Supporting Information

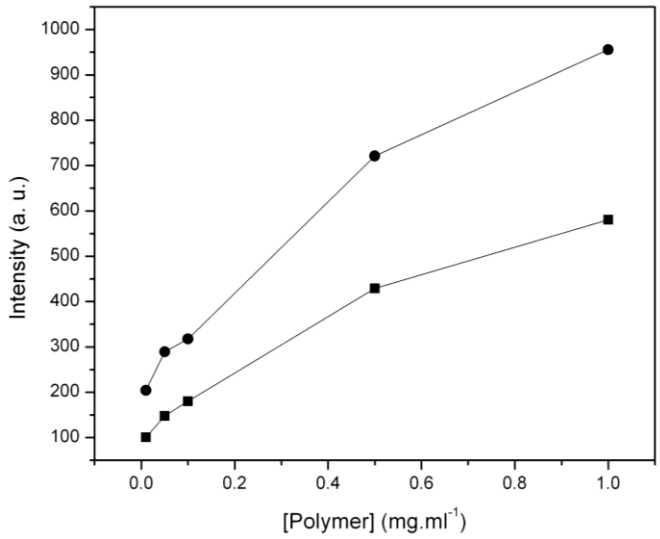

Figure S1: Fluorescence intensity from ONB linker vs. polymer concentration, excitation wavelength is 320 nm. (●): excitation slit set to 5; (■) excitation slit set to 10.

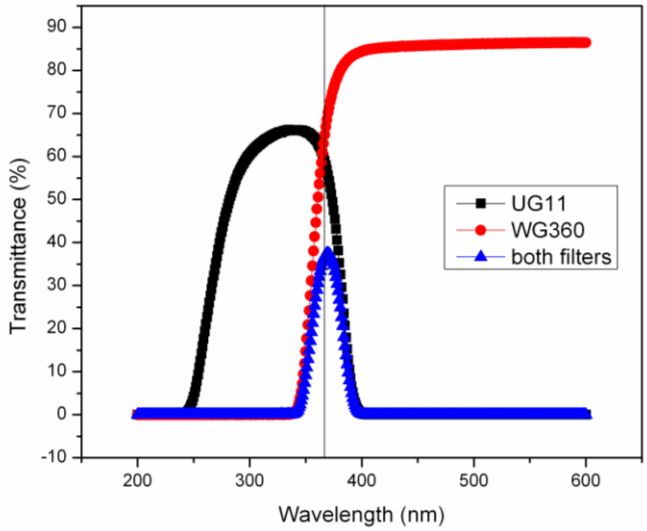

Figure S2: UV transmittance with UV filters to isolate the 365 nm band.

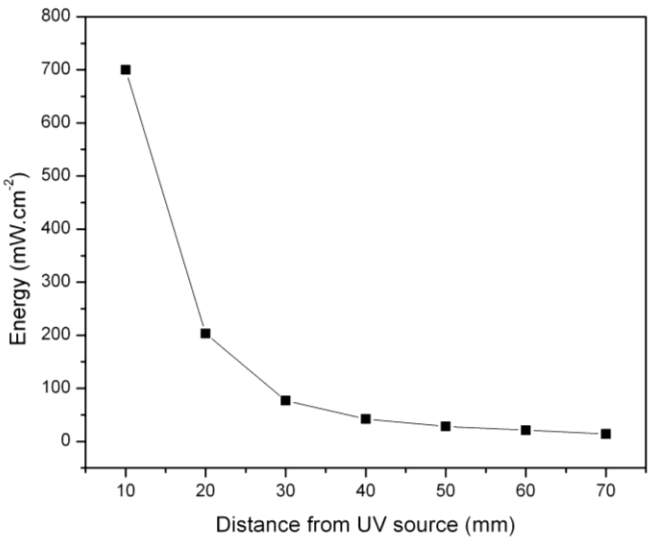

Figure S3: Estimated UV intensity delivered to the samples as a function of distance from UV light source.

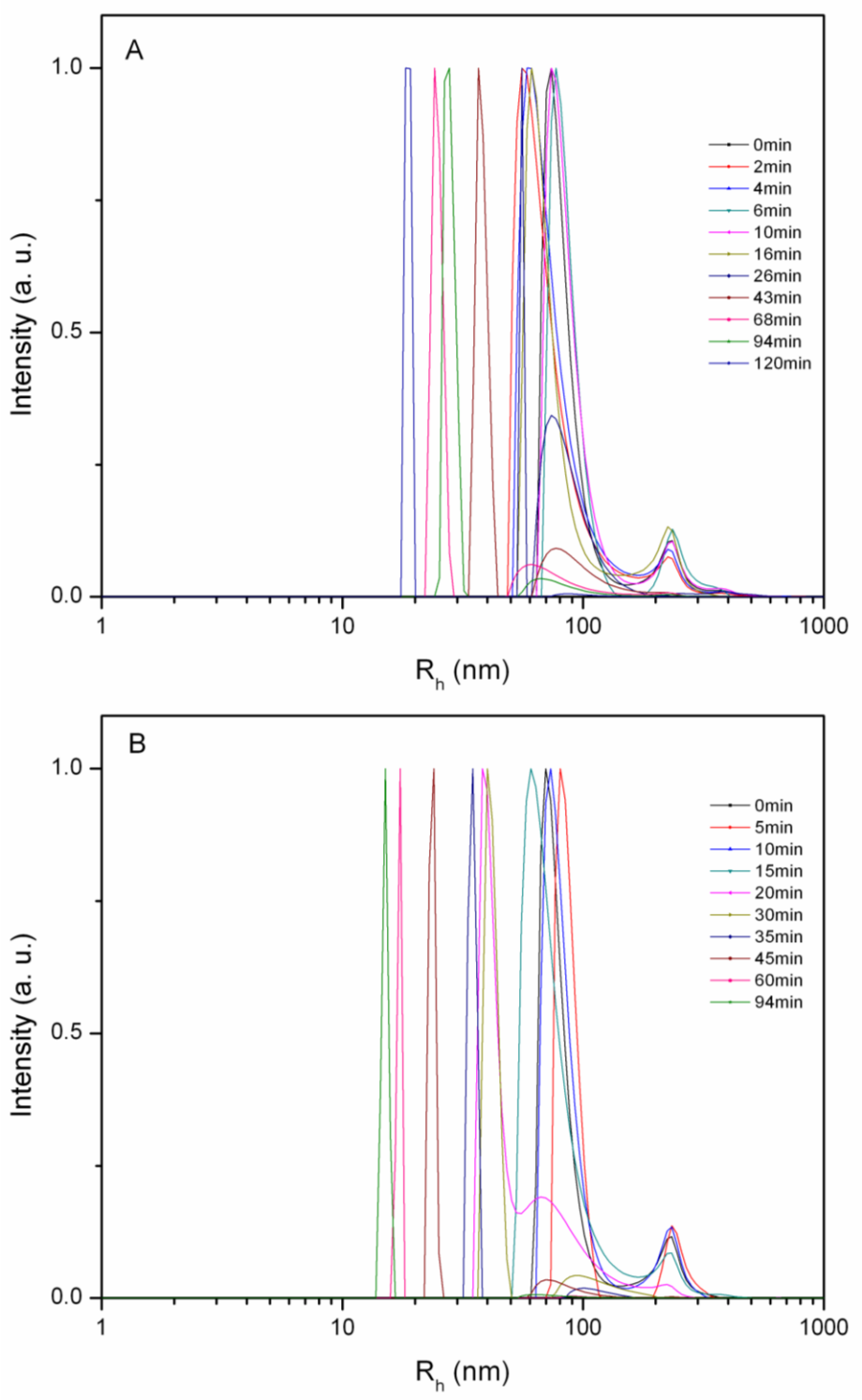

Figure S4: Number-averaged size distributions obtained by dynamic light scattering (DLS). A) 20 $mW.cm^{-2}$; B) 200 $mW.cm^{-2}$. Three measurements at 90° for 300 seconds each were performed on samples irradiated for different amounts of time. The correlation curves were fitted using the CONTIN method.

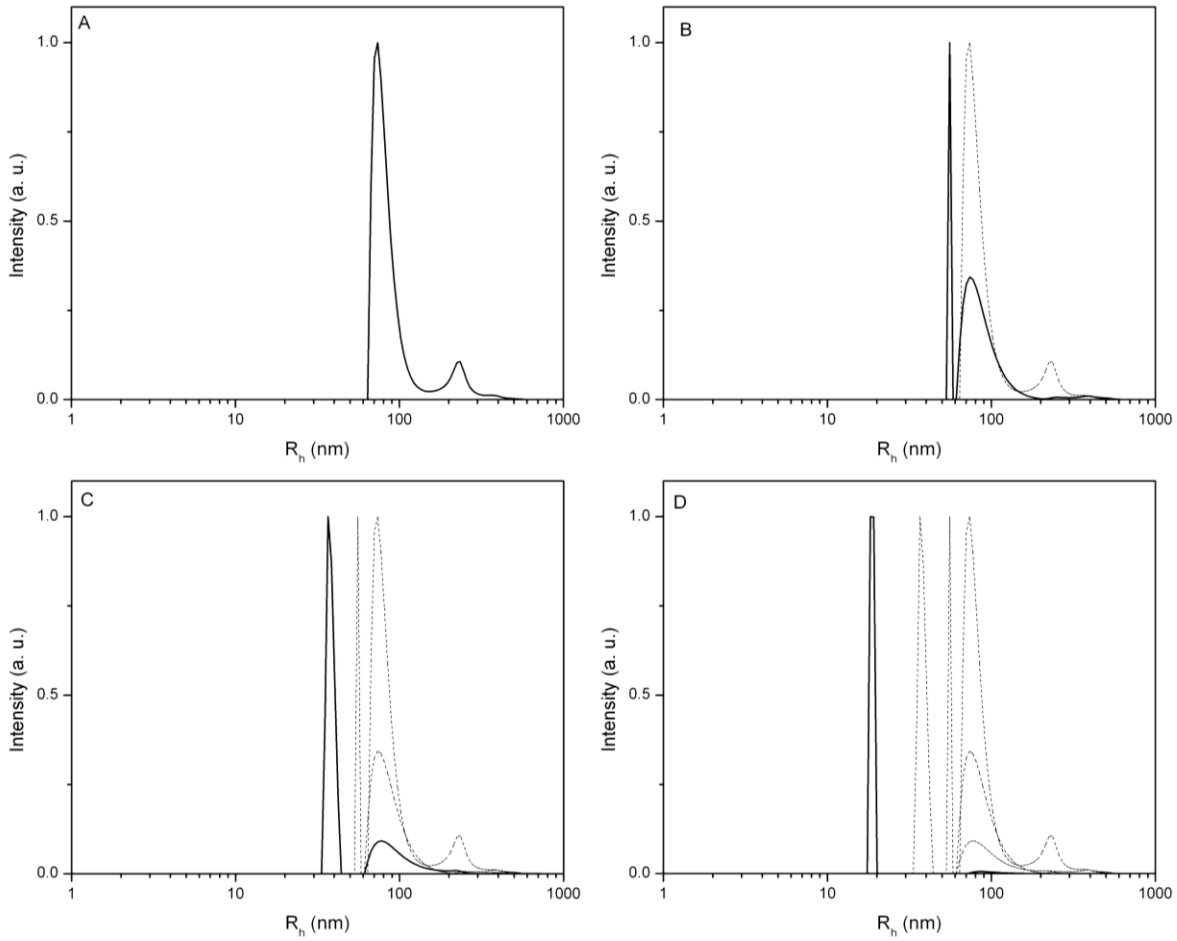

Figure S5: Number averaged size distributions obtained by dynamic light scattering (DLS). Three measurements at 90° for 300 seconds each were performed on samples irradiated (20 $mW.cm^{-2}$) for different amounts of time. The correlation curves were fitted using the CONTIN method. A) 0 minutes, B) 26 minutes UV, C) 43 minutes UV, and D) 120 minutes UV.

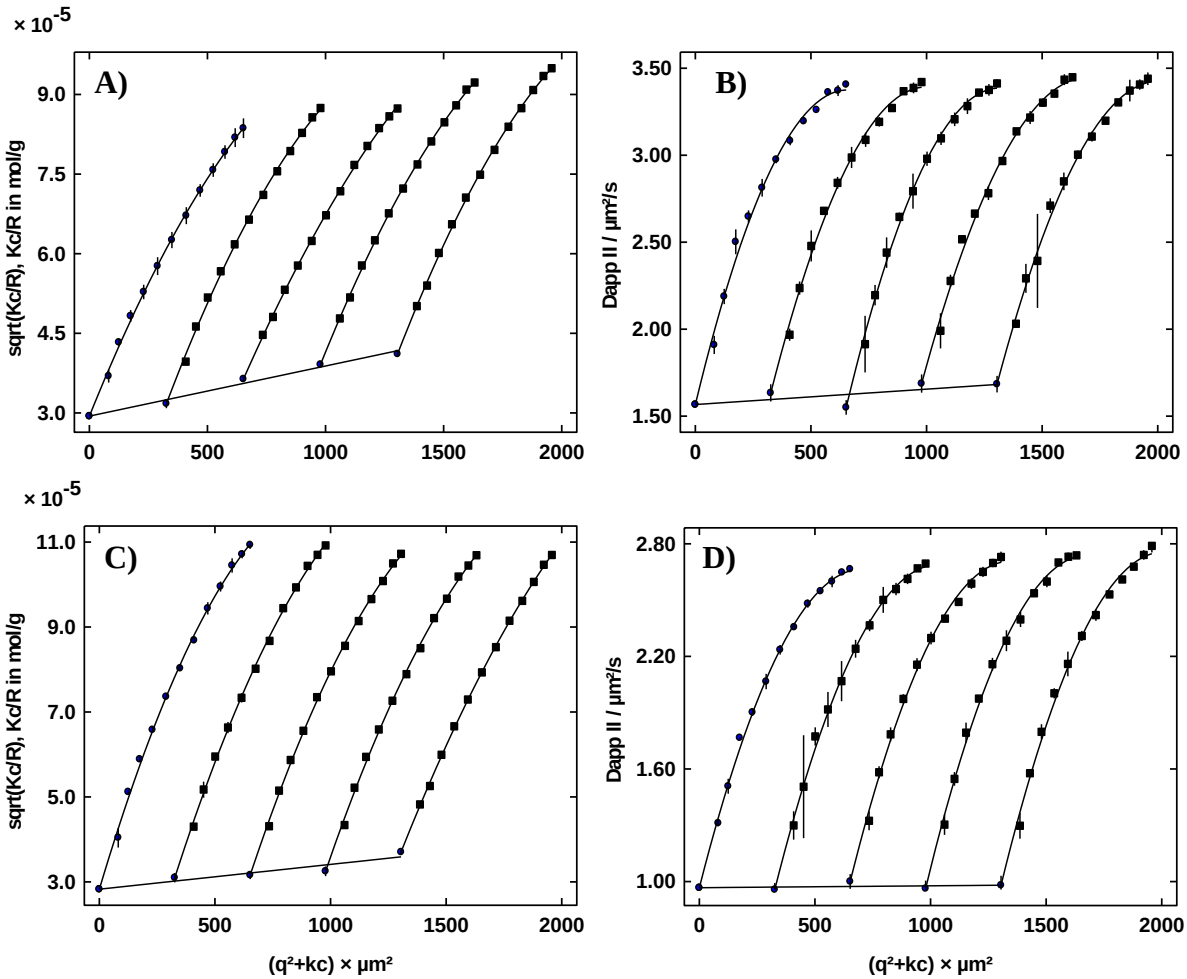

Figure S6: Berry and cumulant plots for a solution of AB2 vesicles before and after UV exposure: A) Berry plot before UV, B) cumulant analysis before UV, C) Berry plot after UV, and D) cumulant analysis after UV. UV irradiation with 200 $mW.cm^{-2}$.

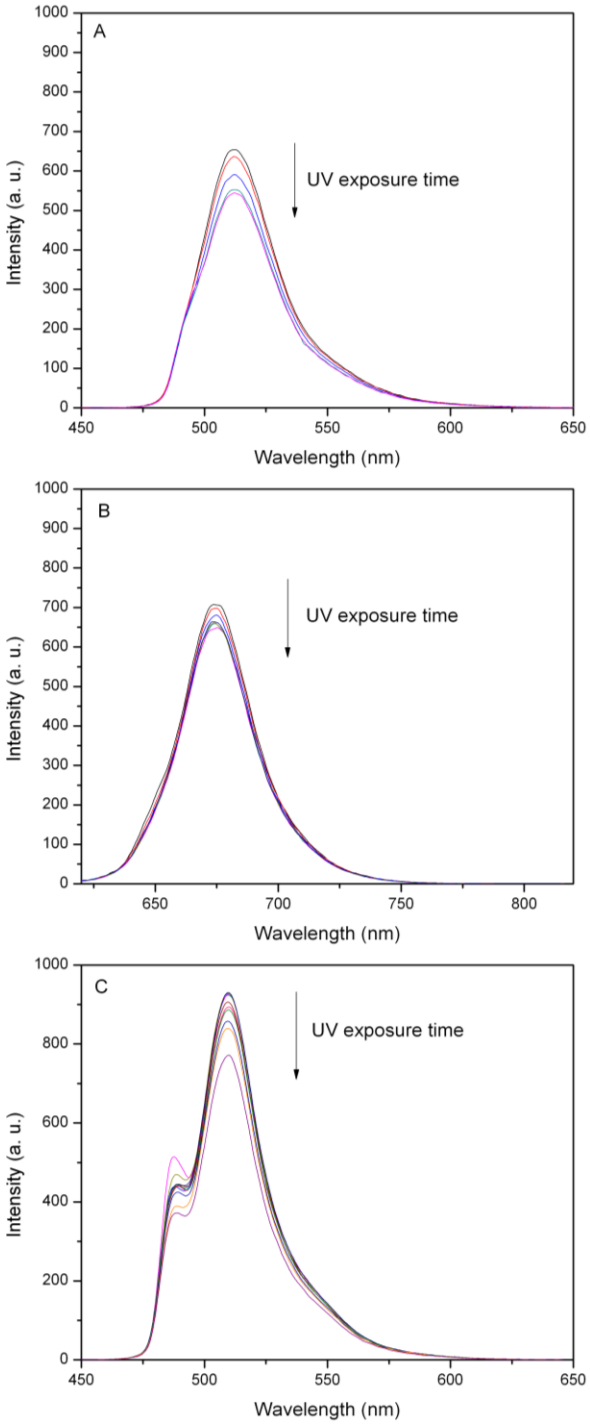

Figure S7: Photobleaching of model molecules used in encapsulation experiments: A) fluorescein, B) ATTO655, and C) eGFP. All experiments were performed for 0 to 100 minutes of irradiation (200 $mW.cm^{-2}$).

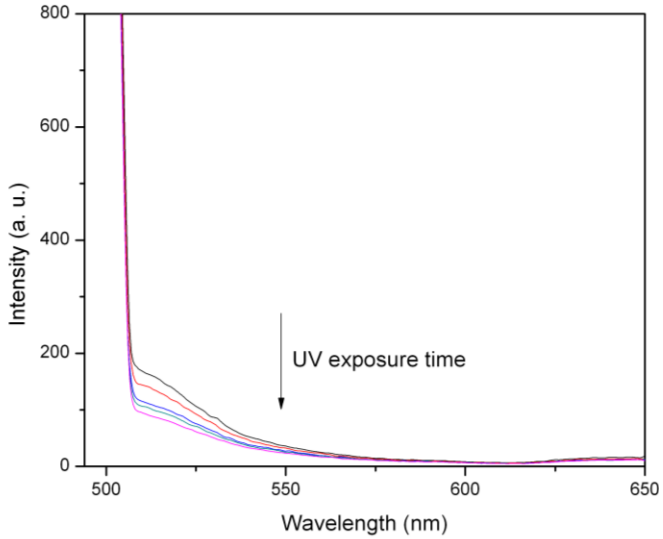

Figure S8: Fluorescence spectra of empty AB2 vesicles in PBS buffer, showing a small decrease of fluorescence intensity over exposure time: the observed decrease is due to photodegradation of the fluorescent ONB moieties comprised in AB2 polymer chains (irradiated with 200 $mW.cm^{-2}$).

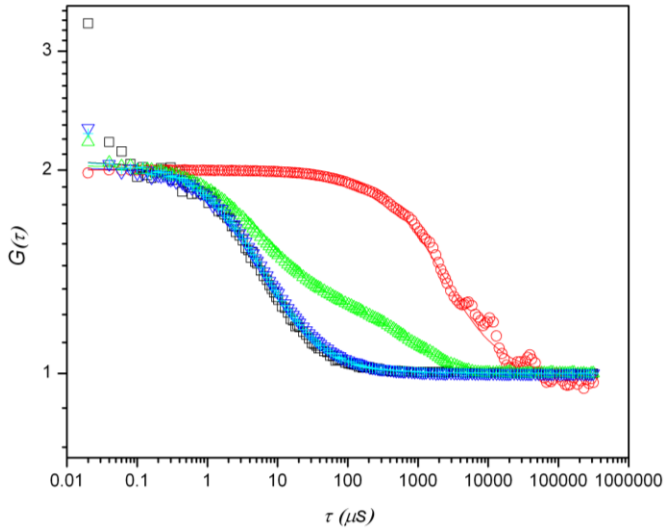

Figure S9: Experimental FCS autocorrelation curves (symbols) and fitted curves (plain lines) for ATTO655 release experiment.

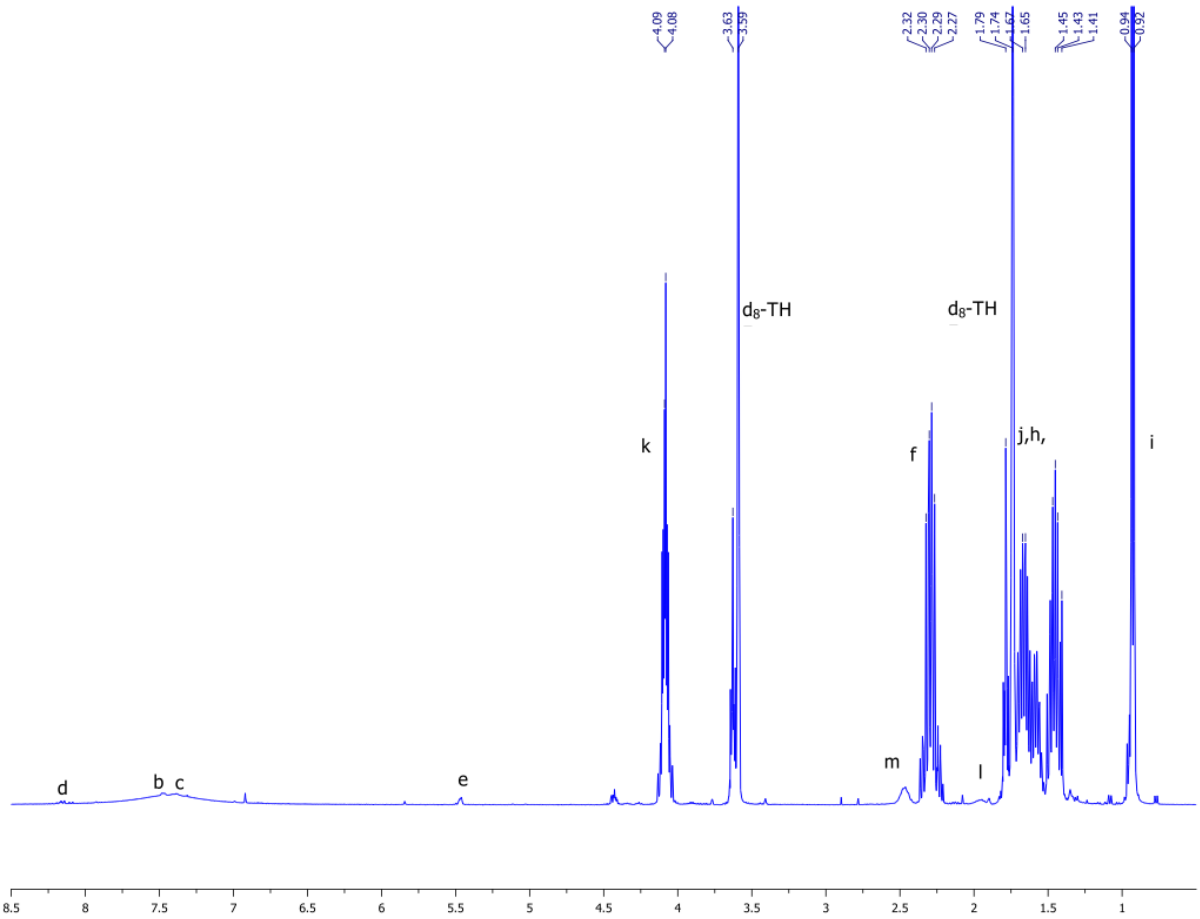

Figure S10: ^{1}H NMR spectrum of AB2, $PMCL_{76}$-*ONB*-PAA_{16} in deuterated THF.

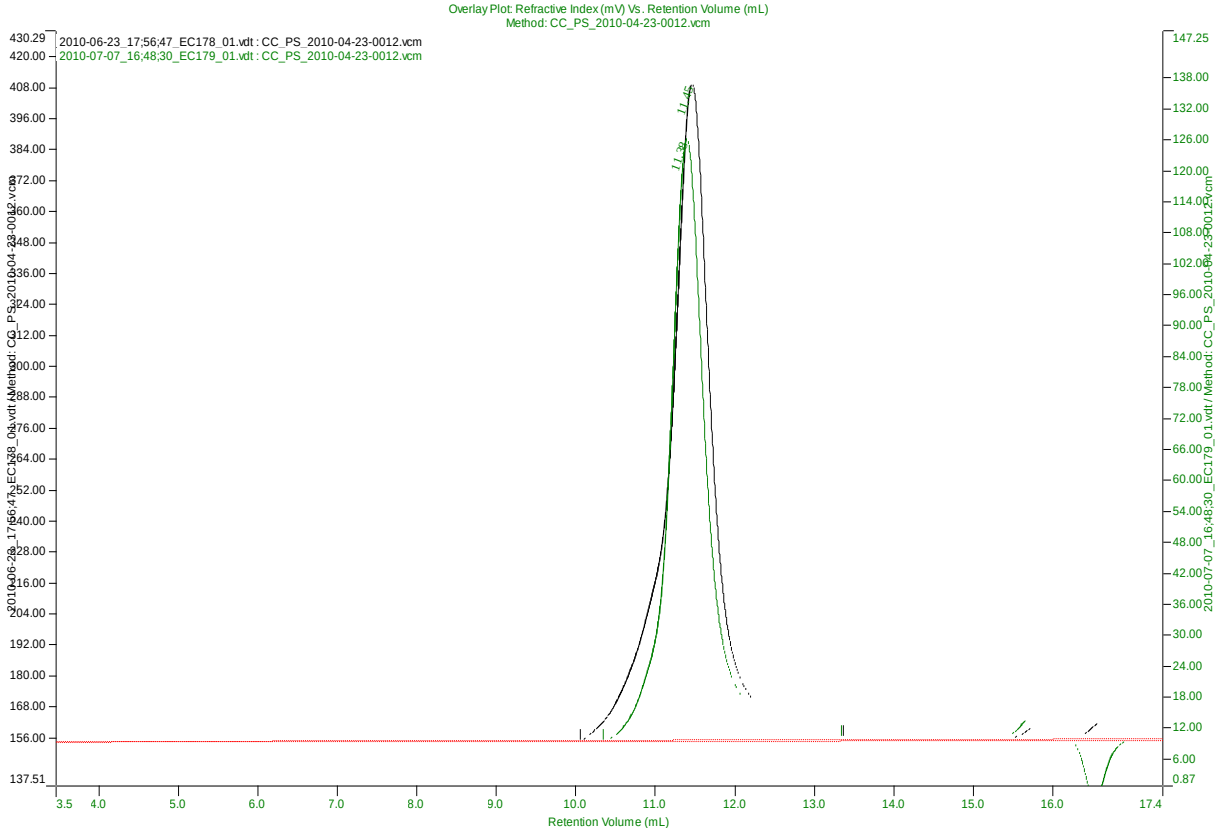

Figure S11: GPC traces of $PMCL_{76}$-*ONB*-Br (prior to ATRP of *tert*-butyl acrylate) in black, and $PMCL_{76}$-*ONB*-$PtBA_{16}$ (protected block), green line. The shoulder observed for the PMCL block chromatogram is due to high molecular weight chains initiated by water traces. Those chains do not initiate ATRP in the next synthesis step, and are easily removed by precipitation.

Calculations for encapsulation efficiency

The encapsulation efficiency *e* may be defined as the ratio between the number of encapsulated eGFP molecules inside the inner volume of one vesicle (N_{exp}) and the theoretical maximal number of eGFP molecules fitting inside the same volume (N_{th}).
The experimental number of encapsulated eGFP molecules can be calculated from FCS data using the following equation:

$$N_{exp} = \frac{CPM_{encapsulated\ \ eGFP}}{CPM_{free\ eGFP}} \qquad \text{equation (1)}$$

where *CPM* stands for counts per molecule. The $CPM_{free\ eGFP}$ is obtained from the brightness measurement of a solution of free eGFP. $CPM_{encapsulated\ eGFP}$ can be calculated from the total CPM (CPM_{total}, measured by brightness measurement for a solution of loaded vesicles, i.e. it contains CPM for encapsulated eGFP and free eGFP). CPM_{total} is defined as follows:

$$CPM_{total} = \sum i * CPM_{free\ eGFP} + (100 - \sum i) * CPM_{encapsulated\ \ eGFP} \quad \text{equation (2)}$$

where i represents the percentage of a population of free eGFP. According to our fitting, only one population of eGFP is free in solution. Therefore, equation (2) is written as follows:

$$CPM_{encapsulated\ \ eGFP} = \frac{CPM_{total} - i * CPM_{free\ eGFP}}{(100 - i)} \quad \text{equation (3)}$$

Using equations 1 and 3, we obtained N_{exp} = 26.
With an initial concentration of 1 mg/ml in protein, N_{th}, the theoretical maximal number of eGFP molecules (MW ca. 32.7kDa) entrapped in a vesicle with a R_h of 115nm (as given by FCS, see table 3), can be approximated as 117 molecules.
Therefore, we can calculate e:

$$e = \frac{N_{exp}}{N_{th}} = \frac{26}{117} \sim 22\%$$

This value seems to be a reasonable estimation when compared to other systems of polymersomes that encapsulate proteins.