A bis-pyrene polyamine receptors for fast optical detection of ketoprofen: synthesis, characterization and application in all-solid-state fluorescent sensors

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Supporting Information

Section S1.

Reagents

High-molecular-weight poly(vinyl chloride) (PVC), bis-ethyl hexyl sebacate (DOS) and tris(2-ethylhexyl) phosphate (TOP) plasticizers, tetradecylammonium chloride (TDACl) and tridodecylmethyammonium chloride (TDMACl) anion-exchangers, trifluoroacetic acid (TFA), anhydrous tetrahydrofuran (THF) highly volatile solvent were used for optode membranes preparation, and were purchased from Sigma-Aldrich. All other chemicals employed for optodes characterization, such as ketoprofen acid form (KP-H), sodium salts of perchlorate ($NaClO_4$), thiocyanate (NaSCN), benzoate (NaBenzoate), ibuprofen sodium salt (NaIBU), ketoprofen sodium (NaKP), naproxen sodium salt (NaNPX) and ketoprofen lysine (Lys-KF) salt, 2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS) were of analytical grade, purchased from Sigma-Aldrich (Italy) and used without further purification. All chemicals and solvents employed for synthesis and characterization of ligands were purchased from Sigma-Aldrich.

Section S2.

Syntheses of L1·3HCl and L1.

N1,N3-bis(1-pyrenylmethyl)diethylenetriamine trihydrochloride salt (L1·3HCl). A solution of 1-pyrenecarboxaldehyde (0.23 g, 1 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of diethylenetriamine (0.05 g, 0.48 mmol) in CH_2Cl_2 (30 mL), in presence of activated molecular sieves (3 g) and refluxed for 3 h under nitrogen atmosphere. The solution was recovered by filtration and concentrated by evaporation. The resulting oil was dissolved in dry EtOH (40 mL) and stirred with $NaBH_4$ (0.46 g, 12 mmol) at 333 K for 4 h. The resultant was concentrated by

evaporation, dissolved in CH_2Cl_2 (50 mL) and washed with an aqueous NaOH solution (1mol/L, 30 mL x 3). The organic layer collected was dried with anhydrous Na_2SO_4 and the solvent was removed under vacuum. The semisolid residue was dissolved in EtOH (20 mL) and precipitated by addition of an aqueous HCl (37%) solution, to afford three-hydrochloride salt of the receptor (L1·3HCl), which was washed with EtOH and CH_2Cl_2, and dried under vacuum (0.10 g, yield 30%). Anal. calcd. for $C_{38}H_{36}N_3Cl_3·3H_2O$: C 65.66, H 6.09, N 6.05. Found: C 67.00, H 5.32, N 5.96. ^{1}H-NMR (400 MHz, DMSO-d_6): δ(ppm) 8.60 (d, *J*=9.2 Hz, 2H, ArH), 8.40-8.34 (m, 10H, ArH), 8.29-8.22 (m, 4H, ArH), 8.14 (t, *J*=7.6 Hz, 2H, ArH), 5.04 (s, 4H, 1_{AL}), 3.65-3.57 (m, 4H, 2_{AL}), 3.56-3.48 (m, 4H, 3_{AL}). ^{13}C-NMR (400 MHz, DMSO-d_6): δ (ppm): δ(ppm) 132.57, 131.79, 131.27, 130.34, 130.11, 129.33, 128.35, 127.70, 126.96, 126.76, 126.39, 125.89, 124.98, 124.69, 124.40, 48.47, 44.22, 43.99. ESI MS (m/z) 532.27427 (m/z = 1, $[L1·3HCl + H]^+$).

N1,N3-bis(1-pyrenylmethyl)diethylenetriamine (L1). L1·3HCl (0.10 g) were dissolved in aqueous NaOH solution (5 mol/L, 50 mL) and extracted with $CHCl_3$ (30 mL x 3). The organic layer collected was dried with anhydrous Na_2SO_4 and the solvent was removed under vacuum. (0.05 g, yield 67%). Anal. calcd. for $C_{38}H_{33}N_3$: C 85.84, H 6.26, N 7.90. Found: C 85.32, H 5.89, N 7.45. ^{1}H-NMR (400 MHz, CDCl3): δ(ppm) 8.80 (d, *J*=7.6 Hz, 2H, ArH), 8.04-7.93 (m, 16H, ArH), 4.44 (s, 4H, H4, 1_{AL}), 2.92 (t, *J*=6.0 Hz, 4H, 2_{AL}), 2.74 (t, *J*=6.0 Hz, 4H, 3_{AL}). ^{13}C-NMR (400 MHz, CDCl3): δ(ppm) 131.85, 131.39, 131.31, 129.64, 128.32, 127.97, 127.74, 126.50, 125.76, 125.66, 125.53, 125.35, 125.26, 123.60, 51.81, 28.79, 48.47.

Section S3.

Optical redout acquisition with a smartphone

For quantification of optical response and its correlation to the KP content in calibration and tested solutions, the optode images were acquired with a smartphone from a fixed distance of 10 cm upon excitation at 365 nm. During the experiments at least 3 different smartphone models have been used; it was found that the smartphone model and camera settings do not influence the results of analysis. The sensor optical output was treated for unique image obtained by any tested smartphone, and had the same baseline for data analysis. Since the calibration procedure was " incorporated" in the measurement procedure (the part of the sensing spot on the same image are used for calibration, other – for measurement. The suggested approach has permitted to keep the developed analytical procedure universal and independent from the settings of signal acquisition device, such as differences in white balance and color rendition.

Section S4.

KP assessment in Okitask

The standard addition method was used to estimate the KP amount in Okitask by Dompé pharmaceutical composition. The total content of an OkiTask 40 mg pocket was weighed in analytical balances, ground in a mortar, and then 1.4 mg of OkiTask powder were sampled and dissolved in 1 mL of 0.005 M TRIS/EtOH 1:1 v/v solution to obtain the stock solution 1 of 2×10^{-4} M concentration of KP. Diluted in 10 times this solution was deposited on optode spot 1 and considered as unknown sample. Other two solutions were prepared from solution 1 through mixing of equal volumes of 100 µL of solution 1 and 100 µL 4×10^{-5} M KP or 100 µl 8×10^{-5} M KP giving correspondingly final solution 2 of KP concentration 3.0×10^{-5} M and solution 3 of 5.0×10^{-5} M. The concentration of KP in test solution 1 was estimated from calibration curve of fluorescence intensity on blue channel plotted versus -log[KP] in solutions 2, 3. The accuracy of KP assessment was estimated through the percentage of known initial concentration recovery, R %, and the relative error of analysis, RSD%.

Section S5.

All-solid state optodes selectivity evaluations

For selectivity tests the 5 µL solutions of primary KP ion, and interfering species in concentrations 4×10^{-5} M prepared on 0.005 M TRIS pH 7.0 background were spiked in triplicate on sensing spots of membrane Mb5 deposited on FP support, see Figure S14 for experimental details. The acquired image of optode was splitted in 3 channels, red, green and blue and the relative luminescence intensity for every ion was calculated as percentage according to the equation (1):

$$I_{lum} = \frac{(I_{lum\ BLUE}^{ION} - I_{lum\ BLUE}^{L1})}{I_{lum\ BLUE}^{L1}} \times 100\% \qquad (1)$$

Where $I^{ION}_{lumBLUE}$ and $I^{L1}_{lumBLUE}$ are the luminescence intensity of membrane Mb5 sensing spots spiked with 4×10^{-5} M in 0.005 M TRIS pH 7.0 background solution of primary KP or interfering ion and only 0.005 M TRIS pH 7.0 background respectively on blue channel.

The optode selectivity coefficients, $K_{KP/ION}$, were evaluated according to the equation (2) by a modified separate solution method (SSM)[1]. For this method, the differences in the sensor luminescence intensities with respect to the blue channel for primary KP ion, $I^{KP}_{lumBLUE}$ and for interfering species, $I^{ION}_{lumBLUE}$, in concentrations 8×10^{-5} M prepared on 0.005 M TRIS pH 7.0

background were subtracted and normalized to the slope of the semi logarithmic calibration curve toward KP on FP support, $S^{KP}_{lumBLUE}$, reported in Figure S14. The difference in interfering ion charge, z_{ION}, was also considered:

$$logK_{KP/ION} = \frac{(I^{KP}_{lum\,BLUE} - I^{ION}_{lum\,BLUE})}{S^{KP}_{lum\,BLUE}} + \left(1 - \frac{z_{KP}}{z_{ION}}\right) \times \log [ION] \quad (2)$$

The sketch on selectivity tests of L1-based membrane Mb5 deposited on FP support is shown in Figure S16. The It should be noted that the presented estimated selectivity coefficient values may be dependent on experimental conditions. No influence of all parent compounds such as naproxen, ibuprofen and benzoate anions which can simultaneously be present for instance in pharmaceutical formulations, as well as few influence of other tested ions were registered to the optical response of L1-based membrane Mb5 towards KP, as it is shown in Figure 7 of main text.

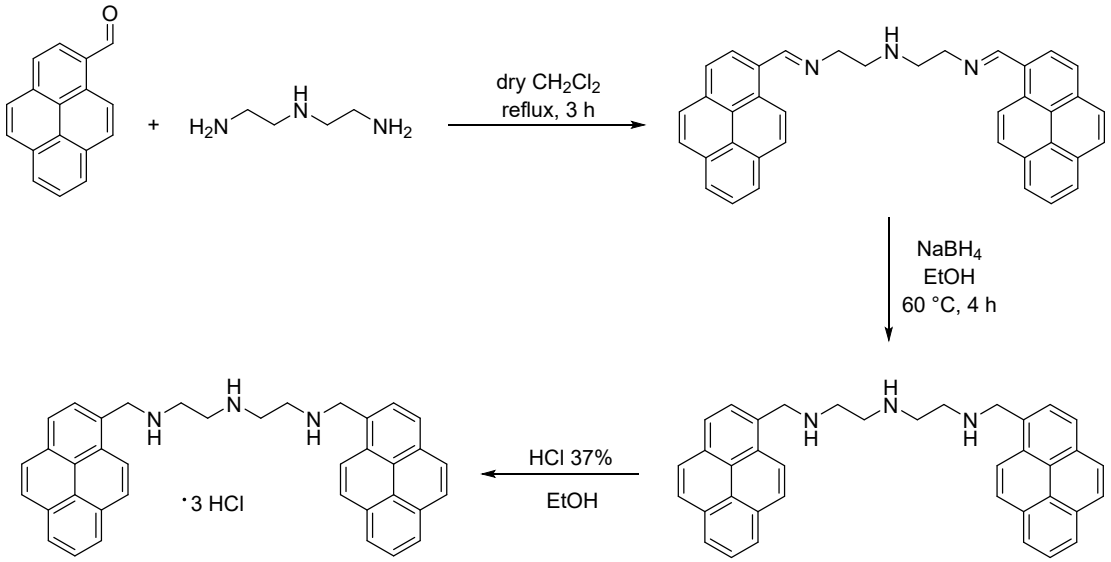
Section S6.

Multisensor approach for KP low detection limit lowering.

The low detection limit of KP detection from PLS1 model was estimated by 3σ method according to the equation (3):

$$LOD = 3\sigma/S \quad (3)$$

where σ is the RMSEC recalculated in mg/L and S is the slope of the regression line at calibration stage. In particular, the RMSEC value was 0.133 of -log[KP] units, and S = 0.944. The σ value was recalculated in mg/L and further the low LOD for ketoprofen was estimated as 0.21 mg/L (0.84 μM).



Scheme S1. Synthetic route to obtain L1·3HCl.

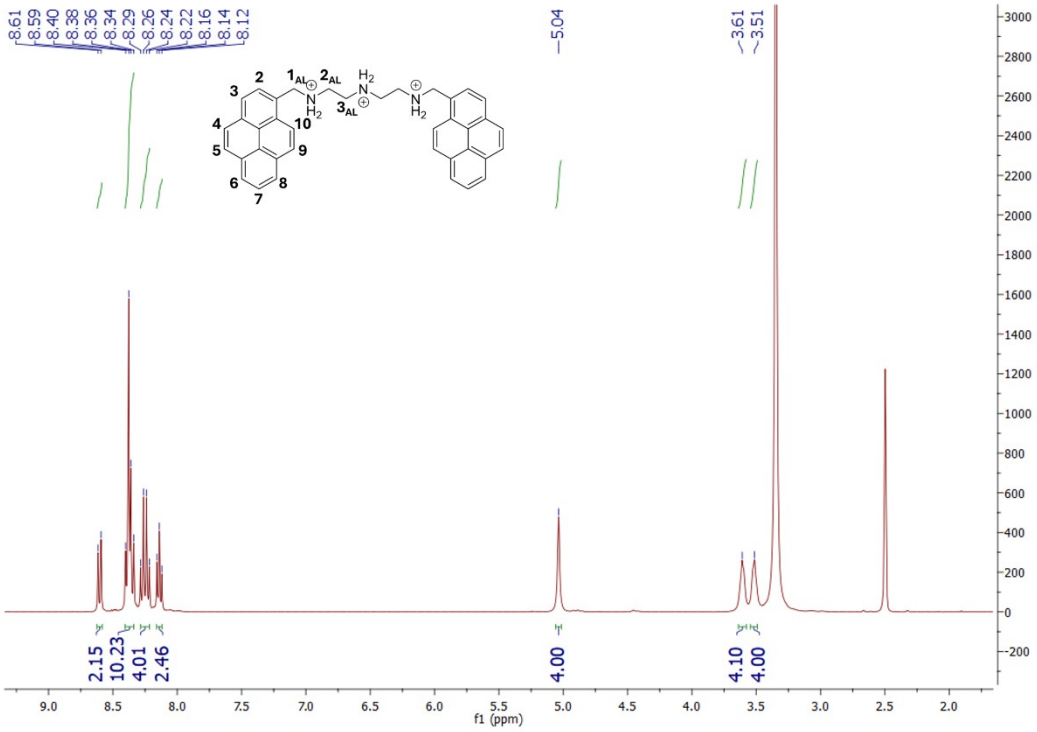


Figure S1. ^{1}H NMR spectrum of L1·3HCl (DMSO-d_6, 400 MHz, 298 K): δ(ppm) 8.60 (d, *J*=9.2 Hz, 2H, ArH), 8.40-8.34 (m, 10H, ArH), 8.29-8.22 (m, 4H, ArH), 8.14 (t, *J*=7.6 Hz, 2H, ArH), 5.04 (s, 4H, 1_{AL}), 3.65-3.57 (m, 4H, 2_{AL}), 3.56-3.48 (m, 4H, 3_{AL}).

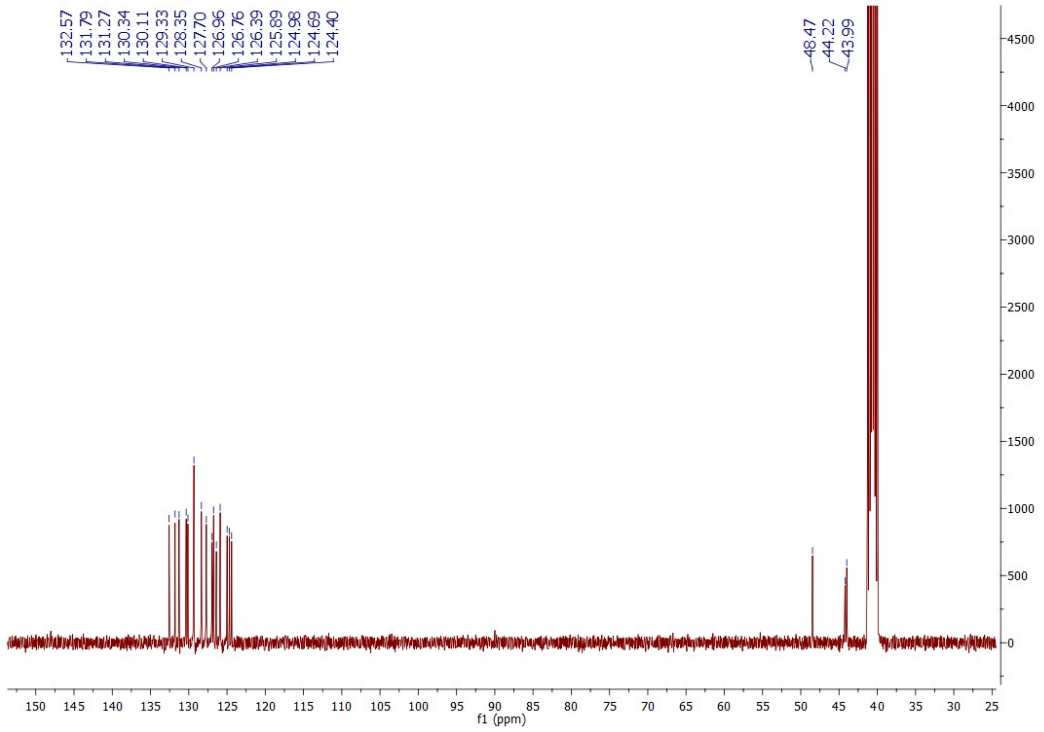


Figure S2. ^{13}C NMR spectrum of L1·3HCl (DMSO-d_6, 400 MHz, 298 K): δ(ppm) 132.57, 131.79, 131.27, 130.34, 130.11, 129.33, 128.35, 127.70, 126.96, 126.76, 126.39, 125.89, 124.98, 124.69, 124.40, 48.47, 44.22, 43.99.

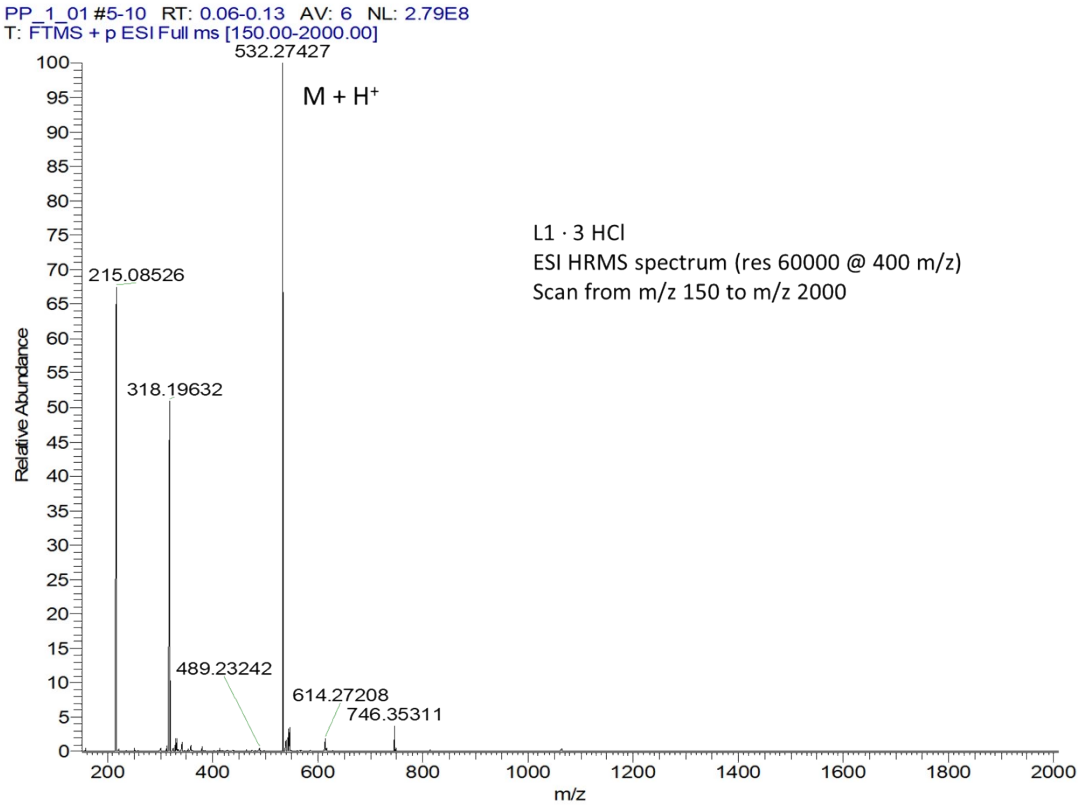


Figure S3. High resolution ESI MS spectrum of L1·3HCl; 532.27427 (m/z = 1, $[L1 \cdot 3HCl + H]^+$).

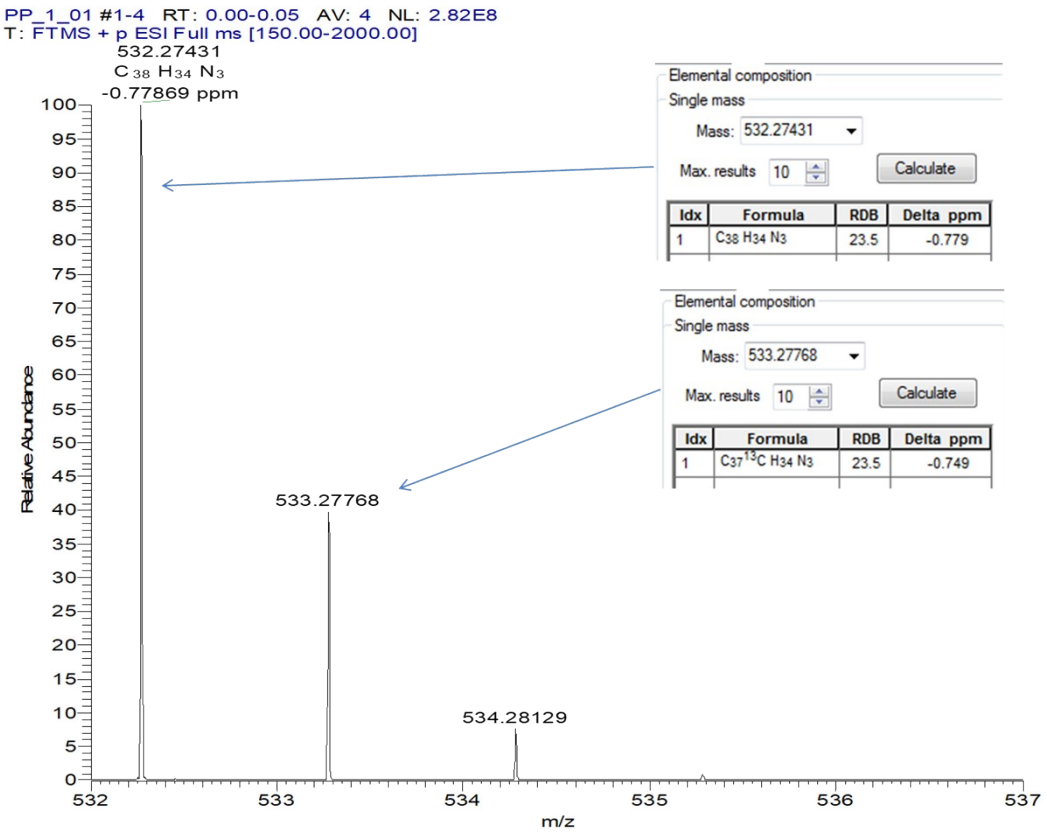


Figure S4. Isotopic pattern of the $[L1 \cdot 3HCl+H]^+$ (m/z = 1) ion, 532-537 Da region.

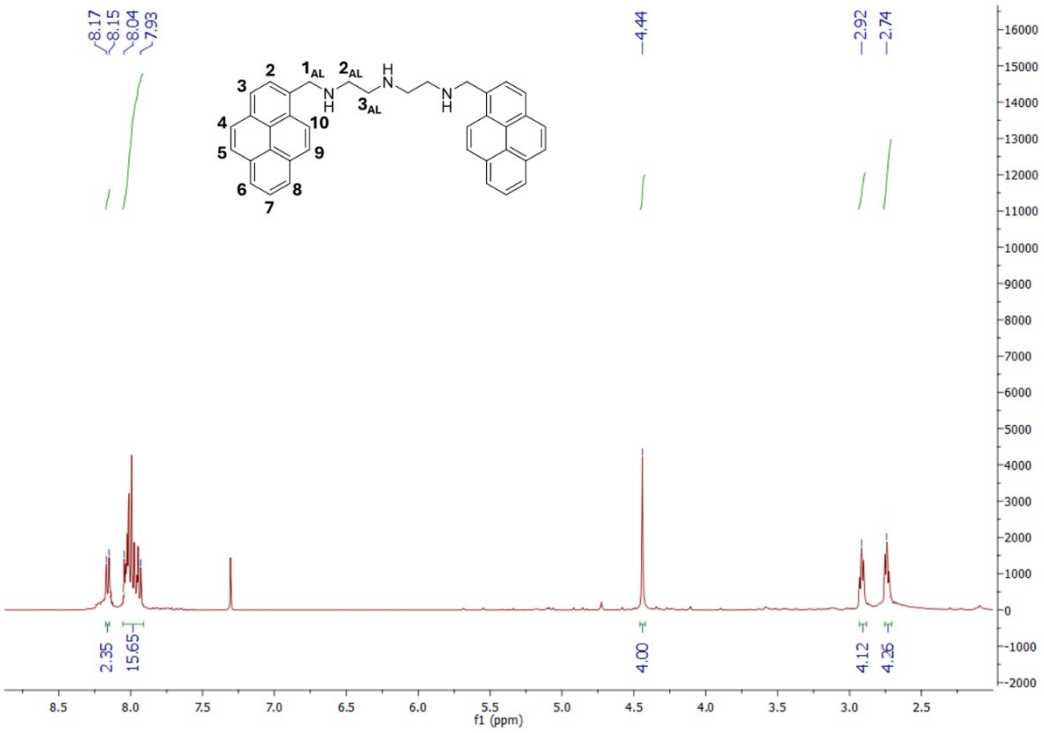


Figure S5. ^1H NMR spectrum of L1 ($CDCl_3$, 400 MHz, 298 K): δ(ppm) 8.80 (d, *J*=7.6 Hz, 2H, ArH), 8.04-7.93 (m, 16H, ArH), 4.44 (s, 4H, H4, 1_{AL}), 2.92 (t, *J*=6.0 Hz, 4H, 2_{AL}), 2.74 (t, *J*=6.0 Hz, 4H, 3_{AL}).

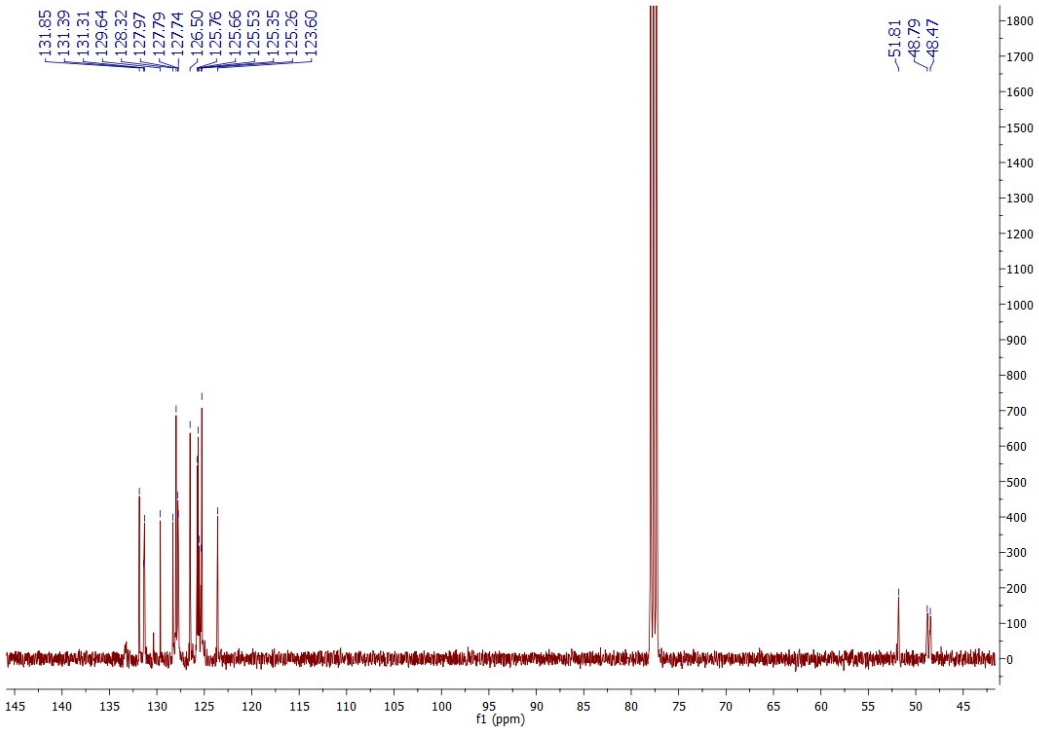


Figure S6. ^{13}C NMR spectrum of L1 ($CDCl_3$, 400 MHz, 298 K): δ(ppm) 131.85, 131.39, 131.31, 129.64, 128.32, 127.97, 127.74, 126.50, 125.76, 125.66, 125.53, 125.35, 125.26, 123.60, 51.81, 28.79, 48.47.

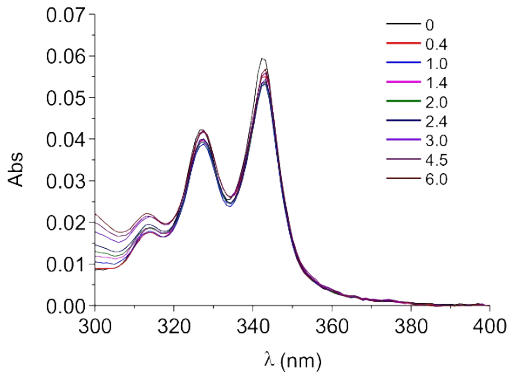


Figure S7. Absorption spectra of L1 at pH 7 in presence of increasing amounts of KP ([L1] = 5 x 10^{-7} M, H_2O/EtOH 1:1 v/v, 298 K)

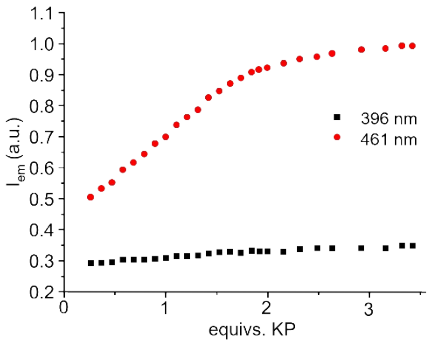


Figure S8. Plot of the emission intensity of L1 at 396 nm and 461 nm in presence of increasing amount of KP (H_2O/EtOH 1:1 v/v, pH = 7, TRIS 0.005 M, [L1] = 5 × 10^{-7} M, λ_{exc} = 360nm).

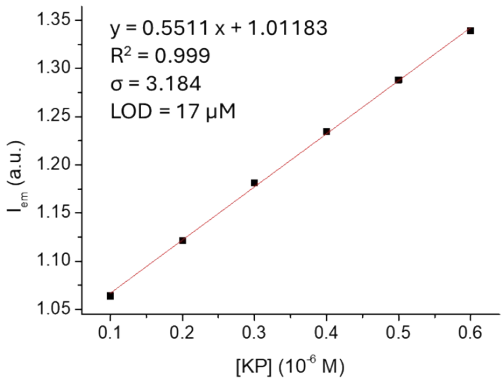


Figure S9. Plot of fluorescence emission of L1 ([L1] = 10^{-7} M, λ_{exc} = 360 nm, σ = 3.184) at pH 7 (0.005 M TRIS buffer) in H_2O/EtOH 1:1 (v/v) in the presence of increasing amount of KP at 298 K. The detection limit was calculated to be 17 μM.

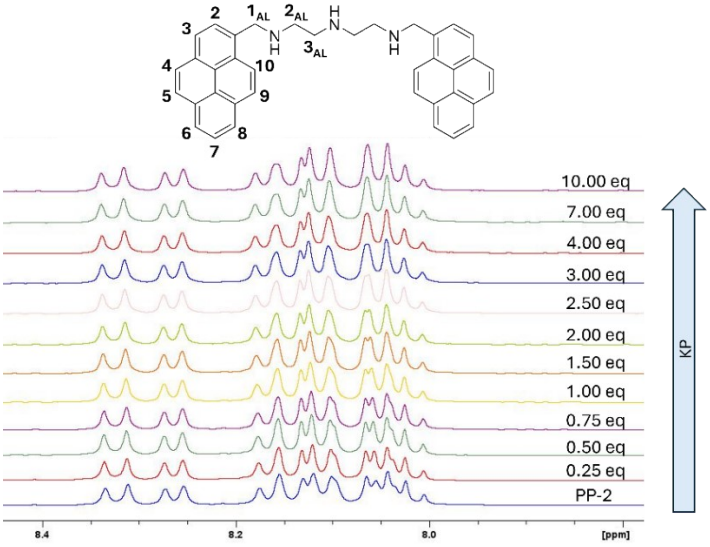


Figure S10. Aromatic signals of the ^{1}H NMR spectra of L1 at pH 7 in the presence of increasing amounts of KP. (D_2O/DMSO-d_6 1:4 v/v, [L1] = 10^{-3} M, 298 K).

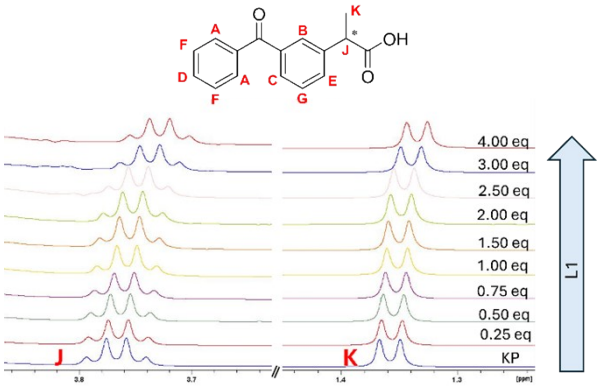


Figure S11. Aliphatic signals of the ^{1}H NMR spectra of KP at pH 7 in the presence of increasing amounts of L1. (D_2O/DMSO-d_6 1:4 v/v, [KP] = 10^{-3} M, 298 K).

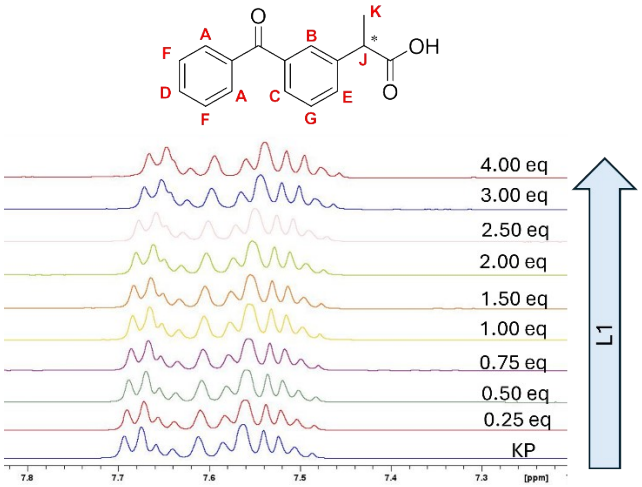


Figure S12. Aromatic signals of the ^{1}H NMR spectra of KP at pH 7 in the presence of increasing amounts of L1. (D_2O/DMSO-d_6 1:4 v/v, [KP] = 10^{-3} M, 298 K).

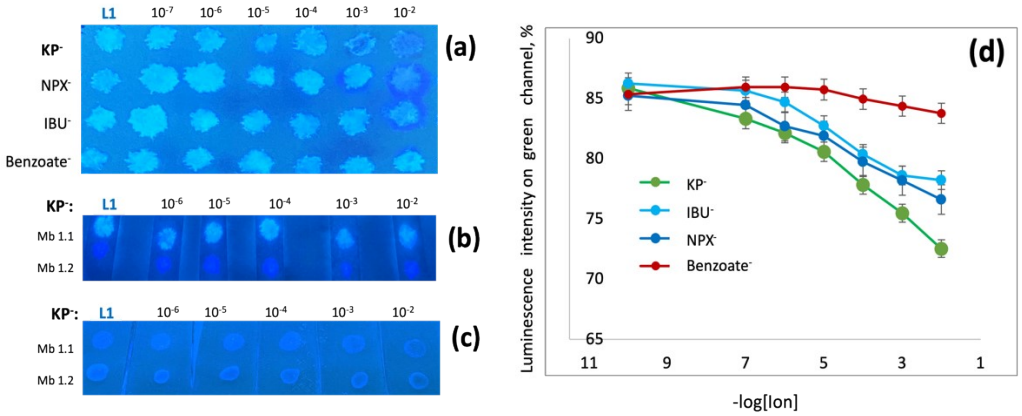


Figure S13. The photograms of L1-based all-solid-state optodes with membranes Mb1.1 and Mb1.2 respectively deposited on (a) CC; (b) FP, and (c) GS solid support and (d) calibration curves in fluorescence redout mode to KP^-, NPX^-, IBU^- and $Benzoate^-$ ions in concentration range 1×10^{-7} to 1×10^{-2} M, λ_{exc} = 365 nm.

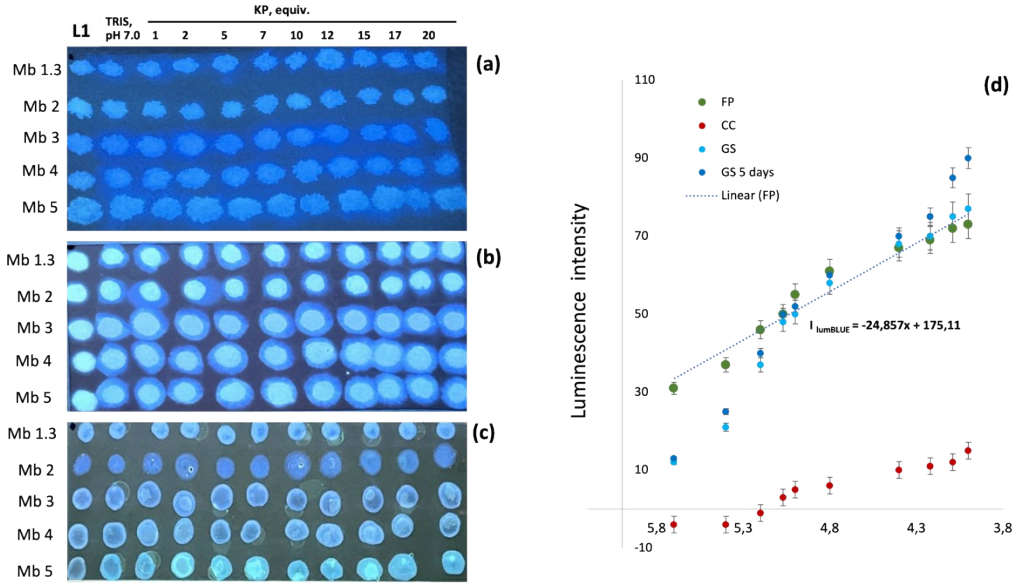


Figure S14. The photograms of L1-based all-solid-state optodes deposited on (a) CC; (b) FP, and (c) GS solid support and (d) calibration curves in fluorescence redout mode to KP in concentration range 2×10^{-6} to 1×10^{-4} M, (1-20 equiv. in respect to L1 amount in membrane). λ_{exc} = 365 nm.

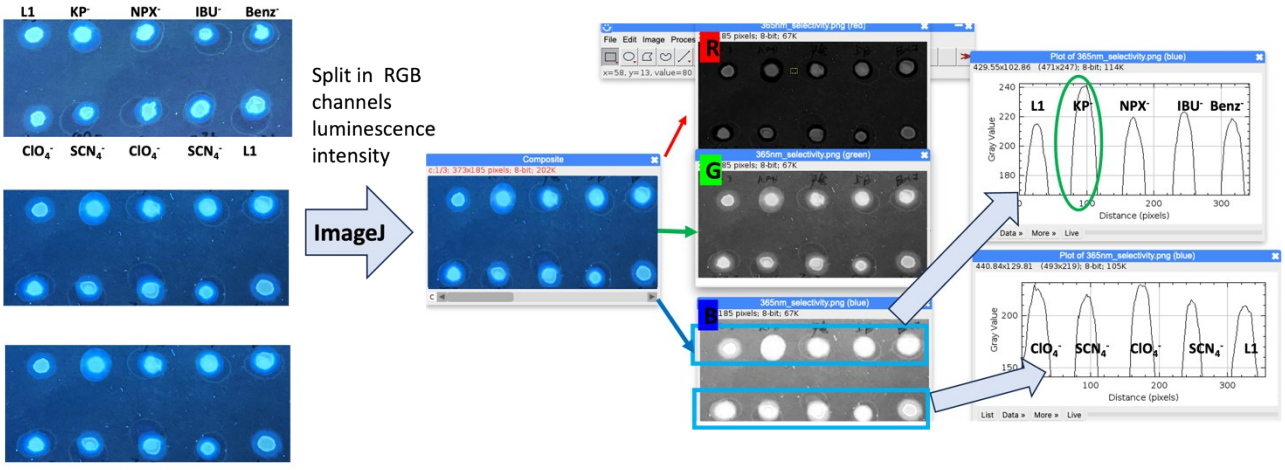


Figure S15. The sketch of selectivity tests of L1-based membrane Mb5 deposited on FP support. The images were treated with ImageJ freeware, split on RGB channels and the intensity on blue channel was considered for selectivity estimation. [Ion] = 4×10^{-5} M, λ_{exc} = 365 nm, n = 6.

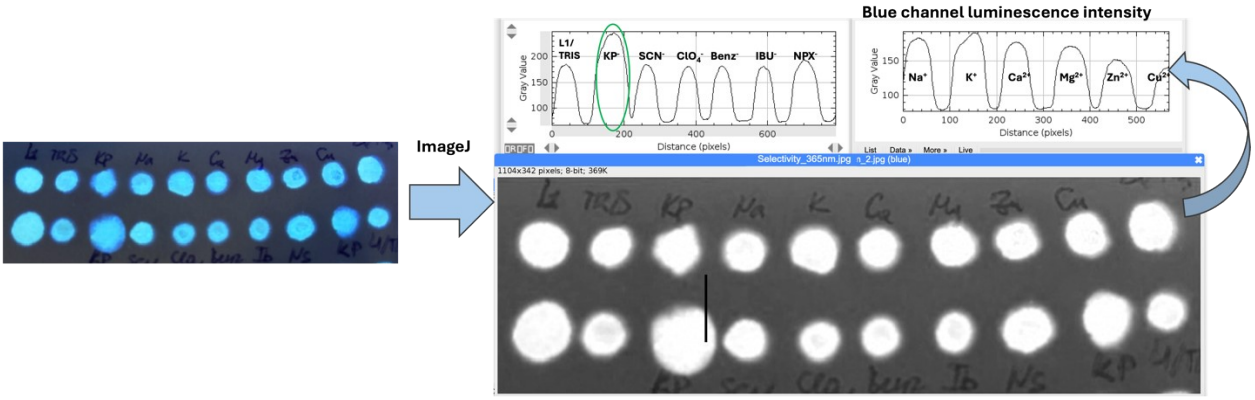


Figure S16. The sketch on selectivity tests of L1-based membrane Mb5 deposited on FP support. The images were treated with ImageJ freeware, split on RGB channels and the luminescence intensity on blue channel was considered for selectivity estimation. [Ion] = 8×10^{-5} M, λ_{exc} = 365 nm, n = 3.

References

1. E. Bakker, P. Bühlmann and E. Pretsch, *Chem. Rev.,* 1998, **98**, 1593-1687.