



Time Resolved Single Molecule Fluorescence Spectroscopy on Surface Tethered and Freely Diffusing Proteins

Diaa Atta

Forschungszentrum Jülich GmbH
Institute of Complex Systems (ICS)
Molekulare Biophysik (ICS-5)

Time Resolved Single Molecule Fluorescence Spectroscopy on Surface Tethered and Freely Diffusing Proteins

Diaa Atta

Schriften des Forschungszentrums Jülich
Reihe Schlüsseltechnologien / Key Technologies

Band / Volume 35

ISSN 1866-1807

ISBN 978-3-89336-763-4

Abbreviations and symbols	iii
1 Introduction	1
2 Methods and materials	7
2-1 Fluorescence.....	7
2-1.1 Basic concepts.....	8
2-2 High resolution fluorescence microscopy.....	15
2-2.1 Wide-field microscopy.....	18
2-2.2 Confocal microscopy.....	21
2-3 Fluorescence correlation spectroscopy (FCS).....	24
2-3.1 Theoretical concepts	25
2-4 Photoinduced electron transfer (PET).....	31
2-5 Anisotropy.....	34
2-5.1 How to measure the anisotropy.....	35
2-5.2 Effects of rotational diffusion on the anisotropy.....	37
2-5.3 Time dependent anisotropy	38
2-6 Glass slide preparation for FCS and imaging.....	40
2-6.1 Preparing the glass slide for imaging.....	41
2-6.2 Protocol for slide preparation and treatment.....	41
2-7 Proteins and labeling.....	42
3 Results and discussion	54
3-1 Setting up the microscopes for single molecule measurements.....	54
3-1.1 Selection of filters and dichroic mirrors for single and dual color imaging.....	54
3-1.2 Wide-field microscope.....	56
3-1.3 Confocal microscope.....	63
3-2 Monitoring protein synthesis and protein folding.....	70
3-2.1 Fast biosynthesis of GFP molecules in a cell free expression system.....	72
3-2.2 Attempts to establish a FRET pair to monitor co-translational folding...	80
3-3 Observing proteins as single molecules encapsulated in surface-tethered polymeric nanocontainer.....	83
3-3.1 Protein encapsulation inside polymerosomes.....	85

3-3.2	Characterizing folded and unfolded states of encapsulated proteins.....	86
3-3.3	Application of photoinduced electron transfer (PET) to monitor the unfolding/refolding of PGK	90
3-4	Native and unfolded states of multidomain proteins studied by FCS.....	95
3-4.1	Determination of hydrodynamic radii for GdnHCl induced unfolded states.....	100
3-4.2	Analyzing structural dynamics and compactness by employing PET.....	103
4	Conclusion and outlook	116
5	Summary	122

Schlüsseltechnologien / Key Technologies
Band / Volume 35
ISBN 978-3-89336-763-4

