Published in final edited form as:

Soft Matter. 2010 ; 6(5): 862–865. doi:10.1039/b915800f.

Neutron reflectometry of supported hybrid bilayers with inserted peptide

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Abstract

The insertion of a synthetic amphiphilic, α -helical peptide into a supported hybrid bilayer membrane (HBM) was studied by neutron reflectometry to elucidate the resulting nanostructure. The HBM consisted of a self-assembled monolayer of perdeuterated octadecanethiol on gold and an overlying leaflet of acyl-deuterated phosphatidylcholine (d-DMPC). Using contrast variation, several reflectivity spectra were recorded for each step of film fabrication, and simultaneously modeled. This analysis indicated that peptide insertion into the DMPC lipid leaflet is the likeliest mode of incorporation.

Introduction

The ability to engineer structurally well-defined transmembrane channels to control mass transfer through otherwise tightly sealing synthetic membranes is of great scientific and technological interest. We formed gold-supported hybrid bilayer membranes (HBMs) that consist of a self-assembled monolayer (SAM) of perdeuterated octadecanethiol (d-ODT) and an overlying leaflet of acyl-deuterated 1,2-dimyristoyl-*sn*-phosphatidylcholine (d-DMPC) and characterized these constructs with specular neutron reflectometry (NR).¹ These HBMs were subsequently exposed to a cysteamine-terminated amphiphilic α -helical peptide, custom-synthesized based on a class of antimicrobial peptides, that includes alamethicin and melittin, known to form transmembrane aggregates in lipid bilayers.² Modeling of the NR data acquired before and after peptide incubation of the HBM excluded several possible modes of peptide inclusion and permitted us to identify trans-bilayer insertion as the primary mode of peptide incorporation. The results are consistent with previously reported electrochemical impedance spectroscopy (EIS) and near-edge X-ray absorption fine structure (NEXAFS) spectroscopy results collected for similar peptide-exposed HBMs, which indicated that the peptide changes significantly the dielectric and ion conductivity

properties of the HBM, accompanied by retention of ordering of the surrounding acyl chains.³

Materials and methods

d₅₄-DMPC from Avanti Polar Lipids (tail perdeuterated, product #860345) was used as supplied. A 23-residue peptide (2776SH), designed using the method of Schiffer and Edmundson⁴ to form an amphiphilic α -helix, was synthesized by Biopeptide Co., LLC (San Diego, CA). The amino acid sequence and circular dichroism characterization of 2776SH are published.³ Perdeuterated d-ODT was from D. Vanderah (NIST CSTL).

NR experiments were conducted on the Advanced Neutron Diffractometer/Reflectometer (AND/R)⁵ at the NIST Center for Neutron Research (NCNR) with a neutron wavelength of 5.0 Å. The effective momentum transfer (Q_z) range was ~ 0.01 to 0.25 Å^{-1} . Experiments were carried out under phosphate-buffered saline (PBS) subphases. Contrast variation was employed by using pure D₂O, pure H₂O, or 1 : 1 (v/v) H₂O : D₂O for the PBS solutions. Data were modeled using software developed at NCNR⁶ based upon optical matrices.⁷

SAMs were formed on Si wafers covered with freshly sputtered gold films (thickness $\approx 70 \text{ Å}$) on top of chromium bonding layers (thickness $\approx 30 \text{ Å}$) by overnight incubation in a 0.26 mM ethanolic solution of d-ODT. Subsequent formation of the HBM was accomplished by exposing the dried d-ODT SAM to a 5.0 mg mL^{-1} ethanolic solution of d-DMPC for 10 min, followed by rapidly flushing the surface with PBS solution, similar to the procedure described by Cornell *et al.*⁸ After NR data collection for the HBM with various solvent contrasts, the overlying aqueous phase was exchanged with a 0.5 mg mL^{-1} solution of 2776SH in PBS, the HBM incubated for 30 min and flushed with PBS buffer. These manipulations, as well as all buffer exchanges for solvent contrast variation, were performed *in situ* on the AND/R sample stage, so that the same area on one physical Si/SiO_x/Cr/Au wafer was examined throughout the extended experiment.⁹ This permits refinement of all NR spectra with one consistent set of parameters for the substrate fine structure and justifies treatment of the contrasts as originating from one isomorphous structure.

Scattering lengths were obtained from published data.¹⁰ A scattering length density (SLD) of $2.07 \times 10^{-6} \text{ Å}^{-2}$ was assumed for Si and an SLD of $3.47 \times 10^{-6} \text{ Å}^{-2}$ was calculated for silicon oxide assuming a mass density of 2.196 g cm^{-3} .¹¹ For modeling of the organic surface structure, the phospholipid leaflet was treated as two distinct layers: the acyl chain region and the head group region. The head group layer was assumed to consist of two components: the head groups themselves and associated water molecules. An SLD of $1.84 \times 10^{-6} \text{ Å}^{-2}$ was calculated for the d-DMPC head groups using molecular volumes obtained from molecular dynamics simulations.¹² To simplify the fitting, a single global interfacial roughness, σ , was assumed for each system modeled.

Results

NR data for the d-ODT SAM in contact with aqueous solution were best modeled as a single organic layer with an interfacial layer of reduced density^{9,13} attributed to gas adsorption,¹⁴ and models excluding this effect yielded unreasonable fits. For the d-ODT layer, this model yielded a thickness of 19.9 Å and an SLD of $7.53 \times 10^{-6} \text{ Å}^{-2}$, consistent with tightly packed polymethylenes in the SAM.

Fig. 1 depicts NR data collected for the HBM, formed on a similar d-ODT SAM at three levels of contrast utilizing D₂O, 50 : 50 D₂O : H₂O and H₂O. Also shown in the inset is the slab model used for each contrast condition. The HBM is modeled as three layers: the d-ODT, the lipid acyl chains and the hydrated lipid head groups (inset), with model parameters

presented in Table 1. The total thickness of the hydrophobic layer formed by d-ODT plus lipid acyl chains, $d \approx 35.5 \text{ \AA}$, is as expected, but the thickness of the d-ODT slab alone is lower than the all-*trans* chain length with polymethylenes normal to the gold. This may be due to tilting of the ODT chains from the interface normal¹⁵ and/or to interdigitation of lipid acyls between ODT alkyl chains. The lipid head group layer includes water at a volume fraction of ≈ 0.57 and is somewhat larger than expected from molecular models. It has been earlier reported that layer models of the 'slab' type tend to overestimate this thickness,^{16,17} and that more realistic estimates may be obtained with more complex models.¹⁶ Since the lipid fine structure is of secondary interest here, we nevertheless chose to use the simpler 'slab' models throughout this study.

Fig. 2 depicts NR data collected for the HBM after incubation with 2776SH under PBS with three isotopic compositions, along with modeled neutron reflection curves, and the inset depicts the SLD profiles associated with the model using the parameters in Table 1. Incubation of the HBM with peptide produces subtle changes in the overall spectra which are most pronounced between $Q_Z = 0.03$ and $0.05 \text{ \AA}^{-1}$. Significantly, the reflectivity is predicted to be higher in this region than for the peptide-free HBM, and this is indeed experimentally observed. SLDs of the peptide were estimated from the molecular amino acid volumes¹⁸ to be $1.5 \times 10^{-6} \text{ \AA}^{-2}$ in H_2O and $3.0 \times 10^{-6} \text{ \AA}^{-2}$ in D_2O . All substrate and ODT monolayer parameters were maintained at the values obtained from the modeling of the bare HBM prior to peptide exposure.

Fig. 3 presents schematically a variety of possible association modes of the peptide with the HBM: formation of a peptide overlayer (O), peptide intercalation into the HBM with partial protrusion of the peptide from the lipid head group region (P), full peptide incorporation throughout the entire d-DMPC leaflet (I), formation of a bundle of peptides throughout the entire d-DMPC leaflet (BI). Versions of the I and BI models in which the peptide was allowed to penetrate partially into the underlying ODT SAM were also considered. In testing these models, the best fits to the NR data were consistently obtained with the I or BI models with a peptide incorporation in the acyl tail region of 13 vol%. The optimum fit in Fig. 2 (detailed in Table 1) yielded a χ^2 of 2.1 across the six datasets, comparable to the best-fit χ^2 of the NR data of the as-prepared HBM ($\chi^2 = 2.0$). In this model, we see no perturbation of the SAM by the peptide, nor any overlayer of peptide associated with the HBM.

While we can confidently rule out the P and O models, we cannot discriminate between the I and BI models on the basis of the NR data because the method averages density laterally over a macroscopic area, and thus cannot detect any bundling. However, a characteristic feature of the BI model is that the bundle core should be hydrophilic and incorporate water. In fact, scenario I is not easy to comprehend in terms of its energetics, because of the penalty associated with the burial of hydrophilic peptide residues deeply in the aliphatic lipid chains. While the best-fit model to the NR data suggests there is no water incorporated, the amount of water in the lipid leaflet is weakly anticorrelated with the peptide volume fraction. Thus, it is feasible that a modest amount of water is indeed incorporated with the peptide bundle.

If one examines the BI model in terms of steric constraints, one arrives at a lower limit of 4 helices in a bundle that can form a hydrated pore within the DMPC overlayer. A recent computer simulation of a similar system where 4 peptide helices formed a pore through a DOPC bilayer¹⁹ showed ≈ 22 water molecules within the transmembrane pore. Judging from these results, ≈ 10 water molecules in a 4-helix bundle appear a realistic lower bound for water incorporation into the lipid (mono)layer in the BI model. An upper limit is more difficult to determine. In our experience,²⁰ one would with confidence detect water at 3 vol% in the lipid overlayer due to the isotopic sensitivity of neutrons. In a simplistic geometric model, we expect the ratio of water and peptide in the lipid overlayer to grow linearly with

the number of individual helices that form one pore. In this scaling, it would still take on the order of 40 helices to form such large pores in the DMPC overlayer that the contained water would exceed this detection limit.

In conclusion, NR data and modeling indicate that insertion of 2776SH into the DMPC lipid leaflet is the principal mode of interaction of the peptide with the HBM. This contrasts with other scenarios in which the peptide inserts into both the hydrophobic portions of the ODT monolayer and the DMPC leaflet, or protrudes from the bilayer and/or overlays on the head groups. While it is likely that full details of the molecular architecture are more complex than captured in these simplistic scenarios, the fact that the data can be well described in a simple layer model suggests a laterally homogeneous lipid-peptide layer architecture at the interface.

Acknowledgments

Dr Ursula Perez-Salas (NCNR) provided invaluable assistance with initial experimental design and NR data collection. The deuterated ODT was a generous gift of Dr David Vanderah (NIST Chemical Sciences and Technology Laboratory). This work was supported by NSF (CBET 0403535), and the NIH (1 P01 AG032131). DJM gratefully acknowledges the support of an AINSE Research Fellowship.

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Fig. 1.

Neutron reflectivity ($R \cdot Q_z^4$) and fitted models for a deuterated HBM. The inset shows the SLD profiles that led to the modeled reflectivity curves (lines in the main panel).

Fig. 2. Neutron reflectivity ($R \cdot Q_z^4$) and best fit “I” model (peptide inserted into DMPC leaflet, see Fig. 3) for an HBM incubated with 2776SH peptide. The inset shows the SLD profiles that led to the modeled reflectivity curves (lines in the main panel).

Fig. 3. Schematic molecular depiction of models of peptide incorporation in hybrid bilayer membrane.

Table 1

Model parameters of the SLD profiles shown in Fig. 1. Scenarios (I) and (BI) in Fig. 3 were used to describe the HBM after incubation with 2776SH. Values of adjusted parameters are shown in *italic*. Parameters printed across columns were determined by requiring that they fit the respective datasets simultaneously

#	Layer	<i>d/Å</i>	No peptide, $\rho/10^{-6} \text{ Å}^{-2}$			Peptide, $\rho/10^{-6} \text{ Å}^{-2}$		
			ρ_{D2O}	$\rho_{1:1}$	ρ_{H2O}	ρ_{D2O}	$\rho_{1:1}$	ρ_{H2O}
1	Si	—	2.07					
2	SiO _x	10.9	3.47					
3	Cr	34.4	3.93					
4	Au	67.8	4.44					
5	d-ODT	15.6	7.88					
6	Acyl	19.8	5.8			5.42	5.32	5.23
7	Heads	11.3	4.37	2.48	0.05	4.13	2.24	0.02
8	Water	—	6.22	2.95	-0.56	6.22	2.95	-0.56
$\sigma = 5.0 \text{ Å}$			$\chi^2 = 2.11$					