Förster resonance energy transfer (FRET)

Subject of this lab experiment is the radiationless transfer of absorbed light energy between two different chromophores. This phenomenon is applied in biophysical chemistry research in order to measure distances well below the optical diffraction limited resolution.

Dye labelled single DNA strands are hybridized, and the optical properties of the double strands are investigated by absorption and fluorescence spectroscopy. The experimentally determined energy transfer efficiency will then be compared to the theoretical value resulting from the spectral overlap of absorption and fluorescence spectra.

Colloquium Topics

- Lambert-Beer law of absorption
- Luminescence, fluorescence, phosphorescence
- Jablonski diagram, singlet and triplet states
- Radiative and radiationless transitions, energy transfer mechanisms
- Fermi's Golden Rule
- Transition dipole moment, oscillator strength
- Fluorescence and absorption spectrometers (components, principles of operation)
1. Theoretical Considerations

1.1 Absorption and fluorescence

Absorption and fluorescence processes play an important role in research as well as in everyday life. By absorption of a photon of suitable wavelength (or energy) a molecule (or an atom) is promoted into a state of higher energy. If the absorption of a photon has promoted the molecule from its electronic ground state $S_0$ into the first excited electronic state $S_1$ (the most common case), then the subsequent de-excitation can occur by one of the following processes:

1) **Heat**: The excitation energy is distributed to the environment as heat by collisions with the environment in a radiationless process.

2) **Fluorescence**: A direct emission of a photon occurs with transition back into the electronic ground state: $S_1 \rightarrow S_0$. The emitted photon generally carries less energy than the absorbed photon (Stokes shift) with the remainder being released as heat (as in 1).

3) **Phosphorescence**: Before emission of a photon the molecule undergoes a radiationless transition to a triplet state ($T_1$), e.g. by collisions (intersystem crossing). In a second step the molecule can return to the electronic ground state by emission of a photon: $T_1 \rightarrow S_0$.

4) **Energy Transfer**: The energy is directly transferred to a molecule (acceptor) in the immediate vicinity of the excited molecule (donor). This transfer occurs radiationless, as in process 1, but in contrast to process 1, the acceptor molecule will be excited electronically. This direct radiationless energy transfer can occur as Förster or as Dexter transfer.

The subject of the lab experiment is the radiationless Förster (or fluorescence) energy resonance transfer (FRET). Conditions under which FRET will occur are discussed below.

1.2 Energy transfer mechanisms

1.2.1 Radiative energy transfer

While the focus of the experiment is the radiationless resonance energy transfer, the same amount of energy can be transferred to the same molecule by a "trivial" form of energy transfer, i.e. in a two-step process in which first a photon is emitted by the donor D and then absorbed by the acceptor A. Upon photon emission the excited donor $D^*$ returns to its ground state D while the ground state acceptor A will be promoted to its excited state $A^*$ by the absorption process:
1) \( D^* \rightarrow D + h\nu \)  
2) \( h\nu + A \rightarrow A^* \)

The efficiency of this process depends on the following four parameters:

a) the quantum yield \( \Phi_e^D \) of \( D^* \) for emitting a photon

b) the number density \( n_A \) of ground state \( A \) molecules that can absorb the emitted photon

c) the absorption cross section \( \sigma_A(\lambda) \) (or, if you prefer the molar absorption coefficient - sometimes still called extinction coefficient - \( \varepsilon_A(\lambda) \)) of \( A \), i.e. the probability with which \( A \) will absorb an emitted photon

d) the overlap of the fluorescence emission spectrum of \( D^* \) and the absorption (or excitation) spectrum of \( A \).

### 1.2.2 Radiationless energy transfer

In addition to the "trivial" of radiative transfer of energy from a donor molecule \( D^* \) to an acceptor molecule \( A \), the energy can also be transferred in a radiationless way. This can occur in two ways:

a) by collision (more precisely: by exchange interaction) (Dexter energy transfer)

b) by Coulomb interaction (Förster energy transfer)

For Dexter energy transfer the involved electronic orbitals "collide" and the excited donor electron is exchanged with an acceptor electron. Dexter energy transfer changes the spin of donor and acceptor:

\[ ^3D^* + ^1A \rightarrow ^1D + ^3A^* \]

The Dexter mechanism requires very small donor-acceptor distances with an interpenetration of their orbitals. Since spin interaction decays exponentially with the distance \( r \) between donor and acceptor (in contrast to the \( r^6 \) dependence for the Förster mechanism, see below), it occurs efficiently only for distances \( r < 10 \, \text{Å} \). Therefore, Dexter energy transfer will not be subject of the lab experiment.

In contrast, the Förster mechanism occurs for larger distances and is therefore of much larger relevance for the lab experiment. In the following it will be discussed in some detail.

Fig. 1 illustrates the Förster mechanism schematically: The two integral parts deactivation of \( D^* \) and excitation of \( A \) are coupled to each other by Coulombic dipole-dipole interaction. Other than for the collisional Dexter mechanism, a direct contact between the two molecules is not required. \( D^* \) can rather be compared to a molecular broadcasting antenna transferring energy onto the molecular receiver \( A \).
1.2.3 Fermi's Golden Rule

The rate constant $k_r$ for spectroscopic transition described by perturbation theory can be expressed by Fermi's "Golden Rule":

$$k_r = \frac{2\pi}{\hbar} |\langle \Phi \mid \hat{H} \mid \Phi_f \rangle|^2 \rho(E)$$  \hspace{1cm} (1)

Here, $\rho$ is the density of the final states relevant for the transition (i.e. the density of isoenergetic donor acceptor states), $\Phi_i$ is the wave function of the initial state, $\Phi_f$ is the wave function of the final state, and $\hat{H}$ is the interaction operator.

Initial and final wavefunctions for Förster transfer can be expressed as:

$$\Phi_i = \psi_D(1)\psi_A(2) \quad \text{and} \quad \Phi_f = \psi_D(1)\psi_A^*(2)$$

The numbers indicate that in the Förster case no electrons are exchanged between donor and acceptor molecules. A final state resulting from such an electron exchange would be described by $\Phi'_{f} = \psi_D(2)\psi_A^*(1)$, which is why this competing process is called exchange mechanism. Note that donor and acceptor need to be considered together in the quantum mechanical initial and final states $\Phi_i$ and $\Phi_f$. 

Fig. 1: The Förster mechanism
1.2.4 Dipole-dipole interaction

The probability for a radiative transition between an excited state and the ground state is given by the transition dipole moment $R$. It is defined as:

$$R = \int \varphi_2^* \cdot \hat{r} \cdot \varphi_1 \, dV$$

(2)

Here, $\varphi_1$ is the electronic wavefunction of the molecule in the excited state, $\varphi_2$ is the conjugate complex wavefunction of the molecule in the ground state, $e$ is the electric charge, and $\hat{r}$ is the spatial coordinate. In a slightly simplified view, the integral $\int \varphi_2^* \cdot \hat{r} \cdot \varphi_1 \, dV$ describes the displacement of the charge distribution between ground and excited states upon inducing an optical transition by the alternating electromagnetic field of the light wave. The transition dipole moment of the donor can now interact in a radiationless way with the transition dipole moment of the acceptor. Thus, a transfer of the excitation energy between the two molecules can take place.

Classically, the interaction energy between two dipoles is given by:

$$E_{\text{dipole-dipole}} = \frac{\kappa}{4\pi \varepsilon_0} \frac{\mu_D \mu_A}{r_{DA}^3}$$

(3)

$\kappa$ is a geometric factor (depending on the orientation of the dipoles with respect to each other), $\varepsilon_0$ is the electric field constant ($8.854 \cdot 10^{-12}$ F/m), $\mu_D$ is the value of the electric dipole moment of the donor, $\mu_A$ is the value of the electric dipole moment of the acceptor, and $r_{DA}$ is the (mean) distance between the dipoles.

Treating the problem quantum mechanically, one finds that

a) the $r^{-3}$ dependence is maintained if the classic energy term is replaced by the Hamilton operator

b) $\mu_D$ and $\mu_A$ are given by the oscillator strengths for the transitions $D^* \rightarrow D$ and $A \rightarrow A^*$.

Altogether one obtains for the matrix element in eq. (1):

$$|\Psi_I \langle \hat{H} | \Psi_F \rangle |^2 = \left| \langle \varphi_D^* \varphi_A \rangle \left( \frac{\kappa}{4\pi \varepsilon_0} \frac{\mu_D \mu_A}{r_{DA}^3} \right) \right|^2 = \frac{\kappa^2}{16 \pi^2 \varepsilon_0^2} \frac{R_D^2 R_A^2}{r^6}$$

(4)

where $R_A$ and $R_D$ are transition dipole moments of the $D^* \rightarrow D$ and $A \rightarrow A^*$ transitions. Here one can already recognize the characteristic $r^{-6}$ dependence of the Förster mechanism.

In eq. (1) the energy value $E$ is discrete. In reality, owing to the uncertainty of the excited $D^*$ state this is not true. Likewise, the energy value of the acceptor also exhibits some uncertainty. Therefore, eq. (1) needs to be written differentially at first and then to be integrated.
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\[ dk_i = \frac{k_r^2}{4h\epsilon_0} \frac{\rho(E)}{r^6} dR_A dR_D \]  \hspace{1cm} (5)

The density of states \( \rho(E) \) can be experimentally determined by the so-called overlap integral

\[ \int \epsilon_A(\lambda) I_F^D(\lambda) \lambda^4 d\lambda \]

which is calculated from the normalized fluorescence intensity \( I_F^D(\lambda) \) of the donor and the absorption coefficient \( \epsilon_A(\lambda) \) of the acceptor. Taking this into account, one obtains for a medium of refractive index \( n \):

\[ k_r = \frac{9k^2\ln 10}{128\pi^2 n^6 N_A \tau_D} \frac{\phi_F^D}{r^6} \int \epsilon_A(\lambda) I_F^D(\lambda) \lambda^4 d\lambda \]  \hspace{1cm} (6)

where \( \tau_D \) is the mean lifetime of \( D^+ \) and \( N_A \) is Avogadro’s constant.

1.2.5 Distance dependence

If all constants in eq.(6) are combined in a single constant one obtains the Förster radius \( r_0 \), and eq. (6) becomes the following relationship which is also known as Förster equation:

\[ k_r = \frac{1}{\tau_D} \left( \frac{r_0}{r} \right)^6 \]  \hspace{1cm} (7)

The Förster radius is the distance at which 50% of the excited donor molecules will be deactivated by the Förster mechanism. Förster radii are characteristic for a specific donor acceptor pair and are compiled in the literature for several such pairs.

The Förster equation is useful when measuring the energy transfer efficiency \( E \), i.e. the portion of all photons absorbed by the donor which have been transferred to the acceptor. The energy transfer efficiency is given by:

\[ E = \frac{k_r}{k_r + \tau_D^{-1}} = \frac{r^{-6}}{r_0^{-6} + r^{-6}} = \frac{r_0^6}{r^6 + r_0^6} \]  \hspace{1cm} (8)

It can be determined from the relative donor fluorescence yields in presence (\( F_{DA} \)) and in absence (\( F_D \)) of the acceptor:

\[ E = 1 - \frac{F_{DA}}{F_D} \]  \hspace{1cm} (9)
2. Förster energy transfer in biophysical chemistry

Owing to the pronounced $r^6$ dependence of the Förster mechanism and Förster radii typically of the dimensions of biologically relevant macromolecules (20-90 Å) the method is ideally suited for obtaining steric information about such molecules. The method is therefore applicable to measuring distances well below the diffraction limited spatial resolution in optical spectroscopy of $\lambda/2$, corresponding to ca. 200 nm for short wave visible (blue) light. Examples for objects in the of a size comparable to typical Förster radii are many proteins, DNA structures, the thickness of biological membranes and distances between subunits of large proteins. Any variation of the distance between donor and acceptor will change the FRET efficiency $E$ which can then be used for the determination of molecular structures.

For example, if a protein is labelled with matching donor and acceptor dyes at different positions, structural changes of the molecule (e.g. due to solvation effects) can easily be monitored. To this end the energy transfer efficiency from eq. (9) must be determined in the presence and in the absence of the acceptor. If the Förster radius of the donor acceptor pair used is know, then the distance between donor and acceptor can be calculated from eq. (8) at any time.

3. Fluorescence spectroscopy

The general principle of operation of a fluorescence spectrometer is shown in Fig. 2

![General principle of a fluorescence spectrometer](image)

Fig. 2: General principle of a fluorescence spectrometer

The source for the excitation light can be a high pressure xenon lamp, e.g. Instead of one monochromator as in a simple optical absorption spectrometer, a fluorescence spectrometer uses two monochromators, the first for the excitation light and the second one for the emitted fluorescence light. In order to
minimise the influence of scattered excitation light, the two beam paths for excitation and emission are normally oriented perpendicular to each other. Since the fluorescence light is weak and emitted isotropically in all directions, special mirrors are required for the collection of as many photons as possible. The emitted light is normally registered by a photomultiplier.

While a normal UV/VIS spectrometer is usually designed in a two beam configuration, fluorescence spectrometers are usually one-beam devices. Another important difference concerns the slit widths of the monochromators. For UV/VIS absorption spectroscopy it is desirable to use as narrow a slit as possible (e.g. 1 nm), for optimum spectral resolution. In fluorescence spectroscopy slit widths are generally larger (e.g. 2 to 5 nm for the excitation light, 5 to 20 nm for the emitted fluorescence), so that sufficiently much light can be collected by the detector. The somewhat lower spectral resolution is tolerable as fluorescence spectra of dissolved molecules in the liquid phase are generally spectrally broad and only weakly structured.

Note that fluorescence spectroscopy can be performed in two modes:

a.) For a fixed excitation wavelength an emission spectrum is measured.
b.) For a fixed emission wavelength an excitation spectrum is measured.

It is also possible to scan both excitation and emission wavelengths, resulting in an array of spectra known as two-dimensional spectra.

4. The experiment

The goal of the experiment is to determine the energy transfer efficiency of a matching pair of donor and acceptor dyes (the donor (D) Cy3 and the acceptor (A) Cy5) after hybridized of dye labelled single DNA strands. The donor strand sequence is

\[
\text{Cy3} \\
\text{CCC AAA CTA AAC TTA ACT AAA CTA AAC CCC}
\]

and the acceptor strand sequence is

\[
\text{GGG TTT GAT TTG AAT TGA TTT GAT TTG GGG} \\
\text{Cy5}
\]

* The physical slit width of the monochromator is much larger than 1 nm, of course. The nm value refers to the achieved spectral resolution and not the physical slit size.
Note that here for reasons of clarity the second strand is not written in the usual notation from the 5´ end to the 3´ end.

1) Your assistant will issue stock solutions (ca. 10μM) of both labelled and unlabelled single strand DNA solutions. From these, first all possible combinations of DNA double strands need to be hybridised: a) donor/acceptor (Cy3/Cy5), b) donor/naked (Cy3/N), c) acceptor/naked (Cy5/N), d) naked/naked (N/N).

2) Register absorption spectra of all double strands using a UV/VIS absorption spectrometer. This will allow you a) to exactly determine the concentration of the solutions and b) to choose the best excitation wavelength for the fluorescence measurements in 3). You will also need to monitor two reference spectra of the cuvette, one of the cuvette filled with buffer solution only, and another one for the empty (air-filled) cuvette. Thus, altogether you will register six absorption spectra.

3) Register two fluorescence spectra for each of the dye containing double strand solutions (Cy3/Cy5, Cy3/N, and Cy5/N), one for the absorption maximum \( \lambda_A^A \) of the acceptor and one for the absorption maximum \( \lambda_D^D \) of the donor. This will result in a set of also six fluorescence spectra.

4) Determine the Förster radius experimentally and theoretically and compare the values to each other. From the measured absorption and fluorescence spectra of donor and acceptor dyes you can calculate the overlap integral of eq. (6) which will in turn let you determine a theoretical value for the Förster radius from the optical properties of the individual dyes. Experimentally, the Förster radius can be obtained from the measured energy transfer efficiency and the known geometry of the hybridized double strands.

With respect to 1)  The sample preparation

Sample preparation requires the use of micropipettes (Eppendorf pipettes). Consult your lab assistant if you have never worked with a micropipette before. Mix 30 μL of each single strand solution in a micro test tube that can be used for the Peqlab Primus 25 thermostyler. Place the four mixtures into the thermostyler. Switch the thermostyler on, select and start the program "Hybridisierung Cyc." by pressing the RUN button. First, the samples will be kept at a temperature of 25 °C for 30 s, then they will be heated to 95 °C for 2 minutes, next they will slowly be cooled to 25 °C at a rate of 0.2 °C/s, and last they will be kept at 25 °C for another 8 minutes. After passing this procedure which will take about 20 minutes the samples will be hybridized.

With respect to 2)  Registration of absorption spectra

The optical cuvette has an optical pathlength of 3 mm. Prior to each use it needs to be thoroughly cleaned. Remove any remaining liquid from the cuvette using the micropipette set to a volume of ca. 80 μL and return it to the micro test tube. Then fill the cuvette with 100 μL double-distilled water...
(ddH2O). Clean the cuvette by repeated (three times) aspirating and dispensing of the pipette tip. Pour the water out and remove the remaining water from the bottom of the cuvette with the micropipette. Repeat this procedure three times with ddH2O and another three times with ethanol. After drying the cuvette with nitrogen it is ready to be refilled with the next sample. Take care when changing pipette tips in order not to contaminate or dilute the samples. Do not touch the optical surface of the cuvette with your fingers, and make sure you leave a small air bubble in the cuvette when applying the stopper.

Try to avoid too many cleaning steps of the cuvette by taking absorption and fluorescence spectra of the same sample. Only the first dye containing sample you will have to prepare twice as you do not yet know the absorption maximum of the second dye that you need to know for the fluorescence measurements as the second excitation wavelength.

Register the six absorption spectra as described in appendix 1 in the wavelength range from 200 nm to 750 nm. Do not use the automatic background correction, as manual background correction (and its discussion) will be part of the data analysis. Save the data on a memory stick or the like.

With respect to 3) Registration of fluorescence spectra

Determine the wavelengths $\lambda^A_A$ and $\lambda^D_A$ of the acceptor and donor absorption maxima from the double strands consisting of one naked and one dye labelled strands each. Use $\lambda^A_A$ and $\lambda^D_A$ as excitation wavelengths for the registration of fluorescence emission spectra for all three dye containing double strand solutions. The procedure for registration of fluorescence spectra is described in the appendix 2.

5. Data analysis and discussion

1) Display the absorption spectra graphically. Discuss the transmission behaviour of the empty cuvette, of the buffer solution, of the naked DNA, and of the dyes. Which spectrum is the best reference spectrum for background correction? Perform the background correction for the absorption spectra of the dye containing double strands and display the background corrected spectra in the relevant wavelength range.

Determine the concentrations $c$ of your samples. Use the Lambert-Beer law

$$A = -\log\left(\frac{l_i}{l_0}\right) = \varepsilon c s$$  \hspace{1cm} (10)

with the decadic molar absorption coefficients $\varepsilon_D = 150000$ M$^{-1}$cm$^{-1}$ for Cy3 (at 550 nm) and $\varepsilon_A = 250000$ M$^{-1}$cm$^{-1}$ for Cy5 (at 650 nm).
Here, $A$ is the absorbance, $\epsilon$ is the decadic molar absorption coefficient, $s$ is the absorption path length, $c$ is the concentration, $I_t$ is the intensity of the transmitted radiation, and $I_0$ is the intensity of the incoming radiation. Estimate the relative and absolute concentration of 1:1 (Cy3/Cy5) complexes.

2) Display the fluorescence spectra graphically. Determine the energy transfer efficiency from eq. (9). In eq. (9), $F_{DA}$ and $F_D$ are integrated fluorescence intensities of the donor-naked hybrid and the donor-acceptor hybrid. Comparing the amplitudes of the fluorescence spectra at their respective maxima yields an approximate value for the ratio $F_{DA}/F_D$, but is not exact enough since the two fluorescence spectra overlap. Suggest a better procedure for determining this ratio.

3) Evaluate the spectral overlap integral $J$. In the integral, the fluorescence spectrum of the donor needs to be normalized with respect to its area (such that $\int I(\lambda) d\lambda = 1$) and the absorption spectrum of the acceptor has to be described by the decadic molar absorption coefficient $\epsilon_A$. Note that the dimension of the integrand is an inverse (wave)length. Owing to the discrete (non-continuous) character of your experimental data, the numeric normalization is in reality a summation over all measured points multiplied by the interval size. Therefore, the interval size needs to be taken into account for determining $I(\lambda)$.

Calculate the products $I(\lambda)\epsilon_A(\lambda) \lambda^4$ and integrate (sum) them over the wavelength. This sum represents the overlap integral $J$. The unit of $J$ is a length to the power of 6 per amount of substance, e.g. cm$^6$/mol, L/(mol·cm)$^2$·nm$^4$, L/mol·cm$^3$, or the like. Use the overlap integral to calculate the Förster radius $r_0$ (by comparing eq. (6) to eq. (7)).

How much smaller would $J$ be if the donor fluorescence spectrum and the acceptor absorption spectrum were shifted further apart by 50 nm? What would be the effect on the Förster radius and the energy transfer efficiency?

4) Estimate the distance $r_{geo}$ between the two dyes of the donor acceptor pair from the geometry of the Cy3/Cy5 double strand. Use a value of 0.34 nm as the mean distance between two neighbouring base pairs. Then use the energy transfer efficiency $E$ determined in 2) and the Förster radius $r_0$ determined in 3) to obtain an experimental value for the distance $r_{exp}$ between the two dyes. Discuss discrepancies.

Instead of using the value of 0.34 nm per base pair one can also measure the distance for a known DNA structure using a visualization program such as VMD (cf. appendix 3). Choose a suitable structure from the PDB protein data base and print a view where the start and end points of your distance measurements have been marked.
5) The DNA strands used could easily be labelled with Cy3 and Cy5 at other base pairs than the ones you have been working with. List expected energy transfer efficiencies for at least five other distances.

References


Appendix 1

Operation instructions: Absorption spectrometer Perkin-Elmer Lambda 25 UV-Vis

Switch on spectrometer

Switch on PC (consult lab assistant for login and password information)

Start program PerkinElmer UV WinLab

Create own data folder (Auto Save)

Select measurement parameters: scan speed: 120 nm/min wavelength range: 200 nm - 750 nm

Perform automatic background measurement with empty spectrometer (NO cuvette inside, not even an empty one!)

After the background measurement insert the cuvette into the spectrometer.
Appendix 2

Operation instructions: Fluorescence spectrometer Cary Eclipse

Switch on spectrometer

Start program Cary Eclipse, call subroutine Scan

Choose "Setup" in the setup menu: You will see a set of 5 dialogue windows for input. You need to input data in 2 of them: "Cary" and "AutoStore"

- "Cary"

DataMode Fluorescence - ScanSetup; Choose Emission

Excitation: Choose appropriate wavelength (consult lab assistant before)

Start / Stop: Choose 500 nm / 800 nm

Excitation slit / Emission slit: Choose values such that maximum fluorescence intensities lie between 100 and 1000. To find suitable values you need to try out several settings. If the slit values are chosen too large then intensities will be outside the allowed range, and too small values will deteriorate the signal to noise ratio more than tolerable. Important: For a quantitative data analysis all measurements must be made with the same slit settings! Otherwise a determination of the energy transfer efficiency will not be feasible.

ScanControl: Choose Medium

- "AutoStore"

Storage: Choose prompt at start

AutoConvert: Choose ASC II (csv) for ASCII /text) format of the data

Close window with OK button

Insert probe

press Start to begin measurement
Appendix 3

Visualization of biomolecules using the program VMD

Download and install the program VMD from http://www.ks.uiuc.edu/Research/vmd/ Apart from having to register the program is free. The current version is 1.9.1.

The protein data bank PDB is hosted at http://www.rcsb.org/pdb/. It contains crystal structures of a large number of molecules. Enter the name of the compound you want to retrieve into the input line in the header and press "Enter". The search term "serum albumin" yields currently ca. 80 hits. Select the molecules you are interested in and download the PDB file.

In order to view the downloaded file you need to run VMD and select the PDB file (File, New Molecule, Browse, select PDB file, Load). The molecule will appear in the viewing screen. Open the windows "Graphical Representations" and "Color Controls" from the "Graphics" selection in the main menu of the VMD main window. In the "Graphical Representations" window you can view the bases (keyword: resname) and the atoms (keyword: elements) which are contained in the molecule. They will be listed in the "value" field.

By clicking "Create Rep" you create a copy of the selected molecule which is then displayed as a new entry in the "Graphical Representations" window. Molecules can be toggled on and off on and off by double clicking (the entry will be displayed in red when toggled off, in black when on). The displayed elements in the graphics window can be changed for a selected entry, e.g. typing "resname ALA" in "Selected Atoms" and pressing "enter" will let all bases disappear except ALA. In the "Draw Style" window the style and the colors of the graphical representation can be adjusted.

In the "Color Controls" window one can adjust the colour of the selected bases, atoms etc. E.g. the settings Categories: resname, Names:ALA, Colors: red lets all ALA bases be displayed in red (provided in the Graphics Representation "resname" has been selected under "Coloring Method". In the same way the colours of selected atoms can be changed. All other elements of the graphical representations can also be changed, i.e. background, axes etc.

The displayed molecule can be zoomed and rotated using the mouse controls. The displayed representation can be saved in the "VMD Main" window (File, Render, choose options, Start Rendering).