Photocycle Kinetics

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Application Note

Introduction

Many important biological processes are light-driven. The example discussed here is bacteriorhodopsin. This protein couples the absorption of a light quantum to proton-pumping steps that in turn generate an electrochemical potential over the bacteria membrane:

$$bR \xrightarrow{h\nu} K \leftrightarrow L \leftrightarrow M \leftrightarrow N \leftrightarrow O \rightarrow bR$$

Upon illumination bacteriorhodopsin (*bR*) undergoes mechanical transient deformations leading to transient states (*K, L, M, N, O*). While sampling through its states the protein transfers a proton. When finally the initial state bR is again reached, a proton transfer is finished and the protein is ready for the next cycle. The duration of a complete cycle depends on the particular protein and typically between some **ms** and some s.

Experimental Setup

A favorite way to study such processes is to collect the absorption changes accompanying a cycle:

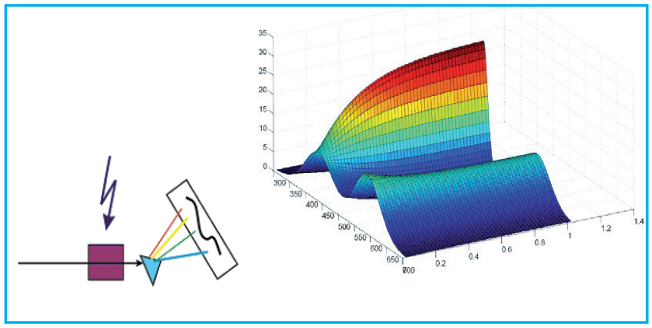

Fig.1, left: experimental setup for collecting transient spectra: The protein solution within the cuvette is illuminated by a short laser flash that starts the photo cycle. While sampling through its states the probe is monitored spectrographically.

The right figure shows a collection of spectra, measured in such a process.

Problems with those measurements are:

- When measuring fast photosystems, the exposure time of the photodetectors is restricted to the sub-µs time domain. Furthermore, it is often imperative to measure light sensitive proteins with weak light. Both demands require high amplification and short exposure times leading in turn to low signal to noise ratios.
- Sometimes the recovery time for a photo system may be very slow. In that case it is not practicable to improve the signal quality by repeated measurements.

In practice two methods for collecting the spectra are established:

The older one combines monochromatic probe light with a photomultiplier. In this case time-dependent spectral data are obtained by choosing a particular wavelength and by measuring at the chosen wavelength the time response of the photo system. This is repeated for all interesting wavelength so that the number of laser flashes required to get the data is determined by the number of discrete wavelengths.

As a second method we use an iStar DH734-18F-A3 ICCD detector (Intensified Charge Coupled Device) attached to a Shamrock SR-303i spectrograph (both from Andor Technology) allowing to measure a complete spectra per laser flash. With this setup the number of repeats is given by the desired number of time-discrete spectra. Both methods have their pros and cons: Under low light conditions the multiplier method give better signal to noise ratios. Furthermore the use of monochromatic measuring light is a gentle way to get the data from sensitive sample. On the other hand it is much easier and much faster to monitor a complete spectrum per excitation flash.

The purpose of analyzing the photo cycle data (those as given in the right fig.1) is to figure out the kinetics of the underlying photo cycle. In many cases such a kinetic can be described mathematically by a series of exponentials. It is clear that parameter estimation in this case is extremely sensitive to noise. Therefore the quality of the data required to solve kinetic problems is the most important criterion for choosing the method of choice. In general we can make the following recommendations:

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- If the biological probe has a fast recovery rate (bacteriorhodopsin has such a short ms-recovery), than it is advantageous to use a system that can monitor complete spectra. In that case a high data quality can be obtained by repeating the measurements.

- If the sample recovers very slowly (e.g. within 30s), then it is also an advantage to use a system monitoring complete spectra. In that case a single excitation flash gives a complete time course and only a few flashes are required to get a good quality of data.

- If the sample is fast and sensitive to the measuring light, then the measurement should be done with monochromatic light and with a multiplier. Although this technique requires more laser flashes per data set, it is gentle because it is often the interaction between probe light and the probe that disturbs the measurement. In these cases the usage of monochromatic detection light clearly is an advantage.

Data Analysis

To mathematically analyze the data the method of global analysis is used [1]. This method represents the measured data set $D(\lambda, t)$ as sum of the products between the intermediate concentrations $c_i(t)$ and their spectra $\varepsilon_i(\lambda)$:

$$D(\lambda, t) = \sum_{i=1}^{n} \epsilon_i(\lambda) * c_i(t) = \mathrm{E}(\lambda)C(t)$$

Under the assumption that the transitions between the intermediates (i) follow first order differential equations the matrix of the concentrations (C(t)) can be written as matrix exponential:

$$C(t) = \begin{bmatrix} c_1(t) \\ \dots \\ c_n(t) \end{bmatrix} = \begin{bmatrix} c_{01} \exp(-k_1 t) \\ \dots \\ c_{0n} \exp(-k_n t) \end{bmatrix}$$

where $E(\lambda)=[\varepsilon_1, \varepsilon_2, \dots, \varepsilon_n]^T$ is the transposed matrix of the intermediate spectra. The global analysis estimates (i) the number of transient components of a cycle; (ii) the kinetics of the data and the model underlying the kinetics and (iii) the spectra of the intermediates. It is well known that global analysis requires high quality data. In this respect the usage of the ICCD spectrometer is a valuable completion of classical measuring techniques.

Reference

[1] Solving complex photocycle kinetics. Theory and direct method

J.F. Nagle; Biophysical Journal, 59, (1991), 478-487

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