Molecular Recognition Using Nanotube-adsorbed Polymer Complexes

By Jingqing Zhang

Molecular recognition is central to the design of therapeutics, chemical catalysis and sensor platforms, with the most common mechanisms involving biological structures such as antibodies and aptamers. The key to this molecular recognition is a folded and constrained heteropolymer pinned, via intra-molecular forces, into a unique three-dimensional orientation that creates a binding pocket or interface to recognize a specific molecule. An alternate approach to constraining a polymer in three-dimensional space involves adsorbing it onto a cylindrical nanotube surface. To date, however, the molecular recognition potential of these structured, nanotube-associated complexes has been unexplored. In this work, we demonstrate three distinct examples in which synthetic polymers create unique and highly selective molecular recognition sites once adsorbed onto a single-walled carbon nanotube (SWCNT) surface. The phenomenon is shown to be generic, with new recognition complexes demonstrated for riboflavin, L-thyroxine, and estradiol, predicted using a 2D thermodynamic model of surface interactions. The dissociation constants are continuously tunable by perturbing the chemical structure of the heteropolymer. The complexes can be used as new types of sensors based on modulation of SWCNT photoemission, as demonstrated using a complex for real time spatio-temporal detection of riboflavin in murine macrophages.

We specifically investigated the selective detection of single nitric oxide (NO) molecules using a specific DNA sequence of d(AT)$_{15}$ oligonucleotides, adsorbed to an array of near infrared fluorescent semiconducting single-walled carbon nanotubes (AT$_{15}$-SWCNT). While SWCNT suspended with eight other variant DNA sequences show fluorescence quenching or enhancement from analytes such as dopamine, NADH, L-ascorbic acid, and riboflavin, d(AT)$_{15}$ imparts SWCNT with a distinct selectivity toward NO. A stepwise fluorescence decrease is observed when the nanotubes are exposed to NO, reporting the dynamics of single-molecule NO adsorption via SWCNT exciton quenching. We describe these quenching traces using a birth-and-death Markov model, and the maximum likelihood estimator of adsorption and desorption rates of NO is derived. Applying the method to simulated traces indicates that the resulting error in estimation is less than 5% under our experimental conditions, allowing for calibration using a series of NO concentrations. As expected, the adsorption rate is found to be linearly proportional to NO concentration, and the intrinsic single-SWCNT-site NO adsorption rate constant is 0.001 s$^{-1}$ µM NO$^{-1}$. The ability to detect nitric oxide quantitatively at the single-molecule level may find applications in new cellular assays for the study of nitric oxide carcinogenesis and chemical signaling, as well as medical diagnostics for inflammation.

Lastly, we explored engineering nanotube sensors for specific protein biomarker detection. Cardiac biomarker troponin I and T have been universally recognized as standard indicators for acute myocardial infarction (AMI), and many diagnostic companies provide assays or point-of-care (POC) devices that are able to detect troponin. However, in-house assays rely on relatively complicated immunoassays, which can take more than 2 hours to finish. POC devices, on the other hand, through integrating in micro-fluidic techniques, are able to significantly shorten the time for diagnostics, but are very expensive. In addition, those devices require a trained and certified professional on-site to take the measurement, significantly limiting the access of the device from many patients. There is an increasing need in developing novel troponin assays that are simple, rapid and sensitive, for POC AMI detections. In this work, we have demonstrated a rapid, quantitative, and label-free assay specific for cardiac troponin T detection, using fluorescent SWCNTs embedded in a chitosan-based hydrogel, with a detection limit of 2.5 nM.

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