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# Contributions to the Hydration Free Energies of Amino Acids

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Molecular solvation is a fundamental factor in biological processes, such as protein folding, receptor binding or enzymatic reactions. Currently, estimates of the hydrophobicity of amino acids are often derived from solvation (or transfer) free energies of side chain analogs. Such an approach implicitly assumes that contributions from the backbone and the side chain to the free energy of solvation are additive. However, it is well known that, in particular for polar amino acids, the properties of side chain analogs and amino acids can deviate significantly. Based on the relative hydration free energies of the amino acid pairs Ala-Ser, Val-Thr, Phe-Tyr, Val-Ala, Thr-Ser, Phe-Ala, and Tyr-Ser determined from molecular dynamics simulations, we quantitatively trace the molecular origin of these deviations to two effects, solvent exclusion and self-solvation. Solvent exclusion accounts for the reduction in solute-solvent interactions as one part of the solute occludes other parts of the solute, e.g., the presence of the backbone lowers the degree of direct interaction possible between the side chain and surrounding water. While solvent exclusion applies to polar and apolar amino acids alike, self-solvation is specific to polar amino acids and results from strong, directed intramolecular interactions between the polar functional groups of the side chains and polar moieties in the backbone, often through hydrogen bond formation. Thus, the contribution of self solvation to the solvation free energy is strongly conformation- and environment-dependent, and, therefore, the correct treatment thereof poses a challenge to applications involving solvation processes. Implications for the utility of hydrophobicity scales and connections to implicit solvent models are briefly discussed.

## 1 Introduction

Proteins, like most other biological macromolecules, function in aqueous solution. Therefore, one has to take into account the influence of solvent on the structure and thermodynamics of proteins, in order to understand their biological function. One fundamental principle for the description of this effect is hydrophobicity, which is often quantified by the partitioning of (model) compounds between water and an apolar medium.

Despite many successes, there are well documented problems associated with the use of hydrophobicity scales derived in this manner: First, very different transfer free energies were obtained depending on the solvent used for the apolar phase<sup>1</sup>. Second, in many approaches the raw data are obtained from side chain analogs, but are applied to the corresponding amino acids. This assumes that solvation free energies are additive, i.e. that the solvation free energy for the amino acid of interest is the sum of the solvation free energy of glycine (accounting for the contribution from the peptide backbone), and the solvation free energy of the side chain analog. However, as can be seen in Fig. 1a there are significant deviations ( $\Delta\Delta\Delta A_{solv}$ ) from this assumed additivity relationship<sup>2-5</sup>. In the most extreme case (Ala-Ser),  $\Delta\Delta\Delta A_{solv}$  is almost 5 kcal/mole. A high  $\Delta\Delta\Delta A_{solv}$  can also be seen in other apolar-polar pairs (Val-Thr). For amino acid pairs of like polarity and relatively similar size (Ala-Val, Ser-Thr), the differences of approximately 1 kcal/mole

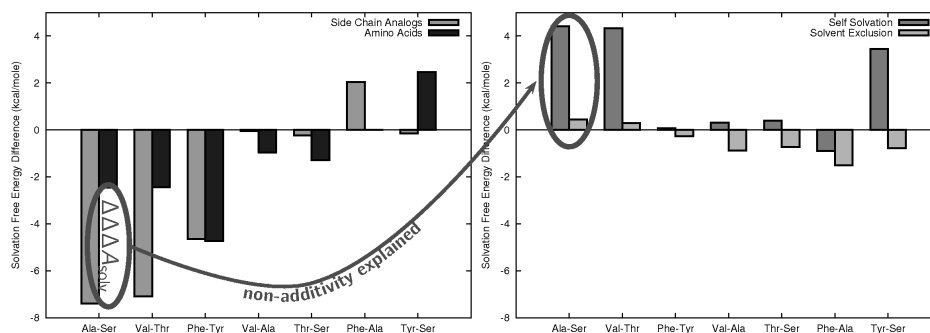


Figure 1. a.) Relative solvation free energy differences of selected amino acid pairs ( $\Delta\Delta A_{solv}^{AA}$ ) and their corresponding side chain analogs ( $\Delta\Delta A_{solv}^{SC}$ ). The resulting difference between  $\Delta\Delta A_{solv}^{AA}$  and  $\Delta\Delta A_{solv}^{SC}$  ( $\Delta\Delta\Delta A_{solv}$ ) is caused by interactions between the amino acid backbone and the side chain (the so-called "non-additivity"). The contributions of the two main effects causing non-additivity in amino acids (self-solvation and solvent exclusion) are depicted in part b.) on the right side of the figure.

between side chain analog and amino acid results are statistically significant, but much smaller than those obtained for the apolar–polar pairs. As the difference in size between two amino acids of similar polarity increases, so does the deviation from the respective side chain analog results (Ala–Phe, Ser–Tyr).

Several effects were assumed to be responsible for these deviations: First, one has to take into account the *solvent exclusion*, since the backbone reduces the solvent accessible surface area (SASA) of the side chain and vice versa. The second important concept has been referred to as *self-solvation*<sup>3,4</sup>. In apolar media, polar side chains interact with the polar groups in the peptide backbone, which effectively lowers the hydrophilicity of the side chain, as well as of the backbone.

The goal of the present study is to determine the molecular origin of any deviations from the additivity relationship, rather than verifying the mere existence of such differences. For this purpose, we concentrated on only a few amino acids which represent a broad range of distinct physicochemical properties (e.g. polarity and size). Furthermore, we (primarily) calculated relative rather than absolute hydration free energy differences.

## 2 Methods

Relative solvation free energy differences ( $\Delta\Delta A_{solv}$ ) were calculated for selected pairs of N-acetyl-X-methylamide amino acids ( $\Delta\Delta A_{solv}^{AA}$  of Ala–Ser, Val–Thr, Phe–Tyr, Val–Ala, Thr–Ser, Phe–Ala, and Tyr–Ser) and pairs of the corresponding side chain analogs ( $\Delta\Delta A_{solv}^{SC}$  of methane–methanol, propane–ethanol, toluene–*p*-cresol, propane–methane, ethanol–methanol, toluene–methane and *p*-cresol–methanol) by using thermodynamic integration and Non-Boltzmann Thermodynamic Integration<sup>6</sup>. The CHARMM22 all-atom protein force field<sup>7</sup> was used.

Gas phase free energy differences were obtained based on Langevin dynamics simulations at 300 K. All gas phase simulations had an overall length of 84 ns and were repeated at least five times. The solvent simulations lengths varied between 2.1 (for side chain analogs) and 42 ns (for full amino acids, using NBTI). Solvent simulations were repeated

at least three times.

### 3 Results and Discussion

To estimate contributions from solvent exclusion we computed solvation free energies for amino acids with the charges of all backbone atoms set to zero ( $\Delta\Delta A_{solv}^{unch. BB}$ ), since the presence of the uncharged backbone prevents a complete solvation of the side chain. Thus, by taking the difference between  $\Delta\Delta A_{solv}^{unch. BB}$  and  $\Delta\Delta A_{solv}^{SC}$ , one can estimate the free energy contribution from solvent exclusion by the backbone on the side chain.

Since the removal of either the backbone or side chain charges ( $\Delta\Delta A_{solv}^{unch. SC}$ ), or all charges ( $\Delta\Delta A_{solv}^{LJ}$ ) also leads to the extinction of all electrostatic interactions between side chain and backbone (thus also eliminating self-solvation), the combination of  $\Delta\Delta A_{solv}$ ,  $\Delta\Delta A_{solv}^{unch. BB}$ ,  $\Delta\Delta A_{solv}^{unch. SC}$  and  $\Delta\Delta A_{solv}^{LJ}$  can be used to estimate the self solvation.

Considering  $\Delta\Delta\Delta A_{solv}$  in Fig. 1a and the self-solvation and solvent exclusion contributions in Fig. 1b, it is possible to distinguish two cases: First, the mutations involving apolar amino acids (Val–Ala, Phe–Ala): Here, the side chain analogs give acceptable approximations, if solvent exclusion is considered. Second, the mutations involving polar amino acids (Ala–Ser, Val–Thr, Tyr–Ser), where self-solvation plays a considerable role. However, there are mutations involving polar amino acids, but without a large self-solvation term (Phe–Tyr and Thr–Ser): In the case of Tyr, the polar groups are too distant from the backbone, and therefore there are no contributions from self-solvation. In Thr–Ser, on the other hand, the side chain hydroxyl groups are almost equidistant from the backbone (2.31 and 2.45 Å), leading to very similar self-solvation contributions, which cancel each other out. Our results imply that a correct treatment of both self-solvation and solvent-exclusion is fundamental for an accurate estimation of the solvation free energy of biomolecules (e.g. in implicit solvent models).

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