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Response to S. H. Gellman, T. S. Haque, and L. F. Newcomb

The experiments of Gellman and co-workers described in the preceding paper (Gellman et al., 1996) and in earlier work (Newcomb and Gellman, 1994a,b) provide an interesting approach to the problem of parsing the various free energy contributions to base stacking. Implicit in the logic of the experiments is that the chemical shifts due to naphthyl-naphthyl (compound 3), naphthyl-adenine (compound 5), and adenine-adenine (compound 1) stacking are approximately equal. Given this assumption, the observation that naphthyl-naphthyl stacking clearly produces smaller shifts than in the other two molecules, each of which contains at least one polar ring system, leads Gellman et al. (1996) to conclude that hydrophobic interactions do not drive base stacking and, rather, that polar interactions are implicated. This clearly contradicts our study, which argues that because of desolvation effects polar interactions oppose base stacking, whereas nonpolar interactions drive ring systems to aggregate (Friedman and Honig, 1995). However, the assumption that the chemical shifts produced by stacking in each of the three compounds are equal is unlikely to be correct. Indeed once the different sources of the chemical shift expected from stacking in compounds 1, 3, and 5 are considered, the measurements of Gellman and co-workers may actually support our theoretical calculations.

As naphthyl groups are nonpolar, ring-current effects presumably are responsible for the chemical shifts in compound 3 relative to compound 4. The magnitude of the shift depends on the unknown relative orientation and distance of the two rings in the stacked conformation. When one of the stacked moieties is polar (adenine in this case) another factor needs to be taken into account. Specifically, it is well known that polar molecules undergo large changes in dipole moment on transfer from polar to nonpolar solvents (because of changes in the reaction field of the solvent). Moreover, the change in dipole moment is proportional to the original dipole moment. Stacking of an adenine against either another adenine or a naphthyl group effectively removes solvent from one face of the ring and will inevitably lead to a reduction in the adenine dipole moment. This should result in significant chemical shifts because the charge distribution near each nucleus would be affected. Determining the relative magnitudes of dipole-moment changes and ring-current effects as well as their dependence on stacking geometry is essential if the data of Gellman and co-workers are to be interpreted properly. In the absence of

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this information, the assumption that dipole-moment changes produce larger chemical shift effects than ring currents, taken together with our theoretical work, yields a consistent explanation of the entire set of experiments reported by Gellman and co-workers. The following points summarize our argument:

1) The larger chemical shifts resulting from adenine-adenine and adenine-napthyl stacking relative to naphthyl-naphthyl stacking are consistent with our assumption about chemical shifts. Specifically, adenine and naphthyl should exert similar effects on another adenine because both desolvate one face of the adenine ring. In contrast, the small dipole moment of naphthalene implies that this molecule should undergo only negligible changes in dipole moment upon phase transfer or stacking.

2) The absence of a shift for compound 1 in DMSO (Newcomb and Gellman, 1994a,b) is consistent with a hydrophobic, but not an electrostatic, mechanism for stacking, because the lower dielectric constant of DMSO relative to water would be expected to enhance electrostatic effects in DMSO.

3) The observed stacking in compound 5 argues against an electrostatic driving force, because the naphthyl ring is nonpolar. Stacking in this case can only be caused by nonpolar interactions, which is consistent with our model.

4) The reduced temperature dependence for the unstacking of compound 5 relative to compound 1 (Newcomb and Gellman, 1994a) suggests that electrostatic effects in compound 1 weaken stacking, as we suggest. That is, stacking appears to be stronger in compound 5 where inter-ring electrostatic effects are absent, than in compound 1, where they are present.

5) It is clear that nonpolar interactions are responsible for the fact that the dinaphthyl compound is the most insoluble in the series. The same type of interactions that affect solubility must affect stacking, and it is then difficult to understand why solubility and stacking should be inversely correlated, as required by the interpretation of Gellman and co-workers.

Finally, we consider the spectra of compounds 3 and 4, which Gellman et al. contend argue against a role for nonpolar interactions in base stacking. In our paper (Friedman and Honig, 1995) we noted a broadening in the spectrum of compound 3 relative to compound 4, which we took as evidence for stacking. In their letter Gellman et al. (1996) acknowledge that this broadening was due to instrument malfunction in their original study (Newcomb et al., 1994a). Focusing entirely on the new data contained in their Fig. 2, Gellman et al. "detect no significant difference between dinaphthyl compound 3 and mononaphthyl compound 4 for any of the seven naphthyl ring protons". In looking at the same data we note four lines in the multiplet centered around 7.9 ppm in compound 4 and five lines in compound 3. Clearly, the peak at 7.911 ppm in compound 4 splits into a doublet located at 7.908 and 7.897 in compound 3. In going from the monomer to the dimer, the peaks within the various multiplets are shifted systematically, depending on the multiplet, indicating intramolecular interactions between the napthyl groups. Whether these shifts are "significant" or not depends on one's expectation for the effects of ring currents in these compounds. In the absence of this information we note that the shifts in compound 3 relative to compound 4 are larger than the individual line widths and are on the order of the chemical shift differences between peaks. We regard this as evidence for stacking and we do not believe that the spectral differences between compounds 3 and 4 are insignificant.

An alternate explanation for the data is that the shifts in compound 3 are due to through-bond effects. However data provided in another publication argue against this interpretation. The dibenzyl compound analogous to compound 3 is not shifted relative to the monobenzyl compound analogous to 4 (Fig. 5 of Newcomb and Gellman (1994b)). If through-bond interactions could lead to the kinds of shifts observed in the napthyl compounds, they would be observed for the benzyl compounds as well.

In summary, most available data supports rather than eliminates nonpolar interactions as the major driving force for base stacking. Further knowledge of the magnitude of various chemical shift contributions would be extremely useful in the interpretation of experimental results. Of course, structural determination based on two-dimensional spectra would provide a definitive solution to the questions that have been raised in this exchange.

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