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Association energetics of membrane spanning α-helices

Kevin R MacKenzie¹ and Karen G Fleming²

Since Popot and Engelman proposed the ‘two-stage’ thermodynamic framework for dissecting the energetics of helical membrane protein folding, scientists have endeavored to measure the free energies of helix–helix associations to better understand how interactions between helices stabilize and specify native membrane protein folds. Chief among the biophysical tools used to probe these energies are sedimentation equilibrium analytical ultracentrifugation, fluorescence resonance energy transfer, and thiol disulfide interchange experiments. Direct and indirect comparisons of thermodynamic results suggest that differences in helix–helix stabilities between micelles and bilayers may not be as large as previously anticipated. Genetic approaches continue to become more quantitative, and the propensities for helices to interact in bacterial membranes generally correlate well with in vitro measurements.

Address

¹ Rice University, Department of Biochemistry & Cell Biology, 6100 Main Street, Houston, TX 77251-1892, USA
² Johns Hopkins University, T.C. Jenkins Department of Biophysics, 3400 North Charles Street, Baltimore, MD 21218, USA

Introduction

Helical membrane proteins represent nearly a quarter of open reading frames in genomes [1], yet little is known about the dynamical nature of their folding and stability. In 1990 the Popot and Engelman ‘two-stage’ model proposed a simplification of the membrane protein-folding problem for helical membrane proteins [2]. It suggested that membrane protein stability could be divided into two independent thermodynamic events: (i) initial establishment of a helix in a membrane environment and (ii) subsequent side-to-side interactions of helices within the membrane. While the crystal structures of some membrane proteins show that this thermodynamic model may be somewhat oversimplified, the parallel alignment of a remarkable number of membrane protein helices provides strong support for this thermodynamic approach to understanding the physical origins of membrane protein folds. Interactions between independently stable helices, this second step of membrane protein assembly, have been intensively investigated using biophysical tools in recent years. We highlight the most useful methods as well the insights they have revealed for this challenging biological problem.

Biophysical methods used to measure transmembrane helix–helix interactions

Three principal methods for measuring the energetics of transmembrane helix–helix interactions have taken prominent positions in the field: sedimentation equilibrium analytical ultracentrifugation (SE), fluorescence resonance energy transfer (FRET), and thiol disulfide interchange (TDI). SE provides a direct measure of mass, unlike any of the other methods. The ‘density matching’ SE protocol pioneered by Reynolds and Tanford [3,4] eliminates the mass contributions of bound lipids or detergents and therefore enables the molecular weight of solely the protein to be measured. Reversibility of membrane protein self-associations in detergent micelle environments can be experimentally verified, which means that both the stoichiometry and equilibrium constants can be rigorously determined [5**]. The main disadvantage of SE experiments in measuring transmembrane domain (TMD) association free energies is that SE is limited to membrane proteins solubilized in detergent micelles, which means that micelles must be chosen that support native protein properties. SE is therefore not useful for experiments in lipid vesicles, since the measured mass in vesicles would be the sum of the masses of all membrane proteins residing within any one vesicle, whether or not there existed preferential interactions between them.

Some of the limitations of SE can be addressed using FRET [6–9,10*] or TDI [11**]. Both these methods rely on proximity as a measure of interactions between TM helices, and both can be applied in either detergents or bilayers. FRET reports on dipolar overlap between donor and acceptor fluorophores on TM domains, while TDI relies on the formation of disulfide bonds between cysteines on different subunits at redox potentials set by varying the ratio of reduced to oxidized glutathione. FRET returns molar ratios for self-association reactions, which means that the reaction stoichiometry must be the prior information assumed in the data analysis. In cases where suspected interactions cannot be observed and the molecules of interest are monomeric, FRET will result in a lack of signal, a negative result, whereas SE experiments will return the monomer molecular weight.
TDI returns mole fractions of disulfide crosslinked dimers; for oligomeric orders other than dimer the fraction oligomer must be inferred from fraction dimer. Both FRET and TDI require the introduction of a label: if a natural cysteine is unavailable TDI requires the incorporation of one; FRET experiments generally involve the introduction of both donor and acceptor fluorophores on two different samples. Since fluorophores are typically large hydrophobic groups, there are a number of controls to quantify labeling efficiency and ensure that the labels do not influence the reaction in undesired ways [10,12]. Despite these many caveats, all three methods work remarkably well and are complementary, orthogonal tools for addressing the energetics of helix–helix stabilities.

There are two molecular issues related to energetic measurements. The first is ‘adventitious’ interactions between TM helices, which are a concern for both FRET and SE experiments. This phenomenon is especially problematic for studying the interactions between weakly interacting TMDs (such as growth factor receptors TMDs). To populate associated states, relatively high protein concentrations are required. In SE experiments, micelles become limiting when the protein:micelle mole ratio approaches unity; under these conditions there is a nonzero probability that two proteins will occupy the same micelle even if there are no preferential interactions between them [13]. FRET or TDI measurements made in micelles suffer from this same problem. For FRET measurements in bilayers, long Forster distances for donor/acceptor pairs can allow energy transfer to occur even when the TM helices are not ‘binding’ to each other. There are analytical solutions to correct for both types of these adventitious interactions [13,14].

A second issue with stability measurements of TMDs is the choice of units in which to express the thermodynamic parameters of interest. Since integral membrane proteins are not soluble in water, they do not partition out of lipid vesicles (or micelles) into the aqueous phase, and lipid vesicles do not mix their contents with other vesicles on the time scale of typical experiments. Therefore, bulk aqueous concentrations of protein and lipids are not appropriate: the relevant concentration scale for thermodynamic parameters is the one that relates the amount of protein to the amount of its available solvent. A mole fraction scale has been generally adopted, although it has been justifiably argued that a Molar scale in which the volume was that of the hydrophobic phase would also be appropriate [15]. The size of the hydrophobic phase in vesicle experiments is set by the vesicle dimensions and would not normally be adjustable during an experiment. By contrast, the size of the hydrophobic volume can be more easily modulated in detergent micelle experiments since increasing the bulk detergent concentration increases the number of micelles. Over 20 years ago, Tanford postulated that micellar solutions could be thermodynamically considered as one single micellar phase as long as there is dynamic exchange of micelle contents [16]; Fleming has shown how to normalize the apparent equilibrium constant for the size of this phase [5**]. The ability to vary the size of the hydrophobic phase means that the experimentalist can deliberately tune the oligomeric population for a particular membrane protein by changing either the protein or the micelle concentration.

How stable are interactions between transmembrane helices?

Thermodynamic free energy values provide a numerical index of population distributions that leads to enormous insight into how biological activities are encoded and regulated by protein self-association. Figure 1a shows the oligomeric distributions for representative helix–helix interactions from the literature, including the WT glycoporphin A (GpA) TMD dimer, and sequence variants [17,18*,19,20], the M2 TMD tetramer and sequence variants [21*,22*,23,24], the HER [25] and erythropoietin receptor TMD dimers [26], and the myelin protein zero dimer (MP0) and tetramer species [27] all as a function of mole fraction protein. Population distribution plots facilitate comparisons of stabilities between proteins of different stoichiometries and show that the GpA WT dimer interaction is quite stable relative to other helical membrane proteins studied to date in micelles. Figure 1c shows that POPC lipid bilayers strongly stabilize both the M2 TM tetramer [11**] and designed ‘serine zipper’ peptide dimers [28].

Thermodynamic measurements can be used to determine the energetic consequences of mutations. The dimer stabilities of over 50 sequence variants of GpA have been determined [17,18*,19,20]; a representative few are shown in Figure 1a. Despite the limited diversity of amino acids available to a transmembrane segment, the GpA sequence shows remarkable plasticity in its ability to tune its energetics, which can vary almost continuously over a range of nearly 4 kcal mol$^{-1}$ [17,18*]. Using structural modeling, a correlation between GpA TMD dimer structural features and energetics suggests that van der Waals interactions explain most of the thermodynamic consequences for the sequence variants; remarkably, a common set of structure-based parameters explains the free energy consequences of amino acid substitutions in helix B of bacteriorhodopsin determined by protein unfolding experiments [29]. By contrast, sequence changes in the M2 TMD tetramer from influenza A virus modulate its tetramer stability over a much narrower concentration range [22*]. Unlike GpA, in which all sequence substitutions (except one) destabilized its dimer, several substitutions in the M2 TMD stabilize its tetramer in micelles [22*] (Figure 1a).
Figure 1

Oligomeric species distributions. The labels indicate the identity of each protein. Most proteins form only dimers, and this is the implied species unless otherwise marked, with the exception of M2, for which the implied species is tetramer. All data in this panel were collected at 25°C, which means that each 10-fold decrease in mole fraction protein is equivalent to a free energy change of \(-1.36 \text{ kcal mol}^{-1}\). Panel (a) shows the species distributions for naturally occurring TMD sequences and some engineered sequence variants in micelles. Panel (b) shows species distributions for designed peptides.
**GxG motifs, polar groups, and designer TMD interactions in micelles**

Analyses of membrane protein structures and of predicted TM helical segments from sequenced genes have identified a plethora of motifs that may be important for stabilizing helix–helix interactions in membranes, and thermodynamic measurements are uniquely suited to test these ideas. A well-known motif, GxG, is overrepresented in open reading frames of TMD regions, and it was hypothesized that it may represent a dimerization code [30]. Energetic measurements of the role of GxG motifs in natural TMDs have returned a mixed verdict. Mutations to large aliphatic groups at either site in the GxG motif in GpA are strongly destabilizing, indicating the importance of these glycines for the most stable GpA TMD dimer, but similar changes at other interfacial sites, particularly at position T87, cost about as much association free energy. By contrast, the human HER TMDs, particularly at position T87, cost about as much association free energy as the myelin protein zero TMD, which contains a GxG motif. The wide range of interaction energies exhibited by GxG-containing TMDs is illustrated by the population distributions in Figure 1, where an asterisk identifies proteins containing the motif. Note that this range is consistent with the observation that sequence context of the GpA TMD GxG motif can strongly modulate dimerization free energy [17,18].

Roles for polar residues in driving helix–helix interactions have also been vetted using thermodynamic measurements. In the low dielectric environment of a membrane, polar interactions and hydrogen bonding may be expected to have significant energetic contributions. Ser is the most frequently occurring polar amino acid in TM helix sequences [30], and it occurs at TMD interaction surfaces [33,34], yet North et al. found that ‘serine zippers’ in designed TM peptides promote dimerization no better than the GpA G84V sequence variant [27]. The wide range of interaction energies exhibited by GxG-containing TMDs is illustrated by the population distributions in Figure 1, where an asterisk identifies proteins containing the motif. Note that this range is consistent with the observation that sequence context of the GpA TMD GxG motif can strongly modulate dimerization free energy [17,18].

**How do stability measurements in micelles translate to those in lipid bilayers or cell membranes?**

Helix–helix interactions should be enhanced in lipid bilayers as compared with detergent micelles simply because of the reduced dimensionality of the bilayer as compared with a micellar phase. Grasberger et al. estimated that this effect, combined with lateral localization and crowding, could result in a million-fold increase in the association constant for a monomer–dimer reaction and considerably more for higher order assemblies [38]. Experiments addressing this question, however, suggest that pre-orientation effects in a bilayer may not be the main enhancement mechanism. North et al. found that the association propensities of serine zippers in distearoylphosphatidylcholine (DIPC) vesicles were comparable to those in DPC micelles [28] (compare Figure 1b and c), whereas the same serine zipper dimers did not dissociate at mole fractions as low as 1:1000 palmitoyl-oleoyl phosphatidylcholine (POPC) lipids (upper limit in micelles. Panel (c) shows species distributions of both designed and naturally occurring sequences that were measured in lipid bilayers. The three panels are vertically aligned with the same absissa scale to enable direct comparisons. Names with an asterisk indicate sequences containing a GxG motif. The species fraction for each protein was calculated using the binding partition function [64] from the reported mole fraction $\Delta G$, or $K_a$ values (where $\Delta G = -RT \ln K_a$) as referenced in the main text with the following exceptions: (1) $\Delta G_{12}$ for HER TMD was reported as no greater than 1 kcal mol$^{-1}$ [25], and a value of 0.5 kcal mol$^{-1}$ was used in this graph; (2) $\Delta G_{12} = -3.38$ kcal mol$^{-1}$ and $\Delta G_{14} = -9.79$ were calculated for MPD using the standard state equation [55] and the reported equilibrium constants and detergent concentration [27]; (3) The serine zipper POPC dimer distribution in panel (c) was calculated using $\Delta G_{12} = -8$ kcal mol$^{-1}$ that represents an upper distribution limit based on the reported observation that only dimers were observed at mole fractions as low as 1:1000 and assuming 3% dimer at MF = .001 is not detectable [28]. For clarity, fraction monomer is not shown, but equals 1 – (sum of all species fractions) for each protein.
EpoR TMDs are more stable than HER TMD dimers by relatively weakly in POPC vesicles using FRET [39]. In factor receptor 3 (FGFR3) were also shown to dimerize pre-dimerization of the EpoR before ligand binding. thanatophoric dysplasia associated G370C [40] nor the found to stabilize FGFR3 dimers, whereas neither the same study the pathogenic mutation A391E was appreciated. The direct comparisons between the conformational freedom may also depend on bilayer plays a modest energetic role in helix–helix interactions as well as how membrane protein consequences of bilayer thickness for a larger number of FGFR3, M2, and serine zipper peptides in POPC vesicles suggest that conformational freedom may also depend on the TM sequence. As methodologies continue to improve it will be of interest to determine the energetic consequences of bilayer thickness for a larger number of helix–helix interactions as well as how membrane protein sequestration by lateral lipid domains may influence the reaction.

How do in vitro and in vivo stabilities of helix–helix interactions compare?

Biological assays of helix–helix interactions offer a complementary approach for addressing TMD interactions in vivo and have contributed significantly to our understanding of how TMDs associate in bilayers (reviewed in reference [42]). In these assays, reporter gene expression in E. coli is controlled by TMD-mediated interactions between fusion proteins. The ToxR [43] and related TOXCAT [44] assays give increased reporter gene activity with increasing self-association of a fusion protein, whereas the GALLEX assay gives increased repression of reporter gene activity with increased association of two heterologous fusion proteins [45]. Two groups have recently reported using dominant negative effects in either the ToxR [46] or the TOXCAT [47] assays to detect heteromeric interactions between TMDs, and the ToxR assay has been extended to allow positive or negative selection schemes using a suite of promoters and reporter genes [48].

The strong dependence of assay signals on the identities of the fusion protein TMDs permits rank ordering of TMD interaction strengths, with the strongly self-associating TMD of glycophorin A usually serving as a positive control. For GpA and sequence variants, results from ToxR, TOXCAT, and SE show similar rank orders [20,43,49**].

An Asn side chain drives tight association of TMDs in TOXCAT [50,51] in agreement with thermodynamic data in micelles [35]. The HER TMDs show moderately strong interactions in TOXCAT (especially HER4) [52]. However, FRET and SE data in detergent micelles indicate that self-association of the HER TMDs is quite weak [25,31], although the SE study did find that the HER4 TMD dimerized more strongly than the TMDs of HER1, 2, or 3 [25], consistent with the TOXCAT rank order. Roles for GxxG motifs, single strongly polar residues, sets of small polar residues, and leucine zippers in driving association of TMDs have been identified or confirmed using biological assays (reviewed in reference [42]). More recently, selection from a randomized library and mutations of selected and designed sequences using the POSSYCAT variant of the ToxR assay led to the conclusions that tryptophan can support helix–helix interactions in membranes [53] and that interaction of TMDs bearing a GxxG motif can be enhanced by an appropriately positioned phenylalanine [54]. Roles for aromatic or cation–π interactions in driving TMD association have been inferred using the TOXCAT assay [55].

In vivo assays are now routinely used to determine if the TMDs of biologically important molecules interact in isolation (reviewed in reference [42]). Weakly self-associating TMDs were recently identified in this manner in the anthrax toxin receptor, whose TMD contains no previously identified interaction motif and probably forms higher order oligomers [56] and in the receptor tyrosine kinase RET, which contains an SxxxS motif [57]. The TMD of the class II MHC receptor Ii protein self-associates strongly using a polar glutamine [58], and the TMD of neuropilin-1 (the semaphorin receptor ligand-binding subunit) self-associates strongly by virtue of a GxxxGxxG ‘glycine zipper’ motif [59]. The four human syndecan TMDs exhibit a wide range of self-association
tendencies despite strong sequence similarity and a conserved GxXG motif, again affirming the importance of sequence context for this dimerization motif [60]. Determining the biological significance of interactions observed in these assays is not straightforward: although the SARS coronavirus spike protein TMD interacts in TOXCAT and a GxXG motif is implicated in this interaction [61], mutations to the GxXG motif do not affect spike protein trimerization in vitro or spike protein mediated cell–cell fusion, and only one G to L substitution significantly affects spike protein mediated cell entry [62].

Two recent studies have attempted to develop quantitative formalisms for interpreting TMD interaction data from biological assays; such efforts could identify differences between in vitro and in vivo measurements. Finger et al. report apparent in vivo free energies of association in the GALLEX assay for wild type (and five mutant) GpA fusion proteins obtained by determining the expression level for each construct at which the reporter gene is half repressed and using this concentration to calculate apparent Kd and ΔGapp values [63]. Duong et al. report changes in apparent association free energy due to mutations in the glycophorin A TMD (ΔΔGmut) without direct measures of ΔGapp [49]; in this approach, TOXCAT reporter gene activity for each mutant glycoporphin A fusion protein is scaled to wild type, corrected for fusion protein expression levels (again relative to wild type), and ΔΔGapp is calculated from an expression that assumes a dilute limit [49]. The two methods each achieve some level of agreement with physical data for the GpA TMD and mutants in detergents, suggesting that biological assays may be able to provide quantitative measures of helix–helix interaction energies in vivo.

The ΔGapp values measured by Finger et al. for wild type and mutant GpA TMDs provide ΔΔGmut values [63] that can be compared with the measurements from SE [18,20]. The rank order of the effects is largely the same, but mutations have a more modest impact on the free energy of association in membranes than they do in detergents. The apparent ΔΔGmut values measured by Duong et al. using TOXCAT [49] also give largely the same rank order as the SE (and the GALLEX) data, but the TOXCAT data show roughly the same extent of disruption as the SE data: a regression analysis of the biological against the physical ΔΔGmut data gives a slope of essentially 1.

The number of caveats associated with either of these calculations makes it remarkable that any agreement at all can be achieved with experimental data from purified proteins in detergents. Both methods rely on only a handful of data points and are thus very sensitive to errors, and both require assumptions about the order of oligomerization. The behavior of a culture of E. coli from one day to the next is not highly reproducible, and although testing fusion protein expression levels in every sample helps control for such variations [49], it must be noted that stochastic differences between cells within populations can affect the biological measurements, which treat whole extracts and not individual cells. Small amounts of misfolded or aggregated fusion protein could also make varying different contributions to reporter gene expression, and if these amounts were below the detection limits of the controls they could not be accounted for quantitatively. Indeed, this last point must be considered crucial to both qualitative and quantitative analyses of biological TMD interaction data. The wide range of apparent ΔΔGmut values reported by Duong et al. for GpA derives from over a 100-fold difference in reporter gene activity [49]; most other studies of TMD interactions using ToxR, GALLEX, or TOXCAT rarely show even a 10-fold change in signal caused by single point mutations. For some TMDs the extent of association may be so high (e.g. the systems are at the right of the population distribution curves in Figure 1) that mutations lower the fraction of oligomeric fusion protein by only a small amount. However, given the wide range of effects that can be achieved in SE by point mutations in the strongly associating GpA TMD [17,18], it seems prudent to exercise caution when making even qualitative interpretations of signals from biological TMD interaction assays that cannot be significantly lowered by point mutations.

Conclusions
Biological assays and biophysical free energy measurements of TMD interactions provide experimental tests of bioinformatics-derived motifs and quantitatively probe the physical basis of helix–helix interactions. Despite the many differences between detergent micelle and in vivo hydrophobic environments, the tendencies for transmembrane domains to self-associate agree remarkably well. Comparisons of measurements in multiple hydrophobic environments suggest that the dimensionality of the bilayer may play a more modest role in promoting helix–helix interactions than previously anticipated.

Acknowledgements
The authors gratefully acknowledge funding from the National Institutes of Health (R01 GM 067850 to KRM) and the National Science Foundation (MCB 0423807 to KGF).

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

● of special interest
◆ of outstanding interest

1. Wallin E, von Heijne G: Genome-wide analysis of integral membrane proteins from eubacterial, archaeal, and eukaryotic organisms. Protein Sci 1998, 7:1029-1038.

2. Popot JL, Engelman DM: Membrane protein folding and oligomerization: the two-stage model. Biochemistry 1990, 29:4031-4037.
3. Reynolds JA, Tanford C: Determination of molecular weight of the protein moiety in protein-detergent complexes without direct knowledge of detergent binding. Proc Natl Acad Sci U S A 1976, 73:4467-4470.

4. Tanford C, Reynolds JA: Characterization of membrane proteins in detergent solutions. Biochim Biophys Acta 1976, 457:133-170.

5. Fleming KG: Standardizing the free energy change of transmembrane helix-helix interactions. J Mol Biol 2002, 323:565-571. This paper proves the physical basis of the mole fraction scale for micelles.

6. Adair BD, Engelman DM: Glycophorin A helical transmembrane domains dimerize in phospholipid bilayers: a resonance energy transfer study. Biochemistry 1994, 33:5539-5544.

7. Choma C, Gratkowski H, Lear JD, DeGrado WF: Asparagine-mediated self-association of a model transmembrane helix. Nat Struct Biol 2000, 7:161-166.

8. Fisher LE, Engelman DM, Sturgis JN: Detergents modulate dimerization, but not helicity, of the glycophorin A transmembrane domain. J Mol Biol 1999, 293:639-651.

9. Fisher LE, Engelman DM, Sturgis JN: Effect of detergents on the association of the glycophorin A transmembrane helix. Biophys J 2003, 85:3097-3105.

10. Li E, You M, Hristova K: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and forster resonance energy transfer suggest weak interactions between fibroblast growth factor receptor 3 (FGFR3) transmembrane domains in the absence of extracellular domains and ligands. Biochemistry 2005, 44:352-360. This paper shows the interactions of FGFR3 in bilayers and discusses controls for FRET studies.

11. Cristian L, Lear JD, DeGrado WF: Use of thiol-disulfide equilibria to measure the energetics of assembly of transmembrane helices in phospholipid bilayers. Proc Natl Acad Sci U S A 2003, 100:14772-14777. This study measures interaction free energies in bilayers and directly compares them to data from micelles.

12. You M, Li E, Wilmley WC, Hristova K: Forster resonance energy transfer in liposomes: measurements of transmembrane helix dimerization in the native bilayer environment. Anal Biochem 2005, 340:154-164.

13. Kobus FJ, Fleming KG: The GxxxG-containing transmembrane domain of the CCK4 oncogene does not encode preferential self-interactions. Biochemistry 2005, 44:1464-1470.

14. Wolber PK, Hudson BS: An analytic solution to the Forster energy transfer problem in two dimensions. Biophys J 1979, 28:197-210.

15. Lomize AL, Pogozheva ID, Mosberg HJ: Quantification of helix-helix binding affinities in micelles and lipid bilayers. Protein Sci 2004, 13:2600-2612.

16. Tanford C: Chapter VI: Micelles. In The Hydrophobic Effect: Formation of Micelles and Biological Membranes, edn 2. Edited by Krieger Publishing Company; 1980:42-59.

17. Doura AK, Engelman DM: Complex interactions at the helix-helix interface stabilize the glycophorin A transmembrane dimer. J Mol Biol 2004, 343:1487-1497.

18. Doura AK, Kobus FJ, Dubrovslyk L, Hibbard E, Fleming KG: Sequence context modulates the stability of a GxxxG mediated transmembrane helix-helix dimer. J Mol Biol 2004, 341:991-998. This paper describes the free energy changes for a large number of GpA sequence variants.

19. Fleming KG, Ackerman AL, Engelman DM: The effect of point mutations on the free energy of transmembrane α-helix dimerization. J Mol Biol 1997, 272:266-275.

20. Fleming KG, Engelman DM: Specificity in transmembrane helix-helix interactions defines a hierarchy of stability for sequence variants. Proc Natl Acad Sci U S A 2001, 98:14340-14344.
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40. You M, Spangler J, Li E, Han X, Ghosh P, Hristova K: Effect of pathogenic cysteine mutations on FGFR3 transmembrane domain dimerization in detergents and lipid bilayers. Biochemistry 2007, 46:11039-11046.

41. You M, Li E, Hristova K: The achondroplasia mutation does not alter the dimerization energetics of the fibroblast growth factor receptor 3 transmembrane domain. Biochemistry 2006, 45:5551-5556.

42. Mackenzie KR: Folding and stability of alpha-helical integral membrane proteins. Chem Rev 2006, 106:1931-1977.

43. Langosch D, Brosig B, Kolmar H, Fritz H-J: Dimerisation of the glycoporphin a transmembrane segment in membranes probed with the ToxR transcription activator. J Mol Biol 1996, 263:525-530.

44. Russ WP, Engelman DM: TOXCAT: a measure of transmembrane helix association in a biological membrane. Proc Natl Acad Sci U S A 1999, 96:863-868.

45. Schneider D, Engelman DM: GALLEX, a measurement of heterologous association of transmembrane helices in a biological membrane. J Biol Chem 2003, 278:3105-3111.

46. Lindner E, Langosch D: A ToxR-based dominant-negative system to investigate heterotypic transmembrane domain interactions. Proteins 2006, 66:303-307.

47. Yin H, Slusky JS, Berger BW, Walters RS, Vilaine G, Litvinov RI, Laar JD, Caputo GA, Bennett JS, DelGrado WF: Computational design of peptides that target transmembrane helices. Science 2007, 315:1817-1822.

48. Lindner E, Unterreitmeier S, Ridder AN, Langosch D: An extended ToxR POSSYCCAT system for positive and negative selection of self-interacting transmembrane domains. J Microbiol Methods 2007, 69:298-305.

49. Duong MT, Jaszewski TM, Fleming KG, MacKenzie KR: Changes in apparent free energy of helix-helix dimerization in a biological membrane due to point mutations. J Mol Biol 2007, 371:422-434. This paper shows a ΔΔG_app correlation between micelles and biological membranes.

50. Zhou FX, Cocco MJ, Russ WP, Brunger AT, Engelman DM: Interhelical hydrogen bonding drives strong interactions in membrane proteins. Nat Struct Biol 2000, 7:154-160.

51. Zhou FX, Merianos HJ, Brunger AT, Engelman DM: Polar residues drive association of polyleucine transmembrane helices. Proc Natl Acad Sci U S A 2001, 98:2250-2255.

52. Mendrola JM, Berger MB, King MC, Lemmon MA: The single transmembrane domains of ErbB receptors self-associate in cell membranes. J Biol Chem 2002, 277:4704-4712.

53. Ridder A, Skupien P, Unterreitmeier S, Langosch D: Tryptophan supports interaction of transmembrane helices. J Mol Biol 2005, 354:894-902.

54. Unterreitmeier S, Fuchs A, Schaffler T, Heym RG, Frishman D, Langosch D: Phenyllalanine promotes interaction of transmembrane domains via GxxxG motifs. J Mol Biol 2007, 374:705-718.

55. Johnson RM, Hecht K, Deber CM: Aromatic and cation-pi interactions enhance helix-helix association in a membrane environment. Biochemistry 2007, 46:9208-9214.

56. Go MY, Kim S, Partridge AW, Melnyk RA, Rath A, Deber CM, Mogridge J: Self-association of the transmembrane domain of an anthrax toxin receptor. J Mol Biol 2006, 360:145-156.

57. Kjaer S, Kurokawa K, Perrinjaquet M, Abrescia C, Ibanez CF: Self-association of the transmembrane domain of RET underlies oncogenic activation by MEN2A mutations. Oncogene 2006, 25:7086-7095.

58. Dixon AM, Stanley BJ, Matthews EE, Dawson JP, Engelman DM: Invariant chain transmembrane domain trimerization: a step in MHC class II assembly. Biochemistry 2006, 45:5228-5234.

59. Roth L, Narasre C, Dirrig-Grosch S, Aunis D, Cremel G, Hubert P, Bagnard D: Transmembrane domain interactions control biological functions of neuropilin-1. Mol Biol Cell 2008, 19:646-654.

60. Dews IC, Mackenzie KR: Transmembrane domains of the syndecan family of growth factor coreceptors display a hierarchy of homotypic and heterotypic interactions. Proc Natl Acad Sci U S A 2007, 104:20782-20787.

61. Arbel E, Granot Z, Kass I, Orly J, Arkin IT: A trimerizing GxxxG motif is uniquely inserted in the severe acute respiratory syndrome (SARS) coronavirus spike protein transmembrane domain. Biochemistry 2006, 45:11349-11356.

62. Corver J, Broer R, van Kasteren P, Spaan W: GxxxG motif of severe acute respiratory syndrome coronavirus spike glycoprotein transmembrane domain is not involved in trimerization and is not important for entry. J Virol 2007, 81:8352-8355.

63. Finger C, Volkmer T, Prodohl A, Otzen DE, Engelman DM, Schneider D: The stability of transmembrane helix interactions measured in a biological membrane. J Mol Biol 2006, 358:1221-1228.

This paper describes a method for determining ΔG_app using biological assays.

64. Wyman J, Gill S: Binding and Linkage: Functional Chemistry of Biological Macromolecules Mill Valley, CA: University Science Books; 1990.