

Supporting Information

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Live-Cell Quantitative Imaging of Proteome Degradation by Stimulated Raman Scattering**

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Supporting Information

Methods

Stimulated Raman Scattering Microscopy We use picoEmerald laser system (Applied Physics and Electronics) as light source for SRS microscopy. It produces Pump (tunable from 720 nm – 990 nm) and Stokes (1064 nm) beams simultaneously with 80 MHz repetition. For 968, 986 and 1004 cm^{-1} , the wavelengths of Pump laser are 964.5, 962.8 and 961.1 nm, respectively. Pulse durations for two beams are 6 ps and 5-6 ps, respectively, (measured bandwidth for OPO laser is 0.2 nm), corresponding to 5 cm^{-1} in excitation spectral resolution. Stokes beam is modulated at 8 MHz by an internal electro-optic modulator. The spatially and temporally overlapped Pump and Stokes beams are introduced into an inverted multiphoton laser scanning microscopy (FV1200MPE, Olympus), and then focused onto the sample by a 60X water objective (UPlanAPO/IR, 1.2 N.A., Olympus). Transmitted Pump and Stokes beams are collected by a high N.A. condenser lens (oil immersion, 1.4 N.A., Olympus) and pass through a bandpass filter (890/220 CARS, Chroma Technology) to filter out Stokes beam. A large area (10×10 mm) Si photodiode (FDS1010, Thorlabs) is used to measure the remaining Pump beam intensity. We apply 64 V DC voltage on the photodiode to increase saturation threshold and reduce response time. The output current is terminated by a 50 Ω terminator and pre-filtered by an 8-MHz band-pass filter (KR 2724, KR electronics) to reduce laser and scanning noise. The signal is then demodulated by a lock-in amplifier (SR844, Stanford Research Systems) at the modulation frequency. The in-phase X output is fed back to the analog channel (FV10-ANALOG) of the microscope. Image acquisition speed is limited by 20 μs time constant set for the lock-in amplifier. Correspondingly, we use 100 μs pixel dwell time, which gives a speed of 25 s/frame for a 512-by-512-pixel field of view. Laser powers on sample are measured to be 130 mW for modulated Stokes beam and 120 mW for Pump beam. Laser powers are monitored through image acquisition by an internal power meter and power fluctuation are controlled within 2% by the laser system. 16-bit grey scale images are acquired by Fluoview software.

Spontaneous Raman Spectroscopy Spontaneous Raman spectra were acquired using an upright confocal Raman spectrometer (InVia Raman microscope; Renishaw). A 532 nm YAG laser is used to illuminate the sample with a power of 27 mW on sample through a 50 \times , N.A. 0.75 objective (NPLAN EPI; Leica). Data acquisition was performed after 80-second integration by the WiRE software. Fixed HeLa cells are used and glass background from the coverslip is subtracted by measuring signal from non-cell region near the target cell. The spectra are normalized according to Amide I band.

¹³C-Phe labeling in mammalian cell lines and mouse hippocampal neuron cells

¹³C-Phe supplemented DMEM for HeLa (ATCC), HEK293T (ATCC) cells were made using ¹³C₉,¹⁵N-L-phenylalanine (Sigma) (final concentration: 0.8 mM) and other regular amino acids, salts and vitamins, with 10% FBS and penicillin-streptomycin (Invitrogen). ¹³C-Phe supplemented DMEM for PC12 (ATCC) differentiation is similar except for low serum concentration (1% FBS) and a supplement of 50 ng/mL NGF-β (murine recombinant, Sigma). HeLa or HEK293T or PC12 cells were seeded onto a coverslip in regular medium and then subjected to medium change or induction to differentiation. For incubation time longer than 48 hrs, the medium is changed again to prevent exhaustion of nutrition. For experiments with mouse hippocampal neuron culture, ¹³C-Phe supplemented Neurobasal Medium was used for labeling ~8 day old neurons. After an intended incubation period, the coverslips were taken out and used to make a chamber filled with PBS for SRS imaging. Cells for spontaneous Raman spectra measurement were fixed using cold 4% paraformaldehyde in PBS for 15 min, then washed twice with PBS.

¹³C-Phe labeling in HEK293T cells to study huntingtin protein aggregation

For studying huntingtin aggregation, HEK293T cells were transfected with 3 μg Htt-Q94 (Addgene, tagged with mEos2 or SNAP) plasmid using Transfection Reagent (FuGene, Promega) in regular DMEM. After 24 hr incubation, the medium is switched to ¹³C-Phe supplemented DMEM. The formation of inclusion body was confirmed by mEos2 fluorescence, which is taken with the same laser scan confocal microscope upon illumination by 488 nm laser (400 μW).

Image processing and data analysis Images are assigned color by ImageJ. Ratio maps are calculated by Matlab2012. Single exponential fitting is performed in Origin8.0. Student t test and P value calculation is performed in Prism5.0.

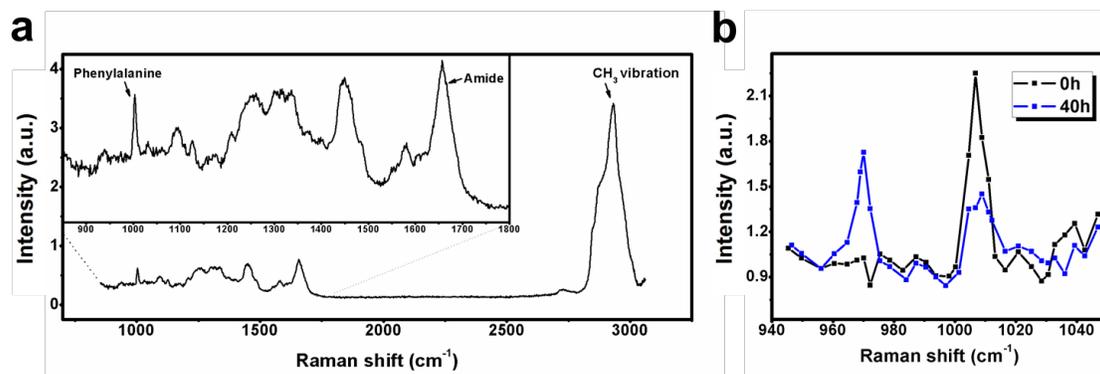


Figure S1 | (a) Spontaneous Raman spectrum of HeLa cell. A spontaneous Raman spectrum of a non-labeled fixed HeLa cell. Non-cellular background has been subtracted. The broad band from 2800 cm⁻¹ to 3050 cm⁻¹, which is often assigned to protein CH₃, contains overlapping signals from lipid, protein, nucleic acids, and water. Inset shows part of the fingerprint region from 850 cm⁻¹ to 1800 cm⁻¹. Amide I band, centered at 1655 cm⁻¹, is also a broad band with interference from unsaturated lipid and water. In contrast, phenylalanine is an isolated sharp peak at 1004 cm⁻¹, sitting on a flat baseline from 950 cm⁻¹ to 1050 cm⁻¹. (b) SRS spectra of HeLa cells incubated with ¹³C-Phe for 0 and 40 hrs. Spectra were acquired by tuning Pump wavelength from 957 to 967 nm (corresponding to 1050 cm⁻¹ ~ 950 cm⁻¹) and obtaining average intensities of resultant images.

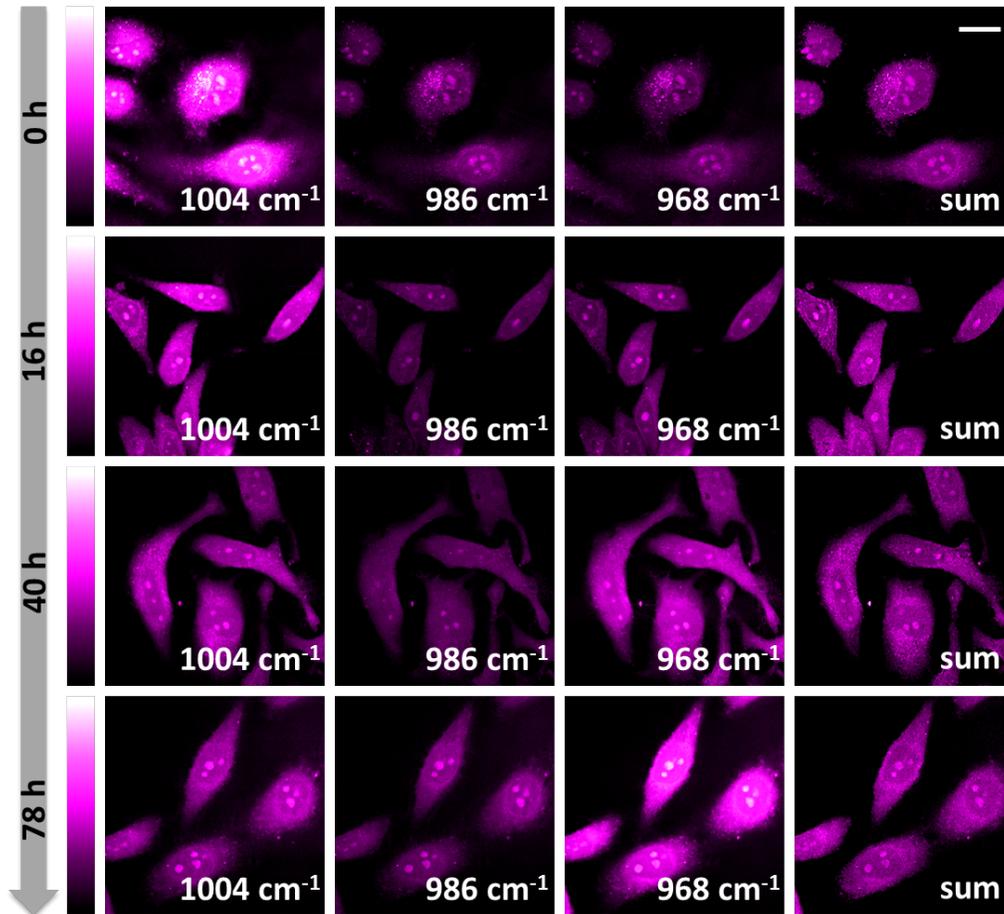


Figure S2 | Raw SRS images from live HeLa cells. HeLa cells incubated in DMEM supplemented with 0.8 mM ¹³C-phenylalanine for 0, 16, 40, 78 hrs. Images were taken at 1004 cm⁻¹, 986 cm⁻¹, 968 cm⁻¹ sequentially. Color bar is the same for all. Images at 986 cm⁻¹ denote the cellular background around 960~1000 cm⁻¹. The last column shows that under quasi steady state, the sum of ¹²C and ¹³C channels, which indicates the amount of total proteome, almost remains constant over time. Scale bar, 20 μm.

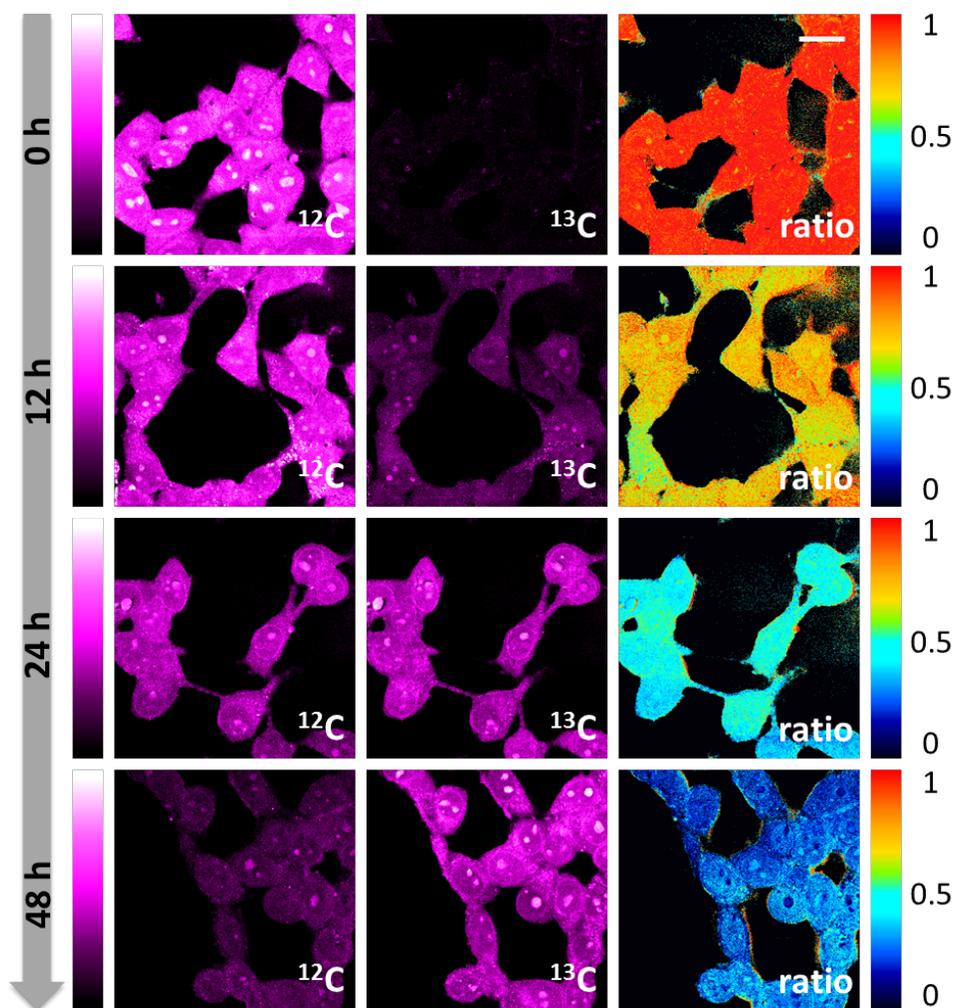


Figure S3 | Time dependent SRS images and ratio maps for HEK293T cells incubated with ^{13}C -Phe for 0, 12, 24, 48 hrs. Scale bar 20 μm .

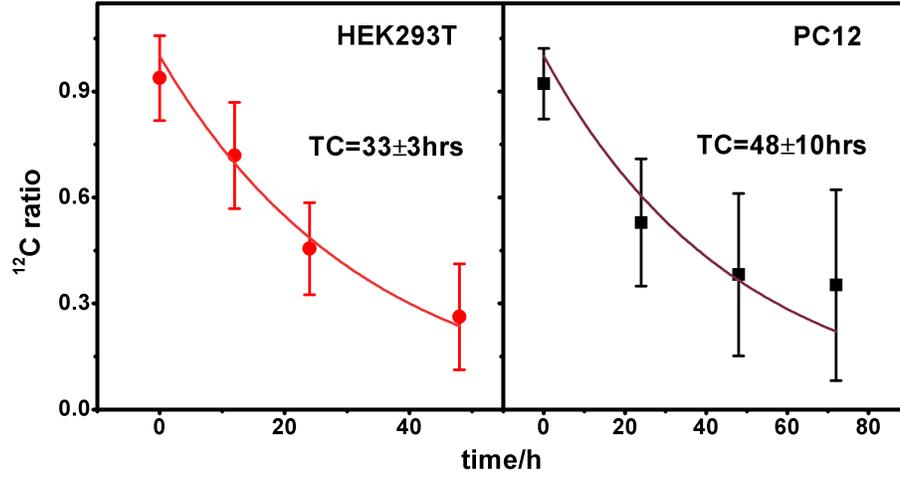


Figure S4 | Single exponential fitting of averaged $^{12}\text{C}/(^{12}\text{C}+^{13}\text{C})$ ratio in HEK293T cells and PC12 cells. Error bar, standard deviation of average ratio.

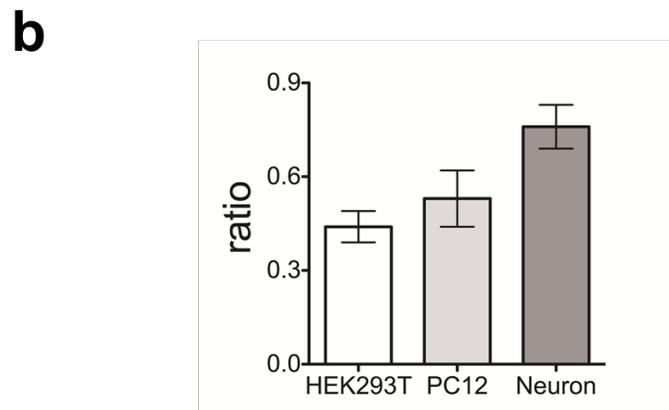
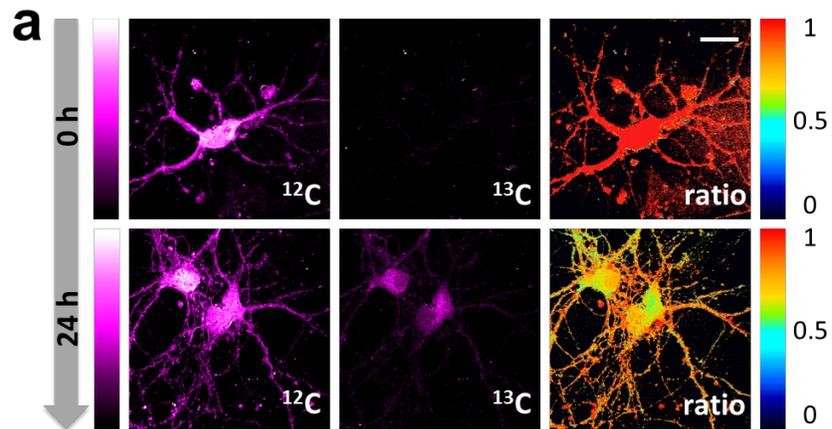


Figure S5 | ^{13}C -Phe labeling reveal slower kinetics in mouse hippocampal neurons. a) SRS images of ^{12}C and ^{13}C channels and calculated ^{12}C ratio images for mouse hippocampal neurons incubated with ^{13}C -Phe for 0 hr and 24 hrs. Scale bar, 20 μm . b) Comparison of ^{12}C ratios at 24 hr incubation among HEK293T, PC12, neurons, indicating that neurons have a slower protein degradation.

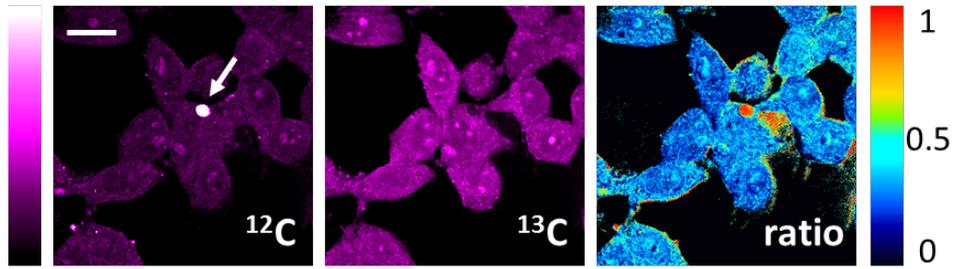


Figure S6 | SRS images and ratio maps for HEK293T cells transfected with SNAP-Htt-Q94. As two-photon absorption may interfere with SRS signal, SNAP sequence tagged Htt-Q94 plasmid was used as a non-chromophore control. Background subtracted ^{12}C and ^{13}C channel SRS images and calculated ratio map are shown here. Inclusion body, indicated by an arrow, still exhibits impaired protein degradation.