

Water influences the stickiness of hyaluronan

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Credit: Ecole Polytechnique Federale de Lausanne

Hyaluronic acid, also known as hyaluronan, is a polysaccharide that helps establish the viscosity of bodily fluids. According to a new study by EPFL scientists, it also influences the behavior of far more water molecules than previously thought. Their findings—just published in *Science Advances*—open new avenues of research on the role water plays in the human body.



While water has long been known as a crucial component of biological systems, it is only recently that scientists are beginning to discover the intricate ways in which it drives the structuring of biological compounds such as proteins, membranes, DNA and sugars That is also the case for hyaluronan, a polysaccharide found around cells and in parts of our body where lubrification and viscosity are important, like in our joints. Hyaluronan is a key determinant of the texture of the aqueous fluids in these areas. Using a new method developed in their lab, the LBP scientists found that hyaluronan influences the orientation of many more water molecules than previously thought. Their research, appearing in *Science Advances*, marks a breakthrough in how scientists perceive water's role in biology.

A new way of understanding hydration

The LBP scientists probed at the nanoscale to better understand how hyaluronan interacts with water. Hyaluronan molecules contain many anions, or negatively charged ions, while the water molecules (H2O) are neutral but positively charged on one end and negatively charged on the other end. This charge distribution orients the water molecules when they 'see' the negative charge of the hyaluronan. Previously, charges were thought to influence water over a distance of 3 water molecules, involving only this interaction. However, using their new method, the LBP scientists found the influence actually extends up to 1,600 water molecules. They also discovered a second mechanism that orients water, namely that the electrostatic field of the anions changes slightly the way in which water molecules connect to one another. This mechanism is also at play in hyaluronan solutions. This groundbreaking discovery could challenge conventional ways of thinking about water and how it interacts with complex molecules. Hyaluronan is known for its viscosityenhancing properties, which has always been thought to arise only from interactions between the hyaluronan molecules. However, this work shows that water and how it is influenced plays an important role as well.



Testing the orientation of water molecules

Hyaluronan orients water molecules by enhancing water-water correlations. It acts as a "flexible chain surrounded by extended shells of orientationally correlated water, which fluctuates depending on the hyaluronan molecule's movements," says Sylvie Roke, head of the LBP. Her team of scientists measured the spatial correlations over nanoscopic length scales.

Their method differs from standard techniques, such as light scattering, which measures variations in hyaluronan rather than water molecules. What's more, existing techniques are not sensitive enough to work at very low concentrations. The LBP method, called femtosecond elastic second-harmonic scattering, offers 1000 times greater sensitivity, allowing to measure the tiny structural correlations that result from the changes in water structure. Illuminating a solution with a femtosecond near infrared laser pulse results in the generation of photons that have the double energy of the incoming photons. Such second harmonic photons can only be generated from regions in the liquid that have a broken symmetry compared to the isotropic structure of the neat bulk liquid. They therefore report in a very sensitive manner on structural differences. By contrast in regular light scattering methods the same color photons are emitted from every molecule so that structural differences are only detectable by making a difference measurement. This results in the 1000 x higher sensitivity, as well as—in this case—the sensitivity to water.

Roke explains: "The ability to observe how water superstructures change in response to molecules like <u>hyaluronan</u> opens up a whole new field of research. Our method could be used in combination with other, nonlinear optical approaches to better investigate the complexity of aqueous systems, which we are just now starting to discover."



More information: J. Dedic et al. Hyaluronan orders water molecules in its nanoscale extended hydration shells, *Science Advances* (2021). DOI: 10.1126/sciadv.abf2558

Provided by Ecole Polytechnique Federale de Lausanne

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