Supporting Information

Copolymer Brush-Based Ultralow-Fouling Biorecognition Surface Platform for Food Safety

Hana Vaisocherová-Lísalová[*1], *František Surman*[2], *Ivana Víšová*[1], *Milan Vala*[1], *Tomáš Špringer*[1], *Maria Laura Ermini*[1], *Hana Šípová*[1], *Petr Šedivák*[3], *Milan Houska*[2], *Tomáš Riedel*[2], *Ognen Pop-Georgievski*[2], *Eduard Brynda*[2], *and Jiří Homola*[*1]

[1] *Institute of Photonics and Electronics of the CAS, Chaberská 57, Prague, Czech Republic*
[2] *Institute of Macromolecular Chemistry of the CAS, Heyrovského nám. 2, Prague, Czech Republic*
[3] *Police of the Czech Republic, Kapucínská 214/2, Prague, Czech Republic*

Prof. Jiří Homola, Ph.D., DSc., Institute of Photonics and Electronics, Czech Academy of Sciences, Chaberská 57, 18251 Prague, Czech Republic; Tel.: +420 266773404, E-mail: homola@ufe.cz,
Dr. Hana Vaisocherová-Lísalová, Institute of Photonics and Electronics, Czech Academy of Sciences, Chaberská 57, 18251 Prague, Czech Republic; Tel.: +420 266773547, E-mail: lisalova@ufe.cz.

Abstract: Functional polymer coatings that combine the ability to resist non-specific fouling of species from complex media with high biorecognition element (BRE) immobilization capacity represent an emerging class of new functional materials for a number of bioanalytical technologies for medical diagnostics, security, and food safety. Here we report on a random copolymer brush surface coating – poly(CBMAA-ran-HPMAA) – providing high BRE immobilization capacity while simultaneously exhibiting ultra-low fouling behavior in complex food media. We demonstrate that both the functionalization and fouling resistance capabilities of such copolymer brushes can be tuned by changing the surface molar ratio of the two monomer units: non-ionic N-(2-hydroxypropyl) methacrylamide (HPMAA) and carboxy-functional zwitterionic

carboxybetaine methacrylamide (CBMAA). It is demonstrated that the resistance to fouling decreases with the surface content of CBMAA; poly(CBMAA-*ran*-HPMAA) brushes with CBMAA molar content up to 15 mol% maintain excellent resistance to fouling from a variety of homogenized foods (hamburger, cucumber, milk, and lettuce) even after covalent attachment of BREs to carboxy-groups of CBMAA. The poly(CBMAA 15 mol%-*ran*-HPMAA) brushes functionalized with antibodies are demonstrated to exhibit fouling resistance from food samples by up to three orders of magnitude better when compared with the widely used low-fouling carboxy-functional oligo(ethylene glycol) (OEG)-based alkanethiolate self-assembled monolayers (AT SAMs) and furthermore, by up to two orders of magnitude better when compared with the most successful ultra-low fouling biorecognition coatings - poly(carboxybetaine acrylamide), poly(CBAA). It is also demonstrated that the antibody-functionalized poly(CBMAA 15 mol%-*ran*-HPMAA) brush exhibits superior biorecognition properties over the poly(CBAA).

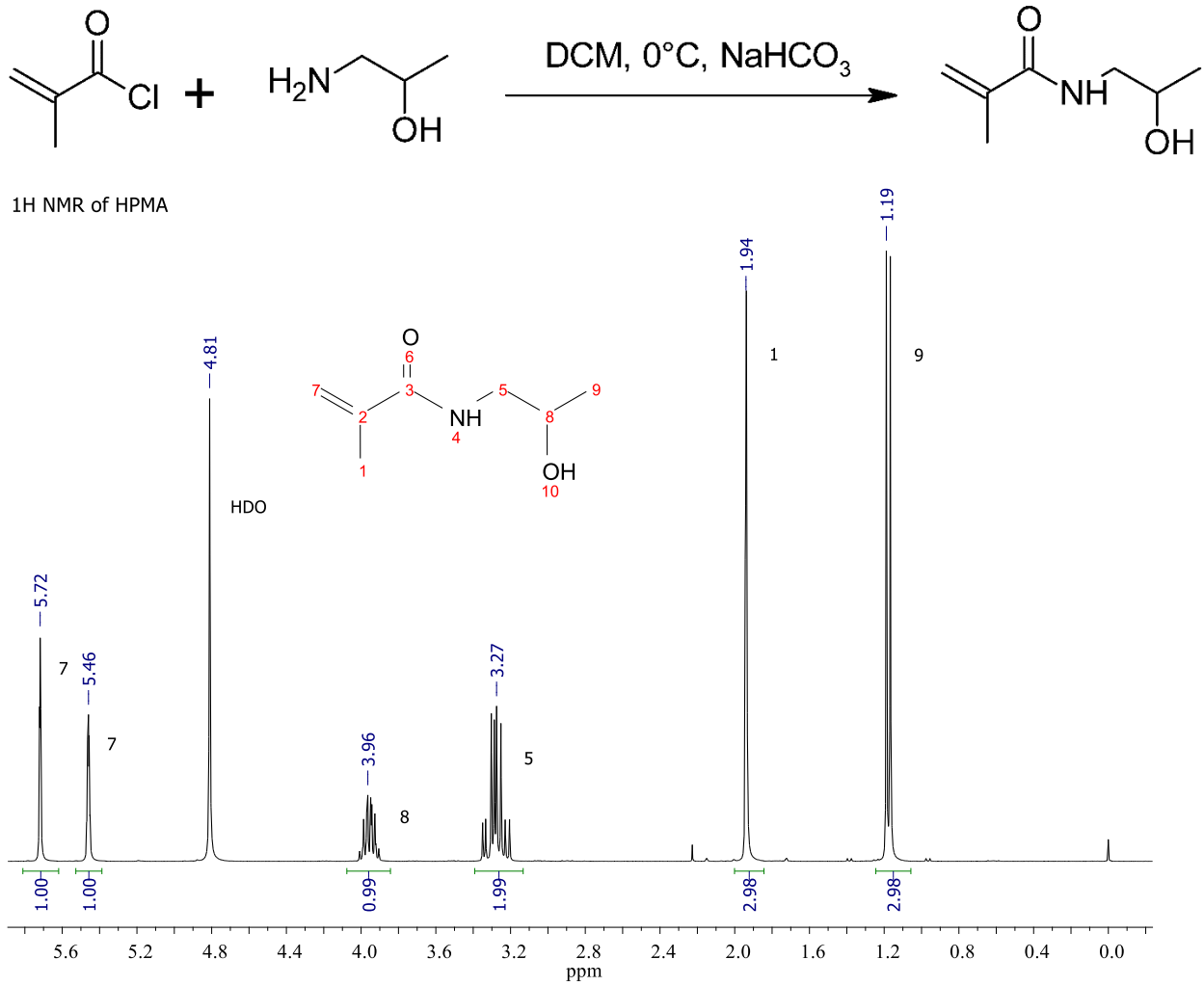
Preparation of food samples

The crude food samples for the detection experiments were prepared from lettuce, sprout, spinach, cucumber, and fresh milk purchased from a local food store and hamburger supplied by a local fast food restaurant. The pieces of vegetables were washed with water and sliced prior to homogenization. The samples were homogenized for 2 min using a Masticator (IUL Instruments, BioTech, Czech Republic) following a standardized procedure. The samples were then centrifuged for 2 min at 1,200 rpm to remove any residual large pieces of foods. The supernatant above the sediment was frozen until used. To confirm that these food extracts were free of tested bacteria, a series of culture-based reference experiments were performed in the Food control laboratory of the Police of the Czech Republic following standard protocols (ČSN ISO 7251 and ČSN EN ISO 6579).

Synthesis of *N*-(2-hydroxypropyl)methacrylamide (HPMAA):

34 g (340 mmol) of anhydrous sodium hydrogen carbonate was suspended in a solution of 23 g (300 mmol) of 1-aminopropan-2-ol in 85 ml of freshly distilled anhydrous methylene chloride. The suspension was cooled to 0 °C, and a solution of 32 g (290 mmol) of methacryloyl chloride in 40 ml of methylene chloride was added dropwise under cooling and vigorous stirring over a period of 1 h. The reaction mixture was stirred for another 30 min at 15 °C, then 10 g of

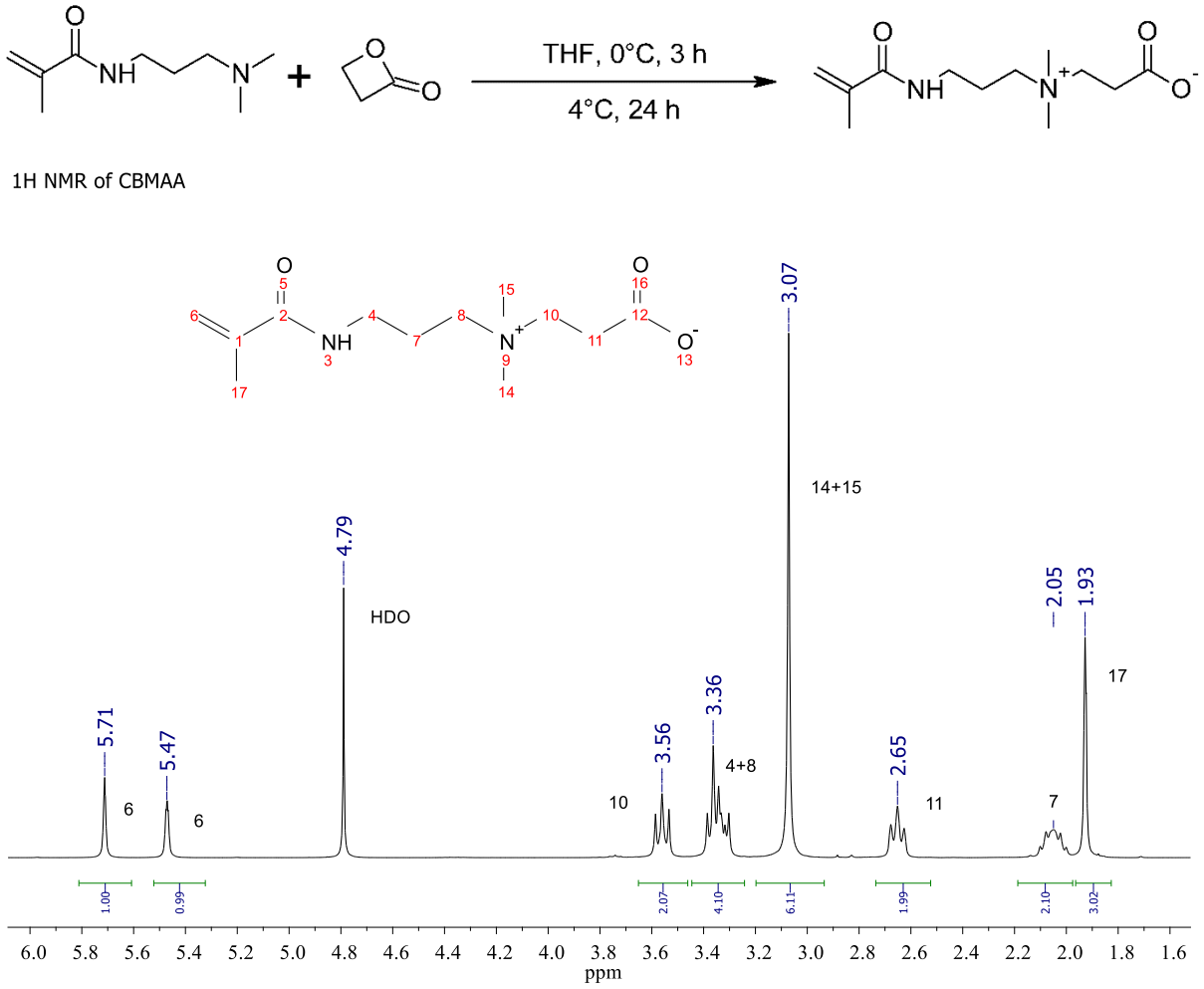
anhydrous sodium sulfate was added, the solid was filtered off, and the dry filtrate was concentrated to half of the original volume under reduced pressure. HPMAA was obtained by crystallization from methylene chloride at −20 °C and purified by recrystallization from acetone to yield 33 g (80%). The structure was confirmed by 1H NMR (Bruker 300 MHz in D_2O) (Scheme S1).



Scheme S1. Scheme of synthesis of HPMAA monomer and ^{1}H NMR spectrum of HPMAA monomer.

Synthesis of 3-methacryloylaminopropyl-2-carboxyethyl-dimethylammonium betaine (CBMAA)

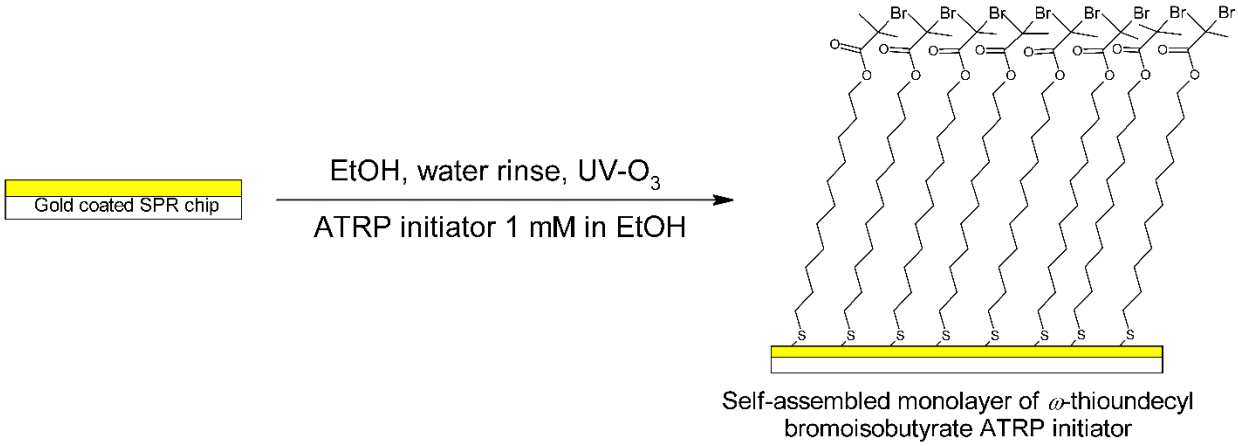
3-methacryloylaminopropyl-2-carboxyethyl-dimethylammonium betaine (carboxybetaine methacrylamide, CBMAA) was synthesized according to a previously published procedure for a similar monomer [1]. DMAPMA (19.4 g, 114 mmol) was dissolved in 100 mL of anhydrous THF in a round bottom flask under vigorous stirring and cooled to 0 °C. Subsequently, β-propiolactone (11.5 g, 160 mmol) was dissolved in 30 mL of anhydrous THF and added dropwise under argon for a period of about 1 h. The reaction was allowed to proceed for 24 h at 4 °C in a refrigerator. The white precipitate was filtered off, washed with anhydrous THF and ether, and dried under high vacuum to yield 17 g of CBMAA (yield 60%). The product was confirmed by 1H NMR (Bruker 300 MHz in D_2O) (Scheme S2).



Scheme S2. Scheme of synthesis of CBMAA monomer and ^{1}H NMR spectrum of CBMAA monomer.

Preparation of initiator SAM for synthesis of copolymer brushes

The initiator SAM for preparation of polymer brushes was prepared according to a procedure described in [2]. The scheme of the initiator SAM preparation is depicted below (Scheme S3).



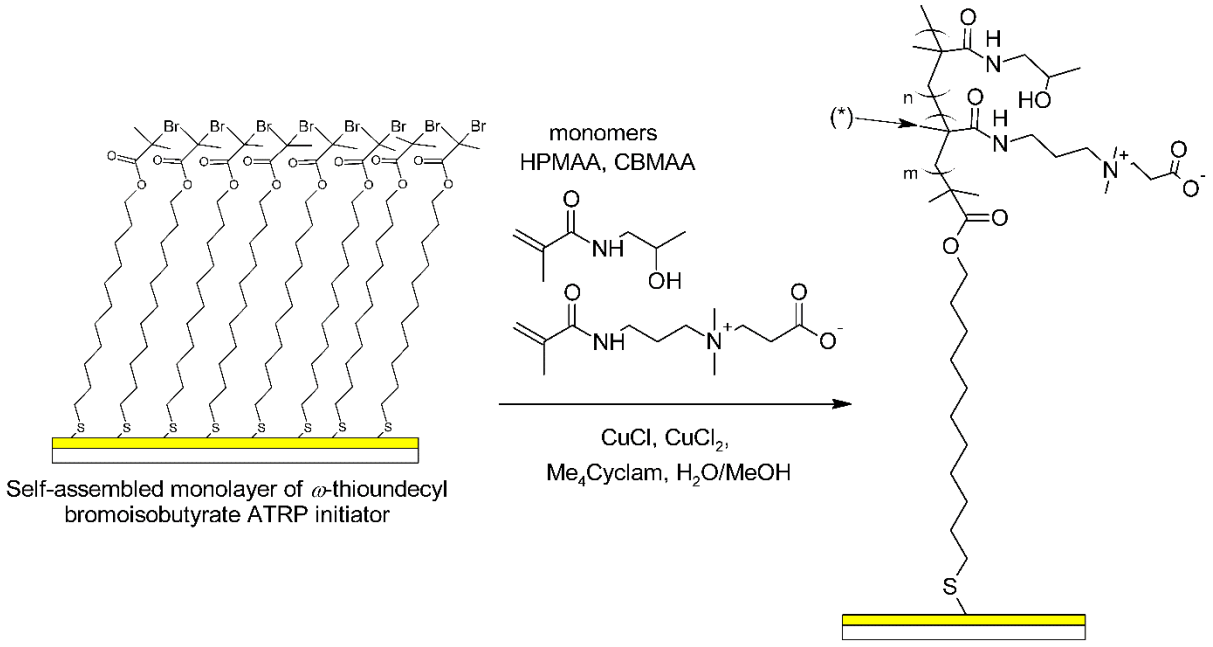
Scheme S3. Scheme of preparation of initiator self-assembled monolayer (SAM) for surface initiated atom transfer radical polymerization.

Preparation of polymer brushes on gold films

Gold-coated glass slides (used in SPR sensor) and gold-coated Si-wafers (used in ellipsometry) were rinsed twice with ethanol and deionized Milli-Q water, blown dry with nitrogen, and cleaned in a UV/Ozone cleaner (Jelight) for 20 min. Immediately after cleaning, the chips were immersed in a 1 mM solution of ω-mercaptoundecyl bromoisobutyrate in ethanol and kept overnight in the dark at a room temperature to allow the formation of a self-assembled monolayer (SAM), see also Scheme S3. Methanol (7 mL) was degassed via six freeze-pump-thaw cycles and subsequently transferred under argon atmosphere to a Schlenk tube containing CuCl (35 mg, 354 µmol), CuCl2 (10.5 mg, 78 µmol), and Me4Cyclam (121 mg, 472 µmol). The catalyst mixture was stirred until all solids were dissolved. In a second Schlenk tube, HPMAA and CBMAA monomers were dissolved in 12 mL of degassed water. The molar ratios of CBMAA/(CBMAA+HPMAA) in mixed CBMAA and HPMAA solutions were: 0, 7.5, 15, 30, 60, 80, and 100 mol%. For the preparation of poly(CBMAA-ran-HPMAA) (see Scheme S4) with 15 mol% CBMAA, HPMAA (2.4 g, 16.6 mmol) was mixed with CBMAA (0.7 g, 2.9 mmol). During the process, the monomer mixture was kept in a water/ice bath. Once the dissolution was completed, a catalyst solution was

added to the monomer solution using a gastight syringe. Subsequently, the homogenized polymerization mixture was cannulated into the reactor containing the substrates coated with the initiator SAM. The polymerization was carried out at 30 °C for 2 h. Then, the samples were washed with water and stored in water/ethanol mixture (95/5 vol%) until used.

The polymer brushes of poly(carboxybetaine acrylamide) (poly(CBAA)) used as reference coatings were prepared on gold substrates via surface-initiated atom transfer radical polymerization [3]. The prepared poly(CBAA) coatings exhibited wet thicknesses of ~25 nm. The mixed COOH-/OH- (3:7) OEG-based AT SAMs were prepared on gold substrates according to the procedure described in [4].



Scheme S4. Synthesis of poly(HPMAA-*ran*-CBMAA) brushes via surface-initiated atom transfer radical polymerization (SI ATRP). It should be noted that for poly(CBAA) brushes there is –H instead of $–CH_3$ in the polymer brush main chain (indicated by asterisk).

Dynamic contact angle measurement

Dynamic contact angles were measured with a contact angle goniometer OCA 20 (DataPhysics Instruments, Germany) equipped with SCA 21 software. Water drops were deposited on the surfaces to be tested, and dynamic changes of the drop profiles were recorded on 10 µL advancing and receding volumes. The profiles were fitted with the tangent leaning method. Reported values are averages of at least three measurements recorded at different positions on each substrate.

Table S1. Advancing Θ_a and receding contact angles Θ_r

	Poly(HPMAA)	Poly(CBMAA 15 mol%-*ran*-HPMAA)	Poly(CBMAA 30 mol%-*ran*-HPMAA)	Poly(CBMAA)
	[deg]			
Θ_a	49.3 ±1.4	33.3±2.9	31.7±0.4	11,9±3.7
Θ_r	19.53 ±0.7	12.00±2.4	11.5±0.9	*N/A*

N/A - Θ_r could not be measured due to very high wettability of polyCBMAA.

PM-IRRAS measurements

In order to determine the relative surface molar content of CBMAA in poly(CBMAA-ran-HPMAA) layers with respect to the molar ratios of monomers in polymerization solution, a series of poly(CBMAA-ran-HPMAA) coatings with molar CBMAA/(CBMAA+HPMAA) ratios of 0, 15, 30, 60, 80, and 100% (in solution) were prepared and analyzed by PM-IRRAS method. PM-IRRAS spectra were recorded on a commercial NICOLET 6700 spectrometer with photoelastic modulation (PEM) module (Thermo Scientific, USA). The external beam was focused on the sample with a mirror at an incident angle of 82°. A ZnSe grid polarizer and a ZnSe photoelastic modulator, modulating the incident beam between p and s polarizations (HINDS Instruments, PEM 90, modulation frequency = 37 kHz), were placed in front of the sample. The light reflected at the sample was focused on a nitrogen-cooled MCT (Mercury–Cadmium–Telluride) detector. The PM-IRRAS signal is given by the differential reflectivity $\Delta R/R = (Rp - Rs)/(Rp + Rs)$. The spectra resulted from the co-addition of 100 scans recorded with 4 cm^{-1} resolution.

The dried chips were stored in a glass vial at 4 °C – 8 °C. Prior to measurement, the chips were cleaned of any debris with a stream of pure nitrogen and immediately mounted to PEM module of spectrometer.

The spectra were normalized using the 1650 cm^{-1} band of the amide C=O stretching vibration (amide I) common to both CBMAA and HPMAA [5]; we used the 1607 cm^{-1} band of the betaine carboxylate group to determine the HPMAA/CBMAA ratio in the copolymer brushes. The areas of both peaks ($A^{amide\ I}$ and A^{1607}) were determined by multiple peak fitting tool in Origin software (Gauss functions) (Figure S1).

We confirmed that the surface molar ratios of CBMAA/HPMAA monomer units in poly(CBMAA-*ran*-HPMAA) were in a good agreement with the ratios in CBMAA/HPMAA polymerization solution (Figure S2). These results suggest similar polymerization rates of CBMAA and HPMAA in the surface-initiated polymerization reaction and furthermore, indicate a random distribution of CBMAA and HPMAA monomer units in the copolymer brush structure.

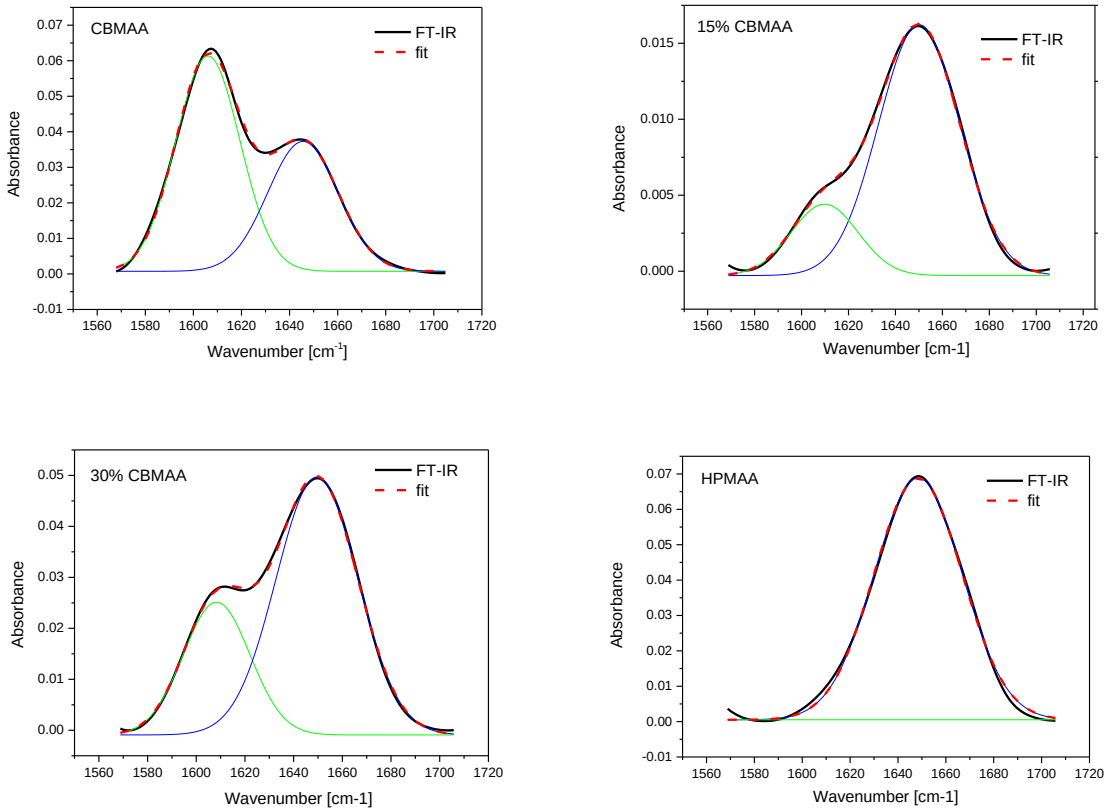


Figure S1. Gaussian fit of IR absorption spectra of CBMAA and HPMAA copolymers on gold surfaces.

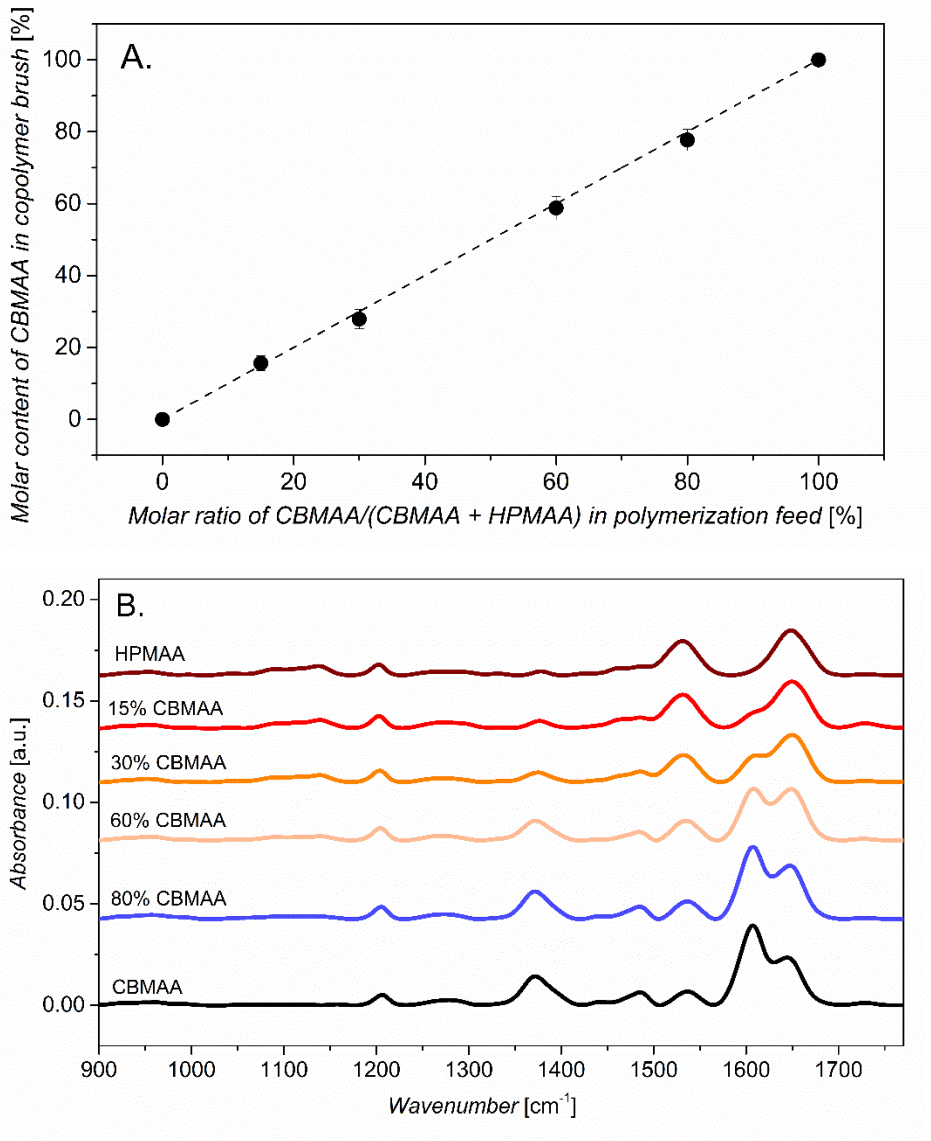


Figure S2. Dependence of surface molar content of CBMAA in copolymer brush on CBMAA/(CBMAA+HPMAA) molar ratio in polymerization solution (A), determined from PM-IRRAS spectra (B).

Preparation of SA-AuNPs

Bare AuNPs were modified with carboxy-terminated OEG-containing AT SAMs, to which streptavidin was covalently bound via amine coupling chemistry. A solution of bare AuNPs (200 mL, absorbance of 0.28) was mixed with a solution of alkanethiols (1 mM, 4 mL, in ethanol). This mixture was sonicated in a water bath (50 °C, 1 h) and shaken at a room temperature (2 h). The unreacted carboxy-thiols were then removed from the solution using four washing cycles: the

solution with thiolated AuNPs was centrifuged (9500 g, 10 min), the supernatant was discarded, and the pellet was dissolved in Milli-Q water. To activate the carboxyls, thiolated AuNPs (250 µL, absorbance of 1.68) were mixed with NHS/EDC solution (1 mM NHS and 5 mM EDC in Milli-Q water, 120 µL, 2 min). This mixture was then centrifuged (9500 g, 3 min) and the supernatant was removed. The pellet was dissolved in streptavidin solution (12 µg streptavidin in 12 µL SA + 88 µL PBS + 400 µL Milli-Q water) and the solution was shaken for 1 h. Then ethanolamine (50 µL, 1 M) was slowly added during 5 min period in order to deactivate all of the non-reacted esters. The free streptavidin was removed from the solution in 6 washing cycles (9500 g, 10 min). The cross-linked streptavidin-coated AuNPs (SA-AuNPs) were removed from the solution in 2 "soft" centrifugation cycles (210 g, 5 min), the supernatant was kept and the pellet was discarded. This solution of SA-AuNPs was stored in a refrigerator until use. Before SPR measurement, the SA-AuNPs solution was centrifuged (9500 g, 10 min) and the pellet was dissolved in a PBS/Tween/Casein solution. To determine the concentration of the SA-AuNPs, the absorption at a wavelength of 528/531 nm was measured using a NanoPhotometer Pearl UV–Vis absorption spectrophotometer (Implen, Germany).

SPR sensor response to binding of bacterial pathogens in food samples measured using direct and SA-AuNP-enhanced assay

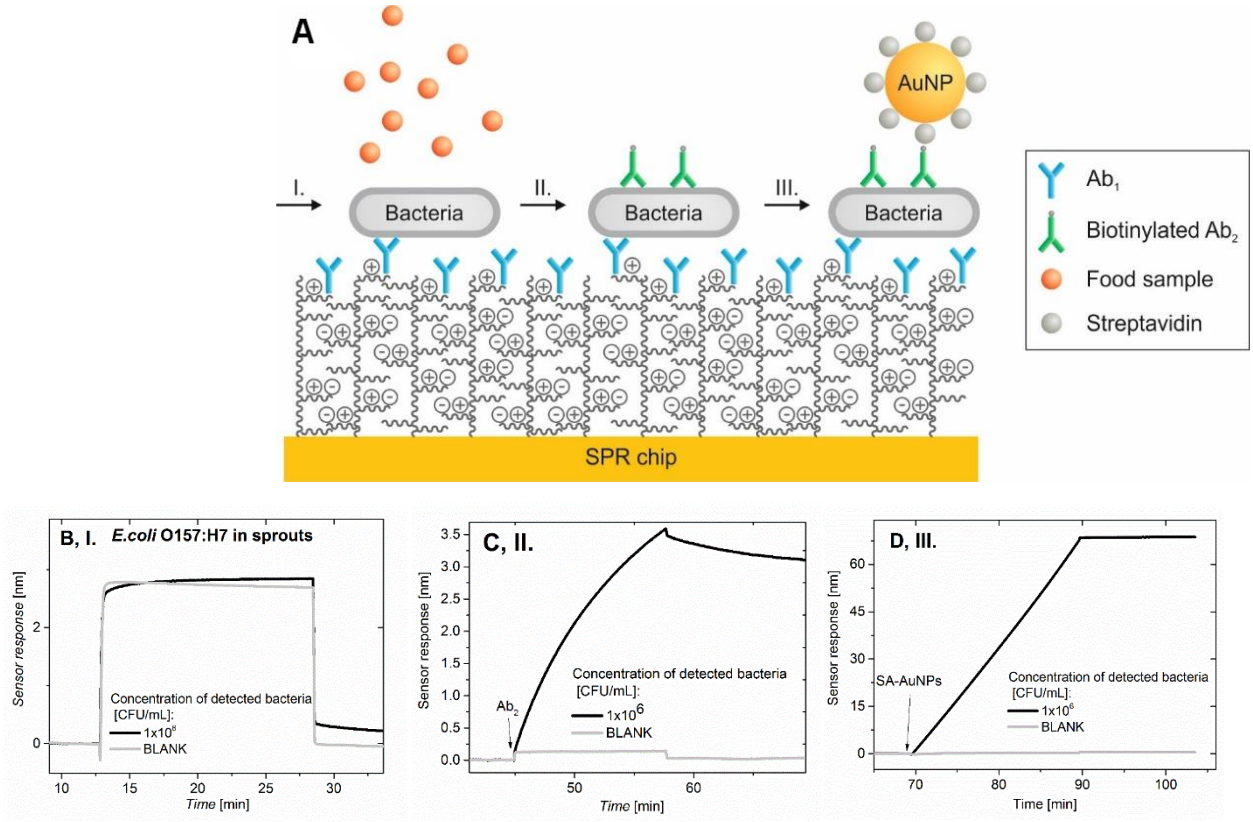


Figure S3. A scheme of the three-step detection assay (A): a direct coupling of bacteria to antibody-functionalized polymer brush (I.) followed with the binding of secondary biotinylated antibody (II.) and SA-AuNPs (III.); SPR sensor response corresponding to the binding of *E.coli* O157:H7 (1×10^6 CFU/mL) in sprouts sample using direct (B, I.), secondary antibody-enhanced (C, II.), and SA-AuNP-enhanced detection formats (D, III.) for measuring and reference (blank) surfaces of poly(CBMAA 15 mol%-*ran*-HPMAA).

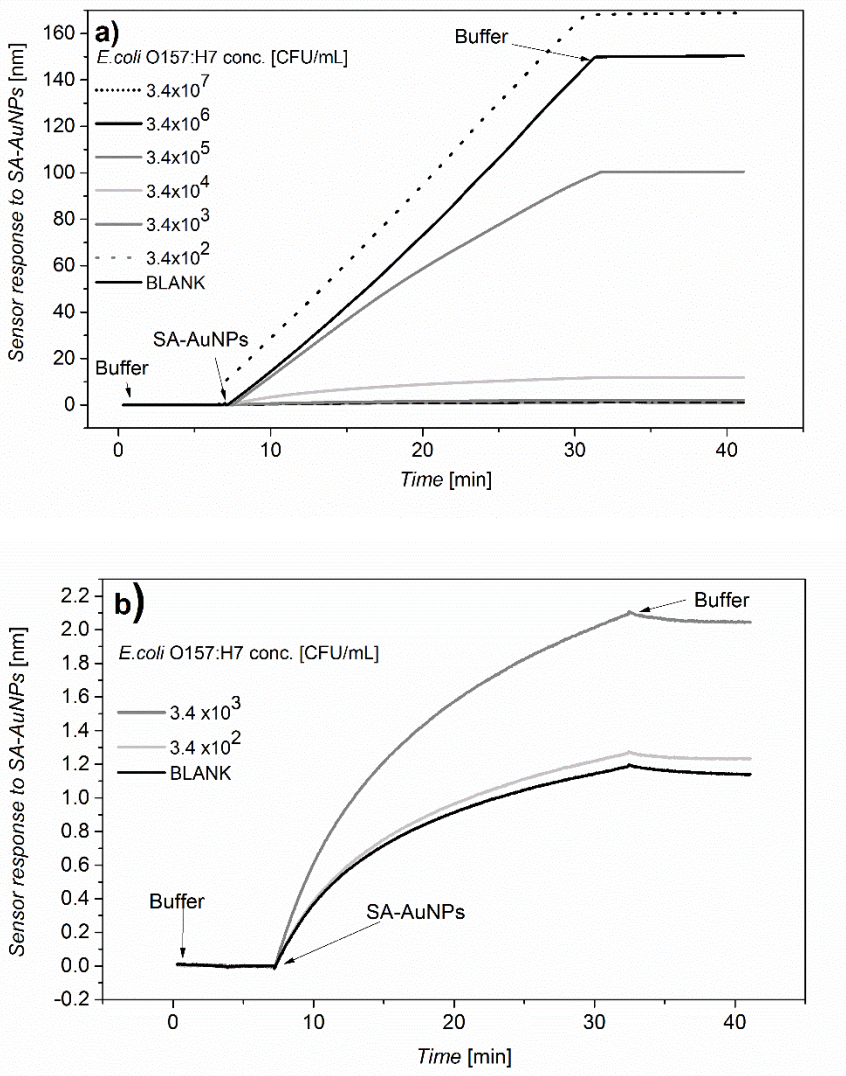


Figure S4. SPR sensor response to the binding of *E.coli* O157:H7 in hamburger sample via the SA-AuNP-enhanced detection format, with variation of the *E.coli* O157:H7 concentrations (a). SPR sensor response to the binding of *E.coli* O157:H7 in hamburger sample via the SA-AuNP-enhanced detection for low *E.coli* O157:H7 concentrations and blank hamburger sample (reference surface) (b).

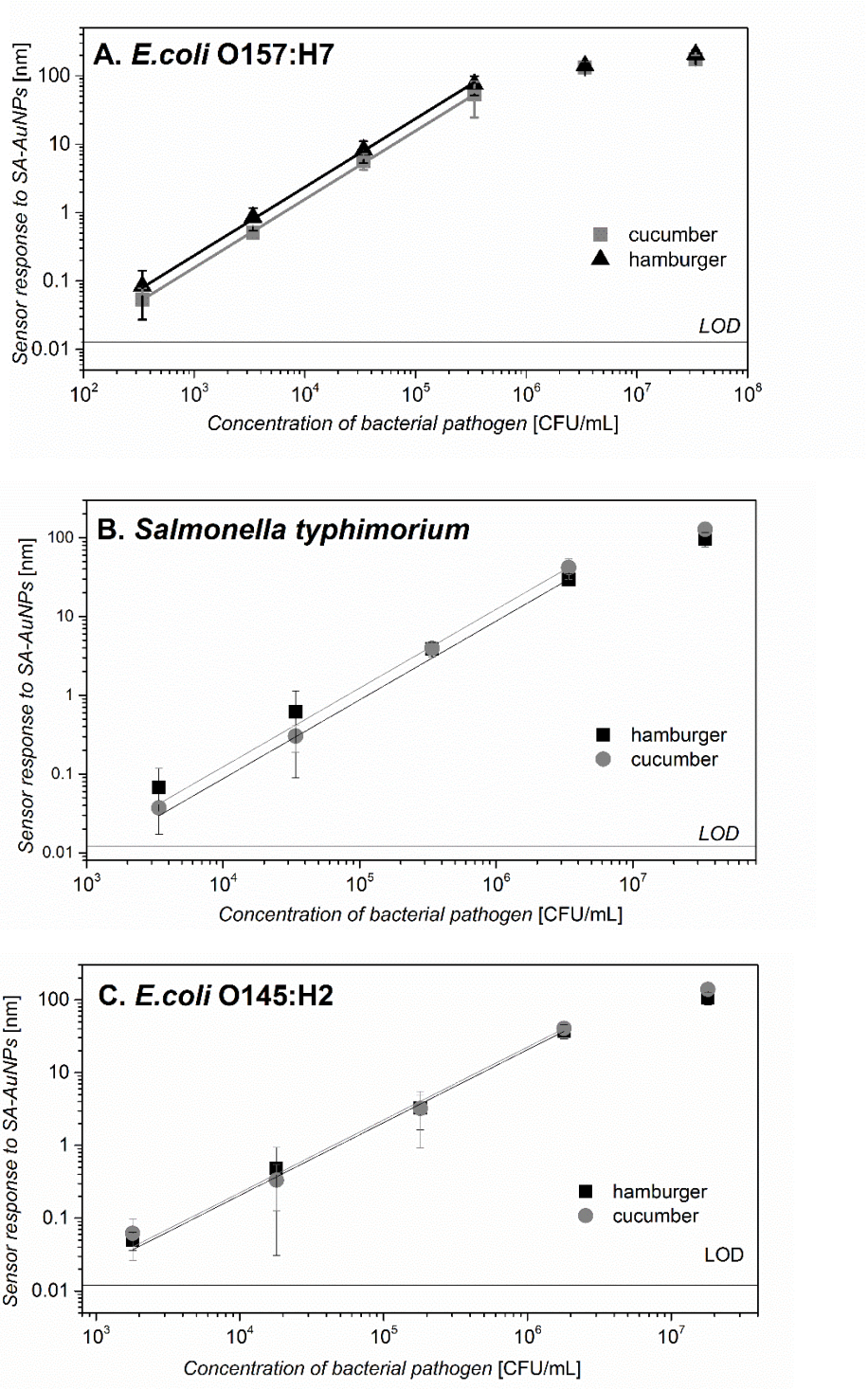


Figure S5. SPR reference-compensated responses to the detection of *E.coli* O157:H7 (A), *Salmonella* (B), and *E.coli* O145:H2 (C) in hamburger and cucumber samples in dependence on bacterial pathogen concentration using antibody-functionalized poly(CBMAA 15 mol%-*ran*-HPMAA) coatings via SA-AuNP-enhanced detection formats.

Table S2. SPR reference-compensated responses to measurements of concentrations of two bacterial pathogens in complex hamburger and cucumber samples. The results were compared to results determined from the respective SPR date obtained using a single bacterium detection ("individual pathogen response").

Food sample	Bacterial pathogen	Concentration [CFU/mL]	Sensor response from mixed sample [nm]	Individual pathogen response [nm]	Recovery (%)
BURGER-1	*E.coli* O157:H7	6×10^2	0.23 ± 0.08	0.20 ± 0.05	**116**
	E.coli O145:H2	9×10^4	2.80 ± 0.25	1.96 ± 0.18	**143**
CUCUMBER-1	*E.coli* O157:H7	6×10^2	0.06 ± 0.03	0.08 ± 0.03	**67**
	E.coli O145:H2	9×10^4	2.03 ± 0.28	2.00 ± 0.36	**101**
BURGER-2	*E.coli* O157:H7	3×10^2	0.10 ± 0.06	0.10 ± 0.04	**94**
	Salmonella t.	3×10^4	0.29 ± 0.31	0.29 ± 0.08	**101**
CUCUMBER-2	*E.coli* O157:H7	6×10^2	0.11 ± 0.06	0.09 ± 0.04	**128**
	Salmonella t.	9×10^3	0.12 ± 0.09	0.11 ± 0.05	**104**

Molar surface concentration of BREs immobilized to poly(CBMAA-ran-HPMAA) and poly(CBAA) in dependence on CBMAA content

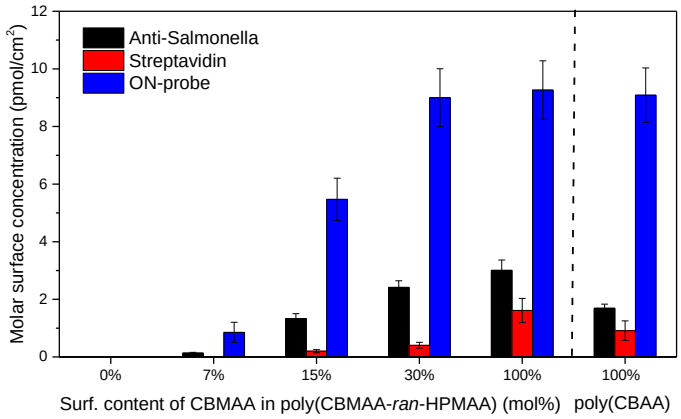


Figure S6. Molar surface concentration of BREs (anti-Salmonella, streptavidin, oligonucleotide probe) immobilized to poly(CBMAA-*ran*-HPMAA) and poly(CBAA) in dependence on CBMAA content.

Detection of *E.coli* O157:H7 in buffer

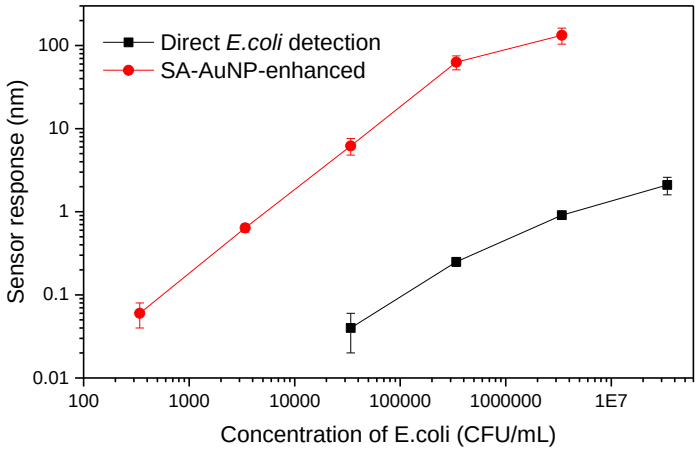


Figure S7. Reference-compensated SPR sensor response to the binding of *E.coli* O157:H7 to antibody-functionalized poly(CBMAA 15%-*ran*-HPMAA) in PBS buffer using the direct and SA-AuNP-enhanced detection formats.

References

(1) Banerjee, I.; Pangule, R. C.; Kane, R. S. *Adv Mater* **2011**, *23*, 690-718.

(2) H. Vaisocherova, K. Mrkvova, M. Piliarik, P. Jinoch, M. Steinbachova, J. Homola, *Biosens. Bioelectron.* **2007**, *22*, 1020.

(3) Vaisocherova, H.; Yang, W.; Zhang, Z.; Cao, Z.; Cheng, G.; Piliarik, M.; Homola, J.; Jiang, S. *Analytical chemistry* **2008**, *80*, 7894-7901..

(4) Rodriguez-Emmenegger, C.; Schmidt, B. V.; Sedlakova, Z.; Subr, V.; Alles, A. B.; Brynda, E.; Barner-Kowollik, C. *Macromolecular rapid communications* **2011**, *32*, 958-965.

(5) D. Lin-Vien, N. B. Colthup, G. Fateley, J. G. Grasselli, *The handbook of infrared and Raman characteristic frequencies of organic molecules*., Elsevier, **1991**.