Identification of glycosaminoglycans with different sulfation degrees with a biological nanopore

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Glycosaminoglycans (GAGs) are acidic polysaccharides that play crucial roles in various physiological and pathological processes. However, their high structural complexity has made comprehensive characterization difficult, often limiting analyses to short fragments. Nanopore technology offers a powerful single-molecule approach and has shown promise for monosaccharide and glycan analysis, yet distinguishing GAGs in complex mixtures remains challenging. In addition, the rapid translocation of GAGs through nanopores limits temporal resolution and hampers sequencing efforts. Here, we present a strategy that utilizes electrolyte-mediated, GAG-specific interactions within biological nanopores to overcome these limitations. The sulfate and carboxyl groups of GAGs interact with acidic residues within the aerolysin channel via divalent cations, thereby slowing GAG translocation by more than 100-fold. Mutating the interacting residues abolishes these effects, confirming their specificity. The interaction mechanism was further validated using all-atom molecular dynamics simulations and cryo-electron microscopy. Notably, similar sulfate-specific signatures were also observed in commonly used biological nanopores, inlcuding a-HL and MspA. Leveraging this mechanism, we successfully distinguished different GAG species within a mixed GAG sample, including long GAG polymers over 20 kDa. Furthermore, machine-learning analysis enabled high-accuracy identification of the regioselective desulfated HP variants. Together, these results represent a valuable step toward GAG identification and sequencing at the single-molecule level.