SINGLE-MOLECULE OPTICS - 2020

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1.1. Introduction

At the end of the 19th century, accumulated evidence had started to convince many physicists and chemists of the existence of atoms and molecules. The final hard proofs appeared at the beginning of the 20th century, with the quantization of charge, Brownian motion, and most importantly the diffraction of X-rays by crystals. Already at that time, Jean Perrin had the idea to observe single fluorescent molecules by eye in an optical microscope. He used black soap films, which gave a very low background of light scattering (only Rayleigh scattering was known at the time, Raman scattering was discovered later), with low concentrations of fluorescent molecules. Unfortunately, the sensitivity of the human eye, the quality of sources (spectral lamps) and filters (colored glass), and the durability of the fluorescent dyes in Perrin's time were all largely insufficient for him to see single molecules by eye. Instead, he performed a very interesting study of fluorescent black films, where he found another evidence for a molecular structure, the quantized thickness of soap multilayers (see exercise 1.1). Thanks to huge progress in optical microscopes, light sources (lasers), filters (multidielectric coatings) and dyes (laser dyes and fluorescent markers), Perrin's thought experiment can be done today in a straightforward manner.

Long after these first heroic attempts, it was thought that molecules were much too weak emitters to be detected optically. The progress of electron microscopy led to observe single rows of atoms in crystals already in the 1950's. A field ion microscope showed atomic structure of a tungsten tip, but the resolution was too low, and the irradiation damage too high, to observe individual molecules under the electron microscope. More recently, however, higher selectivity and better collection of secondary electrons have made it possible to observe individual isolated heavy

atoms in a sample made of light atoms with an electron microscope. The detection of single atoms and single molecules on surfaces first became a reality in the early 1980's with the invention of the scanning tunneling microscope (STM), and later of its variant for insulating substrates, the atomic force microscope (AFM). These two techniques have opened the way to microscopic investigations of matter at the single-atom and single-molecule levels. They are widely used today in many laboratories.

Meanwhile, thanks to lasers and to general progress in optical techniques, it became possible in the 1970's to observe single atoms in the gas phase, in dilute atomic beams. The atoms were detected via the short bursts of fluorescence light they emitted when crossing the laser focus. Single trapped ions were also detected by their fluorescence. Yet, observing a single fluorescent atom in gas phase is a very different problem from observing a single molecule in condensed phase, for the main two following reasons:

- First, condensed matter produces strong optical background via Rayleigh and Raman scattering. Moreover, other fluorescent impurities in the sample may easily dominate the fluorescence from a single molecule, therefore the purity of the sample is crucial, a problem which does not arise in vacuum!
- Second, a single atom usually can fluoresce millions of photons per second, without any degradation. An atom in vacuum is a stable system, even in its excited state. It can thus perform indefinitely many excitation-emission cycles, which simplifies detection considerably. This is unfortunately not the case for most fluorescing systems in condensed matter, which are all subject to irreversible transformations putting an end to luminescence. Those processes, called photobleaching and photoblinking, will be discussed later in this course.

In the late 1980's and early 1990's, progress in sources, optics and detectors have opened the way to detect single molecules and more generally single luminescent objects (nanocrystals, quantum dots, metal particles, color centers, etc.) in condensed matter, and even in such a complex environment as a live cell. This domain of research arises in part from a general change of point of view, brought about by a broad interest for matter at nanometer scales, *nanoscience*. By coupling lasers to optical microscopes, all the spectroscopic techniques developed in the 1970's and 1980's can be transported down to sub-micron scales (the resolution of a confocal optical microscope can be below 200 nm), and even down to nanometer scales if a

single object can be selected. In this course, we will review the motivation, methods, and results of the still relatively new technique of single-molecule optics.

In this introduction, we briefly review the principles of the fluorescence method. We will give further details later. But before starting, it is important to realize how small a molecule is. To give a simple comparison, if a rain drop was magnified to the size of the Earth, a single water molecule would be about the size of a person ! Isolating the signal emitted by a single molecule means separating it from the background of many other molecules. This requirement has two consequences :

- first, to reduce background, the size of the illuminated sample must be reduced as much as possible. For a given light intensity, i.e. for a given signal from the molecule, the background will be proportional to the number of illuminated sample molecules, i.e. proportional to the volume of the illuminated sample. Reducing background requires reducing the illuminated spot, usually by focussing the laser beam as tightly as possible into the sample. This focussing step thus performs a *spatial selection*. Furthermore, the sample itself can be made smaller, either in thickness or in lateral dimensions, with thin films or nanoparticles.
- second, the detection method must in principle have an intrinsic background low enough for the single molecule's signal to dominate the background from sample molecules in the illuminated volume. A diffraction-limited volume of 0.3 μm^3 contains about 10^9 sample molecules. The detection method must therefore have a selectivity ratio, i.e. the ratio of signals from the molecule of interest to that of a sample molecule, higher than 10^9 ! There are not many optical methods presenting such a high selectivity. Fluorescence, compared to Raman scattering, does, but most other methods don't. The high selectivity of fluorescence is achieved through optical *resonance*. Our fluorescent molecule must first absorb a laser photon, which a sample molecule cannot do. More precisely, a fluorescent dye molecule can *really* absorb laser photons, whereas solvent molecules around it only *virtually* absorb photons. The resonance conditions of these two types of processes lead to a difference of many orders of magnitude in their efficiencies or cross sections (see exercise 2.2). Therefore, the laser frequency performs a *spectral selection* on the molecules contained in the sample. It is interesting to compare fluorescence to infrared absorption, for example, which would be interesting to study a single chemical bond. The selectivity of IR absorption is 10^3 at best, because of the width and shape of

vibrational resonances. Therefore, IR absorption could be applicable to study the vibrations of a single bond (in a sample containing bonds with other resonance frequencies), only in combination with spatial selection of less than 1000 molecules. This cannot be achieved by current infrared microscopes, because of the larger wavelength of IR and because of the poor quality of IR objectives.

The optical detection and study of single molecules has now become a standard method, which makes many different experiments possible. In this course, we'll review two kinds of current experiments :

- room-temperature experiments : done with optical microscopes to investigate problems in molecular biochemistry and biophysics and in material science. Some recent results include the dynamics of a single enzyme molecule, or tracking single molecules or single viral particles in living cells;
- cryogenic experiments : done in a cryostat, usually with very high spectral resolution, with possible applications to molecular physics, solid state physics or quantum optics.

The high optical quality of room-temperature microscopy can be combined with the photochemical stability of molecules at cryogenic conditions to improve signals and stability and to obtain structural information with improved accuracy. This is a recent trend in superresolution microscopy.

1.2. Fluorescence and Photophysics of a Dye molecule

Most of the recent work on the optical microscopy and spectroscopy of single objects is based on fluorescence. Therefore, it is important to recall the principal features and properties of fluorescent molecules. All fluorescent organic molecules are conjugated, i.e. they present delocalized electronic pi wavefunctions.

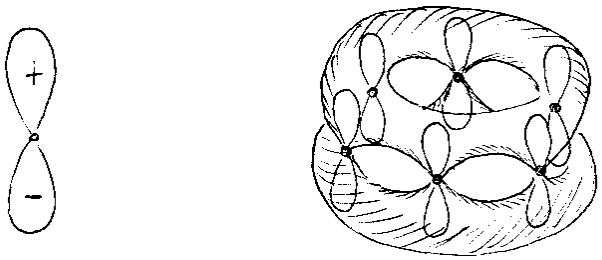

Figure 1.1 : Left, scheme of an atomic p_z orbital ; lowest energy pi molecular orbital of benzene, constructed from a superposition of p-orbitals centered on each carbon atom. The pi-electron can be seen as « moving » in a torus along the conjugation path of the molecule.

Pi wavefunctions are built on C-atom p-orbitals, and have a node in the plane of the bond (we briefly discuss the case of benzene to make this clear). The delocalized p-orbital can be seen as a 1D box-potential along which pi-electrons can move freely. This very simple picture is known as the 1D electron gas model.

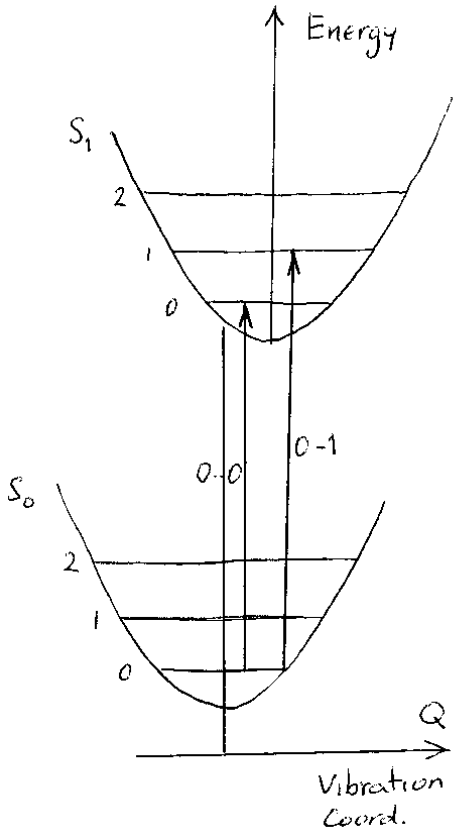

Figure 1.2 : Potential energy curves in the ground and first excited singlet states, as functions of a vibration coordinate. Optical absorption induces transitions between levels, as indicated by the arrows.

When the electronic cloud of a molecule is put into an excited state (after absorption of one photon), the pi-electron density in this new state is changed, which means that the geometry of the molecule, in particular the distances between carbon atoms, is also (slightly) changed. This leads to coupling of the electronic excitation with vibrations. Therefore, at zero temperature, photon absorption leads to an electronic transition accompanied by a series of vibronic bands where 0, 1, 2, etc. vibration quanta are created along with the electronic excitation. In the conjugated photon emission process (called fluorescence), one photon is emitted from the relaxed excited state (0 vibration) to vibrational levels of the ground state. Vibronic coupling will give rise to side-bands at higher energy than the 0-0 transition in the case of absorption, to side-bands at lower energy in the case of fluorescence. For purely harmonic vibrations, this leads to symmetry (mirror image) between normalized absorption and fluorescence spectra as a function of energy (or frequency). At higher temperatures, the molecule may absorb from a vibrational state, or emit from a excited state with vibrational energy, leading to additional bands and to broadening of the bands.

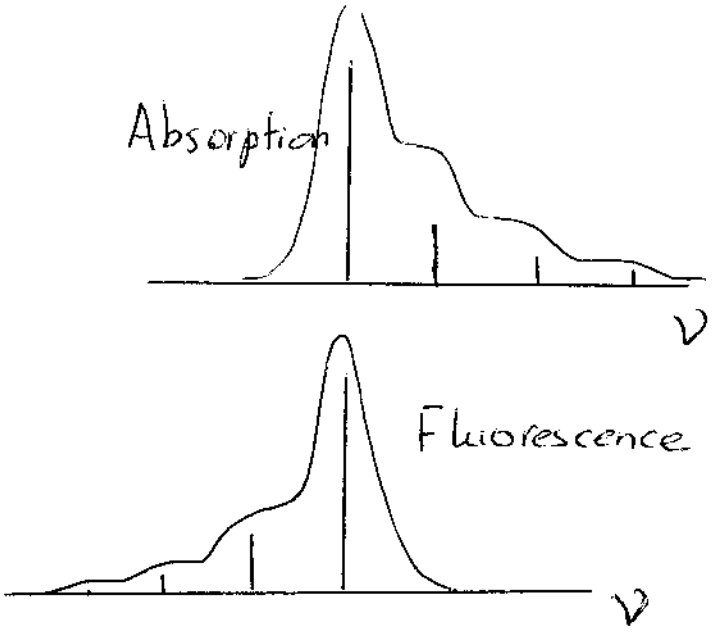

Figure 1.3 : Schematic representation of the absorption and fluorescence spectra of a molecule as functions of the photon frequency. The strengths of the respective transitions are represented by sticks, or by very sharp lines at low temperatures. At room temperatures, all lines are broadened (smooth contours). Note the symmetry between absorption and fluorescence spectra.

Next, it is important to consider how strongly a molecular transition is coupled to radiation. The quantity describing the strength of this coupling is called oscillator strength. It is proportional to the square of the transition dipole moment, a vector homogeneous to an electric dipole and fixed relative to molecular axes.

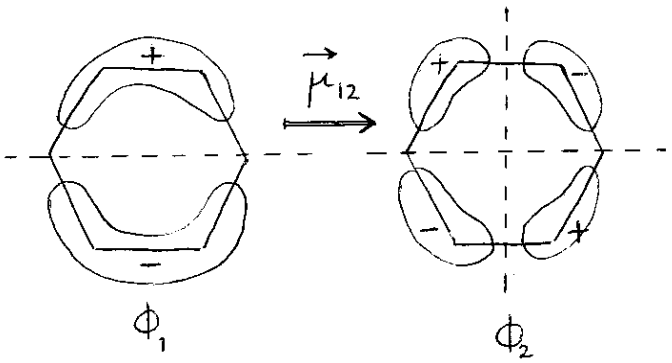

Figure 1.4 : Schematic representation of the pi electron densities for two of the orbitals involved in the lowest optical transition of benzene. The transition dipole moment between these two orbitals, as indicated by the arrow, is a vector with units of C×m.

Being conjugated processes, absorption and fluorescence strengths are closely related, provided the geometry of the molecule does not completely change between absorption and emission. The radiative rate k_r (i.e., inverse of the *radiative* lifetime) of the molecule is proportional to the integral of its absorption coefficient over the spectrum (their relation is called Strickler-Berg formula). However, other channels than the radiative one can be accessible from the excited state of the molecule, thus decreasing the fluorescence intensity. For example, electronic excitation energy may be dissipated into vibrations (or heat), or the molecule can undergo transitions to other

electronic states (triplet, photochemical products, etc.). The total rate of all these non-radiative processes is the non-radiative rate k_{nr}.

The ratio

$$\eta = \frac{k_r}{k_r + k_{nr}}$$

is called the fluorescence quantum yield. Molecules with high radiative rates k_r will therefore tend to be better emitters. This is the case of many dye lasers, who are also good fluorescent probes.

The non-radiative channel is mainly governed by radiationless transitions to lower-lying electronic states, in particular the ground state, and to a lesser extent to the metastable triplet state (see below). Non-radiative transitions are much reduced for planar, rigid molecules. As a rule, therefore, good fluorophores have to be planar and rigid. Figure 1.5 shows the chemical structures of a few representatives of important classes of fluorescent dyes: polycyclic aromatic hydrocarbons, porphyrins, cyanines, rhodamines.

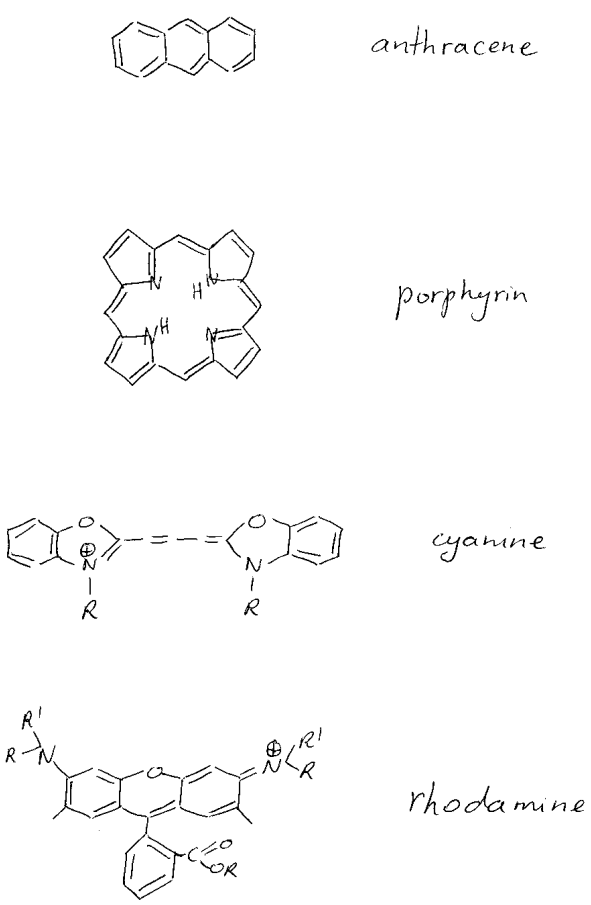

Figure 1.5 : Examples of chemical structures of a few fluorescent molecules as representatives of important classes of fluorophores. Note the alternating single and double bonds (conjugation), the planarity and rigidity of the molecules. Ionic dyes are usually water-soluble. Anthracene and many porphyrins are hydrophobic.

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Strongly fluorescent molecules, called fluorophores, all present conjugated pi-bonds with or without hetero-atoms participating in the conjugation. They are planar, rigid, and chemically stable enough to sustain many excitation-emission cycles in such reactive environments as air or water. The latter is particularly important for biological labelling.

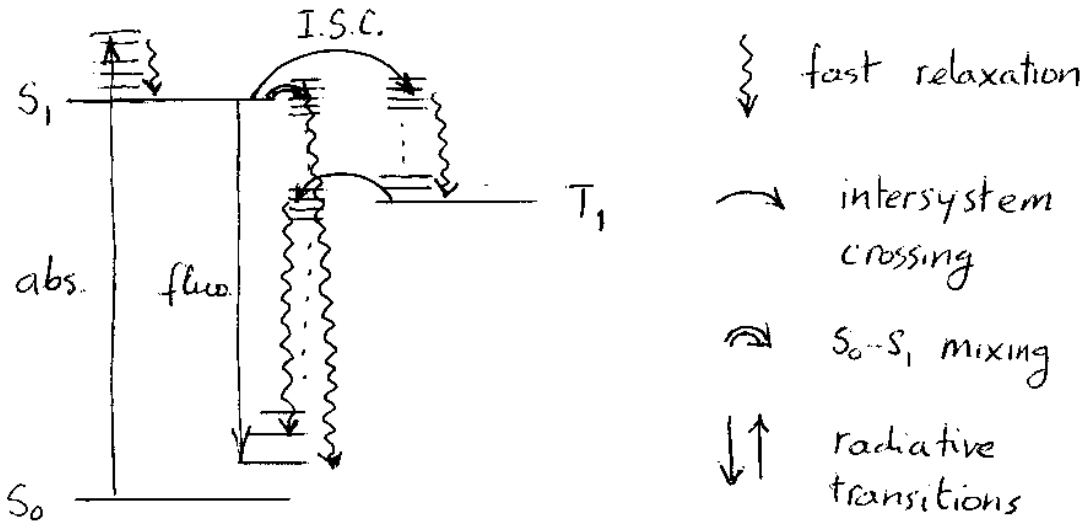

Figure 1.6 : Schematic level scheme of an organic molecule (called Jabłoński diagram), with the three main electronic states involved in absorption, fluorescence, and intersystem crossing (ISC).

The ground state of a large majority of fluorescent molecules is a singlet state, i.e., its electrons are paired into a zero-spin state. The ground state is called S_0. The first excited singlet state S_1 is the one reached after absorption of a photon. The reason for this is that a photon cannot flip spins. This applies to molecules consisting of light atoms, because the magnetic field of a light wave is too weak. But there is another excited state at lower energy than the first excited singlet. It is the triplet state T_1, with total spin 1. This state lies at a lower energy than the singlet S_1 because of a

reduced Coulomb repulsion between electrons (exchange interaction). The triplet state has three spin sublevels, which are usually indistinguishable at room temperature. Transitions between singlet and triplet states are called intersystem crossing, ISC. ISC is spin-forbidden, therefore caused only by relatively weak interactions such as spin-orbit coupling. ISC transition rates are therefore rather low, and the lifetime of the triplet state is rather long (often microseconds to seconds). However, the triplet states play a central role in the photodynamics of the molecule, because they limit the number of resonant absorptions and fluorescence per unit time that a molecule can perform. We'll come back to the consequences of these transitions in later chapters.

It must be realized that aromatic molecules, being conjugated, are rather reactive: the pi-electrons are very polarizable (being far from the carbon nuclei), and therefore readily engage in new chemical bonds. The situation is even worse when the molecule is excited, because then the electronic cloud has been given extra energy, which can help cross photochemical barriers, and which gives access to more new states for the excited electron. Fluorescent molecules, which have long excited state lifetimes, are therefore particularly exposed to photochemistry. Among the possible reactions taking place in excited molecules, we can cite: charge (electron or hole) transfer, proton transfer (intra- or intermolecular), cis-trans isomerization of double bonds, twisted intramolecular charge transfer, photooxidations, photoadditions, etc. The immense majority of these reactions destroy fluorescence. The new product does not absorb light in the same spectral region, or if it still absorbs, does not emit any more, because new non-radiative channels have usually been opened. All fluorescent molecules are therefore subject to photobleaching, that is the "death" of fluorescence after a variable average number of excitation-emission cycles. This number can vary between less than 1 and several hundreds of millions, depending on the molecules and on the conditions of the experiment (presence of small reactive molecules such as oxygen or water, temperature, heavy atoms in the solution or matrix, viscosity of the solvent or rigidity of the environment, etc.).

Fluorescence is a very-low-background technique. The reason is that fluorescence arises only after real excitation of the molecule in its excited state. If a molecule is not excited resonantly, it may still end up in an excited vibrational level of the ground state: this is a Raman scattering process. As compared to fluorescence, the probability of this process is reduced by the extremely short dwell time in the

"virtual" excited state. Raman scattering cross-sections are of the order of $10^{-12}\,\mathring{A}^2$, i.e. more than 10 orders of magnitude smaller than the typical fluorescence cross-section of a good fluorescent dye, $3\,\mathring{A}^2$. This explains why spectral selection by fluorescence is so efficient and so selective.

Exercise 1.1: Macroscopic indications of molecular sizes. Several physical phenomena strongly suggest the existence of atoms and molecules and some even provide estimates for their sizes. Among the latter are surface effects. Here are three examples:

- thermodynamics: by comparing the vaporization energy of water (2.3×10^6 J/kg) to the surface tension (70 mN/m), estimate the molecular size of water. Hint: calculate the total area of water droplets with a variable radius.

- Langmuir films: this argument is due to Benjamin Franklin, who noticed that a given volume of oil was able to cover a given area of water in a pond, and made the hypothesis of a monomolecular layer. Estimate the thickness of the layer knowing that a droplet (0.02 cm^3) covers about 10 m^2.

- Ellipsometry: Jamin's careful measurements (Ann. Chim. 1851) of light reflection by a water surface around Brewster's incidence suggested that the air-water interface is not mathematically sharp. Jamin measured an elliptically polarized reflection for light polarized at 45° from the incidence angle. Use Fresnel's reflection formulas to discuss the origin of the elliptical polarization.

Exercise 1.2: Write the Raman scattering probability per unit time in second-order perturbation theory, treating electron-vibration coupling as a small perturbation. Write a similar expansion for the fluorescence probability per unit time under the same conditions. Derive the ratio of the cross sections of these two processes, and simplify its expression with quantities such as the spontaneous emission rate. Give an order of magnitude for this ratio and relate it to the ratio of cross sections discussed in the course.