Supplementary Material

A. Interaction energy of magnesium with tRNA bases

The hexahydrated magnesium-base complex [$Mg(H_2O)_6^{+2}$] was extracted from the anticodon region of the crystal structure of $tRNA^{phe}$ (Protein Data Bank (PDB) ID:1tra) (Figure 1). The magnesium site occurs in solution as well as in gas phase.[1] The two bases we have studied are Adenine and Guanine and 5MC (5-methylcytidine-5'-monophosphate) and Uracil.

The magnesium-base complexes are capped with hydrogen atoms to balance the valence and the overall charge. The complexes are energy minimized in the gas phase at HF/6-31G(d, p) level[2] without altering the positions of the heavy atoms. In other words, we have carried out minimization of only the hydrogen atoms positions in the complex while freezing the co-ordinates of all other atoms in the complex. This way, the conformations of the minimized complexes mimic the crystal environment. For Guanine hexahydated magnesium ion complex, the basis set superposition errors using counterpoise correction in the gas phase,[3] are known to be approximately one kcal/mol.[1b]

To account for the effects of polar solvent on the stabilization and energetic of the interaction of hexahydrated magnesium with RNA bases, we have minimized the geometries in the isotropic reaction field of water within the framework of the polarized continuum model.[2] In this model, solvation energy was calculated using the optimized geometry. The polar media dielectric constant was taken to be 78.4 (bulk water). The total interaction energy consist of (a) the solvation energy of the magnesium-base complex and its monomers, and (b) a term that describes the change in energy in going from gas as a reference state to water as the new reference state.[2]

To quantitatively assess the non-electrostatic contribution to the total interaction energy of the magnesium-base complex, the optimized geometries in the gas-phase and in solvent, were subjected to energy decomposition analysis at the HF/6-31G(g) level (Table 1).[4] Deformation energy of the components was neglected.[1b]

B. Magnesium binding sites

Consider the μVT ensemble, where the chemical potential μ, the volume V, and the T temperature of the system are held fixed. Grand canonical Monte Carlo simulation simulations combine configuration space sampling via Monte Carlo methods with multiple-particle moves that involve randomly inserting and deleting particles.

In the grand canonical Monte Carlo algorithm employed in this work,[5] the interactions between the solvent and the particles are described by the primitive model in which one assumes an average dielectric constant of the medium. Furthermore, one ignores polarization and explicit solvent effects as well as interactions between three or more particles. The interaction between pair of particles i and j , $V_{ij}(r)$, is sum of $V_{ij}^{C}(r)$ and $V_{ij}^{LJ}(r)$, where $V_{ij}^{C}(r)$ and $V_{ij}^{LJ}(r)$ are respectively the Coulombic interactions and the 6-12 Lennard-Jones potential arising from steric interactions between the particles. The ions are modeled as charged hard spheres.[5] The interactions between pair of ions is sum of $V_{ij}^{C}(r)$ and $V_{ij}^{HS}(r)$, where the later is the hard-sphere potential. $V_{ij}^{HS}(r)$ is zero for $r_{ij} > \sigma_i + \sigma_j$ and infinity for $r_{ij} \le \sigma_i + \sigma_j$, where σ_i is the hard-sphere radius. Finally, there is an external potential on each particle coming from its interaction with the fixed solute, i.e, the coordinates, charge and radius of the macromolecule.[5] The electrostatic interactions between the ions and the solute neglects reaction field and is given by the product of charge q of the ion and the electrostatic potential ϕ, i.e., $V_i(r_i) \approx q_i \phi(r_i)/2$, where ϕ satisfies the Poisson equation.[5]

Several parameters appear in the simulation.[5] One set of parameter determines the frequency of Metropolis steps for changing particle configurations. Another set of parameter describes the maximum displacement of the particles. This quantity is needed before the decision of acceptance or rejection of a move is made based on energy evaluation.[5] The maximum displacement of the particles is taken to be 2.0 Å.[5] The excess chemical potential governs the probability of deleting and inserting particles in the system. Characterization of the excess chemical potential involves the third set of parameters.[5]

The aqueous solution consists of 1 mM Mg^{2+}, 52 mM Cl^{-} and 50 mM Na^{+}. The temperature of the system was taken to be 298.0 K. Dielectric constant of the solvent was

78.36. The radius of cations (Na^+, Mg^{2+}) and anion (Cl^-) as well as depths of Lennard-Jones potentials were taken from the literature.[5-7] The simulation consists of over 250,000 steps. Each step involves several cycles, where each cycle is an attempt to create and destroy one particle.[5] We made use of the parameterization scheme for calibrating the excess chemical potential and the bulk ion concentrations by Vitalis et al.[5] The atom coordinates for the ternary complex, E. coli elongation factor EF-Tu complexed with kirromycin and $tRNA^{Phe}$, were obtained from the crystal structure (Protein Data Bank (PDB) ID: 1OB2).[8] All monovalent and divalent ions were removed from the crystal structure.

C. Flexibility of ternary complex

The model for the ternary complex assumes (a) one interaction site per residue located on the alpha carbon atom; (b) two sites located on the O4* and the P atoms, per nucleotide, for tRNA; (c) the total potential energy E of the sites is Gaussian. E is expressible in terms of the deviation of the instantaneous coordinates of the atoms from the crystallographic coordinates, a force constant, and a matrix Ω whose elements are –1 for distances between sites i and j within a cutoff distance, and is zero otherwise.[9]

The cutoff distance for EF-Tu(GTP) is taken to be 7 Å; this is typically the range of the inter-residue contacts. For tRNA, the cutoff distance is taken to be 20 Å.[9] The coordinates of EF-Tu(GTP)aa-tRNA are obtained from the crystal structure (Protein Data Bank (PDB) ID: 1OB2).[8] In the model there is a single harmonic force constant (γ) for tRNA and another one for EF-Tu(GTP).[9] These quantities are calculated by normalizing the predicted B factors with the B factors obtained from x-ray crystallography.[9] The force constants are γ = 0.142 kcal/mol Å^2 and 0.491 kcal/mol Å^2 for tRNA and protein, respectively.

The quantity of relevance is the mean-square fluctuation of i^{th} nucleotide or residue, $<(\Delta R_i)^2>$, where <(..)> denote an ensemble average. This quantity, related to the Debye-Waller factor, is a measure of the flexibility of i^{th} nucleotide or residue. In fact, $<(\Delta R_i)^2>$ scaled by the factor k_BT is proportional to the diagonal elements of the inverse of Ω, where k_B is the Boltzmann constant and T is the absolute temperature.[9]

The inverse of Ω is obtained numerically by normal mode analysis of the total potential energy.[9] Figure 1 in the text depicts the normalized mean-square fluctuations of aa-tRNA and the ternary complex averaged over two of the slowest normal modes. The term normalized denotes that the mean mean-square displacement of a nucleotide is divided by the sum of mean-square fluctuations of all bonded and non-bonded atoms within the cutoff distance.

Table of contents image was drawn[9c] depicting the flexibility of ternary complex. The color code is such that the flexibility increases as goes from deep blue to green to yellow and red.

D. FRET conformation states

Single-molecule FRET techniques have been developed to study the mechanism of ribosome-tRNA action.[10] Various conformational (FRET) states during initial selection process of aminoacyl-tRNA by the ribosome have been identified. Low-FRET state is identified with initial codon recognition, while mid-FRET state is associated with GTPase activated state, for example.[10] By counting the transitions between kinetically distinct no-FRET, low-FRET, and mid-FRET states, the rate constants of initial selection process are determined.[10]

The experimental forward rate constants of cognate ternary complex from a state corresponding to codon-recognition, to a stable GTPase activated state, at 15 mM Mg^{2+} and 5 mM Mg^{2+} are $14s^{-1}$ and $11s^{-1}$, respectively. For single base pair mismatch (near cognate), the corresponding rate constants at 15 mM Mg^{2+} and 5 mM Mg^{2+} are $2.0s^{-1}$ and $0.095s^{-1}$.[10c]

FRET results suggest that contact between the ternary complex and 30S leads, after correct codon-anticodon recognition, to induced wrapping of the ternary complex around the decoding site.[10] The conformation process associated with induced wrapping is schematically shown in Figures 2a and b below.

References

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Figure 1a. Optimized geometry of $Mg[H_2O]_6^{+2}$ interacting with Adenine and Guanine in the gas phase in which the coordinates of the heavy atoms were frozen.

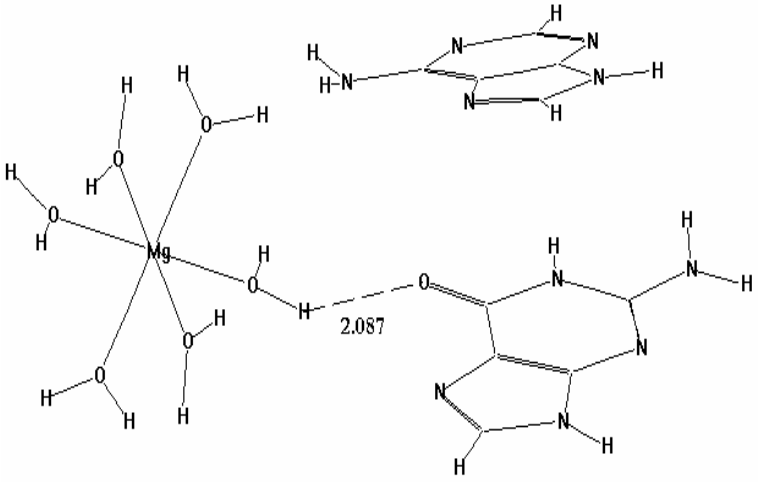

Figure 1b. Optimized geometry of $Mg[H_2O]_6^{+2}$ interacting with 5MC and Uracil in the gas phase in which the coordinates of the heavy atoms were frozen.

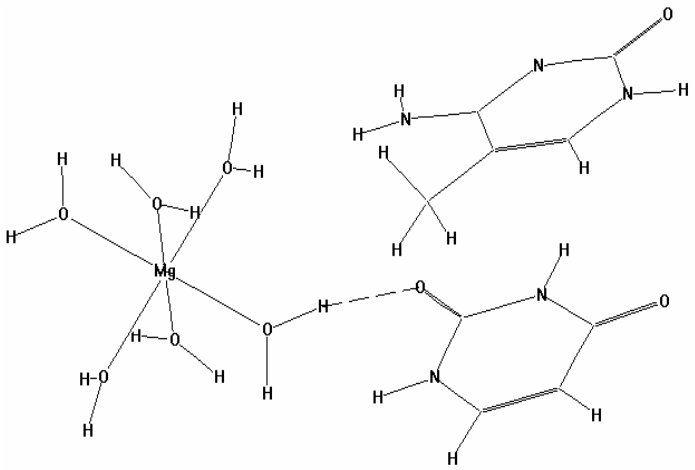

Table 1a. Energy decomposition of the optimized geometry of $Mg[H_2O]_6^{+2}$ interacting with Adenine and Guanine in the gas phase and in polar solvent.

	KCAL/MOLE (gas phase)	KCAL/MOLE (solvent)
ELECTROSTATIC ENERGY(ES)	-35.91	-33.09
EXCHANGE REPULSION ENERGY(EX)	3.95	3.49
POLARIZATION ENERGY(PL)	-11.41	-9.94
CHARGE TRANSFER ENERGY(CT)	-3.65	-3.17
HIGH ORDER COUPLING ENERGY(MIX)	0.14	-0.04
TOTAL INTERACTION ENERGY	-46.88	-42.67

Table 1b. Energy decomposition of the optimized geometry of $Mg[H_2O]_6^{+2}$ interacting with 5MC and Uracil in the gas phase and in polar solvent.

	KCAL/MOLE (gas phase)	KCAL/MOLE (solvent)
ELECTROSTATIC ENERGY(ES)	-7.37	-5.67
EXCHANGE REPULSION ENERGY(EX)	18.46	16.54
POLARIZATION ENERGY(PL)	-12.69	-11.17
CHARGE TRANSFER ENERGY(CT)	-7.61	-6.55
HIGH ORDER COUPLING ENERGY MIX	-0.73	-0.54
TOTAL INTERACTION ENERG	-9.95	-7.39

Figure 2a. Single-molecule FRET studies (ref. 10) suggest that codon-anticodon recognition of cognate ternary complex (aa-tRNA and EF-TU(GTP) is shown) with the mRNA at the decoding site leads to induced wrapping of the ribosome. This leads to pivot-like rotational motion that places the cognate ternary complex near the GTPase-activated state.

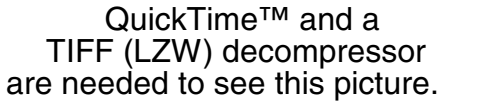

Figure 2b. The ternary complex (blue) is attached to a tether. The tether is modeled as a polymer chain consisting of beads (black spheres), of mass m, connected to springs (orange lines). The other end of the tether is attached to 30S subunit, the later is taken to be a rigid wall. Induced wrapping of the ribosome around the decoding site exerts an external force on the tether. The force perturbs the friction that acts on the ternary complex, as it performs Brownian motion on a potential energy landscape V(q), where q is an appropriate reaction coordinate.