Supplementary Material for *Biochemistry* MS# bi051193b

AN EXPERIMENT–BASED ALGORITHM FOR PREDICTING THE PARTITIONING OF UNFOLDED PEPTIDES INTO PHOSPHATIDYLCHOLINE BILAYER INTERFACES†

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Keywords: Wimley-White hydrophobicity scales, N-terminal acetylation, C-terminal amidation, prediction of partitioning, peptide design, antimicrobial peptides

Running Title: Terminal-group effects on peptide partitioning

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Wimley and White (*1*) determined the free energies of transfer (ΔG_{if}^{aa}) from the POPC interface to buffer of whole amino acid residues in unfolded pentapeptides of the form Ac–WL–X–LL–COOH, where X is any of the twenty natural amino acids. The resulting hydrophobicity scale provides a starting point for a general algorithm for predicting the free energy of transfer any unfolded peptide between the bilayer interface and water. The scale has only limited value, however, in the absence of values for the partitioning of the various N– and C–terminal groups commonly encountered. The present paper provides useful data on the partitioning of these endgroups.

The data presented describe the energetic consequences of deprotonating or acetylating the N–terminus, and amidation of the C–terminus. Wimley and White (*1*) determined the free energy cost of protonating the C–terminal carboxyl group. But these are relative changes. Prediction of absolute partitioning free energies requires absolute values for the various endgroups. We describe in this supplement how we determined absolute reference values for the algorithm shown in Figure 7.

We need two absolute reference values, one for an N–terminal group and one for a C–terminal group. Given those reference values, then absolute values for the other endgroups can be computed from the data in Table 2.

Absolute reference value for COOH: –2.2 kcal mol^{-1}

We estimated the value for partitioning the COOH group by two methods, using the partitioning free energies (ΔG) for AcWL$_m$ (m = 1 … 6) determined by Wimley and White (*1*). The general approach for both is based upon the idea of Wimley and White's Figure 1 in which ΔG is plotted against m. Because the C–terminus is COOH, the m^{th} is missing a peptide bond. But an additional peptide bond is provided by acetylation of the N–terminus, meaning that the number of peptide bonds $n = m$.

Method 1

The ΔG data (bilayer to water) for the AcWL$_m$ peptides with charged carboxy termini (pH 8.0) were used (Table 2 of (*1*)). The hydrophobic free energy contributions of the Trp and Leu sidechains, α–carbons, and the acetyl methyl were computed from the accessible surface areas (*2*) using a solvation parameter of 13.1 cal mol^{-1} Å^{-2} (*1*). These free energies were subtracted from the measured partitioning free energies of

the $AcWL_m$ peptides. Then cost of protonating the COO^- group (2.68 kcal mol^{-1}) was subtracted from each peptide to arrive at values for the partitioning free energies of $(CONH)_nCOOH$, which were plotted as a function of n (Figure S1). The linear fit of the data extrapolates to –2.23 (±0.22) kcal mol^{-1} for $n = 0$, corresponding to the water–to–bilayer ΔG value for the COOH group. The slope of the line, 1.19 (±0.05) kcal mol^{-1} per residue, is cost ΔG_{CONH} of partitioning the peptide bond into the bilayer interface. The published value, derived from the same data in a somewhat different manner is 1.2(±0.1) kcal mol^{-1} (the larger error results from propagation of experimental errors from original data).

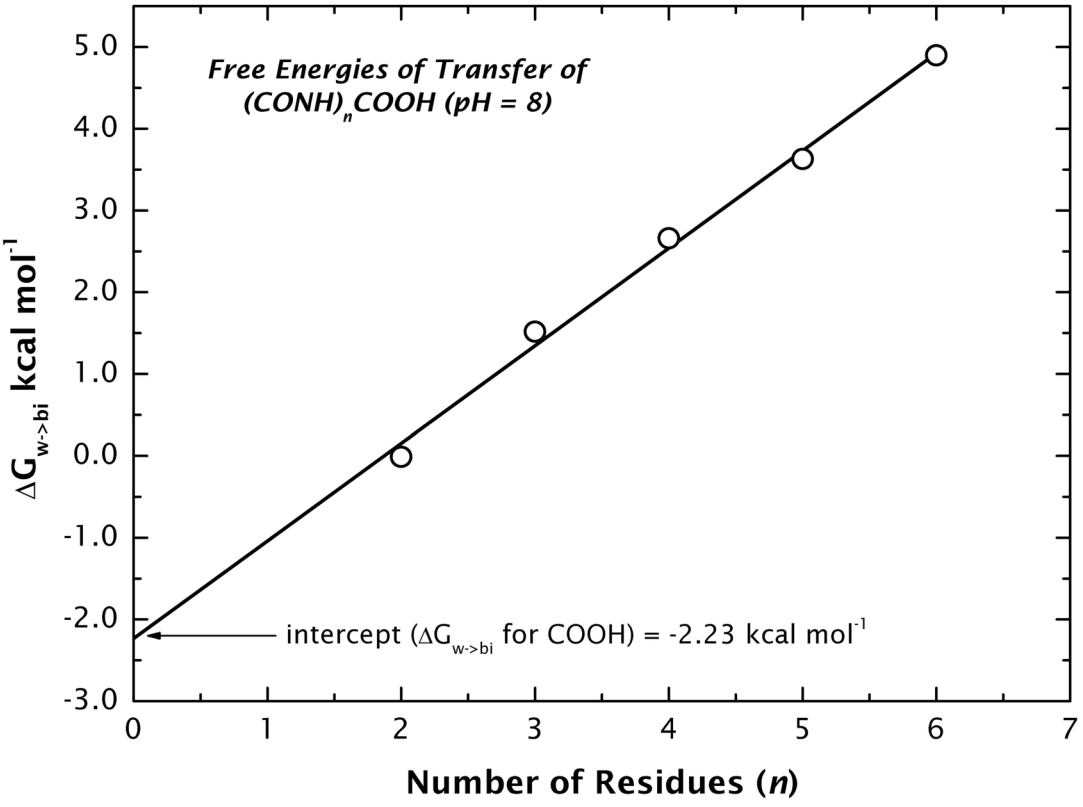

Figure S1

Method 2

The cost of partitioning Ac–COO^- was determined using the same $AcWL_m$ data used in Method 1. In this case, the $AcWL_m$ values were plotted against the number of residues $n = m + 1$, and extrapolated to $n = 0$ (Figure S2). The extrapolated free energy of partitioning for $n = 0$ is –1.91 (±0.19) kcal mol^{-1}. However, this value does not equal

the ΔG for Ac–COO$^-$ because one of the residues is Trp rather than Leu. The correct value is obtained by subtracting the difference $\Delta G_{if}^{Trp} - \Delta G_{if}^{Leu} = -1.29$ kcal mol^{-1} (*1*), which yields –0.62 kcal mol^{-1} for Ac–COO$^-$. The value of Ac–COOH is obtained by subtracting the cost of deprotonating the carboxyl group. The COOH value is obtained from that value by subtracting the methyl group free energy contribution of –1.11 kcal mol^{-1}, computed from its accessible surface area and the interface solvation parameter (*1*) (85 Å^2 × 13.1 cal mol^{-1} Å^{-2}). The final value obtained for COOH is –2.19 kcal mol^{-1}, which validates the value obtained by Method 1. We take the transfer free energy of the COOH group as –2.2 kcal mol^{-1}.

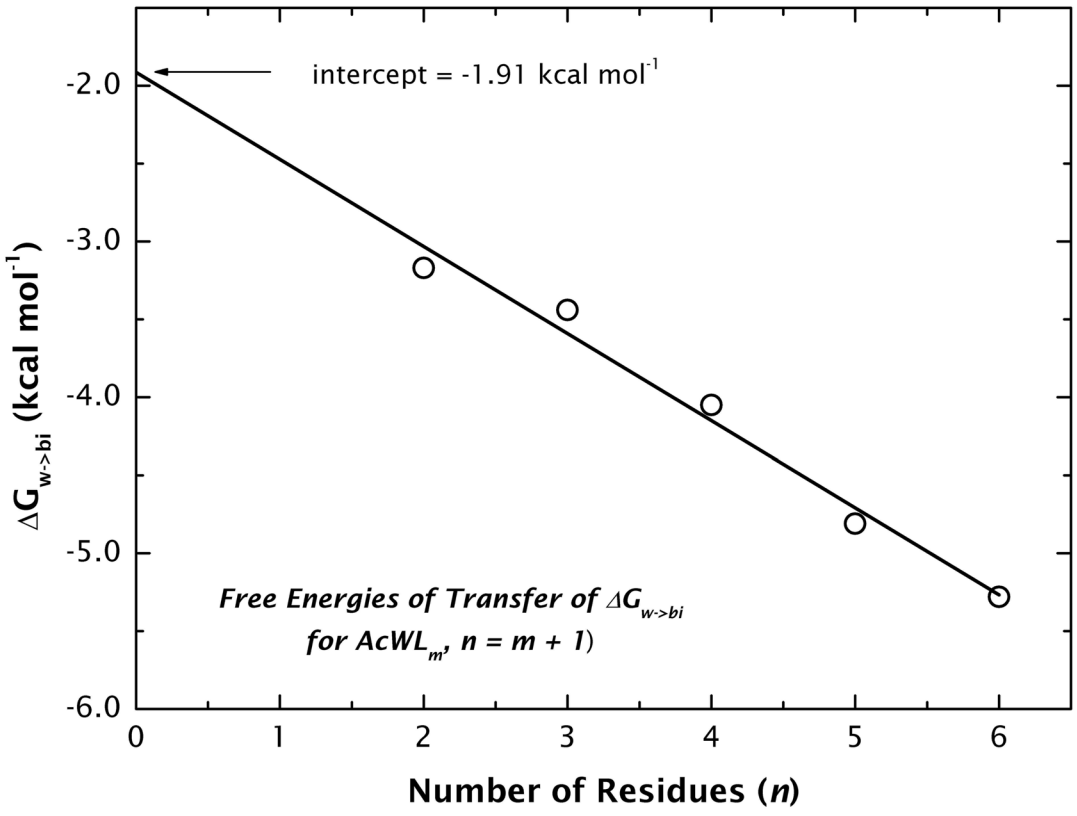

Figure S2

Absolute reference value for CH_3–CONH (Acetyl group): 0.1 kcal mol^{-1}

The free energy of transfer into the interface for the methyl group (above) is –1.1 kcal mol^{-1}, and the free energy of transfer of the peptide bond is 1.2 kcal mol–1. The net value is +0.1 kcal mol^{-1}.

References

1. Wimley, W. C. and White, S. H. (1996) Experimentally determined hydrophobicity scale for proteins at membrane interfaces, *Nature Struct. Biol. 3*, 842-848.

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