# Supporting information

# High salt compatible oxyanion receptors by dual ion imprinting

Sudhirkumar Shinde,[1] Mona Mansour,[2] Anil Incel, Liliia Mavliutova, Celina Wierzbicka and Börje Sellergren*

Department of Biomedical Sciences, Faculty of Health and Society, Malmö University 20506 Malmö, Sweden.

1) Current address: School of Chemistry and Chemical Engineering, Queens University Belfast, Northern Ireland, UK
2) Current address: Analysis and evaluation department, Egyptian petroleum research institute, 1 ahmed el zomor street, nasr city, Cairo, Egypt

* Corresponding author

## Experimental Section

### Materials

Phenyl phosphonic acid (PPA) (98%) and phenyl sulfonic acid (PSA) (90%) came from Aldrich (Milwaukee, USA). PPA was used after recrystallization from water. The base 1,2,2,6,6-pentamethylpiperidine (PMP) was purchased from Fluka (Buchs, Switzerland). Pentaerythritol triacrylate (PETA) and acrylamide (AAm) were purchased from Sigma-Aldrich (Steinheim, Germany). The initiatior *N*,*N*´-Azo-bis-(2,4-dimethyl)valeronitrile (ABDV) was purchased from Wako Chemicals (Neuss, Germany). 18-crown-6, 4-Vinylbenzo-18-crown-6 (**2**), methanol (MeOH) of HPLC grade and MeCN of HPLC grade, were purchased from Acros (Geel, Belgium). All anhydrous solvents were stored over appropriate molecular sieves. p-Toluene sulfonic acid methyl ester (98%) was received from Sigma Aldrich (Milwaukee, USA) and p-toluene sulphonic acid monohydrate was received from Merck. Sodium phenyl phosphate dibasic dihydrate was received from Sigma, Japan. The host functional monomer N-3,5-bis(trifluoromethyl)-phenyl-N'-4-vinylphenylurea (**1**) was synthesized from 4-vinyl aniline (97%, Aldrich) and 3,5-Bis (trifluoromethyl)-phenyl isocyanate (98%, Aldrich) as reported in the literature.[1] The water used in all experiments is Milli-Q water with resistivity equal 18.2 MΩ.cm.

### Apparatus

The HPLC measurements were carried out on Hewlett-Packard HP 1100 instrument (Agilent Technology, Waldbronn, Germany). Elemental analysis were performed at the Department of Organic Chemistry, Johannes Guttenberg Universität Mainz using a Heraeus CHN-rapid analyser (Hanau, Germany). FT-IR spectroscopy was performed using a NEXUS FT-IR spectrometer (Thermo Electron Corporation, Dreieich, Germany) equipped with an Attenuated Total Reflection (ATR) accessory unit and ITR diamond (smart ITR) experimental set up.

Conductivity measurements: CON 510, Bench Conductivity/TDS Meter, Oakton Instruments was used for the measurement of inorganic phosphate and sulfate.

**Preparation of mono- and di- sodium salt of PPA (PPA·Na and PPA·2Na)**

PPA·Na and PPA·2Na were prepared by equilibrating 1 and 2 equivalent sodium hydroxide respectively in methanol with 1 equivalent of PPA followed by removal of solvent and drying. The resulting white solid was dried in oven at 40°C for 12 hrs. The mono-sodium salt of PSA was prepared in a similar way.

**Studies of complex formation by $^1$H NMR spectroscopy**

$^1$H NMR spectroscopic titrations were performed in dry deuterated DMSO. The 18-crown-6 sodium PPA complex was first prepared by mixing monosodium salt of PPA with a stoichiometric amount of 18-crown-6 in methanol followed by evaporation to dryness. The association constant $K_a$ for the interaction between hosts and guests was then determined by titrating an increasing amount of the 18-crown-6-Na·PPA complex into a constant amount of functional urea monomer **1** ($C_0$=1 mM) with the amount of added guest being 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0 and 10.0 equivalents with respect to host. The complexation induced shifts (CISs) of the host urea protons were followed and titration curves were constructed of CIS versus guest concentration. The raw titration data was fitted to a 1:1 binding isotherm by non-linear regression using Prism 7 (Graphpad software Inc) from which association constants were calculated.

**Polymer preparation**

Polymers were prepared using the compositions shown in Table S1. P1 was prepared as follows: PPA in the form of the bis-PMP (PPA·2PMP, prepared in situ) (0.5mmol), **1** (1 mmol), AAm (1 mmol) and PETA (13.3 mmol) were dissolved in dry MeCN (6.1 mL). The initiator ABDV (44 mg, 1% w/w of total monomers) was added to the solution which was subsequently transferred to a scintillation vial, cooled to 0°C and purged with a flow of dry nitrogen for 10 minutes. The vials were sealed with silicon insulating tape and kept in oven at 50 $^o$C for 24h and subsequently at 80 $^o$C for 3h. The vials were thereafter broken and the polymers crushed in a mortar with pestle for further use. P1,2 was prepared identically but using the disodium salt of PPA (PPA·2Na) as template and monomer 2 instead of AAm as second functional monomer. In P2 monomer 1 was omitted and AAm replaced by monomer 2.

Non-imprinted polymers ($P_N1$, $P_N1,2$, $P_N2$) were prepared in the same way as described above, but with the omission of the template molecule from the pre-polymerization solution. To promote template removal the polymers were shaken overnight in MeOH: 1N HCl (80:20), followed by centrifugation and HPLC-analysis of the supernatant for released template. The extraction was repeated twice for 3h and 1h respectively while monitoring the extent of template removal. Thereafter, the polymers were washed in Milli-Q water and MeOH. All wash fractions were analysed by HPLC for released template.

**Reversed phase HPLC detection of unbound PPA and PSA**

Chromatographic analysis was carried out with an HPLC 1100 instrument (Agilent) equipped with a quaternary pump auto-injector and DAD detector. The analytical column was a polar end capped C18 reversed phase Luna (250 mm x 4.6 (i.d.) mm). The mobile phase consisted of methanol/water (0.1 % TFA): 32/68 (v/v) and the flow rate was 1 mL/min. The injection volume was 5 μL. The column was kept at room temperature. The absorbance wavelength was 205 nm for PSA and 225 nm for PPA. For quantification purposes calibration standards were made up using the same solvent as used in the binding experiments. The calibration curve comprised the 0-1.2 mM concentration range and was recorded after incubation of the anions for more than 24 hours.

**Conductometric detection of unbound phosphate and sulfate**

Inorganic phosphate and sulfate were detected by conducometric measurements using a CON 510, Bench Conductivity/TDS Meter, Oakton Instruments.

The instrument was calibrated with 0.01 M KCl according to the instrument user manual followed by rinsing with millipore water until a stable conductivity reading was obtained. Calibration was then performed by measurements of phosphate or sulfate standards from low to high concentration (10, 30, 50, 70, 100, 300, 500, 700, 1000 $\mu$M) with intermediate rinsing with millipore water until a stable conductivity was reached. This was followed by measurement of the supernatants of the polymer incubations. Note that the standards were made up in the same buffer as used in the polymer incubation experiment.

**Tests of polymer uptake of PPA·2Na or PSA·Na from aqueous solutions**

The imprinted and nonimprinted polymers (10 mg) were incubated by shaking in a solution of PPA·2Na (1mL, 0.6 mM) in water (±1M NaCl) for 15 h. The solutions were centrifuged and the supernatant analysed by HPLC for detecting unbound PPA·2Na. The amount of bound analyte (B μmol/g) was determined as:

(1) $$B = (C_0 - F)\, V/m$$

Where $C_0$ (mM) is the initial concentration of PPA·2Na, F (mM) is the free concentration of free PPA or PPA·2Na in the supernatant, V (mL) is the volume of analyte, and m the weight of polymer (g). The imprinting factors IF were calculated as given in eq. 2.

(2) $$IF = B_{MIP}/B_{NIP}$$

Where BMIP and BNIP are the amount of bound analyte by the MIP and NIP respectively. Finally, the selectivity factor S were calculated as given in eq. 3:

(3) $$S = B_{SO3}/B_{PO3}$$

Where $B_{PO3}$ and $B_{SO3}$ are the amount of bound phosphonate and sulfonate anions respectively.

In order to investigate the effect of pH on the anion binding the uptake test was repeated in aqueous buffers of different pH (1,3,5,7.4, 9). These were as follows: pH1 – Millipore water adjusted to pH 1 with 0.1 M HCl; pH 2 and 5: sodium citrate buffer (0.1M sodium citrate/citric acid); pH 7.4: sodium phosphate buffer (0.1 M); pH9: Sodium carbonate (0.1M). Binding of $Na_2HPO_4$ and $Na_2SO_4$ at the optimum pH 9 with and without 1M NaCl were investigated by conductometry as mentioned above.

**Binding isotherms**

The polymers (10 mg each) were incubated in 1 mL solutions of the anions (PPA, PSA, $Na_2HPO_4$, $Na_2SO_4$) at different concentrations in pH 9 buffer as described above (see above). The vials were shaken for 24 hours followed by centrifugation and quantification of unbound analyte by HPLC or conductometry as described above. The amount of bound analyte per unit mass of polymer (*B*) was calculated according to

equation (1). Each experiment was performed at least twice. Binding curves were constructed by plotting *B* against free concentration *c* and were subsequently fitted by non-linear regression using the GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA) to a Langmuir mono-site model (Eq. 4):

(4) $$B = B_{max} \cdot \frac{c}{K_d + c}$$

where $B_{max}$ is the maximum amount of solute bound by the polymer particles at saturation. The association constants $K_a$ were calculated as the inverse of the dissociation constants ($K_d$).

Table S1. Prepolymerization solution composition used to prepare imprinted and nonimprinted polymers

| Polymer | Template | Host monomers |
|---|---|---|
| P1 | PPA·2PMP | 1 |
| $P_N1$ | - | 1 |
| P1,2 | PPA·2Na | 1,2 |
| $P_N1,2$ | - | 1,2 |
| P2 | PPA·2Na | 2 |
| $P_N2$ | - | 2 |

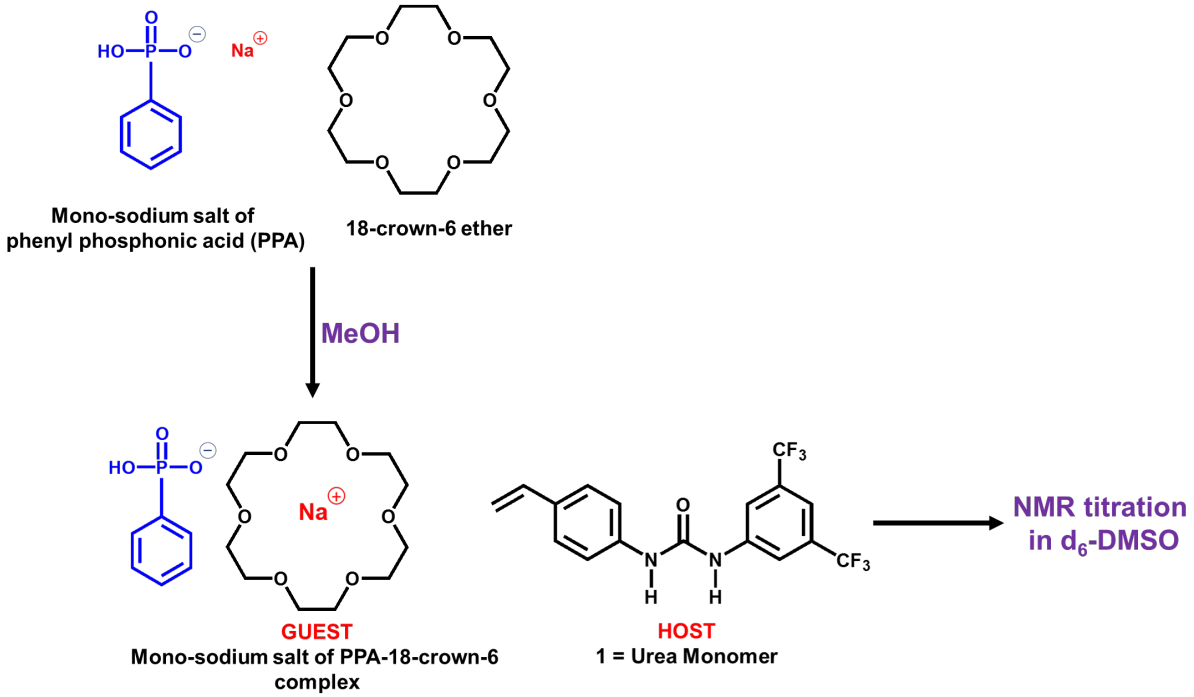
All polymers were prepared using the indicated templates and monomers, ABDV as initiator by thermal homolysis at 50°C, PETA as crosslinker and MeCN as porogenic solvent.

Table S2. Association constants ($K_a$) and binding capacities ($B_{max}$) of PPA imprinted polymers

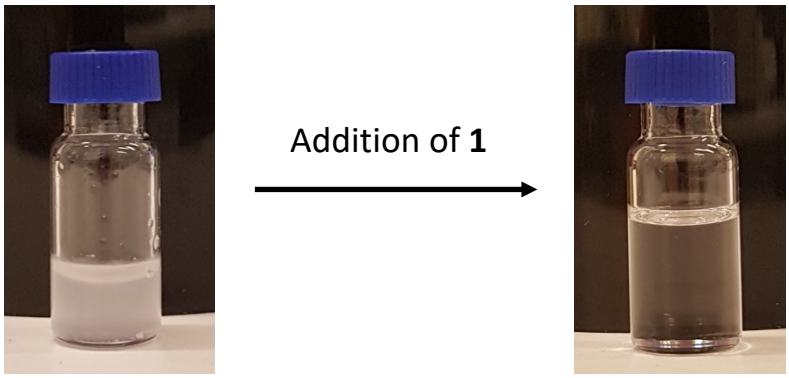
| | Poly | Anion | $K_a$ ($\times 10^3$ $M^{-1}$) | $B_{max}$ (µmol/g) | $R^2$ |
|---|---|---|---|---|---|
| No salt | P1 | PPA | 2.2 ± 0.6 | 34 ± 3 | 0.966 |
| | | PSA | 1.0 ± 0.3 | 24 ± 3 | 0.959 |
| | $P_N1$ | PPA | 1.5 ± 0.5 | 18 ± 2 | 0.950 |
| | | PSA | 0.3 ± 0.2 | 27 ± 11 | 0.940 |
| | P1,2 | PPA | 1.6 ± 0.4 | 45 ± 4 | 0.975 |
| | | PSA | 0.75 ± 0.1 | 22 ± 2 | 0.994 |
| | $P_N1,2$ | PPA | 0.95 ± 0.2 | 28 ± 3 | 0.985 |
| | | PSA | 0.08 ± 0.07 | 53 ± 45 | 0.984 |
| 1M NaCl | P1 | PPA | 1.6 ± 0.2 | 35 ± 1 | 0.995 |
| | | PSA | 2.1 ± 0.4 | 22 ± 1 | 0.978 |
| | $P_N1$ | PPA | 0.5 ± 0.1 | 25 ± 3 | 0.989 |
| | | PSA | 1.8 ± 0.4 | 19 ± 2 | 0.966 |
| | P1,2 | PPA | 3.7 ± 0.5 | 45 ± 2 | 0.987 |
| | | PSA | 1.2 ± 0.1 | 24 ± 1 | 0.994 |
| | $P_N1,2$ | PPA | 2.3 ± 0.6 | 18 ± 2 | 0.985 |
| | | PSA | 0.06 ± 0.09 | 88 ± 128 | 0.970 |

The binding parameters were obtained by fitting of the binding data in Figure S6 to a Langmuir mono-site binding model.

A.




B.




Mono sodium salt of PPA-18-crown-6 complex in d6-DMSO

C.

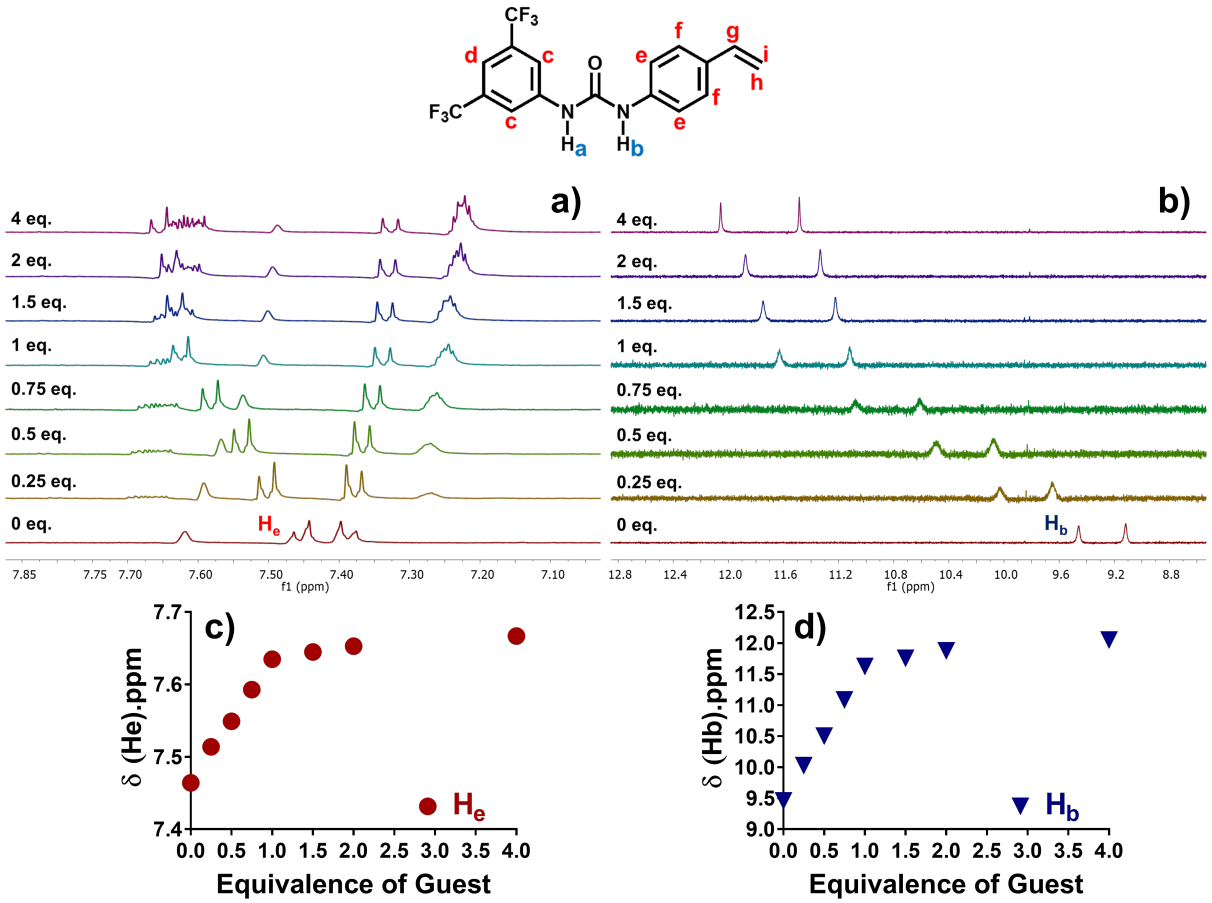
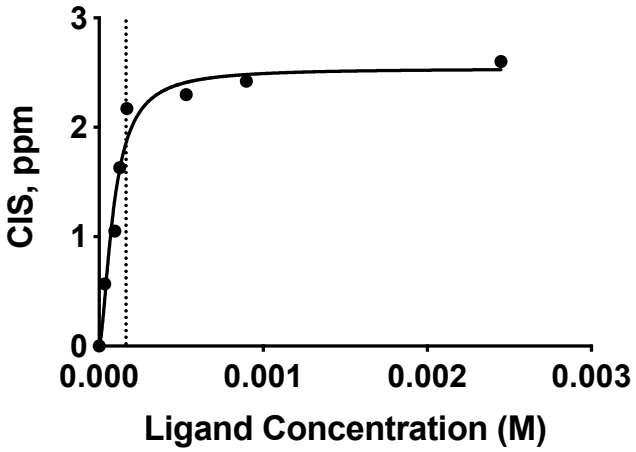



Figure S1. A. Procedure for sample preparation of PPA · Na-18-crown-6 and titration with host monomer 1. B. Image showing the solubility enhancement upon addition of monomer 1 to PPA · Na-18-crown-6 in DMSO-$d_6$. C. $^1$H-NMR complexation induced shift changes of host monomer 1 $H_e$ (a,c) and $H_b$ (b,d) protons upon addition of PPA·Na-18-crown-6. The enlarged graphs show the CIS of the protons of monomer 1 plotted against the number of guest equivalents.




| Specific binding with Hill slope | |
|---|---|
| Best-fit values | |
| Bmax | 2,537 |
| Ka | 11181 |
| h | 1,656 |
| Std. Error | |
| Bmax | 0,1494 |
| Ka | 1655 |
| h | 0,4566 |
| 95% CI (asymptotic) | |
| Bmax | 2,153 to 2,921 |
| Ka | 6926 to 15436 |
| h | 0,4818 to 2,829 |
| Goodness of Fit | |
| Degrees of Freedom | 5 |
| R squared | 0,9668 |
| Sum of Squares | 0,2127 |
| Sy.x | 0,2063 |
| Number of points | |
| # of X values | 16 |
| # Y values analyzed | 8 |

Figure S2. $^{1}$H NMR complexation induced shifts (CIS) of the urea proton Ha recorded over the course of the titration of a 1 mM solution of urea host monomer 1 in DMSO-$d_6$ with PPA·Na-18-crown-6.

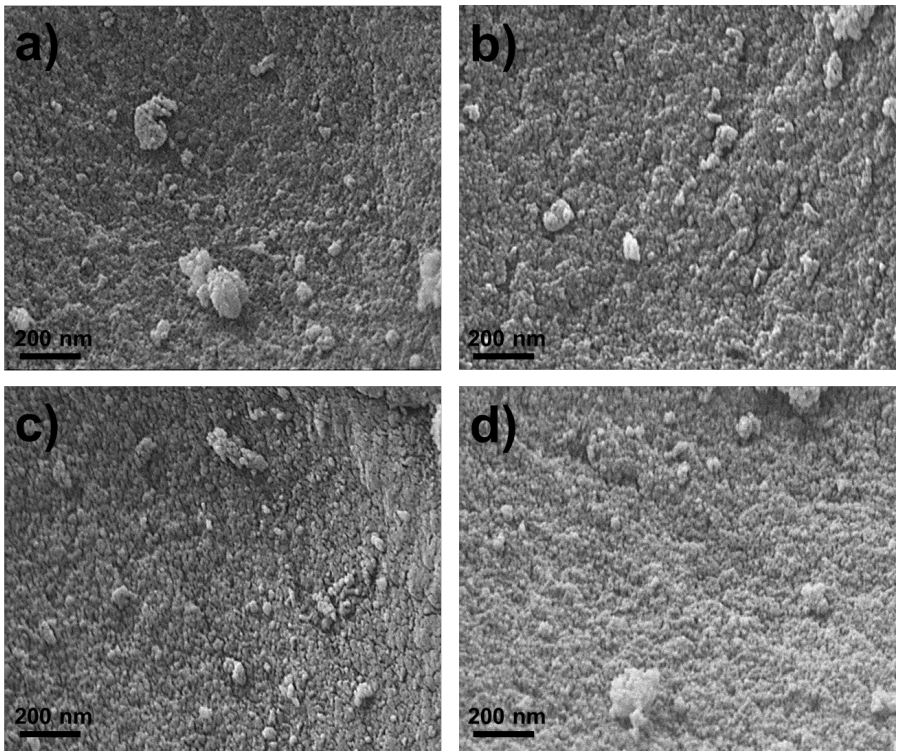



Figure S3. Representative scanning electron micrographs (SEM) of 25-36 μm particle size fractions of a) P1, b) $P_N1$, c) P1,2 and d) $P_N1,2$. Scale bar = 200 nm.

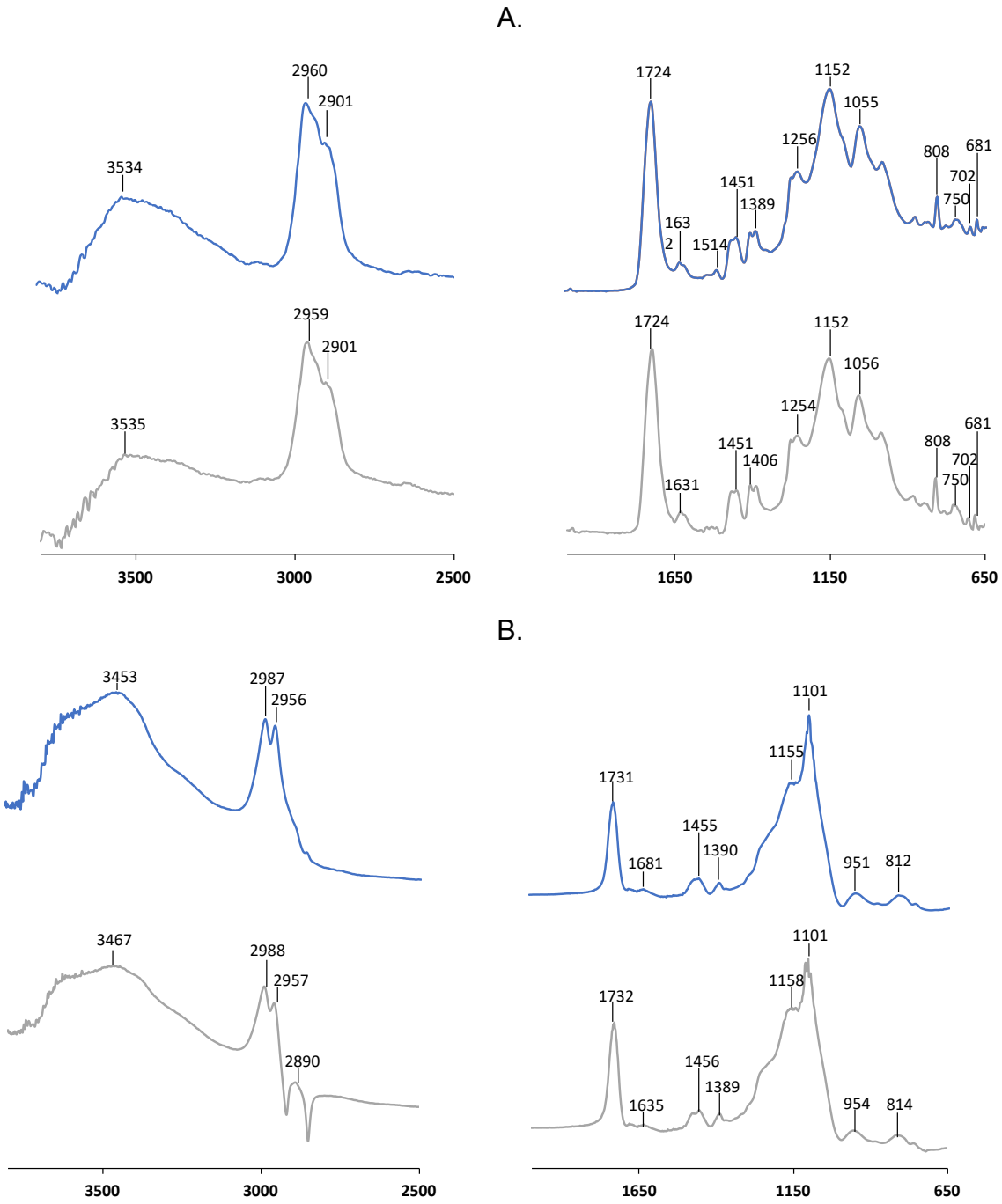



Figure S4. Transmission infrared spectra (KBr) of (A) P1,2 (Top) and $P_N$1,2 (Bottom) and (B) P1(Top) and $P_N$1(Bottom). The spectra show the following characteristic bands: 3300-3700 $cm^{-1}$, CO-NH, -COH stretching, 2850-2990 $cm^{-1}$: C-H stretch, 1700 $cm^{-1}$: C=O stretch, 1630 $cm^{-1}$: unconverted double bonds, ca 1260 $cm^{-1}$ C-O bend, ester, ca 1150 $cm^{-1}$, ester.

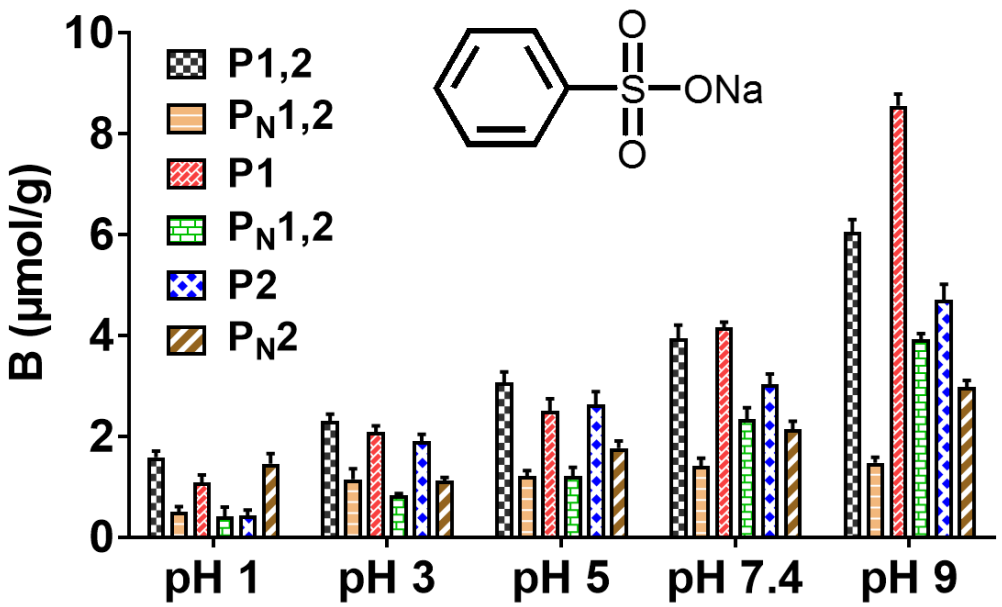



Figure S5. Binding of PSA (0.6 mM) on PPA imprinted polymers in buffers of different pH.

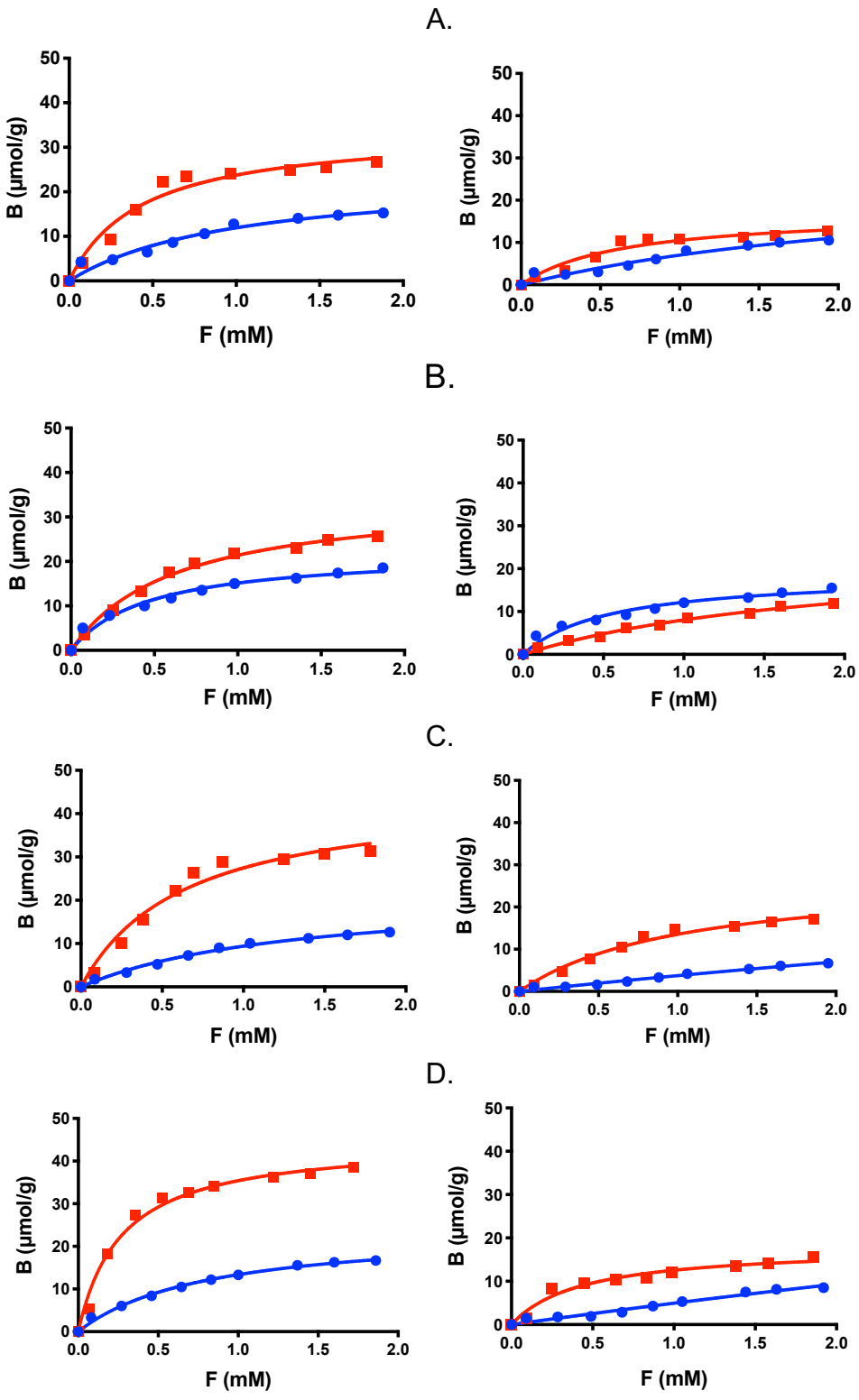



Figure S6. Equilibrium binding isotherms of PPA (red curves, squares) and PSA (blue curves, circles) adsorbed to P1 (left graph) and $P_N1$ (right graph) (A, B) and P1,2 (left graph) and $P_N1,2$ (right graph) (C, D) in 0.1 M sodium bicarbonate pH 9 in absence (A,C) and presence (B,D) of 1 M NaCl. The isotherms were fitted to a one site host guest model resulting in the binding parameters listed in Table S2.