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Development of a Biomolecular Analyzer Based on Ion-conducting Nanopores: The *iNAPO* Project

Wolfgang Ensinger
Technical University of Darmstadt
Department of Materials Science
Alarich-Weiss-Str. 2, 64287 Darmstadt, Germany
ensinger@ma.tu-darmstadt.de

Abstract **–** Analyzing biologically relevant molecules is an important aspect of medical diagnostics. The *iNAPO* project aims at developing a device that is able to specifically detect and quantitatively analyze biomolecules. An example is tumor markers that are emitted by the tumor at elevated levels and can be found in body liquids. The analyzer device core consists of a polymer foil containing a nanopore. In analogy to biological nanopores that control e.g. mass transfer into and out of a cell, the biomimetic artificial nanopore allows the passage of ions of an aqueous electrolyte in an electrochemical two-compartment cell from one compartment to the next one under the influence of an electrical field. When the nanopore wall contains certain immobilized molecules that specifically react with the biomolecules to be analyzed, the ionic current is influenced. The difference in the current can directly be correlated to the presence and the quantity of the biomolecule. As an example, the measurement of small quantities of histamine, the well known neurotransmitter, is shown.

Keywords**:** Nanopores, Ion Conduction, Track Etching Technique, Biomolecular Sensor, Neurotransmitter, Histamine.

1. Introduction

Ion conducting nanopores (*iNAPOs*) regulate the flow of ions and small molecules through the cell membrane into and out of the cells of living organisms [1]. Often, ion transport through the nanopore is regulated by means of an external stimulus, such as light, temperature, or a biomolecule. This feature allows using nanopores as molecular sensor. Biological nanopores, however, are carried by the fragile lipid bilayer membrane that is not suitable for technical application and are, therefore, replaced by solid state nanopores [2,3]. The *iNAPO* project aims at fabricating nanopores in polymer foil, in a biomimetic approach, for use in biomedical sensing and analyzing devices [4]. In the *iNAPO* analyzer device, the ionic transport is measured electrochemically. The ionic flux through the nanopore is dependent on the nanopore dimension and the electrical surface charge. These, in turn, depend on the presence of certain biomolecules to be analyzed, when a suitable set-up and chemical reaction is used. The principles of this method are explained in the following.

As an example of biomolecular sensing, results on quantitative measurements of histamine are shown [5]. Histamine constitutes an important neurotransmitter in the central nervous system [6]. It plays a role in physiological functions such as inflammation, allergic reactions, thermoregulation, gastric acid secretion, sleep/wake and arousal [7,8]. Reduced levels of histamine are found in case of Alzheimer, while Parkinson's patients show enhanced levels. It is, therefore, of interest to be able to analyze small quantities of histamine.

2. Experimental

12 µm thick polyethylene terephthalate (PET) foils (Hostaphan RN 12, Hoechst) were irradiated with single highly charged gold ions of a kinetic energy of 2.2 GeV at the linear heavy ion accelerator UNILAC of GSI Helmholtz-Center of Heavy Ion Research, Darmstadt. The ion damage track, a cylindrical linear zone through the polymer foil with reduced density and changed chemical composition, was etched in a NaOH solution into a conical pore with a large aperture of several 100 nanometers and a small one of a few nanometers diameter. The electrochemically controlled etch procedure has been described elsewhere [4]. Scanning Electron Micrographs of both apertures of a conical nanopore are shown in Fig. 1.

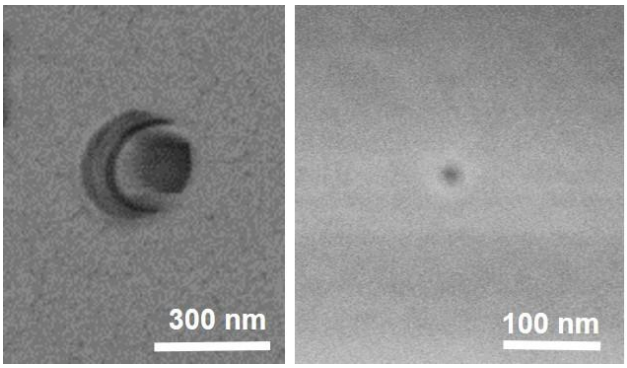

Fig. 1: Scanning electron micrograph of large and small aperture of a conical nanopore in PET foil.

The biochemical analyzer setup is schematically shown in Fig. 2. It consists of a two-compartment electrochemical cell with the polymer foil ***PF*** as a separator. The nanopore ***NP*** within the foil acts as a channel between the two compartments. The cell is filled with a 0.1 molar aqueous solution of potassium chloride (KCl). Each compartment contains a silver / silver chloride electrode ***E***. The electrodes are connected to a picoammeter/voltage source (Keithley 6487, Keithley Instruments), delivering a triangular voltage ***V*** between -1 and +1 V across the cell. The ionic current ***I*** through the nanopore is measured with a sensitive current meter.

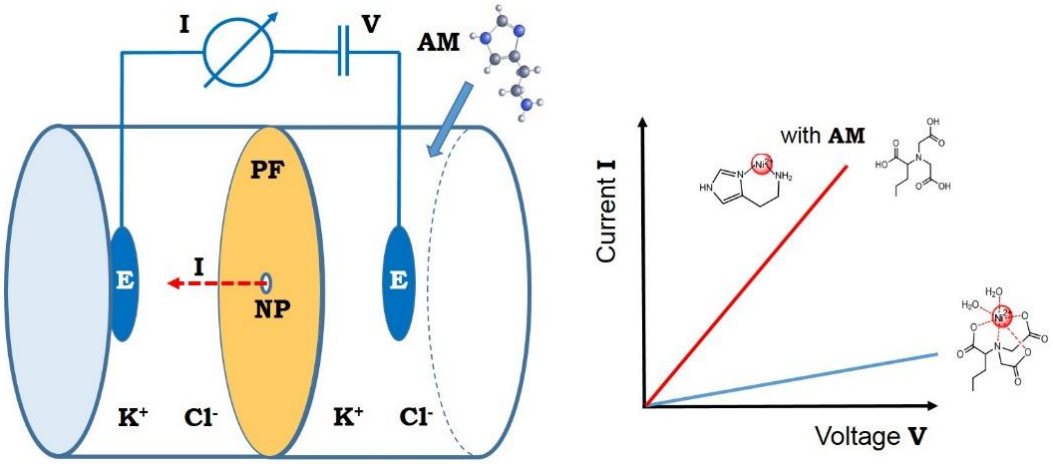

Fig. 2: left: Schematic presentation of electrochemical two-compartment cell: I: current, V: voltage, AM: analyte molecule Histamine, PF: polymer foil, NP: nanopore, E: electrode, K^+ Cl^-: electrolyte ions in water.

In order to have a sensor for Histamine, the nanopore surface has to be functionalized in such a way that that it reacts in a specific way with Histamine. The surface of the polymer is terminated with carboxylate groups (COOH) that have been formed by the nanopore etching process due the hydrolysis of ester bonds in the back-bone of polymer chains. As schematically shown in Fig. 3, they were chemically activated by an ethanolic solution of N-(3-dimethyl-aminopropyl)-N-ethylcarbodiimide (EDC; 100 mM) / pentafluorophenol (PFP; 200 mM). Next, the polymer foil was exposed to a solution of Nα,Nα-bis(carboxymethyl)-L-lysine hydrate (BCML) of 25 mM. After several hours of reaction, the foil was washed several times with ethanol and deionized water. For complexation of Ni(II) an alkaline aqueous $NiSO_4$ solution (100 mM, pH10) was employed. The nickel ions form the chelate complex (NTA)-Ni^{2+} with the nitrilotriacetic acid of BCML (Fig. 3 center). For the analytical measurements, 1 mM aqueous solutions of different neurotransmitters, as well as a series of Histamine solutions from 1 mN down to 1 nM concentration were used.

3. Results and Discussion

Fig. 3 (right hand side) shows the reaction going on inside the nanopore when Histamine is present. It removes the Nickel from the (NTA)-Ni^{2+} chelate, leaving back NTA with its 3 carboxylate groups. This reaction changes the electrolyte ion flux through the nanopore.

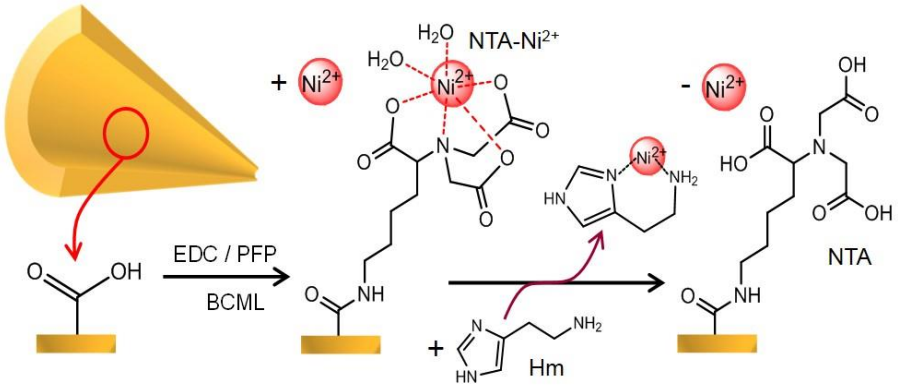

Fig. 3: left: EDC/PFP mediated coupling of BMCL with the COOH groups at the nanopore surface, followed by reaction with Ni^{2+} ions, leads to the NTA-Ni^{2+} chelate complex. In the presence of Histamine Hm, the nickel is displaced from it, leaving back the NTA moiety.

The resulting current/voltage (I/V)-curves are shown in Fig. 4. The nanopore with the NTA-Ni^{2+}-complex (blank) is shown in red. When the control neurotransmitters glycine (Gly), serotonin (5-HT), gamma-aminobutyric acid (GABA) and dopamine (DA) were added, they led to a certain increase of the current, with the current values being similar. In contrast, histamine (Hm) increased the current considerably more than all other neutrotransmitters.

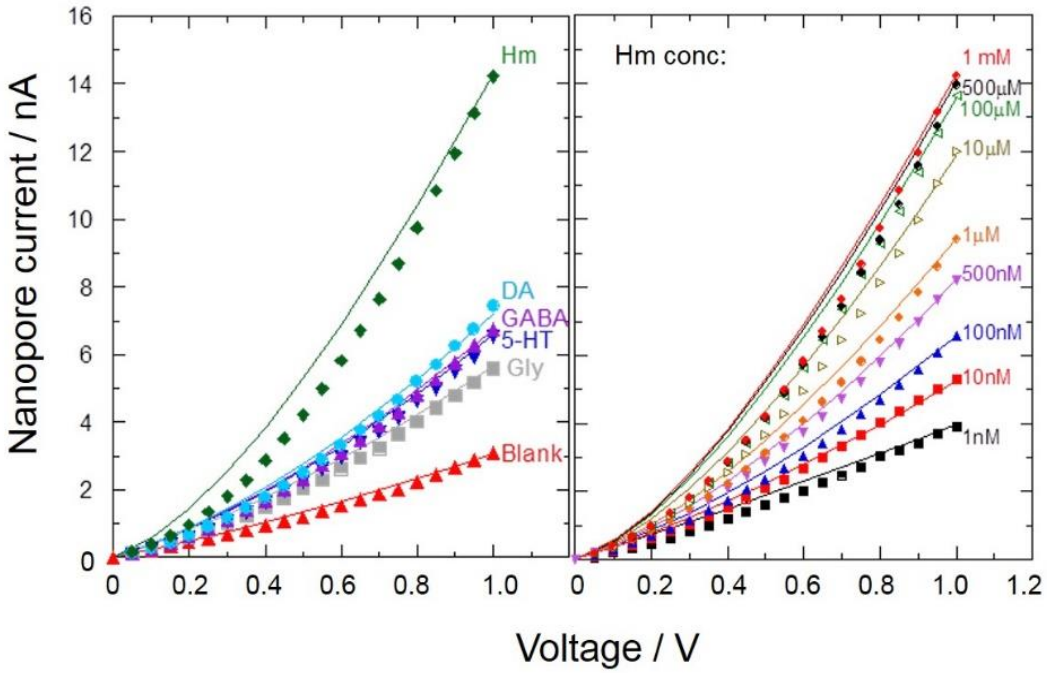

Fig. 4: Current/voltage curves of: left: neurotransmitters histamine (Hm), glycine (Gly), serotonin (5-HT), gamma-aminobutyric acid (GABA) and dopamine (DA) at a concentration of 1 mM; right: histamine at different concentrations; adapted from [5].

The transport of electrolyte ions through the nanopore is influenced by the electrostatic charges located on the nanopore walls that interact with the electrolyte ions. Those are given by the nitrilotriacetic (NTA)-Ni^{2+} complex.

The resulting charge distribution leads to a certain ion current (blank curve). Histamine with its full name 2-(1H-imidazol-5-yl)ethanamine (IUPAC), see Fig. 1, is composed of the imidazole ring with its two nitrogen atoms and a third one on an ethane sidechain with a terminal amine. Two of the nitrogen atoms act as bidentate ligands coordinating the nickel ion to a six membered ring [9]. Since the interaction between the Ni ions and Histamine is stronger than the one to NTA, a decomplexation of the Ni^{2+} ion with the NTA takes place when Hm is present. This leaves back a nanopore surface with changed surface charge, thus allowing the electrolyte ions to pass the nanopore more easily. As a consequence, the current is increased more than 4 times (at a voltage of 1 V). Serotonin does also contain two nitrogen atoms but they do sterically not fit to coordinate nickel. Dopamine, glycine and GABA have only one nitrogen atom. In other words, only Histamine is able to react with the nickel fixed at the nanopore wall and set the NTA moieties free. The I/V-curve shows that Gly, 5-HT, GABA and DA affect the ion current by their presence, but much less than Hm since they are not able to displace Ni from the NTA complex. Hence, the sensor is able to distinguish Hm from other neurotransmitters. The extent of the NTA-Ni^{2+}-decomplexation depends on the concentration of Hm in the solution. This is depiected in Fig. 4, right hand side. The concentration of Hm was changed over 6 orders of magnitude from 1 nM to 10E6 nM (1 mM). The result shows that the analyzer is very sensitive. It is able to analyze the neurotransmitter in the nanomolar range.

4. Conclusion

Robust polymer-based ion conducting nanopores show a large potential as core part of biomolecular sensors or analytical devices for applications in e.g. medical diagnostics or environmental analyses. Nature's nanopores in cell membranes are an excellent model of functionality of switches that control mass transport. Their procedural method can be copied by inverting it: the measured mass transport, here ionic conduction, can be used to monitor the presence of a certain biomolecule of interest and, in a step further, to quantify it. The *iNAPO* project aims at developing biomolecular sensors by fabricating molecular-selective sensitive solid state nanopores and integrating them in microfluidic devices combined with suitable electronics. Thus, a new sensor generation will become available.

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