

## *Supplementary Information*

### **BIOCOMPATIBLE, HYPERBRANCHED NANOCARRIERS FOR THE TRANSPORT AND RELEASE OF COPPER IONS**

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## A. Experimental

**A.1. General.**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Jeol ECX 400 apparatus (400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ ). Calibration was performed using the chloroform peak at 7.26 ppm for  $^1\text{H}$  and 77.0 ppm for  $^{13}\text{C}$ . IR spectra of neat samples were recorded on a Nicolet Avatar 320 FT-IR spectrometer (Thermo Fisher Scientific, Dreieich, Germany). Dialysis was performed with Dialysis tubing benzoylated 10 FT from Sigma-aldrich (molecular weight cut-off 2000 g mol $^{-1}$ ). Ultrafiltration was performed with a 300 mL solvent-resistant stirred cell with regenerated cellulose membranes (molecular weight cut-off 5000 or 10000 g mol $^{-1}$ ), both from Millipore. TLC was performed on Merck aluminium sheets with silica (corn size 60) and fluorescence marker (F254). Flash column chromatography was performed on Merck silica (corn size 60).  $\text{CuSO}_4$  was purchased from Acros Organics Co. Ltd. and  $\text{ZnSO}_4$ ,  $\text{MgSO}_4$ ,  $\text{MnSO}_4$ ,  $\text{CaSO}_4$ ,  $\text{CoSO}_4$ ,  $\text{NiSO}_4$ , Zincon monosodium salt (2-Carboxy-2'-hydroxy-5'-sulfoformazyl-benzene monosodium salt) were purchased from Sigma Aldrich Co. Ltd. at highest purity available ( $\geq 99\%$ ) and used as received. All reagents and solvents were purchased from Acros, Fluka or Aldrich and used as received. Hyperbranched PG was synthesized according to literature.<sup>1</sup>

Water was purified through a filter and ion exchange resin using a Purite device (resistivity 18.2 M $\Omega$ ·cm).

## A.2. Synthesis

### hPG<sub>10k</sub>-(DMEDA)<sub>0.7</sub>

The synthesis of *boc*-DMEDA was described elsewhere.<sup>2</sup> In a sealed tube, PG<sub>10k</sub>-OMs (1.8 g, 13.5 mmol/g, 24.3 mmol OM groups) was dissolved in 5 ml DMF and 3.46 g *boc*-N,N'-dimethylethylene diamine (18.23 mmol, 0.75 equiv.) was added and heated to 120°C for 3 days. After removal of the solvent the crude product was dialyzed in methanol. hPG<sub>10k</sub>-(*boc*-DMEDA)<sub>0.7</sub> was dissolved in DMF:TFA (4:1) and stirred for 3 h. The solvent was evaporated yielding 0.96 g of the slightly yellow product, which was used without further purification.

$^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  = 4.18-3.21 (PG-groups), 3.20-3.14 (-N-CH $_2$ -), 3.10-2.87 (-N-CH $_2$ -), 2.67-2.37 (-N-CH $_3$ ) ppm.

### CMS: hPG<sub>10k</sub>-(DMEDA-PEG<sub>1000</sub>)<sub>0.7</sub>

hPG<sub>10k</sub>-(DMEDA)<sub>0.7</sub> (0.96 g, 1 equiv.) was dissolved in DMF.  $\text{NEt}_3$  (2.1 equiv.) was added and stirred for 1 h. mPEG<sub>1000</sub>-OMs (1.1 equiv.) was added and stirred at room temperature for 5 days. Dialysis of the crude product in methanol yielded 2.3 g hPG<sub>10k</sub>-(DMEDA-PEG<sub>1000</sub>)<sub>0.7</sub>. For the physical-chemical characterization, a mean molar mass of 83 kDa was used (deduced from  $^1\text{H}$ -NMR).

$^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  = 3.67-3.51 (m, PEG and PG), 3.48-3.42 (m, -N-CH $_2$ -) ppm.

IR:  $\tilde{\nu}$  = 3424, 3015, 2873, 2490, 2337, 2320, 1682, 1558, 1540, 1465, 1419, 1342, 1279, 1199, 1172, 1107, 962, 839, 799, 775, 719 cm $^{-1}$ .

### hPG<sub>10k</sub>-(TMEDA)<sub>0.7</sub>

In a sealed tube, PG<sub>10k</sub>-OMs (0.5 g, 6.75 mmol OM groups) was dissolved in 5 ml DMF and *N,N,N'*-trimethylethylene diamine (517 mg, 5.06 mmol, 0.75 equiv.) was added and heated to 120°C for 3 days. After removal of the solvent the crude product was dialyzed in methanol to yield 320 mg of the brown hard paste-like product.

$^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  = 4.41-3.41 (PG groups), 3.40-2.98 (-CH(OH)-CH $_2$ -NMe-CH $_2$ -) 2.87-2.57 (m, PG groups), 2.63-2.48 (m, -N-CH $_2$ -), 2.42-2.10 (-N-CH $_3$ ) ppm.

### hPG<sub>10k</sub>-(TMEDA)<sub>0.7</sub>(N<sub>3</sub>)<sub>0.3</sub>

hPG-(TMEDA)<sub>0.7</sub> (320 mg, 4.05 mmol OM groups) was dissolved in DMF. After addition of NaN<sub>3</sub> (1.32 g, 20.25 mmol, 5 equiv. regarding OM groups), the suspension is heated for 3 days to 60°C. After cooling, the precipitate is filtered off and the filtrate was concentrated. The residue was dialyzed in methanol to yield 235 mg product.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 4.41-3.41 (PG groups), 3.40-2.98 (-CH(OH)-CH<sub>2</sub>-NMe-CH<sub>2</sub>-) 3.87-2.57 (m, PG groups), 2.63-2.48 (m, -N-CH<sub>2</sub>-), 2.42-2.10 (-N-CH<sub>3</sub>) ppm.

### CRS: hPG<sub>10k</sub>-(TMEDA)<sub>0.7</sub>(Triazol-mPEG<sub>5000</sub>)<sub>0.3</sub>

DIPEA (30 mol% per triple bond) was added to 1.0 equiv. of mPEG<sub>5000</sub>-acetylene and 1.1 equiv. of PG-(TMEDA)<sub>0.7</sub>(N<sub>3</sub>)<sub>0.3</sub> per triple bond dissolved in THF (PEG<sub>5000</sub> was used to compensate that only 30% of the hPG surface can be modified, instead of 70% in case of CMS). After the mixture had been stirred for 5 min, 30 mol% per triple bond of sodium ascorbate was added, followed by 10 mol% of CuSO<sub>4</sub>·5H<sub>2</sub>O per triple bond. (A stock solution of sodium ascorbate and CuSO<sub>4</sub>·5H<sub>2</sub>O in water was prepared in concentration 100 mg/mL). THF/H<sub>2</sub>O ratio was 1/1 (v/v). The heterogeneous mixture was stirred vigorously for 5.5 days. The precipitate was removed by centrifugation for 2 h at 11,000 rpm. The product was ultrafiltrated in H<sub>2</sub>O/MeOH = 1:1 + TFA at pH 3 (MWCO = 10000 g mol<sup>-1</sup>). Afterwards, the product was ultrafiltrated in H<sub>2</sub>O/MeOH with NaOH (pH 10, MWCO 5000 g mol<sup>-1</sup>). For the physical-chemical characterization, a mean molar mass of 224 kDa was used (deduced from <sup>1</sup>H-NMR).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 4.20-3.35 (PG groups), 3.01-2.95 (-CH(OH)-CH<sub>2</sub>-NMe-CH<sub>2</sub>-) 2.89-2.77 (m, PG groups), 2.75-2.41 (m, -N-CH<sub>2</sub>-), 2.22-2.08 (-N-CH<sub>3</sub>) ppm.

IR:  $\tilde{\nu}$  = 2946, 2880, 2740, 2694, 1960, 1466, 1455, 1360, 1340, 1279, 1240, 1145, 1097, 958, 948, 840 cm<sup>-1</sup>.

**Table 1. Summary of physico-chemical properties of CS, CMS, CRS.**

	CS	CMS	CRS
M <sub>w</sub> (g/mol)	22,000	83,000	224,000
Grafting ratio	70%	49%	49%
No. of theoretical complexation sites	47	33	33

**A.3. Cu-loading by UV/Vis spectroscopy.** UV/Vis spectra were recorded with Scinco S-3150 (range: 190-1100 nm, resolution 1024 points) in fast mode. Calibration was performed at 360.85 and 453.55 nm with holmium oxide glass. The spectra were recorded at room temperature. To assess the maximum metal cargo capacity of the polymers, a solution of the nanocarriers (80  $\mu$ M) in water were prepared in 2 mL. Successive micro-additions (0.5 - 2  $\mu$ L) of concentrated CuSO<sub>4</sub> solutions (0.4 M and 2 M) were added every 6 min to yield a distinct Cu/nanocarriers molar ratio within the range of 0-125. Absorbance versus the molar Cu/nanocarriers molar ratio was then fitted by a simple model based on the hypothesis that each polymer contains N complexing sites and that the binding constant of Cu(II) is the same for each site, whatever the number of loaded sites may be.

**A.4. Cu-loading kinetics by UV/Vis spectroscopy.** Cu-loading kinetic was monitored by UV/Vis spectroscopy by adding 4  $\mu$ L of CuSO<sub>4</sub> (2 M) into a 2 mL solution of nanocarriers (80  $\mu$ M) (Cu/nanocarriers ratio = 50:1). Measurements were performed at room temperature. The variation of DO with time was adjusted to an exponential function (see below) leading to the characteristic time  $\square$  (given in Table 1).

**A.5. Zincon titration procedure.** Solutions of nanocarriers (6.2  $\mu$ M) with increasing amount of Zn(II) or Cu(II) were prepared (M(II)/nanocarriers ratio from 0 to 150 :1) into 2mL. These samples were then filtrated with centrifugal filters (molecular weight cut-off 3000 g mol<sup>-1</sup>) (Ultracel®-3K - Millipore Ireland Ltd). 160  $\mu$ L of filtrate were mixed with 30  $\mu$ L Zincon (2.8 mM) and completed at 2 mL with B.R. buffer

pH 9. The analysis was performed by UV-Vis spectroscopy to assess free metal ions concentrations. (Calibration curves perform with Zincon (42  $\mu\text{M}$ ) and concentrations of Cu(II) and Zn(II) from 0 to 40  $\mu\text{M}$ ).

**A.6. pH-triggered release study.** Solutions of Cu-loaded nanocarriers were prepared with Cu/nanocarriers ratio = 50:1 by adding an appropriate amount of a  $\text{CuSO}_4$  stock solution (2 M) to a solution of nanocarriers (80  $\mu\text{M}$ ) dissolved in water. Then supplemental non-loaded Cu was removed through dialysis (molecular weight cut-off 2000 g  $\text{mol}^{-1}$ ) in water. The dialyzed product was then dried under vacuum conditions and redissolved into 7 aliquots of 80  $\mu\text{M}$  (pH 2 to 8) in Britton-Robinson Buffer (40 mM  $\text{H}_3\text{BO}_4$ , 40 mM  $\text{H}_3\text{PO}_4$ , 40 mM  $\text{CH}_3\text{COOH}$ ) with 100 mM NaCl. The solutions were incubated for 2 h at 37 °C and UV/Vis spectroscopy measurements were also performed at 37 °C.

**A.7. pH-triggered release kinetic.** Cu pH-triggered release kinetic was monitored by UV/Vis spectroscopy by adding 10  $\mu\text{L}$  of HCl (2 M) into a 2 mL solution of nanocarriers (80  $\mu\text{M}$ ) (Cu/nanocarriers ratio = 50:1). Measurements were performed at 37 °C.

**A.8. DLS and Zeta potential** Dynamic light scattering (DLS) measurements and Zeta potential were conducted using a Zetasizer Nano-ZS (Malvern Instruments, Ltd, UK) with integrated 4 mW He-Ne laser,  $\lambda$  = 633 nm. Nanocarriers were investigated with and without pre-incubation with  $\text{CuSO}_4$  (Cu/nanocarriers ratio = 50:1). The polymer concentration was 80  $\mu\text{M}$  and samples were all filtered with a 20 nm filter unit except for CRS, filtered at 400 nm for aggregates reasons. The correlation function was analyzed *via* the general purpose method (NNLS) to obtain the distribution of diffusion coefficients of the solutes. The apparent equivalent hydrodynamic diameter (d) was then determined using the Stokes–Einstein equation. Mean diameter values were obtained from three different runs of the number plot. Standard deviations were evaluated from diameter distribution. For Zeta potential measurements, Doppler anemometry technique was used whereby electric field was applied across the sample solution. All measurements were carried out at 25 °C using folded capillary cells (DTS 1060).

### **A.9. Metal ions competition**

Solutions of Cu-loaded nanocarriers were prepared with Cu/CS ratio = 20:1 by adding an appropriate amount of a  $\text{CuSO}_4$  stock solution (2 M) to a solution of CS (6.2 $\mu\text{M}$ ) dissolved in water. Then competitor metal ions (Mg, Ca, Mn, Co, Ni, Zn) were added such as M(II)/CS ratio = 100:1 and let to incubate during 24 h at 37 °C. UV-Vis measurements were performed at 37 °C.

### **A.10. Biological studies**

**Cell Culture.** A human neuroblastoma SH-SY5Y cell line were cultured as described previously ([www.lgcpromochem-atcc.com](http://www.lgcpromochem-atcc.com)). Cells at a density of 60,000 cells/well in 96-well plates were used at the time of the experiment. Stock solutions of nanoparticles with Cu were prepared in Milli-Q water by thorough mixing.

For toxicity assays, SH-SY5Y-cells were incubated for 24 h with different concentrations of Cu-saturated and unloaded nanoparticles ranging from 6 nM to 100  $\mu\text{M}$ .

**MTT Assays.** MTT assays were purchased from Promega (Mannheim, Germany) and performed in 96-well plates. This assay is based on tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) that is taken up into cells and reduced to yield a purple formazan product, which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of cells was normalized, and cells were cultured in 96-well plates, incubated for 48 h, and washed three times in 1x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM  $\text{Na}_2\text{HPO}_4$ , and 2 mM  $\text{KH}_2\text{PO}_4$ ) followed by treatment with OptiMEM medium (Invitrogen) supplemented with individual carrier in the absence (control) or presence of Cu for 24 h. The volume of tissue culture medium in each well was 100  $\mu\text{L}$ , to which 20  $\mu\text{L}$  of CellTiter 96 Aqueous One Solution Reagent was added. Plates were incubated for 3 h at 37 °C in a humidified, 5%  $\text{CO}_2$  atmosphere. Then the absorbance was measured at 570 nm.

### A.11. Fit equations

**UV-Vis Cu-loading.** For a system  $V + M \rightleftharpoons VM$  (K), with V: vacant site in nanocarriers, M: free metal ion and VM: complex nanocarriers-Metal. We assumed the two following hypothesis: all the complexation sites are equivalents and the adsorption of a metal ion does not modify the further complexation constants.

After resolution of this system, we found  $\alpha = \frac{\rho + \gamma - \sqrt{(\rho + \gamma)^2 - 4\rho}}{2}$

where  $OD_{Cubind} = \alpha OD_{\infty}$ ; n the number of vacant sites in a nanocarrier;  $\rho = \frac{[M]_0}{n \cdot [CS]_0}$  ;  $\alpha = \frac{[VM]}{[V]_0}$  ;  $\beta = \frac{[VM]}{[M]_0}$  ;  $\gamma = 1 + \frac{1}{K[V]_0}$  ;  $\frac{\alpha}{\beta} = \rho$ .

#### Back titration $Cu_{free}$ concentration.

The back titration fit calculation is based on same hypothesizes than above and calculated as below:  $[M] = n \cdot [CS]_0 (\rho - \alpha)$  with  $\alpha = \frac{\rho + \gamma - \sqrt{(\rho + \gamma)^2 - 4\rho}}{2}$ .

#### Intake and outtake kinetics.

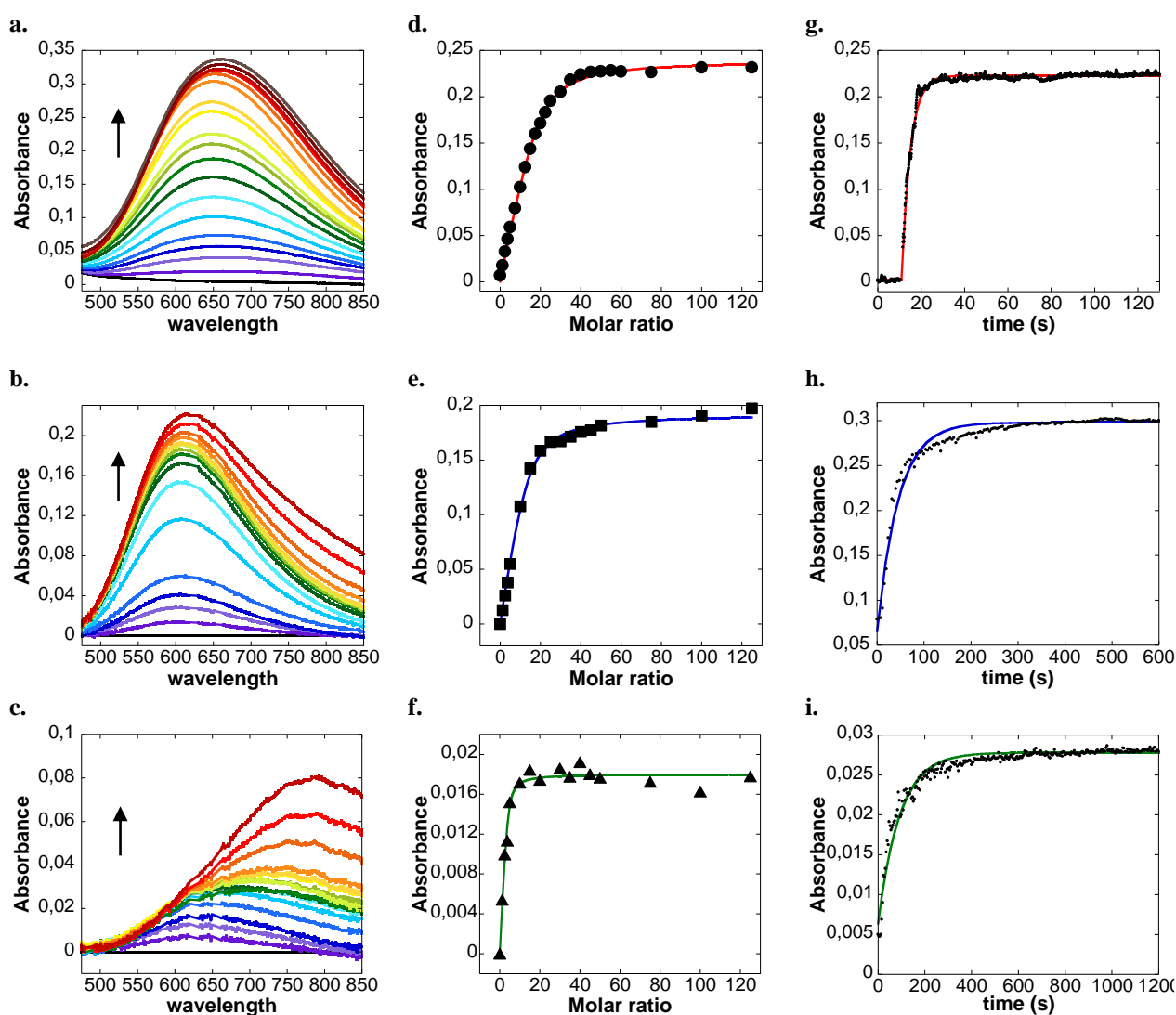
Intake and Outtake kinetics are based on simple exponential fits  $(A + B \cdot (1 - e^{-C(x-x_0)}))$  and  $A + B \cdot (e^{-C(x-x_0)})$ , respt.) with  $\tau = \frac{-\ln(0.37)}{C}$  and  $x_0$  the delay after which Cu is injected (10sec.)

## B. Results and discussion

### B.1. Cu and Zn loading capacity

UV-Vis spectroscopy was used to assess the loading capacity of CS, CMS, and CRS towards Cu ions (Figure S1 a-f). For each nanocarrier (80  $\mu\text{M}$ ), increasing amount of Cu was added in order to reach the maximum Cu-loading observed through the absorbance of Cu binding band. Absorbance at 580 nm was then plotted as a function of Cu:nanocarrier molar ratio and Cu capacity loading was determined using a fit calculation. Binding kinetics was also investigated by observing the absorbance of Cu-binding band at 580 nm by UV/Vis time-dependently (Figure S1 g-i). All those measurements were performed in water at room temperature.

**Figure S1. UV/Vis of Cu loading capacities and loading kinetics of CS, CMS, and CRS.**



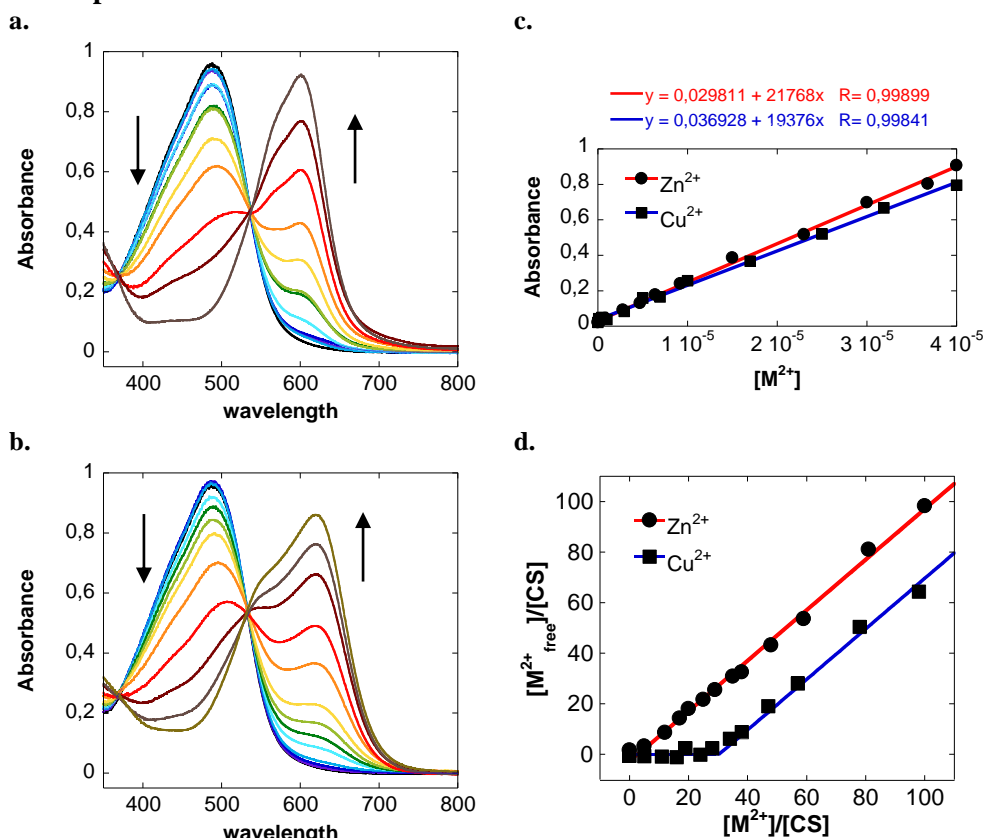
**Figure S1. UV/Vis spectra of titration of a. CS, b. CMS, c. CRS with  $\text{CuSO}_4$ ; respective absorbance at 580 nm over Cu/nanocarrier molar ratio for d. CS, e. CMS, f. CRS; Cu intake kinetic at 580nm of g. CS, h. CMS, i. CRS nanocarriers concentration 80  $\mu\text{M}$  at room temperature.**

As a result for Cu-loading capacity, CS is able to carry  $22 \pm 4$  Cu which correspond to 46% active sites (47 theoretical complexation sites for CS) (Figure S1 d). CMS possess a similar behavior by carrying around  $14 \pm 3$  Cu, corresponding to 42% of active sites over 33 theoretical complexation sites (Figure S1 e). CRS appears to be less effective than the other structures by carrying  $5 \pm 2$  Cu (15% of active sites over the 33 theoretical complexation sites) (Figure S1 f).

Concerning the kinetics properties, absorbance was plotted over time and the resulting fitting equation offered  $\tau = 4$  s for CS,  $\tau = 43$  s for CMS and  $\tau = 141$  s for CRS (Figure S1 g-i) representing the time after which 63% of the plateau value is reached.

In order to check those last Cu-loading capacity results, we performed a back titration based on a centrifugal filtration of the metal ion/polymer solution followed by metallic indicator analysis. As Zinc complexes are colorless ( $d^{10}$  element) and as Zincon (our metallic indicator) is well employed for Cu and Zn determination, we took benefit of this back titration to also assess Zn-loading capacity of CS nanocarriers. Thus Zincon procedure<sup>3</sup> was used as followed: solutions of nanocarriers incubated with increasing amount of metal ions were filtered to remove loaded nanocarriers using centrifugal filtration device (cut off 3 kDa). Then free metal ions concentration containing in the filtrate was determined through Zincon indicator.

**Figure S2. Zincon procedure**



**Figure S2. a. calibration spectra of Zincon-Cu, b. calibration spectra of Zincon-Zn, c. calibration curve for Zincon-Cu and Zincon-Zn, d. free Zn and Cu ions as a function of Cu/Zn:CS molar ratio (concentration determined through Zincon indicator). CS concentration 62  $\mu$ M, room temperature.**

Zincon-Cu and Zincon-Zn calibration curves were performed (Figure S2 a-c). Then free metal ions concentration were plotted as a function of overall metal ions:nanocarrier molar ratio. As a result, free Cu ions appeared for Cu/CS molar ratio equals 30 evidencing the saturation of CS towards Cu-loading. This result coincides with the former result received with the direct UV-Vis analysis. Moreover, free zinc ions increase linearly as a function of overall Zn ions added which indicates the inability of CS towards Zn-loading. CRS and CMS structures have shown similar behavior towards the complexation with  $Zn^{2+}$  and  $Cu^{2+}$ .

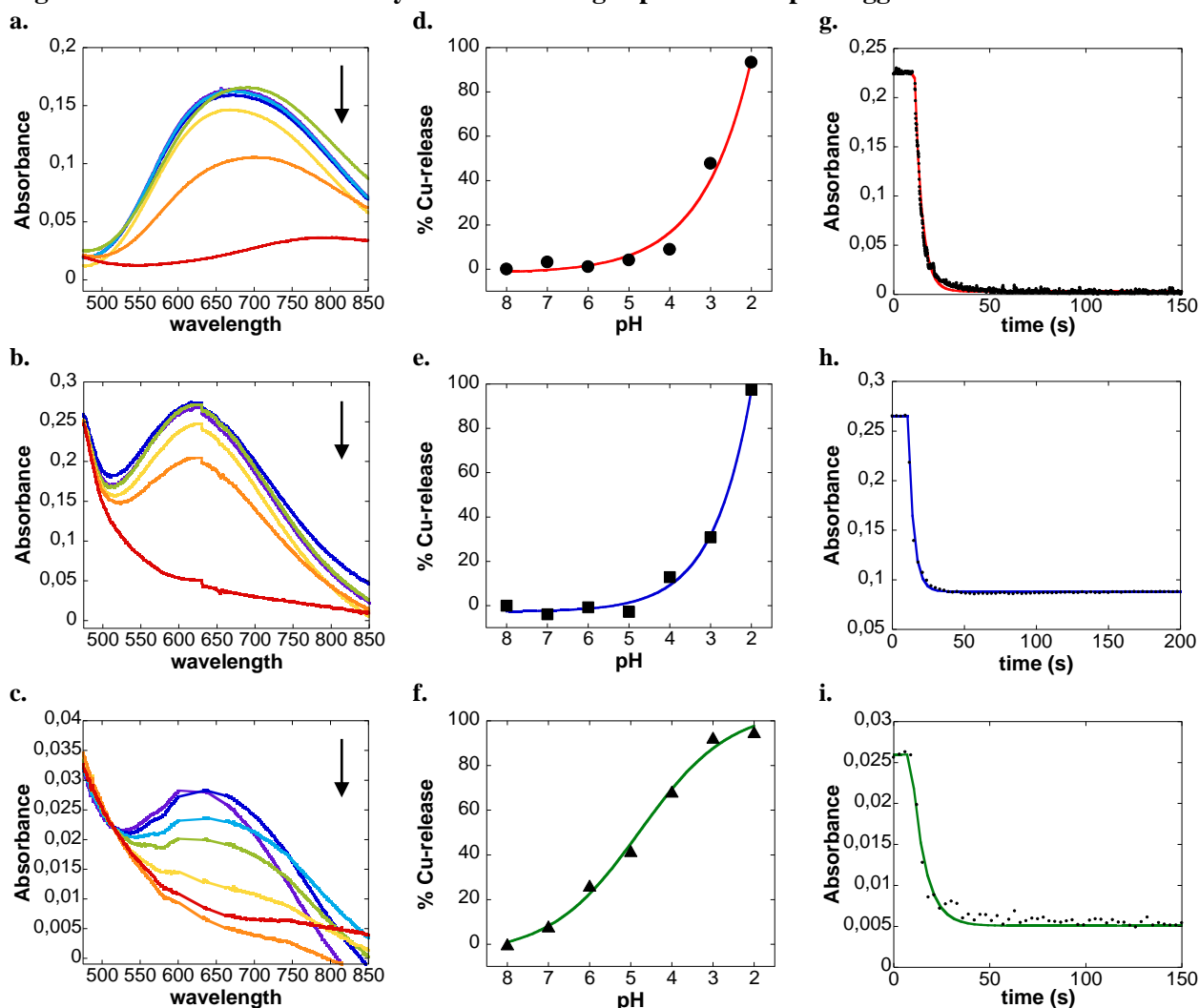
## B.2. pH-triggered Cu release

pH-triggered release of Cu was also evidenced by UV-Vis spectroscopy (Figure S3 a-f). Solutions of nanocarriers with excess of Cu were dialyzed to remove free Cu ions, then dry under high vacuum and redispersed in aliquots of Britton Robinson buffers at pH 8, 7, 6, 5, 4, 3, 2. The sample was left to equilibrium for 2 hours at 37°C and then analyzed by UV-Vis at 37 °C. Release kinetics was also investigated by



observing the absorbance of Cu-binding band after addition of HCl at 580 nm by UV/Vis time-dependently (Figure S3 g-i).

**Figure S3. UV/Vis and ITC analysis of Cu loading capacities and pH triggered release of CS.**



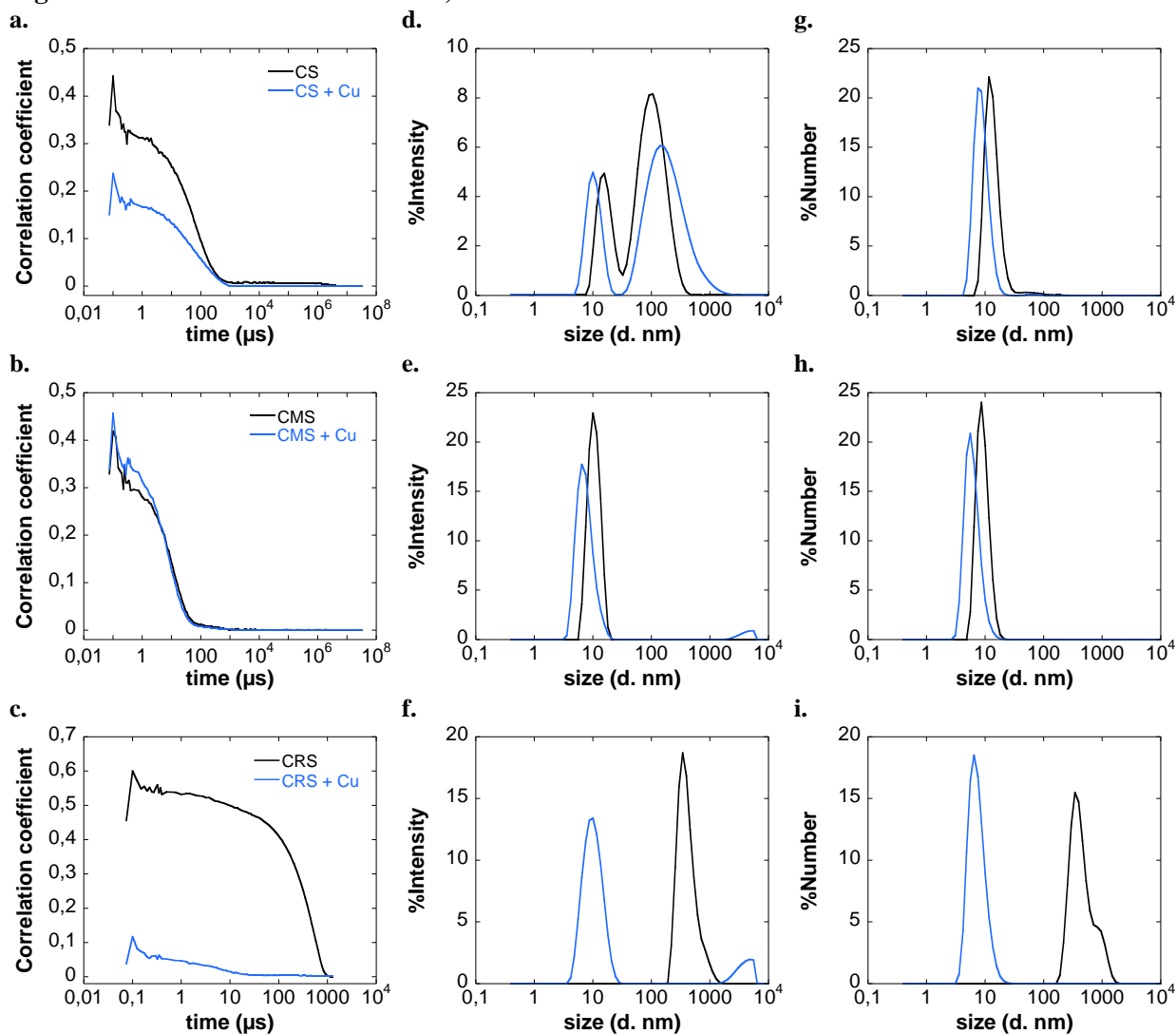
**Figure S3. UV/Vis spectra of a. CS, b. CMS, c. CRS (80  $\mu$ M) with  $\text{CuSO}_4$ , dialyzed, dried and redispersed in Britton Robinson Buffers pH 8, 7, 6, 5, 4, 3, and 2 and 0.1 M NaCl at 37°C; respective absorbance at 580 nm for d. CS, e. CMS, f. CRS; Cu outtake kinetic at 580 nm of g. CS, h. CMS, i. CRS (80  $\mu$ M) in water at 37°C.**

pH-triggered release evidenced a similar behavior of CS and CMS (Figure S3 a, b) with a retention of Cu until pH5 followed by a sharp release for lower pH values. CRS structure possesses a weaker release profile by starting releasing Cu from pH7 (Figure S3 c). Release kinetics equation offered  $\tau = 4$  s for CS,  $\tau = 8$  s for CMS and  $\tau = 9$  s for CRS (Figure S3 g-i) representing the time after which 63% of the plateau value is reached. The time  $t = 5 \cdot \tau$  depicts the time after which the equilibrium is reached. Already after  $t = 19$  s the Cu pH-release was finished in case of CS,  $t = 37$  s for CMS and  $t = 47$  s for CRS.

### B.3. Colloidal behavior in water solution

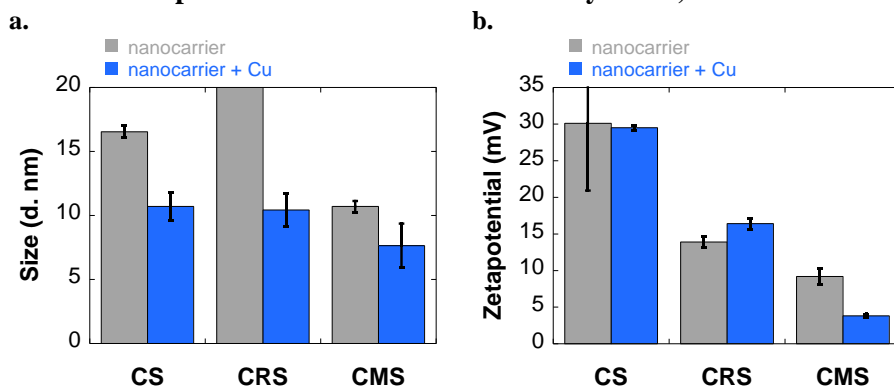
DLS and zeta potential measurements were performed to assess the size and the interfacial electric potential of the empty and Cu-loaded nanocarriers.

**Figure S4. DLS measurements of CS, CMS and CRS.**



**Figure S4. Correlogram of a. CS, b. CMS, c. CRS; size(%intensity) of d. CS, e. CMS, f. CRS; size(%number) of g. CS, h. CMS, i. CRS \_nanocarriers (80 μM) at room temperature, all samples filtered at 20 nm except CRS, filtered at 400 nm.**

**Figure S5. DLS and zeta potential measurements summary of CS, CMS and CRS.**



**Figure S5. a. DLS and b. zeta potential measurements summary of CS, CMS, CRS at pH 7.**

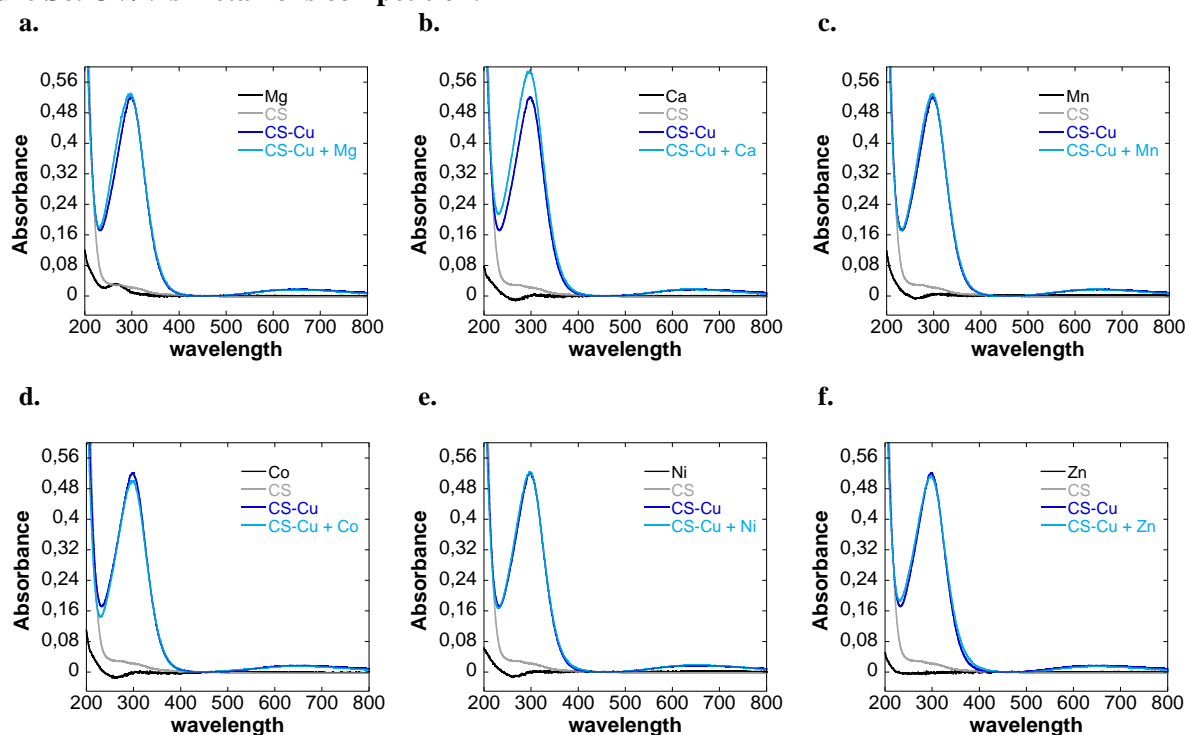
As a result, empty CS and CMS nanocarriers were found in solution as unimer with a hydrodynamic diameter close to  $12 \pm 1$  nm and  $8 \pm 1$  nm respectively (negligible amounts ( $<1\%$  in number) of aggregates for CS) (Figure S4). However, CRS nanocarriers were mainly present as polydisperse aggregates in solutions ( $R_h$  above 100 nm). When Cu was stabilized inside the nanocarriers, a significant decrease of  $R_h$  was observed and  $R_h$  values were found around  $6 \pm 1$  nm.

At pH 7, zeta potential of empty CS was found at  $30 \pm 9$  mV. As expected, grafting of PEG chains induces a significant decrease of zeta potential that remains slightly positive for both CRS ( $+13.9 \pm 0.8$  mV) and CMS ( $+9.2 \pm 1.0$  mV). Partial protonation of amine groups may be responsible for this observation. Therefore, highest zeta potentials were measured at pH 3 due to the increase of protonation level at lower pH.

## B.4. Metal ions competition

Finally, to evaluate the ability for such structures to interact with other ions, Cu-loaded CS nanocarriers were studied in the presence of competitor ions. To perform this study, excess of different metal ions (Mg, Ca, Mn, Co, Ni, Zn) were separately incubated with Cu-loaded CS nanocarriers during 1 day at 37 °C. UV-Vis measurements were monitored before and after the addition at 37 °C.

**Figure S6. UV/Vis metal ions competition.**



**Figure S6. UV-Vis spectra of CS such as Cu:CS (20 : 1) , Metal ions:CS (100:1) Metal ions : a. Mg, b. Ca, c. Mn, d. Co, e. Ni, f. Zn. CS concentration (62  $\mu$ M), incubation with competitor ions during 24 h at 37 °C.**

As a result, none of the metal ions are able to displace copper ions from CS dendritic structure. Similar results were observed in the case of CMS and CRS.

## References

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