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GEORGE OLAH DOCTORAL SCHOOL

Ion-selective electrodes and potentiometric sensing schemes for protein assays

Summary of the PhD thesis

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1. Introduction

Nowadays, there is a growing demand for low-cost, easy-to-use analytical devices, especially in the field of diagnostic assays. As such point-of care diagnostic devices and *in vitro* diagnostic platforms undergone a vigorous development and expansion. The cost-effective fabrication of these devices, the simplification of the analytical methodologies and the use of robust reagents are essential for their wide spread application in the everyday life. Accordingly, the focus of my doctoral research was to explore various new sensing methodologies, chemical reagents and materials to enable such cost-effective assays for protein targets of diagnostic relevance.

Proteins are generally detected via binding to an affinity ligand. Conventionally antibody-antigen interactions are preferred due to their exquisite selectivity. Owing to their higher sensitivity and exceptional selectivity caused by their double recognition process, *sandwich-type assay formats* are the most popular choice. The primary (capture) antibody is usually immobilized to a solid surface; most often to the wells of a microtiter plate or to the surface of the sensor transducer. The label on the second (tracer) antibody ensures quantitative analysis and via the possible signal amplification high sensitivity as well.

Enzyme-linked immunosorbent assay (ELISA)[1-2] remains up to now the gold standard in protein detection, with detection limits in the picomolar range. Conventionally it is coupled with optical detection methods which enable high sample throughput via the highly parallel measurements in the microtiter wells.

Although the optical detection is wide-spread in immunoassays the utilization of electrochemical methods[3-4] is often more suited for meeting the portability and miniaturization requirements of point-of-care testing or field detection of bioreagents. We explored the opportunities to use potentiometry for readout which instrumental-wise necessitates only a high impedance voltmeter (pH meter) ubiquitous in any analytical laboratory. Potentiometry offers several essential advantages, such as cost-effectiveness, simple and fast readout, it is compatible with modern miniaturization/microfabrication technologies; and it is suitable for measurements in minute volumes. The challenge was to adapt this technique, by developing novel ion-selective electrodes (ISEs) and general sensing schemes, to the detection of protein targets.

[1] E. Engvall, P. P, *Immunochemistry* 1971, *8*, 871-874.
[2] B. K. Van Weemen, A. H. W. M. Schuurs, *FEBS Lett.* 1971, *15*, 232-236.
[3] W. Heineman, C. Anderson, H. Halsall, *Science* 1979, *204*, 865-866.
[4] J. M. Fowler, D. K. Y. Wong, H. Brian Halsall, W. R. Heineman, in *Electrochemical Sensors, Biosensors and their Biomedical Applications* (Ed.: X. Z. J. Wang), Academic Press, San Diego, 2008, pp. 115-143.

Although research on potentiometric immunoassays had started already in the 1970s[5-6], little follow-up work was done on the field, due, partially, to the limited availability of commercial reagents needed for the unconventional ELISA systems as well as to the poor detection limit and dynamic range of the early potentiometric ion sensors.

At the end of the 1990s the ISE field experienced a new boost inspired by the significant improvements of the lower detection limit (LDL)[7-8]. This lead to the initiation of new membrane matrices[9-10], introduction of solid-contact ion-selective electrodes (SCISEs)[11-12] and the extensive use of ion-selective sensors in commercial automatic blood-gas analysers based on potentiometric detection. Thanks to these remarkable improvements, potentiometry became rediscovered as a viable alternative for bioanalytical measurements, as detection method in protein assays[13].

Therefore, the aim of the work summarized in my thesis was threefold:

(1) Utilizing the feasibility of potentiometric detection to replace the conventional optical detection methods in bioaffinity assays;
(2) Improving the analytical performance of solid-contact ion-selective electrodes used for the potentiometric measurements;
(3) Fabricating and utilizing novel 3D nanostructured conducting polymer layers both as ion-to-electron transducer and as biorecognition element.

[5] P.W. Alexande, G.A. Rechnitz, *Anal. Chem*. 1974, 46, 1253
[6] M. Meyerhoff, G.A. Rechnitz*, Science* 1977, 195, 494
[7] T. Sokalski, A. Ceresa, T. Zwickl, E. Pretsch, *J. Am. Chem. Soc*. 1997, 119, 11347
[8] S. Mathison, E. Bakker, *Anal. Chem*. 1998, 70, 303
[9] Y. Qin, S. Peper, E. Bakker*, Electroanalysis* 2002, 14, 1375
[10] E. Malinowska, V. Oklejas, R.W. Hower, R.B. Brown, M.E. Meyerhoff, *Sens. Actuators*, B 1996, 33, 161
[11] N. Oyama, T. Ohsaka, F. Yoshimura, M. Mizunuma, S. Yamaguchi, N. Ushizawa, T. Shimomura, *J. Macromol. Sci. Chem.* 1988, A25, 1463
[12] A. Cardogan, Z.Q. Gao, A. Lewenstam, A. Ivaska, D. Diamond, *Anal. Chem*. 1992, 64, 2496
[13] K.Y. Chumbimuni-Torres, Z. Dai, N. Rubinova, Y. Xiang, E. Pretsch, J. Wang, E. Bakker, *J. Am. Chem. Soc*. 2006, 128, 13676

2. Methods

2.1. Potentiometric immunoassays

Two different potentiometric measuring schemes were developed for immunoassay detection (Figure 1 and Figure 2). In both assays, i.e. in the enzyme immunoassay carried out in microtiter plates and in the nanoparticle-based immunoassay in paper, the label (enzyme and nanoparticle, respectively) of the tracer reagent ensures the signal amplification and in the same time via generating ions the possibility of the potentiometric detection. The results measured by the optical and potentiometric techniques were compared side-by-side.

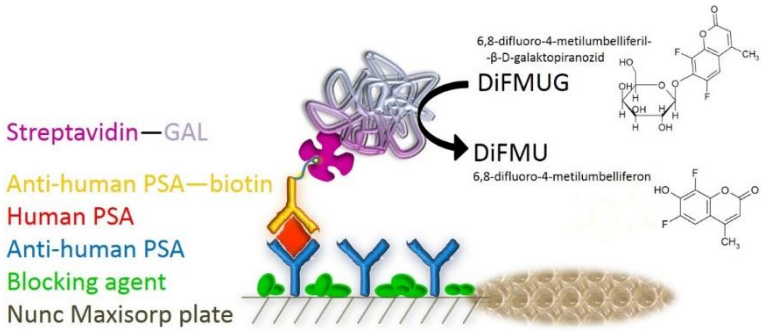

Figure 1 *Schematics of the potentiometric sandwich prostate specific antigen (PSA) assay performed in microtiter plates. The potentiometric detection is based on measuring anions generated by the enzymatic reaction. A simple anion-exchanger based microelectrode is used for this purpose.*

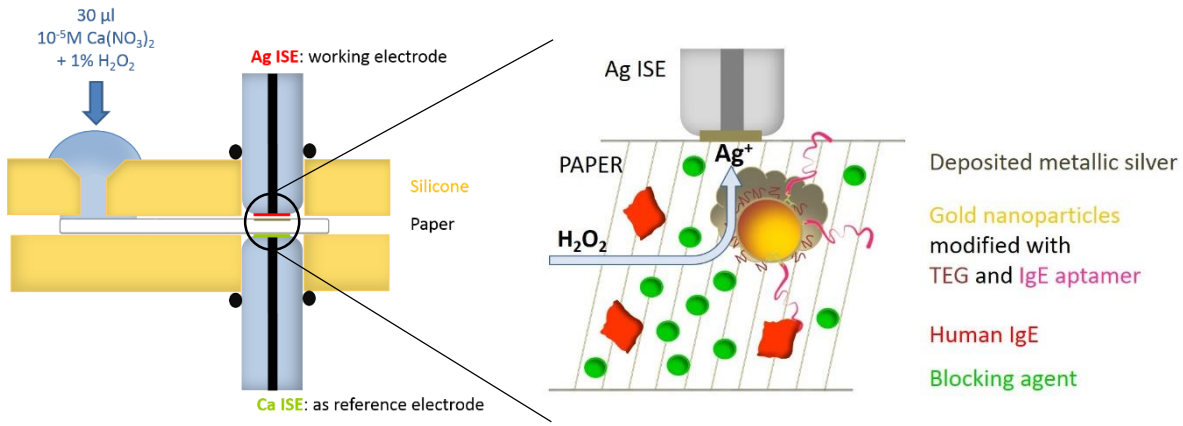

Figure 2 *Potentiometric measuring setup and schematics of the dot-blot assay for IgE detection. For the detection of silver ions generated in paper the paper strip is sandwiched between two silicone rubber sheets with the dot positioned in the midst of the silver-selective electrode and the pseudo-reference calcium-selective electrode. The potentiometric detection is based on measuring silver ions directly in the paper matrix. The ions are released by oxidative dissolution from a silver layer deposited on gold nanoparticle-conjugated bioreagents.*

2.2. Preparation of solid-contact silver-selective electrodes

Various possibilities were explored to fabricate solid-contact silver ion-selective electrodes (AgSCISEs) with low detection limits, good long-term potential stability and reproducible electrode-to-electrode E^0 standard potentials. The solid-contact silver-selective electrodes

were fabricated using silicone rubber (SR)-based ion-selective membrane. As ion-to-electron transducer two different conducting polymer, i.e. polyaniline (PANI) and PEDOT(PSS) ((poly(3,4-ethylenedioxythiophene) with poly(styrenesulfonate)) was utilized.

2.3. Nanosphere lithography

In the respective studies ordered conducting polymer nanostructures were fabricated by nanosphere lithography. 2D and 3D ordered PEDOT(PSS) films with 746 and 750 nm interconnected pores, respectively, were synthesized as shown in Figure 3. Polystyrene (PS) beads were deposited on the substrate under controlled conditions, followed by the potentiostatic deposition of PEDOT(PSS) within the voids of the particle array. After the electropolymerization, the PS template was dissolved away in toluene.

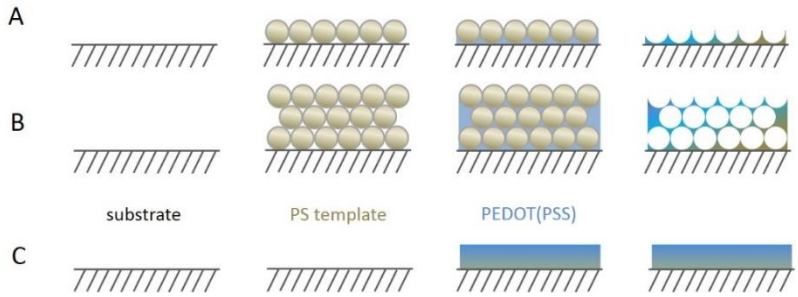

Figure 3 *Scheme of (A) 2D and (B) 3D ordered, and (C) compact PEDOT(PSS) film synthesis*

3. Results

3.1. Potentiometric enzyme immunoassay

A potentiometric ELISA assay was worked out to detect human prostate specific antigen (PSA) in real serum samples in microtiter plate wells (see also Figure 1). The measuring scheme was based on the idea that if sufficiently lipophilic anions are generated by an enzymatic reaction, a simple and cost-effective anion-exchanger electrode could be used for their detection. The choice of measuring anions was determined by the fact that cations in bioassays often get adsorbed and/or complexed by biomolecules, leading to decreased ion activities and thus to insufficient detection limits. The sandwich assay involved the potentiometric detection of the anion 6,8-difluoro-4-methylumbelliferone (DIFMU), product of the hydrolysis reaction catalysed by the galactosidase (GAL) enzyme label of the tracer antibody.

The $DIFMU^-$ calibration curves were recorded in 150 µl volume in the microtiter plate wells (Figure 4a). Since the $DIFMU^-$ detection is performed after the enzymatic reaction is terminated with a stop reagent, calibrations were done in the presence of both 1 mM NaOH

(Figure 4b) and 2 mM $CuSO_4$ (Figure 4c). The use of NaOH is less favourable as it led to sub-Nernstian response slopes (possibly due to anion interference) and much higher LDL. In contrast, the addition of $CuSO_4$ resulted in a linear super-Nernstian response of $DIFMU^-$ (~105 mV/decade), which has proved to be reproducible, and which considerably increased the sensitivity of the determination.

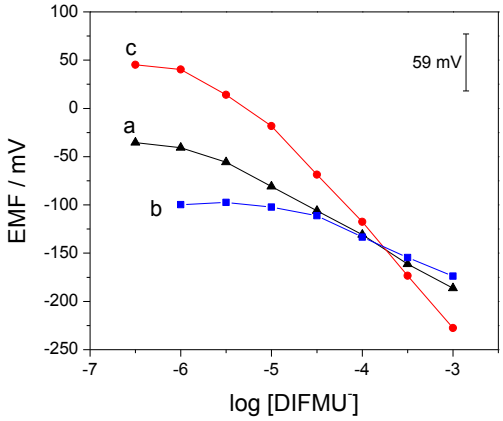

Figure 4 *Calibration curves for $DIFMU^-$ in microtiter plates, i.e. in 150 µl sample volume: (a) in the working buffer (1 mM phosphate buffer with 1 mM $MgSO_4$ at pH7.7), (b) in the same working buffer but with 1 mM NaOH and (c) in the same working buffer but with 2 mM $CuSO_4$.*

The potentiometric ELISA detection of PSA from human serum gave linear semilog calibration in the range of 0.1–50 ng/ml, as shown in Figure 5B. It provides a sufficiently low detection limit (≤0.1 ng/ml), which complies with the requirements of *in vitro* diagnostic PSA assays. When comparing these results with the conventional optical detection of the same assay (Figure 5A), there was no significant difference at the 95 % confidence level between the two methods. Although the multiplexation capabilities of potentiometric detection can hardly compete with the highly parallel manner in which optical assays are performed in the laboratory, they can easily have a niche for point-of-care diagnostic applications.

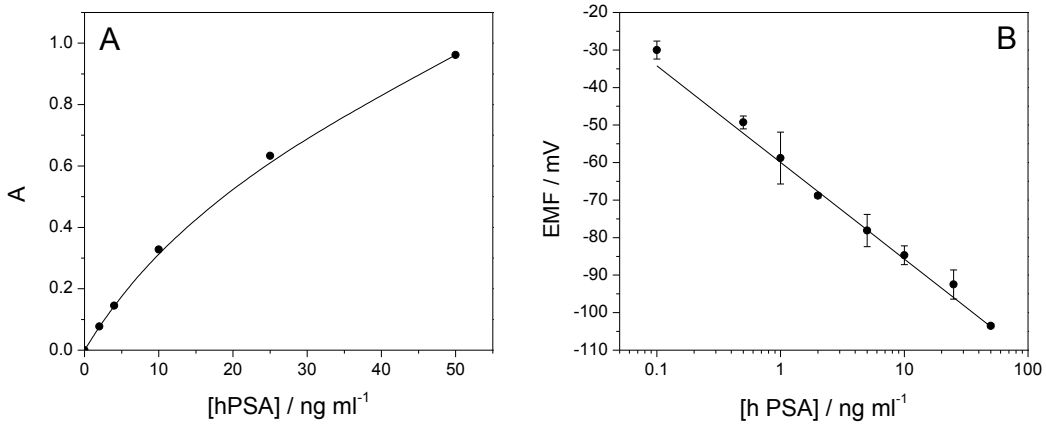

Figure 5 *Calibration curves for PSA in human serum background using ELISA assays with (A) optical absorbance and (B) potentiometric detection.*

3.2. Paper-based potentiometric bioassay

The proof of concept of potentiometric transduction for quantitative paper-based bioassays was demonstrated by placing the ISEs directly on the surface of the wet paper and measuring ions in the solution phase entrapped within the paper (see Figure 2).

As proof of principle a dot-blot model assay was developed for the quantitative determination of human IgE. The target protein is detected by the labelled, highly specific IgE aptamer – gold nanoparticle conjugate. As gold nanoparticles are used light pink to reddish dots are developed on the paper in proportion to the protein concentration, perceptible even by the naked eye and quantifiable after recording with a flatbed scanner. To further amplify the signal a silver enhancement method is used, based on the selective autocatalytic deposition of a metallic silver layer nucleated by the gold nanoparticles and resulting in dark brown to black dots (Figure 6). On the other hand, by oxidative dissolution silver ions can be generated from the metallic silver enabling the potentiometric detection of the assay as well. During the two different detection methods the exact same dots are measured consecutively, and thus the differences in analytical performance reflects solely the performance differences of the respective techniques.

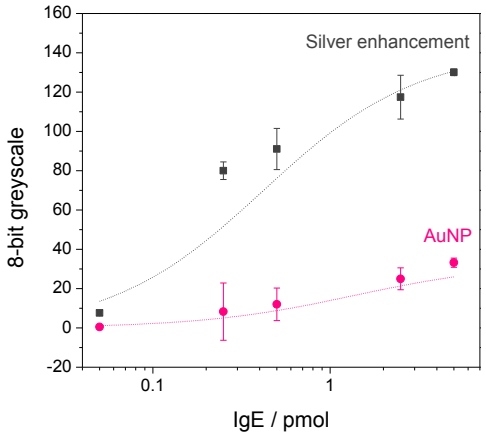

Figure 6 *Eight bit greyscale values versus the IgE amount in the calibration spots recorded with a flatbed scanner after application of the IgE aptamer-linked AuNP and further amplification with the silver enhancement reagent. The data were fitted with a 4-parameter logistic curve.*

For studying the quantitation of silver ions directly in the nitrocellulose, calibrations were performed by wetting the paper strips, sandwiched between the electrodes, with solutions containing different $AgNO_3$ concentrations but a steady 10^{-5} M $Ca(NO_3)_2$ and 1 % H_2O_2 background. The resulting calibration curve (Figure 7Ab) was Nernstian, however the detection limit was somewhat better than 10^{-5} M, which result lag by 2.5 orders of magnitude behind that of calibrations measured with the same electrodes in stirred 100 ml solutions (Figure 7Aa).

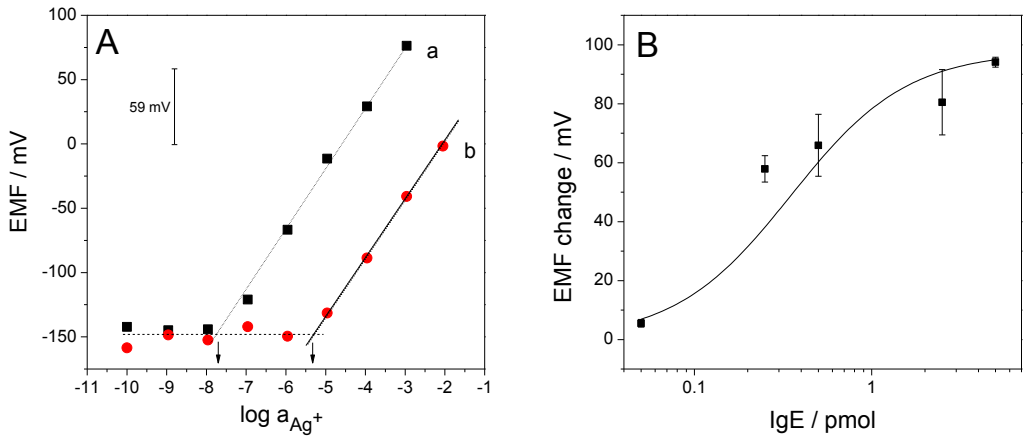

Figure 7 *(A) Potentiometric calibration curves for silver ion (a) in stirred solution phase and (b) in paper. (B) Calibration curve for the potentiometric IgE dot-blot assay.*

The result of the potentiometric dot-blot assay for human IgE is shown in Figure 7B**Error! Reference source not found.**. Despite of the higher detection limit for the calibration in paper, the lowest detectable amount of IgE for both potentiometric and optical detection was the same, 50 fmol, in the exact same conditions. This suggests that potentiometric detection can be a viable alternative to the conventionally used optical detection.

3.3. Silicon rubber-based solid-contact ISEs

In SCISEs the elimination of the inner filling solution can avoid leaching of the primary ion and hence help to lower the detection limit. Possible leakage, however, from the ISM itself still remains. In this respect, to lower the LOD, it is especially important to use membranes characterized by low ion diffusion rates. In this work, for the first time, conducting polymer based solid-contact electrodes with silicone rubber-based membranes were studied. As the solid contact of AgSCISEs a polyaniline nanoparticle dispersion, PANI D1003, was utilized, while as membrane matrix room temperature vulcanising silicon rubber, RTV 3140, was used. First, the unbiased selectivity coefficients were determined as summarized in

Table **1**. Although previous studies for PVC[14] and MMA-DMA-based[15] membranes using the same silver ionophore (last two columns) reported on excellent selectivities, mostly around 10^{-10}, the SR-based electrodes showed significantly better results, approaching 10^{-16} for divalent and 10^{-14} for monovalent ions. These results are outstanding, exceeding any selectivities reported before.

Table 1 *Unbiased selectivity coefficients ($\log K_{Ag,j}^{pot}$) of the SR and plasticized PVC-based SCISEs, measured by the separate solution method at 1 mM level. *Typical standard deviations for the selectivities were ≤0.6 units.*

	SR	PVC		MMA-DMA
	SCISE*	SCISE	ISE †	SCISE ‡
J	5% DOS	56% DOS	56% o-NPOE	-
Na^+	-14.9	-10.4	-11.5	-10.7
K^+	-14.6	-6.6	-7.7	-10.2
Mg^{2+}	-16.6	-8.4	-10.9	
H^+	-13.5	-6.1	-10.9	-10.2
Ca^{2+}	-16.5	-7.7	-12.9	-12.3
Cu^{2+}	-13.9	-9.7	-8.2	-11.1

† The values were taken from reference[14] for the same ionophore, measured with liquid contact electrodes with PVC membrane plasticized with o-NPOE.
‡ The values were taken from reference[15] for the same ionophore, measured with solid-contact electrodes with MMA-DMA membrane and POT solid-contact.

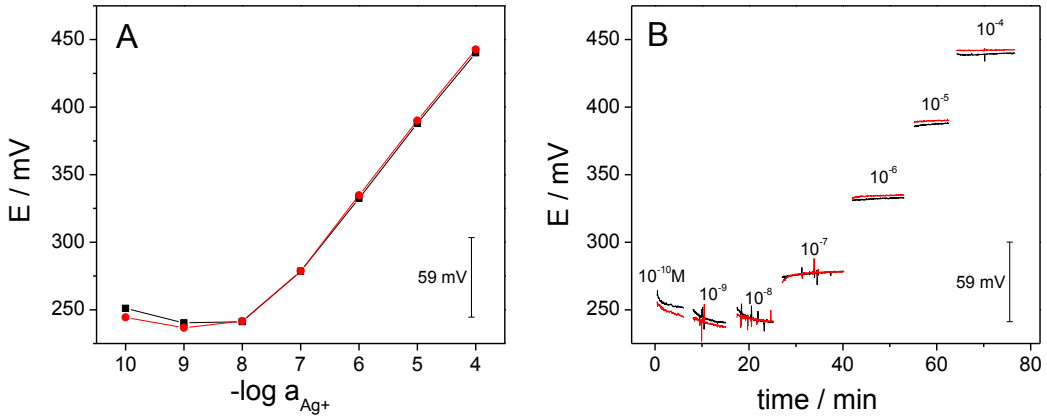

Figure 8 *(A) Calibration curves and (B) the corresponding potential traces of two identically prepared AgSCISEs conditioned in 1 nM $AgNO_3$.*

Figure 8A and B shows the potential traces and the corresponding calibration curves of two identically prepared SCISEs. The detection limit was 2×10^{-8} M. Most remarkably, the solid-contact electrodes showed excellent potential reproducibility between the electrodes. The potential traces of the SCISE calibration curve (Figure 8B) showed, that the potential in $AgNO_3$ solutions stabilize within a minute to ≤ 1 mV at concentrations ≥ 10^{-7} M. The almost noiseless potentials of the SCISEs at higher concentrations show the efficiency of the PANI-based SC as ion-to-electron transducer.

[14] Z. Szigeti, A. Malon, T. Vigassy, V. Csokai, A. Grun, K. Wygladacz, N. Ye, C. Xu, V. Chebny, I. Bitter, R. Rathore, E. Bakker, E. Pretsch, *Analytica Chimica Acta* 2006, *572*, 1-10.
[15] K. Y. Chumbimuni-Torres, N. Rubinova, A. Radu, L. T. Kubota, E. Bakker, *Analytical Chemistry* 2006, *78*, 1318-1322.

3.4. Ion-selective electrodes with 3D ordered solid contact

Solid contact materials with high surface area have subsequently high capacitances leading to remarkable long-term potential stabilities of the SCISEs. The feasibility of unifying the benefits of large surface area nanostructures with the well-defined ion-to-electron transduction and well controllable and rapid fabrication that conducting polymers offer was explored. Therefore, 3D ordered PEDOT(PSS) nanostructures were prepared using nanosphere lithography as shown in Figure 3 (Figure 9). The voids created in the 3D polymer film were loaded by an equimolar ratio of the oxidized and reduced form of a redox mediator 1,1'-dimethylferrocene (DMFe) to control the phase boundary potential at the SC/ISM interface, to achieve good electrode-to-electrode reproducibility of E^0. The properties of both the nanostructured conducting polymer and that of the AgSCISEs with the redox couple infiltrated 3D PEDOT(PSS) solid contacts were investigated.

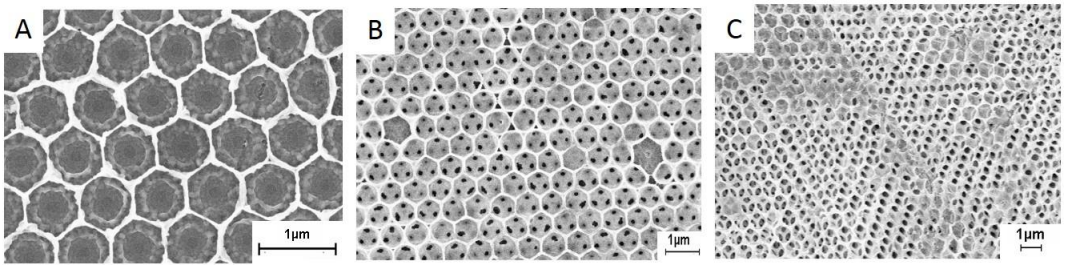

Figure 9 *SEM images of (A) one layer, (B) 3 layers and (C) 30 layers thick PEDOT(PSS) films after removal of PS beads (ø 746 nm) used as template.*

As Figure 10B shows, in contact with a non-aqueous solvent and bulky counterions able to penetrate the porous 3D structure of PEDOT(PSS), which is of relevance for the SCISE systems, 3D ordered polymer films exhibit a linear increase of their capacitance as a function of the film thickness. The thickest 3D films have about 7 times higher capacitance than their compact counterparts, which shows a clear advantage for the use of nanostructured PEDOT(PSS) layers.

To test the analytical performance of the different AgSCISE structures, three identically prepared electrodes were made for each of the 4 following electrode types: (i) without redox couple GC/3D PEDOT(PSS)/ISM, GC/compact PEDOT(PSS)/ISM and (ii) with redox couple GC/3D PEDOT(PSS)/DMFe/ISM, GC/compactPEDOT(PSS)/DMFe/ISM. For comparison coated wire electrodes (CWEs) were also prepared by applying the membrane cocktail directly onto the GC electrodes: GC/ISM, GC/redox couple/ISM.

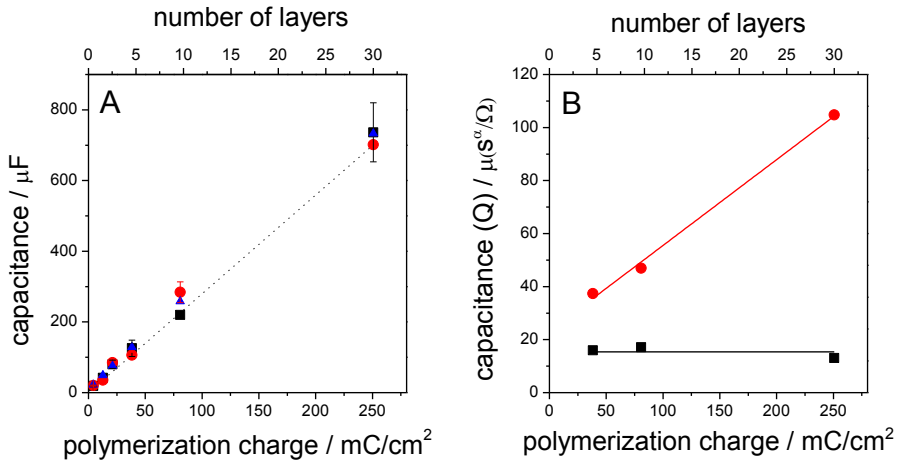

Figure 10 *Capacitances as a function of polymerization charge and polymer thickness for GC/PEDOT(PSS) electrodes measured in (A) 1 mM KCl and (B) acetonitrile solution of 1 mM ETH500. Electrodes with (square) compact and (circle) 3D nanostructured PEDOT(PSS) conducting polymer. The values are obtained by fitting EIS experimental data to the corresponding equivalent circuits. (A) The capacitance values measured in 1 mM KCl do not show significant difference between the two types of PEDOT(PSS) layers, and they are in good agreement with (triangle) the literature[16]. (B) Meanwhile in acetonitrile using bulky electrolyte ions there is a clear capacitance difference in the advance of the 3D ordered polymer.*

The redox couple infiltrated in the 3D polymer structure acts as an internal reference standard, controlling the interfacial potential between the SC and the ISM. DMFe loading thus provides better electrode-to-electrode reproducibility of the E^0 values for SCISEs with 3D PEDOT(PSS) layer, as shown in Figure 11 c and d. The standard deviation of E^0 for electrodes with PEDOT(PSS) polymerized with 21.2 and 250.7 mC/cm^2 was ± 5.4 and 3.9 mV, respectively. These values are 8 times smaller than those for the same 3D ordered SCs but without DMFe loading, ± 43.4 and ± 31.4 mV, respectively (Figure 11 a and b).

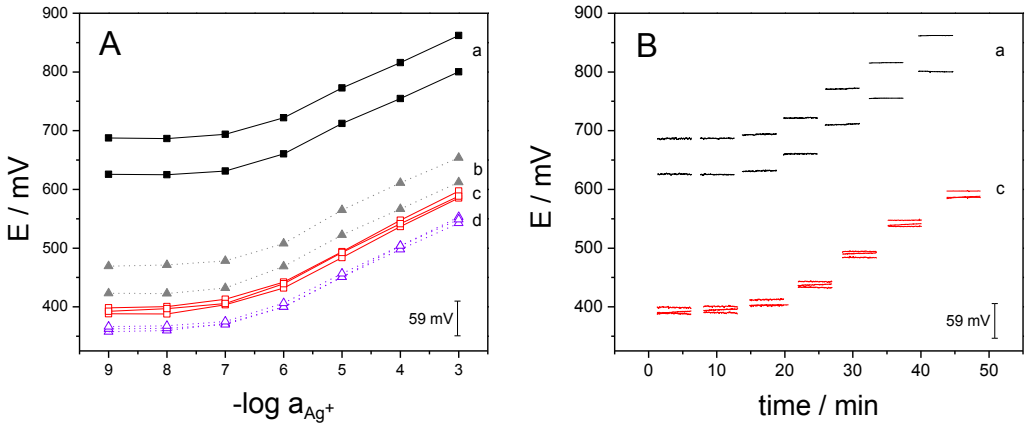

Figure 11 *(A) Calibration curves and (B) their corresponding potential traces of identically prepared GC/3D PEDOT(PSS)/ISM SCISEs with different polymer thicknesses, (a, c) 3 layers and (b, d) 30 layers tick; (a, b) without and (c, d; 3-3 identical) with filling the nanostructured polymer with redox couple.*

[16] J. Bobacka, A. Lewenstam, A. Ivaska, *Journal of Electroanalytical Chemistry* 2000, *489*, 17-27.

Due to their ill-defined phase boundary potentials CWE electrodes were exhibiting large potential drifts, up to 476 µV/s. Meanwhile, electrodes with 3D ordered PEDOT(PSS) filled with the redox couple showed good stability. The drift of GC/3D PEDOT(PSS)/DMFe/ISM electrodes was 4.4 times smaller than their DFM-free counterparts (19.8 and 10.6 vs. 87.7, 46.9 µV/s , respectively) and 1.5 times smaller than those with DMFe but compact polymer film (19.8 and 10.6 vs. 28.7, 15.4 µV/s, respectively). The electrodes with the thickest nanostructured PEDOT(PSS) showed the smallest potential drift, 10.6 ± 2.3 µV/s.

3.5. 2D ordered PEDOT(PSS) polymer for protein recognition

The aim was to create surface-imprinted polymeric nanostructures by nanosphere lithography, as selective recognition sites for the target protein on the enlarged surface of the polymer film. To obtain maximum imprinted to non-imprinted surface ratio, based on simple geometrical calculations and assuming uniform growth of the film, a polymer thickness of 395 nm, slightly higher than the half-height of the beads (375 nm) is desired. The optimal experimental conditions for the polymerization were determined empirically. Films of different thicknesses were prepared by controlling the electrical charge during the polymerization and examining the patterned polymer layers with atomic force microscopy (AFM) after the dissolution of the beads, as shown in Figure 12.

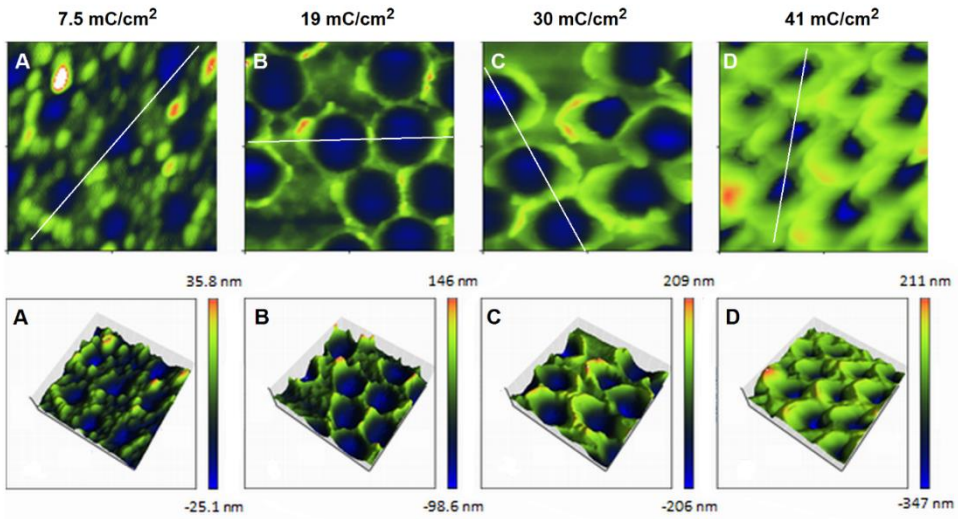

Figure 12 *AFM images showing the surface topography of PEDOT(PSS) films prepared by nanosphere lithography using 750 nm diameter beads. Scans (A-D) are representative of 2.2 µm × 2.2 µm areas of patterned PEDOT(PSS) films prepared using 7.5, 19, 30, and 41 mC/cm^2 surface charge densities, respectively.*

AFM measurements revealed preferential growth of the PEDOT(PSS) film alongside the particles, so the average polymer thicknesses (Figure 13) were calculated based on the exposed diameter of the cavity, measured on several AFM images of different parts of the corresponding

layers. As Figure 13 shows, the control over the polymer thickness between 250 and 500 nm is the most critical. To ensure a better control and be less affected by preferential growth around the beads, a somewhat lower charge density, 17 mC/cm^2, was chosen.

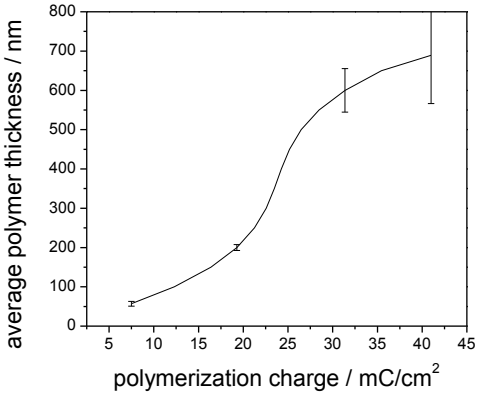

Figure 13 *Theoretical correspondence between the average polymer thickness and the applied polymerization charge based on the AFM measurements, assuming uniform growth of the film.*

4. Thesis points

1. I developed a novel potentiometric enzyme-linked immunosorbent assay (ELISA) for the detection of prostate specific antigen in human serum samples. The assay scheme was based on measuring an anionic product of the enzyme label with anion-exchanger based minielectrodes. [Paper I.]

2. I made the proof of principle for potentiometric detection of microfluidic paper-based bioaffinity assays by measuring silver ions directly in the wet paper matrix. The silver ions were generated from dissolution of the metallic silver layer deposited on the gold surface of the aptamer-gold nanoparticle conjugates used as biorecognition element in the human IgE assay. [Paper II.]

3. I prepared solid-contact silver-selective electrodes with outstanding selectivities exceeding any selectivities reported before, good E^0 reproducibility and ultratrace detection limit. As ion-to-electron transducer layer I used polyaniline nanoparticles and for the ion-selective membrane matrix I utilized room temperature vulcanizing silicon rubber. [Paper III.]

4. I fabricated two- and three dimensionally ordered polymeric nanostructures of PEDOT(PSS) conducting polymer by nanosphere lithography for the first time. I optimized the thickness of the 2D patterned polymer for subsequent application in surface imprinted polymers as biorecognition element and utilized the 3D ordered PEDOT(PSS) as high surface area ion-to-electron transducer in ion-selective electrodes. [Paper IV. and V.]

5. I found the use of 3D ordered PEDOT(PSS) conducting polymer, loaded with a lipophilic redox mediator as large surface area solid-contact in silver-selective electrodes beneficial in terms of E^0 reproducibility and long term potential stability of the electrodes. [Paper IV.]

5. List of publications

Full papers:

I. **J. Szűcs**, E. Pretsch, R.E. Gyurcsányi, *Potentiometric enzyme immunoassay using miniaturized anionselective electrodes for detection,* Analyst, 2009, 134 (8), 1601-1607 (IF: 3.272, I: 15)

II. **J. Szűcs**, R. E. Gyurcsányi, *Towards protein assays on paper platforms with potentiometric detection,* Electroanalysis, 2012, 24 (1), 146-152 (IF: 2.817, I: 11)

III. T. Lindfors, **J. Szűcs**, F. Sundfors, R. E. Gyurcsányi, *Polyaniline Nanoparticle Based Solid-Contact Silicone Rubber Ion-Selective Electrodes for Ultra-Trace Measurements,* Analytical Chemistry, 2010, 82 (22), 9425-9432 (IF: 5.874, I: 29)

IV. **J. Szűcs**, T. Lindfors, J. Bobacka, R. E. Gyurcsányi, *Ion-selective electrodes with 3D nanostuctured polymer solid contact,* Electroanalysis, 2015, DOI: 10.1002/elan.201500465 (IF [2014]: 2.138, I: -)

V. J. Bognár, **J. Szűcs**, Zs. Dorkó, V. Horváth, R. E. Gyurcsányi, *Nanosphere Lithography as a Versatile Method to Generate Surface-Imprinted Polymer Films for Selective Protein Recognition,* Advanced Functional Materials, 2013, 23 (37), 4703-4709 (IF: 10.439, I: 14)

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1. **J. Szűcs**, G. Lautner, R. E. Gyurcsányi, V. Bardóczy, T. Mészáros, K. Tóth, *Development of biochips for surface plasmon resonance imaging of aptamer-ligand interactions,* YISAC, 14th Young Investigators' Seminar on Analytical Chemistry, June 2007, Pardubice, Check Republic
2. **J. Szűcs**, R. E. Gyurcsányi, T. Lindfors, *Potentiometric immunoassay based on sequential flow injection analysis,* 17th Young Investigators' Seminar on Analytical Chemistry, June 2010, Venice, Italy
3. **J. Szűcs**, T. Lindfors, R. E. Gyurcsányi, *Nanosphere lithography patterned solid contact ion-sensors,* 10th International Conference on Colloid Chemistry, Aug. 2012, Budapest, Hungary
4. R. E. Gyurcsányi, **J. Szűcs**, L. Höfler, T. Vigassy, E. Pretsch, *Potentiometric bioassays,* PITTCON, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 2008, New Orleans, USA

5. R. E. Gyurcsányi, Gy. Jágerszki, L. Höfler, **J. Szűcs**, G. Lautner, K. Tóth, I. Bitter, A. Grün, *Ion Transport and Nanostructures-Assisted Potentiometry,* Mátrafüred '08, International Conference on Electrochemical Sensors, Oct. 2008, Dobogókő, Hungary
6. R. E. Gyurcsányi, G. Lautner, Gy. Jágerszki, **J. Szűcs**, L. Höfler, A. Menaker, V. Syritski, *Nanostructures and Synthetic Ligands Assisted (Bio)sensing,* 10th International Symposium on Applied Bioinorganic Chemistry, Sept. 2009, Debrecen, Hungary
7. J. Bognár, **J. Szűcs**, Zs. Dorkó, V. Horváth, R. E. Gyurcsányi, *Nanosphere lithography as a versatile method to generate surface-imprinted polymer films for selective protein recognition,* Graduate Student Symposium on Molecular Imprinting, Aug. 2013, Belfast, U.K

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2. **J. Szűcs**, G. Lautner, R. E. Gyurcsányi, K. Tóth, *Biopolimereken és szintetikus analógjaikon alapuló bioszenzorok fejlesztése,* VII. Balatonfüredi Téli Iskola, Jan-Feb. 2007, Balatonfüred, Hungary
3. **J. Szűcs**, Zs. Balogh, R. E. Gyurcsányi, K. Tóth, *Biokatalitikus jelerősítésen alapuló nagyérzékenységű felületi plazmonrezonanciás immunoassay,* Oláh György Doktori Iskola, IV. Doktoráns Konferencia, Feb. 2007, Budapest, Hungary
4. **J. Szűcs**, T. Lindfors, F. Sundfors, R. E. Gyurcsányi, *Polianilin nanodiszperziós szilárd belső-elvezetésű szilikongumi membránú ionszelektív elektródok nagy érzékenységű ezüstion mérésre,* Kémiai Szenzorok Kutatásának Eredményei IV. Workshop, Oct. 2010, Pécs, Hungary
5. **J. Szűcs**, T. Lindfors, R. E. Gyurcsányi, *Nanoszféra litográfiával mintázott szilárd belső elvezetésű ion-szenzorok,* A szenzorkutatás újabb eredményei Workshop V., Apr. 2013, Pécs, Hungary
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7. R. E. Gyurcsányi, Gy. Jágerszki, G. Lautner, V. Syritski, **J. Szűcs**, L. Höfler, *Nanoszerkezeteken és szintetikus ligandumokon alapuló miniatürizált analitikai rendszerek,* Kémiai Szenzorok Kutatásának Eredményei II. Workshop, Nov. 2006, Pécs, Hungary
8. J. Bognár, **J. Szűcs**, Zs. Dorkó, V. Horváth, R. E. Gyurcsányi, *Szelektív fehérje-felismerés nanoszféra-litográfián alapuló molekuláris lenyomatképzéssel,* Oláh György Doktori Iskola, X. Doktoráns Konferencia, Febr. 2013, Budapest, Hungary
9. J. Bognár, **J. Szűcs**, Zs. Dorkó, V. Horváth, R. E. Gyurcsányi, *Szelektív fehérje-felismerés nanoszféra-litográfián alapuló molekuláris lenyomatképzéssel,* A szenzorkutatás újabb eredményei Workshop V., Apr. 2013, Pécs, Hungary

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1. **J. Szűcs**, E. Pretsch, R. E. Gyurcsányi, *Potentiometric ELISA for the determination of PSA in human serum,* Mátrafüred '08, International Conference on Electrochemical Sensors, Oct. 2008, Dobogókő, Hungary
2. **J. Szűcs**, N. Batjargal, P. Stangl, R. E. Gyurcsányi, *Towards potentiometric dot-blot assay,* Mátrafüred '11, International Conference on Electrochemical Sensors, June 2011, Dobogókő, Hungary
3. **J. Szűcs**, J. Bobacka, T. Lindfors, R. E. Gyurcsányi, Ion-selective *electrodes with three-dimensionally ordered conducting polymer- and redox couple based solid contact,* 4th International Conference on Bio-Sensing Technology, May 2015, Lisbon, Portugal
4. G. Lautner, **J. Szűcs**, R. E. Gyurcsányi, Zs. Balogh, V. Bardóczy, B. Komorowska, T. Mészáros, *Selective Detection of Plant Virus Coat Proteins by Aptamer Based Biochips,* Mátrafüred 08, International Conference on Electrochemical Sensors, Oct. 2008, Dobogókő, Hungary
5. G. Lautner, **J. Szűcs**, R. E. Gyurcsányi, Zs. Balogh, V. Bardóczy, B. Komorowska, T. Mészáros, *Selective Detection of Plant Viruses by Aptamer Based Biochips,* Oláh György Doktori Iskola, VI. Doktoráns Konferencia, Feb. 2009, Budapest, Hungary
6. G. Lautner, **J. Szűcs**, R. E. Gyurcsányi, Zs. Balogh, V. Bardóczy, B. Komorowska, T. Mészáros, *Selective Detection of Plant Viruses by Aptamer Based Biochip,* Mátrafüred 11, International Conference on Electrochemical Sensors, June. 2011, Dobogókő, Hungary
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8. J. Bognár, **J. Szűcs**, Zs. Dorkó, V. Horváth, R. E. Gyurcsányi, *Selective protein recognition with surface molecularly imprinted polymer films prepared by nanosphere lithography,* Mátrafüred '14, International Conference on Electrochemical Sensors, June 2014, Visegrád, Hungary

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1. G. Lautner, **J. Szűcs**, R. E. Gyurcsányi, V. Bardóczy, T. Mészáros, *Aptamereken alapuló SPR biochipek fejlesztése növényi vírusok meghatározására,* Oláh György Doktori Iskola, V. Doktoráns Konferencia, Feb. 2008, Budapest, Hungary

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