The winners of the 2015 CAP Best Student Presentation Competition at the CAP Annual Congress, 2015 June 15–19, in Sudbury are listed on pages 139–140. The extended abstracts of those winners of the CAP prizes who submitted them for publication are reproduced below. Ed.

THE FORMATION OF ALZHEIMER'S PLAQUES IN SYNTHETIC MEMBRANES

BY JENNIFER TANG, RICHARD J. ALSOP, MATILDA BACKHOLM, HANNAH DIES, AND MAIKEL C. RHEINSTÄDTER

According to the Alzheimer's Society, 200,000 people in Ontario suffer from some type of dementia; 1 million are expected by 2030. One of the hallmarks of Alzheimer's disease is the formation of senile plaques in the parenchyma or functional tissue of the brain through the aggregation of amyloid-β peptides. While the role of these plaques in the pathology of the disease is not clear at this point, the mechanism behind peptide aggregation is a topic of intense research and discussion. Membranes are believed to play a key role in this process and serve as a nucleation point for amyloid-β aggregation [1]. Soluble amyloid-β peptides in the α-helical conformation undergoes a transformation into an insoluble, β-sheet structure, which provides a site for further plaque growth.

Because of their simplicity, synthetic membranes are promising model systems to identify the elementary processes involved [2]. In order to mimic the composition of membranes in the human brain, we prepared unsaturated zwitterionic/anionic lipid membranes made of 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (POPC) and 1,2-dimyristoyl-*sn*-glycero3-phospho-L-serine (DMPS) at concentrations of POPC/3 mol% DMPS containing 0 mol%, 3 mol%, 10 mol% and 20 mol% amyloid-β_{25-35} peptides. Multi-lamellar stacks of membranes were prepared on silicon wafers. The constituents were mixed in the appropriate ratios and dissolved in a solvent. The mixture was then applied to 1×1 cm^2 silicon wafers and allowed to dry. The membrane stacks were left in vacuum overnight to remove all traces of the solvent and incubated in a 97% H_2O atmosphere for 3 hours before taking measurements. The membrane samples were studied using optical microscopy and X-ray diffraction.

SUMMARY

This article reports the fabrication of synthetic membranes in which amyloid-β peptides form aggregates similar to neurotoxic plaques found in the brain tissue of Alzheimer's patients.

Amyloid-β (Aβ) consists of a polypeptide with 42 amino acids, 11 of which (the segment 25–35) comprise the transmembrane segment of the amyloid precursor protein (APP) and also comprise part of the full length Aβ peptide. As such, this short transmembrane segment is often used in studies of the protein interactions and partitioning in the membrane. $A\beta_{25-35}$ was recently reported to embed into the hydrophobic core of anionic lipid bilayers [3] and form monomeric α-helices at low peptide concentrations. This suggests any interaction between the peptides must be modelled as a membrane-mediated elastic interaction with strong dependence on the membrane environment [4], rather than a direct peptide-peptide interaction.

Van der Waals and electrostatic interactions alone are too weak to overcome thermal fluctuations and cause proteins to come together and aggregate. However, when embedded in a membrane, the physical properties of the lipid bilayer, determined by the membrane composition, contribute to how well proteins are able to interact with each other. The local distortion created by the proteins is what drives aggregation, or a lack thereof, as a result of trying to make the membrane conformation more energetically favourable [5].

Fig. 1 shows optical microscopy images of an anionic lipid membrane and a membrane containing 20 mol% $A\beta_{25-35}$. While the pure lipid membrane shows a smooth surface, plaques are visible in membranes containing more than 10 mol% peptides, indicating that there is a threshold to plaque formation. Plaque densities were determined to be 59 ± 3 and 920 ± 64 plaques/mm^2 for peptide concentrations of 10 and 20 mol%, respectively. The average plaque diameter stayed relatively constant at 11 ± 0.1 μm which indicates that plaque size does not depend on peptide concentration.

Jennifer Tang, <tangj43@mcmaster.ca>, Richard J. Alsop, Matilda Backholm, Hannah Dies, and Maikel C. Rheinstädter, <rheinstadter@mcmaster.ca>

Department of Physics and Astronomy, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4M1

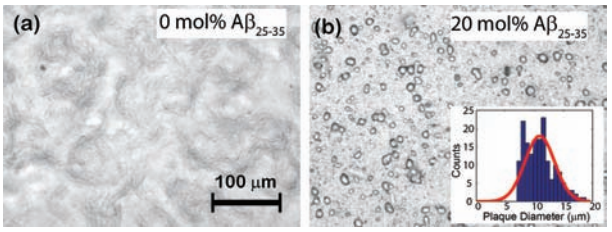

Fig. 1 Optical microscope images of an anionic lipid bilayer in (a). (b) shows a membrane containing 20 mol% $A\beta_{25-35}$ peptides, Peptide plaques were found in membranes containing more than 10 mol% peptide. Plaque size distribution and density were determined from these measurements.

X-ray diffraction experiments were conducted under near-physiological conditions, at 30°C and 97% hydration, in the fluid phase of the synthetic membranes. By preparing highly oriented membranes, in-plane ($q_{||}$) and out-of-plane (q_z) structures can be studied separately but simultaneously.

While monomeric, single peptides were found in α-helical states when embedded in membranes at low peptide concentrations [3], signals of coiled-coil α-helical peptides and β-sheets were observed in X-ray diffraction experiments at high peptide concentrations, as shown in Fig. 2. Different signals can be assigned on the 2-dimensional diffraction maps. The stacking of the membranes leads to a series of well-defined reflectivity Bragg peaks along the out-of-plane axis, q_z. α-helical peptides form a coiled-coil phase with a characteristic spacing in the membrane plane of 8.5 Å ($q_{||}$). The peptide polymer chains form a more densely packed, hydrogen bonded structure in β-sheets, with a characteristic smaller distance of 4.7 Å. The volume fraction of peptides in the different states can be determined from the corresponding X-ray signals, as well as their angular orientation with respect to the membrane normal.

The ratio of number of peptides in α and β-states was found to be approximately 50:50; half of the peptides in plaques underwent a transition from the coiled α into the denser, hydrogen-bonded β-sheet structure. From the appearance of powder rings in the 2-dimensional X-ray image, the presence of the peptides is accompanied by an elastic distortion of the bilayers.

This membrane distortion can result in a long-range interaction between the peptides. The bending of the monolayer will arise, to some extent, due to thermal fluctuations in the membrane, but the most dominant energy cost associated with bending arises when there is an inclusion, such as a peptide, in the membrane. Hydrophobic mismatch, as depicted in Fig. 2(c), occurs when the hydrophobic region of the peptide is larger, or smaller, than the bilayer thickness, which causes each monolayer leaflet to

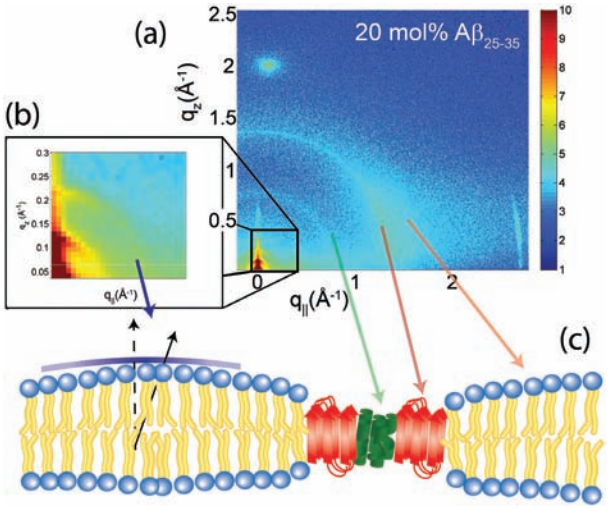

Fig. 2 (a) Two-dimensional X-ray diffraction image of a membrane with 20 mol% Aβ peptides. The various features can be assigned to lipid and peptide structures in the membrane. Peptides in red are in α-helical conformation and the ones in green are in β-pleated sheets. (b) An inset of the image at low-q, highlighting the presence of more isotropic scattering and elastic distortions. (c) A model of the plaque in the membrane. Hydrophobic matching by the membranes to the peptide leads to distortions in the membrane structure.

distort in order to ensure that the entire hydrophobic region of the peptide is contained within the hydrophobic core of the membrane.

To consolidate the experimental findings and investigate the microscopic origin behind the aggregation process, we conducted Monte Carlo simulations and the results are shown in Fig. 3. The system consisted of 2,000 lipid and peptide molecules, with peptide concentrations of 3, 10 and 20 mol% peptide. The lipid bilayer was modelled by an attractive lipid-lipid force of 0.5 k_BT. An attractive peptide-peptide force of 1.5 k_BT was included and simulation runs at different interaction distances were conducted. The most important finding was that a direct peptide-peptide interaction, as shown in Figures 3 (a)-(c), leads to plaque formation at all peptide concentrations and that plaque size increases with peptide concentration, in conflict with the experimental findings. A long range peptide-peptide interaction of up to 20 molecular distances in Figures (d)-(f) was found to best reproduce the threshold effect observed in experimental results: no plaques form at low peptide concentrations and plaque size is independent of the amount of peptides at higher concentrations.

In summary, we managed to fabricate synthetic membranes in which amyloid peptides formed aggregates, similar to

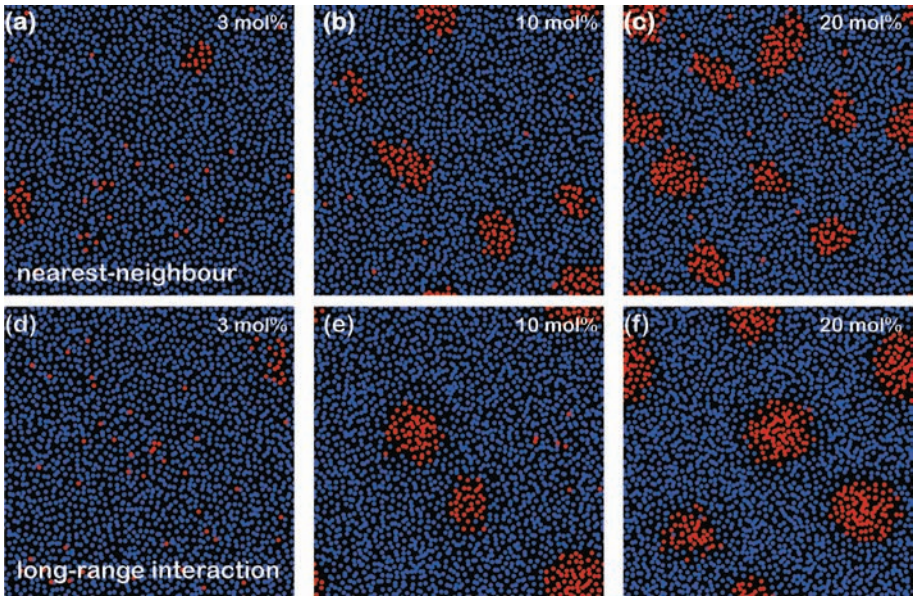

Fig. 3 Monte Carlo Simulations of a lipid bilayer containing different peptide concentrations. A nearest-neighbour interaction between peptides was simulated in (a), (b) and (c). A long-range interaction including up to 20 molecular distances in (d), (e) and (f) was found to best mimic the experimental findings.

Alzheimer's plaques in the brain tissue of Alzheimer's patients. The nature and properties of these plaques were studied using microscopy and X-ray diffraction and computer modeling. We find that the plaques consist of a mix of peptides forming α-helices and β-sheets. Our results suggest that peptide aggregation is driven by a long-range interaction mediated by the lipid membrane. This novel technique provides a promising platform to test new anti-Alzheimer's drugs *in-vitro*. Drugs can be tested in a safe environment where their effects can be quantitatively analyzed before proceeding to clinical trials.

ACKNOWLEDGEMENTS

This research is funded by the Canada's Foundation for Innovation (CFI) and the Natural Sciences and Engineering Research Council of Canada (NSERC). Jennifer Tang is the recipient of an NSERC USRA, Richard Alsop is the recipient of an NSERC PGSD, Maikel Rheinstädter is the recipient of an Early Researcher Award of the Province of Ontario.

REFERENCES

1. Clare L. Armstrong, Erik Sanqvist, Karin Schmalzl, and Maikel C. Rheinstädter, "Membrane Mediated Protein-Protein Interactions", *Physics in Canada*, **66**(3), 189 (2010).
2. Maikel C. Rheinstädter, Laura Toppozini, and Hannah Dies, "The Interaction of Bio-Molecules with Lipid Membranes Studied by X-ray Diffraction", *Zeitschrift für Physikalische Chemie*, **228**, 1105–1120 (2014).
3. Hannah Dies, Laura Toppozini, and Maikel C. Rheinstädter, "The interaction between amyloid-β peptides and anionic lipid membranes containing cholesterol and melatonin", *PLOS ONE*, **9**, e99124 (2014).
4. Clare L. Armstrong, Erik Sandqvist, and Maikel C. Rheinstädter, "Protein-protein interactions in membranes", *Protein & Peptide Letters*, **18**, 344–353 (2011).
5. Maikel C. Rheinstädter, Karin Schmalzl, Kathleen Wood, and Dieter Strauch, "Protein-Protein Interaction in Purple Membrane", *Phys. Rev. Lett.*, **103**, 128104 (2009).