BACHELOR THESIS

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Fluorescence excitation spectra of photosynthetic antennae

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This thesis is dedicated to my father, who has guided me towards appreciation of the beauty in nature and supported me throughout my education.
Thank you for helping me get this far.

I would also like to thank Tomáš Mančal and Jakub Pšenčík for the constructive critique and all the tremendous help.

Title: Fluorescence excitation spectra of photosynthetic antennae

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Abstract: The aim of this thesis is to offer a brief overview of energetic transfer taking place in photosynthetic antenna systems by studying an example antenna and trying to simulate its behavior. Utilising modern literature, we describe the structure of photosynthetic antenna systems, their properties and the methods used to study them. We follow this by proposing a model of the kinetics of energetic states in the studied system. The theoretical section is followed by analysis of measured absorption and fluorescence excitation spectra of the actual chlorophyll + carotenoid dimers with use of OriginPro2020 and Python3. With this knowledge, we have developed a simulation to fit our model to the experimental values. We utilize the Quantarhei Python3 package to calculate the absorption spectra and the FES spectrum is constructed by solving a system of differential equations proposed from the kinetics model and fitting the measured data. From there, we extract the efficiency of energy transfer in the dimer.

Keywords: Photosynthesis, Excitation energy transfer, Photosynthetic antennae

Contents

Introduction

Photosynthesis (*phōs* - "light" and *synthesis* - "putting together"[1]) is a complicated biochemical process which plants and other organisms use for capturing solar energy to form energy rich molecules from simpler abundant ingredients by creating chemical bonds. Before being stored, the energy has to be absorbed and transferred to the specialised organs that are able to manage and execute the necessary chemical processes. Photosynthetic organisms have developed a system designed specifically for this part of the procedure called the photosynthetic antenna system. The aim of this work is to offer a brief overview of the mechanisms of energetic transfer taking place in the these systems by studying an example antenna and trying to simulate its properties.

Understanding the structure of the environment where these phenomena take place and the way these systems function and interact with the outside is key, and many methods have been developed to study them. One popular method is fluorescence excitation spectroscopy. It uses the absorption and the fluorescence response of the system to allow us to study how it interacts with the incoming light. We use it to obtain the fluorescence excitation spectra of a chlorophyll-carotenoid dimer, from which we can extract information on how the dimer is excited by photons of different frequencies and how this excitation travels between the chlorophyll and the carotenoid. This information can be translated into a phenomenological model, taking the populations of the different excited states and describing how and how efficiently energy is transferred between them after all is done. The execution of the measurements themselves was originally planned to be a part of the thesis, however, after running into trouble regarding the stock of available molecular samples, the data was obtained from previous measurements taken by Jakub Pšenčík and Tomáš Malina. For that reason, the only part that is discussed in the experimental section is the analysis of the given spectra.

A large part of this thesis lies in the simulation of the aforementioned spectra. We use the extracted experimental data and the theoretical model to create a program to simulate these spectra in Python3. We utilize a special package designed by Tomáš Mančal called Quantarhei [2] to calculate the absorption spectra and we use the equations from the theoretical part to calculate the fluorescence excitation spectra. The accuracy of this simulation is then checked by comparing the simulation to the obtained experimental spectra. This allows us to determine to what extent our model is valid.

Theoretical section

1. Photosynthetic antenna systems

Photosynthesis uses solar energy to form energy-rich molecules from simpler ingredients by creating chemical bonds - the products typically being sugars synthesized from CO_2 and water.

According to [3], we may divide Photosynthesis into 4 phases - three "light-dependent" and one "light-independent". These are (1) Photon absorption and energy delivery by the antenna system, (2) primary electron transfer in reaction centers, (3) energy stabilization by secondary processes, and (4) synthesis and export of stable products. The only processes that truly concern us in this thesis are the initial photon absorption and the following transfer of energy to the reaction centers via the antenna system.

1.1 Primary photon absorption

Although photosynthesis is performed by many different forms of life, be it vascular plants or tiny bacteria, a substantial amount of them use some form of chlorophyll and carotenoid molecules. These molecules are further organized into more complicated structures, for example, the Photosystem I and II in plants. These structures are composed of two different actors: (1) the reaction centers, where the actual separation of electrons happens, and their further transport begins, and (2) the antenna pigment-protein [4] complexes .

This group of molecules harvests incoming photons and then transfers the received energy in the form of excitation to the reaction centers [5]. Antenna pigments collect energy and concentrate it in a receiver - allowing for a larger photon collection area by specialized molecules. No photochemistry happens in these pigments. They are simply a medium for the transfer process involving the migration of electronic excitation between molecules utilizing the energetic coupling between them.

Carotenoids also take part in this process. They function as accessory sensors of light and protect the system from the dangerous effects of oxidation. They rapidly quench triplet states of connected chlorophylls before they are able to react with surrounding oxygen to form their highly reactive singlet state. Carotenoids have also been shown to take part in the regulation of energy transfer in antennas [6].

1.2 Funnel concept

In the following sections, we shall discuss the physical mechanisms of the energy transfer from one antenna to another. First, let us discuss **the funnel concept**[3], the basic principle which is illustrated in figure 1.1. This principle gives the traveling excitation a vector . Having the parts of the antenna system, which are further away from the reaction center, maximally absorb photons in a higher energy domain than the parts closer to the reaction center creates an energy gra-
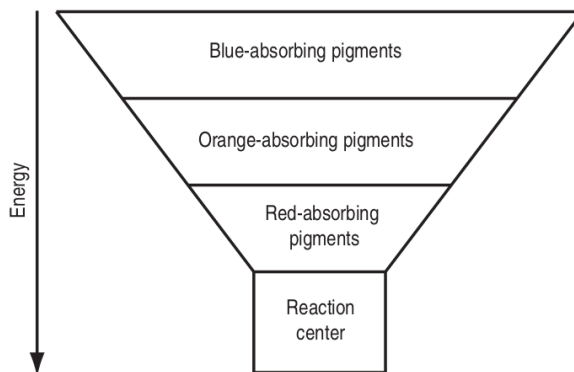
Figure 1.1: Basic principle of the funnel concept in antenna systems. Excitation travels from higher-energy pigments absorbing in the blue area to the red-absorbing lower-energy pigments, which are closer to the reaction center [3]

dient, which drives the collected energy to the reaction center. Such transfer is a dissipative process - every time excitation jumps from one antenna to another, a small amount of energy is lost as heat, making the process thermodynamically irreversible.

This basic principle is applicable in many systems. Photosynthetic antenna systems utilize carotenoids as short wavelength absorbers, which with the use of the aforementioned funneling, gives the system a much broader coverage of the solar spectrum than if it only used chlorophylls.

What allows for the energy to travel as it does?

1.3 Förster theory of resonance energy transfer

The Förster resonance energy transfer is a non-radiative energy transfer between a weakly coupled donor and an acceptor molecule. It has first been described by Theodor Förster in 1948 [7]. The derivation can be approached from a classical and a quantum mechanical approach, both leading to the same conclusion. We shall be following the latter from [8].

In deriving Förster's FRET mechanism, we use the *Fermi's Golden Rule* to describe the rate of transition from donor D to acceptor A. The transfer itself arises from the dipole-dipole coupling of the two molecules, no photon exchange is needed. The two molecules can be in their ground states $|D\rangle$, $|A\rangle$ or their excited states $|D*\rangle$, $|A*\rangle$. If the potential of our system is that of weak coupling through the dipole-dipole interaction, the Hamiltonian can be written as:

$$H = H_0 + V, \tag{1.1}$$

where H_0 is given as:

$$H_0 = |D^*A\rangle H_D \langle D^*A| + |A^*D\rangle H_A \langle A^*D|. \tag{1.2}$$

The first Hamiltonian H_D is that of the system with the donor excited and the second H_A is the Hamiltonian of the system with the acceptor excited. The states represent the electronic and nuclear states of the two molecules.

The potential V is of the dipole-dipole interaction which is well known to correspond to:

$$V = \frac{3(\vec{\mu_A} \cdot \hat{r})(\vec{\mu_D} \cdot \hat{r}) - \vec{\mu_A} \cdot \vec{\mu_D}}{\vec{r}^3}, \qquad (1.3)$$

where r is the distance between the two molecules and the $\vec{\mu}$ members are transition dipole moments, coupling the ground and excited electronic states of the donor and the acceptor. We can rewrite them as

$$\vec{\mu_i} = \hat{u}_i \mu_i \qquad (1.4)$$

with μ_i being the dipole operator and $\hat{\mu}_A$ being the unit vector in the direction of the orientation of the dipole. We can for ease of manipulation rewrite the potential member as

$$V = \mu_A \mu_B \frac{K}{r^3} [|D^*A\rangle\langle A^*D| + \langle A^*D||D^*A\rangle], \qquad (1.5)$$

where K has all the orientational factors:

$$K = 3(\hat{u}_A \cdot \hat{r})(\hat{u}_D \cdot \hat{r}) - \hat{u}_A \cdot \hat{u}_D \qquad (1.6)$$

.

The general formula for Fermi's Golden Rule is:

$$w_{kl} = \frac{2\pi}{\hbar^2} |V_{kl}|^2 \delta(\omega_k - \omega_l). \qquad (1.7)$$

In our case, the initial state is $l = |D^*A\rangle$ and the final state is $k = |A^*D\rangle$. The rule can also be expressed as a correlation function in the interaction Hamiltonian.

$$w_{kl} = \frac{2\pi}{\hbar^2} \sum_l p_l |V_{kl}|^2 \delta(\omega_k - \omega_l) = \frac{1}{\hbar^2} \int_{-\infty}^{+\infty} dt < V_I(t)V_I(0) >, \qquad (1.8)$$

where $p_l = e^{\frac{-\beta E_l}{Z}}$, $\beta = (K_B T)^{-1}$ and Z is the partition function. Here we can plug in the formula for V from eq.(1.5) to obtain:

$$w_{ET} = \frac{1}{\hbar^2} \int_{-\infty}^{+\infty} dt \frac{K^2}{r^6} \langle D^*A| \mu_D(t)\mu_A(t)\mu_D(0)\mu_A(0) |D^*A\rangle. \qquad (1.9)$$

The time dependant dipole operators are $\mu_i(t) = e^{\frac{+iH_i t}{\hbar}} \mu_i e^{\frac{-iH_i t}{\hbar}}$. Since each of the them acts only either on $|D\rangle$ or $|A\rangle$, we may rewrite the experession as:

$$w_{ET} = \frac{1}{\hbar^2} \int_{-\infty}^{+\infty} dt \frac{K^2}{r^6} \langle D^*| \mu_D(t)\mu_D(0) |D^*\rangle \langle A| \mu_A(t)\mu_A(0) |A\rangle. \qquad (1.10)$$

The two matrix elements correspond respectively to the donor fluorescence correlation function $C_{D^*D^*}(t)$ and the acceptor absorption correlation function $C_{AA}(t)$. The expression for w_{ET} is a time integral over a product of correlation functions and since we can express time-correlation functions as inverse Fourier transforms over lineshapes, we can express w_{ET} as an overlap integral between the donor fluorescence and the acceptor absorption spectra:

$$w_{ET} = \frac{1}{\hbar^2} \frac{K^2}{r^6} |\mu_{DD^*}|^2 |\mu_{AA^*}|^2 \int_{-\infty}^{+\infty} d\omega \sigma_{abs}^A(\omega) \sigma_{fluor}^D(\omega), \qquad (1.11)$$

where $\sigma_{abs}^A(\omega)$ is the absorption lineshape of the acceptor molecule and $\sigma_{fluor}^D(\omega)$ is the fluorescence lineshape of the donor molecule. The lineshapes are normalized to $|\mu|^2$.

To put it into words, the energy transfer rate scales with r^{-6}, it depends on the strengths of the electronic transitions for the molecules and requires resonance between the fluorescence of the donor and absorption of the acceptor. For brevity's sake, the formula is often written with the use of effective distance R_0 and the τ_{fluor} lifetime of the donor fluorescence:

$$w_{ET} = \frac{1}{\tau_{fluor}} \left(\frac{R_0}{r} \right)^6. \tag{1.12}$$

1.4 Spectroscopic properties of chlorophylls

Chlorophylls exhibit two major absorption bands, first in the blue/UV region of the spectrum and the second in the red/IR region - hence the green coloring of most plants. Both of these absorption bands are $\pi \rightarrow \pi^*$ transitions. As an example, let us use Fig 1.2, which we borrowed from [3]. In the illustration we can see the typical shapes of the absorption and the fluorescence spectra of *bacteriochlorophyll c*. One can also see the third minor absorption band in the green area of the spectrum. The standard model with which we describe these spectra is called the "four orbital" model, originally proposed by Martin Gouterman in 1961[9] when describing the spectra of porphyrins. The central structure of chlorophylls is an aromatic porphyrin or chlorin ring system with a magnesium atom in the middle.
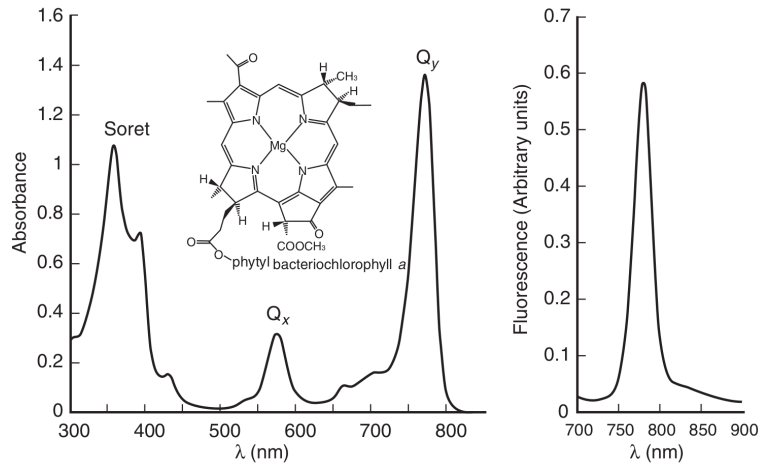

Figure 1.2: Absorption (left) and fluorescence (right) spectra of *bacteriochlorophyll c* in diethyl ether taken from [3].

In Figure 1.3, we can see the orbital model of a simple porphyrin, chlorin and bacteriochlorin. Two of the four π orbitals are the Highest Occupied Molecular Orbitals (HOMOs) and the other two are the Lowest Unoccupied Molecular Orbitals (LUMOs). We also see the electronic transitions, the lowest of the two called the **Q bands** and the remaining ones are the **Soret bands**.

The diagram is a gross oversimplification of the actual situation, ignoring the complex web of possible configurations, the contribution from higher energy
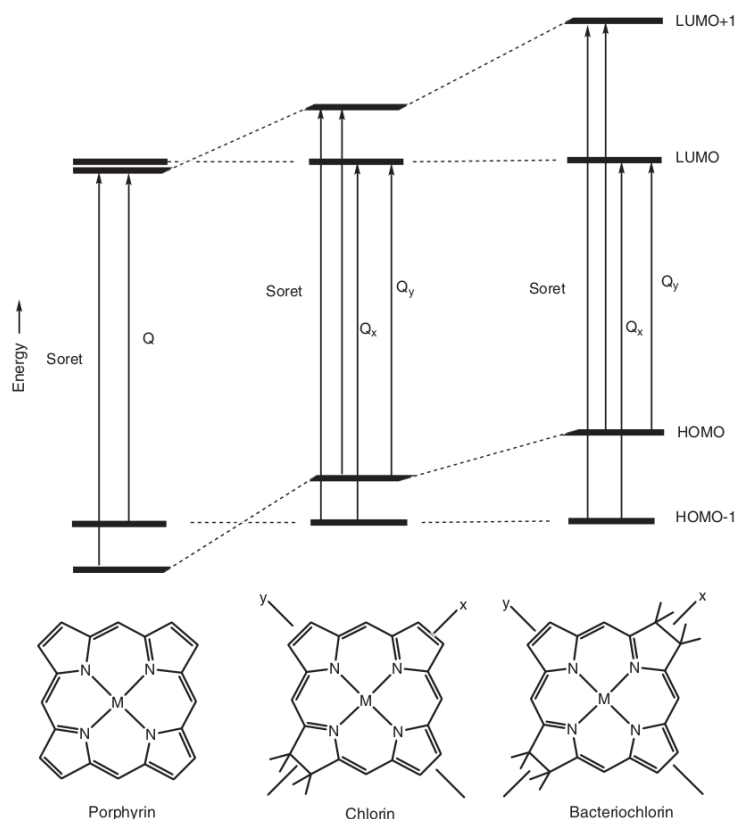
Figure 1.3: A very simplified molecular orbital energy level diagram of porphyrin, chlorin and bacteriochlorin taken from [3].

states, and exciton interaction. It does, however, serve as a sufficient model for explaining the spectra we see when we look at the absorption line shape.

The lower indexes of the Q bands signify the direction of their transitional dipole moments - Q_y being polarized along the y-axis of the molecule while Q_x being polarized along the x-axis. This means that absorption at the specific wavelengths of the transitions will be strongest if the light coming into contact with the molecule is polarized parallel to that specific axis of the chlorophyll. Soret bands (often noted as B) have a mixed polarization.

Vibrational state transitions are another thing to keep in mind, being especially notable on the Q_y band. Combined with the electronic excitation, the product is an excited vibrational state of the excited electronic state.

1.5 Spectroscopic properties of carotenoids

There are a considerable number of different carotenoids in nature, displaying a multitude of different functions in a large number of organisms, usually while being bound to a protein. In photosynthesis, they often play the double role of a secondary light harvester and of a photoprotective molecule.

Carotenoids are tetraterpenoid derivatives that are initially formed of eight isoprene molecules. [12] They split into two main categories, carotenes and xanthophylls. We shall only interest ourselves in the carotenes, which are pure hydrocarbons. The two most noticeable excited states that carotenoids exhibit are
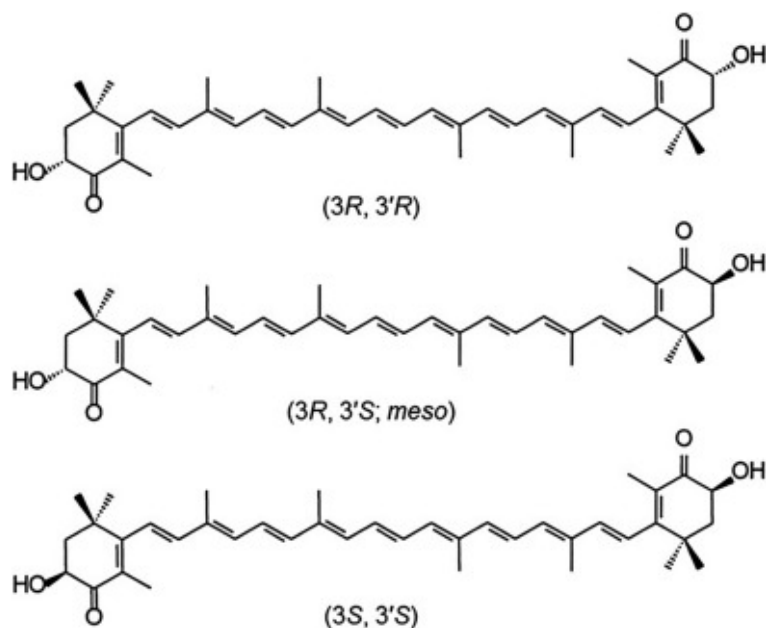
Figure 1.4: The skeletal formula of an astaxanthin molecule, taken from [10]. There are three possible stereoisomers of astaxanthin as seen in the figure, all present in nature in various distributions depending on specific organisms that possess them [11].

the singlet $\mathbf{S_1}$ and $\mathbf{S_2}$ states. The $\mathbf{S_1}$ state is the lowest-lying excited state. The $S_0 \to S_1$ transition is forbidden. The only way the carotenoid by itself can get to this state is by first absorbing $S_0 \to S_2$ and then relaxing to the S_1 state by internal conversion, which in turn relaxes to the ground state almost completely by internal conversion, too (fluorescence occurs with extremely low yield [12]). The transition from the ground state to S_2 is strong and when viewed in the absorption spectrum, exhibits three noticeable peaks corresponding to the three lowest vibrational states of the excited molecule.

2. Fluorescence excitation spectra

Fluorescence is a phenomenon that is very commonly taken advantage of in studying biophysical systems. Through the optical properties of different molecules we may build ourselves a better idea of the structure of their orbitals and the energy transfer mechanisms. Fluorescence takes place during a transition from the lowest excited electronic state to one of the ground electronic states with the emission of a photon. Fluorescence also requires that the excited state has the same multiplicity as the ground state (usually a singlet). If the initial state has a different multiplicity than the final state and occurs on a much longer timescale, the effect is called phosphorescence.

Since we may excite the measured object to multiple possible states, we can then study how much of that absorbed energy is converted to emitted photons through internal conversion followed by fluorescence. The spectrum we use to study this is called the **fluorescence excitation spectrum**.

2.1 Road to the Fluorescence excitation spectrum

When constructing the fluorescence excitation spectrum, we have to take several steps. The first is getting an emission spectrum.

To form it, we choose an arbitrary excitation wavelength with which we illuminate the studied system. Then we measure the intensity of light emitted by fluorescence with respect to the emitted wavelength. The spectrum we obtain by this is commonly in the shape of a broadened peak (as seen in figure 1.2. on the right). Taking that peak as a reference of the fluorescence emission wavelength, we can then build an excitation spectrum by exciting the system with a spectrum of wavelengths and then monitoring the intensity of emission. In this way the excitation spectrum effectively reflects the amount of energy that is converted into fluorescence at different wavelengths of excitation.

Further more, we can combine the absorption spectrum with the emission spectrum. Normalising at the fluorescence maximum (the place in the graph where fluorescence is the most intense), we get a combined graph of two curves. First - the original absorption spectrum, the second with the exact same intensity at the fluorescence maximum but different relative magnitudes to absorption - thus we get how much of the light shined at the object that is absorbed actually goes into fluorescence - the "efficiency spectrum".
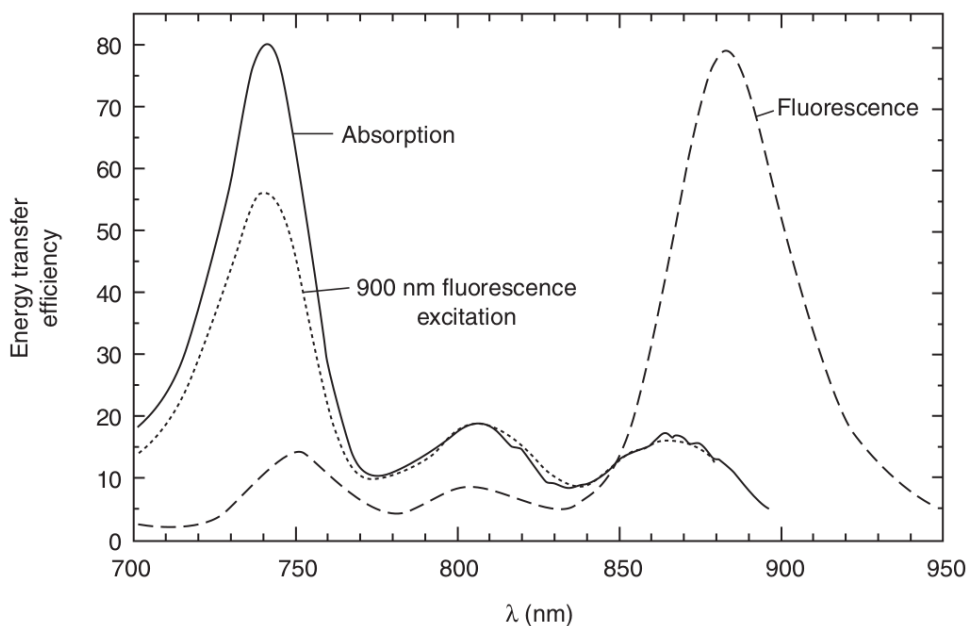
Figure 2.1: Taken from [3]: Measurement of energy transfer efficiency in whole cells of the green bacterium Chloroflexus aurantiacus. Solid line: absorption spectrum; dotted line: fluorescence excitation spectrum, monitored at 900 nm; dashed line: fluorescence emission spectrum. The energy transfer efficiency of transfer from the *bacteriochlorophyll c* absorbing at 740 nm to the *bacteriochlorophyll a* emitting at 900 nm is 70%, while the transfer efficiency for *bacteriochlorophyll a* absorbing at 808 nm is 100%. The curves were normalized at 870 nm. Adapted from [3].

To better understand the energy transfer between the levels, let us now look at the diagram in figure 2.2 (taken from [3]) and describe what processes we can observe. There are multiple events that take place between the initial absorption of a photon and the return of the molecule to the ground state.

In (a), we see that the system originally starts at the least energetic state of the ground electronic state - the baseline. When the incoming photon is absorbed, dependent on its energy, it is possible for the electron to jump to multiple different vibrational states of the excited electronic state. What follows is the *internal conversion* or *vibrational relaxation* (loss of energy in absence of light emission). This excess energy is converted into heat, which is absorbed by the surrounding molecules, leaving us with an electronic excited state with the least energetic vibrational state. The excited molecule exists in this state for a period of nanoseconds before relaxing to a lower state. If this relaxation process is accompanied by the emission of a photon, it is called *fluorescence*. Because higher excited states are usually very short-lived, according to **Kasha's rule** photon emission occurs most prominently from the lowest excited state, since only then, there is enough time for fluorescence to take place.

As we can see in (b), the emission is possible *to* a whole spectrum of different vibrational states of the ground electronic state, many being very closely distributed, more so the closer we get to the ground state. For this and other reasons (the Doppler shift is a major one), the observed emission spectra take the appearance of a broadened peak.
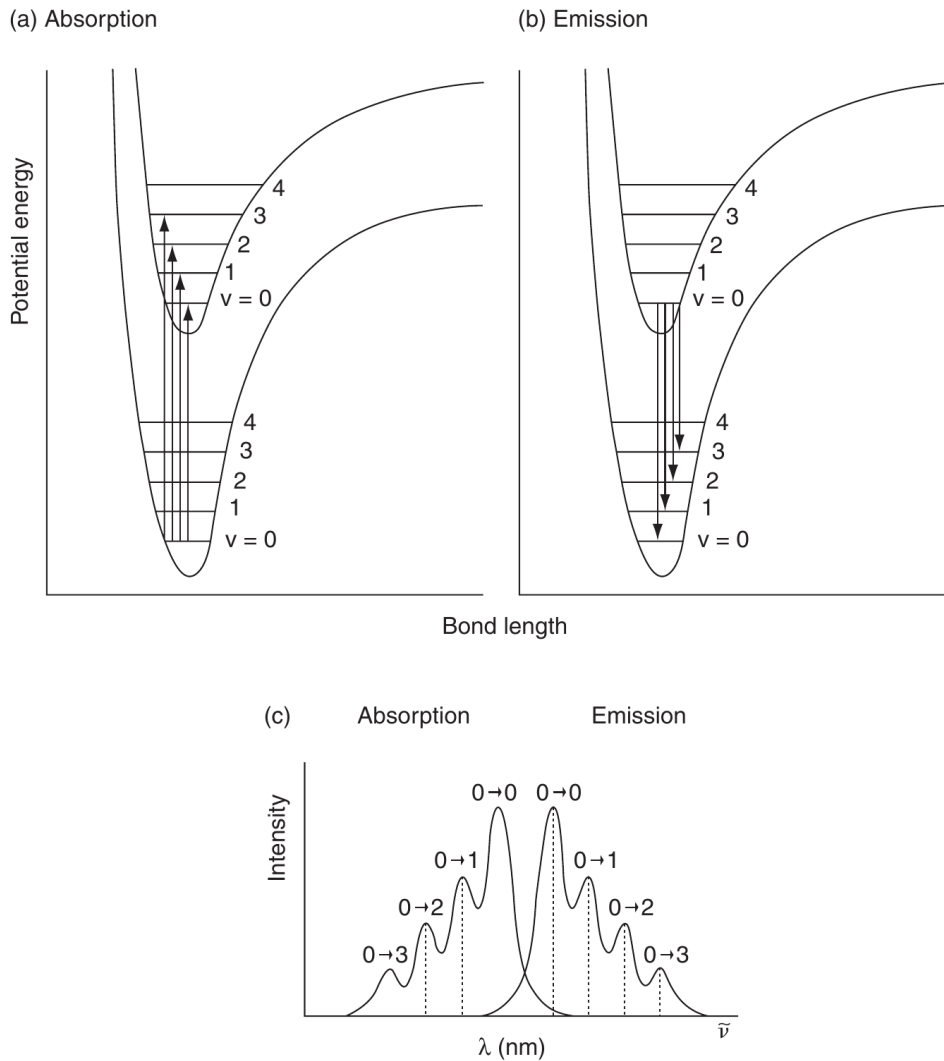
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Figure 2.2: Potential energy diagrams and spectra for fluorescent transitions taken from [3]. (a) Absorption transitions from the least energetic ground electronic state to different possible vibrational states of the electronic excited state. (b) Emission transitions from the lowest energy vibronic state of the excited electronic state to different possible vibronic states of the electronic ground state. (c) Absorption and fluorescence spectra that result from these transitions and their overlap

As we can see from (c), when we put the absorption and emission spectra in one graph, they are typically mirror images of one another. The difference between positions of the absorption and emission maxima is called the **Stokes Shift**. It is for this reason that if we were to look at the absorption and emission spectra of most chlorophylls and we were to excite the Soret band in the blue spectral region, the emission would be observed in the red region.

In general, the relationship between the excitation and emission was first described by Einstein and for that reason, the quantity that quantizes it is called the **Einstein coefficients**. Fluorescence is a phenomenon that takes place in interaction between the ground state and the lowest excited state and for that reason, a simple two-state model suffices. The probability that the absorption of

a photon will cause a transition from a lower state to an upper state is given by the population of the lower level (N_l) and the intensity of light at the frequency at which it is possible to cause the jump $I(\nu)$. The relevant constants times the square of the transition dipole moment B then give us the final piece with which we may formulate the following:

$$P_{l \to u} = N_l I(\nu) B \qquad (2.1)$$

The intrinsic probability for the photon-induced downward emission transition is given by the same relationship and the same Einstein coefficient B.

This **stimulated emission** is a process in which a photon interacts with the excited state and causes the emission of a second photon at the same frequency.

If that were all there was to it, eventually an equilibrium would establish with half of the molecules being in the upper state, half in the lower state. We know that is not the case, and the reason for that is **spontaneous emission**, the probability of which is given by another Einstein coefficient A. It is the source of most of the fluorescence usually measured in biochemistry, it is independent of outside light and when added together with stimulated emission we get

$$P_{u \to l} = N_u \left[I(\nu) B + A \right] \qquad (2.2)$$

The relationship between these two coefficients is given by

$$A = (const) \nu^3 B \qquad (2.3)$$

Einstein coefficient A can also be understood as the intrinsic rate constant for fluorescence k_f, which, similarly to the energy transfer rate constant, is dependant on the radiative lifetime τ_0 of the excited state if fluorescence were the only pathway out of excitation possible. This constant competes with the energy transfer rate constant for where the energy shall flow and it shall flow only when the speed of the excitation traveling is faster than that of a fluorescent decay.

2.2   Fluorescence excitation spectrum

A typical FES of a two-molecular structure can take the following form (see Figure 2.3.). The first molecule A absorbs a photon, energy is transferred to the second molecule B from where it is emitted as fluorescence.

Through analysis of the specific FES for our studied system, we can extract information about how much of the energy absorbed into the carotenoid travels into the chlorophyll. We know from previous studies [13] that the efficiency of the internal conversion from the Soret band to Q_y is nearly 100% so we can safely say that all energy absorbed into the Soret band eventually ends up in the Q_y. One may use the fluorescence excitation spectrum to extract the information about the evolution of the energy absorbed into the S_2 state of the carotenoid, thus getting an approximation of how much energy ends up in the carotenoid and how much of it ends up in the chlorophyll. We shall further discuss the state kinematics in the following section.
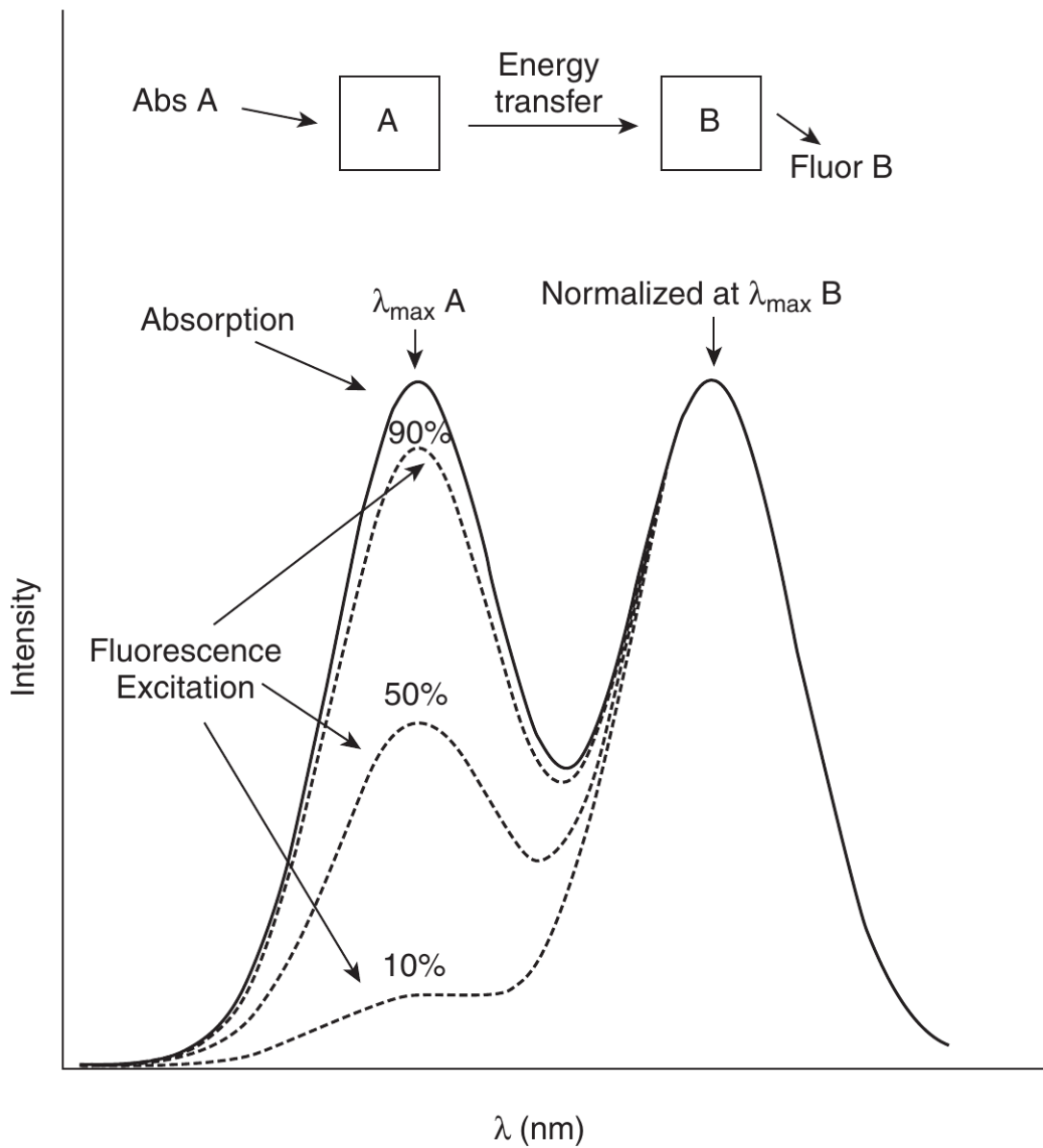
Figure 2.3: Energy transfer efficiency given by a fluorescence excitation spectrum normalized at $\lambda_{max}B$ maximum of emission. The whole line represents the absorption based on excitation wavelength, every dotted line is a possible shape of normalized fluorescence excitation. Taken from [3].

3. Phenomenological description of transport phenomena

In this part, we shall focus on the phenomenological description of the state kinetics we have discussed in the previous chapters. One of the goals of this thesis is to obtain energy transfer efficiency, which is related to the coefficients of the kinetic equations for state transitions in our system.

3.1   Kinetic equation

The kinetic equation for the transition from state 1 to state 2 is

$$\frac{d}{dt}P_2 = K_{21}P_1. \tag{3.1}$$

The coefficient K_{21} stands for the rate of transition and P_1 and P_2 represent the probability we find the system in the respective states.
For a system of multiple possible states, we can generalize the description as follows

$$\frac{d}{dt}P_n = \sum_n K_{mn}P_n \tag{3.2}$$

Equation (3.2) is also sometimes called the *master equation* [14]. We can simplify the equation and separate the diagonal members. Since the change in population is given as the sum of the influx and outflux, the influx being given by the sum $\sum_n K_{mn}P_n$, outflux by $\sum_n K_{nm}P_m$. This gives us:

$$\frac{d}{dt}P_m = \sum_n (K_{mn})P_n = \sum_{n \neq m}(K_{mn}P_n) + K_{mm}P_m = \sum_{n \neq m}(K_{mn}P_n - K_{nm}P_m) \tag{3.3}$$

The last equality is valid since the sum of probabilities must be 1, the derivative of that gives us zero:

$$0 = \frac{d}{dt}\sum_n P_n = \sum_n (\sum_m (K_{nm}P_m)), \tag{3.4}$$

This gives us $\sum_n K_{nm} = 0$, if the equation (3.4) is to hold for all P_m [14].
For the nondiagonal members representing the contribution of the element m to the probability of getting to n, we also get

$$dP_n(t) = K_{nm}P_m(t)dt, \tag{3.5}$$

the probability of jumping $m \to n$ in the interval $< t, t+dt >$. Probability of this jump must be positive, giving us another condition $K_{nm} \geq 0$ for $n \neq m$, which when combined with the condition of probability conservation gives us

$$K_{nn} = -\sum_{m \neq n} K_{mn}. \tag{3.6}$$

Solving such a system of linear differential equations is best done by diagonalization of the rate constants matrix. By finding the k_i eigenvalues and the eigenvectors $\vec{v}_i$ of the K matrix with elements K_{nm} we also get the transformation matrix

given by the eigenvectors $S = (v_1, v_2...)$. With the inverse, we can transform the probability vector $P_n(t) \rightarrow \tilde{P}_n(t) = S^{-1}P_n(t)$. The kinetic equation then transforms into

$$\frac{d}{dt}\tilde{P}_n(t) = k_n P_n(t) \tag{3.7}$$

The solution of this simple equation is

$$\hat{P}_n(t) = e^{k_n t}\hat{P}_n(O) \tag{3.8}$$

We then transform the result into the original basis by inverse transformation

$$P(t) = S\hat{P}(t) \tag{3.9}$$

and the solution takes the form of

$$P(t) = U_K(t)P(0) \tag{3.10}$$

where $U_K(t) = \exp{(Kt)}$ is sort of a stand-in for the quantum evolution operator. Since it follows the rule $U_K(t)U_K(t') = U_K(t + t')$, it suffices to get $U_K(\Delta t)$ for some chosen step Δt and with that we can get the probability vector in times $t = n\Delta t$ by recurrently utilizing this "evolution" operator $U_K(\Delta t)$ on the probability vector.

We shall use the conditions we can pose on our system through this derivation, there is however another way to obtain the K_{mn} matrix for out specific problem. One that uses the phenomenological knowledge that we know about our specific system with no need to find the appropriate basis.

3.2 Application of said description on photosynthetic aggregates

In our specific case, we are dealing with the transport of excitation in a system comprised of bacteriochlorophyll + carotenoid dimers. The molecular orbital energy diagram of the bacteriochlorophyll has 4 levels corresponding with the ground state and the 3 notable transitions. Put in order from the most energetic to the least, those are the **Soret band**, $\mathbf{Q_x}$ and $\mathbf{Q_y}$. The carotenoid has 3 notable energy levels - the ground state, $\mathbf{S_1}$ and $\mathbf{S_2}$ with $S_0 \rightarrow S_1$ being a forbidden transition. In general, taking all the different energetic states of the aggregate into account, we could model the system using the equation (3.2) with the dimension of P being 8:

$$\vec{P} = \begin{pmatrix} P_{S_2} \\ P_{S_1} \\ P_{S_0} \\ P_B \\ P_{Q_x} \\ P_{Q_y} \\ P_{g_0} \\ P_{g_1} \end{pmatrix} \tag{3.11}$$

From the top, we have the three possible energy levels of the carotenoid, followed by the Soret band, the Q_x band, the Q_y band, and then the two possible ground states, that may arise with the excitation traveling from the higher energy levels - the ground state that comes to be through a nonradiative transition g_0 and the ground state we get to by a radiative transition g_1, relaxing from the Qy band, which is the only place we can obtain fluorescence from because of Kasha's rule. The matrix $K(t)$ from the equation (3.2) would then be a square 8 by 8 matrix. The possible channels that one would have to account for are illustrated in Figure 3.1:
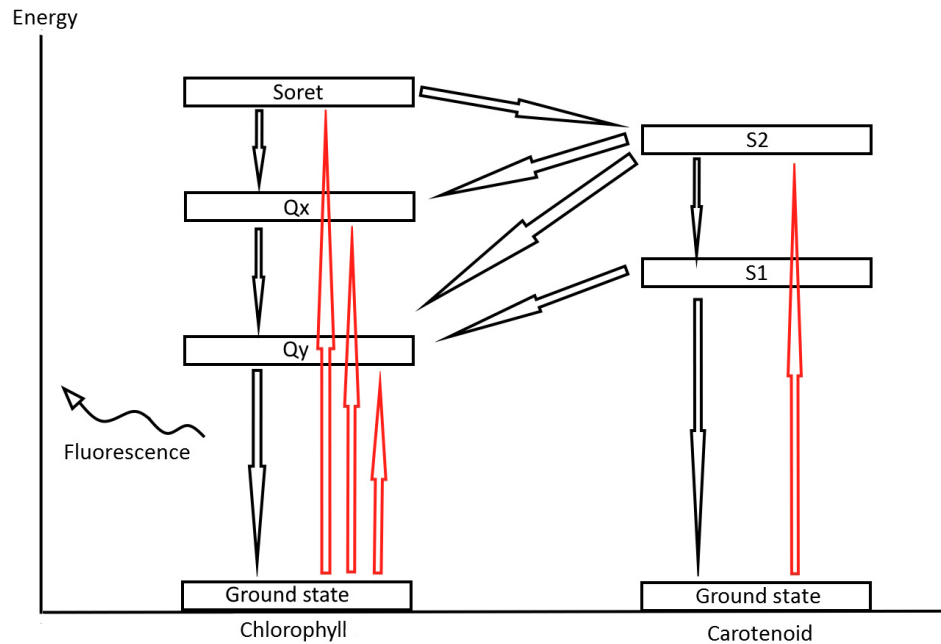
Figure 3.1: The state kinematics of the chlorophyll-carotenoid aggregate. The red arrows represent the possible excitation pathways, the black arrows represent transitions to lower energy states and the undulating line represents the emission of a photon during fluorescence.

Figure 3.1 does not take into account the vibrational states of the electronically excited states as shown for example in Figure 2.2. Modeling such a system would prove strenuous to say the least and is well beyond the scope of this thesis. Working with the obtained fluorescence-excitation spectrum of the *bacteriochlorophyll c* and *astaxanthin* aggregate, we will limit ourselves to working only with the situation shown in figure 3.2.
This is because the given spectra are normalized at the Q_y band, meaning that our fluorescent excitation spectrum is to be interpreted not as "how much energy at λ is then transferred into fluorescent radiation" but rather "how much of the energy at λ is transferred into the Q_y band". The absorption into the Q_x is minimal by comparison to the Soret band. There is almost no absorption into the carotenoid in that region so the spectra are superimposed in a way, that does not exactly provide any new information. There is an existing channel of energy travel from the chlorophyll to the carotenoid but it is also ignored because the method of the experiment does not allow us to monitor it. Taking all of this into
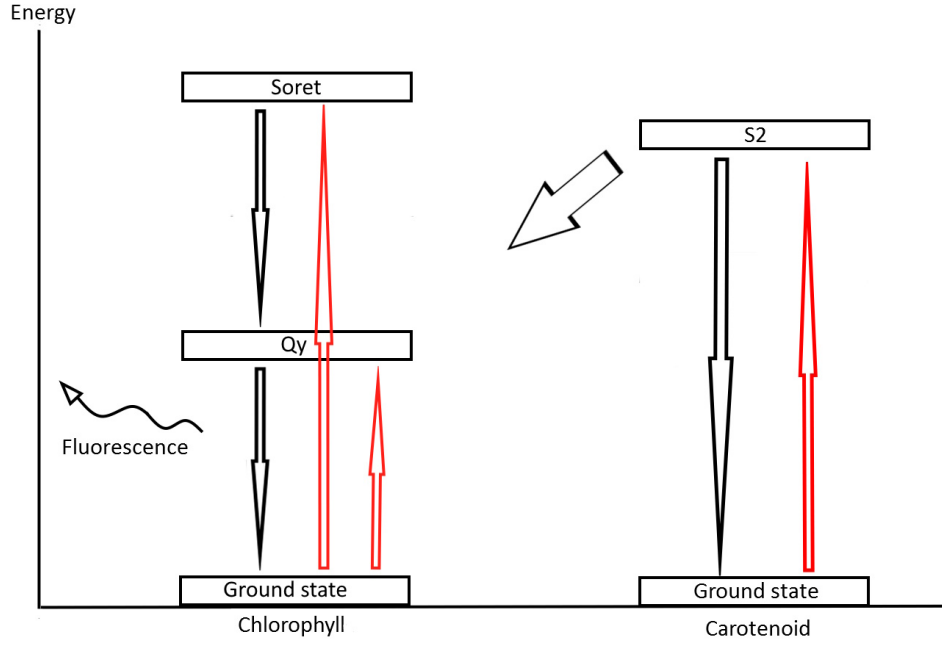
Figure 3.2: The state kinematics of the chlorophyll-carotenoid aggregate that we are modeling. The red arrows represent the possible excitation pathways, the black arrows represent transitions to lower energy states and the undulating line represents the emission of a photon during fluorescence. The arrow pointing from S2 to the chlorophyll represents the transition from S2 to any of the chlorophyll levels.

account, we are left with a much simpler system:

$$\vec{P} = \begin{pmatrix} P_{S_2} \\ P_B \\ P_{Q_y} \\ P_{S_0} \end{pmatrix}. \tag{3.12}$$

We can extract the information of the relative values of populations at $t = 0$ directly from the absorption spectrum. Setting the value of $t \to \infty$, we are left with a system, where all the energy has relaxed into either the ground state of the carotenoid or has gone to the Q_y band and then either relaxed by fluorescence or by nonradiative means. The actual rate of fluorescence for the Q_y relaxation is very low [15] but we cannot tell just by working with the given FES. The relative values of the final populations of the S_0 state and Q_y state can be extracted from the excitation spectrum with the knowledge that from the Soret band, essentially 100% has relaxed into the Q_y, so we can simply add the data from the absorption and when it comes to the carotenoid, the efficiency of transfer is what gives us the relative number to be added to the Q_y population, the rest goes into S_0. The evolution equation 3.2 for our problem takes the shape of

$$\frac{d}{dt} \begin{pmatrix} P_{S_2}(t) \\ P_B(t) \\ P_{Q_y}(t) \\ P_{S_0}(t) \end{pmatrix} = \begin{pmatrix} K_{11} & K_{12} & K_{13} & K_{14} \\ K_{21} & K_{22} & K_{23} & K_{24} \\ K_{31} & K_{32} & K_{33} & K_{34} \\ K_{41} & K_{42} & K_{43} & K_{44} \end{pmatrix} \begin{pmatrix} P_{S_2}(t) \\ P_B(t) \\ P_{Q_y}(t) \\ P_{S_0}(t) \end{pmatrix}. \tag{3.13}$$

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With all that has been stated above, we may simplify the expression into

$$\frac{d}{dt}\begin{pmatrix} P_{S_2}(t) \\ P_B(t) \\ P_{Q_y}(t) \\ P_{S_0}(t) \end{pmatrix} = \begin{pmatrix} -(K_{31}+K_{41}) & 0 & 0 & 0 \\ 0 & -K_{32} & 0 & 0 \\ K_{31} & K_{32} & 0 & 0 \\ K_{41} & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} P_{S_2}(t) \\ P_B(t) \\ P_{Q_y}(t) \\ P_{S_0}(t) \end{pmatrix}. \tag{3.14}$$

The matrix equation is simplified into a system of 4 differential equations, the first one being:

$$\frac{dP_{S_2}(t)}{dt} = -(K_{31}+K_{41})P_{S_2}(t) \tag{3.15}$$

The solution to this equation is well known to be achievable by the separation of variables and can be written as:

$$P_{S_2}(t) = e^{-(K_{31}+K_{41})t}C_1 \tag{3.16}$$

We choose $C_1 = P_{S_2}(0)$ to obtain the exact solution

$$P_{S_2}(t) = e^{-(K_{31}+K_{41})t}P_{S_2}(0) \tag{3.17}$$

The same solution holds for the equation for P_B:

$$P_B(t) = e^{-K_{32}t}P_B(0). \tag{3.18}$$

In the case of P_{S_0}, we must plug in the solution for P_{S_2} and integrate from 0 to time t that we are going to be sending to infinity:

$$\frac{d}{dt}P_{S_0}(t) = K_{41}P_{S_2}(t) = K_{41}e^{-(K_{31}+K_{41})t}P_{S2}(0) \tag{3.19}$$

By direct integration, we get:

$$\int_0^t \frac{d}{dt'}P_{S_0}(t')dt' = P_{S_0}(t) - P_{S_0}(0) = \frac{K_{41}}{K_{31}+K_{41}}P_{S_2}(0)(1-e^{-(K_{13}+K_{41})t}) \tag{3.20}$$

Setting the value of $P_{S_0}(0) = 0$ thus gives us the final formula for $P_{S_0}(t)$:

$$P_{S_0}(0) = \frac{K_{41}}{K_{31}+K_{41}}P_{S_2}(0)(1-e^{-(K_{13}+K_{41})t}) \tag{3.21}$$

This makes sense because the ratio of energy that gets into S_0 from S_2 is dependent on the original population of the S_2 band, the rate at which the population increases in the time given by the $1-e^{\cdots}$ exponential member and the ratio of the kinetic constants for the depletion of S_2 into this channel and the depletion of the state into both of the open channels.

The last equation and the one we are most interested in is solved in a similar manner. It takes the shape:

$$P_{Q_y}(t) = \frac{K_{31}}{K_{31}+K_{41}}P_{S_2}(0)(1-e^{-(K_{13}+K_{41})t})+P_B(0)(1-e^{-K_{32}t})+P_{Q_y}(0). \tag{3.22}$$

To determine the values of the constants K_{nm} we have to fit the model with the data in the following experimental section of the thesis. Then we can decide whether our model is an accurate one or not.

Experimental section

4. Bacteriochlorophyll and carotenoid aggregates

The specific chlorophyll-carotenoid aggregates that were used to obtain the experimental values provided by Jakub Pšenčík and Tomáš Malina were made off *bacteriochlorophyll c* and *astaxanthin* connected with a covalent bound .

   Bacteriochlorophyll c is one of the many types of photosynthetic pigments used by bacteria. They are found in green photosynthetic bacteria containing the *chlorosome* antenna complex [3]. Its structure plays very well into aggregation and
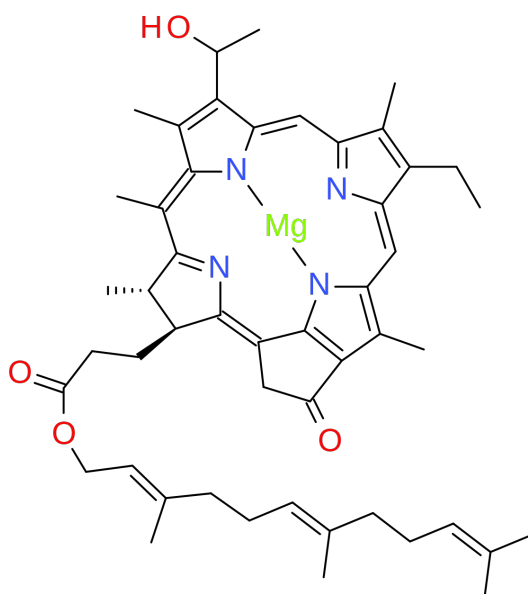
Figure 4.1: The skeletal formula of an *bacteriochlorophyll c* molecule from [16].

in chlorosomes, they indeed form large oligomeric complexes with little protein [3].

   Astaxanthin is a keto-carotenoid with red coloring properties. In nature, it is produced in certain algae and fungi. Larger animals like salmon, crustaceans, and even flamingos feed on them, obtaining the reddish pigmentation that we are familiar with as a result.
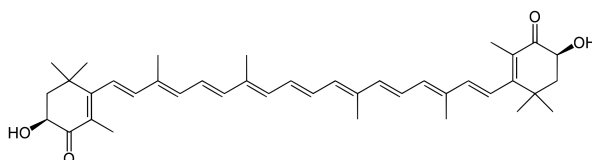
Figure 4.2: The skeletal formula of an 3S, 3S' *astaxanthin* molecule. One may find the other stereoisomers in Figure 1.5., taken from [17]

4.1 Results

We obtained a data set of the 1-T absorption spectrum of a *bacteriochorophyll c + astaxanthin* aggregate, the absorption spectrum of the bacteriochlorophyll by itself, of the carotenoid by itself and the fluorescence excitation spectrum normalized at the Q_y band of the chlorophyll. A graphical representation is displayed in Figure 4.3.
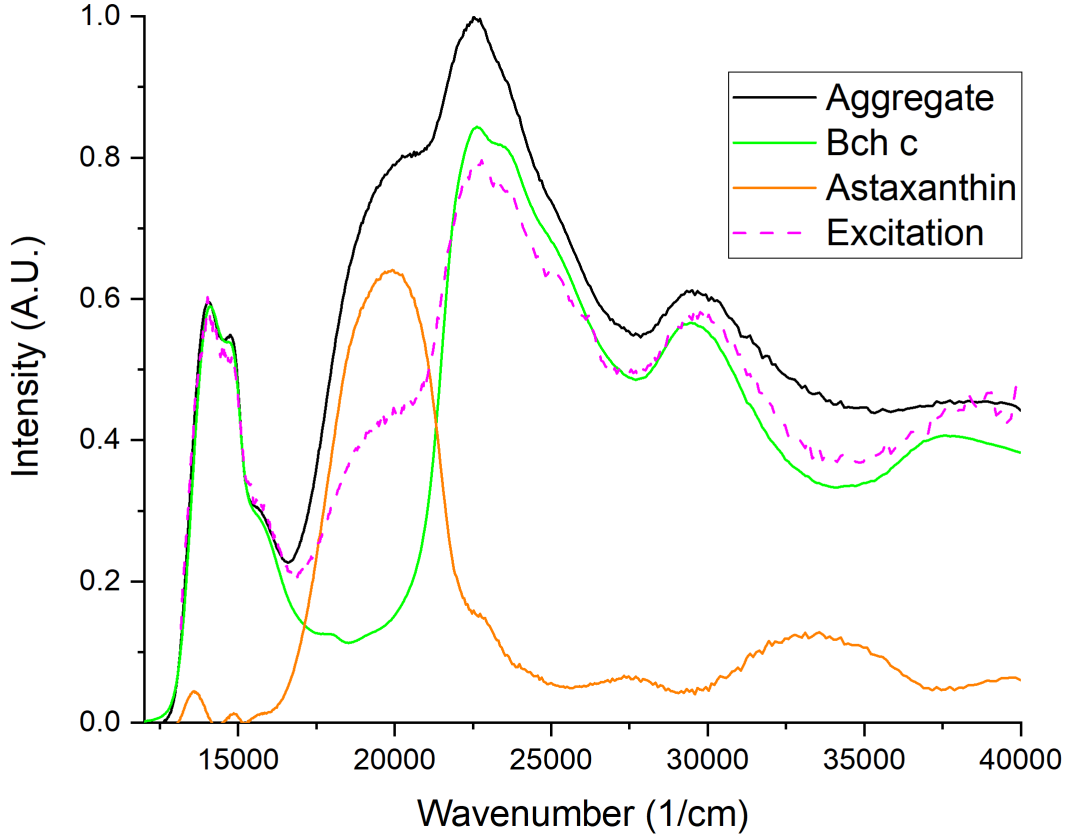
Figure 4.3: The fluorescence excitation spectrum of a bacteriochlorophyll c + astaxanthin aggregate. The black curve is the $1 - T$ spectrum of the aggregate, the green curve is $1 - T$ of just the chlorophyll, the orange is the $1 - T$ of the carotenoid and the dashed magenta line is the excitation curve normalized at the Q_y band of the chlorophyll. The data was provided by Jakub Pšenčík from previous measurements [18]
.

The use of the experimental data in this thesis is to (1) extract the initial values of the populations for the calculation of the exact values of the $\vec{P}$ from Eq. (3.14) for different wavelengths and (2) to see whether the theoretical model, used to simulate the behavior of our dimer in the simulation section of the thesis, is valid.

The matrix K_{nm} from Eq.(3.2) should contain the same values, no matter the excitation wavelength, meaning that we can use any point in the spectrum to

determine the values of our constants and then we can use the equation (3.21) to model the evolution of the Q_y population in $t \to \infty$. If our model is an accurate one, the values of the Q_y population for different wavelengths at $t \to \infty$ should mimic the fluorescence excitation spectrum.

The limit gives us

$$\lim_{t \to \infty} P_{Q_y}(t) = \frac{K_{31}}{K_{31} + K_{41}} P_{S_2}(0) + P_B(0) + P_{Q_y}(0). \tag{4.1}$$

This means that from fitting, we will be able to extract the value for the ratio between K_{31} and K_{41}. The obtained value is:

$$\frac{K_{31}}{K_{41}} = 0.831 \tag{4.2}$$

That means the efficiency of transition from the S_2 band of the carotenoid to the chlorophyll is

$$\frac{\frac{K_{31}}{K_{31}+K_{41}} P_{S_2}(0)}{P_{S_2}(0)} = \frac{K_{31}}{K_{31} + K_{41}} = (45 \pm 7)\% \tag{4.3}$$

The fitting and the error calculation were done in Python 3.10.10 with the use of the *curve_fit* method from the *scipy.optimize* v1.10.0 package. The initial values of constants were guessed to be $K_{31}^{guess} = 2$ and $K_{41}^{guess} = 3$. Through this method we obtained the value in eq.(4.2). With this ratio determined, we can now plug the value into the equation (4.1).

Simulation section

5. Quantarhei package

In parallel with the analysis of the experimental data, our goal is to simulate the absorption and excitation spectra of the photosynthetic antenna system. To get lineshapes of the absorption and excitation, a modified version of the Quantarhei package available on GitHub [2] was used. The original package has a variety of uses however we make use only of the demo_006_Absorption_1.py file used to simulate the absorption of a molecule. It establishes a class called *Molecule*. We can specify its parameters to fit the situation we are trying to model; like the energies of the different transitions and dipole moments. We can then get the correlation function using a built-in method. The **correlation function** is the key object here. Describing the method itself in detail is beyond the scope of this thesis but in general, it follows the theory in [19].

We utilize the semi-classical Liouville-Maxwell description. Within this framework, we obtain the correlation function of the electronic energy gap of the molecule (in the package modeled as a two-level system). This function contains all the necessary microscopic information we need to calculate the linear response of a two-level system. With a combination of a number of such *"molecules"*, we can try to simulate the absorption lineshape of our dimer. The absorption lineshape describes the intensity of absorption corresponding to an energy change in the observed object, usually given as a graph of absorption as a function of either wavelength λ or the wavenumber ω.

In our program, we define the environment the molecule is interacting with as a multimode overdamped Brownian oscillator. We set up the appropriate parameters: the correlation time, the reorganization energy, temperature, and the number of Matsubara frequencies. Now, we may extract the simulated absorption curves for such molecules.

5.1 Absorption spectra

We construct the absorption spectrum of the *molecule* to emulate the absorption curve of a *bacteriochlorophyll c* and *astaxanthin* aggregate as shown in figure (4.3). Our program can give us a simulation of the absorption of a single molecule in a two-level system. Thus, to obtain a complex spectrum such as the one measured, we treat each peak as representative of its separate two-level system with its own set of parameters. We simulate the absorption for a *molecule* object representing the system with the S_2 transition in the carotenoid and the Soret and the Q_y band for the chlorophyll. This is a very large simplification of the actual physical absorption of our dimer. That is not a problem for us since the simulated absorption curve is not necessary for any of our calculations. We describe the process of the simulation in order to demonstrate how one would approach such a problem with the use of the Quantarhei package.

A generic absorption spectrum given by our program can take the following shape:
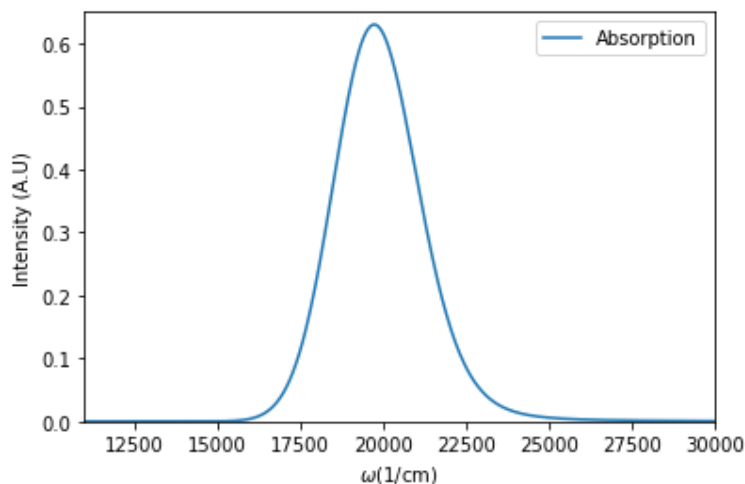
Figure 5.1: The shape of an absorption peak as produced by the Quantarhei package. On the x-axis, we have the wavenumbers in cm^{-1}, on the y-axis, we have an arbitrary absorption scale

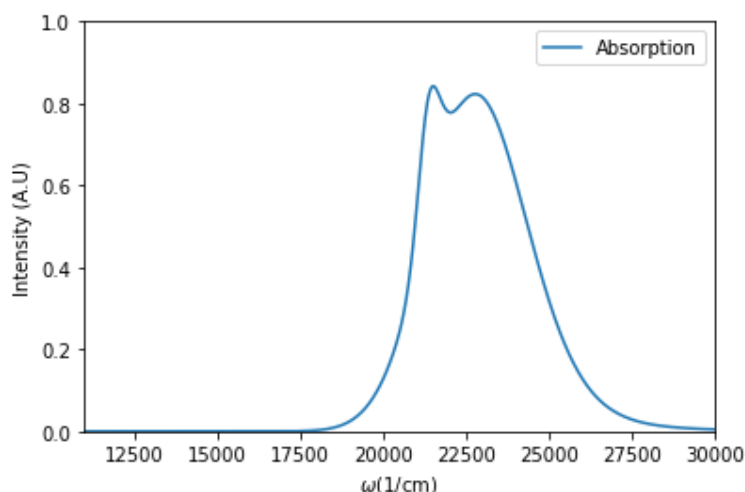We can add multiple absorption lines as such:

Figure 5.2: The shape of an absorption curve created by adding two peaks of the absorption spectra of two molecules in Quantarhei together. On the x-axis we have wavenumbers in cm^{-1}, on the y axis an arbitrary absorption intesity

This way we can simulate more complicated spectra, such as the one in Figure 5.3.

The figure encompasses only a reduced area of the spectrum because, in higher energies, the simulation cannot take into account the processes taking place, such as higher energy state kinetics or different higher vibrational state transitions. Two other inadequacies of the model are visible in the 16000 to 21000 cm^{-1} range. The way our spectrum is constructed does not allow us to fill in the area between two peaks in an adequate way. There is always the option to add more "molecule" objects but that would not reflect the physical reality of what we are attempting to study. Another issue is with the general limitation of the shapes
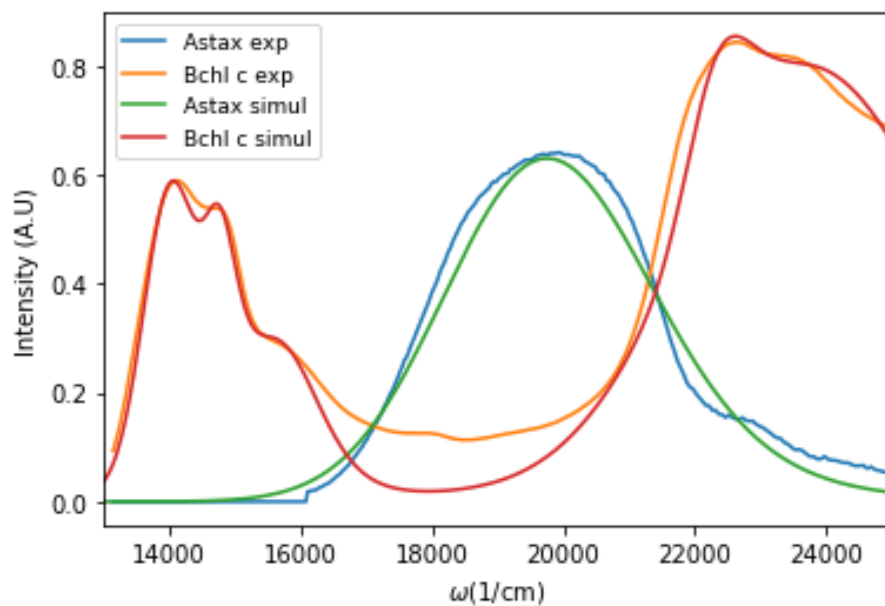
Figure 5.3: Simulated absorption curve of the bacteriochlorophyll a + astaxanthin dimer in the range of 13500 cm^{-1} to 25000 cm^{-1}. The green and red lines are created by our program, and the blue and orange lines are experimental data.

the Quantarhei package allows us to create. The slope of the experimentally measured peak would have us adjust the energy of the molecule object to either (a) have too thin of a shoulder but hit the general area of the absorption or (b) be appropriately wide in the shoulder but encompass too large of a spectral area. We also do not take into consideration many phenomena that are observed in the experimental data, such as dispersion.

5.2   Fluorescence excitation spectra

Now we finally arrive to the simulation of the fluorescence excitation spectrum itself. By fitting the experimental data we obtained the coefficients in the table at the end of chapter 4. We used them and the $P_n(0)$ values from the absorption curves to enumerate the equation (3.22) along the whole studied spectrum from $13000cm^{-1}$ to $40000cm^{-1}$. The results can be seen in the following figure:
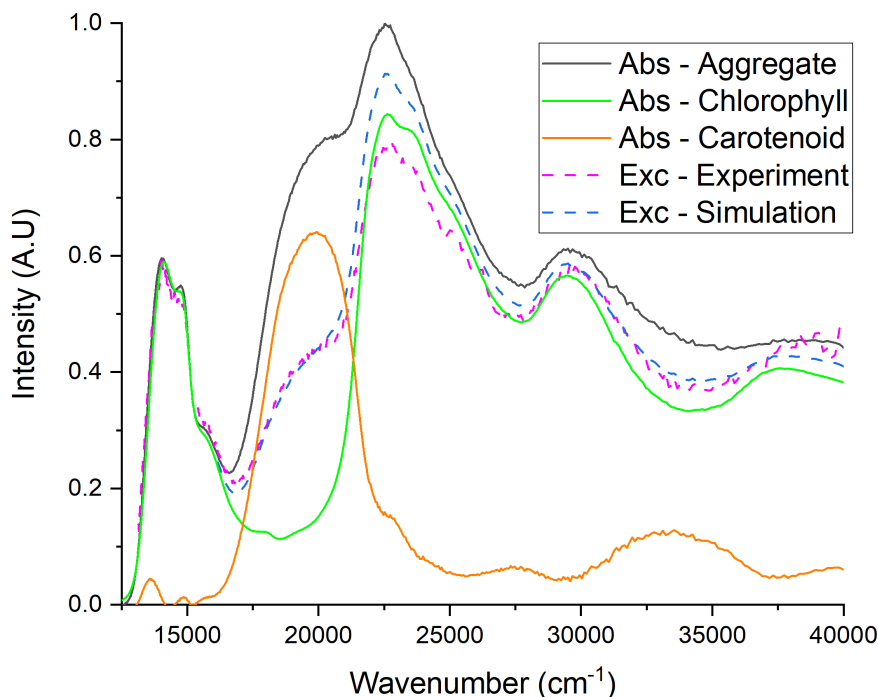
Figure 5.4: Simulated fluorescence excitation spectra with the use of 3.22 at $t \to \infty$ in the range of $13000cm^{-1}$ to $40000cm^{-1}$. The absorption data comes from the experiment while both the experimental and the simulated curves are visible to compare.

From studying the figure, we see that our model seems to follow the experiment rather well in the areas of the Q_y and the S_2 peaks and the higher energies but not as much at the Soret band. There are a number of factors that might contribute to this. The internal conversion in this particular subject might be slightly less than 100%, the transition from the carotenoid to the chlorophyll is sure to be too complicated for our simple model to reflect all of its nuances. However, it does work rather well around the main area of S_2, where the kinetic coefficients in our equation come into play the most.

Conclusion

We started by looking at the structure of photosynthetic antenna systems, which are central to the first light phase of photosynthesis. Afterward, we described the mechanism of energy transfer between them and what molecules they are composed of. Then we studied these light-harvesting molecules composed of different types of chlorophylls and carotenoids by introducing a specific dimer of *bacteriochlorophyll c + astaxanthin* connected by a covalent bond. We studied the absorption spectra of these dimers, mainly in the most noticeable Q_y and Soret bands of the chlorophyll and the S_2 band of the carotenoids. We also described the method of constructing the fluorescence excitation spectrum which is used to map the efficiency of energy transfer to the lowest excited state of the chlorophyll. We proposed a phenomenological model of energy transfer between these states in the dimer and constructed equations (3.16), (3.18), (3.21), and (3.22) to describe the kinematics of this model.

Through the analysis of the experimental data provided by Jakub Pšenčík, we determined the value of the ratio between the kinetic coefficients $K_{31}/K_{41} = 0.831$. This ratio was necessary to construct a functioning simulation of the fluorescence excitation spectrum in our model by using eq. (3.22) in $t \to \infty$. This makes sense because the only energy that is transferred into fluorescence by Kasha's rule is the energy that travels into the Q_y state. We also determined the efficiency of energy transfer from the carotenoid to the bacteriochlorophyll to be, with a rather large error, $(45 \pm 7)\%$.

We then used the Python3 package *Quantarhei* provided by Tomáš Mančal to simulate the absorption spectrum of the *Bchl + Astx* dimer, determined along what range this simulation is of use and the extent of its limitations. We also simulated the fluorescence excitation curve using Python3 3.9.13 and OriginPro2020. We found that our model gives us a rather accurate estimate of the physical reality around most of the studied spectrum except for the peak of the Soret band, where it overtakes the experimental spectrum by a remarkable amount. The graph can be seen in Figure 5.4.

The absorption spectra simulated by Quantarhei however give a rather crude depiction of the reality of the experiment. The comparison with measured data can be seen in Figure 5.3.

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List of Figures

List of Abbreviations

Bchl - Bacteriochlorophyll
Car - Carotenoid
FES - Fluorescence excitation spectrum
HOMO - Highest occupied molecular orbital
LUMO - Lowest unoccupied molecular orbital

A. Attachments

A.1 Transcript of the program used to model the absorption and the fluorescence excitation spectra

```python
# -*- coding: utf-8 -*-
"""
Created on Wed Jan  4 11:20:41 2023

@author: filip materna
"""

import numpy as np
import matplotlib.pyplot as plt
import quantarhei as qr

"""

    Setting up the values of the coefficients

"""
ta=qr.TimeAxis(0.,1000,0.5,atype="upper-half",
               frequency_start=0.)
NormQy = 0.57
NormSoret = 0.23
NormS2 = 0.63
NormQy2 = 0.28
NormQx = 0.27
NormSoret2=0.78
NormSoret3=0.6
left =   11000
right = 50000
enQy = 14150
enQy2=14900
enQx = 15800
enSoret = 22500
enS2 = 19920
enSoret2=24000
enSoret3=26000
k31 =0.2219
k41 =0.2670


"""
```

Setting up the parameters for the molecule objects

```python
"""



"""
Qy
"""
cfce_params1 = dict(ftype="OverdampedBrownian",
                    reorg=500.0,
                    cortime=60.0,
                    T=300,matsubara=30)

cfce_params4 = dict(ftype="OverdampedBrownian",
                    reorg=900.0,
                    cortime=60.0,
                    T=300,matsubara=30)

"""
Qx
"""
cfce_params3 = dict(ftype="OverdampedBrownian",
                    reorg=180.0,
                    cortime=60.0,
                    T=300,matsubara=30)



"""
S2
"""


cfce_params5 = dict(ftype="OverdampedBrownian",
                    reorg=5500.0,
                    cortime=60.0,
                    T=300,matsubara=30)



"""
Soret
"""
cfce_params2 = dict(ftype="OverdampedBrownian",
                    reorg=600.0,
                    cortime=60.0,
                    T=300,matsubara=30)


cfce_params6 = dict(ftype="OverdampedBrownian",
```

```
                    reorg=8000.0,
                    cortime=60.0,
                    T=300,matsubara=30)


cfce_params7 = dict(ftype="OverdampedBrownian",
                    reorg=10000.0,
                    cortime=60.0,
                    T=300,matsubara=30)



"""
    The function used to model the fluorescence excitation
    curve

"""
def func(X, a,b):
    x,y,z = X
    return a/(a+b)*x+y + z



"""
    Function to make the data gathered from the Quantarhei
    absorption spectra compatible with one another

"""



def compatibilize(input):
    div = input[1,0] - input[0,0]
    leftborder = input[0,0]
    if leftborder-left > 0:
        sizetoleft = int((leftborder - left)/div-1)
        lefadded = np.empty([sizetoleft,2])
        input = np.append(lefadded,input, 0)
    else:
        while input[0,0] <= left:
            input = np.delete(input, 0, axis = 0)
    sizetoright = int((right - input[len(input)-1,0])/div)
    last = input[len(input)-1,0]
    righadded = np.empty([sizetoright,2])
    for i in range (0,int(len(righadded))):
        righadded[i]=[last+(i*div),0]
    input = np.append(input, righadded, 0)
    return(input)
```

```python
"""



                              MAIN   BODY

"""



"""
        Getting  the  absorption  spectra
"""




e_units = qr.energy_units("1/cm")
with e_units:
    mQy = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enQy])
    mSoret = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enSoret])
    mQy2 = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enQy2])
    mQx = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enQx])
    mS2 = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enS2])
    mSoret2 = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enSoret2])
    mSoret3 = qr.Molecule(name="Molecule",
                        elenergies=[0.0,enSoret3])

    with qr.energy_units("1/cm"):
        cfce1 = qr.CorrelationFunction(ta,cfce_params1)
        cfce2 = qr.CorrelationFunction(ta,cfce_params2)
        cfce3 = qr.CorrelationFunction(ta,cfce_params3)
        cfce4 = qr.CorrelationFunction(ta,cfce_params4)
        cfce5 = qr.CorrelationFunction(ta,cfce_params5)
        cfce6 = qr.CorrelationFunction(ta,cfce_params6)
        cfce7 = qr.CorrelationFunction(ta,cfce_params7)


mQy.set_egcf((0,1),cfce1)
mQy.set_dipole(0,1,[1.0, 0.0, 0.0])
mSoret.set_egcf((0,1),cfce2)
mSoret.set_dipole(0,1,[1.0, 0.0, 0.0])
mQy2.set_egcf((0,1),cfce3)
mQy2.set_dipole(0,1,[1.0, 0.0, 0.0])
```

```
mQx. set_egcf ((0,1), cfce4)
mQx. set_dipole (0,1,[1.0, 0.0, 0.0])
mS2. set_egcf ((0,1), cfce5)
mS2. set_dipole (0,1,[1.0, 0.0, 0.0])
mSoret2. set_egcf ((0,1), cfce6)
mSoret2. set_dipole (0,1,[1.0, 0.0, 0.0])


acQy = qr.AbsSpectrumCalculator(ta,mQy)
acSoret = qr.AbsSpectrumCalculator(ta,mSoret)
acQy2 = qr.AbsSpectrumCalculator(ta,mQy2)
acQx = qr.AbsSpectrumCalculator(ta,mQx)
acS2 = qr.AbsSpectrumCalculator(ta,mS2)
acSoret2 = qr.AbsSpectrumCalculator(ta,mSoret2)

with qr.energy_units("1/cm"):
    acQy.bootstrap(rwa=enQy)
    a1Qy = acQy.calculate()
    a1Qy.normalize2([NormQy])
    darray = np.column_stack((a1Qy.axis.data,
                              a1Qy.data))
    darray = compatibilize(darray)
    xQy = darray[:,0]
    yQy = darray[:,1]

    acSoret.bootstrap(rwa=enSoret)
    a1Soret = acSoret.calculate()
    a1Soret.normalize2([NormSoret])
    darray = np.column_stack((a1Soret.axis.data,
                              a1Soret.data))
    darray = compatibilize(darray)
    xSoret = darray[:,0]
    ySoret = darray[:,1]

    acQy2.bootstrap(rwa=enQy2)
    a1Qy2 = acQy2.calculate()
    a1Qy2.normalize2([NormQy2])
    darray = np.column_stack((a1Qy2.axis.data,
                              a1Qy2.data))
    darray = compatibilize(darray)
    xQy2 = darray[:,0]
    yQy2 = darray[:,1]

    acQx.bootstrap(rwa=enQx)
    a1Qx = acQx.calculate()
    a1Qx.normalize2([NormQx])
    darray = np.column_stack((a1Qx.axis.data,
                              a1Qx.data))
```

```python
        darray = compatibilize(darray)
        xQx = darray[:,0]
        yQx = darray[:,1]

        acS2.bootstrap(rwa=enS2)
        a1S2 = acS2.calculate()
        a1S2.normalize2([NormS2])
        darray = np.column_stack((a1S2.axis.data,
                                  a1S2.data))
        darray = compatibilize(darray)
        xS2 = darray[:,0]
        yS2 = darray[:,1]

        acSoret2.bootstrap(rwa=enSoret2)
        a1Soret2 = acSoret2.calculate()
        a1Soret2.normalize2([NormSoret2])
        darray = np.column_stack((a1Soret2.axis.data,
                                  a1Soret2.data))
        darray = compatibilize(darray)
        xSoret2 = darray[:,0]
        ySoret2 = darray[:,1]

"""

    Calculating the fluorescence  excitation spectrum

"""


wavenumExp = []
QyExp = []
SoretExp = []
ExcSimul = []
S2Exp = []
ExcExp = []
with open('data.txt') as fobj:
    for line in fobj:
        row = line.split()
        wavenumExp.append(float(row[0]))
        QyExp.append(float(row[1]))
        SoretExp.append(float(row[2]))
        S2Exp.append(float(row[3]))
        ExcExp.append(float(row[4]))



wavenumExp=np.array(wavenumExp)
QyExp=np.array(QyExp)
```

```python
SoretExp=np.array(SoretExp)
S2Exp=np.array(S2Exp)
ExcExp=np.array(ExcExp)
ChlExp = QyExp + SoretExp

PQy=[]
for i in range(len(QyExp)):
    PQy.append(k31/(k31+k41)*S2Exp[i]+SoretExp[i]
               + QyExp[i])

PQy=np.array(PQy)
np.savetxt("excSimul.csv", PQy, delimiter ='\n')
xCelk = xSoret
yCelk =  ySoret+yQy+yQy2+yQx+ySoret2
yAggreg = yCelk + yS2

"""

    Toggling what to plot

"""
plt.plot(wavenumExp,S2Exp, label = "Astax exp")
plt.plot(wavenumExp, ExcExp, label="Excitation - exp")
plt.plot(wavenumExp,ChlExp, label = "Bchl c exp")
#plt.plot(wavenumExp,PQy, label="Excitation - simul")
#plt.plot(wavenumExp,ChlExp + S2Exp, label = "Aggreg exp")
#plt.plot(xCelk,yS2, label ="Astax simul")
#plt.plot(xCelk,yCelk+0.01, label = "Bchl c simul")
#plt.plot(xCelk,yAggreg, label = "Aggreg")
plt.xlim(13000,25000)
#plt.xlim(12000,40000)
plt.legend(loc="upper left",prop={'size': 9})
plt.xlabel("$\omega$(1/cm)")
plt.ylabel("Intensity (A.U)")
plt.show
```