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Using Rapid Kinetics and Molecular Dynamics Simulations to Study Biomolecular Information Processing and Design

Evelina Ines De Laurentiis, Dylan Girodat, Evan Mercier,
Hans-Joachim Wieden
Alberta RNA Research and Training Institute
University of Lethbridge
hj.wieden@uleth.ca

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1 Introduction

Translation, the ribosome dependent synthesis of proteins, is the last step of gene expression. It is targeted by a large number of antibiotics that modulate, in one way or the other, the dynamic properties of the involved biomolecules. Protein synthesis is a highly conserved multistep process facilitated by a large ribonucleoprotein complex (the ribosome) acting as a biomolecular assembler, converting genetic information provided as RNA transcripts (mRNA) into proteins. This process occurs with high speed and incredible accuracy within any living system (1). As such, accuracy is the key to maintaining the integrity of the genetic information to be reflected in the resulting proteins. The

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underlying design principles that enable the translation machinery to achieve these performance characteristics is of great interest for a large number of fields and applications, naming antibiotics design and the rational engineering of biomolecular machines just as two examples.

2 Background

Classical approaches to study enzymatic processes typically involve a combination of biochemical and structure determining techniques which ultimately provide the enzymatic properties such as binding affinities (e.g Kd) or rate constants (e.g. kcat) in correlation to the primary sequence of the respective enzyme or RNA. These findings are then typically complemented with the determination of the 3 dimensional structure of the respective bimolecular component via X-ray crystallography or nuclear magnetic resonance. Due to the importance of the bacterial translation machinery as an antibiotic target, a large number of biochemical and structural studies have provided a wealth of information regarding the makeup and function of the translation machinery, including the ribosome itself and a plethora of proteins and RNAs involved in regulating and coordinating the enzymatic activities of the ribosome. Although this information is available, the rational design of enzymatic properties or the rational modulation of an existing enzyme beyond just simple inhibition by competition is still a challenge. Therefore, the description and rational design of dynamic properties of bimolecular components is one of the key challenges of biophysical research today. Understanding the underlying design principles of information processing and decision making in biomolecular machines will be pivotal for success towards the engineering of novel designer biomolecules and small molecular modulators of biomolecular function, which will in turn enable us to develop novel therapies and biomedical strategies. This is in particular reflected in the ever-increasing number of structures of the bacterial and more and more so of the mammalian ribosomes bound to modulating factors, enabled through the technical advances in cryo-electron microscopy.

3 Protein synthesis

Although significant differences exist between the translation machinery in eukaryotes and prokaryotes, the overall steps are similar (1). Translation of a given mRNA follows the general scheme of: Step 1: initiation, Step 2: elongation, Step 3: termination and Step 4: recycling. Of these 4 steps only Step 2 is essential for maintaining the genetic information and is highly conserved among all kingdoms of life. In one way or the other, the ribosome engages with 2 protein factors and two RNAs during this process. Besides the mRNA as the carrier of the translated information and the corresponding aminoacyl (aa)-tRNAs required for decoding of the mRNA in a codon dependent manner, the rapid and accurate decoding process is facilitated by three additional protein factors, Elongation Factor (EF) Tu, EF-G, and EF-Ts in bacteria (the corresponding eukaryotic factors are eEF1alpha, eEF2 and eEF1beta). EF-Tu is of great interest, as it serves as a gatekeeper of the decoding process. Following correct codon anticodon interaction in the decoding centre of the ribosome, located on the small ribosomal subunit (30S), the GTPase activity of EF-Tu is greatly accelerated by the GTPase activating centre located on large subunit (50S) of the ribosome. For efficient decoding, EF-Tu has to integrate a number of different signals originating from different regions of the ribosome in order to only activate its GTPase activity when the correct codon corresponding to the anti-codon of its bound aa-tRNA is present in the ribosomal decoding site. EF-Tu then has to facilitate rapid GTP hydrolysis followed by the release of the generated inorganic phosphate, triggering a conformational rearmament of the protein that will result in the release and subsequent accommodation of the bound aa-tRNA (2). Interestingly, little is known regarding the processes following this event. EF-Tu is believed to dissociate from the elongating ribosome to enable the encounter of a new ternary complex containing GTP-bound EF-Tu and an aa-tRNA. In order for EF-Tu-GDP to participate again in this cycle, the bound GDP has to be replaced by a new GTP molecule. Although the cellular concentration of GTP is higher than GDP, biochemical studies have revealed that GDP

dissociation is too slow to facility translation rates observed in vivo (3). Both prokaryotes and eukaryotes use an additional elongation factor to facilitate rapid exchange of the bound nucleotide. Interestingly, although EF-Tu and eEF1alpha resemble each other structurally, the respective exchange factors have no structural similarities and even engage differently with the respective EF-Tu/eEF1alpha. Little is known about the exact order of events during the exchange factor stimulated nucleotide exchange reaction and how the overall structure of both factors contributes to the observed rates and the reaction in general. Despite the fact that several X-ray structures of the bacterial EF-Tu, alone, in complex with aa-tRNA, different nucleotides, in complex with the exchange factor, and the ribosome, exist, we do not understand the design principles and their application, giving rise to the enzymatic properties. In particular, how they are fine-tuned within the respective biomolecule to achieve the particular properties is only poorly understood. Ultimately, we are addressing the question of whether general design principles exist. Although the structural information available has shed light on the molecular details of the observed interactions, the dynamic properties and their respective engineering have largely been overlooked. However, the wealth of structural information in conjunction with biochemical data and biophysical techniques available, provide a solid foundation for the investigation of the structural dynamics and the dynamic properties of biomolecular design features with translation factors such as EF-Tu as an excellent example.

4 A combined approach

To further investigate the dynamic properties of biomolecular switches such as EF-Tu, we have begun to use molecular dynamics simulations in conjunction with rapid kinetics approaches to investigate the effect that structural dynamics has on the enzymatic properties with the intention to answer the question if design modules exist that can be used for rational engineering and fine-tuning of enzymatic properties in EF-Tu. We have focused on the modulation of the nucleotide binding properties in EF-Tu as a function of its functional cycle. Our previous

work primarily focusing on the dissection of the nucleotide exchange reaction has allowed us to develop a fluorescence based system that enables the measurement of rates of reactions in real time using the stoppedflow technique. Based on these experiments we have been able to dissect the mechanism of EF-Ts stimulated nucleotide exchange in the common bacteria *Escherichia coli*. (3-5). This approach has also been successful used by others in the yeast *Saccharomyces cerevisiae* (6). In the past, several single amino acid variants of EF-Tu and its exchange factor have been investigated, and the effect of the respective alteration on the nucleotide binding properties and speculations about their mechanistic causes have been reported. However, no detailed and rigorous analyses of the respective structural dynamics have been used. As direct observation of the dynamic proprieties are difficult, the use of computational methods simulating the dynamics of biomolecules based on Newtonian physics (Molecular Dynamics simulations) provides a powerful alternative, easily accessible to molecular systems of less then 1,000,000 atoms such as EF-Tu and its exchange factor in explicit solvent simulations (7). The use of stopped-flow technique to determine the rates of conversion between the different states of EF-Tu during its functional cycle provides easy access to the thermodynamics of the respective conversion, which in turn can be interpreted using the dynamics data derived from the corresponding molecular dynamics (MD) simulations.

5 The nucleotide exchange mechanism in P-Loop NTPases

I. Nucleotide binding in P-loop Nucleoside triphosphate hydrolases (NTPases) is modulated by the structural dynamics of the P-loop (7). Using a combined MD simulations, rapid kinetics and molecular biology approach we were able to demonstrate that the nucleotide binding properties of EF-Tu depend on the structural dynamics of the P-loop, an evolutionary very successful sequence element also know as the Walker A motif (consensus sequence GxxxxGK(S/T)), contributing to

the binding of phosphate containing small ligands such as nucleotides. Our kinetic analysis and MD simulation of EF-Tu variants engineered to contain P-loops that exhibit different structural dynamics revealed that one end of this short loop serves as an anchor, and that disrupting this so called P-loop anchor can be used to modify the nucleotide binding properties. This feature of the P-loop is not limited to EF-Tu, but instead can be found in a wide range of P-loop NTPases, demonstrating the generalizability of this finding. Interestingly, we also were able to demonstrate that interaction with this module is used by a large number of nucleotide exchange factors with a wide range of biological functions and evolutionary backgrounds to accelerate nucleotide dissociation. Furthermore, the use of reaction rate information obtained by rapid kinetics provides access to the thermodynamics of the transitions state of the particular reaction, information that is structurally very difficult to obtain. Using the Eyring equation, we were able to correlate the changes in transition state entropy to the structural dynamics of EF-Tu using so called steered molecular dynamics simulations (7). This approach allowed us not only to characterize the P-loop as a structural element designed for modulating the nucleotide binding properties, but also identified a region in EF-Tu that likely is important for modulating nucleotide binding properties, and which has previously been overlooked as a putative contributor (7).

II. Interestingly, this region comprised of EF-Tus helix E and F exhibits the highest evolutionary speeds observed within EF-Tu (8) and might therefore provide EF-Tu with some flexibility with respect to modulation of nucleotide binding during its functional cycle and among different EF-Tu species. With this in mind, we investigated the role of a non-conserved element of EF-Ts that interacts with this region of EF-Tu using the approach described above. Consistent with our original hypothesis we were able to demonstrate that this region is indeed important for the EF-Ts mediated nucleotide exchange reaction in EF-Tu by modulating the structural dynamics of the nucleotide-binding pocket. Our results also provide critical information regarding the mechanism of nucleotide association to the EF-Tu-EF-Ts binary complex, not accessible to purely structure based

approaches. Although several recent X-ray structures of this complex exist (9) with and without bound nucleotides, the question regarding the order of events leading to binding of a new nucleotide molecule and subsequent dissociation of EF-Ts were still unclear. In particular the question if the incoming nucleotide first interacts with the nucleotide binding pocket via its base (base-side first mechanism) or the phosphates (phosphate-side first mechanism) has been discussed for over 20 years. Our rapid kinetics analysis reveal strong evidence that the nucleotide exchange mechanism applied by EF-Tu proceeds via a base-first entry into the binding pocket which also provides a mechanistic explanation for how nucleotide binding properties can be fine-tuned and how EF-Tu efficiently selects against the other nucleotides present in the cell. (DeLaurentiis, under review).

6 Significance

Our findings not only demonstrate the critical role structural dynamics and its respective modulation play for the function and design of biomolecular machines, but also that structural dynamics modules exist that have the potential to be used for the engineering of specific properties. We contribute to the experimental and theoretical framework required for describing and analyzing the structural dynamics of biomolecular information processing, and provide parameters for the rational design of small molecular modulators of biomolecular function, as well as the design of biomolecular machines in general.

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