

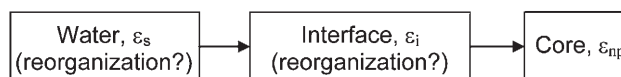
Free Energy Profile of H-ras Membrane Anchor upon Membrane Insertion**

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Ras GTPases mediate signaling pathways in cell proliferation, development, and apoptosis. They undergo isoprenylation at a C-terminal *CaaX* signal (*a* usually represents aliphatic and *X* any amino acid) followed by proteolysis of aaX and carboxymethylation. In the case of H-ras, a subsequent dual palmitoylation of cysteines adjacent to the site of farnesylation produces a mature anchor for plasma membrane targeting.^[1,2] Atomistic information, such as the structure of membrane-bound ras and the free energy of complex formation, are vital in research efforts geared towards designing ras-isoform-selective anticancer agents. The most common experimental techniques are not yet able to provide such information. Here we present computational results on the free energy profile for the transfer of the H-ras membrane anchor from water to a bilayer of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (1,2-DMPC) lipids. We find that there is no significant barrier for insertion, and that once a few carbon atoms of the ras lipid chains cross the membrane–water interface, the free energy displays a steeply downhill profile. Insertion into the hydrocarbon core of the ras lipids and the interfacial localization of the backbone together produce a gain in free energy of up to 30 kcal mol^{−1}. Additionally, using the recently reported computationally derived structures of full-length H-ras in a DMPC bilayer,^[3] we explain how a small difference in free energy would enable modulation of H-ras membrane binding by the linker and the catalytic domain.

Molecular dynamics (MD) simulations of the H-ras anchor (residues 180–186) were carried out using the CHARMM27 force field^[4] and the program NAMD.^[5] Details of the simulation have been published elsewhere.^[3] The potential of mean force (PMF) was computed using the adaptive-biasing-force (ABF) method.^[6] The reaction coordinate was defined by the distance along the membrane normal between the geometric centers of the lower leaflet phosphorus atoms and the peptide backbone C and O atoms (see the Supporting Information).

The thermodynamic path depicted in Scheme 1 was used to describe the insertion process. The main idea is that the hydrophobic effect^[7] drives the spontaneous transfer of the



Scheme 1. A thermodynamic model for the insertion of the ras peptide into a DMPC bilayer.

highly nonpolar peptide from a region of high dielectric (water, ϵ_s) to an intermediate one (bilayer–water interface, ϵ_i), and subsequently to the hydrophobic core (core, ϵ_{np}). The first two processes could involve structural reorganization, such as stretching of the ras lipid tails and adjustment of the backbone, respectively.

Figure 1 shows the free energy profile (PMF). The initial flat region prior to peptide–membrane contact shows that there is no significant barrier between the aqueous phase and the water–membrane interface. This remarkable result suggests that insertion is spontaneous and nonspecific; this is comparable to the spontaneous insertion of mono-S-acylated and -isoprenylated lipopeptides into lipid vesicles.^[8] Furthermore, when ras is microinjected into a mammalian cell, it first binds to all cellular membranes nonspecifically before it gets sorted in the Golgi apparatus.^[9] In addition, the initial flat profile is expected from a chemical standpoint for a peptide located within about three layers of water molecules (ca. 9 Å) from the membrane surface, which was the case in the simulations. If the peptide is further away, an entropy-controlled barrier—arising from thermal fluctuations and the peptide’s reorganization so as to present its hydrophobic face to the bilayer—might be encountered. For example, it has been shown that antimicrobial peptides bind to the interface and subsequently penetrate the bilayer if their hydrophobic faces, and not their positively charged faces, are directed toward the interface.^[10] Thus, the reorganization in water is not fully accounted for, although the agreement between the computed and experimental free energy change

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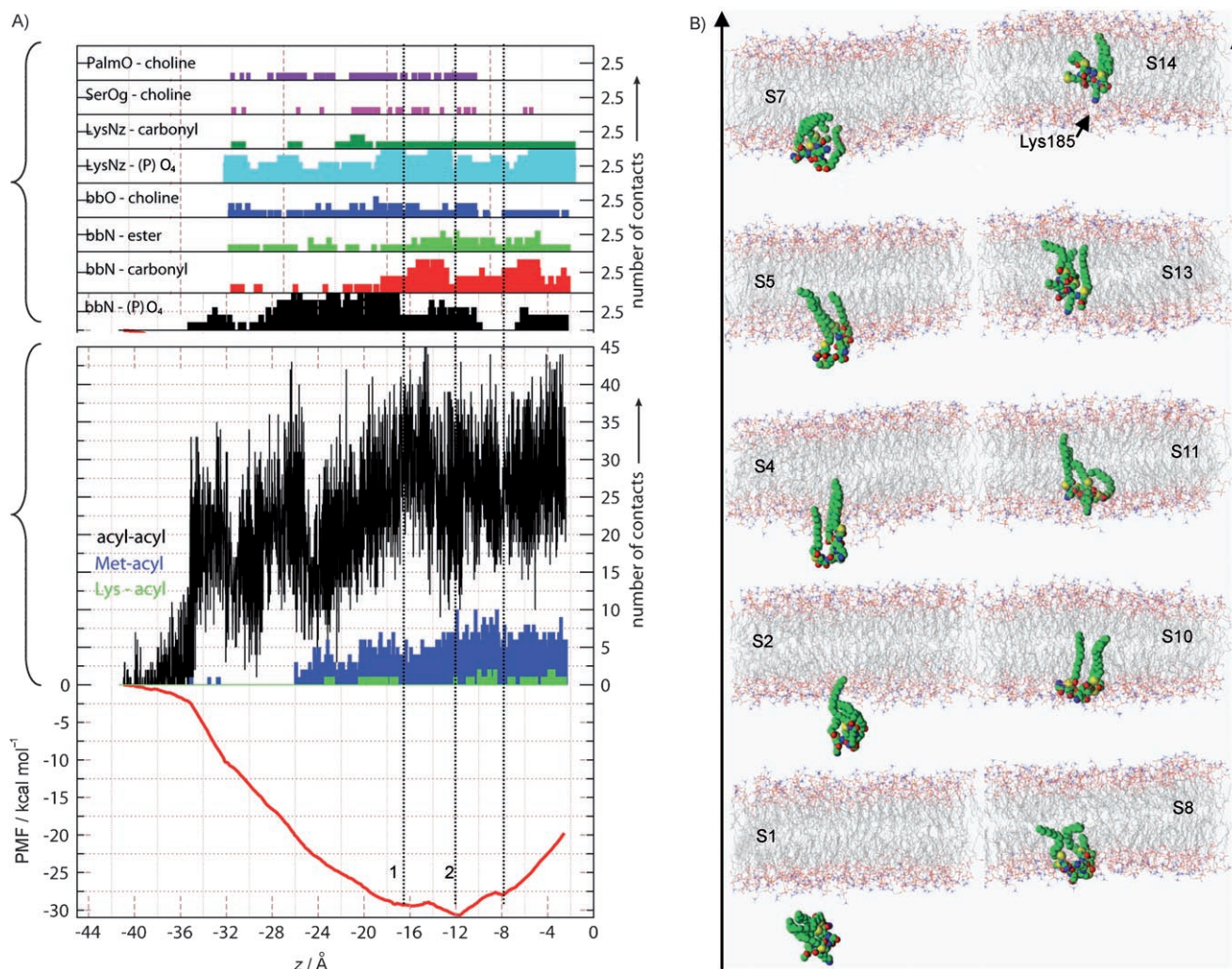


Figure 1. A) Bottom: ABF-calculated PMF for the insertion of H-ras anchor into a DMPC bilayer plotted against the distance of C and O atoms' center-of-geometry from the bilayer center (z , see Supporting Information). Also plotted are the number of peptide–DMPC contacts for polar (top) and carbon–carbon (middle) interactions; bbO = carbonyl, bbN = amide, PalmO = palmitoyl carbonyl, Nz = the amino group of Lys. B) Snapshots illustrating peptide localizations in 10 of the 14 sections of independent ABF runs (see the Supporting Information). Sections S1 and S2 are associated with the initial flat region and section S11 with the start of rise in the PMF.

(ΔG , see below) suggests that its effect on thermodynamic stability is small.

Formation of a few initial contacts between ras and DMPC acyl carbons triggers a steep descent of the PMF (Figure 1A). This critical number of five to seven contacts corresponds to a free energy gain of roughly $-2.5 \text{ kcal mol}^{-1}$. Remarkably, the same number of contacts was previously estimated to be essential for a subsequent “spontaneous” insertion in MD time scales.^[3,11]

To underline that the computed free energy captures all relevant contributions, we compare it with two experimental observations. The equilibrium free energy for the partition of a 16-carbon free fatty acid (FFA) in heptane–water was determined to be approximately $9.0 \text{ kcal mol}^{-1}$.^[12] Shahinian and Silvius determined effective dissociation constants (K_d^{eff}) for the association of lipopeptides with egg yolk phosphocholine/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (90:10) vesicles.^[13] They measured, depending on the fluorophore and amino acid composition, a K_d^{eff} value of 0.196,

0.60, and $0.126 \mu\text{M}$ (or, using $\Delta G = -RT \ln K_d^{\text{eff}}$, 9.51, 8.82, and $9.78 \text{ kcal mol}^{-1}$) for palmitoyl, and 12.5 and $18.5 \mu\text{M}$ (6.68 and $6.95 \text{ kcal mol}^{-1}$) for farnesyl. Note, however, that the hexadecyl chain used here may have stronger membrane affinity than farnesyl. The insertion free energy for a combination of two palmitoyl and one hexadecyl chain would be approximately $-27.0 \text{ kcal mol}^{-1}$ using the FFA data of Kampf et al.^[12] and approximately $-25.6 \text{ kcal mol}^{-1}$ using the mean values of K_d^{eff} .^[13] Both are remarkably close to our computed overall free energy of -29.5 to $-30.6 \text{ kcal mol}^{-1}$. The small discrepancy can be attributed to the additional hydrogen bonding and van der Waals contributions. An example of the former is the interaction of the backbone with the DMPC carbonyls (see below), and of the latter, the interaction of Met 182 side chain with the tails (Figure 1), which contributes approximately $-1.24 \text{ kcal mol}^{-1}$.^[11,14]

During the insertion, other van der Waals interactions, including the snorkeling of Lys 185 side chain, also form until a free energy minimum (1 in Figure 1A, $\text{PMF} = -29.5 \text{ kcal}$

mol^{-1}) is reached at $z = -15.9 \text{ \AA}$. However, a narrower and deeper minimum (2, $\text{PMF} = -30.6 \text{ kcal mol}^{-1}$) appears at $z = -11.7 \text{ \AA}$. The energy difference between the two minima of $1.1 \text{ kcal mol}^{-1}$ ($\approx 2k_B T$), which is roughly equivalent to the energy of a single hydrogen bond, originates from small changes in the interaction of the backbone with the bilayer; the backbone amide interacts with the phosphates in minimum 1, but with the glycerol carbonyls in minimum 2 (Figure 1).

The trend of the profile reverses following the translocation of the backbone into the hydrocarbon core (Figure 1), which has been estimated to cost approximately $5.6 \text{ kcal mol}^{-1}$.^[11,15] Note that in addition to the hydrogen bonds by the amide groups (Figure 1 and Figure 2), contacts by the peptide

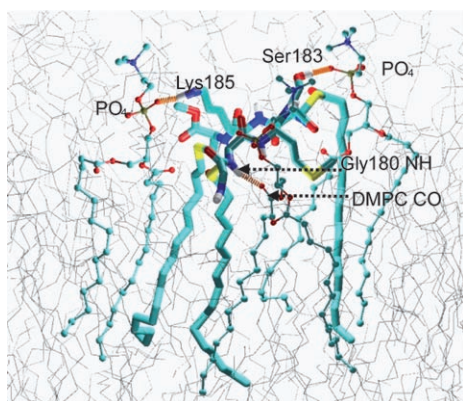


Figure 2. Hydrogen bonds between H-ras anchor and DMPC. The peptide is represented as a stick figure, and the DMPC lipids involved in hydrogen bonding are shown as ball-and-stick structures (the rest of the lipids are given as gray lines). The orange dotted lines represent hydrogen bonds.

carbonyls suggest small contributions whose disappearances coincide with the rise of the PMF. The hydroxy group of Ser183 interacts with the DMPC carbonyls (Figure 2), while the charge on Lys185 is “solvated” by two to six phosphate oxygen atoms even after the backbone has lost its hydrogen bonds (Figure 1 and Figure 2). Thus, the polar and charged residues in the anchor maintain the peptide’s orientation.

The large gain in free energy by the insertion of the triply lipidated H-ras anchor implies that spontaneous dissociation is unlikely, as suggested before.^[13] The contributions of the individual lipid modifications to membrane affinity may be inferred from general microscopy results, which showed that the cytosolic pool of H-ras G12V is much smaller than the doubly lipid-modified N-ras G12V or variants of H-ras G12V with either Palm 181 or Palm 184 removed.^[16]

Two structural models have been computationally predicted and experimentally supported for the GDP- (H-ras-GDP) and GTP-bound (H-ras-GTP) full-length H-ras in a DMPC bilayer.^[3] One of the main differences between the two structures was the insertion depth of the anchor.^[3,17] The average z -location of the anchor backbone was 12 and 16 $\text{\AA}$ in H-ras-GDP and H-ras-GTP, which almost exactly match the location of minima 1 and 2, respectively (Figure 1). One consequence of this was that H-ras-GTP increased membrane

thickness in its vicinity (the change in the average phosphorus atom location across the bilayer, $D_{pp} = 2.7 \text{ \AA}$) while H-ras-GDP reduced it ($D_{pp} = -1.4 \text{ \AA}$). Summarizing these data, Figure 3 illustrates that ras can switch between the two modes

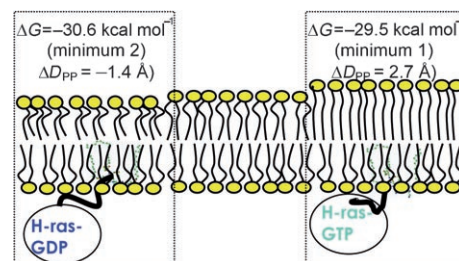


Figure 3. A schematic representation illustrating two modes of membrane binding by ras and their effects on membrane structural deformation and insertion free energy. Note that membrane perturbations at the lower leaflet are damped by the catalytic domain.

of membrane binding with a cost or gain in free energy that can be compensated for by formation or destruction of as few as one hydrogen bond or a couple of van der Waals contacts. This fine balance of free energy and the associated conformational change, which is modulated by the linker and the catalytic domain, may enable ras to undergo alterations in membrane affinity,^[16,18] lateral segregation,^[19] and nanoclustering.^[3,17,20]

The presented structural and energetic data, which were not accessible by common experimental techniques, provide new opportunities for the design of H-ras selective anticancer drugs such as ligands that inhibit the conformational change associated with the membrane binding of the activated protein. Note that changes in protein conformation can open previously uncharacterized binding sites; targeting these sites has led to useful drugs, such as the HIV integrase inhibitor Raltegravir. Finally, the work lies the foundation for future computational and experimental investigations of the thermodynamics of membrane binding by lipidated proteins. We are currently investigating the contribution of individual lipid modifications and entropy to membrane affinity.

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