Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Ultra-low fouling alkylimidazolium modified surfaces to detect HER2 in breast cancer cell lysate

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GENERAL INFORMATION

List of abbreviations

EtOH: Ethanol 100%

DMF: N,N-Dimethylformamide

EtOAc: Ethyl acetate

DCM: Dichloromethane

TBAB: Tetrabudylammonium bromide

MeOH: Methanol

$LiNTf_2$: Bis(trifluoromethane)sulfonimide lithium salt

Materials

HBr, TBAB, K_2CO_3, KOH, $CDCl_3$, 3-bromopropionic acid, 6-bromohexanoic acid, NaBr, $NaBH_4$, $NaClO_4$, $NaPF_6$ and $LiNTf_2$ were purchased from Sigma-Aldrich (St. Louis, MO). Potassium thioacetate, 1,2-dibromododecane, imidazole, were purchased from Alfa Aesar. Sulfuric acid, DCM, DMF, EtOAc, were purchased from Fisher Scientific. Dimethyl-d6 sulfoxide was purchased from C/D/N isotopes (Pointe-Claire, QC). The silica used to purify the compounds by chromatography was purchased from Silicycle Chemical division (40-63 nm; 230-240 mesh). The thin-layer chromatography (TLC) was performed on glass-backed silica gel. Visualization of TLC plates was performed by $KMnO_4$ stain. All mixed solvent eluents are reported as volume/volume solutions. NMR spectra were acquired with a Bruker AV-400 instrument, except for the 13C spectra that were acquired with a Bruker AV-300 instrument. Multiplicity in the reported spectra analysis is indicated as follow: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet) and br (broad). Exact MS spectra were acquired with a Synapt G2-Si instrument fromWaters.

Fabrication of SPR chips

Dove glass prisms were washed in piranha solution (3:1 sulfuric acid : hydrogen peroxide 30%) during 1 hour at room temperature. They were then thoroughly rinsed with deionized water and rinsed once with ethanol afterwards. Prior to gold deposition, the glass prisms were air dried and placed into a sputtering instrument (Cressington 308R sputter coater, Ted Pella Inc. Redding, CA) where approximatively 0.7 nm (35 seconds) of chromium and 50 nm (1 minute and 40 seconds) of gold were deposited on the surface to form a continuous film.

IONIC LIQUID SYNTHESIS

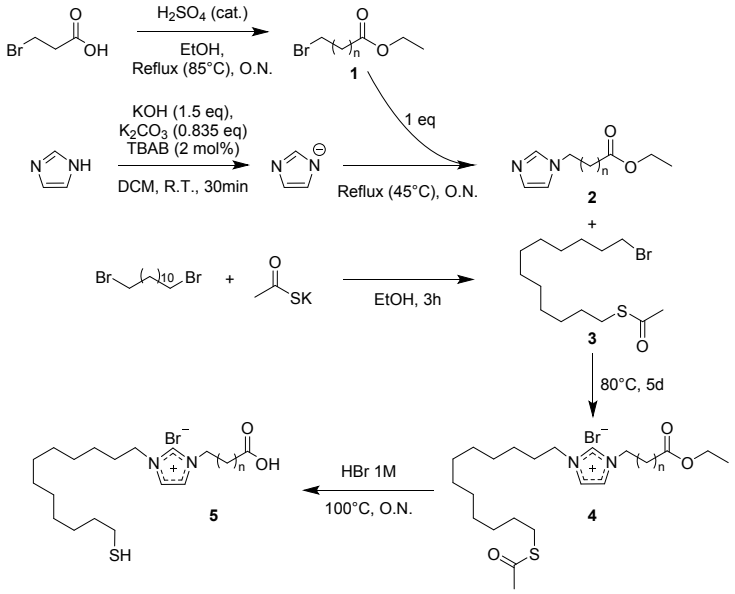
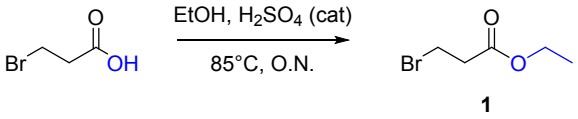
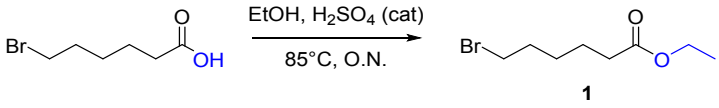


Figure S1. Synthesis of 1-(2-carboxyethyl)-3-(12-mercaptododecyl)-1H-imidazol-3-ium bromide (n=1) and 1-(5-carboxypentyl)-3-(12-mercaptododecyl)-1H-imidazol-3-ium bromide (n=4) (The same figure is found in the paper (Figure 2). It was repeated here for clarity.)



1

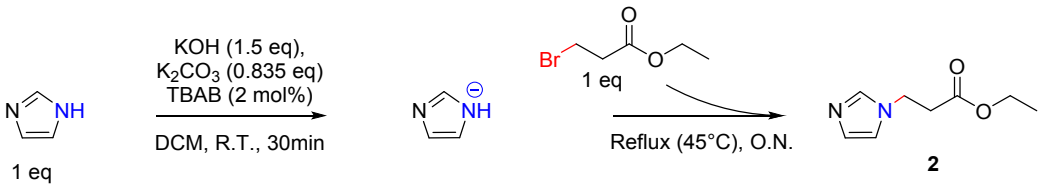
Ethyl-3-bromopropionate (1, n=1). 3-bromopropionic acid (7.6 g, 50 mmol) was added to 150 mL of EtOH in a round-bottom flask. The mixture was agitated until the acid is well dissolved and 50 drops of H_2SO_4 was added. The light orange mixture was agitated at reflux (85°C) overnight. The EtOH was evaporated to yield a dark orange solution and 120 mL of water and 30 mL of $NaHCO_3$ saturated solution was added. The mixture was agitated for a few seconds and transferred in a separatory funnel to be extracted 6 times with 80 mL of DCM. The organic phase was dried on $MgSO_4$ and filter on cotton. Solvent was evaporated and the product was purified by gel column chromatography (100% hexanes to 2% ether/hexanes). A yellowish liquid compound was obtained with a 44% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.224 (q, 2H, J=7.2), 3.615 (t, 2H, J=6.8), 2.941 (t, 2H, J=6.8), 1.313 (t, 3H, J=7.2).



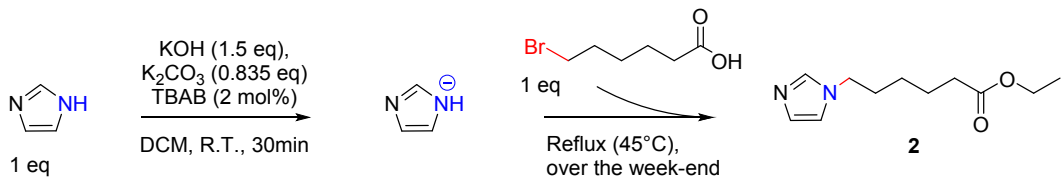
1

Ethyl-3-bromohexanoate (1, n=4). 3-bromohexanoic acid (12.7 g, 65 mmol) was added to 200 mL of EtOH in a round-bottom flask. The light orange mixture was agitated until the acid is well dissolved and 30 drops of H_2SO_4 was added. The mixture was agitated at reflux (85°C) overnight. The EtOH was evaporated to yield a dark orange solution and 40 mL of water and 10 mL of $NaHCO_3$ saturated solution was added. The mixture was agitated for a few seconds and transferred in a separatory funnel to be extracted 6 times with 50 mL of DCM. The organic phase was dried over $MgSO_4$ and

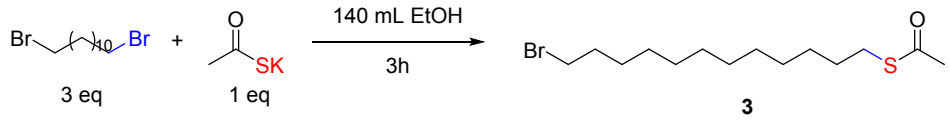
filter on cotton. Solvent was evaporated and the product was purified by gel column chromatography (5% ether/hexanes). A yellowish liquid compound was obtained with a 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.163 (q, 2H, J=7.2), 3.441 (t, 2H, J=6.8), 2.347 (t, 2H, J=7.2), 1.913 (m, 2H), 1.690 (quin, 2H, J=7.6), 1.511 (m, 2H), 1.290 (t, 3H, J=7.2).



Ethyl 3-(1H-imidazol-1-yl)propanoate (2, n=1). Imidazole (3.0 g, 44 mmol) was added to a round-bottom flask and dissolved in 25 mL of DCM by sonication. KOH (3.7 g, 66 mmol), K_2CO_3 (5.1 g, 36.74 mmol) and TBAB (0.283 g, 2 mol%) were added to the mixture with another 8 mL of DCM. The mixture was agitated 30 minutes at reflux (45°C). Compound 1 (n=1) was added dropwise to the mixture while stirring and the mixture was agitated overnight. The mixture was filtered on Buckner and the flask and residue were rinsed 3 times with DCM. The mixture was then filtered on cotton and transferred in a separatory funnel. It was washed 6 times with 50 mL H2O (until the water pH=7). The aqueous phase was then transferred in a separatory funnel to recover all the DCM. The organic phase was dried over $MgSO_4$ and the solvent was evaporated. A translucent oil was obtained, with a yield of 85%. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 7.613 (s, 1H), 7.171 (s, 1H), 6.872 (s, 1H), 4.210 (t, 2H, J=6.4), 4.069 (q, 2H, J=7.2), 2.830 (t, 2H, J=6.4), 1.168 (t, 3H, J=7.2).

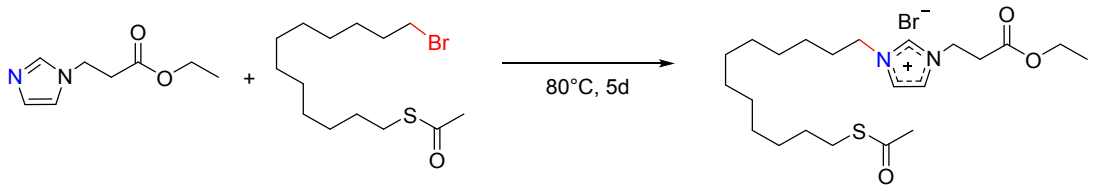


Ethyl 6-(1H-imidazol-1-yl)hexanoate (2, n=4). Imidazole (3.4 g, 50 mmol) was added to a round-bottom flask and dissolved in 25 mL of DCM by sonication. KOH (4.2 g, 75 mmol), K_2CO_3 (5.8 g, 41.75 mmol) and TBAB (0.322 g, 2 mol%) were added to the mixture with another 5 mL of DCM. The mixture was agitated 30 minutes at reflux (45°C). Compound 1 (n=4) was added dropwise to the mixture while stirring and the mixture was agitated over-the-week-end (overnight is probably enough). The mixture was filtered on Buckner and the flask and residue were rinsed 5 times with DCM. The organic phase was washed 4 times with 30 mL of brine solution and dried over $MgSO_4$. The solvent was evaporated yielding in a dark orange liquid which was purified by gel column chromatography (2% MeOH/DCM) to obtain a yellow oil (with traces of TBAB that were not separated). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 7.607 (s, 1H), 7.161 (s, 1H), 6.878 (s, 1H), 4.047 (q, 2H, J=7.2), 3.945 (t, 2H, J=7.2), 2.279 (t, 2H, J=7.6), 1.707 (quin, 2H, J=7.2), 1.543 (quin, 2H, J=7.6), 1.214 (m, 2H), 1.178 (t, 3H, J=7.2).



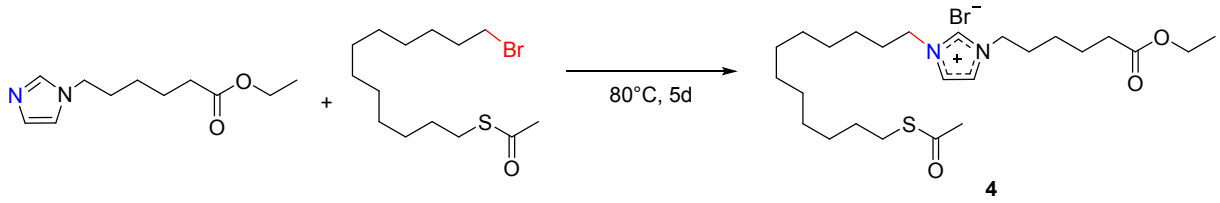
S-(12-bromododecyl) ethanethioate (3). 1,2-dibromododecane (66.0 g, 201 mmol) was added in a round bottom flask and heated to 85°C until the compound is liquefied. Potassium thioacetate (7.7 g, 67 mmol) was dissolved in a minimum of EtOH (140 mL) by sonication (10 minutes). The potassium thioacetate solution (not the solid

residue) was added dropwise to the liquefied 1,2-dibromododecane (the addition took 3 hours). After the addition, the reaction was followed by TLC and was finished. The hot mixture was filtered on cotton and rinsed with a small volume of EtOAc. The solvent was evaporated and the compound was allowed to cool-down at room temperature, leading to a solid white compound. A dry-pack was prepared in order to purify the product by gel column chromatography (100% hexanes until the first product (1,2-dibromododecane) is out of the column, then 2% EtOAc/hexanes). The 1,2-dibromododecane was recovered for further use. After evaporating the solvent from the second product (desired product) (small volumes in large flasks since the product is a surfactant and bumps in the rotary evaporator), the white product has been re-purified by gel column chromatography (100% hexanes to 3% EtOAc/hexanes). A white solid was obtained with a 55% yield. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 3.536 (t, 2H, J=6.0), 2.832 (t, 2H, J=6.0), 2.327 (s, 3H) 1.797 (quin, 2H, J=6.8), 1.503 (quin, 2H, J=7.2), 1.383 (br m, 2H), 1.259 (br m, 14H).



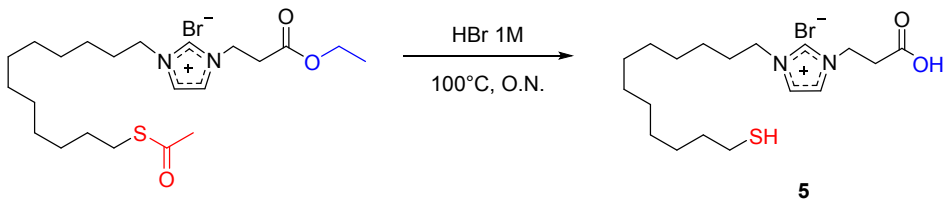
3-(12-(acetylthio)dodecyl)-1-(3-ethoxy-3-oxopropyl)-1H-imidazol-3-ium bromide (4, n=1). Compounds 2 (n=1) and 3 were added in a round-bottom flask, heated to 80°C and agitated 5 days. 60 mL of hexanes were added in the flask and agitated 2 minutes wash the compound. The hexanes is discarded (the desired compound is very viscous and stays in the flask) and the washing procedure is repeated once. The residual

hexanes is evaporated, yielding in a light orange opaque viscous oil with a quantitative yield. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 9.206 (s, 1H), 7.799 (s, 2H), 4.408 (t, 2H, J=6.8), 4.169 (t, 2H, J=6.8), 4.083 (q, 2H, J=6.8), 3.007 (t, 2H, J=6.8), 2.830 (t, 2H, J=6.8), 2.330 (s, 3H), 1.780 (m, 2H), 1.499 (m, 2H), 1.245 (br m, 16H), 1.179 (t, 3H, J=7.2).



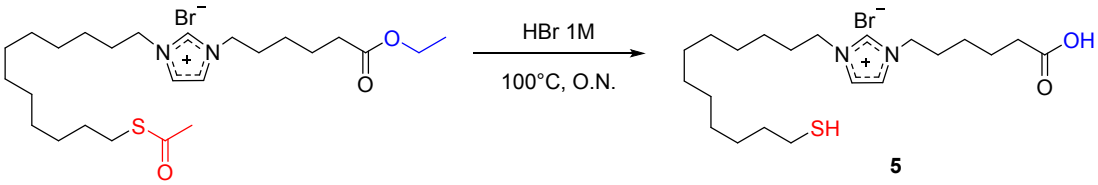
4

3-(12-(acetylthio)dodecyl)-1-(6-ethoxy-6-oxohexyl)-1H-imidazol-3-ium bromide (4, n=4). Compounds 2 (n=4) and 3 were added in a round-bottom flask, heated to 80°C and agitated 5 days. 60 mL of hexanes was added in the flask and agitated 2 minutes wash the compound. The hexanes is discarded (the desired compound is very viscous and stays in the flask) and the washing procedure is repeated once. The residual hexanes is evaporated, yielding in a dark orange translucent viscous oil with a quantitative yield. . ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 9.197 (s, 1H), 7.811 (s, 2H), 4.163 (m, 4H), 4.055 (q, 2H, J=6.8), 2.827 (t, 2H, J=8.0), 2.329 (s, 3H), 2.303 (t, 2H, J=7.6), 1.795 (m, 4H), 1.560 (m, 2H), 1.499 (m, 2H), 1.246 (br m, 18H), 1.182 (t, 3H, J=7.2).



5

1-(2-carboxyethyl)-3-(12-mercaptododecyl)-1H-imidazol-3-ium bromide (5, n=1). Compound 4 (n=1) was kept in the same flask (from last reaction) and 80 mL of HBr 1M was added. The mixture was agitated at reflux (100°C) overnight. The liquid was transferred to a separatory funnel and the aqueous phase was extracted with 3 times 100 mL DCM. The organic phase was dried over $MgSO_4$ and the solvent was evaporated, yielding in a light orange solid with 83% yield. Melting point: 88°C. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 9.242 (s, 1H), 7.808 (s, 2H), 4.370 (t, 2H, J=6.8), 4.170 (t, 2H, J=7.2), 2.926 (t, 2H, J=7.2), 2.469 (q, 2H, J=7.6), 2.239 (t, 2H, , J=6.8), 1.780 (br quin, 2H, J=7.6), 1.527 (quin, 2H, , J=7.2), 1.252 (m, 14H). ^{13}C NMR (300 MHz, DMSO-d6) δ ppm: 172.13, 136.89, 122.98, 122.75, 49.26, 45.23, 34.14, 33.82, 29.79, 29.40 (3C), 29.25, 28.95, 28.80, 28.19, 25.89, 24.21. Experimental exact mass (m/z): 341.22750 Calculated exact mass (m/z): 341.22630



5

1-(5-carboxypentyl)-3-(12-mercaptododecyl)-1H-imidazol-3-ium bromide (5, n=4). Compound 4 (n=4) was kept in the same flask (from last reaction) and 80 mL of HBr 1M was added. The mixture was agitated at reflux (100°C) overnight. The liquid was transferred to a separatory funnel and the aqueous phase was extracted with 3 times 100 mL DCM. The organic phase was dried over $MgSO_4$ and the solvent was evaporated, yielding in a white powder with 91% yield. Melting point: 104°C. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 12.025 (s, 1H), 9.214 (s, 1H), 7.814 (s, 2H), 4.168-4.052 (m, 4H),

2.471 (q, 2H, J=7.2), 2.224 (m, 2H), 1.803 (m, 4H), 1.532 (m, 4H), 1.251 (m, 18H). ^{13}C NMR (300 MHz, DMSO-d6) δ ppm: 174.74, 136.41, 122.91 (2C), 49.31, 49.10, 33.81, 33.79, 29.71, 29.47, 29.40 (2C), 29.35, 29.26, 28.95, 28.78, 25.93, 25.43, 24.20, 24.17. Experimental exact mass (m/z): 383.27360 Calculated exact mass (m/z): 383.27320

SPR MEASUREMENTS

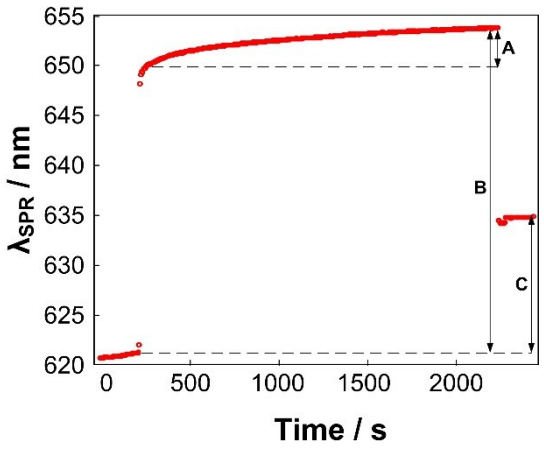


Figure S2. Different calculation techniques for the measurement of the nonspecific binding shift on a SPR sensorgram

The nonspecific adsorption shifts that are reported in the article correspond to shifts labeled A and C. Shift A represents the measurement of real-time adsorption of nonspecific material on the surface, termed *real-time nonspecific adsorption* in the main text. Shift C corresponds to the shift from the nonspecific adsorption of strongly bonded molecules, termed *irreversible nonspecific adsorption* in the main text. To an average of the last 30 seconds of the cell lysate exposition, the mean of the 375th second to the 400th second was subtracted. Seconds 375 to 400 were selected to be able to compare as precisely as possible every sensorgram (with the shift A). Even if some of the lipids nonspecific adsorption may occur during the bulk refractive index change, this does not affect the real-time nonspecific adsorption measurements. If real-time measurement of the binding of a protein, a biomarker for example, were to be made, the protein adsorption would be much slower than the adsorption of lipids. Therefore, the protein binding signal would be distinguishable from the nonspecific adsorption and this is what is important for the quantitation of a biomarker. Also, we observe on Figure 3 that the

bulk refractive index change is smaller for the ionic liquid monolayer than the one for the polymer or the bare gold. Thus, we are confident that the nonspecific adsorption of cell lysate in decreased significantly by ionic liquids monolayers.

The SAMs were also classified for their ability to reduce nonspecific binding after washing the surface with buffer (irreversible nonspecific adsorption). The shifts B was also measured, but the results were not use to compare one SAM to another. Shift B represents the bulk refractive index change, due to the difference in the refractive index between PBS and concentrated cell lysate, plus the nonspecific adsorption of material during the surface exposition to cell lysate.

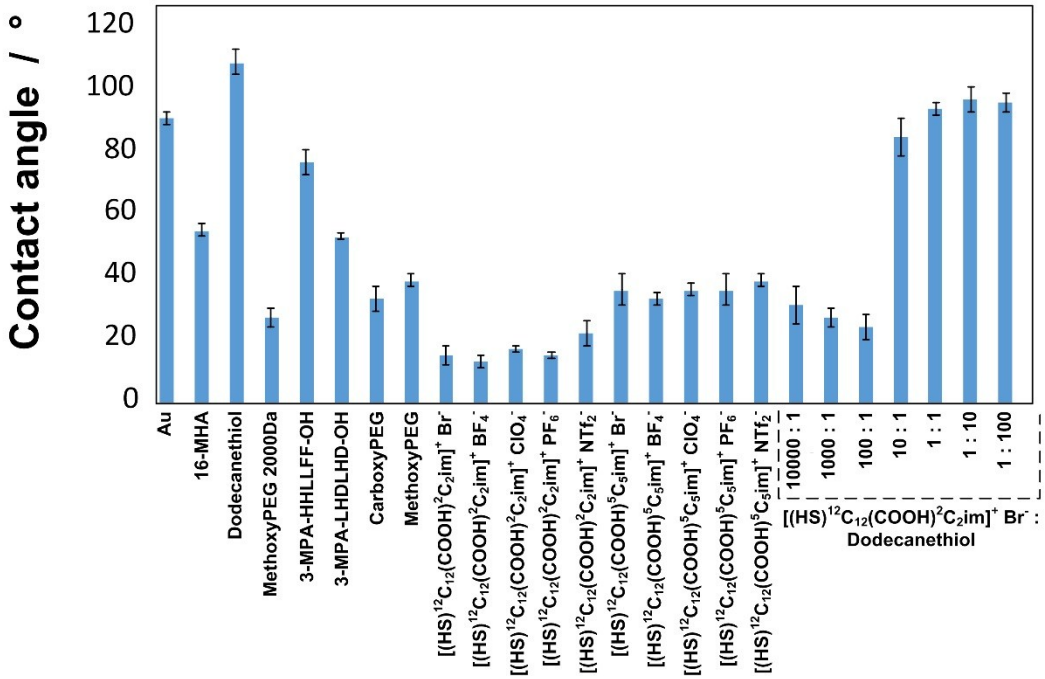


Figure S3. Contact angle of the different monolayers used to reduce nonspecific binding of cell lysate

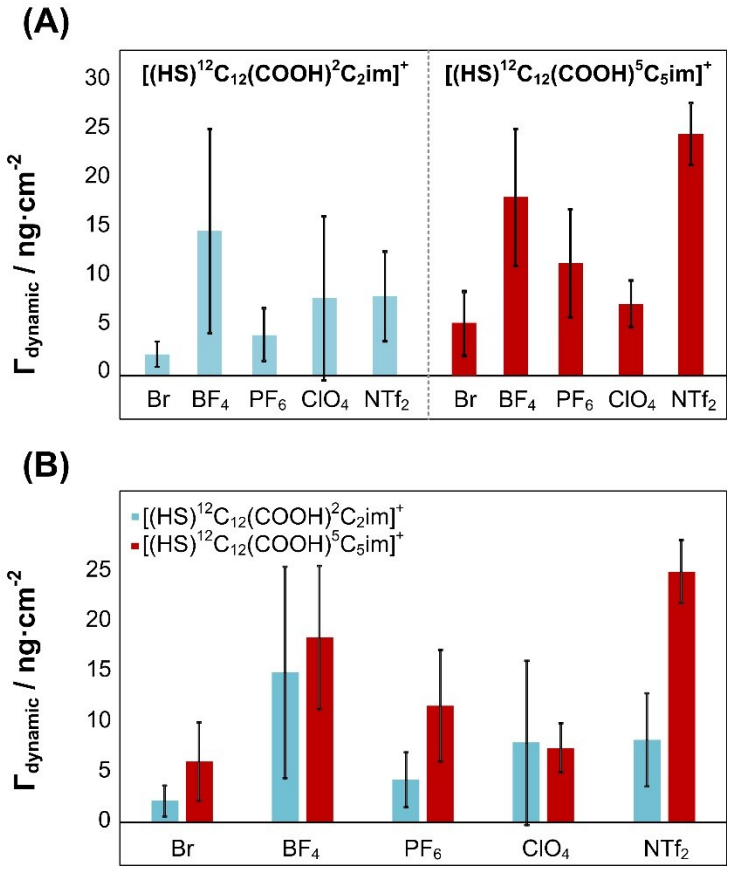


Figure S4. Real-time nonspecific adsorption of SK-BR-3 crude cell lysate on ionic liquid monolayers. (A) Effect of the chain length and (B) effect of the nature of the counterion.

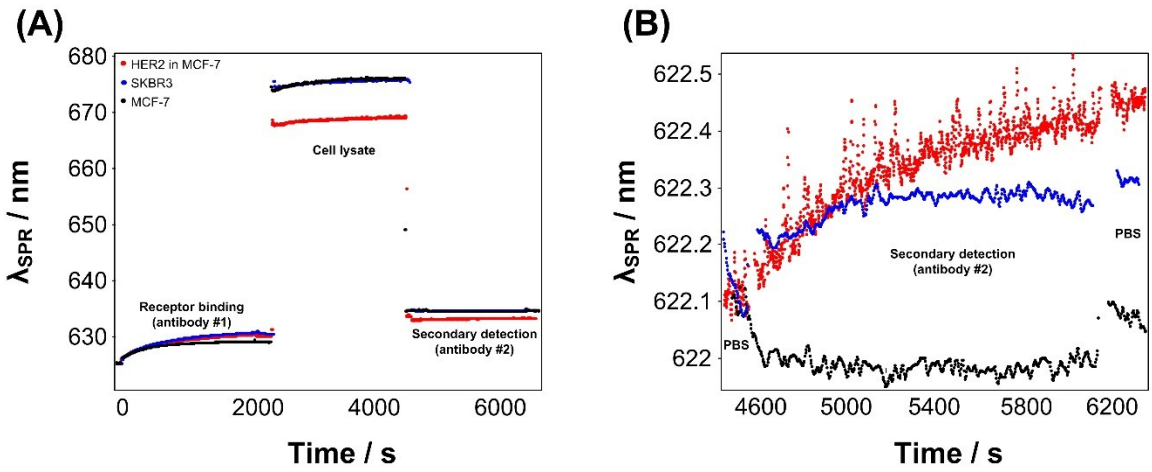


Figure S5. SPR sensorgram of HER2 detection in undiluted cell lysate by a sandwich assay on [(HS)12C12(COOH)5C5im]+ Br- SAM. Complete sensorgram (A) and zoom on the secondary detection with a Savitzki-Golay smoothing (x ± 2) (B).