**The effect of protein dynamics on quenching in the terminal emitter domain of LHCII**

**Aims**

To determine how movements within the Chla610-Chla611-Chla612-Lut620 domain of LHCII correlate with excitation quenching. Ultimately we hope to identify key degrees of freedom on which to base a meta-dynamical probe of the unquenched state.

**The three stage model**

The basic model will consist of five states, four of which will represent the Chla610-Chla611-Chla612-Lut620 *terminal emitter cluster* with the remaining state representing the remaining pool of Chla pigments. The system dynamics will be defined by the redistribution of energy between these states and their interconversion to the ground state. What these state are physically and how we treat the energy transfer dynamics will depend on model assumptions. We will employ three models which differ in the degree to which we account for coherent effect.

***1. Förster Theory***

This is the simplest approach which we don’t expect to give a very accurate description of the precise dynamics of the system. Interestingly it probably gives a reasonable description of the overall dynamics of excitation quenching. The basic assumption of Forster theory is that the inter-molecular couplings are far weaker than the system-bath interaction. As such the eigenstates of our system are simply the electronic transitions of the individual pigments. Transitions between these states are induced by the inter-pigment coupling which serves as a 1st order perturbation. The system-bath interaction is treated phenomenologically in terms of the instantaneous Stokes shift of the pigment excited states.

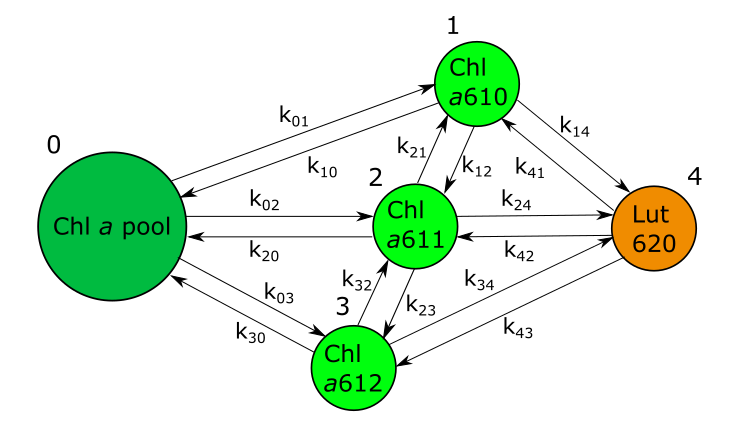
*1.1 The Structure of the Model*

The model is illustrated in Fig. 1. For convenience we will adopt a numbering convention for our states where represents the excited Chl a pool (which contains a total of 5 pigments) and , , and . Since we have 5 sites the dynamics are totally defined by 5 inter-connected (or ‘coupled’) differential equations. We encapsulate these 5 equations into a single matrix equation,

where is a column vector of occupation probabilities

is known as the ***transfer matrix*** which encodes the kinetic connectivity of the sites as shown in Fig. 1. Even for 5 sites it is too large to write out in full but we can write general expressions for the diagonal and off-diagonal elements. Since we are working in the site basis we are going to use the indices and . The diagonal elements are,

Since we are not explicitly considering the ground states of our pigment molecules we add in de-excitation via the addition of phenomenological rate constants where or depending on the site. These ***deactivation rate constants*** are taken from experimental measurement of the excited state lifetimes of the pigments.



**Figure 1:** The 5-site Forster model of quenching in the terminal emitter cluster. In addition to the first order rate constants shown each site is associated with a decay constant characterizing interconversion to their respective ground states. The Chl *a* decay rate is assumed to be while for Lutein we assume .

Note that Eqn. (3) is a very general expression and some of the rate constants in the summation will be zero due to a lack of connection. For example, considering the first diagonal element, Eqn. (3) suggests,

However, if we look at Fig. 1 we see there is no connection between and and so ,

The off diagonal elements are defined,

and again some of these may be zero. For example,

while,

due to a lack of connection between and .

*1.2 Assigning the rate constants*

Defining the rates constants, , is relatively straight forward. Firstly, we realize that a particular site cannot transfer energy to itself,

Nor can it donate energy to or accept energy from a site to which it is not connected in the model. Next we consider the rates of transfer between the 3 explicit Chl *a* sites and the rest of the Chl *a* pool. In the LHCII monomer, following photo-excitation of any of the pigments, excitation energy equilibrates across the 8 Chl *a* pigments within . This is a due to the Chl *a* -band being significantly lower in energy than that of Chl *b* and transfer between Chls being quite fast. The 8 Chl *a* pigments are ***approximately*** iso-energetic but the Chl *a*610- Chl *a*611- Chl *a*612 cluster is slightly lower energy than the others (Chl *a*610 is the lowest) and so it tends to have a larger share of the population after equilibration. However, we are not really interested in these dynamics so a very simple model is reasonable. Let us assume that the 8 Chl *a* ***are iso-energetic*** and that the characteristic timescale for transfer between two Chl *a* pigments is There is one subtlety to consider. The laws of thermodynamics dictate that there must be an entropic penalty associated with energy transfer from a large pool of pigments to a single pigment. Therefore,

While,

where is Boltzmann’s constant and,

where the comes from the fact that the Chl *a* pool contains 5 Chl *a*. This simplifies to,

meaning back-transfer to the Chl *a* pool is 5 times faster than transfer from the Chl *a* pool.

For transfer among the pigments being treated explicitly we use Fermi’s Golden Rule,

where is the fluorescence response function of the donor molecule, is the absorption response function of the acceptor and the asterisk indicates the complex conjugate. The response functions are defined as follows,

where are the ***site energies*** and is the ***organization energy*** of the donor. These parameters are generally well known or can be obtained by spectral fitting. are the ***line-broadening functions***. Line broadening is due to the coupling of the pigment electronic transition to fluctuations in the positons of the nuclei. It is therefore related to the ***spectral density function***, ,by the ***fluctuation-dissipation theorem***,

may seem like an odd concept. It arises from a statistical treatment of the interactions between our (observable) electronic degrees of freedom and the unobservable, essentially random vibrational degrees of freedom. The vibrational bath performs fluctuations of various frequencies. How strongly these frequencies effect the energy of our electronic transitions is given by . For a Chl these fluctuations are ***under-damped***. This means that following a fluctuation the nuclear positions will oscillate a little before returning to equilibrium. Imagine flicking a stiff pendulum that was initially at rest. The spectral density of such fluctuations is given by the phenomenological function,

By *phenomenological function* we mean a function that gives the correct line shape but isn’t really based on a detailed molecular model. The meaning of the various parameters are not terribly important but we can think of and as being the ***natural frequencies*** of two optically-coupled vibrational modes. After a fluctuation the Chl tends to oscillate with these frequencies a few times before rapidly coming to rest. Note, the dependent variable, , is probably better thought of as an energy, , rather than a frequency as not all fluctuations oscillate before returning to equilibrium. The following values are obtained by fitting the absorption spectrum,

For Chl *a* and *b*,

The reorganization energy (needed for computing the fluorescence response function is given generally by,

The frequencies and are very small and therefore they can be said to represent overall banding and flexing of the Chl head group rather than bond vibrations. This spectral density translates into a single Lorentzian spectral line. The position of this spectral line depend on the electronic transition energy, . For the three Chls in the excitonic cluster we will assume the values calculated by Muh and Renger (2010),

For Carotenoids the situation is more complicated as we need to consider two types of fluctuation. The first are the low frequency/energy fluctuations due to bending/twisting of the molecule as a whole. These we assume are ***overdamped***, meaning that after the fluctuation the system returns straight to equilibrium without any oscillation. However, the electronic transition is also strongly-coupled to two high-frequency vibrational modes. These are the ***optically-coupled*** C-C and C=C modes. These modes are very ‘stiff’ and therefore following a fluctuation they undergo damped oscillations before returning to equilibrium. We say these modes are ***under-damped***. This leads to the familiar three-component spectral density,

The first term is the ***over-damped*** part while the two terms in the summation are ***under-damped***. The parameters have a physical interpretation. As before, and are the ***natural frequencies*** of the under-damped modes. We notice that the over-damped mode does not have a natural frequency because, being over-damped, it does not oscillate. , and are ***correlation/damping times***. They characterize the time it takes for the system to return to equilibrium after a fluctuation. Lastly, , and are the reorganization energies associated with each mode. The is one subtlety, only the reorganization of the under-damped mode contributes to the Stokes shift. By fitting the 2-photon absorption spectrum we were able to obtain the following parameters for lutein,

For lutein S1,

This spectral density translates into a very broad spectral line with three vibronic peaks. The first peak is the 0-0 line and this appears at the purely electronic transition energy,

The second peak is higher than this and the third peak higher again.

Lastly, all that is left to do is assign the phenomenological decay constants and . These are obtained from experiment. The excitation lifetime of Chl in solution lies in the range and we assign a typical value of . Similarly, the lifetime of the S1 state of xanthophylls you find in LHCII is around 10-20 ps. We use a representative value of .

*1.2 Solving the equations of motion*

Assuming that one has calculated/assigned all of the necessary rate constants and use them to define the transfer matrix, **,** then one is in a position to solve the dynamics and calculate the mean excitation lifetime. The general solution to Eqn. (1) is,

We will discuss how we assign the various terms. Firstly, we can define the initial conditions . Our basic assumption will be that at the energy is equilibrated over the Chl *a* pool. The initial population of each Chl a state is given by the Boltzmann distribution,

where is the site energy of the ith site, is Boltzmann’s constant and is the temperature. is the so-called ***partition function*** which here simply ensures that all of the initial probabilities sum to 1,

We assume that the lutein is not initially populated,

Before we can proceed we need to assign a value for . This site represents the collective population of 5 additional Chl *a* and so we will average over the values reported by Muh and Renger,

The next thing to assign is the eigenvalue matrix, , and the matrix of eigenvectors, . This is simply obtained by diagonalizing using whichever linear algebra library you prefer,

You will be able to use a matrix inversion function from the same library to obtain and (we will use later on). We are now in a position to compute the excitation dynamics. Firstly, we define some time range that we are interested in and some time-step. Our time range must cover the dynamics of excitation decay and as such should be approximately equal to the longest timescale in our set-up. In our case this would be . We may want to reduce this if there is significant quenching. The time-step must be sufficiently short to resolve the initial redistribution of energy but since we are using Forster theory there will be no non-trivial coherent dynamics on the ultra-fast timescale. should be sufficient. For this full set of time points we can then compute,

**Remember:** These eigenvalues are simply eigenvalues of our transfer matrix, . They are completely unrelated to the energy eigenvalues that we compute when considering excitonic interactions. In Firster theory we assume that there are no excitonic states.

In the MD run we treat each uncorrelated snap shot as a configuration within the ***microcanonical ensemble***. For each one we shall first compute the evolution of the populations of our 5 sites. Next we compute the ***total decay kinetics***,

This will be a ***not quite exponential*** curve with several components. The next thing to do will be to determine the lifetime components by fitting a sum of 2 (or maximum 3) exponential decay functions to the trace,

where and are the amplitudes and lifetimes of these components respectively. We use 3 components only in the event that a good fit cannot be obtained for 2. Lastly, we calculate the ***mean excitation lifetime*** for the snap-shot,

This should be approximately equal to,

is our key parameter. It effectively defines whether the snap-shot represents a quenched or unquenched conformation. Understanding which conformation degrees of freedom are coupled to is an essential goal.