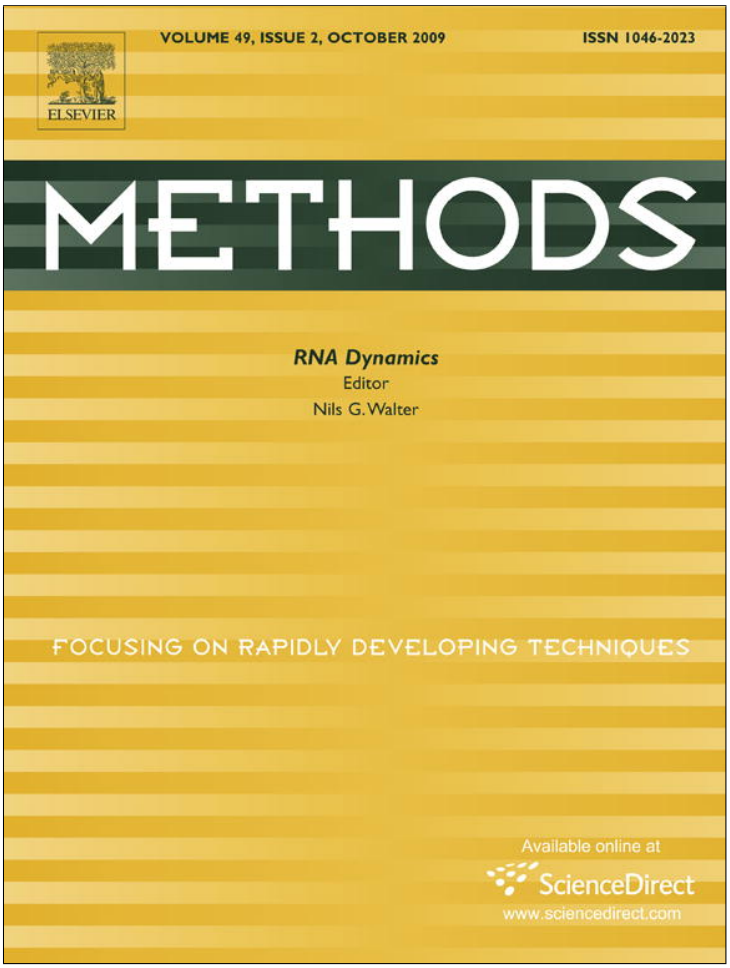

Methods 49 (2009) 85–86

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Guest Editor's Introduction

The blessing and curse of RNA dynamics: past, present, and future

RNA is a dynamic biopolymer that forms very stable and quite predictable secondary structures of highly polyanionic character, imparting strongly solvent dependence behavior. We now understand that all cellular processes involving the processing, regulation, and coordination of gene expression critically depend on the ability of RNA to fold into specific secondary, tertiary, and quaternary structures that are sufficiently dynamic to undergo rearrangements ranging from small, fast (picosecond), local to large, slow (second), global scales [1]. In addition, recent single molecule studies have revealed a surprisingly rugged folding free energy landscape of RNA that, in at least some cases, leads to multiple functional, yet distinct, structures with extraordinary resistance to interconversion [2]. While inherent structural dynamics are thus key to the biological function of RNA, they also present a significant challenge for modern biophysical and (bio)chemical tools that aim at their dissection.

In this special issue, reviews were selected to provide a broad, if regrettably incomplete, overview of current successful examples and future challenges for approaches studying RNA dynamics. First, Spitale and Wedekind describe how classic high-resolution X-ray crystallography yielding static structures can be used to explore RNA conformational changes by comparing specifically designed analogs of reaction intermediates and paying attention to conformational heterogeneity [3]. The authors suggest that picosecond timescale and single molecule diffraction techniques are on the horizon, promising to revolutionize the way RNA dynamics can be visualized. Next, Bevilacqua, Carey, Golden and colleagues show that single RNA crystals can alternatively be utilized for probing the protonation state of functionally relevant nucleotides under ambient conditions and, by future extension, local conformational changes [4]. RNA crystals here provide a way to reduce conformational heterogeneity and isolate a defined state or starting structure known from X-ray crystallography.

The next set of reviews focuses on modern optical spectroscopy tools that probe RNA dynamics in solution. Zhao and Rueda highlight the unique insights afforded by single molecule fluorescence spectroscopy and provide details for building and utilizing a total internal reflection fluorescence microscope (TIRFM) that observes fluorescence resonance energy transfer (FRET) changes when single RNA molecules undergo large-scale conformational rearrangements at the tens-of-millisecond or slower timescale [5]. Next, Marino and colleagues show that site-specific labeling the HIV-1 dimerization initiation site (DIS) with the fluorescent nucleotide analog 2-aminopurine provides for a local steady-state fluorescence probe of the kinetics and thermodynamics of DIS kissing loop formation [6]. Applying an ultrafast fluorescence up-conversion technique with femtosecond time resolution to 2-aminopurine in conjunction with specific quencher nucleotide analogs, Zhao and Xia are further able to extract detailed snapshots of the conformational heterogeneity around the local probe/quencher pair [7].

Given the magnitude of the challenge, many complementary techniques will be required to eventually solve the mysteries of the RNA structure–dynamics–function relationship. One recent addition to the arsenal is quantitative mass spectrometry. In their review, Bunner and Williamson describe how pulse-chase experiments with stable-isotope labeled proteins can be used to dissect the *in vitro* self-assembly kinetics of RNA–protein (RNP) complexes such as the *Escherichia coli* 30S ribosomal subunit [8]. The authors suggest that the technique in the future can be applied to the reconstitution of other stable RNP complexes. A more classic tool available to the RNA researcher is chemical footprinting, as exemplified by the article by Schlatterer and Brenowitz that focuses on time-resolved hydroxyl radical footprinting [9]. This technique offers a global view at single nucleotide resolution of the compaction and increasing solvent protection of a polyanionic RNA that undergoes folding in response to addition of divalent metal cations. The authors suggest that such information is complementary to that on global size and shape available from small angle X-ray scattering (SAXS) and analytical ultracentrifugation. In the following paper, Frederiksen and Piccirilli describe how site-specifically bound metal ions can be probed using chemical modification of specific ligands on the RNA [10]. For example, sulfur or nitrogen can be used to replace a suspected oxygen ligand of a Mg^{2+} ion, providing evidence for a direct (inner-sphere) contact if the chemical substitution is rescued structurally or functionally by one of the softer metal divalents Mn^{2+}, Zn^{2+}, Cd^{2+}, or Co^{2+}.

To fully describe RNA function, RNA dynamics ultimately needs to be resolved at the atomic level. Combining experimental with computational tools arguably presents the most efficient way to flesh out the (limited) observables from a given experiment and integrate the results from diverse experimental techniques. Roughly the second half of this special issue is therefore dedicated to a range of computational approaches for studying RNA dynamics. The first such review, by Al-Hashimi, Andricioaei and colleagues, gives a protocol for combining molecular dynamics (MD) simulations with experimental residual dipolar coupling (RDC) measurements from NMR to map structural ensembles of RNA at the atomic level [11]. RDCs are essentially used to "weed out" overly unrealistic snapshots from the MD simulation. An approach to integrating high-resolution X-ray crystal or NMR structures with lower-resolution cryo-electron microscopy (cryo-EM) snapshots of conformational intermediates of a reaction pathway is described by Schulten and colleagues as molecular dynamics flexible fitting [12]. Here, a guiding potential is added to the standard force field to "mold" high-resolution component structures into a cryo-EM map of, for example, the translating ribosome.

1046-2023/$ - see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.ymeth.2009.09.002

Fulle and Gohlke go on to describe the flexibility of RNA through counting constraints imparted by both covalent and strong non-covalent bonds [13]. In this fashion the internal conformational degrees of freedom of even large RNPs such as the ribosome can be quickly assessed. Another coarse grained approach to predicting RNA dynamics is summarized in the review by Isambert [14]. The author exploits the separation of timescales between small-scale local motions and large-scale global rearrangements to predict, using a simulation server made available on the internet, the folding, misfolding, and unfolding pathways of RNAs including pseudoknots, a notoriously difficult problem for computational approaches. Another internet available tool is presented by Pande and colleagues in the form of a software package that builds Markov State Models (MSMs) to identify metastable states in Generalized Ensemble (GE) simulations [15]. This approach allows one to quickly map out an RNA conformational space.

The ultimate test for how well we understand Nature is to ask whether we can explain biological behavior from first principles. We are a far cry from achieving this goal, but the last (but not least) review of this special issue by Otyepka, Šponer, Walter and colleagues epitomizes the current state-of-the-art in using quantum mechanical (QM) calculations for describing RNA functions such as chemical reactions catalyzed by ribozymes [16]. One goal here is to integrate the rigor of QM with the ease of MD (or molecular mechanical, MM) treatments to pinpoint how RNA dynamics couples to function. Another goal, and certainly a goal of this issue as a whole, is, as the authors put it, "to foster mutual appreciation and facilitate collaboration between experimentalists and theorists to jointly advance our understanding of RNA" function at the atomic level. The future of studies on RNA dynamics is wide open, yet a rising interest as evident from symposia such as that held at the American Chemical Society meeting [17], new books published on RNA Biophysics [18,19], and a recently instituted Telluride workshop on RNA dynamics all signal that the future is also bright and promising.

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Nils G. Walter
Department of Chemistry, Single Molecule Analysis Group,
University of Michigan,
930 North University Ave., Ann Arbor, MI, USA
Phone: +1 734 615 2060
E-mail address: nwalter@umich.edu