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Conjugated polymer nanoparticles hybridized with the peptide aptamer

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Preparation of Hyp01/hypPPV nanoparticles. The hypPPV was synthesized according to a previous report.[1] The number average molecular weight and polydispersity index, determined by gel permeation chromatography using polystyrene standards, were 1400 and 1.2, respectively. All C-terminal amidated peptides used in this paper were synthesized by standard solid-phase synthesis, as described in our previous report.[2] These peptides were purified by reverse-phase high-performance liquid chromatography and identified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a single species.

A 6 μL aliquot of a N,N-dimethylformamide (DMF) solution of hypPPV (333 μM) was added under sonication, into 1994 μL of aqueous peptide solution to give a final concentration of [hypPPV] = 1.0 μM in a total volume of 2 mL. Mixed solutions of hypPPV and Hyp01 peptide in glass vials were mildly sonicated for 1 h at 25 ± 5 °C in a normal bath-type ultrasonic cleaner (55 W, USK-1R, AS ONE). Next, the samples were transferred into polypropylene tubes and centrifuged at 10000 *g* for 5 min at 25 °C to remove any precipitated hypPPV. The upper 70% of the supernatants were collected for further analysis.

Spectroscopy. The fluorescence spectra excited at 320 nm were recorded on a spectrofluorometer (FP-6500, Jasco). The slit width was 5 nm for both excitation and emission. The absorption spectra were recorded on a spectrophotometer (V-550, Jasco). The peptide solution concentrations were determined by their absorbance at 280 nm using molar absorption coefficients calculated from their Trp, Try, and Cys contents.[3]

TEM Observation. Aliquots of 10 μL of the nanoparticle solution were cast onto collodion-coated 200-mesh copper grids. Some of these grids were negatively stained by placing them onto a droplet of 2% (w/v) uranyl acetate solution for 30 sec. Next, the grids were blotted with filter paper and allowed to dry. The observations were performed with an electron microscope (Hitachi H-7500) operating at 100 kV.

Fluorescamine Assay. An 8.4 mL solution of the prepared nanoparticle was passed through a Centricut mini V-10 ultrafiltration membrane (molecular weight cut off (MWCO): 10 kDa) and washed with milliQ water 5 times to remove any unbound peptides. The concentrated nanoparticle solution was diluted up to a total volume of 840 μL, added onto a $CHCl_3$ layer in a glass vial, and vigorously stirred over night. Six hundred microliters (600 μL) of the aqueous layer was then carefully separated and subjected to the fluorescamine assay.[4] Briefly, 200 μL of fluorescamine solution in DMSO (3 mg mL^{-1}) was added to the aqueous peptide solution under vortex mixing for 30 sec, and rotated for 30 min. The fluorescence intensity at 475 nm (excited at 390 nm) of this solution was then recorded. The concentration of Hyp01 peptide was determined from a separately constructed standard calibration curve.

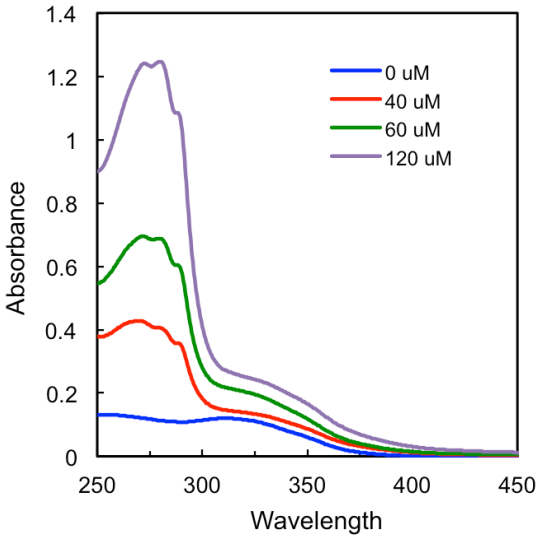

Fig. S1 Absorption spectra of dispersed hypPPV in aqueous solution when the indicated concentrations of Hyp01 were present.

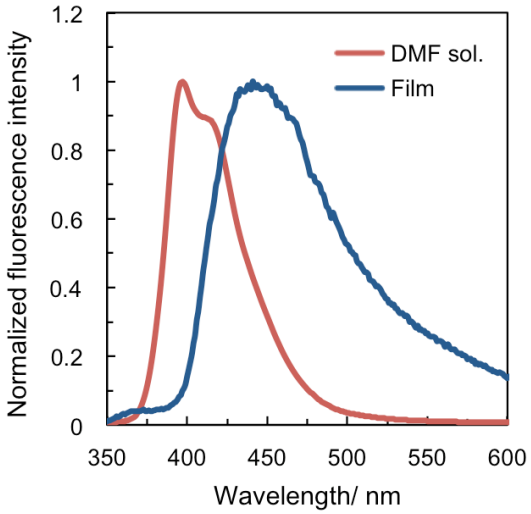

Fig. S2 Fluorescence spectra of hypPPV in DMF solution and film state.

References

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