New and Notable

Setting the Stage for Predicting RNA Thermodynamic Properties and Their Structural Components

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In this issue of the *Biophysical Journal*, Singh and Kollman (1996) present an article in which a free energy simulation is employed to compute the free energy difference between a pair of RNA hairpin loops and to delineate the structural origins to the stability differences.

"Neutrinos have mass. Great, now why does my mother care?" is how Hazel O'Leary, the sometimes controversial Secretary of the Department of Energy has called on the scientific community to explain the rationale behind its research objectives (Lawler, 1995). It is in this spirit that a larger context for the work by Singh and Kollman may be worth offering. The application of rational drug design comes to mind. In particular, computer assisted molecular modeling has begun to show great promise for reducing the time and treasure needed to develop new pharmaceuticals (Marshall, 1995). A key feature necessary to determine relative specificities of selected potential drugs is the capability to quantitatively determine binding-free-energy differences between the biological target and a range of possible pharmaceutical ligands. Such knowledge should reveal which substance binds most tightly and why. Of course this information can be garnered experimentally, but the laborious synthetic, thermodynamic, and structural studies make the computational alternative an exceedingly attractive goal. Here, free energy simulations can play a major role.

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Biological targets of potential drugs are often proteins, and free energy simulations between protein substrates and drug ligands have been undertaken with reasonable success (Kollman, 1993). DNA as a biological target for drugs has a long history, but a successful free energy simulation evaluating the relative binding energies of a pair of drugs has only been carried out recently for the first time (Singh et al., 1994). RNA has now also come to the fore as a potential drug target, especially in wake of human diseases, including AIDS, that result from RNA virus infection.

RNAs, being predominantly single stranded, have been known to contain hairpin loops since the cloverleaf model of tRNA was verified with crystal structures (Kim, 1978). Now the loops are attracting even more attention because they are known to play important roles in a growing list of biological activities, including RNA catalysis, and some loops manifest unusual stability (Varani, 1995). In the case of the HIV-1 virus, a stable hairpin loop structure in the trans-activation response (TAR) element of the viral mRNA is the target of the virus' *tat* protein. TAR plays a key role in activating transcription and replication. "Thus, the TAR loop is an appealing target for antiviral drugs" (Chang and Tinoco, 1994). Targeting this loop with a drug that competes successfully with *tat* is a possible strategic approach for disrupting the viral life cycle.

Considerable effort is currently being devoted to understanding RNA hairpin loop structures and the sources of their relative stabilities through experimental structural and thermodynamic studies. Recent reviews on RNA structures have been presented by Varani (1995) and by Shen et al. (1995). The computational community is also beginning to address these challenging entities, and the work of Singh and Kollman is the first-published free energy perturbation/molecular dynamics simulation with solvent and salt for an RNA system. It tests whether such a simulation can yield the thermodynamic and structural data that till now has been the province of experiment.

Scientific problems in carrying out reliable free energy simulations are still targets on the research frontier (Kollman, 1993). Issues include the adequate sampling of accessible states, which encompasses the time-step problem in molecular dynamics that limits current simulations with solvent and salt to the nanosecond range; adequate treatment of environment; proper evaluation of the important electrostatic interactions; the ever present quality of force field issue; and the significant problem of protocol design. Applying molecular dynamics and free energy calculations to nucleic acid systems with full representation of water and ions is particularly challenging. The large electrostatic effects in these multiply charged systems make them especially refractory. In contrast to protein systems, even succeeding to generate stable molecular dynamics trajectories is difficult, let alone using such trajectories in free energy calculations. In addition, there is current controversy concerning whether force field components, the nonbonded Lennard-Jones and electrostatic terms, can be employed to aid in understanding relative stabilities, because these, in contrast to relative free energies, are path dependent (see references in Singh and Kollman, this issue).

The Singh and Kollman paper is new and notable in the light of the present state of the art. It employs for the first time the free energy perturbation method to assess relative loop stabilities and their underlying structural origins. Solvent and counterions were treated explicitly, and a necessary evaluation of alternate simulation protocols was carefully made. With this strategy Singh and Kollman were able to reproduce the observation that in the hairpin loop GGAC(UUCG)GUCC, mutating C7 to U7 destabilized the loop structure by 1–2 kcal/mol (Varani

et al., 1991). Moreover, the free energy component analyses that were carried out in this case provided insight to the factors governing the loop stability that were in harmony with experimental understanding. In addition, the component analyses suggested that specific chemical modification to the phosphate between U5 and U6 would reduce the stability of the C7 loop, and this prediction is, of course, testable. Recent results using continuum electrostatic theory by Friedman and Honig (1995) also calculated relative stability of this pair of loops in agreement with experiment. Their method is simpler and more rapid, but does not simulate structural changes.

Thus, the paper by Singh and Kollman suggests that difficulties in RNA free energy perturbation simulations are being overcome. Their free energy simulation can be viewed as a very promising pilot project, a test-bed that supports the ultimate use of computation for obtaining reliable free energy differences and their structural underpinnings. Recent improvements in the computation of the electrostatic force field components in polynucleotide molecular dynamics, notably application of the particle mesh Ewald method (Darden et al., 1993), indicate that all atom simulations can become reliable in delineating both thermodynamic quantities and structural details. As force fields, computational algorithms that enhance sampling, and computational resources continue to improve, the promise for computational reliability accompanied by feasibility in reasonable time frames should come to fruition. Of course, reasonable starting structures from experiment, or from modeling if necessary, will be required. Nonetheless, in the future we should see exciting applications of free energy calculations to RNA systems. One could then envision computationally evaluating relative binding free energies of potential drug ligands for the HIV-1 TAR element.

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