Supplementary Information

TEM Images

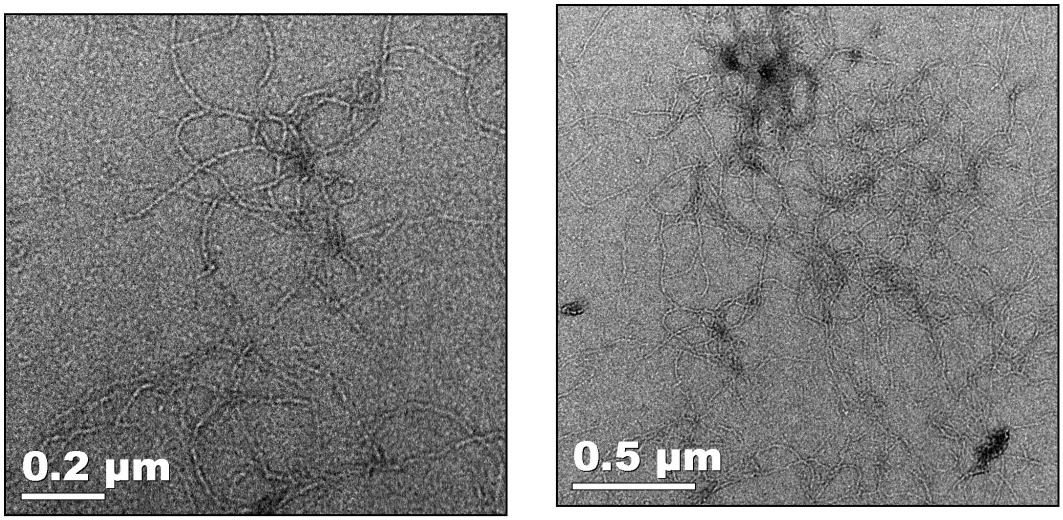

Figure S1 – Left: 0.5wt% MAX8 without vincristine Right: 0.5wt% MAX8 with 100µmol/L of vincristine encapsulated. The TEM images show that the addition and presence of the drug does not significantly alter the local structure of the hydrogel consistent with the SANS results.

MAX8 Synthesis Details

The methods used for solid phase peptide synthesis and purification have been discussed in previous publications (e.g. Haines-Butterick, et al., PNAS, vol. 104, 2007, pp 7791–7796,

supplemental information). The specific protocol for amino acid addition is the same as previously published using RINK amide resin and standard Fmoc-protocol and HCTU activation. However, the following description of double coupling and capping during the solid phase peptide synthesis may provide additional guidance for researchers looking to synthesize MAX8 peptides in their own laboratories.

The charged nature of lysine and glutamic acid, the hydrophobic nature of valine, and the overall length of the MAX8 peptide requires double coupling during the solid phase synthesis in order to ensure that the intended next amino acid attaches to the growing peptide chain. Double coupling repeats the amino acid solution addition during the synthesis protocol to better ensure a desired amino acid addition occurs and that sequences with deleted/missing amino acids are minimized. The second addition provides for unbound chain ends to be re-introduced to the desired amino acid for coupling, helping to ensure the desired product. If even a double coupling step does not succeed in adding a desired amino acid to the growing chain, then capping is used to seal the chain ends that were unsuccessfully reacted with the desired amino acid. Capping ensures that if the intended amino acid does not attach to a chain end, subsequent amino acids will not attach to the chain either, removing the chance for similar, but improper, sequences.

V K V K V K V K V ^{D}P P T K V E V K V K V

The use of double coupling and capping reaction steps help ensure the successful synthesis of the desired MAX8 sequence with simple separation of capped, shorter peptide impurities. The MAX8 amino acid sequence is shown above with standard abbreviations: V for valine, K for lysine, P for proline, ^{D}P for d-proline, T for threonine, and E for glutamic acid. During a typical MAX8 synthesis, the yellow, underlined amino acids are added once for coupling during peptide growth and have a capping solution added after the respective single amino acid addition. Blue amino acids are also added once for coupling without any subsequent capping solution addition. Black, double underlined amino acids are double coupled (added twice) and then subsequently capped. Capping solutions are 5% acetic anhydride in N-Methyl-2-pyrrolidone (NMP). While the above protocol is one that is successful, slight alterations of double coupling and/or capping steps (as well as different activation agents, solvents, etc.) may be found to also be successful depending on individual laboratory protocols, solid phase synthesizer, and individual user.

MAX8 Purification

After the MAX8 peptide sequence is created, the resin bound peptides are dried, cleaved, and side-chain protected using a trifluoroacetic acid (TFA): thioanisole: ethanediol: anisole (90:5:3:2) cocktail. After ether precipitation and subsequent filtration, the crude peptide is lyophilized and then purified by reverse phase HPLC. The crude peptides are dissolved in water (1mg/mL), and a flow rate of 8mL/min was used. The elutants used were solvent A (0.1% TFA in water) and solvent B (90% acetonitrile, 10% water, 0.1% TFA). The elutants are held at 0% B for 2 minutes, then a there is a linear gradient from 0 to 24% B for minutes 2 to 5, and finally, ramped up to 100% for the remaining elution. Electrospray ionization mass spectrometry (ESI-MS) was used to test the purity of the eluted MAX8 product. The three major peaks seen in

Figure S2—1116.1, 744.6, 558.9, correspond to the calculated mass to charge ratio (m/z) for $[M+2H]^{2+}$ of 1116.5, $[M+3H]^{3+}$ of 744.6, and $[M+2H]^{2+}$ of 558.7, respectively.

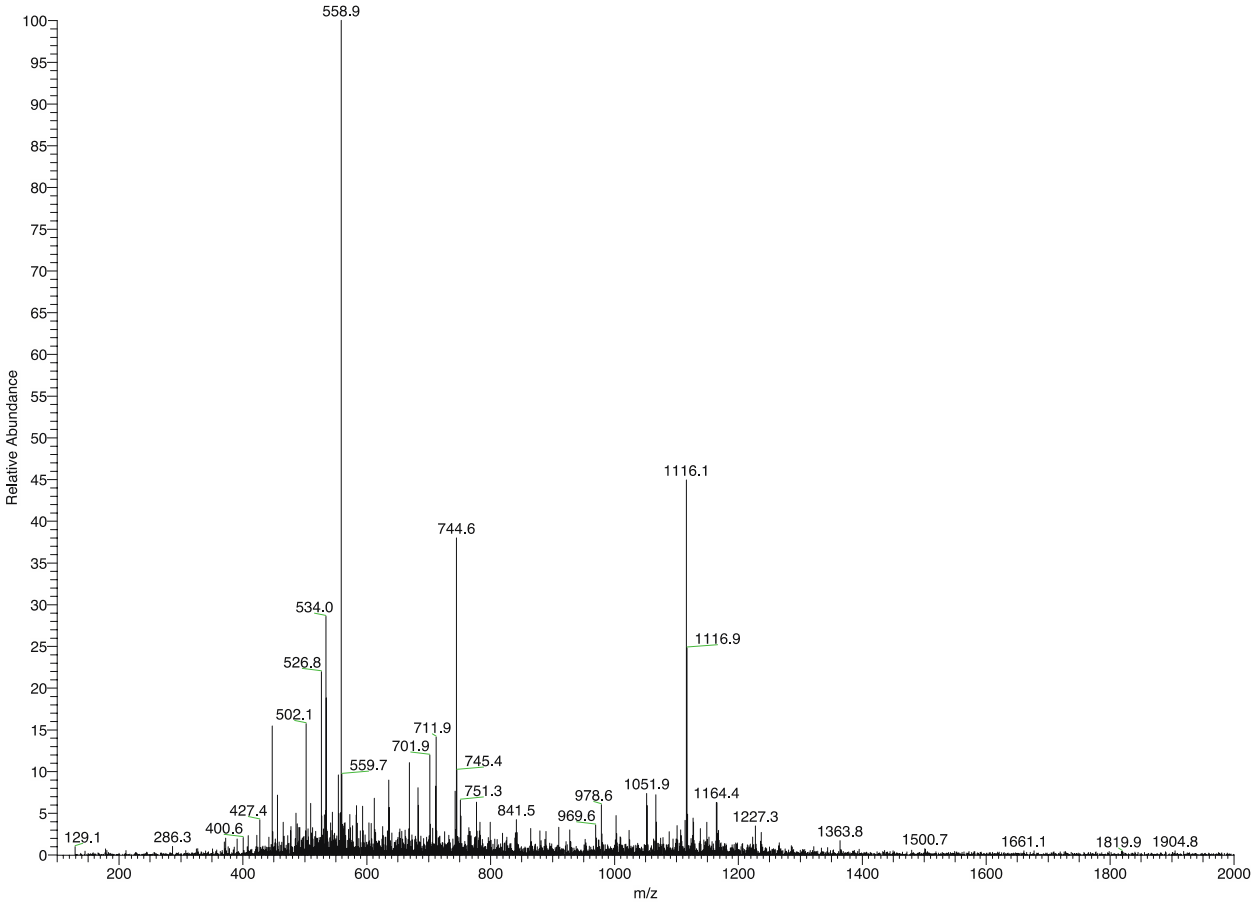

Figure S2 – ESI-MS of purified MAX8 from Figure S2.