Electronic Supplementary Information

Modulating Water-Responsive Actuation Energy of Regenerated Silk Fibroin via Tyrosine Modification

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1. *Water-Responsive (WR) Energy Density Calculation*

WR energy density was calculated as reported previously[1]. WR energy density was calculated by dividing the energy stored in the bilayer film by the volume of RSF. The stored energy, *U*, was determined by the following equation:

$$U = \frac{(E_1 I_1 + E_2 I_2)L}{R^2} \qquad (1)$$

where E_1 and E_2 are the Young's modulus of silk and polyimide, and I_1 and I_2 are their area moments of inertia, and *R* is radius of curvature of the film. The area moments of inertia for silk and polyimide layers are defined by the following equations:

$$I_1 = \frac{bt_1^3}{12} \qquad (2)$$

$$I_2 = \frac{bt_2^3}{12} + bt_2\left(\frac{t_2}{2} - H\right)^2 \qquad (3)$$

In these equations, t_1 refers to the thickness of the silk layer, b is the width of the film, t_2 is the thickness of the polyimide layer, and *H* indicates the location of the neutral axis of the substrate, described by the Stoney's equation as follows:

$$H = t_2 - E_1^2 t_1^4 + \frac{E_1 E_2 t_1 t_2^2 (3t_1 + 4t_2)}{6E_1 E_2 t_1 t_2 (t_1 + t_2)} \qquad (4)$$

2. *Calculation of Modification Degree of Tyrosine Residues*

The degree of the tyrosine modification was estimated from the UV-vis spectra by using the Beer's law[2]:

$$A_{325nm} = \varepsilon l c_{azo} \qquad (5)$$

where A_{325nm} is the absorbance at 325 nm corresponding to the azobenzene peak, ε is the molar extinction coefficient, l is the path length, and c_{azo} is the concentration of the azo species. We used a molar extinction coefficient of 22,000 M^{-1} cm^{-1}[3], and the path length of our quartz cuvette of 1 cm. Using 277 tyrosines per silk protein[4] and the known concentration of RSF in our solution, c_{RSF}, we can then calculate the percentage of tyrosines modified as follow:

$$\%\ tyrosines\ modified = \frac{c_{azo}}{277 c_{RSF}} * 100 \qquad (6)$$

3. *^{1}H NMR Spectra*

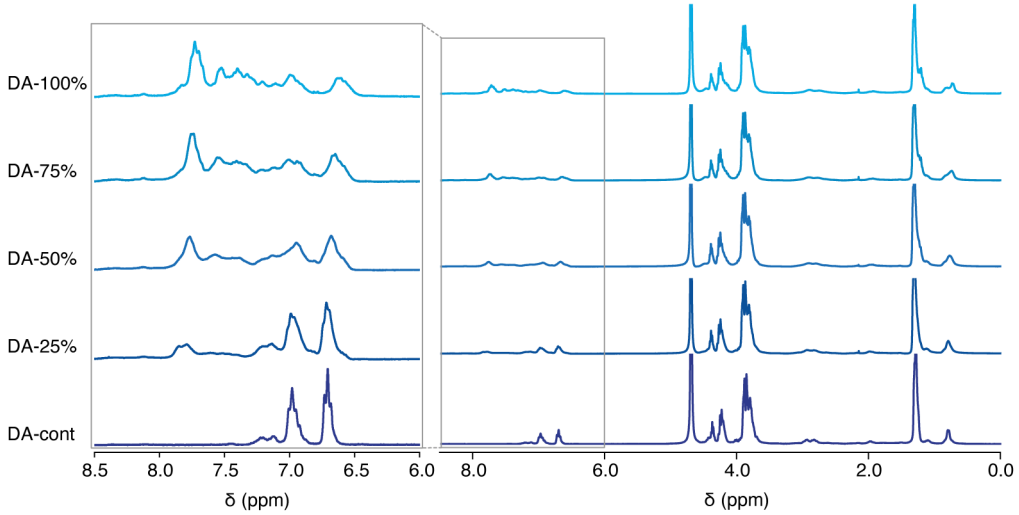

Figure S1. ^{1}H NMR spectra for DA-RSF. The right panel shows the spectra from 0.0 to 8.5 ppm with the left panel highlighting the aromatic region between 6.0 and 8.5 ppm. As the degree of modification increases the tyrosine peaks between 6.5 and 7.0 ppm shift upfield and broaden, while the new azo aromatic peaks appear downfield between 7.3 and 8.0 ppm.

4. FTIR Amide I Peak Deconvolution

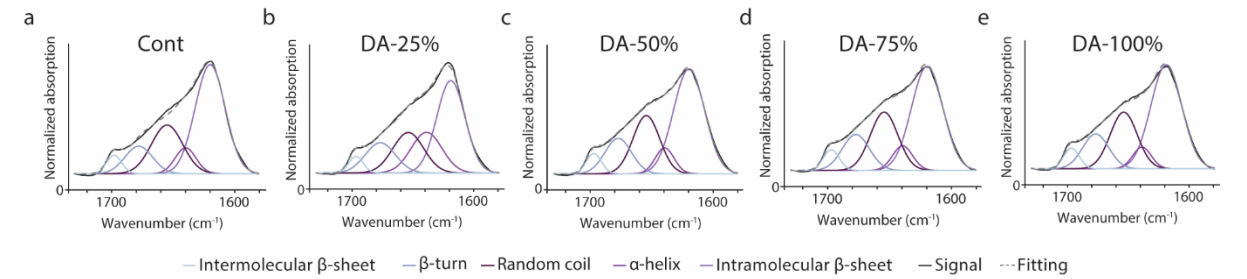

Figure S2. Amide peak deconvolution for a) Control b) DA-25% c) DA-50% d) DA-75%, and e) DA-100%.

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