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Much more than a vitamin: Folate mediated targeting of nanoparticles to cancer

I arrived at UW determined to be an oncologist, as my mother had passed away from cancer just months before the first day of school. This propelled me into bioengineering over the other more focused life sciences because I wanted a more holistic view of medicine, and I knew in the future this would involve developing and employing new research. When I first discovered the field of bioengineering I was ecstatic. Its breadth seemed the perfect fit for me, offering a wide breadth of knowledge in all areas of science and engineering, while complementing this foundation with insightful depth into core areas and the numerous research projects focused on clinical applications. I knew that these were the skills I wanted to carry with me into the next stage of my career.

However, I did not understand how valuable independent undergraduate research could be in preparing me for being a doctor. I am fortunate that my faculty mentor, Dr. Daniel Ratner – knowing my diligence, persistence, and zest for knowledge – urged me to apply to the Clinical Research Experience for Engineers (CREE) program. Dr. Ratner planted the seed that research done in bioengineering, at the forefront of nanotechnology, can be a cornerstone of clinical medicine as it is translational research that ends up *actually* impacting the real-world (i.e. clinical bedside). Dr. Ratner also showed me that being intimately involved in such research as an undergraduate will allow me to utilize more fully, and even participate in, translational research when I become a doctor. Integrating nanotechnology research with clinical care offers a unique and compelling opportunity for me to contribute to science in a way that may reduce the burden of disease and illness.

My CREE project involved the collaboration between a UW Bioengineering lab specializing in inorganic nanoparticle technology led by Dr. Xiaohu Gao and a UW Radiology lab led by Dr. Xiaoming Yang. The clinical need was simple: many cancers are more effectively combated if detected early, but early detection remains a challenge. For instance, magnetic resonance imaging (MRI) has low sensitivity and contrast in comparison with other imaging modalities, therefore precluding its use in early detection of tumors. Recently, targeted magnetic nanoparticles (MNPs) have been explored as so-called contrast agents to overcome this limitation. Dr. Yang and

I intended to explore just that: targeting MNPs made in the Gao lab to cancer cells with high specificity in the hope that early cancers may be better visualized on MRI. It excited me to work in a new area that I initially knew little about. Indeed, undergraduate nanotechnology textbooks are not readily available. All that I did not know drove me to dive into the subject head-first, where I dissected the literature, independently outlined the synthetic scheme, and learned how to conduct the research to achieve our research aims.

I was handed an open-ended project involving the implementation of a new nanotechnology developed by the Gao lab – a gold shell deposited around a 20-30 nm MNP which would serve as the MRI contrast agent. The three-fold challenge was to select an appropriate targeting moiety, a conjugation strategy and a method to stabilize the nanoparticles *in vivo*. Poly(ethylene glycol) (PEG) has been used successfully to increase the blood half-life of nanoparticles. To specifically target cancer cells, we decided to employ the targeting ligand folic acid (FA), because its receptor is greatly overexpressed on the surface of many cancer cells (Fig. 1). The FA receptor mediates a natural endocytotic pathway and can be used to obtain preferential uptake of the targeted nanoparticle to cancer cells which enables contrast agent sequestration for improved detection sensitivity. My task, therefore, was to synthesize and conjugate a FA-PEG linker to the surface of gold shelled MNPs and test their effectiveness in targeting cancer cells *in vitro* and *in vivo*.

Ambitiously, I began the summer as a novice researcher thinking it was reasonable to synthesize and conjugate the FA-PEG linker to the nanoparticles within my first month. Then I could smoothly transition to *in vitro* uptake studies and possibly even *in vivo* biodistribution studies in the Yang lab. What I lacked in experience I thought I could make up for with hard work. Indeed, I ended up working many 50+ hour weeks in the lab, in addition to the summer coursework I was doing. The biggest hurdle I faced was finding out that published synthetic schemes often lack critical details or are only vaguely reproducible. Making headway required frequent and evolving discussions with Dr. Gao and his senior graduate student Shivang Dave to constantly improvise upon existing experimental procedures. Dr. Ratner was also instrumental in providing practical organic synthesis advice, without which I would never have made the progress I did. By the end of the summer and countless

synthetic routes later I was amazed to have synthesized my very first linker molecule and conjugated nanoparticle (Fig. 2). Unfortunately, time did not allow me to verify the targeting effectiveness of my linker and we were not yet ready for an *in vivo* biodistribution study. This taught me a valuable lesson: meaningful research that can be replicated and validated by other scientists takes time.

The possibility of improving early detection of cancer is tantalizing and draws me to move hastily forward before my technology is fully characterized or optimized. However, this is fundamentally hazardous, as recent nanotechnology publications have demonstrated the critical role of the surface of a nanoparticle on its behavior in the body. Thus, I had to learn to be patient and realized a more mature researcher would exhaustingly analyze the abilities and limitations of their technology by quantitatively verifying and optimizing its targeting effectiveness. More than ever, I now appreciate the practical nature of translational research. At a basic level I understand what it takes to develop new drugs and imaging agents for medicine.

Unexpectedly, it has become apparent that this project complements my required Bioengineering Capstone Design project in the Ratner lab. The first aim of my Capstone project involves redesigning an instrument that performs surface plasmon resonance imaging (SPRi). Although the detailed physics of SPRi are too advanced for this discussion (see Supplementary Material), basically SPRi is an optical imaging method capable of monitoring molecular events on a surface. Changes at the surface of a gold chip can be monitored with high sensitivity by flowing a specific ligand over a receptor immobilized on the gold surface, or vice versa (Figs. 3 and 4). This type of analysis can examine the kinetics of a binding interaction, as well as the effects of altering surface chemistries, quantitatively and in real-time. This is directly applicable to the aim of thoroughly characterizing my nanoparticle.

Preliminary data suggests that my linker molecule specifically binds to the folate receptor (Fig. 3). However, the current flow channel does not have well defined and replicable flow behavior across the gold surface. In contrast, microfluidic flow channels – having micron scales – allow for precise control of fluid dynamics, making experimentation repeatable and robust. I am working closely with a graduate student in the

Ratner lab to incorporate microfluidics into the SPR imager. Additionally, we are retrofitting the imager to allow for multiplexing, whereby multiple analytes can be flowed across a single gold chip. These expansions of functionality in the SPR imager will greatly increase the throughput of our lab's experimentation and directly aid me in optimizing the surface chemistry of my nanoparticle, bringing my nanoparticles closer to clinical application and, hopefully, towards the improvement of lives through early detection of cancer.

Even though I did not plan for it, these ongoing research projects have opened my eyes to research as a process, which when fully struggled through and overcome yields meaningful benefits to society. As a bioengineer, the thrill of research is its tangible applications. I am not interested in 'science for the sake of science'. Rather, I want to be involved in science that has direct promise to improve quality of life for those facing illness or disease. The research I am immersing myself in for the next year is just that. The Mary Gates Research Scholarship will help me pursue this research, as it is nearly impossible for young labs such as ours to financially support ambitious undergraduate work. I believe that my research may ultimately help to save lives. In this way, this scholarship will be an enabling force to achieve these goals – not possible with the extra burden of holding a job.

Conducting serious undergraduate research has opened the door to really understanding the *process* of experimentation, which in turn has made me a more creative and critically thinking student. These experiences have forced me to internalize the complexities at the nano- and micro-scale, giving me a unique perspective compared to more conventional viewpoints of already-trained physicians. I will not be limited in that manner; rather I am now committed to being an ardent advocate of translational research at the forefront of nanotechnology.

I respectfully request your consideration.

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Supplementary Material

Fig. 1

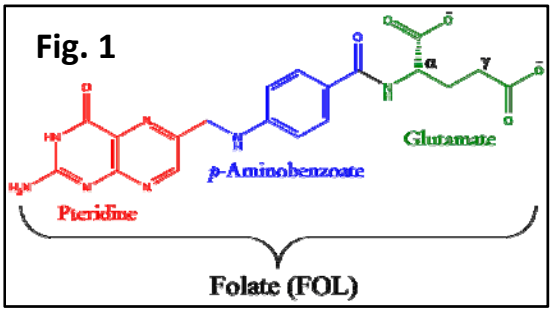


Fig. 2

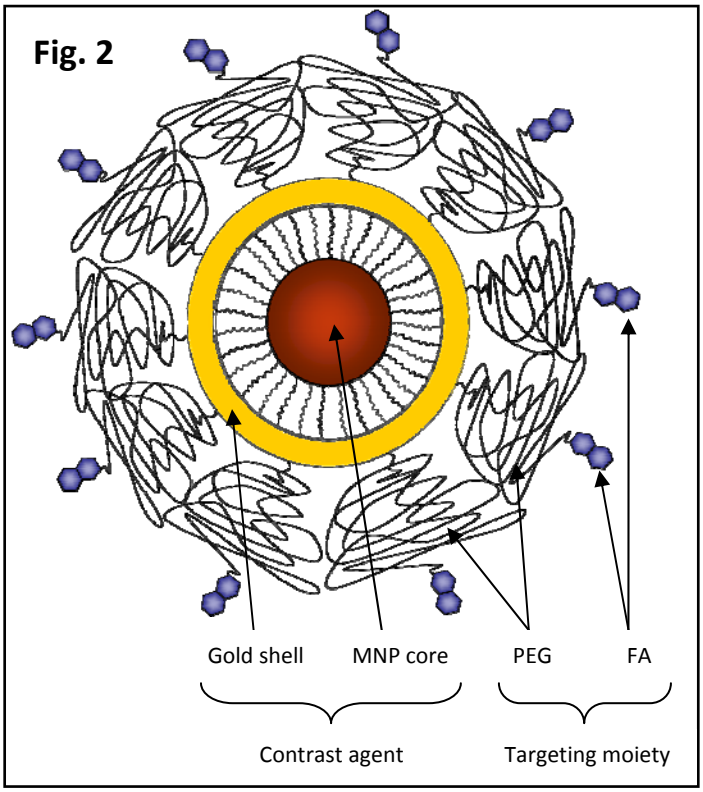
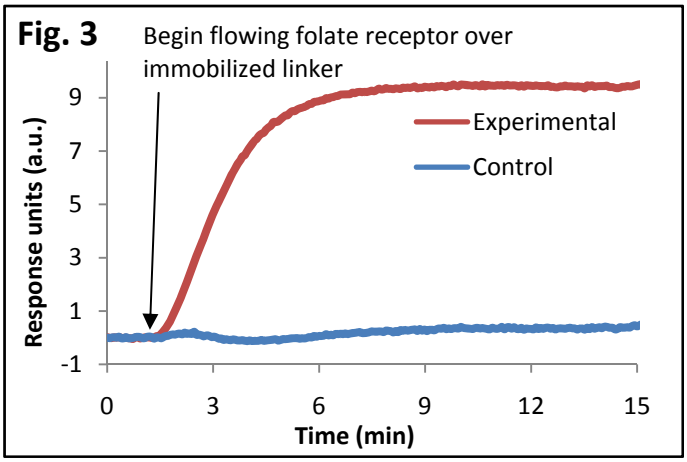


Fig. 3



Important Terminology

***In vivo/In vitro*:** inside/outside of an organism
Biodistribution: processes of monitoring where a substance of interest migrates in a living subject
Magnetic resonance imaging (MRI): employs radio-frequency waves and intense magnetic fields to excite atoms in the object under evaluation. Patterns in this excitation are observed and displayed
Nanoparticle: a particle 1 – 100 nm in diameter
Magnetic nanoparticles (MNPs): nanoparticles, usually containing iron oxide, that in the presence of an external magnetic field respond quickly and align to that field. This phenomenon is known as superparamagnetism and produces significant imaging contrast under MRI

Poly(ethylene glycol) (PEG): a polymer of repeated ethylene glycol units that, when conjugated to drugs and nanoparticles, slows clearance from the blood and imbues water solubility
Folic acid (FA): a light sensitive B-vitamin whose deprotonated form (folate) is slightly water soluble
Folate receptor: a glycosylphosphatidylinositol anchored cell surface receptor with high affinity for FA (K_d = 0.1 – 1.0 nM), found overexpressed in >90% of ovarian cancers, and capable of mediating endocytosis of the FA ligand and its reduced derivatives
Endocytosis: process whereby cells engulf and internalize material with their cell membrane into endosomes
Surface plasmon resonance imaging (SPRi): see below

What is Surface Plasmon Resonance imaging (SPRi)?

"The surface plasmon resonance (SPR) phenomenon occurs when polarized light, under conditions of total internal reflection, strikes an electrically conducting gold layer at the interface between media of different refractive index: the glass of a sensor surface (high refractive index) and a buffer (low refractive index) . . . An electric field intensity, known as an evanescent wave, is generated when the light strikes the glass. This evanescent wave interacts with, and is absorbed by, free electron clouds in the gold layer, generating electron charge density waves called plasmons and causing a reduction in the intensity of the reflected light. The resonance angle at which this intensity minimum occurs is a function of the refractive index of the solution close to the gold layer on the opposing face of the sensor surface."

The coupling of an optical detection unit to a light source producing SPR is known as SPRi. The SPR signal, which is expressed in resonance units, is a measure of mass concentration at the sensor chip surface. The analyte and ligand association and dissociation can be observed in real-time. Ultimately, rate constants as well as equilibrium constants can be calculated with quantitative precision.

Fig. 4

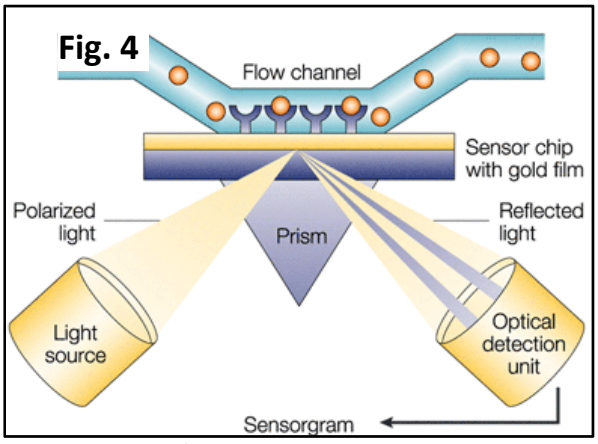


Image: Matthew Cooper. *Nature Reviews Drug Discovery*. 2002 (**1**), 515-528.
SPRi Quote: Biacore Life Sciences ©2008.