Objective and Methodology

Alzheimer's Disease (AD) pervades worldwide, affecting many seniors regardless of race, nationality and creed. Thus far, it is a disease without a cure. Researchers are seeking the understanding of the assembling mechanisms of beta-amyloid (A-beta) proteins, with the hypothesis that new insights in this area will provide the underlying causes of AD. Whereas it has been known that the existence of fibril assemblies of A-beta is related to neuronal loss associated with AD, more recent studies have shown that much smaller aggregates, called A-beta oligomers, may be more directly related to the toxicity of the neuronal cells than the fibrils. In most of these studies, detergent resistant membrane fragments, often called lipid rafts, are implicated in the developing neuronal cytotoxicity. Given the many new observations by various research groups worldwide, which I will briefly describe below, our thinking is that a label-free approach that has ultrahigh quantitative sensitivity (nearing single molecule level) and possessing temporal sensing capability may be able to provide new information about the complex interplay amongst the dynamic membrane molecules and these complex A-beta proteins, especially during the early phase of their interactions. I will provide the linking expertise between one of the world's most advanced optical sensing laboratories at Chang Gung University (CGU), Taiwan, led by Professor Chien Chou, and the unique laboratory at UC Davis (UCD), USA, led by Professor Atul Parikh, providing membrane configurations, namely supported lipid bilayers, for biomolecular reaction studies using the optical sensing platforms developed at CGU. We recognize that this topic is clearly a much more complex one than what we will be able to complete in a single Fulbright project period. We will use this grant to establish the basis of what we anticipate to be long-termed research collaboration between CGU and UCD on this very timely topic of AD resolution.

Background

In the not-to-distant past, amyloid-beta (A-beta) fibrils were thought to be the primary entities causing the onset of the Alzheimer's disease (AD). It was conjectured that if a platform upon which A-beta preferentially aggregates can be identified, the ability to modulate this A-beta peptides' aggregation process, hence to intervene and inhibit this fibril forming process, can be found. Within the last few years, much has come to light to suggest that there is a major role played by the A-beta oligomers, rather than mature fibrils, in AD (Walsh, Klyubin et al. 2002); (Lesne, Koh et al. 2006). Furthermore, the pathway for disease initiation may be only associated with the oligomeric form of A-beta (Matsumura, Shinoda et al. 2011), and not the fibrils. There has also been a strong correlation found between A-beta oligomer initiated reactive oxidative species (ROS) (Cecchi, Fiorillo et al. 2007) within the cytoplasm and the onset of AD. Further studies have indicated that lipid raft domains serve in a protective role against membrane damage caused by these A-beta oligomers (Cecchi, Nichino et al. 2009; Zampagni, Evangelisti et al. 2010);(Pensalfini, Zampagni et al. 2011). Another research group also identified a different A-beta oligomer, called annular protofibrils (Kayed, Pensalfini et al. 2009) that seem to have the role of pore formation on membrane surfaces, a feature first noted by (Lashuel, Hartley et al. 2002). Together,

these new findings point toward a distinct pathway for AD infection, and implicate the dynamic assembly of "lipid rafts" in the cellular membrane as a key process for molecular transport toward onset of this infection.

Monitoring the state of lipid rafts using fluorescence microscopy is a relatively common approach to probing lipid dynamics. However, as is with all external probe studies of complex biological phenomena, the concern that the probe may influence the process being monitored, especially where subtle conformational changes may be important, is a significant question. What we propose here are label-free methods designed to probe this dynamic lipid raft assembly, coupled with the process of pore formation and A-beta oligomer transport into neuronal cells.

Methodology and Time Frame

Professor Chien Chou's research efforts at Chang Gung University, Taiwan, developing ultra-high sensitivity optical methods to monitor molecular reaction is well known world-wide (Hsieh, Chang et al. 2007; Li, Chang et al. 2008; Chang, Chen et al. 2009; Chang, Wang et al. 2010). The method of paired surface plasmon wave biosensor, an ultrasensitive version of surface plasmon resonance (SPR) has been shown to have the sensitivity of single molecule detection capability hitherto achievable only using fluorophores. Because surface plasmon waves (SPW) are sensing molecular mass, its ability to identify biochemical reactions near surfaces where receptors reside allows for label-free measurements of reactions involving A-beta proteins initiating its critical assembly for the purpose of transduction into cellular interior. Recently, Professor Chou's group has further perfected a paired beam dynamic scattering method that allows for lateral dynamics to be quantitatively measured in a label-free environment (Chou 2011). This approach will be used to monitor the dynamic process of lipid raft assembly on well-cushioned supported bilayer surfaces, models of which have been extensively developed in an on-going effort at UC Davis by Professor Atul Parikh's group (Yee, Amweg et al. 2004; Brozell, Muha et al. 2006; Sanii and Parikh 2008; El-Khouri, Bricarello et al. 2011) (Yee, Ulman et al. 2003; Brozell, Muha et al. 2005; Wu, Hollars et al. 2007) . Parikh lab's have also demonstrated lateral dynamics and protein interactions with these membrane constructs (Forstner, Yee et al. 2006; Wu, Hollars et al. 2007) as well as developed methods to study membrane poration induced by photo-induced reactive oxygen species (Smith, Howland et al. 2009; Howland and Parikh 2010), of relevance to the present work. As an active member of the US NSF sponsored Center for Biophotonics Science and Technology, I propose to interface with both the groups of Professor Parikh (UCD) and Professor Chou (CGU) in advancing the capabilities of these techniques toward defining experiments aimed at the fundamental understanding of the processes associated with the onset of AD. These include: i) the dynamic assembly of A-beta oligomers in vitro, identifying the structural origin for aggregation pathways; ii) the source of A-beta signaling that initiates the dynamic lipid raft re-organization, and the dynamic re-organization of lipid raft domains so as to capture fundamental A-beta entities for annular protofibril formation, which are the postulated precursors preparing the pores for A-beta oligomer's transport into the neuronal cells; and finally iii) how the A-beta oligomers interact with entities within the cytoplasm for initiating toxic reactive

oxidative species (ROS) production and cell death.

- Pre-fellowship phase – *2011-2012* – Identify the distinct capabilities of the techniques in our collective repertoire and associate specific objective to defined instruments. Most specifically, we will focus on the ability to create and identify the distinctive characteristics of the many states of A-beta aggregation in vitro (i).
- Fellowship phase – 2012-2013 – Couple the necessary complex membrane dynamics with annular protofibril construct, using both the photon correlation spectroscopy (PCS) for lateral lipid dynamics and dual beam SPR for pore construction (ii).
- Post-fellowship phase – 2013-2015 – Creation of a Center for AD research focusing on these definable processes AND initiate the studies to examine intracellular signal transduction processes related to toxicity and cellular apoptosis (iii).

What research facilities and resources are found in Taiwan?

The unique facilities that Professor Chou's group has in paired wave probing, either in the surface plasmon mode or dynamic light scattering mode, are one of a kind. It is the culmination of over ten years of dedicated efforts by Professor Chou and his research team to achieve the type of sensitivity that would make SPW technique truly competitive as a label-free method for single molecule biochemical reaction kinetics studies. The method of paired wave dynamic scattering further enhances the capability to sense velocity of unlabeled lipid species in lateral motion. As the original developer of the technique of PCS (Cummins, Yeh et al. 1964; Alpert, Yeh et al. 1965) and the related laser Doppler anemometry (LDA) (Yeh and Cummins 1964), I am capable of contributing the specific, detailed, analytical approaches to advance the frontiers of these methods using Professor Chou's instruments. Furthermore, Professor Chou, in the College of Engineering of CGU, has strong and supported research collaborations with Professor Yu-Sun Chang, Director of the CGU Molecular Medicine Research Institute (MMRI) and Professor J.C. Chen, Director of the Health and Aging Research Institute (HARI) of CGU.

Professional Experience

My entire career in academia has been focused on developing and exploring new uses of optical techniques for probing molecular systems, in solution or more complex environments such as biological cells. Besides the techniques of PCS and LDA, I have also developed more precise label-free approaches aimed at probing of complex mechanisms in biological cells. This optical diffraction ellipsometry (Yeh, Baskin et al. 1985; Yeh and Baskin 1987; Yeh and Baskin 1988; Sidick, Baskin et al. 1994) method was used to probe structural and dynamic changes of protein assemblies within single muscle cells between resting and contracting states. I have explored many elements of fluorescence spectroscopy and microscopy for studying biological cells and molecules in their functional states (Bianco, Brewer et al. 2001; Fore, Laurence et al. 2005; Lincoln, Boling et al. 2006). Culminating this career effort is the joining together with Professors James Boggan and Dennis Matthews (UCD) in the establishing of the NSF funded Center for Biophotonics Science and Technology (CBST), where the focus has been to develop ultrahigh spatial resolution optical microscopy instruments to complement these dynamic probes. Professor Parikh and I are both Associate Directors with CBST and

Professor Chou is a participating foreign member. The recently published a chapter on "Fluorescence Spectroscopy" with my former students is used by our Biophysics graduate students under the title of "Techniques for Biophysical Research" (Yeh 2009). I was the creator and founding Chair of the Designated Emphasis in Biophotonics (2004 – 2008), providing a conduit for graduate students to conduct highly interdisciplinary research on biophysical and biomedical issues. Annually, Professor Parikh and I co-teach a course called "Optical Methods for Biophysical Research". I feel comfortable giving these lectures at CGU, in English or in mixed English and Chinese, if needed. With my retirement, I see the opportunity to extend my broad base of biophotonics knowledge to CGU, where the research institutes for molecular medicine (MMRI) and health and aging (HARI) are collaborating with Professor Chou's College of Engineering.

Significance of This Research

Alzheimer's disease (AD) and many of the other neurodegenerative diseases of the brain are known to be associated with the alteration of neuronal physiology. There is strong evidence that the initiation activities of A-beta oligomer formation and its associated cytotoxicity relate to the interaction of A-beta derived groups of molecules specifically at the site of membrane microdomains, the lipid rafts. Many recent studies indicate that the process of disease initiation is more than just extracellular fibril formation. Indeed, the chief culprit remains A-beta proteins. However, there are several new findings that will benefit from our contributions into this field:

1. (Pre-fellowship phase) Why do these A-beta monomeric molecules, A-beta 40 or 42, start to self-aggregate in solution (Nag, Sarkar et al. 2011)? Are there distinct driving forces favoring aggregation, and are there different conformations of this assembly process?
    a. Oligomeric form assembly of A-beta are stabilized in solution by Apo-E4 proteins (Cerf, Gustot et al. 2011; Yang, Ji et al. 2011). Why is that an important element of the assembly process? It has also been determined that *amylospheroids* are the toxic species that can transport across membrane structures. Why? Are these like the nanolipoproteins?
    b. *Annular protofibrils* of A-beta tend to form only on lipid structures that resemble the lipid rafts (Kayed, Pensalfini et al. 2009). These protofibrils are again distinct from *marcofibrils* (Glabe 2008), and they form structures in lipid rafts that includes pores. Are these the A-beta oligomers entry points?
    c. What condition will favor the macrofibril formation? What is the significance of this formation? Are the molecular structures of the macrofibrils distinct from the annular protofibrils or the amylospheroids?
2. (Fellowship phase) Upon formation of amylospheroids (Matsumura, Shinoda et al. 2011) and annular protofibrils, this system is self-propelling toward transport across the membrane of the cell.
    a. Prion proteins (PrP), are known to compete for the membrane raft's region necessary for A-beta derived diffusible ligands (*ADDL*) transport (Zou, Xiao et al. 2011). Does PrP function via a competitive inhibition mechanism on the membrane (Caetano, Beraldo et al. 2011)?

b. Raft dynamics is likely the key that creates the mediating element against A-beta's intracellular oxidative attacks. This is postulated to be due to ADDL's ability to enter the cell under various raft stability conditions. Do these ADDL's enter the cell through the annular pores? Why does raft structure favor these pore formation? How do the ADDLs get impeded from entering the cell if the lipid constitution is rendered unfavorable (Cecchi, Nichino et al. 2009)? E.g., via the introduction of methyl-β-cyclodextran (MβCD).

3. (Post-fellowship collaborations) How do the ADDLs, which may be these amylospheroids, create the downstream finding of ROS?
    a. Apparently *fln*, a non-receptor tyrosine kinase on the raft membrane (Williamson, Usardi et al. 2008), works to transmit signal for onset of reactions leading to ROS.
    b. How are these associated with the annular protofibrils? Finally, what is the significance of the macrofibril structure, the hallmark of AD?

Benefits for CGU, UCD, the Applicant and the Discipline

We do not pretend to be able to solve all of the issues outlined above in this brief period of the Fulbright Fellowship. However, we have a group of highly experienced individuals on both sides of the Pacific Ocean who are dedicated to making some major inroads in this complex biology problem using advanced photonics methods. I as the applicant for the Fulbright Fellowship, feel that the timing is right for us to seriously relate the fundamentals of biophysical research with the focus needed to access a fuller understanding of the mechanism of AD initiation.

Recent studies on this subject form a very interesting line of thinking, steering researchers toward the innovative idea that biological membranes are key players in driving biological functions. Structural visibility being accomplished with the modern imaging and spectroscopic tools, often used in conjunction, interest is high on visualizing the dynamic assembly of these membrane-protein complexes, changing the surface from a resting set of molecules to an assembly of functioning complexes that further signal downstream intracellular functions. Quantitative studies of these dynamic assemblies are made possible by the new research tools being developed in Professor Chien Chou's laboratories at Chang Gung University, Taiwan. These dynamic probe results will be correlated with the structural data we can obtain now using innovative and highly specific spectral microscopy being used at CBST.

We expect the results of these interdisciplinary research efforts to be of great interest in the biophysical, cellular biology, and molecular medicine communities. We expect to participate in many international conferences and symposia, and to publish accordingly in internationally reputed journals.

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