**Supplementary Information** 

# INFORMATION-BASED DESIGN OF POLYMERIC DRUG FORMULATION ADDITIVES

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#### **1** Materials

Monomers & monomer synthesis: 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid used as chain transfer agent (CTA, Sigma-Aldrich), 4-dimethylaminopyridine (DMAP, Fluka,  $\geq$ 98.0% (NT)) and *N,N'*-dicyclohexylcarbodiimide (DCC, Iris Biotech GmbH) and mPEG5000 (Sigma-Aldrich). Phthalimide potassium salt (Sigma-Aldrich, 98%), 4-vinylbenzyl chloride (Sigma-Aldrich, 90%), maleic anhydride (MA, Sigma-Aldrich, 99%), isopropyl amine (Sigma-Aldrich,  $\geq$ 99.5%), isobutyl amine (Sigma-Aldrich, 99%). Dichloromethane (DCM) was dried by calcium hydride (Sigma-Aldrich, reagent grade, 95%) and dry dimethylformamide (DMF, Sigma-Aldrich, 99%) was used as received.

Polymer synthesis: 1,1'-azobis(isobutyronitrile) (AIBN, Sigma-Aldrich, 98%) was recrystallized twice from methanol prior to use. 1,4-Dioxane (Sigma Aldrich, 99%), Styrene (Sigma-Aldrich, ReagentPlus®, 99.9%) was filtered over Al<sub>2</sub>O<sub>3</sub> to remove stabilizers and chloroform (CHCl<sub>3</sub>, VWR, ≥99.5% for HPLC) was used as received.

#### 2 Instrumentation and sample analysis procedures

Nuclear magnetic resonance spectroscopy (NMR): <sup>1</sup>H (400, 500 MHz) and <sup>13</sup>C NMR (100, 125 MHz) spectra in CDCl<sub>3</sub> or DCM-d2 were acquired from Bruker spectrometers and calibrated to residual solvent peaks ( $\delta$  7.26 and  $\delta$  77.13 for CDCl<sub>3</sub> and  $\delta$  5.32 for DCM-d2). NMR spectra were analyzed via MestReNova software. All chemical shifts are recorded in ppm ( $\delta$ ) and determined relative to the residual solvent absorption peaks. The multiplicities were explained using the following abbreviations: s for singlet, d for doublet, t for triplet, m for multiplet, bs for broad signal and dd for doublet of doublets.

**Gel permeation chromatography (GPC)** .GPC measurements were conducted using WGE Dr. Bures-Systems (WGE Dr. Bures GmbH & Co. KG, Dallgow Doeberitz, Deutschland) and with the application of refraction index detectors (Knauer RI K-2301). Tetrahydrofuran (THF) was used as eluent and PSS columns (50 - 1000 Å) were used for size exclusion. Polystyrene standards with low dispersity were used for calibration. 2,4-di-tert-butyl-4-methoxy-phenol was used as an internal standard. The measurement was conducted at 60 °C with a flow rate of 1 ml/min.

**Fourier transformation infrared spectroscopy (FT-IR).** FT-IR spectra were recorded on a Bruker Vertex 70v FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) with an evacuable optics bench in a range from 4000-400 cm<sup>-1</sup>. Samples were measured in solid form in ATR-IR modus under vacuum system.

**Ultraviolet–visible spectroscopy (UV-Vis).** UV-Vis spectroscopy was performed using a Shimadzu UV-2501 PC spectrometer (Shimadzu Corp., 604-8511 Kyoto, Japan) using PS-cuvettes with 10 mm path.

**Fluorescence.** Steady-state fluorescence emission spectra and kinetics were recorded on a Synergy MXmicroplate reader (BioTek, Winooski, VT 05404, USA) in black polystyrene 96-well plates.

**Dynamic Light Scattering (DLS)** data were collected on a Malvern Zetasizer nano-ZS (Malvern Instruments Ltd., Worcestershire, UK) equipped with a He-Ne-Laser ( $\lambda = 632.8$  nm) on 400 µL, aliquots of polymer-solutions with and without *m*-THPC incubation. DLS experiments were repeated three times. Hydrodynamic radii RH were calculated using Stokes-Einstein equation (eq 1).

$$R_H = \frac{kT}{6\pi\eta\mu} \qquad (\text{eq 1})$$

with k = 1.38 x 10-23, T = 293 K,  $\eta = \text{Viscosity}$ ,  $\mu = \text{Diffusion coefficient}$ .

### 3. Synthesis of monomers

#### 3.1 Synthesis of p-vinyl benzyl-phthalimide (VBP)



20.4 g Potassium phthalimide (110.2 mmol) and 12 mL 4-Vinylbenzyl chloride (85.2 mmol) was dissolved in 100 mL DMF. The suspension was heated and stirred at 110 °C for 5 h. After cooling, 400 mL water and 200 mL ethyl acetate were added. The aqueous phase was extracted three times with 200 mL ethyl acetate. The united organic phases were washed, dried over MgSO4 and evaporated. The beige residue was dissolved in 50 mL chloroform at 40 °C. The solution was added to 500 mL ice cooled pentane, giving sediment. The crude product was filtered and washed with cold pentane. The beige residue was dried in high vacuum, giving 18.1 g *p*-vinyl benzyl phthalimide (68.0 mmol, 81%).

 $\frac{1}{\text{H NMR}} (300 \text{ MHz, CDC1}_3) \delta \text{ [ppm] 7.68 (dd, } J = 5.5, 3.1 \text{ Hz, 2H}), 7.53 (dd, } J = 5.5, 3.0 \text{ Hz, 2H}), 7.27 (d, } J = 8.3 \text{ Hz, 2H}), 7.23 - 7.20 (m, 2H), 6.53 (dd, } J = 17.6, 10.9 \text{ Hz, 1H}), 5.58 (dd, } J = 17.6, 0.7 \text{ Hz, 1H}), 5.09 (dd, } J = 10.9, 0.7 \text{ Hz, 1H}), 4.69 (s, 2H).$ 

<sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 168.02, 137.17, 136.32, 135.90, 134.01, 132.07, 128.86, 126.50, 123.45, 123.33, 114.17, 41.32.



**Figure S1.** <sup>1</sup>H NMR of *p*-vinyl benzyl phthalimide.



**Figure S2.** <sup>13</sup> C NMR of *p*-vinyl benzyl phthalimide.



1.0 g (3.57 mmol) of PEG 4-cyan-4(phenylcarbonothioylthio)pentanoic acid and 0.08 g (0.71 mmol) of DMAP were added to a solution of PEG (5000 g / mol) 1.79 g (0.35 mmol) in 20 mL of dry DCM. The reaction flask was cooled in an ice bath and the solution were stirred for 10 min, followed by dropwise addition of 0.738 g (3.58 mmol) of DCC in 5 mL of dry DCM. The reaction mixture was stirring for 24h at room temperature. The raw product was filtered and dried. Afterwards, the raw product was purified by precipitating it in cold diethyl ether and dissolved in dichloromethane (3x).1.15 g 82 % yield (97 % conversion).

 $\frac{1}{H \text{ NMR}} (300 \text{ MHz, CDC1}_3) \delta \text{ [ppm]}: 7.91 (s, 2H), 7.58 (s, 1H), 7.38 (s, 2H), 4.27 (d, J = 13.4 \text{ Hz}, 2H), 3.87 (dd, J = 5.6, 4.2 \text{ Hz}, 3H), 3.63 (s, 472H), 3.40 (dd, J = 5.6, 4.2 \text{ Hz}, 3H), 3.37 (s, 3H), 2.70 (s, 3H), 2.43 (s, 1H), 1.93 (s, 3H).$ 



**Figure S3.** <sup>1</sup>H NMR of *m*PEG-CTA.



Figure S4. GPC data for the series of PEG and RAFT-PEG

## 4. Synthesis of polymers

4.1 Synthesis of  $\alpha$ -mPEG- $\omega$ -RAFT-Poly(Styrene)-alt-Poly(Maleic anhydride): P[S-alt-MA]<sub>n</sub>-PEG



0.447 g (4.56 mmol, 20 equiv.) maleic anhydride, 0.474 g (4.56 mmol, 20 equiv.) styrene, 0.0037 g (0.0228 mmol, 0.1 equiv.) AIBN and 1.2 g (0.228 mmol, 1 equiv.) RAFT-PEO (5000 g/mol) was dissolved in 14 mL 1,4-dioxane. After freeze-pump-thaw degassing of the reaction mixture (3x) the reaction mixture was heated at 60°C for 15 h (P[S-*alt*-RMA]<sub>2</sub>-PEG 18% conversion), 24 h (P[S-*alt*-MA]<sub>4</sub>-PEG 28% conversion) and 35 h (P[S-*alt*-RMA]<sub>6</sub>-PEG 39% conversion). Considering the uncertain fractionation occurring during polymer precipitation, the DP of the functional segments was determined by NMR on the stage of the final products. The different polymers were purified by precipitating it in cold diethyl ether and dissolved in dichloromethane (3x).

Polymer	Mn	Ð	DP*
P[S-alt-MA]2-PEG	5800	1.02	2
P[S-alt-MA]4-PEG	6100	1.02	4
P[S-alt-MA]6-PEG	6550	1.08	6



**Figure S5.** GPC data for the series of P[S-*alt*-MA]<sub>n</sub>-PEG (\* functional block).



Scheme S1. Reaction pathway between anhydride structure with primary amines.

Functionalization of P[S-alt-MA]<sub>n</sub>-PEG to P[S-alt-RMA]<sub>n</sub>-PEG



#### • For P[S-alt-RMA]<sub>2</sub>-PEG:

0.038 g (0.036 mmol, 50 equiv.) of the corresponding amine was dissolved in 1 mL of CHCl<sub>3</sub> and cooled in ice-bath. To this solution was added a solution of 0.062 g (0.011 mmol, 1 equiv.) of P[S-*alt*-MA]<sub>2</sub>-PEG that was dissolved in 2 mL of CHCl<sub>3</sub>. The reaction mixture was stirred for 10 min. at room temperature and heated subsequently to 70 °C for 30 min., forcing amide formation and thio-carbonylthio aminolysis to completion. The crude was precipitated 10 times\* in diethyl ether and dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The product was obtained as pink powder.

#### • For P[S-alt-RMA]<sub>4</sub>-PEG:

0.029 g (0.024 mmol, 50 equiv.) of the corresponding amine was dissolved in 1 mL of CHCl<sub>3</sub> and cooled in ice-bath. To this solution was added a solution of 0.057 g (0.009 mmol, 1 equiv.) of P[S-*alt*-MA]<sub>4</sub>-PEG that was dissolved in 2 mL of CHCl<sub>3</sub>. The reaction mixture was stirred for 10 min. at room temperature and heated subsequently to 70 °C for 30 min. where amide formation and thio-carbonylthio aminolysis was forced to completion. The crude was precipitated 10 times\* in diethyl ether and dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The product was obtained as pink powder.

<sup>\*</sup> Commonly the purification of the polymer products by 3-4 times precipitation was sufficient, but careful removal of the excess amines required more cycles.

#### • For P[S-alt-RMA]<sub>6</sub>-PEG:

0.023 g (0.040 mmol, 50 equiv.) of the corresponding amine was dissolved in 1 mL of CHCl<sub>3</sub> and cooled in ice-bath. To this solution was added a solution of 0.054 g (0.008 mmol, 1 equiv.) of P[S*alt*-MA]<sub>6</sub>-PEG that was dissolved in 2 mL of CHCl<sub>3</sub>. The reaction mixture was stirred for 10 min. at room temperature and heated subsequently to 70 °C for 30 min. where amide formation and thio-carbonylthio aminolysis was forced to completion. The crude was precipitated 10 times\* in diethyl ether and dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The product was obtained as pink powder.



R	Mn	Đ	DP
iBu	5872	1.03	2
iPr	5667	1.03	2
Me	5610	1.02	2

P[S-alt-RMA]<sub>4</sub>-PEG

R	Mn	Ð	DP
iBu	6178	1.07	4
iPr	6170	1.08	4
Me	6076	1.07	4
DEC	0 1	• 1	

D	<b>IC</b> <i>a</i>	14 D	лл	A 1	D	FC
I I	S-a	<i>и-</i> г		A 1 (	ς <b>-Γ</b> Ι	ĽG

R	Mn	Ð	DP
iBu	6934	1.06	6
iPr	6785	1.07	6
Me	6637	1.05	6

Figure S6. GPC data of P[S-alt-RMA]n-PEG after aminolysis.

\* Commonly the purification of the polymer products by 3-4 times precipitation was sufficient, but careful removal of the excess amines required more cycles.

4.2 Synthesis of  $\alpha$  – mPEG -  $\omega$ - RAFT – Poly (p-vinylbenzylphthalimide)-alt-Poly(Maleic anhydride):P[VBP-alt-MA]<sub>2</sub>-PEG



0.447 g (4.56 mmol, 20 equiv.) maleic anhydride, 1.2 g (4.56 mmol, 20 equiv.) *p*-vinyl benzyl phthalimide, 0.0037 g (0.0228 mmol, 0.1 equiv.) AIBN and 1.2 g (0.228 mmol, 1 equiv.) RAFT-PEO (5000 g/mol) was dissolved in 14 mL 1,4-dioxane. After freeze-pump-thaw degassing of the reaction mixture (3x) the reaction mixture was heated at 60°C for 15 h (P[VBP-*alt*-MA]<sub>2</sub>-PEG 17% conversion). The polymer was purified by precipitating it in cold diethyl ether and dissolved in dichloromethane (3×).

#### Functionalization P[VBP-alt-MA]2-PEG to P[VBP-alt-RMA]2-PEG



#### • For P[VBP-alt-RMA]<sub>2</sub>-PEG

0.026 g (0.036 mmol, 50 equiv.) of the corresponding amine was dissolved in 1 mL of CHCl<sub>3</sub> and cooled in ice-bath. To this solution was added a solution of 0.048 g (0.007 mmol, 1 equiv.) of P[VBP-*alt*-MA]<sub>2</sub>-PEG that was dissolved in 2 mL of CHCl<sub>3</sub>. The reaction mixture was stirred for 10 min. at room temperature and heated subsequently to 70 °C for 30 min. where amide formation and thio-carbonylthio aminolysis was forced to completion. The crude was precipitated 10 times\* in diethyl ether and dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The product was obtained as pink powder.

Polymer	Mn	Đ	DP*
P[VBP-alt-MA]2-PEG	6200	1.03	2
P[VBP-alt-iBuA]2-PEG	6438	1.08	2
P[VBP-alt-iPrMA]2-PEG	6221	1.02	2
P[VBP-alt-MeMA]2-PEG	6195	1.03	2



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Figure S7. GPC data of P[VBP-alt-MA]<sub>2</sub>-PEG.



# 5. Characterization

## 5.1 Infrared spectroscopy (IR)

# 5.1.1. P[S-alt-RMA]<sub>n</sub>-PEG

• a) P[S-*alt*-RMA]<sub>2</sub>-PEG



Polymer	vs(CO)MA	vas(CO)MA	v (CO)	ν (NH <sub>2</sub> )	v (CN)
P[S-alt-MA]2-PEG	N.O	1778	1734	-	-
P[S-alt-iBuMA]2-PEG	-	-	1734	1651	1601
P[S-alt-iPrMA]2-PEG	-	-	1734	1651	1601
P[S-alt-MeMA]2-PEG	-	-	1734	1651	1601

## • b) P[S-*alt*-RMA]4-PEG



Polymer	vs(CO)MA	vas(CO)MA	v (CO)	ν (NH <sub>2</sub> )	v (CN)
P[S-alt-MA]4-PEG	1851	1778	1730	-	-
P[S-alt-iBuMA]4-PEG	-	-	1730	1644	1605
P[S-alt-iPrMA]4-PEG	_	-	1730	1644	1605
P[S-alt-MeMA]4-PEG	-	-	1730	1644	1605

# c) P[S-alt-RMA]6-PEG



Polymer	vs(CO)ma	vas(CO)MA	v (CO)	v (NH2)	v (CN)
P[S-alt-MA]6-PEG	1852	1776	1726	-	-
P[S-alt-iBuMA]6-PEG	-	-	1726	1641	1558
P[S-alt-iPrMA] <sub>6</sub> -PEG	_	-	1726	1641	1558
P[S-alt-MeMA]6-PEG	-	-	1726	1641	1558

## 5.1.2. P[VBP-alt-RMA]2-PEG

• a) P[VBP-*alt*-RMA]<sub>2</sub>-PEG



Polymer	vs(CO)MA	vas(CO)MA	v (CO)	v (NH2)	ν (CN)
P[VBP-alt-MA]2-PEG	1848	1774	1711	-	-
P[VBP-alt-iBuMA]2-PEG	-	-	1711	1658	1585
P[VBP-alt-iPrMA] <sub>2</sub> -PEG	-	-	1711	1658	1585
P[VBP-alt-MeMA]2-PEG	-	-	1711	1658	1585

# 5.2 Nuclear magnetic resonance (NMR)

• a) P[S-*alt*-MA]<sub>n</sub>-PEG



Figure S8. <sup>1</sup>H NMR spectra of the polymer P[S-alt-MA]<sub>2</sub>-PEG



Figure S9. <sup>1</sup>H NMR spectra of the polymer P[S-*alt*-MA]<sub>4</sub>-PEG



Figure S10. <sup>1</sup>H NMR spectra of the polymer P[S-*alt*-MA]<sub>6</sub>-PEG.

• a.1) P[S-alt-RMA]n-PEG



Figure S11. <sup>1</sup>H NMR spectra of the polymers P[S-*alt*-iBuMA]<sub>n</sub>-PEG, n= 2, 4, 6.



Figure S12. <sup>1</sup>H NMR spectra of the polymers P[S-*alt*-iPrMA]<sub>n</sub>-PEG, n= 2, 4, 6.



**Figure S13.** <sup>1</sup>H NMR spectra of the polymers P[S-*alt*-MeMA]<sub>n</sub>-PEG, n= 2, 4, 6.

# • b) P[VBP-alt-MA]<sub>2</sub>-PEG



**Figure S14.** <sup>1</sup>H NMR of P[VBP-*alt*-RMA]<sub>2</sub>-PEG.

• b.1) P[VBP-alt-RMA]<sub>2</sub>-PEG



Figure S15. <sup>1</sup>H NMR of P[VBP-*alt*-RMA]<sub>2</sub>-PEG.

#### 6. Solubilization of *m*-THPC

*m*-THPC was dissolved in 0.5 mL of EtOH (1.47 µmol *m*-THPC) and added to an aqueous solution of each polymer (0.5 mL, concentrations are summarized in Table S1). The resulting solutions were shaken at 200 rpm at RT for 1 h, followed by freezing in liquid nitrogen and evaporated *in vacuo*. Residues were dissolved in deionized water (0.5 mL) and the drug-loaded nanoparticles were separated from non-encapsulated drug by centrifugation (30 min., 13000 rpm). UV-Vis absorption spectra of each supernatant diluted in ethanol (1: 99 v/v) were recorded on a Shimadzu UV-2501 PC spectrometer (Shimadzu Corp., 604-8511 Kyoto, Japan). Concentration of m-THPC solubilized by each carrier was calculated through comparison of the absorption maximum at 650 nm to a calibration curve of the free drug in EtOH (0.0001, 0.0005, 0.001, 0.005, 0.01 mg/mL). Maximum payload capacity of each carrier is summarized in Table S1. Drug/polymer complexes were analyzed by Dynamic Light Scattering (DLS)(Figures S17-S18).



Figure S16. DLS-analyzed of P[S-alt-iBuMA]<sub>n</sub>-PEG.

• P[S-alt-RMA]n-PEG



**Figure S17.** DLS-analyzed hydrodynamic radii and dispersity of *m*-THPC loaded nanoparticles of a) P[S-*alt*-iBuMA]<sub>n</sub>-PEG, b) P[S-*alt*-iPrMA]<sub>n</sub>-PEG and a) P[S-*alt*-MeMA]<sub>n</sub>-PEG.

• P[VBP-alt-RMA]2-PEG



**Figure S18.** DLS-analyzed hydrodynamic radii and dispersity of *m*-THPC loaded nanoparticles of P[VBP*alt*-RMA]<sub>2</sub>-PEG.

## 7. Drug release studies

For fluorescence-measurement, drug-loaded polymer solutions were dissolved in Millipore water to obtain 280  $\mu$ L of 0.1  $\mu$ M *m*-THPC concentrations that were filled in a 96-well plate. The release was initiated by adding 20  $\mu$ L aqueous BSA-solution (100  $\mu$ M). Additionally, same drug loaded solutions were treated with 20  $\mu$ L aqueous Triton-X solution (0.1%) to get a top fluorescence release-experiment. Top-up experiments were normalized to fluorescence measurement of 0.1  $\mu$ M *m*-THPC solutions with 0.1% Triton-X solution. Samples were exited at 417 nm and emission was recorded at 654 nm every 2 min for 18 h (gain 80, slit 20, sample width 8.00 mm). For each polymer the release was measured 3 times and the arithmetic mean was calculated to give the fluorescence release curve.

Table S1. Solubilization of <i>m</i> -THPC by using standard solubilization experiment with indicated
used polymer, molar mass, and resulting end concentrations of polymer solutions and molar ratio
of drug to polymer.

	sidechain functionalities				conc. n [mM] <sup>a)</sup>	conc.	Upload with m-THPC			Initial release <sup>d)</sup>
polymer						b)	[g] <sup>c)</sup>			
[VBP- <i>alt</i> -RMA]₅ -PEG	VBP	CC	OH	Bu	2	0.29	1: 13.6	0.044	±0.003	11 500
	VBP	СС	OH	Pr	2	0.29	1: 5.8	0.109	±0.021	11 100
	VBP	СС	OH	Me	2	0.29	1: 3.4	0.126	±0.042	9800
[Sty- <i>alt</i> -RMA]ո -PEG					2	1.57	1: 23.9	0.045	±0.004	40 000
	Sty	СООН	Bu	4	1.50	1: 3.2	0.157	±0.015	8 300	
					6	1.53	1: 1.7	0.205	±0.011	6 300
	Sty	СООН		Pr	2	1.50	1: 19.4	0.036	±0.008	25 500
					4	1.51	1: 4.4	0.118	±0.002	13 500
					6	1.52	1: 2.5	0.147	±0.011	12 300
					2	1.52	1: 55.5	0.020	±0.008	37 400
	Sty	СС	OH	Ме	4	1.51	1: 18.4	0.034	±0.012	12 500
					6	1.53	1: 7.2	0.063	±0.022	14 400
Pep-QFFLFFQ	QF	F	L	FF	Q	1.47	1:3.9	0	.26	2200
Pep-QFFVFFQ	QF	F	V	FF	Q	1.47	1:8.9	0	.11	1000
Pep-QFFAFFQ	QF	F	А	FF	Q	1.47	1: 41.1	0	.02	2700

a) polymer concentration used in solubilization experiment, b) mol ratio drug:carrier, c) mass of solubilized drug per g of functional segment [g], d) determined by 1st order derivative at t = 0 min.



Figure S19. Original data sets of the release kinetics from drug loaded polymer-PEG solubilizers. These curves constitute the arithmetic mean release curves. Repetition experiments are presented in the same color.