Preface

Time-resolved optical spectroscopy has been a rapidly developing technique that is widely applied in the field of Biophysical Chemistry. Time and frequency domain methods have resulted in state-of-the-art instrumentation and modern approaches of data analysis. It is the purpose of this issue to show the reader that sophisticated optical spectroscopic methods have led to very detailed insights in functionally relevant aspects of proteins, nucleic acids, membranes and living cells. The dynamics of these systems indeed cover a time span between femtoseconds and seconds and can be monitored by the use of optical methods described in this issue. The papers are more or less chronologically arranged from ultrashort to longer times with a rather high density in the nanosecond region. The content of this issue is merely a snapshot of our current information on biochemical and biophysical phenomena available from optical spectroscopy. Let me briefly address the topics covered by this issue. The results of femtosecond fluorescence upconversion spectroscopy of bacteriorhodopsin contribute to a deeper understanding of the primary events after photoexcitation and they assist in unraveling the complex excited state dynamics. The measurement and analysis of Raman vibrational bandwidths of polyriboadenylic acid provide an observable to investigate subpicosecond macromolecular and solvent dynamics of nucleic acids. The process of protein unfolding and denaturation pathways can be extremely well followed using time-resolved fluorescence detection of energy transfer (nuclease mutants) or by using a combination of time-resolved fluorescence and anisotropy (molten globules in human superoxide dismutase). A more complex system is formed by the intrinsic membrane protein Na,K-ATPase in which nanosecond dipolar relaxation of the immediate (multi)tryptophan surroundings could be determined from fluorescence spectroscopy at a range of temperatures. The natural fluorophores bound to proteins and enzymes of the bacterial bioluminescent system have excellent long fluorescent lifetime properties to investigate protein–protein complexes using time-resolved fluorescence anisotropy. These findings were related to the excitation mechanism of bioluminescence. The most widely used intrinsic fluorescent probe in proteins is tryptophan. However, this amino acid possesses complex photophysics and is not selective in multitryptophan-containing proteins. The introduction of tryptophan analogs with a more red-shifted fluorescence and its biosynthetic incorporation in proteins can be considered as potential intrinsic probes for protein structure and dynamics. Specificity of the interaction of proteins with membrane-mimetic interfaces (protein kinase C with mixed micelles) could be monitored by time-resolved detection of resonance energy transfer from tryptophan residues in the protein to specific pyrene-labeled phospholipids in mixed micelles. This issue also contains studies of membrane bilayers with membrane embedded fluorescent probes. One study describes the fluorescence quenching of perylene in small unilamellar vesicles by several ions like Co^{2+}, Ni^{2+} and Cr^{3+} added to the aqueous phase. Not only classical Förster-type energy transfer was found but also additional quenching mechanisms had to be invoked. It has been demonstrated further in this issue that the external refractive index (of the solvent) influences the natural fluorescence lifetime of the frequently used fluorescent membrane probe diphenylhexatriene in phospholipid bilayers. The observed change of fluorescence decay with refractive index (by adding viscogens like glycerol) followed the theoretical prediction and, in addition, enabled the determination of the second rank orientational order parameter of diphenylhexatriene in membranes. A frequency domain fluorescence lifetime imaging microscope has been extensively detailed. The versatility of this microscope has been proven by several applications like fluorescence lifetime measurements in picoliter solution, lifetime imaging of single cells and image enhancement of cells by phase suppression of certain lifetime components. We

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then approach relaxation measurements on slower time scale. A fluorescence depletion method is able to measure slow protein rotational diffusion times in the microsecond time domain. Procedures are given to carry out these measurements in cuvet and microscope geometries permitting evaluation of both absorption and emission anisotropies and the interdipole angle. A comprehensive survey of laser flash induced electron transfer methods (mediated by flavin in the triplet state) within or between redox proteins has been given. Both millisecond kinetics and mechanisms of electron transfer have been elucidated for various redox protein couples like cytochrome c/cytochrome peroxidase or plastocyanin, ferredoxin/ferredoxin NADP + reductase, etc. Room temperature time-resolved tryptophan phosphorescence of seconds duration turned out to be a very selective method to follow the denaturation of alkaline phosphatase after addition of denaturants. The patterns of enzymatic activity and phosphorescence intensity followed on the minutes time scale show characteristics which depend on the nature of denaturant added. Finally the application of time-resolved optical spectroscopy has also spurred the development of advanced physical models which satisfactorily describe the experimental data. Two examples are given. The first example describes fractal analysis of time-resolved fluorescence in lipid aggregates which yields a time-dependent reaction rate. The second approach is an extension of a previous study on intramolecular excited state reactions using global compartmental analysis of a fluorescence decay surface in the sense that now specific bounds on rate constants can be set.

It is almost needless to state that the methods are being developed further to enhance our knowledge of biosystems of large diversity and increasing complexity.

I wish to thank the contributors and many anonymous referees who made the appearance of this issue possible within rigid time limits.

Finally, it is necessary to make a small confession. Almost two years ago Professor Michel Mandel kindly requested me to undertake this endeavour of composing a special issue. In may innocence I agreed to give it a try. The result of this concerted effort happens to coincide with Professor Mandel's retirement as Principal Editor of Biophysical Chemistry. I would like to dedicate this issue to him.

Antonie Visser

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