Supporting Information for:

One-Component Supramolecular Filament Hydrogels as Theranostic Label-Free Magnetic Resonance Imaging Agents

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S1. Synthesis and molecular characterization

S1.1 Materials

Fmoc amino acids and Fmoc-Glu(OtBu)-Wang resin were purchased from Advanced Automated Peptide Protein Technologies (AAPPTEC, Louisville, KY). Pemetrexed (**Pem**) was purchased from AvaChem Scientific (San Antonio, TX). Lauric acid was purchased from Fisher Scientific (Pittsburgh, PA). Hydrochloric acid was purchased from EMD Millipore (Billerica, MA), and sodium hydroxide was purchased from Fisher Scientific (Fair Lawn, NJ).

S1.2 Electrospray ionization-mass spectra (ESI-MS)

ESI mass spectra were acquired using a Finnigan LCQ Deca ion-trap mass spectrometer equipped with an electrospray ionization source (Thermo Finnigan, San Jose, CA). Samples were dissolved in water with 5% of acetonitrle containing 0.1% v/v NH_4OH and introduced into the instrument at a rate of 10 μL/min using a syringe pump via a silica capillary line. The heated capillary temperature was 250 degC and the spray voltage was 5kV.

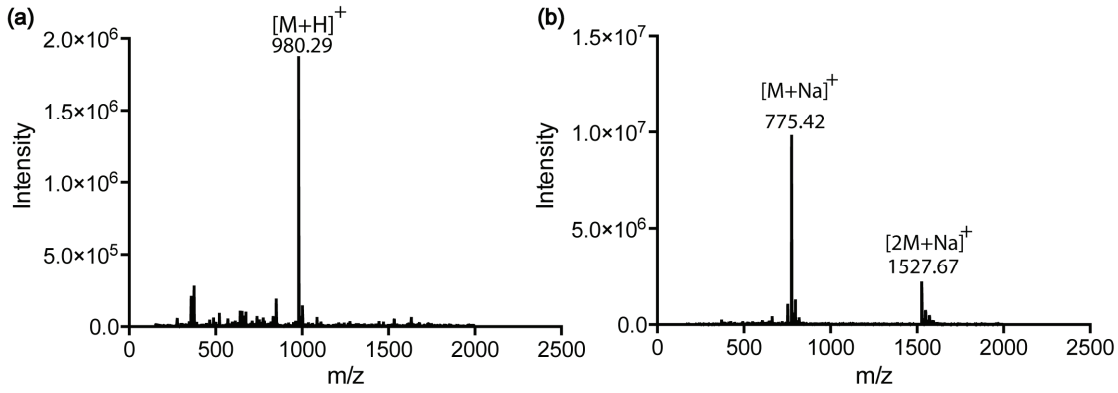


Figure S1. ESI-MS of (a) **PemFE** and (b) **C12FE** molecules**.**

S1.3 Analytical HPLC characterization

Analytical RP-HPLC was performed using a Varian polymeric column (PLRP-S, 100 Å, 10 µm, 150 × 4.6 mm) with 20 µL injection volume. A water/acetonitrile gradient 5%-100% was ran for 30 minutes containing 0.1% v/v NH_4OH at a flow rate of 1 mL/min.

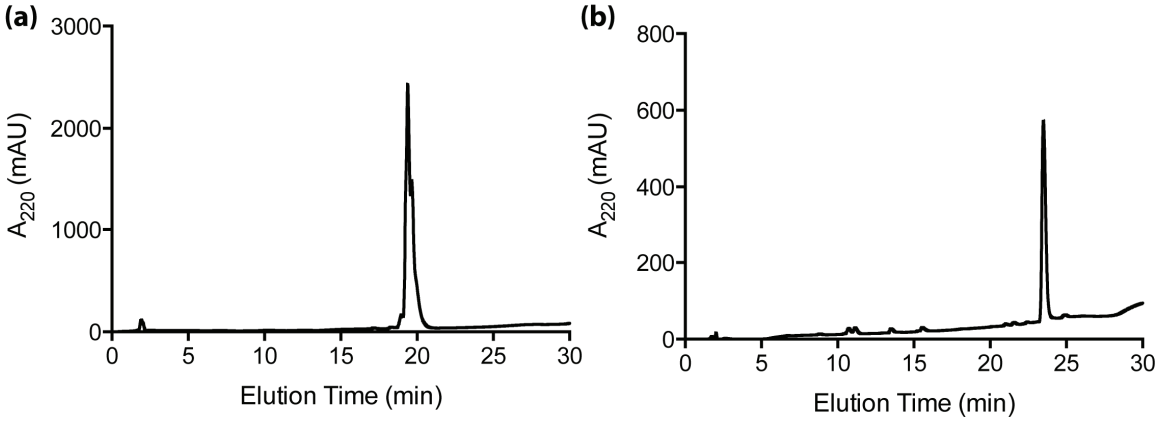
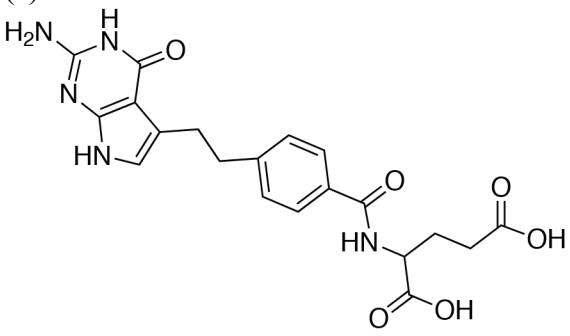


Figure S2. Analytical RP-HPLC of (a) **PemFE** and (b) **C12FE. PemFE** isomer resulted in a shoulder peak in plot (a).

S1.4 Chemical structures of Pem, PemFE isomer and C12FE

(a)



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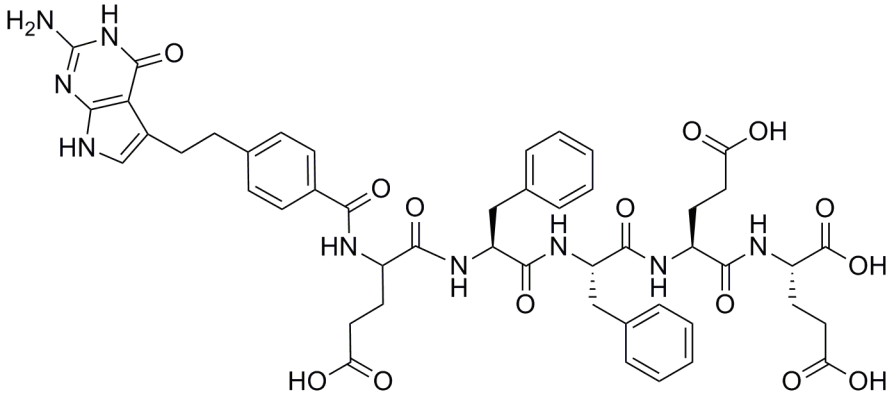

(c)

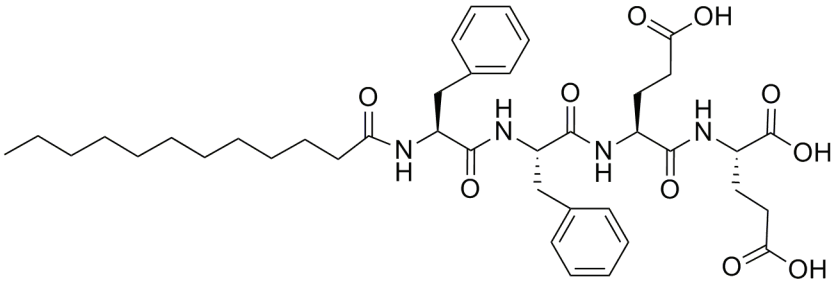

Figure S3. Chemical structure of (a) **Pem**, **PemFE** Isomer, and (c) **C12FE**.

S1.5 Drug loading for PemFE

Pem mass = 427.4 g/mol – 17 g/mol (-OH) = 410.4 g/mol
PemFE mass = 979.3 g/mol

PemFE drug loading = $410.4/979.3 \times 100\% = 41.9\%$

S2. Self-assembly characterization protocols

S2.1 Transmission electron microscopy (TEM) imaging protocol

4 mM solutions of **C12FE** and **PemFE** in 1xDPBS were prepared by directly dissolving lyophilized powders, and allowed to age overnight. A sample for imaging was prepared by depositing 5 μL of the solution onto a carbon-coated copper grid (Electron Microscopy Services, Hatfield, PA, USA), wicking away the excess solution with a small piece of filter paper. Next, 5 μL of a 2 wt % aqueous uranyl acetate solution was deposited and the excess solution was carefully removed as above to leave a very thin layer. The sample grid was then allowed to dry at room temperature prior to imaging. Bright-field TEM imaging was performed on a FEI Tecnai 12 TWIN Transmission Electron Microscope operated at an acceleration voltage of 100 kV. TEM images were recorded by a 16 bit 2K × 2K FEI Eagle bottom mount camera.

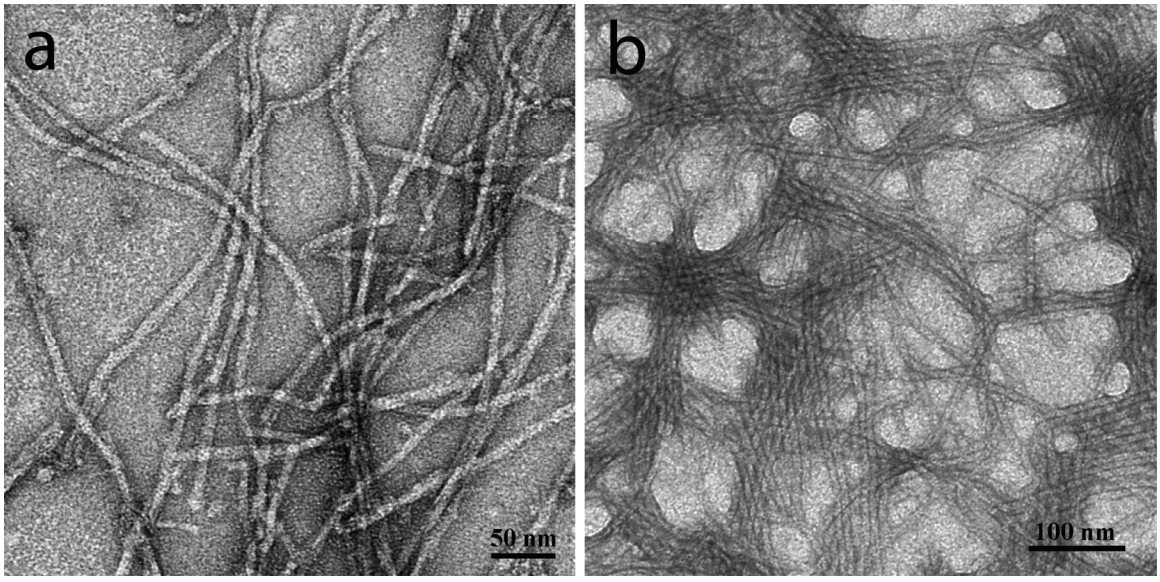

Figure S4. TEM micrographs of (a) **PemFE** (4mM) and (b) **C12FE** (10mM) dissolved in 1xDPBS, aged 1 day. **PemFE** and **C12FE** respective diameters are 9.1 ± 1.4 nm and 8.5 ± 0.9 nm.

S2.2 Cryogenic TEM protocol

To visualize nanostructures in its solution-state through a vitrified thin-film, cryo-TEM imaging was performed on the FEI Tecnai 12 TWIN Transmission Electron Microscope, operating at 80 kV. **C12FE** and **PemFE** samples were prepared at 4mM in PBS, the same concentration as conventional TEM. Prior to sample preparation, all TEM grids were treated with plasma air to make the lacey carbon film hydrophilic. The Vitrobot with a controlled humidity chamber (FEI) was used to generate a thin film of sample on grid. The lacey carbon grid (Electron Microscopy Services, Hatfield, PA) was blotted and plunged into liquid ethane pre-cooled by liquid nitrogen. All vitrified samples were transferred to a cryo-holder filled with liquid nitrogen to prevent sublimation of vitreous water. The images were recorded by a 16 bit 2K × 2K FEI Eagle bottom mount camera.

S3. Cytotoxicity assay and drug release experiments

S3.1 Cytotoxicity assay

The cytotoxicity of **PemFE**, **C12FE** and free pemetrexed was evaluated on a mouse glioma cell line (GL-261). Cells were seeded into 96-well plates at 1500 cells/well in the medium DMEM containing 10% FBS and 1% antibiotics solution (penicillin and streptomycin), and incubated overnight in 37 ^{o}C incubator with 5% CO_2 (Oasis CO_2 incubator, Caron, Marietta, OH).The three molecules with different concentrations, 0.01 μM, 0.1 μM, 0.5 μM, 1 μM, 2 μM, 4 μM, 10 μM and 50 μM were added to corresponding well with final volume of 200 μL. All samples were triplicated. After 4 days of incubation in 37 ^{o}C, cell cytotoxicity was evaluated using SRB based (Sigma- Aldrich, MO) in vitro Toxicology Assay Kit, and procedures were carried as described in manufacturer's protocol. The IC_{50} (the half maximal inhibitory concentration) values for each molecule were obtained by fitting the data using the non-linear regression (curve fit) analysis functions with Prism software.

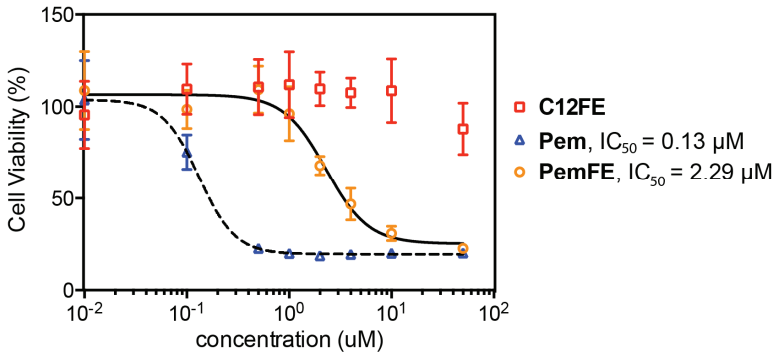

Figure S5. *In vitro* dose-response relationship study of the **PemFE**, **C12FE**, and free **Pem** molecules against mouse glioma GL-261 cells.

S3.2 Drug release experiments

The release of **PemFE** from its hydrogel form was evaluated using RP-HPLC. A certain amount of **PemFE** owder was added to 200 µl of a PBS solution to reach a final concentration of 30 mM. After gelation, another 100 µl PBS solution was added onto the top of the hydrogels. The mixture was then incubated at 37 °C in a water bath. Samples were collected at a series of desired time points and stored at −30 °C until HPLC analysis. The amounts of released **PemFE** were determined by measuring the peak areas of the respective signals at 220 nm. Data were plotted as a percentage of the total **PemFE** concentration.

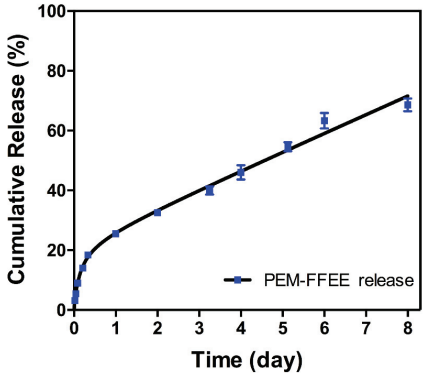

Figure S6 Cumulative drug release of **PemFE** from its hydrogel of 30 mM in a phosphate buffer (pH 7.4, 37 °C). The data are given as mean ± s.d. (n = 3).

S4. In vitro MRI CEST measurement

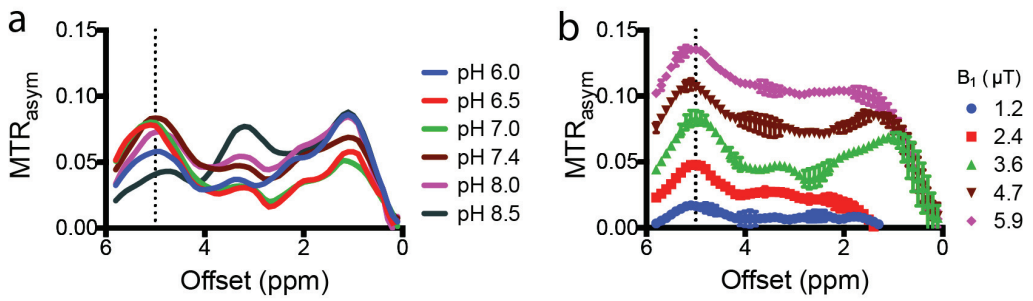

Figure S7. The MTR_{asym} plots of **PemFE** a) at different pH (using a B_1=3.6μT), and b) acquired using different B_1 values (pH=7.4). The vertical line shows the offset at 5.2 ppm.

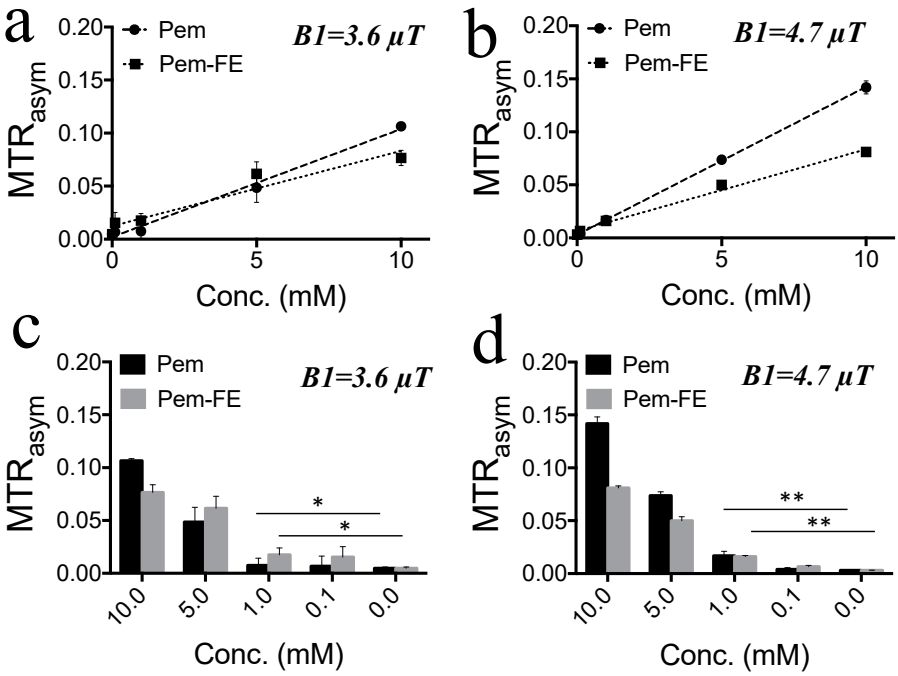

Figure S8. *In vitro* measurement of the CEST MRI detectability of **PemFE** filaments. **a**) and b) The concentration dependence of CEST MRI signal (MTR_{asym} at 5.2 ppm) of **Pem** and **PemFE** filaments at concentrations ranging from 100 μM to 10 mM were measured using a saturation pulse with its B1= 3.6 and 4.7 μT respectively. **c**) and **d**) The corresponding bar plots suggest that a CEST MRI detection sensitivity of < 1 mM could be accomplished at both B1 levels. * P< 0.05 and ** P< 0.01. Statistical analysis was performed using a two-tailed Student's t-test. The P values are 0.012 and 0.0048 between 1 mM **Pem** and PBS solution for B1= 3.6 and 4.7 μT respectively; 0.025 and <0.0001 between 1 mM **PemFE** and PBS solution for B1= 3.6 and 4.7 μT respectively. At both B1 levels, all P values between 100 μM samples and PBS solution are > 0.05 except **PemFE** at B1= 4.7μT (P=0.009).

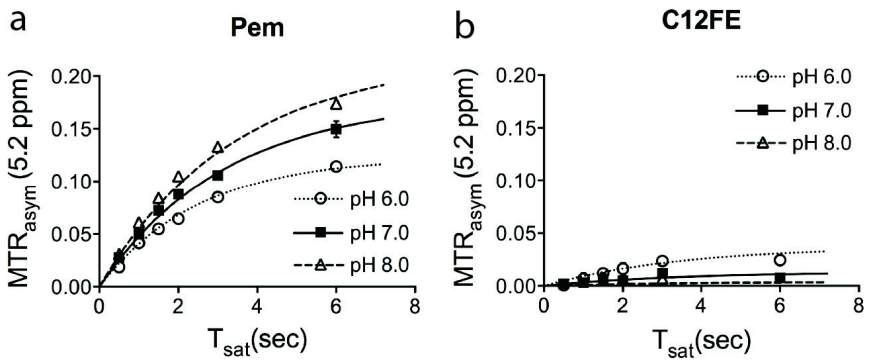

Figure S9. The effect of pre-saturation time on MTR_{asym} at 5.2 ppm offset for (a) **Pem** and (b) **C12FE**. Data were fitted by QUEST method for different pH values as shown by dotted lines (pH 6.0), solid lines (pH 7.0) and dashed line (pH 8.0).