How to address the effect of pH on protein dimerization: A constant-pH MD study of beta-lactoglobulin

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Protein–protein association is an essential part of the function of many proteins, being often mediated by electrostatic interactions that are physiologically modulated by pH. Nonetheless, although protein–protein association has been widely studied using experimental and computational methods, the effects on protonation upon association have been often overlooked.

In this work, we present a methodological approach to obtain a detailed description of pH-dependent protein association, and we apply it to study the dimerization of beta-lactoglobulin. The approach consists in performing constant-pH MD simulations of the monomeric and dimeric forms, followed by a set of analyses aiming to integrate and synthesize the huge amount of data relating protonation and structure, monomer and dimer, and their dependence on pH. These analyses include: (1) identification of dimer association modes using principal component analysis; (2) characterization of the fluctuating protein charge through ionic densities; (3) calculation of the pH dependency of the dimerization free energy from a linkage relation; (4) identification of protonation correlation pairs and networks.

Dimerization is found to be higher near the isoionic point, in agreement with experiments, and seems to be partly mediated by electrostatic complementarity. The dimer configuration exhibits a transition between two forms (compact and relaxed) around pH 3 and 5. We observe correlated protonations for many pairs of sites, some quite distant, which tend to form large networks of correlated sites in both the monomer and the dimer. Interestingly, the sites involved in these networks are also those that mostly affect the pH dependency of the dimerization free energy, suggesting that protonation correlations are determinant to the dimerization of beta-lactoglobulin.