Modeling and simulation from T7 RNA polymerase to yeast RNA polymerase II transcription

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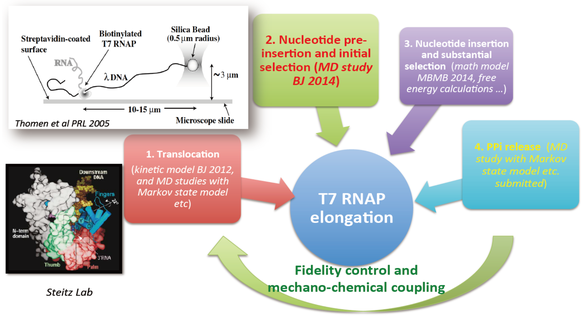
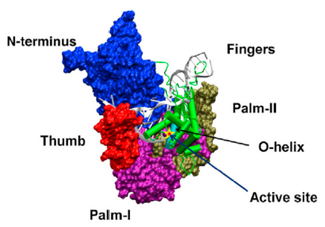
Our computational work on T7 RNAP and Pol II

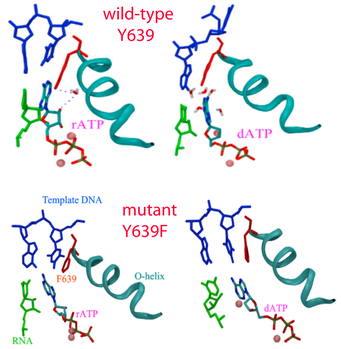
RNA polymerase (RNAP) is the key enzyme that directs gene transcription. We have systematically studied mechanochemical coupling and fidelity control during bacteriaophage T7 RNAP elongation. Based on the single molecule measurements, our kinetic modeling revealed that a small translocation bias of T7 RNAP aids nucleotide selection during its ratcheting. Next we constructed a theoretical framework to analyze how stepwise nucleotide selection proceeds efficiently for fidelity control through multiple kinetic checkpoints in the absence of proofreading. To substantiate the previous ideas and findings, we recently performed atomistic molecular dynamics (MD) simulation of T7 RNAP, and discovered how a critical residue aids the nucleotide selection from the nucleotide pre-insertion to insertion and therefore selectively ratchets the RNAP for the template-based elongation. Furthermore, we constructed the Markov state model for the reaction product PPi release and further translocation of T7 RNAP, implementing a large number of short MD simulations.



Comparatively, we also built a structure-based kinetic model of eukaryotic polymerase II elongation, taking into account transition rates and conformational changes characterized from single molecule experimental studies and atomistic MD simulations. Our model shows that it is an essential conformational change of a trigger loop opening prior to translocation that is slow and force dependent in the elongation. Moreover, our model suggests that better characterization of the NTP binding process is necessary for obtaining more accurate descriptions of the elongation. Overall, the study provides a working model of the polymerase II elongation under a generic Brownian ratchet mechanism.

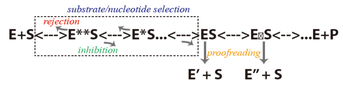
A critical residue Tyr639 selectively ratchets T7 RNAP by selecting against non-cognate nucleotides at pre-inserton [2]





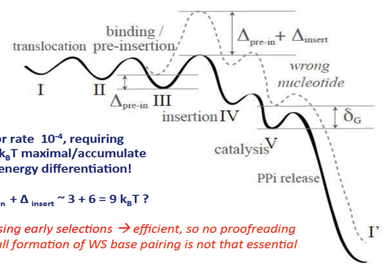
Through atomistic MD simulations, we show that Tyr639 is stabilized at the active site upon non-cognate nucleotide pre-insertion (dATP: Tyr639-end bp stacking strengthens; wrong rNTP: strong Tyr639-rNTP interaction). Thus, the non-cognate NTP is hard to insert into the active site [2].

Efficient fidelity control by stepwise nucleotide selection , and calculations on the T7 RNAP selection free energetics [3]

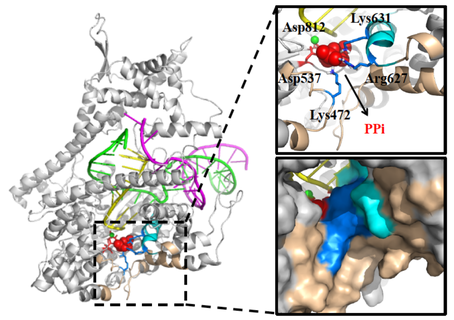


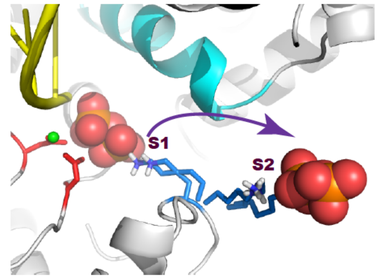
We show that selection without proofreading can also have differentiation free energy accummunated through multiple checkpoints for error reduction [3].

1) Inhibition and rejection of the wrong substrate from a same kinetic state perform equivalently in error reduction;
2) The error reduction performance never improves down the reaction path: the earlier the better !
3) Initial screening is indispensible to maintain speed high

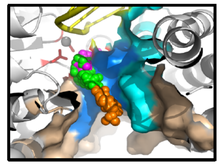


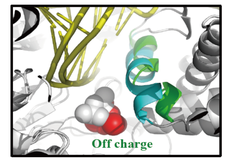
A ump-from-cavity PPi release assisted by Lys472 in T7 RNAP: unlikely to drive translocation; may be a general mechainsm for similar polymerases [4]

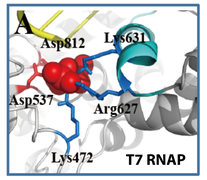


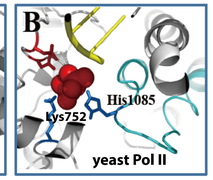


Lys472 swings to assist PPi release

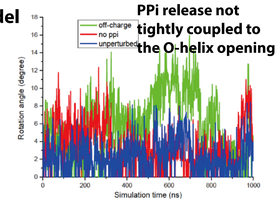




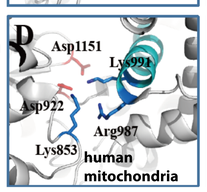


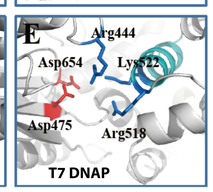


3-state Markov state model

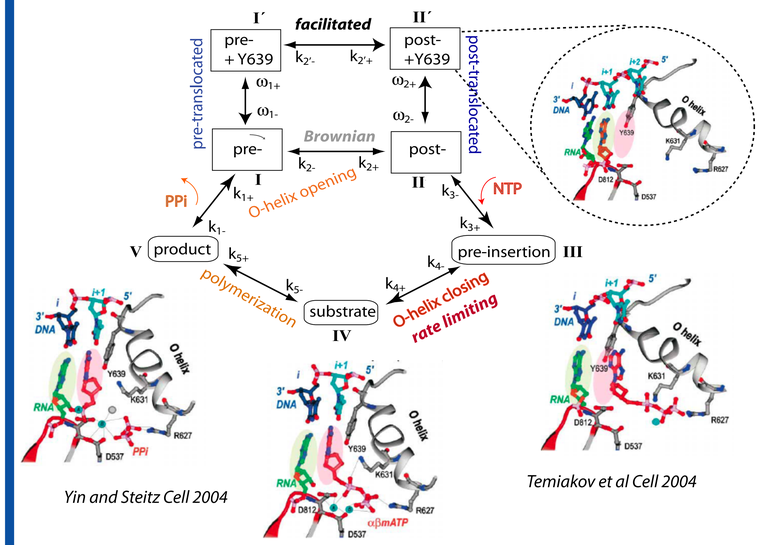


PPi release not tightly coupled to the O-helix opening



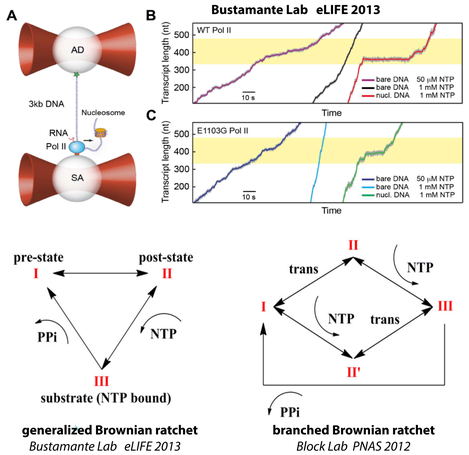


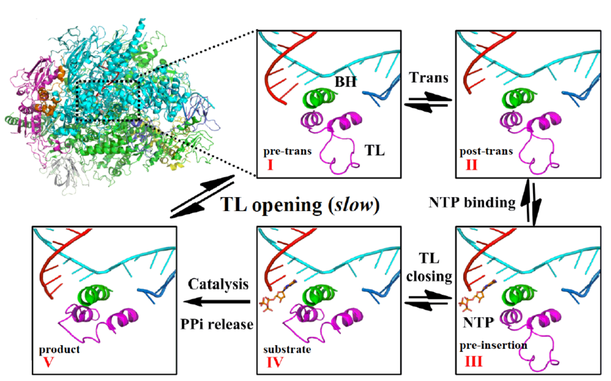
A small post-transloction free energy bias aids nucleotide selection by stabilizing Tyr639 in the active site [1]



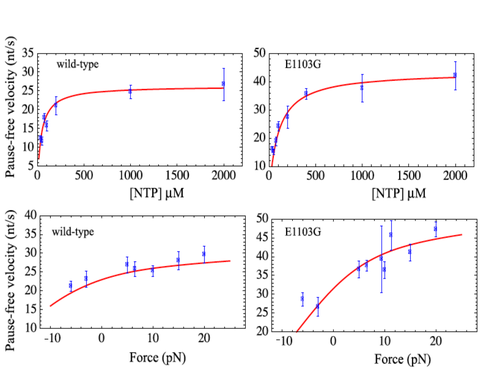
Based on previous single molecule measurements of T7 RNAP elongation, as well as on high-resolution structural studies, we built a kinetic model of T7 RNAP [1], highlighting an interesting property that the post-translocation free energy bias can be manifested through a critical residue Tyr639, stabilized in the active site for further nucleotide selection.

Constructing kinetic models to elucidate structural dynamics of a complete RNA polymerase (Pol) II elongation cycle [5]





Structure-based model of Pol II elongation kinetis [5] non-branched Brownian ratchet model in five states



Fitting of our model with single molecule measurement data from Bustamante Lab and Block Lab

[1] Jin Yu* and George Oster* (2012) A small post-translocation energy bias aids nucleotide selection in T7 RNA polymerase transcription. *Biophysical Journal* 102:532-541 **(NSF grant DMS 1062396)**
[2] Baogen Duan, Shaogui Wu, Lin-Tai Da, and J Yu* (2014) A critical residue selectively recruits nucleotides for T7 RNA polymerase transcription fidelity control. *Biophysical Journal* 107:2130-2140
[3] Jin Yu* (2014) Efficient fidelity control by stepwise nucleotide selection in polymerase elongation. *Molecular Based Mathematical Biology* 2: 141-160
[4] Lin-Tai Da, Chao E, Baogen Duan, Chuanbiao Zhang, Xin Zhou, and Jin Yu* (2015) A jump-from-cavity pyrophosphate ion release assisted by a key lysine residue in T7 RNA polymerase transcription elongation. *PLos Computational Biology* 11(11): e1004624
[5] Jin Yu*, Lin-Tai Da, and Xuhui Huang* (2015) Constructing kinetic models to elucidate structural dynamics of a complete RNA polymerase II elongation cycle. *Physical Biology* 102: 016004

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