Rapid Capillary Mixing Experiments for the Analysis of Hydrophobic Membrane Complexes Directly from Aqueous Lipid Bilayer Solutions

John W. Patrick, Breanna Zerfas[†], Jianmin Gao[†], and David H. Russell^{*} Department of Chemistry, Texas A&M University, College Station, TX 77843 [†]Department of Chemistry, Boston College, Chestnut Hill, MA 02467

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Figure S1 – Collision Cross Section profiles for $[2GA3K + 2Na]^{2+}$ ions electrosprayed from (**A**) isobutanol and (**B**) isobutanol containing 10% water. The predominant conformer adopted by GA3K is shown in purple at *ca*. 635 Å². Profile B demonstrates that the addition of 10% water to the sample depletes the abundance of the extended GA3K conformer. Conformers have been labeled according to the following scheme: purple, compact conformation (635 Å²); green, extended conformations (encompassing PDH, ADH, SSHH) (~700 Å²)

Lipid Vesicle Preparation for VCFD and MT-ESI Experiments

For the preparation of lipid vesicles, aliquots of lipid were dissolved in chloroform, dried under nitrogen gas, and desiccated under vacuum to remove residual solvent. Samples were rehydrated to 1.0 mg/mL lipid in 18 M Ω distilled water and sonicated for 30 min. Ten freeze–thaw cycles were performed on each sample using liquid nitrogen and ~50 °C water. Samples were then extruded through a size-controlled polycarbonate filter (100 nm pore size) to produce uniformly sized vesicles. GA containing samples for use in VCFD experiments were prepared by addition of the peptide prior to drying the sample under nitrogen. VCFD samples were prepared by freeze-drying using liquid nitrogen and a vacuum desiccator. After freeze-drying the samples were dissolved in isobutanol for immediate ESI analysis. For all mixing tee experiments aliquots of GA were added, allowed to incubate for an indicated time period, and subsequently analyzed using a 1:1 ratio of aqueous vesicle containing solution and isobutanol.