Finding the determinants of outer membrane protein insertion is a task of intrinsic scientific interest, as a missing piece in a mechanistic model of bacteria. It is also of potentially great technological value. GFP would be a great tool for neuroscience experiments if it could be made to insert into membranes (Blunck et al., 2005). GFP is a β-barrel, a structure it shares with bacterial outer membrane proteins. Understanding why outer membrane β-barrels insert may allow the design of transmembrane mutants of water-soluble β-barrels such as GFP.

Outer membrane proteins (OMPs) are insoluble in water, and if unfolded can spontaneously refold and insert into vesicles (Surrey and Jähnig, 1992). Together this suggests that at equilibrium, OMPs are in membranes, rather than being held there by some kinetic barrier. It is likely, then, then negative of folding is a necessary condition for insertion.

Under the hypothesis that for a whole OMP is a sum of contributions from solvent-exposed residues on its surface, the problem of estimating for an arbitrary OMP becomes the problem of finding free energies of transfer for individual amino acids. Once this is achieved, the calculation of is as simple as summing the transfer energies. There have been three broad categories of approaches to this problem.

One is experimental. 's for each amino acid have been derived through a mutation study (Moon and Fleming, 2011). Another is through simulation. Molecular dynamics simulations have been used to derive 's which are very close to those estimated from experiment (Gumbart and Roux, 2012).

This study is concerned with the development and application of the knowledge-based approach to the problem. In a knowledge-based approach, one desires to know the *energy* of a particular state; and, from a structure database, one knows the *frequency* of that state. Under the hypothesis that the frequency and the energy are related by Boltzmann's law, the energy of each state is derived (Sippl, 1993). The resulting energy function is called a *knowledge-based potential*.

This work builds upon the knowledge-based Ezβ potential (Hsieh et al., 2012). Unlike the above referenced experiment- and simulation- based values, the Ezβ potential is *depth-dependent*: it estimates the energy of transfer to a given depth in the membrane, not just to the center. Depth is represented by a number, z, that represents the distance from the center: at z=0, Ezβ should, and does, correlate with the experimental values. This depth dependence is likely to increase accuracy because the membrane has been experimentally shown to have varying hydrophobicity; and because each residue has a distinct depth-dependent frequency profile, sometimes with a smooth transition or even a peak partway through, that seem to reflect a dependence of insertion energy upon depth that is more complicated than a simple distinction between "in" and "out" (Hsieh et al., 2012).