DYNAMIC HYALURONAN-ARGININE INTERACTIONS MODULATE SOLUTION PROPERTIES AT THE MOLECULAR LEVEL

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Glycosaminoglycans (GAGs) are complex polysaccharides found in the extracellular matrix. They are vital for regulating cellular processes, such as cell-cell communication and molecular recognition. All GAGs have high negative charge, which facilitates interactions with positively charged regions of proteins. These interactions are of both industrial and biological interest, particularly for tuning hydrogel properties and GAG-protein binding.
In this study, we focus on the interactions between hyaluronan (HA), one of the most prevalent GAGs, and polyarginines or polylysines—positively charged peptides that serve as simplified interaction models, while also being of medical interest as cell-penetrating drug carriers. First, we investigated small HA molecules of well-defined length (octasaccharides) interacting with short (tetra-) peptides to identify characteristic interaction fingerprints. Using a combination of nuclear magnetic resonance (NMR) experiments and molecular dynamics (MD) simulations, we demonstrate that electrostaticsalone are insufficient to justify their different interaction patterns and strength. Polyarginines are favored over polylysines due to the dual polar/hydrophobic nature of the arginine side chain, unlike the only polar lysine. Furthermore, we identify a highly dynamic interaction pattern characterized by short-lived HA-peptide contacts, contrasting with the conventional view of stable complex formation [1].
We extended our study to larger, polydisperse HA and longer (nona-) peptides. In this case, the formation of insoluble molecular clusters hindered the straightforward use of NMR methods. To overcome this, we developed a novel approach combining MD simulations with second harmonic scattering (SHS), an experimental technique that probes solvent structure and is effective at much lower concentrations than NMR. This enabled us to study interactions at biologically relevant submicromolar concentrations where molecular complexes remain in solution. Using SHS, we confirmed polypeptide-HA interactions and aggregation even at micromolar concentrations. Extensive MD simulations provide an atomistic view of these interactions, revealing that the clusters are maintained through multivalent, dynamic contacts rather than single, strong binding events.
Overall, our study characterizes the interactions of industrially relevant polymers, potentially improving their applications, and introduces a new methodology for investigating such interactions at physiologically relevant concentrations. Furthermore, we provide a consistent molecular picture of the interactions, comprehensively addressing arginine selectivity and the effects of HA polymer length.

References:
1. M. Riopedre-Fernandez et al., *Carbohydr. Polym.* 2024, 325, 121568.