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Fluorescence correlation spectroscopy on electron transfer reactions : probing inter- and intramolecular redox processes

Sen, S.

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Author: Sen, S.

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Stellingen

Behorend bij het proefschrift

“Fluorescence Correlation Spectroscopy on Electron Transfer Reactions Probing Inter-and Intramolecular Redox Processes”

- I. While studying a fluorescently labeled protein under redox conditions using fluorescence correlation spectroscopy, blinking of the fluorescent dye must be considered in analyzing the autocorrelation data.

Chapter 3 of this thesis
- II. For labeled redox enzymes, the photo-induced electron transfer between dye and redox-active centres should be taken into account together with fluorescence-resonance energy-transfer for quantitative analysis of autocorrelation data.

Chapter 4 of this thesis
- III. To distinguish between the intramolecular and intermolecular electron-transfer reactions in labeled oxido-reductases using fluorescence correlation spectroscopy, the viscosity of the solution and the concentration of the reductant/oxidant must be varied.

Chapter 4 of this thesis
- IV. In fluorescently labeled oxido-reductases, photo-induced intramolecular electron transfer between the label and the active center is not restricted to situations where the label is in Van-der-Waals contact with the redox center.

Chapter 4 and Chapter 5 of this thesis
- V. Morishima and coworkers documented the occurrence of electron-transfer reactions in a single dye-labeled protein molecule. Besides intramolecular electron-transfer between the

dye and the redox center, intermolecular electron-transfer processes should have been considered as well.

Isao Morishima et al., *J. Am. Chem. Soc.*, **127** 2098 (2005).

- VI. Tinnefeld and coworkers introduced a reducing and oxidizing system to minimize photobleaching and blinking of a dye (ATTO647N). This method is not relevant for ATTO655 due to its different chemical structure and photophysical properties.

Philip Tinnefeld et al., *Angew. Chem. Int. Ed.*, **47** 5465 (2008).

- VII. In contrast to the conclusions drawn by Cordes *et al.* and Grimm *et al.*, the ideal fluorescent probe still does not exist.

Thorben Cordes et al., *Nat. Methods*, **9** 426 (2012); Jonathan B Grimm et al., *Nat. Methods*, **12** 244 (2015).

- VIII. The standard calibration technique proposed by Rüttinger *et al.* for the determination of effective volume by fluorescence correlation spectroscopy is not applicable in viscous solvent.

Steffen Rüttinger et al., *J. Microscopy*, **232** 343 (2008).

- IX. Criticism may not be agreeable, but it is necessary to aid and discover new talent.

- X. To facilitate free and open exchange of new ideas for the growth of our society, the connection between academic institutions/research centers and private companies/pharmaceuticals is indispensable.

Saptaswa Sen

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