IDENTIFYING PEPTIDE SENSORS FOR HIGHLY CURVED MEMBRANES AND LIPID COMPONENTS

by

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

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IDENTIFYING PEPTIDE SENSORS FOR HIGHLY CURVED MEMBRANES AND LIPID COMPONENTS

Thesis directed by Associate Professor Hang (Hubert) Yin, Ph.D.

Membrane curvature is a vital function in several significant biological processes. Indeed, this behavior is critical for activating certain signaling processes, membrane budding for endocytosis and exocytosis, membrane fusion and transporting molecules across the membrane. Membrane curvature triggers the activation of specific proteins that specifically target positively curved membranes, i.e. Caveolin-1, Amphiphysin, Synaptotagmin-1, ARF GTPase activating protein (ARFGAP1), and other proteins containing Bin/Amphiphysin/Rvs (BAR) domains. These proteins carry out important behaviors for proper cellular function. There is currently a need to further investigate the biophysical interactions involved between these proteins and highly curved membranes to further understand their biological behaviors. Furthermore, lipid composition has also been reported to influence membrane curvature targeting as its shape and charge gives it a specific behavior within the bilayer. Lipids have an important role of maintaining the cellular mobility and shape, where certain lipids can generate bilayer accessibility, initiating bilayer insertion of specific moieties of proteins and peptides.

Highly curved membranes are not only found as undulations and indentions on the surface of cells and cellular organelles but also can be identified as nano-sized extracellular vesicles (EVs) that shed from cells. These shed EVs are commonly recognized as microvesicles (d = 100-1000 nm) and exosomes (d = 30-100 nm). Their primary function involves traveling to distal parts of the body for cellular communication via protein-protein or membrane-membrane interaction, carrying proteins and nucleic acids from the cells they were derived from. Most interestingly, these EVs are highly expressed in bodily fluids of patients with metastatic cancer. Currently, there is a need to develop a noninvasive tool to effectively and specifically target these EVs. With an understanding of membrane curvature, we identified a peptide derived from the effector domain (ED) of the membrane protein Myristoylated Alanine-Rich C-Kinase Substrate, formally known as MARCKS-ED, which was observed to target highly curved membranes similar to known protein curvature sensors. Following this observation, we focused on applying this peptide to targeting biological EVs as a potential probe to study cancer progression as well as gaining more knowledge on the biophysical interactions involved with membrane curvature sensing.

In chapter 1, membrane curvature is introduced and its significance is thoroughly discussed. Specific proteins known to target membrane curvature are also described as well as our interest in further understanding these protein-membrane interactions. Our approach to use the MARCKS-ED peptide is explained as well as our project goal to use its potential in

translating its curvature sensing behavior to a biotechnology application. In chapter 2, a detailed protocol on how to effectively prepare and extrude synthetic lipid vesicles is described. This technique is performed prior to any *in vitro* binding assay and is critical in preparing homogenous vesicle solutions. Producing distinct vesicle sizes using a pressured extruder gives validity and confidence to our curvature sensing studies. This established protocol is thus significant to this work. Chapter 3 discusses our novel findings of identifying curvature sensing behavior by the MARCKS-ED peptide using *in vitro*, *in vivo* and *ex vivo* experiments. Fluorescence assays display MARCKS-ED's distinct curvature sensing behavior comparable to known curvature sensing proteins using synthetic lipid vesicles. Using a robust technique to track and analyze nanoparticles in real time further revealed MARCK-ED's ability to bind to stress-induced rat secreted positively curved vesicles, e.g. EVs. These findings led us to further investigate how MARCKS-ED interacts and prefers highly curved vesicles to flatter ones since this behavior was not fully understood. In chapter 4, our approach focuses on biophysically characterizing the specific factors such as Phe residue insertion and lipid composition that contribute to curvature sensing behavior in hopes of further understanding this preferential targeting observed by MARCKS-ED. These results were based on biophysical evidence supporting a molecular dynamics simulation model of how MARCKS-ED may be inserting into the membrane of highly curved synthetic vesicles. Chapter 5 discusses the current conclusions of this work as well as the future works that will be explored as it describes the potential directions

of this project to further understand membrane curvature in hopes of significantly contributing to this field.

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