Label-free Carbon Nanotube Sensors for Glycan and Protein Detection

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Nanoengineered glycan sensors may help realize the long-held goal of accurate and rapid glycoprotein profiling without labeling or glycan liberation steps. Current methods of profiling oligosaccharides displayed on protein surfaces, such as liquid chromatography, mass spectrometry, capillary electrophoresis, and microarray methods, are limited by sample pretreatment and quantitative accuracy. Microarrayed platforms can be improved with methods that better estimate kinetic parameters rather than simply reporting relative binding information. These quantitative glycan sensors are enabled by an emerging class of nanoengineered materials that differ in their mode of signal transduction from traditional methods. Platforms that respond to mass changes include a quartz crystal microbalance and cantilever sensors. Electronic response can be detected from electrochemical, field effect transistor, and pore impedance sensors. Optical methods include fluorescent frontal affinity chromatography, surface plasmon resonance methods, and fluorescent single walled carbon nanotubes (SWNT). Advantages of carbon nanotube sensors include their sensitivity and ability to multiplex. The focus of this work has been to develop carbon nanotube based sensors for glycans and proteins. Before detailing the development of these new sensors, the thesis will begin with a very brief primer on glycobiology, its connection to medicine, and the advantages and limitations of existing tools for glycan analysis. In the second chapter we model the use of quantitative nanosensors in a weak affinity dynamic microarray (WADM) to simulate practical uses of these sensors in bioprocessing and clinical diagnostics.

There is significant interest in developing new detection platforms for characterizing glycosylated proteins, despite the lack of easily synthesized model glycans or high affinity receptors for this analytical problem. In the third chapter we experimentally demonstrate ‘proof of concept’ of carbon nanotube-based glycan sensors. This is done with a sensor array employing recombinant lectins as glycan recognition sites tethered via Histidine tags to Ni\(^{2+}\) complexes that act as fluorescent quenchers for SWNT embedded in a chitosan hydrogel spot to measure binding kinetics of model glycans. We examine as model glycans both free and streptavidin-tethered biotinylated monosaccharides. Two higher-affined glycan-lectin pairs are explored: fucose (Fuc) to PA-III and N-acetylglucosamine (GlcNAc) to GafD. The dissociation constants (K\(_d\)) for these pairs as free glycans (106 and 19 \(\mu\)M respectively) and streptavidin-tethered (142 and 50 \(\mu\)M respectively) were found. The absolute detection limit for the first-generation platform was found to be 2 \(\mu\)g of glycosylated protein or 100 ng of free glycan to 20 \(\mu\)g of lectin. Glycan detection (GlcNAc-streptavidin at 10 \(\mu\)M) is demonstrated at the single nanotube level as well by monitoring the fluorescence from individual SWNT sensors tethered to GafD lectin. Over a population of 1000 nanotubes, 289 of the SWNT sensors had signals strong enough to yield kinetic information (K\(_d\) of 250 ± 10 \(\mu\)M). We are also able to identify the locations of “strong-transducers” on the basis of dissociation constant (4 sensors with K\(_d\) < 10 \(\mu\)M) or overall signal modulation (8 sensors with > 5% quench response). We report the key finding that the brightest SWNT are not the best transducers of glycan binding. SWNT ranging in intensity between 50 and 75% of the maximum show the greatest response. The ability to pinpoint strong-binding, single sensors is promising to build a nanoarray of glycan-lectin transducers as a high throughput method to profile glycans without protein labeling or glycan liberation pretreatment steps.
In the fourth chapter we move from detection of model glycoproteins (streptavidin with biotinylated glycans) to a more applied problem: detection of antibodies and their glycosylation. We do this with a second generation array of SWNT nanosensors in an array format. It is widely recognized that an array of addressable sensors can be multiplexed for the label-free detection of a library of analytes. However, such arrays have useful properties that emerge from the ensemble, even when monofunctionalized. As examples, we show that an array of nanosensors can estimate the mean and variance of the observed dissociation constant (K_D), using three different examples of binding IgG with Protein-A as the recognition site, including polyclonal human IgG (K_D = 19 μM, σ^2 = 1000 mM^2), murine IgG (K_D = 4.3 nM, σ^2 = 3 μM^2), and human IgG from CHO cells (K_D = 2.5 nM, σ^2 = 0.01 μM^2). Second, we show that an array of nanosensors can uniquely monitor weakly-affinned analyte interactions via the increased number of observed interactions. One application involves monitoring the metabolically-induced hypermannosylation of human IgG from CHO using PSA-lectin conjugated sensor arrays where temporal glycosylation patterns are measured and compared. Finally, the array of sensors can also spatially map the local production of an analyte from cellular biosynthesis. As an example we rank productivity of IgG-producing HEK colonies cultured directly on the array of nanosensors itself.

One great limitation to these practical applications, common to other new sensor developments, are the constraints of large, bulky, and capital-intensive excitation sources, optics, and detectors. In the fifth chapter we detail the design of a lightweight, field-portable detection platform for SWNT based sensors using stock parts with a total cost below $3000. The portable detector is demonstrated with antibody detection in our lab and onsite at a commercial facility 3700 miles away with complex production samples.

Along the course of developing these sensors, there was a need to analyze noisy data sets from signal nanotubes (Chapter 3) to determine distinct binding states. NoRSE was developed to analyze high-frequency data sets collected from multi-state, dynamic experiments, such as molecular adsorption and desorption onto carbon nanotubes. As technology improves sampling frequency, these stochastic data sets become increasingly large with faster dynamic events. More efficient algorithms are needed to accurately locate the unique states in each time trace. NoRSE adapts and optimizes a previously published noise reduction algorithm (Chung et al., 1991) and uses a custom peak flagging routine to rapidly identify unique event states. The algorithm is explained using experimental data from our lab and its fitting accuracy and efficiency are then shown with a generalized model of stochastic data sets. The algorithm is compared to another recently published state finding algorithm and is found to be 27 times faster and more accurate over 55% of the generalized experimental space. This work is detailed in Chapter 6.

Future uses of these sensors include in vivo reporters of protein biomarkers. In Chapter 7, three-dimensional tracking of single walled carbon nanotubes (SWNT) with an orbital tracking microscope is demonstrated for this purpose. We determine the viscosity regime (above 250 cP) at which the rotational diffusion coefficient can be used for length estimation. We also demonstrate SWNT tracking within live HeLa cells and use these findings to spatially map corral volumes (0.27-1.32 μm^3), determine an active transport velocity (455 nm/s), and calculate local viscosities (54-179 cP) within the cell. With respect to the future use of SWNTs as sensors in living cells, we conclude that the sensor must change the fluorescence signal by at least 4-13% to allow separation of the sensor signal from fluctuations due to rotation of the SWNT when measuring with a time resolution of 32 ms.

In the final chapter we draw conclusions from the development of this carbon nanotube-based sensor for glycan analysis and show the start of future work with arrays of SWNT sensors for glycoprofiling.

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