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. 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 little side effects as possible it is useful to look at as many compound structures as

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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 seconds. 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 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

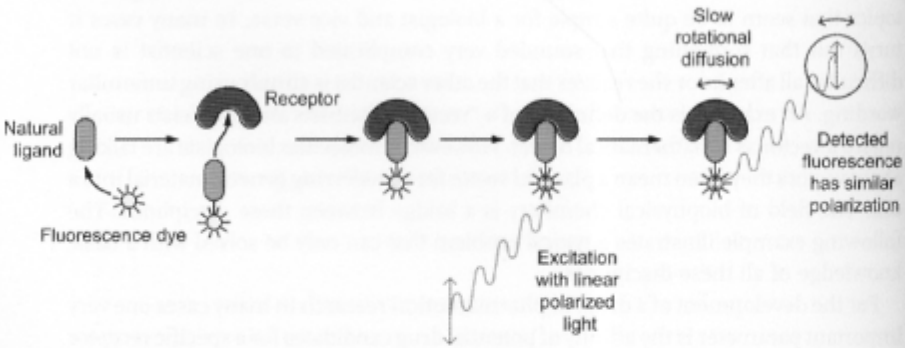

Figure I.1 Schematic representation of a typical fluorescence polarization assay. In a first step a small ligand is labelled with a fluorescent marker and forms a stable receptor–ligand complex with a receptor. Owing to the large mass of the receptor–ligand complex its rotational diffusion in solution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

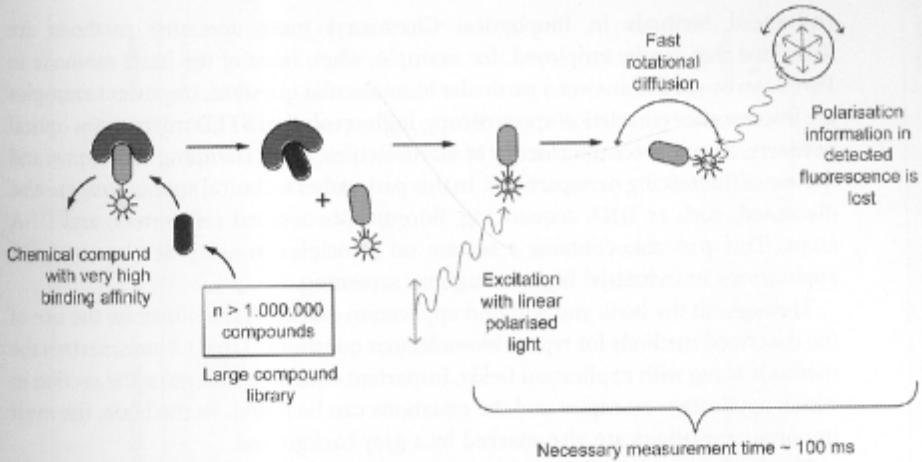

Figure I.2 If a chemical compound out of a large pool of synthetic compounds has a significantly higher affinity for the receptor it replaces the labelled ligand. The free ligand exhibits very fast rotational diffusion. This can be measured very quickly because now the polarization orientation of emitted 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.

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 than 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 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. In the book, the most important equations are also marked by a grey background.

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 topic presented in this book since this would be clearly beyond the scope and also not very didactic. I am very happy to receive any comments or criticism that will help improve any future editions of this book.

Finally, I thank all the friends and colleagues whole helped to improve this book with their very useful and often essential comments. Special thanks are to Stefan Bode, Anna Cypionka, Dr Christian Eggeling, Jan Frähmcke, Professor Dr Karl-Heinz Gericke, Dr Ulrich Haupts, Dr Hendrik Hippchen, Professor Dr Henrike Heise, Dr Martin Michels, Professor Dr Filipp Oesterhelt, Wiebke Pohl, Professor Dr Christoph Schmidt, Dr Jakob Sørensen, Professor Dr Dirk Schwarzer and finally Dr Andreas Volkmer.

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