Detection of intermediate states of the streptavidin-biotin bond along its energy landscape by atomic force spectroscopy with high temporal resolution

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The streptavidin-biotin intermolecular bond is often used for the immobilization of biomolecules on biosensors because of its extraordinarily low dissociation constant (K_D of 10^{-15} M). However, its binding mechanism is not well understood.

This system is considered the strongest noncovalent interaction occurring in nature, which originates from multiple hydrogen-bond interactions as suggested by the system's chemical structure revealed by its crystallographic images. Therefore, we can expect the presence of several intermediate states along its intermolecular energy potential landscape. Through experimental studies, two energy barriers were revealed along the course of bond breakage. However, results from computer simulation studies suggested the presence of six intermediate states between the system's bound and unbound states. To explain this discrepancy, along with providing experimental information on the simulation results, we performed single-molecule force spectroscopic measurements of the streptavidin-biotin system using atomic force microscopy (AFM). We employed a quasi-static process of slowly loading force onto the bond (loading rate = 20 pN/s) to provide a meaningful description of the system in near equilibrium through thermodynamic assumptions.

Moreover, by utilizing a fast sampling rate for AFM detection (20 μs/data point), several transient states of the system were clearly resolved in our force spectroscopic measurements. These key strategies allow the determination of the states' relative positions and free energy levels along the pulling reaction coordinate for the illustration of an energy landscape of the system.

This is a great leap towards uncovering the binding mechanism of the ligand molecule, biotin to its receptor, streptavidin. Several studies have been focusing on the investigation of the streptavidin-biotin system showing discrepancies in results. The findings presented in our study bridge the gaps, showing that the results of several experimental and simulation works are pieces of information from the overall sophisticated nature of this receptor-ligand system. In the paper of Pincet and Husson, it was suggested that the history of the bond might be the origin of the streptavidin-biotin paradox. This goes in parallel with our findings and could be a consequence of the stochastic behaviour of the system, that is, several intermediate metastable states are present where the system can possibly reside during the recognition process of streptavidin and biotin enhancing the stability of the bond.

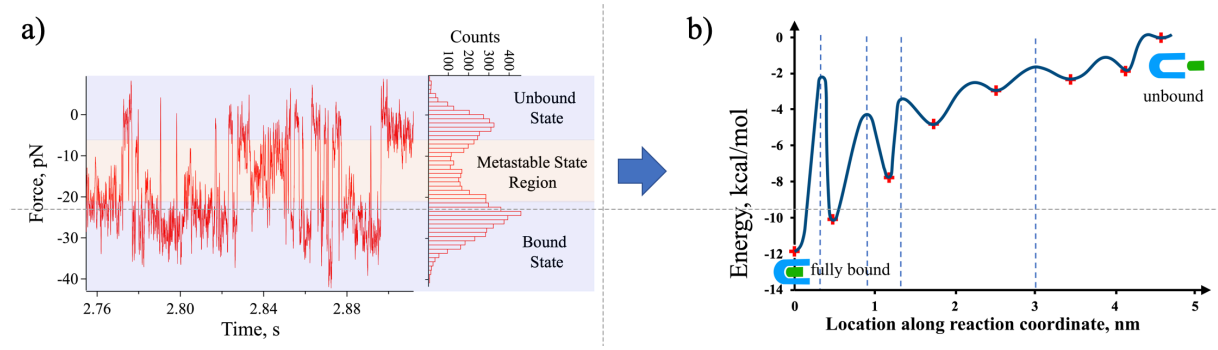

Figure 1. a) Force plotted as a function of time showing streptavidin-biotin bond dynamics in near-equilibrium probed at slow loading rate. Transient events were spotted (green arrows as representatives) suggesting the presence of intermediate metastable states. The histogram displays the distribution of the force values observed in the force-time plot. b) Generated energy landscape of the system.

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