Electronic Supplementary Information (ESI)

Amphiphilic hyperbranched polyglycerols in a new role as highly efficient multifunctional surface active stabilizers for poly(lactic/glycolic acid) nanoparticles

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CONTENT

Synthesis of hyperbranched polyglycerol via ring-opening multibranching polymerization of glycidol with dodecyl and octadecyl alcohol initiators (C12-HbPG; C18-HbPG)

For this work, the amphiphilic hyperbranched polymers were synthesized with different monomer/initiator ratios to the weight fraction of hydrophobic alkyl chains to be around 10% of the resulting macromolecules. The appropriate amount of initiator (dodecyl alcohol: 1.2202 g, 6.56 mmol; octadecyl alcohol: 1.2346 g, 4.57 mmol; both form Sigma Aldrich, 99%) was placed into a dry 100 mL three-neck, round-bottom flask with a mechanical stirrer, connected to a vacuum line and the third neck was sealed by a septum. In the first step, 10% of hydroxyl groups of initiator were deprotonated by potassium methoxide (Sigma Aldrich, 25% in methanol) at 40 °C, under nitrogen for 30 minutes. Subsequently, the methanol was removed by vacuum and the reaction mixture was heated to 95 °C. Then, 10 mL (11.17 g, 150.9 mmol, Sigma Aldrich, 97%, purified by vacuum distillation prior to use) monomer was added slowly into the reaction flask by a syringe pump. The feed rate of the monomer was changed in tree steps (1.5 mL with 1 ml/h; 2.5 mL with 2 ml/h; 6 mL with 4 ml/h) to avoid chain transfer to the monomer. After the monomer addition completed, the stirring was continued at least for 3 more hours. The cooled crude product was dissolved in 60 mL of MeOH, passed throw a column filled with a cation exchange resin (Amberlite IR120, hydrogen form) and precipitated two times into large excess of diethyl ether. The collected polymers were dried in vacuum oven at 50 °C until constant weight. (*yields C12-HbPG: 10.2838 g, 83%; C18-HbPG: 9.7996 g, 79%*)

Characterization methods for the HbPG samples

Acetylated HbPG derivatives were synthesized to be able to investigate the molecular weights and molecular weight distribution by GPC chromatography. General procedure: acetylation was carried out in a round-bottom flask connected with a condenser. 200 mg of HbPG sample was placed in a 25 mL round-bottom flask, dissolved in 10 mL pyridine and 2.5 mL acetic acid was added. The reaction mixture was stirred at 80 °C overnight. Then the solvent and the byproducts were removed by rotary evaporator. The crude product was dissolved in DCM, washed three times with water. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure and precipitated two times into large excess of hexane. The collected products were dried at 45 °C in vacuum until constant weight.

The acetyl derivatives of the HbPG macromolecules were characterized by GPC equipped with differential refractive index detector (Agilent 390), with Waters Styragel

columns (HR 1, 2 and 4) and Waters Styragel HR guard column connected in series and thermostated at 35 °C. The eluent was THF with a flow rate of 1 mL/min. The evaluation of molecular weights and polydispersity indexes was made on the basis of a conventional calibration curve, obtained from linear polystyrene standards (PSS Polymer Standards Service GmbH).

For the purpose to the determination of DP_n and number average molecular weight of the produced HbPGs, the samples were analyzed by ^1H NMR spectroscopy. The measurements were performed on a Bruker Avance 500 equipment operating at 500 MHz ^1H frequency in DMSO-*d6* at 30 °C.

Characterization results of the synthesized HbPG samples

The synthesized HbPG samples and acetyl derivatives were investigated by NMR spectroscopy in DMSO. The recorded ^1H NMR spectra can be seen in Fig. S1 and S2. The DP_ns were calculated as the ratio of the integral of the methyl protons of the initiator (observed in the 0.78–0.89 ppm region) and hydroxyl protons (4.25–4.82 ppm). This ratio gives one-unit higher result than the DP_n because every incorporated monomer increases the number of the hydroxyl groups by one regardless the forming branched structure. The following equation was applied for M_n determination:

$$M_n = M_I + DP_n \bullet M_{mon} = M_I + (I_{OH}/I_{CH3} - 1) \bullet M_{mon} \quad \text{(Eq. S1)}$$

where M_I and M_{mon} stand for the molar weights of the initiator and the monomer, respectively. I_{OH} and I_{CH3} are the integral values of hydroxyl and methyl protons. The calculated number average molecular weights based on DP_n values are represented in Table S1.

On the basis of the evaluation of the of recorded ^1H NMR spectra of the acetyl derivatives of the HbPG samples, it can be stated that the acetylation of the macromolecules in all cases were quantitative. Based on the ratio of the integral values of acetyl protons (~1.9 ppm) and the methyl group of the initiator, the molecular weights of polymers were also determined by Equation S2.

$$M_n = M_I + DP_n \bullet M_{mon} = M_I + (I_{Ac}/I_{CH3} - 1) \bullet M_{mon} \quad \text{(Eq. S2)}$$

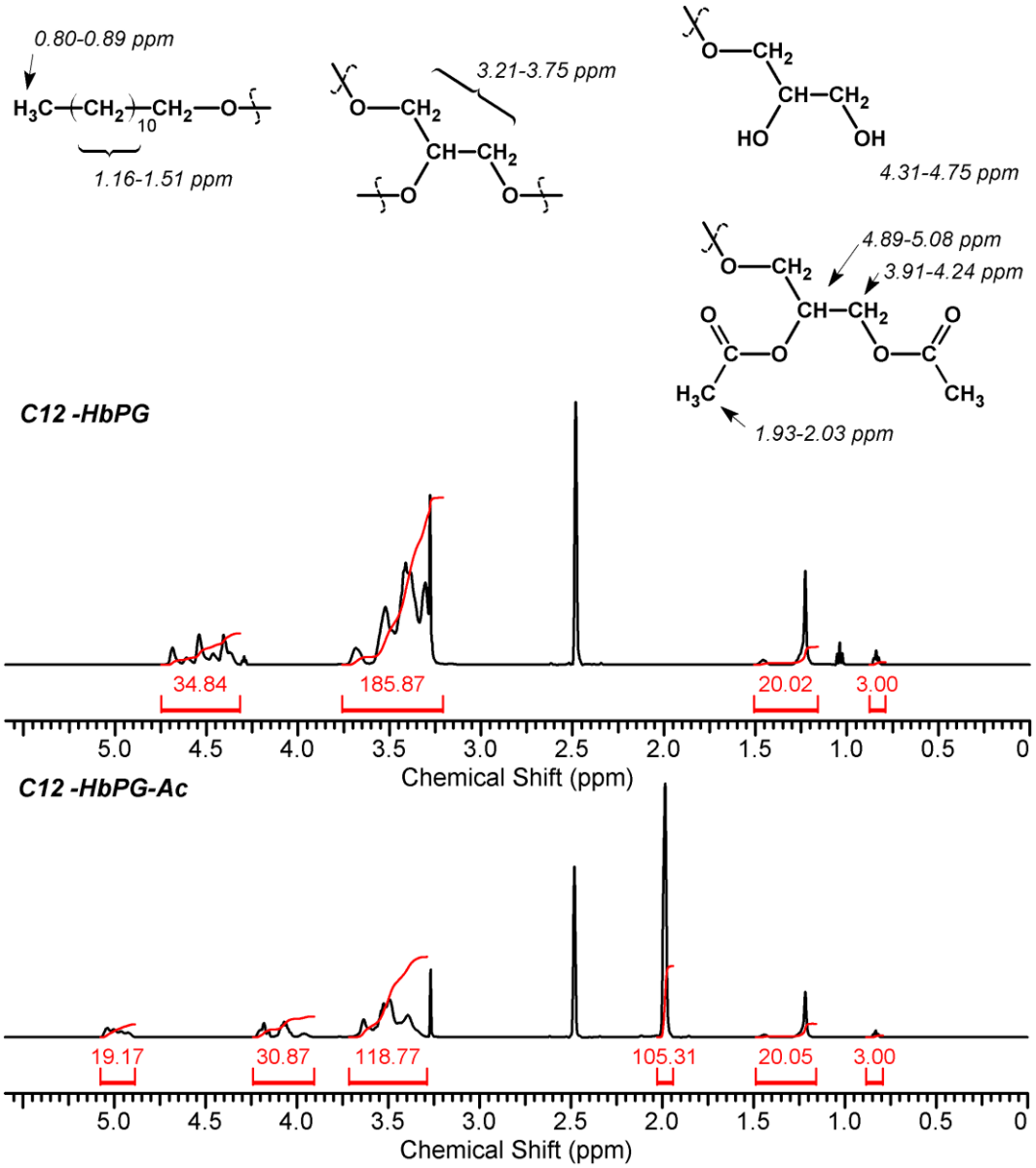

Fig. S1 ^{1}H NMR spectra of C12-HbPG (top) and its acetyl derivative (bottom) in DMSO-*d6*.

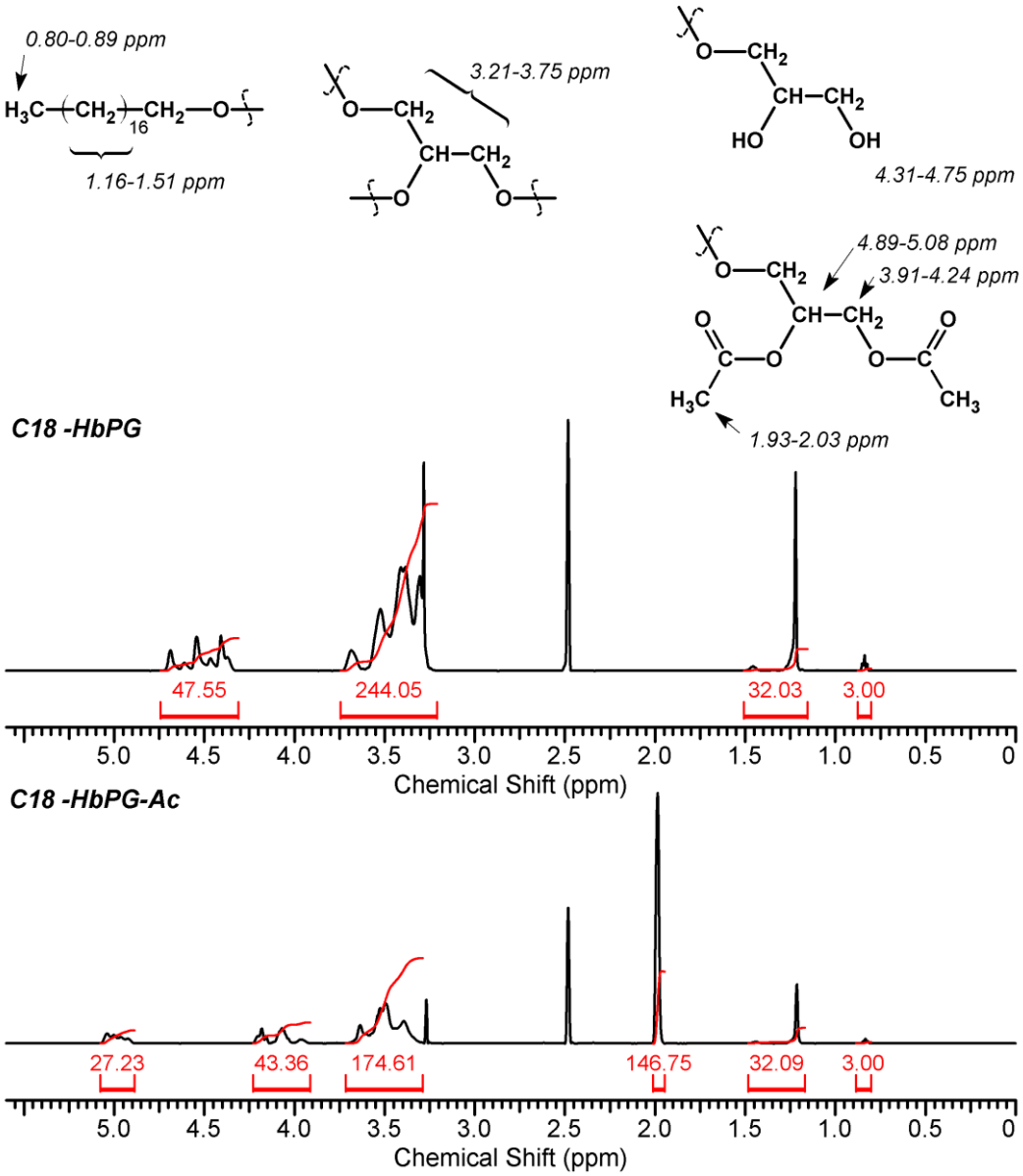

Fig. S2 ^{1}H NMR spectra of C18-HbPG (top) and its acetyl derivative (bottom) in DMSO-*d6*.

The average molecular weights and polydispersity values of the acetyl derivatives of the HbPG samples were determined by GPC. The molecular weight distribution curves are shown in Fig. S3. Since the acetylation was quantitative, it can be assumed that the polydispersity has not changed during the acetylation and the molecular weights of the unmodified polymers can be calculated by the following equation:

$$M_n = M_i + (M_{n,Ac} - M_i - M_{Ac})/(M_{mon} + M_{Ac}) \bullet M_{mon} \quad \text{(Eq. S3)}$$

where M_n, M_i, $M_{n,Ac}$, M_{Ac} and M_{mon} stand for the molecular weight of the polymer, the molecular weight of the initiator, the molecular weight of the acetylated polymer determined

by GPC, molecular weight of the acetylated end groups (42 g/mol) and the molecular weight of the monomer, respectively.

The resulting average molecular weights and polydispersities (PD) of the acetylated HbPG samples and the calculated molecular weights of the unmodified HbPGs are presented in Table S1. The molecular weights determined by GPC are lower than the molecular weights determined by ^{1}H NMR. Since GPC separation is based on the hydrodynamic volume (V_h) and branched polymers having lower V_h than the corresponding linear analogs with same molecular weight, these GPC results are proved the compact branched structure of the synthesized polymers.

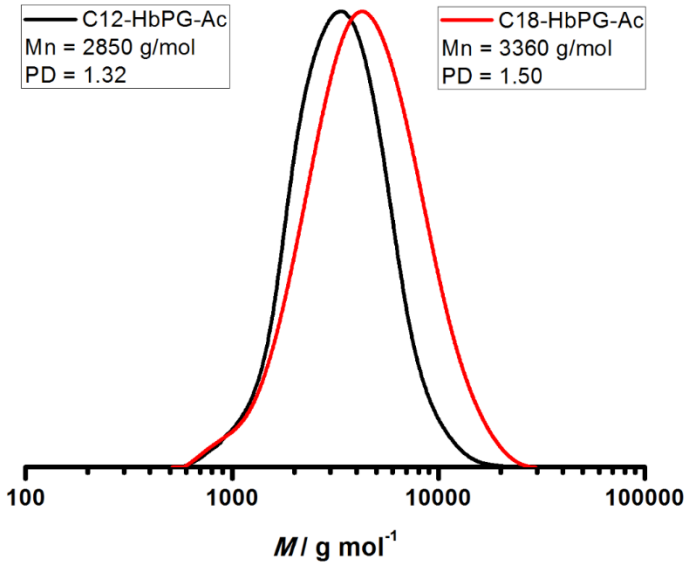

Fig. S3 Molecular weight distribution curves of acetyl derivatives of HbPG samples.

Table S1 Polydispersity (PD), M_n values and hydrophobic contents of the alkyl-HbPG samples and their acetyl derivatives.

	PD	Molecular weight (g/mol)				Hydrophobic content[×] (%)
		M_n* Acylated	M_n[#]	$M_{n,NMR}$	$M_{n,NMR}$ Acylated	
C12-HbPG	1.32	2850	1860	2690	2710	6.9
C18-HbPG	1.50	3360	2210	3750	3820	7.2

* M_n of acylated derivatives of HbPG samples determined by GPC
[#] Calculated molecular weight based on GPC results by Eq. S3
[×] Calculated by the ratio of molecular weight of initiator and $M_{n,NMR}$

The hydrophobic content of the synthesized polymers was determined by the ratio of molecular weight of the initiator and molecular weight of the polymer determined by ^{1}H NMR (see Table S1). The hydrophobic contents of the synthesized polymers are approximately equal (~7%), so these polymers can be utilized and compared to each other in the preparation and stabilization of PLGA nanoparticles.

Synthesis and characterization of carboxyfluorescein functionalized C18-HbPG (C18-HbPG-CF)

The octadecyl-hyperbranched polyglycerol (C18-HbPG) was functionalized by carboxyfluorescein via an esterification reaction to prove our concept that the modified HbPG can be also applied for the preparation PLGA nanoparticles with functionalized surface. C18-HbPG (0.3001 g, 0.08 mmol), 5(6)-carboxyfluorescein (0.0753 g, 0.20 mmol, from Sigma Aldrich, 95%+), N,N′-dicyclohexylcarbodiimide (0.0413 g, 0.20 mmol, from Sigma Aldrich, 99%) and 4-(dimethylamino)pyridine (0.0244 g, 0.20 mmol, from Sigma Aldrich, 98%+) were dissolved in 10 mL distilled DMF (from Molar Chemicals Ltd.). The reaction mixture was stirred for 5 hours at room temperature. Then the reaction flask was cooled to 4 °C for 6 hours, and the formed urea was removed by filtration. The crude product was purified two times by precipitation into large excess of diethyl ether followed by centrifugation, and the resulting C18-HbPG-CF was dried until constant weight in vacuum at 45 °C. The functionalization was investigated by ^{1}H NMR spectroscopy (500 MHz ^{1}H frequency in DMSO-*d6* at 30 °C), the recorded spectrum is displayed in Fig. S4. The functional group assignation: 4.17-4.31 (m, 0.7H, OCH*CH2*OCO), 5.26-5.43 (m, 0.67H, O*CH*OCO), 6.37-6.48 (m, 2H, Ar-H), 6.51-6.73 (m, 2H, Ar-H), 6.91-7.08 (m, 1H, Ar-H), 7.26-7.50 (m, 1H, Ar-H), 7.63-7.78 (m, 1H, Ar-H),

8.08-8.27 (m, 1H, Ar-H),, 8.31-8.56 (m, 1H, Ar-H), 10.20 (s, 2H, Ph-OH). Based on the integrated spectra it can be concluded that the functionalization was successful and the dye content was approximately one CF per macromolecule.

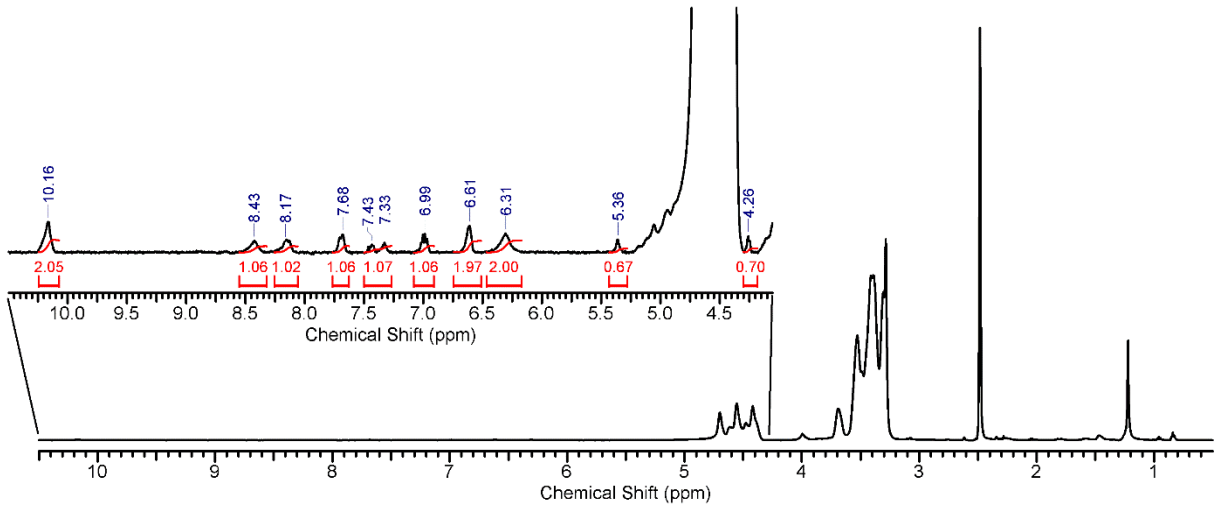

Fig. S4 The ^{1}H NMR spectrum of CF functionalized C18-HbPG in DMSO-*d6*

Morphological characterization of PLGA nanoparticles

The morphology and shape of the nanoparticles were investigated by atomic force microscopy (AFM). Mica with adsorbed polyethylene imine was used as a substrate to get a layer with tightly fixed and separated particles. The samples were imaged in air at room temperature using a XE-100 AFM system (Psia, South Korea). Measurements were carried out in noncontact mode using NSC15 (Micromash, Estonia) cantilevers.

Protein adsorption on alkyl-HbPG covered surface, Quartz Microbalance (QCM) measurements

A QCM200 (Stanford Research Systems, USA) equipment was used to establish C18-HbPG layer on a gold coated quartz crystal surface (5 MHz AT-cut, diameter: 1 inch, sensitivity factor at room temperature: 56.6 Hz × μg^{-1} × cm^2) and study the interaction with bovine serum albumin (BSA). The apparatus consists of a flow-through cell, an oscillator and an analyzer. The flow cell was connected to a peristaltic pump applying a flow rate of 0.2 mL/min. The different solutions were added using a tap system. The experiments were performed at 25 ± 0.1 °C. The gold crystals were cleaned by UV/ozone for 30 min followed by washing with anhydrous ethanol and double distilled water. Immediately before measurements, they were treated again with UV/ozone.

After achieving a stable baseline with continuous flow of a 2 mM NaCl solution in the liquid flow cell, 2.5 mL 2 g/L C18-HbPG solution was added and the peristaltic pump was switched off for 15 min. Subsequently, 1 mL of BSA solution with a concentration of 10 µg/mL and with 2 mM NaCl content was injected. The peristaltic pump was turned off for 10 min so the interactions between the C18-HbPG and the protein molecules can take place.

During measurements, the resonance frequency (f) of the sensor crystal was recorded. The adsorbed mass (Δm) on the sensor surface can be related to changes in resonance frequency (Δf) through the Sauerbrey equation [1]:

$$\Delta f = -C_f \cdot \Delta m \qquad \text{(Eq. S4)}$$

where C_f corresponds to the sensitivity factor for the crystal. These measurements were repeated three times.

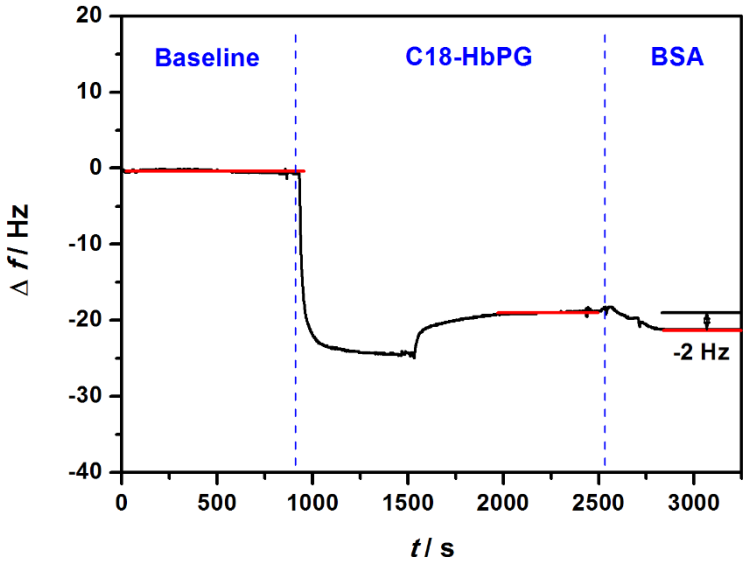

Fig. S5 Changes in the resonance frequency (Δf) of the QCM sensor crystal over time.

Frequency change characterizes the C18-HbPG adsorption on gold crystal sensor and the interactions between the sample and BSA protein solution. According to the large frequency drop C18-HbPG shows a high affinity to gold forming a stable adsorbed layer. The frequency value of BSA adsorption was only -2 Hz, which means a reduced adsorption comparing to the corresponding reference value of -9 Hz [2] detected for gold surface. The measurement demonstrated the effect of surface alkyl-HbPG layer to suppress BSA adsorption significantly.

References

(1) G. Sauerbrey, *Z. Phys.*, **1959**, *155*, 206–222.

(2) Muratsugu, M.; Ohta, F.; Miya, Y.; Hosokawa, T.; Kurosawa, S.; Kamo, N.; Ikeda, H.. *Anal. Chem.*, **1993**. *65(20)*, 2933–2937.