A Heptad Motif of Leucine Residues Found in Membrane Proteins Can Drive Self-assembly of Artificial Transmembrane Segments*

(Received for publication, October 2, 1998, and in revised form, January 15, 1999)

Rolf Gurezka, Rico Laage, Bettina Brosig, and Dieter Langosch‡

From the Universität Heidelberg, Neurobiology Department, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

Specific interactions between α-helical transmembrane segments are important for folding and/or oligomerization of membrane proteins. Previously, we have shown that most transmembrane helix-helix interfaces of a set of crystallized membrane proteins are structurally equivalent to soluble leucine zipper interaction domains. To establish a simplified model of these membrane-spanning leucine zippers, we studied the homophilic interactions of artificial transmembrane segments using different experimental approaches. Importantly, an oligoleucine, but not an oligoalanine, sequence efficiently self-assembled in membranes as well as in detergent solution. Self-assembly was maintained when a leucine zipper type of heptad motif consisting of leucine residues was grafted onto an alanine host sequence. Analysis of point mutants or of a random sequence confirmed that the heptad motif of leucines mediates self-recognition of our artificial transmembrane segments. Further, a data base search identified degenerate versions of this leucine motif within transmembrane segments of a variety of functionally different proteins. For several of these natural transmembrane segments, self-interaction was experimentally verified. These results support various lines of previously reported evidence where these transmembrane segments were implicated in the oligomeric assembly of the corresponding proteins.

In any type of cell, a multitude of integral membrane proteins is simultaneously synthesized and integrated into various membranes followed by association to homo- or heterooligomeric complexes. To ensure specific assembly, their subunits must present complementary recognition domains to each other. These domains may be located on the ectodomains and/or the transmembrane segments (TMSs). Interactions between TMSs are currently intensely studied, since they usually form autonomous α-helices and have been found to direct subunit assembly or support correct folding of many membrane proteins (1, 2). Biochemical and functional analyses, molecular modeling, and structural studies indicated that the self-assembly of transmembrane helices is driven by a close packing of their characteristically shaped surfaces. These packing interactions may result in pairs of α-helices with a right-handed twist as exemplified by glycoporin A (3, 4) and probably by synaptobrevin II (5). Other TMS interactions involve a leucine zipper type of side-chain packing as known from certain soluble proteins. Within soluble leucine zippers, the interacting residues form repeated heptad (abcdefg) motifs. Residues at α- and δ-positions constitute the hydrophobic core of the interfaces; side-chains at the e- and g-positions are frequently charged, form salt bridges to each other, and make hydrophobic contacts to the core (6). Heptad motifs were also suggested to form the TMS interfaces of phospholamban (7, 8) and the M2 proton channel (9). Based on a quantitative evaluation of high resolution structures, we recently confirmed previous observations (10, 11) in demonstrating that TMSs primarily interact via a leucine zipper type of packing within bacteriorhodopsin, the photosynthetic reaction center, and cytochrome c oxidase. There, the heptads are repeated on average 2–3 times, and the motif gaxdxgaxdxgaxg covers the central parts of the membrane-spanning interfaces. Salt bridges are absent due to the hydrophobic nature of most membrane-embedded residues (12).

To establish a simplified model of membrane-spanning leucine zipper domains, we designed artificial TMSs on the basis of leucine and alanine residues. We show that an oligoleucine sequence or a gaxdxgaxdxgaxg motif of leucine residues elicits specific self-assembly in membranes and in detergent solution. Interestingly, variants of this motif are found within the TMSs of a diverse set of natural membrane proteins, where they appear to be important for oligomeric assembly.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—Construction of plasmids pToxR4TM and pSNIR4TM was described previously (5, 13). All other pToxR constructs were made by ligating synthetic oligonucleotide cassettes encoding the desired sequences into the plasmid pHKToxR(TMIL4)MalE (14) previously cut with Nhel and BamHI. For the nuclease A fusions, the oligonucleotide cassettes were ligated into plasmids pSNIR (5) or pSNIR2 previously cut with Nhel and BamHI. Details on the pSNIR and pSNIR2 plasmids will be described elsewhere. All constructs were verified by dideoxy sequencing.

ToxR Activity Assays—Transcription activation was determined upon expression of the pToxR constructs in the indicator strain FHK12 as described (15). 0.4 mM isopropyl 1-thio-β-D-galactopyranoside was added to the cultures to enhance the dynamic range of the produced β-galactosidase signals (in Miller units (MU), means ± S.D.) elicited by the different constructs in several independent experiments. This effect is thought to result from isopropyl 1-thio-β-D-galactopyranoside-induced expression of an F- plasmid-encoded truncated β-galactosidase, which competes with full-length enzyme in the formation of functional tetramers. The previously (15) described construct pToxR/GFA13 elicited 1240 ± 296 MU under these conditions.

Gel Filtration Chromatography—pSNIR and pSNIR2 fusion proteins were expressed in BL21(DE3)pLysS cells (Novagen), solubilized in 25 mM HEPES, pH 7.9, 0.5 mM NaCl, 2% CHAPS, 1 mM EDTA and quantitated as described (5). Volumes of 300 μl at concentrations of 4 or 20 μM fusion protein were separated on a Superdex 200HR 10/30 column.
The cytoplasmic domain of ToxR is linked via a TMS of choice to the periplasmic MalE moiety. Upon dimerization, ToxR binds to the ctx promoter, thus initiating lacZ transcription in the indicator cells. TM, transmembrane segment; MalE, maltose-binding protein; OM, outer membrane; IM, inner membrane.

(Amersham Pharmacia Biotech FPLC system) using a flow rate of 0.5 ml/min and 25 mM HEPES, pH 7.9, 0.5 mM NaCl, 1% CHAPS, 1 mM EDTA as running buffer. Fractions of 0.5 ml were collected and analyzed for fusion protein with a dot blot procedure (16) using the 9E10 monoclonal antibody directed against the c-myc marker epitope for detection. The elution profiles were constructed from the antigen content, and the apparent molecular weights were calculated with reference to standards given in the legend to Fig. 3.

**RESULTS**

**A Model of Membrane-spanning Leucine Zipper Domains**—Leucine is the most prevalent amino acid within the interface of leucine zippers (18), which is probably related to its ability to adopt multiple conformations (19). We therefore reasoned that the flexible leucine side chain may be particularly well suited to form a well packed membrane-spanning leucine zipper. The methyl side chain of alanine, in contrast, is expected to be too small for efficient interaction with other alanine residues. This prediction was tested by comparing the self-association of oligoleucine and oligoalanine sequences, which are known to form stable α-helices (20, 21).

One of the experimental approaches used is based on an engineered version of the ToxR transcription activator. This protein is anchored by a single TMS of choice within the inner membrane of expressing Escherichia coli cells, where it is thought to exist in a monomeric/dimer equilibrium. The dimeric form binds to the choler toxin promoter, thus activating expression of a downstream lacZ gene in a reporter strain (Fig. 1; Ref. 14). β-Galactosidase expression is therefore diagnostic of ToxR self-assembly in the membrane. We previously established this system as a sensitive tool to study TMS interactions using the structurally well-characterized glycophorin A TMS dimer for reference (13, 15).

Here, we found that a sequence of 16 leucine residues (designated L16) elicited strong transcription activation (924 ± 209 MU; mean ± S.D.). In contrast, a sequence of 16 alanine residues (designated A16), elicited only a weak signal (210 ± 53 MU) (Fig. 2, A and B). This suggests that the oligoleucine sequence self-assembles in the membrane, whereas the oligoalanine sequence stays largely monomeric. Thus, the latter can be used as host for a leucine zipper motif. Based on the gaxdexgaxdexga motif representing the central parts of most transmembrane helix-helix interfaces within crystallized membrane proteins (12), a simplified version of a membrane-spanning leucine zipper interaction domain was designed. In this model, the a, d, e, and g positions are occupied by leucine and all others by alanine. The construct with this hybrid sequence (AZ2) self-interacted to a similar degree (929 ± 186 MU) as the parental L16 protein (Fig. 2, A and B). To demonstrate that the leucine residues contained within AZ2 constitute the helix-helix interface, we mutated some of them to alanine and assessed the consequences for self-interaction. None of the single mutations made (L2A, L5A, L9A) significantly reduced the signal (data not shown). However, when either four a and d (L2A/L5A/L9A/L12A) or four g and e (L6A/L8A/L13A/L15A) positions were simultaneously mutated, the signal dropped by about 50% (516 ± 106 or 596 ± 102 MU). Thus, the leucine residues are critical for the interaction and, hence, most likely make up the interface. Further, ad- and eg-positions seem to be of similar importance for helix-helix packing. Introducing a glycine-proline pair into the center of the AZ2 sequence (L9G/A10P) similarly affected the interaction (584 ± 100 MU), consistent with the known destabilization of α-helices by glycine (22) and their kinking by proline (23) residues. We also replaced the leucines of AZ2 by three different random sequences consisting of the most abundant residues found within TMSs (leucine, isoleucine, valine, phenylalanine, alanine) (24) while maintaining total hydrophobicity and side-chain surface (25).

Compared with AZ2, these random sequences also self-assembled much less efficiently, thus emphasizing the superior suitability of the leucine side chain for helix-helix packing (e.g., “random,” 446 ± 72 MU; Fig. 2, A and B, and data not shown). The reductions in signal strength of the mutants compared with AZ2 are statistically highly significant (two-tailed Student’s t test, p < 0.001).

Comparing the concentrations of our ToxR constructs by Western blot analysis indicated that most of them were expressed at similar levels, whereas consistent overexpression was noted for the A16 construct (Fig. 2C). When we extracted the cells with NaOH to separate membrane proteins (pellet) from soluble proteins (alkali supernatant) (17), all constructs cosedimented quantitatively with the membranes as expected except A16, which could be partially alkali-extracted (Fig. 2C). Thus, a fraction of the A16 protein seems to remain in a soluble compartment, which is probably due to the comparably low hydrophobicity of the oligoalanine sequence. This fraction is thought not to interfere with the assay. To assess correct integration of the proteins into the inner membrane, we tested their ability to functionally complement the MalE deficiency of PD28 cells. Due to a MalE deletion, this E. coli strain is unable to grow in minimal medium with maltose as the only carbon source (26). In cells expressing correctly inserted ToxR membrane proteins with the ToxR moiety facing the cytoplasm and the MalE domain exposed to the periplasmic space (see Fig. 1), however, the MalE domain allows maltose uptake and thus cell growth (13, 14). Here, expression of all constructs including A16 complemented the MalE deficiency of PD28 cells to comparable degrees (Fig. 2D). In contrast, a control construct...
where the TMS is deleted (ToxR\textsuperscript{DM}) proved unable to support cell growth as expected from its presumed cytoplasmic localization. In sum, equivalent amounts of all ToxR proteins analyzed here for self-assembly appear to be correctly integrated into the inner bacterial membrane, and the obtained \( b\)-galactosidase activities can thus be directly compared.

To examine self-assembly of our artificial TMSs by an independent approach, their oligomeric states were directly compared in detergent solution (Fig. 3). The L16, A16, A22, D\textsuperscript{TM}, L9G/A10P, and “random” sequence segments were genetically fused to the C terminus of a fusion moiety based on Staphylococcus aureus nuclease A, a monomeric soluble protein. The fusion proteins were overexpressed in \( E.\text{coli} \), solubilized with CHAPS, and subjected to gel filtration chromatography at concentrations of 20 \( \mu\text{M} \). When injected at 20 \( \mu\text{M} \), both L16 and AZ2 fusion proteins eluted as broad peaks with mean apparent molecular masses of \( \approx 300 \text{kDa} \) plus minor peaks probably representing monomers. At 4 \( \mu\text{M} \), the major fractions of all other analyzed proteins migrated at apparent molecular weights consistent with monomers containing different amounts of bound detergent. Elution profiles are compared with the positions of marker proteins (vitamin B12, 1.35 kDa; carbonic anhydrase; 29 kDa; bovine serum albumin, 67 kDa; alcohol dehydrogenase, 150 kDa; thyroglobulin, 669 kDa). The chromatograms are normalized relative to their highest peaks.

where the TMS is deleted (ToxR\textsuperscript{DM}) proved unable to support cell growth as expected from its presumed cytoplasmic localization. In sum, equivalent amounts of all ToxR proteins analyzed here for self-assembly appear to be correctly integrated into the inner bacterial membrane, and the obtained \( b\)-galactosidase activities can thus be directly compared.

To examine self-assembly of our artificial TMSs by an independent approach, their oligomeric states were directly compared in detergent solution (Fig. 3). The L16, A16, AZ2, D\textsuperscript{TM}, L9G/A10P, and “random” sequence segments were genetically fused to the C terminus of a fusion moiety based on Staphylococcus aureus nuclease A, a monomeric soluble protein. The fusion proteins were overexpressed in \( E.\text{coli} \), solubilized with CHAPS, and subjected to gel filtration chromatography at concentrations of 20 \( \mu\text{M} \). When injected at 20 \( \mu\text{M} \), both L16 and AZ2 fusion proteins eluted as broad peaks with mean apparent molecular masses of \( \approx 300 \text{kDa} \) plus minor peaks probably representing monomers. At 4 \( \mu\text{M} \), the major fractions of all other analyzed proteins migrated at apparent molecular weights consistent with monomers containing different amounts of bound detergent. Elution profiles are compared with the positions of marker proteins (vitamin B12, 1.35 kDa; carbonic anhydrase; 29 kDa; bovine serum albumin, 67 kDa; alcohol dehydrogenase, 150 kDa; thyroglobulin, 669 kDa). The chromatograms are normalized relative to their highest peaks.
22, 31, and 41 kDa at both concentrations. These peaks are consistent with monomers (calculated masses: 19.5, 20.5, 20.9, and 20.9 kDa, respectively) whose migration may be influenced by different amounts of bound detergent depending on the presence and the hydrophobicity of the hydrophobic segments.

Taken together, two independent experimental approaches indicate that both the oligoleucine sequence and the model leucine zipper motif AZ2 self-assemble in a sequence-specific way in membranes as well as in detergent solution.

**Self-assembly of Leucine-rich Natural Transmembrane Segments**—Given the self-assembly of the AZ2 model, we assessed whether TMSs with similar leucine patterns exist in naturally occurring proteins. The Swiss-Prot data base was searched for occurring proteins. The Swiss-Prot data base was searched for

Given the self-assembly of the AZ2 model, we assessed way in membranes as well as in detergent solution.

indicate that both the oligoleucine sequence and the model presence and the hydrophobicity of the hydrophobic segments.

consistent with monomers (calculated masses: 19.5, 20.5, 20.9, and 20.9 kDa, respectively) whose migration may be influenced by different amounts of bound detergent depending on the presence and the hydrophobicity of the hydrophobic segments.

Taken together, two independent experimental approaches indicate that both the oligoleucine sequence and the model leucine zipper motif AZ2 self-assemble in a sequence-specific way in membranes as well as in detergent solution.

**Self-assembly of Leucine-rich Natural Transmembrane Segments**—Given the self-assembly of the AZ2 model, we assessed whether TMSs with similar leucine patterns exist in naturally occurring proteins. The Swiss-Prot data base was searched for hydrophobic sequence segments with the motif LXXLL-LXXLLL allowing for up to three mismatches. This search yielded 38 predicted N-terminal signal sequences, 30 TMSs predicted within polytopic membrane proteins, and 15 predicted TMSs from bitopic membrane proteins when homologous sequences from different species were counted only once. Whereas the signal sequences and TMSs of polytopic proteins were not further investigated here, the TMS sequences corresponding to the bitopic proteins are shown in Table I. Self-interaction of a subset was examined with the ToxR system. The TMS eliciting the strongest signal was derived from the erythrophatoxin receptor followed by the TMSs of the Friend spleen focus-forming virus; EPOR_MOUSE, mouse erythropoietin receptor; GPBB_HUMAN, human platelet glycoprotein Ib-β-chain; HEMA_CDVO, hemagglutinin-neuraminidase from canine distemper virus; LECH_CHICK, chick hepatic lectin; PVR_MOUSE, mouse poliovirus receptor homolog; SRPB_MOUSE, mouse signal recognition particle receptor β-subunit; TNRC_MOUSE, mouse lymphotixin-β receptor; VE5A_BPV1, E5 protein from bovine papilloma virus; VGLX_HSBS, glycoprotein GX from bovine herpesvirus.

**Sequences** representing those parts of the TMSs that cover the query pattern. The sequence positions of the N-terminal residues are stated, and leucine residues within the leucine zipper pattern given above the sequences are in boldface type.

**β-Galactosidase activity as determined with the ToxR system (MU, mean ± s.d.)**

*Not determined. The following Swiss-Prot identifiers denote polytopic proteins with the search pattern: ALG3 YEAST, BTUC_ECOLI, BVGS_BORBR, CCKR_CAVPO, CLC5_HUMAN, CO3_RAT, COMT_HUMAN, COP_CLOPE, CYB_BOVIN, DCDR_XENLA, DHSD_FROPU, FL01_HUMAN, GLR_MOUSE, HYFPECOLI, I12A_FIG, I18B_HUMAN, I18B_HUMAN, I223_HUMAN, ILM7, ILM8, KNOB_MOUSE, LEVIN_nick, M2_MOUSE, LM2_MOUSE, NPT2_HUMAN, NRT1_NTHER1, NU2M_CHICK, NU4M_BRACM, OL1C_HUMAN, PP2R_HUMAN, PM22_MOUSE, PSBC_MAIZE, ROM1_BOVIN, TSHR_HUMAN, VMSA_PBGS.

considered in interpreting the results.

A leucine zipper type of side-chain packing also accounts for the query pattern. The sequence positions of the N-terminal residues are stated, and leucine residues within the leucine zipper pattern given above the sequences are in boldface type.

**β-Galactosidase activity as determined with the ToxR system (MU, mean ± s.d.)**

*Not determined. The following Swiss-Prot identifiers denote polytopic proteins with the search pattern: ALG3 YEAST, BTUC_ECOLI, BVGS_BORBR, CCKR_CAVPO, CLC5_HUMAN, CO3_RAT, COMT_HUMAN, COP_CLOPE, CYB_BOVIN, DCDR_XENLA, DHSD_FROPU, FL01_HUMAN, GLR_MOUSE, HYFPECOLI, I12A_FIG, I18B_HUMAN, I18B_HUMAN, I223_HUMAN, ILM7, ILM8, KNOB_MOUSE, LEVIN_nick, M2_MOUSE, LM2_MOUSE, NPT2_HUMAN, NRT1_NTHER1, NU2M_CHICK, NU4M_BRACM, OL1C_HUMAN, PP2R_HUMAN, PM22_MOUSE, PSBC_MAIZE, ROM1_BOVIN, TSHR_HUMAN, VMSA_PBGS.

considered in interpreting the results.

A leucine zipper type of side-chain packing also accounts for the query pattern. The sequence positions of the N-terminal residues are stated, and leucine residues within the leucine zipper pattern given above the sequences are in boldface type.

**β-Galactosidase activity as determined with the ToxR system (MU, mean ± s.d.)**

*Not determined. The following Swiss-Prot identifiers denote polytopic proteins with the search pattern: ALG3 YEAST, BTUC_ECOLI, BVGS_BORBR, CCKR_CAVPO, CLC5_HUMAN, CO3_RAT, COMT_HUMAN, COP_CLOPE, CYB_BOVIN, DCDR_XENLA, DHSD_FROPU, FL01_HUMAN, GLR_MOUSE, HYFPECOLI, I12A_FIG, I18B_HUMAN, I18B_HUMAN, I223_HUMAN, ILM7, ILM8, KNOB_MOUSE, LEVIN_nick, M2_MOUSE, LM2_MOUSE, NPT2_HUMAN, NRT1_NTHER1, NU2M_CHICK, NU4M_BRACM, OL1C_HUMAN, PP2R_HUMAN, PM22_MOUSE, PSBC_MAIZE, ROM1_BOVIN, TSHR_HUMAN, VMSA_PBGS.

considered in interpreting the results.

A leucine zipper type of side-chain packing also accounts for the query pattern. The sequence positions of the N-terminal residues are stated, and leucine residues within the leucine zipper pattern given above the sequences are in boldface type.

**β-Galactosidase activity as determined with the ToxR system (MU, mean ± s.d.)**

*Not determined. The following Swiss-Prot identifiers denote polytopic proteins with the search pattern: ALG3 YEAST, BTUC_ECOLI, BVGS_BORBR, CCKR_CAVPO, CLC5_HUMAN, CO3_RAT, COMT_HUMAN, COP_CLOPE, CYB_BOVIN, DCDR_XENLA, DHSD_FROPU, FL01_HUMAN, GLR_MOUSE, HYFPECOLI, I12A_FIG, I18B_HUMAN, I18B_HUMAN, I223_HUMAN, ILM7, ILM8, KNOB_MOUSE, LEVIN_nick, M2_MOUSE, LM2_MOUSE, NPT2_HUMAN, NRT1_NTHER1, NU2M_CHICK, NU4M_BRACM, OL1C_HUMAN, PP2R_HUMAN, PM22_MOUSE, PSBC_MAIZE, ROM1_BOVIN, TSHR_HUMAN, VMSA_PBGS.

considered in interpreting the results.

A leucine zipper type of side-chain packing also accounts for the query pattern. The sequence positions of the N-terminal residues are stated, and leucine residues within the leucine zipper pattern given above the sequences are in boldface type.

**β-Galactosidase activity as determined with the ToxR system (MU, mean ± s.d.)**

*Not determined. The following Swiss-Prot identifiers denote polytopic proteins with the search pattern: ALG3 YEAST, BTUC_ECOLI, BVGS_BORBR, CCKR_CAVPO, CLC5_HUMAN, CO3_RAT, COMT_HUMAN, COP_CLOPE, CYB_BOVIN, DCDR_XENLA, DHSD_FROPU, FL01_HUMAN, GLR_MOUSE, HYFPECOLI, I12A_FIG, I18B_HUMAN, I18B_HUMAN, I223_HUMAN, ILM7, ILM8, KNOB_MOUSE, LEVINNick, M2_MOUSE, LM2_MOUSE, NPT2_HUMAN, NRT1_NTHER1, NU2M_CHICK, NU4M_BRACM, OL1C_HUMAN, PP2R_HUMAN, PM22_MOUSE, PSBC_MAIZE, ROM1_BOVIN, TSHR_HUMAN, VMSA_PBGS.
cell-cell adhesion molecules. Their function depends on lateral clustering within the plasma membrane (34), which is believed to involve interactions between extracellular (35) and juxtamembrane domains (36). On the other hand, leucine-rich heptad motifs are evolutionarily conserved in the TMSs of different cadherin families, and our data demonstrating self-interaction of the E-cadherin TMS suggest a role of TMS interactions in clustering. Strong support for this hypothesis is provided by our recent experimental evidence indicating that mutations reducing the TMS interaction likewise affect the adhesive properties of full-length E-cadherin expressed in eukaryotic cells.2

Erythropoietin Receptor—The erythropoietin receptor (EpoR) is required for erythrocyte maturation. In analogy to other growth factor receptors, erythropoietin binding is thought to trigger homo-dimerization followed by receptor activation (37). Apart from the case of the Neu oncogene product, where a point mutation within the TMS triggers ligand-independent receptor activation (38), the role of the TMS in growth factor receptor activation is currently not clear. Since ligand binding is translated into activation of cytoplasmic domains, it has been postulated that the subunit-subunit interface of growth factor receptors extends across the membrane and that TMS interactions contribute to ligand-induced subunit assembly in a non-specific way (1). Our finding that the EpoR TMS is capable of self-interaction indeed suggests its contribution to ligand-induced receptor assembly. Alternatively, the EpoR may exist as a preformed dimer activated by ligand binding. Precedence for the latter model is given by the insulin receptor or the asparagine residue within the TMS are important for activity (54), we speculate that the leucine-rich surface of its TMS aids in homodimer formation and/or binding to the various growth factor receptor TMSs.

Asialoglycoprotein Receptor—The hepatic asialoglycoprotein receptor (ASGPR) removes abnormally glycosylated proteins from blood circulation (55). The chick homolog exists as a homotrimer (56). Both the E5 protein extracellular region and the glutamine residue within the TMS are important for activity (54), we speculate that the leucine-rich surface of its TMS aids in homodimer formation and/or binding to the various growth factor receptor TMSs.

These examples demonstrate that assembly of several different natural membrane proteins depends on their TMSs as predicted by the presence of leucine-rich heptad repeats. Future studies will show whether these TMS interactions are based on the leucine zipper type of packing as inferred for our self-assembling model TMSs L16 and A22. TMS interactions may be modulated by the lipid composition of the respective host membrane. Further, they may not be the exclusive cause of subunit-subunit recognition but may be complemented by interactions between extramembraneous domains in particular cases.

Acknowledgments—We thank Drs. W. Hoch, G. Schönrich, and A. Skerra for critically reading the manuscript; Dr. H. Bedouelle for providing strain PD28; M. Seiler for constructing pSNiR2; A. Szabowski for initial experiments; and Dr. W. B. Huttner for continuous support.

REFERENCES
1. Lemmon, M. A., and Engelman, D. M. (1994) Q. Rev. Biophys. 27, 157–218
2. Dieckmann, G. R., and DeGrado, W. F. (1997) Curr. Opin. Struct. Biol. 7, 486–494
3. Lemmon, M. A., Flanagan, J. M., Treutlein, H. R., Zhang, J., and Engelman, D. M. (1992) Biochemistry 31, 12719–12725
4. MacKenzie, K. R., Prestegard, J. H., and Engelman, D. M. (1997) Science 276, 131–133
5. Laage, R., and Langosch, D. (1997) Eur. J. Biochem. 249, 540–546
