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[5] 'Ionic Partition Diagram: A potential pH Representation', F. Reymond, H.H. Girault, P.-A. Carrupt, G. Steyaert, B. Testa, *J. Am. Chem. Soc.*, in press.
[6] 'Electrochemical Study of Phase Transfer Catalysis Reactions: The Williamson Ether Synthesis', S.N. Tan, R.A. Dryfe, H.H. Girault, *Helv. Chim. Acta* **1994**, *77*, 231.
[7] 'Spectroelectrochemical Studies of $Ru(bpy)_3^{2+}$ at the Water/1,2-Dichloroethane Interface', Z.F. Ding, R.G. Wellington, P.-F. Brevet, H.H. Girault, *J. Phys. Chem.* **1996**, *100*, 10658.
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[9] 'Coplanar Interdigitated Band Electrodes for Electrosynthesis, Part 2: Methoxylation of Furan', C. Belmont, H.H. Girault, *J. Appl. Electrochem.* **1994**, *24*, 719.

*Chimia 50 (1996) 590–592*

*ISSN 0009–4293*

# Laboratoire de Chimie Physique des Polymères et Membranes

Prof. Dr. **Horst Vogel***

The institute concentrates on three major research topics ranging from fundamental studies on the **structure and dynamics of membrane proteins** to the investigation of the **formation of supramolecular structures at interfaces** and applied research in the field of **biosensor development.** All the projects concern interfaces both in a literal sense and in terms of combining different disciplines such as chemistry, physics, and biology. The highly multidisciplinary team works on the synthesis of lipids and polypeptides, the chemical modification of surfaces, the isolation of proteins, the application of recombinant DNA technology, the use of optical spectroscopy (FTIR, fluorescence, circular dichroism) combined with molecular-dynamics calculations as well as with surface-sensitive techniques (surface plasmon resonance, integrated optics, *Langmuir* film balances, scanning probe microscopies).

In the following, we give some representative examples of our research.

## 1. Fundamental Studies on the Structure and Dynamics of Membrane Proteins

### 1.1. *Membrane Insertion and Folding of Proteins*

Protein integration into or translocation across membranes is a fundamental process in procaryotic and eucaryotic cells. The biosynthesis of almost all cellular proteins begins in the cytoplasm. The insertion of newly synthesized membrane proteins into cellular membranes is in many cases directed by *N*-terminal signal (leader) sequences, that are removed from the mature protein after insertion. Complicated protein insertion and translocation machineries composed of water-soluble and membrane-bound proteinaceous components have been characterized genetically and biochemically in prokaryotic and eukaryotic cells, but the detailed molecular mechanism by which the nascent polypeptide chain is driven through the membrane is unknown. However, there are several examples of relatively small proteins which insert into cellular membranes independently of a translocation machinery. Classical examples are the coat proteins of the filamentous phages M13 and Pf3. This suggests that such small proteins must have the structural properties necessary for targeting and translocating within their primary amino-acid sequence.

The project concerns fundamental studies of membrane protein insertion and structural folding using the coat proteins of the filamentous phages M13 and Pf3. The 50-residue-long M13 coat protein is synthesized in *Escherichia coli* cells as a precursor, termed procoat protein, with a classical 23-residue leader sequence at its *N*-terminus. Procoat protein inserts into the plasma membrane as a loop structure in the presence of a transmembrane electrical potential and is finally processed by leader peptidase, yielding the mature transmembrane M13 coat protein. In contrast to M13 coat protein, the 43-residue-long Pf3 coat protein is synthesized without a leader sequence and the protein directly transfers its *N*-terminus across the membrane.

*Horst Vogel* studied Chemistry at the University of Würzburg/Germany. After his diploma thesis in Physical Chemistry he went to the *Max-Planck* Institute for Biophysical Chemistry in Göttingen, where he performed his Ph.D. work on the 'Structure of lipid membranes' in the Departments of Profs. *M. Eigen* and *A. Weller*. In the following, he worked at the *Max-Planck* Institute for Biology in Tübingen/Germany, at the Biocenter of the University of Basel/Switzerland, and at the Karolinska Institute in Stockholm/Sweden, studying the structure and dynamics of membrane proteins. In 1994, he was appointed as a Professor of Physical Chemistry at the EPFL, where he is director of the Institute for Physical Chemistry of Polymers and Membranes.

**Correspondence*: Prof. Dr. H. Vogel
Département de chimie
Institut de chimie physique
Laboratoire de chimie physique des polymères et membranes
EPFL
CH–1015 Lausanne

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A combination of biochemical and biophysical techniques are applied to obtain details of the protein structure as the proteins translocate across the membrane. Genetic studies with mutant proteins have allowed dissection of the membrane insertion process into distinct steps. This offers the possibility to observe and characterize the conformational details of translocation intermediates of the wild type and of different mutant proteins. The project focusses on the following topics:

- The thermodynamics and kinetics of the membrane insertion of the two wild type and different mutant proteins are investigated by spectroscopic and calorimetric techniques in order to distinguish different membrane translocation intermediates.
- Structural and dynamic details of the translocation intermediates as well as the final membrane-inserted state of the proteins are investigated by fluorescence spectroscopy using site-specific chemical labelling of suitable reporter groups.

Of particular interest for the present project is the investigation of how transmembrane potentials influence the membrane insertion process and subsequent protein folding. Fluorescence measurements allow observation of the translocation process *in situ* and thus provide important information on how an electrical transmembrane potential influences the translocation of charged residues across lipid membranes.

### 1.2. *Protein Structure Determination by Fluorescence Spectroscopy*

Detailed structural information of receptor proteins is important for the understanding of the mechanisms of ligand recognition and signal transduction and in turn for the design of novel therapeutic agents. In the case of membrane-integrated receptor, the determination of the three-dimensional protein structure represents a formidable task because of the difficulties of obtaining and purifying sufficiently large amounts of protein to produce crystals for high-resolution X-ray or electron diffraction. We are developing a new and general method for protein structure determination by fluorescence spectroscopy. Here, a fluorescent, nonnatural amino acid is introduced at a known site into the receptor protein by molecular biological methods. Subsequently, intermolecular distances are determined by measuring the fluorescence resonance energy transfer between the fluorescent, nonnatural amino acid in the receptor and a suitably fluorescently labeled ligand molecule which binds to the receptor. Using a variety of different receptor mutant proteins, allows the determination of distances, which permit to fix the ligand in space and define the three-dimensional structure of the receptor. The method can be generally applied to understand the molecular architecture and conformational changes of integral membrane proteins such as channels, G-protein coupled receptors as well as protein-protein or ligand-protein interactions, either in reconstituted systems, membrane fragments or even intact living biological cells.

## 2. Formation of Supramolecular Structures by Selective Self-assembly Processes

This project deals with the formation of two-dimensional micro-scale structures on solid surfaces. The preparation of these structures makes use of the combined application of the *Langmuir-Blodgett* technique and the formation of self-assembled monolayers of sulfur-bearing molecules. This combination allows the generation of well-defined surface patterns with contrasting properties. Since the fabrication is based on self-organizing principles, there is no theoretical limit on the size of the patterns created. Our motivation in creating these surface structures is to develop micro- and nano-sized multichannel sensor arrays. However, the potential for creation of structures below optical limits makes this technique of great intrinsic interest for all lithographic applications.

## 3. Development of Novel Biosensors Based on Natural and Artificial Receptors

Biosensors are devices based on the specific recognition of an analyte of interest by a biological component (enzymes, antibodies, receptors, whole cells), and the subsequent transformation of this event into an electrical, optical, or other signal. Although it is only recently that biological signal recognition, transduction, and amplification principles have been applied to develop new analytical tools, biosensors have already attracted intense interest in many different fields such as medical diagnostics and control, environmental analysis, and monitoring of biotechnological processes.

The potential for biosensor technology is enormous and it will revolutionize certain areas of analysis and control. However, in order to fulfill the high expectations and to make biosensors practical, a number of obstacles must be overcome.

Most of the existing biosensing principles rely on enzymatic reactions or antigen-antibody interactions in order to detect the analytes of interest. Although such sensors have been quite successful in certain areas such as measuring the sugar concentration in blood, in enzyme-based analytics and immunodiagnostics, the fields of application are presently still quite limited. In order to overcome the limitations of traditional biosensor applications, novel concepts have to be found to improve the biosensors' stability to increase their sensitivity by applying novel physical detection techniques and, most importantly, to open biosensors to other biological signal recognition, transduction, and amplification principles. Our research has entered a novel field of biosensors by applying biological signal recognition and amplification processes which are based on either natural membrane protein receptors or tailor-made sensing molecules, that is artificial membrane-active receptors, derived from natural precursors.

Many central biological signal transduction processes such as synaptic transmission occur at the level of cell membranes. It is therefore not surprising that the biological function of membrane proteins in general and neuroreceptors in particular can be modulated by pharmacologically active substances. Both the functional understanding of molecular interactions of receptors as well as their respective use in screening for effector compounds are important aspects in drug discovery. At present, the screening for new pharmacologically active compounds still follows traditional routes applying time-consuming ligand binding studies and receptor function tests separately. It is a major objective of our research to develop novel principles for functional tests to detect ligand-receptor interactions with higher efficiency and sensitivity.

A central problem which has to be solved in the project is the immobilization of membrane receptor proteins in a functionally active form on sensor surfaces. In this context, we are developing a new generation of biosensors by combining improved membrane-immobilization procedures with novel ways of enriching the concentration of receptor proteins on the sensor surface by affinity binding.

For the project to be accomplished, a multidisciplinary approach is necessary, combining the expertise of membrane biochemists and molecular biologists, chemists, physicists, and silicon technologists

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in a collaborative effort between the EPFL and the Universities of Lausanne, Geneva, Neuchâtel, and Bern. Collaboration with leading pharmaceutical companies as partners within the project helps to focus on developing novel analytical techniques in general and drug screening in particular. Collaboration with small- and medium-sized companies with experience in the development of optical and electronical devices and equipment allows us to transfer the know-how of the feasibility studies for the construction of future sensing devices.

Within the project, three different classes of receptor systems are under investigation: *i*) Ligand-gated ion channels such as the $5HT_3$ (serotonin), $GABA_A$, and nicotinic acetylcholine receptors as examples of pharmacologically important neuroreceptors. Here the intrinsic signal amplification of a channel protein is used as a detection principle. *ii*) G-Protein coupled receptors such as the NK2 receptor for introducing new principles for the sensing of ligand-receptor interactions for this important class of receptor proteins. Both *i*) and *ii*) might be applied to develop new drug screening tests using a functional assay. *iii*) Ligand-receptor interactions at the membrane surface for investigating the specific binding of antibodies to these proteins. As representative examples, relatively small lipid-attached polypeptides (repeating NANP sequences which represent the major epitope of the protein covering the surface of the sporosite state of the human malaria parasite *Plasmodium falciparum*) and larger lipid-anchored proteins such as the promastigote surface protease (PSP) of the parasite *Leishmania* are studied. Artificial ligand-gated channel proteins based on synthetic template-assembled proteins are also used for developing a new concept of immunosensors.

In order to detect different ligand-receptor interactions, surface sensitive techniques are applied which can be divided into two groups according to their sensor supports: *i*) methods using gold surfaces (impedance spectroscopy, piezoelectric devices, surface plasmon resonance spectroscopy and microscopy) and *ii*) methods which use hydrophilic substrates such as glass or $TiO_2$ surfaces (integrated optics, fluorescence spectroscopy). The two different groups of techniques require different strategies to immobilize the biological components which are developed within the different projects. It is important for the success of sensors that the different techniques deliver complementary information and sensitivity which offers the possibility to combine the different detection techniques within one sensor device in order to improve its performance in practical applications.

## 4. Selective Recent Publications on the Three Different Areas of Research

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