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## **Towards a single-molecule FRET study of Frauenfelder's nonexponential rebinding of CO in myoglobin**

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# Stellingen

behorende bij het proefschrift

## **'Towards a single-molecule FRET study of Frauenfelder's nonexponential rebinding of CO in myoglobin'**

1. Although very small, the difference between the absorption spectra of MbCO and deoxy-Mb in the far-red area causes a substantial difference in FRET efficiency for a far-red dye donor.

*Chapters 3 and 4 of this thesis.*

2. Contrary to what most (if not all) labeling protocols suggest, i.e., to use gel filtration columns for the purification of dye-labeled proteins, the best way is the use of simple membrane centrifuges.

*Chapter 3 of this thesis.*

3. Although two donor dyes may have the same estimated FRET efficiency, after attachment to an acceptor protein, they may be quenched completely differently.

*Chapters 3 and 4 of this thesis.*

4. There is strong heterogeneity of decay rates in ensembles of donor-acceptor systems, even when they present apparently weak deviations from single-exponentiality.

*Chapter 5 of this thesis.*

5. Single-molecule observables, such as rotation, diffusion and fluorescence lifetime, can provide details on dynamic heterogeneity. However, it is still an important challenge to discriminate spatial and temporal heterogeneity, and detailing the molecular length scales and timescales of heterogeneity.

*Laura J. Kaufman, Annu. Rev. Phys. Chem. 64, p.177 (2013).*

6. Although single-molecule spectroscopy provides much information about the heterogeneous composition of an ensemble, it is sometimes preferable to use ensemble techniques.

*Zondervan et al. J. Phys. Chem. A 107, p.6770 (2003) en J. Phys. Chem. A 108, p. 1657 (2004).*

7. To fully understand the complex dynamics of biological molecules, it may be indispensable to study individual molecules both at room temperature and at low (cryogenic) temperature.

*Kulzer and Orrit, Annu. Rev. Phys. Chem. 55, p.585 (2004).*

8. Contrary to what is often thought, CO is not always toxic for the body.

*Kim, H. P et al. Annu. Rev. Pharmacol. Toxicol. 46, p.411 (2006).*

9. Artificial-meat consumption can reduce greenhouse gas emissions.

*Tuomisto, H. L. et al. Environ. Sci. Technol. 45, p.6117 (2011).*

10. Humans should get inspiration from Nature by mimicking it, not only for technological applications, but also for efficient recycling of resources.