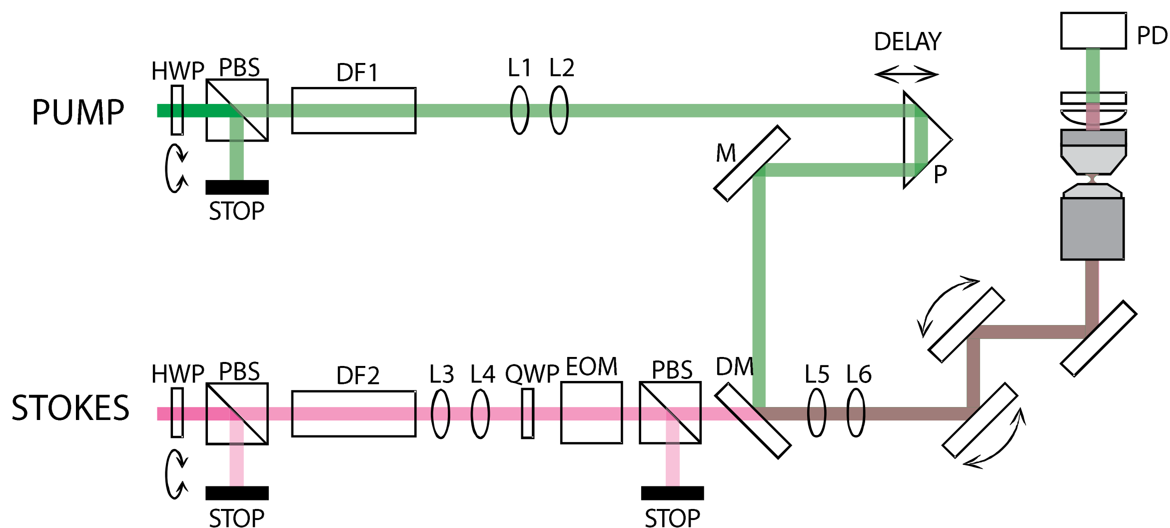
Supplementary Methods

Stimulated Raman scattering microscope

The design of the SRS microscope is primarily determined for the reproducibility of SRS intensity between repeated retuning of the pump beam wavelength, and full automation of acquisition of the three Raman bands. The light source is dual-output tunable femtosecond laser (Spectra-Physics, Insight X3). Tunable beam (680-1040 nm) and fixed wavelength beam (1045 nm) are used as pump beam and Stokes beam, respectively. The laser power is reduced by a pair of attenuators consisting of motor controlled half wave plates and cube polarizers. The pump beam power and Stokes beam power at the sample was 52-60 mW and 25 mW, respectively. Beam heights from the optical table was reduced by using periscope to increase the long term stability of the optomechanics. Pump beam and Stokes beams are chirped by passing through high dispersion dense flint glass rod (Casix, SF57). Stokes beam intensity is modulated by electro-optical modulator (EOM, Thorlabs EO-AM-R-20-C2) at 20 MHz. A motor-controlled retroreflector controls the optical path length of the pump beam. Stokes beams are co-linearly combined with pump beams using dichroic mirror. The combined beams enter a scanning microscope to image the sample by point scanning. The stimulated Raman loss of the pump beam is measured by a high speed photodiode (Thorlabs, FDS1010) on the trans-side of the sample. The photo current is converted to voltage by a 50 ohm resister, filtered by a 3-30 MHz bandpass filter and amplified by a lock-in amplifier (Zurich Instruments, HF2LI) at the EOM modulation frequencies. The demodulation amplitude of the lock-in amplifier is mapped to images in real time by analog to digital data acquisition board (Olympus, Analog Box) synchronized to the scanning microscope (Olympus, FV3000).



Supplementary Figure 1. Schematics of SRS microscope. HWP, half waveplate; PBS, polarizing beam splitter; STOP, beam stop; DF1-2, SF57 glass rod; L1-6, relay lenses for adjusting the beam diameter; QWP, Quarter wave plate; EOM, electro optical modulator; DM, dichroic mirror; M, mirror; P, retroreflector prism; PD, photo diode.

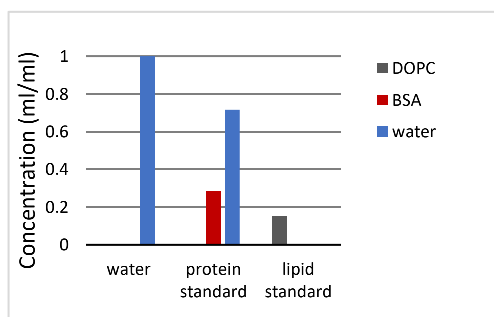
Image acquisition settings

All SRS images are acquired with 2 microsecond per pixel dwell time. As such, a single 512x512 frame of SRS intensity image take 1 second acquisition time. In a typical NORI acquisition, three Raman bands are acquired

at 2853, 2935, and 3250 cm^{-1}. Raman band change requires wavelength change of tunable laser, which takes 7-11 seconds to finish. When scanning large sample area, we acquired images of at one Raman band for approximately 30 minutes before imaging the same area in other Raman bands to avoid image misalignment problem.

Calibration standards

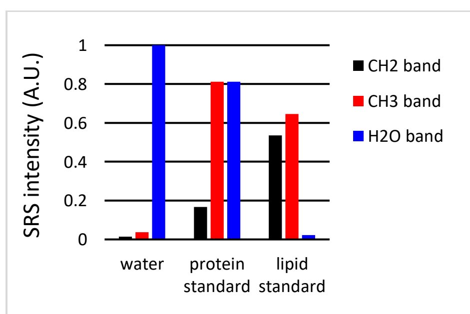
Calibration standards are 30% BSA solution in water, 15 or 35% DOPC solution in 4-deuterated methanol, pure water and pure 4-deuterated methanol. All solution samples are mixed by measuring the weights of the components. BSA powder (Sigma Aldrich, A7638) is weighed in a conical tube and 150 mM pH7.6 phosphate buffer is added to make the concentration of 33%(w/w). The tube is tightly closed and placed in 40 degrees shaker until BSA is fully dissolved in buffer, while occasionally spinning down undissolved chucks that are stuck on the tube wall. DOPC powder is similarly weighted in a conical tube to which 4-deuterated methanol is added by weight. The final concentration in volume fraction is calculated from the mass fractions and the density of the pure components. The density of BSA is 1.364 g/ml and DOPC is 1.010 g/ml.



Supplementary Figure 2. Concentration of calibration standard samples.

Measuring SRS intensity images of the calibration standards

We assembled sample holder on a glass slide by creating 4 sample holding slots using a double-sided tapes or adhesive sheets (Grace Biolabs, SA-S-1L). We find it convenient to laser cut the adhesive sheets to have regular distance between the sample slots for automation of data acquisition. To prevent mixing of the adjacent samples, there were empty slots between sample slots. The samples are injected to the holding slots by pipet. The sample volume is approximately 7 μl with the thickness of 120 μm. We acquired z stack of each sample with 2-5 μm step size. In order to capture the z plane of highest intensity immediately past the coverslip, the z stack started outside of the sample.



Supplementary Figure 3. SRS intensity of calibration standards.

Image processing of SRS intensity images

To prepare SRS intensity images for light scattering normalization algorithm, we applied background subtraction and flat-field correction. To ensure the linearity of the SRS intensity to concentration, dark noise is measured and subtracted from SRS images. Due to the aberration of optical system, the SRS intensity is 2D Gaussian function whose strongest intensity is in the center of the field of view. The spatial inhomogeneity is stable for a given wavelength over repeated retuning of the pump beam. However, the position of the intensity centroid changes between different wavelengths of the tunable pump beam. The images of calibration standard samples capture this 2D intensity inhomogeneity. We created flat-field correction mask from BSA sample images at CH3 and H2O Raman bands and from the DOPC sample image at CH2 Raman band. The background level is subtracted from the images, then the intensity is normalized by dividing with the maximum intensity. Flat-field correction was applied by dividing sample images post-background subtraction with the intensity 2D mask of matching Raman band.

Animal protocols

Wild type mouse tissues are collected from 8-12 week male mice in accordance with the procedure approved by Institutional Animal Care and Use Committee (IACUC) at Harvard University. Transgenic female APP-PS1 mice and age-matched WT female mice were purchased from Jackson Laboratory. All animal experiments were approved by the Institutional Animal Use and Care Committee at Massachusetts General Hospital. For live imaging of zebrafish embryo, AB strain wild type fish was used. All fish were kept at 28˚C on a 14-hour-light/10-hour-dark cycle. Embryos were collected from natural crosses. The chorion was removed manually prior to imaging. All fish-related procedures were carried out with the approval of Institutional Animal Care and Use Committee (IACUC) at Harvard University.

Sample preparations

HeLa cell was cultured on cover glass and fixed with 4% formaldehyde for 20 minutes in room temperature. The fixed cell is washed with phosphate buffered saline and assembled to a glass slide for imaging.

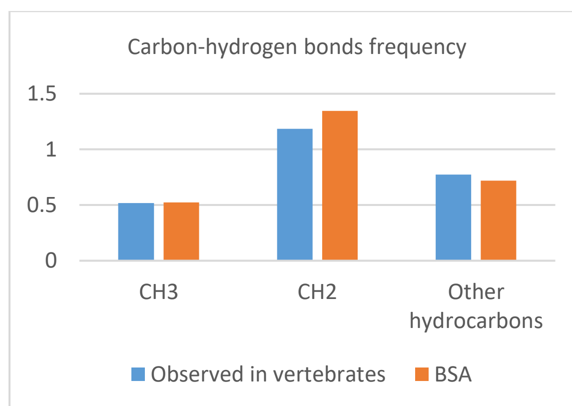
Mouse tissues including brain, kidney, liver, pancreas, cartilage and skeletal muscle are dissected from the euthanized animal and immersion fixed in 4% formaldehyde in 4°C for 24 hours. Mouse cerebellum was perfusion fixed with 4% formaldehyde in 4°C for 24 hours.

Fixed tissues except for cartilage and APP-PS1 and WT brains are embedded in 2% agarose and sectioned to 40-100 μm thickness using a vibrating microtome (Precisionary Instruments, VF-300-0Z). Fixed brains were transferred into 30% sucrose at 4°C until tissue sinks. Then the tissue was embedded in OCT and the OCT embedded frozen tissue blocks was sectioned into 25-µm slice in cryostat. The brain sections were washed of sucrose with phosphate buffered saline. Cartilage tissues were embedded in BSA-gelatin gel cured with formaldehyde in room temperature overnight, and sectioned by a vibrating microtome (Leica).

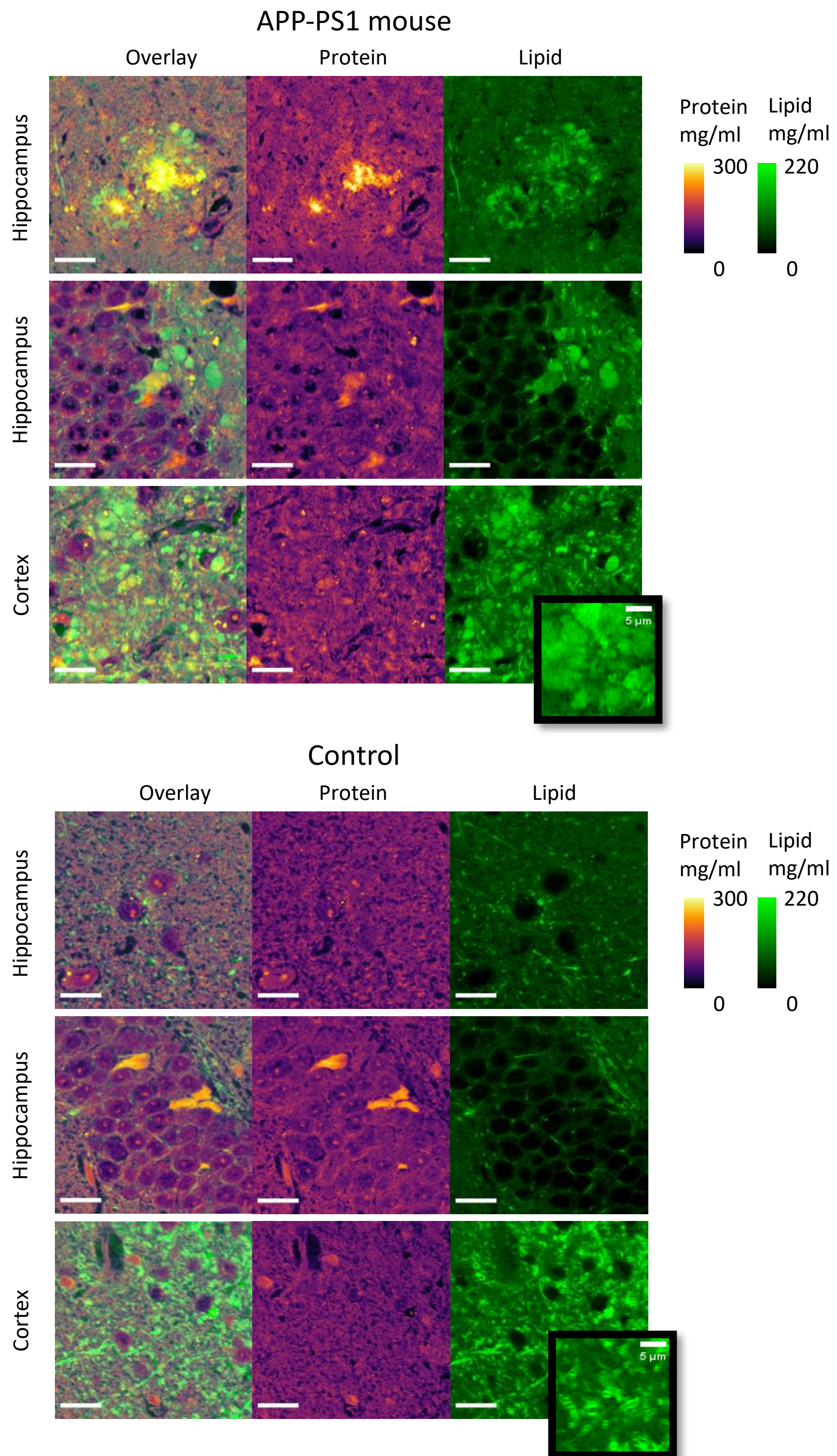
The tissue sections are transferred to phosphate buffered saline bath and stored in 4°C until imaging. For imaging, a tissue section is sealed between a coverglass and a slide glass with phosphate buffered saline using a double-sided tape spacer (Grace Biolabs, SS1X13) or using a nail polish as sealant.

Table 1. Frequency of methyl and methylene groups in bovine serum albumin and average vertebrate proteome

Amino acids	Amino acids	CH3 in side group	CH2 in side group	Other C-H bonds	Observed Frequency in Vertebrates	Frequency in BSA
Alanine	A	1			7.4%	8.2%
Arginine	R		3		4.2%	4.4%
Asparagine	N		1		4.4%	2.4%
Aspartic Acid	D		1		5.9%	7.0%
Cysteine	C		1		3.3%	6.0%
Glutamic Acid	E		2		5.8%	0.0%
Glutamine	Q		2		3.7%	9.9%
Glycine	G			1	7.4%	2.9%
Histidine	H		1	2	2.9%	2.7%
Isoleucine	I	2	1	1	3.8%	2.6%
Leucine	L	2	1	1	7.6%	11.1%
Lysine	K		4		7.2%	10.2%
Methionine	M	1	2		1.8%	0.9%
Phenylalanine	F		1	5	4.0%	5.1%
Proline	P		2		5.0%	4.8%
Serine	S		1		8.1%	5.5%
Threonine	T	1		1	6.2%	5.8%
Tryptophan	W		1	5	1.3%	0.5%
Tyrosine	Y		1	4	3.3%	3.6%
Valine	V	2		1	6.8%	6.5%



Supplementary Figure 4. Comparison of carbon-hydrogen bonds frequency in vertebrates and bovine serum albumin (BSA)



Supplementary Figure 5. Protein and lipid concentration images of APP-PS1 mouse brain and control mouse brain. Scale bars, 20 µm. Inset scale bars, 5 µm.