Electronic Supporting information (ESI)

RAFT coupling chemistry: a general approach for post-functionalizing molecularly imprinted polymers synthesized by radical polymerization

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EXPERIMENTAL SECTION

***Materials**:*

Methacrylic acid (MAA, 99%), 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%), ethyleneglycol dimethacrylate (EGDMA, 98%), 1,1,1-trimethylolpropane trimethacrylate (TRIM, technical grade), 4-[[[(2-carboxyethyl)thio]thioxomethyl]thio]-4-cyano-pentanoic acid (CETPA, 95%), 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPADB, 98%), salicylic acid (SA, 99%), ethyleneglycol methacrylate phosphate (EGMP, 98%), N-ilsopropylacrylamide (NIPAm, 99%), acetonitrile (anhydrous, 99.8%), atenolol (98%) and (±)-propranolol hydrochloride were from Sigma. DMAEMA and EGDMA were filtered over basic alumina for removing the inhibitor. Methanol (MeOH), ethanol (EtOH) and diethyl ether were from VWR. (±)-Propranolol hydrochloride was converted into its free-base through extraction from a sodium carbonate solution at pH 9 into chloroform. 2,2'-Azobis-(2,4-dimethylpentanenitrile) (Vazo-52) (ABDV) was from Dupont Chemicals (Wilmington, USA.)

***Polymer Synthesis**:*

Synthesis of propranolol imprinted (MIP) and non-imprinted (NIP) nanoparticles (NPs) via free radical precipitation polymerization (FRP). In a 40 mL glass vial sealed with a silicone septum, MMA (15.5 mg, 0.18 mmol), propranolol (5.6 mg, 0.022 mmol), TRIM (205 mg, 0.58 mmol) and ABDV (5 mg, 0.02 mmol) were dissolved in acetonitrile (20 mL). Upon cooling on an ice-water bath, the mixture was purged with nitrogen for 10 min. The vial was immersed into an oil bath preheated at 60 °C and the polymerization was allowed to run for 24 h. The reaction was stopped upon exposure to air. The resulting polymer particles were collected by centrifugation and then washed thoroughly with methanol/acetic acid (9:1 v/v, 3 x 10 mL) and methanol (3 x 10 mL) until no template could be detected in the washing solution. After drying under high vacuum, a white powder (MIP) was obtained in a gravimetric yield of 40%. Reference non imprinted (NIP) NPs were prepared and purified under identical conditions except that the template was omitted (gravimetric yield: 42%).

Synthesis of reference, propranolol imprinted (MIP) particles via RAFT-mediated precipitation polymerization. In a 40 mL glass vial sealed with a silicone septum, MAA (15.5 mg, 0.18 mmol), propranolol (5.6 mg, 0.022 mmol), TRIM (205 mg, 0.58 mmol), CPADB (5.36 mg, 0.019 mmol) and ABDV (0.94 mg, 3.8 µmol) were dissolved in acetonitrile (20 mL). Upon cooling on an ice-water bath, the mixture was purged with nitrogen for 10 min. The vial was immersed into an oil bath preheated at 60 °C and polymerization was allowed to run for 72 h. The reaction was stopped upon exposure to air. No precipitated particles could be seen. However, the drop-wise precipitation of this polymerization solution into an excess of cold diethyl ether afforded some insoluble polymer (gravimetric yield: 3%)

Functionalization of propranolol imprinted and non-imprinted NPs via RAFT coupling chemistry. In a 4 mL glass vial sealed with a screw cap and an air-tight septum, CPADB (11.52 mg 0.041 mmol) or CETAP (12.60 mg 0.041 mmol) and ABDV (0.47 mg *1.92* µmol) were dissolved in a suspension of MIP or NIP NPs in ethanol (1 mL, 30mg/mL). Upon cooling on an ice-water bath, the dispersion was purged with nitrogen for 10 min, and immersed in an oil bath at 60 °C for 24 h under vigorous magnetic stirring.

The reaction was stopped by exposure to air. The resulting particles were collected by centrifugation and washed thoroughly with ethanol (3 x 10 mL) and DCM (3 x 10 mL). After drying under high vacuum, a pale yellow powder (CETAP@MIP/NIP-NPs) or a pinkish powder (CPADB@MIP/NIP-NPs) was obtained. The gravimetric yield based on CETAP for CETAP@MIP was 76 %, whereas that of CETAP@NIP was 73 %. The gravimetric yield for CPADB@MIP was of 75 % based on CPADB, whereas that of CPADB@NIP was 73 %.

Surface-initiated synthesis of PEGMP brushes on imprinted and non-imprinted (CPADB@MIP-NIP) nanoparticles (NPs) via surface-initiated RAFT polymerization. In a 4 mL glass vial sealed with a screw cap and an air-tight septum, CPADB-decorated (CPADB@MIP-NIP) NPs (30 mg) were mixed with CPADB (2.94 mg, 0.01 mmol), EGMP (106.12 mg, 0.50 mmol) and ABDV (0.83 mg, 3.33 µmol) in 2 mL of ethanol. Upon cooling on an ice-water bath the mixture was purged with nitrogen for 10 min and immersed into an oil bath at 60 °C for 24 h under vigorous stirring. The resulting polymer particles in the reaction solutions were collected by centrifugation and washed with methanol (5 x 1 mL). After drying, a pale pink powder (PEGMP-CPADB@MIP-NIP) was obtained with a gravimetric yield (based on EGMP) of 31 % for PEGMP-CPADB@MIP and 38 % for PEGMP-CPADB@NIP.
Surface-initiated synthesis of PNIPAm brushes on imprinted and non-imprinted (CETAP@MIP-NIP) nanoparticles (NPs) via surface-initiated RAFT polymerization. In a 4 mL glass vial sealed with a screwcap and an air-tight septum, CETAP-decorated (CETAP@MIP or CETAP@NIP) NPs (30 mg) were mixed with CETAP (2.94 mg, 0.01 mmol), NIPAm (178.2 mg, 1.58 mmol) and ABDV (0.83 mg, 3.33 µmol) in 2 mL of ethanol. Upon cooling on an ice-water bath the mixture was purged with nitrogen for 10 min and immersed into an oil bath at 60 °C for 24 h under vigorous stirring. The resulting polymer particles in the reaction solutions were collected by centrifugation and washed with ethanol (3 x 10 mL). After drying, a pale yellow powder (PNIPAm-CETAP@MIP/NIP-NPs) was obtained with a gravimetric yield (based on NIPAM) of 61 % for PNIPAm-CETAP@MIP and 58 % for PNIPAm-CETAP@NIP.

Synthesis of salicylic acid imprinted and non-imprinted polymers via free radical bulk polymerization. In a 1 mL glass vial sealed with a screwcap and an air-tight septum, DMAEMA (10.18 mg, 0.065 mmol), salicylic acid (2.98 mg, 0.022 mmol) EGDMA (85 mg, 0.43 mmol) and ABDV (1.5 mg, 9.28 µmol) were dissolved in acetonitrile (0.25 mL). Upon cooling on an ice-water bath, the mixture was purged with nitrogen for 10 min and immersed in a thermostated oil bath at 60 °C for 24 h. The polymerization was stopped by exposure to air. The resulting bulk polymer was ground in a mortal, transferred into microcentrifuge tubes and crushed with zirconia beads in methanol on a Precellys 24 homogenizer (Bertin Technologies, Montigny le Bretonneux, France). The template was extracted with methanol/0.1M HCl (3 x 10 mL), methanol/0.1 M NH4OH (3 x 10 mL), water (2 x 10 mL), and methanol (2 x 10 mL). The MIP was obtained in a yield of 78%. The relatives NIP bulk particles were prepared and purified under the identical conditions except that the template was omitted (gravimetric yield: 75%).

Functionalization of salicylic acid imprinted and non-imprinted bulk polymers via RAFT coupling chemistry. In a 1 mL glass vial sealed with a screwcap and an air-tight septum, CETAP (12.69mg 0.041 mmol) and ABDV (0.99 mg 0.0039 mmol) were added to a suspension of bulk polymer (MIP or NIP) in ethanol (1 mL, 30 mg/mL). Upon cooling on an ice-water bath the mixture was purged with nitrogen for 10 min. The vial was then immersed in a thermostated oil bath at 60 °C for 24 h under vigorous stirring. The resulting polymer particles were collected by centrifugation and then washed with ethanol (3 x 10 mL) and DCM (3 x 10 mL). After drying under high vacuum, a pale yellow powder was obtained for both MIP and NIP, which afforded a gravimetric yield based on CETAP of respectively 70 % and 68 %.

Surface-initiated RAFT polymerization of PNIPAm brushes on imprinted and non-imprinted RAFT-coupled bulk polymers. In a 4 mL glass vial sealed with a screwcap and an air-tight septum, CETAP-decorated bulk polymer (CETAP@MIP or CETAP@NIP) (1 mL, 30 mg/mL) was mixed with CETAP (2.94 mg, 0.01 mmol), NIPAm (178.2 mg, 1.58 mmol) and ABDV (0.83 mg, 3.33 µmol) in 2 mL of ethanol. Upon cooling on an ice-water bath, the mixture was purged with nitrogen for 10 min. The vial was then immersed in a thermostated oil bath at 60 °C for 24 h under vigorous stirring. The reaction was stopped upon exposure to air. The resulting polymer was collected by centrifugation and then washed with ethanol (3 x 10 mL). After drying, a pale yellow powder was obtained for both MIP and NIP in a gravimetric yield, based on NIPAm, of respectively 63 % and 60 %.

RAFT polymerization of PNIPAm in presence of uncoupled, propranol imprinted and non-imprinted NPs. In a 4 mL glass vial sealed with a screwcap and an air-tight septum, previously synthesized NPs by precipitation polymerization (MIP or NIP, 30 mg) were mixed with CETAP (2.94 mg, 0.01 mmol), NIPAm (178.2 mg, 1.58 mmol) and ABDV (0.83 mg, 3.33 µmol) in 2 mL of ethanol. Upon cooling on an ice-water bath, the mixture was purged with nitrogen for 10 min. The vial was then immersed in a thermostated

oil bath at 60 °C for 24 h, under vigorous stirring. The resulting polymer particles were collected by centrifugation and then washed with ethanol (3 x 10 mL). After drying, a whitish powder was obtained for MIP and NIP with a gravimetric yield, based on NIPAm, of respectively 6 % and 5 %.

	Template (propranolol)	Functional monomer (MMA)	Cross-linker (TRIM)	Initiator (ABDV)	RAFT	T (°C)	Time (h)	ACN (mL)
MIP NPs	5.6 mg	MMA 15.5 mg	TRIM 205mg	ABDV 5 mg	x	60	24	20
NIP NPs	x	MMA 15.5 mg	TRIM 205mg	ABDV 5 mg	x	60	24	20
NPs reference	5.6 mg	MMA 15.5 mg	TRIM 205mg	ABDV 0.1 mg	CPADB 5.36 mg	60	72	20

Table S1. Recipes for the synthesis of nanoparticles by precipitation polymerization.

	Polymer [mg/mL]	Initiator (ABDV)	RAFT	T (°C)	Time (h)	EtOH (mL)
CPADB@MIP-NIP NPs	30	0.47 mg	CPADB 11.52 mg	60	24	1
CETAP@MIP-NIP NPs	30	0.47 mg	CETAP 12.60 mg	60	24	1

Table S2. Recipe for the decoration of nanoparticles with CPADB or CETAP RAFT agents.

	polymer	Initiator (ABDV)	RAFT	monomer	T (°C)	Time (h)	EtOH (mL)
PEGMP-CPADB@MIP-NIP	30	0.83mg	CPADB 2.94 mg	EGMP 106.2mg	60	24	2
PNIPAm-CETAP@MIP-NIP	30	0.83mg	CETAP 3.07 mg	NIPAM 178.2mg	60	24	2

Table S3. Recipe for the chain extension with pNIPAm of decorated nanoparticles.

	Template (salicylic acid)	Functional monomer (DMAEMA)	Cross-linker (EGDMA)	Initiator (ABDV)	T (°C)	Time (h)	ACN (mL)
bulk	2.99 mg	10.18 mg	85 mg	1.5 mg	60	24	0.25

Table S4. Recipes for the synthesis of bulk polymers.

	polymer	Initiator (ABDV)	RAFT	Time (h)	T (°C)	EtOH (mL)
CETAP@MIP-NIP bulk	30	0.99 mg	CETAP 12.69 mg	24	60	1

Table S5. Recipe for the decoration of bulk particles with CETAP RAFT agents.

	polymer	Iniziator (ABDV)	RAFT (CETAP)	NIPAM	T (°C)	Time (h)	EtOH (mL)
PNIPAm-CETAP@MIP-NIP bulk	30	0.85 mg	2.94 mg	178.2	60	24	2

Table S6. Recipe for the chain extension with pNIPAm of decorated bulk particles.

Binding experiments:

Equilibrium binding assays on nanoparticles. The binding properties of the propranolol MIP/NIP-NPs, CETAP@MIP/CETAP@NIP-NPs and PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-NPs were studied by equilibrium binding experiments.[2] Polymer concentrations ranging from 0.05 to 1 mg/mL in either acetonitrile or water were incubated overnight in 1 µM propranolol solution on a Stuart Tube Rotator at room temperature. After centrifugation for 30 minutes at 20000 rpm, 700 µL of the supernatant was withdrawn and analyzed on a Fluorolog-3 fluorescence spectrophotometer (Horiba Jobin Yvon, Longjumeau, France) at 20°C *(λ_{exc}=300 nm and λ_{em} = 335, silt 3)*. The amount of bound propranolol to the polymers was calculated by subtracting the amount of unbound propranolol from the initial amount of propranolol added.

Selectivity assays on nanoparticles. The selectivity of propranolol MIP/NIP-NPs, CETAP@MIP/CETA@NIP-NPs and PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-NPs was studied by equilibrium binding experiments, as previously described for propranolol. The experimental conditions were exactly the same, except that propranolol was replaced with atenolol, and the fluorescence excitation and emission were modified to *λ_{exc}=298 nm and λ_{em} = 327*, slit 3.5

Equilibrium binding assays of bulk polymers. The binding characteristics of the salicylic acid imprinted MIP/NIP-B, CETAP@MIP/CETAP@NIP-B and PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-B were studied by equilibrium binding experiments. Polymer concentrations ranging from 0.5 – 10 mg/mL were incubated overnight with 1µM salicylic acid solution in either acetonitrile or water on a Stuart Tube rotator, at room temperature. After centrifugation for 30 minutes at 20000 rpm, 700 µL of supernatant was withdrawn and analyzed on a Fluorolog-3 fluorescence spectrophotometer (Horiba Jobin Yvon, Longjumeau, France) at 20°C *(λ_{exc}=306 nm and λ_{em} = 408, slit 1.5 nm).*[3]
Selectivity assays on bulk polymers:

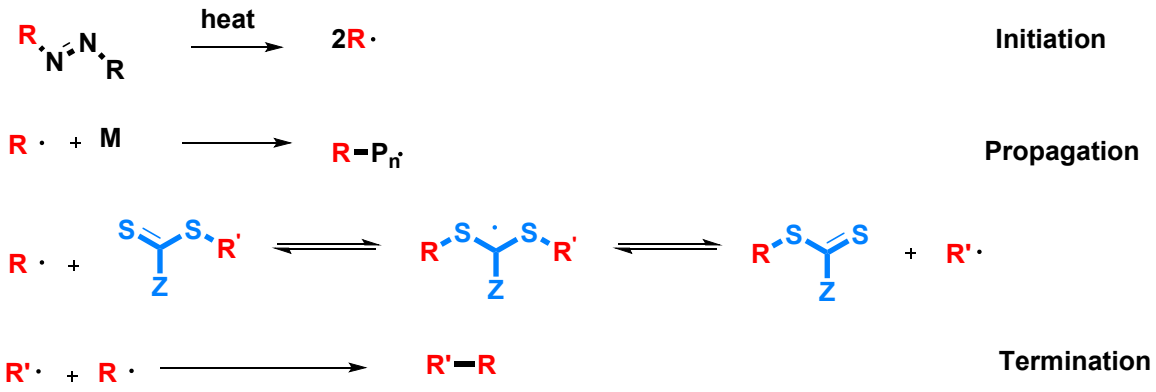
The binding characteristics of the salicylic acid imprinted MIP/NIP-B, CETAP@MIP/CETAP@NIP-B and PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-B were studied by equilibrium binding experiments. Polymer concentrations ranging from 0.5 – 10 mg/mL were incubated overnight with 1µM atenolol solution in either acetonitrile or water on a Stuart Tube rotator, at room temperature. After centrifugation for 30 minutes at 20000 rpm, 700 µL of supernatant was withdrawn and analyzed on a Fluorolog-3 fluorescence spectrophotometer (Horiba Jobin Yvon, Longjumeau, France) at 20°C *λ_{exc}=298 nm and λ_{em} = 327*, slit 3.5.

***Polymer Characterization**:*

Scanning Electron Microscope (SEM) images and Energy Dispersive X-ray (EDX) spectroscopy were recorded on a QUANTA FEG 250. Upon EDX analysis, the samples were sputtered with gold before SEM imaging. The particles' size and size distribution were measured on SEM images using ImageJ and averaged on at least 100 particles.

FT-IR transmission spectra were recorded on a Nicolet iS5 Thermo Scientific on KBr discs (from 4000 cm^{-1} to 500 cm^{-1}, resolution 4 cm^{-1}, 32 scans).

DLS measurements were recorded on disposable PS cells using a Zetasizer Nano ZS at 20°C (Malvern Instruments, Orsay, France).



Scheme S1. Mechanism of reversible addition-fragmentation chain-transfer (RAFT) polymerization. Adapted from [1]

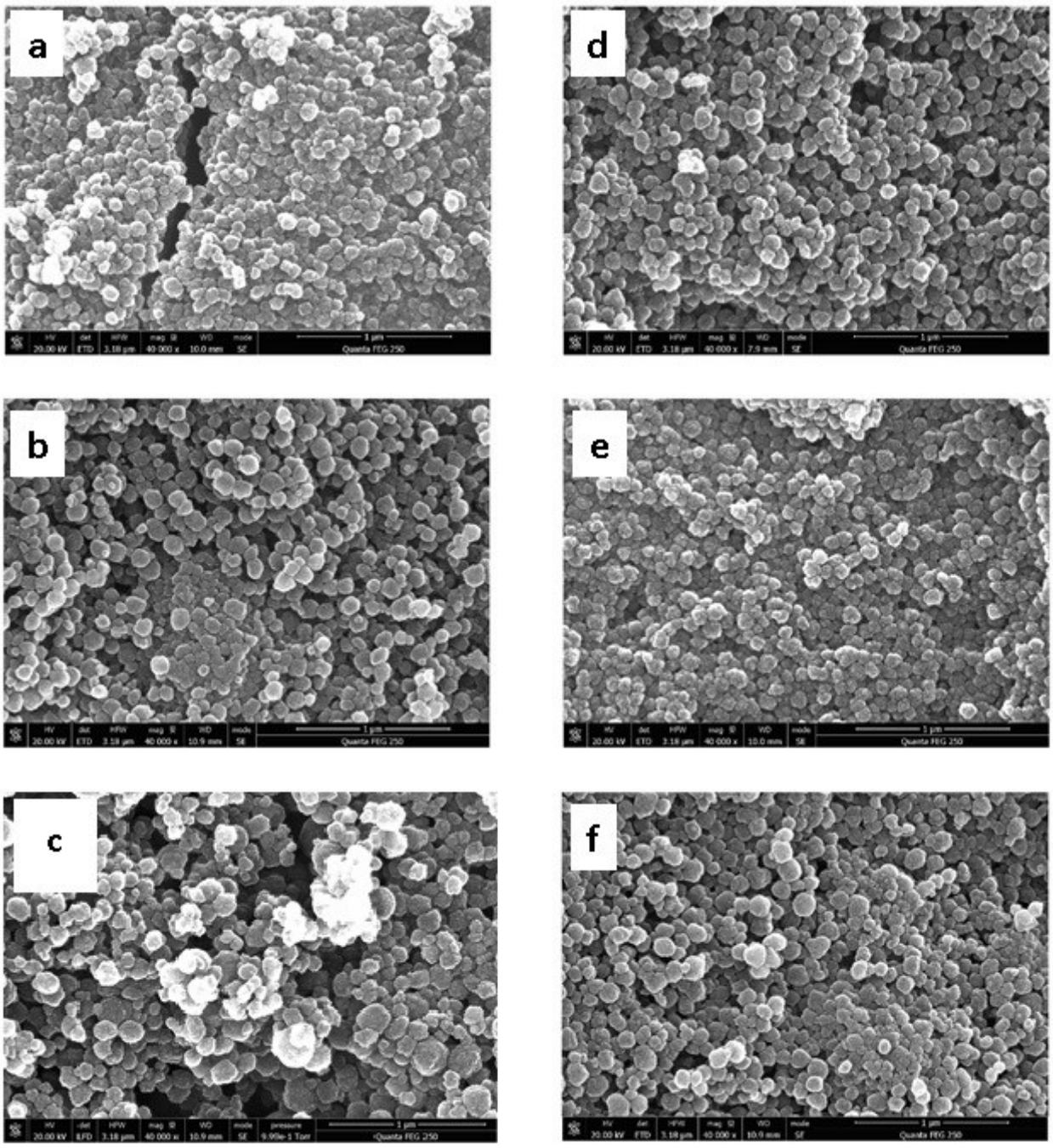


Figure S1. Representatives SEM images of (a) propranolol imprinted and (b) non-imprinted NPs by free-radical precipitation polymerization. (c) Surface-modified, CETAP-coupled imprinted (CETAP@MIP) and (d) non-imprinted (CETAP@NIP) NPs. (e) PNIPAm surface extended imprinted (PNIPAm-CETAP@MIP) and (f) non-imprinted (PNIPAm-CETAP@NIP) NPs.

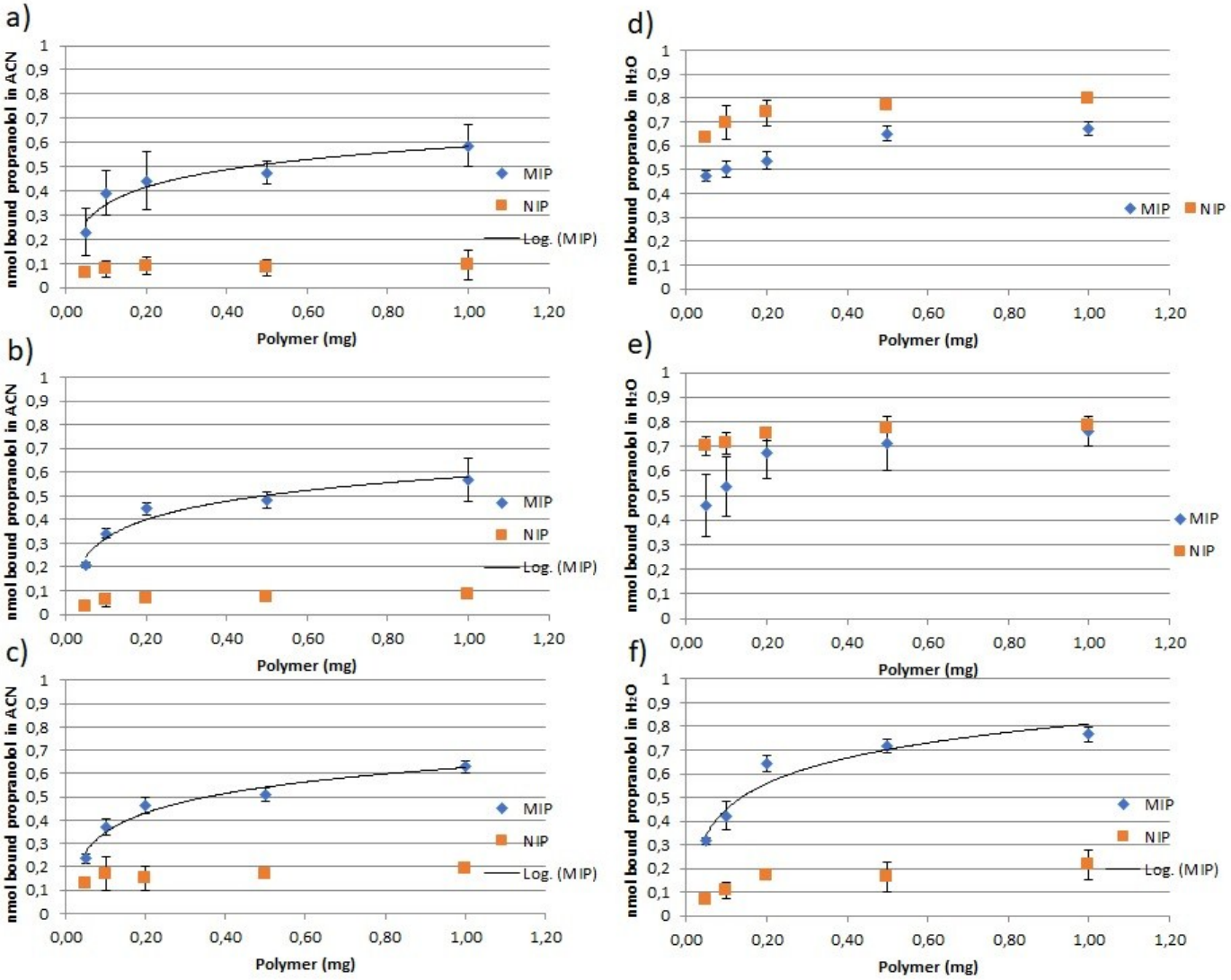


Figure S2. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) NPs in 1µM propranolol in acetonitrile: (a) bare MIP and NIP NPs, (b) CETAP@MIP/CETAP@NIP-NPs and (c) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-NPs. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) NPs in 1µM propranolol in water: (d) bare MIP and NIP NPs, (e) CETAP@MIP/CETAP@NIP-NPs and (f) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-NPs.

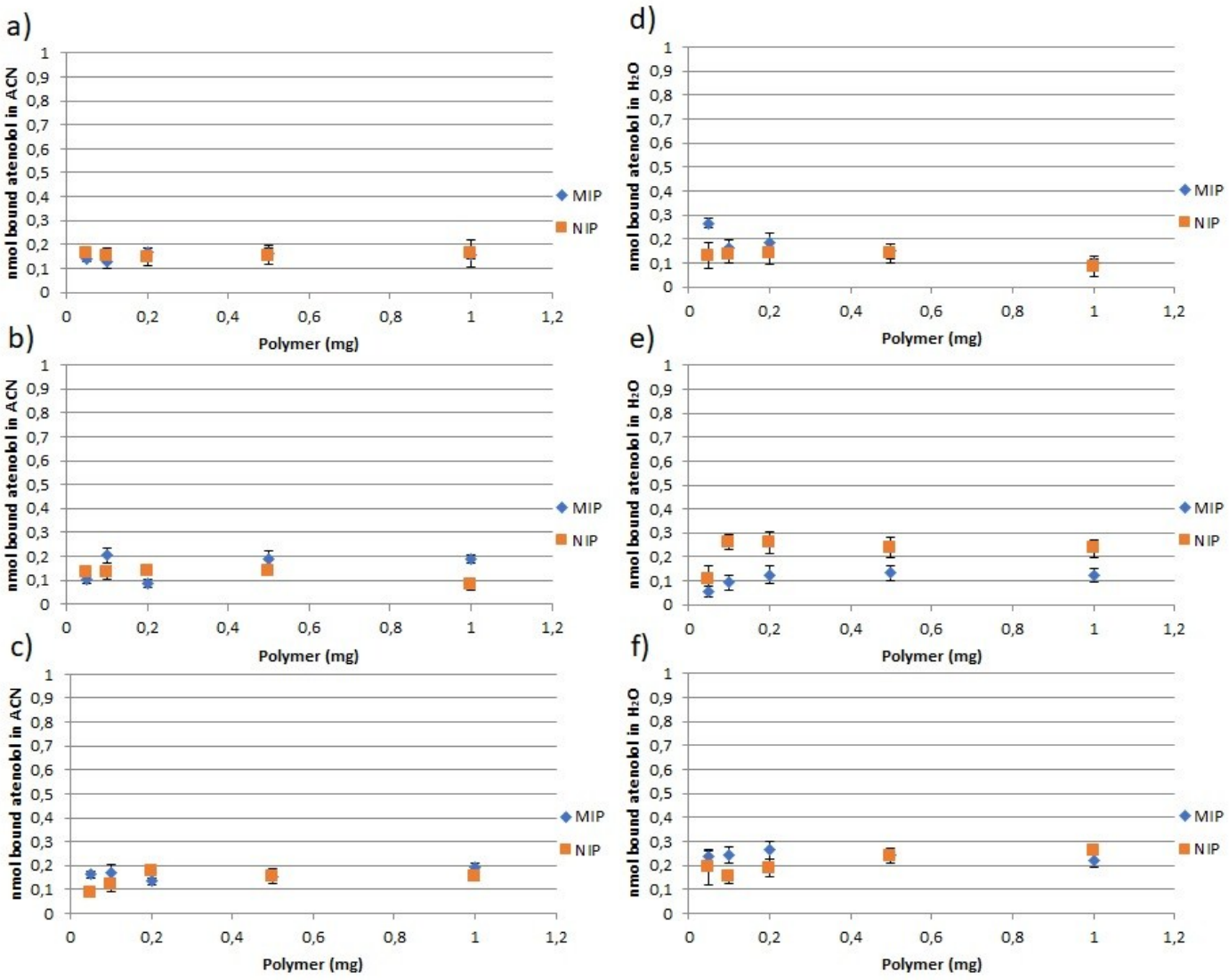


Figure S3. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) NPs in 1µM atenolol in acetonitrile: (a) bare MIP and NIP NPs, (b) CETAP@MIP/CETAP@NIP-NPs and (c) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-NPs. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) NPs in 1µM atenolol in water: (d) bare MIP and NIP NPs, (e) CETAP@MIP/CETAP@NIP-NPs and (f) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-NPs.

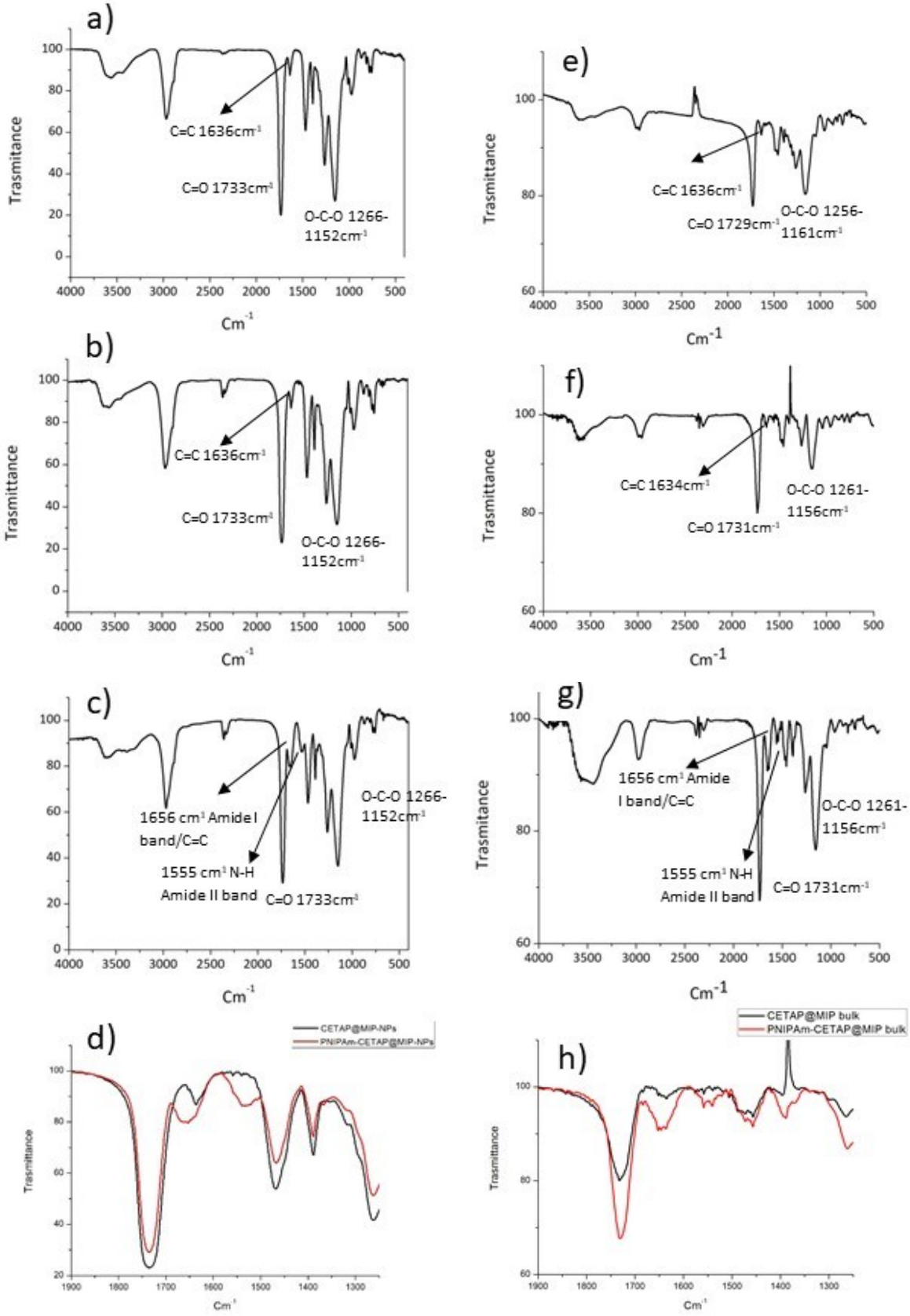


Figure S4. FTIR spectra of (a) imprinted NPs (b) CETAP@MIP-NPs and (c) PNIPAm-CETAP@MIP-NPs, (d) overlap and magnification of CETAP@MIP-NPs (black) and PNIPAm-CETAP@MIP-NPs (red), (e) imprinted bulk, (f) CETAP@MIP bulk and (g) PNIPAm-CETAP@MIP bulk, (h) overlap and magnification of CETAP@MIP bulk (black) and PNIPAm-CETAP@MIP bulk (red).

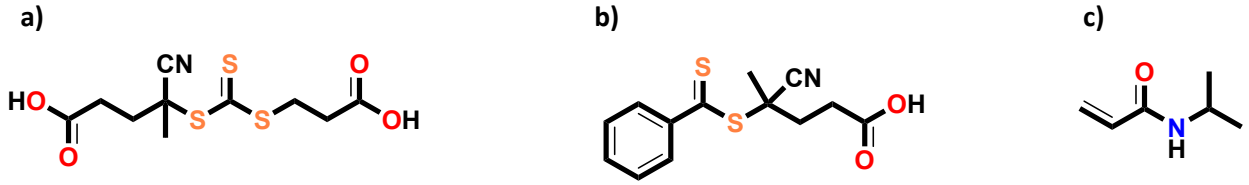


Figure S5. Molecular structures of: **a**) 4-[[[(2-carboxyethyl)thio]thioxomethyl]thio]-4-cyano- pentanoic acid (CETAP), **b**) 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPADB) and **c**) N-isopropylacrylamide (NIPAm).

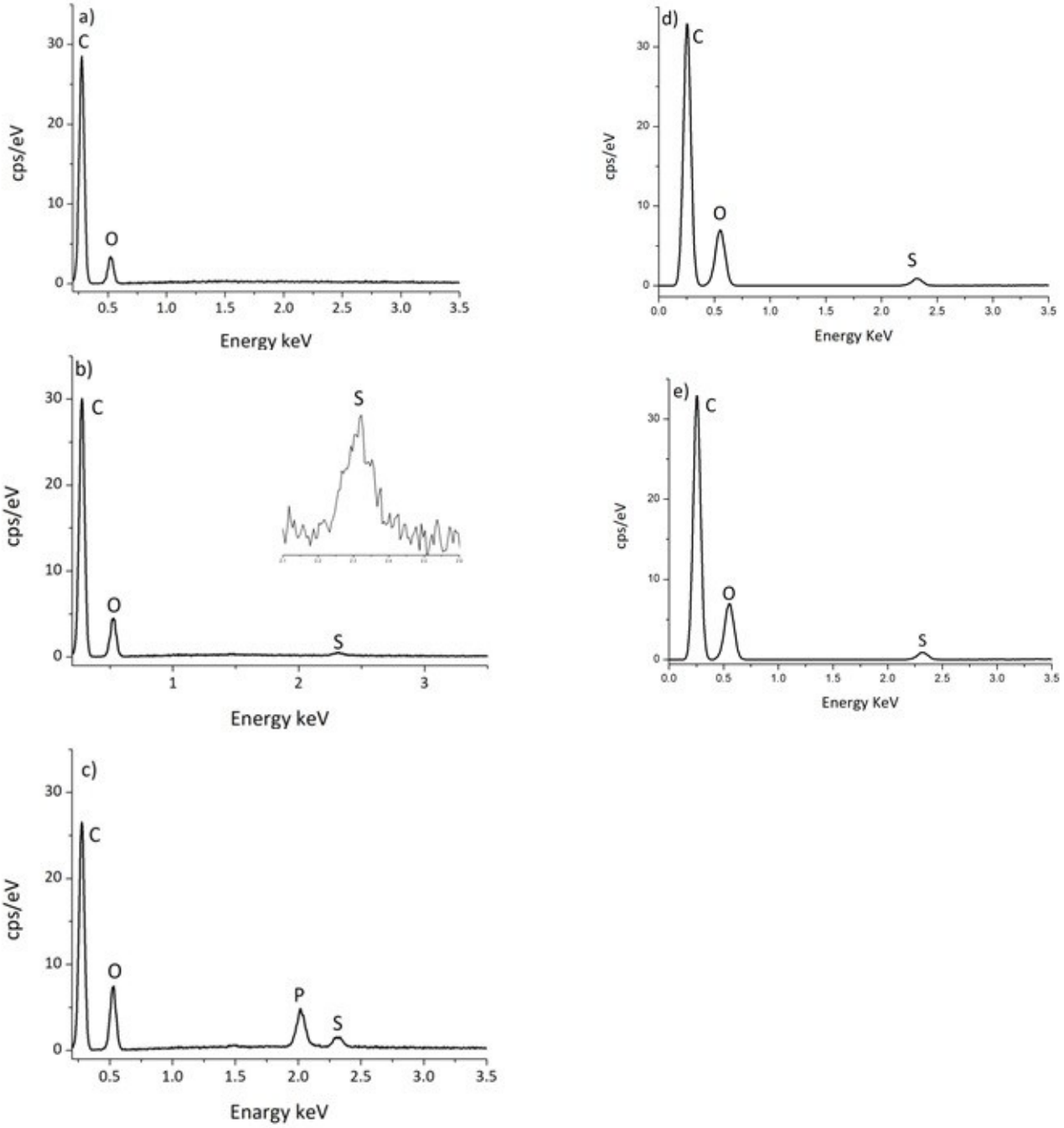


Figure S6. EDX analysis of (a) imprinted NPs (b) CETAP@MIP-NPs and (c) PEMP-CPADB@MIP-NPs, (d) NPs negative control, (e) bulk negative control.

Langmuir model

Template affinity (K_{50}) and maximum binding (B_{max}) were calculated assuming that the binding to the MIP could be modelled as a Langmuir adsorption isotherm.

$$\theta = \frac{B_{max}[MIP]}{K_{50} + [MIP]}$$

where θ = fraction of bound template, B_{max} = maximum binding capacity, [MIP] = MIP concentration and K_{50} = apparent binding affinity.[4]

	K_{50} (mg/mL) in ACN	B_{max} (nmol/mg) in ACN	K_{50} (mg/mL) in H_2O	B_{max} (nmol/mg) in H_2O
NPs	0.067	0.59	--	--
CETAP-NPs	0.083	0.60	--	--
pNIPAm@CETAP-NPs	0.083	0.65	0.083	0.84
Bulk	2.326	0.56	--	--
CETAP-bulk	0.829	0.58	--	--
pNIPAm@CETAP-bulk	1.483	0.59	1.676	0.47
CPADB-NPs	0.082	0.60	--	--
pNIPAm@CPADB-NPs	0.080	0.64	--	--

Table S7 K_{50} and B_{max} values for the different polymer formulations: NPs by precipitation polymerization (yellow and green) and particles by bulk polymerization (orange).

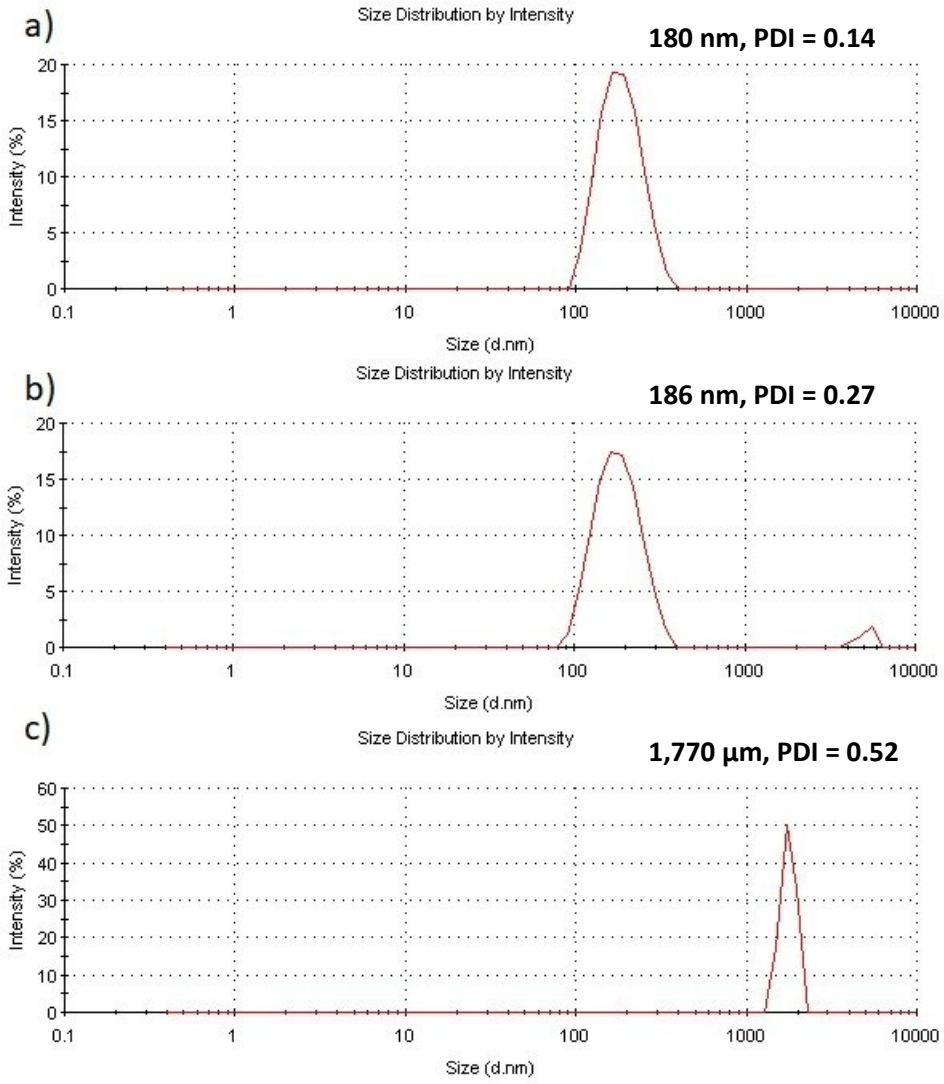


Figure S7. DLS in phosphate buffer (pH = 7.0, 25 mM) of (a) bare MIP NPs, (b) CETAP@MIP-NPs and (c) PNIPAm-CETAP@MIP-NPs (0.1 mg/mL, 20°C).

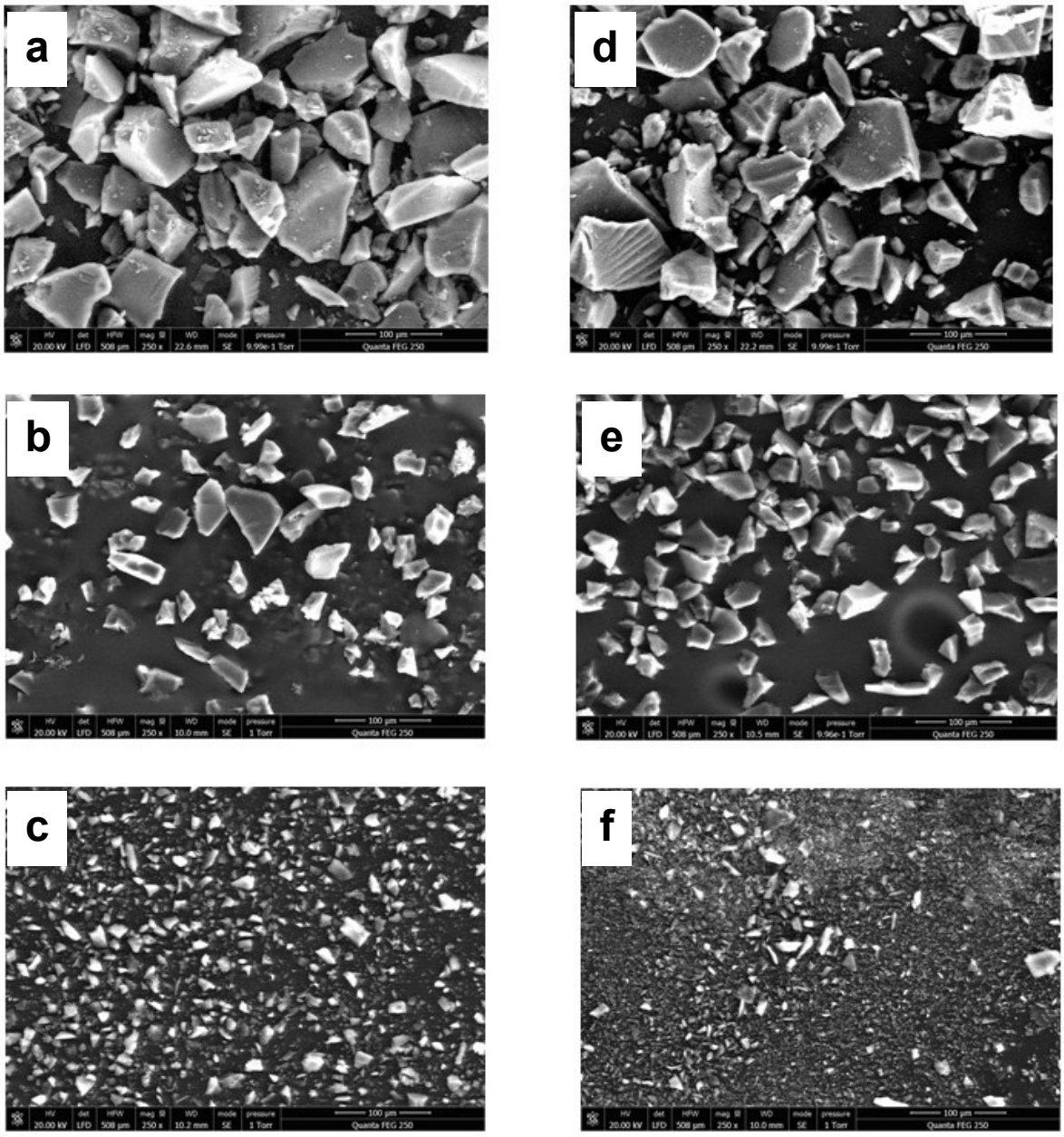


Figure S8. Representatives SEM images of (a) salicylic acid imprinted and (b) non-imprinted bulk polymers by free-radical precipitation polymerization. (c) Surface-modified, CETAP-coupled imprinted (CETAP@MIP) and (d) non-imprinted (CETAP@NIP) bulk polymers. (e) PNIPAm surface extended imprinted (PNIPAm-CETAP@MIP) and (f) non-imprinted (PNIPAm-CETAP@NIP) bulk polymers.

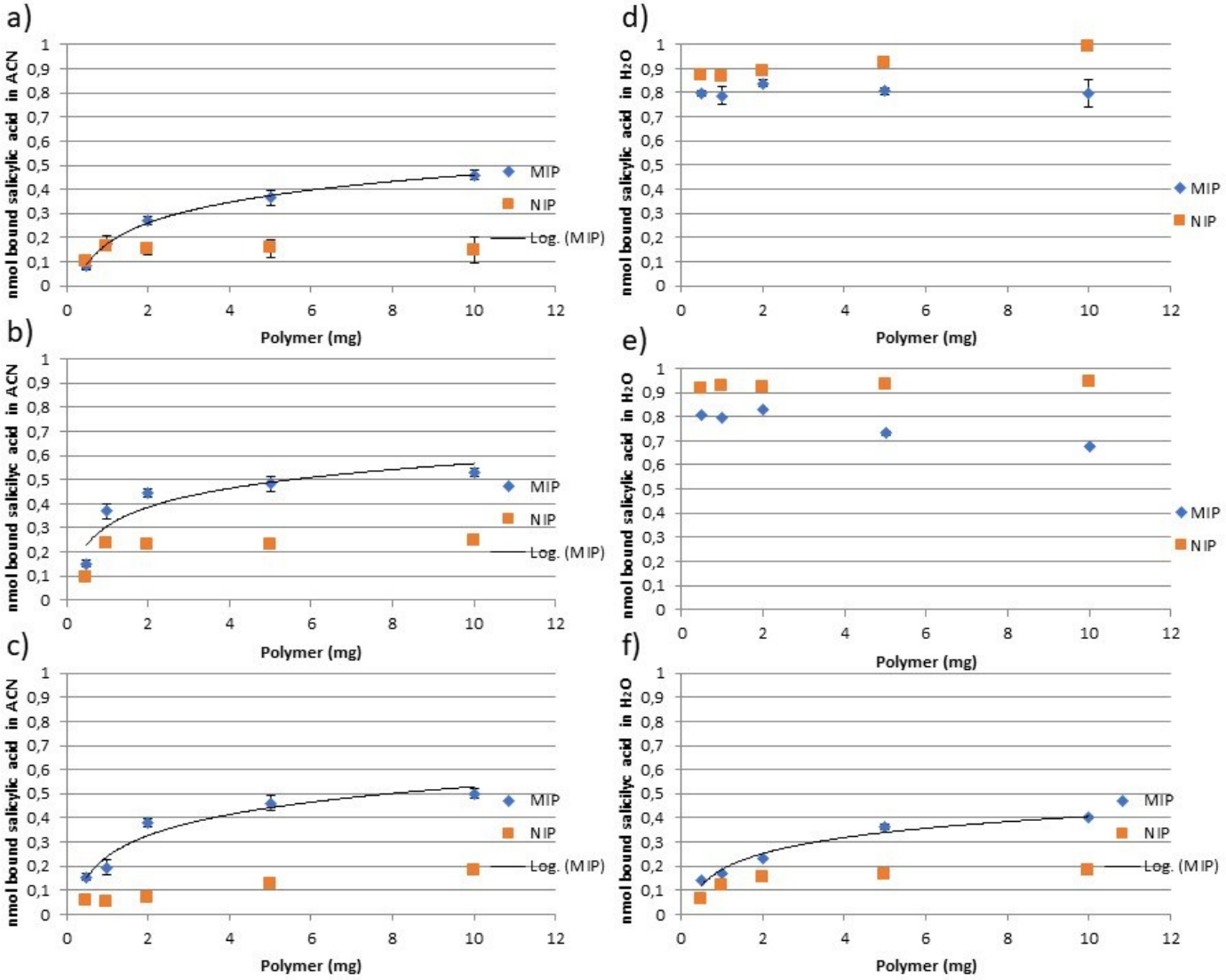


Figure S9. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) bulk polymers in 1µM salicylic acid in acetonitrile: (a) bare MIP and NIP bulk, (b) CETAP@MIP/CETAP@NIP-bulk and (c) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-bulk. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) bulk polymers in 1µM salicylic acid in water: (d) bare MIP and NIP bulk, (e) CETAP@MIP/CETAP@NIP-bulk and (f) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-bulk

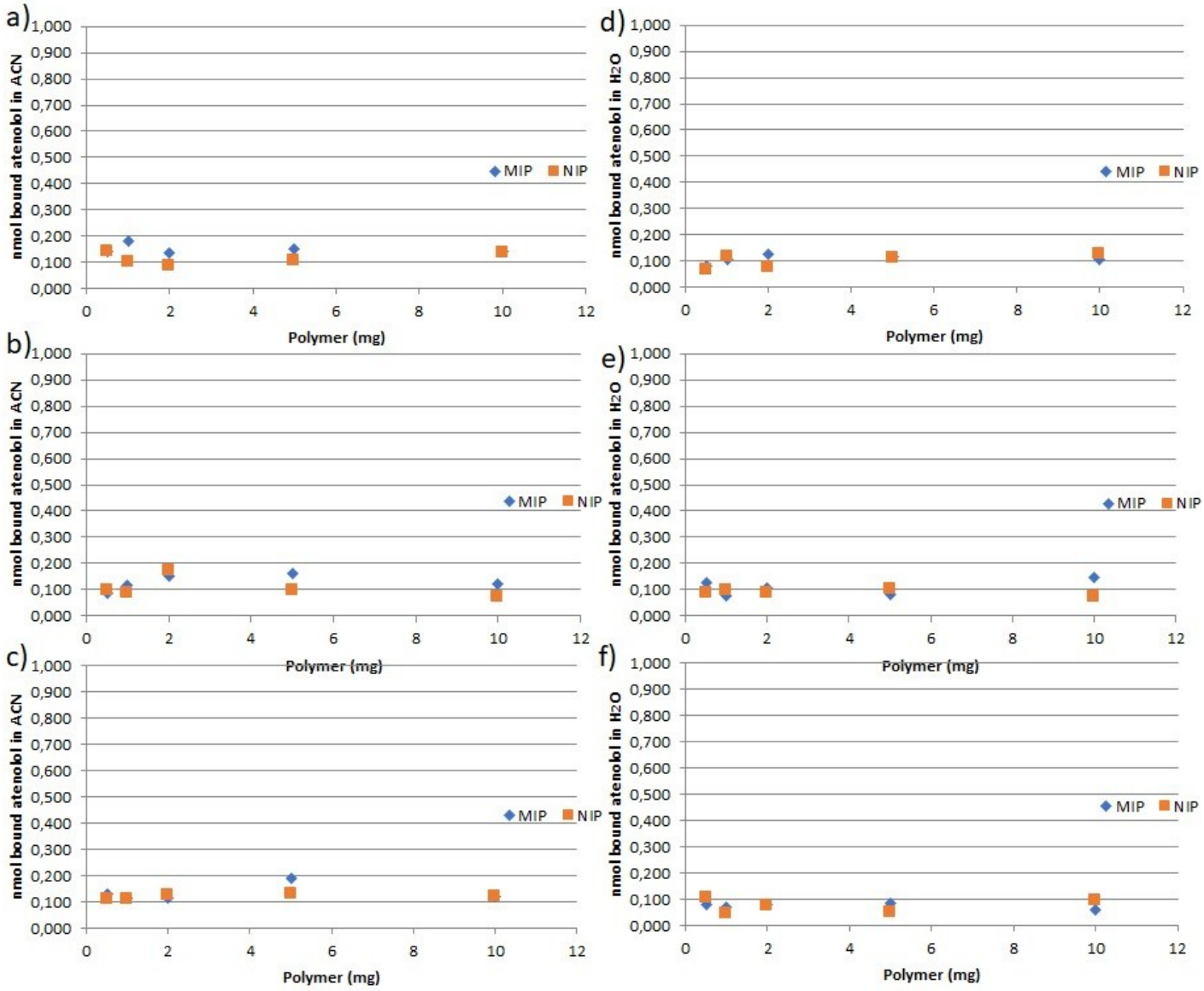


Figure S10. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) bulk polymers in 1µM atenolol in acetonitrile: (a) bare MIP and NIP bulk, (b) CETAP@MIP/CETAP@NIP-bulk and (c) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-bulk. Equilibrium binding essays for imprinted (blue) and non-imprinted (red) bulk polymers in 1µM salicylic acid in water: (d) bare MIP and NIP bulk, (e) CETAP@MIP/CETAP@NIP-bulk and (f) PNIPAm-CETAP@MIP/PNIPAm-CETAP@NIP-bulk.

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