for high molecular weight PEGs are extremely high, a comparison based on molal concentration is not practically relevant for pharmaceutical purposes, which is the primary reason why mass-based concentration is used in this presentation. However, stabilization during freeze-thaw is reported to be superior for high molecular weight PEGs, even when comparing stabilizers at constant weight percent (31). Moreover, the trends in stability during freeze-thaw of PFK given in Figure 4 are not consistent with the data given in Table 2. At constant molar concentration of stabilizer (0.5 M), the order of free energies of transfer are (for lysozyme) glycine > proline. For BSA, the order is inositol > lactose > glucose > glycerol. For retention of activity on freeze-thaw (of PFK), the order is proline >> glycine, and disaccharide > glucose = glycerol > inositol. While the correlation with the preferential hydration coefficient is somewhat better, the theory is really based on a correlation with free energies of transfer.

The lack of a quantitative correlation between the thermodynamics of solute exclusion and stability during freeze-thaw described above may have several contributing factors. First, the discussion above is limited to only a few proteins and includes only those excipients that are excluded solutes. That is, a correlation would certainly appear better if one were to include known denaturing solutes such as urea, although the pharmaceutical significance of such a comparison is questionable. Even the sparse data in Table 2 demonstrate that the relative effectiveness of the PEGs varies somewhat with the protein, so a wider protein database could improve the correlation. Also, the data in Table 2 refer to free energies of transfer at room temperature. Free energies are obviously temperature dependent, and whether the rank order of free energies at room temperature will persist at the subzero temperatures encountered during freezing is far from obvious. One must also question the validity of assuming the relationship between the increase in chemical potential of the native form and the denatured form are coupled by a k value that is really a constant for a variety of stabilizers. It must also be recognized that other factors may be controlling *instability* and stability. One possibility that has been largely ignored is the role of denaturation at the aqueous-ice interface and the role of the stabilizer in minimizing protein adsorption on the ice surface. While minimization of protein adsorption is a thermodynamic mechanism, it is a mechanism that does not directly involve thermodynamic stabilization of the aqueous protein. An additional factor, likely important if the stress occurs late in freezing, is the increasing viscosity of the freeze concentrate. Near the end of freezing, the protein is dispersed in an excipient matrix where the viscosity becomes very high (Fig. 1). Both dilution of the protein in the matrix and the low molecular mobility generally associated with high viscosity would tend to slow the rate of denaturation or at least retard aggregation of partially denatured protein, assuming that the mobility critical for degradation is coupled, at least to some extent, to viscosity. Finally, there is at least one example of the stabilizer operating, at least in part, through its ability to prevent crystallization of a buffer component, thereby preventing a large pH shift that destabilizes the protein (79,80). Thus, while solute exclusion is likely a major factor in stabilization during freezing, other stabilization mechanisms are possible and even probable in some cases. It is not the purpose of this discussion to refute the concept of stabilization during freezing via the excluded solute concept, at least as a significant factor. Indeed, in this author's opinion, the concept does have merit and