Supporting Information for

Genetically Encoded Aryl Alkyne for Raman Spectral Imaging of Intracellular α-Synuclein Fibrils

Matthew D. Watson and Jennifer C. Lee*

Table S1. Raman shifts‡ of the F_{CC} alkyne stretching band in α-syn constructs in the soluble and aggregated states shown in **Fig. 4**.

Protein	Soluble ν_{max} (cm^{-1})	Fibrillar ν_{max} (cm^{-1})	$\Delta\nu_{max}$ (cm^{-1})
$F4F_{CC}$	2109.6	2108.9	−0.7
$Y39F_{CC}$	2109.3	2107.8	−1.5
$F94F_{CC}$	2108.9	2105.9	−3.0
$Y125F_{CC}$	2108.7	2107.7	−1.0

‡ Raman shifts were determined by fitting the alkyne stretching band to a Lorentzian function, which was found to have an accuracy of ±1 cm^{-1}.

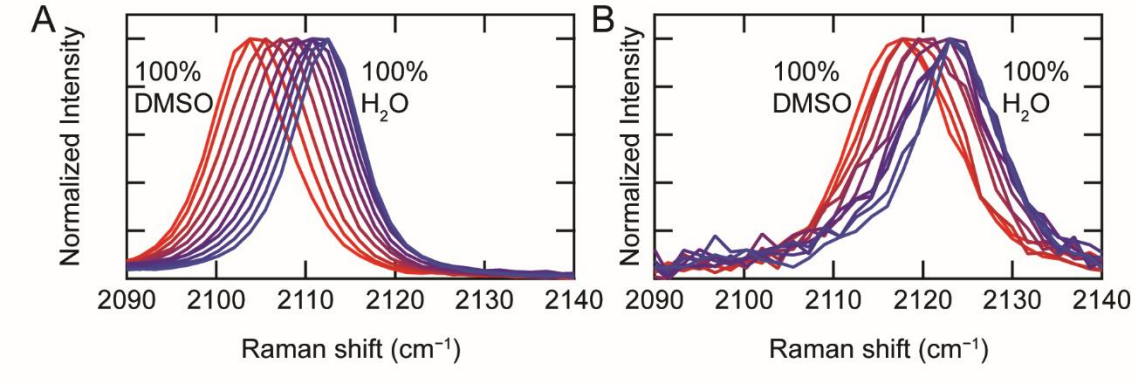

Figure S1. C≡C stretching bands of 10 mM **(A)** F_{CC} and **(B)** HPG in solvent mixtures from 100% DMSO (red) to 100% phosphate buffer (20 mM sodium phosphate, 100 mM NaCl, pH 7.4, blue).

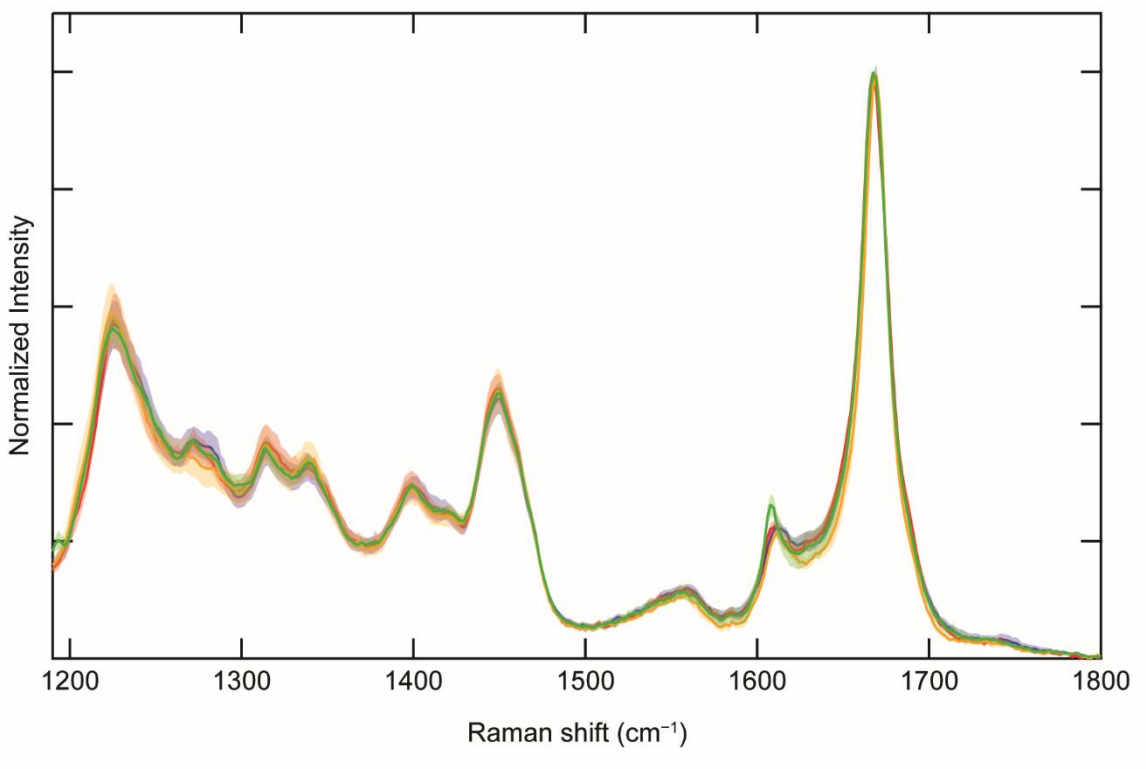

Figure S2. Fingerprint region from the Raman spectra of fibrillar $F4F_{CC}$ (blue), $Y39F_{CC}$ (green), $F94F_{CC}$ (orange), and $Y125F_{CC}$ (red). Solid lines represent averages across multiple aggregates with shaded areas indicating the standard deviation (n = 8).

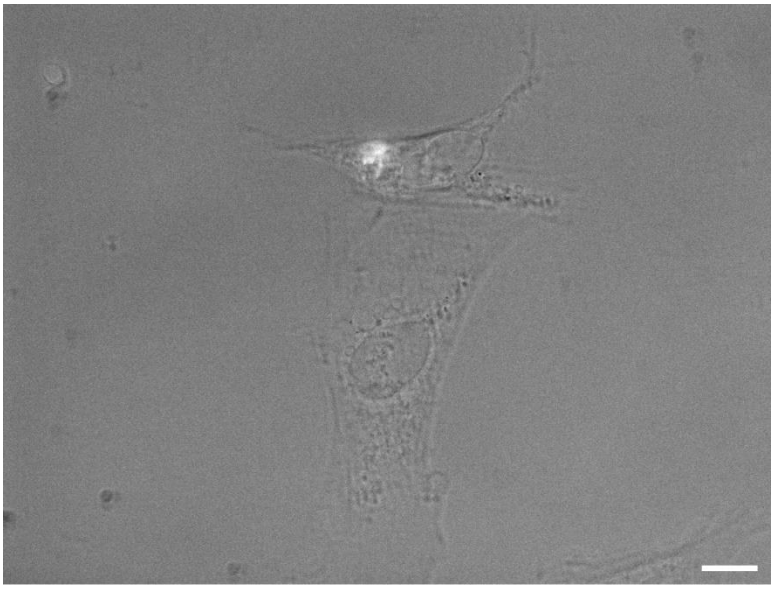

Figure S3. Combined bright-field and wide-field fluorescence image of the cell shown in **Figure 5**. The fluorescence signal represents ThT emission from internalized F_{CC} fibrils.

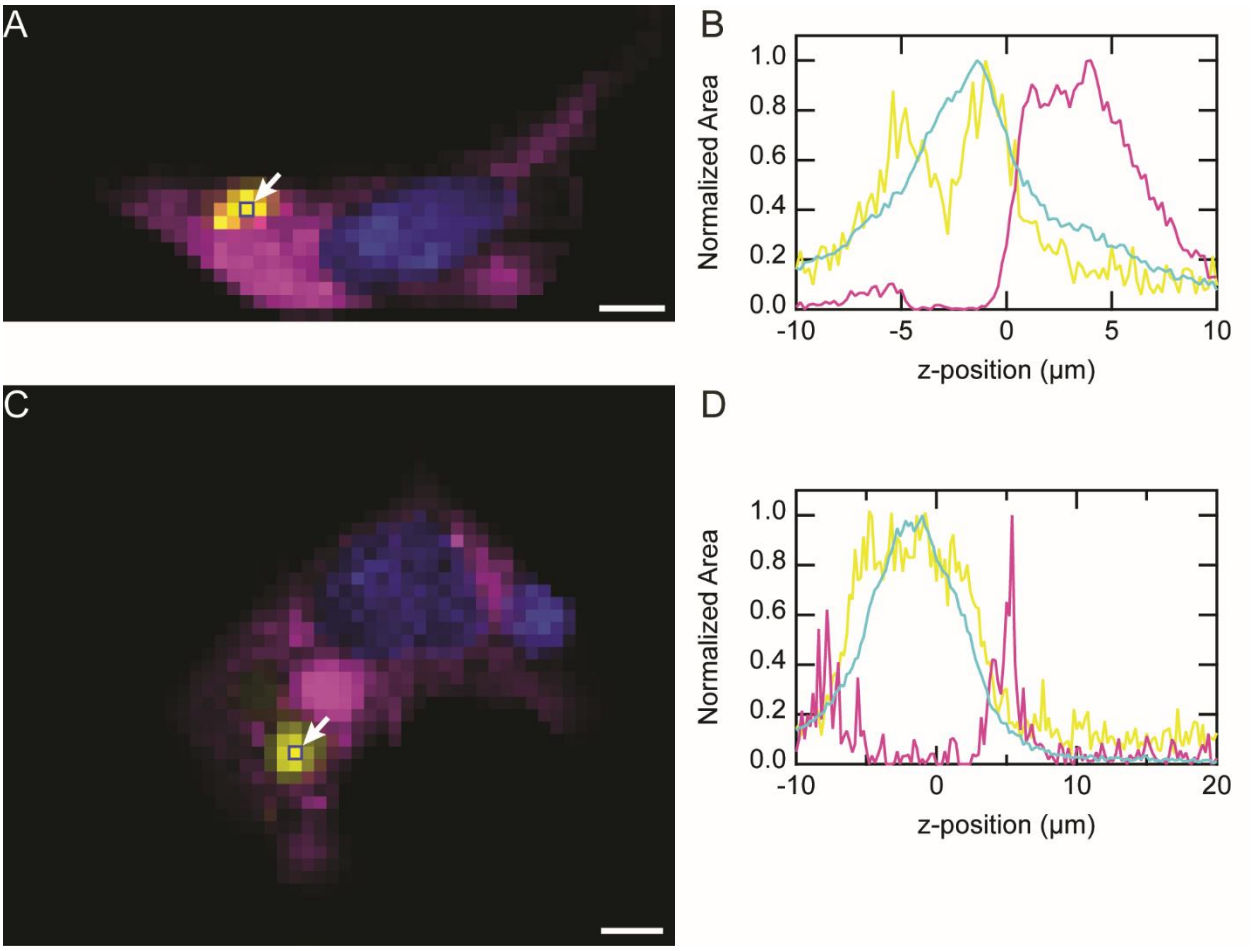

Figure S4. Fibril clusters are fully internalized within SH-SY5Y cells. **(A, C)** Raman maps of lipids (magenta, methylene C–H stretching band 2825–2860 cm^{-1}), nucleotides (blue, nucleotide ring breathing mode 1557–1587 cm^{-1}), and F94F_{CC} fibrils (yellow, C≡C stretching band 2090–2130 cm^{-1}). Scale bars are 5 μm. **(B, D)** *z*-Position scans recorded at the fibril clusters marked in **(A)** and **(C)** at a single location (blue squares indicated by white arrows) showing lipids (magenta, 2825–2860 cm^{-1}), proteins (cyan, 2900–2950 cm^{-1}), and F94F_{CC} fibrils (yellow, 2090–2130 cm^{-1}). Data were collected with 1-s accumulation at each *z*-position with 0.2 μm steps. The cell in **(A)** is the same cell shown in **Figure 5**.

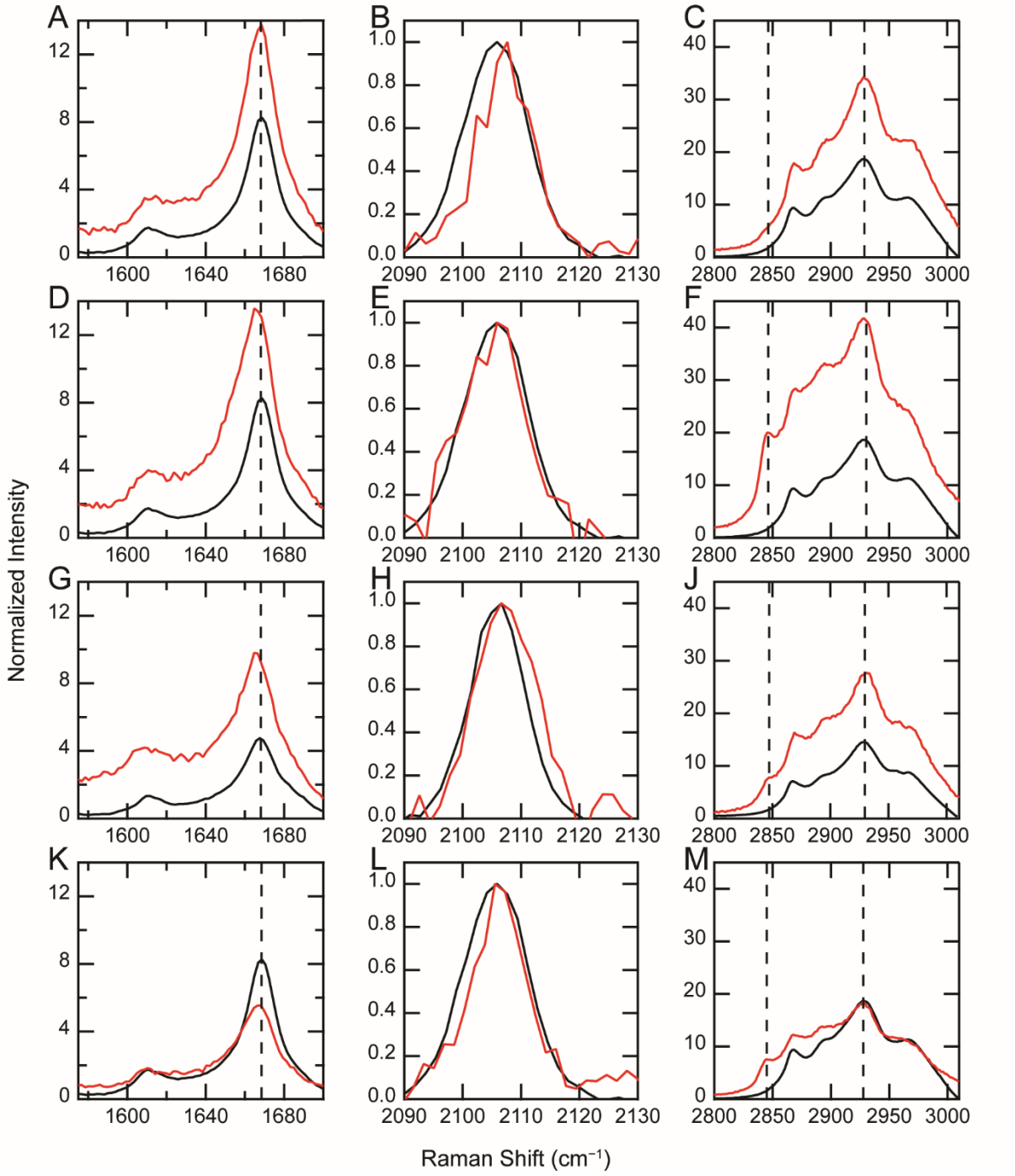

Figure S5. Raman spectra from the individual pixels (blue boxes) as indicated in the corresponding panels in **Figure 7 A**, **D**, **G**, and **K**. Amide-I (**A**, **D**, **G**, **K**), C≡C stretching (**B, E, H, L**), and C–H stretching (**C, F, J, M**) regions are shown as red curves. The *in vitro* fibril spectrum is also shown as a reference (black curves) in each panel. Dashed lines serve as guides for comparison.

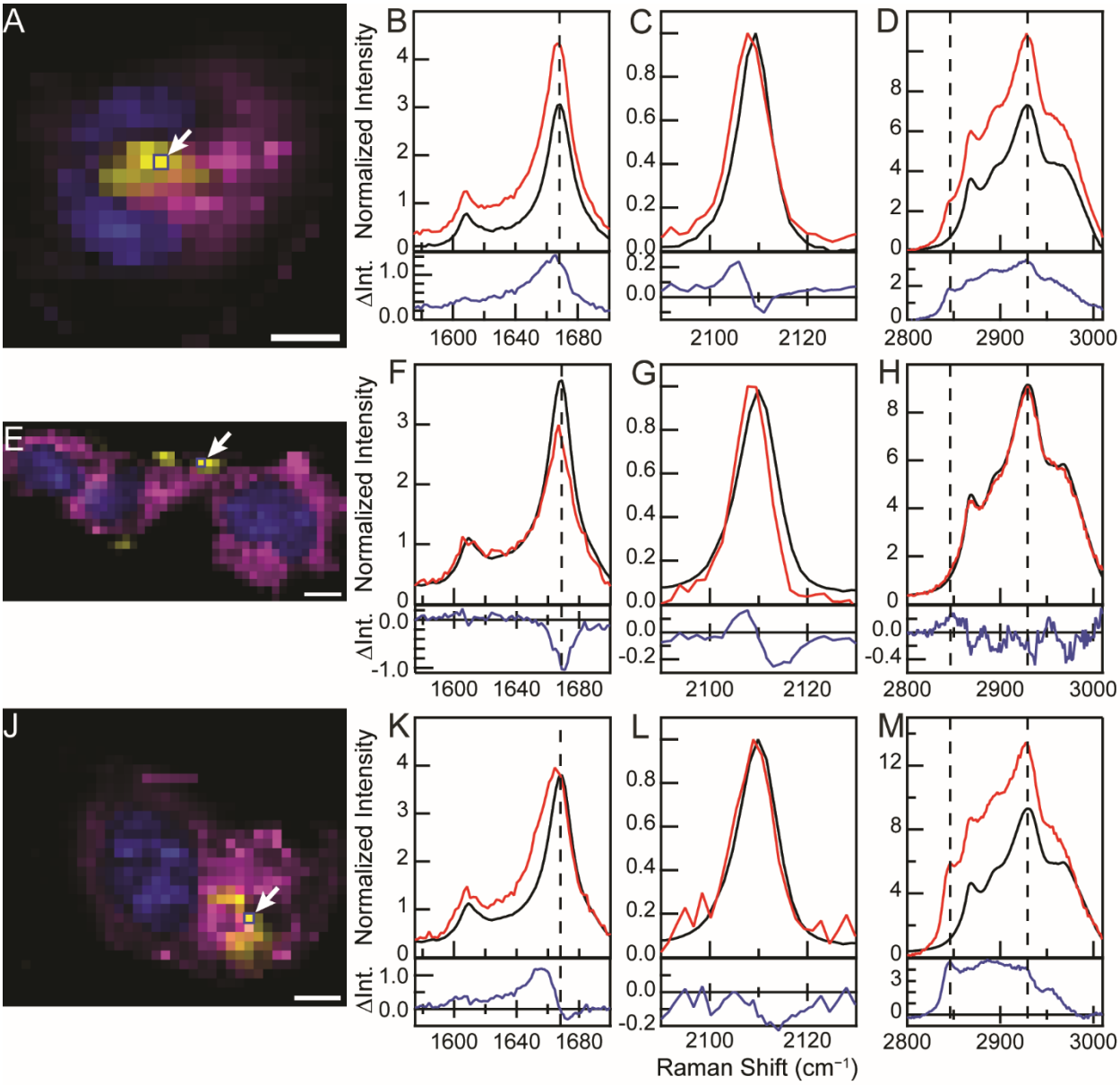

Figure S6. Cellular treatments with Y39F$_{CC}$ fibrils. **(A, E, J)** Raman maps of lipids (magenta, methylene C–H stretching band 2825–2860 cm^{-1}), nucleotides (blue, nucleotide ring breathing mode 1557–1587 cm^{-1}), and Y39F$_{CC}$ fibrils (yellow, C≡C stretching band 2090–2130 cm^{-1}). Scale bars are 5 μm. Raman spectra (red) of the amide-I **(B, F, K)**, C≡C stretching **(C, G, L)**, and C–H stretching regions **(D, H, M)** collected from the individual pixels in **(A**, **E**, and **J**, blue boxes indicated by white arrows**)** are shown. Raman spectra collected for *in vitro* Y39F$_{CC}$ fibrils are also shown for comparison (black) with the difference spectra (cell−*in vitro* fibril, blue) shown below. Data are normalized to the intensity of the C≡C stretching band. Dashed lines are shown as guides for comparison.