A simple model of the electrostatic environment around the catalytic center of the ribosome and its significance for the elongation cycle kinetics

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INTRODUCTION

Ribosome Catalyzes Peptide Bond Formation

- The mechanisms of peptide bond formation at the ***peptidyl transferase center (PTC)*** have been studied for decades [1]
- There is a great ***variation in peptide bond formation rate*** during mRNA translation
- ***The kinetics and the factors*** that ***impact*** on the peptide bond formation rates ***are not fully understood***

Key determinants of Peptide Bond Formation Rate

- ***Nature of the amino acid*** at A-site and ***C-terminal amino acid*** of the nascent chain ***at the P-site***
- ***Charged amino acids*** interaction with ***electric field at the catalytic center*** of the ribosome
- Electrostatic interaction of the nascent chain with the ***ribosome exit tunnel***
- ***Proline*** at the A site and/or the P-site

Approaches of this study

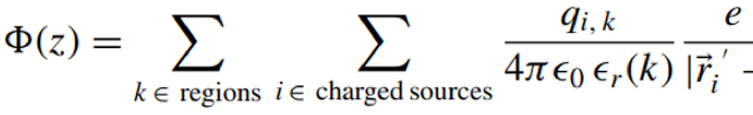
- ***3D-Map*** the charged groups that are close to the PTC from X-Ray crystallography
- ***Relate*** the ***electrostatic potential profile*** around the catalytic center of the ribosome to X-Ray solved structures
- Incorporate ***mechano-chemistry*** into the ***transition state energy barrier*** of the catalytic reaction and the kinetics calculations

MATERIALS & METHODS

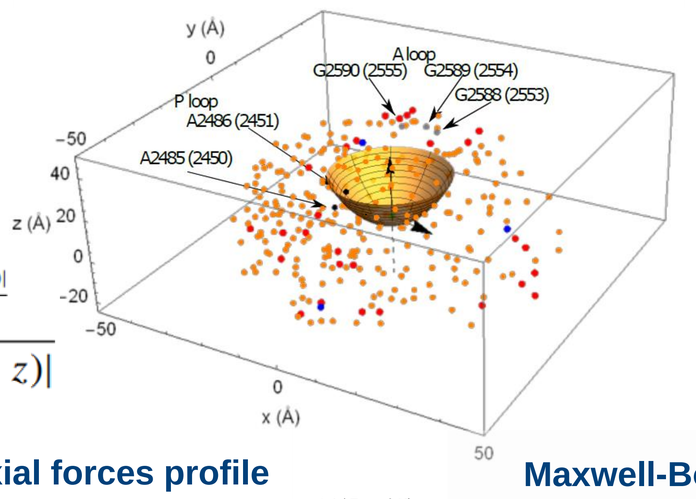
Ribosome large subunit X-Ray crystallography data mining and queueing time theory of the elongation cycle

- ✓ Our approach is to 3D map the charged groups (phosphate moieties of 23S/28S rRNA and charged amino acids from ribosomal proteins)
- ✓ Empirical parameters for dielectric response and screening lengths are estimated [2, 3]
- ✓ The electrostatic potential around the PTC cavity is calculated by the Yukawa-Debye-Hückel theory from the charged sources positions
- ✓ Comparison with previous experimental results of *Wohlgemuth et al.* [4] of elongation rate with the use of puromycin as A-site acceptor
- ✓ The elongation cycle queueing time is analyzed as the convolution product of 3 queueing times of the 3 sequential steps of elongation.

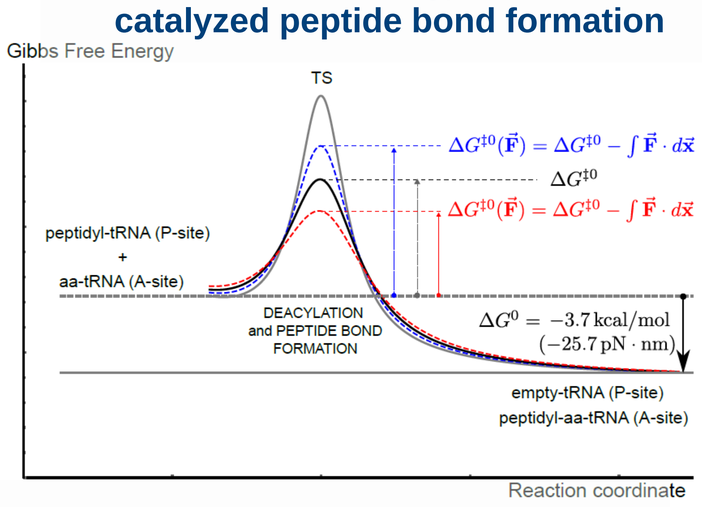
$$\Phi_{Yuk}(\vec{\mathbf{r}}) = \iint_S \frac{\sigma^*(\vec{\mathbf{r}}')\,da}{4\pi\,\epsilon\,\epsilon_0} \cdot \frac{e^{-\frac{|\vec{\mathbf{r}}-\vec{\mathbf{r}}'|}{\xi}}}{|\vec{\mathbf{r}}-\vec{\mathbf{r}}'|}.$$



Mapping the phosphate moieties (orange spheres) and amino acids (red+, blue- spheres) around the PTC

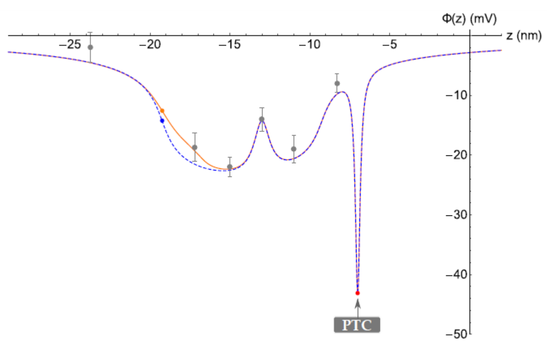


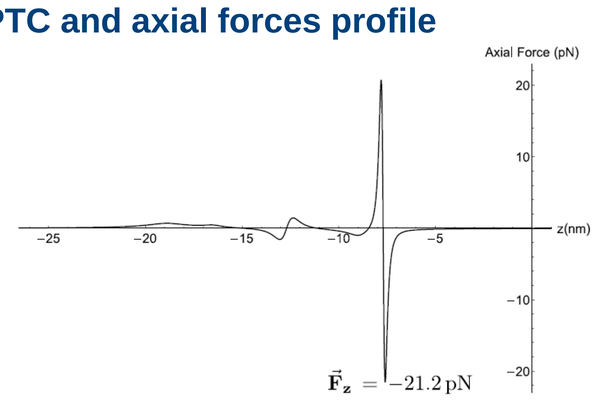
Mechano-chemistry modulates the transition state energy barrier of the catalyzed peptide bond formation



$$\Phi(z) = \sum_{k\,\in\,\text{regions}} \sum_{i\,\in\,\text{charged sources}} \frac{q_{i,k}}{4\pi\,\epsilon_0\,\epsilon_r(k)} \frac{e^{-\frac{|\vec{r}_i{}' - (0,\,0,\,z)|}{\xi_k}}}{|\vec{r}_i{}' - (0,\,0,\,z)|}$$

Electrostatic potential model around the PTC and axial forces profile





Maxwell-Boltzmann factor incorporating mechanical effects into the catalyzed reaction rate constant

$$k(\vec{F}) = k(0) \cdot e^{-\frac{\Delta G^{0\dagger}}{k_B \cdot T}} \cdot e^{+\frac{\int \vec{F} \cdot dx^{\dagger}}{k_B \cdot T}}$$

RESULTS AND PERSPECTIVES

Biophysics and biochemistry

- ✓ The calculated electric field order of magnitude at the catalytic center of the ribosome (PTC) is 143 MV/cm, similar to the one observed in typical protein enzyme-substrate configuration [3, 5]
- ✓ The axial force around the PTC towards the exit tunnel is 21.2 pN for a positive unit test charge [3].
- ✓ The fold change in the peptide bond formation rate between positively and negatively charged amino acid as C-terminal residue at the P-site is consistent with the Maxwell-Boltzmann factor accounting for the mechanical forces acting upon the oligopeptide backbone at the P-site.

Computational Biology Perspectives for Ribo-Seq analysis

We suggest that the deconvolution of ribosome residence time or normalized footprint counts from ***RiboSeq*** data [6] can provide valuable information on the kinetics of the three sequential reaction steps of the elongation cycle.

REFERENCES

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