Published in final edited form as:

J Mol Biol. 2012 February 24; 416(3): 328–334. doi:10.1016/j.jmb.2011.12.037.

THERMODYNAMIC MEASUREMENTS OF BILAYER INSERTION OF A SINGLE TRANSMEMBRANE HELIX CHAPERONED BY FLUORINATED SURFACTANTS

Alexander Kyrychenko¹, Mykola V. Rodnin¹, Yevgen O. Posokhov¹, Andrea Holt², Bernard Pucci³, J. Antoinette Killian², and Alexey S. Ladokhin^{1,*†}

¹Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160-7421, U.S.A ²University of Utrecht, Utrecht, Netherlands ³Université d'Avignon et des Pays du Vaucluse, F-84000, Avignon, France

Abstract

Accurate determination of the free energy of transfer of a helical segment from aqueous into a transmembrane conformation is essential for understanding and predicting of the folding and stability of membrane proteins. Until recently, direct thermodynamically sound measurements of free energy of insertion of hydrophobic transmembrane peptides were impossible due to peptide aggregation outside the lipid bilayer. Here we overcome this problem by using fluorinated surfactants that are capable of preventing aggregation, but, unlike detergents, do not themselves interact with the bilayer. We have applied FCS methodology to study surfactant-chaperoned insertion into preformed POPC vesicles of the two well-studied dye-labeled transmembrane peptides of different lengths: WALP23 and WALP27. Extrapolation of the apparent free energy values measured in the presence of surfactants to a zero surfactant concentration yielded free energy values of -9.0 ± 0.1 and -10.0 ± 0.1 kcal/mole for insertion of WALP23 and WALP27, respectively. Circular dichroism measurements confirmed helical structure of peptides in lipid bilayer, in the presence of surfactants and in aqueous mixtures of organic solvents. From a combination of thermodynamic and conformational measurements we conclude that the partitioning of a 4-residue L-A-L-A segment in the context of a continuous helical conformation from aqueous environment into the hydrocarbon core of the membrane has a favorable free energy of one kcal per mole. Our measurements, combined with the predictions of two independent experimental hydrophobicity scales, indicate that the per-residue cost of transfer of the helical backbone from water to the hydrocarbon core of the lipid bilayer is unfavorable and equals $+2.13 \pm 0.17$ kcal/mole.

Keywords

free energy; WALP peptides; transmembrane helices; surfactants; bilayer insertion; helical backbone

© 2012 Elsevier Ltd. All rights reserved.

*To whom correspondence should be addressed: Phone: 913-588-0489, FAX: 913-588-7440, aladokhin@kumc.edu.

Permanent address for Alexander Kyrychenko and Yevgen Posokhov is Institute for Chemistry at V.N. Karazin Kharkiv National University, Kharkiv, 61077, Ukraine

†This research was supported by NIH grant GM-069783.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Folding and stability of membrane proteins in their native lipid environment remains one of the most elusive problems in physical biochemistry, with various methods often leading to widely variable answers. Generally, membrane protein folding and bilayer insertion are managed by complex multi-protein assemblies, such as the endoplasmic reticulum translocon^{1; 2; 3}. For non-constitutive proteins (e.g., bacterial toxins^{4; 5; 6; 7; 8}, colicins^{9; 10; 11}, tail-anchor^{12; 13} and Bcl-2 proteins^{14; 15; 16}), however, such insertion is achieved post-translationally and often in response to changes in the environment. Despite the obvious structural and mechanistic differences between the two, recent thermodynamic evidence indicates that the underlying physicochemical principles for these processes are likely to be the same^{17; 18; 19}. Increasing evidence indicates that post-translational insertions and changes in membrane topology are important features of folding^{20; 21; 22; 23} and possibly misfolding²⁴ of membrane proteins. A rather striking example of how lipid properties modulate this process is the variation in topogenesis of LacY, which requires phosphatidylethanolamine (PE) to act as a lipid chaperone for proper folding²⁵. Moreover, after PE is synthesized in initially PE-less cells, the fully inserted LacY undergoes a massive conformational change, resulting in several helices inserting into the bilayer or flipping so their termini switch their *cis/trans* topology within the membrane. Yet we know little of the free energy profiles along such transitions, primarily because of the experimental challenges inherent to thermodynamic studies with membrane proteins in the cell. An accurate experimental determination of the free energy of TM insertion for an isolated helical segment, presented in this study, is an important benchmark for understanding the thermodynamics of membrane proteins.

Direct experimental exploration of the folding and stability of membrane proteins has been hindered by their insolubility. But because membrane proteins are equilibrium structures, their folding and stability can be examined by studying various aspects of the membrane interactions of peptides^{26; 27; 28}. Such studies resulted in establishing the whole-residue absolute free energy scale for interfacial partitioning²⁶, the rules for interplay of electrostatic and hydrophobic interactions^{29; 30}, and the energetics of secondary structure formation on membrane interfaces^{31; 32; 33; 34}. While various model peptides were extremely useful experimental models for studying interfacial binding and folding, deciphering the energetics of transbilayer insertion turned out to be much more elusive. Systematic studies of membrane interactions of designed peptides of the TMX series^{35; 36} demonstrated that the interfacial folded state is the most thermodynamically stable one for a self-inserting helical peptide, which remains monomeric in solution. This trend can't be reversed by an increase in sequence hydrophobicity, since the latter results in peptide precipitation in solution (rendering thermodynamic analysis impossible) prior to any noticeable increase in insertion. Monomeric self-inserting peptides of the pHLIP (pH (Low) Insertion Peptide) family³⁷ are notable exceptions, yet the analysis of their insertion is complicated by the substantial refolding (from random coil in solution to TM helix) and the presence of the interfacial intermediate state. The solution to this conundrum suggested here is not in the design of the peptide but in the mode of its membrane delivery, namely via application of fluorinated surfactants^{38; 39}.

Peptides of the WALP family have been used in numerous studies and are perhaps the best understood helical TM peptides^{40; 41}. They are composed of a hydrophobic core of variable length, made of alternating Leu and Ala residues, flanked by a pair of Trp residues needed for proper positioning of the termini at each of the bilayer interfaces. As the result of this design, WALP peptides have a unique conformation in the lipid bilayer, namely a monomeric TM helix, which makes them convenient models for studies of lipid-protein interactions and for development and calibration of new methods. Because of their high hydrophobicity, WALPs precipitate in aqueous solution, preventing accurate

thermodynamic measurements under equilibrium conditions, which require the existence of a measurable fraction of peptide in solution. Generally, solubility can be amended by detergents, but, because they will partition into the membrane, this will not work for equilibrium measurements. To enable measurements of WALP partitioning into lipid vesicles, we take advantage of unique properties of fluorinated surfactants that are both hydrophobic (and can help maintain peptide in solution) and lipophobic (do not penetrate into the bilayer themselves). Previously we have used FTACs to chaperone the insertion of the translocation domain of diphtheria toxin^{42; 43} and developed a protocol for measurements of its TM penetration using FCS spectroscopy^{44; 45}.

FCS, which measures intensity fluctuations of a small number of fluorescent molecules diffusing through a small focal volume, is a very useful technique to quantitate membrane binding^{44; 45; 46; 47; 48}. Detection of membrane interaction is based on the difference in diffusion of fluorescently labeled peptide free in solution and inserted into LUV (See Supplemental Materials and Methods for more details). Because of the extremely high sensitivity of the FCS experiment, reliable data can be obtained using sub-nanomolar concentrations of protein. The main advantage of this approach is that a single autocorrelation curve, measured at a particular lipid concentration, contains all the information needed to calculate free and bound protein fractions⁴⁵.

The results of the application of FCS to interactions of WALP peptides with POPC LUV are presented in Fig. 1A, B (examples of original autocorrelation data are shown in Supplemental Results Figs. S1 and S3). Titration curves measured in the presence of various concentrations of FTAC-C6 follow a simple thermodynamic scheme, with the entire population of peptides being in membrane-competent form⁴⁵. This differs from membrane insertion of such proteins as annexin B12⁴⁸ and diphtheria toxin T-domain⁴⁹ and simplifies further thermodynamic analysis. The apparent free energies are about -8 to -9 kcal/mole, which is a convenient range for FCS determination. The true values of the ΔG are obtained from linear extrapolation of the apparent ΔG , measured at three different FTAC concentrations; these equal -9.0 ± 0.1 and -10.0 ± 0.1 kcal/mole for WALP23 and WALP27, respectively (Fig. 1C). The reported deviations are standard errors of linear regression weighted by the standard deviation of each apparent ΔG at individual surfactant concentration calculated using Origin software package (OriginLab, Northampton, MA).

The slopes of the linear dependence of apparent ΔG vs. surfactant concentration is somewhat higher for WALP27, which is consistent with the expectation that its greater length will allow it to interact with more molecules of surfactant.

The obtained ΔG values for WALP partitioning in the membranes are a few kcal/mole higher than typical helical peptides undergoing interfacial partitioning into neutral membranes, normally ranging from -6 to -8 kcal/mole^{34; 50}. On the other hand, they are lower than the free energy of insertion of diphtheria toxin T-domain ($\Delta G = -12$ kcal/mole⁴⁴), which has several TM helices. The latter estimate, however, contains a potentially large unknown contribution arising from the change in the protein fold from solution to membrane.

To characterize the true reference state for WALP partitioning, which is required for further analysis, we need to establish its folding properties outside the lipid bilayer (note that bilayer-inserted WALP peptides are 100% helical). For hydrophobic peptides this can be achieved by CD measurements in a series of water/alcohol mixtures as described in^{33; 51}. Here we use the same approach, with the only difference being that mixtures containing less than 2 M of trifluoroethanol (TFE) are excluded from the analysis, as at low organic solvent concentrations the peptide becomes less soluble (for detailed description of data collection

and analysis see Supplemental Methods). Application of CD spectroscopy to estimate the folding of WALP peptides in water/TFE mixtures is summarized in Fig. 2. At high concentrations of TFE both peptides are completely helical and undergo a partial unfolding transition around 6 M TFE (~43% by volume). The folding free energies are practically identical for WALP27 (4.8 ± 0.2 kcal/mole) and WALP23 (4.6 ± 0.2 kcal/mole). These experiments also allowed us to estimate the amount of secondary structure projected to the pure water sample based on the limiting molar ellipticity $\Theta_{\min}$. Both peptides maintain the folded core of 7 residues and 11 residues for WALP23 and WALP27, respectively, which means they both have an equal number of unfolding residues (presumably in terminal regions).

We summarize our results and provide further analysis using the scheme in Fig. 3. The application of FCS and chaperoned insertion allowed us to measure the free energy of insertion of fluorescently labeled WALP peptides. While the absolute values of ΔG (-9 to -10 kcal/mole) provide a useful reference point, the differential value of $\Delta G(\text{LALA}) = \Delta G(\text{WALP27}) - \Delta G(\text{WALP23}) = -1.0$ kcal/mole, which corresponds to partitioning of the Leu-Ala-Leu-Ala region, is even more useful for the reasons discussed below.

First, taking the difference in ΔG for two peptides allows us to eliminate the unknown unfavorable contribution of the polar Alexa probe residing on the membrane interface. One might argue that the exact contribution of the probe could be somewhat dependent on peptide length. This effect can't be substantial, however, as the depth of the interfacial region ($\sim 15 \text{ \AA}^{28; 52}$) is much larger than the difference in peptide length. In addition, the measurements of the spectral position of Trp fluorescence in either peptide were exactly the same (330 nm maximum) and independent of the presence of the probe (this is consistent with the interfacial region of the bilayer being largely disordered and chemically heterogeneous^{28; 52}, and thus providing the necessary polar interactions for the probe). Second, the same argument holds against any noticeable contribution of the difference in hydrophobic matching in two peptides. Both of these peptides match well to the hydrophobic core of POPC, and one can expect the matching to become ideal with only a slight tilt of the longer WALP27. Third, using the differential number $\Delta G(\text{LALA})$ eliminates the contribution of the membrane-induced folding of the terminal regions that are slightly unfolded in solution (Fig. 2). As discussed above, the number of unfolded residues, and hence the contribution of their folding, is the same for both peptides.

Thus, the value of -1.0 ± 0.2 kcal/mole accurately represents the partitioning of the LALA sequence from aqueous environment into the hydrocarbon core in the context of a continuous helical segment (Fig. 3, central scheme). Note that this definition coincides with the one used in hydrophobicity predictions of the TM helical segments of membrane proteins, which are critically dependent on precise knowledge of the contribution of the backbone $\Delta G(\text{BB})$ ²⁸. We can estimate the latter from our measurements by accounting for the free energies of side chains according to the following: $\Delta G(\text{BB}) = (\Delta G(\text{LALA}) - 2\Delta G(\text{Leu}) - 2\Delta G(\text{Ala}))/4$. We will use two independent experimental hydrophobicity scales to estimate $\Delta G(\text{Leu})$ and $\Delta G(\text{Ala})$, a whole residue Wimley-White scale derived from octanol partitioning of model pentapeptides²⁶ and the side-chain only scale of Moon and Fleming⁵³ derived from guanidine unfolding studies of the transmembrane β -barrel protein, outer membrane phospholipase A (OmpA). Note that, because of the different natures of these two scales, their estimates need to be brought to a common reference point before meaningful comparison with our data can be made. The Wimley-White scale is a whole-residue scale and contains the contribution of the side-chain and that of the peptide bond. The latter equals $+2.0$ kcal/mole and must be subtracted from the whole residue ΔG value for each amino acid as illustrated by White and Wimley⁵⁴ on P. 327. The resulting side-chain values are $\Delta G(\text{Leu}) = -3.25 \pm 0.11$ kcal/mole and $\Delta G(\text{Ala}) = -1.50 \pm 0.12$ kcal/mole. These values

combined with our results lead to this value for the helical backbone contribution of $\Delta G(\text{BB}) = +2.13 \pm 0.17$ kcal/mole.

The OmpA-based scale⁵³ is a differential scale with Ala being a zero reference point and $\Delta G(\text{Leu}) - \Delta G(\text{Ala}) = -1.75$ kcal/mole. Moon and Fleming had estimated the ΔG value for the Ala side-chain by comparing ΔG values for a series of substitutions with hydrophobic residues to the values of accessible surface area calculated for each guest residue in the context of Gly-X-Gly tripeptide. The resulting reference-free scale has the following values (Table S1 of the supplement to⁵³): $\Delta G(\text{Leu}) = -3.32$ kcal/mole and $\Delta G(\text{Ala}) = -1.57$ kcal/mole. Application of these values results in $\Delta G(\text{BB}) = +2.2$ kcal/mole which coincides with the value obtained when the Wimley-White octanol scale was used to account for side-chain contribution.

The resulting unfavorable contribution of the backbone coincides very closely with the estimate based on the statistical analysis of the predictions of known TM helical segments⁵⁵. In the latter study, the whole-residue octanol scale was utilized and the difference between backbone contributions for unfolded peptide in octanol and helical peptide in the bilayer was used as a variable correction factor to optimize the overall prediction score. Based on the thermodynamic measurements presented here this correction factor (denoted as CONH value) should be set at +0.13 kcal/mole when TM hydrophaty analysis is performed using the web-based program MPEx (<http://blanco.biomol.uci.edu/mpex/>)⁵⁶. To verify the consistency of such an approach, we use this number together with the whole residue Wimley-White octanol scale to estimate the free energy for the entire unlabeled peptide, $\Delta G(\text{WALP23}) = -12$ Kcal/mole. This value is in reasonable agreement with the experimentally measured value of -9 kcal/mole for the Alexa-labeled peptide reported here; the difference is explained by the penalty for partitioning of the bulky and charged fluorescent probe to the membrane interface.

The free energy estimates for TM insertion presented here (Fig. 3) add an important quantitative aspect to our understanding of thermodynamic laws that govern membrane protein insertion (Fig. 3), while the methodology described will undoubtedly be instrumental in further deciphering of the complex nature of membrane protein folding and stability in the native lipid environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Dr. W.C. Wimley for helpful discussions and to Mr. M.A. Myers for his editorial assistance.

ABBREVIATIONS

FTAC-C6	fluorinated surfactant $\text{C}_6\text{F}_{13}\text{C}_2\text{H}_4$ - <i>S</i> -poly-Tris-(hydroxymethyl)aminomethane with a critical micelle concentration (CMC) of 0.33 mM
LUV	extruded large unilamellar vesicles of 100 nm diameter
POPC	palmitoyl-oleoyl-phosphatidylcholine. (from Avanti Polar Lipids, Alabaster, AL)

WALP23	(Acetyl-CGWWLALALALALALALALWWA-Amide) and WALP27 (Acetyl-CGWWLALALALALALALALALALWWA-Amide) model TM peptides labeled with Alexa-488 fluorescent probe attached at C1 residue
TM	transmembrane
TFE	trifluoroethanol
FCS	fluorescence Correlation Spectroscopy

REFERENCE LIST

1. Johnson AE, van Waes MA. The translocon: A dynamic gateway at the ER membrane. *Annu Rev Cell Dev Biol.* 1999; 15:799–842. [PubMed: 10611978]
2. Alder NN, Johnson AE. Cotranslational membrane protein biogenesis at the endoplasmic reticulum. *J Biol Chem.* 2004; 279:22787–22790. [PubMed: 15028726]
3. Rapoport TA, Goder V, Heinrich SU, Matlack KES. Membrane-protein integration and the role of the translocation channel. *Trends Cell Biol.* 2004; 14:568–575. [PubMed: 15450979]
4. Oh KJ, Senzel L, Collier RJ, Finkelstein A. Translocation of the catalytic domain of diphtheria toxin across planar phospholipid bilayers by its own T domain. *Proc Natl Acad Sci USA.* 1999; 96:8467–8470. [PubMed: 10411898]
5. Miller CJ, Elliott JL, Collier RJ. Anthrax protective antigen: Prepore-to-pore conversion. *Biochemistry.* 1999; 38:10432–10441. [PubMed: 10441138]
6. Shatursky O, Heuck AP, Shepard LA, Rossjohn J, Parker MW, Johnson AE, Tweten RK. The mechanism of membrane insertion for a cholesterol-dependent cytolysin: A novel paradigm for pore-forming toxins. *Cell.* 1999; 99:293–299. [PubMed: 10555145]
7. Senzel L, Gordon M, Blaustein RO, Oh KJ, Collier RJ, Finkelstein A. Topography of diphtheria toxin's T domain in the open channel state. *J Gen Physiol.* 2000; 115:421–434. [PubMed: 10736310]
8. Collier RJ, Young JA. Anthrax toxin. *Annu Rev Cell Dev Biol.* 2003; 19:45–70. [PubMed: 14570563]
9. Zakharov SD, Lindeberg M, Cramer WA. Kinetic description of structural changes linked to membrane import of the colicin E1 channel protein. *Biochemistry.* 1999; 38:11325–11332. [PubMed: 10471282]
10. Tory MC, Merrill AR. Adventures in membrane protein topology: A study of the membrane-bound state of colicin E1. *J Biol Chem.* 1999; 274:24539–24549. [PubMed: 10455117]
11. Parker MW, Tucker AD, Tsernoglou D, Pattus F. Insights into membrane insertion based on studies of colicins. *Trends Biochem Sci.* 1990; 15:126–129. [PubMed: 2187292]
12. Rabu C, Schmid V, Schwappach B, High S. Biogenesis of tail-anchored proteins: the beginning for the end? *J Cell Sci.* 2009; 122:3605–3612. [PubMed: 19812306]
13. Renthal R. Helix insertion into bilayers and the evolution of membrane proteins. *Cell Mol Life Sci.* 2010; 67:1077–1088. [PubMed: 20039094]
14. Hsu YT, Wolter KG, Youle RJ. Cytosol-to-membrane redistribution of Bax and Bcl-X(L) during apoptosis. *Proc Natl Acad Sci U S A.* 1997; 94:3668–3672. [PubMed: 9108035]
15. Antignani A, Youle RJ. How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? *Curr Opin Cell Biol.* 2006; 18:685–689. [PubMed: 17046225]
16. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol.* 2008; 9:47–59. [PubMed: 18097445]
17. Hessa T, Kim H, Bihlmaler K, Lundin C, Boekel J, Andersson H, Nilsson I, White SH, von Heijne G. Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature.* 2005; 433:377–381. [PubMed: 15674282]
18. White SH, von Heijne G. Do protein-lipid interactions determine the recognition of transmembrane helices at the ER translocon? *Biochem Soc Trans.* 2005; 33:1012–5. [PubMed: 16246034]
19. Bowie JU. Border crossing. *Nature.* 2005; 433:367–369. [PubMed: 15674274]

20. Zhang W, Bogdanov M, Pi J, Pittard AJ, Dowhan W. Reversible topological organization within a polytopic membrane protein is governed by a change in membrane phospholipid composition. *J Biol Chem.* 2003; 278:50128–50135. [PubMed: 14525982]
21. Lu Y, Turnbull IR, Bragin A, Carveth K, Verkman AS, Skach WR. Reorientation of aquaporin-1 topology during maturation in the endoplasmic reticulum. *Mol Biol Cell.* 2000; 11:2973–2985. [PubMed: 10982394]
22. Rapp M, Granseth E, Seppala S, von Heijne G. Identification and evolution of dual-topology membrane proteins. *Nat Struct Mol Biol.* 2006; 13:112–6. [PubMed: 16429150]
23. Bogdanov M, Xie J, Dowhan W. Lipid-protein interactions drive membrane protein topogenesis in accordance with the positive inside rule. *J Biol Chem.* 2009; 284:9637–9641. [PubMed: 19074771]
24. Sanders CR, Myers JK. Disease-related misassembly of membrane proteins. *Annu Rev Biophys Biomol Struct.* 2004; 33:25–51. [PubMed: 15139803]
25. Dowhan W, Bogdanov M. Lipid-dependent membrane protein topogenesis. *Annu Rev Biochem.* 2009; 78:515–40. [PubMed: 19489728]
26. Wimley WC, White SH. Experimentally determined hydrophobicity scale for proteins at membrane interfaces. *Nature Struct Biol.* 1996; 3:842–848. [PubMed: 8836100]
27. White SH, Wimley WC, Ladokhin AS, Hristova K. Protein folding in membranes: Determining the energetics of peptide-bilayer interactions. *Methods Enzymol.* 1998; 295:62–87. [PubMed: 9750214]
28. White SH, Ladokhin AS, Jayasinghe S, Hristova K. How membranes shape protein structure. *J Biol Chem.* 2001; 276:32395–32398. [PubMed: 11432876]
29. Ladokhin AS, White SH. Protein chemistry at membrane interfaces: Non-additivity of electrostatic and hydrophobic interactions. *J Mol Biol.* 2001; 309:543–552. [PubMed: 11397078]
30. Posokhov YO, Gottlieb PA, Morales MJ, Sachs F, Ladokhin AS. Is lipid bilayer binding a common property of inhibitor cysteine knot ion-channel blockers? *Biophys J.* 2007; 93:L20–L22. [PubMed: 17573432]
31. Wimley WC, Hristova K, Ladokhin AS, Silvestro L, Axelsen PH, White SH. Folding of β -sheet membrane proteins: A hydrophobic hexapeptide model. *J Mol Biol.* 1998; 277:1091–1110. [PubMed: 9571025]
32. Ladokhin AS, White SH. Folding of amphipathic α -helices on membranes: Energetics of helix formation by melittin. *J Mol Biol.* 1999; 285:1363–1369. [PubMed: 9917380]
33. Fernandez-Vidal M, Jayasinghe S, Ladokhin AS, White SH. Folding amphipathic helices into membranes: amphiphilicity trumps hydrophobicity. *J Mol Biol.* 2007; 370:459–470. [PubMed: 17532340]
34. Almeida PF, Ladokhin AS, White SH. Hydrogen-bond energetics drive helix formation in membrane interfaces. *Biochim Biophys Acta.* 2011
35. Wimley WC, White SH. Designing transmembrane α -helices that insert spontaneously. *Biochemistry.* 2000; 39:4432–42. [PubMed: 10757993]
36. Ladokhin AS, White SH. Interfacial folding and membrane insertion of a designed helical peptide. *Biochemistry.* 2004; 43:5782–5791. [PubMed: 15134452]
37. Reshetnyak YK, Segala M, Andreev OA, Engelman DM. A monomeric membrane peptide that lives in three worlds: in solution, attached to, and inserted across lipid bilayers. *Biophys J.* 2007; 93:2363–2372. [PubMed: 17557792]
38. Chabaud E, Barthelemy P, Mora N, Popot JL, Pucci B. Stabilization of integral membrane proteins in aqueous solution using fluorinated surfactants. *Biochimie.* 1998; 80:515–30. [PubMed: 9782390]
39. Breyton C, Chabaud E, Chaudier Y, Pucci B, Popot JL. Hemifluorinated surfactants: A non-dissociating environment for handling membrane proteins in aqueous solutions? *FEBS Lett.* 2004; 564:312–318. [PubMed: 15111115]
40. Killian JA, Nyholm TK. Peptides in lipid bilayers: the power of simple models. *Curr Opin Struct Biol.* 2006; 16:473–479. [PubMed: 16828281]
41. Holt A, Killian JA. Orientation and dynamics of transmembrane peptides: the power of simple models. *Eur Biophys J.* 2010; 39:609–621. [PubMed: 20020122]

42. Palchevskyy SS, Posokhov YO, Olivier B, Popot JL, Pucci B, Ladokhin AS. Chaperoning of Insertion of Membrane Proteins into Lipid Bilayers by Hemifluorinated Surfactants: Application to Diphtheria Toxin. *Biochemistry*. 2006; 45:2629–2635. [PubMed: 16489756]
43. Rodnin MV, Posokhov YO, Contino-Pepin C, Brettmann J, Kyrychenko A, Palchevskyy SS, Pucci B, Ladokhin AS. Interactions of fluorinated surfactants with diphtheria toxin T-domain: testing new media for studies of membrane proteins. *Biophys J*. 2008; 94:4348–4357. [PubMed: 18310255]
44. Posokhov YO, Rodnin MV, Das SK, Pucci B, Ladokhin AS. FCS study of the thermodynamics of membrane protein insertion into the lipid bilayer chaperoned by fluorinated surfactants. *Biophys J*. 2008; 95:L54–L56. [PubMed: 18708456]
45. Ladokhin AS. Fluorescence spectroscopy in thermodynamic and kinetic analysis of pH-dependent membrane protein insertion. *Methods Enzymol*. 2009; 466:19–42. [PubMed: 21609856]
46. Rusu L, Gambhir A, McLaughlin S, Radler J. Fluorescence correlation spectroscopy studies of Peptide and protein binding to phospholipid vesicles. *Biophys J*. 2004; 87:1044–1053. [PubMed: 15298909]
47. Rhoades E, Ramlall TF, Webb WW, Eliezer D. Quantification of alpha-synuclein binding to lipid vesicles using fluorescence correlation spectroscopy. *Biophys J*. 2006; 90:4692–700. [PubMed: 16581836]
48. Posokhov YO, Rodnin MV, Lu L, Ladokhin AS. Membrane insertion pathway of annexin B12: thermodynamic and kinetic characterization by fluorescence correlation spectroscopy and fluorescence quenching. *Biochemistry*. 2008; 47:5078–5087. [PubMed: 18407663]
49. Kyrychenko A, Posokhov YO, Rodnin MV, Ladokhin AS. Kinetic intermediate reveals staggered pH-dependent transitions along the membrane insertion pathway of the diphtheria toxin T-domain. *Biochemistry*. 2009; 48:7584–7594. [PubMed: 19588969]
50. Fernandez-Vidal M, White SH, Ladokhin AS. Membrane partitioning: “classical” and “nonclassical” hydrophobic effects. *J Membr Biol*. 2011; 239:5–14. [PubMed: 21140141]
51. Hirota N, Mizuno K, Goto Y. Group additive contributions to the alcohol-induced α -helix formation of melittin: Implication for the mechanism of the alcohol effects on proteins. *J Mol Biol*. 1998; 275:365–378. [PubMed: 9466915]
52. White, SH.; Wiener, MC. The liquid-crystallographic structure of fluid lipid bilayer membranes. In: Merz, KM.; Roux, B., editors. *Membrane Structure and Dynamics*. Birkhäuser; Boston: 1996. p. 127-144.
53. Moon CP, Fleming KG. Side-chain hydrophobicity scale derived from transmembrane protein folding into lipid bilayers. *Proc Natl Acad Sci U S A*. 2011; 108:10174–7. [PubMed: 21606332]
54. White SH, Wimley WC. Membrane protein folding and stability: Physical principles. *Annu Rev Biophys Biomol Struct*. 1999; 28:319–365.
55. Jayasinghe S, Hristova K, White SH. Energetics, stability, and prediction of transmembrane helices. *J Mol Biol*. 2001; 312:927–934. [PubMed: 11580239]
56. Snider C, Jayasinghe S, Hristova K, White SH. MPEx: a tool for exploring membrane proteins. *Protein Sci*. 2009; 18:2624–8. [PubMed: 19785006]

Figure 1.

Thermodynamic analysis of the bilayer insertion of model transmembrane WALP peptides chaperoned by fluorinated surfactant FTAC-C6. Lipid titration isotherms for WALP23 (A) and WALP27 (B) measured by FCS in the presence of 0.1, 0.2 and 0.27 mM FTAC-C6 (red, green and blue symbols, respectively). Large unilamellar vesicles made of POPC were used in the titration. FCS data collection, binding analysis and ΔG calculation were as described in ⁴⁸ and in Supplemental Methods. FTACs that do not interact with the membrane themselves ⁴³ are used to prevent aggregation and precipitation of hydrophobic peptides and keep them in solution. The chaperone-like action of FTAC allows determination of free energy of insertion under equilibrium conditions (see text and Supplemental Results Fig.

S2). (C) Free energies of membrane insertion in the absence of surfactants (solid symbols) are estimated by the extrapolation (arrows) of the apparent ΔG measurements in the presence of surfactants.

Figure 2.

Folding of WALP peptides in the aqueous mixtures of TFE. The fraction of helical content for WALP23 (squares) and WALP27 (circles) was estimated from CD measurements as described in Supplement. Solid lines correspond to fit with Eq. S11 yielding the estimates for free energy of folding and the fraction of helical content in aqueous solution (see Supplemental Methods). Note that the latter can't be measured directly due to aggregation and precipitation of hydrophobic WALP peptides.

Thermodynamics of α -helix insertion into lipid bilayer

Figure 3.

Summary of thermodynamic analysis of WALP insertion in the lipid bilayer. We have applied previously introduced FCS-based methodology^{44; 45} to study surfactant-chaperoned insertion into preformed POPC vesicles of the well-studied dye-labeled transmembrane peptides of WALP family, known to form single TM helices. Interpolation of the apparent free energy values measured in the presence of surfactants to a zero surfactant concentration (Fig. 1) yielded free energy values of -9.0 ± 0.1 and -10.0 ± 0.1 kcal/mole for insertion of WALP23 and WALP27, respectively (left panels). From a combination of thermodynamic and conformational measurements (e.g., Fig. 2) we conclude that the partitioning of a 4-residue L-A-L-A segment in the context of a continuous helical conformation from aqueous environment into the hydrocarbon core of the membrane has a favorable free energy of one kcal per mole (center panels). This estimate combined with the predictions of the hydrophobicity scales of Wimley and White²⁶ or Moon and Fleming⁵³ indicates that the per-residue cost of transfer of helical backbone from water to hydrocarbon core of the lipid bilayer is unfavorable and equals $+2.13 \pm 0.17$ kcal/mole (right panels).