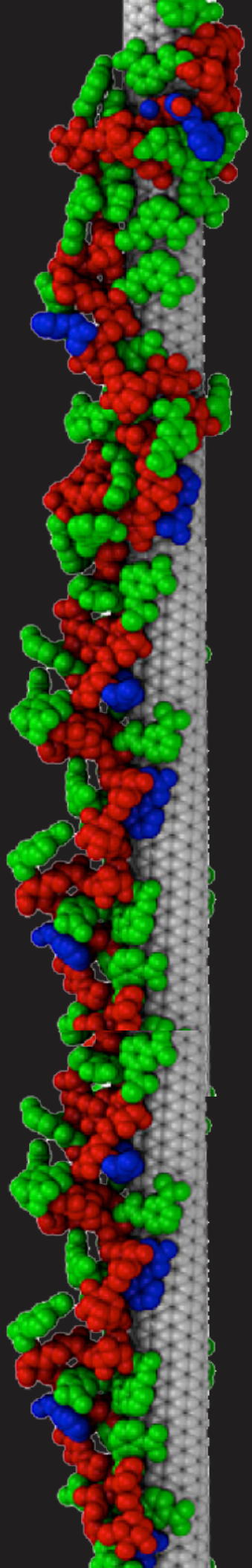

NEUROTRANSMITTER IMAGING AND PLANT NANOBIONICS

Interview with Professor Markita Landry

BY CATRIN BAILEY, ISABEL CRAIG, NIKHIL CHARI, YANA PETRI, ELENA SLOBODYANYUK

Dr. Markita Landry is an Assistant Professor of Chemical and Biomedical Engineering at the University of California, Berkeley. Professor Landry's laboratory focuses on understanding and exploiting optical nanomaterials to access information about biological systems. In this interview, we discuss semiconducting single-walled carbon nanotubes (SWNTs) and their applications in the detection of dopamine in the brain and biological cargo delivery to plant systems.

Professor Markita Landry
[Source: UC Berkeley College of Chemis-

BSJ: How did you first get involved in the field of Chemical and Biomedical Engineering?

ML: I trained in Physics for my undergraduate degree and Ph.D. The focus of my Ph.D. work was to study molecular interactions. To do so, our lab developed high spatial and temporal resolution instruments, which were well-suited for the systems that we were studying. When I graduated, I felt that these instruments were more broadly applicable and wanted to translate their use into nanotechnology. For my postdoc, I planned to come back to physics and then apply nanotechnology tools, but biophysics tools ended up being really useful for nanotechnology. That's how I was introduced to Chemical and Biomolecular Engineering: by building biophysics tools in engineering space. That's how I ended up here as well.

BSJ: What has made you so interested in optical nanomaterials and nano-sensor design?

ML: There is a lot of opportunity in developing nanosensors, especially for molecules that are otherwise very difficult to access information from. For example, when we diagnose something like cancer, we use quantitative methods: typically, a blood screen for biomarkers and then an assay that shows how many cytokines are in the blood. For behavioral disorders like psychosis and depression, we have only very qualitative methods. That's where my interests are: in the more challenging areas to develop sensors for. I'm trying to make diagnosis

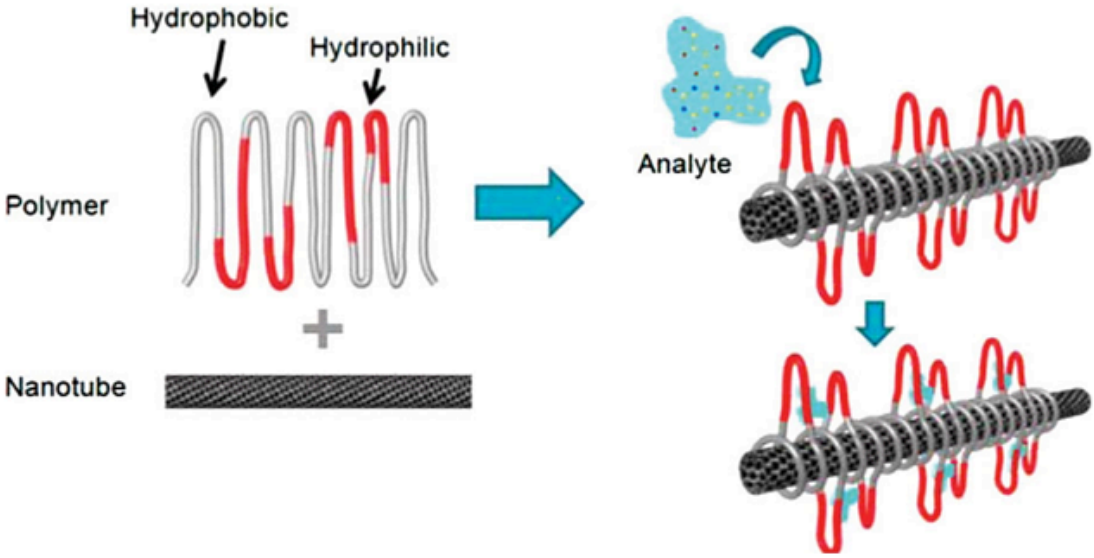

Figure 1. Polymers with hydrophobic and hydrophilic segments are pinned to the surface of a SWNT. The polymer-SWNT conjugate is able to detect a molecular analyte such as dopamine by selectively enabling the analyte to access the SWNT[1].

more quantitative by developing sensors for modulatory neurotransmitters, which govern behavior and disease.

BSJ: Semiconducting single-walled carbon nanotubes (SWNTs) have been used in your laboratory for a variety of applications. Neurotransmitter detection[1,2], recognition of riboflavin[2], and sensing of nitric oxide[3] are only a few examples. What challenges are associated with traditional methods of single-molecule detection?

ML: One of the main challenges is in the photostability of traditional probes. If we consider organic fluorophores, green fluorescent proteins (GFPs), or even quantum dots, the fluorescence of these materials can deteriorate over time. For single fluorophores, it can be as short as a few seconds. For quantum dots, we can get out to tens of minutes. SWNTs don't photobleach. If we are looking to study something like behavior, we want an experimental time window that's much more than a few seconds. What we aim to do is study behavior over the course of multiple days. The physics behind why SWNTs don't photobleach goes back to the unique way they provide infrared (IR) fluorophores of light that we use for imaging. It's really unique to SWNTs and that's why we chose them for these sensors.

"Non-photobleaching fluorescence [of SWNTs] can be modulated selectively by the presence of molecular analytes"

BSJ: What exactly are SWNTs and which properties make them so suitable for selective recognition of a broad range of molecules?

ML: They are, conceptually, sheets of graphene that are rolled up. They are very high aspect-ratio nanomaterials, which means that they are about 1 nm wide and several hundreds long. They are very non-biological in their structure and in their shape. That makes them easy to interface with biological systems because they are relatively small and can be inserted into the extracellular space of the brain or into the extracellular space of plant tissues fairly noninvasively. What makes them well-suited for biological imaging and molecular recognition is that the non-photobleaching fluorescence emission can be modulated selectively by the presence of molecular analytes. By performing some chemistry on the surface of the carbon nanotube, we can make it selective for molecular analytes that will change the fluorescence intensity only when that analyte is present.

BSJ: Why is detecting a fluorescent signal in the IR region particularly advantageous?

ML: Photons that are emitted in the visible wavelength range are scattered by biological tissues; it's the reason that we can't see through hands, skin, and bone. And when we try to do microscopy, especially high-resolution single molecule or single cell microscopy, any photons that are emitted by fluorophores or probes in the visible wavelengths will be scattered by tissues, blood, and bone. And on the opposite side of the spectrum, water starts absorbing photons past 1800 to 1900 nanometers. So between these two scattering and absorption regimes, we have this really nice dip, at around 1,000 nm, where photons can go through water without being

absorbed and through bones without being scattered. SWNTs emit in this nice wavelength range that we can use to minimize interference with biological samples so that we can insert these probes deep into tissues and perform imaging studies without, for example, having to open the skull.

BSJ: What guides your selection of nanoparticle-adsorbed organic phases for SWNT libraries?

ML: We started with a fundamental proof-of-principle assay. We wanted to see if we could replicate the mechanism by which proteins have evolved to recognize antibodies. A protein is just a chain of amino acids, and it's really not functional until it folds and adopts a nice globular 3D form that can then do biocatalysis or molecular recognition. Much in the same way, these polymers, in their 1D sequence or the way that they're synthesized, don't have any affinity for any analyte, but it's only once they fold onto the nanotube structure that they adopt a globular conformation to recognize an analyte. That was the design principle behind our assay. Proteins have had several billions of years to evolve this structure-function relationship, and we were hoping that we could at least somewhat replicate it synthetically. Initially, we just designed polymers that would partially adsorb to the tube and partially remain desorbed, where the adsorbed phase would be something that would tether the molecule to the tube, and the desorbed phase would be the molecular recognition phase. We made a library of these polymers with slight chemical variations and then started screening to show that we could achieve a good level of molecular cell activity with just these synthetic polymers.

BSJ: How are the polymer-wrapped carbon nanotubes synthesized?

ML: The nanotubes now have become a popular starting material for many applications beyond biological sensing. Given their popularity, they can now be commercially procured. We typically purchase the tubes, purify them, and do post-processing to ensure consistency amongst batches. We also have an ongoing collaboration with Ron Zuckermann's lab at the Molecular Foundry at LBNL (Lawrence Berkeley National Laboratory]). Zuckermann's lab has a robot that synthesizes polymer sequences.

BSJ: How is analyte recognition achieved on a molecular level? What role does corona phase molecular recognition [CoPhMoRe] play in this process?

ML: We would love to know the answer to that question. We're working towards it. Initially, we just started by building an almost random library

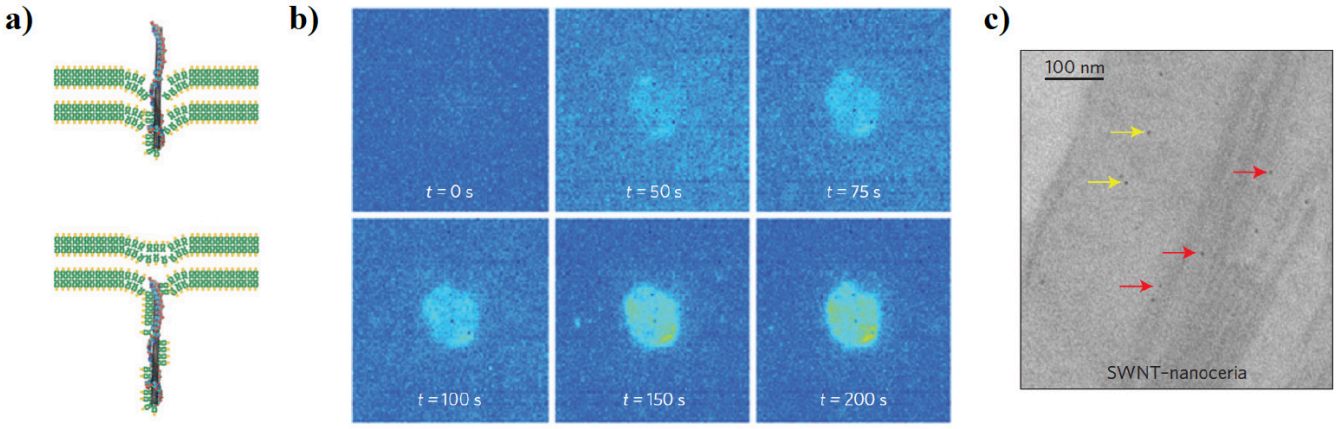

Figure 2. a) Near-infrared photo indicating rapid penetration of ss(AT)15-SWNTs through chloroplast lipid bilayer b) SWNT transport through chloroplast double membrane envelope via kinetic trapping by lipid exchange c) Chloroplast TEM after incubation in SWNT-NC suspension.

"We would eventually like to accomplish neurotransmitter detection in awake and behaving animals"

of polymers and an almost random library of analytes to show that this could work. As we move towards different spaces - neuroscience, protein detection - we are getting a little bit smarter about our approaches. The way that we develop a corona phase for proteins is different than what we use for neurotransmitters. For neurotransmitters, now we know that the important part is to have polymers that will wrap in loops as opposed to helices. That's just one of the discoveries that we made by trial and error. For proteins, what's important is either using protein-like molecules such as peptoids that have loops for protein recognition, or by using phospholipid coatings so that the corona phase resembles the membrane of a cell.

BSJ: In one of your articles, SWNTs were used for neurotransmitter detection. Why did you specifically focus on dopamine for further sensor optimization?

ML: It was a bit of luck. That was the best sensor we found within our screen. But we were lucky because dopamine is one of the three primary modulatory neurotransmitters that govern behavior. Dopamine is a molecular target that's been used by the pharmaceutical industry for over sixty years to treat depression, psychosis, and ADHD.

BSJ: What polymers were the most effective sensors for dopamine?

ML: For dopamine, nucleic acid polymers worked very well. We tried many different sequences, and, counterintuitively, as soon as we started changing the bases within a sequence we got very different response profiles to dopamine and other molecules. One of the key findings we made recently is that the original (GT)15 DNA polymer on the tube creates about a 90% dopamine response. If we cut that roughly in half and make a (GT)6, then instead of making helices the polymer makes rings. That does funny things to the nanotube excitons, which provide light output and increase signal by over an order of magnitude. So these (GT)6 rings end up being probably what we'll be pursuing now for in vivo studies of dopamine.

BSJ: Another exciting area of research in your laboratory is plant nanobionics[3]. What has motivated you to attempt to engineer plant function with SWNTs?

ML: The plant nanobionics area of the group, which also looks at delivering biological cargo into plants, was motivated by some frustrations we had in the neuroscience space. We were having issues with sensors going inside cells, which is not where we wanted to measure dopamine. But we found that there's a lot of very easy internalization of these nanotubes through biological membranes. Although we can now fix this penetration issue with chemistry, we wanted to exploit this phenomenon known as "barrier crossing" to deliver useful biological cargo to systems. And one of the more difficult systems to deliver biological cargo to is plants. In addition to a cell membrane, they also have a cell wall, which evolved to be very stiff to provide the turgor pressure that the plants need to stay upright. We're motivated by the introduction of foreign genes, for example, into mature plants. We can develop nanomaterials in which a gene vector for a certain transgene is introduced passively into the plant. Then we observe that the test vector that codes for GFP expression, for example, will see cells produce protein at the GFP injection site. So that's a proof of principle that, not only is the gene vector getting into the plant, but that protein expression is also happening after the delivery.

BSJ: How do SWNTs have the ability to modify photosynthetic activity of plants?

ML: We don't know that yet. SWNTs have these unique photonic properties in the way excitons travel through them, so that they can absorb light not just in the visible range but also in the infrared range. Photosynthetic pigments can only absorb visible light. So what we're thinking is that there might be some ability of the carbon nanotube to absorb photons of light within a very broad range. The sun emits in the IR as well, and that energy is somehow transferred to the pigment of the plant that can then increase photosynthetic efficiency.

BSJ: Do you thing that SWNT-mediated photosynthesis could serve as an ex vivo source of renewable energy?

ML: We are currently working on recomposing the plant from its species. One of the things we are doing is extracting chloroplasts (main photosynthetic element of the plant) and looking at its interactions with the carbon nanotube. One challenge there is that the chloroplast is a plastid, or not an independently living organism. Keeping it viable when extracted is a bit of a challenge. We are exploring with a few synthetic chemistry approaches that mimic the native environment of the chloroplast in a tissue culture.

BSJ: What are the future directions of your research and how will the Zuckerberg award allow you to expand into more high-risk directions?

ML:In addition to neurotransmitter imaging, we would eventually like to accomplish neurotransmitter detection in awake and behaving animals. We would like to start probing the relationship between different social environments and how these affect neurotransmission in the brain. We would also like to start validating some clinical therapies. If we dose a mouse with an antidepressant and employ our technology, that can be a quantitative measure of how dopamine is actually changing in the brain. For plants, we would also like to move forward with material plant transformation. Currently, if you want a transgenic plant, you need to start with a seedling, wait 4-5 weeks until it grows, and see whether the resistance element that was introduced is actually working. A method for direct modification of just a subset of a plant tissue would allow us more spatial control over what parts of the plants are transgenic. That can be very interesting if, for example, you wanted to grow a non-GMO fruit, but still wanted to confer disease resistance to the roots and the leaves, thus creating locally transformed plant tissue. These are the types of projects that we are mainly pursuing under the Zuckerberg initiative. The ability to change directions if we find something more exciting than originally expected is part of what makes the Zuckerberg award so powerful.

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