Electronic Supplementary Material

Active targeted drug delivery system constructed from functionalized pillararenes for chemo/photodynamic synergistic therapy

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Materials

Unless stated otherwise, all the chemical reagents and solvents were obtained commercially and used without further purification. Compound 1, MPyTPP and G_M were synthesized via the previously reported methods [S1~ S3].

Fluorescence titration experiments

Fluorescence titration experiments were conducted to estimate the association constant (*K*a) and the stoichiometry of the complexation. The fixed initial concentration of WP5-PEG-TPP in DMF is 10 μM. The fluorescence spectra of the WP5-PEG-TPP solution mixed with Py-Por of different concentrations were obtained. The fluorescence intensities at 325 nm were recorded respectively.

The association constant was calculated based on the following equation:

$$\Delta F = (\Delta F\infty/[H]0)(0.5[G]0 + 0.5([H]0 + 1/Ka) - (0.5([G]_0^2 + (2[G]0(1/Ka - [H]0)) + (1/Ka + [H]0)2)0.5))$$

Where ΔF is the fluorescence intensity changes at 325 nm; $\Delta F\infty$ is the fluorescence intensity changes at 325 nm when BDP-CN is completely complexed; [G]0 is the fixed initial concentration of WP5-PEG-TPP and [H]0 is the initial concentration of Py-Por.

Preparation of TPZ-loaded NPs

TPZ (0.356 mg) and Py-Por (1.56 mg) were dissolved into DMF (1 mL). The obtained solution was dropwise injected into WP5 (5.84 mg) aqueous solution (9 mL) under vigorous stirring for 6 h. The prepared nanoparticles were purified by dialysis (molecular weight cutoff 10000) in distilled water for 48 h.

Photodynamic experiments

NPs aqueous solution (25 μg/mL, 2.97 mL) with different concentrations in quartz cuvettes were mixed with DPBF in ethanol (10.0 mM, 30 μL), which was then irradiated by lasers (660 nm, 1.0 W/cm^2) for a period of 120 s. The absorbance at 415 nm of the solution was recorded at the pre-set time points during the process.

***In vitro* experiments**

Cellular Uptake: HeLa cells were incubated in DMEM. The medium was supplemented with 10% FBS and 1% Penicillin-Streptomycin. HeLa cells were seeded in 96-well plates (1×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37 ºC in 5% CO_2. Then DMEM containing NPs (25 μg/mL), MitoTracker green and Hoechst 33342 was introduced to replace the original medium. The fluorescence was observed using fluorescence microscopy at the pre-set time points during the process.

Intracellular total ROS detection: HeLa cells were incubated with NPs (25 μg/mL) for 4 h followed by incubation with 10 μM DCFH-DA for 15 min. After being washed by PBS buffer for three times, cells were irradiated with 660 nm laser at a power density of 1.0 W/cm^2 for 15 min. Then, the fluorescence was immediately observed using fluorescence microscopy (λ_{ex}: 460 – 495 nm, $\lambda_{em:}$ > 510 nm).

Cell viability test: Cancer cells were incubated in DMEM. The medium was supplemented with 10% FBS and 1% Penicillin-Streptomycin. Cells were seeded in 96-well plates (1×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37 ^{o}C in 5% CO_2. Then DMEM containing different concentrations of NPs was introduced to replace the original medium. Four hours later, the cells were treated with or without a 660 nm laser (1.0 W/cm^2). After 10 min irradiation, cells were cultured for the next 24 h. The relative cellular viability was determined by the MTT assay.

Live-dead cell staining: The same density of cancer cells (3×10^5 cell mL^{-1}) were distributed into three confocal dishes (35 mm) for 12 h. Then the 2-plate cells were cultured with new DMEM containing NPs (25 μg/mL). After 4 h, the cells were subjected to dark or laser irradiation (660 nm, 1.0 W/cm^2, 10 min). After 48 h, the cells were stained with a calcein AM/propidium iodide mixture for 30 min and washed twice using PBS. The fluorescence images eventually acquired via fluorescence (AM: λ_{ex}: 460 – 495 nm, $\lambda_{em:}$ > 510 nm; propidium iodide: λ_{ex}: 510 – 550 nm, $\lambda_{em:}$ > 575 nm).

Intracellular hypoxia: HeLa cells were incubated in DMEM with 10% FBS and 1% Penicillin-Streptomycin. For hypoxia, the original culture medium was replaced

by 1 mL fresh medium containing DFO (100 μM). Then the cells were returned to incubator (5% CO_2, 20% O_2 at 37 °C) and incubated for 8 h. Next, the cells were washed thoroughly with PBS three times.

Mitochondrial membrane potential evaluation: The mtitochondrial membrane potential assay kit with JC-1 was used to indicate the changes in mitochondrial membrane potential. When the mitochondrial membrane potential is high, JC-1 aggregates in the matrix as a polymer and generates red fluorescence. On the contrary, JC-1 forms a monomer when the potential is low and produces green fluorescence. The decrease of cell membrane potential is regarded as a sign of mitochondrial damage in terms of the proportional changes of JC-1 by the transition of red to green fluorescence.

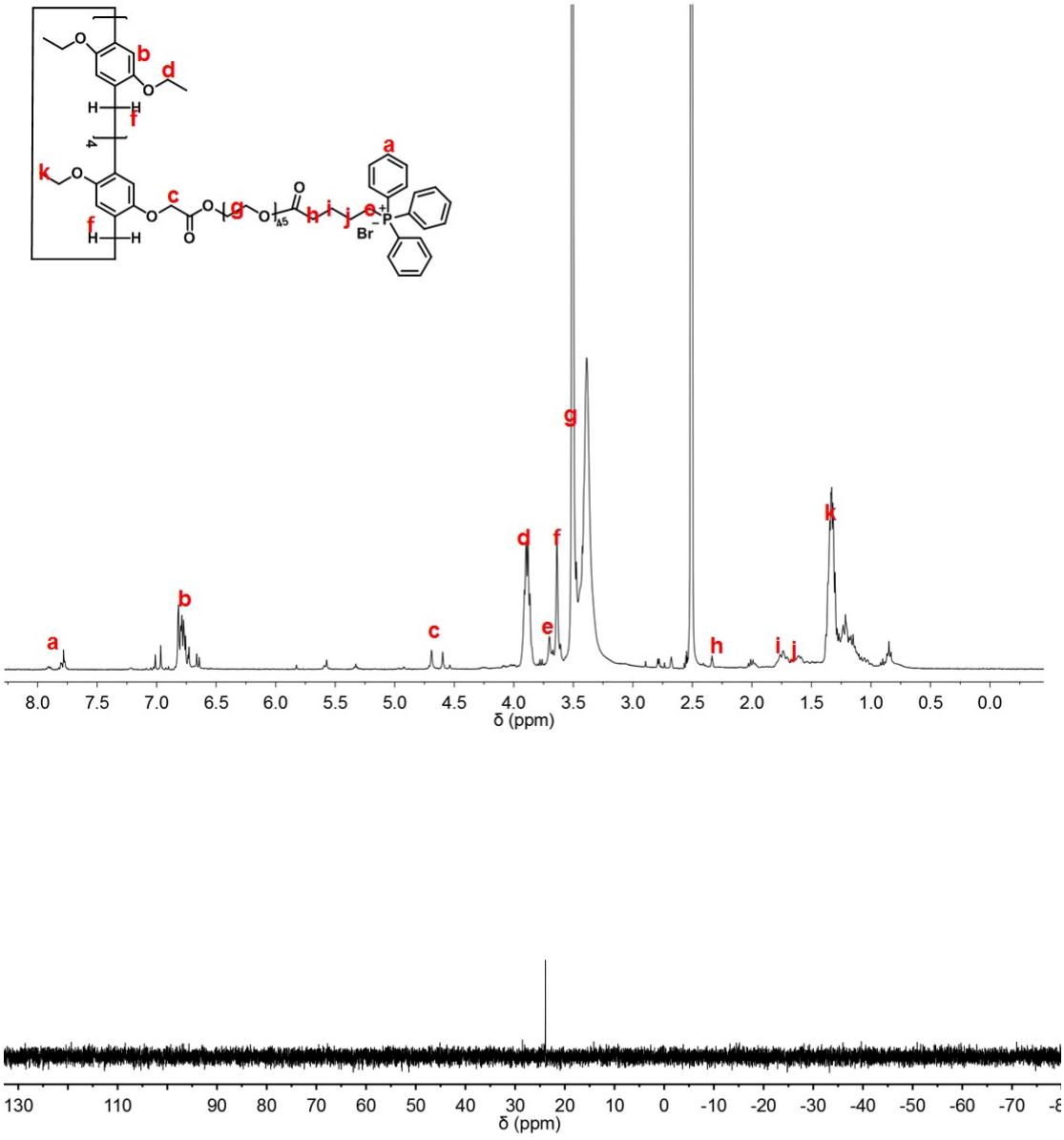

Fig. S3. The ^{1}H NMR and ^{31}P NMR spectra (DMSO-d6, 293 K) for WP5-TPP-PEG

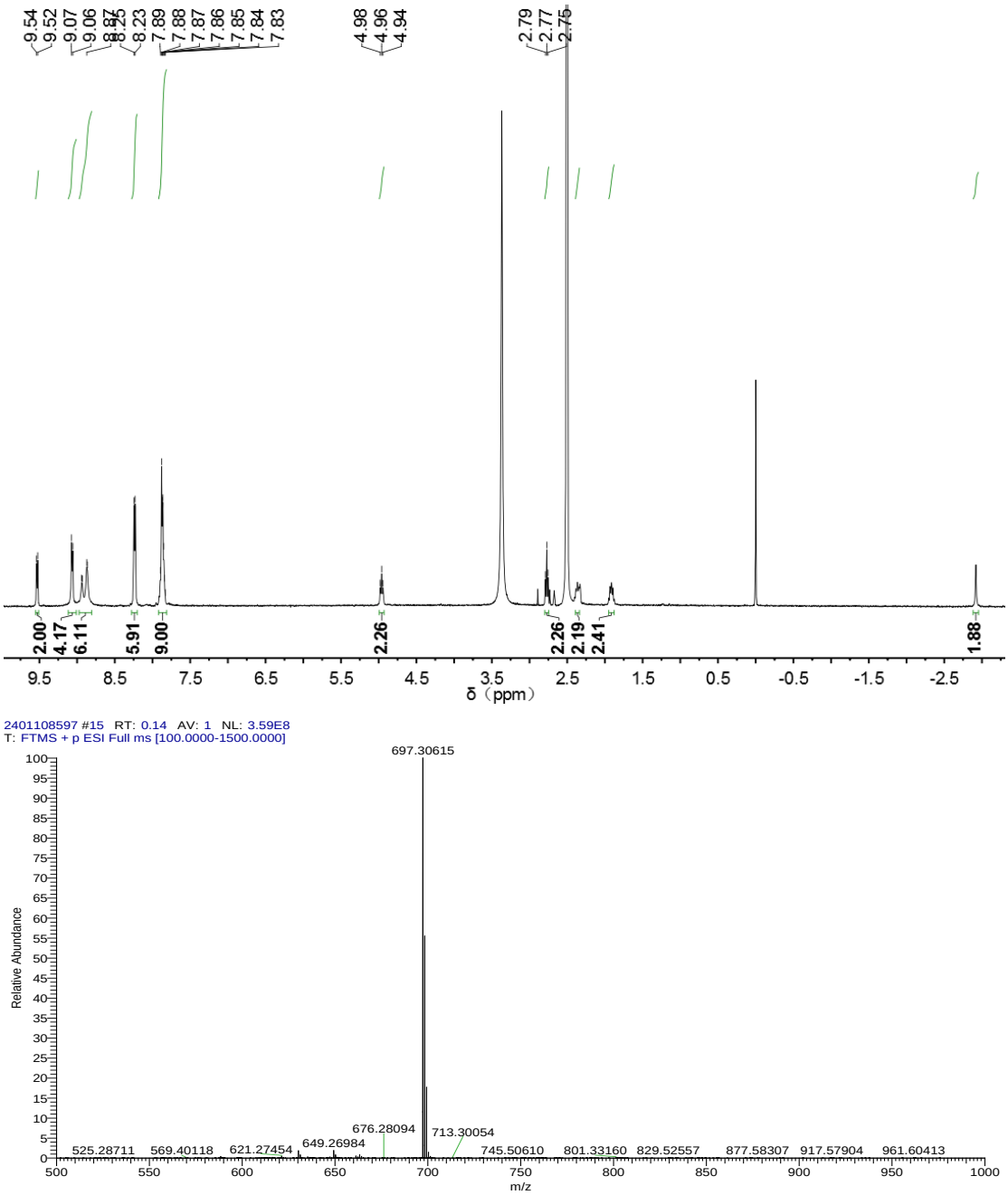

Fig. S4. The ^{1}H NMR (DMSO-d6, 293 K) and HRMS spectra for Py-Por

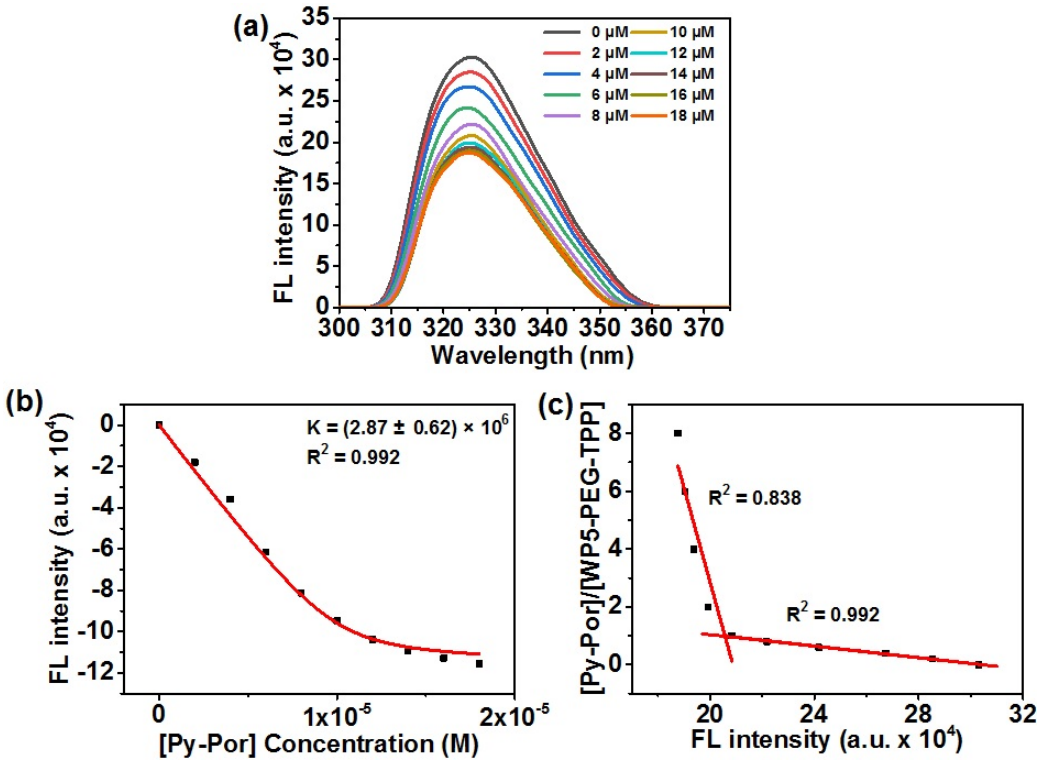

Fig. S5. (a) The fluorescence spectra of WP5-PEG-TPP with the addition of different concentrations of Py-Por. (b) The fitting curves of ΔF versus concentration of Py-Por. (c) The stoichiometry of host and guest complex.

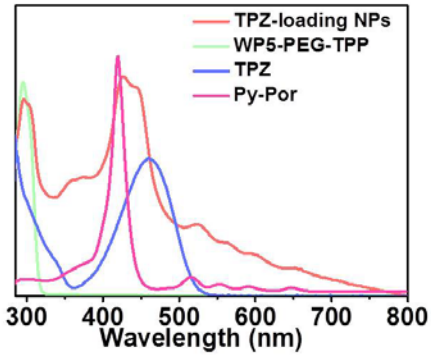

Fig. S6. The UV-vis absorption spectra of TPZ-loading NPs, WP5-PEG-TPP, TPZ and Py-Por.

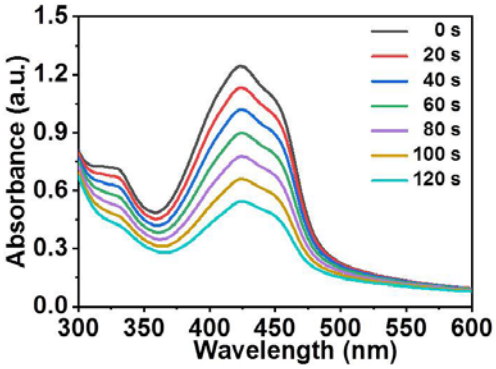

Fig. S7. The UV-vis absorption spectra of the mixture of DPBF and TPZ-loading NPs aqueous solution (25 μg/mL) after laser irradiation (660 nm, 1.0 W/cm^2) of different time.

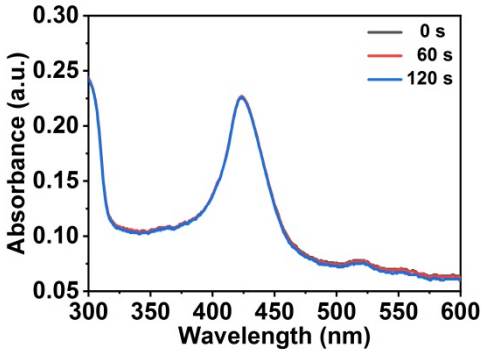

Fig. S8. The UV-vis absorption spectra of the TPZ-loading NPs aqueous solution (25 μg/mL) before and after laser irradiation (660 nm, 1.0 W/cm^2).

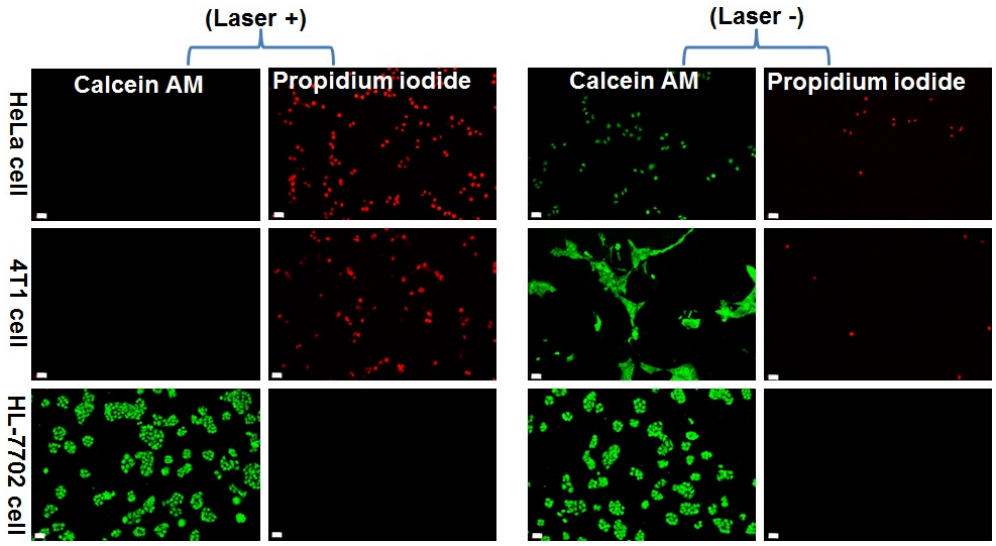

Fig. S9. The staining images of live and dead HeLa, 4T1 and HL-7702 cells received different treatments using calcein AM (green) as live cells staining dyes and propidium iodide (red) as dead cells staining dyes (Scale bar: 50 μm).

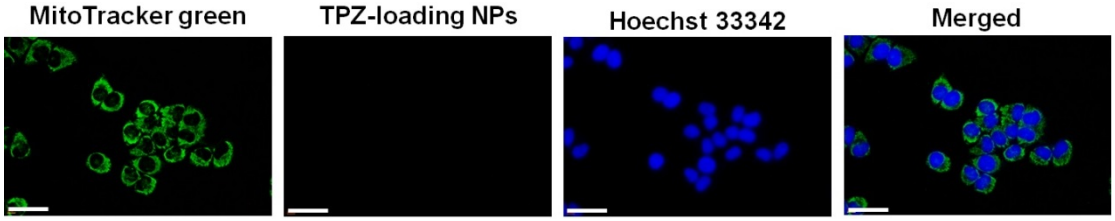

Fig. S10. The confocal images of HL-7702 cells incubated with TPZ-loading NPs aqueous solution (25 μg/mL) by using Hoechst 33342 to stain cell nucleus and MitoTracker green to stain mitochondria (Scale bar: 50 μm).

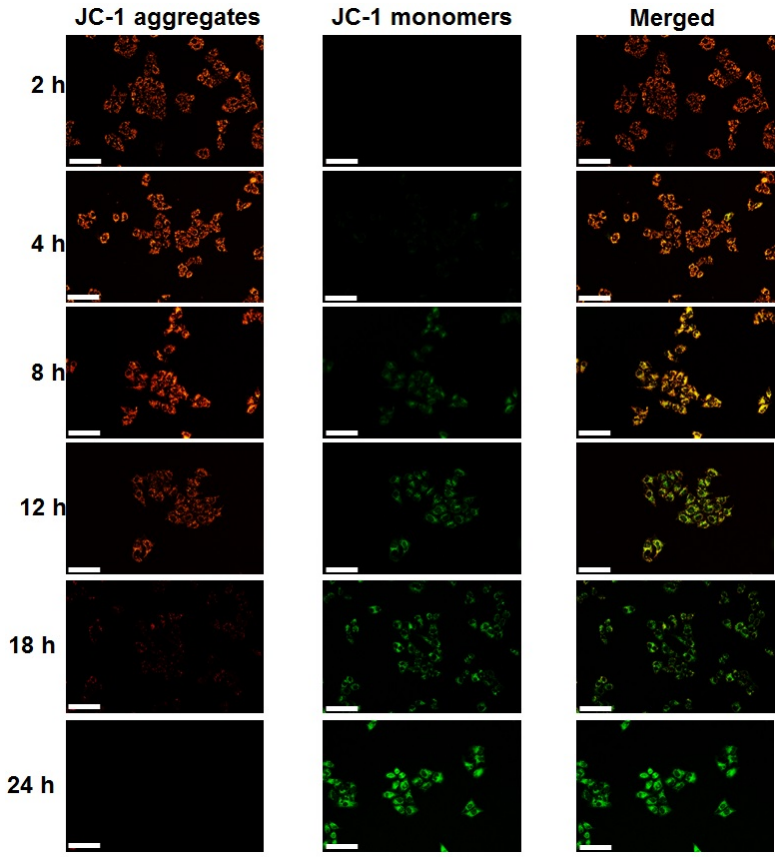

Fig. S11. The changes of mtitochondrial membrane potential for HeLa cells after the chemo/photodynamic therapy of different time (Scale bar: 50 μm).

S1 Xia B, Xue M, *Chem. Commun*., 2014, 50, 1021−1023.

S2 Ferro S, Ricchelli F, Monti D, Mancini G, Jori G, *Int. J. Biochem. Cell B*, 2007, 39, 1026−1034.

S3 Lethesh K C, Hecke K V, Meervelt L V, *J. Phy. Chem. B*, 2011, 115, 8424−8438.