Elucidating Enzymatic Dynamics and Function using Carbon Nanotube Circuits

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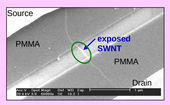
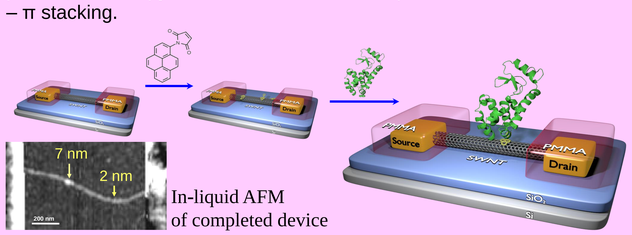
Abstract

By combining molecular biology with the capabilities of solid-state electronics, we devised a new chip-based technique based on single-walled carbon nanotube (SWNT) field effect transistors (FETs) to explore the dynamics and functions of enzymes at the single molecule level. This electronic technique measures the time trajectory of the conformational dynamics of an individual enzyme, revealing its instantaneous dynamic and stochastic behaviors during binding and enzymatic catalysis that are inaccessible using traditional bulk techniques. To show the generality of this technique, we applied it to three enzymes: Lysozyme, DNA Polymerase I, and Protein Kinase A. By investigating the transduction mechanism, we established general design rules to enhance the signal of the protein under observation.

Device Fabrication



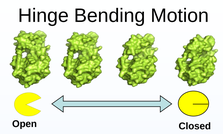
Using standard semiconductor techniques such as chemical vapor deposition and photolithography, we fabricated SWNT FETs. Individual proteins were then attached to the SWNTs using pyrene-maleimide, noncovalent linker molecules. The maleimide group covalently bonds to a cysteine amino acid of the target protein, and the pyrene serves to adhere the protein to the SWNT via π – π stacking.



Lysozyme

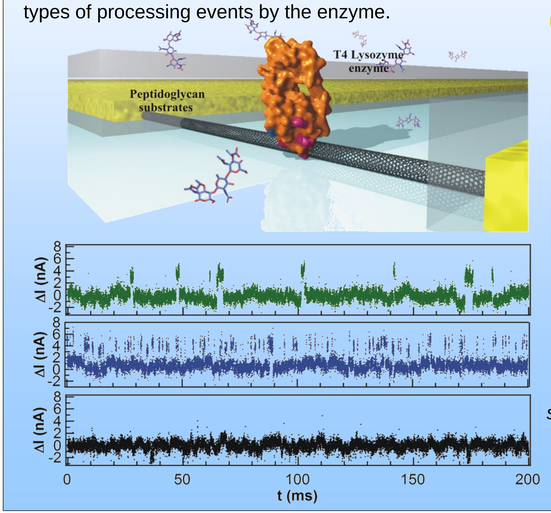
Lysozyme is a critical part of our immunological defenses. Its function is to enzymatically hydrolyze peptidoglycan, a polysaccharide making up the cell wall of pathogenic, gram-negative bacteria. Successful repeated hydrolysis can cut open the cell wall and destroy a bacteria.

The mechanisms behind lysozyme's processivity and dynamics remain poorly understood. One aspect that is well known, though, is that a mechanical, hinge-like motion of the protein is critical to the catalytic process.



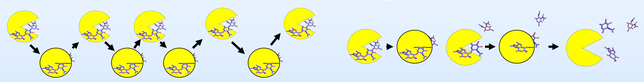
Electronic readout of lysozyme activity

Lysozyme was attached to SWNT FETs and then allowed to freely interact with peptidoglycan in solution. Fluctuations in the electrical conductance reported two types of processing events by the enzyme.



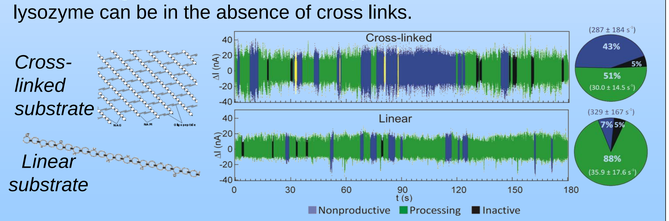
All of lysozyme's activity could be categorized as either a fast rate, non-catalytic binding (blue), or a slower rate, enzymatic turnover (green).

Non-catalytic Binding: k = 330 s^{-1} **Enzymatic Turnover: k = 15 s^{-1}**



Discerning the cause of non-catalytic binding

Using a linear form of peptidoglycan with no cross links and long-duration recordings of single molecule activity, we proved that the fast, non-catalytic activity of lysozyme is associated with transits past those cross links. The graphs below show examples of how much more effective lysozyme can be in the absence of cross links.



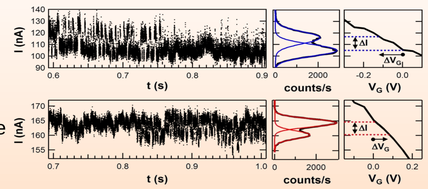
Y. Choi, I. Moody, P. Sims, *et. al. Science* 335, 319 (2012)
Y. Choi, I. Moody, P. Sims, *et. al. JACS* 134, 2032 (2012)

Transduction mechanisms

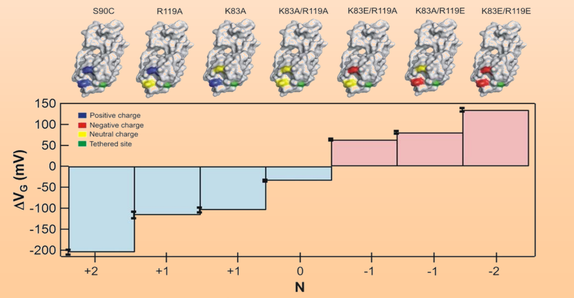
We investigated how the motion of the lysozyme, or any other protein, causes the conductance change in SWNT FETs by synthesizing lysozyme variants with different surface charges. Two charges near the SWNT attachment site (K83 and R119) were selected and mutated. By tailoring these charges from positive to neutral and then negative, we manipulated the signal sign and magnitude, demonstrating that the transduction mechanism is electrostatic gating of the SWNT FET by these two amino acids.

Positive charges produced positive current spikes

Negative charges produced negative current spikes



Summary of Signal Magnitude versus Charge



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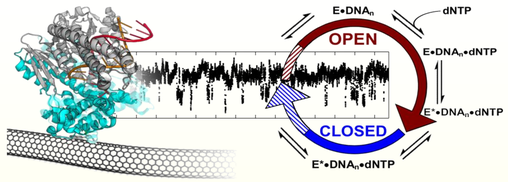
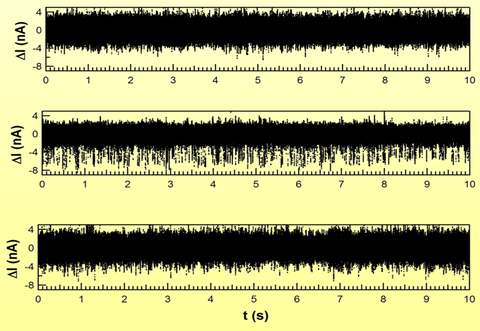
DNA Polymerase I (KF)

The Klenow fragment of DNA polymerase 1 (KF) is a model enzyme for investigating DNA replication. KF consists of a "fingers" domain where nucleotides bind, a "thumb" domain where the DNA template binds, and a "palm" domain where incorporation occurs. Upon binding, the fingers domain closes in order to position the correct, complementary nucleotide for incorporation into the template strand. After extending one nascent pair on the template, KF either proceeds to the next nucleotide on the template or else dissociates from the template entirely.



Electronic readout of KF activity

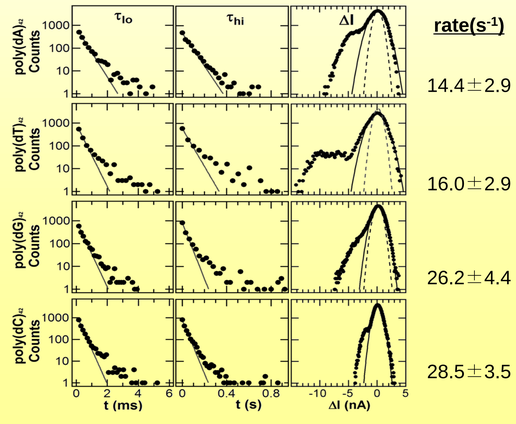
KF was attached to SWNT FETs and then allowed to freely interact with template and nucleotides in solution. Electronic signals directly monitored the open-to-closed and closed-to-open conformational transitions that occurred during processing. Experiments using non-complementary nucleotides confirmed that the electronic signals truly resulted from KF's processive replication activity.



T. Olsen, Y. Choi, P. Sims, *et. al. JACS, in press (2013)*

Nucleotide incorporation rates

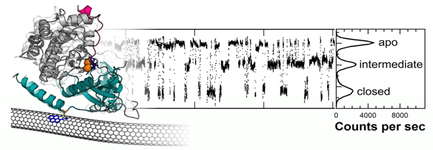
Recording KF for long durations enabled good statistical analysis. The durations spent in the high- and low-current states (τ_{hi} and τ_{low}) characterize KF's activity for each possible pair of template and complementary nucleotide. Probability distributions fit simple Poisson distributions.



rate(s^{-1})
14.4±2.9
16.0±2.9
26.2±4.4
28.5±3.5

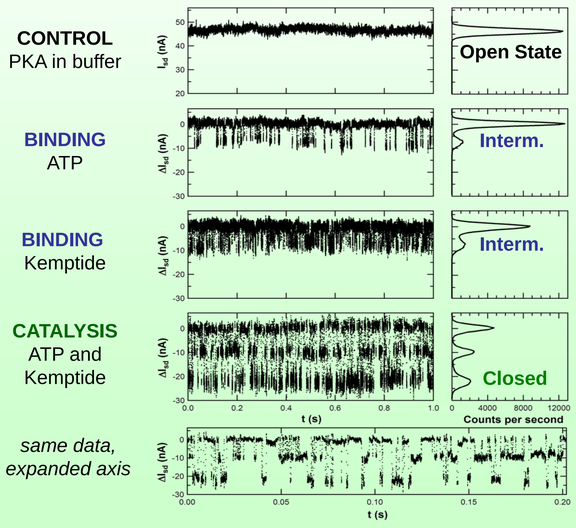
Protein Kinase A (PKA)

PKA regulates cell signaling, transcription, and metabolism by phosphorylating a wide range of other signaling proteins. Successful phosphorylation requires three binding partners: a target protein, Mg^{2+}, and adenosine-5'-triphosphate (ATP). Our experiments investigate phosphorylation kinetics of Kemptide, a synthetic peptide substrate for PKA.



Electronic readout of PKA activity

PKA was attached to SWNT FETs and then monitored as it interacted with ATP and/or Kemptide in solution. Electronic signals reported binding of each to form an intermediate conformation, and then the successful formation of the fully-closed, ternary complex that leads to successful phosphorylation.

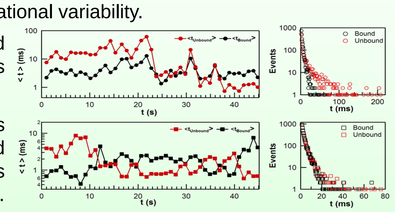


Enzyme variability and dynamic disorder

Dynamic behaviors were observed in long-duration recordings of single-molecule activity. In the presence of just one binding partner, binding and unbinding times were varied widely from moment to moment, presumably due to conformational variability.

ATP binding: mean bound and unbound durations varied 100-fold each minute

Kemptide binding: less variable rates, , but bound and unbound durations were strongly anticorrelated.

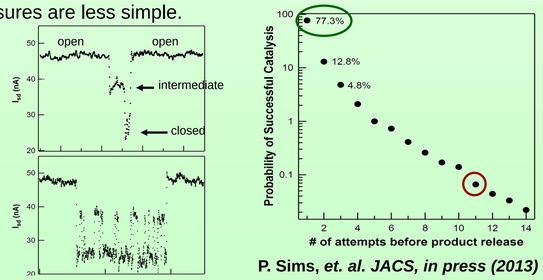


Closure without phosphorylation

In 77% of attempts, PKA motion proceeds along the simplest possible catalytic cycle: open-intermediate-closed-open. However, the remaining 23% of closures are less simple.

Simplest cycle – 1 closure

Inefficient cycle – numerous closures



P. Sims, *et. al. JACS, in press (2013)*