Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information for:

Relative Size Selection of a Conjugated Polyelectrolyte in Virus-like Protein Structures

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Materials and Methods

Materials

The purification of the CCMV virus, removal of RNA and isolation of the coat protein (CP) were carried out according to literature procedures. The purity of the viral CP was characterized by size exclusion chromatography (FPLC; VCP = 18 mL) and UV-Vis spectroscopy.[i]

Poly[5-methoxy-2-(3-sulfopropoxy)-1,4-phenylenevinylene] potassium salt solution (MPS-PPV) 0.25 wt. % in H_2O was purchased from Sigma Aldrich, Netherlands.

Methods

FPLC sample preparation

Size exclusion chromatography was performed using the following buffers: 50 mM Tris-HCl, 0.3 M NaCl and 1 mM DTT (pH 7.5). The FPLC column used for preparative purposes was superose 6 10/100 GL (GE Healthcare) with a 100 µL injection volume. All chemicals used for the preparation of buffers were of analytical quality. Buffers were prepared in ultrapure (Milli-Q) water. UV-Vis spectra were recorded using a Perkin Elmer Lambda 850 UV spectrophotometer

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) was performed as follows: onto Formvar-carbon coated grids 5 µL of the desired sample was applied. After leaving the sample for 1 minute, the excess of liquid was drained using a piece of filter paper. Uranyl acetate (5 µL, 1% w/v) was then applied and the drying procedure was repeated.

Encapsulation of MPS-PPV into CCMV

Different solutions of MPS-PPV (stock solution: 2500 µg/ml) in 50 mM Tris-HCl buffer (0.3 M NaCl, 1 mM DTT, pH 7.5) were prepared, and a solution of the CP (8 mg/ml) in the same buffer was added. The resulting solutions were incubated for 1 h at 4 °C on a roller bank. After the incubation step, the sample as well as a reference solution (only CP) were purified and analyzed by size exclusion chromatography. 100 µl samples were injected into the FPLC column at room temperature and monitored using UV-Vis detection at λ = 260 nm, 280 nm and 451 nm. Fractions of 200 µL were collected, and those corresponding to AP, FP or CP were further analyzed by TEM, UV-Vis spectroscopy, DLS and fluorescence spectroscopy.

DLS of AP and FP

A cell inside the DLS-setup was filled with 600 µl of solution containing either AP or FP. The size distribution of the capsids was determined by measuring their diameter for more than 80 counts unless otherwise stated.

Figures:

S1 A and B: Transmission electron microscopy (TEM) images of FP and AP

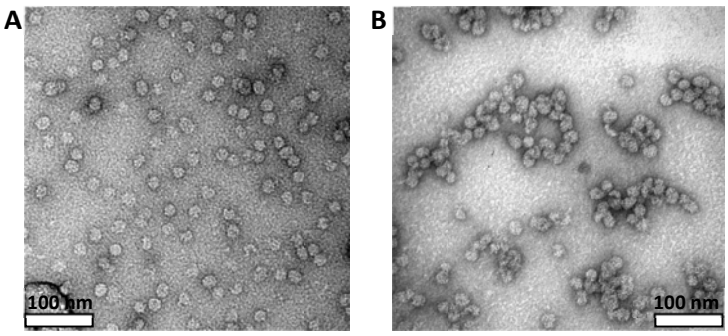

Fig. S1. TEM measurements of the corresponding size exclusion fractions of FP, 12.5 ml (A) and AP, 8.5 ml (B)

S2 A and B: Absolute values of AP, FP and CP calculated from size exclusion **results**

Absolute values of AP, FP and CP formation during the encapsulation process, by changing either the concentration of CP or MPS-PPV.

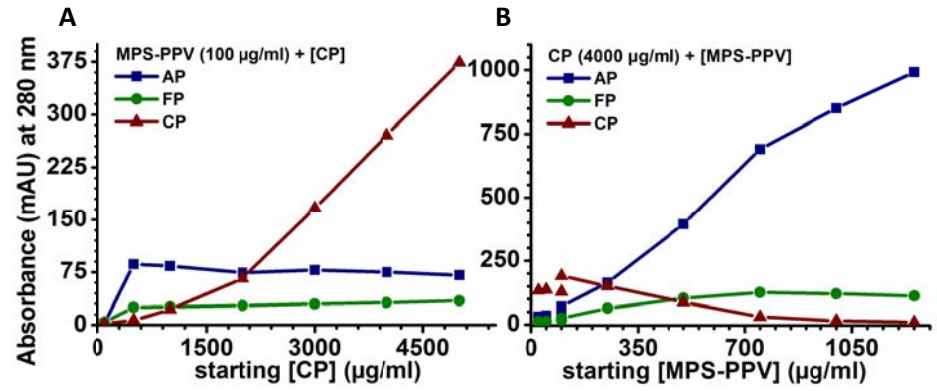

Fig. S2. The absolute values of each species, AP, FP and CP by either increasing the concentration of CP and constant MPS-PPV (A) or by increasing the concentration of MPS-PPV and constant CP (B) during the encapsulation process.

S3: Size exclusion **chromatogram of reinjection of AP and FP after 30 days**

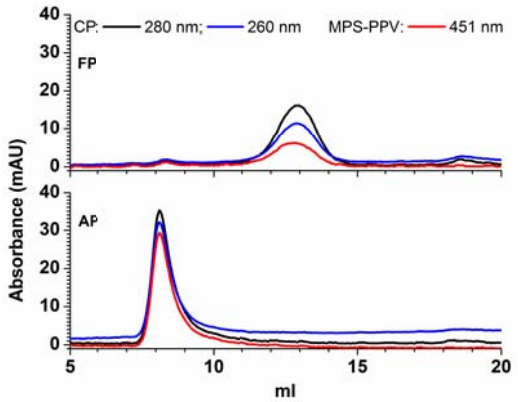

Fig. S3. Size exclusion chromatography run of AP and FP after storing the solution of separated AP and FP 30 days at 4°C.

Separation of different chain lengths of MPS-PPV by FPLC

In order to compare the nature of the chain length of MPS-PPV, encapsulated in FP and AP, we characterized the MPS-PPV polymer itself. The MPS-PPV polymer was purchased from Sigma Aldrich, (0.25 wt.% in H_2O) no further information such as the polydispersity index was given.

Figures:

S4: Size exclusion **chromatogram of free MPS-PPV**

To obtain more detailed information about the size distribution of the chain length of MPS-PPV, we separated the free polymer MPS-PPV by size exclusion chromatography. The obtained fractions were analyzed by UV-Vis spectroscopy (Fig. S5).

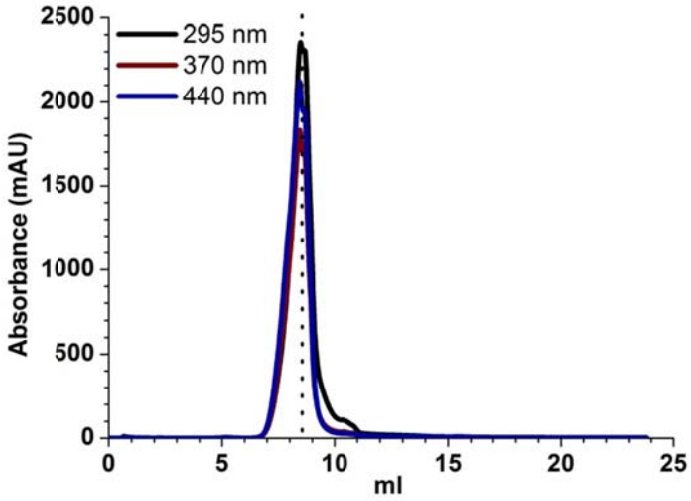

Fig. S4. Size exclusion -chromatograms of MPS-PPV.

S5: UV-Vis absorbance spectra of MPS-PPV fractions after size exclusion **separation**

In the early fractions (7.1 ml – 8.5 ml) longer chains are expected to be the dominant species, which is also shown by the higher absorbance at λ = 440 nm compared to the absorbance at λ = 370 nm. After 8.5 ml, the absorbance at λ = 440 nm decreased during the next fractions (8.5 ml – 9.5 ml) until the same height of the absorbance at λ = 370 is achieved. The latter results indicate the elution of shorter MPS-PPV chains.

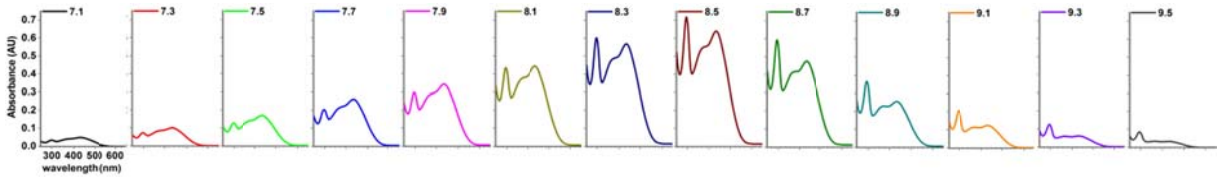

Fig. S5. UV-Vis absorbance spectra of MPS-PPV fractions after size exclusion separation.

S6: Plot of peak ratio from MPS-PPV against size exclusion**-fractions**

This process (see Fig. S4 & S5) was further visualized by plotting the peak ratio of λ = 295 nm to λ = 440 nm or λ = 370 nm against the elution volume. Figure S5 illustrates the increase in the peak ratios upon increasing elution volume. A peak ratio value below 1.5 points to higher molecular mass, whereas a peak ratio value above 1.5 indicates the elution of shorter chains.

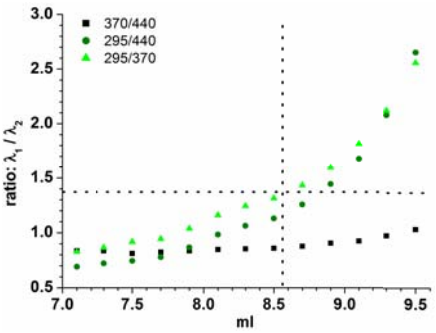

Fig. S6. Plot of the peak ratio of λ = 440 nm or λ = 370 nm to λ = 295 nm as a function of the elution volume of MPS-PPV fractions after size exclusion separation.

S7: Determination of the extinction coefficient of MPS-PPV

The extinction coefficient for MPS-PPV was determined at λ = 451 nm and λ = 280 nm.

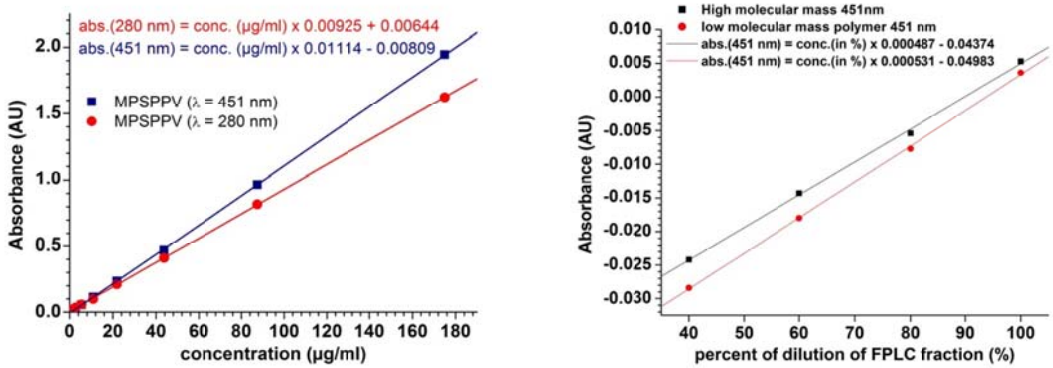

Fig. S7. Determination of the extinciton coefficient for MPS-PPV at λ = 451 nm and λ = 280 (left) and effect of dilution of polymer fractions with relatively high and low molecular weight on the absorption. The straight lines with a comparable slope point to similar extinction coefficients of short and long MPS-PPV fractions.

Based on the UV-Vis absorbance (Fig. S7) the composition of FP of 0.10-0.14 CP dimers per polymer unit and 0.02-0.05 CP dimers per polymer unit for AP can be estimated. This is in line with the encapsulation of polymers with different molecular mass and/or differences in loading efficiency.

S8: DLS of AP and FP after size exclusion purification

DLS results show that the AP range in diameter from 20 -120 nm (A) and FP are 10-30 nm (B), independent of the starting concentration of MPS-PPV (100 – 1250 µg/ml). The measuring range of the instrument is divided into fixed "channels" or particle sizes. Particles sizes are identified on the x-scale in units of nanometers sizes. Cumulative data values are on the same line as the particle size and are read as "percent smaller (%passing) than". For data presented as "percent smaller than", volume percent-in-channel (%channel) values are read as volume percent between the particle size on the same line and the line below.

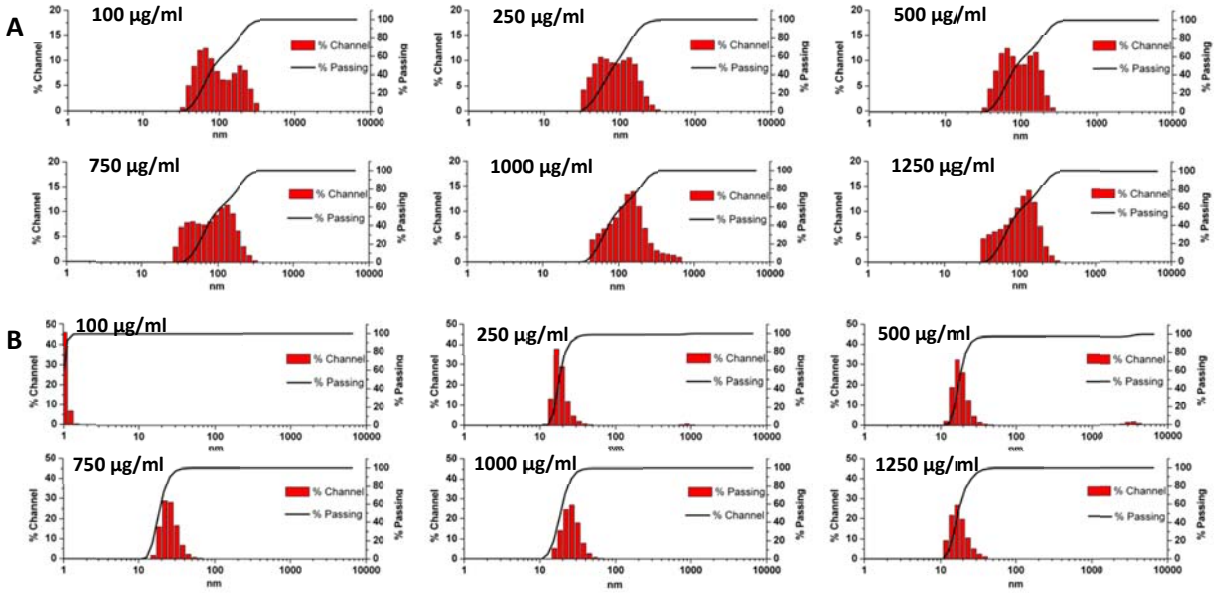

Fig. S8. DLS measurements of AP (A) and FP (B) samples (starting [MPS-PPV] 100-1250 µg/ml). The DLS measurements were performed after purification of the samples by size exclusion chromatography.

S9: Fluorescence quenching studies of MPS-PPV with different concentrations of methyl viologen (MV^{2+})

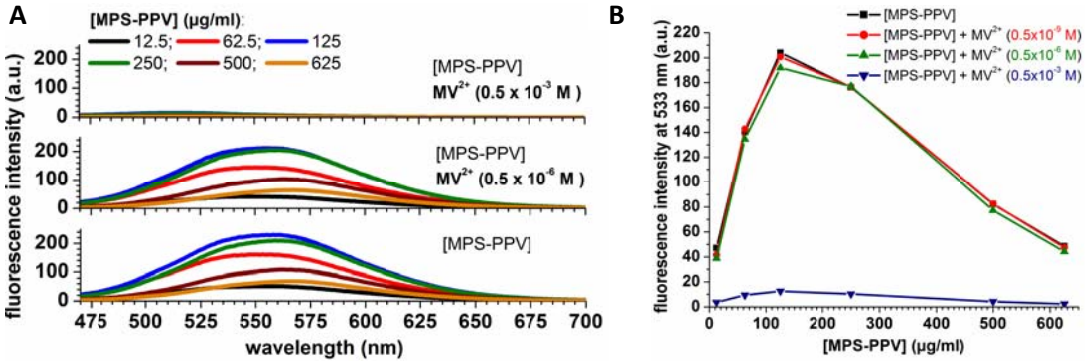

Fig. S9. (A) Emission spectra of MPS-PPV (concentration range from 12,5 µg/ml to 625 µg/ml) before adding MV^{2+} (bottom), after adding MV^{2+} (0.5 x 10^{-6} M) (middle), after adding MV^{2+} (0.5 x 10^{-3} M) (top). A successful quenching process of almost 90% was obtained by adding a concentration of 0.5 x 10^{-6} M MV^{2+} to a solution of MPS-PPV. Furthermore a blue shift in the emission spectra of MPS-PPV from 560 nm to 512.5 nm was observed after the quenching process. (B) Plot of [MPS-PPV] (µg/ml) vs. fluorescence intensity at 533 nm (a.u.). Different concentration of MV^{2+} were added to show the most efficient quenching of MPS-PPV. A concentration of 0.5 x 10^{-3} M MV^{2+} shows the highest efficiency and was used for all further quenching studies.

S10: Stern-Volmer plot for fluorescence quenching of MPS-PPV in AP and FP

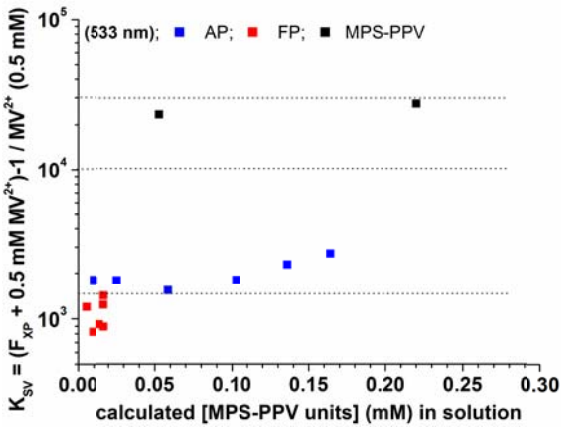

Fig. S10. Stern-Volmer plot for quenching of the fluorescence of MPS-PPV (repeating units) encapsulated in AP and FP. The Stern-Volmer fluorescence quenching constant (K_{sv}) was calculated for AP and FP with values of 2 x 10^6 M^{-1} and 1 x 10^6 M^{-1}, respectively. The K_{sv} for MPS-PPV had a value of ca. 2.7 x 10^7 M^{-1}.

S11: Scheme of quencher interaction with encapsulated MPS-PPV in AP and FP

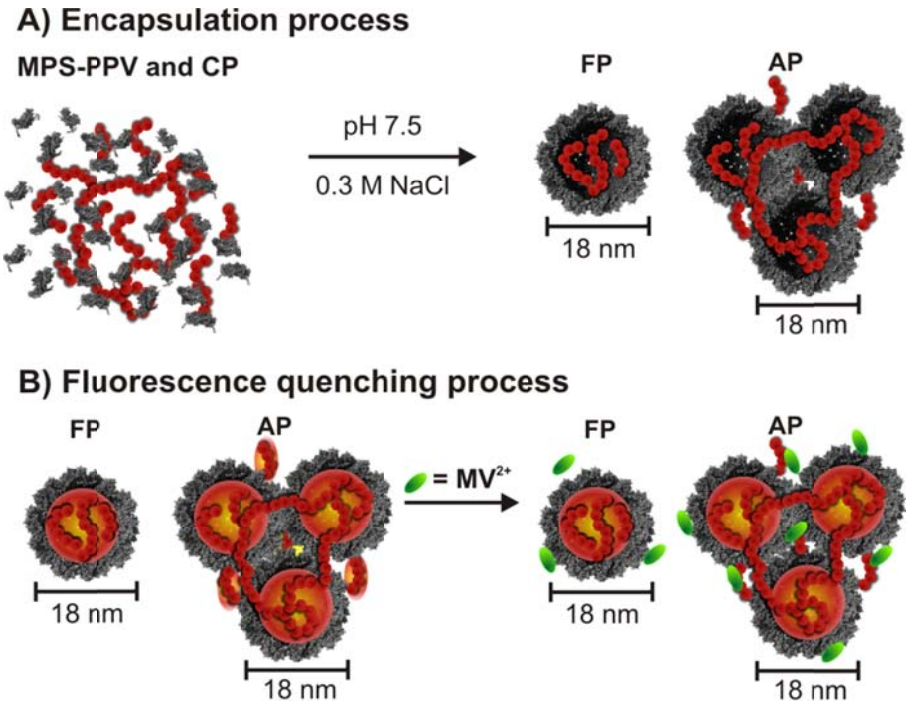

Fig. S11. Encapsulation of different chain length of MPS-PPV in AP and FP (A). MPS-PPV is not accessible for MV^{2+} in the case of FP but it is accessible in the interface of two particles in the case of AP (B).

S12: Fluorescence quenching studies of MPS-PPV, AP and FP with methyl viologen (MV^{2+}) at pH 5

The morphology and structure/porosity of the virus-like particles might be pH dependent. Therefor the FP and AP formed at pH 7.5 were purified by SEC and subsequently dialyzed to pH 5. After dialysis, SEC and TEM (Fig. S13) were used to confirm the integrity of the FP and AP, and next the fluorescence spectra of the obtained materials were measured in the absence and presence of 0.5 M methyl viologen as a quencher at pH 5. In the case of the free polymer the addition of the quencher at this concentration resulted in a decrease of about 70% of the fluorescence intensity (λ_{max} = 550 nm) with a concomitant blue shift of about 20 nm. Contrary to the data obtained at pH 7.5 the fluorescence emission maxima of the FP and AP both are λ_{max} = 535 nm. Addition of the viologen (0.5 M) did not give the same degree of quenching as observed as pH 7.5, which can be explained by a reduced porosity but also other factors related to a different degree of protonation of polymer and capsid protein might play a role.

Experimental description:

To perform fluorescence quenching studies of MPS-PPV, AP and FP at pH 5 the polymer MPS-PPV (stock solution: 2500 µg/ml) was added to the coat protein at pH 7.5 in 50 mM Tris-HCl buffer (0.3 M NaCl, 1 mM DTT, pH 7.5) in a ratio of 70 µl MPS-PPV stock solution to 180 µl CP (12 mg/ml). The resulting solutions were incubated for 1 h at 4 °C on a roller bank. After the incubation step, the samples were purified and analyzed by size exclusion chromatography. 100 µl samples were injected into the FPLC column at room temperature and monitored using UV-Vis detection at λ = 260 nm, 280 nm and 451 nm. Fractions of 60 µL each were collected and those corresponding to AP or FP were dialyzed to a pH 5 buffer (50 mM Sodium-Acetate, 1 M NaCl and 1 mM Sodium-Azide) for 24 h. The dialyzed samples were re-injected into the SEC column and analysed to be sure that the FP or AP stay intact during the buffer exchange from pH 7.5 to pH 5. The samples show the same elution volume as observed before at pH 7.5, which indicate that both samples keep their size during the dialysis. The corresponding fractions of AP or FP form the size exclusion purification were further used for fluorescence and quenching studies as well as for TEM imaging (Fig. S13).

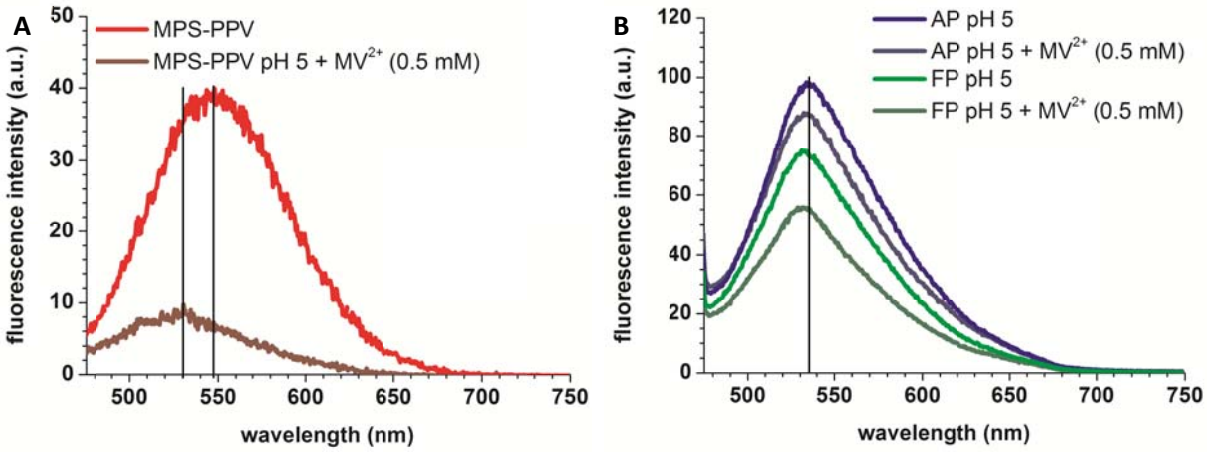

Figure 12 (A) Emission spectra of MPS-PPV at pH 5 before adding MV^{2+} (red line) and after adding MV^{2+} (0.5 x 10^{-3} M) (brown line). (B) Emission spectra of AP and FP at pH 5 before adding MV^{2+} and after adding MV^{2+} (0.5 x 10^{-3} M).

S13: Transmission electron microscopy (TEM) images of FP and AP at pH 5

The presumed T = 1 structure of FP and AP at pH 5 was confirmed by TEM. Preliminary analysis of the AP suggested a slightly larger diameter as expected for the T = 1 particles, suggesting the presence of some pseudo 'T = 2' morphologies.

[i] (a) B. J. M. Verduin, *FEBS Lett.* **1974**, *45*, 50. (b) M. Comellas-Aragones, H. Engelkamp, V. I. Claessen, N. A. J. M. Sommerdijk, A. E. Rowan, P. C. M. Christianen, J. C. Maan, B. J. M. Verduin, J. J. L. M. Cornelissen, R. J. M. Nolte, *Nature Nanotech.* **2007**, *2*, 635.