University of Illinois at Urbana-Champaign
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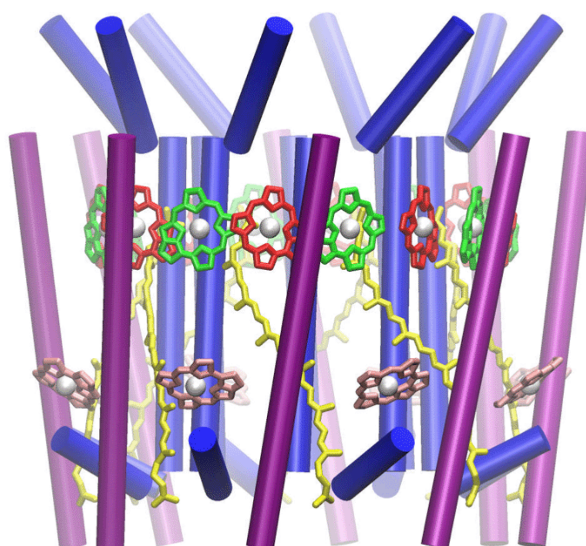
Theoretical and Computational Biophysics Group

Excitation and Electron Transfer



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A web version, in color, is available at
http://www.ks.uiuc.edu/Training/SumSchool03/Tutorials/photo

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Introduction

Life on earth is sustained through the harvesting of the energy of sunlight through photosynthesis, where the energy of an absorbed photon is first converted to a (short lived) electronic excitation, which is then transferred to a reaction center, and later stored in a (longer lived) charge gradient across the membrane via an electron transfer. These two fundamental processes in photosynthetic light-harvesting form the subject matter of this tutorial. The first section of this tutorial studies the **excitation transfer** process in the pigment complex of the peripheral light-harvesting complex, LH-II, of the purple bacterium *Rhodospirillum molischianum*. The second section introduces **electron transfer** in a a protein that shuttles electrons between different membrane proteins, namely cytochrome c_2 from *Rhodobacter sphaeroides*.

Getting started

In order to set up your local workspace for these tutorials, begin by typing in the terminal window:

```
tbss> cd ~/tbss.work
```

Now you will copy all necessary files into the tbss.work directory. Instead of typing `TOP_DIR`, type the location of the Summer School directory tree:

```
tbss>cp -r TOP_DIR/sumschool03/tutorials/10-electron-transfer/photo-tutorial-files ./photo-tutorial-files
```

For instance, if the materials are located at `/mnt/cdrom` or at `~/Desktop`, replace `TOP_DIR` by `/mnt/cdrom` or `/Desktop`. Use this copy of the tutorial files. Change your current directory to `photo-tutorial-files` by typing:

```
tbss>cd ~/tbss.work/photo-tutorial-files
```

The two subdirectories

`~/tbss.work/photo-tutorial-files/1-excitation`

and

`~/tbss.work/photo-tutorial-files/2-electron`

contain the files required for the two sections of this tutorial.

If you ever get lost.... Both tutorial directories contain an `example-output` directory that has all intermediate and target files in it. You can restore your session to any intermediate stage by copying the files from this directory to your workspace. Simply type `cp example-output/filename .` in your working directory.

1 Tutorial on Excitation Transfer

In this part of the tutorial, you will learn about the transfer of excitation energy between pairs of bacteriochlorophyl molecules (BChls) in light harvesting complex II (LH-II). The tutorial consists of two sections. In the first section, you will use VMD to investigate the structure of LH-II in order to see how proteins and pigments are organized in the complex. Then, in the second section you will calculate the rate of excitation energy transfer between pairs of BChls in LH-II using VMD and Mathematica.

1.1 File Setup

The working directory for this tutorial is at
`photo-tutorial-files/1-excitation`
It contains five files and one directory.
1. `lh2.pdb` is a pdb file for LH-II.
2. `TwoDimer.vmd` is a VMD state file for two LH-II structural units.
3. `EightDimer.vmd` is a VMD state file for LH-II ring.
4. `lh2-dipole.vmd` is a VMD state file for showing direction of transition dipole moments of BChls in LH-II.
5. `transfer.nb` is a Mathematica file for excitation transfer rate calculation.
6. `example-output` is a directory containing example output files e.g. `OneDimer.vmd` is a VMD state file for an LH-II structural unit.

1.2 Architecture of Light Harvesting Complex II (LH-II)

In this section, you are going to view and investigate the three dimensional structure of the light harvesting complex II from Rhodospirillum (Rh.) molischianum using VMD.

Your objective in this section is to see how proteins and pigments (BChls and carotenoids) are arranged in LH-II by using VMD. It should take you about 45 minutes to complete.

LH-II from *Rh. molischianum* is a membrane protein complex which absorbs light and transfers its energy to a reaction center in a photosynthetic unit. It consists of eight copies of a basic structural unit (building block) containing a heterodimer of two protein subunits (α-apoprotein and β-apoprotein), three bacteriochlorophyls (BChls named B850a, B850b and B800, according to their absorption wavelength in nm) and one carotenoid. Eight of these units are assembled into the LH-II ring as shown in Figure 1. In total, there are 16 protein segments, 24 BChls (each contains a Mg^{2+} ion at its center) and 8 carotenoids.

You will start from viewing a building block of LH-II. From there, you will then examine how two heterodimers fit together in LH-II. Finally, you will investigate

the complete LH-II ring which is made of eight of these heterodimers.

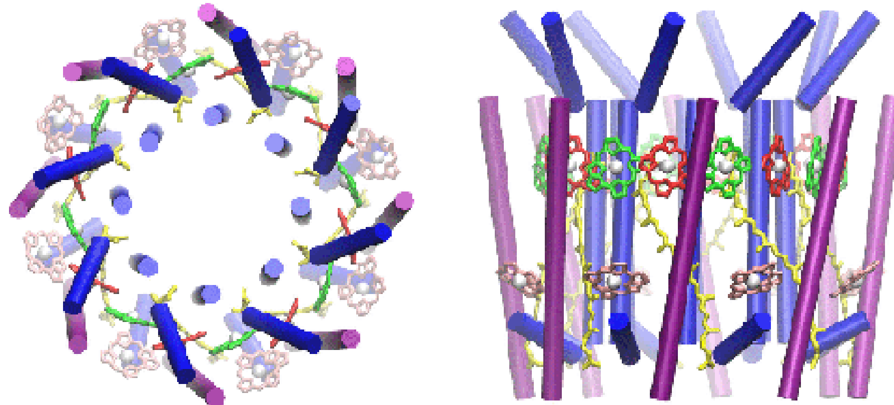
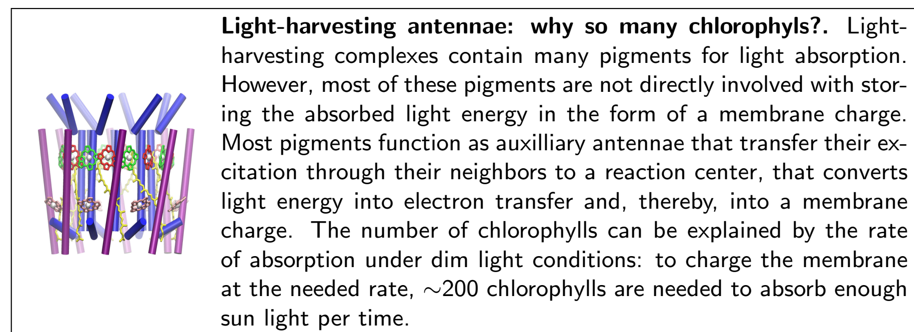


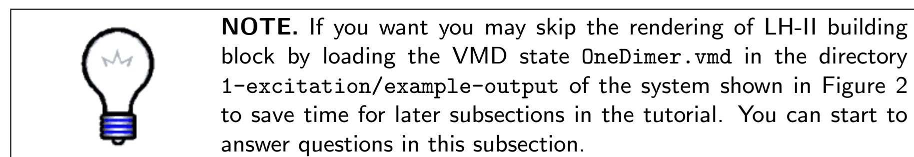
Figure 1: Top and side view of a light harvesting complex II from *Rh. molischianum.*



Light-harvesting antennae: why so many chlorophyls?. Light-harvesting complexes contain many pigments for light absorption. However, most of these pigments are not directly involved with storing the absorbed light energy in the form of a membrane charge. Most pigments function as auxilliary antennae that transfer their excitation through their neighbors to a reaction center, that converts light energy into electron transfer and, thereby, into a membrane charge. The number of chlorophylls can be explained by the rate of absorption under dim light conditions: to charge the membrane at the needed rate, ∼200 chlorophylls are needed to absorb enough sun light per time.

1.2.1 Building block of LH-II

In this subsection, you will examine the building block of LH-II as shown in Figure 2 and investigate how the two proteins and the four pigments are arranged.



NOTE. If you want you may skip the rendering of LH-II building block by loading the VMD state `OneDimer.vmd` in the directory `1-excitation/example-output` of the system shown in Figure 2 to save time for later subsections in the tutorial. You can start to answer questions in this subsection.

In the pdb file of LH-II (`lh2.pdb`), there are eight copies of the α-apoprotein with segment names `ALP1, ALP2,.. .., ALP8` and eight copies of the β-apoprotein with segment names `BET1, BET2,.. .., BET8`. The corresponding eight groups of BChls have segment names `BCA1, BCA2,.. .., BCA8` and each consists of three individual BChls with residue identification number (ResID) `57, 58` and `59` corresponding to BChls B850b, B800 and B850a respectively. Finally there are eight carotenoids with segment names `LYC1, LYC2,.. ..,` `LYC8`.

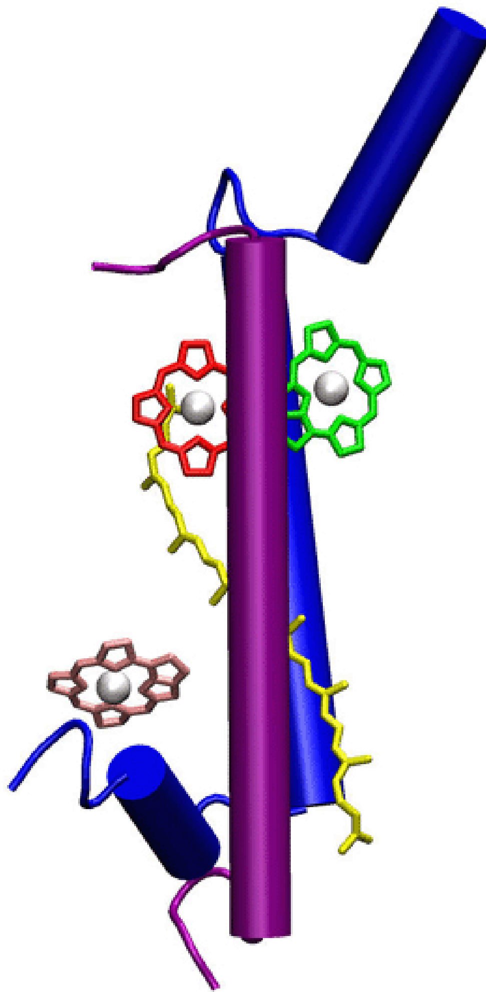


Figure 2: Building Block of LH-II.

You will first render a heterodimer of the two proteins made of the segment ALP1 and BET1 in Cartoon representation and color the α- and β-apoproteins in blue and purple respectively according to their segment names. You will then render the four pigments contained in the heterodimer in Licorice representation using various ways of coloring methods which allow you to color the carotenoid in yellow and color BChl B850a, B850b and B800 (resid 59,57 and 58) in red, green and pink, respectively, to show their locations relative to each other. Finally, you will render the two proteins in Surf representation and color them so that you can learn how they pack with each other and with the four pigments. Here is one way how to do this all.

1 **Open VMD and load the LH-II coordinate file lh2.pdb.** Open a terminal and change directory to the Excitation Transfer tutorial directory.

```
tbss> cd ~/tbss.work/photo-tutorial-files/1-excitation
```

Open a VMD session and load the LH-II coordinate file lh2.pdb.

```
tbss> vmd lh2.pdb
```

2 **Select the α-apoprotein with segment name ALP1 to be displayed in the VMD OpenGL Window.** In the Selected Atoms text entry of the Graphical Representation Window, delete the word all and type segname ALP1 and then press Enter.

3 **Render the α-protein in Cartoon representation and color it in blue according to its segment name.** In the Draw style tab of the Graphical Representation Window, select Cartoon as Drawing Method and Segname as Coloring Method. The α-apoprotein should be colored in blue by default.

4 **Reduce the distortion of the displayed protein due to Perspective viewing mode by changing it to Orthographic.** In the VMD Main Menu Window, click the third pull-down menu Display and click Orthographic in the second box. Orthographic mode renders three dimensional structure of the proteins by projecting them normally onto the screen.

5 **Make a new representation and select the β-apoprotein with segment name BET1.** In the Graphical Representation Window, click Create Rep to create a new representation. In the Selected Atoms text entry, delete ALP1 and type BET1 and then press Enter. Make sure that the β-apoprotein is drawn in Cartoon representation.

6 **Color the β-apoprotein in purple according to its segment name.** In the VMD Main Window, click the third pull down menu Graphics and select the second option Colors to open a Color Controls Window. In the first column Categories of the Color Controls Window, scroll down and click Segname. In the second column Names, click BET1. In the third column Colors, select 11 purple.

Q1: What is the main secondary structure of α- and β-apoproteins?

7 Change the drawing method for the two proteins to Surf **representation to see how both proteins are packed with each other.** NOTE: Surface rendering may take a-little while.

Q2: Where do α- and β-apoproteins make contact with each other?

8 Change the coloring method for the two proteins to ResType **to see how polar and non-polar residues are distributed on the two proteins.**

Q3: Can you tell that α- or β-apoproteins are transmembrane proteins?

9 Change the Drawing Method **for the two proteins to** Trace **representation and use** Bonds **labelling to measure the distance between the transmembrane helices on the α- and β-apoproteins.** To use Bonds Llabelling, click Mouse in the VMD Main Window. Then click and hold Label and select `Bonds 2`. The mouse arrow should change to a cross sign +. This will provide a separation distance of any two atoms you click. A quick and easy way to use this Bonds Labelling is to press the button 2 on your keyboard.

Q4: What is the approximate distance between the transmembrane helices of α- and β-apoproteins?

Q5: Can you show that the N-terminals of both proteins are on the same side of the membrane while their C-terminals are on the other side.

You can see that α- and β-apoproteins are transmembrane proteins which consist mainly of α-helices. You also might have noticed that there is sufficient space in the middle of the heterodimer to accommodate 3 BChls and 1 carotenoid molecule. Now, let's have a look at how pigments are arranged in the heterodimer.

NOTE: To delete the labels of atoms and distances on the VMD OpenGL Display, Click Graphics in the VMD Main Window and select Labels. Select Atoms to see a list of atom labels. Select atom names, then click Delete. Select Bonds to see a list of distance labels. Select pairs of atom names, then click Delete.

10 Change the Drawing Method **for the two proteins to** Cartoon **representation and color them according to** SegName

11 **Create a new representation and select the BChl B850a with segment name BCA1 and Residue ID 59 to be displayed.** In the Graphical Representation Window, click Create Rep to create a new representation. In the Selected Atoms text entry, delete all the words and type `segname BCA1 and resid 59`. Press Enter.

12 **Render the BChl B850a in** Licorice representation **and color it in red according to its** Chain ID**.** In the Draw style tab, select `Licorice` as Drawing Method and `Chain` as Coloring Method. In the first column Categories of the Color Controls Window, scroll down and click on `Chain`. In the second column Names, click on `X`. In the third column Colors, select `1 Red`.

13 **Create a new representation and select the BChl B850b with segment name BCA1 and residue ID 57 to be displayed.**

14 **Render the BChl B850b in Licorice representation and color it in green according to its Residue Type.** In the Draw style tab, select `Licorice` as Drawing Method and `ResType` as Coloring Method. In the first column Categories of the Color Controls Window, scroll down and click on `ResType`. In the second column Names, click on `Unassigned`. In the third column Colors, select `7 green`.

15 **Create a new representation and select the BChls B800 with segment name BCA1 and Residue ID 58 to be displayed.**

16 **Render the BChl B800 in Licorice representation and color it in pink according to its Residue Name.** In the Draw style tab, select `Licorice` as Drawing Method and `ResName` as Coloring Method. In the first column Categories of the Color Controls Window, scroll down and click on `Resname`. In the second column Names, click on `BCA`. In the third column Colors, select `9 pink`.

17 **Create a new representation and select the Carotenoid with segment name LYC1 to be displayed.**

18 **Render the Carotenoid in Licorice representation and color it in yellow according to its Residue Name.** In the Draw style tab, select `Licorice` as Drawing Method and `ResName` as Coloring Method. In the first column Categories of the Color Controls Window, scroll down and click on `Resname`. In the second column Names, click on `LYC`. In the third column Colors, select `4 yellow`.

19 **Create another representation and select the three magnesium ions in the segment name BCA1 to be displayed.** In the Graphical Representation Window, click Create Rep to create a new representation. In the Selected Atoms text entry, delete all the words in the entry and type `name MG and segname BCA1` and then press Enter.

20 Render the three magnesium ions on the BChls in VDW representation and color them in white according to atom name. In the Draw style tab, select `VDW` as Drawing Method and `Name` as Coloring Method. In the first column Categories of the Color Controls Window, scroll down and click on `Name`. In the second column Names, click on `M`. In the third column Colors select `8 white`.

Your LH-II structural unit should look like what is shown in Figure 2 except that your BChls are rendered in their full chemical structure.

21 Use the Bonds Labelling to measure the distances between BChls.

Q6: What are the distances between (1) BChls B850a and B850b; (2) BChls B850a and B800; (3) BChls B850b and B800?

22 Change the Drawing Method for the two proteins to VDW representation and color them according to Residue Type.

Q7: What are the polar protein residues which make close contact with the three BChls?

You can see that the BChls B850a (red), B850b (green) and the carotenoid (yellow) are packed in the space between the α- and β-apoproteins (blue and purple) while BChl B800 make a contact to the N-terminal helix of the α-apoprotein. It can be seen that there are two histidine side chains: one (αHis34) contacta BChl B850a and the other (βHis35) contacts BChl B850b. BChl 800 (pink) is coordinated by an aspartate (αAsp6). A very important is how two of these building blocks assemble together. Experimentally, it has been shown that these proteins actively self-assemble into LH-II.

23 Before you proceed to the next subsection, save your work as a VMD state `my-lh2-unit.vmd` and delete the LH-II unit system. From the VMD Main Window, click File and then select Save State. Type `my-lh2-unit.vmd` and click `OK`. In the VMD Main Window, highlight `lh2.pdb`. Click Molecule and then select Delete Molecule.

1.2.2 Packing of two LH-II structural units

In this section, you will study two LH-II structural units from a VMD state prepared by us. You will then investigate the packing of proteins and pigments between them.

24 Load the VMD state `TwoDimer.vmd` of two LH-II structural units. In the VMD Main Window, click File and then select Load State to open a

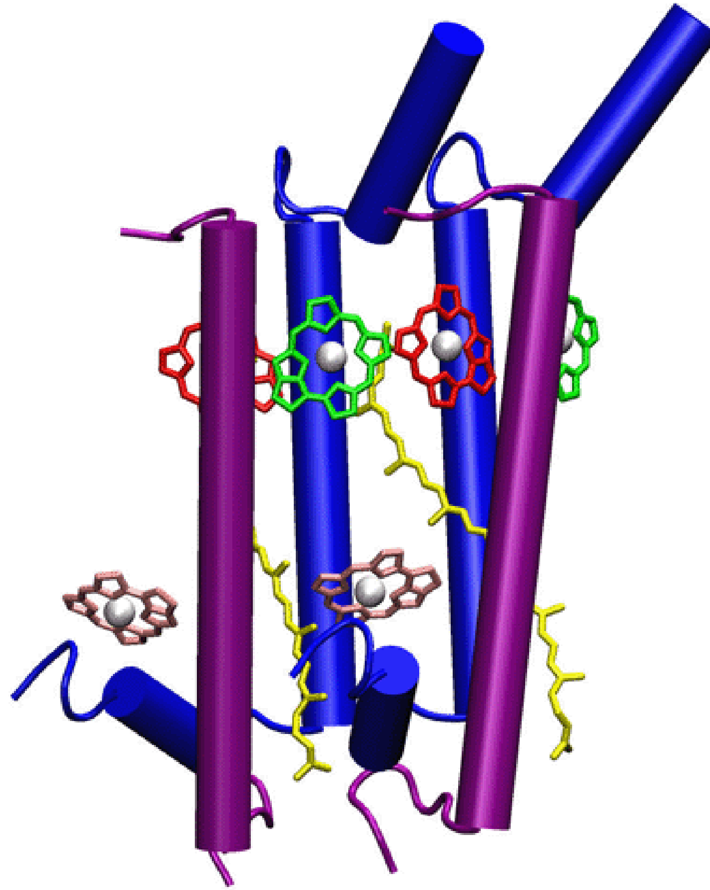


Figure 3: Packing of two LH-II structural units.

file selection window. Click `TwoDimer.vmd` and then click `OK`. You should see two copies of the LH-II structural unit which you rendered in the previous section (see Figure 3). All the tails and branches of the BChls have been removed to make it easier for viewing the system.

25 Change the drawing method for all the proteins to Surf representation. Take a look at the system particularly at the interface between two proteins to see how they pack.

Q1: How well do the two α-apoproteins pack with each other? How about the packing between the two β-apoproteins?

26 Now render all the proteins in Trace representation.

Q2: What is the distance in Å (1) between two α-apoproteins; (2) between two β-apoproteins?

27 Hide all the proteins and carotenoids.

Q3: What is the distance in Å between (1) two BChls B850a (red); (2) two BChls B850b (green); (3) BChls B850a (red, BCA1) and B850b'(green, BCA2) ; (4) B800 (pink, BCA1) and B850b'; (5) B800 and B850a' (red, BCA2)?

It can be seen that the transmembrane helices of the α-apoproteins are packed closely while the transmembrane helices of the β-apoproteins make very little contact with each other. The termini of both proteins also make extensive contact with each other. Can you imagine how a ring of eight of these structural units looks like?

1.2.3 LH-II ring

In this subsection, you will investigate an LH-II as a ring of 8 heterodimers containing 24 BChls and 8 carotenoids.

28 Delete the previous system and load the VMD state `EightDimer.vmd`. The structure of the LH-II will be loaded as 8 copies of the LH-II structural unit. One can readily recognize that this system forms a ring. (see Figure 1)

29 Have a look at how proteins are organized in LH-II by hiding all the pigments and vice versa.

Q1: What geometrical shape do the proteins and the pigments in LH-II form? What are the advantages of having such an arrangement?

30 Draw the protein in Trace representation and hide all the pigments.

Q2: What are the diameters of the rings of α- and β-apoproteins in LH-II?

31 Create a new representation. Make a selection of amino acids Tyrosine (`TYR`) and Tryptophan (`TRP`) on all the proteins in LH-II. Render them in VDW according to ResType. These two amino acids are usually used to identify the positions at which a membrane protein is aligned with a lipid bilayer. They are usually located at the interface between the fatty acids and the lipid head groups in the lipid bilayer.

Q3: Can you estimate the thickness of the membrane (in Å) in which the LH-II is embedded?

32 Hide all the proteins and show all the pigments. See how they are arranged relative to one another.

Q4: What are the diameters of the B850 ring and the B800 ring?

Q5: What are the distances between BChls B850a (red) and its two adjacent BChls B850b (green)? Are they equal?

An LH-II is made up of eight heterodimers. They are assembled into a circular ring with eight α-apoproteins (blue) forming an inner ring and eight β-apoprotein (purple) forming an outer ring. Pigments are embedded within the proteins and are placed roughly in the middle of the transmembrane part of the protein away from the bulk water at the interfaces of the membrane. Sixteen BChls (B850a and B850b) and eight carotenoids are packed between the rings of α- and β-apoproteins forming a ring of 16 BChls. Eight BChls B800 are held between β-apoproteins and supported by short helices from α-apoproteins. They form a larger ring of 8 BChls.

In LH-II, light is absorbed by pigment molecules such as BChls and carotenoids while proteins function as a scaffolding structure that hold all the pigments in their places. In the next section you will do some calculations to see how fast excitation energy is transfered between pairs of BChls when their separation is larger than their molecular sizes.

1.3 Excitation Transfer between two Bacteriochlorophyls in LH-II

In this section, you are going to determine the rate of excitation transfer between pairs of BChls within LH-II.

Your objective in this section is to learn how the rate of excitation transfer between a pair of BChls is calculated. This should take about half an hour to complete.

To do this, you will need a few mathematical formulae that are used to calculate the excitation transfer rate. You will start by determining the orientations of the transition dipole moments of BChls in LH-II and represent them as cones using VMD. Then you will use this structural information to compute the excitation transfer rate between a pair of BChls using Mathematica.

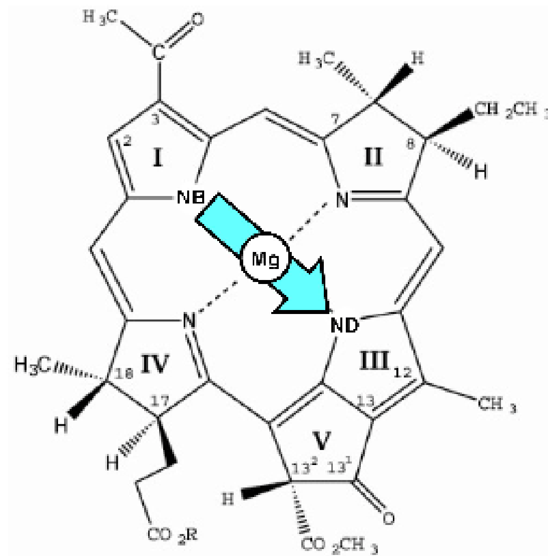


Figure 4: BChl and its transition dipole moment

Figure 4 shows the ring part of a BChl in LH-II. Magnesium ions (Mg^{2+}) are shown as white circles at the center. The direction of the transition dipole moment is shown as a cyan arrow. It points from the nitrogen atom of ring I (NB) to that of ring III (ND).

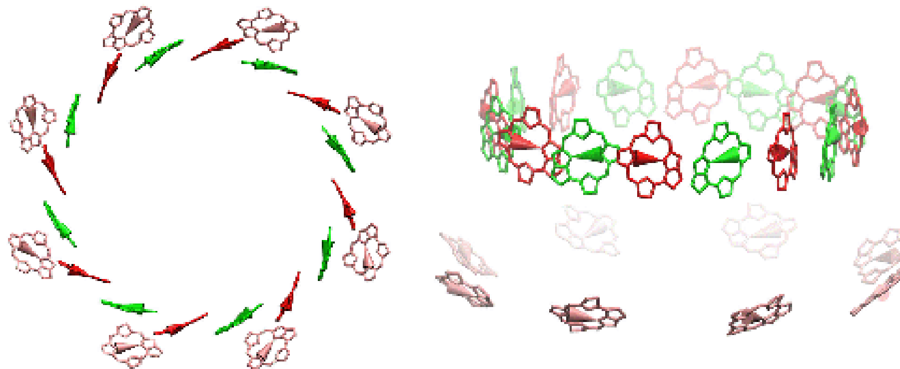


Figure 5: Top and side view of 24 BChls in an LH-II from *Rhodospirillum molischianum*.

Figure 5 shows the top and side view of 24 BChls in LH-II forming two rings of

BChls. The inner ring consists of sixteen BChls (eight BChls B850a (red) and eight BChls B850b (green) arranged alternatively). The outer ring consists of eight BChls B800 colored in pink. Direction of the transition dipole moments are represented by cones connecting two nitrogen atoms `NB` and `ND` (see Figure 5). First, you will learn how to compute an excitation transfer rate.

The rate k_{ij} of excitation energy transfer from BChl i to BChl j can be determined via

$$k_{ij} = \frac{2\pi}{\hbar} |U_{ij}|^2 J_{ij}$$

where U_{ij} is the Coulomb coupling between the excited state of BChl i and the ground state of BChl j; J_{ij} is an overlap integral between the emission spectrum of BChl i and the absorption spectrum of BChl j which can be determined spectroscopically. For a pair of BChls B850a $J_{ij} = 1.05 \times 10^{-3}$ cm; $\pi = 3.1416$ and $\hbar = 5.31$ ps cm^{-1}.

When the distance between the two BChls is larger than their molecular sizes, the Coulomb interaction term U_{ij} can be approximated as an induced dipole-induced dipole interaction involving the transition dipole moments $\vec{d_i}$ and $\vec{d_j}$ of the two BChls whose central Mg^{2+} ions are separated by a distance r_{ij}.

$$U_{ij} = C \frac{\hat{d_i} \cdot \hat{d_j} - 3(\hat{d_i} \cdot \hat{r}_{ij})(\hat{d_j} \cdot \hat{r}_{ij})}{r_{ij}^3}$$

where $\hat{d_i}$, $\hat{d_j}$ and $\hat{r}_{ij}$ are unit vectors along $\vec{d_i}$, $\vec{d_j}$ and $\vec{r}_{ij}$ respectively; and C = 505,644 Å^3.cm^{-1} is a constant.

Let's ask a more specific question. *What is the excitation energy transfer rate between a pair of BChls B850a (say segment names BCA1 and BCA2)?* Let's call them the first and the third BChls in the ring of 16 BChls. To be able to compute the excitation energy transfer k_{13} between the two BChls, we need to determine the transition dipole moment $\vec{d_1}$ and $\vec{d_3}$ of the BChls and the relative position vector $\vec{r}_{13}$. These vectors can be determined from the positions of nitrogen atoms `NB` and `ND` and Mg^{2+} ions (see Figure 4).

1.3.1 Orientation of BChl Transition Dipole Moments in LH-II

In this subsection, you will use VMD to measure the positions of the nitrogen atoms `NB`, `ND` and the Mg^{2+} ions named MG of the BChls B850a from the segments `BCA1` and `BCA2` in the pdb file `lh2.pdb`. Their positions will be used to determine the directions of the transition dipole moments of the two BChls and the relative position vectors between them. You will then draw the transition

dipole moments as cones to show how they are oriented in LH-II.

1 **From the previous VMD session, hide all the proteins, the carotenoids and the Mg^{2+} ions.**

2 **Locate the positions of nitrogen atoms NB, ND and magnesium ions MG on a pair of two BChls B850a (red) from segments `BCA1` and `BCA2`. Read out their coordinates and copy them into a file.** In the VMD Main Menu, click on the pull down menu Mouse, then click Label and hold the button and then move your mouse to select `Atoms 1`. The mouse arrow should change to a cross sign +. This will provide a label and coordinates of any atom you click (Coordinates will be shown in the VMD Console Window). Now use the figure of a BChl (Fig. 4) to help you identify the two nitrogen atoms `NB` and `ND` and the Mg^{2+} ions on the pair of BChls B850a (red, ResID 59) in segments `BCA1` and `BCA2`. Click on them, see the labels and the VMD console output to check that they are the atoms you are looking for. Then read out their (x, y, z) coordinates from the VMD Console Window as `rNB1 = (xNB1, yNB1, zNB1), rND1 = (xND1, yND1, zND1), rMG1 = (xMG1, yMG1, zMG1) and rNB3 = (xNB3, yNB3, zNB3), rND3 = (xND3, yND3, zND3), rMG3 = (xMG3, yMG3, zMG3)`. Copy these numbers into a text file using `nedit`.

3 **Set the drawing color to red.** At the prompt command in the VMD Console Window type `draw color red` and press Enter.

4 **Draw a cone pointing from atom NB to atom NA on each BChl.** Read the coordinates of atoms NB and ND from your text file. Then type `draw cone {xNB1 yNB1 zNB1} {xND1 yND1 zND1} resolution 20` where `xNB1, yNB1, zNB1` and `xND1, yND1, zND1` are the actual coordinates (e.g. (1.23, 4.56, 7.89)) of the two nitrogen atom `NB` and `ND` on the BChl 850a with segment `BCA1` and resid `59`. A red cone pointing from atom `NB` to atom `ND` will be drawn on the VMD OpenGL Window. Repeat the process by drawing another cone on the other BChl using the same command. Type `draw cone {xNB3 yNB3 zNB3} {xND3 yND3 zND3} resolution 20` where `(xNB3, yNB3, zNB3) and (xND3, yND3, zND3)` are the actual coordinates (e.g. (1.23, 4.56, 7.89)) of the two nitrogen atom `NB` and `ND` on the BChl 850a with segment `BCA2` and resid `59`.

5 **Load a VMD state `lh2-dipole.vmd` to view the directions of all BChl transition dipole moments as cones in LH-II. Look at how the transition dipole moments of BChls in LH-II are oriented.** To save your time, we have already drawn all the rest of the BChls as cones and save it as a VMD state for you to investigate. You should see all the BChls drawn in `Licorice` representation with red, green and pink cones on BChls B850a, B850b and B800 respectively. (see Figure 5

Q1: How is the transition dipole moment of a BChl in the LH-II oriented relative to that of its neighboring BChls.?

It can be seen that the BChls in LH-II have eight-fold symmetry. The transition dipole moment of BChl B850a (red) points roughly opposite to its neighboring BChl B850b (green) and it points in the same direction to its adjacent BChl B800. Knowing the positions of nitrogen atoms `NB` and `ND` and Mg^{2+} ions, you can start to calculate the excitation transfer rate in the next subsection.

1.3.2 Calculation of Excitation transfer rate

In this final part, you will use the atomic positions obtained from the previous subsection to calculate the excitation transfer rate k_{13} between two nearest BChls B850a (the first and the third BChls in the ring of 16 BChls B850) using Mathematica. You will also compute the excitation transfer rate from BChl B800 to BChl B850a and the reverse transfer rate from BChl B850a to BChl B800.

First, you need to calculate the directional vectors of the BChl transition dipole moments $\vec{d_1}$, $\vec{d_3}$ and the relative position vector $\vec{r}_{13}$ (both magnitude r_{13} and direction $\hat{r}_{13}$) between the two BChls from segments `BCA1` and `BCA2` in the LH-II coordinate file `lh2.pdb`. You will use these information to compute the induced dipole-induced dipole interactions term U_{13} between them. Then you will compute the transfer rate k_{13} between two BChls B850a. Finally, you will determine the excitation transfer rate k_f from BChl B800 to BChl B850a and the reverse transfer rate k_r.

6 Open a Mathematica notebook file `transfer.nb`. Open a terminal window and change directory to your working directory for this tutorial.

```
tbss> cd ~/tbss.work/photo-tutorial-files/1-excitation
```

Open the mathematica file `transfer.nb`.

```
tbss> mathematica transfer.nb
```

This should open the mathematica file as a window shown in Figure 6.

STEP 1: Execute commands to define constants used and load a Vector Analysis package. Click anywhere within the cell covered by a blue bracket on the right which has a semi-arrow. Then hold down the Shift key and press Enter. This will execute the command lines in the cell which define some physical constants to be used in the calculation and load a package called Vector Analysis into the program.

NOTE: Please ignore "Possible spelling error" message due to the naming of variables used.

Figure 6: Mathematica Graphics User Interface for the file `transfer.nb`

STEP 2: Calculate the directional vector $\hat{d}_1$ of the transition dipole moment $\vec{d}_1$ of the BChl B850a in segment BCA1. In the Mathematica window, replace the text {`xNB1, yNB1, zNB1`} and {`xND1, yND1, zND1`} by the actual coordinates of nitrogen atoms `NB` and `ND` of the BChl B850a in segment `BCA1` read from the previous subsection in the same format e.g. {1.23, 4.56, 7.89}. Then execute the commands to calculate the directional vector $\hat{d}_1 = \frac{\vec{r}_{ND1}-\vec{r}_{NB1}}{|\vec{r}_{ND1}-\vec{r}_{NB1}|}$ by holding down the Shift key and then press Enter.

STEP 3: Calculate the directional vector $\hat{d}_3$ of the transition dipole moment $\vec{d}_3$ of the BChls B850a in segment BCA2. Repeat the previous step by replacing the text {`xNB3, yNB3, zNB3`} and {`xND3, yND3, zND3`} with the actual coordinates of nitrogen atoms `NB` and `ND` of the BChl B850a in segment `BCA3` read from the previous subsection in the same format e.g. {1.23, 4.56, 7.89}. Then execute the commands to calculate the directional vector $\hat{d}_3 = \frac{\vec{r}_{ND3}-\vec{r}_{NB3}}{|\vec{r}_{ND3}-\vec{r}_{NB3}|}$ by holding down the Shift key and then press Enter.

STEP 4: Compute the separation vector $\vec{r}_{13}$ and the distance r_{13} (Å)

between the the central Mg^{2+} ions of the two BChls B850a. (NOTE: In the mathematica script, the distance r_{13} is denoted as L13). Replace the text `{xMG1, yMG1, zMG1}` and `{xMG3, yMG3, zMG3}` by the actual coordinates of the two Mg^{2+} ions, named `MG` , on the two BChls B850a read from the previous subsection in the same format e.g. {1.23, 4.56, 7.89}. Then execute the commands to calculate the relative position vector of $\vec{r}_{13} = \vec{r}_1 - \vec{r}_3$ and the separation distance $r_{13} = \sqrt{\vec{r}_{13}.\vec{r}_{13}}$ (measured in Å) by holding down the Shift key and then press Enter.

Q1: What is the separation distance between two BChls B850a? How large is it compare with the size of a BChl? Why is this important?

STEP 5: Calculate the directional vector $\hat{r}_{13}$ of the relative position vector $\vec{r}_{13}$. Execute the commands to calculate the directional vector $\hat{r}_{13}$ of the relative position vector $\vec{r}_{13}$ i.e. $\hat{r}_{13} = \frac{\vec{r}_{13}}{r_{13}}$ by holding down the Shift key and then press Enter.

STEP 6: Calculate the induced dipole-induced dipole interaction term U_{13}. (cm^{-1}) Execute the commands to compute the induced dipole-induced dipole interaction term U_{13} calculated according to the second equation above by holding down the Shift key and then press Enter.

STEP 7: Calculate the excitation transfer rate k_{13} (per ps). Execute this section to compute the the excitation transfer rate k_{13} according to the first equation above by holding down the Shift key and then press Enter.

Q2: What is the rate of excitation transfer between two BChls B850a?

Now let's calculate the excitation transfer rate k_f from BChl B800 to Bchl B850a and the reverse rate k_r.

STEP 8: Calculate the forward rate k_f of excitation transfer from a BChl B800 to its nearest BChl B850a. Up to here we have determined all the quantities required for the calculation of the transfer rate from the BChl B800 of segment `BCA1` to its nearest BChl B850a of the same segment for you. You just need to execute the commands to calculate the rate of forward energy transfer by holding down the Shift key and then press Enter.

STEP 9: Calculate the rate k_r of excitation transfer from a BChl B850a to BChl B800. Similarly in this step, we have determined all the quantities required for the calculation of the transfer rate from the BChl B850a of segment `BCA1` to its nearest BChl B800 of the same segment for you. You just need to execute the commands to calculate the backward transfer rate by holding down the Shift key and then press Enter.

Q3: Compare the forward excitation transfer rate k_f with the reverse transfer

rate k_r. Which direction of excitation transfer is more favorable?

You can see that the excitation transfer rate from BChl B800 to BChl B850a is six orders of magnitude higher than that of the reverse direction. So it is much more favorable for excitation energy to be transferred from a ring of 8 BChls B800 to a ring of 16 BChls B850a within an LH-II. The factor that directly affects the forward and reverse rates is the overlap integral J_{ij} which depends on the energies of the emission peak E_i and absorption peak E_j. When E_i is more than E_j you get large value of overlap integral and vice versa.

2 Tutorial on Electron Transfer

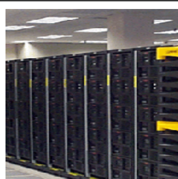
In this part of the tutorial you will construct an energy gap function through the molecular dynamics study of an electron transport protein.
The computational demands of this section of the tutorial are rather large compared to what you have studied in previous tutorials. You may want to immediately get started on your first molecular dynamics run of the day and then read on further while your simulation is running.

Switch to the directory required for this section by typing

```
tbss> cd ~/tbss.work/photo-tutorial-files/2-electron
```

This will henceforth be referred to as your working directory.

2.1 Starting your simulation



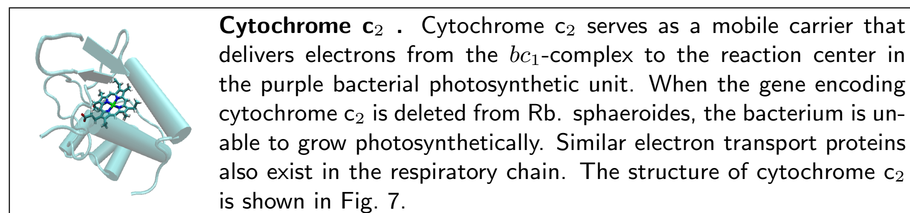
Running a molecular dynamics simulation of cytochrome c_2 . As in the previous tutorials and as described by the NAMD handout start a simulation with the configuration file `cytreduced.namd` and output file `cytreduced.out`. Your simulation will last 200 time steps. Do not forget to keep an eye on your simulation while you read on.

The system you are currently simulating is the electron transport protein cytochrome c_2 from the purple bacterium *Rhodobacter sphaeroides*. You have three main steps to perform in this section:

- **NAMD Run 1.** Run a MD simulation of cytochrome c_2 starting from a restart file. This will generate a trajectory file describing the dynamics of a solvated cytochrome c_2 molecule in reduced charge state.
- **NAMD Run 2.** Use NAMD to read the previous trajectory in order to compute an energy gap function at each frame. At this stage you will use a new PSF parameter file containing the charge differences between the reduced and oxidized states of the molecule. This will let you compute the energy cost of removing an electron from the system.
- **Analysis.** Use *Mathematica* to examine the thus computed energy gap function.

After starting your simulation, let us first take a closer look at cytochrome c_2.

2.2 Interlude: Structure of cytochrome c_2



Cytochrome c_2 . Cytochrome c_2 serves as a mobile carrier that delivers electrons from the bc_1-complex to the reaction center in the purple bacterial photosynthetic unit. When the gene encoding cytochrome c_2 is deleted from Rb. sphaeroides, the bacterium is unable to grow photosynthetically. Similar electron transport proteins also exist in the respiratory chain. The structure of cytochrome c_2 is shown in Fig. 7.

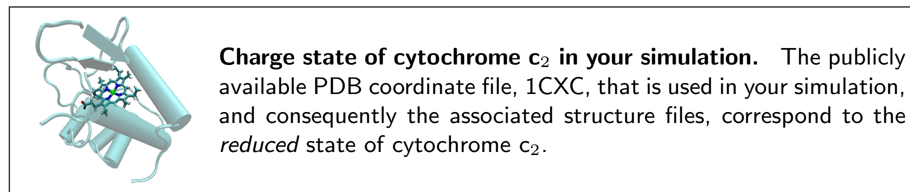
To explore the structure of cytochrome c_2 we will use VMD. Start VMD and load the required coordinate and parameter files by typing

```
tbss> vmd -e cytexamine.vmd
```

in your working directory. This loads VMD with a state file that allows you to view the equilibrated cytochrome c_2 structure in the beginning of the simulation. (You do not need to type these lines as they are taken from the state file you have just loaded.)

```
...
mol new cyt_reduced.psf type psf
mol addfile SPH_1CXCequi.pdb type pdb
mol representation Cartoon 2.100000 12.000000 5.000000
mol color ColorID 7
mol selection protein
...
mol representation Bonds 0.300000 6.000000
mol color Name
mol selection resname HEMP
...
```

This should give you a picture that resembles Fig. 7. You may also turn on the water molecules to note that this system is solvated in a water box. Indeed your current simulation has started from an already minimized and equilibrated state with periodic boundary conditions. Let us now take a closer look at the simulation configuration you are currently running.



Charge state of cytochrome c_2 in your simulation. The publicly available PDB coordinate file, 1CXC, that is used in your simulation, and consequently the associated structure files, correspond to the *reduced* state of cytochrome c_2.

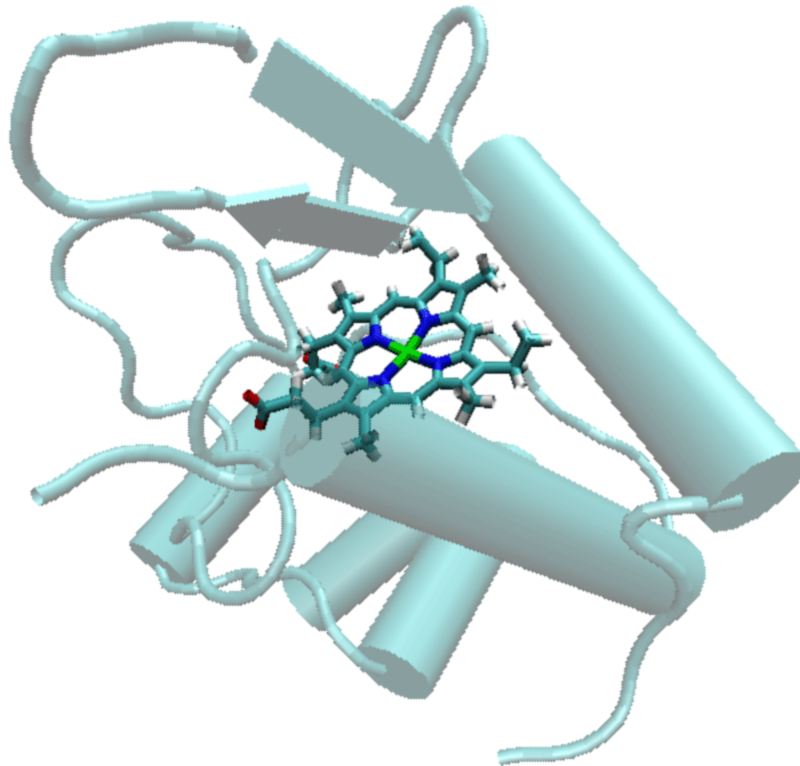


Figure 7: Cytochrome c_2 in the cartoon representation with its heme group highlighted.

2.3 Interlude: a closer look at your configuration files

The NAMD configuration file `cytreduced.namd` you executed earlier continues your simulation from a restart point.

```
...
set inputname cyt_red_init
bincoordinates $inputname.rst.coor
ExtendedSystem $inputname.rst.xsc
binvelocities $inputname.rst.vel
...
```

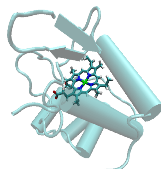
Without a restart point you would have had to worry about velocity relaxations and discard the initial part of your already short simulation.

Closer examination of the configuration file will reveal use of periodic boundary conditions. Also of interest is the unusual and ordinarily costly fact that

you are writing a DCD frame at each and every time step.

```
...
outputEnergies 1
dcdfreq 1
...
```

This is needed to be able to probe the time dependence of the energy gap function you will compute below.



Energy gap function $\epsilon(t)$. The electrostatic energy difference at a given time between the two charge states of cytochrome c_2 gives the energy gap function, $\epsilon(t) = E_{ox}(t) - E_{red}(t)$, where $E_{red}(t)$ and $E_{ox}(t)$ describe the interaction energy of the HEME group with the rest of the system for the oxidized and reduced states respectively.

The output of the first namd run will be written to a DCD file called `cytreduced.dcd`, which in turn will be read by a second NAMD run described below.

2.4 Re-running NAMD to read your previous trajectory

Now you will start a new simulation to read the trajectory created by the first one to compute the interation between two sets of atoms in your simulation. In section 2.1.5 of the NAMD tutorial of past week, you have seen how to compute the specific heat of a protein by a similar technique.

Running NAMD a second time . As before, start a new simulation, this time with the configuration file `cytrun2.namd` and output file `cytrun2.out`. Again remember to keep an eye on your simulation while you read on.

The configuration file you just executed, `cytrun2.namd`, refers to a new structure file, `cyt_deltaQ.psf`, which contains the information about the charge differences between the reduced and oxidized forms of the HEME group. Hence, the total electrostatic energy computed by this run, appearing in the `ELECT` column of the output file, will correspond to the energy gap function defined above.

Parameter files. The `.psf` structure files as well as the modified `.inp` parameter files provided for your simulation are simplistic representatives and should not be used beyond the scope of a tutorial. For example, the difference between the charge states of the reduced and oxidized forms of the HEME group are approximated by removing a total of one electron charge from Fe and N atoms at the center of the HEME without modifying the partial charges in the rest of the system.

Some details on the second configuration file `cytrun2.namd`. In addition to loading a new `.psf` file containing the charge differences of the HEME atoms between the reduced and oxidized states, this configuration file also employs a second `.pdb` file `cyt_ox_beta.pdb` which divides the atoms in your simulation into two groups by the labels in the BETA column. The first group is the HEME, where the second group comprises everthing else. Since `pairInteraction on` is specified the energy output in the output file `cytrun2.out` corresponds to only the interaction energy between these two groups. If you wish you may compare this configuration file to that in sec. 2.1.5 of the NAMD tutorial, where a similar method was employed for specific heat computations.

2.5 From the trajectory to the energy gap function

In order to extract the gap function information, after your second NAMD run is over, type:

```
tbss> namddat ELECT cytrun2.out
```

This writes the gap function to the file `data.dat`. Now cut the header (i.e. the first line) of the output `data.dat` with an editor, so we can read it easily with a script. For example, you may use `pico`

```
tbss> pico data.dat
```

Delete the first line `TS ELECT` so that the file consists only of two columns of numbers.

As in the previous part of the tutorial you will use *Mathematica* to display your results. In the limited time frame of a tutorial session, it is not possible to produce and analyze a long molecular dynamics trajectory. For your convenience, a longer 10 ps version of the energy gap function data file, `dataLONG.dat`, is provided for you. In order to use *Mathematica* to extract the energy gap function from the data file. First type

```
tbss> mathematica energygap.nb &
```

and then select Kernel → Evaluation → Evaluate Notebook (See Fig. 8.). This will read the data file containing the energy gap function you have created earlier, as well as a longer data file containing the data from a 10 ps simulation. You can now examine your data more closely. In particular, note that the histogram of the energy gap function over time gives a Gaussian distribution. This concludes the last section of this tutorial.

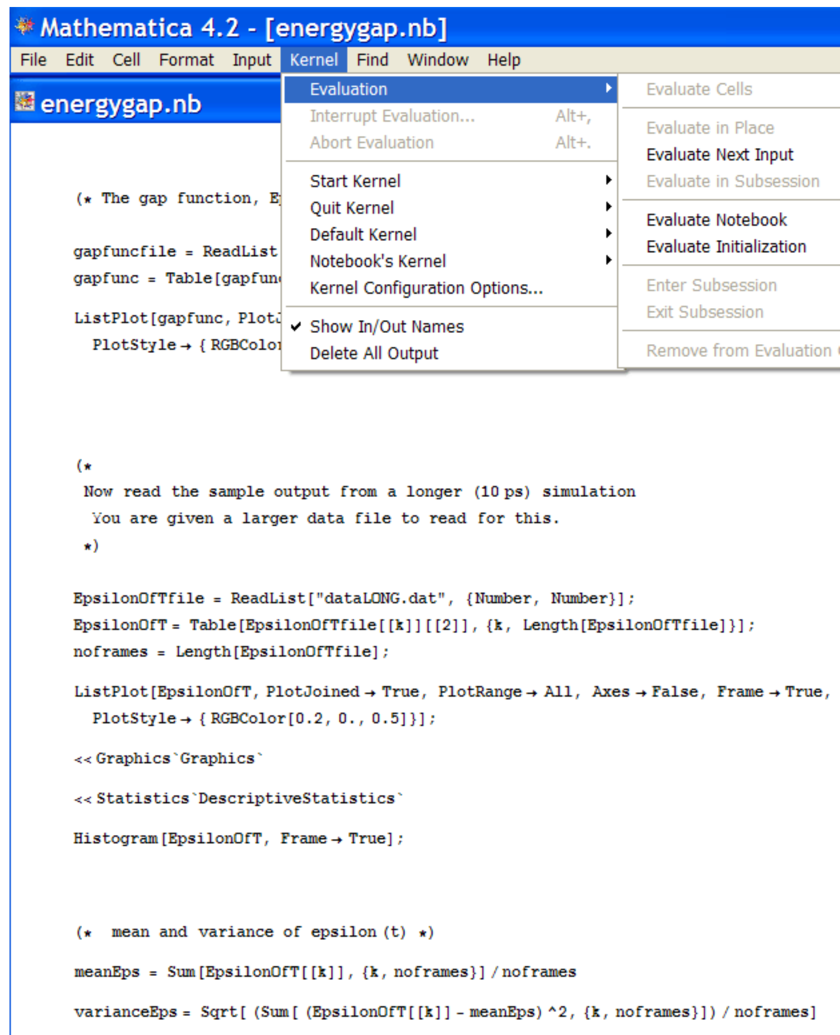


Figure 8: Running a *Mathematica* script to study the energy gap function.

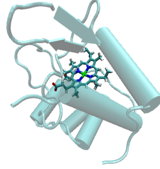
Ornstein-Uhlenbeck model for the energy gap function. The stochastic process of the fluctuations of the energy gap function, $\epsilon(t)$, can be modelled by a so-called Ornstein-Uhlenbeck process. Such a process is characterized through three properties:

1 The average value of the distribution of $\epsilon(t)$,

$$\langle\epsilon\rangle = \frac{1}{N_t}\sum_{j=1}^{N_t}\epsilon(t_j),$$

where N_t denotes the number of time steps.

2 The distribution over time of $\epsilon(t)$ is Gaussian, as such it is characterized through the variance, σ of the distribution



$$\sigma^2 = \frac{1}{N_t}\sum_{j=1}^{N_t}\left(\epsilon^2(t_j) - \langle\epsilon\rangle^2\right).$$

The validity of the assumption of Gaussian distributions for $\epsilon(t)$ can be seen in Fig. 10

3 The time dependence $\epsilon(t)$ is characterized by the normalized correlation function of the energy gap, or just the *energy gap correlation function* for short, defined by

$$C_{\epsilon\epsilon}(t) = \frac{1}{\sigma^2}\left(\langle\epsilon(t)\epsilon(0)\rangle - \langle\epsilon\rangle^2\right).$$

Practically, this function is evaluated through a windowed averaging procedure

$$C_{\epsilon\epsilon}(t) = \frac{1}{\sigma^2}\frac{1}{M}\sum_{\alpha=1}^{M}\left(\epsilon(t+t_\alpha)\epsilon(t_\alpha) - \langle\epsilon\rangle^2\right),$$

where M denotes the number of windows through which the trajectory is divided for sampling.

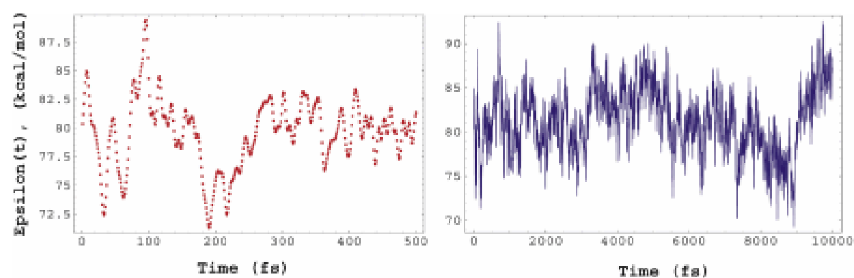


Figure 9: The energy gap function for cytochrome c_2 obtained through a MD simulation. (Left: for a 500 fs trajectory; Right: for a 10 ps trajectory)

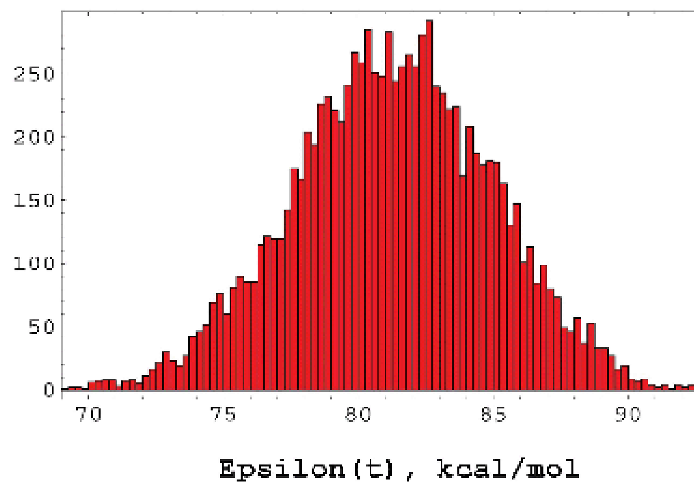


Figure 10: The histogram of the the energy gap function, $\epsilon(t_j)$, sampled over a time interval of 10 ps.