Bridging the gap between ribosome structure and biochemistry by mechanistic computations

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Abstract

The wealth of structural and biochemical data now available for protein synthesis on the ribosome present major new challenges for computational biochemistry. Apart from technical difficulties in modeling ribosome systems, the complexity of the overall translation cycle with a multitude of different kinetic steps presents a formidable problem for computational efforts where we have only seen the beginning. However, a range of methodologies including molecular dynamics simulations, free energy calculations, molecular docking and quantum chemical approaches have already been put to work with promising results. In particular, the combined efforts of structural biology, biochemistry, kinetics and computational modeling can lead towards a quantitative structure-based description of translation.

Introduction

Computational studies of the translation machinery based on experimental 3D structures actually date back to the first crystallographic structure of a ribosomal component, namely the C-terminal domain of the L12 protein [1]. Several molecular dynamics (MD) simulations of this molecule were published [2-5] showing a harmonic-like collective motion of the \( \alpha \)-turn-\( \alpha \) motif that is today believed to be involved in recruiting translation factors to the ribosome [6]. Otherwise, not much could be learned from such simulations of isolated components, except that the L12 CTD turned out to be an unusually stable protein in solution MD simulations with an \( \alpha \)-carbon RMSD below 0.7 Å with respect to the crystal structure, commensurable with experimental B-factors [4]. However, the situation of course changed drastically after the first crystal structures of entire ribosomal subunits at medium-high resolution were published in 2000 [7-9], that were soon to be followed by full 70S ribosome structures [10,11]. In principle, the mechanisms of the key events taking place on the ribosome such as the peptidyl transfer reaction and the mRNA decoding by tRNAs could now be addressed by quantitative computational methods based on molecular mechanics/dynamics force field calculations or even quantum mechanical approaches. However, the size and complexity of these molecular assemblies presented computational biochemists with major challenges that, in many respects, still remain to be overcome.
One obvious problem is the sheer size of any realistic simulation system that includes entire ribosomes. Another problem is the comparatively low resolution of the crystal structures available for ribosome, which are of the order of 3 Å. Not only are there conformational uncertainties about local conformation in structures derived from electron density maps having resolutions that low, the location of bound water molecules and counterions cannot be reliably determined. The higher resolution structures of the 50S subunit with transition state (TS) analogs bound [12••,13] are notable exceptions in this regard, reaching as far as 2.2 Å where the level of detail is considerably higher.

Anyone who has tried to analyze detailed structural and energetic convergence in biomolecular MD simulations, e.g., by free energy calculations, realizes that inaccurate solvent or ion positions can completely invalidate the results. In this respect, longer simulations usually do not solve the problem since waters and ions can be trapped on very long time scales and the likelihood that a large number of them will find their stable “equilibrium” positions within a reasonable time is small. This problem would seem to cast some doubt over the reliability of large scale ribosome MD simulations, e.g., of entire 70S structures in aqueous solution, where the details are likely to be wrong in many places. However, even though detailed mechanistic predictions at the atomic level may be beyond the scope, such calculations could still yield relevant information on larger scale movements and conformational dynamics. A parallel can perhaps be drawn to the significant insight into many of the ribosome states during translation obtained by the relatively lower resolution cryo-EM reconstructions [14,15]. An alternative is to break down the translation cycle into sub-problems that can be computationally investigated with high precision. These processes can then be analyzed with different types of methods, such as MD, docking, free energy calculations and chemical reaction simulations, applied to limited structural regions of the ribosome. The drawback is, of course, that larger scale motions cannot be treated with truncated systems, so that the two above strategies are rather complementary to each other.

**Peptide bond formation**

The crystallographic determination of the 50S ribosomal subunit from *Haloarcula marismortui* [7] opened up the possibility of computationally analyzing the peptidyl transfer reaction (Figure
Although an early attempt of modeling a peptidyl transfer reaction with semiempirical quantum chemistry had already been made [16], that study did not include any elements of the ribosome and obtained energy barriers of over 100 kcal/mol. The first two computational papers addressing peptidyl transfer in the ribosomal environment, including water and counter ions, were published in 2005 [17••,18•]. Both of these studies utilized the empirical valence bond (EVB) method and obtained reasonable activation free energies and similar transition states. This demonstrated that the ribosome can catalyze a proton shuttle type of mechanism where protons are relayed between the A-site amine nucleophile and O3’ leaving group via the P-site A76 O2’ hydroxyl (it was also shown that A2451 cannot act as a general base [17••,19]). This type of mechanism had also been suggested based on experimental results with 2’-deoxy or chemically modified substrates [20,21]. These computational studies as well as kinetic experiments [22••,23] further showed that a neighboring hydroxyl group (2’OH), per se, does not confer any catalytic advantage. Hence, the term “substrate-assisted mechanism” is probably preferable to “substrate-assisted catalysis”. The surprising entropy effect reported by Sievers et al. for peptide bond formation with the puromycin substrate analog [22••] indicated that something unusual is going on in the ribosomal peptidyl transferase center (PTC). That is, a huge difference in activation entropy was observed between the uncatalyzed and catalyzed reactions [22••]. This was also confirmed for the reaction with full length tRNAs where for $k_{cat}$ (ribosome vs. solution) is about 18 kcal/mol at 25°C [24]. The corresponding prediction from our computer simulations was 19 kcal/mol [17••] but considerably smaller in the work by Sharma et al. [18•]. However, the two studies were in agreement with regard to the relatively small contribution from alignment and proximity of the substrates (~4 kcal/mol) [17-19], as also indicated by the small entropy effect on binding [22••]. The similarity between the absolute activation parameters for the small puromycin and large tRNA substrates [24] is also noteworthy in this respect.

There has been some recent controversy regarding the magnitude of the 2’OH contribution to the rate of peptide bond formation, or rather the rate reduction caused by its removal [25,26], where its initially estimated large effect of about $10^6$ [21] now seems to have been revised to about 100-1000 [26]. However, the key issue is what the actual reaction mechanism is on the native ribosome, rather than how big an effect removal of the 2’OH causes. If, for example, the 2’OH
can be effectively substituted by a water molecule, which is not unlikely, then the viability of such a perturbed system in peptide bond formation is not inconsistent with the proposed role of the 2’OH.

Computer simulations [17••] also made detailed predictions regarding stereochemistry, product structure and key water molecules in the PTC, that act as bridges in an H-bond network involving the substrates and universally conserved bases. This H-bond network was further identified as the main factor responsible for the favorable activation entropy in that it, unlike bulk solvent molecules, does not need to reorganize significantly during the reaction. Subsequent analysis of new high-resolution 50S structures with bound TS analogs [12••,13] validated the MD predictions [19]. Attempts to model the peptidyl transfer reaction with density functional theory (DFT) methods without including any solvent molecules or parts of the ribosome have also been made [27-29]. Typical of such minimal gas-phase models is the spurious H-bonding often occurring between whatever donors and acceptors the model includes. Hence, both H-bonding between the P-site 2’OH and the A-site aminoacyl-tRNA ester carbonyl [27] and between the 2’OH and the P-site substrate carbonyl [28,29] were predicted to exist, in compact four-membered transition states with unrealistically high energies. These types of transition states, however, are not supported by the crystal structures with TS analogs [12••,13] (Figure 1a).

The question of whether the ribosome is really a ribozyme surfaced again after publication of a 2.8 Å resolution Thermus thermophilus 70S complex [11], which showed ribosomal protein L27 protruding into the PTC. As that structure, however, had no visible electron density for the A-site tRNA the possible role of L27 in peptidyl transfer remained unclear. This problem was addressed by modeling and MD/EVB simulations of both the tRNA and puromycin reactions with and without L27 present [30]. The results showed only a minor rate reduction upon deletion of L27, in agreement with biochemical data [31], but that its N-terminus could affect tRNA binding as it was predicted to interact with the phosphate groups of A76 and C2452. Very similar interactions were seen in a subsequent 70S structure with both P-site and A-site tRNAs bound [32]. The calculations further predicted that the ionization state of the L27 N-terminus has a marked effect on the puromycin reaction that could reflect its particular pH-dependence with an
unknown group (in addition to the attacking amine) ionizing in the physiological pH range [33,34].

The relevance of the puromycin reaction for peptide bond formation with tRNAs has been the subject of some debate. That is, apart from the fact that the puromycin reaction has a different pH-dependence with seemingly two ionizing groups affecting the rate [33,34], the response of the reaction rate to active site mutations also differs significantly from the native tRNA reaction [35•]. Possible explanations for this behaviour come both from crystal structures with TS analogs [12••,13] and molecular simulations [19,30], where the non-native amide ribose linkage of puromycin was seen to engage in H-bonding that would not be possible with a natural tRNA substrate. Puromycin and derivatives thereof have nevertheless long been used to study the mechanistic details of the peptidyl transfer reaction. A pioneering attempt to measure kinetic isotope effects (KIEs) for peptide bond formation on the ribosome showed that $^{15}$N substitution of the attacking (CC-puromycin derivative) amine somewhat surprisingly gave rise to a normal KIE ($>1$), in contrast to nonenzymatic aminolysis reactions in solution [36]. This experiment, carried out in a very slow 50S assay, indicated that the TS is early rather than late and associated with N–C bond making rather than C–O bond breaking.

To shed further light on the transition state for ribosomal peptidyl transfer, quantum chemical ab initio calculations were carried out on relatively large model systems containing, not only substrates but also key elements of the PTC, including water molecules [37••]. It was found that unconstrained geometry optimizations converged to structures very close to those obtained experimentally with TS analogs, and that no early transition states exist on the corresponding potential energy surface. Both for six- and eight-membered mechanisms, the TS was predicted to be late and mainly associated with C–O bond breaking. The eight-membered mechanism corresponds to a double proton shuttle, with an extra intervening water molecule that was earlier predicted from MD simulations [17••,19] and found in high-resolution 50S structures [12••,13]. This mechanism also gave an activation enthalpy in excellent agreement with experiment [37••]. As expected for this type of TS, however, the $^{15}$N isotope effect was predicted to be inverse rather than normal. A recent study also measured (H/D) kinetic solvent isotope effects (KSIEs) on the 70S puromycin reaction [38•], where the proton inventory suggests three protons in flight
in the TS and a corresponding large KSIE of about 8. These results are in very good agreement with the eight-membered proton shuttle which involves three H-bonds with a predicted KSIE of 7.1 (Wallin & Åqvist, unpublished results).

Strobel and coworkers also recently reported additional heavy atom KIEs for the same 50S reaction used earlier [39•]. These results are still consistent with an early TS, showing a normal $^{15}\text{N}$ KIE that is stronger than the corresponding $^{18}\text{O}$ effect for the leaving 3'-oxygen, but are not easy to reconcile with the proton inventory studies. They have also attempted to model the observed KIEs with DFT calculations on minimal vacuum models by fitting observed and calculated isotope effects in terms of geometry [36,39•]. However, transition states cannot really be determined in this way, but must be obtained by unconstrained search and optimization of saddle points with vanishing gradients on the relevant potential energy surface. In this sense, no true TS consistent with an $^{15}\text{N}$ KIE > 1 has yet been shown to exist. Further, the TS suggested in [39•] does not appear to be on the reaction path to products as it gives no clue of how the O3’ would become protonated. Clearly, these isotope effect experiments are extremely elegant and carefully executed but it should be remembered that they do not pertain to the native ribosome reaction. That is, apart from reported differences in pH-dependence and the effect of substituting the $\alpha$-amino group for a hydroxyl [36, 40], the rate of reaction for the model substrate with only large ribosomal subunits is over a 1000-fold slower than the native 70S reaction with tRNAs [24,41,42]. This situation would seem to call for considerable caution in extrapolating detailed conclusions regarding transition states to the native reaction.

Peptidyl transfer was further probed by measuring Bronsted coefficients for a series of puromycin derivatives in both 50S and 70S assays [42]. Linear free energy relationships between nucleophile pKa and the logarithm of the peptidyl transfer rate showed Bronsted coefficients near zero, i.e. essentially no rate dependence on the nucleophilicity of the attacking amine. This is usually qualitatively interpreted such that either the nucleophilic attack is not rate-limiting (implying a late TS associated with leaving group departure), or that there is no charge build-up on nucleophile in a rate-limiting transition state. While several types of TSs are compatible with these results one can note that deprotonation of the attacking amine as the N–C bond forms provides a possible explanation, in agreement with the ab initio DFT calculations [37••] that do
not show any significant charge build-up on the amine in the TS. In fact, these calculations also predicted little charge development on the substrate “oxyanion” as the reaction proceeds towards the TS (Fig. 5 of [37••]). The interaction of the oxyanion with a (single) conserved water molecule [12••,17••,37••] is, however, clearly favourable and could therefore be said to stabilize the oxyanion compared to the (gas-phase like) situation with no water present. Adding more waters around the oxyanion, approaching the solution situation, was seen to polarize the oxyanion in the TS and lower the activation enthalpy of the reaction towards the value observed for the uncatalyzed reaction (presumably with a concomitant decrease in the activation entropy) [37••]. The fact that the activation enthalpy is larger for the ribosome reaction than in solution [22] is thus consistent with the role of this key water molecule (that, however, by itself does not provide as much solvation of the oxyanion as bulk solvent). Attempts to estimate the corresponding interaction by binding of TS mimics [43] also appear to be consistent with this interpretation. A recent DFT study using a different functional confirms the importance of including waters and ribosomal hydroxyl groups for approaching realistic energetics [44].

Another proton shuttle variant was suggested there [44] that is high in energy and hard to reconcile with crystal structures [12••,13]. Interestingly, none of the quantum mechanical calculations published so far supports any early rate-limiting TS.

**tRNA accommodation**

The issue of whether tRNA accommodation into the ribosomal A-site, after GTP hydrolysis and release of EF-Tu, actually limits the rate of peptide bond formation has also been debated. Rodnina and coworkers have reported rates of tRNA accommodation, based on fluorescence measurements, that are essentially identical to their measured rates of dipeptide formation with full-length tRNAs [45,46], suggesting that accommodation is rate-limiting for the overall process. On the other hand, the reported rate constant for dipeptide formation at saturating conditions (174 s$^{-1}$) is about four times faster than the maximal reported rate of accommodation (40 s$^{-1}$), casting some doubt on the latter value [46]. Further evidence in support of a rate-limiting accommodation step also seemed to be provided by the reported pH-independence of peptidyl transfer with Phe-tRNA$^{\text{Phe}}$, suggesting that the expected ionization of the α-amino group was masked by a slower step [46]. However, pH-dependence of the reaction with six different tRNAs
was recently measured, instead suggesting that the chemical step is rate-limiting [47•]. MD simulations further showed that the observed pKa-shifts were clearly correlated with the electrostatic interactions of the α-amino groups of the aminoacyl-tRNAs, as would be expected if their ionization is reflected in the pH-rate profiles. In this respect, the similarity of and between the full-length tRNA [24] and puromycin reactions (for which there is no accommodation) [22] also speaks against accommodation being rate-limiting. Sanbonmatsu and coworkers have addressed the accommodation process by computer simulations in a series of papers [48•,49,50]. Initially they used targeted MD simulations (gradual restraining to target structure) based on low-resolution structures to elucidate the overall path and proposed the existence of an accommodation gate comprised of three universally conserved bases [48]. Mutation of C2573 to a purine was suggested to cause inhibition of accommodation but was, interestingly, found experimentally to affect termination but not peptide bond formation [51]. Later, both so-called structure based simulations (i.e., utilizing Go-like potential energy functions biased to the target structure with no solvent) and all-atom endpoint MD with explicit solvent have been used to explore the time-dependence of accommodation [49,50]. These simulations were also based on low-resolution models and the more recent atomic structures of A/A and A/T states [11,52••,53] would be interesting to use for validation.

**Codon reading and GTPase activation**

The initial tRNA selection process preceding peptide bond formation has also been addressed in some computational studies. Sanbonmatsu and Joseph carried out MD simulations of the decoding center based on the crystal structures of the 30S subunit with mRNA and tRNA antistem loops, focusing on structural fluctuations of cognate and near-cognate codon-anticodon pairs and their possible relation to tRNA discrimination [54]. Almlöf et al. used the same type of system to evaluate binding free energies for six codon-anticodon pairs by MD simulations, in combination with the linear interaction energy method [55]. They found that the ribosome environment with the three monitoring bases A1492, A1493 and G530 amplifies the intrinsic stability differences of codon-anticodon complexes in aqueous solution. With binding energies in good agreement with experimental data for the first and third codon positions, they also predicted the strongest discrimination at the second codon position which is consistent with its
generally more important role in determining amino acid properties [56,57•]. These results were recently confirmed by free energy perturbation (FEP) simulations based on more recent crystal structures [58•]. That work also used free energy calculations to address the effect of the cmo5U34 modification in tRNAVal and proposed alternate mismatched base-pair conformations from those observed in the crystal structure [59]. The binding energetics obtained, however, does not seem to explain the observed reading of all four synonymous codons by the tRNA. In general, computational evaluation of base-pairing energetics on the ribosome, including effects of tRNA modifications, is of tremendous interest as it could allow an energetic interpretation of the code table by quantifying the codon recognition contribution to the initial tRNA selection fidelity [57]. We have probably only seen the beginning of such efforts and they are likely to be very challenging, particularly if also possible keto-enol tautomerization of base-pairs have to be taken into account [53].

A key step in initial selection is the irreversible hydrolysis of GTP on EF-Tu following ternary complex binding to the ribosome. The presumably universal process by which the translational GTPases become activated has been enigmatic, although components such as the universally conserved ribosomal sarcin-ricin loop (SRL) and invariant His84 (EF-Tu numbering) of the translational GTPases have been identified as being important for GTPase activation [60]. A major breakthrough was the recent determination of the crystal structure of an EF-Tu ternary complex bound to the 70S ribosome [52••], showing the key interaction between the SRL and His84 in the activated conformation. This led Vorhees et al. to suggest a GTP hydrolysis mechanism where His84 acts a general base in the reaction. An analysis of this crystal structure by Liljas et al., however, instead led to the suggestion that a substrate-assisted mechanism is more likely [61]. Such a mechanism was also explored by Warshel and coworkers in a simulation study that concluded that the histidine contributes to an allosteric effect [62]. While they did not directly compare the different mechanistic possibilities, recent FEP simulation results from our lab show the His84 general base mechanism to be strongly disfavoured [Åqvist et al. unpublished].

**Translocation**
Following peptide bond formation, translocation of the ribosome relative to the mRNA and bound tRNAs is the step that moves the next codon to be read into the ribosomal A-site. Like accommodation, translocation involves large conformational changes and is thus difficult to model in atomic detail. Translocation is rendered unidirectional by the hydrolysis of GTP on EF-G, which induces a large conformational change in that factor that appears to force its fourth domain into the A-site on the small subunit, pushing the message and its attached tRNAs across the ribosome. A 3.6 Å crystal structure of the post-translocational state was obtained by Ramakrishan and coworkers [63] but the pre-translocational configuration remains unknown. However, several attempts have been made to model the translocation process computationally using cryo-EM data [64•,65]. MD simulations have also been done on free EF-G•GDP in solution [66]. The combined use of MD fitting and cryo-EM techniques hold considerable promise for further elucidating the mechanics of translocation.

**Termination and peptide release**

Termination of translation is catalyzed by class-1 release factor (RF) proteins that decode stop codons and trigger hydrolysis of the ester bond between the nascent polypeptide and P-site tRNA. The first low resolution (~6Å) crystal structures of 70S complexes with RF1 and RF2 [67] showed no atomic detail for either of the two key loop motifs involved in stop codon reading and peptide-tRNA hydrolysis. Trobro and Åqvist used molecular docking of a heptapeptide, containing the universally conserved GGQ motif responsible for promoting hydrolysis, to predict how this motif could interact with the PTC [68•]. Docking solutions were then subjected to MD/EVB/FEP simulations in order to gauge their catalytic potency. A single solution emerged from these calculations, where the glutamine sidechain inserts deep into the A-site and can coordinate a water molecule for attack on the P-site ester carbon, where again the 2’-OH group plays a key role [68•,69] (Figure 1b). This model explained the effects of several mutants and the predicted position and conformation of the GGQ motif turned out to be in very good agreement with subsequently determined medium-resolution (3-3.5Å) structures [70••,71•,72]. The N-methylation of the catalytic Gln sidechain is important for peptide release and its effect could also be rationalized [68•,73].
The new crystal structures of 70S ribosomes with bound RFs [70●,71●] also made it possible to decipher the energetics of stop-codon reading by computational analysis and to clarify the origin of the high RF binding accuracy. Sund et al. used MD/FEP calculations to predict the relative binding affinities of cognate and non-cognate termination complexes with RF1 and RF2 [74●]. These simulations quantitatively explained the basic principles of decoding in all three codon positions and revealed that stop codon reading is considerably more complex than the tripeptide anticondon model [75●] and has little to do with tRNA mimicry. Interaction networks were identified that explain the high RF specificity and these were also found to be reflected by strong conservation patterns among bacterial RFs (Figure 2a). Simulations of complexes with tRNA\textsuperscript{Trp} further rationalized the observation of the “leaky stop codon” UGA (Figure 2b).

A remarkable feature of translation termination is the fact that prokaryotic and eukaryotic release factors are completely different, except for the universally conserved GGQ motif involved in peptidyl-tRNA hydrolysis. The bacterial RF1 and RF2 are specific for the UAA/UAG and UAA/UGA stop codon pairs, respectively. In eukaryotes the single class-1 release factor eRF1 decodes all three stop codons and contains conserved NIKS and YxCxxxF motifs involved in the recognition. Using the crystal structure of free eRF1 [76], Vorobjev and Kisselev have attempted to model the eukaryotic termination complex based on the bacterial 70S complexes with tRNAs and RFs, including some MD refinement [77,78●]. Although these works were not based on the higher resolution RF complexes and do not address the details of stop codon reading, the overall structural models seem reasonable and consistent with experimental cross-linking data. The emergence of the first eukaryotic ribosome structures [79,80] could now open the way for more detailed modeling of the enigmatic stop codon reading by eRF1.

**Outlook**

Computational modeling and simulation of the key events in protein synthesis on the ribosome is a relatively new field and, naturally, lagging behind the enormous progress in structural biology of the translation system. Nevertheless, this area of computational biochemistry holds considerable promise for the future, not the least because computations sometimes seem to be the only way to really translate the multitude of 3D structures into coherent functional mechanisms.
In this respect, calculation of energetics is central since it provides the most important link between structure and function that can be used to evaluate hypotheses based on structures alone. Further, since the translational machinery occupies a dominant mass fraction of growing cells, the ribosome and its auxiliary factors are under extraordinary selection pressure for optimal performance. This means that many of the fundamental binding and rate constants of the system will be closely connected to bacterial fitness. In this respect, computational techniques have the potential to anchor kinetics in the structures of the macromolecular complexes and rationalize the evolutionary design principles of the translation system.
References and recommended reading


A very elegant crystallographic work where a series of 50S structures with substrate and TS analogs are reported at unusually high resolution. Key water molecules likely to be important for the peptidyl transfer reaction are identified and different mechanistic possibilities discussed.


The first computer simulation of the peptidyl transfer reaction making detailed structural predictions that were later verified by experiments. The calculations showed the viability of a 2’OH proton shuttle mechanism and that A2451 could not function as a base. A preorganized H-bond network was identified as responsible for the unusual entropy effect of the reaction.

An elegant computational study addressing the catalytic advantage of the A76 2’OH group both in the uncatalyzed water reaction and in the PTC. It was shown that the vicinal hydroxyl group confers no rate acceleration in solution but is effective only in the ribosomal environment.


A groundbreaking study of the temperature dependence of ribosomal peptidyl transfer in comparison to an uncatalyzed reference reaction. The results showed an unprecedented reduction of the entropy contribution to the activation free energy. The similarity between for $k_{cat}/K_M$ and for $k_{cat}$ indicates that substrate alignment and proximity is not a major part of the entropy effect.


35. Youngman EM, Brunelle JL, Kochaniak AB, Green, R: The active site of the ribosome is composed of two layers of conserved nucleotides with distinct roles in peptide bond formation and peptide release, Cell 2004, **117**: 589-599. The role of universally conserved bases in the PTC was investigated by mutagenesis and kinetic measurements. Mutations in the innermost layer of the active site had large effects on peptide bond formation with puromycin, but not with aminoacyl-tRNA, and show defects in peptide release.


This high-level quantum chemical study could locate and characterize global transition states for the reaction, described by a molecular model encompassing all the key elements of the reaction center (~80 atoms), pointing to the feasibility of an eight-membered “double proton shuttle” mechanism.


Kinetic solvent isotope effects show that different numbers of protons are in motion in the TSs of the peptidyl transfer and termination reactions. Three H-bonds are predicted to be involved in the former TS but the proposed eight-membered structure seems difficult to reconcile with the 1VQN and 1VQP analog structures [12,13]. Only one proton is predicted to be in flight in the TS for hydrolysis which is consistent with the early TS predicted in [68]. The observed pH-dependence indicates that a group with pKa > 9 is active in its ionized form, suggesting that deprotonation of either a water molecule, the A76 2’OH or another group in the PTC contributes to catalysis.


An elegant heavy atom KIE analysis of peptide bond formation with a puromycin derivative on 50S subunits. The proposed early TS of a two-step mechanism has a doubly protonated A76 2’-oxygen, which would seem highly unfavourable in terms of pKa.


   The first demonstration of the pH-dependence of the reaction with native substrates showed downshifted pKa values of the aminoacyl-tRNAs that correlated well with those predicted from MD simulations.

48. • Sanbonmatsu KY, Joseph S, Tung CS: Simulating movement of tRNA into the ribosome during decoding, *Proc Natl Acad Sci USA* 2005, **102**: 15854-15859.

   This large all-atom MD simulation of a solvated 70S ribosome used targeted MD to drive the structure between models of the A/T (unaccomodated) and A/A (accommodated) states in order to elucidate the conformational pathway.


Groundbreaking crystal structure of an EF-Tu ternary complex, with a non-hydrolyzable GTP analog, bound to the ribosome. The structure shows how the sarcin-ricin loop on the ribosome positions the universally conserved histidine of the translational GTPases, thereby activating EF-Tu for GTP hydrolysis.


An extraordinary detailed kinetic analysis of the maximal accuracies that can be attained in tRNA selection, showing differential accuracies at the different codon positions.

Extensive MD free energy calculations were carried out to explore the effects of tRNA modifications and the reading of the four synonymous codons by tRNAVal. Many interesting points are brought up in this study, which illustrates that the energetics of codon reading is far from trivial.


An elegant analysis of structural substates from cryo-EM where pre- and post-translocational intermediates are used to interpret the mechanics of the process.


A surprisingly accurate structural prediction from computer simulations of how the catalytic GGQ motif inserts into the ribosomal A-site to promote the termination reaction.

The first medium resolution crystal structure of a release factor bound to the ribosome showed the positioning of the GGQ loop and the unexpected mRNA stop-codon conformation in complex with RF1.

This medium resolution structure of a 70S complex with RF2 gave valuable clues as to how RF2 can read both A and G in the second stop-codon position.

72. Trobro S, Åqvist J: **Mechanism of the translation termination reaction on the ribosome.** *Biochemistry* 2009, **48**: 11296-11303.

73. Andér M, Åqvist J: **Does glutamine methylation affect the intrinsic conformation of the universally conserved GGQ motif in ribosomal release factors?** *Biochemistry* 2009, **48**: 3483-3489.

These pioneering free energy perturbation calculations gave a quantitative picture of the structural mechanisms underlying stop-codon reading and could also explain sequence conservation patterns among bacterial RFs.

An impressive genetic engineering approach was used to identify characteristic RF tripeptide motifs that later were verified to indeed be in the immediate vicinity of the stop-codons.

76. Song H, Mugnier P, Das AK, Webb HM, Evans DR, Tuite MF, Hemmings BA, Barford D: **The crystal structure of human eukaryotic release factor eRF1-mechanism of stop codon recognition and peptidyl-tRNA hydrolysis.** *Cell* 2000, **100**: 311-321.

78. Vorobjev YN, Kisselev LL: **Modeling of the positioning of eRF1 and the mRNA stop codon explains the proximity of the eRF1 C domain to the stop codon in the ribosomal complex**, *Mol Biol* 2008, **42**: 302-311.

A very challenging docking problem was attacked by computational modeling and MD simulation guided by experimental cross-linking data and bacterial RF complex structures. Although no detailed model for stop-codon reading was presented the overall positioning of the eukaryotic RF appears largely consistent with experiments.


Illustration of the two reactions taking place in the ribosomal peptidyl transferase center: (a) peptide bond formation and (b) peptidyl-tRNA hydrolysis. The depicted geometry of the peptidyl transfer reaction (a) corresponds to that observed in the high-resolution 1VQP crystal structure [12], while the model for the RF catalyzed hydrolysis reaction is based on computer simulations [68, 72] and medium-resolution crystallographic complexes of RFs bound to the ribosome [70, 71]. Water molecules are depicted as red spheres and possible H-bond interactions are indicated by dashed lines.
(a) Prokaryotic RFs show a strong conservation of the residues involved in the stop-codon reading mechanisms. Residues involved in the first, second and third position reading mechanisms are coloured in green, cyan and magenta, respectively, while the characteristic PxT and SPF tripeptide motifs [75] are in yellow. The inset shows the detailed interaction network in RF2 that allows dual reading of A and G in the second codon position. (b) Comparison of the predicted near-cognate interaction between the UGA stop-codon and tRNA\textsuperscript{Trp} from MD simulations [74] (green carbons) with the recent crystallographic structure of the same type of complex [81]. The third position A–C mismatch was predicted not to involve a very strong energetic penalty.
**Highlights**

- Computational modeling and simulation are powerful tools for deriving functional mechanisms from ribosome structures.

- Evaluation of energies is most important for linking structure and function.

- Methods ranging from quantum chemistry to MD simulation can be used to explore the steps of translation.

- Several predictions about ribosome function from computations have been confirmed experimentally.

- Quantitative structure-based descriptions of protein synthesis can be obtained by combining computations with experimental kinetics.