Single Molecule Bioelectronics

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Abstract

Nanoscale electronic devices like field-effect transistors have long promised to provide sensitive, label-free detection of biomolecules. In particular, single-walled carbon nanotubes have the requisite sensitivity to detect single molecule events, and they have sufficient bandwidth to directly monitor single molecule dynamics in real time. Recent measurements have demonstrated this premise by monitoring the dynamic, single-molecule processivity of three different enzymes: lysozyme (Fig. 2) [1, 2], protein Kinase A (Fig. 3) [3], and the Klenow fragment of DNA polymerase I (Fig. 4) [4]. With all three enzymes, single molecules were electronically monitored for 10 or more minutes, allowing us to directly observe rare transitions to chemically inactive and hyperactive conformations. The high bandwidth of the nanotube transistors further allow every individual chemical event to be clearly resolved, providing excellent statistics from tens of thousands of turnovers by a single enzyme. Besides establishing values for processivity and turnover rates, the measurements revealed variability, dynamic disorder, and the existence of intermediate states. Initial success with three different enzymes indicates the generality and attractiveness of the nanotube devices as a new tool to complement other single molecule techniques. Furthermore, our focused research on transduction mechanisms provides the design rules necessary to further generalize this architecture [5]. This presentation will summarize these rules, and demonstrate how the purposeful incorporation of just one amino acid is sufficient to fabricate effective, single molecule nanocircuits from a wide range of enzymes or proteins (Fig. 1).

References

[1] Y. Choi, I. Moody, P. Sims, S. Hunt, B. Corso, G. Weiss, P. Collins, Science **335** (2012) 319.
[2] Y. Choi, I. Moody, P. Sims, S. Hunt, B. Corso, D. Seitz, L. Blaszcazk, P. Collins, G. Weiss, J. Am. Chem. Soc. **134** (2012) 2032.
[3] P. Sims, I. Moody, Y. Choi, C. Dong, M. Iftikhar, B. Corso, O. Gul, P. Collins, G. Weiss, J. Am. Chem. Soc. **135** (2013) 7861.
[4] T. Olsen, Y. Choi, P. Sims, O. Gul, B. Corso, C. Dong, W. Brown, P. Collins, G. Weiss, J. Am. Chem. Soc. **135** (2013) 7855
[5] Y. Choi, T. Olsen, P. Sims, I. Moody, B. Corso, M. Dang, G. Weiss, P. Collins, Nano Lett. **13** (2013) 625.

Figures

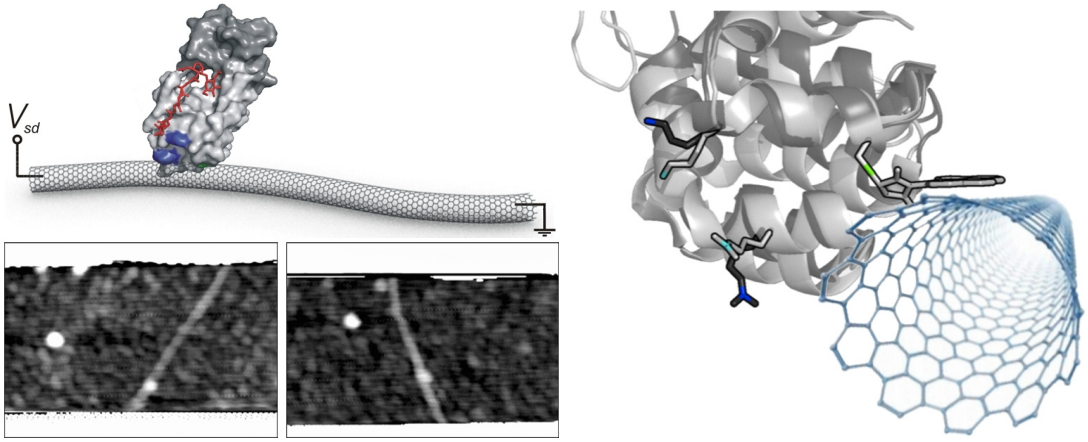

Fig 1. Single biomolecule nanocircuits. *(left) Schematic representation of a device, showing the relative size of a carbon nanotube to lysozyme. Lysozyme's two active domains (light and dark grey) move with respect to each other when processing substrate (red). Example AFM images show carbon nanotube transistors labeled with single T4 lysozyme molecules. (right) Detail of a pyrene-maleimide linker molecule attaching a protein to the nanotube sidewall. A cysteine (C90) has been introduced to a cysteine-free variant of T4 lysozyme to provide a single and reproducible attachment site. Charged amino acids K83 and R119 are highlighted because they move substantially during catalytic processing and are believed to play important roles affecting the SWNT device.*

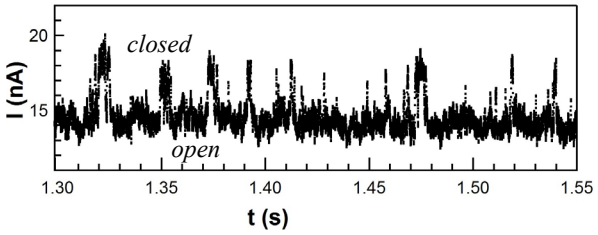

Fig. 2. Lysozyme activity. *Electronic signals transduced by a single T4 lysozyme molecule during processing of its substrate, peptidoglycan. The device current fluctuates between two levels in sync with the enzyme domains opening and closing on substrate, producing a real-time electrical recording of the enzyme's activity.*

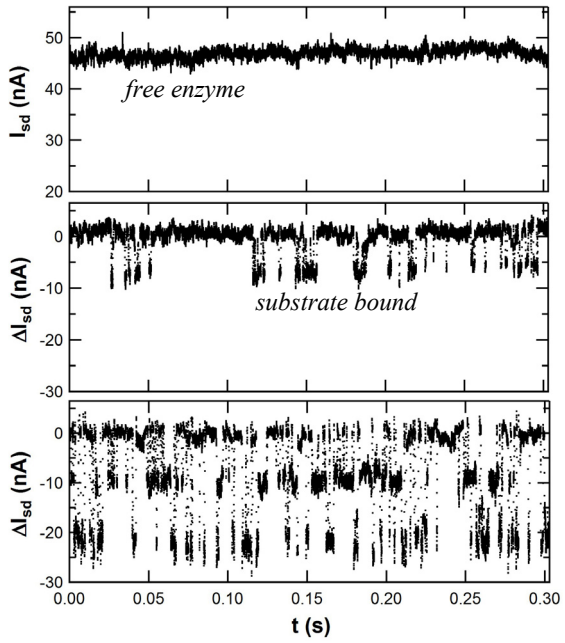

Fig. 3. Protein Kinase A activity. *Electronic signals transduced by a single PKA molecule without substrate (top), with Kemptide substrate (middle), and with both substrate and ATP present (bottom). The current difference ΔI in the bottom two panels is relative to the free enzyme level of the top panel. When both substrate and ATP are present, two independent binding events produce a three-molecule complex that allows for substrate phosphorylation. The electronic signal easily distinguishes the intermediate state with substrate bound from the fully closed state when phosphorylation can occur.*

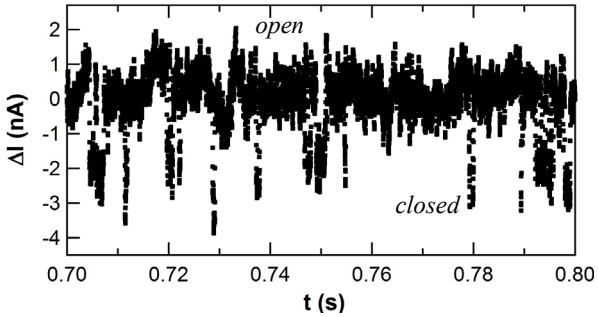

Fig. 4. Klenow fragment activity. *Electronic signals transduced by a single molecule of the Klenow fragment of DNA polymerase I. When measured in the presence of homopolymeric, single-stranded DNA (poly(dT)$_{42}$) and complementary dATP nucleotides, brief current excursions below the baseline ΔI=0 correspond to single nucleotide incorporation events.*