

# Two-dimensional infrared spectroscopy measures the structural dynamics of a self-assembled film only one molecule thick

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Since its first demonstration, many comparisons have been made between 2D infrared (2D IR) and NMR spectroscopy (1, 2). Both techniques use a series of pulses to manipulate and measure free induction decays that resolve couplings and dynamics through their characteristic multidimensional plots. The timescales are very different, with 2D IR spectroscopy using femtosecond pulses to measure the picosecond coherence times of vibrational modes, whereas NMR spectroscopy uses microsecond or millisecond pulses to measure nuclear spins that can last seconds. Thus, the two spectroscopies provide vastly different perspectives on structural dynamics. The article by Yan et al. (3), published in PNAS, uses the fast time resolution of 2D IR spectroscopy to study the structural dynamics of a functionalized self-assembled monolayer, providing insights into structural motions that are intimately related to chemistry. However, their experiments are also demonstrating an aspect of the technique that is only now being recognized as exceptional: the sensitivity of the technique to incredibly small amounts of material.

NMR spectroscopy is often said to be insensitive (4). Although the level of sensitivity depends on many factors, such as the type of nuclei being measured and the magnetic field strength of the spectrometer, the statement itself refers to the differences in population between the nuclear spin states that create the free induction decays, given by the Boltzmann distribution. At room temperature, the energy difference between nuclear spin states is much smaller than  $kT$ , resulting in nearly equal populations of states. The difference in population between two  $^1\text{H}$  proton states in an 11.7-Tesla magnetic field is about 1 in  $10^5$  (4). In comparison, the energy difference between the  $\nu = 0$  and  $\nu = 1$  vibrational states is about eight-times larger than  $kT$ , resulting in an enormous difference in population between the states. In other words, vibrational states have almost the entire population in the ground state.

Signal strength, and hence the sensitivity, are related to population differences because free induction decays are generated by unequal numbers of transitions between

eigenstates (5). An ensemble of molecules with statistically equally populated eigenstates will produce no signal because there are as many absorption as emission transitions. (In fact, a commonly used trick in NMR is to purposely equilibrate eigenstates to eliminate unwanted signals.) Thus, the ultimate sensitivity of 2D IR spectroscopy is inherently much higher than NMR spectroscopy, because nearly all of the molecules in the ensemble are available for excitation.

## Dynamics of a Self-Assembled Film only One Molecule Thick

In their article, Yan et al. (3) demonstrate and use the sensitivity of 2D IR spectroscopy to measure a monolayer of molecules on a surface. The authors tethered a rhenium carbonyl vibrational probe to a monolayer of undecanethiol alkyl chains. Vibrational modes are sensitive to hydrogen bonding and electrostatics, and so the frequencies of the rhenium carbonyls reflect their surrounding environment (6, 7). As the probe and environment move, the electric field surrounding the probe changes, causing the frequency to shift. This phenomenon is called "spectral diffusion." Spectral diffusion is measured in a 2D IR experiment by collecting a series of spectra at increasingly large "waiting times" between the first two pulses (sometimes called pump pulses) and a third pulse (sometimes called the probe), which the authors term  $T_w$ . Spectral diffusion broadens the 2D spectra (8). By measuring the breadth as a function of time, one then obtains the timescales for the structural motions of the probe and its environment.

For this self-assembled monolayer, Yan et al. (3) found a time constant for spectral diffusion of  $\sim 80$  ps when the maximum number of headgroups are attached to the alkyl chains. Interestingly, when the alkyl chains are tethered with fewer headgroups, the timescale for spectral diffusion slows to  $\sim 100$  ps. In addition, polarized IR measurements found a concomitant tilt in the orientation of the headgroup to point more parallel to the surface at lower headgroup coverages. Thus, Yan et al.'s experiments reveal

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that functionalization alters both the structures and the dynamics of the catalytic headgroups.

The ability to simulate observables in 2D IR spectra from molecular dynamic simulations is another strength of the technique (9, 10), whether it is correlations of motions that match the measured timescales or simulations of the spectra themselves. The connection between experiment and theory is facilitated by the 100-ps timescale measured in these 2D IR experiments, which can easily be covered by molecular dynamics simulations. Thus, after generating an ensemble or several ensembles of possible structures, each can be tested against an experiment. In the article by Yan et al. (3), the experimental timescales match the headgroup motions, which the authors learn are dictated by the dynamics of the alkyl chains. The 80-ps timescale is caused by dihedral flips of the alkyl chains and rearrangement of the triazole linker that is used to attach the headgroup to the chains. In other words, the probe dynamics report on the dynamics intrinsic to the alkyl chains, and that functionalization causes a structural and dynamical change that permeates the monolayer.

### Methods and Applications of 2D IR with Monolayer Sensitivity

Two-dimensional IR spectroscopy has often been used to measure femto- to picosecond dynamics of liquids, proteins, catalysts, and many other bulk systems at high concentrations or surface areas (11–13). When 2D IR was first invented 15 y ago, it was considered an achievement to measure spectra at millimolar protein concentrations, because that is the typical concentration used for protein NMR spectroscopy. At that time, measuring 2D IR spectra for a single monolayer of molecules was not in the realm of feasibility, but now there are several efforts underway. My group and others have been developing a 2D version of sum-frequency generation spectroscopy (14, 15). Sum-frequency generation spectroscopy is well known for its ability to measure signals from interfaces and surfaces. By adding infrared pump pulses, a spectrum analogous to a 2D IR spectrum is

generated, but with different selection rules. In a separate approach, Hamm and coworkers modified a 2D IR spectrometer to work in an attenuated total reflection geometry (16). They deposited a monolayer onto the attenuated total reflection cell and used an azide reporter group. Rezus and coworkers enhanced 2D IR surface signals using mid-IR plasmons (17). Of all of these, Yan et al.'s (3) implementation is the most elegant and straightforward to employ. In the PNAS article, they simply reflect the 2D IR pulses off of the gold interface; in their prior report, a few years ago, Yan et al. used a transparent substrate so that the standard transmission geometry for data collection could be used (18). Even though the optical density for a monolayer is one-thousandth that of a bulk sample, Yan et al. (3) obtain excellent data with their reporter group, enabling dynamics of molecules on surfaces to be measured on new timescales.

Determining molecular structures and dynamics is difficult under any circumstance, but even more so on a surface. Monolayer-sensitive 2D IR spectroscopy makes possible many new experiments. Molecular structures associated with sensor technologies might be investigated. Structures and orientations of molecules on semiconductors could be studied, such as used in next-generation photovoltaics. Voltages can now be applied, enabling 2D IR studies of electrochemistry. My dream is to study the structural dynamics of voltage-gated ion channels.

The often-used comparison between 2D IR and NMR spectroscopies is driven by similarities in pulse sequences, and the 2D IR community has drawn inspiration from the historic evolution of NMR spectroscopy in chemistry. Looking to the future, perhaps inspiration should be drawn from other high-sensitivity techniques. After all, infrared microscopies are now reaching sensitivities sufficient to measure single molecules (19).

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