High-Throughput Engineering of DNA-wrapped Single-Walled Carbon Nanotube Sensors for Single-Molecule Optical Detection of Nitric Oxide

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1 State of the art

As of now, numerous researchers are dedicated to achieving high-resolution, fast and precise detection of biological analytes linked to disease and infection. Enhanced biosensors could give rise to early diagnosis, broaden intervention possibilities as well as enhance the effectiveness of therapeutics. Amongst the various types of biosensors, single-walled carbon nanotubes (SWCNTs) stand out for their nanoscale resolution, single molecule sensitivity and ability to be used reversibly over extended periods. Although their use has been proven in rodents, they remain currently at the research stage for larger animal models [1]. We here plan to draw on strategies developed for protein engineering to optimally design DNA-SWCNTs in a more systematic, neat and directed fashion.

2 Aims

SWCNTs have proven to be promising optical biosensors owing to their intriguing structural and functional material properties. These complexes emit sensitive and photostable near-infrared fluorescence. This, hence, allows the in-vivo and continuous analysis of molecules while minimizing interference from biological tissues [2].

Non-covalently functionalizing SWCNTs with oligonucleotides (DNA-SWCNTs) determines the former's fluorescence response and interfacial interactions. They furthermore grant them biocompatibility, enhanced colloidal stability as well as the ability to sense a plethora of analytes [2–4].

Nonetheless, the dearth of understanding of how the adsorbate's structure influences said biosensors' fluorescent properties, hinders the predictive design of sequences tailored to target specific analytes [5]. This, therefore, constitutes a bottleneck in developing analyte-specific sensors.

Thus, my research aims to optimally design an optical bio-compatible sensor to spatio-temporally detect nitric oxide and quantify its concentrations within bovine joints. This will be performed by monitoring the fluorescence quench of SCWNTs wrapped with specific single-stranded DNA chains.

I am thrilled to tackle this research subject given my keen interest in the Health Sciences field and the pivotal role new technologies may play in it. The development of these biosensors could do just that; by enabling the single-molecule detection of such a prominent post-surgery inflammatory agent.

3 Methods

The general approach is to start from a DNA-SWCNT complex displaying a weak optical signal. We then make use of a protein engineering process, called directed evolution and winner of the 2018 Nobel Prize in Chemistry, so as to enhance its optical behavior [6]. The former involves three key steps:

1. Random mutation of the DNA sequence
2. Wrapping the SWCNTs with the mutated DNA and screening the resulting complex's fluorescence
3. Selecting the DNA-SWCNT complexes showing an improved optical signal

After iterating such evolutionary cycles, we should successfully evolve towards nanosensors that exhibit significantly improved optical properties while maintaining their analyte's selective detection.

This study focuses on investigating the fluorescent emissions of (7,6) and (9,4) SWCNT chiralities as well as their interactions with 44 DNA sequences comprising 30 oligonucleotides. Using machine learning methods, 1 million DNA sequences are firstly randomly generated in a library and divided into 100 classes based on multiple sequence alignment. The goal is then to identify which DNA sequences exhibit fluorescence quenching or enhancement upon interaction with the SWCNTs.

This research project particularly aims to spatially image the adsorbed nitric oxide by monitoring SWCNT exciton quenching [5–7]. We eventually aim to establish equations that translate the latter step-wise decrease into accurate measurements of NO concentration.

4 Impact and further interdisciplinary opportunities

The importance of this research project is best illustrated by the fact that nitric oxide, a free radical present in biological systems, intervenes in various physiological processes and pathologies. Its short half-life renders its detection and quantification difficult [2, 8]. The latter hurdles have hindered researchers' understanding of its impact in both healthy and diseased scenarios.

This research aspires to advance the field of optical biosensing and open doors for novel applications in detecting specific molecules in vivo and at the single-molecule level. The latter has not been allowed by other assay techniques. These sensors' nano-size and acute reactivity could indeed allow tracing extracellular products to a resolution exceeding that of contemporary instruments [9, 10].

These advances may eventually have practical applications in clinical diagnostics for nitric oxide inflammation and carcinogenesis [2]; hence trailblazing promising avenues for improved disease detection and public healthcare.

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