Molecular dynamics simulations of chemically modified ribonucleotides

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Abstract. Post-transcriptional modifications are crucial for RNA function, with roles ranging from the stabilization of functional RNA structures to modulation of RNA–protein interactions. Additionally, artificially modified RNAs have been suggested as optimal oligonucleotides for therapeutic purposes. The impact of chemical modifications on secondary structure has been rationalized for some of the most common modifications. However, the characterization of how the modifications affect the three-dimensional RNA structure and dynamics and its capability to bind proteins is still highly challenging. Molecular dynamics simulations, coupled with enhanced sampling methods and integration of experimental data, provide a direct access to RNA structural dynamics. In the context of RNA chemical modifications, alchemical simulations where a wild type nucleotide is converted to a modified one are particularly common. In this Chapter, we review recent molecular dynamics studies of modified ribonucleotides. We discuss the technical aspects of the reviewed works, including the employed force fields, enhanced sampling methods, and alchemical methods, in a way that is accessible to experimentalists. Finally, we provide our perspective on this quickly growing field of research. The goal of this Chapter is to provide a guide for experimentalists to understand molecular dynamics works and, at the same time, give to molecular dynamics experts a solid review of published articles that will be a useful starting point for new research.

Keywords: RNA, chemical modification, molecular dynamics simulations
List of abbreviations:

- A: adenosine
- C: cytidine
- G: guanosine
- i^6A: N6-isopentenyladenosine
- I: inosine
- LNA: locked nucleic acid
- m^1A: N1-methyladenosine
- m^1G: N1-methylguanosine
- m^2G: N2-methylguanosine
- m^2,2G: N2-dimethylguanosine
- m^6A: N6-methyladenosine
- m^6,6A: N6-dimethyladenosine
- MD: molecular dynamics
- mRNA: messenger RNA
- NMR: nuclear magnetic resonance
- Ψ: pseudouridine
- PT: Phosphorothioate
- rRNA: ribosomal RNA
- s^2U: 2-thiouridine
- s^4U: s42-thiouridine
- siRNA: small interfering RNAs
- tRNA: transfer RNA
- U: uridine
1 Introduction

RNA molecules are sequences of four common nucleotides: adenosine (A), uridine (U), cytidine (C), and guanosine (G). However, a number of naturally occurring or artificially synthesized nucleotides can be incorporated as well (see Fig. 1). Naturally occurring modifications are often chemical marks on cellular RNA, are regulated and recognized by proteins known as writers and readers, respectively, and are usually referred to as post-transcriptional modifications. After the first modification was discovered [Davis and Allen, 1957], more than a hundred of them have been identified. Transfer RNAs (tRNAs) are known to be heavily modified [Nachtergaele and He, 2017], with a variety of modifications found both in the anticodon region and in the tRNA-body region [Ramos and Fu, 2019; Suzuki, 2021]. Ribosomal RNA (rRNA) is also extensively edited after transcription [Jiang et al., 2016]. Recent technical advances revealed widespread modifications also on messenger RNAs (mRNAs) [Gilbert et al., 2016]. In general, post-transcriptional modifications have two roles: (i) they allow correct folding of noncoding RNAs (e.g., tRNA and rRNA) into their functional structure and (ii) they affect the target specificity of RNA–RNA and RNA–protein interactions. In addition to naturally occurring modifications, a number of artificially modified nucleotides have been studied, mostly in the context of oligonucleotide design [Wan and Seth, 2016]. Synthetic oligonucleotides hold great potential as innovative therapeutic strategies, including small interfering RNAs (siRNAs), antisense oligonucleotides, microRNAs, and aptamers. However, intrinsic limitations in terms of instability, immunogenicity, and poor pharmacokinetic properties hamper their use. Therefore, artificial modifications of RNA nucleotides were explored to overcome these limitations and optimize the oligonucleotide properties. A remarkable example in this respect, though through artificial insertion of a natural modification, is pseudo-uridine (Ψ) in mRNA-vaccine technology [Karikó et al., 2008].

Fig. 1. Schematic representing some of the nucleotide modifications that are discussed in this Chapter.
Although the research on RNA modifications has been exponentially increasing in the past years, computational studies on modified RNAs are still limited, even in the relatively simpler context of secondary structure prediction (Tanzer et al., 2019). Additional complexity is present in the study of the impact of modifications on tertiary structure. Models for tertiary structure predictions are typically trained on available structural datasets (Parisien and Major, 2008; Townshend et al., 2021), and the statistics available on modified nucleotides is scarce. Furthermore, methods trained on static structures give limited access to structural dynamics. In this respect, molecular dynamics (MD) simulations (Dror et al., 2012; Šponer et al., 2018) are a promising tool since (a) they give direct access to dynamics and (b) they are grounded in physics-based models, which could possibly be capable to describe systems for which the amount of reference experimental structures is limited.

The aim of this Chapter is to review recent applications of MD simulations to the study of chemically modified ribonucleotides. In particular, we will first describe the basic principles of MD simulations, including advanced methods to enhance sampling and compute mutation free energies. Then, we will review selected applications of MD to the study of the effect of modifications on RNA structural dynamics and RNA recognition. Finally, we will provide our perspective on the field.

2 Molecular dynamics simulations

2.1 Standard molecular dynamics simulations

Molecular dynamics simulations are a natural tool to characterize RNA structural dynamics (Šponer et al., 2018). A key ingredient of any molecular dynamics (MD) simulations is the employed force field, that is a function that computes the forces on the atoms given their positions. Since evaluating the force field is the computational bottleneck of MD simulation, its functional form has to be chosen with compromises, so as to be accurate enough to describe the relevant chemistry but not too expensive. The functional form of the commonly used AMBER (Cornell et al., 1995) force field is the following one:

\[
E = \sum_{\text{bonds}} \frac{1}{2} k_b (r - r_0)^2 + \sum_{\text{angles}} \frac{1}{2} k_a (a - a_0)^2 + \sum_{\text{torsions}} \frac{V_n}{2} (1 + \cos(n\phi - \delta)) + \\
\sum_{\text{LJ}} 4\epsilon_{ij} \left( \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right) + \sum_{\text{electrostatics}} \frac{q_i q_j}{r_{ij}} \tag{1}
\]

Here, \(k_b\), \(k_a\), and \(V_n\) control the so-called bonded interactions (bonds, angles, and torsional angles, respectively), \(\sigma\) and \(\epsilon\) control Lennard-Jones potentials, and charges \(q\) control electrostatic interactions.

The parameters of the force field are heavily system dependent and are derived using a mixture of accurate quantum chemical calculations and of experimental data (see Frohling et al., 2020 for a recent review). The two main
families of force fields used for nucleic acids are AMBER (Cornell et al., 1995) and CHARMM (MacKerell Jr et al., 1995), both of which have evolved in multiple revised versions during the past decades.

2.2 Force fields for chemically modified nucleotides

Specific force-field parameters should be derived for each type of modification. The AMBER family of force fields offers a well-defined recipe for arbitrary molecules. In particular, charges are obtained by fitting the electrostatic potential, and torsional parameters by fitting the energy profiles associated to bond rotations. For the CHARMM force field, the procedure is more complex and targets both quantum mechanical data on nucleosides and experimental data on nucleosides or oligonucleosides. Luckily, parameters obtained using these procedures have been published for approximately 100 naturally occurring modified nucleotides, both in the AMBER (Aduri et al., 2007) and in the CHARMM (Xu et al., 2016b) frameworks.

In Aduri et al. (2007), parameters were validated performing standard MD simulations of a tRNA containing a fraction of the modifications for which parameters were reported. These force-field parameters have been used in several later MD simulations using the AMBER force field (see below), and are also used in modelling tools (see, e.g., Stasiewicz et al. (2019)).

Xu et al. (2016b) provided force-field parameters for 112 modified nucleotides and tested in detail 13 of them. Tautomers and protonation variants have also been included. The charge-fitting strategy aims at reproducing interactions with water and correct dipole moments. Torsions were fitted computing potential energy surfaces with quantum mechanical methods. Simulations of nucleosides and trimucleotides were compared with nuclear-magnetic-resonance (NMR) data, when available. These force-field parameters have been used in several later MD simulations using the CHARMM force field (see below).

In addition to these two works, it is relevant to mention that for many of the applications discussed below the authors developed and tested new sets of force-field parameters specific for a single or a few modifications.

2.3 Enhanced sampling methods

RNA molecules are often characterized by conformational ensembles composed of multiple partly heterogeneous structures or substates that are relevant for function (Ganser et al., 2019). MD simulations can access at most the multi-microsecond timescale with current resources. Changes in tertiary interactions and base-pairing pattern cannot thus be directly simulated with MD. To circumvent this problem, enhanced sampling methods can be used.

Enhanced sampling methods (Miłynski and Bussi, 2018; Hénin et al., 2022) are roughly classified in two categories. One category includes methods based on heating the system so as to accelerate the exploration of the conformational space. Representatives of these methods are parallel tempering, also known as temperature replica exchange (Sugita and Okamoto, 1999), and solute tempering
Molecular dynamics simulations of chemically modified RNA (Wang et al., 2011). These methods are typically very expensive and can thus be fruitfully applied only for sampling small oligomers. The other category includes methods based on adding biasing forces on specifically chosen degrees of freedom, or collective variables. Representatives of these methods are umbrella sampling (Torrie and Valleau, 1977), often performed combining multiple windows (Kumar et al., 1992) so as to progressively convert the system from an initial conformation to a final one, and metadynamics (Laio and Parrinello, 2002). These methods can be used to accelerate relevant events if sufficient prior information about the process is given.

### 2.4 Alchemical methods

Alchemical methods allow to simulate trajectories where molecular species are mutated to different ones, and the free-energy associated to the transformation can be computed (Mey et al., 2020). Most MD code support these methods, but setting up the simulations is usually more complex than for standard MD. The intermediate states might be simulated independently of each other or with a more robust replica-exchange procedure (Meng et al., 2011). Simulations should be then repeated in different structural contexts. For instance, the conversion between (unmodified) A and (modified) m$^6$A can be performed in a single strand and in a duplex. The difference between the free-energy changes computed in the two contexts corresponds to the stabilization of the duplex resulting from the additional methylation (see Fig. 2). Similarly, the impact of the modification on the affinity between the studied RNA and a protein can be estimated.

Results of alchemical simulations should be judged with care. Specifically, if there are slow degrees of freedom coupled with the alchemical change, the result might be affected by artifacts. A typically difficult situation arises when one or both the alchemical states correspond to flexible conformations whose extensive sampling is difficult. A possible way to alleviate this problem consists in combining alchemical simulations with enhanced sampling methods, as done for instance in alchemical metadynamics (Hsu et al., 2022).

### 3 Applications

#### 3.1 Validation and fitting of force fields against experimental data

Quantitative validations are crucial to assess the capability of force-field parameters to generate structural ensembles compatible with experiments. Deb et al. (2014) showed that the Aduri parametrization is not able to reproduce substate populations for a number of modified uridines. Later, they provided a reparametrization for $\Psi$, $s^2$U and $s^4$U (Deb et al., 2016), and discussed how changes in the Lennard-Jones parameters could affect the relative population of different sugar conformations. Dutta et al. (2020) later confirmed these parameters to be transferable to other modified uridines. More recently, Dutta et al. (2022) reparametrized charges for $\Psi$ and three different methylated
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Fig. 2. Schematic representation of alchemical simulation protocols. Transformation A↔m6A (vertical red arrows) can be used to compute the free-energy change in different structural contexts (duplex, $\Delta G_{\text{dup}}$, single strand, $\Delta G_{ss}$, and RNA–protein complex, $\Delta G_{\text{com}}$). The Hamiltonian function ($H$) is interpolated between the two physical systems. Experiments (horizontal blue and orange arrows) report hybridization free energies ($\Delta G_{\text{hyb}}$) and free energies of binding ($\Delta G_{\text{bind}}$). $\Delta \Delta G$s can be directly compared between simulation and experiment. E.g., $\Delta G_{\text{dup}} - \Delta G_{ss} = \Delta G_{\text{hyb,m6A}} - \Delta G_{\text{hyb,A}}$

versions of $\Psi$, obtaining parameters that were then validated simulating single-stranded oligonucleotides, obtaining conformational and hydration properties in agreement with experimental data. Hurst and Chen (2021) have validated Aduri parameters for m6A using alchemical simulations, confirming their capability to reproduce melting experiments. However, a later work from our group (Piomponi et al., 2022) using a similar protocol on a larger validation set showed that modifications to the charges are necessary to simultaneously reproduce thermodynamic data and syn/anti balance for the methyl group.

3.2 Effects of post-transcriptional modifications on RNA structure and dynamics

Here we review MD simulation studies aimed at elucidating the impact of post-transcriptional modifications on the structure and dynamics of tRNAs, rRNAs, and other systems.

Molecular simulations of anti-codon stem loops and of entire tRNAs. A number of works used MD simulations to investigate the structural role of modifications on entire tRNAs, suggesting them to be crucial for the stabilization of the functional structure. A comprehensive study on 3 tRNAs was reported by Zhang et al. (2014), comparing all-modified with nonmodified tRNAs.
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Modifications were shown to increase the rigidity of the anti-codon stem-loop, presumably facilitating pairing with mRNA during translation. Overall, the effect of the modifications was suggested to be non-trivial, making tRNA more rigid in some regions and more flexible in other regions. Similar results were obtained by Xu et al. (2016a). The effect of single modifications was analyzed by Sonawane et al. (2016) (G vs m<sub>2</sub>G and m<sub>22</sub>G) and by Prabhakar et al. (2021) (G or A vs a total of 9 modifications), both showing that a single modified position could crucially stabilize the functional structure of the anticodon loop (see Fig. 3A and B).

Fig. 3. (A) Stacking interactions between unmodified A37 and modified i<sup>6</sup>A37 and neighboring nucleobases in the anticodon loop. (B) Hydrogen-bonding interactions (with occupancies) between base A37/i<sup>6</sup>A37 and Watson-Crick base U33 as obtained with MD simulations. Adapted with permission from Prabhakar et al. (2021) (C) Detailed interactions between m<sup>6</sup>A and pocket of YTHDC1. Adapted with permission from Li et al. (2019). The full structure of this complex can be seen in Fig. 2.

Other works directed the effort on the simulation of the anticodon stem loop. Galindo-Murillo et al. (2016) showed that a hypermodified nucleotide stabilized interaction with a model mRNA fragment. Sambhare et al. (2014) studied the dynamics of the hypermodified nucleotide. Xiao et al. (2016) discussed how this modification modulated the interaction with a designed peptide, and Vangaveti et al. (2022) analyzed the interaction with insulin mRNA, specifically monitoring how the modification tuned the van-der-Waals interactions and the hydration of the stem loop. The effect of anticodon modifications was also studied by Sambhare et al. (2014) and by Sonawane and Sambhare (2015). Vangaveti et al. (2020) reported how modifications at the wobble position of the anticodon loop affect pairing with mRNA and codon recognition. Finally, Wang et al. (2016) presented a synthesis protocol for geranylated nucleotides. Their work also includes MD simulations of geranylated nucleotides in RNA and DNA duplexes and in anti-codon–codon pairs, including the ribosomal subunit with all its associated proteins, showing how geranylation of uridine affects U·G pairing.
Molecular simulations of entire ribosomes. Ribosomes contain a large number of post-transcriptional modifications. Therefore, MD simulations of the ribosome usually take these modifications into account. However, in many cases the modifications are not directly involved in the process of interest. Pavlova et al. (2017) performed simulations of the *E. coli* ribosome in multiple variants to characterize the relationship between mutations and modifications and resistance to macrolides, a widely prescribed class of antibiotics. Several mutations and modifications of an adenine in the exit tunnel were simulated (G, m^6^A, and m^6^6^A) in complex with a number of macrolides. The mutations and modifications were found to weaken the interaction of the ribosome with the macrolides, thus providing a microscopic explanation for the observed antibiotic resistance.

Molecular simulations of model systems and of other post-transcriptionally modified RNAs. A number of papers focused on the effect of post-transcriptional modifications on the energetics and structure of other systems, ranging from individual base pairs to duplexes.

Some works addressed the dynamics of individual or paired nucleotides. Bavi et al. (2013) reported short MD simulations of modified nucleotides (m^2^G and m^2^2^G) and compared them with crystal structures. Vendeix et al. (2009) performed umbrella-sampling simulations to characterize the effect of modifications on base-pairing free energy. Hopfinger et al. (2020) computed the thermodynamic stability of multiple modified nucleotides using a combination of MD simulations and quantum mechanical calculations.

Several works specifically studied pseudouridine (Ψ). A few of them addressed the impact of U to Ψ modification in the structure and stability of duplexes containing CUG or CΨG repeats. deLorimier et al. (2014) reported enhanced-sampling calculations to estimate the free-energy change associated to the opening of the central base pair in the repeat, which was found to be larger in the presence of one or two Ψs, relating the result to a change in the water coordination of the modified nucleotide. A later work from the same group (deLorimier et al., 2017) showed that the rigidity of Ψ in the context of CUG and CCUG repeats resulted in lower affinity with a RNA–binding protein. Similar results were obtained by Deb et al. (2019) using a different force field and thereby confirmed by NMR experiments. Another modification of uridine, s^2^U, has been studied by Sarkar et al. (2020), where it was shown that intercalation of the sulfur atom can induce order in single stranded RNAs with consecutive uridines.

Another commonly found modification is m^6^A. Hurst and Chen (2021), after validating the employed force field using alchemical calculations on duplexes (see Section 3.1), computed the free-energy landscape of a hairpin composed of a tetraloop and three base pairs with or without modifications, including m^6^A. Simulations were accelerated using temperature replica-exchange MD. Remarkably, methylations at different positions were shown to stabilize or destabilize the hairpin structure in specific contexts, which may provide a tool for RNA nanotherapeutic design.
The consequences of N1 adenosine and guanosine methylation (m\(^1\)A and m\(^1\)G) on duplex dynamics was studied by Zhou et al. (2016). In particular, this work shows that this modification changes the energetic balance between Watson–Crick and Hoogsteen pairing, with the result of being not tolerated in RNA, where it can induce duplex melting. We note that, compared with other common nucleobase methylations, m\(^1\)A shifts the nucleotide charge by +1, and that the destabilization of a RNA duplex by m\(^1\)A has been reported to be sufficient to induce a completely different secondary structure in a tRNA (Voigts-Hoffmann et al., 2007).

A few papers addressed inosine (I). Given the similarity in the hydrogen bond pattern formed by I and G, I can pair with both U and C, though the I-C pair only forms two hydrogen bonds. Krepl et al. (2013) studied the thermodynamics of I-C pairs using MD simulations of RNA and DNA duplexes subjected to alchemical calculations. The thermodynamic cycle was performed converting a G-C pair to a I-C pair and computing the corresponding destabilization of the duplex, and results were correlated with experimental thermodynamic data. Sakuraba et al. (2020) reported a more systematic study including more sequences as well as new experimental data, confirming that MD can correctly predict differential stability of G-C and I-C pairs. Less stable I-U pairs were investigated using quantum mechanical calculations by Jolley et al. (2015). Špačková and Réblrová (2018) reported MD simulations of I-U pairs, showing that dynamics of tandem I-U pairs depends on the neighboring base, with UII being the most rigid sequence, with a limited impact on structure with respect to unmodified nucleotides.

Finally, not only tRNA and rRNA can be modified, as discussed in previous Sections, but also mRNA. Simulations of tRNA in complex with portions of rRNA and mRNA (Elliott et al., 2019) showed that 2′-O-methylation in mRNA could hinder interactions that are crucial during translation.

### 3.3 Effects of post-transcriptional modifications on RNA recognition

**RNA recognition by reader domain YTHDC1.** The YTHDC1 is one of most studied m\(^6\)A readers. The RNA–protein complex has been solved for different oligonucleotide sequences, invariably showing that m\(^6\)A is captured in an aromatic cage, with the flanking nucleotides laying at the RNA–protein surface. Several MD studies have characterized the hydrogen-bond networks formed in the complex (Li et al., 2019, 2021, 2022; Krepl et al., 2021; Zhou et al., 2022). Li et al. (2019) reported simulations with the CHARMM force field using a 5-nucleotides RNA single strand (5′-GG(m\(^6\)A)CU-3′), highlighting the role of two tryptophan residues in the pocket, respectively stacking on m\(^6\)A and stabilizing its methyl group (see Fig. 3C). The role of the flanking nucleotides was studied by both analyzing their fluctuations in the simulations and performing experiments with shorter variants. Li et al. (2021) continued this work by performing alchemical calculations to estimate the complex stabilization induced by m\(^6\)A, using as a reference an isolated nucleoside. Stabilization was slightly over-
estimated with respect to reference experimental data. A crucial water molecule was identified in the binding site and also studied with alchemical methods. Krepl et al. (2021) published a related study with a similar RNA sequence (5′-CG(mA)CAC-3′) using AMBER parameters and specifically developed charges. The authors performed alchemical calculations, identifying a water molecule entering the binding site in a position occupied by the methyl group. The stabilization of the complex was overestimated when compared to experiment, as in (Li et al. 2021). Results were shown to be dependent on the protocol used to initialize the alchemical calculation.

Other RNA–protein interactions. Gonzalez-Rivera et al. (2020) characterized the interaction between RNA strands with a single oxidized G (8-oxoG) and a polynucleotide phosphorylase implicated in RNA turnover. Interestingly, they developed a protocol based on relatively short MD simulations to assess the affinity of mutated protein sequences, validating the optimized sequences by affinity measurements.

3.4 Molecular dynamics simulations of synthetic nucleic acids

Synthetic nucleic acids often display backbone modifications that are introduced to facilitate their therapeutic use, often aimed at modulating degradation and/or affinity with the target sequences. MD simulations have been used to understand how the modifications impact backbone flexibility and hybridization energies. Gore et al. (2012) synthesized siRNAs including a 4′-C-aminomethyl-2′-O-methyl modification. They then used MD simulations to show that the modification induces changes in the sugar puckering, flexibility in groove dimension, and disturbances in hydrogen bonding and base stacking, resulting in lower duplex stability as confirmed experimentally. In a later work, Harikrishna and Pradeepkumar (2017) used plain MD simulations of siRNA in complex with Argonaute 2 to elucidate how a number of synthetic modifications affect protein–RNA interactions. Simulations were performed on the microsecond timescale, showing that the minimum timescale to see this conformational variability is of the order of 300 ns. Masaki et al. (2010) characterized a number of 2′-O-modified nucleotides. These modifications affect the sugar flexibility and thus the fluctuations of duplex helical parameters. The authors identified linear relationships between the predicted fluctuations and the duplex thermal stability, suggesting the possibility of predicting the thermal stability of 2′-O-modified duplexes at the computer-aided molecular design stage. This idea was then generalized to other modifications, also covering the nucleobase (Masaki et al. 2012).

Seio et al. (2012) developed a modified nucleotide that can be used at the 5′ termini of oligonucleotides so as to distinguish complementary polynucleotide depending on their length, for instance to distinguish microRNAs and pre-microRNAs. In this work, they used MD simulations to construct structural models of the resulting double helix and to characterize the interaction of the modified nucleotide with the terminal phosphate of the recognized RNA.
Phosphorothioate (PT) modification of RNA backbone is generally considered an artificial modification, though it has been recently reported to occur naturally. Zhang et al. (2021) studied the effect of PT in a riboswitch in which the modification was artificially included and compared the resulting trajectories with NMR data. The authors observed that existing force-field parameters cannot recapitulate the interactions seen in experiment, and suggested that polarizable force fields might be necessary to describe this modification. Interestingly, Jing et al. (2019) proposed an extension of the AMOEBA polarizable force field to a number of modified nucleotides, including PT. Alchemical calculations were used to compute changes in duplex hybridization free energy induced by the modification, reporting results in general agreement with experimental data.

Other interesting synthetic backbone modifications are locked nucleic acids (LNAs). Simulations with these modifications have been mostly conducted on LNAs within DNA or fully modified sequences and are thus not covered here.

4 Discussion and challenges ahead

In this Chapter we reviewed selected applications of molecular dynamics (MD) simulations to modified RNAs, covering validation of force-field parameters, effects of modifications on dynamics or recognition, and synthetic nucleotides. A recent related review complements ours, by providing a different perspective (D’Esposito et al., 2022). A number of issues can be highlighted from our survey.

MD simulations of non-modified RNAs are routinely performed by many groups, thus providing a significant mass of reference work. On the contrary, simulations of modified nucleotides are sparse, with most modifications simulated in a handful of papers. Hence, force fields has not been validated to the same extent. Researchers approaching the field should carefully validate parameters and be ready to develop new ones. Integration of experimental data (Bernetti and Bussi, 2022) could provide a significant step forward.

Advanced sampling techniques are used rarely in this field. However, flexible and structurally heterogeneous RNA molecules require enhanced sampling methods to be faithfully characterized. Possibly, this shortfall is a consequence of the fact that being able to carefully design studies on systems with complex modified nucleotides require a deep biochemistry knowledge that is not commonly available in the community using enhanced sampling methods, and vice versa. Collaborative works could open the way to the application of state-of-the-art simulation methods in this field.

Nevertheless, the presented applications show that MD simulations are at the level of being useful in the interpretation and design of experiments. Given the growing biological relevance of RNA modifications, we are confident that synergy between simulation and experiment will become even stronger in the coming future.
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Bibliography

R. Aduri, B. T. Psciuk, P. Saro, H. Taniga, H. B. Schlegel, and J. SantaLucia. AMBER force field parameters for the naturally occurring modified nucleosides in RNA. *J. Chem. Theory Comput.*, 3:1464–1475, 2007.

R. S. Bavi, S. B. Sambhare, and K. D. Sonawane. MD simulation studies to investigate iso-energetic conformational behaviour of modified nucleosides m2G and M22G present in tRNA. *Comput. Struct. Biotechnol. J.*, 5:e201302015, 2013.

M. Bernetti and G. Bussi. Combining simulations and experiments to investigate RNA dynamics. *arXiv:2207.08622*, 2022.

W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, and P. A. Kollman. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.*, 117:5179–5197, 1995.

F. F. Davis and F. W. Allen. Ribonucleic acids from yeast which contain a fifth nucleotide. *J. Biol. Chem.*, 227:907–915, 1957.

I. Deb, J. Sarzynska, L. Nilsson, and A. Lahiri. Conformational preferences of modified uridines: comparison of AMBER derived force fields. *J. Chem. Inf. Model.*, 54:1129–1142, 2014.

I. Deb, R. Pal, J. Sarzynska, and A. Lahiri. Reparameterizations of the χ torsion and Lennard-Jones σ parameters improve the conformational characteristics of modified uridines. *J. Comput. Chem.*, 37:1576–1588, 2016.

I. Deb, L. Popenda, J. Sarzyńska, M. Malgowska, A. Lahiri, Z. Gdaniec, and R. Kierzek. Computational and NMR studies of RNA duplexes with an internal pseudouridine-adenosine base pair. *Sci. Rep.*, 9:16278, 2019.

E. deLorimier, L. A. Coonrod, J. Copperman, A. Taber, E. E. Reister, K. Sharma, P. K. Todd, M. G. Guenza, and J. A. Berglund. Modifications to toxic CUG RNAs induce structural stability, rescue mis-splicing in a myotonic dystrophy cell model and reduce toxicity in a myotonic dystrophy zebrafish model. *Nucleic Acids Res.*, 42:12768–12778, 2014.

E. deLorimier, M. N. Himman, J. Copperman, K. Datta, M. Guenza, and J. A. Berglund. Pseudouridine modification inhibits muscleblind-like 1 (mbnl1) binding to CCUG repeats and minimally structured RNA through reduced RNA flexibility. *J. Biol. Chem.*, 292:4350–4357, 2017.

R. O. Dror, R. M. Dirks, J. Grossman, H. Xu, and D. E. Shaw. Biomolecular simulation: a computational microscope for molecular biology. *Annu. Rev. Biophys.*, 41:429–452, 2012.

N. Dutta, J. Sarzynska, and A. Lahiri. Molecular dynamics simulation of the conformational preferences of pseudouridine derivatives: improving the distribution in the glycosidic torsion space. *J. Chem. Inf. Model.*, 60:4995–5002, 2020.
N. Dutta, I. Deb, J. Sarzyńska, and A. Lahiri. Data-informed reparameterization of modified RNA and the effect of explicit water models: application to pseudouridine and derivatives. *J. Comput. Aided Mol. Des.*, 36:205–224, 2022.

R. J. D’Esposito, C. A. Myers, A. A. Chen, and S. Vangaveti. Challenges with simulating modified RNA: Insights into role and reciprocity of experimental and computational approaches. *Genes*, 13:540, 2022.

B. A. Elliott, H.-T. Ho, S. V. Ranganathan, S. Vangaveti, O. Ilkayeva, H. Abou Assi, A. K. Choi, P. F. Agris, and C. L. Holley. Modification of messenger RNA by 2′-O-methylation regulates gene expression in vivo. *Nat. Commun.*, 10:1–9, 2019.

T. Fröhling, M. Bernetti, N. Calonaci, and G. Bussi. Toward empirical force fields that match experimental observables. *J. Chem. Phys.*, 152:230902, 2020.

R. Galindo-Murillo, D. R. Davis, and T. E. Cheatham III. Probing the influence of hypermodified residues within the tRNA^{Lys} anticodon stem loop interacting with the A-loop primer sequence from HIV-1. *Biochim. Biophys. Acta*, 1860:607–617, 2016.

L. R. Ganser, M. L. Kelly, D. Herschlag, and H. M. Al-Hashimi. The roles of structural dynamics in the cellular functions of RNAs. *Nat. Rev. Mol. Cell Biol.*, 20:474–489, 2019.

W. V. Gilbert, T. A. Bell, and C. Schaeining. Messenger RNA modifications: Form, distribution, and function. *Science*, 352:1408–1412, 2016.

J. C. González-Rivera, A. A. Orr, S. M. Engels, J. M. Jakubowski, M. W. Sherman, K. N. O’Connor, T. Matteson, B. C. Woodcock, L. M. Contreras, and P. Tamamis. Computational evolution of an RNA-binding protein towards enhanced oxidized-RNA binding. *Comput. Struct. Biotechnol. J.*, 18:137–152, 2020.

K. R. Gore, G. N. Nawale, S. Harikrishna, V. G. Chittoor, S. K. Pandey, C. Höbartner, S. Patankar, and P. Pradeepkumar. Synthesis, gene silencing, and molecular modeling studies of 4′-c-aminomethyl-2′-o-methyl modified small interfering RNAs. *J. Org. Chem.*, 77:3233–3245, 2012.

S. Harikrishna and P. Pradeepkumar. Probing the binding interactions between chemically modified siRNAs and human argonaute 2 using microsecond molecular dynamics simulations. *J. Chem. Inf. Model.*, 57:883–896, 2017.

J. Hénin, T. Lelièvre, M. R. Shirts, O. Valsson, and L. Delemotte. Enhanced sampling methods for molecular dynamics simulations. *arXiv:2202.04164*, 2022.

M. C. Hopfinger, C. C. Kirkpatrick, and B. M. Znosko. Predictions and analyses of RNA nearest neighbor parameters for modified nucleotides. *Nucleic Acids Res.*, 48:8901–8913, 2020.

W.-T. Hsu, P. T. Merz, G. Bussi, and M. R. Shirts. Adding alchemical variables to metadynamics to enhance sampling in free energy calculations. *arXiv:2206.01329*, 2022.

T. Hurst and S.-J. Chen. Deciphering nucleotide modification-induced structure and stability changes. *RNA Biol.*, 18:1920–1930, 2021.

J. Jiang, H. Seo, and C. S. Chow. Post-transcriptional modifications modulate rRNA structure and ligand interactions. *Acc. Chem. Res.*, 49:893–901, 2016.
Z. Jing, R. Qi, M. Thibonnier, and P. Ren. Molecular dynamics study of the hybridization between RNA and modified oligonucleotides. *J. Chem. Theory Comput.*, 15:6422–6432, 2019.

E. A. Jolley, M. Lewis, and B. M. Znosko. A computational model for predicting experimental RNA nearest-neighbor free energy rankings: Inosine-uridine pairs. *Chem. Phys. Lett.*, 639:157–160, 2015.

K. Karikó, H. Muramatsu, F. A. Welsh, J. Ludwig, H. Kato, S. Akira, and D. Weissman. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol. Ther.*, 16:1833–1840, 2008.

M. Krepl, M. Otyepka, P. Banáš, and J. Šponer. Effect of guanine to inosine substitution on stability of canonical DNA and RNA duplexes: molecular dynamics thermodynamics integration study. *J. Phys. Chem. B*, 117:1872–1879, 2013.

M. Krepl, F. F. Damberger, C. von Schroetter, D. Theler, P. Pokorná, F. H.-T. Allain, and J. Šponer. Recognition of N6-methyladenosine by the YTHDC1 YTH domain studied by molecular dynamics and NMR spectroscopy: The role of hydration. *J. Phys. Chem. B*, 125:7691–7705, 2021.

S. Kumar, J. M. Rosenberg, D. Bouzida, R. H. Swendsen, and P. A. Kollman. The weighted histogram analysis method for free-energy calculations on biomolecules. I. the method. *J. Comput. Chem.*, 13:1011–1021, 1992.

A. Laio and M. Parrinello. Escaping free-energy minima. *Proc. Natl. Acad. Sci. U.S.A.*, 99:12562–12566, 2002.

Y. Li, R. K. Bedi, L. Wiedmer, D. Huang, P. Sledz, and A. Caflisch. Flexible binding of m^6A reader protein YTHDC1 to its preferred RNA motif. *J. Chem. Theory Comput.*, 15:7004–7014, 2019.

Y. Li, R. K. Bedi, L. Wiedmer, X. Sun, D. Huang, and A. Caflisch. Atomistic and thermodynamic analysis of N6-methyladenosine (m^6a) recognition by the reader domain of YTHDC1. *J. Chem. Theory Comput.*, 17:1240–1249, 2021.

Y. Li, R. K. Bedi, F. Nai, V. von Roten, A. Dolbois, F. Zálešák, R. Nachawati, D. Huang, and A. Caflisch. Structure-based design of ligands of the m^6A-RNA reader YTHDC1. *Eur. J. Med. Chem.*, 5:100057, 2022.

A. D. MacKerell Jr, J. Wierckiewicz-Kuczera, and M. Karplus. An all-atom empirical energy function for the simulation of nucleic acids. *J. Am. Chem. Soc.*, 117:11946–11975, 1995.

Y. Masaki, R. Miyasaka, A. Ohkubo, K. Seio, and M. Sekine. Linear relationship between deformability and thermal stability of 2′-O-modified RNA hetero duplexes. *J. Phys. Chem. B*, 114:2517–2524, 2010.

Y. Masaki, R. Miyasaka, K. Hirai, H. Tsunoda, A. Ohkubo, K. Seio, and M. Sekine. Prediction of the stability of modified RNA duplexes based on deformability analysis: oligoribonucleotide derivatives modified with 2′-O-cyanoethyl-5-propynyl-2-thiouridine as a promising component. *Chem. Commun.*, 48:7313–7315, 2012.

Y. Meng, D. Sabri Dashti, and A. E. Roitberg. Computing alchemical free energy differences with hamiltonian replica exchange molecular dynamics (H-REMD) simulations. *J. Chem. Theory Comput.*, 7:2721–2727, 2011.
Molecular dynamics simulations of chemically modified RNA 17

A. S. J. S. Mey, B. K. Allen, H. E. Bruce McDonald, J. D. Chodera, D. F. Hahn, M. Kuhn, J. Michel, D. L. Mobley, L. N. Naden, S. Prasad, A. Rizzi, J. Scheen, M. R. Shirts, G. Tresadern, and H. Xu. Best practices for alchemical free energy calculations [article v1.0]. LiveCoMS, 2:18378, 2020.

V. Mlýnský and G. Bussi. Exploring RNA structure and dynamics through enhanced sampling simulations. Curr. Opin. Struct. Biol, 49:63–71, 2018.

S. Nachtergaele and C. He. The emerging biology of RNA post-transcriptional modifications. RNA Biol., 14:156–163, 2017.

M. Parisien and F. Major. The MC-Fold and MC-Sym pipeline infers RNA structure from sequence data. Nature, 452:51–55, 2008.

A. Pavlova, J. M. Parks, A. K. Oyelere, and J. C. Gumbart. Toward the rational design of macrolide antibiotics to combat resistance. Chem. Biol. Drug. Des., 90:641–652, 2017.

V. Piomponi, T. Fröhliking, M. Bernetti, and G. Bussi. Molecular simulations matching denaturation experiments for N6-methyladenosines. ACS Cent. Sci., 8:1218–1228, 2022.

P. S. Prabhakar, N. A. Takyi, and S. D. Wetmore. Posttranscriptional modifications at the 37th position in the anticodon stem–loop of tRNA: structural insights from MD simulations. RNA, 27:202–220, 2021.

J. Ramos and D. Fu. The emerging impact of tRNA modifications in the brain and nervous system. Biochim. Biophys. Acta Gene. Regul. Mech., 1862:412–428, 2019. mRNA modifications in gene expression control.

S. Sakuraba, J. Iwakiri, M. Hamada, T. Kameda, G. Tsuji, Y. Kimura, H. Abe, and K. Asai. Free-energy calculation of ribonucleic inosines and its application to nearest-neighbor parameters. J. Chem. Theory Comput., 16:5923–5935, 2020.

S. B. Sambhare, B. V. Kumbhar, A. D. Kamble, R. S. Bavi, N. M. Kumbhar, and K. D. Sonawane. Structural significance of modified nucleosides k^c and t^6A present in the anticodon loop of tRNA^lle. RSC Adv., 4:14176–14188, 2014.

A. K. Sarkar, J. Sarzynska, and A. Lahiri. Ensemble allosteric model for the modified wobble hypothesis. J. Phys. Chem. Lett., 11:6337–6343, 2020.

K. Seio, S. Kurohagi, E. Kodama, Y. Masaki, H. Tsunoda, A. Ohkubo, and M. Sekine. Short-RNA selective binding of oligonucleotides modified using adenosine and guanosine derivatives that possess cyclohexyl phosphates as substituents. Org. Biomol. Chem., 10:994–1006, 2012.

K. D. Sonawane and S. B. Sambhare. The influence of hypermodified nucleosides lysidine and t^6A to recognize the AUU codon instead of AUG: a molecular dynamics simulation study. Integr. Biol., 7:1387–1395, 2015.

K. D. Sonawane, R. S. Bavi, S. B. Sambhare, and P. M. Fandilolu. Comparative structural dynamics of tRNA^Phe with respect to hinge region methylated guanosine: a computational approach. Cell Biochem. Biophys., 74:157–173, 2016.

N. Špačková and K. Rěhlová. Role of inosine–uracil base pairs in the canonical rna duplexes. Genes, 9:324, 2018.

J. Šponer, G. Bussi, M. Krepl, P. Banáš, S. Bottaro, R. A. Cunha, A. Gil-Ley, G. Pinamonti, S. Pobelete, P. Jurečka, N. G. Walter, and M. Otyepka. RNA
structural dynamics as captured by molecular simulations: a comprehensive overview. *Chem. Rev.*, 118:4177–4338, 2018.

J. Stasiewicz, S. Mukherjee, C. Nithin, and J. M. Bujnicki. QRNAS: software tool for refinement of nucleic acid structures. *BMC Struct. Biol.*, 19:1–11, 2019.

Y. Sugita and Y. Okamato. Replica-exchange molecular dynamics method for protein folding. *Chem. Phys. Lett.*, 314:141–151, 1999.

T. Suzuki. The expanding world of tRNA modifications and their disease relevance. *Nat. Rev. Mol. Cell Biol.*, 22:375–392, 2021.

A. Tanzer, J. L. Hofacker, and R. Lorenz. RNA modifications in structure prediction – status quo and future challenges. *Methods*, 156:32–39, 2019.

G. M. Torrie and J. P. Valleau. Nonphysical sampling distributions in monte carlo free-energy estimation: Umbrella sampling. *J. Comput. Phys.*, 23:187–199, 1977.

R. J. Townshend, S. Eismann, A. M. Watkins, R. Rangan, M. Karelin, R. Das, and R. O. Dror. Geometric deep learning of RNA structure. *Science*, 373:1047–1051, 2021.

S. Vangaveti, W. A. Cantara, J. L. Spears, H. DeMirci, F. V. Murphy IV, S. V. Ranganathan, K. L. Sarachan, and P. F. Agris. A structural basis for restricted codon recognition mediated by 2-thiocytidine in tRNA containing a wobble position inosine. *J. Mol. Biol.*, 432:913–929, 2020.

S. Vangaveti, S. V. Ranganathan, and P. F. Agris. Physical chemistry of a single tRNA-modified nucleoside regulates decoding of the synonymous lysine wobble codon and affects type 2 diabetes. *J. Phys. Chem. B*, 126:1168–1177, 2022.

F. A. Vendeix, A. M. Munoz, and P. F. Agris. Free energy calculation of modified base-pair formation in explicit solvent: A predictive model. *RNA*, 15:2278–2287, 2009.

F. Voigts-Hoffmann, M. Hengesbach, A. Y. Kobitski, A. van Aerschot, P. Herdewijn, G. U. Nienhaus, and M. Helm. A methyl group controls conformational equilibrium in human mitochondrial tRNA-Lys. *J. Am. Chem. Soc.*, 129:13382–13383, 2007.

W. B. Wan and P. P. Seth. The medicinal chemistry of therapeutic oligonucleotides. *J. Med. Chem.*, 59:9645–9667, 2016.

L. Wang, R. A. Friesner, and B. Berne. Replica exchange with solute scaling: a more efficient version of replica exchange with solute tempering (REST2). *J. Phys. Chem. B*, 115:9431–9438, 2011.

R. Wang, S. Vangaveti, S. V. Ranganathan, M. Basanta-Sanchez, P. Haruehanroengra, A. Chen, and J. Sheng. Synthesis, base pairing and structure studies of geranylated RNA. *Nucleic Acids Res.*, 44:6036–6045, 2016.

X. Xiao, B. Zhao, P. F. Agris, and C. K. Hall. Simulation study of the ability of a computationally-designed peptide to recognize target tRNA-Lys and other decoy tRNAs. *Protein Sci.*, 25:2243–2255, 2016.

Y. Xu, A. D. MacKerell Jr, and L. Nilsson. Structural effects of modified ribonucleotides and magnesium in transfer RNAs. *Bioorg. Med. Chem.*, 24:4826–4834, 2016a.
Y. Xu, K. Vanommeslaeghe, A. Aleksandrov, A. D. MacKerell Jr, and L. Nilsson. Additive CHARMM force field for naturally occurring modified ribonucleotides. *J. Comput. Chem.*, 37:896–912, 2016b.

X. Zhang, R. C. Walker, E. M. Phizicky, and D. H. Mathews. Influence of sequence and covalent modifications on yeast tRNA dynamics. *J. Chem. Theory Comput.*, 10:3473–3483, 2014.

Z. Zhang, J. Vögele, K. Mráziková, H. Kruse, X. Cang, J. Wöhnert, M. Krepl, and J. Šponer. Phosphorothioate substitutions in RNA structure studied by molecular dynamics simulations, QM/MM calculations, and NMR experiments. *J. Phys. Chem. B*, 125:825–840, 2021.

H. Zhou, I. J. Kimsey, E. N. Nikolova, B. Sathyamoorthy, G. Grazioli, J. McSally, T. Bai, C. H. Wunderlich, C. Kreutz, I. Andricioaei, et al. m1A and m1G disrupt A-RNA structure through the intrinsic instability of Hoogsteen base pairs. *Nat. Struct. Mol. Biol.*, 23:803–810, 2016.

W. Zhou, Z. Han, Z. Wu, W. Gong, S. Yang, L. Chen, and C. Li. Specific recognition between YTHDF3 and m6A-modified RNA: An all-atom molecular dynamics simulation study. *Proteins: Struct. Funct. Bioinf.*, 90:1965–1972, 2022.