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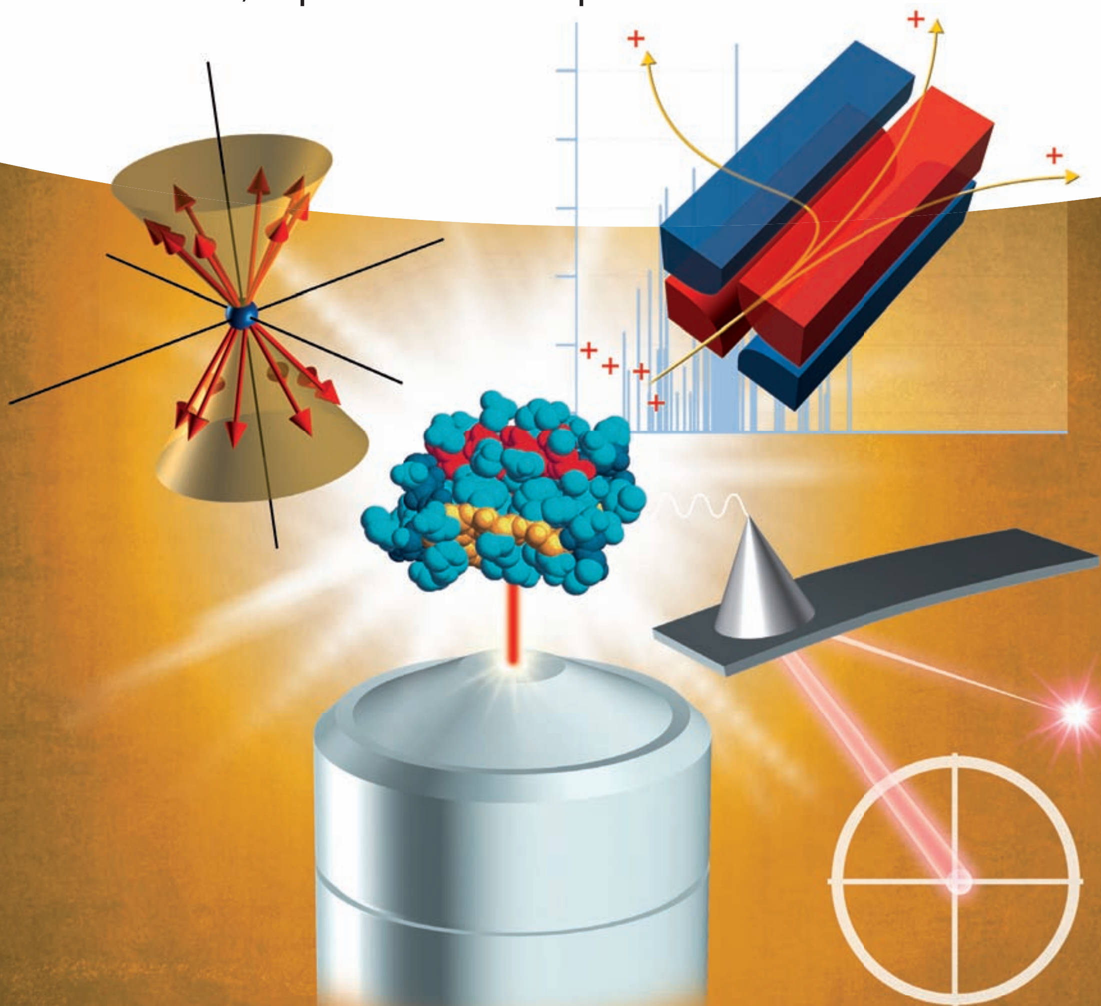
Modern Biophysical Chemistry

Detection and Analysis of Biomolecules

Second, Updated and Expanded Edition



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Second, Updated and Expanded Edition

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*To my wonderful wife Uli and my great kids Christian,
Maike, Paul and Johanna*

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Foreword to the Second Edition

It has now been five years since the first edition of this book entitled "Modern Biophysical Chemistry" appeared and of course it was high time that this book was updated with the latest exciting developments that are now established enough to be included in a text book. As already mentioned in the first edition, the field of biophysical chemistry is huge, covering aspects of chemistry, biology, physics and even medicine and so it is not easy to assess which aspects are really the most important ones that must be included. As for the first edition, I have tried to make a selection of methods and application examples that contain general concepts that also cover the basis for most of the techniques and applications that are not considered explicitly in the book. Since this selection can be done in many different ways I ask those who are disappointed that their method or application is not explicitly included here to excuse me. The goal was a book that allows a comparatively quick insight to be gained into the very large range of possibilities provided by modern biophysical chemistry, while still being detailed enough to use this knowledge for the first steps in actually applying it for research.

Amongst the important developments of recent years are certainly significant improvements achieved in DNA sequencing by next-generation methods as well as super-resolution microscopy that goes beyond the resolution of conventional, diffraction-limited microscopy. Therefore, these two developments are now represented by two, entirely new chapters in the book. Also, other new developments have been included, such as light-sheet microscopy, introduced now in Chapter 7, and DNA origami techniques in Chapter 12. In addition, new problems have been added. To further improve the clarity many figures have been coloured and, in general, we have made major efforts to optimize the clarity and conciseness throughout the entire book. To help readers focus on the important equations in all mathematical subjects they are now marked by black boxes throughout the entire book.

I would like to thank again all persons who helped me to improve the first edition and of course also those who helped me with the second edition with their very useful and often essential comments.

Special thanks go to Dr Stefan Bode, Dr Anna Cypionka, Professor Dr Christian Eggeling, Professor Dr Jörg Enderlein, Dr Jan Frähmcke, Professor Dr Karl-Heinz

Gericke, Matthias Grunwald, Dr Ulrich Haupts, Dr Hendrik Hippchen, Professor Dr Henrike Heise, Christoph-Peter Holleboom, Chao-Chen Lin, Dr Martin Michels, Professor Dr Filipp Oesterhelt, Dr Wiebke Pohl, Professor Dr Christoph Schmidt, Sabrina Schröder, Professor Dr Jakob Sørensen, Professor Dr Dirk Schwarzer, Dr Michael Teufel, Dr Andreas Volkmer and finally Laura van den Heuvel. In addition, I especially thank Silke Lubahn for generating most of the figures in this book and Julia Lüttich for her extremely valuable support throughout the entire time this second edition was being prepared. Gratitude is also expressed to Dr Pen-Nan Liao for his assistance with the solutions to the questions asked in the book. Last, but not least, I would like to thank all persons at Wiley-VCH who helped with the production of this book and especially Lesley Fenske, Dr Peter Capper, Mamta Pujari and Dr Frank Weinreich for their patience regarding my special requests and wishes.

Braunschweig and Göttingen, May 2014

Introduction

What is Biophysical Chemistry? – An Example from Drug Screening

Biophysical chemistry is a fascinating field of research because it combines aspects of chemistry, biology, physics and sometimes even medicine in one discipline. Owing to this diversity it is difficult to give an exact definition of biophysical chemistry. In principle, everything in biology or medicine is based on a chemical or physical foundation. For a physical chemist, one reasonable definition is 'Biophysical chemistry is the application of principles known from physical chemistry to elucidate biomolecular and biochemical questions'. For a biologist a reasonable definition might be 'Biophysical chemistry is the description of the physicochemical properties of biomolecules'.

Actually, it makes a lot more sense to answer the question 'Why do we need biophysical chemistry?'. In recent years more and more questions relevant to biology have been answered using methods originating from the field of physics or physical chemistry. These problems require at least some basic understanding in all three disciplines. However, often a physicist or chemist feels uncomfortable talking about topics that seem to be quite simple for a biologist and vice versa. In many cases it turns out that something that sounded very complicated to one scientist is not difficult at all after he or she realizes that the other scientist is simply using unfamiliar wording. An example is the definition of a 'vector'. Chemists and physicists usually regard a vector as a mathematical object. However, if molecular biologists are talking about vectors they often mean a plasmid vector for transferring genetic material into a cell. The field of biophysical chemistry is a bridge between these disciplines. The following example illustrates a typical problem that can only be solved with a basic knowledge of all these disciplines.

For the development of a drug, in pharmaceutical research in many cases one very important parameter is the affinity of potential drug candidates for a specific receptor or enzyme. The mechanism by which many drugs act is simply based on their ability to selectively block the active site of specific biomolecules.

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For example, the biomolecular targets of many antibiotics are enzymes responsible for the cell-wall synthesis of bacteria. Since it is very hard to find such compounds that also have as few side effects as possible it is useful to look at as many compound structures as possible. Pharmaceutical companies often have a very large pool – up to millions – of already synthesized compound structures. Often, in a first step in the process of industrial drug development, many of these compound structures are tested for their affinity to a specific target using high-throughput screening (HTS). If a compound structure with a high affinity can be found (a 'Hit') it can be used as starting point for further drug development. But how can the affinity of a million compounds be measured with sufficient speed and accuracy? A day lasts 86 400 s. If the accurate measurement of the binding affinity takes only one second per compound, then more than 11 days of constant measurements are required for one million compounds.

One possibility for a fast and robust solution to this problem is to use fluorescence polarization anisotropy (FPA) assays. The mathematical and technical aspects of such assays are described in detail in Chapter 3 but here it serves as a first insight into what the field of biophysical chemistry can mean.

The problem is *biological* or medical – for example, you want to find a molecule that blocks specifically the binding site of a certain receptor that is closely associated with the development or progression of a certain disease. In a fluorescence polarization assay you could first label a small natural ligand of the receptor *chemically* with a fluorescent molecule (Figure I.1). When you subsequently mix the labelled ligand with the receptor they form a stable receptor–ligand complex. Often, this complex has a large molecular mass and is therefore tumbling and rotating very slowly in solution. If this complex is excited with

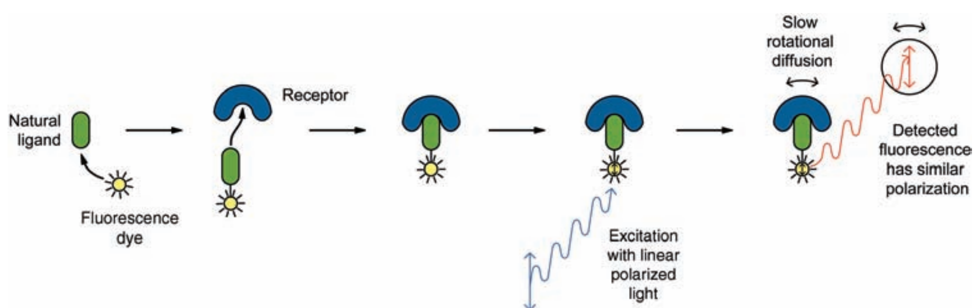

Figure I.1 Schematic representation of a typical fluorescence polarization assay. In a first step a small ligand is labelled with a fluorescent marker and forms a stable receptor–ligand complex with a receptor. Owing to the large mass of the receptor–ligand complex its rotational diffusion in solution is very slow. Consequently, the orientation of the polarization of the emitted photons is very similar to the orientation of the polarization of the laser excitation.

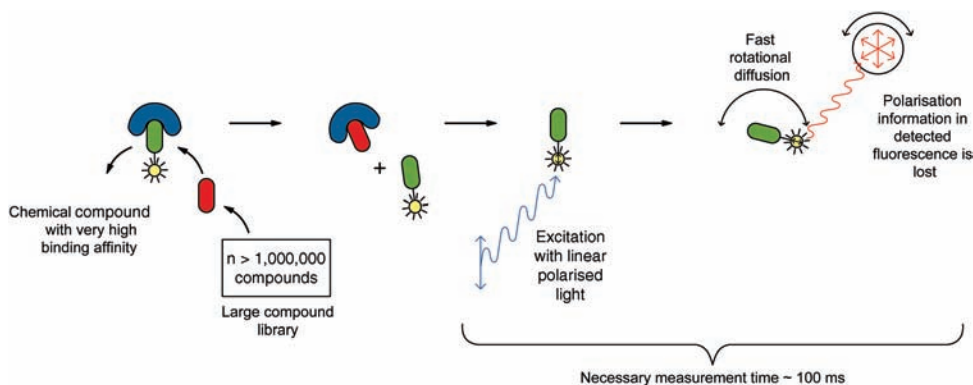

Figure I.2 If a chemical compound out of a large pool of synthetic compounds has a significantly higher affinity for the receptor it replaces the labelled ligand. The free ligand exhibits very fast rotational diffusion. This can be measured very quickly because now the polarization orientation of emitted photons is very different from that of the laser excitation. A hit is found that can be used for further tests or modification to evaluate it as a potential drug structure.

polarized laser light, most of the fluorescing molecules emit light with a very similar polarization. This polarization can be measured for instance with polarization filters (here comes the *physics*!).

In a next step, a chemical compound to be tested is added to the solution. If the compound is capable of binding more strongly to the receptor than the natural labelled ligand then the labelled ligand will be forced out of the binding site and the chemical compound itself blocks the site (Figure I.2). Now, however, the fluorescently labelled natural ligand is diffusing and rotating freely in the solution. Since the fluorescently labelled ligand is small its rotational diffusion is faster than the time it typically takes for the label to emit fluorescence. Therefore, most photons are now emitted with an arbitrary, very different polarization from that of the exciting laser light. By measuring this different polarization the binding constant of each chemical compound can be determined very accurately even within a few hundred milliseconds.

This assay principle is an example of a competitive binding assay measured by fluorescence polarization anisotropy. Since this experiment requires knowledge from all three disciplines – chemistry, biology and physics – it is a good example of what biophysical chemistry can mean. Of course, biophysical chemistry methods are not restricted to industrial applications and are probably even more important in basic research.

The intention of this book is to give an insight into the most important basic, as well as modern, biophysical chemistry methods and their application in both basic and industrial science. The book is divided into two parts. In Part 1 (Basic Methods in Biophysical Chemistry) essential traditional, but still

modern, methods are described for the investigation of biomolecular processes and the characterization of biomolecules. Important examples are fluorescence methods such as fluorescence polarization and Förster energy transfer (FRET) techniques as well as more modern methods for the characterization of biomolecules by mass spectroscopy or pulsed NMR. In addition, an insight into important labelling techniques and techniques for linking biomolecules with each other or to immobile supports is given. In Part 2 (Advanced Methods in Biophysical Chemistry) more specialist methods are described that can be employed, for example, when none of the basic methods in Part 1 can be used to answer a particular biomolecular question. Important examples are fluorescence correlation spectroscopy, high-resolution STED microscopy, optical tweezers, atomic force microscopy of biomolecules, patch clamping techniques and the use of fluorescing nanoparticles. In this part, rather technical applications are also discussed, such as DNA sequencing, fluorescence-assisted cell sorters, and DNA chips. This part also contains a section on principles in assay development and applications in industrial high-throughput screening.

Throughout the book you will find application examples that illustrate the use of the described methods for typical biomolecular questions. Table I.1 summarizes the methods along with application fields, important equations and gives the section in which application examples and the equations can be found. As already mentioned, the most important equations are also marked by a box throughout the entire book.

This book was originally based on my lectures on biophysical chemistry. The field of biophysical chemistry is huge and so it is difficult to encapsulate the most relevant methods in a single book. I ask all of those who feel that their methods or application examples should have been included or who think that their method was not described in sufficient detail to excuse me. For the same reasons also the given literature references constitute only selected examples for further reading. It was not our intention to present the entire literature relevant for the topics presented in this book since this would be beyond the scope and also not very didactic. Of course, I am always very happy to receive any comments or criticism that will help improve any future editions of this book.

Table I.1 Methods described in this book.

Method	Section	Examples of Typical Applications Fields	Selected Examples of Specific Applications Presented in this Book	Section	Important Equation	Section
Absorption spectroscopy	Section 2.2	Determination of concentration of certain biomolecules	Determining DNA purity	Section 2.3.2	Optical density: $\mathrm{OD}(\lambda) = \log \frac{I_0(\lambda)}{I(\lambda)} = \varepsilon(\lambda) \cdot C \cdot l$	Section 2.2
			Measuring oxygen saturation in blood Measuring cooperativity of haemoglobin	Section 2.4.1		
Intrinsic fluorescence spectroscopy	Section 2.2	Identification of the presence of fluorescing or fluorescently labelled biomolecules	Determination of metabolic activity from NADPH-fluorescence	Section 2.4.2		
			Determination of plant fitness by chlorophyll fluorescence	Section 2.4.4		
Fluorescence labelling	Section 3.2	Identification of labelled biomolecules in complex biological environments				
Fluorescence polarization anisotropy (FPA)	Section 3.4	Determining binding processes or other processes that can affect the rotational diffusion of labelled species	Receptor–ligand binding; molecular mass estimate; enzyme kinetics; enzyme inhibition	Sections 3.4.2–3.4.5	Fluorescence anisotropy: $r = \frac{I_{\mathrm{II}} - I_{\perp}}{I_{\mathrm{II}} + 2I_{\perp}}$	Section 3.4.1

(*continued*)

Table I.1 (*Continued*)

Method	Section	Examples of Typical Applications Fields	Selected Examples of Specific Applications Presented in this Book	Section	Important Equation	Section
Förster resonance energy transfer (FRET)	Section 3.5	Determining binding processes, conformational transitions and biomolecular distances	Receptor–ligand binding. Conformational changes in DNA. Determining distances during protein unfolding. Membrane fusion	Section 3.5.2	FRET efficiency: $\Phi_{ET} = \frac{R_0^6}{R_0^6 + r^6}$ $I_{Fl}^{D}/I_{Fl}^{D,0} = 1 - \Phi_{ET}$	Section 3.5.1
Fluorescence kinetics	Section 3.6	Sensing changes in the environmental polarity of biomolecules or other processes that can affect the excited state lifetime of fluorescence markers	Detecting FRET or FPA via fluorescence kinetics	Section 3.6	Time-dependent fluorescence intensity: $I_{\mathrm{Fl}}(t) \alpha P_{S_1}(t)$ $I_{\mathrm{Fl}}(t) = I_{\mathrm{Fl}}^{0} \cdot \mathrm{e}^{-t/\tau_{S_1}}$	Section 1.6
Fluorescence recovery after photobleaching	Section 3.7	Membrane diffusion				
Biochemilumine-scence	Section 3.8	Monitoring ATP concentrations. Monitoring protein expression				
Circular dichroism (CD), optical rotation dispersion	Section 4.1.1	Determination of amount of secondary structure elements in proteins			Molar ellipticity: $[\Theta](\lambda) = \frac{100 \cdot \Theta(\lambda)}{C \cdot l} = 3298 \cdot \Delta\varepsilon(\lambda)$. Molar circular birefringence/optical rotation: $[\Phi](\lambda) = \frac{100\Phi(\lambda)}{C \cdot l}$	Sections 4.1.1 and 4.1.2

Light scattering	Section 4.2	Determination of molecular mass of biomolecules. Aggregation and shape of biological objects			Rayleigh ratio: $R_\Theta = \frac{I_{\text{Scat}}}{I_0} \frac{r^2}{V(1+\cos^2\Theta)} = K \cdot c \cdot M$	Section 4.2.1
Vibrational spectroscopy (infrared/Raman spectroscopy)	Section 4.3	Determination of secondary structure elements. Label-free identification of chemical composition				
Nuclear magnetic resonance (NMR)	Section 5.1	Structure determination under physiological conditions. Determination of structural flexibility of biomolecules. Label-free observation of biomolecular processes	Comparison of NMR structure of insulin and severin with X-ray structure. Flexibility of severin structure under physiological conditions	Section 5.1.8	Chemical shift: $\delta = \frac{\omega_{\text{signal}} - \omega_{\text{reference}}}{\omega_{\text{reference}}} \cdot 10^6$ ppm	Section 5.1.3
Electron paramagnetic resonance (EPR)	Section 5.2	Observation of redox reactions in photosynthesis or haemoglobin. Determination of rotational diffusion using spin labels	Determination of membrane structure and dynamics	Section 5.2		
Mass spectrometry	Chapter 6	Identifying biomolecules from mixtures. Determining biomolecular structures from fragments	Peptide sequencing	Section 6.4		
Fluorescence microscopy	Chapter 7	Imaging of labelled proteins in a whole cell context. Real-time imaging of biological processes			Diffraction-limited resolution: $r_0 \approx \frac{\lambda}{n \cdot \sin\varphi}$	Section 7.2.1

(continued)

Table I.1 (*Continued*)

Method	Section	Examples of Typical Applications Fields	Selected Examples of Specific Applications Presented in this Book	Section	Important Equation	Section
Super-Resolution Fluorescence Microscopy	Chapter 8	High resolution imaging of fluorescently labelled biological objects and/or under more physiological conditions	High resolution images of neuronal actin filaments	Section 8.2		
Optical single-molecule detection	Section 9.2	Real-time observation of biomolecular mechanisms	Mechanism of ATPase. Mechanism of motor proteins	Sections 9.2.1 and 9.2.2		
Fluorescence correlation spectroscopy	Section 9.3	Observation of binding and aggregation processes. Determination of microscopic concentrations. Determination of diffusional properties	Receptor–ligand binding. Determination of microscopic concentration. Determination of aggregation. Estimate of molecular mass	Sections 9.3.1, 9.3.3.1, and 9.3.3.2	Correlation function: $G(\tau) = \frac{\langle I_{\mathrm{Fl}}(t) \cdot I_{\mathrm{Fl}}(t+\tau)\rangle}{\langle I_{\mathrm{Fl}}(\nu)\rangle^2} - 1\ G_{\mathrm{Dif}}(\tau)$ $= \frac{1}{\langle C\rangle \pi^{\frac{3}{2}} r_0^2 z_0} \cdot \frac{1}{1+\frac{\tau}{\tau_{\mathrm{D}}}} \cdot 1 \sqrt{1+\left(\frac{r_0}{z_0}\right)^2 \frac{\tau}{\tau_{\mathrm{D}}}}$ $G_{\mathrm{Dif}}(0) = \frac{1}{\langle C\rangle V_{\mathrm{eff}}} = \frac{1}{\langle N\rangle},$	Sections 9.3.1–9.3.3
Fluorescence cross-correlation spectroscopy	Section 9.3.5	Binding processes	Determination of binding degree	Section 9.3.5	Cross-correlation function: $G_{\mathrm{X}}(\tau) = \frac{\langle I_{\mathrm{Fl}}^{\mathrm{G}}(t) \cdot I_{\mathrm{Fl}}^{\mathrm{R}}(t+\tau)\rangle}{\langle I_{\mathrm{Fl}}^{\mathrm{R}}(t)\rangle} - 1,$ $C_{\mathrm{RG}} = \frac{G_x(0)}{G_{\mathrm{R}}(0) G_{\mathrm{G}}(0) \cdot V_{\mathrm{eff}}}$	Section 9.3.5
Optical tweezers	Section 9.4	Force–distance measurements with single biomolecules	Unfolding RNA-hairpins. Forces exerted by RNA-polymerase. Forces exerted by DNA-polymerase	Sections 9.4.2.1–9.4.2.3	Trap stiffness: $k_{\mathrm{Trap}} = -\frac{\Delta \vec{x}_{\mathrm{Trap}}}{\vec{F}_{\mathrm{Trap}}}$	Section 9.4.1

Atomic force microscopy	Section 9.5	Determination of surface topology of membranes containing transmembrane proteins. Force–distance measurements with single biomolecules	Unfolding DNA-hairpins. Receptor–ligand forces. Unfolding titin	Sections 9.5.2.1–9.5.2.3	Forces exerted at cantilever: $\vec{F}_{\text{AFM}} = -k \cdot \Delta z$	Section 9.5.1
Patch clamping	Section 9.6	Measuring ion currents through single ion channels	Measuring open and closed times at a ligand gated ion channel as a function of ligand concentration	Section 9.6.1		
Multiphoton excitation	Section 10.2.1	3D laser scanning microscopy. Fluorescence cross-correlation spectroscopy	Two-photon microscopy. Selective carotenoid excitation in photosynthetic proteins	Sections 10.2.2 and 10.2.4	Probability of two-photon excitation: $P_{\text{Absorption}}^{\text{Two-Photon}} \propto I_{\text{Excitation}}^2$	Section 10.2.1
Second-harmonic generation; third-harmonic generation	Section 10.2.5	Label-free microscopy of structures like collagen, muscle fibres, cell membranes or lipid bodies			Generated frequency: $\nu_{\text{wavemixing}} = \pm\nu_1 \pm \nu_2 \pm \nu_3 \ldots$	Section 10.2.5
CARS microscopy	Section 10.2.5	Label-free microscopy of biological objects based on vibrational frequencies of chemical bonds			Generated frequency: $\nu_{\text{Anti-Stokes}} = 2 \cdot \nu_{\text{Pump}} - \nu_{\text{Stokes}}$ Direction of detected anti-Stokes beam: $\vec{k}_{\text{Anti-Stokes}} = -(2 \cdot \vec{k}_{\text{Pump}} - \vec{k}_{\text{Stokes}})$	Section 10.2.5
Ultrafast spectroscopy	Section 10.3	Observation of fastest processes in biology	Investigation of ultrafast light-harvesting and energy conversion in photosynthesis	Section 10.3.2		
DNA sequencing	Chapter 11	Determining and identifying genetic code				
Fluorescing nanoparticles	Section 12.2	Fluorescence labelling with superior signal-to-noise ratio	Encoded microspheres	Section 12.8		

(*continued*)

Table I.1 (*Continued*)

Method	Section	Examples of Typical Applications Fields	Selected Examples of Specific Applications Presented in this Book	Section	Important Equation	Section
Surface plasmon resonance detection	Section 12.3	Determination of receptor–ligand binding and dissociation kinetics				
DNA chips	Section 12.5	Determining gene expression as a function of regulators or drugs				
Flow cytometry	Section 12.6	Sorting cells as a function of distinct cell properties monitored by fluorescence or light scattering				
Fluorescence *in situ* hybridization	Section 12.7	Identifying certain genes in genomes				
Micro- and nanospheres	Section 12.8	Many different application possibilities				
Microtitre plates, fluorescence readers	Section 13.3	Measurements in dilution series and high-throughput screening, assay development	High-throughput screening for finding lead-structures in drug design	Section 14.4	Quality of assay: $Z' = 1 - 3\frac{\delta^+ + \delta^-}{\lvert\bar{x}^+ - \bar{x}^-\rvert}$	Section 13.2

Part One
Basic Methods in Biophysical Chemistry

In Part 1 of this book the more established modern techniques are presented that are nowadays very broadly applied in many labs throughout the world. It starts with a chapter that introduces the basic principles governing the interactions of electromagnetic radiation ("light") with molecules and biomolecules. These interactions are the basis for many of the methods presented not only in Part 1 but also in some chapters of Part 2. Important examples are general methods such as absorption spectroscopy and a plethora of advanced fluorescence techniques (Chapters 2 and 3) as well as very important techniques such as NMR studies of biomolecules (Chapter 5), but also methods such as super-resolution microscopy (Chapter 8), single-biomolecule detection (Chapter 9), nonlinear spectroscopy (Chapter 10) and next-generation DNA-sequencing methods (Chapter 11).

The introductory chapter (Chapter 1) aims at providing a brief and concise but still thorough review of the interaction of electromagnetic radiation with molecules for readers with a more chemical or physical background and serves as an introduction into these interactions for readers with a more biological background. Readers who require a rather quick overview and who have at least some basic knowledge about orbitals of molecules might focus on Sections 1.1, 1.3, 1.5 and the beginning of Section 1.6 only. These parts should suffice to have enough background information to understand the subsequent chapters. For example, they will already give an indepth idea about transition dipole moments and the polarization of light, which are central for quite a number of the methods presented in this book.

In Chapters 2 and 3, absorption and fluorescence properties in the visible spectral range of important biomolecules and fluorescence markers are presented as well as the application of the corresponding techniques to investigate biomolecules and biomolecular processes. Important examples are the determination of biomolecule concentrations or purities as well as investigations of receptor–ligand equilibria, enzymatic conversions and activators and inhibitors. In Chapter 3 some very central concepts in modern biophysical chemistry are also discussed in more detail, such as labelling and linking techniques, Förster

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resonance energy transfer (FRET) and fluorescence polarization assays. Also, the mathematical framework for the latter two is discussed in more detail, but readers who require a quick overview can also focus only on the description of the equations that are marked by boxes, which represent, in general, the central equations throughout this book.

Chapter 4 summarizes some very important optical methods that do not primarily rely on the absorption and emission of unpolarized or linearly polarized light in the visible spectral range but rather on scattering of light, the use of left- and right-handed circular light or light in the infrared spectral region. These techniques are, in general, of great use to derive information about the size, shape and secondary structural elements of biomolecules without any labelling of the samples.

Chapter 5 gives an overview of the application of nuclear magnetic resonance (NMR) techniques, which work with electromagnetic radiation in the radiowave range. NMR allows for the analysis of biomolecular structures under more physiological conditions than is possible with classical electron or X-ray microscopies. Chapter 5 also aims at providing an insight into the physical basics behind multidimensional NMR that are essential for such studies and how the information derived from such techniques can be used to derive a final 3D structure of proteins. Also, the use of electron paramagnetic resonance (EPR) for investigating biomolecules is described in this chapter.

Chapter 6, finally, presents techniques that allow very accurate determination of one of the most central parameters for characterizing biomolecules, namely their molecular weight. In this chapter it is discussed how mass spectrometry can be applied to biomolecules without harming their structural integrity and what further very useful information can be derived using advanced mass spectrometric methods.

1
Basic Optical Principles

1.1
Introduction

To understand important optical methods used to investigate biomolecules, such as fluorescence polarization anisotropy, Förster resonance energy transfer, fluorescence lifetime techniques (Chapter 3), optical single-molecule detection techniques (Chapter 9), fluorescing nanoparticles (Chapter 12) or high-resolution fluorescence microscopy such as STED and PALM (Chapter 8), a basic understanding of the interaction of light with molecules and biomolecules is required. Such basic knowledge is also necessary in understanding the use of magnetic resonance techniques (Chapter 5). In the following sections we discuss a few fundamental principles of the physical processes that govern the interaction of light with biomolecules, optical markers or other relevant matter such as nanoparticles. This chapter provides a brief but wide-ranging insight into important physical and quantum mechanical basics of these processes also for, but not limited to, readers without a physical or chemical background, while being mathematically and physically as exact and concise as possible. Readers that look for a rather quick overview and that have at least some basic knowledge about orbitals of molecules might focus on sections 1.1, 1.3, 1.5 and 1.6 only. Please be aware that a complete, indepth treatment of quantum mechanics and molecular spectroscopy is beyond the scope of this chapter. Only the fundamentals that are needed for the biophysical chemistry methods presented in the following chapters are discussed. For further information about quantum mechanics and molecular spectroscopy, the reader might refer to the more specialized books given in the bibliography at the end of this chapter.

Light is a wave of oscillating electric and magnetic fields propagating through space (Figure 1.1). The electric- and magnetic-field components of this electromagnetic radiation are oscillating in phase perpendicular to the propagation direction and with respect to each other. The smallest possible units of light are elementary particles called photons. These possess a specific amount of energy, E,

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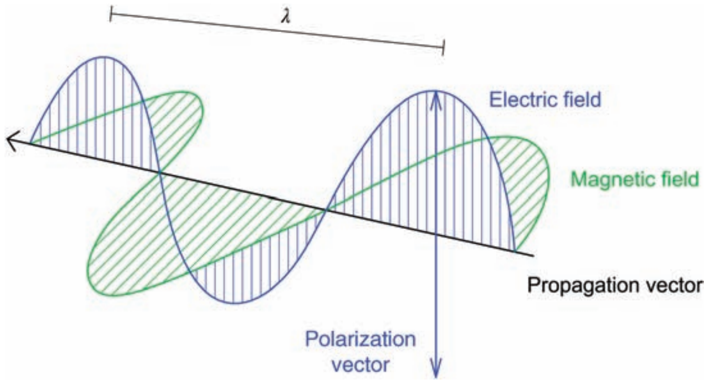

Figure 1.1 Light is a wave of oscillating electric and magnetic fields propagating through space.

which depends on the frequency or wavelength of the light radiation:

$$E = h\nu = \frac{hc}{\lambda} = hc\overline{\nu} \tag{1.1}$$

Here, h is Planck's constant, ν is the frequency of the electric- and magnetic-field oscillation, c is the speed of light, λ is the wavelength of the oscillation and $\overline{\nu}$ is the corresponding wave number. The energy E of a photon is linear proportional to the frequency ν and wave number $\overline{\nu} = \frac{1}{\lambda}$. The wavelength, in turn, is inversely proportional to the energy.

Figure 1.2 shows the spectrum of electromagnetic radiation. Optical methods make use of electromagnetic radiation having wavelengths from ~200 nm to a few μm. This interval can be subdivided into the ultraviolet (UV) region (~200–400 nm), the visible region (~400–700 nm) and the near infrared (NIR) region (~700 nm to a few μm). The UV range, for example, is important for the characterization of proteins and DNA, which is described in Chapter 2. The visible region is important for most fluorescence techniques, which are described in

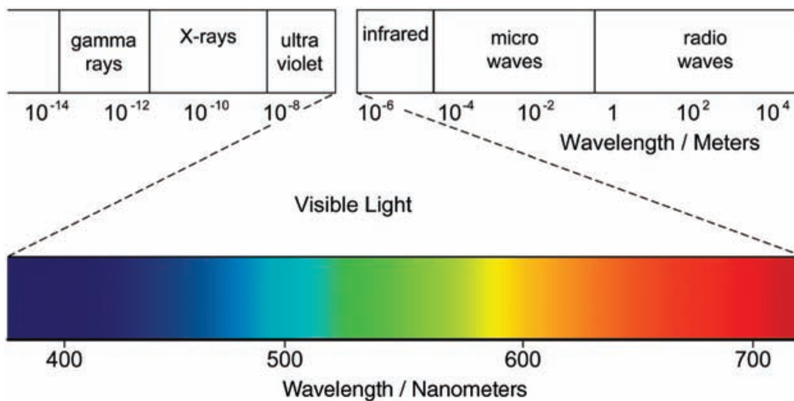

Figure 1.2 Spectrum of electromagnetic radiation.