Cellular membranes have long served as an inspiration for nanomaterial research. The preparation of ultrathin polydopamine (PDA) films with integrated protein pores containing phospholipids and an embedded domain of a membrane protein glycophorin A as simplified cell membrane mimics is reported. Large area, ultrathin PDA films are obtained by electropolymerization on gold surfaces with 10–18 nm thickness and dimensions of up to 2.5 cm². The films are transferred from gold to various other substrates such as nylon mesh, silicon, or substrates containing holes in the micrometer range, and they remain intact even after transfer. The novel transfer technique gives access to freestanding PDA films that remain stable even at the air interfaces with elastic moduli of ≈6–12 GPa, which are higher than any other PDA films reported before. As the PDA film thickness is within the range of cellular membranes, monodisperse protein nanopores, so-called “nanodiscs,” are integrated as functional entities. These nanodisc-containing PDA films can serve as semipermeable films, in which the embedded pores control material transport. In the future, these simplified cell membrane mimics may offer structural investigations of the embedded membrane proteins to receive an improved understanding of protein-mediated transport processes in cellular membranes.

1. Introduction

Nature has evolved cellular membranes with various remarkable features to control material transport within cells with great selectivity. Proteins serve as important components of cellular membranes that impart functionality and ligand-specificity thus regulating, for example, many transport processes[1] or bioenergy production.[2] There has been much interest in the fabrication of synthetic cell membrane mimics with comparable properties, and different materials have been considered including lipid bilayers,[3] block copolymer membranes,[4] vesicles,[5] synthetic channels,[6] or nanoporous membranes.[7] Distinct nanosized objects such as phospholipid nanodiscs with inner lipid bilayers also provide certain properties of cellular membranes. Thus, host membrane proteins could be embedded that retain their structure integrity and functionality.[8,9] Nanodiscs consist of self-assembled lipid bilayers encircled by two amphipathic membrane scaffold proteins (MSP) leading to a discoidal structure with varying diameters from ≈8 to 17 nm.[10]

It would be desirable to generate ultrathin polymeric films with thicknesses comparable to lipid bilayer membrane ranging from 5 to 8 nm[11] containing nanodiscs that could host membrane proteins. However, the preparation of ultrathin and stable polymeric films with such
dimensions is very challenging,[12] and film transfer to other substrates[13–15] without damage as well as the introduction of biological entities into such films still remains elusive. Polydopamine (PDA) is a highly cross-linked polymer formed by the oxidative polymerization of dopamine revealing several similarities to the naturally occurring biopolymers, melanins, and eumelanins.[16] PDA (Figure 1A) is considered biocompatible, it is resistant to a number of reagents, highly adhesive to various materials and surfaces[17] and hence, it has been used for functionalization of many substrates.[18,19] There is a rich chemistry available, via which PDA surfaces could be functionalized, for example, with Schiff base reactions or Michael additions. Freestanding PDA films with a thickness of several hundreds of nanometers or even micrometers have been synthesized before.[20–27] Ultrathin and homogeneous PDA films attached to gold surfaces with thicknesses of 5–20 nm have been prepared by electropolymerization.[28] Electropolymerization is considerably faster than the often applied dip coating procedure, allowing precise control of the film thickness and homogeneity.[20,28–30] Although this mild polymerization technique is restricted to conductive materials, it provides the opportunity to embed non-conductive materials, for example, biomolecules into the PDA film. Until now, applying positive potentials for film removal after electropolymerization only resulted in delamination and destruction of the PDA films.[31] To the best of our knowledge, electropolymerization has never been used to prepare ultrathin freestanding PDA films.

Herein, we present the electropolymerization of large area, ultrathin PDA films with 10–18 nm thickness and dimensions of up to 2.5 cm², exhibiting high elastic moduli (between 6 and

---

**Figure 1.** A) PDA polymerization. The first oxidations steps are also present in the electropolymerization (violet stars) representing the starting reaction for PDA formation. The highly cross-linked PDA is obtained by covalent bonds and supramolecular interactions. B) Typical cyclic voltammogram of PDA electropolymerization on gold. The potential is sweep between −0.5 and +0.5 V with a scan rate of 0.01 V s⁻¹ using a gold counter electrode and an Ag/AgCl reference electrode. The typical catechol to quinone oxidation peak, observed at +0.3 V (violet star), the second oxidation peak (two violet stars) is present −0.2 V. The reduction peaks are assigned at 0.15 and −0.3 V (one and two blue stars, respectively). The peak intensity decreases over the cycles until it disappears in the fourth cycle, due to the deposition of the insulating PDA layer. C) Electrochemical removal procedure of PDA. All three curves show a cyclic voltammogram of gold with a typical gold oxidation peak at 1 V (red star) and the reduction at 0.5 V (green star). The decrease of current intensity (indicated with an arrow) from the first curve to the second one and the presence of a peak at 0.6 V, which afterward disappears, suggested reduced interactions between the PDA film and the gold substrate, hence further oxidation only arises from the gold surface.
12 GPa) facilitating their transfer to various substrates. This approach also allowed the preparation and transfer of patterned PDA structures. Due to the PDA film thickness in the range of lipid bilayers, phospholipid nanodiscs were introduced into the PDA films containing the membrane domain of glycophorin A, an antigen-presenting membrane protein found at the surface of human erythrocytes. Such nanodisc-containing ultrathin PDA films offer potential for the selective separation of organic molecules, nanopore-based DNA-sequencing, cascade reactions, or sensing. We also envision that they could serve as very simplified cell membrane models offering structural investigations of the embedded membrane proteins or to study protein-mediated transport processes in cellular membranes.

2. Results and Discussion

2.1. Preparation of Ultrathin, Free-Standing PDA Films

Ultrathin PDA films were prepared on a gold substrate by cyclic voltammetry (CV) (Figure 1B) immersing the gold substrate in dopamine solution and sweeping the potential between −0.5 and +0.5 V for several cycles. In Figure 1A, a simplified representation of one of the possible mechanisms of polydopamine formation and structure is depicted. The first step is the oxidation of catechol to quinone followed by cyclization and another oxidation step from leucodopaminechrome to dopaminechrome. Oxidative polymerization of dopaminochrome affords the highly cross-linked polydopamine film. Polydopamine growth and the chemical composition of the film was proven by IR spectroscopy (Figure S4, Supporting Information) and the chemical signature corresponded to previous reports. In order to remove the film, we adapted a method developed by Greco et al., where a sacrificial polymeric layer, polyvinyl alcohol (PVA), was used as mechanical support for the removal of a polystyrene sulfonate (PEDOT/PSS) nanofilm from the substrate as depicted in Figure 2A. Prior to removal, the PDA film at the gold surface was immersed into carbonate buffer at pH 10 for 30 min to allow further cross-linking of the dopamine monomers and oligomers absorbed into the film (Figure S1, Supporting Information), which renders the film more stable and compact and was essential to facilitate film removal (Figure 2A). The sample was then dipped into phosphate buffer and three additional cycles were applied over a wider potential range −800 to 1200 mV to overoxidize the system by forming an oxide layer on gold that reduces the adhesion between the film and the gold substrate. According to previous reports, this procedure reduces the Au–N coordination thus allowing detachment of the PDA film.

Figure 2. A) Top: Schematic procedure of the removal and transfer procedure of freestanding PDA films (gray) on gold substrate (yellow); three main steps are shown: 1) Formation of the PDA film, 2) Stripping of the PDA film off the gold substrate by applying PVA (light blue), 3) Transfer of the PDA film onto a different substrate (brown) by dissolving PVA with water. Bottom: Photos recorded of each sample during the corresponding procedure; 1′) the PDA film on the gold substrate; 2′) the removed PDA film on the transparent PVA support; 3′) the PDA film transferred onto a glass slide by dissolving the PVA layer. B) Cyclic voltammetry (CV) curves recorded between −0.5 and +0.5 V of equimolar solutions of 5 mM K₃[Fe(CN)₆] and K₄[Fe(CN)₆] for an uncoated gold electrode of 1 × 1.5 cm (green curve) and the CV curve of the same electrode coated with the PDA film after 15 cycles at scan rate of 0.01 V s⁻¹ (blue curve). The CV curve of the clean electrode after the removal of the PDA film (gray curve). C) SEM micrograph of a freestanding PDA film on a nylon mesh. The PDA film was obtained after 15 cycles by cyclic voltammetry with a thickness of ≈17 nm and covers the mesh homogeneously.
depicts the electrochemical removal cycles; at 1000 mV, the corresponding oxidation peak of gold is clearly present.

The peak intensity decreased after the first cycle due to over oxidation of the PDA film and the gold surface thus weakening the bonds between these two materials. Consequently, the following cycles revealed oxidation and reduction of bare gold indicating the reduced interactions of the PDA film and the gold surface. The area under the curve (AUC) of the oxidation and reduction peaks of gold (Table S1, Supporting Information) was calculated. A decrease of the AUC was observed after the first electrochemical cycle for the oxidation peak but all following AUC remained constant. No changes were detected for the AUC of the gold reduction peaks. Next, the PDA film was coated with a sacrificial PVA layer, which served as an adhesive tape of the gold reduction peaks. Next, the PDA film was coated electrochemical cycle for the oxidation peak but all following calculated. A decrease of the AUC was observed after the first cycle due to over oxidation of the PDA film and the gold surface thus weakening the bonds between these two materials. Consequently, the following cycles revealed oxidation and reduction of bare gold indicating the reduced interactions of the PDA film and the gold surface. The area under the curve (AUC) of the oxidation and reduction peaks of gold (Table S1, Supporting Information) was calculated. A decrease of the AUC was observed after the first electrochemical cycle for the oxidation peak but all following AUC remained constant. No changes were detected for the AUC of the gold reduction peaks. Next, the PDA film was coated with a sacrificial PVA layer, which served as an adhesive tape of the gold reduction peaks. Next, the PDA film was coated electrochemical cycle for the oxidation peak but all following calculated. A decrease of the AUC was observed after the first cycle due to over oxidation of the PDA film and the gold surface thus weakening the bonds between these two materials. Consequently, the following cycles revealed oxidation and reduction of bare gold indicating the reduced interactions of the PDA film and the gold surface. The area under the curve (AUC) of the oxidation and reduction peaks of gold (Table S1, Supporting Information) was calculated. A decrease of the AUC was observed after the first electrochemical cycle for the oxidation peak but all following AUC remained constant. No changes were detected for the AUC of the gold reduction peaks. Next, the PDA film was coated with a sacrificial PVA layer, which served as an adhesive tape of the gold reduction peaks. Next, the PDA film was coated electrochemical cycle for the oxidation peak but all following calculated. A decrease of the AUC was observed after the first cycle due to over oxidation of the PDA film and the gold surface thus weakening the bonds between these two materials. Consequently, the following cycles revealed oxidation and reduction of bare gold indicating the reduced interactions of the PDA film and the gold surface. The area under the curve (AUC) of the oxidation and reduction peaks of gold (Table S1, Supporting Information) was calculated. A decrease of the AUC was observed after the first electrochemical cycle for the oxidation peak but all following AUC remained constant. No changes were detected for the AUC of the gold reduction peaks. Next, the PDA film was coated with a sacrificial PVA layer, which served as an adhesive tape of the gold reduction peaks. Next, the PDA film was coated electrochemical cycle for the oxidation peak but all following calculated. A decrease of the AUC was observed after the first cycle due to...
transfer protocol depicted in Figure 2A. The image shows that the masking and transfer method allowed a fine shape control of the PDA film.

### 2.3. Embedding of Phospholipids Nanodiscs

The controlled dopamine polymerization only at the conductive areas of the gold substrate offers the opportunity to embed non-conductive molecules or supramolecular structures such as phospholipid bilayers into the thin film, which is of great interest to access nanocomposite functional materials. In this case, the current should not flow at positions, where the non-conductive elements have been placed and PDA polymerization should only occur at the conductive surface areas. Lipid nanodiscs were selected due to their rich functionality and defined dimensions such as the formation of precise nanopores filled with lipids and the opportunity to incorporate membrane proteins into the discs.

Phospholipid nanodiscs were prepared according to a published protocol using Glycophorin A transmembrane domain (GpAtm) as a model membrane protein. This model protein domain was used in a molar ratio of 1:1 relative to the membrane scaffold protein (MSP) to realize one GpAtm dimer per nanodisc. The nanodiscs were characterized by AFM revealing uniform nanoobjects with discoidal shapes providing a diameter of 10 nm and a height of ≈3 nm (Figure 4B). These dimensions correlate well with previous reports. GpAtm incorporation into the nanodiscs was verified using a biotin labeled GpAtm derivative that induces a significant size increase after the addition of streptavidin, which also allows nanodiscs separation during size exclusion chromatography (Figure S9, Supporting Information). Then, the nanodisc solution with a concentration of 1.6 µg mL⁻¹ was drop cast on a gold substrate, which was prefunctionalized with 3,3'-dithio bis(sulfosuccinimidyl propionate) (DTSSP) to enhance binding of the nanodiscs to the gold surface (Figure S6, Supporting Information). The nanodiscs adsorbed at the gold surface (Figure S7, Supporting Information) were then embedded into the PDA film.
Figure 4. A) Procedure for embedding the nanodiscs with the integrated transmembrane domain of glycophorin A into the PDA film. B) AFM maps of the nanodiscs. C) AFM maps of the nanodiscs embedded into the PDA film on gold. The red cross indicates where the nanodiscs are present, the blue cross indicates where only PDA is detected. D) IR spectrum of the nanodiscs embedded into the PDA film.

during electropolymerization (Figure 4A). Five CV cycles were performed with a faster scan rate of 0.05 V s\(^{-1}\) yielding sharper peaks in the CV (Figure S8, Supporting Information) to produce a 6 nm film preventing overgrowth of the nanodiscs by the PDA film. AFM-IR was applied to characterize the films to prove the presence of single nanodiscs within the few nanometer thick PDA film with a chemical spatial resolution of \(\approx 10\) nm (Figure 4C).

Because of the comparable size of the nanodiscs and the film roughness, as expected, we could not directly identify the nanodiscs by conventional morphology imaging. In order to chemically identify the presence and spatial distribution of the nanodiscs, we imaged the chemical differences of the polymeric PDA matrix and the lipid components of the nanodiscs. As depicted in Figure 2B, PDA has a broad absorption at 1600 cm\(^{-1}\). On the other hand, lipids reveal a typical IR absorption at 1725–1228 cm\(^{-1}\), corresponding to the C=O stretching vibration, and at 1228 cm\(^{-1}\), corresponding to the IR absorption of phospholipids PO\(_2\) groups. We simultaneously acquired nanoscale resolved maps of morphology, nanomechanics and IR absorption of the sample by AFM-IR exploiting gold probes to enhance the electromagnetic field, resonance enhanced mode and phase-locked loop to increase the sensitivity of the system\(^{[42,52,53]}\) (Figure 4C).

The nanoscale chemical imaging revealed the enhanced absorption of the nanodiscs at the typical IR absorption bands of phospholipids at 1725 and 1228 cm\(^{-1}\) as bright spots in the IR map (Figure 4C). Consequently, at the location of the nanodiscs, one would anticipate a decreased IR absorption related to the diminished local content of PDA. As expected, chemical imaging of the film at 1590 cm\(^{-1}\), corresponding to IR absorption of the C=C ring vibration of PDA, showed a reduced absorption at the locations of the nanodiscs in the IR map. Furthermore, the regions corresponding to the lipids exhibit significantly lower tip-sample frequency of contact resonance, indicating that the lipid–protein nanodiscs have relative lower stiffness than the polymeric PDA film.

We then acquired nanoscale localized spectra from areas close to the nanodiscs and the PDA film (Figure 4C). In good agreement with the chemical imaging, the spectra acquired on the area of the nanodiscs showed an increase of IR absorption at the IR absorption bands of lipids (1720–30 and 1228 cm\(^{-1}\)) and of protein amide bands I (1700–1600 cm\(^{-1}\)) and II (1580–1500 cm\(^{-1}\))
indicating the presence of the protein in the nanodiscs. In both spectra, there is a dominating contribution arising from the IR absorption of the PDA and of the continuous underlying layer of DTSSP below, which absorbs strongly in the IR at 1400 cm$^{-1}$ (Figure 4D). These contributions could not be minimized because of the larger size of the AFM tip (≈30 nm) compared to the average radius of the nanodiscs (≈5 nm), thermal diffusion or the long-range penetration depth of the IR light sensing also the underlying DTTSP layer. However, these results clearly indicated that the thin disks were actually embedded within the PDA film.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to detect the phosphatidylethanolamine (PE) lipids (57.5 w/w%) from the nanodiscs deposited on the gold surface. The PE molecule consists of a phosphatidylethanolamine headgroup and fatty acid chains. Therefore, fragment signals from both parts of the lipids were expected as shown in Figure 5A. The lateral signal distribution of the PE lipid headgroup fragment $C_{16}H_{32}NO_4P$ reveals the highest signal intensity in the central square-shaped nanodisc-deposition area. C) The contrast for the headgroup fragment $C_{16}H_{32}NO_4P$ is less pronounced after embedding. However, slightly elevated signal intensities compared to the surrounding area can be observed in the central area. D) The fatty acid fragment $C_{16}H_{32}O_2$ of the PE lipid matches the expected distribution with high signal intensities in the central area. E) Signal intensities of the fatty acid fragment $C_{16}H_{32}O_2$ of the PE lipid are reduced compared to the non-embedded sample, but the central square area still shows a notably higher signal intensity.

The PDA film was polymerized after six cycles at 50 mV s$^{-1}$ on the substrate containing the nanodiscs applying the same setup as above. The signal intensities of the fatty acid- and headgroup-related signals decreased significantly as shown in Figure 5C,E, but they were still detectable. A slightly higher signal intensity
relating from C3H10NO4P+ was found in the central square area, where the nanodiscs were deposited suggesting the presence of the lipids bilayers in the PDA film. The intensity of the signals relating to the fatty acid distribution (Figure 5E) was also much lower after embedding the nanodiscs into the PDA film. Nevertheless, a higher signal intensity was observed in the central area, still depicting the square shape from the mask. This indicates that the lipids of the nanodiscs were present within the topmost layers of the PDA surface (<1 nm). The ToF-SIMS experiments indicated that the lipid bilayers were still accessible after embedding, suggesting the formation of channels, which could provide great potential for advanced separations and as cell membrane mimics.

Next, we studied the availability of the nanodisc pores to control ion transport. The ion permeability through ultrathin PDA films on the gold surface with and without the embedded nanodiscs was investigated by cyclic voltammetry as shown in Figure 6A. Ruthenium hexamine chloride, Ru(NH3)6Cl3, was used as redox probe due to its positive charge, which would in principle allow permeation through the film due to the introduction of negatively charged phospholipids bilayers. Figure 6B depicts the resulting cyclic voltammetry graphs of the 6 nm polydopamine films with and without the embedded nanodiscs. By applying a potential between −0.6 and +0.1 V, the typical oxidation and reduction peaks from Ru(NH3)6·Cl3 appear at −0.1 and −0.3 V, respectively, indicating that Ru(NH3)6·Cl3 reached the gold surface, where it further reacted. Compared to the PDA film without the nanodiscs, the PDA film containing the accessible nanodiscs showed a significantly increased signal for both oxidation and reaction peaks, which was indicative for an enhanced permeability of Ru(NH3)6Cl3.

Comparative CV measurements were performed applying K3[Fe(CN)6]/K4[Fe(CN)6] as redox couple with negative charge. Figure 6C depicts the cyclic voltammetry graphs using K3[Fe(CN)6]/K4[Fe(CN)6] by applying a potential between −0.1 and +0.5 V. In both cases, the oxidation and reduction peaks that typically appear at +0.3 and +0.1 V, respectively, are barely distinguishable indicating a weak permeation of the redox probe through the PDA film to the gold electrode. These results indicated the availability of nanodiscs in the film with accessible pores filled with phospholipids that participated in transport through the PDA film rendering it permeable to positively charged Ru(NH3)6Cl3 and much less permeable to K3[Fe(CN)6]/K4[Fe(CN)6].

3. Conclusions

Herein, the first ultrathin, freestanding PDA films were prepared by electropolymerization and application of the
sacrificial polymer PVA. Delamination of the PDA film was accomplished in a non-destructive way yielding PDA films with 10–20 nm thickness that were successfully transferred onto various other substrates such as Si, Si$_3$N$_4$, glass slide, and nylon. This procedure was scaled conveniently up to 2.5 cm$^2$ dimensions and the PDA films revealed outstanding Young moduli of about 12 GPa after applying five and ten polymerization cycles. Moreover, dopamine polymerization could be spatially controlled by applying a non-conducting PDMS mask as well as non-conducting biomolecules. In this way, patterned PDA films were prepared and transferred successfully without deformation. Non-conducting phospholipid nanodiscs containing the transmembrane domain of glycophorin A were successfully embedded into the PDA film and the shapes of the nanodiscs were retained after electropolymerization. The lipid bilayers in the nanopores remained at the surface of the film and accessible after PDA deposition. Increased permeability of Ru(NH$_3$)$_6$Cl$_3$ was observed in the presence of the nanopores, whereas only very low permeability for K$_3$[Fe(CN)$_6$]/K$_4$[Fe(CN)$_6$] was measured for PDA and PDA with nanodiscs.

We believe that the reported procedure solves the long-lasting challenge to fabricate polymeric films with ultrathin geometries, as well as their transfer to other substrates and the incorporation of functional biological entities. Therefore, these ultrathin PDA films could be of great interest in flexible electronics, water purification, separation, and tissue engineering. The presence of protein nanopores within the PDA films provide a unique platform, into which membrane proteins could be integrated, for example, to allow their structure investigations in more native environments. Future work will also focus on elucidating the functionality of the embedded nanopores for, example, the selective separation of organic molecules, nanopore-based DNA-sequencing, cascade reactions, or sensing.

4. Experimental Section

Gold-coated (1000 Å) microscope slide (Sigma-Aldrich), were cut using a diamond tip. Phosphate buffer pH 7, 100 mw was prepared using sodium phosphate dibasic anhydrous (99%) and sodium phosphate monobasic (99%) in Milli-Q water. Carbonate buffer pH 10, 100 mw was prepared using sodium bicarbonate (>99.7%) and sodium carbonate (>99.8%) from Sigma-Aldrich, in Milli-Q water. Polyvinyl alcohol 80% hydrolyzed 9–10 kDa Mw (Sigma-Aldrich) was used to prepare the 10 wt% solution in Milli-Q water. Nylon net 60 µm was purchased from Merck. Potassium ferricyanide (99%) and potassium ferrocyanide trihydrate (99%) were bought from Acros Organics. DTSSP (3,3′-dithiobis(sulfosuccin-imidyl propionate) was purchased from (Chem Cruz). Sodium dihydrogenphosphate was obtained from Sigma-Aldrich and potassium hydrogen phosphate from Appli Chem.

Film Preparation: Electropolymerization and cyclic voltammetry experiments were performed using a Metrohm Autolab PGSTAT 204 with a standard three electrode configuration. A gold covered glass microscope slide was used as the working electrode, a Ag/AgCl, 3 v KCl as the reference electrode, and gold wire as the counter electrode for film preparation while a platinum wire for CV experiments. All reactions were performed in a 35 mL electrochemical cell (Metrohm), in air atmosphere and at room temperature. Pre-treatment of gold working electrode was performed by 10 min in Ar plasma cleaning at the pressure of 6 mbar.

Gold was used as substrate for electrodeposition of PDA due to its inertness, excellent conductivity, and low surface roughness. After cleaning in argon plasma, the gold substrate was immersed in a 1 mg mL$^{-1}$ solution of dopamine hydrochloride dissolved in 100 mM phosphate buffer at pH 7.0. A potential was applied and cycled from +500 mV to −500 mV with a scan rate 0.01 V s$^{-1}$ to induce dopamine polymerization at the interface. The film thickness was controlled by the number of cycles as determined by AFM (Park NX20).

Film Transfer Procedure: The PVA/PDA film was placed gently on the water surface with the polydopamine side facing the liquid side and after a couple of minutes, it could be noticed that the PVA layer expanded and dissolved leaving only the PDA film floating on water. Once the film floated on water, it could be fished from the bottom with a support like a nylon mesh leading in this way to a freestanding polydopamine film. Then the sample was washed in water for 1 h and in 45 °C water for 1 h to completely remove the PVA and subsequently dried at room temperature for 1 h. SEM Images were acquired using LEO Gemini 1530 by attaching the sample with double side tape on the sample-holder.

Deposition of Nanodiscs: In general, 10 µL of a 16 µg mL$^{-1}$ concentrated solution of nanodiscs in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 1.8 mM KH$_2$PO$_4$) was used and incubated on the substrates under different conditions varying in time and temperature. Gold was cleaned with argon plasma for 10 min at 6 mmbar before being used.

Moreover, the nanodiscs were attached to gold surface by using the crosslinker DTSSP. As a cross-linker, DTSSP offers the possibility for a covalent linkage of the nanodiscs MSP to the gold surface, since it creates a self-assembled monolayer (SAM-layer) on the gold surface by chemisorption using interactions between gold and the disulphide linkage of DTSSP. In a second reaction step primary amines of the MSP lysine residues can react with the functional NHS-ester of DTSSP to covalently bind the nanodiscs to the gold surface (Figure S6, Supporting Information).

For the formation of a SAM-layer on gold 200 µL of a 5 mM DTSSP solution was attached to the gold surface and incubated at several conditions. After washing with 2 mL PBS 10 µL of a 16 µg mL$^{-1}$ concentrated solution of Nanodiscs in PBS was added to the gold slide and incubated at 4 °C for 24 h. The gold slides were washed with 2 mL PBS and dried with nitrogen. The six cycles polydopamine film was then performed on the gold with the nanodiscs applying a scan rate of 50 mV s$^{-1}$ and the CV is shown in Figure S7, Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors acknowledge support by the BMBF (Biotechnologie 2020 + initiative, “Selekom” project). This work received funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under the Collaborative Research Center (CRC) Transregio 234 (No. 407426226, B04). T.W., K.W. and T.M. are grateful for the support from the Marie Curie ITN BORGES (No. 813863). T.W. acknowledges support from ERC Synergy Grant BioQ (No. 319130); G.F. and K.W. acknowledge support from ERC Ad G. SmartPhon (No. 694977). Open Access funding provided by the Max Planck Society is acknowledged. The authors also thank Katharina Maisenbacher for support with drawing of the TOC figure.
Conflict of Interest
The authors declare no conflict of interest.

Keywords
freestanding ultrathin films, glycophorin A, membrane mimics, nanodiscs, polydopamine

Received: January 14, 2020
Published online: March 23, 2020