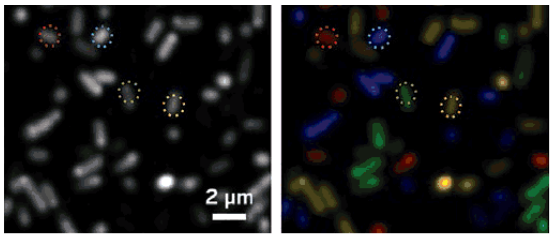


Researchers couldn't distinguish among labeled bacteria cells by fluorescence alone (left), but they could with SREF (right). The red, blue, green, and yellow circles indicate places labeled with dyes with different isotopes in a nitrile group.

CREDIT: *NAT. PHOTONICS* (MICROGRAPHS); CHAO WANG/C&EN (SCHEME)

IMAGING

Combining the best of Raman and fluorescence

New technique provides simultaneous selectivity and sensitivity

Fluorescence spectroscopy has great sensitivity, allowing scientists to detect signals from single molecules. But it has limited chemical specificity, making it hard sometimes for researchers to distinguish between overlapping signals from different fluorescent dyes. For Raman spectroscopy, the reverse is true—great specificity but limited sensitivity. A new method called stimulated Raman excited fluorescence (SREF) combines the strengths of both techniques (*Nat. Photonics* 2019, DOI: 10.1038/s41566-019-0396-4).

In SREF, the researchers, led by Wei Min of Columbia University, use a pair of laser pulses to excite a molecule to a vibrational excited state. Such excitation happens only if the frequency difference between the two laser pulses matches the frequency of the vibrational state. Then, before the molecule can relax back to the ground state, a third laser pulse promotes the molecule to an electronic excited state. From there, the molecule emits a fluorescent photon as it relaxes back to the ground state.

The Columbia team used SREF to detect four isotope-edited versions of the fluorescent dye rhodamine 800. Each dye has nearly identical fluorescence emission but different Raman spectra due to the different incorporated isotopes. The differences in the Raman frequencies allow the researchers to distinguish the dyes when they label cells with them.

Min, who previously demonstrated stimulated Raman with 20 labels, thinks that SREF will allow him to use 100 or more "colors" simultaneously. "Color is now a two-dimensional concept," he says, with each color defined by both the Raman and the fluorescence frequencies.

Adding the Raman selectivity to experiments involving fluorescent tags is the most significant advance with SREF, says John C. Wright, a spectroscopist at the University of Wisconsin–Madison. "There has been great interest in using multiple-color fluorescence tags to image different biological molecules, but that approach is limited by the spectral width of fluorescent peaks," he explains. "Vibrations have very narrow widths, so they can create more selectively vibrationally tagged molecules and perhaps drastically increase the number of molecules that can be distinguished."

Min and colleagues next plan to see if they can combine SREF with superresolution microscopy techniques.—CELIA ARNAUD

COMPUTATIONAL CHEMISTRY

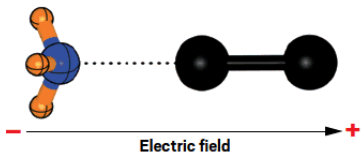
Electric field could spark halide reaction

Applied field could hold reactants in place and stretch halide bond

An electric field could make an unlikely nucleophilic substitution reaction feasible by fixing the reactants in place and lowering the reaction energy barrier, according to a theoretical study (*J. Am. Chem. Soc.* 2019, DOI: 10.1021/jacs.9b02174).

The reaction in question involves a complex held together by a halogen bond. These bonds are akin to hydrogen bonds and exist when a Lewis base coordinates with an electrophilic halogen atom. But an S_N2-like reaction in which the base pulls off the halogen to form two ions is energetically unlikely in either the gas phase or in solvent, says study coauthor Sason Shaik of the Hebrew University of Jerusalem. Shaik previously calculated that electric fields could drive Diels-Alder reactions (*ChemPhysChem.* 2010, DOI: 10.1002/cphc.200900848), work that chemists later confirmed in the lab (*Nature* 2016, DOI: 10.1038/nature16989).

Now Shaik, along with graduate student Chao Wang, has shown that an electric field oriented along the direction of the halogen bond will weaken the halide's bond, making it reactive. In a reaction involving ammonia and dichloride, a field of about 0.5 V/Å lengthened the chloride bond and reduced the reaction energy barrier by more than 30 kcal/mol, making the reaction possible. At higher field strengths, the dichloride dissociates spontaneously. "The electric field is really helping the movement of electrons," Shaik says.



An electric field enables an unlikely substitution reaction involving NH_3 (left) and Cl_2 (right).

Not only does the field stretch out the halide bond, Shaik explains, but it also holds the molecules in the correct orientation to facilitate the reaction. The researchers liken the effect to holding the molecules in place with tweezers. In the applied electric field, the molecules need more than 25 kcal/mol to rotate, higher than the energy barrier of the reaction.

It remains to be seen if the reaction can happen in the laboratory. Jean-Sabin McEwen of Washington State University, who studies catalysis in electric fields, says the study's calculated fields could be replicated in a scanning tunneling microscope, although the reactants may not stay in the gas phase in that environment. But Chérif F. Matta, a theoretical chemist at Mount Saint Vincent University, says whether or not these reactions are feasible, the work "may guide experiments in the future."—SAM LEMONICK