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Review

Low temperature spectroscopy of proteins. Part II: Experiments with single protein complexes ☆

Yuri Berlin [a,1], Alexander Burin [b], Josef Friedrich [c], Jürgen Köhler [d,*]

[a] *Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113, USA*
[b] *Department of Chemistry, Tulane University, New Orleans, LA 70118, USA*
[c] *TUM Physik-Department E14, Lehrstuhl für Physik Weihenstephan, An der Saatzucht 5, 85350 Freising, Germany*
[d] *Universität Bayreuth, Lehrstuhl für Experimentalphysik IV und BIMF, Universitätstr. 30, 95447 Bayreuth, Germany*

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Abstract

In this part of the review we describe aspects of the physics of proteins at low temperature as they are reflected in the spectra of individual pigment–protein complexes. The focus of this review is on the spectral diffusion of chromophores that are naturally embedded in light-harvesting complexes from purple bacteria. From the spectral diffusion behaviour we can deduce details about the organisation of the energy landscape of the protein and discuss the implications for the motions of the protein in conformational phase space.

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Keywords: Protein spectroscopy; Single-molecule spectroscopy; LH2; Spectral diffusion; Energy landscape; Excitons in biomolecules; Conformational substates

Contents

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* Corresponding author.
E-mail addresses: berlin@chem.northwestern.edu (Y. Berlin), aburin@tulane.edu (A. Burin), j.friedrich@lrz.tu-muenchen.de (J. Friedrich), juergen.koehler@uni-bayreuth.de (J. Köhler).
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In part I of this review we focused on high resolution hole burning spectroscopy of protein ensembles at low temperature and the associated physics. In the following part (part II), the focus is on the spectroscopy of single light harvesting complexes, especially their dynamics at temperatures in the Kelvin-range as it is reflected in spectral diffusion.

6. Basic aspects of spectral diffusion experiments with single molecules

As pointed out in part I above the exact energies of the electronically excited states of a chromophore are fine tuned by the interaction with its local surrounding. Accordingly, the spectral lineshape observed for an ensemble reflects the statistical distribution of local environments rather than dynamical properties of the chromophore (Section 2.3 of part I). However, if the local environment of the chromophore is not static but shows temporal fluctuations as well, the absorption frequency of a single-molecule undergoes also temporal fluctuations. In hole burning the ensemble average of these fluctuations show up as waiting time dependent line broadening phenomena. In experiments with single molecules they can be directly observed as a spectral diffusion trajectory in the time domain. Indeed, spectral diffusion of a single molecule was one of the first effects observed by the Moerner group in the pioneering days of this technique [1].

In order to take advantage of a single molecule as a local probe to monitor fluctuations of its truly local environment—which is of particular interest if the "host" is a protein—we have to consider the mutual relationship between the timescales of the experiment and spectral fluctuations. In Fig. 1 we show a schematic plot of a two-dimensional representation of sequentially "recorded" spectra stacked on top of each other. In each "scan" the molecule is supposed to absorb at a distinct wavelength that is given by a black dot and spectral fluctuations over time show up as "spectral trails" in the wavelength versus time pattern. Let us assume that our artificial molecule undergoes

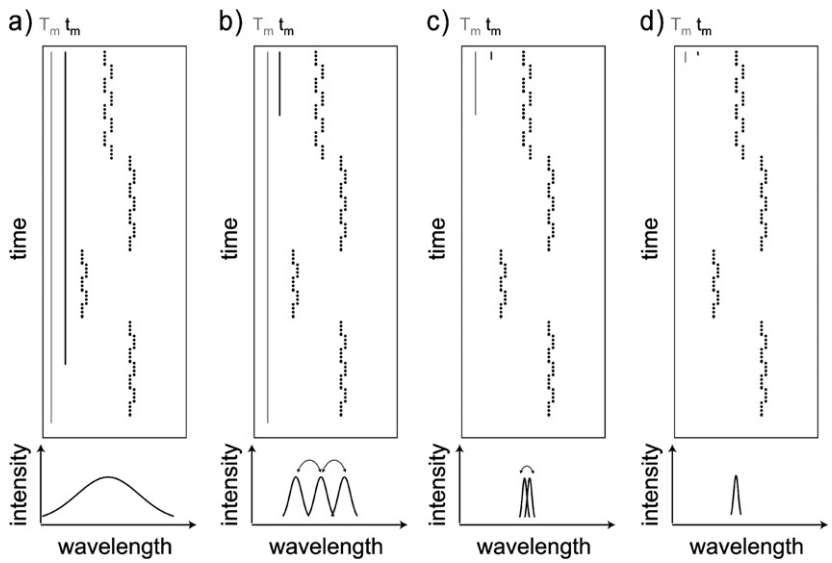

Fig. 1. Sketch of the spectral diffusion of a molecule that features two timescales $t_{\mathrm{fluc}} \ll T_{\mathrm{fluc}}$, together with the spectra that would have been obtained in a hypothetical experiment (a)–(d). In the upper part the same sequence of consecutively recorded spectra is stacked on top of each other. The spectral position of the molecular absorption is indicated by the black dot. The experimental timescales, t_m (temporal resolution), and T_m (duration of the experiment), are indicated by the solid lines in the panels. For details see text.