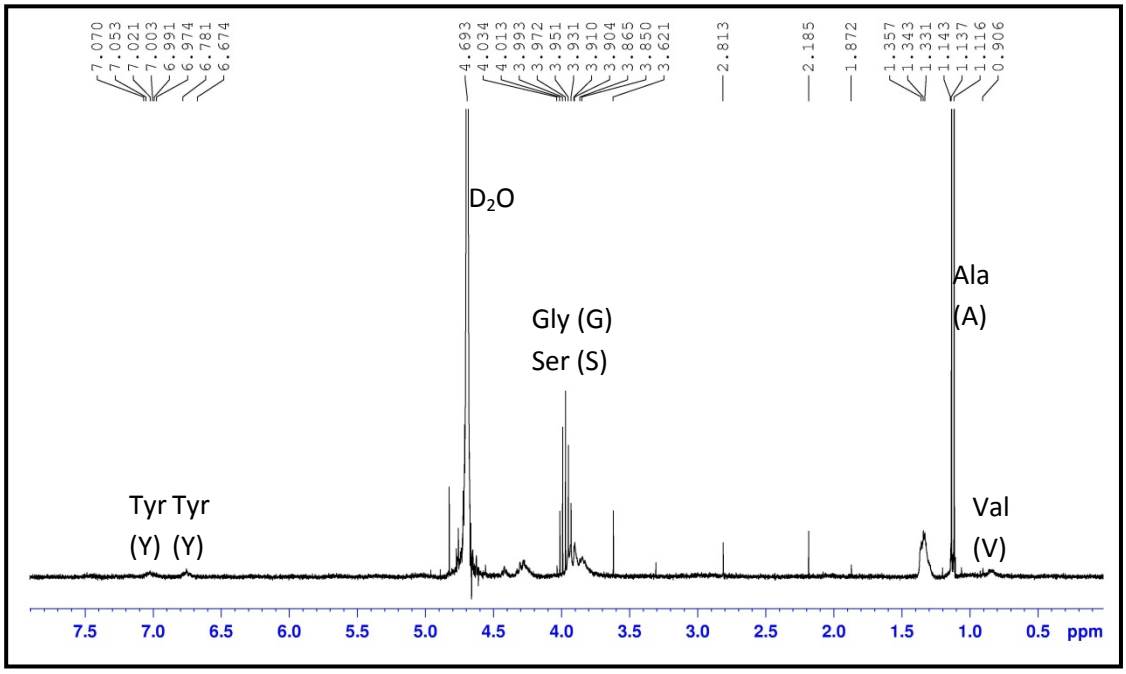
Supporting information

1. NMR analysis of BMWS and regenerated SF in D_2O

Experimental:

Silk fibroin from the raw silk and BMWS powder were analysed to confirm the structure of the protein. The silk fibroin in the $CaCl_2/H_2O/EtOH$ solution was dialysed against distilled water for several days and centrifuged at 10,000 rpm for 20 minutes at room temperature. A small amount of solution was dissolved in D_2O. The BMWS powder was dissolved in deuterated water (D_2O) to a concentration of 0.95 %. ^1H NMR spectra were obtained on a 300 MHz BrukerAvance spectrometer operating at 300.138 MHz. Reference relative to 0 ppm for TMS. The predominant presence of glycine (Gly) and alanine (Ala) that makes up the structure of silk fibroin has been confirmed[1]. In addition, the presence of the tyrosine groups required for crosslinking has also been confirmed through the peaks in the spectra, which were correlated with literature values[1].



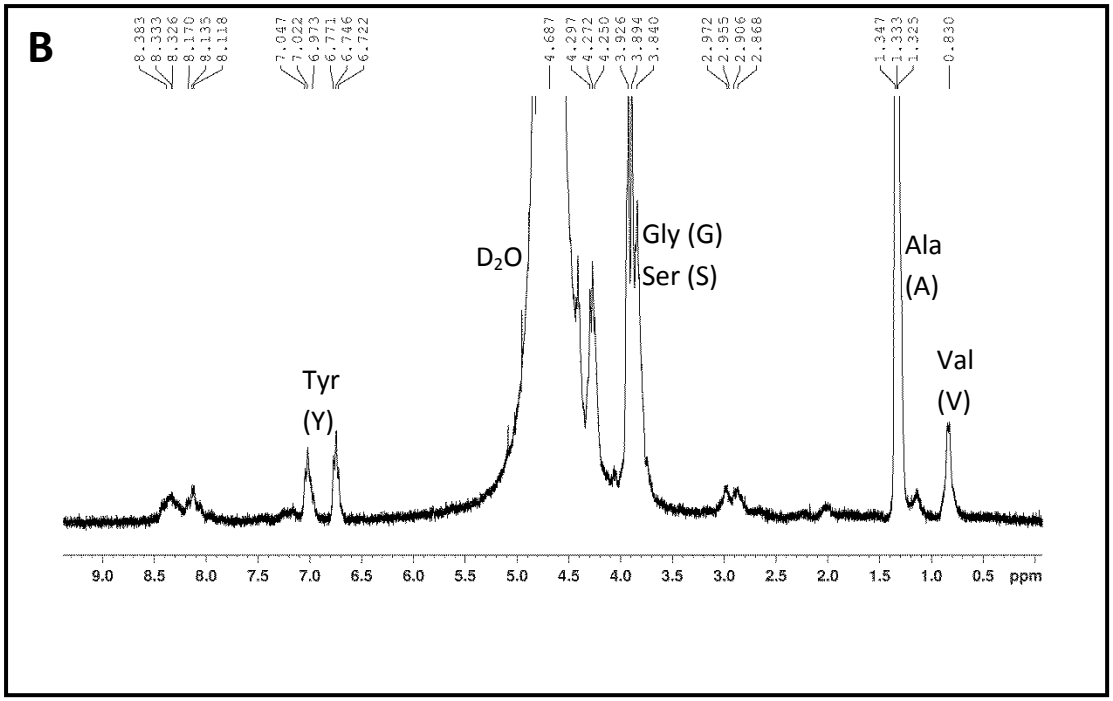


Figure S1: NMR spectrum of A) BMWS dissolved in D_2O; B) silk fibroin aqueous solution dissolved in D_2O (peak positions showing individual residues)[1].

2. Proposed reaction mechanism for the photodynamic crosslinking of silk fibroin

Figure S2 depicts the proposed reaction mechanism for the formation of dityrosine crosslinks tyrosine groups present in the silk fibroin (SF) chains. The $Ru(II)(bpy)_3^{2+}$ and APS compounds act as the catalyst and electron acceptor, respectively. This reaction is similar to our previous work in which the recombinant protein rec1-resilin was crosslinked via the same mechanism.[2]

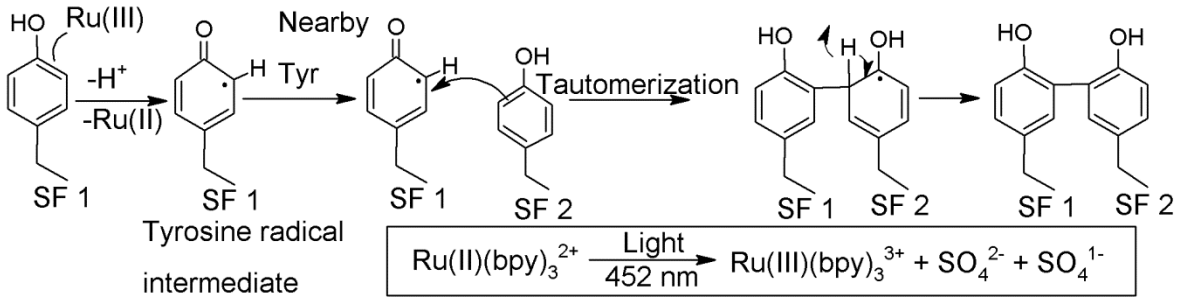


Figure S2: Proposed reaction mechanism for photodynamic crosslinking of silk fibroin

3. Fluorescence spectra indicating presence of dityrosine crosslinks

Experimental:

A sample gel was prepared with a BMWS concentration of 300 mg/ml, $Ru(II)(bpy)_3^{2+}$ concentration of 0.03 mM, and an APS concentration of 20 mM. A low concentration of $Ru(II)(bpy)_3^{2+}$ was used to prepare a gel that was transparent in order to be suitable for fluorescence measurements using the Cary Eclipse Fluorescence Spectrometer. At this concentration of $Ru(II)(bpy)_3^{2+}$, an extended crosslinking time (~12 hours) was required to form a gel. Figure 2 shows the fluorescence spectra of

the gel. The peak at ~405 nm in the emission spectra confirms the presence of dityrosine crosslinks in the gel structure[3].

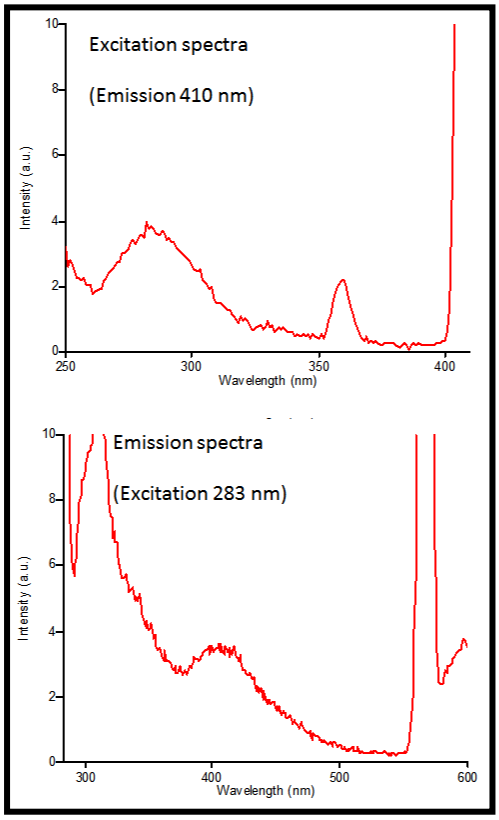


Figure S3: Fluorescence spectra of crosslinked gel.

4. UV-vis spectrometry of crosslinked dilute solution and gel

Experimental:

A Ru5APS28 gel sample was run in the Evolution 201 UV/VIS Spectrophotometer by cutting a thin strip and vertically placing it in the cuvette to obtain the spectra shown in Figure S2 A. This was compared to the spectra of the uncrosslinked silk solution. The Tyr present in the silk solution is evident with the peak occurring at 275 nm. The spectra of the gel shows a broad peak ranging from approximately 250 to 350 nm, indicating a range of structures involving tyrosine are present. It is known that the deprotonated absorption of dityrosine occurs at ~315 nm[3]. However, due to the noisy nature of the gel spectra it is difficult to identify the presence of dityrosine. Thus, a 1% silk solution was mixed with $Ru(II)(bpy)_3^{2+}$ (1 mM) and APS (20 mM) and exposed to light for 180 seconds and the UV-vis spectra was obtained (Figure S2 B). It was easier to identify the presence of dityrosine in the solution spectra with a peak evident at 313 nm, along with different forms of tyrosine at 288 nm and 301 nm. In addition, the presence of $Ru(II)(bpy)_3^{2+}$ in the gel and solution was also confirmed due to the peak at 452 nm. As this peak is present in the gel, which has been washed for several days, it indicates that the gel contains $Ru(II)(bpy)_3^{2+}$ that is bound to the silk molecules. This will be investigated further in the future using surface analysis techniques.

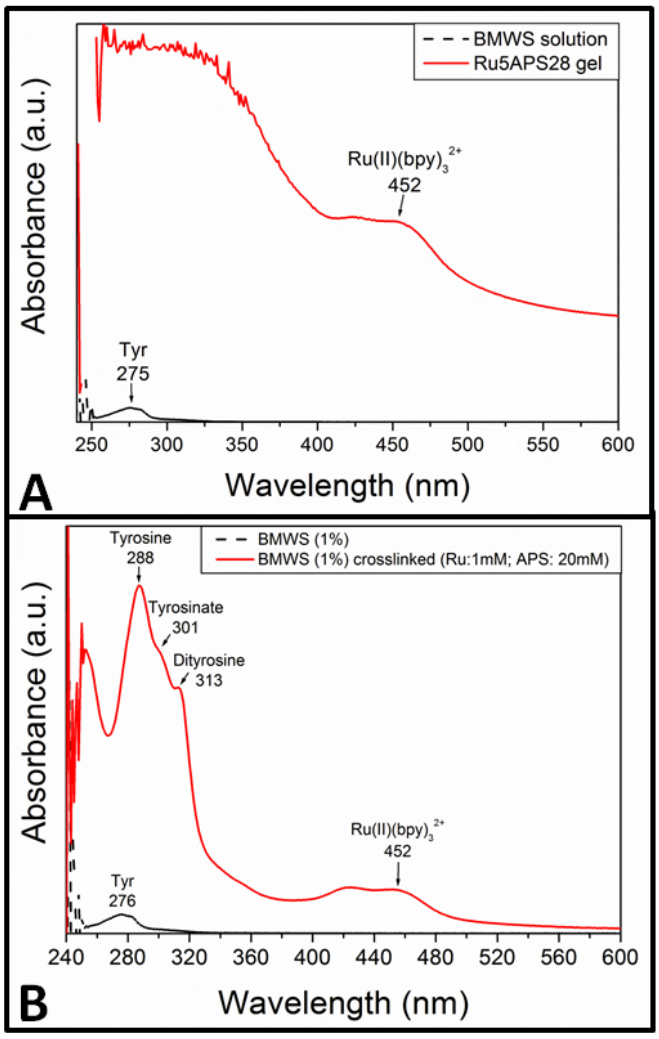


Figure S4: UV-vis spectra of A) Ru5APS28 gel and uncrosslinked silk solution; and B) crosslinked and uncrosslinked 1% silk solutions.

5. Determination of minimum $Ru(II)(bpy)_3^{2+}$ concentration required for crosslinking

Table S1: Ability of BMWS to crosslink at various $Ru(II)(bpy)_3^{2+}$concentrations (APS concentration remained at 20 mM).

$Ru(II)(bpy)_3^{2+}$ concentration (mM)	Crosslinking observations (crosslinking time: 180 seconds)
0.03	No crosslinking – remained as a solution.
0.105	Partially crosslinked – some gel formation, small portion remained in liquid state.
0.16	Crosslinked – gel fragile but able to be removed from mould after leaching for 1 day.
0.28	Crosslinked – gel fragile but able to be removed from mould after leaching for 1 day.
0.5	Crosslinked – gel remained intact when removed from mould.

From Table S1 it can be observed that a minimum $Ru(II)(bpy)_3^{2+}$concentration of 0.5 mM was required to form a gel that was able to support itself on removal from the mould. Crosslinking was

achieved with a Ru(II)$(bpy)_3^{2+}$concentration of 0.105 mM and above, however gels were flimsy and broke easily upon removal from the mould.

References:

1. Zainuddin; Le, T. T.; Park, Y.; Chirila, T. V.; Halley, P. J.; Whittaker, A. K., *Biomaterials* **2008,***29* (32), 4268-4274.
2. Truong, M. Y.; Dutta, N. K.; Choudhury, N. R.; Kim, M.; Elvin, C. M.; Nairn, K. M.; Hill, A. J., *Biomaterials* **2011,***32* (33), 8462-8473.
3. Correia, M.; Neves-Petersen, M. T.; Jeppesen, P. B.; Gregersen, S.; Petersen, S. B., *PLoS ONE* **2012,***7* (12), e50733.