Ionophore-Gold Nanoparticle Conjugates for Ag+-Selective Sensors with Nanomolar Detection Limit

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Chemicals and reagents

The commercial components and solvents used for ion-selective membrane preparation, i.e., poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (*o*NPOE), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), and tetrahydrofuran (THF), were of Selectophore® grade from Fluka. Tetraoctylammonium bromide, $HAuCl_4 \cdot 3H_2O$, $NaBH_4$, toluene, dichloromethane (DCM), *N,N'*-dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP) were obtained from Sigma-Aldrich. Analytical grade $AgNO_3$, $Pb(NO_3)_2$, and $Mg(NO_3)_2$ were from Fluka, Suprapur grade $NaNO_3$ and KNO_3 from Merck. Solutions were prepared with deionized water of 18.2 MΩcm resistivity (Synergy, Syns50001; Millipore, Bedford, MA).

## Chemical synthesis of bridged thiacalixarene-based Ag+-selective ionophore

The IUPAC names of calixarenes are vast and very complicated; therefore, the special calixarene nomenclature is used. The synthesis, eventually, resulted in a mixture of two ligands differing only in the number of dithiolane moieties attached to the thiacalixarene ring (Scheme S1), i.e., ligand **I**: 1,3-*alt*-5,11,17,23-tetra-*tert*-butyl-25,27-dihydroxy-26,28-(3,9-dithia-6-oxaundecane-1,11-diyloxy)thiacalix[4]arene; ligand **II**: 1,3-*alt*-5,11,17,23-tetra-*tert*-butyl-25,27-bis(10-hydroxydecyloxy)-26,28-(3,9-dithia-6-oxaundecane-1,11-diyloxy)thiacalix[4]arene). While the spontaneous self-assembly of ligand **I** onto

the Au surface is straightforward and involves the reduction of the disulfide bond, ligand **II** might react either with only one or both dithiolane moieties with contingent effects on the surface coverage and ion complexing properties. Therefore, to reduce the complexity of the system, ligand **I** was used throughout in this study.
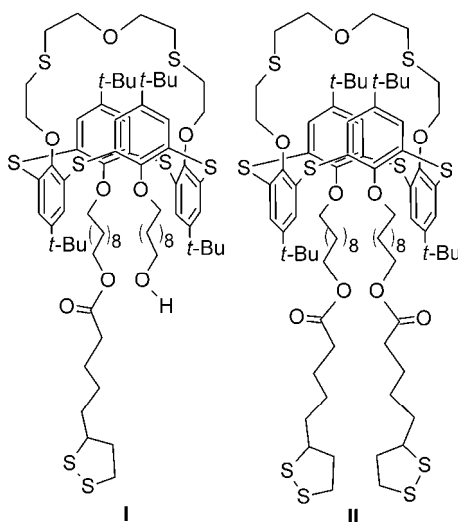
Scheme S1 Chemical structures of ligands **I** and **II**

Synthesis of ligands I and II
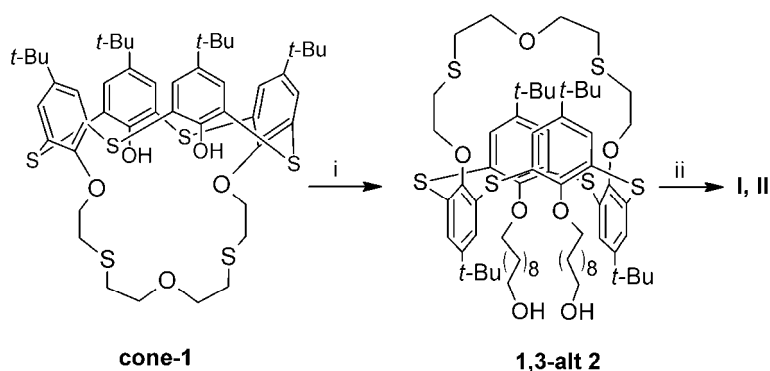
Scheme S2 Synthesis of ligands I and II

The following reagents and conditions were used for the synthesis (Scheme S2): (i) 10-iododecanol, Cs_2CO_3, Δ ; DCC, DMAP, DCM at room temperature. All chemicals were reagent grade and used without further purification. NMR spectra were recorded in $CDCl_3$ at 500/125 MHz on a Bruker Avance DRX-500

spectrometer. Precoated silica gel plates (Merck 60 F$_{254}$) were used for analytical TLC, and Kieselgel 60 for column chromatography. Further details of the synthesis reactions are given below.

Intermediate 1,3-alt 2

The mixture of **cone-1**[1] (0.91 g, 1 mmol), 10-iododecanol (1.14 g, 4 mmol), and Cs$_2$CO$_3$ (1.3 g, 4 mmol) in CH$_3$CN (40 mL) was stirred under reflux for 8 h. After evaporating the solvent, the dry matter was redissolved in DCM, washed with dilute HCl (2 × 40 mL) and dried (Na$_2$SO$_4$) to give the crude product (0.77 g). This was crystallized with CH$_3$OH. The pure intermediate, **1,3-alt 2** (0.40 g, 33%), was finally obtained as a white solid after column chromatography on silica gel using hexane/ EtOAc (6:4 v/v) as eluent.

^{1}H NMR: δ 7.30 (s, 4H, Ar*H*), 7.28 (s, 4H, Ar*H*), 3.88 (t, 4H, *J* = 14.0 Hz, C*H$_2$*O), 3.75 (t, 4H, *J* = 13.0 Hz, C*H$_2$*O), 3.64 (t, 4H, *J* = 10.5 Hz, C*H$_2$*O), 3.46 (t, 4H, *J* = 8.0 Hz, C*H$_2$*O), 2.52 (t, 4H, *J* = 8.0 Hz, C*H$_2$*S), 2.15 (t, 4H, *J* = 15.0 Hz, C*H$_2$*S), 1.56 (m, 4H, C*H$_2$*), 1.34 (s, 18H, C*H$_3$*), 1.28 (s, 18H, C*H$_3$*), 1.26–0.81 (m, 28H, C*H$_2$*). ^{13}C NMR: δ 156.7, 146.2, 146.0, 128.2, 127.8, 127.0, 126.2 (Ar), 74.2, 68.5, 67.5, 63.2 (OC*H$_2$*), 34.6, 34.5 (*C*(CH$_3$)$_3$), 34.1, 33.2 (*C*H$_2$S), 33.0 (*C*H$_2$), 31.7, 31.6 (C(*C*H$_3$)$_3$), 30.3, 30.0, 29.9, 29.7, 28.8, 26.1 (*C*H$_2$).

Anal. Calcd. for C$_{68}$H$_{102}$O$_7$S$_6$ (1223.92): C, 66.73%; H, 8.40%. Found: C, 66.54%; H, 8.44%.

Ligands I and II:

Dicyclohexylcarbodiimide (0.25 g, 1.20 mmol) dissolved in DCM (2 mL) was added to a DCM solution (30 mL) of **1,3-alt 2** (0.37 g, 0.3 mmol), (±)-α-lipoic acid (0.16 g, 0.75 mmol), and DMAP (0.02 g). The mixture was allowed to react at room temperature overnight. After filtering, the solvent was evaporated to dryness and the products were separated by column chromatography on silica gel (eluent: hexane/EtOAc, 8:2 v/v) to give **I** (0.05 g, fraction 2) and **II** (0.1 g, fraction 1) as white solids.

Analytical data of I:

^{1}H NMR: δ 7.30 (s, 4H, Ar*H*), 7.28 (s, 4H, Ar*H*), 4.06 (t, 2H, *J* = 6.5 Hz, C*H₂*O), 3.88 (t, 4H, *J* = 8.0 Hz, C*H₂*O), 3.75 (t, 4H, *J* = 8.0 Hz, C*H₂*O), 3.64 (t, 2H, *J* = 6.5 Hz, C*H₂*O), 3.57 (m, 1H, C*H₂*S), 3.46 (t, 4H, *J* = 5.0 Hz, C*H₂*O), 3.2–3.09 (m, 1+1H, C*H₂*S, C*H*S), 2.52 (t, 4H, *J* = 5.0 Hz, C*H₂*S), 2.45 (m, 1H, C*H₂*), 2.32 (t, 2H, *J* = 7.5 Hz, C*H₂*CO), 2.15 (t, 4H, *J* = 8.5 Hz, C*H₂*S), 1.91 (m, 1H, C*H₂*), 1.73–1.43 (m, 14H, C*H₂*), 1.34 (s, 18H, C*H₃*), 1.27 (s, 18H, C*H₃*), 1.28–0.82 (m, 24H, C*H₂*). ^{13}C NMR: δ 173.7 (C=O), 156.5, 146.1, 145.8, 128.0, 127.6, 126.8, 126.1 (Ar), 74.1, 68.3, 67.3, 64.6, 63.1 (O*C*H₂), 56.4 (S*C*H₂), 40.3 (*C*H₂), 34.6 (S*C*H₂), 34.4, 34.3 (*C*(CH₃)₃), 34.2 (*C*H₂CO), 33.9, 33.0 (*C*H₂S), 32.9 (*C*H₂), 31.5, 31.4 (C(*C*H₃)₃), 30.0, 29.8, 29.7, 29.6, 29.5, 29.3, 28.8, 28.7, 28.6, 26.0, 25.9, 24.8 (*C*H₂).

Anal. Calcd. for $C_{76}H_{114}O_8S_8$ (1412.23): C, 64.64%; H, 8.14%. Found: C, 64.39%; H, 8.20%.

Analytical data of II:

^{1}H NMR: δ 7.30 (s, 4H, Ar*H*), 7.29 (s, 4H, Ar*H*), 4.06 (t, 4H, *J* = 6.5 Hz, C*H₂*O), 3.88 (t, 4H, *J* = 8.5 Hz, C*H₂*O), 3.75 (t, 4H, *J* = 8.5 Hz, C*H₂*O), 3.57 (m, 2H, C*H₂*S), 3.46 (t, 4H, *J* = 5.0 Hz, C*H₂*O), 3.18 (m, 2H, C*H₂*S), 3.11 (m, 2H, C*H*S), 2.52 (t, 4H, *J* = 5.0 Hz, C*H₂*S), 2.46 (m, 2H, C*H₂*), 2.32 (t, 4H, *J* = 7.5 Hz, C*H₂*CO), 2.16 (t, 4H, *J* = 8.5 Hz, C*H₂*S), 1.90 (m, 2H, C*H₂*), 1.72–1.43 (m, 20H, C*H₂*), 1.34 (s, 18H, C*H₃*), 1.27 (s, 18H, C*H₃*), 1.28–0.83 (m, 24H, C*H₂*). ^{13}C NMR: δ 173.6 (C=O), 156.5, 146.1, 145.8, 128.0, 127.6, 126.9, 126.1 (Ar), 74.1, 68.3, 67.3, 64.6 (O*C*H₂), 56.4 (S*C*H₂), 40.3 (*C*H₂), 34.6 (S*C*H₂), 34.4, 34.3 (*C*(CH₃)₃), 34.2 (*C*H₂CO), 33.9, 33.1 (*C*H₂S), 31.5, 31.4 (C(*C*H₃)₃), 30.0, 29.8, 29.7, 29.6, 29.4, 28.9, 28.8, 28.6, 26.0, 25.9, 24.8 (*C*H₂).

Anal. Calcd. for $C_{84}H_{126}O_9S_{10}$ (1600.54): C, 63.03%; H, 7.93%. Found: C, 63.29%; H, 7.86%.

Synthesis of Au nanoparticles in a liquid-liquid system

The colloid Au solution was prepared in a two-phase (toluene/water) system following the method described by Brust et al.,[2] but without adding thiol in the synthesis stage.[3] The aqueous solution of HAuCl₄ (10 mL, 30 mM) was mixed with

the phase transfer catalyst (tetraoctylammonium bromide, 28 mL, 50 mM). The two phases were stirred at room temperature until the water phase became transparent. Then, an aqueous solution of $NaBH_4$ (0.4 M, 8.4 mL) was added under intensive stirring. After 3 h, the two phases were separated and the organic phase was washed three times with deionized water and filtrated using a 0.45- µm PTFE syringe filter. The average diameter of the Au nanoparticles (5.5 nm) was determined by transmission electron microscopy (Fig. S1).

Synthesis and purification of ionophore–Au nanoparticle conjugates (IP–AuNP)

The Ag^+-selective ionophore **I** (2 mg) was dissolved in a toluene solution (7 mL) of AuNP and incubated for 1 h. Subsequently, 1-dodecanethiol (200 µL, 10 mM in toluene) was added and left to react for 1 h. The reaction mixture was diluted tenfold with EtOH, the color of the solution changing from red to grayish. The black precipitate resulting after centrifugation at 2000g for 3 min was washed with EtOH (9 mL altogether) in six consecutive rounds. After drying, the ionophore–Au nanoparticle conjugates (IP–AuNPs) were resuspended in THF (500 µL).
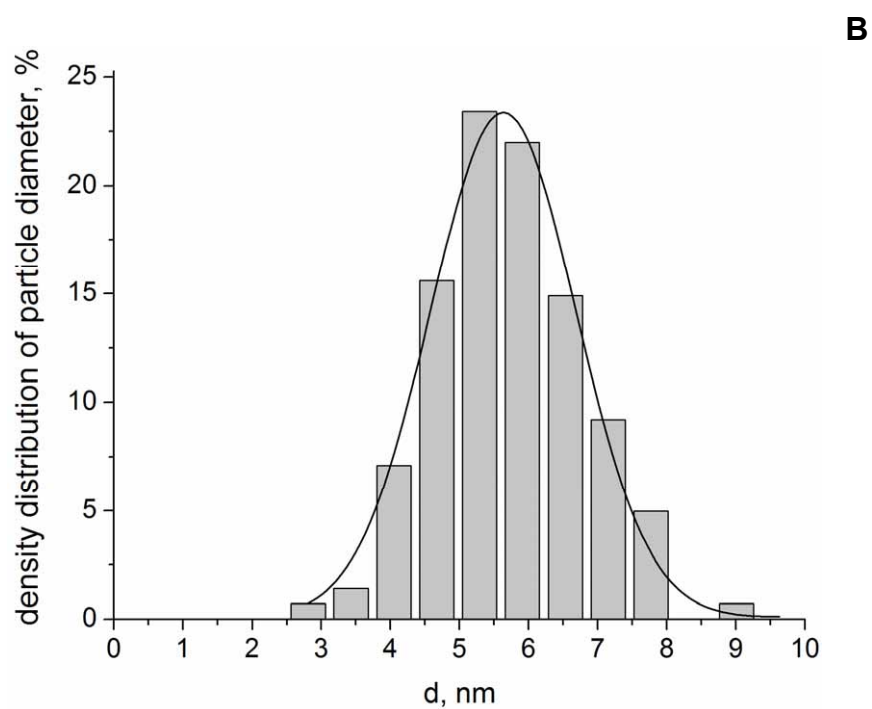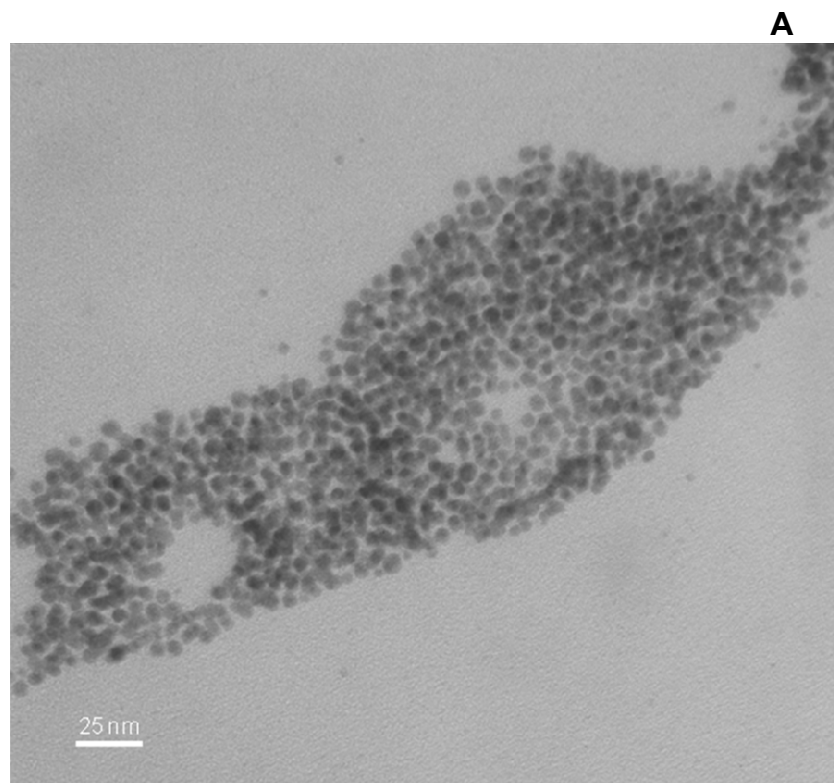
A

B

Fig. S1 A: Transmission electron micrograph of the Au nanoparticles; B: distribution of their diameter (d).

Preparation of ISE membranes and electrodes

The optimal composition of plasticized PVC membranes consisted of PVC (50 mg), oNPOE (150 mg), NaTFPB (0.369 mg), and IP−AuNP (16.68 mg, equivalent to ca. 2 mg of ionophore **I**). The membrane components were dissolved in THF (ca. 2 mL), poured into a glass ring (diameter, 24 mm), and the solvent was allowed to evaporate overnight. Membrane disks (diameter, 7 mm) were placed in commercial Philips electrode bodies using 0.01 M NaCl as inner solution. For spectral imaging the IP-AuNP loaded membranes consisted of PVC (50 mg), oNPOE (148 mg), NaTFPB (0.378 mg), and IP−AuNP (0.19 mg) while the blank, ionophore-free, membranes had the exact same composition except the ionophore.

EMF measurements

Potentiometric responses were measured with a high-input impedance (10^{15} Ω) 16-channel pH meter (Lawson Labs, Inc., Malvern, PA, USA) at room temperature. A double-junction Ag/AgCl reference electrode (No. 60729.100; Metrohm AG, CH-9101 Herisau, Switzerland) with 3 M KCl as electrolyte and 1 mM KNO_3 as salt bridge was used. Potential values were corrected for the liquid-junction potential based on the Henderson equation, and activities were calculated according to Debye−Hückel.

The *unbiased* potentiometric selectivity coefficients were measured by the separate solution method (SSM) at 1 mM level using nitrate solutions of the respective cations (primary: Ag^+ and interfering: J) as described previously.[4] For these experiments, membranes that had never been in contact with the primary ion solution were used. *Conventional* selectivity determinations, on the other hand, were made with electrodes preconditioned in 1 mM $AgNO_3$.[5] Calibrations were done with $AgNO_3$ standard solutions in the range of 1 nM to 10 mM.

 The ionophore-ion complex stability constants were determined with the method described by Mi and Bakker[6]. In addition to the ionophore and IP−AuNP ionophore containing membranes, blank membranes containing no ionophore but otherwise having exactly the same composition were also prepared. The complex formation constants were determined for the primary (Ag^+) and for a representative interferent ion (K^+) by first conditioning the ionophore loaded and ionophore free membranes

in a10 μM nitrate solution of the respective ion (AgNO$_3$ or KNO$_3$) for at least one day. After placing the membranes in Philips electrode bodies their potential was determined in 1 mM AgNO$_3$ or KNO$_3$, respectively. The sandwich membranes was prepared by mechanically pressing together the ionophore loaded and its correspondent blank membrane right after their individual membrane potentials were determined. Then this sandwich membrane was incorporated in a Philips electrode body and the potential was again determined.

The complex formation constants β_{IL_n} were calculated according to the equation:

$$\beta_{IL_n} = (L_T - \frac{n\,R_T}{z_I})^{-n} \exp\left(\frac{E_M\,z_I F}{RT}\right) \qquad\text{Eq. S1}$$

where L_T is the total concentration of the ionophore, R_T is the concentration of lipophilic ionic sites in the ion-selective membrane, n is the stoichiometry of the ion–ionophore complex, R, T and F are the gas constant, the absolute temperature, and the Faraday constant, respectively. E_M is the membrane potential determined by the difference of the cell potentials recorded with the sandwich membrane and the ionophore free membrane.

Optimization of the plasticizer content

As the addition of IP−AuNPs significantly increases the dry matter content of the membrane, the amount of plasticizer had to be optimized with respect to the potentiometric characteristics. In Fig. S2, calibration curves obtained with membranes containing 1 wt % of ionophore, 30 mol % of NaTFPB, and various wt % ratios of oNPOE/PVC are given.
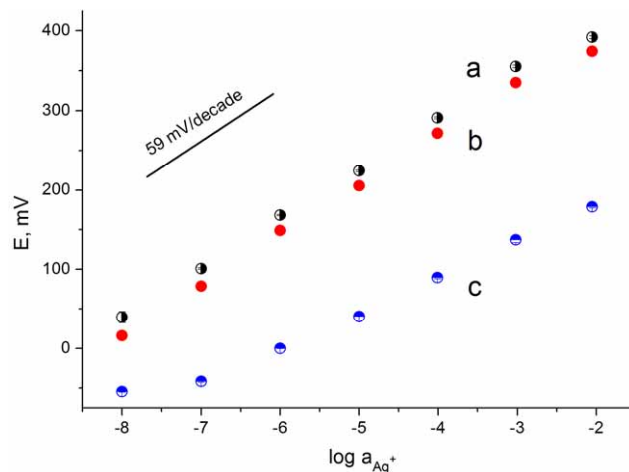
Fig. S2 Calibration curves for membranes based on IP–AuNPs with different wt % ratios of oNPOE/PVC: (a) 5.7, (b) 3.0, and (c) 2.0.

The results show that the conventional membrane composition (oNPOE/PVC, 2:1 w/w) is not optimal for IP–AuNP-based membranes both in terms of lower detection limit and slope. However, the performance characteristics can be efficiently improved by increasing the plasticizer content. Since increasing the oNPOE/PVC ratio above 3.0 w/w did not result in any significant improvement but deteriorated the mechanical stability of the membranes, this ratio was accepted as optimal and used in all subsequent studies.

Membrane resistances

The membrane resistances were determined using a Potentiostat/Galvanostat Autolab Pgstat 12 equipped with FRA2 electrochemical impedance spectroscopy module (Ecochemie B.V. Utrecht, The Netherlands). Membrane disks conditioned previously for 1 d in 10^{-5} M AgNO$_3$ were mounted in a transport cell. The two compartments filled with 1 mM AgNO$_3$ and accommodating a Ag electrode each were separated by a membrane disk exhibiting an area of 3.14×10^{-2} cm^2. All impedance spectra were measured within the frequency range of 1 MHz to 1 Hz at an excitation potential of 50 mV.

Fig. S3 shows that the specific resistance of membranes based on the free ligand **I** or on IP–AuNP, otherwise containing the identical amount of TFPB$^-$ and ionophore, is practically the same. Apparently, the bulk resistance is mainly determined by TFPB$^-$.
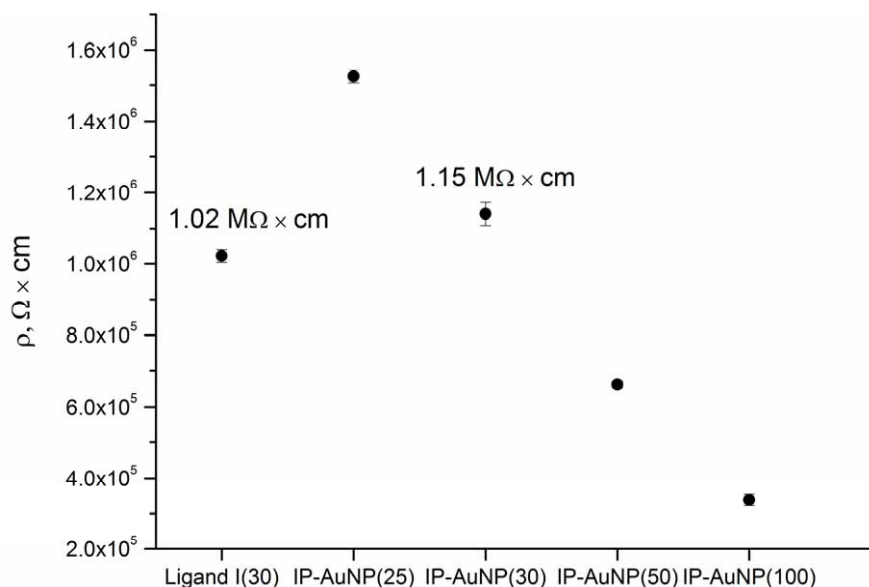
Fig. S3 Specific resistances of ISMs based on the free ligand **I** and IP−AuNP with different mol % TFPB$^-$ (given in parentheses). For all membranes, the wt % ratio of oNPOE/PVC (3.0) and the amount of ionophore were the same.

Spectral imaging measurements to determine the diffusion properties of IP-AuNP conjugates in the ion-selective membrane

To determine the diffusion properties of IP-AuNPs in the ion selective membrane phase an ionophore loaded and ionophore free (blank) membrane segment were merged together on a glass microscope slide. Merging the two membranes induces the diffusion of IP-AuNPs from the ionophore loaded membrane (donor) into the blank membrane (acceptor). The merged membranes were then immediately placed in the observation field of an inverted optical microscope (Olympus IX71 Inverted Research Microscope, Olympus Hungary Kft., Budapest, Hungary) equipped with a PARISS® Spectral Imaging System (http://www.lightforminc.com, Lightform Inc., Hillsborough, NJ, USA).[7] The spectral imaging system has the capability to gather 240 full spectra in the visible range along a selected observation line corresponding to the entrance slit of the spectrometer. This is made by wavelength dispersing the light entering in the spectrometer and projecting it on a Peltier cooled charge coupled device (CCD). Thus the recorded spectral image (240 × 750 pixels) consists of light intensity values with their spatial and wavelength coordinates (Fig. S4). For monitoring the diffusion of IP-AuNPs the joint interface of the merged

membranes was aligned to be perpendicular on the observation line (projection of the entrance slit), i.e., to coincide with the direction of the diffusion. The strong absorption of the IP−AuNPs at 525 nm provides a sensitive means of optically monitoring their diffusion, while the spectral imaging system confers 1 micrometer spatial resolution.
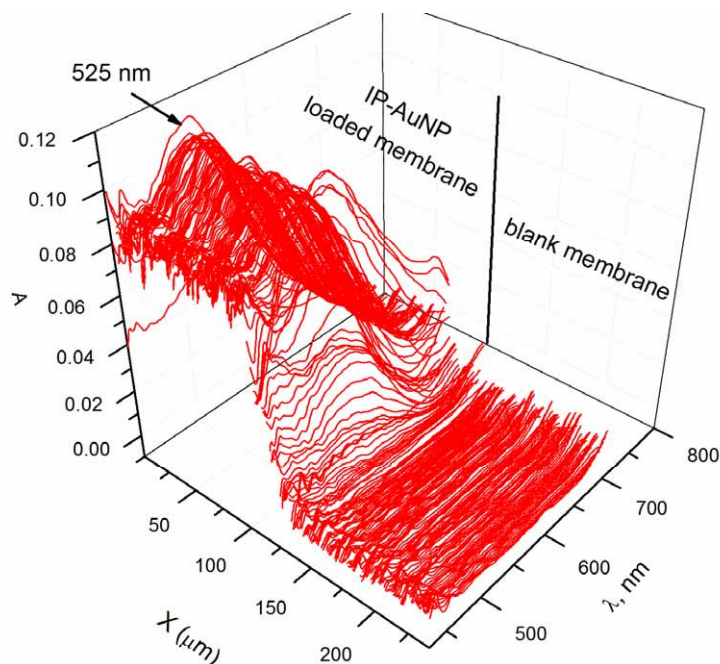

Fig. S4 Full visible spectra recorded simultaneously along the selected observation line during a single 80 ms exposition. The observation line was aligned to be perpendicular on the joint interface of the merged membrane segments. The spectra corresponding to the IP-AuNP loaded membranes showing absorption at 525 nm and those of the blank membranes (showing close to zero absorbance values) are indicated on the X axis.

The diffusion coefficients were determined by fitting Eq. 2 derived from Fick's second law for diffusion into a semiinfinite matrix to the experimentally absorbance profiles corresponding to λ=525 nm:

$$A(x,t) = \frac{1}{2} A_0 \, erfc \, \frac{x}{2\sqrt{Dt}} \qquad \text{Eq.S2}$$

where, A_0 is the initial absorbance (at $t = 0$) and D is the diffusion coefficient of the IP-AuNP conjugate in the donor membrane, x is the distance, t is the time, and erfc is the error function complement. Since no change in the absorption profiles were

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observed during more than 24 h investigations, only the upper limit of the IP-AuNP corresponding to the time frame of the measurements and spatial resolution could be determined.

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