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Vibrational spectroscopy of single molecules/single entities without plasmonic enhancement

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Abstract

Single-molecule vibrational spectroscopy is typically carried out using surface-enhanced Raman scattering and related techniques that rely on plasmonic enhancement. While these techniques are powerful, the need for the molecule of interest to interact with a nanostructured metal surface is a drawback for many applications. This review summarizes methods that have been developed to enable vibrational spectroscopy at the single-molecule level without plasmonic enhancement, which fall into two general categories. Techniques that resolve the vibrational structure in electronic emission spectra are well established and can produce high quality spectra, but require cryogenic temperatures. Newer methods that utilize more than one excitation field to couple vibrational excitation to a strong electronic transition are potentially applicable to room-temperature materials and biological samples, but single-molecule sensitivity has been demonstrated in only a few systems to date. Potential future applications of both classes of techniques are discussed.

Introduction

Spectroscopy in its many forms provides a highly informative, often rapid, and noninvasive probe of the structure of molecules and materials. However, much of the information that might be obtained from measuring spectra in condensed phase environments is often obscured by inhomogeneous broadening caused by different molecules being in different initial quantum states, having different geometric conformations or chemical identities, and/or occupying different local environments. The development of spectroscopic methods that mitigate inhomogeneous broadening has long been an active area of research, leading in the 1990s to techniques that allow measurement of the spectra of individual molecules, one at a time.[1] Single-molecule spectroscopy has developed from a laboratory curiosity to a useful physical and analytical technique widely applied to the study of complex materials and biological systems.[2-4] A majority of these applications, particularly those in biology, probe only electronic transitions by detecting fluorescence at low spectral resolution. This is because fluorescence is inherently a highly sensitive analytical technique; a strongly fluorescent molecule can, under favorable circumstances, emit 10^7-10^8 fluorescent photons per second, most of these protons are produced at wavelengths where there can be very little background light, and detectors for ultraviolet (UV) and visible light have high sensitivity and low noise.

Vibrational spectroscopy is one of the most informative spectroscopic techniques in analytical and physical chemistry. A molecule with N atoms has 3N-6 vibrational normal modes which typically span a wide range of frequencies and have sufficiently narrow linewidths that many of the frequencies can be resolved. The vibrational frequencies and intensities carry information about the geometric structure of the molecule, its charge distribution, and the strengths of the chemical bonds.[5-6] The energy separations between vibrational levels correspond to infrared (IR) light, and detecting these at the single molecule level is much more difficult than for electronic transitions. Spontaneous emission in the IR from excited vibrational states is many orders of magnitude weaker than the visible emission from excited electronic states, largely because of the ν^3 dependence of the spontaneous emission rate on frequency, and detectors that are sensitive in the IR are fundamentally much noisier than detectors for visible light. The most direct way to measure a vibrational spectrum is through absorption of IR light, but the attenuation of the incident beam by one molecule's absorption is so tiny as to be nearly undetectable. Even the stronger absorption of visible light by electronic transitions is barely detectable under favorable conditions.[7] The other common technique for routine vibrational spectroscopy is Raman scattering, where light in the UV to near-IR range is inelastically scattered to excite a molecular vibration. While Raman scattering, like fluorescence, produces its signal at a wavelength where there is little or no background, Raman scattering from a single molecule is extremely weak compared with fluorescence and is usually not detectable. The strength of the Raman signal can be enhanced by several orders of magnitude by making use of resonance enhancement, *i.e*. choosing the excitation wavelength to coincide with a strongly allowed electronic transition. However, the potential advantages of resonance enhancement are often negated by interference from fluorescence occurring at the same wavelength as the Raman scattering, and/or photochemical or thermal damage to the sample at electronically resonant wavelengths.

Most single molecule vibrational spectroscopy experiments utilize Raman spectroscopy with plasmonic enhancement, often in addition to electronic resonance. This is broadly known as surface enhanced Raman scattering (SERS). A surface plasmon is a collective excitation of the conduction band electrons of a metal, which occurs at a frequency determined by the identity of the metal and its dimensions and shape. Nanostructured gold and silver, in particular, exhibit strong surface plasmon resonances at visible wavelengths. Resonant excitation of a surface plasmon leads to strong local enhancement of the applied electromagnetic field, serving to enhance radiation-matter interactions of any molecule on or near the surface of the metal. This field enhancement can be particularly strong at certain geometries such as nanometer-scale gaps between two nanoparticles or sharply curved tips, and Raman enhancement factors of 9-12 orders of magnitude have been reported at such "hot spots". This provides more than enough enhancement to allow Raman spectra of single molecules to be obtained. The sample may be adsorbed to the surface of a metal nanostructure, or techniques adapted from other scanning microscopies may be employed to move a sharply pointed plasmonic metal tip over a sample, enhancing the Raman signal from the molecules closest to the tip. The latter is known as tip-enhanced Raman scattering (TERS). The vast majority of single-molecule vibrational spectroscopy papers published between 2000 and 2024 utilize either SERS or TERS, and several very good review articles on the subject are available.[8-10] However, there are disadvantages to these plasmonically enhanced methods. In SERS, adsorption of the molecule to the metal surface can perturb the molecule's structure, and strong electromagnetic fields and/or local heating may cause undesirable photochemistry or thermal chemistry. TERS can provide very high spatial resolution but only for molecules on surfaces, and cannot be applied to the interiors of materials, cells, or most other biological structures.

This review addresses the relatively limited field of vibrational spectroscopy of single entities (molecules, nanoparticles, or polymers) without plasmonic enhancement. For the purposes of this review, "spectroscopy" refers to interactions of matter with electromagnetic radiation. This excludes some single-molecule techniques that provide information about vibrational states but do not involve electromagnetic radiation. For example, Ho and co-workers have shown that under favorable conditions vibronic states can be probed by modulating the tip-to-surface bias voltage in scanning tunneling microscopy (STM).[11-12] Injection of electrons from an STM tip into a metallic solid can also generate plasmonic light emission that can be modulated by vibronic transitions of a molecular adsorbate, as discussed in a detailed review by Kuhnke *et al*.[13] What constitutes a "single entity" is less straightforward to define. In addition to species that any chemist would consider a molecule, I include semiconductor nanocrystals and organic polymers but omit bulk solids and defects in bulk solids.

Techniques that allow the measurement of single-molecule vibrational spectra without the presence of perturbing plasmonic nanostructures fall primarily into two groups, both based on the vibrational substructure of electronic states. The best developed method, frequency resolved fluorescence or fluorescence excitation spectroscopy, is applicable only at very low temperatures and only with certain types of molecules. Various nonlinear or pump-probe methods have potentially wider ranges of applicability but are at an earlier stage of development and have been demonstrated to have single-molecule sensitivity in only a few cases. These two groups of techniques are addressed separately below.

Vibrationally resolved low-temperature fluorescence spectroscopy

The first experiments that directly resolved vibrational frequencies of single molecules were based on electronic spectroscopy, specifically fluorescence at very low temperatures (Fig. 1). In most single-molecule fluorescence experiments the emitted light is not spectrally resolved apart from some coarse filtering to eliminate scattered excitation light. That is, all of the transitions enclosed in the blue circle in Fig. 1 are detected together. However, strongly allowed electronic transitions of molecules and quantum dots have sufficiently high radiative rates that a single molecule can produce well over 10^3 detected photons per second, which is plenty of signal to yield a high quality dispersed emission spectrum in a fairly short period of time. While molecular fluorescence spectra consist of transitions between discrete vibrational levels of the excited and ground electronic states, this vibronic structure (changes in vibrational level accompanying a change in electronic state) is rarely resolved in complex molecules at ambient temperatures. This is due to a combination of spectral congestion (many overlapping transitions) and spectral diffusion (the electronic energy gap is very sensitive to small changes in the local environment, so the emitted wavelengths move around with time because of thermal motions of the environment). However, in certain classes of molecules at very low temperatures, typically below 10 K, the vibronic transitions are sufficiently well separated and stable that it becomes possible to resolve the vibrational structure of the single-molecule fluorescence spectrum.

To my knowledge, the first measurement of vibrational frequencies of single molecules was published by Tchénio, Myers, and Moerner in 1993 by spectrally dispersing the fluorescence from single molecules of the aromatic hydrocarbon pentacene in a *p*-terphenyl matrix at 1.8 K.[14] The emitted light was sent through a spectrograph and detected with a charge-coupled device (CCD) camera, allowing each of the vibronic transitions (yellow, orange, and red arrows in Fig. 1) to be observed separately. A subsequent study by Fleury et al.[15] obtained higher-quality spectra of this system and found that the main vibronic transitions were very similar from one molecule to another, with some small intensity variations. This work was followed by more detailed studies on the aromatic molecule terrylene in polyethylene[16-18] (Fig. 2). Single molecules were isolated by bringing the excitation beam into the cryostat through a single-mode optical fiber to irradiate a very small volume of a thin, dilute sample, and then tuning the narrow-bandwidth excitation laser through the red edge of the zero-phonon optical transition to select a single-molecule electronic resonance. In the terrylene system, most of the molecules investigated showed vibrational spectra similar to those of the bulk sample, but a few had distinctly different spectra that were tentatively attributed to conformational isomers.

In the above experiments, the number of terrylene molecules in the illuminated volume was large (~10^4) and single molecules were isolated for study spectrally, by tuning the excitation laser into resonance with the electronic origin or "zero-phonon" transition (upward green arrow in Fig. 1) of a single molecule. An alternative approach is to make the sample sufficiently dilute that no more than one molecule is present within the illuminated volume and select single molecules by their location. This enables measurements on single molecules even in the presence of fast spectral diffusion and/or spectrally broader transitions.

Terrylene turned out to be an excellent chromophore for such studies, possessing strong absorption (near-unity oscillator strength) and bright fluorescence (fluorescence quantum yield > 0.5) in a spectral region readily accessible to the single-frequency dye lasers that were at that time the best source for tunable, very narrow-band laser excitation. (Modern, commercially available Ti:sapphire lasers can achieve equally narrow bandwidths, <100 kHz, and are tunable from roughly 700-1000 nm, while dye lasers work best in the 550-650 nm range.) It also has high photostability and well resolved vibronic lines. In the 30+ years since those original studies, a few other groups have carried out related experiments on terrylene and its derivatives at cryogenic temperatures. Kiraz *et al.* obtained vibrational frequencies of electronically excited states of single molecules of terrylenediimide in both hexadecane and poly(methyl methacrylate) matrices at cryogenic temperatures.[19] They did this by preparing dilute samples having only one terrylenediimide molecue within their confocal excitation and detection volume and then detecting the fluorescence while tuning the excitation laser 1100-1700 cm^{-1} above the electronic origin transition. The vibrational frequencies were found to vary by up to 16 cm^{-1} from one molecule to another. Zirkelbach *et al.* used a combination of fluorescence excitation and stimulated emission depletion at a very low temperature (nominally 20 mK) to study both the vibrationally resolved excitation and fluorescence spectra as well as ground-state vibrational relaxation in dibenzoterrylene in *p*-dichlorobenzene.[20] Significant molecule-to-molecule variations in the frequencies, linewidths, and intensities of different vibrational features were observed. They also showed that at high laser powers a coherent stimulated Raman transition could be driven in this system.[21] de Haas *et al*. examined fluorescence spectra of single terrylene molecules adsorbed onto boron nitride and found large molecule-to-molecule differences in vibronic coupling strengths attributed to charge transfer between boron nitride and terrylene.[22]

The other main group of organic molecules for which vibronic spectroscopy has been studied at the single-molecule level are the conjugated polymers polyphenylene (PPP) and poly(phenylene vinylene) (PPV) and their derivatives and relatives. These organic semiconductors are of interest largely because of their applications in organic LEDs and solar cells. While some work has been done on monomers or well-defined oligomers, most "single molecule" studies examine single polymer chains that consist of up to thousands of monomer units. As the electronic excitations are usually delocalized over only 6-12 monomer units, each polymer chain contains tens to hundreds of distinct chromophores, although rapid energy transfer usually results in the fluorescence arising from only one or a few of these chromophores. PPP and PPV have much more conformational flexibility than fused-ring aromatic molecules like terrylene, giving rise to much more spectral congestion in their vibronic spectra. However, reasonably well-resolved vibronic spectra have been obtained at cryogenic temperatures (5K and below) for PPP[23], substituted PPV (Fig. 3),[24-28] and related polymers,[29] and even at higher temperatures there remains some poorly resolved vibronic structure in single-chromophore emission spectra.[24, 30]

Semiconductor quantum nanocrystals (NCs) are another, very different group of systems that have been widely studied using single entity techniques. Semiconductor NCs tend to have very large optical absorption cross-sections, high quantum yields for light emission, and often high photostability, making them very amenable to single entity studies. Their vibronic spectroscopy has several important differences from that of most molecules. Because of strong spin-orbit coupling in semiconductors containing heavy atoms, there is no clear distinction between fluorescence and phosphorescence and emission of light following photoexcitation is usually referred to as photoluminescence (PL). Molecular electronic transitions are usually described in terms of excitation between ground and excited states that differ in the occupation of molecular orbitals. Electronic transitions of semiconductors are described as promotion of an electron from the filled valence band to the empty conduction band to produce two separate particles, an electron and a hole, which together form an exciton. The exciton may recombine by emitting light, often with excitation of vibrations referred to as phonons. Phonons in semiconductor NCs are classified into optical phonons, which involve relative motions of the atoms in a unit cell of the crystal and usually have frequencies above ~30 cm^{-1}, and acoustic phonons, which involve motions of different unit cells relative to each other and usually occur below ~30 cm^{-1}. Both types have been observed in the spectrally resolved PL of many NCs. In addition, because the electrons and holes are delocalized over a large number of atoms, NCs containing more than one exciton (biexcitons, triexcitons) and charged NCs (positive or negative trions) may absorb and emit at nearly the same wavelength as singly excited neutral NCs, often complicating the spectroscopy.

Most of these studies have been performed on CdSe-based NCs, usually with CdS and/or ZnS shells to improve stability,[31-33] or perovskites,[34-41] a class of NCs that have attracted great recent interest for potential applications in solar energy conversion. There has also been some work on InP-based NCs,[33] which are promising materials for photoluminescent and electroluminescent displays. High-resolution photoluminescence spectra of single NCs at cryogenic temperatures exhibit resolved phonon substructure, typically showing the longitudinal optical phonon and/or lower-frequency acoustic phonons. Additional features in the photoluminescence spectrum arise from the complex angular momentum fine structure of the excitonic transitions and/or from biexcitons or trions. In most cases the phonon frequencies of different single NCs exhibit only modest differences but the intensities of the phonon sidebands sometimes show large variations, indicating that the magnitude of the exciton-phonon coupling can vary greatly from one NC to another. Small graphene NCs have also been examined by vibronically resolved luminescence spectroscopy at 7 K.[42]

Two specific examples of single NC PL spectra are presented in Figs. 4 and 5. Figure 4 shows resolved PL spectra of four different "dot-in-rod" nanocrystals that consist of a roughly spherical $Zn_{1-x}Cd_xSe$ core and a rod-shaped CdS shell. When x = 0, the lowest-energy exciton, from which the PL occurs, is expected to have both the electron and the hole largely localized to the "dot" region; for increasing x, the hole remains localized to the dot while the electron spreads out over both dot and rod. Accordingly, one sees from Fig. 4 that when x = 0 (DR-4) the Se-type optical phonon from the dot is much more strongly coupled to the excitonic transition than the S-type phonon of the rod at higher frequency; as the value of x increases from DR-3 to DR-1, the ratio of S-type to Se-type phonon intensity increases. This shows how single-NC PL spectroscopy can reveal the electron and hole charge distributions in the exciton. Figure 5 shows PL spectra of a single NC of $CsPbBr_3$, one of the perovskites. This spectrum shows that the PL from three different excited species assigned as the neutral exciton (XB), the negatively charged trion (X*), and the biexciton (XX) all appear within a narrow spectral window of ~40 meV or 320 cm^{-1}. These features are not resolvable in an ensemble spectrum because of the inhomogeneous broadening of the spectra caused by size variations.

Multiphoton techniques

Pure vibrational spectra are much less sensitive to spectral diffusion and spectral congestion than vibronic spectra, but as discussed in the introduction, they are normally too weak to be measured directly at the single-molecule level without plasmonic enhancement. Recently, several techniques have been demonstrated that combine vibrational and electronic spectroscopy via pump-probe or nonlinear interactions with the goal of achieving single-molecule sensitivity in room temperature, solution phase environments. These techniques are all based on the idea that a vibrationally excited molecule can be brought to a fluorescent excited electronic state by absorbing a photon of lower energy than that required to excite a ground-state molecule. One photon or pair of photons is used to excite a ground-state vibration (the pump), and then a second, probe photon is used to excite the fluorescent electronic state. By recording the fluorescence intensity as a function of pump frequency, a vibrational spectrum of the molecule can be measured.

Fluorescence-encoded infrared (FEIR) spectroscopy[43-48] is a pump-probe technique that operates by the principle shown in Figure 6 (left). An infrared pulse, or pair of pulses, excites a molecule to a higher vibrational level of the ground electronic state, and a second, visible pulse excites these vibrationally hot molecules to the excited electronic state, from which fluorescence is detected. In order for this to work as desired, the large number of ground-state molecules must make a small contribution to the fluorescence compared with the relatively small number of vibrationally excited molecules; this requires that the vibrational level excited be strongly Franck-Condon active and high in frequency, and that it undergo relatively slow vibrational relaxation. By performing fluctuation correlation spectroscopy with the FEIR signals the Tokmakoff group was able to demonstrate single-molecule sensitivity of the technique,[43] but they did not obtain vibrational spectra of single molecules. Vibrational spectra of single molecules were obtained by the Wei group,[48] who demonstrated the single-molecule origin of their signals using the isotopic mixture technique that had been previously utilized by the van Duyne group to demonstrate SERS at the single-molecule level.[49] Here, a mixture of Rhodamine 800 with nitrile isotopologues $^{12}C{\equiv}^{14}N$, $^{12}C{\equiv}^{15}N$, $^{13}C{\equiv}^{14}N$, and $^{13}C{\equiv}^{15}N$ was examined. Under extremely dilute conditions, each spectrum was shown to contain only one of the four distinct nitrile stretching frequencies, indicating the presence of only one isotopologue (Fig. 7). Since it is statistically very unlikely that multiple molecules would all be the same isotopologue, this was interpreted as demonstrating the single-molecule origin of the signals.

A similar double resonance technique (near-IR electronic excitation and vibrational IR excitation) was used by Otomo *et al*. to obtain vibrational spectra of single pigments of bacteriochlorophyll *a* (BChl-*a*) in the light-harvesting 2 complex of a purple photosynthetic bacterium at 1.5 K.[50] In this system individual BChl-*a* chromophores can be observed by fluorescence excitation spectroscopy. Vibrational IR excitation in the C=O stretching range was found to shift the peak wavelengths of the BChl-*a* excitation peaks, and these shifts were interpreted and analyzed using a two-state kinetic model. A plot of the normalized ratio of residence times in the two states for a single chromophore versus IR frequency gave a peak that was narrower and shifted relative to the FTIR spectrum of the ensemble. The observed shifts in the fluorescence excitation peaks were attributed to disruption of hydrogen bonding caused mainly by excitation of one particular carbonyl group on the BChl-*a*.

A technique closely related to FEIR, stimulated Raman excited fluorescence (SREF),[51-53] uses stimulated Raman scattering rather than infrared absorption to create vibrationally excited molecules (Figure 6 (right)). Two pulses, a pump pulse and a Stokes pulse, are used to excite a ground-state vibrational level through a stimulated Raman process, and then an excitation pulse, which may be the same as the pump pulse, brings the vibrationally excited molecule to an excited electronic state, from which the emitted fluorescence is detected. This technique is conceptually similar to FEIR but has some practical differences. Raman scattering is normally a weak process, but by using strong pump and Stokes pulses and taking advantage of electronic preresonance enhancement a significant population of vibrationally excited molecules can be generated. However, if the pump pulse is too close to electronic resonance it can produce unwanted fluorescence background, so very careful selection of the pump and probe wavelengths is required. SREF has the advantage of using only visible wavelengths for excitation, allowing higher spatial resolution than FEIR. This technique has been demonstrated to yield single-molecule vibrational spectra in favorable cases[52] through the isotopologue mixture method discussed above for FEIR. Achieving single-molecule sensitivity requires a delicate balance between preresonance enhancement and pump-induced fluorescence background, and its utility may turn out to be limited.

Summary and Conclusions

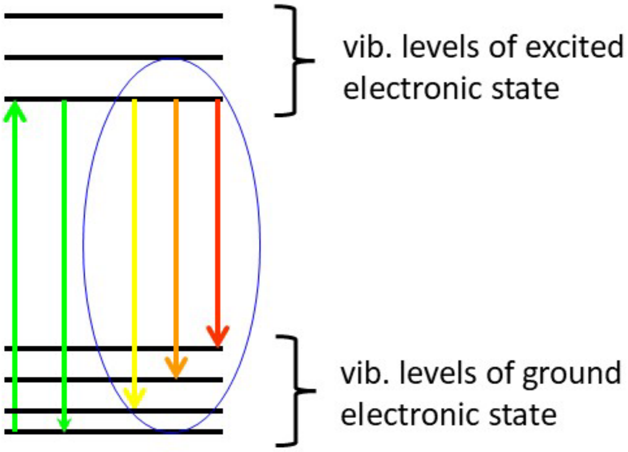
Techniques that obtain single-entity vibrational information by resolving the vibronic structure in fluorescence or fluorescence excitation spectra are well developed, reasonably straightforward to carry out, and work well on certain classes of systems, but the requirement for very low temperatures necessarily limits the scope of their application. Nonlinear and pump-probe spectroscopic techniques that can provide vibrational spectra of single entities in complex room-temperature media without extrinsic plasmonic probes are still in the early stages of development. To date there appear to be only a small subset of molecules for which these methods are feasible, even with carefully optimized excitation and detection wavelengths. It remains to be seen whether these or related techniques can become broadly applicable in the same way as, for example, single-molecule FRET.[54-55] Currently, pump-probe methods for single-molecule vibrational spectroscopy are being developed mainly for applications in biological systems, with optimized chromophores attached as probes to macromolecules of interest. There is still a need in both materials and biological research for methods that can measure vibrational spectra of single entities without the addition of extrinsic labels and without the perturbation of a nearby plasmonic metal.

Disclosure of Interest

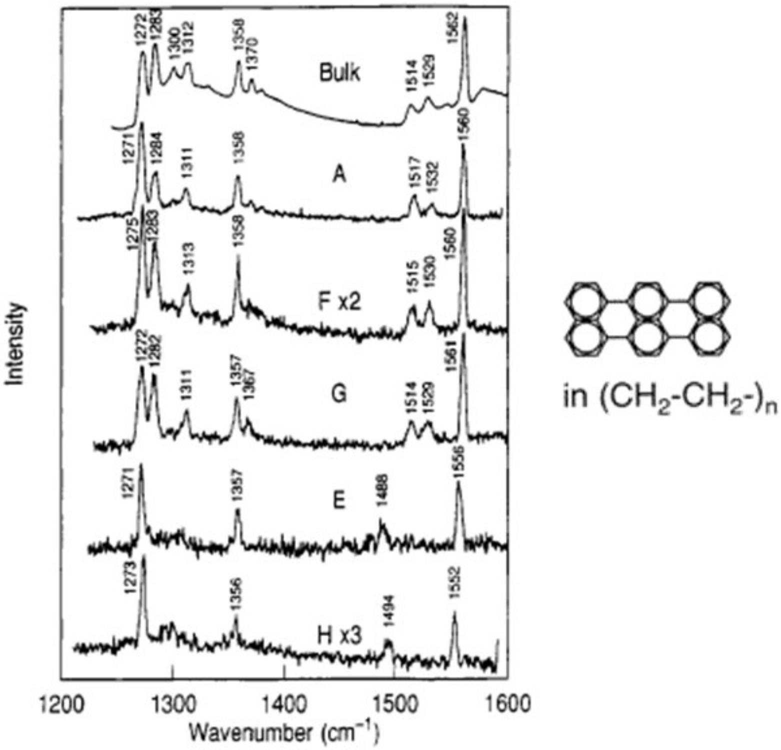
The author reports that there are no competing interests to declare.

Figures

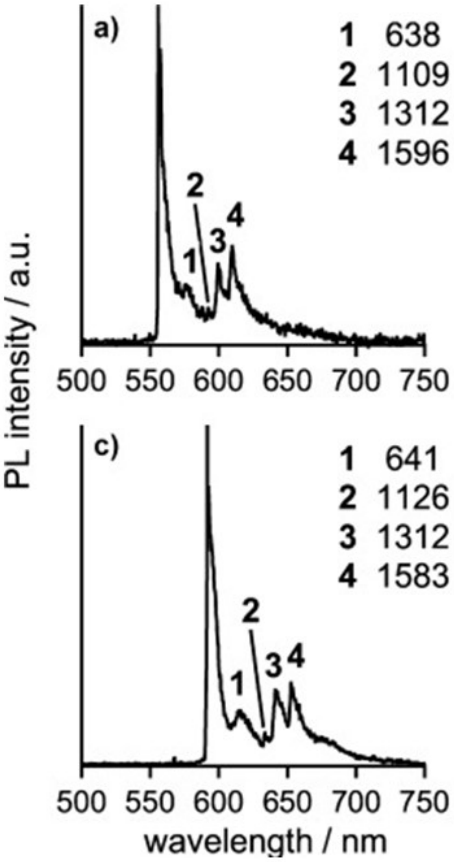
1. Vibrationally resolved fluorescence for single molecule spectroscopy. The electronic origin is excited with a narrow-band laser (green upward arrow) and the Stokes-shifted light that is emitted by spontaneous fluorescence to vibrationally excited levels of the ground state is dispersed in frequency and detected (yellow, orange, and red arrows). When spectral selection is used to isolate single molecules, the excitation wavelength (green upward arrow) is first scanned over narrow ranges while detecting all of the Stokes-shifted emitted light (blue circle) to identify resonances from single molecules.



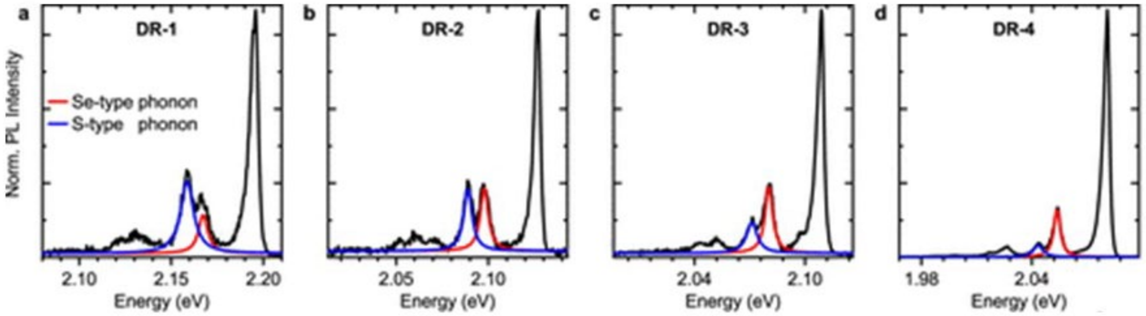
2. Dispersed fluorescence spectra of different single molecules of terrylene in polyethylene showing ground-state vibrational frequencies, compared with the spectrum of a bulk sample. The x-axis is plotted as wavenumber shift from the excitation laser. Adapted with permission from Myers, A. B.; Tchénio, P.; Zgierski, M. Z.; Moerner, W. E., Vibronic spectroscopy of individual molecules in solids, *J. Phys. Chem. 98*, 10377-10390. Copyright 1994 American Chemical Society.



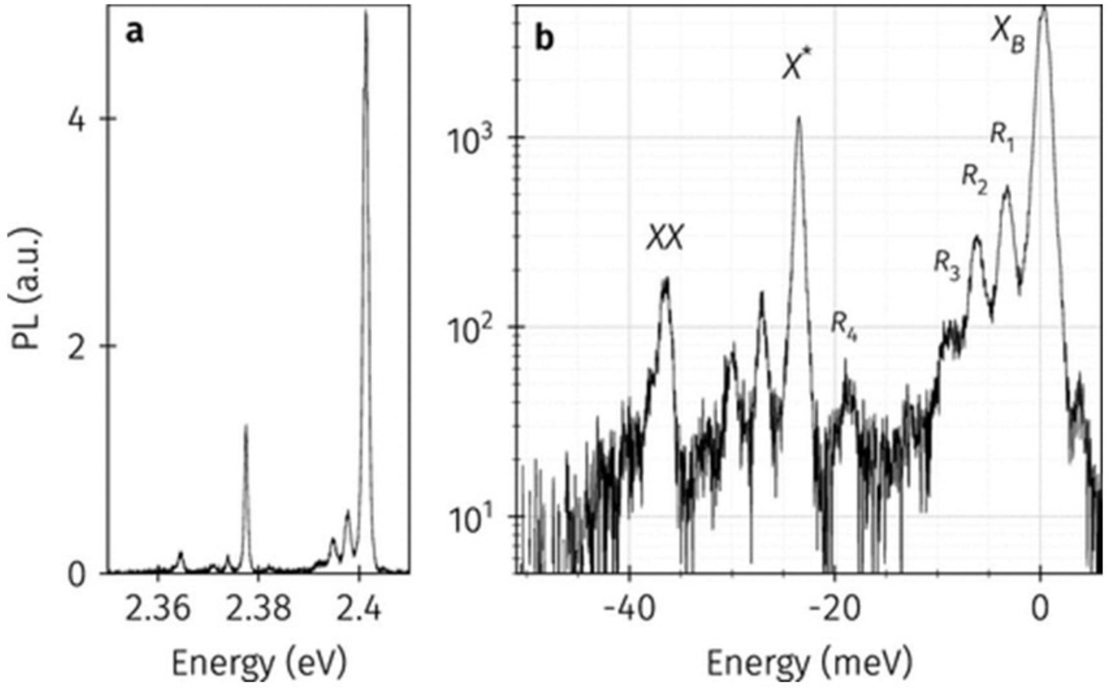
3. Fluorescence spectra of single MEH-PPV polymer chains at 1.2 K, showing emission from both blue-shifted and red-shifted chromophores. The prominent vibrational frequencies in each spectrum are labeled, calculated as the shift (in cm^{-1}) from the sharp electronic origin transition. The redder-shifted chromophores are attributed to larger numbers of conjugated monomer units in more ordered regions of the polymer. Adapted with permission from Feist, F. A. Zickler, M. F., Basché, T., Origin of the Red Sites and Energy Transfer Rates in Single MEH-PPV Chains at Low Temperature, *ChemPhysChem 12*, 1499 – 1508. Copyright 2011 John Wiley & Sons.



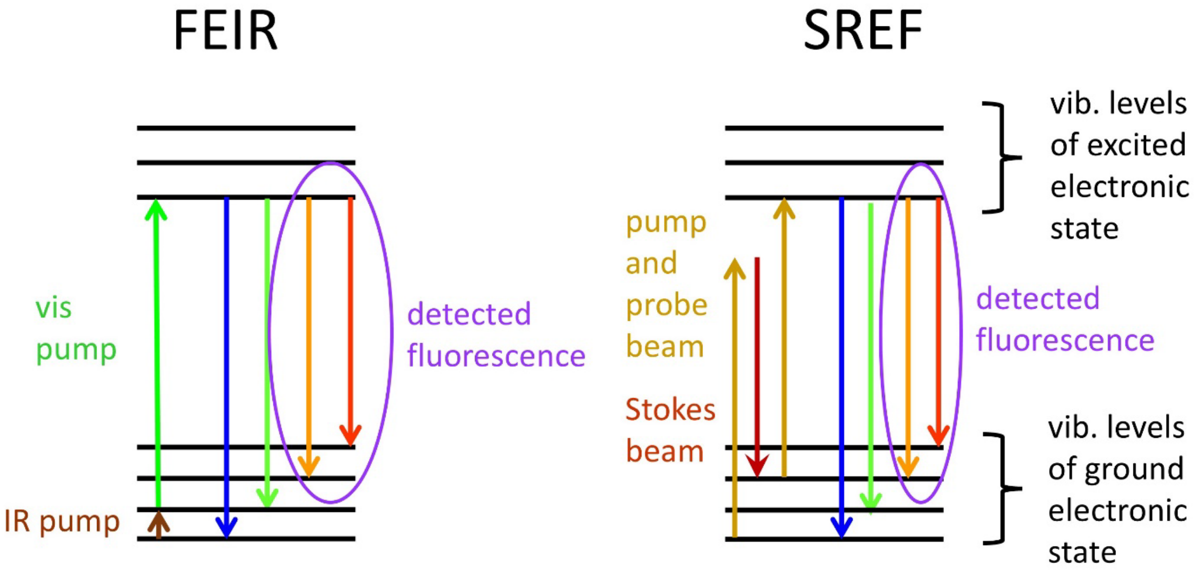
4. Photoluminescence spectra of single $Zn_{1-x}Cd_xSe$/CdS dot-in-rod nanostructures with decreasing fractions of zinc from DR-1 to DR-4, at $T \approx 10$ K. The CdSe-type and CdS-type longitudinal optical phonons are shown with Lorentzian fits. Note the significantly different relative intensities of these two peaks showing different extents of exciton delocalization from the CdSe core to the CdS shell. Adapted with permission from Johst, F.; Rebmann, J.; Werners, H.; Klemeyer, L.; Kesavan, J. K.; Koziej, D.; Strelow, C.; Bester, G.; Mews, A.; Kipp, T., Exciton−Phonon Coupling in Single ZnCdSe-Dot/CdS-Rod Nanocrystals with Engineered Band Gaps from Type-II to Type-I, *ACS Photonics 11*, 3741-3749. Copyright 2024 American Chemical Society.



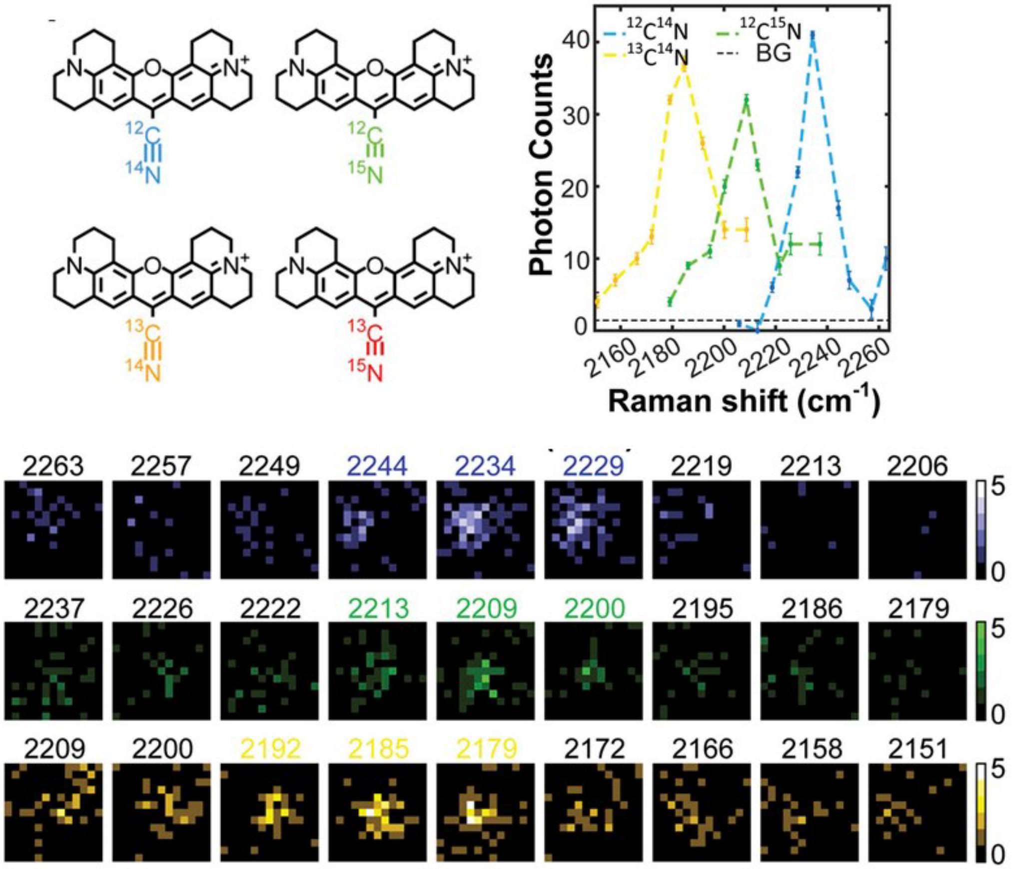
5. Photoluminescence spectrum of a single $CsPbBr_3$ nanocrystal plotted on (a) linear and (b) logarithmic scales. The spectrum shows features assigned to the origin transitions of the bright exciton (X_B), trion (X^*), and biexciton (XX), as well as their optical phonon progressions, labeled R_i for X_B. Reproduced with permission from Amara, M.-R.; Said, Z.; Huo, C.; Pierret, A.; Voisin, C.; Gao, W.; Xiong, Q.; Diederichs, C., Spectral Fingerprint of Quantum Confinement in Single $CsPbBr_3$ Nanocrystals, *Nano Lett. 23*, 3607-3613. Copyright 2023 American Chemical Society.



6. Schematic of the FEIR (left) and SREF (right) methods for obtaining vibrational spectra of single molecules under non-cryogenic conditions.



7. The upper left shows the structure of Rhodamine 800 with different isotope edited nitrile groups. The images at the bottom are SREF images of three different single molecules, showing Raman intensity as a function of Raman shift (difference in cm^{-1} between the pump and Stokes pulses). The plot on the upper right shows the SREF photon counts for each of these three molecules as a function of Raman shift. Each molecule shows a single maximum at a frequency corresponding to a particular isotopomer, confirming the single-molecule origin of the signals. Reprinted from Xiong, H.; Min, W., Combining the best of two worlds: Stimulated Raman excited fluorescence, *J. Chem. Phys.* **2020**, *153*, 210901 with the permission of AIP Publishing.



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