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The Centre for Chemical Sensors/Biosensors and bioAnalytical Chemistry, CCS, at the Interface between Science and the Market Place

Ursula E. Spichiger-Keller*

Abstract: In the following article, a very brief overview describing the general strategies and the main goals tackled by CCS is given. The latest news, as well as projects running in collaboration with companies are not discussed here. For more details see homepage, http://www.chemsens.ethz.ch.

1. Purpose and Origin

The *Centre for Chemical Sensors/Biosensors and bioAnalytical Chemistry, CCS,* is located in the Zürich Science Park in a wing rented to the Swiss Federal Insitute of Technology, ETHZ. The focus of efforts in CCS is on: *1*) making analytical use of thirty years of developments in the field of sensor technology, *2*) developing appropriate instrumentation prototypes, *3*) exploring feasible analytical applications, and along with this mission of technology transfer, *4*) to develop new recognition and transducing processes as well as novel sensor designs. Innovation in such designs tend to be 'driven' by gaps in the sensor technology market and related scientific fields rather than by basic scientific considerations.

CCS was initiated by Prof. *Wilhelm Simon*, Laboratory of Organic Chemistry ETHZ, in the eighties. In 1988, I became involved in planning the office and laboratory areas in the future CCS. At that time, the centre was called 'Swiss Centre for Chemical Sensors'. After a rather difficult period, when Prof. *Simon* passed away unexpectedly in 1992, I was able to open the Centre in March 1994. The Department of Pharmacy under Prof. *G. Folkers* took over the responsibility for CCS.

**Correspondence*:
Assoc. Prof. U.E. Spichiger-Keller
Department of Pharmacy
ETH-Technopark
Technoparkstrasse 1
CH-8005 Zürich
E-Mail: uspi@chemsens.pharma.ethz.ch.

The goals of CCS range from molecular recognition and modelling of ligands to the application of sensors so that the background of the specialists collaborating within the group at CCS is accordingly very diverse. CCS runs a synthetic, an electrochemical, and an optical laboratory as well as the computer equipment needed for molecular modelling and calculations. All of us very much appreciate being allowed, as an ETHZ group, to make use of the services available in the Departments of Chemistry and Pharmacy.

2. Goals and Focus of Efforts

2.1. *Molecular Recognition*

Chemical sensors in the strict sense differ from biosensors in that the active components involved in the molecular recognition process are different. In chemical sensors, synthetic ligands and solubilizing agents act as host molecules. The active components in biosensors, on the other hand, are natural bioactive compounds, and the corresponding substrates are quantified. Since it is becoming increasingly difficult to separate between the two classes of sensors, the more general name 'chemical sensors' is preferred and recommended for both classes.

Typical traditional biosensors make use of the natural selectivity or specificity of enzymes, receptors and antibodies. In all these cases, the recognition process is more likely to be regenerable than reversible. *Immunoassays* are routinely used in environmental analysis [1] and medical applications [2], where specially low concentrations are determined. The most attractive feature of *immunosensors*, where the antibody or antigen is immobilized on a transducer directly, is real-time monitoring of the association and dissociation kinetics [3]. Since antibodies work dissociation controlled, and the dissociation constant is much lower than the association constant for most species, regeneration involves problems in terms of regeneration time. Such virtual sensors are, therefore, used preferably just as one-way probes.

In contrast, *enzymatic sensors* are in widespread use and some have also been commercialized [4]. At CCS, redox-active enzymes, preferably oxidases and reductases, are used in order to metabolize and quantify specific substrates. We have focused on enzymes which need no cosubstrates such as peroxidase (EC 1.11.1.7), L-ascorbate oxidase (EC 1.10.3.3), D-glucose oxidase (EC 1.1.3.4), L-glutamate oxidase (EC 1.4.3.11), sulfite oxidase (EC 1.8.3.1), xanthine oxidase (EC 1.2.3.2) (EC see [5]). In addition, the enzyme is coupled to the electrode by an organic mediator (see below). This has enabled us to develop analyte specific continuous monitoring electrodes, which exhibit a high selectivity at low applied voltage (< 200mV) and which work without adding reagents. Only a single commercial test is known so far to have been performed with this basic principle involved [6].

At CCS, the modelling of new *synthetic ligands* is combined with their synthesis, with the development of a prototype sensor design, and with testing analytical applications. Modelling of ligands is required and very helpful for structures too complex for the human brain to obtain an overview on the likely conformations and interactions with a target analyte. It is also required to estimate and compare interaction energies of a selection of supposed ligands.

In a project aimed at the development of new host compounds for oxoanions [7], the interacting groups L-arginine, L-serine and L-histidine of the active site of the enzyme purine nucleoside phosphorylase (PNP, EC 2.4.2.1) are considered as being the synthon of a synthetic peptide. As an example, the ligand shown in *Fig. 1* will be discussed further. In this ligand, the three natural target amino acids L-serine, L-histidine, and L-arginine are introduced into the model as binding sites. The amino acids are linked by different L-glycyl oligopeptides in this simple case. The modelling procedure aims at comparing different constitutions of ligands involving the

same amino acids as active sites. Relative interaction enthalpies between $H_2PO_4^-$ and the host molecule are modelled by computational methods using the software Sybyl (version 6.2 (1995) and 6.3. (1996), *Tripos Inc.*, St. Louis) and Amber (version 4.1 (1995), University of California, San Francisco). The force-field calculations involve energy minimization of the ligand-ion complex followed by molecular-dynamics calculations (simulated annealing) where the position of the monovalent or divalent phosphate ion is fixed. In the Amber force-field program, point charges for individual atoms are derived from *ab initio* calculations of the electrostatic potential for small fragments such as single amino-acid residues and can be retrieved from a database [8][9]. Formal charges are assigned to phosphate and those amino acids which are expected to exist in an ionic state under physiological conditions.

A relatively simple model involving two sites of interaction of $H_2PO_4^-$ to an arginine and a serine moiety is shown in *Fig. 1*. No specific assumptions were made for the dielectric constant of the environment, which means that calculations refer to the dielectric constant of vacuum. However, in view of the final goal, these situation was considered to be acceptable. In a more advanced state, the ligand will be incorporated into an apolar solvent polymeric membrane which will be exposed to the aqueous phase of a specimen. Assuming a low permittivity of the membrane phase, primary phosphate is more likely to be extracted and formed than HPO_4^{2-}. In addition, the pK_a of L-arginine within the apolar phase raises and the protonated species can be stabilized by solubilized counterions.

The results of these calculations are taken as a basis for solid-phase synthesis of the artificial peptides on a fully automatic synthesizer Syro (MultiSynTech, Bochum). For several of the peptides, an interaction with phosphate in solution could be shown by the ^{31}P-NMR technique in deuterated DMSO. At equimolar concentrations of the ligand and phosphate (see *Fig. 1*), the ligand was observed to induce a chemical shift of the phosphor signal of 2.36 ppm (T = 300 K) to higher field. In further experiments, the stoichiometry of phosphate/ligand complexes will be investigated.

Synthesis involves not only host compounds but also additives, dyes, and polymers which have to be adapted according to the required features such as solubility, lipophilicity and lifetime, spectral absorbance range, permittivity, a.o. In this area, we are competing with a few other groups worldwide [10]. Unfortunately, to date few scientific publications bear witness to CCS activities. This is for two main reasons: patent applications are more important than publications; raising money as well as providing project reports consumes considerable quantities of whole group efforts and time.

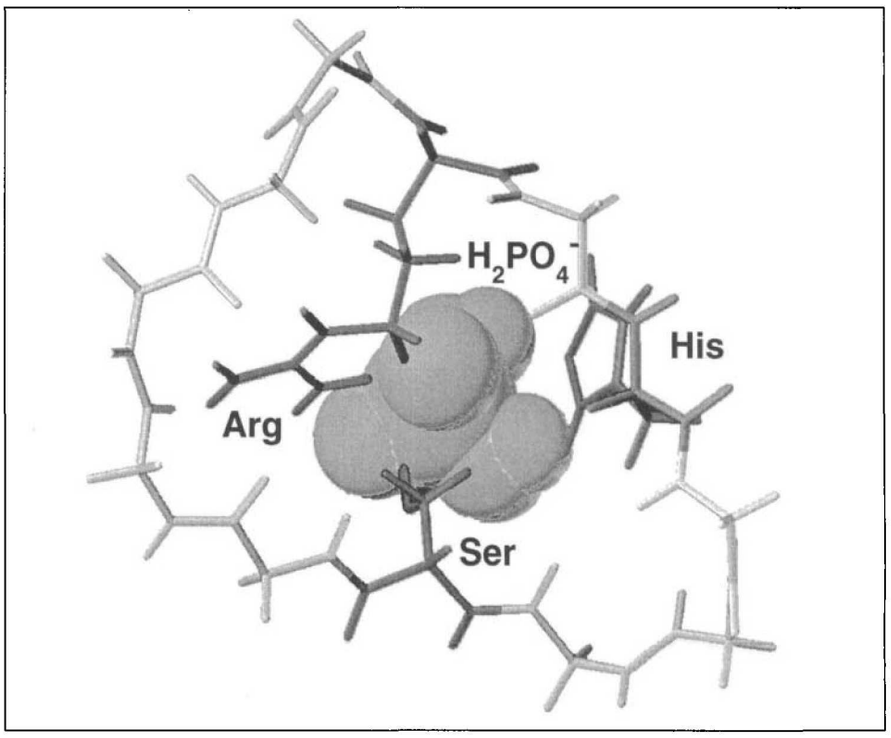

Fig. 1. *Example of a complex between a ligand and an oxoanion* (*e.g.*, phosphate; in spacefill representation, software see text). The ligand, the cyclic peptide GGRGHGGGSGG, consists of the biotic amino acids of the active site of purine nucleoside phosphorylase (PNP), linked by L-glycine (G). (R, L-arginine; H, L-histidine; S, L-serine) [7].

2.2. *Design of Selective Layers and Membranes, and Coupling to Transducers*

Incorporating host compounds in a polar or apolar *polymeric layer*, also called a membrane, results in a striking advantage: Upon partition of the analyte between the layer and the specimen or sample phase, the analyte is not only solubilized and selectively recognized but also separated from the bulk. The solubilization and recognition process of a target compound is largely influenced by such properties of the membrane environment as: the presence of functional groups competitive to the ligand, the lipophilicity of the solvent, the nature of additives, the permittivity and the surface tension of the layer (see [2a]). Based on this knowledge, one area of our research tackles the problem of predicting and quantifying the structure-selectivity relationships (QSSER) of such membrane phases (see *Fig. 2*). The sensing layer is the core of each sensing unit and can have a rather complex structure and composition depending on the characteristics required of typical applications. It is one of the CCS' main goal to design such sensing layers for biological, medical, and environmental specimens along with investigating the response behaviour and, newly, transport characteristics in the gas phase.

The *transducer* coupled to the sensing layer can, basically, be an optical or electrochemical probe, a mass sensitive device or any other suitable physical probe. In redox-active (bio)sensors, an organic salt (TTF/TCNQ: tetrathiafulvalene-*p*-tetracyanoquinodimethane), *e.g.*, is coupled to the metabolic turnover of the substrate by an enzyme and acts as an electron-transfer catalyst. The transducer is an electrode operated in the amperometric mode, where the current corresponds to the concentration of the free analyte in the specimen [12].

2.3. *Applications in Analytical Chemistry*

It would be an oversimplification to picture a sensor as looking similar to a tube-like pH electrode. Since we focus primarily on reversible systems, continuous flow systems are the most attractive and relevant. They allow increasing as well as decreasing free analyte concentrations to be monitored (see *Fig. 3*). The signal changes can be visualized on a display which means the ungoing process

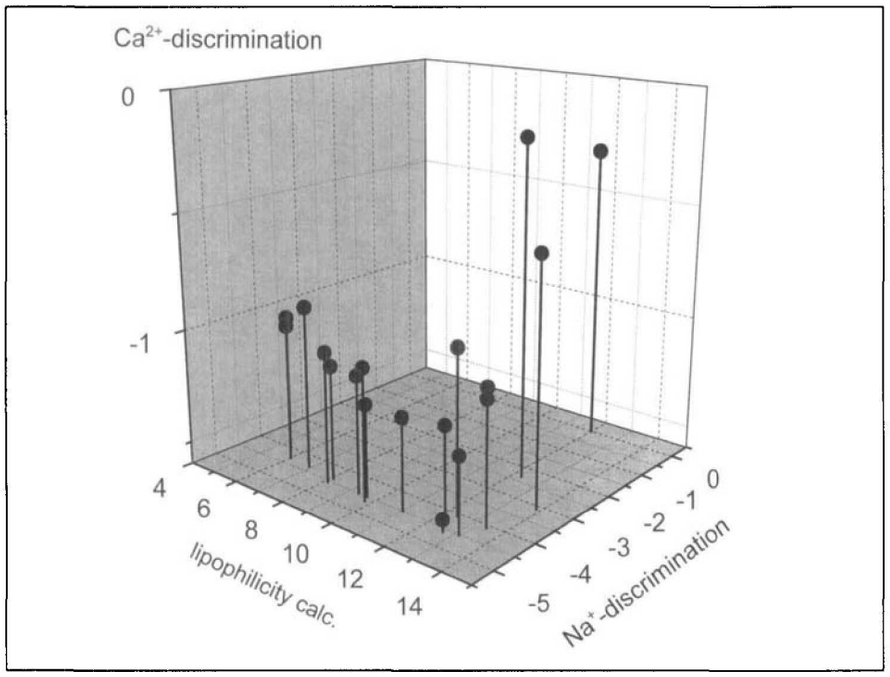

Fig. 2. *Selectivity of the magnesium-selective electrode membranes based on the ligand ETH 7025 (Fluka Chemie AG,* Buchs) *in plasticized PVC.* Influence of the lipophilicity of various derivatives of the plasticizer 4-nitrophenyl octylether (*x* axis) on discrimination of Ca^{2+} (*y* axis) and Na^+ (*z* axis) over Mg^{2+} (at $y = 0, z = 0$). Discrimination factors are given in logarithmic units. (Lipophilicities calculated according to [11].) The discrimination of both ions Ca^{2+} and Na^+ improves with increasing lipophilicity of the plasticizer.

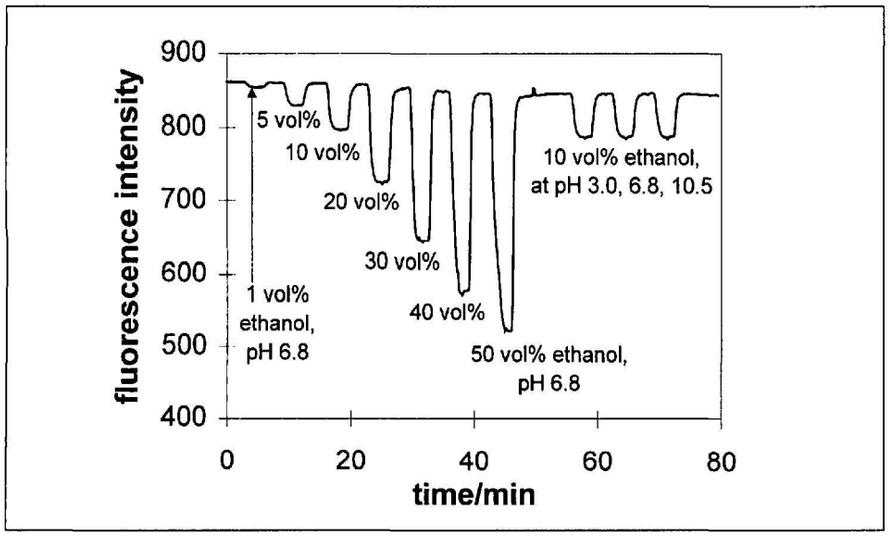

Fig. 3. *Reversible response of a plasticized PVC membrane* (thickness *ca.* 3 mm) *incorporating the ligand ETH^T 4004* (*4*-(trifluoroacetyl-)4'-(dioctylamino)stilbene) *on exposure to aqueous ethanol solutions.* The excitation and emission wavelengths were set to 450 and 550 nm, respectively. The *Teflon*-coated membrane is insensitive to pH from pH 3 to 10.5 [13].

can be continuously controlled. In order to demonstrate and offer the complete range of sensing principles available in our laboratory, a new form and design of a continuous flow system was developed. The idea behind it was to ensure that all types of reversible sensing layers and transducers could be implemented and demonstrated within the same chemical sensor system. One module of the system consists of a polymer body incorporating the sample channel and the sensing layer, where the latter is in contact with the sample on one side and the transducing element on the other side. The sensing layer and the transducing element are independent of the polymer body and can be exchanged. The single modules can be arranged in series or for parallel analysis. The modular system *CHemsens* was presented at the Hannover trade fair in April 1997. A Swiss patent application was submitted. It may be the only continuous measuring sensor system worldwide which is able to cope with several different sensing techniques simultaneously [14].

Besides continuous monitoring and process control, *CHemsens* has many other attractive applications. Typically, analysis by chemical sensors allows interaction and detection to operate simultaneously. The restricted selectivity of sensors, however, means that a careful preevaluation of the required analytical parameters is necessary. Since the 'reagents' are incorporated into the sensing layer, sensors work 'reagentfree' and offer themselves for on-site and point-of-care analysis. This is especially attractive for screening tests where an overall more effective analytical procedure is desirable, for analytes where the yielded information has immediate consequences for a decision or a treatment, and for target compounds that are not stable during transport. This type of analysis is likely to have enormous impact since it offers a more economic and effective analytical process.

The chemical sensor also has potential applications in animal-free toxicity tests as it combines molecular recognition of the target compound and partition with a membrane medium, and responds to a free, biologically active compound. The reversed micellar membranes may be especially suitable for such assays [15].

3. Current Status and Collaborations

CCS is not only involved in research and development but also in education. CCS has never had problems finding Ph.D. students and collaborators. CCS' position between industry and academia has evolved in a way that is specially challenging for the type of student who is more inclined to do applied science than to conduct theoretical or fundamental investigations. In addition to the head of the centre, CCS currently hosts eight Ph.D. students, two assistants, and three academic guests, as well as several associated members located in other institutes. The pressure on office and laboratory space is increasing, and the project work is becoming more and more intense.

Currently, five companies are involved in three different projects. We collaborate with the Engineering Schools in Windisch/Brugg, Wädenswil, and Winterthur. The collaboration with complementary centres and potential competitors is developing very satisfactorily. Such centres include FSRM (Fondation Suisse pour la Recherche en Microtechnique) and CSEM

(Centre Suisse d'Electronique et de Microtechnique SA) including the former group of Paul-Scherrer Institute Zürich; the group of Applied Physics, University of Geneva; Pharmaceutical Chemistry, Department of Pharmacy ETHZ; Institute of Biomedical Engineering ETHZ; Institute for Robotics ETHZ; Institute of Animal Nutrition, University of Zürich. We also enjoy fruitful collaborations with governmental institutions such as the FAM (Forschungsanstalt für Milchwirtschaft) and the BVET (Bundesamt für Veterinärmedizin).

CCS works autonomously in collaboration with industry and companies. Salaries, running costs, and investments are entirely financed by collaborations and specific project funds. Support from collaborating companies (see homepage), from collaborating institutes and engineering schools as well as from the *Swiss Commission for Technology and Innovation* (KTI) and the *Swiss National Foundation* (NF) is gratefully acknowledged.

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Le Centre de Compétence en Chimie et Toxicologie Analytiques. The Centre of Competence in Analytic Chemistry and Toxicology (CCCTA)

Jean-Luc Veuthey*

Abstract. This article presents concisely activities, goals and members of the Centre of Competence in Analytical Chemistry and Toxicology which was created recently in the Lake Geneva region.

1. Introduction

The Centre of Competence in Analytic Chemistry and Toxicology (CCCTA) was created on June 24, 1997 in Geneva, Switzerland. Today, it groups a dozen laboratories or institutes of the Lake Geneva region involved in the analysis of drugs and medicines. The CCCTA is recognised from an administrative point of view by the different departments, universities and hospitals in charge of the laboratories. Members of the CCCTA have recently been appointed as the national reference

Correspondence: Prof. J.-L. Veuthey
Laboratory of Pharmaceutical Analytical Chemistry
University of Geneva
Bd d'Yvoy 20
CH-1211 Genève 4
Tel.: +41 22 702 63 36
Fax: +41 22 781 51 93
E-Mail: Jean-Luc.Veuthey@pharm.unige.ch