Supramolecular co-assembly of water soluble nucleobase-containing copolymers: bioinspired synthetic platforms towards new biomimetic materials

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[1] The ^{1}H-NMR analysis showed some signals with very low intensity (noted as*) which correspond to residual traces (~ 1.2 %) of bromo propyl methacrylate. The % of residual bromo propyl methacrylate was calculated by comparing the signal integral of one of the vinyl protons of the bromo propyl methacrylate (noted as I_{1*}, between 5.72 ppm and 5.75 ppm) to the signal integral of one of the vinyl protons of the AdMA (noted as I_1, between 5.82 ppm and 5.9 ppm), by using the equation: % of residual (non-reacted) bromo propyl methacrylate = $\frac{(\frac{I_{1*}}{1})}{(\frac{I_1}{1})} \times 100$.

[2] The ^{3}C-NMR analysis depicted some traces corresponding to hydroquinone residues. Hydroquinone was added to AdMA in order to avoid the degradation of monomer over time. The hydroquinone residues were removed before to perform the polymerisations, by passing AdMA through a silica column.

Fig. S1 ^{1}H-NMR spectrum[1] (A) and ^{13}C-NMR spectrum (B) of 3-(adenin-9-yl)propyl methacrylate (AdMA).[2]

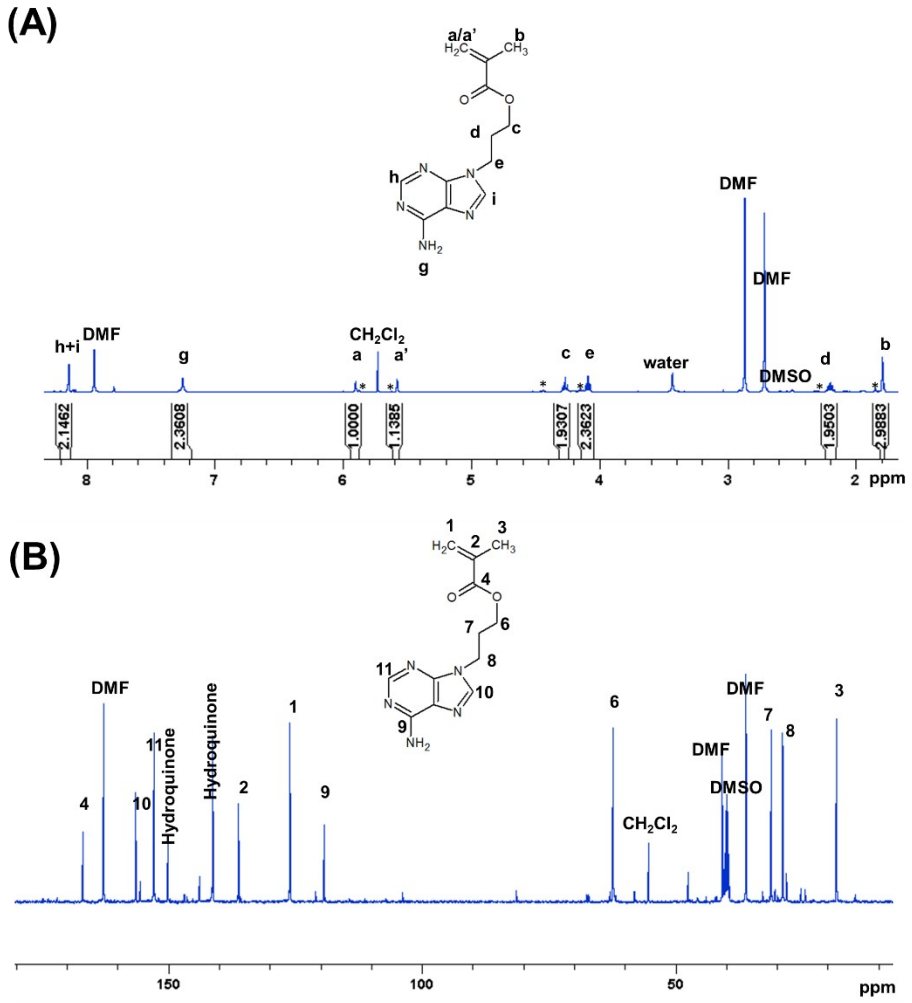
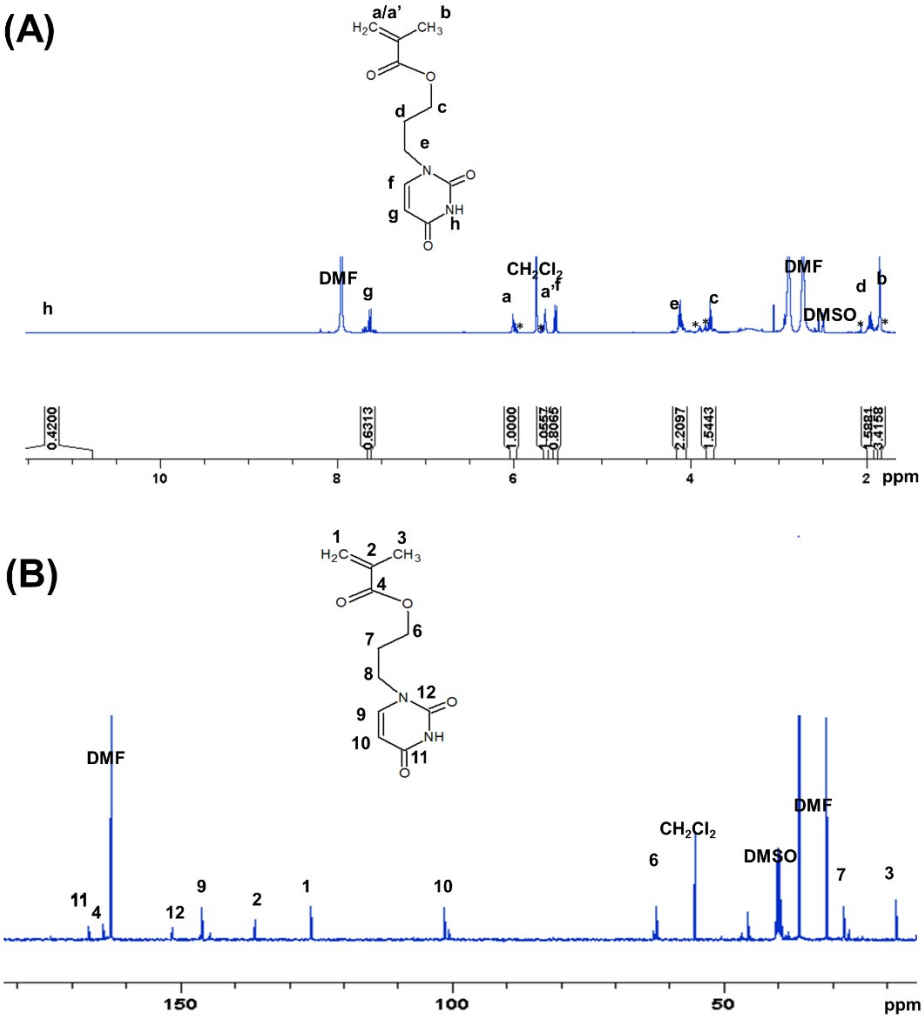
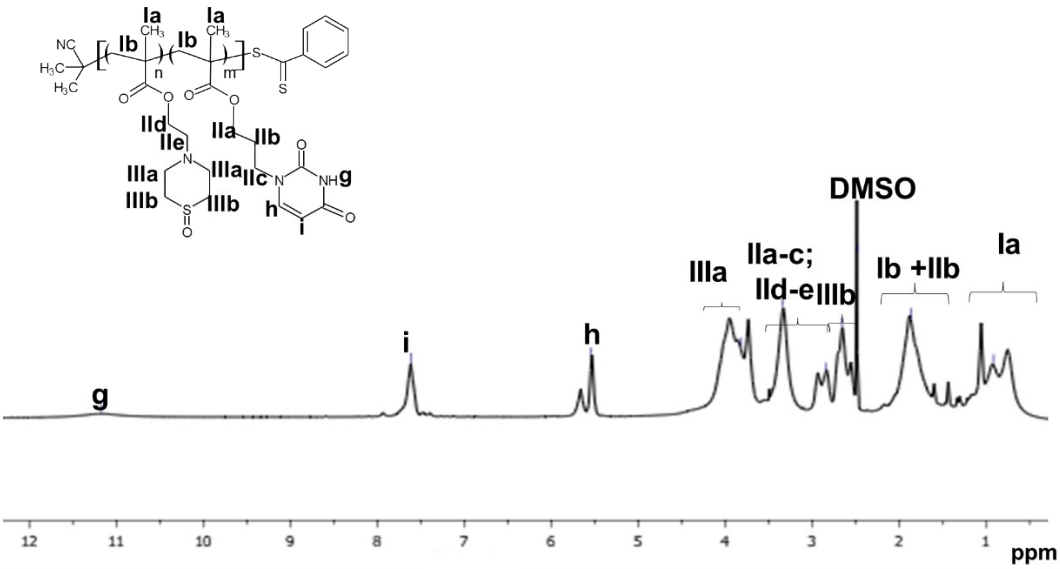


Fig. S2 ^{1}H-NMR spectrum[3] and ^{13}C-NMR spectrum (B) of 3-(uracil-1-yl)propyl methacrylate (UrMA)



[3] The ^{1}H-NMR analysis showed some signals with very low intensity (noted as*) which correspond to residual traces (~ 1 %) of bromo propyl methacrylate. The % of residual bromo propyl methacrylate was calculated by comparing the signal integral of one of the vinyl protons of the bromo propyl methacrylate (noted as I_{1*}, between 5.72 ppm and 5.75 ppm) to the signal integral of one of the vinyl protons of the UrMA (noted as I_1, between 5.9 ppm and 6.01 ppm), by using the equation: % of residual (non-reacted) bromo propyl methacrylate = $\frac{(\frac{I_{1*}}{1})}{(\frac{I_1}{1})} \times 100$.

Fig. S3 ^{1}H-NMR spectrum of Poly((3-(uracil-1-yl) propyl methacrylate) -*stat*-(2-ethyl thiomorpholine oxide methacrylate)) P(UrMA$_n$ -*stat*-THOXMA$_m$)



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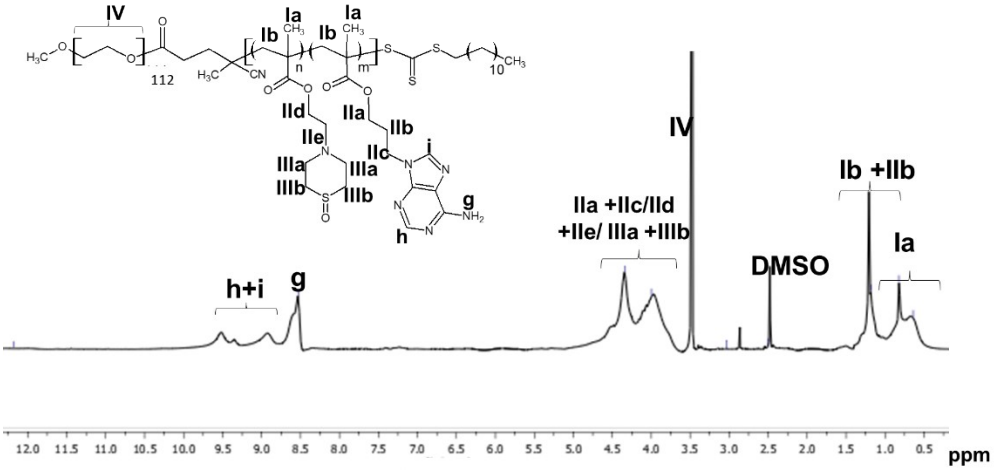


Table S1 Properties co-assembled formulations

Code	Co-assembly formulation	Molar ratio between Ur/Ad units	Aggregation number (N_{agg})[a]	Particle size (D_H)[b]
A	P1+P2	1:1	315	130
B	P3+P2	1:1	194	101
C	P3+P4	1:1	9.95	40
D	P1+P4	1:1	7.8	35
E	P1+P2	0.1:1	44	58
F	P3+P2	0.1:1	47.6	52
G	P3+P4	0.1:1	12	28.5
H	P1+P4	0.1:1	5.2	31
I	P1+P2	10:1	101	81
J	P3+P2	10:1	92	75
K	P3+P4	10:1	33	21
L	P1+P4	10:1	21	29

[a]Evaluated by SLS: for 0.1:1 and 1:1 stoechiometries the measurements were performed at different concentrations (ranging from 1 g/L to 5 g/L), according to the protocol presented in Materials and Methods section; for 10:1 stoechiometry, the measurements were performed at a mass concentration of 5 g/L; [b]Evaluated by DLS, at a concentration of 5 g/L.

Table S2 Molar mass of individual unimers (M_u) vs. apparent molar mass and aggregation number N_{agg} of objects in the starting polymer solutions (evaluated by SLS)

Polymer entry	Molar mass of unimers (M_u, g/mol)*	Apparent molar mass (g/mol, by SLS)	Aggregation number (N_{agg})
P1	12100	20000	1.6
P2	39864	300000	7.5
P3	11550	38000	3.3
P4	8769	100000	11.4

*** M_u determined by multiplying the M_n (determined by end-group analysis from ^{1}H-NMR spectroscopy) by the corresponding M_w/M_n values determined by SEC**

Table S3 Characterisation of nucleobase-containing copolymers

	Polymer name	Experimental DP[a]	Experimental molar composition (%)[b]		Co-monomer conversion (%), by ^{1}H-NMR[c]		M_n (g/mol), by ^{1}H-NMR[d]	M_n (g/mol), by SEC[e]	Dispersity (Đ)[e]	Average number of nucleobases per polymer chain	M_{th} (g/mol)	Theoretical target DP[f]
			Nucleobase	THOXMA	Nucleobase	THOXMA						
P1	P($UrMA_8$-*stat*-$THOXMA_{34}$)	42	20	80	85	92	10,000	10,430	1.21	8	11,840	50
P2	PEG_{112}-*b*-P($AdMA_{30}$-*stat*-$THOXMA_{70}$)	104	30	70	72	88	30,200	32,100	1.32	30	29,400	100
P3	P($UrMA_{22}$-*stat*-$THOXMA_{19}$)	41	53	47	81	90	10,500	11,200	1.1	22	11,950	50
P4	PEG_{112}-*b*-P($AdMA_5$-*stat*-$THOXMA_5$)	10	50	50	70	87	7,900	9,000	1.11	5	7,700	15

[a] Calculated by ^{1}H-NMR performed in DMSO-d6, according to the Eq. S4. and Eq. S9.; [b] Calculated by ^{1}H-NMR performed in DMSO-d6, according to the Eq. S5., Eq. S6., Eq. S12., Eq. S13.; [c] Calculated by ^{1}H-NMR performed in DMSO-d6, according to the Eq. S1. and Eq. S8.; [d] Calculated by ^{1}H-NMR performed in DMSO-d6, according to the Eq. S7. and Eq. S14.; [e] SEC analysis performed in DMF containing 0.1% LiCl and by using PMMA standards. [f]Calculated using the following equation DP_{target} = (([THOXMA]/[chain transfer agent]) x $Conv_{THOXMA}$) + (([AdMA or UrMA]/[chain transfer agent]) x $Conv_{AdMA\ or\ UrMA}$).

Fig. S5 (A) Overall conversion evaluated by ^{1}H-NMR; (B) Representation of ln(C_0/C) versus time for statistical copolymers prepared by RAFT; (C) Evolution of number-average molecular weight M_n and dispersity (Đ) versus global monomer conversion monitored by SEC. Experiments performed for P(UrMA$_n$ -*stat*-THOXMA$_m$)

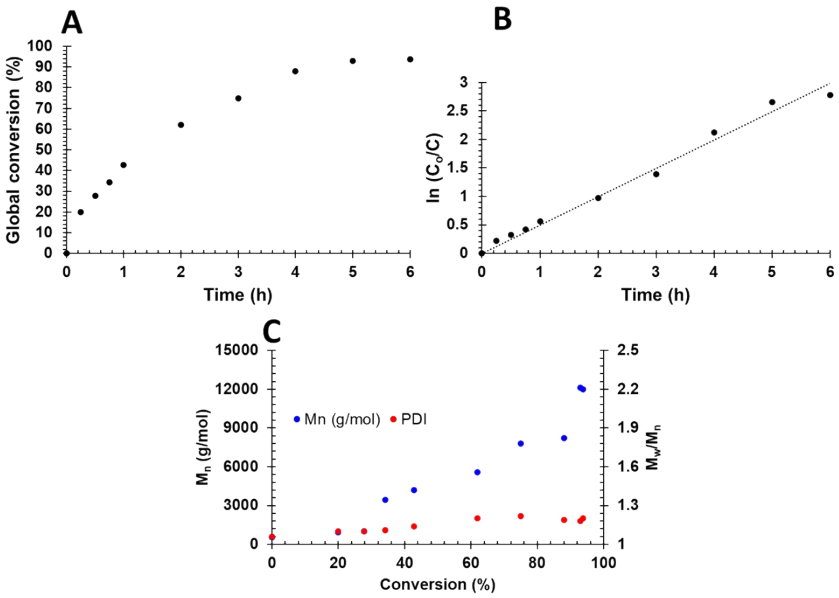


Fig. S6 (A) Overall conversion evaluated by ^{1}H-NMR; (B) Representation of ln(C_o/C) versus time for statistical copolymers prepared by RAFT; (C) Evolution of number-average molecular weight M_n and dispersity (Đ) versus global monomer conversion monitored by SEC. Experiments performed for PEG_{112}-*b*-P($AdMA_n$ -*stat*-$THOXMA_m$)

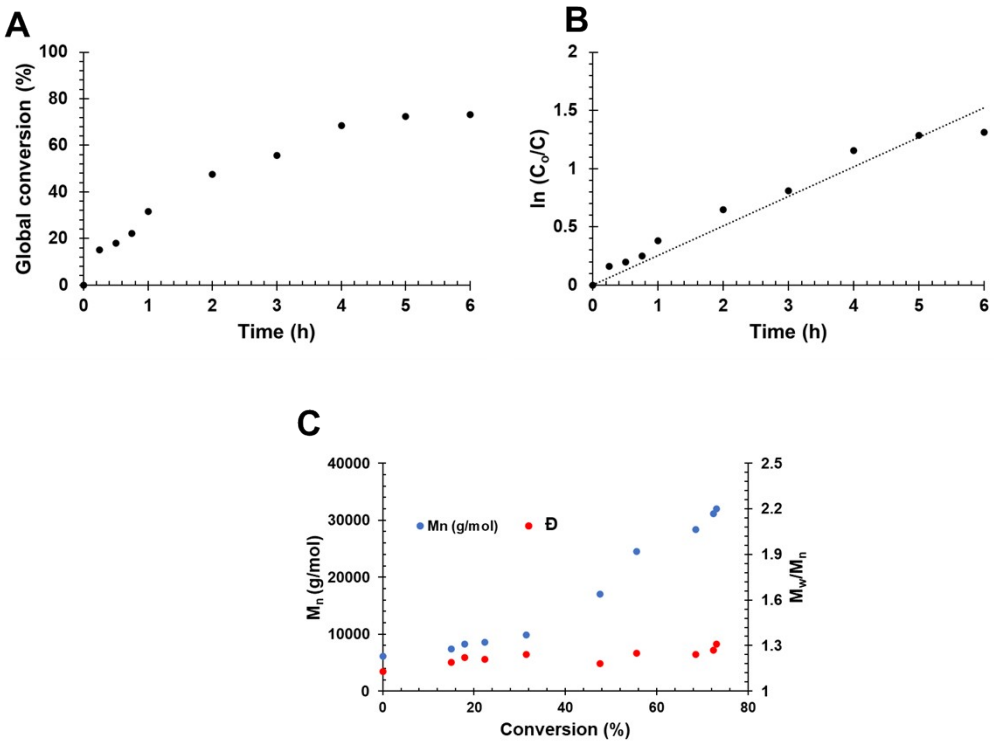


Fig. S7 Urea effect on the co-assembled structures: N_{agg} and D_H evolution data

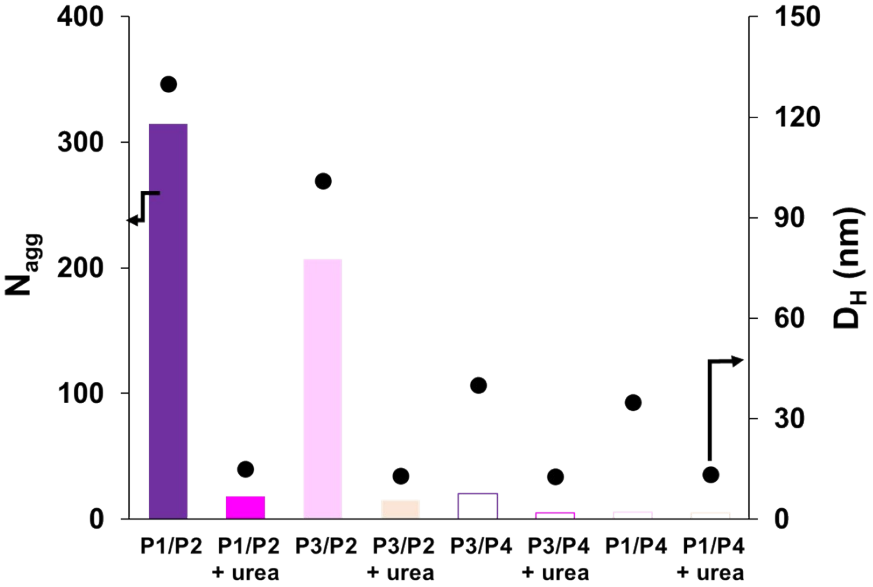


Fig. S8 N_{agg} (determined by SLS) and D_H (determined by DLS) for the co-assembled nucleobase copolymer compositions for a ratio 0.1/1 of Ur/Ad (A) and 10/1 of Ur/Ad (B)

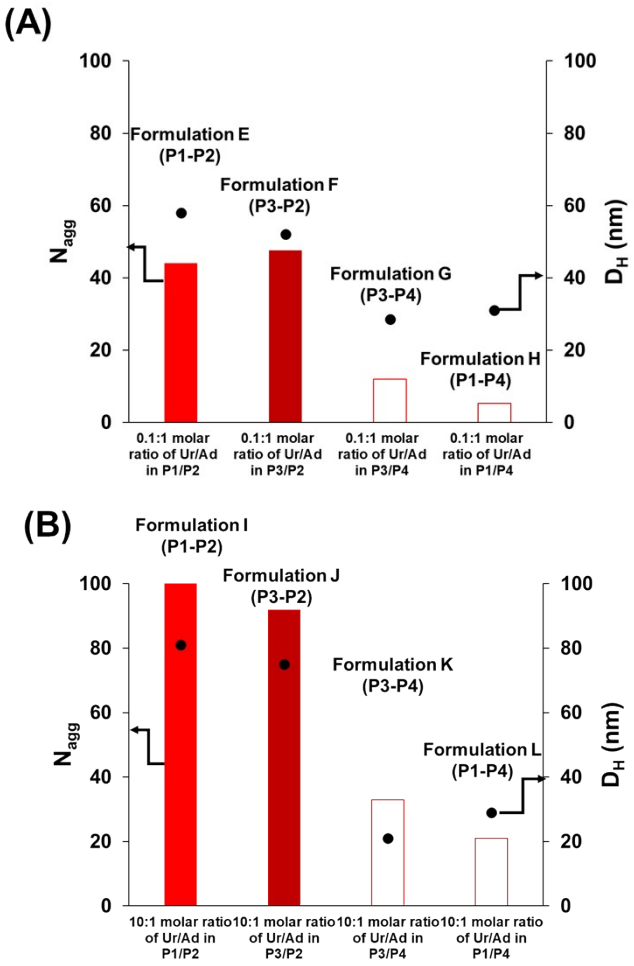
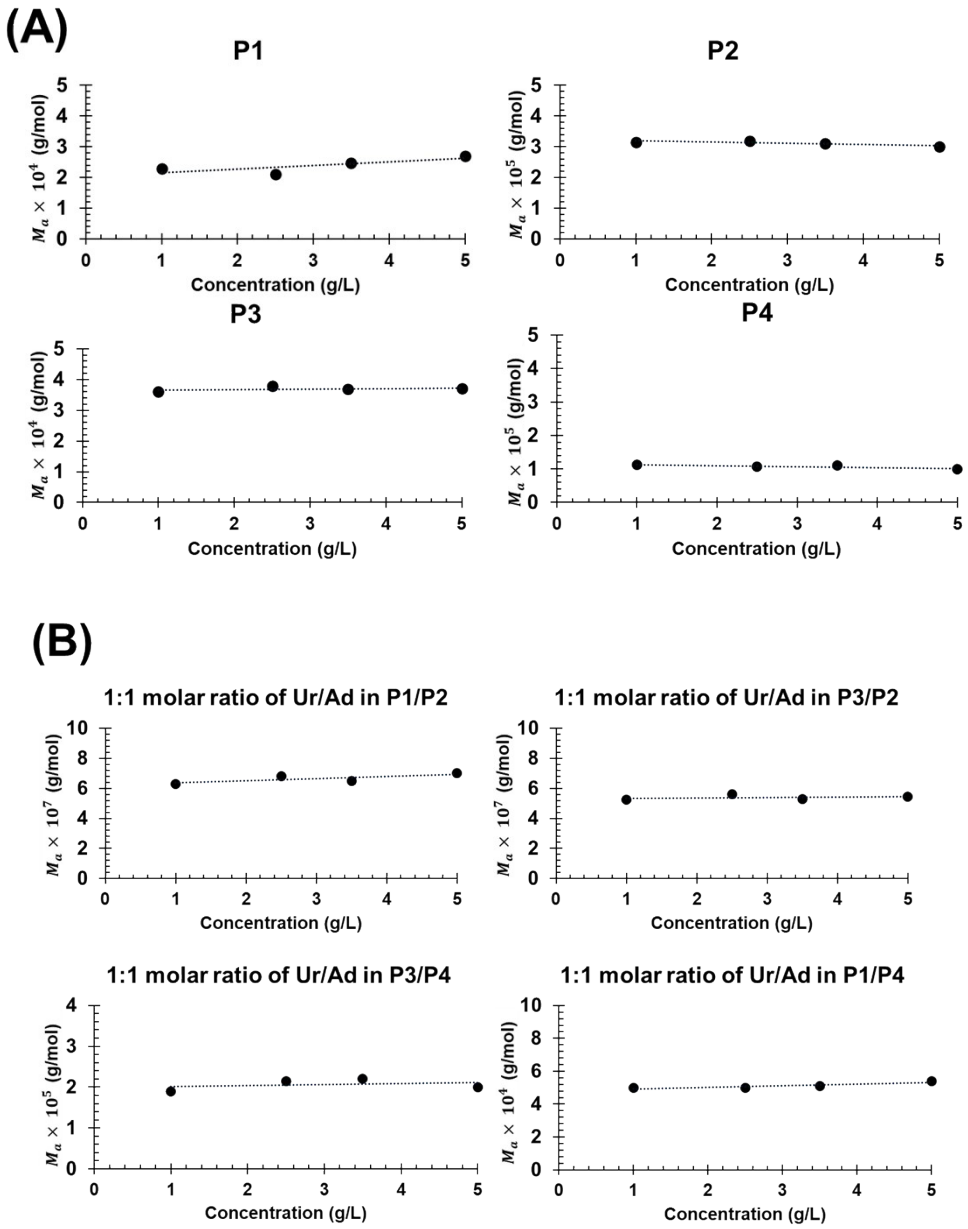


Fig. S9 Evolution of apparent molecular weight (M_a) at different concentrations (g/L) for different formulations (A) individual polymer solutions; (B) formulations using 1:1 molar ratio between uracil and adenine nucleobases; (C) formulations using 0.1:1 molar ratio between uracil and adenine nucleobases.



(C)

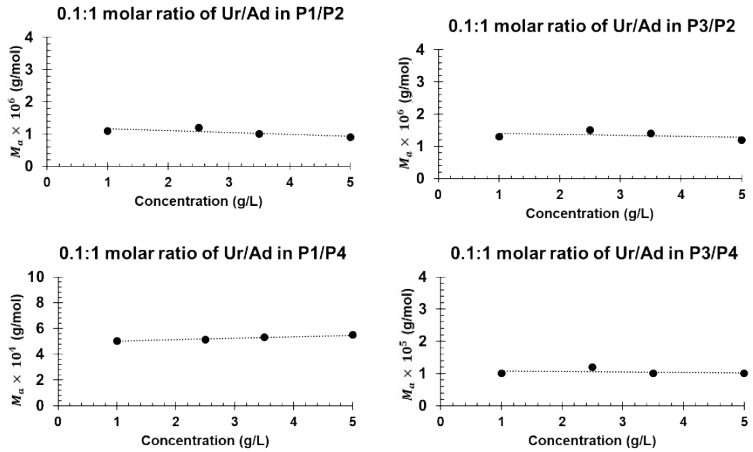
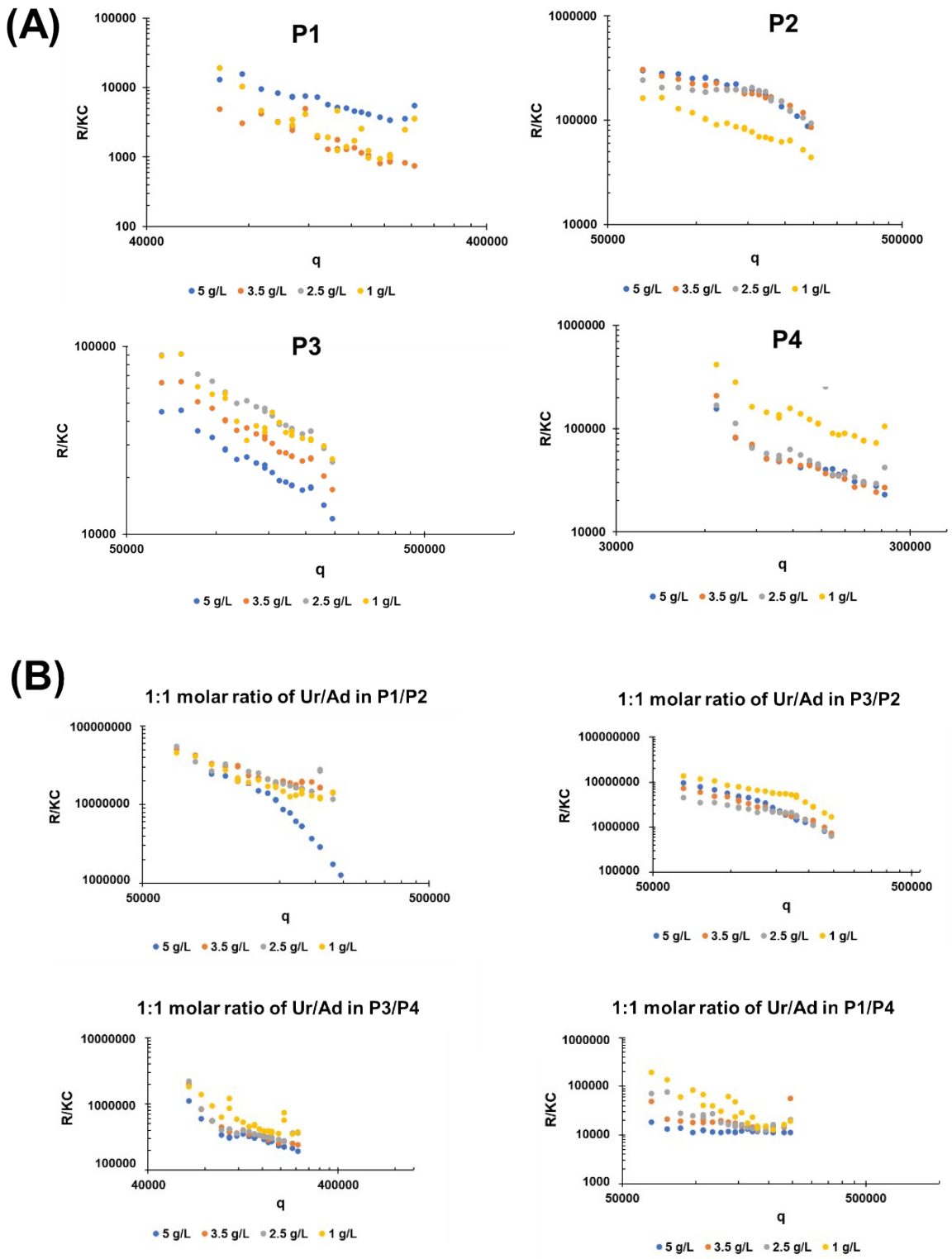
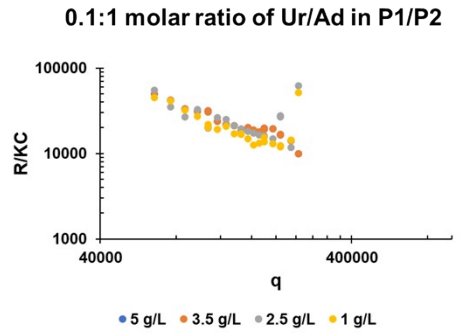

0.1:1 molar ratio of Ur/Ad in P1/P2
$M_a \times 10^6$ (g/mol)
Concentration (g/L)
0.1:1 molar ratio of Ur/Ad in P3/P2
$M_a \times 10^6$ (g/mol)
Concentration (g/L)
0.1:1 molar ratio of Ur/Ad in P1/P4
$M_a \times 10^4$ (g/mol)
Concentration (g/L)
0.1:1 molar ratio of Ur/Ad in P3/P4
$M_a \times 10^5$ (g/mol)
Concentration (g/L)

Fig. S10 Evolution of R/KC over q for different formulations (A) individual polymer solutions; (B) formulations using 1:1 molar ratio between uracil and adenine nucleobases; (C) formulations using 0.1:1 molar ratio between uracil and adenine nucleobases; (D) formulations using 10:1 molar ratio between uracil and adenine nucleobases.



(C)


0.1:1 molar ratio of Ur/Ad in P1/P2
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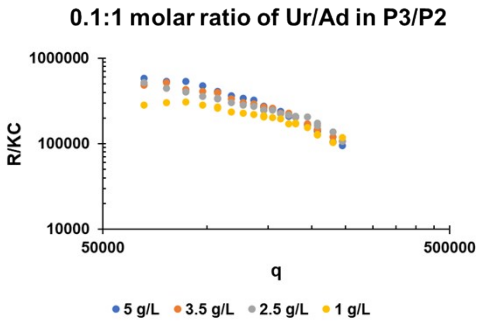
10000
1000
R/KC
40000
400000
q
5 g/L
3.5 g/L
2.5 g/L
1 g/L


0.1:1 molar ratio of Ur/Ad in P3/P2
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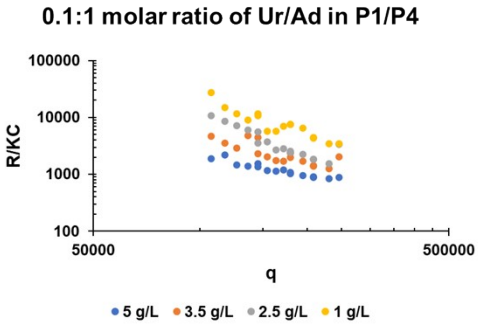
100000
10000
R/KC
50000
500000
q
5 g/L
3.5 g/L
2.5 g/L
1 g/L


0.1:1 molar ratio of Ur/Ad in P1/P4
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R/KC
50000
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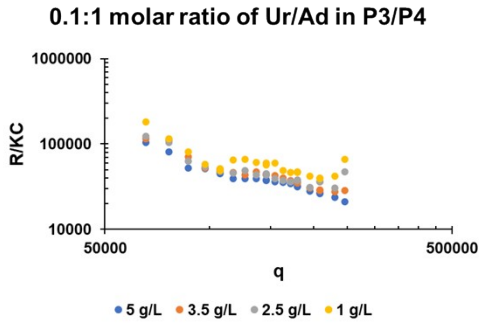
3.5 g/L
2.5 g/L
1 g/L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q
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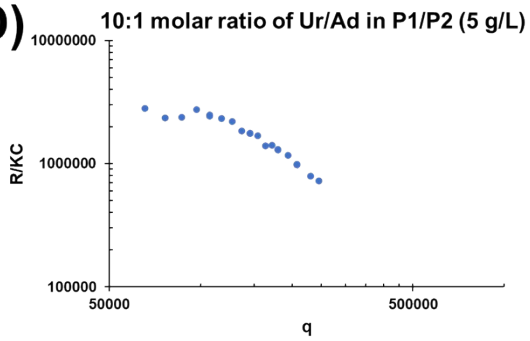
3.5 g/L
2.5 g/L
1 g/L

(D)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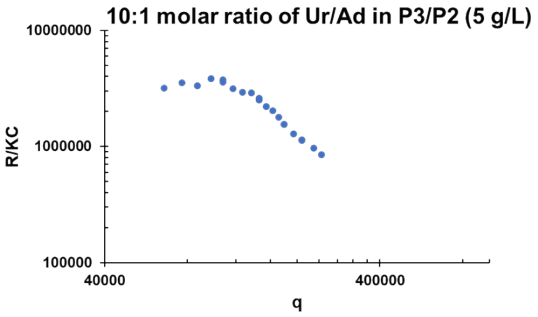
q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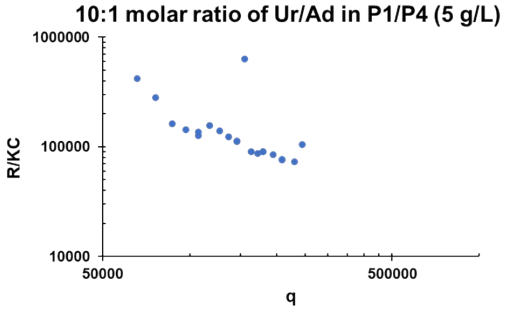
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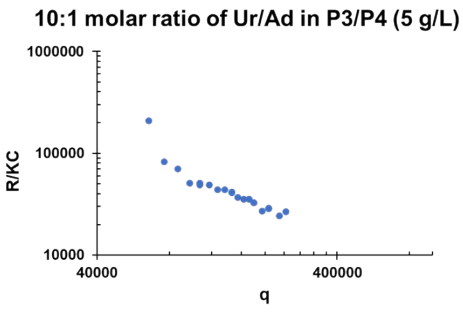
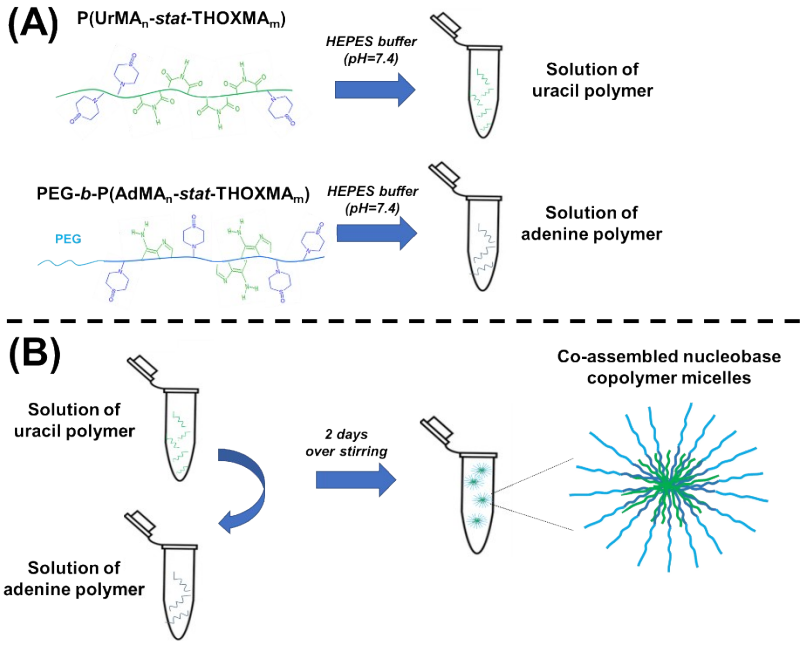

10:1 molar ratio of Ur/Ad in P3/P4 (5 g/L)
1000000
100000
10000
R/KC
40000
400000
q

Scheme S1. (A) Preparation of the solutions of individual uracil- (P1, P3) and adenine-containing (P2, P4) polymers, at physiological pH; (B) Preparation of co-assembled micelles, obtained by adding the solution of uracil-containing polymer (at pH 7.4) to the solution of adenine-containing polymer (at pH 7.4)



Equations used for polymer characterisation

A. Characterisation of P(UrMA$_n$ -stat-THOXMA$_m$) copolymers

- *Conversion of co-monomers*

The conversions of co-monomers were calculated by ^{1}H NMR (Eq. S1.), *via* the comparison of signal integrals of the CPDB (7.4-7.9 ppm) and of the protons of -C=C- double bond of *THOXMA* (6.06-5.68 ppm) and/or UrMA (5.99-5.73 ppm).

$$\text{Conversion (\%)} = \frac{\left(I_{0,\,vinyl\,function}/I_{0,\,CTA}\right) - \left(I_{t,\,vinyl\,function}/I_{t,\,CTA}\right)}{\left(I_{0,\,vinyl\,function}/I_{0,\,CTA}\right)} \times 100 \qquad \text{(Eq. S1.)}$$

Where $I_{0,\,CTA}$ and $I_{t,\,CTA}$ are the values of the integrals of the signal of the aromatic protons of the chain transfer agent (between 7.4 ppm and 7.9 ppm) at t=0 and t respectively; $I_{0,\,vinyl\,function}$ and $I_{t,\,vinyl\,function}$ are the value of the integral of the signal of one of the protons of the vinyl group of methacrylate (5.68 ppm and 6.06 ppm for THOXMA, 5.73 ppm and 5.99 ppm for UrMA), at t=0 and t respectively.

- *Degree of polymerisation*

$DP_{uracil\ copolymer}$ was calculated as a sum between the number of UrMA (noted as $DP_{UrMA\ synthons}$) and THOXMA (noted as DP_{THOXMA}).

The number of UrMA was calculated considering the integral values at 5.56-5.58 ppm and 7.64 ppm that correspond to -C=C- double bond protons of uracil, compared to the two of the protons of CPBD (between 7.88 ppm and 7.9 ppm) as it follows (Eq. S2.):

$$DP_{UrMA} = \left(\frac{(I_1 + I_2)/2}{I_{CTA}/2}\right) \text{ (Eq. S2.)}$$

Where I_1 is the value of the integral of the signal of the proton of double bond of uracil heterocycle (between 7.6 ppm and 7.83 ppm (H_i)), I_2 is the value of the integral of the other signal of the proton of double bond of uracil heterocycle (between 5.31 ppm and 5.71 ppm (H_h)), and I_{CTA} is the value of the integral of the signal of two of the aromatic protons of the chain transfer agent (between 7.88 ppm and 7.9 ppm).

The number of THOXMA were calculated according to Eq. S3.:

$$DP_{THOXMA} = \left(\frac{(I_3 + I_4 + I_5 - 2(I_1 + I_2))/12}{I_{CTA}/2}\right) \text{ (Eq. S3.)}$$

The I_3 and I_4 are the values of the integrals of the proton signals in the field of 3.5 ppm-4.5 ppm and 2.52 ppm- 2.82 ppm respectively that correspond to the protons of thiomorpholine oxide cycle (**III_a and III_b**). I_5 is the value of the integral of the protons of UrMA aliphatic linker (**II_a and II_c**), and the protons of THOXMA aliphatic linker (**II_d and II_e**) in the field of 2.82 ppm-3.48 ppm. To calculate the DP of THOXMA, the sum of integrals corresponding to these signals was assessed. Because this sum includes the integral of the protons of UrMA aliphatic linker, these values (I_1 and I_2) were subtracted in order to correctly evaluate the DP of THOXMA. However, it was impossible to determine exactly the region where the protons of UrMA aliphatic linker were situated, due to signal interferences. Since in UrMA4 protons of aliphatic linker are present in the field of 2.52 ppm- 4.55 ppm, the sum of integrals of uracil double bond protons (that correspond to 2 protons in UrMA) was multiplied by 2, to calculate the integral value of UrMA aliphatic linker protons. Then, this multiplied value (corresponding with the UrMA aliphatic linker) was subtracted and the result was divided by 12 (that correspond to the total of protons of thiomorpholine oxide cycle and THOXMA aliphatic linker).

Then, $DP_{uracil\ copolymer}$ was calculated (Eq. S4.):

$$DP_{uracil\ copolymer} = DP_{UrMA} + DP_{THOXMA} \text{ (Eq. S4.)}$$

- *Experimental molar percentage of co-monomers*

The molar percentage of UrMA and THOXMAwere calculated according to the equations:

$$\% \text{ (molar) of } UrMA = (^{DP_{UrMA}} \times 100) / ^{DP_{uracil\ copolymer}} \quad \text{(Eq. S5.)}$$

$$\% \text{ (molar) of } THOXMA = (^{DP_{THOXMA}} \times 100) / ^{DP_{uracil\ copolymer}} \quad \text{(Eq. S6.)}$$

- *Experimental M_n*

The experimental M_n of P($UrMA_n$-*stat*-$THOXMA_m$) copolymer was calculated as:

M_n = (% (molar) of UrMA × $DP_{\text{uracil copolymer}}$ × $M_{\text{th of UrMA}}$) + (% (molar) of THOXMA × $DP_{\text{uracil copolymer}}$ × $M_{\text{th of THOXMA}}$) + $M_{\text{th,CTA}}$ (Eq. S7.)

Where % (molar) of UrMA was calculated by Eq. S5., % (molar) of THOXMA was calculated by Eq. S6., $DP_{\text{uracil copolymer}}$ was calculated by Eq. S4. $M_{\text{th,CTA}}$ = 221.34 g/mol, $M_{\text{th of THOXMA}}$ = 231 g/mol, $M_{\text{th of UrMA}}$ = 238 g/mol.

B. Characterisation of PEG-*b*-P($AdMA_n$ -stat-$THOXMA_m$) copolymers

- *Conversion of co-monomers*

The conversions of co-monomers were calculated by ^{1}H NMR (Eq. S8.), *via* the comparison of signal integrals of the PEG region (noted as region IV) of the macro-CTA agent (3.48-3.58 ppm) and of the protons of -C=C- double bond of *THOXMA* (6.06-5.68 ppm) and/or AdMA (5.95-5.72 ppm).

$$\text{Conversion (\%)} = \frac{\left(I_{0,\ vinyl\ function}/I_{0,\ macroCTA}\right) - \left(I_{t,\ vinyl\ function}/I_{t,macro\ CTA}\right)}{\left(I_{0,\ vinyl\ function}/I_{0,macro\ CTA}\right)} \times 100 \quad \text{(Eq. S8.)}$$

Where $I_{0,macro\ CTA}$ and $I_{t,\ macroCTA}$ are the values of the integrals of the signal of the methylene protons of the PEG region of the macro-chain transfer agent (between 3.48 ppm and 3.58 ppm) at t=0 and t respectively; $I_{0,\ vinyl\ function}$ and $I_{t,\ vinyl\ function}$ are the value of the integral of the signal of one of the protons of the vinyl group of methacrylate (5.68 ppm and 6.06 ppm for THOXMA, 5.72 ppm and 5.95 ppm for AdMA), at t=0 and t respectively.

- *Degree of polymerisation of the adenine containing block*

$DP_{adenine\ containing\ block}$ was calculated as a sum between the number of AdMA (noted as DP_{AdMA}) and THOXMA (noted as DP_{THOXMA}).

The number of AdMA was calculated considering the integral values at 8.7-9.3 ppm and 9.48-9.56 ppm that correspond to the heterocycle protons of adenine (noted with h and i), compared to the methylene protons of the PEG region (region IV) of the macro-CTA (between 3.48 ppm and 3.58ppm) as it follows (Eq. S9.). The DP of the macro-CTA is equal to 112, so it is assigned to 112 ethylene glycol units. Since 1 unit of ethylene glycol contains 4 protons, in the PEG region (with 112 ethylene glycol units) of macro-CTA we have 4x 112= 448 protons.

$$DP_{AdMA} = \left(\frac{(I_1 + I_2)/2}{I_{macroCTA}/448}\right) \text{ (Eq. S9.)}$$

Where I_1 is the value of the integral of the signal of the first proton of adenine heterocycle (between 8.7 ppm and 9.3 ppm (H_h)), I_2 is the value of the integral of the other signal of the proton of double bond of uracil heterocycle (between 9.48 ppm and 9.56 ppm (H_i)), and $I_{macroCTA}$ is the value of the integral of the proton signals of PEG region the macro-chain transfer agent (between 3.48 ppm and 3.58 ppm).

The number of THOXMA were calculated according to Eq. S10.:

$$DP_{THOXMA} = \left(\frac{(I_3 + I_4 - 2(I_1 + I_2))/12}{I_{macroCTA}/448}\right) \text{ (Eq. S10.)}$$

The I_3 and I_4 are the values of the integrals of the proton signals in the field of 3.7 ppm-4.3 ppm and 4.31 ppm- 4.8 ppm respectively that correspond to the protons of thiomorpholine oxide cycle (**III_a and III_b**), the protons of AdMA aliphatic linker (**II_a and II_c**), and the protons of THOXMA aliphatic linker (**II_d and II_e**). To calculate the DP of THOXMA, the sum of integrals corresponding to these signals was assessed. Because this sum includes the integral of the protons of AdMA aliphatic linker, these values were subtracted in order to correctly evaluate the DP of THOXMA. However, it was impossible to determine exactly the region where the protons of AdMA aliphatic linker were situated, due to signal interferences. Since in AdMA 4 protons of aliphatic linker are present in the field of 3.7 ppm- 4.8 ppm, the sum of integrals of the protons of adenine heterocycle (that correspond to 2 protons in AdMA monomer) was multiplied by 2, to calculate the integral value of AdMA aliphatic linker protons. Then, this multiplied value (corresponding with the AdMA aliphatic linker) was subtracted and the result was divided by 12 (that correspond to the total of protons of thiomorpholine oxide cycle and THOXMA aliphatic linker).

Then, $DP_{adenine\ containing\ block}$ was calculated (Eq. S11.):

$$DP_{\text{adenine containing block}} = DP_{\text{AdMA}} + DP_{\text{THOXMA}} \quad \text{(Eq. S11.)}$$

- *Experimental molar percentage of co-monomers*

The molar percentage of AdMA and THOXMA were calculated according to the equations:

$$\%\ \text{(molar) of } AdMA = (^{DP_{AdMA}} \times 100) / ^{DP_{adenine\ containing\ block}} \quad \text{(Eq. S12.)}$$

$$\%\ \text{(molar) of } THOXMA = (^{DP_{THOXMA}} \times 100) / ^{DP_{\ adenine\ containing\ block}} \quad \text{(Eq. S13.)}$$

- *Experimental M_n*

The experimental M_n of PEG-*b*-P(AdMA$_n$ -stat-THOXMA$_m$) was calculated as:

M_n = (% (molar) of AdMA $\times$ $DP_{\text{adenine containing block}}$ $\times$ $M_{\text{th of AdMA}}$) + (% (molar) of THOXMA $\times$ $DP_{\text{adenine containing block}}$ $\times$ $M_{\text{th of THOXMA}}$) + $M_{\text{n (macro CTA)}}$ (Eq.S14.)

Where % (molar) of AdMA was calculated by Eq. S12., % (molar) of THOXMA was calculated by Eq. S13., $DP_{\text{adenine containing block}}$ was calculated by Eq. S11. $M_{\text{th,CTA}}$ = 5400 g/mol, $M_{\text{th of THOXMA}}$ = 231 g/mol, $M_{\text{th of AdMA}}$ = 261 g/mol.

Equation used for the preparation of co-assembled formulations

The volumes of uracil-containing copolymer solution (noted as V_1) and of adenine-containing copolymer solution (noted as V_2) were calculated according to the following equations (Eq. S15. And Eq. S16):

$$V_1 = \frac{R \times c_2 \times Number_{Ad} \times M_{Ur\ polymer}}{c_1 \times Number_{Ur} \times M_{Ad\ polymer} + R \times c_2 \times Number_{Ad} \times M_{Ur\ polymer}} \quad \text{(Eq. S15.)}$$

Where:

V_1 is the volume (in mL) of the uracil-containing copolymer solution; R is the molar ratio between the molar equivalents of the number of uracil groups and the number of adenine groups; $Number_{Ad}$ is the number of adenine groups in the copolymer; $Number_{Ur}$ is the number of uracil groups in the copolymer; c_1 is the concentration of uracil containing copolymer (in mg/mL); c_2 is the concentration of adenine containing copolymer (in mg/mL); $M_{\text{Ur polymer}}$ is the molecular weight of uracil containing copolymer (calculated by ^{1}H-NMR, according to the Eq. S7.) and $M_{\text{Ad polymer}}$ is the molecular weight of adenine containing copolymer (calculated by ^{1}H-NMR, according to the Eq. S14.).

$$V_2 = V_{total} - V_1 \quad \text{(Eq. S16.)}$$

Where: V_2 is the volume (in mL) of the adenine-containing copolymer solution and V_{total} is the total volume (in mL) of the formulation prepared by using uracil-containing and adenine-containing copolymers.