Molecular dynamics simulations suggest a structural basis for the slow-onset inhibition of KasA by thiolactomycin and provide insights for TB drug discovery

Brian Miranda

Great Neck South High School, New York

Section I

Before I entered high school, my older brother returned from his lab each day with a story about his work. I could always hear his excitement when he talked about his project. I knew I wanted to try my hand at research but the microarray analysis procedures he described did not appeal to me. Volunteering then at a local hospital, I wanted to pursue a hands-on study of disease, but was unsure how to go about it. When I made the decision in the spring of 2008 to do summer research, I consulted one of my teachers; he recommended Dr. Carlos Simmerling's lab, which specialized in computational structural biology at Stony Brook University. And in my first visit to the Simmerling Lab, I was fascinated by the work of two graduate students who were visualizing proteins folding on the computer. The computer offered such a controlled environment for studying biological systems. Working mostly with software tutorials my first summer there, I also found that any experiment required my input at every step—it was rewarding to have complete control. I soon began my own analysis of an enzyme in the tuberculosis (TB) pathogen. The freedom I had to study the complexities of TB under such controlled conditions inspired me to continue my project for the next three years.

It was during those years that my research mentor taught me the value of being an active research participant. By allowing me to take on a project completely independent

of the lab, he helped foster a growing confidence that empowers me to pursue the unfamiliar and study new fields. Once for several hours, I worked together with him to analyze protein crystal structures on the computer. I was inspired by the patient logic he employed to troubleshoot errors and by the organized thought he used when forming predictions. The opportunity to apply the same tireless inquiry and critical thinking I observed to my own work made my research experience that much more appealing.

***

My project has taught me that science is not simply solving questions for the sake of finding answers, but rather improving our approach to real-life challenges by integrating academic disciplines. I had always viewed my classroom interest in physics as academic, merely an exercise in abstract thinking. However, the direct application of vector physics in generating my simulations, which were guided by Newtonian laws, and in examining my data by a quasi-harmonic analysis, has shown me the value of mathematical thinking in the study of disease. As my study progressed, I began to delve into more complex mathematical subjects, particularly linear algebra. Even though the computer programs I ran took only an hour to spew out mass-weighted covariance matrices that described the motions of over 12,000 atoms in three degrees of freedom (over 36,000 motions!), I spent a good deal of time learning about matrix diagonalization, eigenvectors and eigenvalues, and normal modes.

After my constant efforts to place the design and results of my study in the context of experimental findings, I am interested in working with enzymes in a laboratory. Hours of visualizing the atomic motions of proteins on the computer have motivated me to understand biological systems on a more tangible, macroscopic level.

My research has encouraged me to use collaborative science, like biophysics, in the future to further my study of diseases.

***

I certainly advise all those wishing to pursue research that combines science and mathematics to begin early and importantly, to be proactive about your experimental designs. To ensure that you develop true mastery and ownership of your project, you can do yourself no bigger favor than by starting as early as possible. In doing so, you afford yourself the opportunity to understand your own goals and to develop the focuses of *your own* experiments. Even though you may be working in a laboratory setting under supervision, you should aim to build a strong connection to your work—something that cannot exist without deep immersion in your field of study. For example, before I could even begin my research project, I needed to learn how to use the Linux operating system, master the various programs in the Amber biosimulation suite and have a firm grasp of computational structural biology. If I had only worked for one summer, I would not have had the time to fully complete these prerequisites and perform an in-depth analysis of protein dynamics. And finally, I would be remiss if I neglected the significance of enjoying your work. When embarking on any journey—scientific, mathematical or otherwise—it is necessary to strive for passion, rather than success, for without the first, the second is empty and usually unfeasible.

Section II

Tuberculosis (TB) persists as a global pandemic: over two billion people harbor latent infection and more than nine million new cases are acquired each year. The survival of the TB pathogen, *Mycobacterium tuberculosis*, requires the presence of mycolic acids in the cell wall. Constituting about sixty percent of the cell wall, these mycolic acids protect the bacterium against common antibiotics and other chemical agents. In the absence of mature mycolic acids, mycobacteria have been shown to lyse or die. The bacterial enzymes necessary for mycolic acid production thus become key targets for novel drug discovery efforts.

One such enzyme, KasA extends the carbon chain of the growing mycolic acid through a condensation reaction. When the enzyme catalyzes this reaction, the protein's initial structure (A) changes into an intermediate structure (B) [See Figure 2]. Importantly, the novel drug compound thiolactomycin (TLM) more effectively targets this intermediate structure (B) through a process called **slow-onset inhibition**.

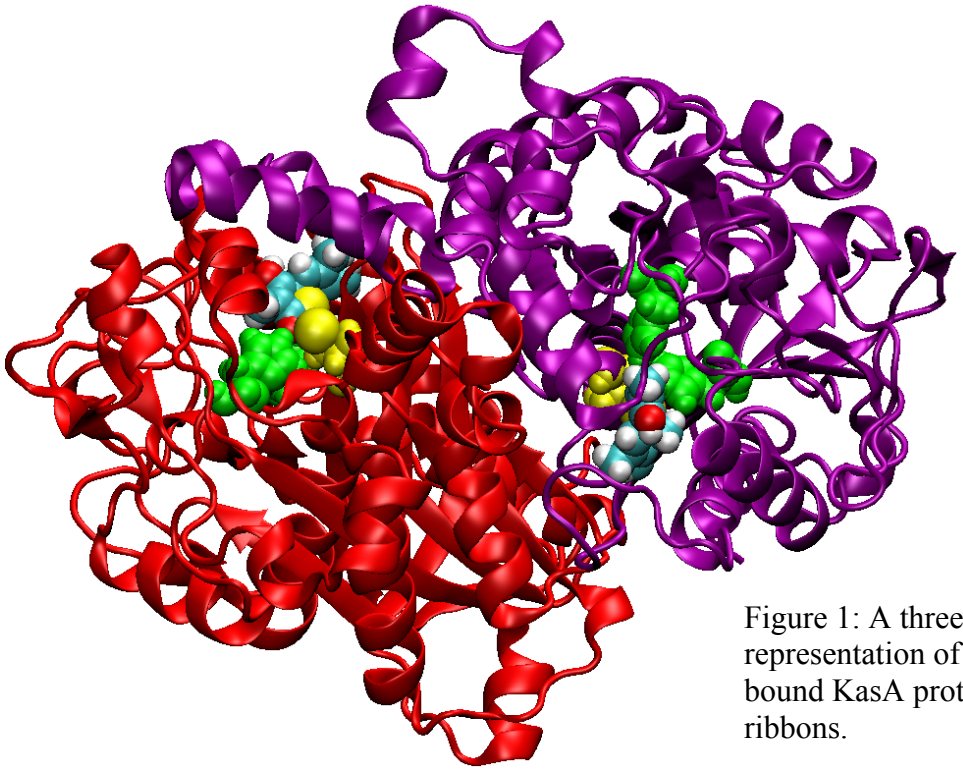

Figure 1: A three-dimensional representation of the TLM-bound KasA protein shown in ribbons.

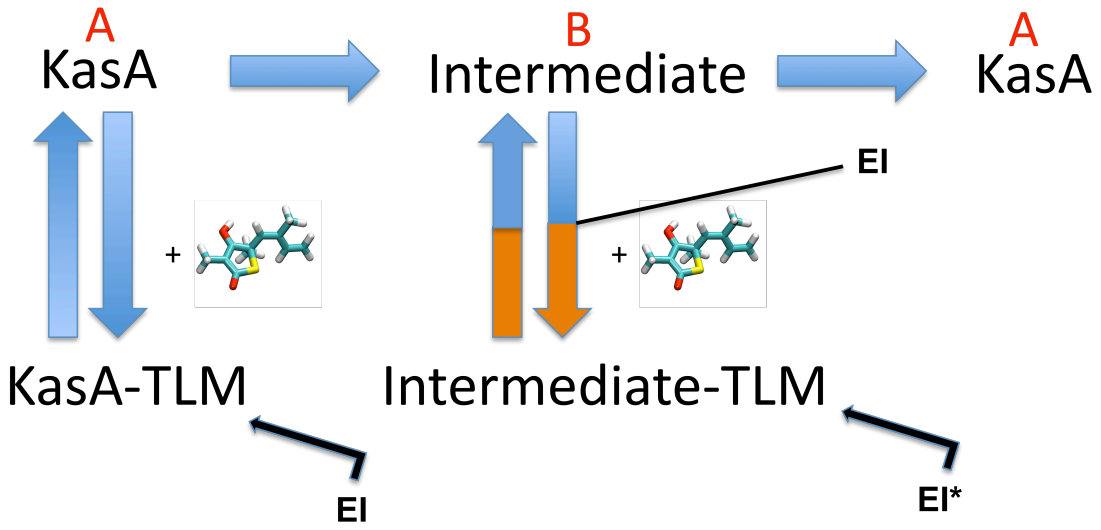

Figure 2: Generalized scheme of the condensation reaction catalyzed by KasA and the two different binding patterns of the initial and intermediate KasA states.

The Slow-Onset Binding Mechanism (Please reference Figure 2 above)

- When TLM binds to KasA, it reversibly forms an enzyme-inhibitor (EI) complex. ("KasA-TLM" on the left-hand side of Figure 2)
- However, when binding to the intermediate structure, TLM shows a different binding pattern (center of Figure 2). Here, the formation of the EI complex is only temporary. (Intersection of blue and orange arrow segments)
- It is followed by a slow binding step (orange) and the formation of a final, more stable EI* complex. ("Intermediate-TLM" in the center of Figure 2)
- **Implications of slow-onset inhibition**: In the field of drug discovery, traditional efforts have aimed to develop inhibitors that fit more precisely within their targets (e.g. the lock-and-key model you may have learned in biology). On the other hand, recent attention is also being given to the amount of time the drug, or inhibitor, resides within its target (i.e. residence time). In other words, an inhibitor with a longer

residence time acts against its protein target for a larger period of time, and shows greater effectiveness. For drug developers, the ability to control the residence times of drugs is the ability to increase the efficacy of drug compounds. As discussed below, **slow-onset inhibition has been shown to increase the residence time of TLM**, and ultimately the drug's success rates.

- The slow binding step in the intermediate structure is presumed to correlate with a slow dissociation step, which does not exist binding interaction for the initial state of KasA. (Orange segment directed upwards toward the unbound "Intermediate" B)
- The EI* complex of intermediate state B has a longer half-life than the EI complex of initial state A. Thus, the drug TLM has greater residence time in the intermediate structure and shows more activity against this target.

Interestingly, experimental studies have not explained why TLM binding interactions are different. These studies, like X-ray crystallography, can only show snapshots in the behaviors of the two proteins, as they exist in different structural conformations. For that reason, molecular dynamics (MD) simulations were employed in my study to model the time-dependent motions of the initial and intermediate states of KasA. Run on supercomputers, MD simulations provide a controlled environment governed by Newtonian laws and model the motions of biological systems over time. In the past, they have aided drug discovery efforts, ranging from HIV/AIDS to cancer and DNA damage.

While I cannot publish the main data of this study, the simulations were designed to reveal any differences in the motions of both protein states (A and B). And overall, they suggest that a region at the protein surface, which has been proposed to allow the entry of

TLM, is very flexible in the initial state (A), but shows less motion in the intermediate state (B). The differential behavior in this region provides an explanation for the different binding interactions in the two states of the protein KasA. This computational model allows for a full examination of the slow-onset binding mechanism and lays the foundation for the synthesis of future slow-onset KasA inhibitors. Ushering in a new generation of protein inhibitors, long residence time drugs will undoubtedly furnish a cost-effective tool for combating deadly diseases, especially the recent worldwide resurgence of TB infection.