the proper functioning of RNA-guided endonucleases in CRISPR-Cas systems where the guide RNA must fully hybridise with its DNA target for cleavage to take place $^{4-6}$. Much longer Rloops (of the order of 1 kilobase) are formed during the replication of mitochondrial DNA and immunoglobulin class-switch recombination³. R-loops play an important role in gene regulation. Errors in their formation and resolution can cause DNA damage, transcription elongation defects, hyper-recombination and genome instabil ity^7 , and they are also implicated in disease^{8,9}. Finally, DNA-RNA hybridisation underlies the action of antisense oligonucleotide (ASO) drugs, a therapeutic modality which has shown great promise in, for example, the treatment of neurological disorders $^{10-12}$.

The specificity and predictability of Watson-Crick base-pairing also makes DNA and RNA excellent candidate materials for the design of synthetic self-assembled nanostructures, underpinning the growing field of nucleic acid nanotechnology¹³. By simply annealing sets of strands with designed patterns of sequence complementarity, DNA has been used to assemble complex shapes^{14,15}, dynamic nanomachines^{16–19} and constructs with potential therapeutic and diagnostic applications^{20–23}. Due to the presence of non-canonical interactions in RNA, its self-assembly is less well characterised. However, the field of RNA nanotechnology is also advancing rapidly, with many examples of functional nanostructures and methods for their assembly^{24–26}. The design of nanostructures comprising DNA hybridised to RNA is under-explored, although interest is increasing with exciting potential uses such as the delivery of therapeutic mRNA and artificial ribozyme fabrication^{27–29}.

Many different approaches have been developed to tackle the problem of nucleic acid modelling and simulation. Analytical mathematical models such as the worm-like chain $(WLC)^{30}$, which treats DNA or RNA as a semi-flexible polymer, can be useful if one is not concerned with details of the structure of the sys-Classical molecular dynamics simulations that tem. consider effective interactions between every atom have yielded useful insights into nucleic acid structure and dynamics, although they can only access microsecond timescales $^{31-33}$. Quantum-chemical calculation is the most fine-grained computational technique used to study nucleic acids³⁴, but this is usually limited to very small systems such as dinucleotides³⁵. Coarse-grained models, in which groups of atoms are represented as single particles, are a viable intermediate which offers a compromise between speed and detail 36,37 . While many coarse-grained models of DNA and RNA have been developed^{38–43}, modelling of hybrid systems, coarsegrained or otherwise, is relatively sparse, and is mostly limited to atomistic simulations^{44–46}. Other examples include a mesoscopic model parameterised to reproduce melting temperatures⁴⁷ and an abstract model for R-loop formation⁴⁸.

Here, we combine the most up-to-date versions of the models for DNA and RNA developed within the oxDNA framework⁴⁹ to enable the simulation of DNA-RNA hybrids. The original average-sequence DNA model⁵⁰ has been extended to introduce sequencedependent thermodynamic properties⁵¹, improved struc-tural properties and salt-dependence⁵². The same coarsegraining methodology has been used to develop an RNA model^{49,53}. A version of the DNA model with sequence-dependent structural and elastic properties is currently under development. The oxDNA family of models has seen tremendous success as tools for the study of nucleic acids and have improved our understanding of DNA and RNA origami⁵⁴⁻⁶⁰ and strand displacement reactions 61,62 , as well as fundamental nucleic acid $biophysics^{63-71}$. The introduction of our hybrid model to include DNA-RNA interactions will further expand the range of systems that can be simulated.

II. THE MODEL

Here we provide a brief overview of the previously developed models for DNA and RNA as well as the introduction of new inter-strand interactions that enable the simulation of hybrids. We then describe in detail how the model was parameterised.

A. oxDNA and oxRNA

In both oxDNA and oxRNA, nucleotides are treated as rigid bodies with interaction sites at the backbone and base. The models take a top-down coarse-graining approach—instead of attempting to exactly replicate the complex intermolecular forces between nucleotides, we use a series of simplified, physically plausible pairwise interactions which we then parameterise so that our model reproduces desired properties of the system. The oxDNA/oxRNA interaction potential takes the following form:

$$U = \sum_{bonded} V_{bb} + V_{stck} + V_{exc} + \sum_{nonbonded} V_{HB} + V_{crstck} + V_{cxstck} + V_{DH} + V'_{exc}.$$
(1)

The first summation runs over all pairs of particles which are connected through covalent bonds, and includes V_{bb} which enforces backbone connectivity, a stacking potential V_{stck} and an excluded volume potential V_{exc} . The second summation runs over all remaining

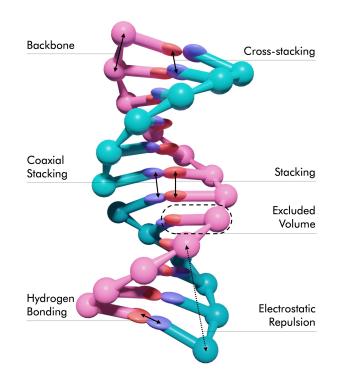


FIG. 1. A nucleic acid duplex as represented by the coarsegrained model, depicting DNA (blue) hybridised to RNA (pink). Interactions between nucleotides are indicated. Linkages between backbone sites indicate strand directionality, getting thinner in the 5' to 3' direction.

pairs of particles—which are not covalently bonded—and includes hydrogen bonding V_{HB} , cross-stacking V_{crstck} , coaxial stacking V_{cxstck} , a Debye-Hückel electrostatic interaction V_{DH} , and excluded volume V'_{exc} . Fig. 1 depicts how nucleic acids are represented in oxDNA and indicates the interactions between nucleotides. Coaxial stacking can be thought of as a modified version of the stacking interaction which is applied across a nick in a backbone. Both the bonded and non-bonded excluded volume terms are implemented as repulsive Lennard-Jones potentials between backbone-backbone, backbone-base and base-base interaction sites. Details of the exact functional forms of individual interactions can be found in the publications which first introduced the models 52,53. oxDNA and oxRNA operate within the same framework and differ only in the relative positions of interaction sites representing DNA/RNA nucleotides and the parameters which govern the strengths of interactions between them.

B. Incorporating DNA-RNA interactions

The hybrid model was implemented by introducing a new DNA-RNA hybrid potential while using existing potentials to handle DNA-DNA and RNA-RNA interactions. The full interaction potential of our hybrid model, for a

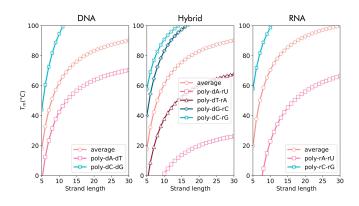


FIG. 2. Duplex melting temperature as a function of strand length for different sequences in DNA, DNA-RNA hybrids and RNA, as predicted by nearest neighbour models. For the average case, the mean melting temperature of 10 000 random sequences was calculated.

system containing both DNA and RNA, now reads

$$U = U_{DNA} + U_{RNA} + U_{hybrid}.$$
 (2)

 U_{DNA} and U_{RNA} include DNA-only and RNA-only interactions, respectively, and have the same general form as Eq. 1. Interactions between DNA and RNA are represented by U_{hybrid} , which uses the non-bonded interstrand potentials—hydrogen bonding, cross stacking, coaxial stacking, the Debye-Hückel interaction and excluded volume. Since we do not allow covalent bonds between DNA and RNA nucleotides, U_{hybrid} does not include any of the bonded interactions in Eq. 2. The forms of these new hybrid interactions are the same as those used in the DNA model and, unless otherwise specified, the same parameters were also used.

C. Parameterisation

Parameterisation of our hybrid model was constrained by the requirement to avoid changes to U_{DNA} and U_{RNA} in order to maintain compatibility with the original oxDNA and oxRNA models; only the hybrid DNA-RNA interactions were modified. In future versions it would be possible to reparameterise all of U_{hybrid} and potentially obtain an even better fit to experimentally measured properties.

As for oxDNA and oxRNA, we parameterise the hybrid model by fitting to the predictions of a nearestneighbour model of thermodynamic properties which has itself been calibrated to reproduce experimental observations. Nearest-neighbour models for nucleic duplex formation are built by first conducting melting experiments for a range of sequences and using these data to estimate the thermodynamic parameters (ΔH and ΔS) associated with the formation of every possible nucleotide pair in the context of its nearest neighbours (as well as initiation parameters). Using these parameters one can estimate the melting temperature (T_m) of an entire duplex, which is defined as the temperature at which the single-stranded (ss) and double-stranded (ds) states are equally probable.

Sugimoto et al. first estimated nearest-neighbour thermodynamic parameters for DNA-RNA hybrids over two decades ago⁷². A more recent set of improved parameters (now also with sequence-specific initiation parameters)⁷³ is employed here. The Sugimoto nearest-neighbour model (SNN) predicts melting temperatures to an accuracy of roughly 1 °C: for the purposes of this work we consider it to be a very good fit to experiment. Fig. 2 highlights the drastic effect that sequence can have on melting temperature in DNA-RNA hybrids, also showing the differences in melting thermodynamics between hybrids, ds-DNA and dsRNA. We used the nearest neighbour model of SantaLucia and Hicks⁷⁴ to estimate dsDNA melting temperatures, and the model of Xia *et al.* for $dsRNA^{75}$. In both cases we employed an empirical salt correction to T_m derived by SantaLucia⁷⁶. Melting temperatures were calculated using Biopython 1.75^{77} . For hybrids it is also noteworthy that, for case of G-C base pairs, there is a dependence on not only sequence but also on the distribution of bases between the DNA and RNA strands, as illustrated by the difference between melting curves for poly-dG-rC and poly-dC-rG. The T_m shown are at a monovalent salt concentration of 1 M, and a total strand concentration of 3.5×10^{-4} M—values which were also used in melting simulations.

To parameterise our model we selected hydrogen bonding strength parameters (i.e. potential well depths) for hybrid A-U, A-T and G-C base-pairs that reproduce the melting temperatures predicted by the Sugimoto model. To estimate the melting temperatures of hybrid duplexes predicted by the model, we simulated duplex dynamics near the melting temperature using the Virtual Move Monte Carlo (VMMC) algorithm⁷⁸ and umbrella sampling⁷⁹. Umbrella sampling weights were chosen to ensure that the transition between the single- and double-stranded state was thoroughly sampled. For any given duplex, simulations were run for 10^9 time-steps (three independent simulations for average-sequence, one for sequence-dependent versions of the model) at the melting temperature predicted by the Sugimoto model. From these simulations we obtain the equilibrium populations of single- and double-stranded states and extrapolate to the temperature at which these states are equally probable—details of the method can be found in the paper introducing oxRNA⁵³. We sought a set of parameters which minimise the following cost function:

$$C = \sum_{i \in S} \Delta T_m(i)^2, \tag{3}$$

where $\Delta T_m(i) = T_m^{VMMC}(i) - T_m^{SNN}(i)$, $T_m^{VMMC}(i)$ and $T_m^{SNN}(i)$ are the melting temperatures predicted by VMMC simulations using our model and the SNN model, respectively, for a given sequence *i*. The sum runs over a training library, *S*. For the sequence-dependent parameterisation, $S = S_{dep}$, comprising data from 4096 6mers and 30000 each of random 8-, 10- and 12-mers. In the average-sequence parameterisation strand length is the only determinant of T_m , in which case we use $S = S_{avg}$, which only contains four training points: strands of length 6, 8, 10 and 12.

For the average-sequence model we assume that all hydrogen bonds have the same strength, ε_{HB} . We ran melting simulations for a range of bond strengths for duplexes of length 6, 8, 10 and 12—in each case we find a linear relationship between $T_m^{VMMC}(i)$ and ε_{HB} . For each duplex length we fit a straight line to the data (Fig. 3(a)), enabling accurate prediction of $T_m^{VMMC}(i)$ from ε_{HB} . We chose the value of ε_{HB} which minimises C over the average-sequence training library, S_{avg} .

In the sequence-dependent case we have four possible types of hydrogen bond, thus four parameters to select: $\varepsilon_{dArU}, \varepsilon_{dTrA}, \varepsilon_{dGrC}$ and ε_{dCrG} . In order to fit sequencedependent parameters, previous iterations of our coarsegrained models used a histogram reweighting technique to calculate melting temperatures and an annealing algorithm to search the parameter space 51-53. This approach is necessary when one is fitting >10 parameters, many of which, e.g. stacking strengths, have quite subtle effects on T_m . Here, since the parameter space to be searched is much smaller, we are able to use a simpler method. We first find an approximate linear mapping between sequence and the melting temperature predicted by our model. We then use this mapping to find the parameters which best reproduce melting temperatures predicted by the Sugimoto model.

In order to search the parameter space, we used the following initial minimisation procedure: (1) Initialise parameters to average-sequence values. (2) For every sequence in S_{dep} , use the current values of ε_{dXrY} to calculate the average bond strength $\overline{\varepsilon}_{HB}(i)$ and, assuming the linear scaling established for the average-sequence model, the corresponding approximation to $T_m^{VMMC}(i)$. Compute C. (3) Randomly perturb parameters to generate new parameters, and repeat step 2 for the new parameter set. (4) If new parameters reduce C, accept them, otherwise repeat step 3. (5) Repeat steps 2 to 4 until Cconverges.

In order to further refine the parameters, we needed a better mapping between sequence and $T_m^{VMMC}(i)$. With this in mind, we used multiple linear regression (MLR) to predict the result of a VMMC calculation of the melting temperature of a sequence i of length k, such that

$$T_m^{VMMC}(i) = \beta_0 + \beta_1 x_1(i) + \dots + \beta_k x_k(i), \qquad (4)$$

where $x_n(i)$ is the hydrogen bonding strength of the n^{th} base-pair within the duplex (read out in a 5' to 3' direction with respect to the DNA strand), and $\beta_0, \beta_1, \dots, \beta_k$ are fitting parameters obtained from a leastsquares minimisation. For example, for a hybrid duplex 5'-dATGC-3'/3'-rUACG-5', we would estimate T_m as $\beta_0 + \beta_1 \varepsilon_{dArU} + \beta_2 \varepsilon_{dTrA} + \beta_3 \varepsilon_{dGrC} + \beta_4 \varepsilon_{dCrG}$.

Using our previously obtained estimates of the bonding parameters, we ran melting simulations for 500 random 4

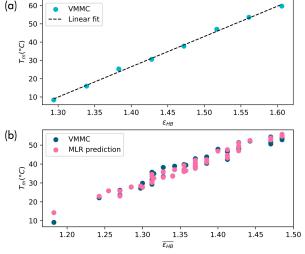


FIG. 3. Dependence of melting temperature of an 8-mer on hydrogen bonding strength, obtained from VMMC simulations. (a) Melting temperature as a function of hydrogen bonding strength calculated using the average-sequence model alongside a fitted straight line. (b) Melting temperatures of 50 random sequences, calculated by VMMC simulation, plotted against the mean hydrogen bonding strength of the sequence, and the corresponding fits to an MLR model.

sequences (125 per duplex length) and used these data to fit an MLR model for each length of duplex (Fig. 3(b)). We then performed the minimisation procedure described above—now with the improved VMMC predictions made using the MLR models—to arrive at a refined parameter set. We repeated all of the above (i.e. melting simulations of 500 random sequences, MLR model fitting, followed by the minimisation) for a final time, but found that by this point the parameters had converged.

Note that initially we also attempted to fit the strength of the cross-stacking interaction for the average-sequence model. We performed preliminary fitting of the hydrogen bonding and cross-stacking (K_{crstck}^{hybrid}) strengths simultaneously, and found that the value which minimised C was $K_{crstck}^{hybrid} = 0.938 K_{crstck}^{DNA}$, where K_{crstck}^{DNA} is the value used by the DNA model (for reference, $K_{crstck}^{RNA} = 1.262 K_{crstck}^{DNA}$). However, we also found that increasing K_{crstck}^{hybrid} while decreasing ε_{HB} accordingly (and vice versa), made little difference to the overall fit. Since the relative strengths of the cross-stacking and hydrogen bonding interactions are not experimentally constrained, different values for K_{crstck}^{hybrid} and ε_{HB} could have been cho-sen without detriment to the model. We chose to set $K_{crstck}^{hybrid} = 0.938 K_{crstck}^{DNA}$ and then selected ε_{HB} using the procedure outlined in Section II C.

The coaxial stacking and Debye-Hückel interactions also could, in principle, have been reparameterised. However, to the best of our knowledge, no data for DNA-RNA hybrids exists which could be used to fit these interactions. We set parameters of Debye-Hückel interaction for hybrids to the values used by the RNA model. The hybrid model uses the same coaxial stacking interaction as the DNA model.

III. PROPERTIES OF THE MODEL

In this section we report the physical predictions of our model. These include the structure of double-stranded DNA-RNA hybrid duplexes, the melting behaviour of both the average-sequence and sequence-dependent versions of our model, and mechanical properties such as persistence length and force-extension characteristics.

A. Structure

The structures of double-stranded DNA and RNA differ significantly—DNA most commonly folds into a B-form helix, whereas RNA takes up an A-form conformation. The A-form helix is characterized by significant slide (displacement of adjacent base pairs along the long axis of the pair) and roll (the angle by which base-pairs open up toward the minor groove), with the result that base pairs are shifted away from the helical axis and inclined to $it^{80,81}$. Fig. 4(d) defines the parameters x-displacement and inclination⁸² which are used to characterise the structure of the double helix in this work.

Reports on the exact structure of DNA-RNA hybrids vary. Thanks to studies of polymeric hybrids it is largely accepted that poly-rA-dT can experience an A- to B-form transition with changes in relative humidity⁸³. Hybrids containing poly-dA-rU or poly-dIrC have been termed *heteromerous*, whereby the DNA and RNA strands possess B- and A-form characteristics respectively⁸⁴. The detailed structure of oligomeric hybrids can depend on sequence—it is known, for instance, that the purine/pyrimidine content of the DNA strand can change the backbone conformation⁸⁵. An NMR study by Gyi et al.⁸⁶ found that the extent of A- or Bform helicity, as well as the major/minor groove widths vary with purine/pyrimidine content. They also found that a high-purine DNA strand results in greater conformational diversity as a result of increased sugar flexibility, compared to the case when the RNA strand of the hybrid duplex is high in purine. More recent crystallography studies of oligomeric DNA-RNA hybrids typically characterise them as A-form^{87–89}, and estimates of their exact x-displacement and inclination obtained from allatom simulations suggest a structure in-between those of DNA and RNA⁴⁶.

To determine the structure of a hybrid duplex in our model, and compare it to DNA and RNA, we generated 10 000 uncorrelated configurations of each class of 16 base-pair duplex by performing average-sequence Monte Carlo simulations at 25 °C, with a monovalent salt concentration of 0.5 M. We then measured the helical parameters of each configuration and calculated their means, shown in Table I. Note that the values for the RNA

^a As reported by Snodin *et al.*⁵²

^b As reported by Snodin *et al.* at 0.5 M salt^{52}

^c As reported by Šulc *et al.* for the first version of α RNA⁵³

TABLE I. Comparison of the inclination, x-displacement, pitch and rise helical parameters for double stranded nucleic acids, obtained from simulations of our model.

Parameter	DNA	Hybrid	RNA
ε_{HB}	1.07	1.50	0.87
ε_{dArU}	N/a	1.21	0.82
ε_{dTrA}	0.89	1.37	N/a
$\varepsilon_{dGrC/dCrG}$	1.23	1.61/1.77	1.06

TABLE II. The hydrogen bonding parameters of the model (in simulation units), compared to analogous parameters for the DNA and RNA models.

model differ from those first reported by Sulc *et al.*⁵³, since the model used here includes salt-dependent effects which were not included in the original model. Representative structures are shown in Fig. 4. We see that the values of inclination, x-displacement and pitch are intermediate with respect to those for DNA and RNA. While our coarse-grained models are not primarily designed to achieve structural accuracy, it is encouraging that the high-level structural features of DNA-RNA hybrids emerge without being explicitly imposed.

In oxRNA, an A-form conformation is imposed on the helix by making the stacking interaction dependent on the angle between the nucleotide orientation vector and the backbone vector connecting neighbouring nucleotides, such that the potential energy of the stacking interaction is minimised if the helix adopts an A-form geometry. This angular dependence is not present in the DNA model and, in hybrids, only the RNA strand has this modified stacking interaction. However, the short range of the hydrogen-bonding interaction forces base pairs to lie approximately in the same plane, resulting in a compromise between A- and B-forms. We find that this intermediate helix geometry has an effect on thermodynamic propertiess which is discussed in the next section.

B. Thermodynamics

The model parameters selected by the fitting procedure described in Section II C, and used below, are shown in Table II. We note that the hydrogen bonding parameters required to reproduce the correct melting temperatures

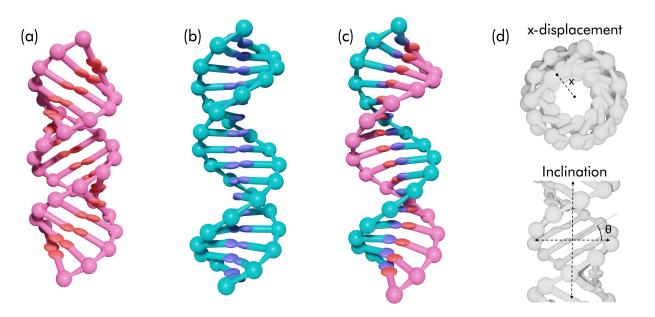


FIG. 4. A comparison of the structures of double-stranded nucleic acids in oxDNA and oxRNA. Shown side-by-side are the structures adopted by a 16-mer of (a) RNA, (b) DNA and (c) a DNA-RNA hybrid, which naturally adopts a conformation somewhere between that of A-form RNA and B-form DNA. (d) An illustration of the x-displacement and inclination helical parameters, which are used to structurally characterise the helices. The shortest distance, x, between the helical axis and the interaction site where bases meet is defined as x-displacement. Inclination is the angle made between a base and the plane perpendicular to the helical axis, indicated as θ .

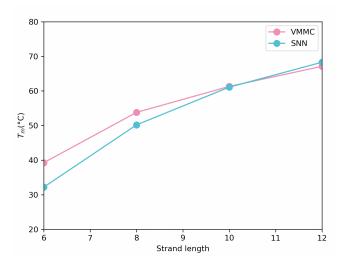


FIG. 5. Melting temperature as a function of duplex length calculated for the average-sequence hybrid model, using VMMC simulations, compared to the target T_m obtained from the Sugimoto nearest neighbour model.

are substantially larger than in either oxDNA or oxRNA: this point is discussed below.

The fit of the average-sequence model to target melting temperatures is shown in Fig. 5. While in general our model reproduces the melting behaviour of short hybrid duplexes quite well, there is a noticeable deviation from target temperatures at short strand lengths—for strands of length 6 and 8 the melting temperature is overestimated by around 7.1 °C and 3.6 °C, respectively. In the average-sequence DNA and RNA models corresponding deviations are typically no more than 1 °C.

In order to investigate how hybridisation between DNA and RNA affects individual interactions, we computed the mean potential energies associated with stacking and hydrogen bonding using a simulation protocol similar to that used in Section III A but with the temperature set to 1°C in order to reduce fluctuations away from the double-stranded ground state. In general, stacking contributes less to the stability of hybrids than of dsDNA or dsRNA duplexes. This is because A- and B-form geometries respectively were imposed onto the RNA and DNA models through the forms of the interaction potentials: when part of a hybrid duplex, neither the DNA nor RNA is in its preferred conformation, which has a destabilising effect. This explains why the fitting procedure described in Section II C increases the hydrogen bonding strengths to compensate (cf. Table II). We also find that, as strand length increases, both stacking and hydrogen bonding interactions become, on average, less stabilising. This can be understood as a consequence of stabilising relaxation of the strained duplex near the ends—which becomes relatively less important as the duplex increases in length. It is also noteworthy that stacking is more disrupted for the RNA strand of a hybrid duplex than for the DNA strand. We propose that this tendency for the (RNA) stacking and hydrogen bonding to weaken with increasing strand length is the reason for the melting temperature overestimation in 6- and 8mers. The model could be further adapted to include a

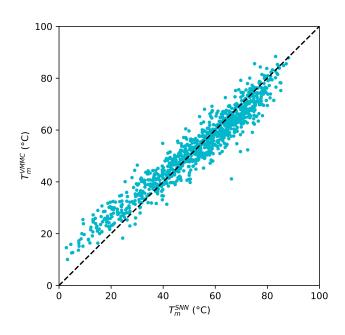


FIG. 6. Performance of the sequence-dependent hybrid model, tested on 1000 random sequences. The plot shows the melting temperature predicted by the model, against the value predicted by the Sugimoto nearest neighbour model. The dashed line indicates y = x.

modified stacking potential which can better accommodate hybrids, enabling an even better fit to experimental melting temperatures. This could be implemented by including a double-well angular/radial dependence in the stacking interactions, such that A- and B-form helicities are maintained in dsRNA and dsDNA respectively, while also allowing a hybrid duplex to inhabit a second potential energy well, mitigating the destabilising effect in the current version of the model.

In order to test the sequence-dependent version of the model, we ran melting simulations on 1000 random duplexes of lengths 6, 8, 10 and 12 (250 per length). Sequences with predicted melting temperatures below 1 °C (short, U-rich sequences) were discarded. Results are shown in Fig. 6. Over this 1000-sequence test set, the model achieves a mean ΔT_m of 0.0926 °C, with a standard deviation of 5.36 °C. While we consider this to be a more than satisfactory fit, we are aware of factors which limit our model's performance. The first is its over-estimation of the stability of short duplexes, as discussed for the average-sequence model. In Fig. 6, there is noticeable overestimation of T_m in the <30 °C region, which is almost certainly a manifestation of this effect. As discussed in the previous section, sequence can affect backbone conformation. Our model does not factor in these structural changes, which likely worsens the overall sequence-dependent fit.

C. Mechanical properties

The mechanical properties of nucleic acids are biologically important⁹⁰ and determine the mechanical behaviour of synthetic constructs like DNA origami⁹¹. For this reason, it is important to check that our model captures the basic mechanics of double-stranded DNA-RNA hybrids. Here we measure the persistence length and force-extension characteristics of hybrid duplexes within our model, and compare the results to available experimental data.

The persistence length L_p of a polymer quantifies its bending stiffness. In a semi-flexible, infinitely long polymer, the persistence length quantifies the correlation between local helix orientations:

$$\langle \mathbf{n}(k) \cdot \mathbf{n}(0) \rangle = \exp\left(\frac{-k\langle r \rangle}{L_p}\right),$$
 (5)

where $\mathbf{n}(k)$ is the local helical axis vector of the k^{th} basepair along the duplex and $\langle r \rangle$ is the rise per base-pair⁹². To measure L_p , we performed molecular dynamics (MD) simulations of a 150 base-pair hybrid duplex with the average-sequence model at 22 °C, with the monovalent salt concentration set to 0.5 M. We ran 10 independent simulations, each for 10^8 time-steps, integrated using Langevin dynamics with a damping constant equal to the time-step. We sampled simulation frames every 10^4 timesteps giving us a total of 10^5 configurations. For each base-pair we computed the centre of mass, and translated it to account for the shift in an A-form helix to give us a point on the helical axis. From these points we calculate local helical axis vectors which are used to obtain $\langle \mathbf{n}(k) \cdot \mathbf{n}(0) \rangle$. We discard the 5 terminal base-pairs to avoid end effects. From the gradient of the line in Fig. 7(a), we obtain an estimate of $L_p = 39$ nm.

A separate set of simulations was performed to measure the force-extension relationship. We used the same settings as before, except that in this case we ran 30 replicas, each for 10^7 time-steps. We applied a uniformly increasing, equal and opposite force of up to 50 pN to terminal nucleotides and sample the distance between them every 10^3 time-steps to measure the extension, which was averaged over independent simulations. In this case, we fit our data to the extensible worm-like chain model⁹³ which predicts that the projected end-to-end distance Lof a polymer along the direction of a force with magnitude F is, in the limit $F > k_B T/2L_p$,

$$L = L_c \left[1 + \frac{F}{K} - \frac{k_B T}{2FL_c} (1 + A \coth A) \right], \qquad (6)$$

where

$$A = \sqrt{\frac{FL_c^2}{L_p k_B T}},$$

K is the stretching modulus, and L_c is the relaxed contour length. The results are shown in Fig. 7(b). Fitting to our data gives $K = 780 \text{ pN}, L_c = 51 \text{ nm}$ and $L_p = 20$ nm. It must be pointed out that the value of K especially is quite sensitive to the size of the fitting window—for example, fitting up to only 30 pN doubles the estimated stretching modulus (L_p is 25% lower and L_c changes very little). Note also that a similar issue was observed for oxRNA (but not oxDNA) and was ascribed to a decrease in the inclination angle as the force increased⁵³. Consequently, the error on these estimates can be assumed to be relatively large, which should be kept in mind when comparing to experimental values, and the persistence length obtained from the tangent-tangent correlation function should be considered to be more accurate.

Experimental data on the mechanics of hybrid duplexes is scarce, and the number of all-atom simulation studies is also low. Zhang $et \ al.^{94}$ performed a series of magnetic tweezer experiments to measure the mechanical properties of a long (>10 kilobase) hybrid duplex at different salt concentrations. They report a stretching modulus of 660 pN, which does not depend strongly on salt concentration. Conversely, salt does have an effect on persistence length which ranges from 49 nm to 63 nm at salt concentrations of 0.5 M and 1 M respectively. An all-atom simulation study performed at 1 M monovalent salt concentration estimated a stretching modulus of $834 \,\mathrm{pN^{46}}$. Given that the model is parameterised to reproduce thermodynamic properties, the agreement between calculated and measured elastic properties is satis factory. We note that for low applied forces (<35 pN)or so) the persistence length is more significant than the stretching modulus in determining the mechanical behaviour of the duplex.

To put this into perspective, the persistence length L_p of dsDNA at moderate to high salt concentration is in the range 45–50 nm, and the stretching modulus K is around 1050–1250 pN at high salt⁵⁰. The first version of the oxDNA model achieves $L_p = 43.8$ nm and K = 2120 pN. For dsRNA, experimental estimates of L_p are in the range 58–80 nm and K = 615 pN, while for the oxRNA model $L_p = 28.3$ nm and K = 296 pN.

IV. APPLICATIONS OF THE MODEL

We provide examples of the application of the model to three hybrid systems—toehold-mediated strand displacement, a short R-loop and RNA-scaffolded wireframe origami, all of which are technologically and/or biologically important.

A. Toehold-mediated strand displacement

Toehold-mediated strand displacement (TMSD) is a process in which one of the strands within a nucleic acid duplex is exchanged for another: displacement of the incumbent strand is initiated by the binding of the invader to a short single-stranded toehold region on the comple-

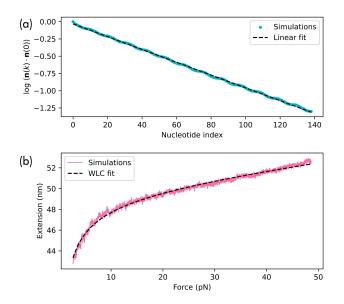


FIG. 7. Measuring the mechanical properties of a 150-mer DNA-RNA hybrid. (a) Natural logarithm of the correlation function $\langle \mathbf{n}(k) \cdot \mathbf{n}(0) \rangle$ against the nucleotide index, k. (b) The force-extension curve obtained from simulations, alongside a fit to the extensible worm-like chain.

mentary strand^{16,95} (Fig. 8(a)). TMSD has many applications in nanotechnology, including in the construction of synthetic molecular circuits⁹⁶. DNA-RNA hybrid TMSD is of particular interest by virtue of its relevance to *in vivo* applications⁹⁷. Strand displacement has also been argued to play important roles in various naturally-occurring RNA systems⁹⁸.

We note that oxDNA has been remarkably successful in reproducing experimental observations related to TMSD, having been used, for example, to study mismatches as a tool for modulating strand displacement kinetics^{99,100}. RNA strand displacement has likewise been simulated using the oxRNA model⁶¹.

Here, we use our newly developed model to study strand displacement systems involving DNA-RNA hvbrids. As in the melting simulations, we use a combination of VMMC and umbrella sampling to explore the state space efficiently. From the simulations, we obtain unbiased estimates of equilibrium populations of states parameterised by the number of substrate-invader hydrogen bonds. In simulations of toehold-mediated strand displacement, we assigned a weight of zero to states with no hydrogen bonds between substrate and invader or substrate and incumbent hydrogen bonds, to prevent dissociation. Umbrella sampling weights were chosen (by trial and error) so that all states have approximately equal occupancy (within an order of magnitude) in the biased ensemble. Assuming that the state space has been adequately sampled, the free energy difference ΔG between states A and B can be written as

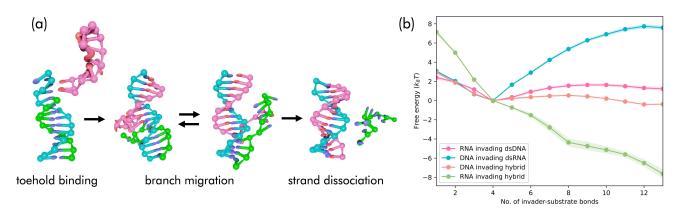


FIG. 8. Simulation of toehold-mediated strand displacement using the average-sequence model. (a) Snapshots of key steps for one of the strand displacement reactions: An RNA strand (pink) invades a DNA duplex (blue and green) by binding initially to a single-stranded toehold. (b) Free energy profiles of the different systems simulated, showing the free energy (set to zero for a fully-occupied toehold) against the number of hydrogen bonds between the substrate and invader strands. Error bars indicate the standard error of the mean.

$$G(A) - G(B) = -k_B T \ln\left[\frac{p(A)}{p(B)}\right],$$
(7)

where p(A) and p(B) are the probabilities of being in state A and B respectively. We can similarly compute free energy profiles for systems with multiple states. For every system studied, we ran 10 independent simulations for 10^9 time-steps each, at $37 \,^{\circ}$ C and a $0.5 \,\text{M}$ monovalent salt concentration using the average-sequence model. We simulated four systems—an RNA strand invading ds-DNA, a DNA strand invading dsRNA, a DNA strand invading a hybrid duplex to displace an RNA incumbent from a DNA substrate, and finally an RNA strand invading a hybrid duplex to displace an DNA incumbent. In each case, the toehold region was 4 nucleotides long, with a 10-nucleotide branch migration domain. Results are shown in Fig. 8(b).

A common feature of all of the free energy profiles is the initial downhill trajectory in the range of 1 to 4 invadersubstrate hydrogen bonds. This is associated with toehold binding, which is always favourable, as there is no competition between strands. Generally, there is an entropic barrier associated with the formation of a branch junction during strand displacement, which is seen as an activation barrier in the branch migration region (for RNA invading dsDNA and DNA invading hybrid). In the case of DNA invading dsRNA, the landscape is steeply uphill, as on average dsRNA is substantially more thermally stable than a DNA-RNA hybrid. Conversely, when RNA invades a hybrid this results in the formation of dsRNA, which is much more thermally stable than a hybrid duplex, resulting in a downhill landscape. This can be understood in terms of the difference in average melting temperature between dsDNA and dsRNA—around 60 °C and 71 °C for a 10 base-pair duplex respectively The difference between the free energy landscapes for RNA invading dsDNA and DNA invading a hybrid is

more subtle because hybrids and dsDNA are quite close in melting temperature (around 61 °C for a 10 base-pair hybrid duplex). It is likely that this relative difference is smaller than the typical effects of varying base sequence.

The simulations performed here only scratch the surface of what can be studied with the model—future work will investigate the effect of sequence on TMSD free energies and kinetics. Preliminary simulations with the model suggest that free energy landscapes, as well as reaction kinetics, are strongly sequence-dependent. We are also looking into how secondary structure in the RNA strand impacts the reaction. Given the success of previous oxDNA models in studying TMSD, we are confident that our DNA-RNA hybrid model will provide useful insights.

B. R-loop resolution

An R-loop is a three-stranded nucleic acid structure consisting of double-stranded DNA which is partially hybridised to complementary RNA. As discussed in Section I, this is possibly the most important naturally occurring DNA-RNA hybrid system.

We use our coarse-grained model to simulate the resolution of an R-loop. While this system appears to be similar to the TMSD studied in Section IV A, as both involve DNA-RNA strand displacement, we observe behaviour which is quite different. The simulation protocol used closely resembles our TMSD simulations. We study a single R-loop consisting of 55 base-pair double-stranded DNA which is hybridised to a 25-nucleotide RNA strand at its centre (Fig. 9 (a), top). As before, in order to prevent strand dissociation we restrict the system to states with at least one DNA-DNA and one RNA-DNA hydrogen bond, and use average-sequence parameters. In this case, we ran separate simulations for two overlapping windows of the order parameter space—one restricted to 1–13 RNA-DNA hydrogen bonds, and another to 13–25 bonds. We performed 10 independent VMMC simulations per window, each for 3×10^8 time-steps. Temperature and monovalent salt concentration were the same as for our TMSD simulations.

Computed free energy profiles are shown in Fig. 9(b). There is a barrier of around $2k_BT$ associated with the transition from 1 to 2 RNA-DNA bonds. The zoomed-in snapshot of the resolved state in Fig. 9(a) suggests an explanation. In the resolved state, the DNA double helix tends to be fully closed, with the RNA strand forming a weak hydrogen bond with one of the DNA strands. As a result, in order to make the transition from one to two RNA-DNA bonds, two DNA-DNA bonds must be broken, which is energetically costly. This is in part an artefact of restricting the simulation to bound states. Without this restriction, the RNA strand would have dissociated completely in the resolved state.

In general, we observe that the formation of the DNA-RNA hybrid in this particular system is significantly less favourable than in the analogous TMSD reaction of RNA invading dsDNA, depicted in Fig. 8(b). Several factors contribute to the difference between the two energy landscapes. In a fully-formed R-loop, displacement of the RNA strand can take place from either end: the DNA loop is tethered at both sides, increasing its proximity to the hybrid, making displacement more likely. Resolving an R-loop is clearly entropically favourable, as it entails exchange of a single strand tethered at both ends for one tethered at only one end in our simulations, or fully displaced in practice, thus having much greater conformational freedom.

We also observe an oscillatory component to the free energy which has minima at R-loop sizes of around 13 and 23 RNA-DNA bonds. When the DNA-RNA hybrid helix is of a size roughly commensurate with its pitch (around 11 base pairs), the ends of the displaced DNA loop are on the same side of the duplex, which entails higher conformational freedom. Conversely, at half a turn away, e.g. around 18, the ends are at opposite sides of the duplex, reducing conformational freedom, and leading to a slight additional increase in free energy cost.

The inset in Fig. 9(b) depicts a 2D free-energy landscape that provides additional information about the system. The presence of the R-loop destabilises the DNA double helix beyond the region of the DNA-RNA hybrid, with states which are not fully hybridised being readily accessible. This is clear from the fact that, at any given number of RNA-DNA bonds, states with numbers of DNA-DNA bonds below what would be expected for a fully hybridised system (55 bonds in total) are sampled.

The stability of an R-loop depends on its length and sequence¹⁰¹. An obvious future application of our model would be a comprehensive study of the effects of these factors. The kinetics of R-loop resolution could also be studied using specialised sampling techniques.

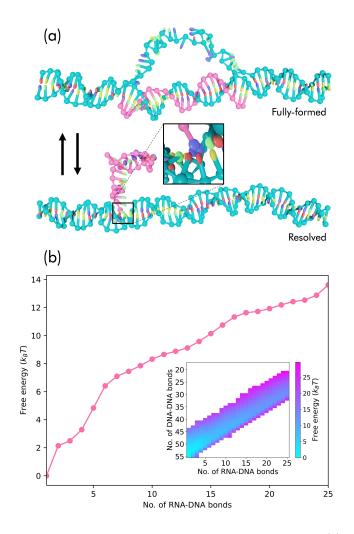


FIG. 9. Studying the resolution of a short R-loop. (a) A fully-formed 25-nucleotide R-loop, consisting of doublestranded DNA (blue) and a single strand of RNA (pink). Through the process of strand displacement, the system can resolve the R-loop by forcing out the RNA strand. In our simulations, this transition is sampled many times in both directions. (b) Free energy of the system as a function of the number of RNA-DNA hydrogen bonds and (inset) the number of both DNA-DNA and RNA-DNA bonds (states with fewer than a total of approximately 45 base pairs are not sampled).

C. RNA-scaffolded wireframe origami

Nucleic acid origami is one of the most common techniques used for assembling single-stranded DNA/RNA building blocks into a target structure. Origami nanostructures consist of a scaffold, which is a long strand running through the entire assembly, and shorter staple strands which hybridise to two or more scaffold domains to control its spatial arrangement. Domains of the scaffold strand which are widely separated in its primary sequence can be held in close spatial proximity in the final structure. This technique has been applied primarily to DNA, although interest in the design of DNA-RNA

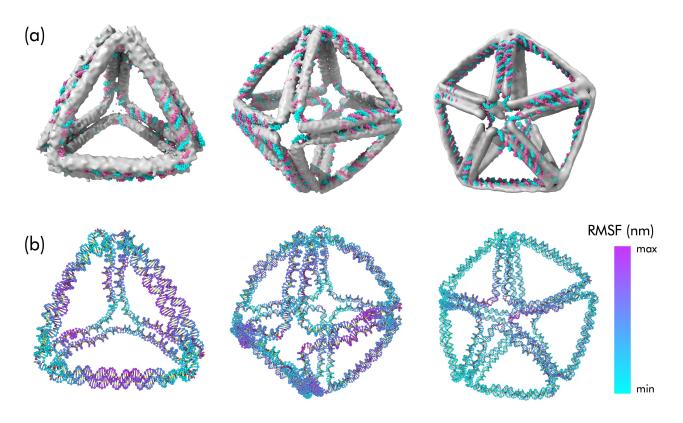


FIG. 10. Mean structures of RNA-scaffolded origami simulated using the model. (a) Atomic models of the tetrahedron (left), octahedron (middle) and pentagonal bipyramid (right), each consisting of an RNA scaffold strand (pink) and DNA staples (blue). Experimentally obtained cryo-EM densities (grey) have been superimposed onto each structure. (b) Structures with colouring to indicate the per-nucleotide RMSF. The structures have different fluctuation ranges: 1.15–1.72 nm, 1.38–2.13 nm and 1.47–3.87 nm respectively.

hybrid nanostructures is increasing.

We have used our model to simulate three hybrid wireframe origami nanostructures from Parsons et al.²⁹ which consist of an RNA scaffold and DNA staples. The structures were designed assuming a double helix with a pitch of 11 base-pairs per turn, which is roughly reproduced by our model. We performed MD simulations at 4 °C and a monovalent salt concentration of 0.3 M, to match the experiments. Each structure was simulated for 10^7 time-steps and the positions of particles were sampled every 10^4 time-steps for analysis. We simulated three nanostructures—a tetrahedron, an octahedron and a pentagonal bipyramid—each having edges 66 base-pairs long. For each, we calculated the mean structure and pernucleotide RMSF (root-mean-square fluctuation). From these mean structures, we reconstructed all-atom models of the nanostructures using the oxDNA-to-PDB converter on TacoxDNA⁷⁰ (by superimposing atomic coordinates onto individual nucleotides) and then aligned them with cryo-EM densities, obtained by Parsons et al. and retrieved from EMDB¹⁰², using ChimeraX¹⁰³.

Fig. 10 compares our results to the experimental data. Our model captures the measured structures reasonably well, with no systematic strain build-up. For the tetrahedron and octahedron it is immediately clear that structural fluctuations are concentrated at edge centres.

V. CONCLUSION

We have introduced a new coarse-grained model, based on existing oxDNA and oxRNA models, which enables the simulation of DNA-RNA hybrids. As with previous models, we parameterised the hydrogen bonding interaction to reproduce the melting temperatures of short duplexes. Quantitative agreement with the experimentallycalibrated nearest-neighbour model of the thermodynamics of hybrid duplexes is nearly as close as that achieved for DNA and RNA duplexes using oxDNA and oxRNA. The persistence length and stretching modulus derived from simulations of longer duplexes are consistent with experimental values, although some uncertainty about their values remains. The conformation of DNA-RNA hybrid duplexes is a compromise between the structures preferred by DNA and RNA alone. As a result, stabilization of the duplex by stacking interactions is reduced, necessitating the increase of hydrogen bonding strength to produce desired melting temperatures. One consequence of this choice is that the model overestimates the stability of short double-stranded helices—something which users

of the model should keep in mind. Nevertheless, the overall performance of our DNA-RNA hybrid model for the systems we studied gives us confidence that it will be able to capture sequence-dependent kinetics/thermodynamics of more complex biophysical processes. A future version of the model will include a modified stacking potential which can accommodate the preferred conformations of dsDNA, dsRNA and DNA-RNA hybrids.

We have demonstrated the versatility and applicability of our model by performing simulations for three different systems. Our study of toehold-mediated strand displacement using the average-sequence model suggests that the relative stabilities of DNA-DNA, RNA-RNA and DNA-RNA duplexes plays a key role in determining the free energy landscapes of hybrid displacement reactions. Our simulations show that the biophysics of R-loop resolution includes geometric effects related to the commensurability of the R-loop length and the pitch of the double helix. Finally, we have shown that our model can help validate DNA-RNA hybrid origami designs.

Future work will focus on DNA-RNA hybrid systems at time and length-scales that are inaccessible to all-atom simulations, including the sequence-dependent kinetics of strand displacement reactions and the effects of RNA secondary structure motifs.

VI. CODE AVAILABILITY

The code implementing the model alongside the supporting documentation can be found at https:// lorenzo-rovigatti.github.io/oxDNA/. A new topology file format supporting DNA-RNA hybrids has been implemented in the official oxDNA code, and the accompanying suite of analysis tools has likewise been extended to enable the analysis of systems containing both DNA and RNA. The online visualization tool oxView.org¹⁰⁴ has been extended to also support viewing of DNA-RNA hybrids. The simulations performed here were run on single CPUs, although a GPU version of the model is a likely future development.

VII. ACKNOWLEDGMENTS

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VIII. REFERENCES

- ¹G. Milman, R. Langridge, and M. J. Chamberlin, "The structure of a DNA-RNA hybrid." Proceedings of the National Academy of Sciences **57**, 1804–1810 (1967).
- ²E. Petermann, L. Lan, and L. Zou, "Sources, resolution and physiological relevance of R-loops and RNA–DNA hybrids," Nature Reviews Molecular Cell Biology **23**, 521–540 (2022).
- ³A. Aguilera and B. Gómez-González, "DNA–RNA hybrids: the risks of DNA breakage during transcription," Nature Structural & Molecular Biology 24, 439–443 (2017).
- ⁴B. Zhang, D. Luo, Y. Li, V. Perčulija, J. Chen, J. Lin, Y. Ye, and S. Ouyang, "Mechanistic insights into the R-loop formation and cleavage in CRISPR-Cas12i1," Nature Communications **12**, 3476 (2021).
- ⁵M. Pacesa, L. Loeff, I. Querques, L. M. Muckenfuss, M. Sawicka, and M. Jinek, "R-loop formation and conformational activation mechanisms of Cas9," Nature **609**, 191–196 (2022).
- ⁶F. Jiang and J. A. Doudna, "CRISPR-Cas9 structures and mechanisms," Annual Review of Biophysics 46, 505–529 (2017).
- ⁷C. Niehrs and B. Luke, "Regulatory R-loops as facilitators of gene expression and genome stability," Nature Reviews Molecular Cell Biology **21**, 167–178 (2020).
- ⁸C. Rinaldi, P. Pizzul, M. P. Longhese, and D. Bonetti, "Sensing R-loop-associated DNA damage to safeguard genome stability," Frontiers in Cell and Developmental Biology 8, 618157 (2021).
- ⁹A. Brambati, L. Zardoni, E. Nardini, A. Pellicioli, and G. Liberi, "The dark side of RNA:DNA hybrids," Mutation Research/Reviews in Mutation Research **784**, 108300 (2020).
- ¹⁰D. D. Fusco, V. Dinallo, I. Marafini, M. M. Figliuzzi, B. Romano, and G. Monteleone, "Antisense oligonucleotide: Basic concepts and therapeutic application in inflammatory bowel disease," Frontiers in Pharmacology **10**, 305 (2019).
- ¹¹J. Lee and T. Yokota, "Antisense therapy in neurology," Journal of Personalized Medicine 3, 144–176 (2013).
- ¹²C. Rinaldi and M. J. A. Wood, "Antisense oligonucleotides: the next frontier for treatment of neurological disorders," Nature Reviews Neurology **14**, 9–21 (2017).
- ¹³Y. Krishnan and N. C. Seeman, "Introduction: Nucleic acid nanotechnology," Chemical Reviews **119**, 6271–6272 (2019).
- ¹⁴P. W. K. Rothemund, "Folding DNA to create nanoscale shapes and patterns," Nature **440**, 297–302 (2006).
- ¹⁵S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf, and W. M. Shih, "Self-assembly of DNA into nanoscale threedimensional shapes," Nature **459**, 414–418 (2009).
- ¹⁶B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel, and J. L. Neumann, "A DNA-fuelled molecular machine made of DNA," Nature **406**, 605–608 (2000).
- ¹⁷A. J. Turberfield, J. C. Mitchell, B. Yurke, A. P. Mills, M. I. Blakey, and F. C. Simmel, "Dna fuel for free-running nanomachines," Phys. Rev. Lett. **90**, 118102 (2003).
- ¹⁸E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf, and J. Kjems, "Self-assembly of a nanoscale DNA box with a controllable lid," Nature **459**, 73–76 (2009).
- ¹⁹A.-K. Pumm, W. Engelen, E. Kopperger, J. Isensee, M. Vogt, V. Kozina, M. Kube, M. N. Honemann, E. Bertosin, M. Langecker, R. Golestanian, F. C. Simmel, and H. Dietz, "A DNA origami rotary ratchet motor," Nature **607**, 492–498 (2022).
- ²⁰S. Li, Q. Jiang, S. Liu, Y. Zhang, Y. Tian, C. Song, J. Wang, Y. Zou, G. J. Anderson, J.-Y. Han, Y. Chang, Y. Liu, C. Zhang, L. Chen, G. Zhou, G. Nie, H. Yan, B. Ding, and Y. Zhao, "A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo," Nature Biotechnology **36**, 258–264 (2018).
- ²¹C. Sigl, E. M. Willner, W. Engelen, J. A. Kretzmann, K. Sachenbacher, A. Liedl, F. Kolbe, F. Wilsch, S. A. Aghvami,

U. Protzer, M. F. Hagan, S. Fraden, and H. Dietz, "Programmable icosahedral shell system for virus trapping," Nature Materials **20**, 1281–1289 (2021).

- ²²Y. Benenson, B. Gil, U. Ben-Dor, R. Adar, and E. Shapiro, "An autonomous molecular computer for logical control of gene expression," Nature **429**, 423–429 (2004).
- ²³S. M. Douglas, I. Bachelet, and G. M. Church, "A logic-gated nanorobot for targeted transport of molecular payloads," Science **335**, 831–834 (2012).
- ²⁴A. Chworos, I. Severcan, A. Y. Koyfman, P. Weinkam, E. Oroudjev, H. G. Hansma, and L. Jaeger, "Building programmable jigsaw puzzles with RNA," Science **306**, 2068–2072 (2004).
- ²⁵C. Geary, P. W. K. Rothemund, and E. S. Andersen, "A singlestranded architecture for cotranscriptional folding of RNA nanostructures," Science **345**, 799–804 (2014).
- ²⁶E. K. S. McRae, H. Ø. Rasmussen, J. Liu, A. Bøggild, M. T. A. Nguyen, N. S. Vallina, T. Boesen, J. S. Pedersen, G. Ren, C. Geary, and E. S. Andersen, "Structure, folding and flexibility of co-transcriptional RNA origami," Nature Nanotechnology 18, 808–817 (2023).
- ²⁷L. Zhou, A. R. Chandrasekaran, M. Yan, V. A. Valsangkar, J. I. Feldblyum, J. Sheng, and K. Halvorsen, "A mini DNA–RNA hybrid origami nanobrick," Nanoscale Advances **3**, 4048–4051 (2021).
- ²⁸X. Wu, Q. Liu, F. Liu, T. Wu, Y. Shang, J. Liu, and B. Ding, "An RNA/DNA hybrid origami-based nanoplatform for efficient gene therapy," Nanoscale **13**, 12848–12853 (2021).
- ²⁹M. F. Parsons, M. F. Allan, S. Li, T. R. Shepherd, S. Ratanalert, K. Zhang, K. M. Pullen, W. Chiu, S. Rouskin, and M. Bathe, "3D RNA-scaffolded wireframe origami," Nature Communications 14, 382 (2023).
- ³⁰A. Marantan and L. Mahadevan, "Mechanics and statistics of the worm-like chain," American Journal of Physics 86, 86–94 (2018).
- ³¹R. Galindo-Murillo and T. E. C. III, "Lessons learned in atomistic simulation of double-stranded DNA: Solvation and salt concerns," Living Journal of Computational Molecular Science 1, 9974 (2019).
- ³²J. Šponer, P. Banáš, P. Jurečka, M. Zgarbová, P. Kührová, M. Havrila, M. Krepl, P. Stadlbauer, and M. Otyepka, "Molecular dynamics simulations of nucleic acids. from tetranucleotides to the ribosome," The Journal of Physical Chemistry Letters 5, 1771–1782 (2014).
- ³³J. Šponer, G. Bussi, M. Krepl, P. Banáš, S. Bottaro, R. A. Cunha, A. Gil-Ley, G. Pinamonti, S. Poblete, P. Jurečka, N. G. Walter, and M. Otyepka, "RNA structural dynamics as captured by molecular simulations: A comprehensive overview," Chemical Reviews **118**, 4177–4338 (2018).
- ³⁴ J. Šponer, J. E. Šponer, A. Mládek, P. Banáš, P. Jurečka, and M. Otyepka, "How to understand quantum chemical computations on DNA and RNA systems? A practical guide for nonspecialists," Methods **64**, 3–11 (2013).
- ³⁵A. Mládek, M. Krepl, D. Svozil, P. Čech, M. Otyepka, P. Banáš, M. Zgarbová, P. Jurečka, and J. Šponer, "Benchmark quantumchemical calculations on a complete set of rotameric families of the DNA sugar-phosphate backbone and their comparison with modern density functional theory," Physical Chemistry Chemical Physics 15, 7295 (2013).
- ³⁶A. E. Hafner, J. Krausser, and A. Šarić, "Minimal coarsegrained models for molecular self-organisation in biology," Current Opinion in Structural Biology 58, 43–52 (2019).
- ³⁷S. Kmiecik, D. Gront, M. Kolinski, L. Wieteska, A. E. Dawid, and A. Kolinski, "Coarse-grained protein models and their applications," Chemical Reviews **116**, 7898–7936 (2016).
- ³⁸T. Sun, V. Minhas, N. Korolev, A. Mirzoev, A. P. Lyubartsev, and L. Nordenskiöld, "Bottom-up coarse-grained modeling of DNA," Frontiers in Molecular Biosciences 8, 645527 (2021).
- ³⁹N. A. Denesyuk and D. Thirumalai, "Coarse-grained model for predicting RNA folding thermodynamics," The Journal of Phys-

ical Chemistry B 117, 4901-4911 (2013).

- ⁴⁰R. V. Reshetnikov, A. V. Stolyarova, A. O. Zalevsky, D. Y. Panteleev, G. V. Pavlova, D. V. Klinov, A. V. Golovin, and A. D. Protopopova, "A coarse-grained model for DNA origami," Nucleic Acids Research 46, 1102–1112 (2017).
- ⁴¹ J. Li and S.-J. Chen, "RNA 3D structure prediction using coarsegrained models," Frontiers in Molecular Biosciences 8, 720937 (2021).
- ⁴²W. K. Dawson, M. Maciejczyk, E. J. Jankowska, and J. M. Bujnicki, "Coarse-grained modeling of RNA 3D structure," Methods **103**, 138–156 (2016).
- ⁴³C. Maffeo, T. T. M. Ngo, T. Ha, and A. Aksimentiev, "A coarse-grained model of unstructured single-stranded DNA derived from atomistic simulation and single-molecule experiment," Journal of Chemical Theory and Computation **10**, 2891–2896 (2014).
- ⁴⁴T. E. Cheatham and P. A. Kollman, "Molecular dynamics simulations highlight the structural differences among DNA:DNA, RNA:RNA, and DNA:RNA hybrid duplexes," Journal of the American Chemical Society **119**, 4805–4825 (1997).
- ⁴⁵A. Noy, A. Pérez, M. Márquez, F. J. Luque, and M. Orozco, "Structure, recognition properties, and flexibility of the DNA·RNA hybrid," Journal of the American Chemical Society **127**, 4910–4920 (2005).
- ⁴⁶J.-H. Liu, K. Xi, X. Zhang, L. Bao, X. Zhang, and Z.-J. Tan, "Structural flexibility of DNA-RNA hybrid duplex: Stretching and twist-stretch coupling," Biophysical Journal **117**, 74–86 (2019).
- ⁴⁷È. de Oliveira Martins, V. B. Barbosa, and G. Weber, "DNA/RNA hybrid mesoscopic model shows strong stability dependence with deoxypyrimidine content and stacking interactions similar to RNA/RNA," Chemical Physics Letters **715**, 14–19 (2019).
- ⁴⁸N. Jonoska, N. Obatake, S. Poznanović, C. Price, M. Riehl, and M. Vazquez, "Modeling RNA:DNA hybrids with formal grammars," in Using Mathematics to Understand Biological Complexity: From Cells to Populations, edited by R. Segal, B. Shtylla, and S. Sindi (Springer International Publishing, Cham, 2021) pp. 35–54.
- ⁴⁹E. Poppleton, M. Matthies, D. Mandal, F. Romano, P. Šulc, and L. Rovigatti, "oxDNA: coarse-grained simulations of nucleic acids made simple," Journal of Open Source Software 8, 4693 (2023).
- ⁵⁰T. E. Ouldridge, A. A. Louis, and J. P. K. Doye, "Structural, mechanical, and thermodynamic properties of a coarse-grained DNA model," The Journal of Chemical Physics **134**, 085101 (2011).
- ⁵¹P. Šulc, F. Romano, T. E. Ouldridge, L. Rovigatti, J. P. K. Doye, and A. A. Louis, "Sequence-dependent thermodynamics of a coarse-grained DNA model," The Journal of Chemical Physics **137**, 135101 (2012).
- ⁵²B. E. K. Snodin, F. Randisi, M. Mosayebi, P. Šulc, J. S. Schreck, F. Romano, T. E. Ouldridge, R. Tsukanov, E. Nir, A. A. Louis, and J. P. K. Doye, "Introducing improved structural properties and salt dependence into a coarse-grained model of DNA," The Journal of Chemical Physics **142**, 234901 (2015).
- ⁵³P. Šulc, F. Romano, T. E. Ouldridge, J. P. K. Doye, and A. A. Louis, "A nucleotide-level coarse-grained model of RNA," The Journal of Chemical Physics **140**, 235102 (2014).
- ⁵⁴B. E. K. Snodin, F. Romano, L. Rovigatti, T. E. Ouldridge, A. A. Louis, and J. P. K. Doye, "Direct simulation of the selfassembly of a small DNA origami," ACS Nano **10**, 1724–1737 (2016).
- ⁵⁵C.-M. Huang, A. Kucinic, J. V. Le, C. E. Castro, and H.-J. Su, "Uncertainty quantification of a DNA origami mechanism using a coarse-grained model and kinematic variance analysis," Nanoscale **11**, 1647–1660 (2019).
- ⁵⁶E. Benson, A. Mohammed, D. Rayneau-Kirkhope, A. Gådin, P. Orponen, and B. Högberg, "Effects of design choices on the stiffness of wireframe DNA origami structures," ACS Nano 12,

9291-9299 (2018).

- ⁵⁷M. C. Engel, D. M. Smith, M. A. Jobst, M. Sajfutdinow, T. Liedl, F. Romano, L. Rovigatti, A. A. Louis, and J. P. K. Doye, "Force-induced unravelling of DNA origami," ACS Nano 12, 6734–6747 (2018).
- ⁵⁸E. Torelli, J. W. Kozyra, J.-Y. Gu, U. Stimming, L. Piantanida, K. Voïtchovsky, and N. Krasnogor, "Isothermal folding of a light-up bio-orthogonal RNA origami nanoribbon," Scientific Reports 8, 6989 (2018).
- ⁵⁹B. E. Snodin, J. S. Schreck, F. Romano, A. A. Louis, and J. P. Doye, "Coarse-grained modelling of the structural properties of DNA origami," Nucleic Acids Research **47**, 1585–1597 (2019).
- ⁶⁰E. Torelli, J. Kozyra, B. Shirt-Ediss, L. Piantanida, K. Voïtchovsky, and N. Krasnogor, "Cotranscriptional folding of a bio-orthogonal fluorescent scaffolded RNA origami," ACS Synthetic Biology **9**, 1682–1692 (2020).
- ⁶¹P. Šulc, T. E. Ouldridge, F. Romano, J. P. Doye, and A. A. Louis, "Modelling toehold-mediated RNA strand displacement," Biophysical Journal **108**, 1238–1247 (2015).
- ⁶²N. Srinivas, T. E. Ouldridge, P. Šulc, J. M. Schaeffer, B. Yurke, A. A. Louis, J. P. K. Doye, and E. Winfree, "On the biophysics and kinetics of toehold-mediated DNA strand displacement," Nucleic Acids Research **41**, 10641–10658 (2013).
- ⁶³F. Romano, D. Chakraborty, J. P. Doye, T. E. Ouldridge, and A. A. Louis, "Coarse-grained simulations of DNA overstretching," The Journal of chemical physics **138** (2013).
- ⁶⁴T. E. Ouldridge, P. Šulc, F. Romano, J. P. Doye, and A. A. Louis, "DNA hybridization kinetics: zippering, internal displacement and sequence dependence," Nucleic Acids Research **41**, 8886–8895 (2013).
- ⁶⁵M. Mosayebi, A. A. Louis, J. P. Doye, and T. E. Ouldridge, "Force-induced rupture of a DNA duplex: from fundamentals to force sensors," ACS nano 9, 11993–12003 (2015).
- ⁶⁶C. Matek, T. E. Ouldridge, J. P. Doye, and A. A. Louis, "Plectoneme tip bubbles: coupled denaturation and writhing in supercoiled DNA," Scientific Reports 5, 7655 (2015).
- ⁶⁷J. S. Schreck, T. E. Ouldridge, F. Romano, P. Šulc, L. P. Shaw, A. A. Louis, and J. P. Doye, "DNA hairpins destabilize duplexes primarily by promoting melting rather than by inhibiting hybridization," Nucleic acids research **43**, 6181–6190 (2015).
- ⁶⁸F. Kriegel, C. Matek, T. Dršata, K. Kulenkampff, S. Tschirpke, M. Zacharias, F. Lankaš, and J. Lipfert, "The temperature dependence of the helical twist of DNA," Nucleic Acids Research **46**, 7998–8009 (2018).
- ⁶⁹S. K. Nomidis, M. Caraglio, M. Laleman, K. Phillips, E. Skoruppa, and E. Carlon, "Twist-bend coupling, twist waves, and the shape of DNA loops," Physical Review E **100**, 022402 (2019).
- ⁷⁰A. Suma, V. Carnevale, and C. Micheletti, "Nonequilibrium thermodynamics of DNA nanopore unzipping," Physical Review Letters **130**, 048101 (2023).
- ⁷¹W. Lim, F. Randisi, J. P. K. Doye, and A. A. Louis, "The interplay of supercoiling and thymine dimers in DNA," Nucleic Acids Research **50**, 2480–2492 (2022).
- ⁷²N. Sugimoto, S. ichi Nakano, M. Katoh, A. Matsumura, H. Nakamuta, T. Ohmichi, M. Yoneyama, and M. Sasaki, "Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes," Biochemistry **34**, 11211–11216 (1995).
- ⁷³D. Banerjee, H. Tateishi-Karimata, T. Ohyama, S. Ghosh, T. Endoh, S. Takahashi, and N. Sugimoto, "Improved nearestneighbor parameters for the stability of RNA/DNA hybrids under a physiological condition," Nucleic Acids Research 48, 12042–12054 (2020).
- ⁷⁴J. SantaLucia and D. Hicks, "The thermodynamics of DNA structural motifs," Annual Review of Biophysics and Biomolecular Structure **33**, 415–440 (2004).
- ⁷⁵T. Xia, J. SantaLucia, M. E. Burkard, R. Kierzek, S. J. Schroeder, X. Jiao, C. Cox, and D. H. Turner, "Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with watson-crick base pairs," Bio-

chemistry 37, 14719-14735 (1998).

- ⁷⁶J. SantaLucia, "A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics," Proceedings of the National Academy of Sciences **95**, 1460–1465 (1998).
- ⁷⁷P. J. A. Cock, T. Antao, J. T. Chang, B. A. Chapman, C. J. Cox, A. Dalke, I. Friedberg, T. Hamelryck, F. Kauff, B. Wilczynski, and M. J. L. de Hoon, "Biopython: freely available python tools for computational molecular biology and bioinformatics," Bioinformatics **25**, 1422–1423 (2009).
- ⁷⁸S. Whitelam and P. L. Geissler, "Avoiding unphysical kinetic traps in monte carlo simulations of strongly attractive particles," The Journal of Chemical Physics **127**, 154101 (2007).
- ⁷⁹G. Torrie and J. Valleau, "Nonphysical sampling distributions in monte carlo free-energy estimation: Umbrella sampling," Journal of Computational Physics **23**, 187–199 (1977).
- ⁸⁰C. Calladine and H. Drew, "A base-centred explanation of the B-to-A transition in DNA," Journal of Molecular Biology **178**, 773–782 (1984).
- ⁸¹R. E. Dickerson and H.-L. Ng, "DNA structure from A to B," Proceedings of the National Academy of Sciences **98**, 6986–6988 (2001).
- ⁸²B. Hartmann and R. Lavery, "DNA structural forms," Quarterly Reviews of Biophysics **29**, 309–368 (1996).
- ⁸³N. N. Shaw and D. P. Arya, "Recognition of the unique structure of DNA:RNA hybrids," Biochimie **90**, 1026–1039 (2008).
- ⁸⁴S. Arnott, R. Chandrasekaran, R. Millane, and H.-S. Park, "DNA-RNA hybrid secondary structures," Journal of Molecular Biology **188**, 631–640 (1986).
- ⁸⁵R. T. Wheelhouse and J. B. Chaires, "Drug binding to DNA·RNA hybrid structures," in *Drug-DNA Interaction Protocols*, edited by K. R. Fox (Humana Press, Totowa, NJ, 2010) pp. 55–70.
- ⁸⁶J. I. Gyi, A. N. Lane, G. L. Conn, and T. Brown, "Solution structures of DNA-RNA hybrids with purine-rich and pyrimidine-rich strands: Comparison with the homologous DNA and RNA duplexes," Biochemistry **37**, 73–80 (1998).
- ⁸⁷G. L. Conn, T. Brown, and G. A. Leonard, "The crystal structure of the RNA/DNA hybrid r(GAAGAGAAGC)·d(GCTTCTCTTC) shows significant differences to that found in solution," Nucleic Acids Research 27, 555–561 (1999).
- ⁸⁸J. C. Cofsky, G. J. Knott, C. L. Gee, and J. A. Doudna, "Crystal structure of an RNA/DNA strand exchange junction," PLOS ONE **17**, e0263547 (2022).
- ⁸⁹Y. Xiong, "Crystal structure of a DNA RNA hybrid duplex with a polypurine RNA r(GAAGAAGAG) and a complementary polypyrimidine DNA d(CTCTTCTTC)," Nucleic Acids Research 28, 2171–2176 (2000).
- ⁹⁰A. Marin-Gonzalez, J. G. Vilhena, R. Perez, and F. Moreno-Herrero, "Understanding the mechanical response of doublestranded DNA and RNA under constant stretching forces using all-atom molecular dynamics," Proceedings of the National Academy of Sciences **114**, 7049–7054 (2017).
- ⁹¹J. Ji, D. Karna, and H. Mao, "DNA origami nano-mechanics," Chemical Society Reviews **50**, 11966–11978 (2021).
- ⁹²M. Doi and S. F. Edwards, *The theory of polymer dynamics*, International Series of Monographs on Physics (Clarendon Press, Oxford, England, 1988).
- ⁹³T. Odijk, "Stiff chains and filaments under tension," Macromolecules 28, 7016–7018 (1995).
- ⁹⁴C. Zhang, H. Fu, Y. Yang, E. Zhou, Z. Tan, H. You, and X. Zhang, "The mechanical properties of RNA-DNA hybrid duplex stretched by magnetic tweezers," Biophysical Journal **116**, 196–204 (2019).
- ⁹⁵D. Y. Zhang and E. Winfree, "Control of DNA strand displacement kinetics using toehold exchange," Journal of the American Chemical Society **131**, 17303–17314 (2009).
- ⁹⁶L. Qian and E. Winfree, "Scaling up digital circuit computation with DNA strand displacement cascades," Science **332**, 1196–

- ⁹⁷H. Liu, F. Hong, F. Smith, J. Goertz, T. Ouldridge, M. M. Stevens, H. Yan, and P. Šulc, "Kinetics of RNA and RNA:DNA hybrid strand displacement," ACS Synthetic Biology **10**, 3066–3073 (2021).
- ⁹⁸F. Hong and P. Šulc, "An emergent understanding of strand displacement in RNA biology," Journal of Structural Biology **207**, 241–249 (2019).
- ⁹⁹R. R. F. Machinek, T. E. Ouldridge, N. E. C. Haley, J. Bath, and A. J. Turberfield, "Programmable energy landscapes for kinetic control of DNA strand displacement," Nature Communications 5, 5324 (2014).
- ¹⁰⁰N. E. C. Haley, T. E. Ouldridge, I. M. Ruiz, A. Geraldini, A. A. Louis, J. Bath, and A. J. Turberfield, "Design of hidden thermodynamic driving for non-equilibrium systems via mismatch elimination during DNA strand displacement," Nature Communications **11**, 2562 (2020).

- ¹⁰¹R. Landgraf, C. hong B. Chen, and D. S. Sigman, "R-loop stability as a function of RNA structure and size," Nucleic Acids Research **23**, 3516–3523 (1995).
- ¹⁰²C. L. Lawson, A. Patwardhan, M. L. Baker, C. Hryc, E. S. Garcia, B. P. Hudson, I. Lagerstedt, S. J. Ludtke, G. Pintilie, R. Sala, J. D. Westbrook, H. M. Berman, G. J. Kleywegt, and W. Chiu, "EMDataBank unified data resource for 3DEM," Nucleic Acids Research 44, D396–D403 (2015).
- ¹⁰³E. F. Pettersen, T. D. Goddard, C. C. Huang, E. C. Meng, G. S. Couch, T. I. Croll, J. H. Morris, and T. E. Ferrin, "UCSF ChimeraX: Structure visualization for researchers, educators, and developers," Protein Science **30**, 70–82 (2020).
- ¹⁰⁴J. Bohlin, M. Matthies, E. Poppleton, J. Procyk, A. Mallya, H. Yan, and P. Šulc, "Design and simulation of DNA, RNA and hybrid protein–nucleic acid nanostructures with oxView," Nature Protocols **17**, 1762–1788 (2022).