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A bifunctional nanocarrier based on amphiphilic hyperbranched polyglycerol derivatives

Indah N. Kurniasih,[a] Hua Liang,[b] Sumit Kumar,[c] Andreas Mohr,[d] Sunil K. Sharma,[e] Jürgen P. Rabe[b] and Rainer Haag*[a]

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Supporting Information

Synthesis of amphiphilic core-shell modified hPG

Materials

Polyglycerol (PG) **1** (Mn≈5.000 g/mol, Mw/Mn = 1.9) was prepared as described above, using 1,1,1- tris(hydroxylmethyl)propane (TMP) as initiator.[14] Acetone dimethylacetal and 4-toluenesulfonic acid (PTSA) were purchased from Fluka. Lewatit K1131 acidic ionic exchange resin was received from Bayer. Novozyme-435 was purchased from Codexis (Juelich, Germany). The solvent tetrahydrofuran and pyridine (ultra dry quality, water <50 ppm, stored over molecular sieve and p.a. quality, respectively) as well as methanol and chloroform (both p.a. quality) were purchased from Acros. All other reagents used were purchased from Aldrich and used without further purification. Dialysis (benzoylated cellulose tubing, Sigma Aldrich, MWCO 1000) was performed in either methanol or chloroform in a 2 L beaker, while changing the solvent 3 times over a period of 24 hours.

Chemical differential strategy

1. Synthesis of polyglycerolacetal

To a mixture of polyglycerol (24.0 g, 97.296 mmol of diol units) and acetone dimethylacetal (116 mL, 97.56 g, 0.936 mol), dry PTSA (231.41 mg, 1.344 mmol) was added. The reaction was performed under ultrasonication over 3 h at 40 °C. After about 15 minutes a homogeneous solution was obtained. The crude product was diluted in chloroform and then extracted three times with saturated Na_2CO_3 solution to remove the remaining PTSA. The organic phase was dried over $MgSO_4$. Dialysis in chloroform was performed for 24 hours in order to remove traces of remaining acetone dimethylacetal and PTSA. The purified product was dried in vacuo. (Yield 89 %, >95 % conversion by ^{1}H NMR) Polyglycerolacetal was obtained as a pale yellow liquid.

^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) = 0.77 [t, 3H, C<u>H</u>$_3$CH$_2$C(CH$_2$O)$_3$-PG], 1.29 [s, 3H, PG-C(CH$_3$)C<u>H</u>$_3$], 1.35 [s, 3H, PG-C(C<u>H</u>$_3$)CH$_3$], 3.35 - 3.80 [m, <u>PG</u>], 3.86 [m, 1H, C<u>H</u>(H)-PG (1,3-dioxolane)], 3.99 [m, 1H, CH(<u>H</u>)-PG (1,3-dioxolane)], 4.19 [m, 1H, C<u>H</u>-PG (1,3-dioxolane)].
^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) = 25.4 [PG-C(CH$_3$)<u>C</u>H$_3$], 26.8 [PG-C(<u>C</u>H$_3$)CH$_3$], 62.1 [PG], 66.7 [<u>C</u>H(H)-PG (1,3-dioxolane)], 69.4, 71.6, 72.5, 74.7, 78.6, 79.7 [<u>PG</u>-C(CH$_3$)CH$_3$], 109.4 [PG-<u>C</u>(CH$_3$)CH$_3$].

2. Core modification

Core functionalization with biphenyl-4-methyl ether groups (1)

Polyglycerolacetal (5.00 g, 23.25 mmol OH groups), dissolved in dry DMF (18 mL), was partially deprotonated using 60% sodium hydride (23.25 mmol) under vigorous stirring and cooling with an ice bath (0 °C) under an argon atmosphere. After hydrogen formation ceased, a solution of 4-phenylbenzyl chloride (2.63 g, 13.02 mmol, 0.56 e.q) in dry DMF (18 mL) was slowly added to the reaction mixture over 4 hours at room temperature. The suspension was then heated at 60 °C for 18 hours in order to reach quantitative conversion. After cooling down to room temperature, the reaction mixture was quenched with methanol and water. All solvents were removed in vacuo; the residue dissolved in chloroform and extracted three times with water. The organic phase was dried over $MgSO_4$, concentrated in vacuo, and purified by dialysis in chloroform. After purification by dialysis in chloroform, core-functionalized polyglycerolacetals was isolated as a pale yellow highly viscous liquid. (52 % core functionalized, Yield 72 %)

^{1}H NMR ($CDCl_3$, 250 MHz): δ (ppm) = 0.81 [t, 3H, $CH_3CH_2C(CH_2O)_3$-PG], 1.33 [s, 3H, PG-$C(CH_3)CH_3$], 1.38 [s, 3H, PG-$C(CH_3)CH_3$], 3.35 - 3.80 [m, PG], 3.90 [m, 1H, CH(H)-PG (1,3-dioxolane)], 3.99 [m, 1H, CH(H)-PG (1,3-dioxolane)], 4.21 [m, 1H, CH-PG (1,3-dioxolane)], 4.53 [m, PG-$O_{prim}CH_2Ar$], 4.69 [m, PG-$O_{sec}CH_2Ar$], 7.28 - 7.45 [m, 5H, 3 -H, 3'-H, 4'-H, 5-H, 5'-H], 7.54 [m, 4H, 2-H, 2'-H, 6-H, 6'-H].
^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) = 25.5 [PG-$C(CH_3)CH_3$], 26.9 [PG-$C(CH_3)CH_3$], 62.2 [PG], 66.8 [CH(H)-PG (1,3-dioxolane)], 69.4, 70.5, 71.7, 72.1, 72.5, 74.7, 78.8, 79.8 [$PGO_{prim/sec}CH_2Ar$], 109.4 [PG-$C(CH_3)CH_3$], 127.1, 127.3, 128.2 [2-C, 2'-C, 3-C, 5-C, 6-C, 6'-C], 128.8 [3'-C, 4'-C, 5'-C], 137.4, 137.9 [4-C], 140.5, 140.9 [1-C, 1'-C].

Core functionalization with biphenyl ester groups (2)

Polyglycerolacetals (5.54 gram, 25.76 mmol) were dissolved in dry pyridine (20 ml, 19.56 gram, 250 mmol). With the help of dropping funnel a solution of 4-biphenyl carbonyl chloride (1.674 gram, 7.73 mmol) in dry THF (14 ml, 12.03 gram, 167 mmol) was added slowly and let the reaction left 2 hour under vigorous stirring and cooling with an ice bath (0 °C) under an argon atmosphere. The reaction then stirred over 16 hours at room temperature. To the reaction mixture, 20 g of ice was added to quench the reaction and let it stirred for 30 minute. Remaining THF and Pyridine were removed in vacuo and the polymer started to precipitate from water. The residue was dissolved in acetone and purified by dialysis in acetone. After purification by dialysis in acetone, core-functionalized polyglycerolacetals were isolated as a pale yellow highly viscous liquid. (51 % core functionalized (2c), Yield 89 %)

^{1}H NMR ($(CD_3)_2CO$, 250 MHz): δ (ppm) = 0.79 [t, 3H, $CH_3CH_2C(CH_2O)_3$-PG], 1.33 [s, 3H, PG-$C(CH_3)CH_3$], 1.38 [s, 3H, PG-$C(CH_3)CH_3$], 3.35 - 3.80 [m, PG], 3.90 [m, 1H, CH(H)-PG (1,3-dioxolane)], 3.99 [m, 1H, CH(H)-PG (1,3-dioxolane)], 4.21 [m, 1H, CH-PG (1,3-dioxolane)], 4.33 [PG-CH(H)-O-C(O)Ar], 4.48 [PG-CH(H)-O-C(O)Ar], 5.35 [PG-CH-O-C(O)Ar], 7.30 - 7.48 [m, 2-H, 2'-H, 6-H, 6'-H], 7.49 - 7.70 [m, 3'-H, 4'-H, 5'-H], 8.07 [m, 3-H, 5-H].
^{13}C NMR ($(CD_3)_2CO$, 125 MHz): δ (ppm) = 25.5 [PG-$C(CH_3)CH_3$], 26.9 [PG-$C(CH_3)CH_3$], 62.2 [PG], 66.8 [CH(H)-PG (1,3-dioxolane)], 69.4, 70.5, 71.7, 72.1, 72.5, 74.7, 78.8 [$PGO_{prim/sec}C(O)Ar$], 109.4 [PG-$C(CH_3)CH_3$], 127.1, 127.3 [2-C, 2'-C, 6-C, 6'-C], 128.8 [4'-C], 130.3 [3-C, 5-C], 139.9 [1'-C], 145.9 [1-C].

3. Acetal cleavage

General Procedure

To a diluted solution of the acetal-protected core functionalized polyglycerol in methanol, acidic ionic exchange resin Lewatit K1131 was added. The mixture was stirred and heated at reflux for 24 h. The crude product was filtered and the clear methanol solution was concentrated in vacuo. For further purification dialysis in methanol and water were performed for 24 hours.

Polyglycerol with 52% biphenyl-4-methyl ether groups in the core (3)

This product was obtained according to general procedure I with 4.5 gram biphenyl functionalized polyglycerolacetal (*1*), 5 gram ion exchange resin Lewatit K1131, and 20 mL methanol (Yield 72.2%).

^{1}H NMR (CD_3OD, 250 MHz): δ (ppm) = 3.30 - 3.85 [m, PG], 4.41 [m, PG-$O_{prim}CH_2Ar$], 4.57 [m, $PGO_{sec}CH_2Ar$], 7.13 - 7.37 [m, 5H, 3-H, 3'-H, 4'-H, 5-H, 5'-H], 7.46 [m, 4H, 2-H, 2'-H, 6-H, 6'-H].
^{13}C NMR (CD_3OD, 125 MHz): δ (ppm) = 62.8, 64.5, 70.6, 71.2, 72.2, 72.4, 72.8, 74.0, 79.8, 81.4 [PG-$O_{prim/sec}CH_2Ar$], 128.0, 128.5, 129.5 [2-C, 2'-C, 3-C, 5-C, 6-C, 6'-C], 130.0 [3'-C, 4'-C, 5'-C], 138.6, 139.1 [4-C], 141.6, 141.9 [1-C, 1'-C].

Polyglycerol with 51% biphenyl ester in the core (4)

This product was obtained according to general procedure I with 5 gram biphenyl ester functionalized polyglycerolacetal (*2*), 5 gram ion exchange resin Lewatit K1131, and 20 mL methanol (Yield 60%).

^{1}H NMR (D_2O, 250 MHz): δ (ppm) = 3.35 - 3.90 [m, PG], 4.21 [m, 1H, CH-PG (1,3-dioxolane)], 4.33 [PG-CH(H)-O-C(O)Ar], 4.48 [PG-CH(H)-O-C(O)Ar], 5.35 [PG-CH-O-C(O)Ar], 7.30 - 7.78 [m, 2-H, 2'-H, 3'-H, 4'-H, 5'-H, 6-H, 6'-H], 8.05 [m, 3-H, 5-H].
^{13}C NMR (D_2O, 125 MHz): δ (ppm) = 62.8, 64.5, 70.6, 71.7, 72.1, 72.5, 74.7, 78.8, 81.4 [$PGO_{prim/sec}C(O)Ar$], 128.3 [2-C, 2'-C, 6-C, 6'-C], 129.4 [4'-C], 130.3 [3'-C, 5'-C], 131.4 [3-C, 5-C], 139.9 [1'-C], 145.9 [1-C], 167.6 [$PGO_{prim/sec}C(O)Ar$].

Enzyme Reaction

Synthesis of PEG acid (5)

Polyethylene glycol monomethyl ether (1kDa) (5.0 g, 5.0 mmol) was dissolved in acetonitrile (60 mL) followed by addition of succinic anhydride (0.52 g, 5.25 mmol) and catalytic amount of DMAP (0.06 g, 0.5 mmol). The reaction mixture was stirred for 24 h at room temperature and the reaction was monitored on TLC (5% MeOH in $CHCl_3$), the solvent was evaporated and 200 mL of chloroform was added to the crude product and washed with brine solution. The organic layer was dried over a hydrous magnesium sulphate. The drying agent was removed by filtration, and the solvent was evaporated to yield the colorless solid **2** in 90% yield (4.95 g).

^{1}H NMR($CDCl_3$, 400 MHz): δ 2.44-2.51 [m, -OC(O)C$\underline{H}_2$C$\underline{H}_2$C(O)O-], 3.20 [s, PEG -OC$\underline{H}_3$), 3.28-3.66 (m,methylene protons of PEG), and 4.07-4.10 (m, OC(O)C$\underline{H}_2$C$\underline{H}_2$O).
^{13}C NMR($CDCl_3$, 101 MHz): δ28.24, 28.60, 58.44, 63.24,68.47, 69.92, 70.01, 71.37, 77.19, 171.68 and 173.59.

Synthesis of PEG coupled PG (hPG-A)

Polyglycerol 5kDa (200 mg) and PEG acid (1 kDa) (2.97 g) were placed in a round bottom flask. To this mixture was added the enzyme (10% by weight w.r.t. total monomers weight) and the reaction mixture was stirred under vacuum (0.001 mm of Hg) at 60^{o}C. The reaction was allowed to proceed for 18 hours; it was then quenched by adding chloroform and filtering off the enzyme. The crude product was dialyzed in chloroform using membrane (MWCO8000). After the completion of dialysis, the solvent was evaporated under reduced pressure to yield the PEGylated PG.

^{1}H NMR(Methanol-*d*4, 400 MHz): δ0.90 (TMP-C$\underline{H}_3$), 1.29(TMP-C$\underline{H}_2$CH3), 2.59-2.67 (m, OC(O)C$\underline{H}_2$C$\underline{H}_2$C(O)O-),3.36-4.24 (PG and PEG protons).
^{13}C NMR(Methanol-*d*4, 101 MHz): δ7.30, 29.90, 59.14,64.89, 64.97, 66.21, 70.0- 72.23, 72.49, 72.98, 73.98,75.32, 76.93 and 173.94.

Synthesis of PEG coupled PG with 52% biphenyl-4-methyl ether groups in the core (hPG-B)

Polyglycerol with 52% biphenyl-4-methyl ether groups in the core (*3*) (1 g) and PEG acid (1 kDa) (18.4 g) were placed in a round bottom flask. To this mixture was added the enzyme (10% by weight w.r.t. total monomers weight) and the reaction mixture was stirred under vacuum (0.001 mm of Hg) at 60^{o}C. The reaction was allowed to proceed for 18 hours; it was then quenched by adding chloroform and filtering off the enzyme. The crude product was dialyzed in chloroform using membrane (MWCO8000). After the completion of dialysis, the solvent was evaporated under reduced pressure to yield the hPG-B.

^{1}H NMR(Methanol-*d*4, 400 MHz): δ0.90 (TMP-C$\underline{H}_3$), 1.29(TMP-C$\underline{H}_2$CH3), 2.59-2.67 (m, OC(O)C$\underline{H}_2$C$\underline{H}_2$C(O)O-), 3.36-4.24 (PG and PEG protons), 3.30 - 3.85 [m, PG], 4.41 [m, PG-O$_{prim}$C$\underline{H}_2$Ar], 4.57 [m, PGO$_{sec}$C$\underline{H}_2$Ar], 7.13 - 7.37 [m, 5$\underline{H}$, 3-$\underline{H}$, 3'-$\underline{H}$, 4'-$\underline{H}$, 5-$\underline{H}$, 5'-$\underline{H}$], 7.46 [m, 4$\underline{H}$, 2-$\underline{H}$, 2'-$\underline{H}$, 6-$\underline{H}$, 6'-$\underline{H}$].
^{13}C NMR(Methanol-*d*4, 101 MHz): δ7.30, 29.90, 59.14,62.8, 64.5,64.89, 64.97, 66.21, 70.0- 72.23, 72.49, 72.98, 73.98, 75.32, 76.93, 81.4, 128.0, 128.5, 129.5, 130.0, 138.6, 139.1, 141.6, 141.9 and 173.94.

Synthesis of PEG coupled PG with 51% biphenyl ester in the core (hPG-C)

Polyglycerol with 51% biphenyl ester in the core (*4*) (1 g) and PEG acid (1 kDa) (18.4 g) were placed in a round bottom flask. To this mixture was added the enzyme (10% by weight w.r.t. total monomers weight) and the reaction mixture was stirred under vacuum (0.001 mm of Hg) at 60^{o}C. The reaction was allowed to proceed for 18 hours; it was then quenched by adding chloroform and filtering off the enzyme. The crude productwas dialyzed in chloroform using membrane (MWCO8000). After the completion of dialysis, the solvent was evaporated under reduced pressure to yield the hPG-C.

^{1}H NMR(Methanol-*d*4, 400 MHz): δ0.90 (TMP-C$\underline{H}_3$), 1.29(TMP-C$\underline{H}_2$CH3), 2.59-2.67 (m, OC(O)C$\underline{H}_2$C$\underline{H}_2$C(O)O-), 3.36-4.24 (PG and PEG protons), 3.35 - 3.90 [m, PG], 4.21 [m, 1H, C$\underline{H}$-PG (1,3-dioxolane)], 4.33 [PG-C$\underline{H}$(H)-O-C(O)Ar], 4.48 [PG-CH($\underline{H}$)-O-C(O)Ar], 5.35 [PG-C$\underline{H}$-O-C(O)Ar], 7.30 - 7.78 [m, 2-$\underline{H}$, 2'-$\underline{H}$, 3'-$\underline{H}$, 4'-$\underline{H}$, 5'-$\underline{H}$, 6-$\underline{H}$, 6'-$\underline{H}$], 8.05 [m, 3-$\underline{H}$, 5-$\underline{H}$].
^{13}C NMR(Methanol-*d*4, 101 MHz): δ7.30, 29.90, 59.14,62.8, 64.5,64.89, 64.97, 66.21, 70.0- 72.23, 72.49, 72.98, 73.98, 75.32, 76.93, 81.4, 128.3, 129.5, 130.3, 131.4, 139.9, 145.9, 167.6 and 173.94.

Degree of functionalization: The degree of PEGylation was calculated by ^{1}H NMR spectroscopy and was found to be nearly 50% with regard to 1^o hydroxylgroups. The pegylation occurred selectively through the primary hydroxyl groups of polyglycerol and this was established on the basis that no peak was observed in the range of δ5.0 ppm to 6.0 ppm. It has been observed by us and others that upon esterification the protons attached to the secondary carbon of glycerol/polyglycerol shift down field in this above-mentioned.[28]

Methods

Gel permeation chromatography (GPC).

Molecular weight and molecular weight distribution M_w/M_n of polymer were determined using a GPC equipped with agilent 1100 pump, refractive index detector, and PL gel and Suprema columns. The eluent was water with flow rate of 1.0 ml/min. the molecular weights were calibrated with pullulan standards. For in vitro aqueous degradability experiment, the polymer hPG-C was dissolved in buffer solutions of pH 5 (30 mM acetate buffer with 70 mM $NaNO_3$) at a concentration of 10 mg/mL and incubated at 37 °C. Samples were withdrawn at 10 and 27 weeks and analyzed by size exclusion chromatography for molecular weight characteristics.

Dynamic Light Scattering (DLS)

The precise size of hPG nanoparticles in aqueous solution were conducted using Zetasizer Nano ZS analyzer with integrated 4 mW He-Ne laser at wavelength 633 nm with back scattering detector angle 173° (Malvern Instruments Ltd, UK.) at 25 °C. For measurement of the size, an aqueous solution of polymer at concentration of 5 g/L was prepared in Milli-Q water and vigorously stirred for 18 hours at room temperature (25 °C). Solutions were filtered via 0.45 µM polytetrafluoroethylene (PTFE) filters and used for dynamic light scattering measurements. For all experiments disposable UV-transparent cuvettes (Sarstedt AG & Co, Germany) were used.

UV-VIS and Fluorescence Measurements

Absorption spectra were recorded using a Scinco S-3150 UV/VIS spectrophotometer. All measurements were carried out in Milli-Q water in a thermostated UV-cell (1cm). Fluorescence emission spectra were taken with a Jasco FP-6500 spectrofluorimeter equipped with a thermostated cell holder at room temperature (25 °C). For nile red, emission spectra were recorded from 575 to 800 nm after excitation at 550 nm. Both excitation and emission slits were set at 5 nm. For pyrene, emission spectra were recorded from 350 to 600 nm after excitation at 317 nm. Both excitation and emission slits were set at 1 nm. For the release study, the intensity of the emission spectrum of nile red or pyrene was monitored as a function of time at pH 5.0 and physiological pH (7.4) by maintaining the temperature at 37.0 ± 0.1 °C. Data were fitted to the relation $I_t = I_0 \cdot \exp(-k_{obs} \cdot t)$ with I_t as the intensity measured at time t, I_0 as the initial fluorescence intensity. The half-life time is given by simple relationship $t_{1/2} = \ln(2)/k_{obs}$.

Atomic Force Microscopy (AFM)

A droplet of polymer solution in chloroform and in water was deposited on a freshly cleaved mica surface and spun off after 5 seconds. The surface was dried and imaged by AFM in tapping-mode under ambient conditions, with a Nanoscope 3a (Veeco, USA), using silicon cantilevers (Olympus, Japan) with a typical resonance frequency of 300 kHz and a spring constant about 42 Nm^{-1}. Both height and phase images were recorded.[39;40]

Calculating guest encapsulation in polymer

Nile red

From the spectroscopic results in presence of hPG-C (λ_{max}=560 nm, Figure 2a) and hPG-A, hPG-B, and hPG-C (SI, Figure S4a) it can be inferred that the nile red probes preferably reside within the poly(oxyethylene) layer of the dendritic scaffold. In fact, this finding was well-supported by an absorption spectrum in ethyleneglycol (λ_{max}=557 nm, ε = 37.7) and a mixture of 60 wt% of dioxane in water (λ_{max}=559 nm ε = 40.9). Since the polarity of the abovementioned mixture generally decreases with increasing fraction of organic solvent, cosolvent/water mixtures can be taken as model systems to probe the local environment of solubilized dye molecules.[41;42] Our particular interest in the present study was to quantitatively determine the amount of solubilized dye molecules. We calculate the concentration of nile red that encapsulated in the polymer using the calibration curves of nile red in a 60 wt% of dioxane in water.

Pyrene

From the spectroscopic results in presence of polymer the λ_{max} is 338 nm which is comparable with the PG-PEG system from Brooks. Using the same method, we calculate the concentration of pyrene using molar absorptivity (ϵ) = 29500 at λ_{max}= 338 nm.[43] We compared the result using the calibation curves pyrene in a 60 wt% of dioxane in water. The result is comparable.

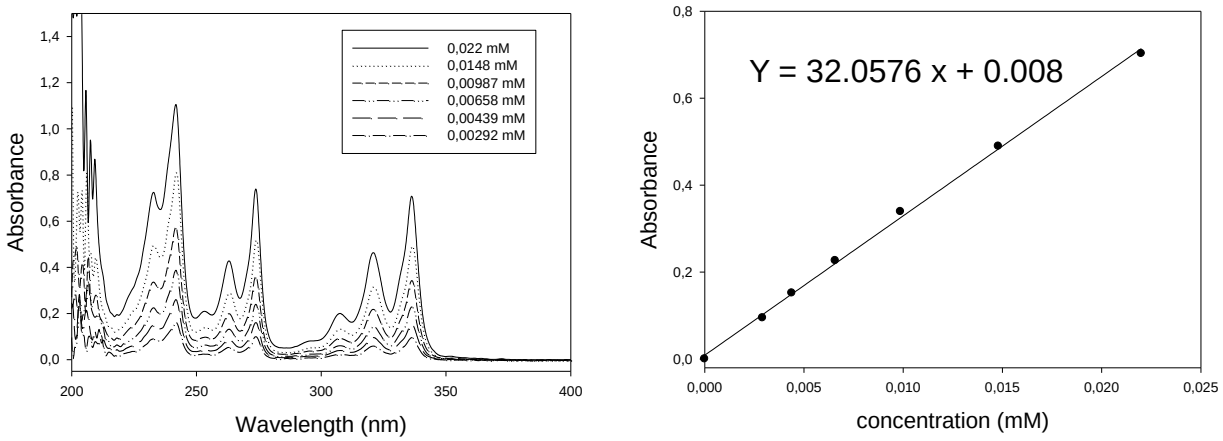


Figure S.1 a) UV-VIS spectroscopy of pyrene in 60 wt% in dioxane and b) Calibration curve of pyrene in 60 wt% dioxane

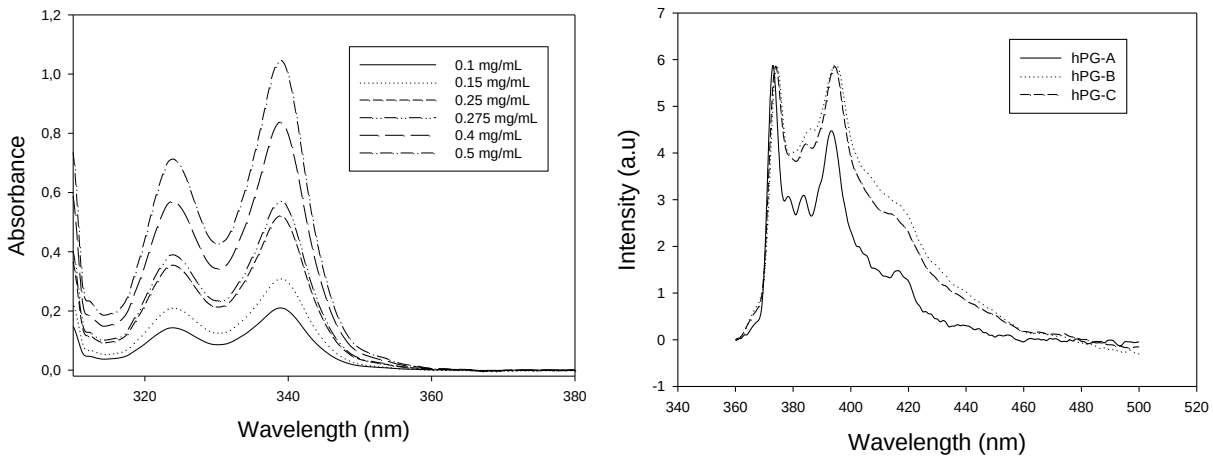


Figure S.2a) UV-VIS spectroscopy of pyrene in hPG-B, b) Fluorescence of pyrene

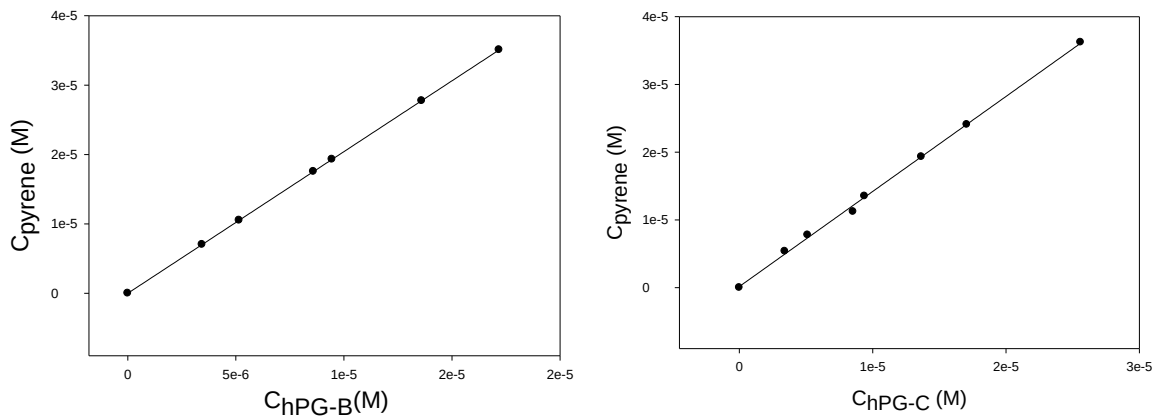


Figure S.3 solubilization of pyrene in polymer a) hPG-B b) hPG-C

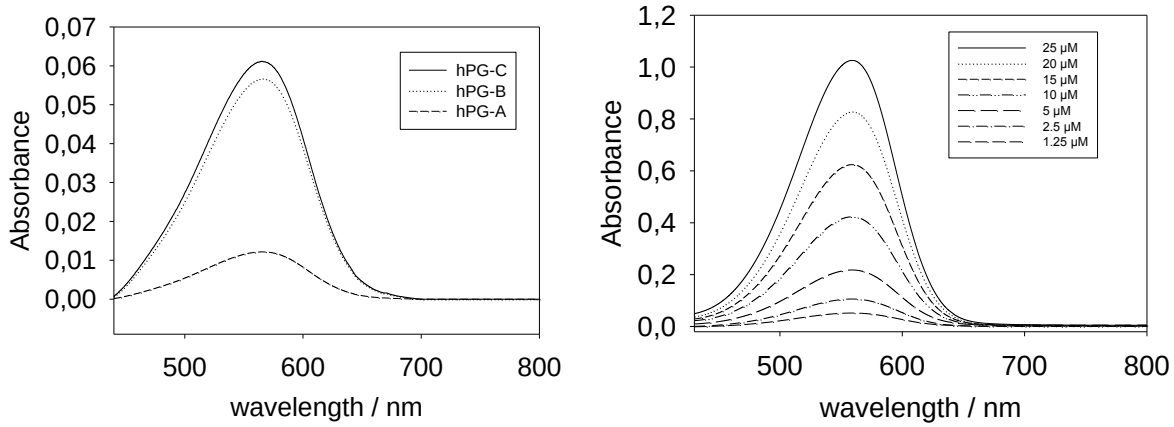
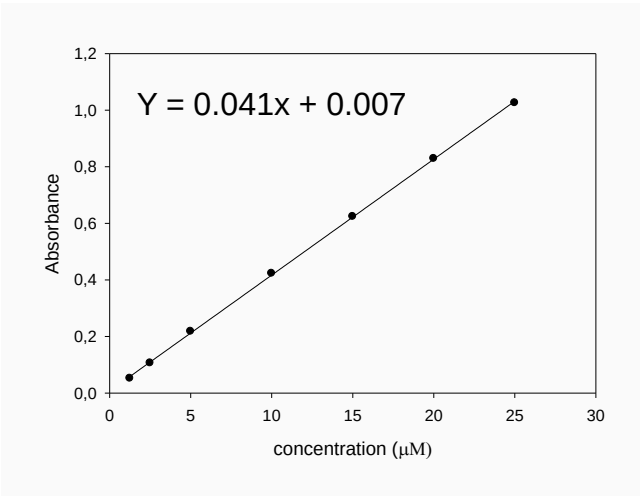


Figure S.4 (a) nile red UV-VIS Spectroscopy for hPG-A, hPG-B, hPG-C, (b) dependence concentration of nile red in 60 wt% of dioxane with λ_{max} = 559



nm

5

Figure S.5 Calibration curves of nile red in 60 wt% of dioxane with λ_{max} = 559 nm

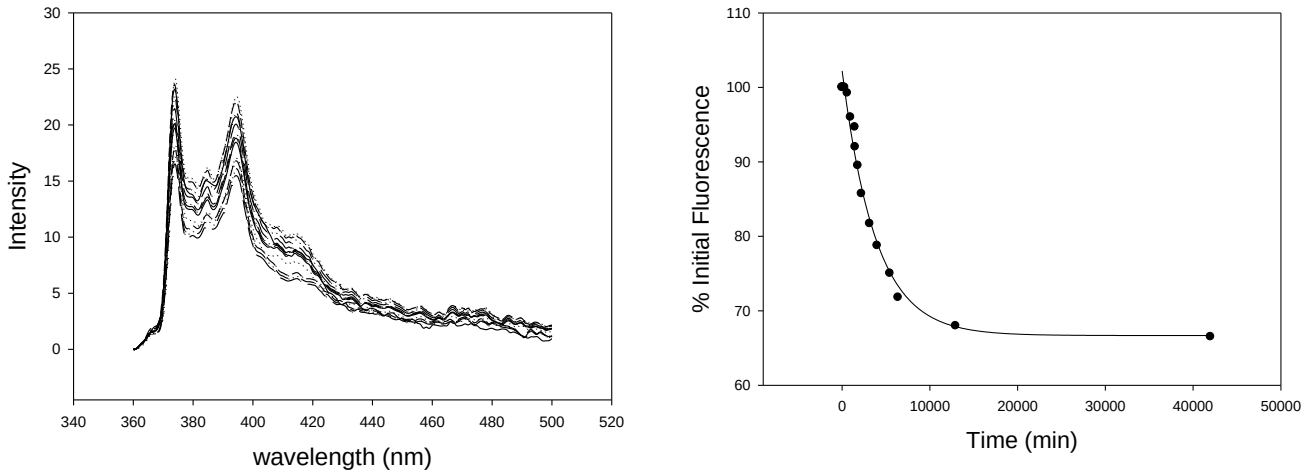


Figure S.6(a) Fluorescence spectroscopy of pyrene for hPG-B enzyme release study, (b) Percentage initial maximum fluorescence of pyrene observed at different time intervals of hPG-B enzyme release at 37 °C

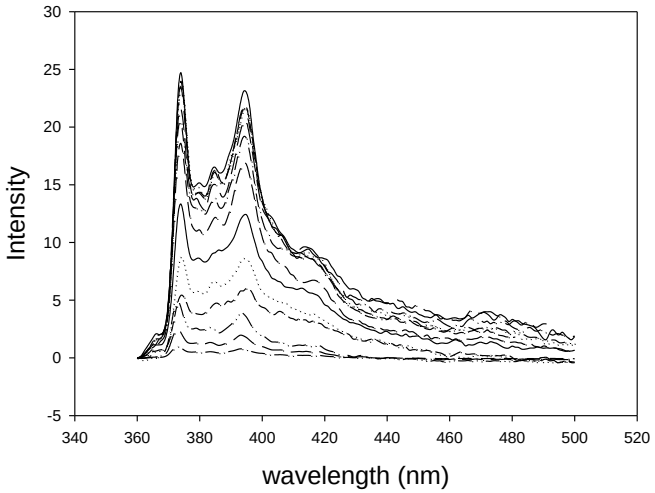


Figure S.7 Fluorescence spectroscopy of pyrene release for hPG-C under enzyme release study

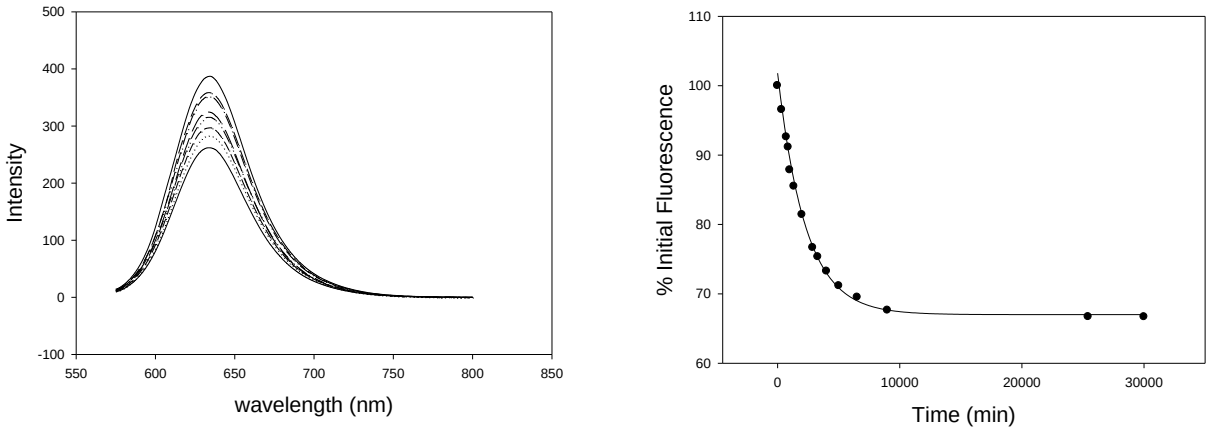


Figure S.8(a) Fluorescence spectroscopy of nile red for hPG-C enzyme release study, (b) Percentage initial maximum fluorescence of nile red observed at different time intervals of hPG-C enzyme release at 37 °C

Table 2. Details of fitted Gaussian peaks of nile red solubilisation in hPG-C

	Peak 1			*Peak 2*			*Peak 3*		
	Center	Height	Area	Center	Height	Area	Center	Height	Area
20 µL	528,7627	0,04265	6,13798	571,82195	0,08255	8,38038	670,1685	0,01738	0,88806
30 µL	535,21649	0,08469	10,81595	574,48048	0,11478	10,13564	671,52191	0,01412	0,68854
40 µL	475,04411	0,02589	6,8453	568,17737	0,12518	14,82351	671,72693	0,03058	1,55545
50 µL	513,19274	0,05844	10,01531	568,83922	0,17069	17,93833	672,67102	0,03309	1,69542
60 µL	533,51498	0,20515	29,80876	574,63135	0,1826	15,61416	673,7025	0,03154	1,76052
80 µL	539,53834	0,47857	69,19932	577,94063	0,18257	12,06725	675,93021	0,0438	2,71366
100 µL	502,97521	0,38608	34,19543	573,58167	0,31833	24,37293	559,62385	0,25008	65,24845
200 µL	511,57266	1,40748	169,22296	577,68747	0,57719	36,20346	634,50303	0,22793	27,74179