Quantifying and visualizing weak interactions between anions and proteins

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The molecular properties of proteins are influenced by various ions present in the same solution. While site-specific strong interactions between multivalent metal ions and proteins are well characterized, the behavior of other ions that are only weakly interacting with proteins remains elusive. In the current study, using NMR spectroscopy, we have investigated anion–protein interactions for three proteins that are similar in size but differ in overall charge. Using a unique NMR-based approach, we quantified anions accumulated around the proteins. The determined numbers of anions that are electrostatically attracted to the charged proteins were notably smaller than the overall charge valences and were consistent with predictions from the Poisson–Boltzmann theory.

This NMR-based approach also allowed us to measure ionic diffusion and characterize the anions interacting with the positively charged proteins. Our data show that these anions rapidly diffuse while bound to the proteins. Using the same experimental approach, we observed the release of the anions from the protein surface upon the formation of the Antp homeodomain–DNA complex. Using paramagnetic relaxation enhancement (PRE), we visualized the spatial distribution of anions around the free proteins and the Antp homeodomain–DNA complex. The obtained PRE data revealed the localization of anions in the vicinity of the highly positively charged regions of the free Antp homeodomain and provided further evidence of the release of anions from the protein surface upon the protein–DNA association. This study sheds light on the dynamic behavior of anions that electrostatically interact with proteins.