The path to my thesis project, which incorporates cell biology, biophysics and neuroscience, is somewhat unconventional. During the summer of 2005, I began working in Ka Yee Lee’s laboratory in the Department of Chemistry. Under the guidance of Eva Chi, a post-doctoral fellow in the lab, I carried out experiments to elucidate the effects of model cell membranes on the aggregation of the Alzheimer’s protein amyloid-beta (Aβ) protein into amyloid fibrils. In light of research suggesting that Aβ interacting with cell membranes “template” Aβ into the fibrillar form seen in the brains of Alzheimer’s patients, the effects of cell membrane components, such as charged phospholipids and gangliosides, on Aβfibril formation were examined. We used a number of analytical techniques, including thioflavin-T fluorescence, a classic amyloid dye used to monitor fibril formation, size exclusion chromatography and dynamic light scattering to determine whether the lipid templated β-sheet structures could lead to fibril formation. I found that in water, anionic PG vesicles uniquely induced the formation of Aβ fibrils whereas cationic and neutral vesicles had no effect on fibril formation [1]. These results yielded a hypothetical mechanism for *in vivo* Aβ fibrillogenesis. Since negatively charged phospholipids reside only in the inner leaflet of the cell membrane, it is possible that as oxidative damage to the membrane accumulates, negatively charged phospholipids are flipped outward and exposed to the extracellular environment. These negatively charged lipids are then able to interact with Aβ in a potentially deadly capacity. Later experiments included testing the ability of the air-water interface to seed Aβ into fibrils [2].

Following my work at the University of Chicago, I sought out an additional research experience that focused on more biological models. Darrell Irvine’s work at MIT captivated me because of its unique blend of immunology and material science. I applied to the Irvine Group for a research position. Fortuitously, Professor Irvine was looking for a technical assistant to aid his graduate student, Yuki Hori, on her project to develop a novel cancer vaccine. I began work on Ms. Hori’s project to generate injectable polysaccharide alginate hydrogels that can attract immune cells to the injection site *in vivo* (mouse models) for immunotherapy. Our system uses a novel technique for gelation: alginate microspheres containing excess Ca2+ were co-injected with soluble alginate to form a gel via divalent cross-linking. Our gels, when co-injected with dendritic cells and other immunostimulatory factors, are designed to circumvent the natural defenses a tumor has against the body’s immune system by locally boosting immune response at the tumor site. For roughly two-thirds of my time I aided Ms. Hori with mammalian cell harvesting and culture, alginate particle synthesis, *in vivo* work and assays such as ELISA, flow cytometry and rheometry [3]. For my independent project, I quantified the amount of Ca2+ bound in our microspheres and characterized the redistribution of calcium from the microspheres to the soluble alginate *in vitro* using a calcium-sensing dye and fluorescence microscopy [4].

After a year at MIT, I switched coasts and began my graduate studies at Stanford. My first rotation was with Sarah Heilshorn, a professor in the department of Material Science and Engineering. I had heard Dr. Heilshorn give an amazing talk at MIT on tunable biomaterials for regenerative medicine and was excited to explore this aspect of bioengineering. For my rotation, I expressed and purified synthetic polypeptides, which were then cross-linked to form hydrogels for cell encapsulation. I then assessed the efficiency of cross-linking reaction using a free amine sensor. The overall goal of the project was to identify cross-linking conditions that would allow cells and growth factors to be safely encapsulated in hydrogels. My next rotation took place in Bianxio Cui’s laboratory in the Department of Chemistry. I was intrigued by the Cui Group’s use of advanced biophysical techniques to image dynamic processes in live cells. For my rotation project, I used total internal reflection fluorescence (TIRF) to image the transport of growth factors tagged with quantum dots in primary neurons. I learned to culture primary rat neurons and work with a laser-based imaging system. I also learned how to make the microfluidic chips used in the lab to control the patterning of neurons. My third and final rotation led me to the Meyer Lab in the Chemical and Systems Biology department. There, I was immersed in the world of cell signaling. I worked on the characterization of a G1 phase sensor developed by the lab. I began to use the lab’s automated epifluorescence microscope and learned MATLAB in order to write a program to detect the translocation of the sensor from the cytosol to the nucleus of neuronal-like PC12 cells.

The Meyer Lab specializes in taking multiple approaches to study dynamic processes and signaling pathways in cells, which I found very exciting. The lab often performs high-throughput screens combined with quantitative imaging to discover proteins involved in a variety of cellular processes. Confocal, TIRF and epifluorescence imaging are used to examine cells transfected with an ever expanding library of biosensors. The lab also uses computation biology to inform and describe experiments and data. The resources available to me, combined with the interdisciplinary environment provided by the Bio-X program at Stanford, made the Meyer lab an easy and enthusiastic choice for my thesis project. Although I took an unconventional path to my thesis project, my prior experiences have enriched and refined my approach to the dynamic process of axon specification. My research at the University of Chicago taught me the power of using biophysical studies to distill biological processes to controllable parameters. However, my studies at MIT have shown me the necessity of verifying the same principles in a biological context. Thus, when addressing question of neuronal polarization, I included both biophysical and live cell experiments in my experimental approach. My proposal also includes techniques acquired over my rotations such as primary neuronal culture, TIRF and protein purification. Taken together, my previous projects have provided me a valuable platform to continue my development as a scientist.

**Publications**

[1] EY Chi,C Ege, **A Winans**, J Majewski, K Kjaer, and KYC Lee. Lipid membrane templates the ordering and induces the fibrillogenesis of Alzheimer’s disease amyloid- β peptide. *Proteins* (2008) **72**(1), 1-24.

[2] EY Chi, SL Frey, **A Winans**, KH Lam, K Kjaer J Majewski, and KYC Lee. Amyloid- β fibrillogenesis seeded by interface-induced peptide misfolding and self-assembly. *Accepted to Biophys. J.*

[3] Y Hori, **AM Winans**, CC Huang, EM Horrigan and DJ Ivine. Injectable dendritic cell-carrying alginate gels for immunization and immunotherapy. *Biomaterials* (2008) **29**(27),3671-3682.

[4] Y Hori, **AM Winans** and DJ Irvine. Modular injectable matrices based on alginate solution/microsphere mixtures that gel in situ and co-deliver immunomodulatory factors. *Acta Biomaterialia* (2009) **5**(4), 969-982.

**Selected Presentations**

* “β-sheet templating of amyloid- β by anionic phosphatidylglycerol lipid membrane”. **Amy M. Winans,** Eva Y. Chi and Ka Yee C. Lee. Chicago Area Undergraduate Research Symposium, Chicago, IL, April 2007, *invited* (presenter)
* “β-sheet templating of amyloid- β protein by anionic phosphatidylglycerol membranes”. **Amy M. Winans**, Eva Y. Chi, Canay Ege, Jaroslaw Majewski, Kristian Kjaer and Ka Yee C Lee. 233rd American Chemical Society National Meeting, Chicago, IL, March 2007  (presenter)