Supporting information: Assessment of Shifted Excitation Raman Difference Spectroscopy in Highly Fluorescent Biological Samples

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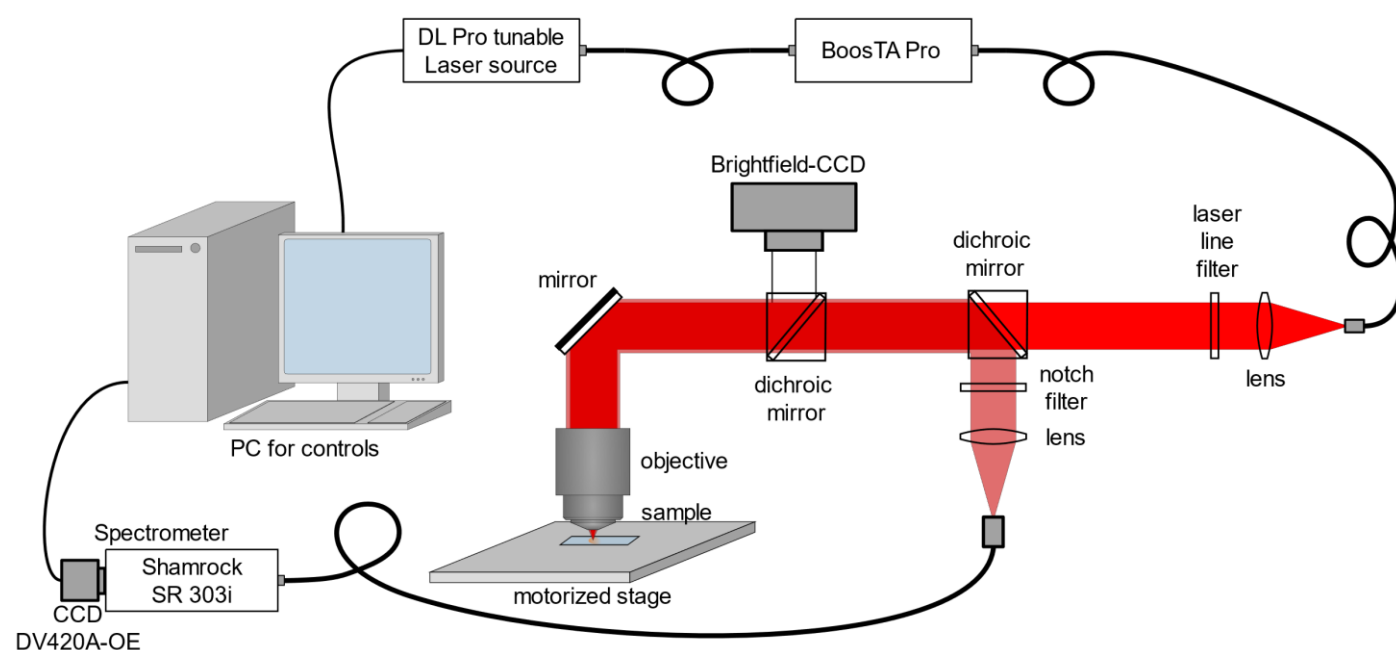c Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich Schiller University, 07743 Jena, Germany.

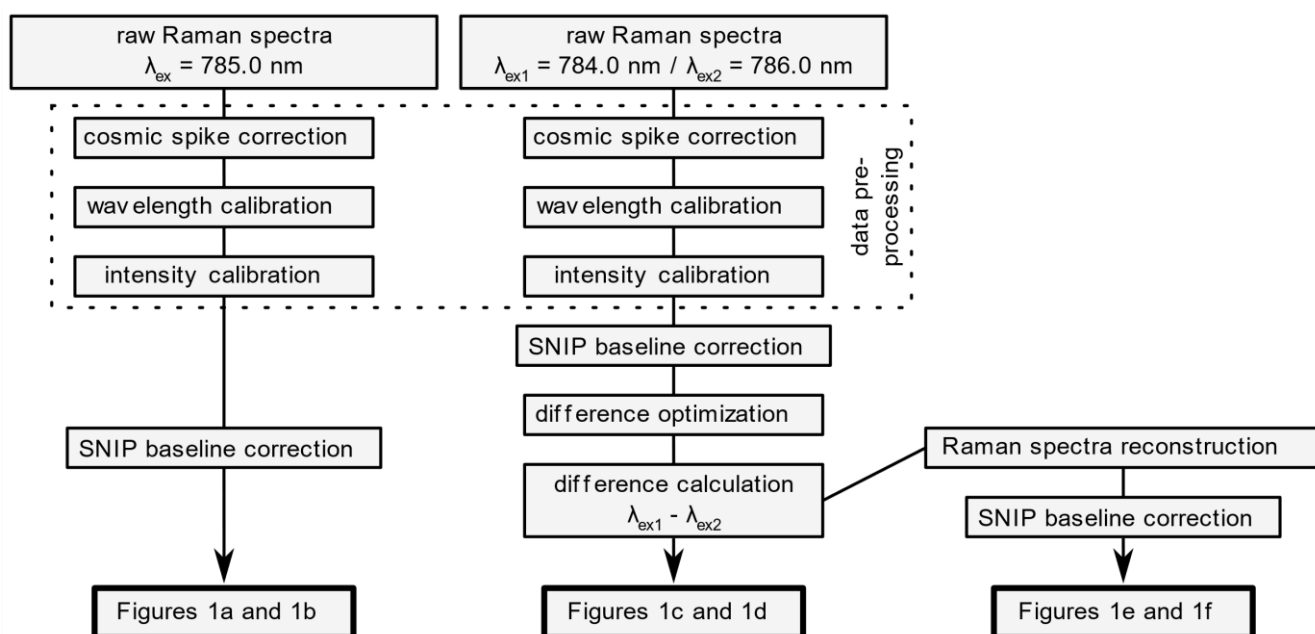*Figure S1. Schematic of the SERDS Setup.*

Figure S2. Overview of the necessary spectral preprocessing steps for the assessment of the SERDS method and the generation of Figure 1.
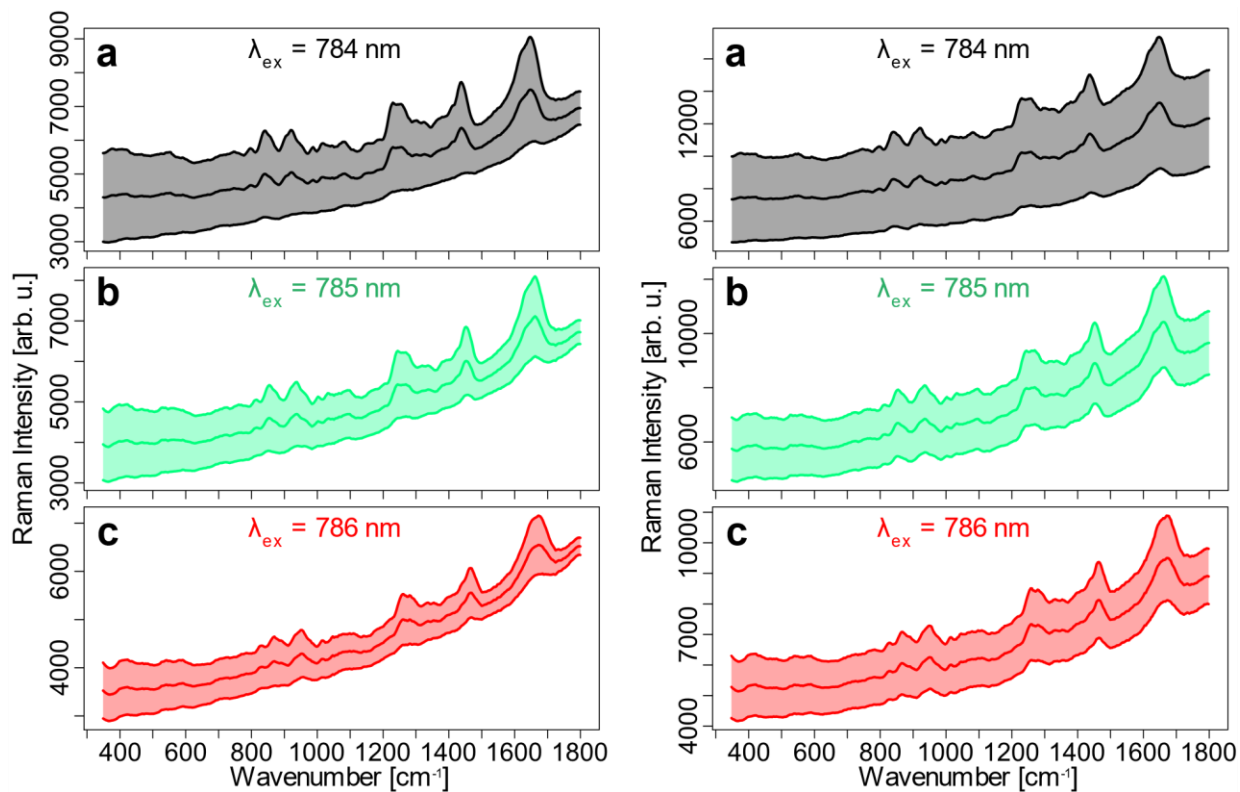
*Figure S3. Raw, calibrated mean Raman spectra (± standard deviation) of untreated control sample (left) and GE cross-linked EP after 0.5 h (right) measured at: **(a)** λ$_{ex}$ = 784 nm; **(b)** λ$_{ex}$ = 785 nm; **(c)** λ$_{ex}$ = 786 nm (Wavenumber axis relative to λ$_{ex}$ = 785 nm for **a**, **b**, **c**).*

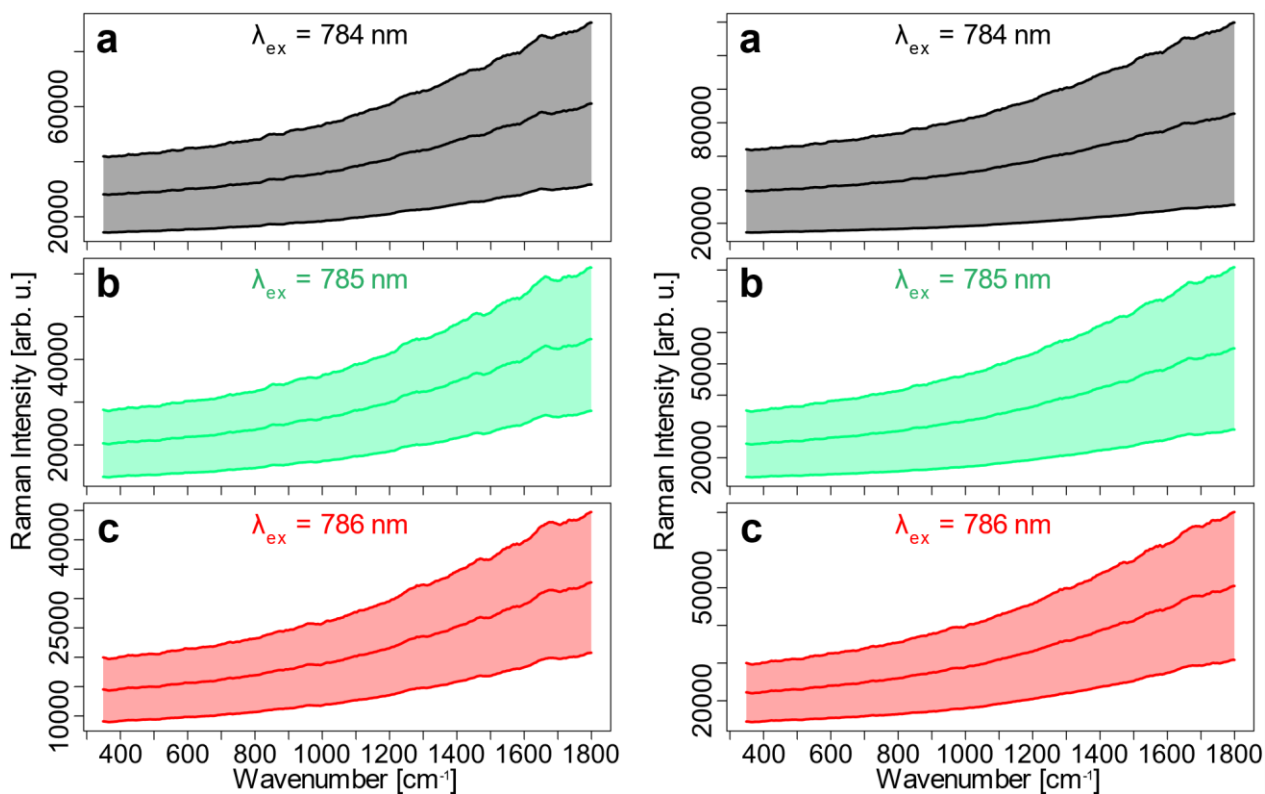*Figure S4. Raw, calibrated mean Raman spectra (± standard deviation) of the GE cross-linked EP after 2.0 h (left) and 4 h (right) measured at: **(a)** λ$_{ex}$ = 784 nm; **(b)** λ$_{ex}$ = 785 nm; **(c)** λ$_{ex}$ = 786 nm (Wavenumber axis relative to λ$_{ex}$ = 785 nm for **a**, **b**, **c**).*
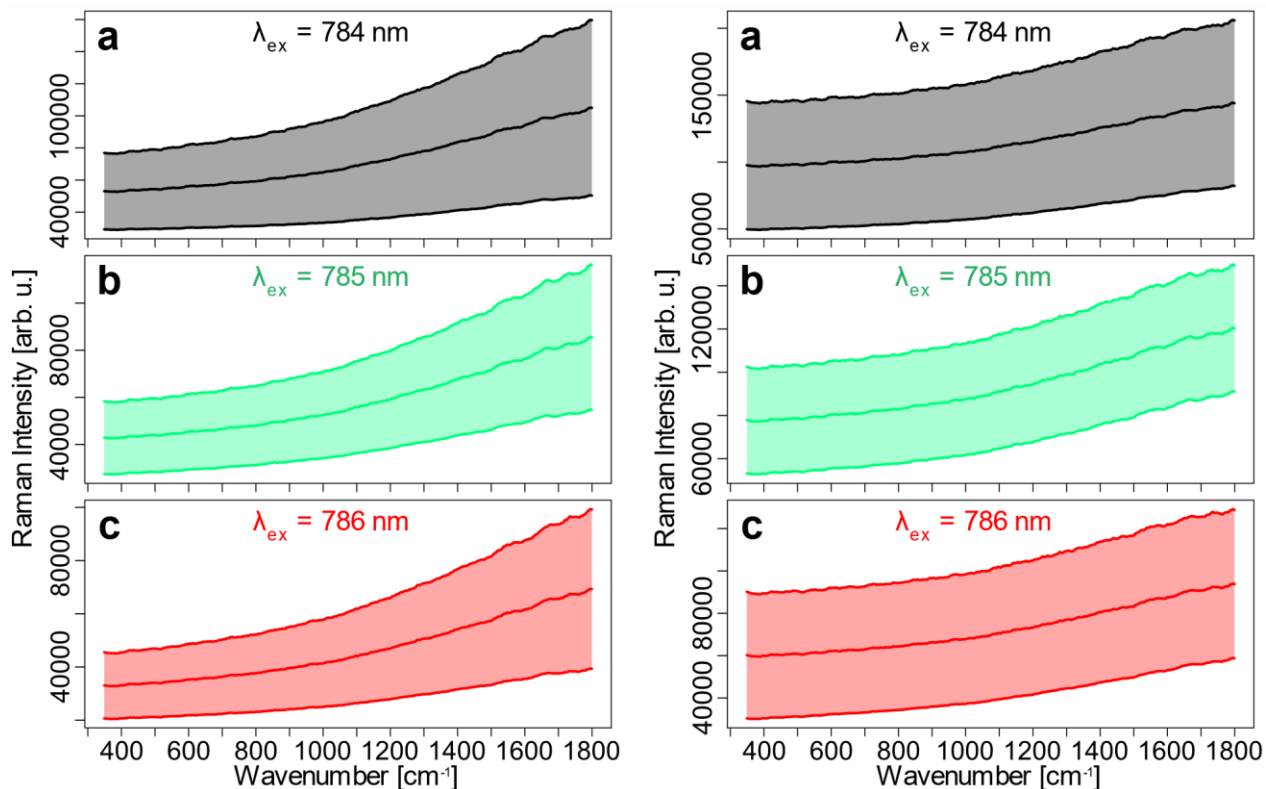
Figure S5. Raw, calibrated mean Raman spectra (± standard deviation) of the GE cross-linked EP after 6.0 h (left) and 12 h (right) measured at: (a) λ_{ex} = 784 nm; (b) λ_{ex} = 785 nm; (c) λ_{ex} = 786 nm (Wavenumber axis relative to λ_{ex} = 785 nm for a, b, c).
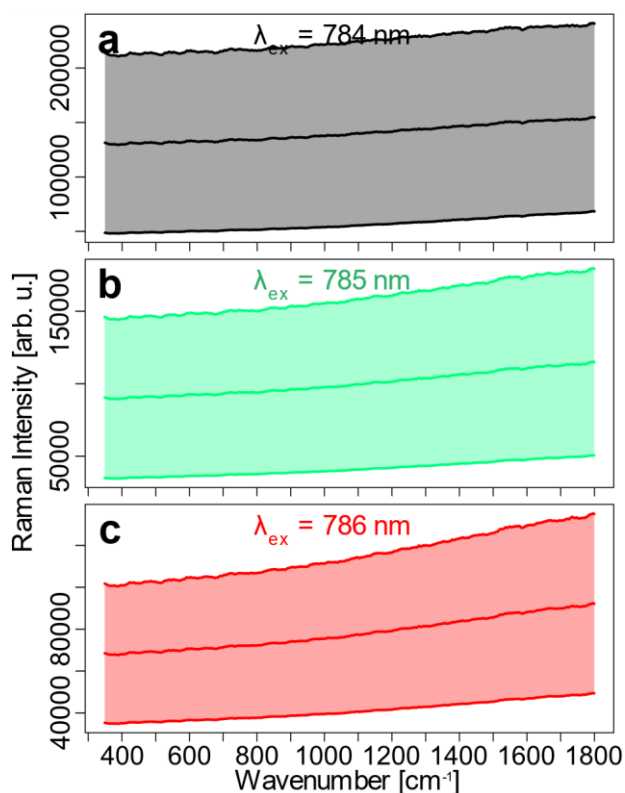

Figure S6. Raw, calibrated mean Raman spectra (± standard deviation) of the GE cross-linked EP after 24 h measured at: (a) λ_{ex} = 784 nm; (b) λ_{ex} = 785 nm; (c) λ_{ex} = 786 nm (Wavenumber axis relative to λ_{ex} = 785 nm for a, b, c).
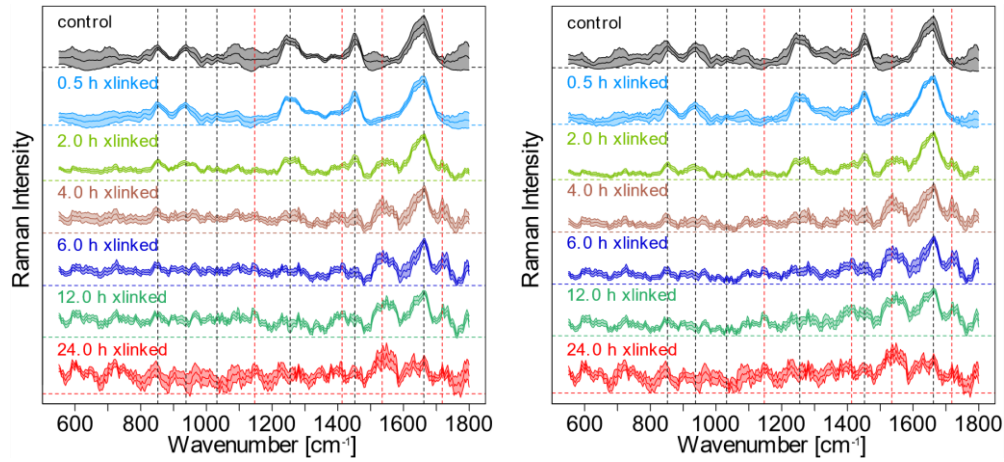
Figure S7. Mean EMSC (left) and rubberband (right) corrected Raman spectra (± standard deviation) of untreated and GE cross-linked EP (0.5 h, 2 h, 4 h, 6 h, 12 h and 24 h) measured at λ_{ex} = 785 nm, normalized on the highest intensity. Black dotted vertical lines correspond to collagen type I Raman bands and red dotted vertical lines correspond to Raman bands associated with cross-linking.

Figure S8. Mean polynomial (left) and SNIP (right) corrected Raman spectra (± standard deviation) of untreated and GE cross-linked EP (0.5 h, 2 h, 4 h, 6 h, 12 h and 24 h) measured at λ_{ex} = 785 nm, normalized on the highest intensity. Black dotted vertical lines correspond to collagen type I Raman bands and red dotted vertical lines correspond to Raman bands associated with cross-linking.
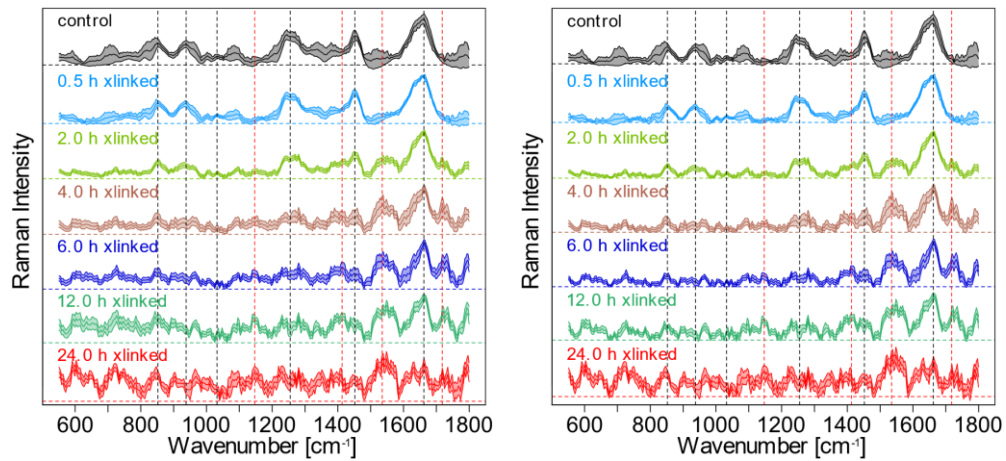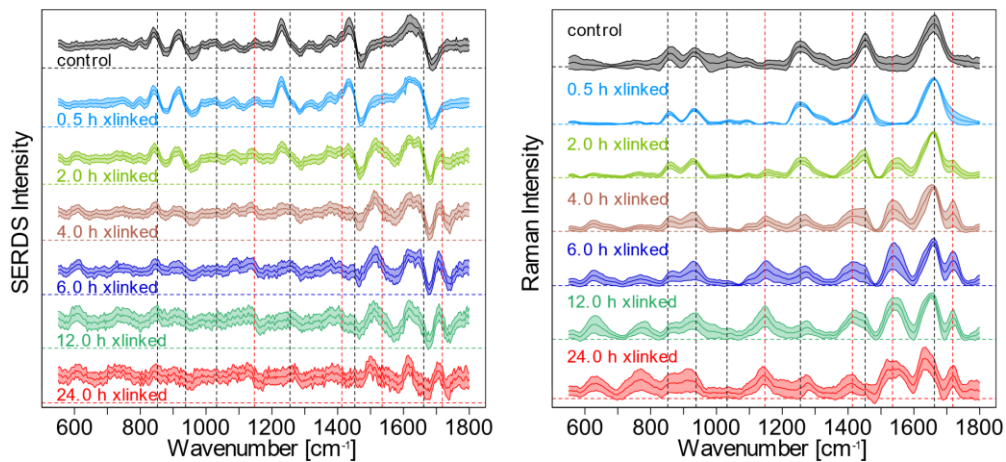

Figure S9. Mean SNIP corrected, difference optimized SERDS spectra (± standard deviation) of λ_{ex1} - λ_{ex2} = 784 nm - 786 nm (left) and mean reconstructed, SNIP corrected Raman spectra (± standard deviation) of the SERDS spectra on the left (right) for untreated and GE cross-linked EP (0.5 h, 2 h, 4 h, 6 h, 12 h and 24 h), normalized on the highest intensity. Black dotted vertical lines correspond to collagen type I Raman bands and red dotted vertical lines correspond to Raman bands associated with cross-linking.