# Hydrogen bonding chromatography of large biomolecules and supramolecular assemblies on monoliths

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Hydrogen bonding is a critical contributor to the architecture of biomolecules, supramolecular assemblies, and interactions among them but its role in the field of adsorption chromatography has been oddly superficial. This presentation will share extensive experimental data from evaluation of a ligand developed expressly to mediate hydrogen bonding. Studies to date indicate that it provides slightly stronger retention than classical ion exchangers for small proteins but roughly doubles retention of large proteins such as IgM. Retention of DNA is enhanced by roughly a factor of 3. Retention of supramolecular assemblies such as exosomes and virus particles is also dramatically enhanced. Since retention of smaller biomolecules is not significantly increased, the degree of separation between large solutes and their usual contaminants is substantially improved. Elution is strongly affected by soluble hydrogen donors and acceptors, as well as by pH, enabling a variety of novel elution strategies. Results can be summarized by saying that hydrogen bonding chromatography enables rapid high capacity fractionation according to solute size. Case studies will be shared demonstrating the application of hydrogen bonding chromatography for rapid analysis of exosome-containing samples and purification of exosomes from various biological sources. Basic strategies for method development will also be discussed.