Unraveling tautomeric mixtures

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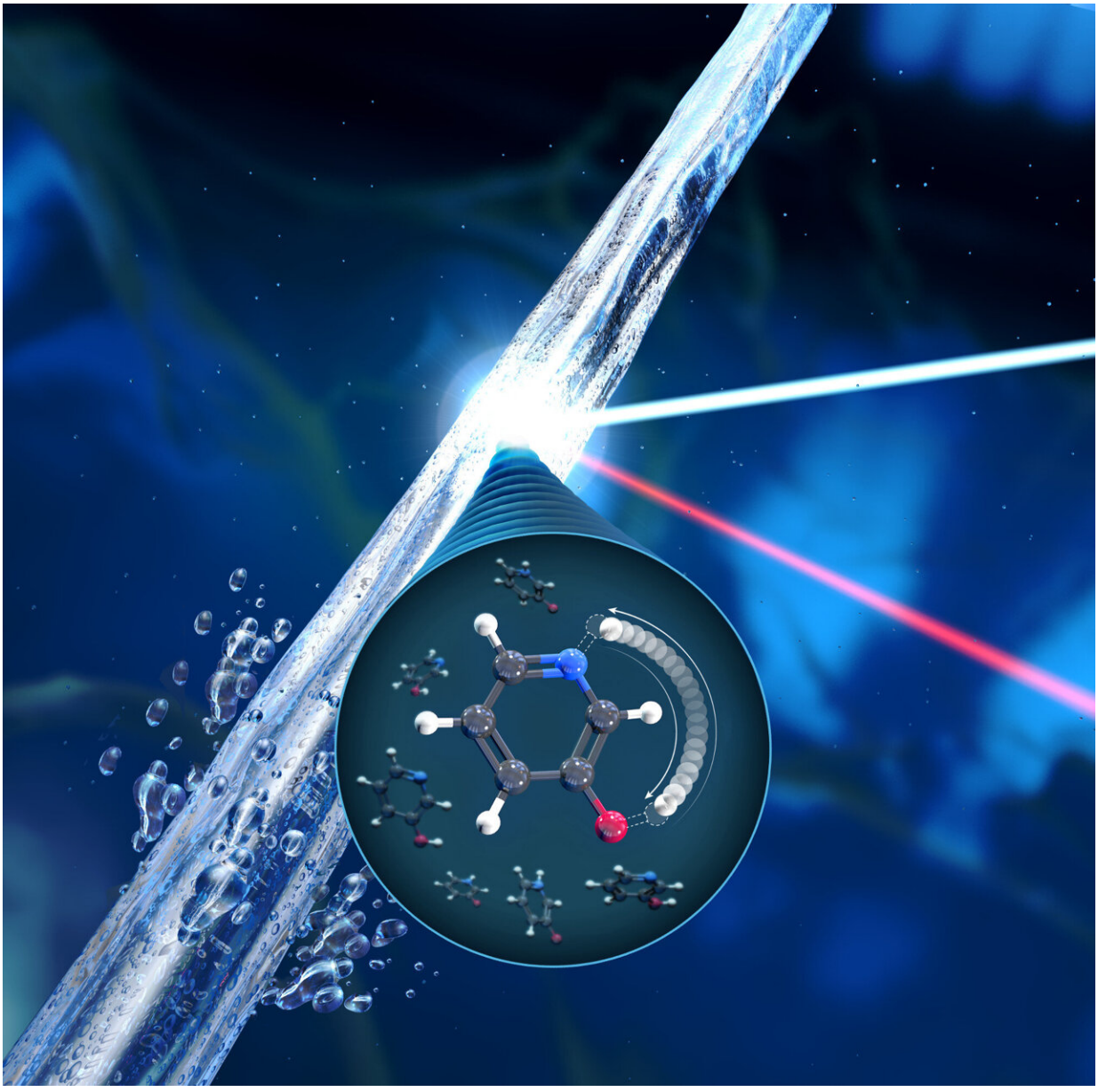

The illustration visualizes the experimental method, here on the prototypical keto-

enol equilibrium. Credit: Martin Künsting /HZB

A team at HZB has developed a method of experimentally unraveling tautomeric mixtures. Based on resonant inelastic X-ray scattering (RIXS) at BESSY II, not only proportions of the tautomers can be deduced, but the properties of each individual tautomer can be studied selectively. This method could yield to detailed information on the properties of molecules and their biological function. In the present study, now advertised on the cover of *The Journal of Physical Chemistry Letters*, the technique was applied to the prototypical keto-enol equilibrium.

Many (organic) molecules exist as a mixture of two almost identical molecules, with the same molecular formula but one important difference: A single hydrogen atom sits in a different position. The two isomeric forms transform into each other, creating a delicate equilibrium, a "tautomeric" mixture. Many amino acids are tautomeric mixtures, and since they are building blocks of proteins, they may influence their shape and function and thus their biological functions in organisms.

Until now: Mission impossible

Until now, it has been impossible to selectively investigate the electronic structure of such tautomeric mixtures experimentally: Classical spectroscopic methods "see" only the sum of the signals of each molecular forms—the details of the properties of the two individual tautomers cannot be determined.

Now at BESSY II: It works

A team led by HZB physicist Prof. Alexander Föhlisch has now succeeded in providing a method of experimentally unraveling tautomeric mixtures. Using inelastic X-ray scattering (RIXS) and a data processing/evaluation method newly developed at HZB, the individual proportions of the tautomers can be clearly deduced from the measured data. "We can experimentally separate the signal of each individual molecule in the mixture by X-ray scattering, which leads to a detailed insight into their functionality and chemical properties," says Dr. Vinicíus Vaz Da Cruz, first author of the paper and postdoc in Föhlisch's team.

"Specifically, we measure a pure spectrum of each tautomer, taking advantage of the element specificity and site selectivity of the method," Vaz Da Cruz explains. This allowed them to fully characterize the components in the tautomer mixture.

New insights into biological processes

In the present study, the technique was applied to the prototypical keto-enol equilibrium of 3-hydroxypyridine in aqueous solution. The data were obtained at the EDAX terminal station at BESSY II.

These results provide experimental evidence for concepts that have previously only been discussed theoretically in the literature. They are particularly interesting for better understanding important biological processes, such as the interaction between nucleoid bases of DNA, metabolic conversion of fructose into glucose, and the folding of proteins.

More information: Vinícius Vaz da Cruz et al, Targeting Individual Tautomers in Equilibrium by Resonant Inelastic X-ray Scattering, *The Journal of Physical Chemistry Letters* (2022). DOI: 10.1021/acs.jpclett.1c03453