

# Modeling Membrane Binding Kinetics of Antimicrobial Peptides

By Alex Kreutzberger

## Introduction:

Amphipathic peptides are involved in numerous biological functions, including the innate immune system, viral infection, fertilization, cellular signaling, and selective membrane lysis. They are referred to as amphipathic due to the segregation of polar and non-polar amino acid residues in their final, folded state which enables them to bind to membrane-water interfaces. Antimicrobial peptides are short, amphipathic peptides that bind specifically to cell membranes of bacteria and other microorganisms. Mastoparan X, a 14 residue antimicrobial peptide with the sequence INWKGIAAMAKKLL-amide, is found in the venom of the Japanese hornet, *Vespa xanthoptera*. The peptide has been shown by NMR and circular dichroism (CD) spectroscopy to be unstructured in aqueous solutions, bind to lipid bilayer membranes, and form an amphipathic  $\alpha$ -helix when bound at the membrane-water interface (3,4).

Previous studies have investigated the association of peptides with model membranes by stopped-flow fluorescence to determine the role of the coil-to-helix transition in the association of the peptide with a lipid membrane (1, 2). In these studies, stopped-flow fluorescence techniques were combined with fluorescence resonance energy transfer (FRET) to examine the coil-to-helix transition of the peptide. During FRET, excitation energy is transferred from a donor fluorophore to an acceptor fluorophore. The technique is highly distance dependent and only occurs efficiently if donor and acceptor are in close proximity. A tryptophan (Trp) residue, in the peptide's amino acid sequence served as the donor fluorophore, and a modified fluorescent phenylalanine, *p*-cyano-L-phenylalanine, at the N-terminus of a peptide fluorescent probe was the FRET acceptor. Upon binding to the membrane, the peptide forms a  $\alpha$ -helix, bringing the phenylalanine and Trp pairs closer together, and increasing the efficiency of FRET (2).

Through the study of the coil-to-helix transition by stopped-flow fluorescence and FRET techniques it has been found that a biphasic kinetic model best fits the experimental FRET signal, which indicates two steps in the kinetics of the peptide binding to a membrane. The fast first step was assigned to the initial binding and helix formation of the peptide, and the second step was hypothesized to indicate further insertion of the peptide in to the membrane bilayer (1).

The goal of the current project is to develop a number of models that could be used to fit the biphasic kinetic model, derive analytical solutions for these models, and determine which of these best describes the experimental data. We have repeated the stopped-flow fluorescence and FRET experiments using a different acceptor fluorophore paired with the intrinsic tryptophan residue in mastoparan X. The acceptor fluorophore used was 7-methoxycoumarin-3-carboxylic acid (7MC) purchased from Molecular Probes/Invitrogen (Carlsbad, CA). Instead of attaching 7MC to the peptide, like had been done with the phenylalanine probe, it was attached to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine (POPE) which was then imbedded into the artificial membrane. A 1:1 ratio of two phospholipids, a neutral lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and an anionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), obtained from Avanti Polar Lipids (Alabaster, Al), was used to make the membrane vesicles. Utilizing this approach and using smaller concentrations of peptide and lipids, the previously observed biphasic model that was seen with mastoparan X was only rarely observed while in most cases a monophasic model best fit the experimental curves.

Several kinetic models were designed and tested, which would describe the experimentally observed biphasic kinetics that was occasionally observed. For each model, a set of coupled differential equations was derived. Their solutions describe the time courses of each species involved in the reaction. Actual rate constants and initial concentrations were taken from experimental measurements and set ups. The model that best described the experimental results is one that includes aggregation of the peptides which must de-aggregate before the binding of the peptide to the membrane can occur. Thus, the published theory that attributes the biphasic

experimental kinetics to deeper insertion of the peptide in the membrane does not hold true when tested theoretically.

**Specific Objective:**

To further study the peptide binding kinetics to a model membrane, a peptide that has more of a tendency to aggregate is required. Lysette-26, will be used. Lysette-26 is a peptide that was designed as a sequence-scrambled variant of  $\delta$ -lysin.  $\delta$ -Lysin is a 26 amino acid peptide with the sequence formyl-MAQDIISTIGDLVKWIIDTVNKFTKK, that is secreted by *Staphylococcus aureus*, cause of the common staph infection. Lysette-26 (IISTIGDLVKWIIDTVNKFTKK) was designed by A. Pokorny and synthesized by New England Peptide (Gardner, Massachusetts). We already found some indication that lysette-26 aggregate in solution, which will aid in studying the effects of aggregation on the observed FRET spectrums.

Our first goal will be to ascertain the amount of aggregation that occurs in solutions of lysette-26. Trp fluorescence changes as a function of aggregation (5) and can thus be used to follow peptide aggregation as a function of concentration. Then, the stopped flow fluorescence with FRET experiments will be repeated using different concentrations of lysette-26 to observe any differences that occur by using a peptide that aggregates. This will help ascertain the effects of aggregation on the kinetics because any increase in fluorescence after the initial binding should be more pronounced. The rates and concentrations from the experimental results for the lysette-26 peptide will be compared with the theoretical models to ascertain if the model that takes into account aggregation provides a good fit to this data.

The next step of our research will then be to explore a method to directly measure the coil-to-helix transition of a peptide as it interacts with a membrane. CD spectroscopy can be used to determine the conformation of a peptide since different secondary structures have distinct signature CD spectra. To observe the effects of the peptide interacting with a membrane as it occurs over time, our stopped-flow apparatus will need to be altered and attached to the CD instrument. This will enable us to measure the direct coil-to-helix transition as it is occurring.

**Justification:**

The goal of our research is to quantitatively describe the interaction of a peptide with a lipid membrane with the goal to understand which forces drive this interaction. This could lead to the ability to design peptides that will fulfill designed biological functions. For instance, the lipid composition of a regular eukaryotic membrane is very different from the one of bacteria and many tumor cells. By understanding exactly which forces drive a peptide into interaction with a membrane, a peptide could be designed to specifically target a specific type of cell membrane while leaving normal cells intact.

**Methods:**

The kinetics of association of lysette-26 with membrane vesicles is observed and recorded on a stopped-flow fluorimeter (model No. SX-18MV; Applied Photophysics, Leatherhead, Surrey, UK). FRET between the intrinsic Trp residue of lysette-26 and 7MC-POPE incorporated in the lipid membrane will be used peptide binding and dissociation with the membrane vesicles. A constant concentration of vesicles will be, while the concentration of peptide will be varied in order to determine the effects of aggregation on FRET.

We will modify the existing CD spectrometer to accommodate a portable stopped-flow mixer. This will allow us to directly follow the time course of the final folded state over time. Both the peptide and vesicles will be suspended in 10mM phosphate buffer and the kinetics of their interaction will be measured by observing the CD signal at 220nm which is characteristic of the helical state.

**Description of the Product:**

The early research I conducted in this area using Mastoparan X was recently presented at the Annual Meeting of the Biophysical Society (2010). The goal for the remainder of the research is to determine a correct kinetic model for the coil-to-helix transition of a peptide binding to a membrane. The next part of the research will be presented again at the next Annual Meeting of the Biophysical Society (2011) in Baltimore and published in 2010/2011.