1
Basic Optical Principles

1.1
Introduction

To understand important optical methods used to investigate biomolecules, such as fluorescence polarization anisotropy, Förster resonance energy transfer, fluorescence lifetime techniques (Chapter 3), optical single-molecule detection techniques (Chapter 9), fluorescing nanoparticles (Chapter 12) or high-resolution fluorescence microscopy such as STED and PALM (Chapter 8), a basic understanding of the interaction of light with molecules and biomolecules is required. Such basic knowledge is also necessary in understanding the use of magnetic resonance techniques (Chapter 5). In the following sections we discuss a few fundamental principles of the physical processes that govern the interaction of light with biomolecules, optical markers or other relevant matter such as nanoparticles. This chapter provides a brief but wide-ranging insight into important physical and quantum mechanical basics of these processes also for, but not limited to, readers without a physical or chemical background, while being mathematically and physically as exact and concise as possible. Readers that look for a rather quick overview and that have at least some basic knowledge about orbitals of molecules might focus on sections 1.1, 1.3, 1.5 and 1.6 only. Please be aware that a complete, indepth treatment of quantum mechanics and molecular spectroscopy is beyond the scope of this chapter. Only the fundamentals that are needed for the biophysical chemistry methods presented in the following chapters are discussed. For further information about quantum mechanics and molecular spectroscopy, the reader might refer to the more specialized books given in the bibliography at the end of this chapter.

Light is a wave of oscillating electric and magnetic fields propagating through space (Figure 1.1). The electric- and magnetic-field components of this electromagnetic radiation are oscillating in phase perpendicular to the propagation direction and with respect to each other. The smallest possible units of light are elementary particles called photons. These possess a specific amount of energy, E,
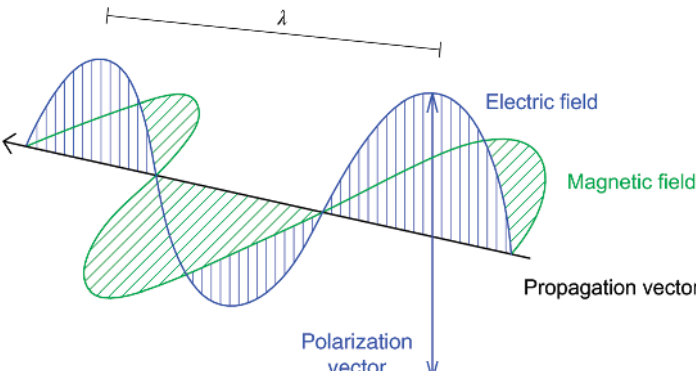
Figure 1.1 Light is a wave of oscillating electric and magnetic fields propagating through space.

which depends on the frequency or wavelength of the light radiation:

$$E = h\nu = \frac{hc}{\lambda} = hc\bar{\nu} \tag{1.1}$$

Here, h is Planck's constant, ν is the frequency of the electric- and magnetic-field oscillation, c is the speed of light, λ is the wavelength of the oscillation and $\bar{\nu}$ is the corresponding wave number. The energy E of a photon is linear proportional to the frequency ν and wave number $\bar{\nu} = \frac{1}{\lambda}$. The wavelength, in turn, is inversely proportional to the energy.

Figure 1.2 shows the spectrum of electromagnetic radiation. Optical methods make use of electromagnetic radiation having wavelengths from ∼200 nm to a few μm. This interval can be subdivided into the ultraviolet (UV) region (∼200–400 nm), the visible region (∼400–700 nm) and the near infrared (NIR) region (∼700 nm to a few μm). The UV range, for example, is important for the characterization of proteins and DNA, which is described in Chapter 2. The visible region is important for most fluorescence techniques, which are described in
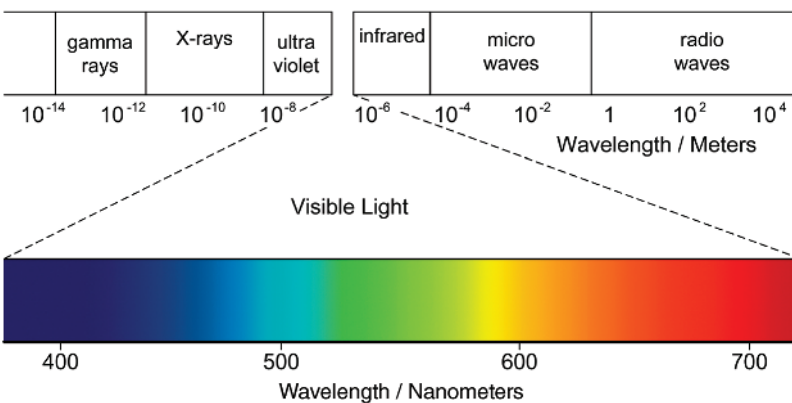

Figure 1.2 Spectrum of electromagnetic radiation.

detail in Chapters 2 and 3 and partly in Chapters 7–13. The infrared region enables the identification and investigation of biomolecules based on their characteristic molecular vibrations (Chapter 4). Radiowaves and microwaves are used for nuclear and magnetic resonance techniques. Chapter 5 describes the application of these techniques for the investigation of biomolecules.

The size of typical molecules or biomolecules is usually on the order of a few nanometres, which is far smaller than the dimensions of the oscillating electric-field waves of visible light, which are on the order of a few hundred nanometres. When molecules are within the waves of the light, the charged components of the molecules, especially the electrons, are affected by the oscillating electric-field component of the light. If a molecule possesses a suitable electronic structure it can absorb a photon of a specific wavelength, resulting in an electronic structure rearrangement. In the next section, we discuss what the electronic structure of molecules looks like.

1.2
What Does the Electronic Structure of Molecules Look Like? Orbitals, Wave Functions and Bonding Interactions

As with photons, electrons posses also both wave- and particle-like properties. Because of the wave-like character of electrons, only probabilities can be given of finding electrons at a certain place around the nuclei of a molecule. This electronic structure is usually visualized by electron clouds or probability densities, which are mathematically described by three-dimensional probability density functions. Depending on the molecular structure, only distinct eigenstates of the electron probability densities are possible. Such a state simply describes a possible three-dimensional standing wave of the negatively charged electron waves in the presence of the positively charged nuclei – similar to a string of an instrument, which can also vibrate only with certain frequencies and overtones. Each of these states corresponds to distinct energies.

During the absorption of a photon the electronic structure of the molecules is usually transferred from an electronic ground state into an energetically higher electronic excited state. In an excited state the three-dimensional, electronic probability densities are different from the ground-state probability densities. Only photons having energies corresponding to the energy difference between these electronic states can be absorbed by the molecules. This is the origin of the colour of a compound: only light of certain wavelengths is absorbed by the compound – light of other wavelengths is either transmitted or scattered. The photon energy corresponding to the absorbed wavelength is equal to the necessary excitation energy for the respective transition.

What does the probability density function, $P(x, y, z)$, corresponding to a certain electronic state look like? In quantum theory, the probability density for finding electrons at a certain place $P(x, y, z)$ is calculated from the electronic wave function, $\psi(x, y, z)$, corresponding to this state:

$$P(x, y, z) = \psi^*(x, y, z)\psi(x, y, z) \tag{1.2}$$

The probability of finding an electron in a certain volume element $x \cdots x + dx$; $y \cdots y + dy$, $z \cdots z + dz$ can be calculated by integrating the probability density $P(x, y, z)$ in the corresponding range. The electronic wave function is also a three-dimensional mathematical function that contains all information about the electrons in a molecule. Some of the most relevant parameters for optical techniques in the biosciences, such as the transition dipole moment, can only be properly derived from electronic wave functions. Therefore, we will briefly introduce some important aspects about wave functions here.

Usually, the wave function is simply written as ψ rather than $\psi(x, y, z)$ but the meaning is the same. In addition, we will use for the coordinates of the electrons and nuclei in the following $\vec{r} = (x, y, z)$:

$$\psi(x, y, z) = \psi(\vec{r}) = \psi \tag{1.3}$$

Electronic wave functions describing single electrons of a molecule in a certain electronic state are called orbitals, $\varphi(x, y, z) = \varphi(\vec{r}) = \varphi$.[1] For example, the simplest possible single electron wave function is an atomic s orbital, φ_s (on the left in Figure 1.3). It corresponds to a spherically symmetrical probability density of a single electron around a single nucleus. An examination of the time dependence of wave functions shows that they are actually oscillating. The sign of positive
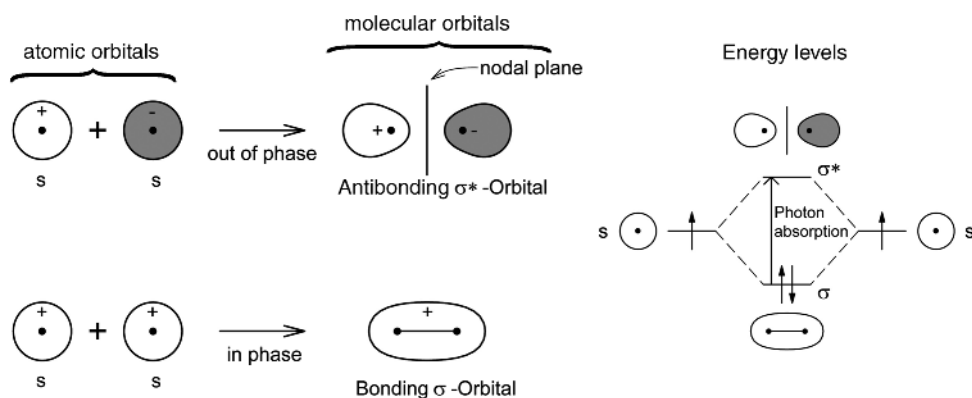

Figure 1.3 Schematic construction of bonding and antibonding molecular orbitals from atomic orbitals along with the resulting electron energy levels. Probability functions for finding an electron at a certain place can be calculated from the orbitals using (1.2) and look qualitatively similar, with the most important difference that probabilities exhibit no negative regions. The height of the horizontal lines on the right represents the energies of electrons in the corresponding orbitals. Electrons occupying certain orbitals are depicted by arrows. The direction of the arrows assigns the spin of the electrons.

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1) The description that an electron occupies orbitals independently from all other electrons is only a very useful approximation, which is close to reality in many cases. Since all charged particles in a molecule influence each other, an exact description would be only possible by wave functions that describe a state of an entire molecule, $\psi_{molecule}$, in a certain environment including all electrons and nuclei simultaneously.

and negative parts of the three-dimensional wave function changes with a certain frequency. The negative and positive signs of the orbitals are not to be confused with negative or positive charges. They are rather a consequence of the wave character of the electrons. For example, the waves of light (Figure 1.1) are also mathematically described by oscillating functions having negative and positive values but of course this has nothing to do with positive or negative charges. However, often, it is sufficient to work with time-independent versions of the wave functions, which represent stationary states or standing waves and in which the oscillating, time-dependent factor is omitted. In Figure 1.3, for example, time-independent wave functions or orbitals are visualized for which negative parts are depicted in grey, whereas the positive parts are unfilled. The oscillation also has no effect on the probability density of finding an electron at certain places, $P(\vec{r}\,)$, negative signs are cancelled out by the multiplication described by (1.2). The shape of the three-dimensional probability densities, $P(\vec{r}\,)$, as calculated by (1.2) are visually often quite similar to the shape of the wave functions, with the most important difference that there are no "negative" regions for the probability density of electrons.

Orbitals, and thus probability densities, never change abruptly. For an electron in an s orbital, for example, the corresponding probability density increases smoothly when approaching the nucleus. Presentations of orbitals, such as shown in Figure 1.3, or probability densities usually symbolize a certain volume of the corresponding probability density in which the electron can be found, for example, with 90% probability. The shown surfaces are called isosurfaces because they correspond to equal values for the probability of finding the electron or to equal absolute values of the wave function.

To contain all information on the electrons, for example the actual phase of the oscillation, wave functions can also contain mathematically complex terms. The symbol "*" in (1.2) simply denotes a transformation in which all imaginary parts of complex numbers in the wave function are multiplied by −1. However, for a qualitative understanding of the following sections, an indepth knowledge about the imaginary parts of the wave functions is not mandatory.

If an atom approaches another atom this affects the possible eigenstates of the electrons because they are then in the vicinity of two positively charged nuclei. The possible wave functions for single electrons in the presence of several nuclei are called molecular orbitals. Molecular orbitals can be approximated by linear combinations of atomic orbitals (LCAO). If the wave functions of two atomic s orbitals with the same sign (corresponding to the situation that they oscillate in phase) are combined they form a new a bonding molecular orbital called a σ-orbital, ($\varphi_\sigma \propto \varphi_s^1 + \varphi_s^2$, in the lower middle of Figure 1.3). The energy of an electron occupying a bonding molecular σ orbital is lower than that of an electron in an atomic s orbital. This is the driving force for molecular bonds. So, if, for example, two hydrogen atoms with a single electron in an s orbital approach, both electrons can then be found in the newly formed bonding σ-orbital. The probability density of finding the electrons at a certain place of the molecular σ-orbital can again be calculated from the corresponding molecular orbital φ_σ

using (1.2). Notably, an orbital can only be occupied by a maximum of two electrons with opposite spins (We will discuss the meaning of electron spins further below.) If two atomic s-orbitals with opposite signs (corresponding to the situation that they oscillate out of phase) are combined they form an antibonding molecular σ^*-orbital, $\varphi_\sigma \propto \varphi_s^1 - \varphi_s^2$ (in the upper middle of Figure 1.3). Electrons in antibonding σ^*-orbitals have higher energies than electrons in bonding σ-orbitals (orbital energies are visualized as horizontal lines on the right in Figure 1.3, the corresponding electrons with opposite spins as arrows on these lines). By absorbing a photon with an energy corresponding to the energy difference between these orbitals one of the electrons in the bonding σ-orbital can be excited into the antibonding σ^* orbital. The large energy gap of the $\sigma \rightarrow \sigma^*$ transition usually requires excitation with photons corresponding to UV light. The antibonding σ^*-orbital has one nodal plane, which constitutes a region of zero electron density. Therefore, a $\sigma \rightarrow \sigma^*$ excitation can lead to bond breakage. Notably, it is a convention to assign antibonding orbitals also by a star, which is not to be confused with the meaning of the star in equations such as (1.2) where it assigns the earlier mentioned transformation of imaginary parts of the wave functions.

The second simplest atomic orbitals are p-orbitals (left-hand side in Figure 1.4). They have a nodal plane at the nucleus and the sign of the real part of the wave function is always opposite across this nodal plane. Two atomic p-orbitals of appropriate relative orientation at neighbouring nuclei can form molecular π-orbitals.[2] In organic molecules and biomolecules most π-orbitals are found at
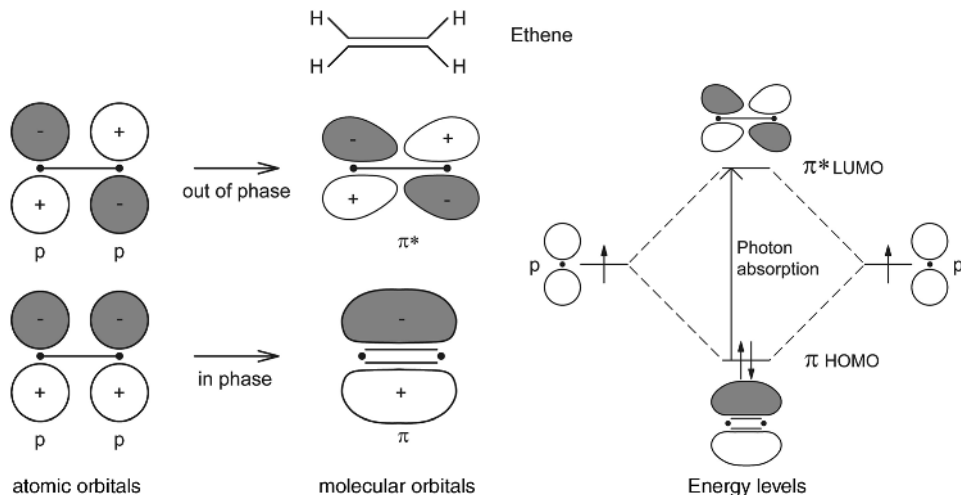

Figure 1.4 Schematic construction of bonding and antibonding molecular π-orbitals from atomic p-orbitals in the case of ethene.

2) Also, a molecular σ-orbital can be constructed from two atomic p-orbitals if the latter are orientated parallel to the bond axis (perpendicular to the ones shown in Figure 1.3). In general, molecular orbitals are defined as a σ-orbital when they are symmetrical with respect to a rotation about the bond axis.
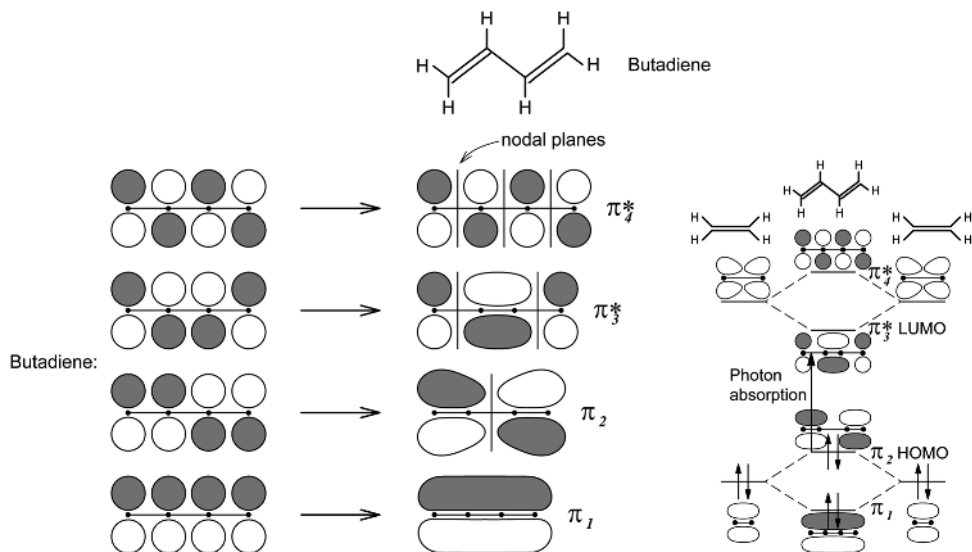
Figure 1.5 Schematic construction of an extended molecular π-orbital system from conjugated atomic p-orbitals. The example corresponds to butadiene. The HOMO and LUMO of butadiene correspond to the π_2 and π_3^* orbitals, respectively. The four molecular orbitals $\pi_1, \pi_2, \pi_3^*, \pi_4^*$ of butadiene can also be thought of as being constructed from the two molecular orbitals, π, π^*, of two ethene molecules. Direct comparison of the energy levels of the HOMO and LUMO of ethene with the HOMO and LUMO of butadiene shows that the energy gap of the latter is smaller.

carbon, oxygen and nitrogen atoms and often constitute double bonds together with simultaneously existing σ-bonds. A simple example is ethene.

Similarly, several p-orbitals can form π-orbitals that are delocalized over multiple nuclei (Figure 1.5). Such molecular orbitals are called conjugated π-systems. In Figure 1.5, four atomic p-orbitals form four molecular orbitals. In a similar manner, n molecular π-orbitals can be constructed from n atomic p-orbitals. The energy of the newly formed molecular orbitals increases with the number nodal planes (see energies of the molecular π-orbitals $\pi_1, \pi_2, \pi_3^*, \pi_4^*$ in Figure 1.5). The electrons from the original atomic p-orbitals (four in Figure 1.5) are then filled into molecular orbitals, starting with the lowest-energy orbitals and obeying the rule that only a maximum of two electrons are allowed per molecular orbital. If orbitals have equal energies, they are first occupied by single electrons before any of them is filled with a second electron.

The highest occupied molecular orbital and the lowest unoccupied molecular orbital are usually assigned by their acronyms HOMO and LUMO, respectively. For most organic molecules, the electronic ground state corresponds to the situation that all orbitals up to the HOMO electrons are occupied by two electrons (see, for example, Figures 1.4 and 1.5). Similarly, for organic molecules the first excited state corresponds often to the situation that one electron has been promoted from the HOMO to the LUMO. The electronic ground state and the first possible
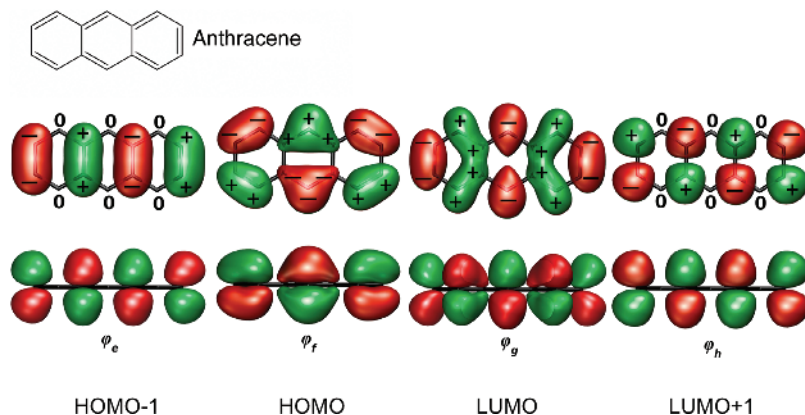
Anthracene

φ_e φ_f φ_g φ_h

HOMO-1   HOMO   LUMO   LUMO+1

Figure 1.6 Schematic top and side views of examples of the π-orbitals of anthracene. HOMO-1 and LUMO+1 assign those orbitals that are the energetically next orbitals below or above the HOMO and LUMO orbitals, respectively. Figure created using VMD (see Bibliography for details).

electronically excited state of molecules are often referred to as S_0 and S_1 states, respectively. The reasons for this nomenclature are explained below (Section 1.5).

For butadiene (Figure 1.5), for example, S_0 corresponds to the situation in which the lowest two molecular orbitals π_1 and π_2 are each occupied by two electrons. As can be seen by a comparison of the orbital energies of ethene in Figure 1.4 and the corresponding orbital energies of butadiene in Figure 1.5 the energy difference between the electrons in the HOMO and the LUMO decreases as the system of conjugated p-orbitals becomes larger. In general, larger conjugated π-systems typically absorb photons of lower energy and thus longer wavelengths than smaller conjugated π-systems [compare with (1.1)].

The molecular π-orbitals of more complicated molecules, like biomolecules or optical markers, naturally look more complicated. Figure 1.6 shows wave functions of the π-system of the organic molecule anthracene as an example. The general rule that electrons in orbitals having more nodal planes have higher energies still applies in most cases.

Electronic transitions involving conjugated π-systems are especially important in the spectroscopy of biomolecules, or optical markers, because absorption and emission of photons of these systems are often strong and correspond to light in the spectroscopically convenient visible region. Since σ-bonds remain, $\pi \rightarrow \pi^*$ transitions usually do not cause bond breakage.

1.3
How Does Light Interact with Molecules? Transition Densities and the Transition Dipole Moment

In the previous section we discussed the electronic structure of molecules before and after they have absorbed a photon and what photon energy is necessary for
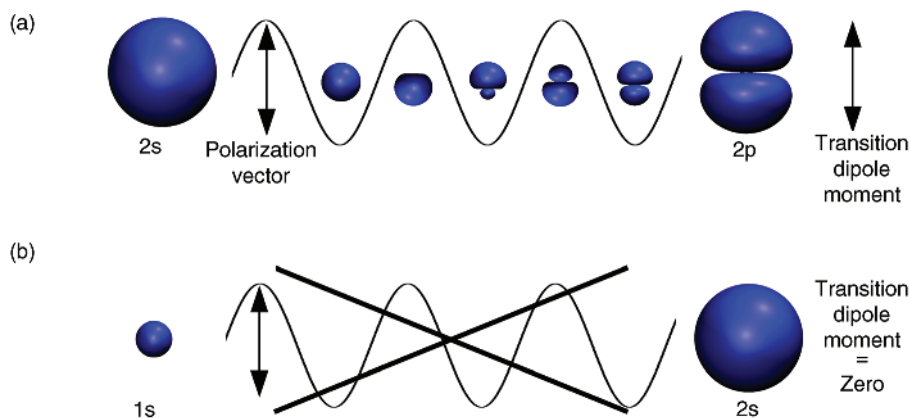
(a)

| 2s | Polarization vector | | 2p | Transition dipole moment |

(b)

| 1s | | 2s | Transition dipole moment = Zero |

Figure 1.7 (a) The oscillating electric field component of light can induce a transition from an s to a p orbital when it has the correct polarization (direction of oscillating electric field component of light). Please note that here probability densities and not wave functions are visualized. The response of the electron probability densities to the oscillating electric field during the transition is also schematically illustrated. (b) In contrast, the oscillating electric-field component of light cannot induce a transition from one s-orbital to another s orbital. Figure created using VMD (see Bibliography for details).

such a transition. Here we take a closer look at the mechanism by which a photon or optical radiation can actually interact physically with molecular orbitals to promote the molecule from one electronic state to another.

The probability by which a photon of correct energy is absorbed by a molecule depends on the ability of the light's oscillating electric field to change the initial electronic state into the final electronic state. For example, light can excite the single electron of an isolated hydrogen atom from an s-orbital into a p-orbital, when the orientation of its oscillation electric field vector is perpendicular to the nodal plane of the p-orbital (Figure 1.7a). In turn, it is not possible to excite the electron from one s-orbital into another s-orbital of higher energy because such a transition cannot be promoted by an oscillating electric field (Figure 1.7b).

As can be seen from this simple example, it is important that the direction of the oscillation of the electric field of the light fits somehow with the orientation and shape of the electron clouds and the wave functions describing the initial and the final electronic state of the atom or molecule. The direction of the oscillating electric field of the light is defined by its polarization vector (Figure 1.1). The direction and magnitude of the change in the charge distribution during the transition is closely related to the so-called transition dipole moment, which can be calculated from the wave functions of the initial and final electronic state (1.4):

$$\vec{M}_{I \to F} = \int \psi_F^*(\vec{r})e\vec{r}\ \psi_I(\vec{r})dV \qquad (1.4)$$

Here, ψ_F is the electronic wave function of the molecule in the final state and ψ_I of the molecule in the initial state; e is the elementary charge of an electron

and $\vec{r} = (x, y, z)$ constitutes coordinates, which have their origin ($x = 0$, $y = 0$, $z = 0$) in the centre of the molecules charge distribution. The integration $\int \dots dV = \iiint \dots dx dy dz$ runs over the entire space. The transition dipole moment is one of the most important optical parameters of optical markers in general and in understanding techniques such as fluorescence polarization or Förster resonance energy transfer. Therefore, we will discuss the transition dipole moment in some more detail.

It can be shown that $\psi_F^* e\vec{r}\, \psi_I = \psi_F^*\psi_I \cdot e\vec{r}$. It is easy to see that the first part of this product, the so-called transition density $\psi_F^*\psi_I$, has some similarities with $\psi^*\psi$ (1.2). However, whereas $\psi^*\psi$ describes the probability density of finding an electron at a certain position in a distinct state $\psi_F^*\psi_I$, describes changes in the electronic structure and charge distributions during the transition from the initial state to the final state. Figure 1.8 visualizes the meaning of the transition density exemplarily with a transition of an electron in anthracene (Figure 1.6) from the orbital φ_f to the orbital φ_h. In this case the transition density can be graphically estimated by simply multiplying the signs of the electrons initial and final orbitals, φ_f and φ_h, close to each atom (indicated in Figure 1.8 by + and −, with the number 0 for zero values at nodal planes). At the atom of anthracene, for example, placed in the upper left-hand corner of Figure 1.8 (arrow 1), φ_f has a negative sign, whereas φ_h has a positive sign. As a result of the multiplication the transition density, $\varphi_f\varphi_h$ must have also a negative sign at this position. At the atom depicted with arrow 5 both φ_f and φ_h have a positive sign and thus the resulting transition density has a positive sign. Repeating this procedure for all atoms helps to generate a graphical estimate of the transition density $\varphi_f^*\varphi_h$ shown on the right-hand side in Figure 1.8. Obviously, the transition density is dominated by positive signs on the right-hand side of the molecule and by
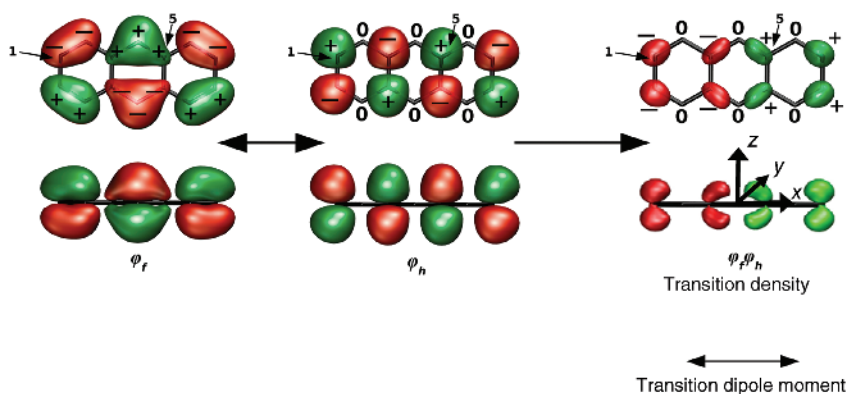

Figure 1.8 Schematic representation of the construction of the transition density and the direction of the transition dipole moment for the transition between the φ_f and φ_h orbitals of anthracene. In this illustration the size of the transition density is reduced in comparison to the orbitals for better visibility (higher isosurface values for the transition density). Figure created using VMB (see Bibliography for details).
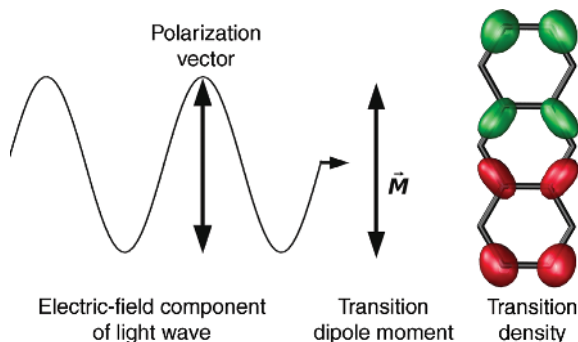
Polarization
vector

$\vec{M}$

Electric-field component
of light wave

Transition
dipole moment

Transition
density

Figure 1.9 Schematic representation of the optimum direction of the oscillating electric-field component of light (polarization vector) in relation to the direction of the transition dipole moment and the corresponding transition density for a $\varphi_f \leftrightarrow \varphi_h$ transition in anthracene. The probability that photons of correct energy can induce this transition is the highest when the polarization vector is parallel to the transition dipole moment. Figure created using VMB (see Bibliography for details).

negative signs on the left-hand side. The consequence is that light with an oscillating electric-field component orientated in the same or a similar direction in which the most dominant difference in the transition density is present has the highest chance of promoting an electron from the molecular orbital φ_f to the molecular orbital φ_h (Figure 1.9).

The direction of the largest difference in the transition density for a certain transition is calculated by integration over the entire transition density using (1.4) and results in a vector $\vec{M}$, which is the transition dipole moment. The highest probability that light excites electrons in a molecule is observed when its oscillating electric-field component, that is its polarization vector, is exactly parallel with the transition dipole moment for the corresponding transition (Figure 1.9). Since the wave functions oscillate, the signs of the orbitals in Figure 1.8 are arbitrary. As a result, it is also arbitrary whether the transition dipole moment vector points in one or the other direction (indicated by the double arrow in Figures 1.8 and 1.9).

The probability that a photon is absorbed is higher the larger the corresponding transition dipole moment vector becomes. It depends quadratically on the absolute value of the transition dipole moment vector:

$$P_{I \to F} \propto \left| \vec{M}_{I \to F} \right|^2 \tag{1.5}$$

Also, the probability of the reverse process, the transition of an electron from an energetically higher state to an energetically lower state with a simultaneous emission of a photon is directly proportional to the square of the transition dipole moment.

Inspecting the time dependence of the transition density reveals that it has one oscillating component that corresponds to the difference frequency between the final and initial wave-function oscillations. This difference frequency corresponds exactly to the frequency and thus the energy of photons that can induce the

transition. A transition thus corresponds to the case where the oscillating electric-field component of light is in resonance with the corresponding transition density oscillation between initial and final states.

A calculation of the transition density and the transition dipole moment between the two s-orbitals (Figure 1.7b) explains why this transition cannot be induced by light. The corresponding transition density is spherically symmetrical around the spherically symmetrical origin at the nucleus (position of the nucleus is at $x = 0$, $y = 0$, $z = 0$). Since the term $e\vec{r}$ in (1.4) switches signs at the origin ($x = 0$, $y = 0$, $z = 0$), in the overall integration different parts around the origin cancel each other out exactly. For example, at the arbitrary position ($x = l$, $y = 0$, $z = 0$) the value of the transition density, $\psi_F^* \psi_I$, is exactly the same as at the position ($x = -l$, $y = 0$, $z = 0$). The term $e\vec{r}$ also has the same absolute value at these two positions but opposite signs [remember that $\vec{r} = (x, y, z)$]. Consequently, $\psi_F^* \psi_I \cdot e\vec{r}$ at these two positions also have the same absolute values but opposite signs and therefore cancel each other out exactly in the integration described by (1.4). The same applies for any other two positions (x, y, z) and ($-x, -y, -z$). As an overall result the transition dipole moment is zero. In general, transitions are called optically forbidden or dipole forbidden when they have a zero transition dipole moment.

Some important selection rules for optical transitions can be deduced directly from the above argument. For instance, the parity selection rule, also called Laporte's rule, that applies for molecules having an inversion symmetry centre. Molecules having an inversion symmetry centre always have identical atoms at the positions (x, y, z) and ($-x, -y, -z$). The centre itself is located in the middle of the molecules and has the coordinates $x = 0$, $y = 0$, $z = 0$. This is the case for ethene (Figure 1.4), butadiene (Figure 1.5) and also for anthracene (Figure 1.6). The corresponding molecular orbitals of these molecules can either have a g (gerade) or a u (ungerade) symmetry. For orbitals with g symmetry the wave functions have identical values for any pair of positions (x, y, z) and ($-x, -y, -z$). Examples are the orbitals π_2 and π_4 of butadiene (Figure 1.5). All other orbitals of such molecules have u symmetry. Laporte's rule states that transitions of electrons between two orbitals that both have either g (gerade) or u (ungerade) symmetry have vanishing transition dipole moments. Therefore, g $\rightarrow$ g and u $\rightarrow$ u transitions are forbidden, while u $\rightarrow$ g and g $\rightarrow$ u transitions are allowed. As an example the π_2(g) $\rightarrow$ π_3^*(u) transition of butadiene (Figure 1.5) is allowed whereas the π_2(g) $\rightarrow$ π_4^*(g) transition is dipole forbidden. In the next chapter we will see that these rules also apply for important biological pigments such as carotenoids.

1.4
Absorption Spectra of Molecules in Liquid Environments. Vibrational Excitation and the Franck–Condon Principle

Having learnt what determines the magnitude of absorption and emission of photons by molecules we now turn to the question of what factors affect the overall shape of an absorption or emission spectrum. An absorption spectrum

describes the frequency or wavelength dependence of the probability that a molecule absorbs photons of the corresponding photon energy. Figures 1.10a and b show schematic absorption spectra of two different molecules in liquid solution. The absorption spectra are composed of various peaks or absorption bands. The absorption band observed for the longest wavelength (lowest photon energy), eq. 1.1 corresponds to the absorption of photons having energies corresponding to transitions from the electronic ground state to the first possible electronically excited state, $S_0 \rightarrow S_1$. The absorption band is not a narrow line, corresponding to an exact defined transition energy, because molecules in liquids are exposed to many different solvent environments. The different momentary solvent charge distributions and other environmental effects affect the electrons of the molecule and result in slight shifts in the S_0 and S_1 state energies. Other effects can also result in an additional line broadening of the observed absorption bands.
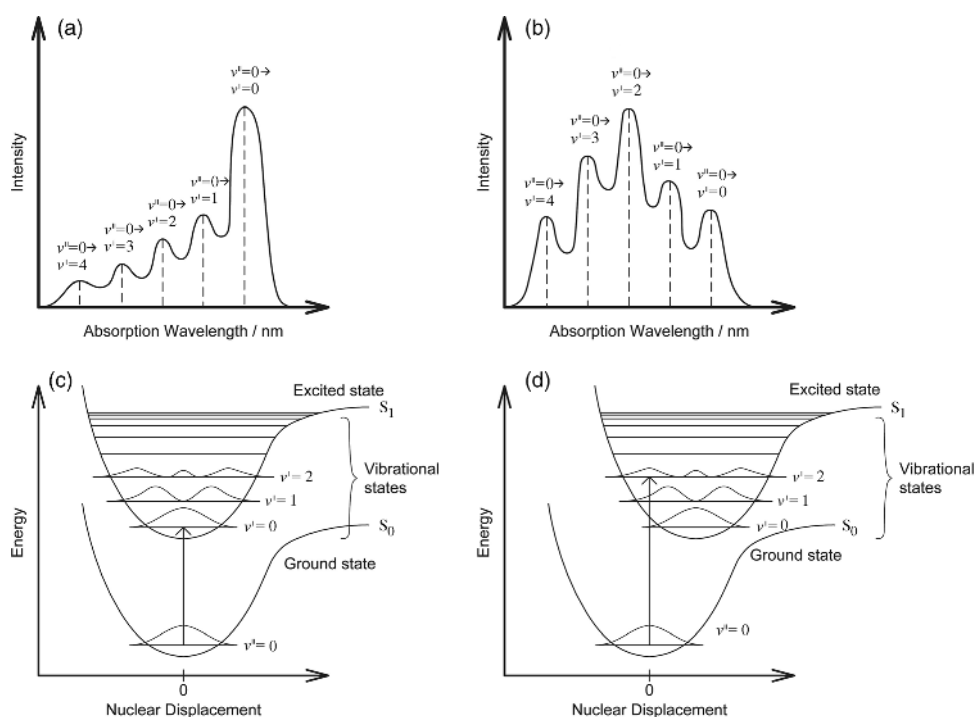

Figure 1.10 (a) and (b) Schematic absorption spectra of two different molecules. Parts (c) and (d) show, schematically, the respective potential curves for the molecules in the ground and in the excited states, S_0 and S_1. For the vibrational eigenstates the probabilities of finding the nuclei at certain positions relative to the equilibrium positions are visualized. Because nuclei are heavier than electrons the nuclei remain mainly stationary during electronic transitions and therefore "vertical" transitions are most likely. For the molecule depicted in (b) and (d), for example, it is most probable that a simultaneous excitation into the third vibrational state ($v' = 2$) occurs during an electronic excitation, $S_0 \rightarrow S_1$. Please note that here probability densities for finding the nuclei at a certain place, $P_v(\vec{r})$ (1.6), are visualized and not the vibrational wave functions, $\chi_v(\vec{r})$, from which the Franck–Condon integrals are actually calculated according to (1.7).

The bands observed at shorter wavelengths or higher photon energies correspond to transitions in which, simultaneously, vibrations of the nuclei in the molecules are excited in addition to the pure electronic excitation (Figures 1.10c and d). Such transitions are called vibronic transitions. What determines the probability that a certain vibrational state is excited simultaneously? As with electrons, for the nuclei of a molecule only probabilities can be given of finding them at a certain position. For a certain vibrational state, v, of molecules this probability can be calculated from vibrational wave functions, $\chi_v(\vec{r})$:

$$P_v(\vec{r}) = \chi_v^*(\vec{r})\chi_v(\vec{r}) \qquad (1.6)^{3)}$$

Again, only certain vibrational eigenstates are possible. At room temperature, molecules are usually in the lowest vibrational state of the electronic ground state, S_0. Vibrational eigenstates are defined by the vibrational quantum number, being zero for the vibrational ground state, $v = 0$. Figure 1.10c and d shows that for a diatomic molecule the probability distribution of finding the two nuclei at a certain position displaced from their equilibrium distance is given by a Gaussian function for $v = 0$. In Figure 1.10 the equilibrium position in the S_0 state is defined by the nuclear displacement 0 on the x-axis. A displacement of the nuclei to longer distances acts against the electronic bonding forces. A displacement of the nuclei to shorter distances acts against the repulsion forces between the positively charged nuclei. Consequently, any displacement leads to an increase in the energy contained in the molecule. This energy increase for the S_0 and in the S_1 states is visualized by the parabolic-like curves in Figure 1.10c and d. Such curves are called potential-energy curves. For very large separations of the nuclei, of course, bond breakage or dissociation occurs. This is indicated by the asymmetry of the curves for high vibrational energies.

Why is the probability for a pure electronic excitation ($v'' = 0 \rightarrow v' = 0$)[4] for some molecules (Figures 1.10a and c) the highest, whereas for other molecules (Figure 1.10b and d) an excitation into the third vibrational state of S_1 ($v'' = 0 \rightarrow v' = 2$) apparently has the highest probability? This can be rationalized by the so-called Franck–Condon principle. Since the timescale of excitation of the light electrons into a higher electronic state is far shorter than the vibrational motions of the heavier nuclei, the molecular geometry remains constant during the excitation process (vertical transitions in Figure 1.10). In the first molecule (Figures 1.10a and c) the equilibrium positions of the atoms in the excited state, S_1, are very similar to those in the S_0 state. As a consequence, no vibrations are

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3) The separate description of vibrational wave functions, χ_v, that are independent from wave functions describing the only the electrons, ψ (1.2), is again only an approximation that is usually close to the real situation. This approximation is called the Born–Oppenheimer approximation. Again, an exact description is only possible by a wave function, $\psi_{molecule}$, that describes the states of an entire molecule in a certain environment including all electrons and nuclei simultaneously: $\psi_{molecule} \approx \psi \cdot \chi_v$.

4) The convention is to define the number of the vibrational quantum states of molecules in the electronic ground state, S_0, by v'', whereas the corresponding number for vibrational quantum states of the excited state, S_1, is defined by v'.

induced by the electronic transition, $S_0 \rightarrow S_1$, and the molecule is preferentially excited into the vibrational ground state of the S_1 state. In the absorption spectrum the band corresponding to this transition dominates. In the other molecule, however, the equilibrium positions of the nuclei in the S_1 state are shifted towards longer internuclei distances (Figure 1.10d). This situation often applies simply because excited states usually have more antibonding character (Figures 1.3–1.5) and, therefore, the equilibrium distances of the nuclei are longer. Because the nuclei are heavier than the electrons, they can change their position very little during the excitation of an electron. As a consequence, the vibrational state observed after this transition is the most probable, in which the nuclear geometry can remain the same or similar to the nuclear geometry in the ground state, S_0, $v'' = 0$. For the molecule in Figure 1.10b and d the third vibrational state ($v' = 2$) of the S_1 state has a very large overlap of its vibrational probability distribution with the vibrational probability distribution in the ground state, or to be more quantitative the nuclear overlap integral

$$\int \chi_{v''}(r)\chi_{v'}(r)\mathrm{d}V \tag{1.7}$$

is the largest for this transition. Here $\chi_{v''}(r)$ describes the vibrational wave function of the vibrational ground state ($v'' = 0$) in the S_0 state and $\chi_{v'}(r)$ the vibrational wave functions in the S_1 state. Integrals described by (1.7) are called Franck–Condon integrals. In the absorption spectrum the relative magnitudes of the vibrational bands are proportional to the square of the corresponding Franck–Condon integrals:

$$P_{v'\rightarrow v'} \propto \left| \int \chi_{v''}(r)\chi_{v'}(r)\mathrm{d}V \right|^2 \tag{1.8}$$

Figures 1.10c and d show only the potential curves, vibrational energy levels and probabilities of finding the nuclei separated by certain displacements for a diatomic molecule. In more complex molecules of course various different vibrational modes are possible that cannot be visualized in a single two-dimensional plot. Nevertheless, the most important conclusions derived here are also valid for more complicated molecules.

1.5
What Happens After Molecules have Absorbed Light? Fluorescence, Nonradiative Transitions and the Triplet State

Let us now turn to the question of what happens to the excitation energy after a molecule has absorbed a photon. The energy absorbed by a molecule can be released via several pathways. For biomolecules or optical markers, which are usually in an aqueous or liquid environment, in most cases first vibrational relaxation is dominant. This process is the quick release of the entire vibrational
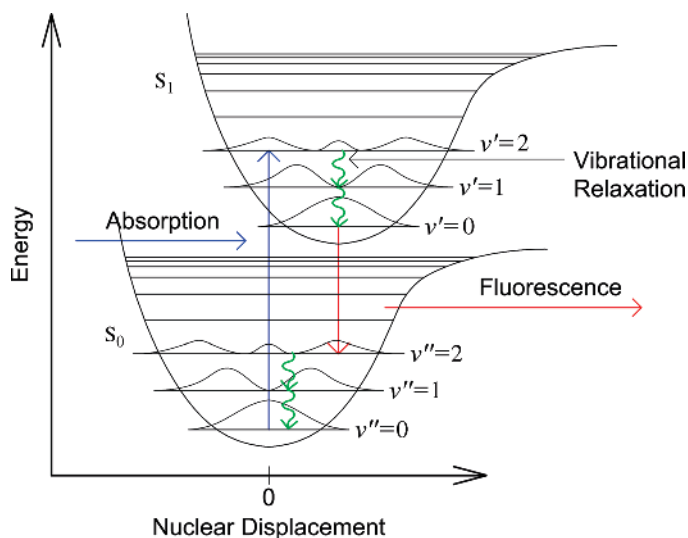
Figure 1.11 After excitation of a molecule (blue) usually very rapid vibrational relaxation (green) occurs in the excited state. As a consequence, fluorescence (red) normally occurs from the lowest vibrational level of the excited state, $S_1(v' = 0)$.

excess energy in the S_1 state, often vibrational quantum by vibrational quantum, to the surrounding solvent molecules. The process is very fast, occurring on the femto- to picosecond (10^{-15}–10^{-12} s) timescale. In Figure 1.11 this rapid vibrational relaxation is indicated by green wavy arrows. It is a general convention that such nonradiative processes are indicated by wavy arrows, whereas radiative processes, which consist of the absorption or emission of a photon, are indicated by straight arrows. The final result of fast vibrational relaxation is that the molecule is now in the lowest vibrational state of the electronic excited state (S_1, $v' = 0$).

The next obvious pathway is the opposite process to absorption. This radiative de-excitation into the ground state, $S_1 \rightarrow S_0$, is called spontaneous emission or simply fluorescence (red arrow in Figure 1.11). Mechanistically, it is governed by very similar processes as absorption. The probability of fluorescence is again proportional to the square of the transition dipole moment. The emitted photon has preferentially a polarization parallel to the transition dipole moment. For the emission, again the Franck–Condon principle applies (Figure 1.10). Because the potential curves and vibrational wave functions for vibrational eigenstates are often very similar and symmetric for both the S_0 and S_1 states, it can be understood from the Franck–Condon principle that the fluorescence spectrum often has a mirror image like shape in comparison to the absorption spectrum (Figure 1.12). Again the Franck–Condon integral (1.7) is the largest for the transition of the lowest vibrational state of the electronically S_1 state to the third vibrational state of S_0 for the example shown in Figures 1.11 and 1.12 [$S_1(v' = 0) \rightarrow S_0(v'' = 2)$]. Consequently, also in the fluorescence spectrum the third vibrational band dominates. The absorption and fluorescence bands with the largest and smallest
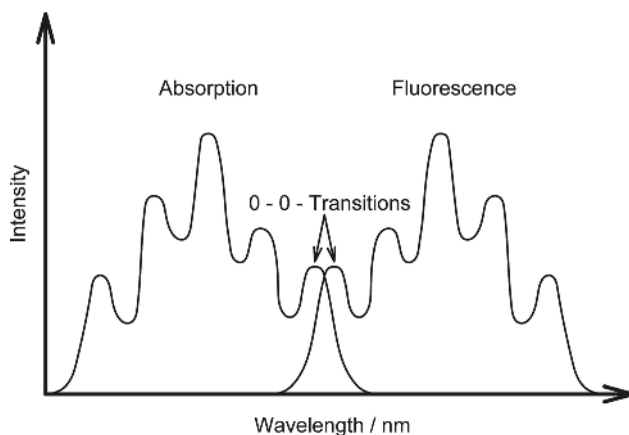
Figure 1.12 Because the potential curves and vibrational wave functions for the electronic ground and excited states of a molecule, are often very similar and symmetric for small v in many cases a mirror symmetry of absorption and fluorescence spectra can be observed.

wavelengths, respectively, define the so-called 0–0 transitions. These correspond to transitions between the lowest vibrational eigenstates in the ground and excited states, $S_0(v'' = 0) \rightarrow S_1(v' = 0)$ and $S_1(v' = 0) \rightarrow S_0(v'' = 0)$. Usually, these bands are not exactly overlapping, because typically the molecule itself and solvent molecules around the solute molecule rearrange themselves as a response to the new electronic structure in excited states. This results in a slight lowering of the S_1 state energy and leads to a shift of the 0–0 transition in the fluorescence spectrum to longer wavelengths in comparison to the 0–0 transition in the absorption spectrum.

The general observation that emitted photons have longer wavelengths than the absorbed photons is called the Stokes shift.

If the energy gap between the S_1 state and the S_0 state is large and the molecular structure is rather rigid, for example because of many double bonds, then often fluorescence dominates the de-excitation of the molecules. However, if the energy gap becomes smaller or if the molecule is very flexible then nonradiative de-excitation of the S_1 state often becomes dominant (energy-gap law). This is shown schematically in Figure 1.13. The smaller the energy gap between S_1 and S_0, the higher becomes the chance that there is a good overlap between the vibrational ground-state wave function of the S_1 state with wave functions of highly excited vibrations in the electronic ground state. If that is the case, the molecule can switch from the electronic excited state into the electronic ground state, $S_1 \rightsquigarrow S_0$, without the emission of a photon (blue horizontal arrow in Figure 1.13) and without a major rearrangement of the nuclear positions during this transition. This process is called internal conversion. Again, the probability for this process can be calculated using overlap integrals similar to (1.7). Once such a high vibrational state of the electronic ground state is populated, fast vibrational relaxation of the vibrational energy into the solvent environment again occurs on the femto- to picosecond timescale.
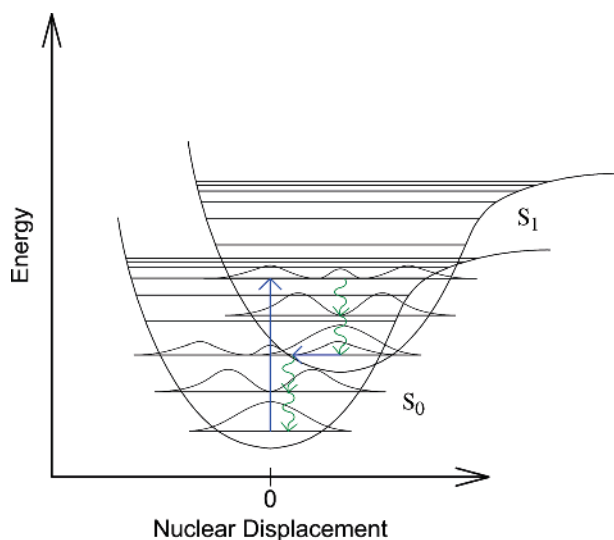
Figure 1.13 Visualization of nonradiative relaxation (internal conversion) of excited states, which often dominates for small ground state–excited state energy gaps.

If originally a higher electronically excited state, S_2, S_3 . . . , has been populated then usually very fast internal conversion into the lowest excited state, S_1, also takes place, because the energy gap between excited states is normally smaller than the energy gap between S_1 and S_0. Thus, in general, fluorescence occurs from the lowest electronically excited state in its lowest vibrational state, regardless of which excited state was originally populated. This general rule is called Kasha's rule.

For the third possible de-excitation path way we have to introduce a further important class of electronically excited states that are named triplet states. Figure 1.14 shows examples of electrons occupying different orbitals in the ground state, S_0, and in various possible excited states of a molecule. For most biomolecules and fluorescence markers in their electronic ground state a situation similar to the S_0 state in Figure 1.14 applies, in which all orbitals up to the HOMO
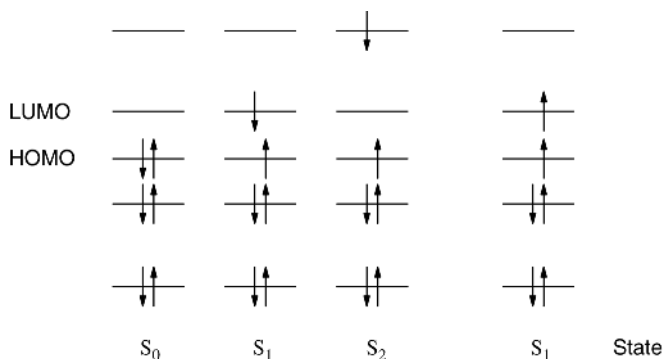

Figure 1.14 Possible excited-state configurations.

are filled with two electrons of opposite spins. The spin of an electron is a purely quantum mechanical property and can be regarded as a kind of angular momentum of the electron. The electron can only occur in two spins, α and β, which are depicted by opposite arrows in Figure 1.14. The quantum mechanical Pauli rule states that no electron in a molecule can have the exact same properties as another electron in the molecule. Therefore, an orbital can be occupied by two electrons only if they have different spins.

However, as can be seen from the right most diagram in Figure 1.14, Pauli's rule is not violated if a single electron excited into a higher molecular orbital has the same spin as the remaining electron in the original HOMO, because they occupy different orbitals. Such electronic states with two unpaired electrons of the same spin are referred to as triplet states. The energetically lowest triplet state is usually depicted by T_1. States in which for each electron another electron with opposite spin is present are called singlet states.

The vector diagrams depicted in Figure 1.15 give us an idea of the origin of this nomenclature. The spin of an electron is a purely quantum mechanical property and has no classical analogue. However, due to the spin, the electron has a small magnetic moment, with the result that an isolated electron acts similarly to a small magnet. In an external magnetic field, the magnetic moment of an electron can be symbolized by a vector representing the orientation of the magnetic moment with respect to the external field (thin arrows in Figure 1.15). Depending on the spin of the electron, the magnetic moment can be either orientated with or against the external magnetic field. These two orientations are assigned α- or β-spin, respectively. Due to quantum mechanical reasons, it is not orientated exactly parallel to the external field but slightly tilted. In addition, it rotates around the axis of this external field. Further details of the properties of spin states will be discussed in Chapter 4. Here, it shall only be noted that the magnetic moments of electrons paired in an orbital sum to a zero total magnetic moment symbolized by the opposite arrows on the left in Figure 1.15. Therefore, only a single state is possible in an external magnetic field, which is defined as singlet state, S. In the triplet state, however, the magnetic moments of the
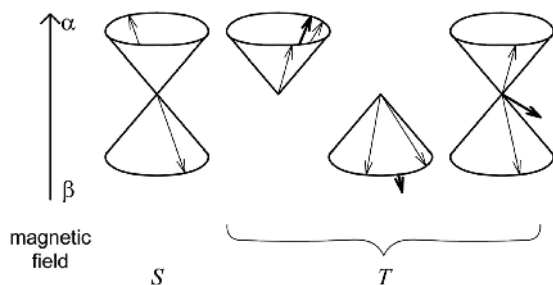

Figure 1.15 Possible orientations of the magnetic moments of the electron spin of two electrons and the corresponding total magnetic moment (thick arrow) in the vector representation. In the singlet state the magnetic moments of the individual electrons (thin arrows) cancel each other out resulting in a zero total magnetic moment. In the triplet state the magnetic moments of the individual electrons always span the same angle an their summation can point in three different directions.

unpaired electrons do not cancel each other out (thick arrows in Figure 1.15). The individual magnetic moments of the two unpaired electrons can be combined in three ways ($\alpha\alpha$, $\beta\beta$, $\alpha\beta$) in an external magnetic field. This is the origin for the name triplet state (T). S and T are also called the multiplicity of an electronic state. Because electrons with the same spin cannot be simultaneously at the same place (Pauli's rule) the repulsive interactions between these electrons are generally smaller. As a consequence, triplet states are usually lower in energy than the respective singlet states.

Figure 1.16 shows how the formation of a triplet state typically takes place. The triplet state is usually also populated from the vibrational ground state of an excited singlet state, $S_1(v'=0)$. The corresponding transition, $S_1 \rightsquigarrow T_1$, is called intersystem crossing. As in the case of internal conversion, intersystem crossing is favoured when a good overlap exists between vibrational wave functions of the excited singlet state, S_1, with vibrational wave functions of the triplet state, T_1 [Figure 1.16, (1.7)]. In comparison to fluorescence or internal conversion, the process of intersystem crossing is usually a lot less probable. Intersystem crossing requires a change of the total spin of the electron that corresponds to a change of the angular momentum. However, it is a fundamental law that the overall angular momentum of an isolated system must remain constant. Because there are only very limited possibilities to compensate for the change in angular momentum such spontaneous flips in the spin of electrons are rather unlikely and are therefore called spin forbidden. Only when the change in the spin angular momentum can be compensated by certain mechanisms can such spin flips happen. An important example fur such a mechanism is spin–orbit coupling in which, in a classical picture, a change in the angular momentum describing the electron movement around the nuclei compensates the spin flip.

After intersystem crossing, vibrational excitation is also usually generated, which is again dissipated within femto- to picoseconds until the molecule
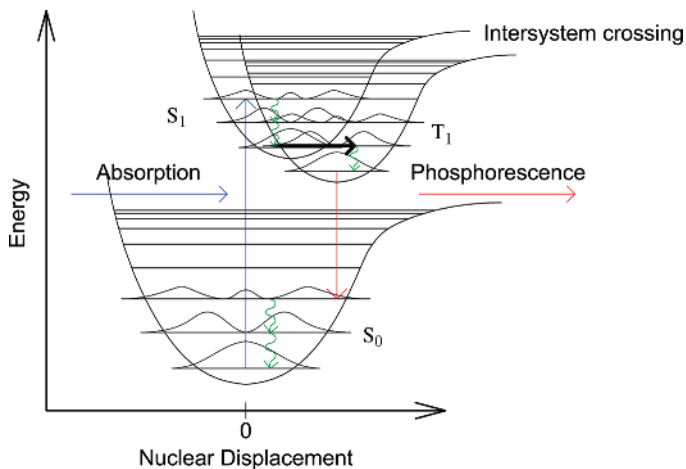

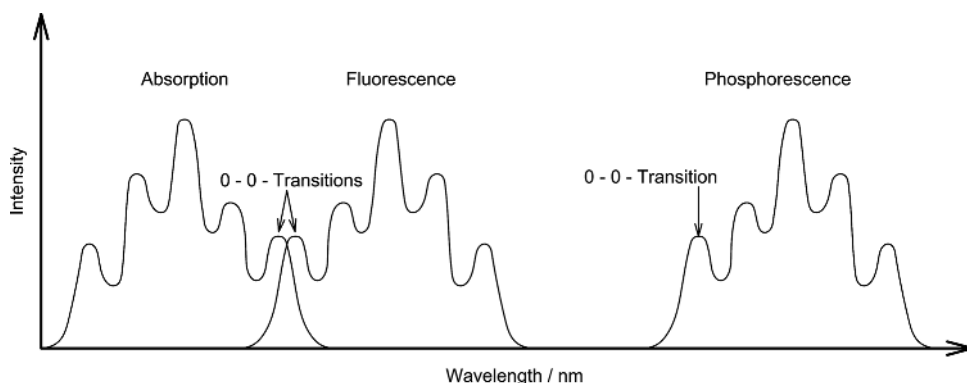**Figure 1.16** Visualization of triplet-state formation (intersystem crossing) and phosphorescence.

Figure 1.17 Schematic representation of typical absorption, fluorescence and phosphorescence spectra. It should be noted that in real spectra with a wavelength scale the phosphorescence spectrum typically appears somewhat broader than the corresponding fluorescence spectrum. The reason is that wavelengths are not linearly proportional to the state energies (compare with (1.1)). Only on a wavenumber or frequency scale really identical spacings of the vibrational bands can be expected.

remains in the vibrational ground state of T_1. From here, two path ways are possible. Radiative relaxation from the triplet state, $T_1 \rightarrow S_0$, is called phosphorescence. Generally, phosphorescence is the luminescence arising from radiative transitions involving a change in multiplicity. Nonradiative de-excitation or intersystem crossing, $T_1 \rightsquigarrow S_0$, is also possible. Similar to the internal conversion, $S_1 \rightsquigarrow S_0$, the probability for this process depends on overlap integrals such as (1.7) of the vibrational ground state of T_1 with highly excited vibrational states of S_1. Both processes also require a spin flip and are thus rather improbable. As an overall result, many biomolecules and fluorescence markers rarely undergo intersystem crossing to the triplet states, but once a triplet state has been formed it is usually quite long lived in comparison to singlet states.

The generally lower energy of the triplet states cause the phosphorescence to appear at longer wavelengths than fluorescence (Figure 1.17). Similarly to fluorescence, the phosphorescence spectrum often appears to be a mirror image of the ground-state absorption, because the vibrational modes are often very similar in all electronic states.

1.6
Quantitative Description of all Processes: Quantum Efficiencies, Kinetics of Excited State Populations and the Jablonski Diagram

All the effects described so far can be summarized in a diagram named after the Polish physicist Aleksander Jabłoński (1898–1980) (Figure 1.18). In the Jablonski diagram only energy levels of the electronic states, and sometimes also vibrational energy levels, are depicted as horizontal lines. As mentioned earlier, all radiative processes (absorption or emission of a photon) are depicted using straight arrows,
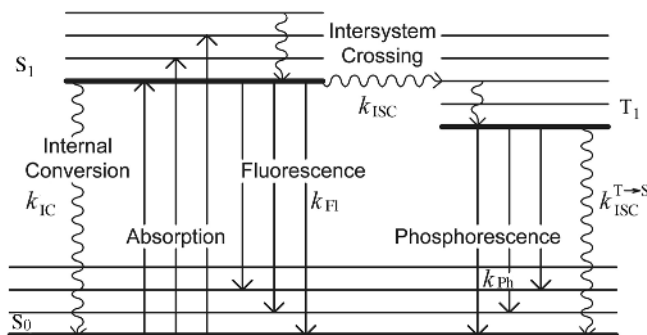
Figure 1.18 A Jablonski diagram.

whereas nonradiative processes are portrayed using wavy arrows. For each process a rate constant k can be given, which is proportional to the probability per time unit that the corresponding process occurs. For example, if the rate constant for internal conversion, $S_1 \rightsquigarrow S_0$, of a molecule is $k_{IC} = 1\,ns^{-1}$ and if the rate constant for fluorescence, $S_1 \rightarrow S_0$, is only $k_{Fl} = 0.1\,ns^{-1}$, the probability that the excitation energy is released via fluorescence is ten times less than via nonradiative internal conversion.

With the Jablonski diagram important parameters like, for example, quantum efficiencies or quantum yields of different processes can be derived. The fluorescence quantum efficiency is the number of photons emitted by a molecule as fluorescence divided by the number of photons that were previously absorbed by the molecule in a larger number of excitation and de-excitation cycles.

$$\Phi_{Fl} = \frac{\text{Number of photons emitted as fluorescence}}{\text{Number of absorbed photons}} \tag{1.9}$$

A high fluorescence quantum efficiency is an important property of fluorescence markers. In general, the quantum efficiency of any process is defined as the number of quanta undergoing the process divided by the number of absorbed photons or quanta originally present in the initial state from which this process occurs. Therefore, the quantum efficiency is a direct measure of the probability of certain processes in a molecule. The quantum efficiency of a process can be calculated by dividing the rate constant of the process of interest by the sum of the rate constants of all processes simultaneously depopulating the initial state. The fluorescence quantum yield, for example, can be calculated by:

$$\Phi_{Fl} = \frac{k_{Fl}}{k_{Fl} + k_{IC} + k_{ISC}} \tag{1.10}$$

The quantum yield for $S_1 \rightsquigarrow T_1$ intersystem crossing can be calculated analogously:

$$\Phi_{ISC} = \frac{k_{ISC}}{k_{Fl} + k_{IC} + k_{ISC}} \tag{1.11}$$

To calculate the phosphorescence quantum yield one has to multiply the quantum yield for $S_1 \rightsquigarrow T_1$ intersystem crossing by the probability for radiative emission from the triplet state, $T_1 \rightsquigarrow S_0$:

$$\Phi_{Ph} = \Phi_{ISC} \frac{k_{Ph}}{k_{Ph} + k_{ISC}^{T \rightarrow S}} \tag{1.12}$$

The sum of all radiative and nonradiative processes must be one:

$$\Phi_{Fl} + \Phi_{Ph} + \sum \Phi_{NR} = 1 \tag{1.13}$$

Here, nonradiative processes can be internal conversion from the singlet or intersystem crossing from the triplet state, $S_1 \rightarrow S_0$ and $T_1 \rightarrow S_0$, or other processes like photochemical reactions or energy-transfer processes (not depicted in Figure 1.18). Energy-transfer processes are described in detail in Chapter 3 (Section 3.5).

A time constant of a process is defined as the inverse of its corresponding rate constant $\tau_X = k_X^{-1}$. A rate constant for fluorescence, $S_1 \rightarrow S_0$, of $k_{Fl} = 0.1\,ns^{-1}$, for example, corresponds to a time constant for fluorescence of $\tau_{Fl}^{rad} = 10\,ns$. The overall lifetime of an electronic state is also a time constant and can be calculated from the inverse of the sum of the rate constants, k_X, of all processes depopulating this state:

$$\tau_{State} = \left(\sum k_X \right)^{-1} \tag{1.14}$$

According to the Jablonski diagram in Figure 1.18 the lifetime of the first excited singlet state of a molecule is for example:

$$\tau_{S_1} = (k_{IC} + k_{Fl} + k_{ISC})^{-1} \tag{1.15}$$

as long as no additional processes depopulate S_1. Often, the rate constant for intersystem crossing, k_{ISC}, is small compared to k_{Fl} and k_{IC}. Then, the S_1 state lifetime depends on the relative magnitudes of k_{Fl} and k_{IC}. The magnitude of k_{Fl} is directly related to the magnitude of the transition dipole moment for the transition $S_1 \rightarrow S_0$:

$$k_{Fl} \propto \left| \vec{M}_{S_1 \rightarrow S_0} \right|^2 \tag{1.16}$$

[see also (1.4)]. Typically, the corresponding time constants, τ_{Fl}, are on the order of a few up to tens of nanoseconds. Consequently, if the corresponding time constant for internal conversion is as short as, for example, 100 ps, for instance due to a small S_1–S_0 energy gap, then the S_1 state lifetime will also be on the order of only a few 100 ps and almost no fluorescence will be emitted by the molecule. In contrast, if the corresponding time constant for internal conversion is >10 ns then the S_1 state lifetime will be on the order of the time constant for fluorescence and fluorescence emission will be dominant.

The kinetic behaviour of the different states also depends on the specific rate constants. Basically, the kinetics of the population of excited states of an ensemble of molecules can be treated by the same mathematical frame work as the kinetics of chemical or biochemical reactions. If the S_1 state of the molecules is populated by a short pulse of excitation light its decay can be calculated using the following approach:

$$\frac{dP_{S_1}(t)}{dt} = -(k_{Fl} + k_{IC} + k_{ISC}) \cdot P_{S_1}(t) \tag{1.17}$$

Here, $P_{S_1}(t)$ is the population of the first excited singlet state. $dP_{S_1}(t)/dt$ is the actual velocity by which the population of molecules in the S_1 state changes; it depends on the rate constants of all depopulating processes and of the actual remaining S_1 population. Integration of Eq. (1.17) shows that the S_1 state population decays monoexponentially after a pulsed population (Figure 1.19):

$$P_{S_1}(t) = P_{S_1}^0 \cdot e^{-(k_{Fl}+k_{IC}+k_{ISC})t} = P_{S_1}^0 \cdot e^{-t/\tau_{S_1}} \tag{1.18}$$

Here, $P_{S_1}^0$ is the initial absolute population generated in the S_1 state due to the pulsed excitation.

The lifetime of the S_1 state, τ_{S_1}, determines how fast its population decreases. Since the fluorescence intensity, $I_{Fl}(t)$, is always proportional to the actual S_1 state population, $P_{S_1}(t)$, it also decays in the same way as the S_1 state population:

$$\boxed{\begin{aligned}I_{Fl}(t) &\propto P_{S_1}(t) \\ I_{Fl}(t) &= I_{Fl}^0 \cdot e^{-t/\tau_{S_1}}\end{aligned}} \tag{1.19}$$

Thus, the lifetime of the first excited state S_1 can be determined experimentally from the fluorescence's decay observed in time-resolved fluorescence
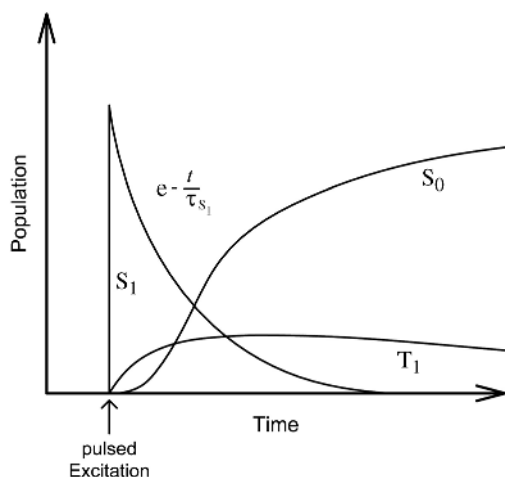

Figure 1.19 Schematic presentation of typical kinetics of excited-state populations.

measurements. The measured fluorescence decay is therefore often also referred to as the fluorescence lifetime of a molecule. Notably, however, this is not to be confused with the time constant for the radiative emission or fluorescence, τ_{Fl}^{rad}. The time constant for radiative emission or fluorescence, $\tau_{Fl}^{rad} = k_{Fl}^{-1}$, is defined for the pure process of depopulation of the first excited singlet state by emission of fluorescence photons (Figure 1.18). This time constant is in general larger than or at most equal to the lifetime of the first excited state, τ_{S_1}: $(\tau_{S_1})^{-1} = (\tau_{IC})^{-1} + (\tau)^{-1} + (\tau_{ISC})^{-1}$ [compare with (1.14) and (1.15)]. In this book the time constant for radiative emission or fluorescence is always depicted by τ_{Fl}^{rad} to clearly differentiate it from τ_{S_1}. However, be aware that in the literature the symbol, τ_{Fl}, is very often used to describe measured fluorescence decays that reflect nothing other than the lifetime of the first excited singlet state, τ_{S_1} (1.19).

Figure 1.19 depicts schematically typical time-dependent changes of the populations of T_1 and S_0. Similarly to the kinetic description of the single state population, changes in the triplet-state population can also be described by differential equations. Because the triplet state is populated from the singlet state via intersystem crossing we have to consider a pair of coupled differential equations:

$$\frac{dP_{S_1}(t)}{dt} = -(k_{Fl} + k_{IC} + k_{ISC}) \cdot P_{S_1}(t)$$

$$\frac{dP_T(t)}{dt} = +k_{ISC} \cdot P_{S_1}(t) - (k_{Ph} + k_{ISC}^{T \to S}) \cdot P_T(t) \tag{1.20}$$

Here, $P_T(t)$ is the population of the first excited triplet state and $dP_T(t)/dt$ is the actual velocity by which the population of molecules in the T_1 state changes as a function of S_1 and T_1 state populations and all rate constants populating or depopulating these states.

Integration of these coupled equations gives:

$$P_T(t) = P_{S_1}^0 \cdot k_{ISC} \left(\frac{-e^{-(k_{Fl}+k_{IC}+k_{ISC})t} + e^{-(k_{ph}+k_{ISC}^{T \to S})t}}{(k_{Fl} + k_{IC} + k_{ISC}) - (k_{Ph} + k_{ISC}^{T \to S})} \right)$$

$$= P_{S_1}^0 \cdot A(-e^{-t/\tau_{S_1}} + e^{-(k_{Ph}+k_{ISC}^{T \to S})t}) \text{ for } \frac{1}{\tau_{S_1}} > (k_{Ph} + k_{ISC}^{T \to S})$$

$$\Rightarrow$$

$$I_{Ph}(t) = I_{Ph}^0 \cdot A(-e^{-t/\tau_{S_1}} + e^{-(k_{Ph}+k_{ISC}^{T \to S})t}) \tag{1.21}$$

In a manner similar to the way fluorescence kinetics describe population changes in the excited singlet state, S_1, (1.19) the observable phosphorescence kinetics describe the kinetics of the triplet-state population. The population curve for T_1 in Figure 1.19 is an exemplary illustration of typical triplet-state kinetics. The triplet-state kinetics are an example of a consecutive reaction scheme, $S_1 \to T_1 \to S_0$, with the T_1 state being an intermediate. According to Eq. (1.21) the intermediate T_1 population kinetics are described by a biexponential function with an exponential rise term, depending on the S_1 state lifetime, τ_{S_1}, and an exponential decay term, depending on the rates for intersystem

crossing from the triplet state and phosphorescence, $k_{Ph} + k_{ISC}^{T \to S}$. The maximum intermediate triplet-state population depends on the relative magnitude of the rate for intersystem crossing, k_{ISC}, in comparison to $k_{Fl} + k_{IC}$ and $k_{Ph} + k_{ISC}^{T \to S}$. If k_{ISC} is large compared to $k_{Fl} + k_{IC}$ and $k_{Ph} + k_{ISC}^{T \to S}$ a large and long-lived intermediate triplet-state population will be observed. In contrast, if k_{ISC} is small compared to $k_{Ph} + k_{ISC}^{T \to S}$ or $k_{Fl} + k_{IC}$ the intermediate triplet-state population will always remain very small.

Problems

1.1 Orbitals have regions in which they have negative and positive signs. Explain the physical background of the signs and how this relates to the probability of finding electrons at a certain place.

1.2 Spectroscopists often describe energies that are necessary to excite certain electronic states by the wave number of the corresponding photons in units of cm^{-1}. Why is this more advantageous than describing the corresponding energies in units of the photons wavelength?

1.3 Try to estimate the shape of the possible molecular π-orbitals of the molecule hexatriene ($CH_2{=}CH{-}CH{=}CH{-}CH{=}CH_2$). How many nodal planes does each orbital have? What is the energetic order of these orbitals? Which orbitals are the HOMO and LUMO orbitals?

1.4 What are important prerequisites before a photon can be absorbed by a molecule?

1.5 Figure 1.6 shows schematically four π-wave functions of the molecule anthracene. Sketch the transition density for a transition between the states f and g. In what direction relative to the molecular axis is the transition dipole moment orientated?

1.6 Figure 1.5 shows schematically the π-wave functions of the molecule butadiene. Which of the following transitions of butadiene are dipole allowed:

a) The $\pi_1 \to \pi_4^*$ transition?
b) The $\pi_1 \to \pi_3^*$ transition?
c) The $\pi_2 \to \pi_3^*$ transition?

1.7 Describe in general the important factors that govern the shape of absorption and fluorescence spectra of molecules in a liquid environment.

1.8 The probabilities of finding nuclei at certain positions can be calculated from vibrational wave functions (1.6) in the same way as the probabilities of finding electrons at certain positions can be calculated from electronic wave functions. Figure 1.10 shows schematically the probabilities of finding nuclei at certain positions relative to their equilibrium position for

$v' = 0$, 1, 2. Try to guess what the corresponding vibrational wave functions look like.

1.9 Describe in general the factors that determine the probabilities for fluorescence, internal conversion and intersystem crossing of molecules in a liquid environment.

1.10 Below are given corresponding wave numbers and time constants for several transitions between various states of a fluorescent molecule:

$S_0 \rightarrow S_1$: $18\,000\,\text{cm}^{-1}$
$S_0 \rightarrow S_2$: $22\,000\,\text{cm}^{-1}$
$T_1 \rightarrow S_0$: $15\,000\,\text{cm}^{-1}$

Radiative $S_1 \rightarrow S_0$ transition (fluorescence):	$\tau_{\text{Fl}}^{\text{rad}} = 5\,\text{ns}$
Nonradiative $S_1 \rightsquigarrow S_0$ transition (internal conversion):	$\tau_{\text{IC}}^{\text{S}} = 25\,\text{ns}$
$S_1 \rightsquigarrow T_1$ intersystem crossing:	$\tau_{\text{ISC}} = 100\,\text{ns}$
Radiative $T_1 \rightarrow S_0$ transition (phosphorescence):	$\tau_{\text{Ph}} = 2\,\text{s}$
Nonradiative $T_1 \rightsquigarrow S_0$ transition (intersystem crossing):	$\tau_{\text{ISC}}^{\text{T} \rightarrow \text{S}} = 20\,\mu\text{s}$

Sketch the Jablonski diagram using the given data. Mark the radiative and nonradiative transitions on the diagram. Which wavelengths are needed to excite the molecule into S_1 and S_2? Calculate for this fluorescent molecule (a) the lifetime of the first excited singlet state, τ_{S_1}. (b) The fluorescence quantum yield Φ_{FL}. (c) The triplet quantum yield Φ_{Triplet}. (d) The triplet-state lifetime τ_{Triplet}. (e) The phosphorescence quantum yield after excitation into S_1.

1.11 Fluorescent molecules with larger conjugated π-systems often absorb and emit at longer wavelengths and have larger transition dipole moments than molecules with smaller conjugated π-systems. Try to explain why.

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