

Supporting Information for Heterodyne-detected Achiral and Chiral Vibrational Sum Frequency Generation of Proteins at Air/Water Interface

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Spectral Fitting

In order to extract band parameters, we fitted a single Lorentzian function to each pair of imaginary and real $\chi^{(2)}$ spectra in Figure 2, 3, and 4. The fitting parameters are summarized in Table S1. The fits to the spectra are shown in Figure S1, S2, and S3.

Table S1. Fitting parameters of the bands in Figure 2, 3, and 4

Protein	Band	Amplitude	Center frequency / cm^{-1}	Bandwidth / cm^{-1}
BSA	Chiral NH	0.26	3333	48
	Achiral amide I	0.98	1646	20
	Chiral amide I	0.10	1628	13
Pepsin	Chiral NH	0.89	3286	54
	Achiral amide I	0.87	1647	25
	Chiral amide I	0.46	1636	16
Concanavalin A	Chiral NH	0.78	3321	40
	Achiral amide I	0.77	1641	29
	Chiral amide I	0.82	1627	17

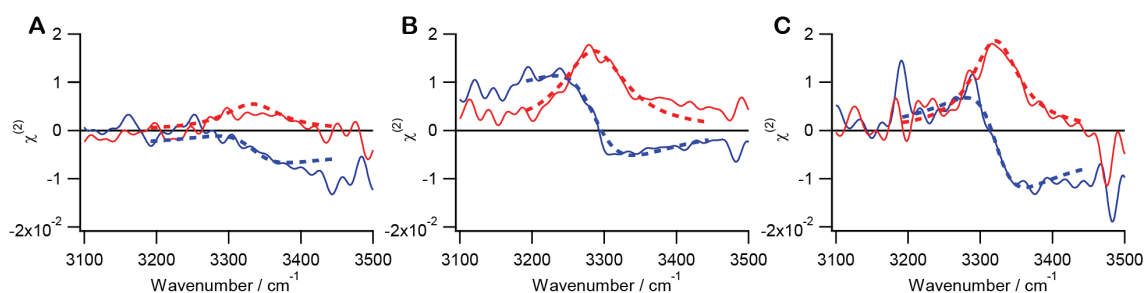


Figure S1 Fits to the complex spectra shown in Figure 2.

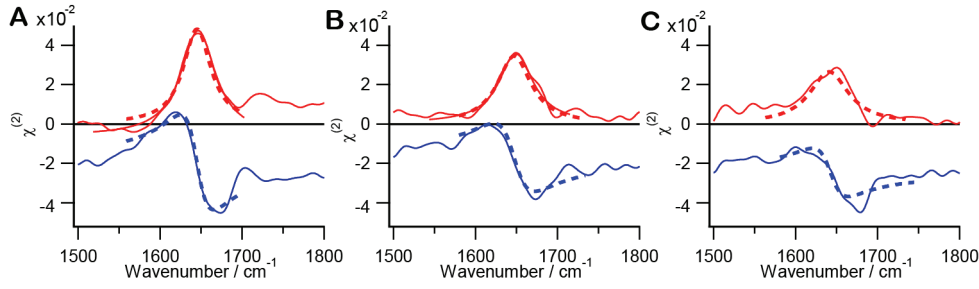


Figure S2 Fits to the complex spectra shown in Figure 3.

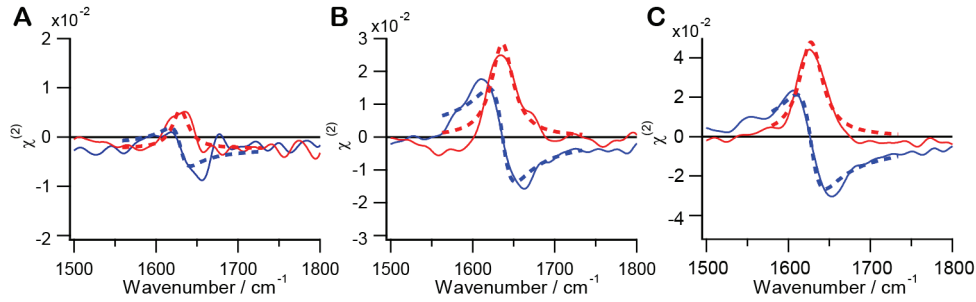


Figure S3 Fits to the complex spectra shown in Figure 4.

Calculation Details

Tensor elements of vibrationally resonant second-order hyperpolarizability were estimated for the amide I mode based on quantum mechanical (QM) calculations. *N*-methylacetamide (NMA) was used as a model molecule, and the QM calculations were performed by Gaussian 09.¹ The B3LYP exchange-correlation function and 6-31G+(d) basis set functions were used for geometry optimization and vibrational analysis. The molecular axes were defined such that the amide plane lies in the *xz* plane and the C=O bond is parallel to the *z* axis. In this scheme, the transition dipole moment of the amide I band is oriented 16 degrees from the C=O bond, The Raman tensor *c*- and *b*-axis are in the amide I plane. The *c*-axis is directed at the angle of 34 degrees with respect to the C=O bond shown in Figure S4. These results were consistent with prior values.²⁻⁴

The transition dipole $\vec{\mu}$ and the Raman tensor $\vec{\alpha}$ in the molecular fixed *xyz* frame are,

$$\vec{\mu} = \begin{pmatrix} 4.20 \\ 0 \\ 14.91 \end{pmatrix}, \vec{\alpha} = \begin{pmatrix} -0.180 & 0.017 & -0.154 \\ 0.017 & 0.153 & 0.008 \\ -0.154 & 0.008 & -0.596 \end{pmatrix} \text{ (units: a.u.).} \quad \text{Equation 1}$$

When the molecular *z* axis and the surface normal, the laboratory *Z* axis, are parallel and the molecular orientation around *Z*-axis is isotropic, χ_{YYZ} is calculated as follows:

$$\frac{(\alpha_{xx} + \alpha_{yy})\mu_z}{2} = -0.20 (< 0) \quad \text{Equation 2}$$

This calculation shows that the sign of the amide I band in the SSP spectrum is negative when the C=O bond is parallel to the laboratory *Z* axis with the O atom pointing up. We calculated likewise

χ_{YYZ} for orientations with a variety of the tilt angle θ (0° to 180°), assuming that the azimuthal angle around z -axis (ϕ) and the twist angle around z -axis (ψ) are isotropic (Figure S5). The calculations showed that χ_{YYZ} is negative for θ from 0° to 70° and positive for θ from 110° to 180° . This result suggests that the positive (negative) sign of the amide I band indicates the O-down (up) orientation unless the C=O bond is almost parallel to the surface.

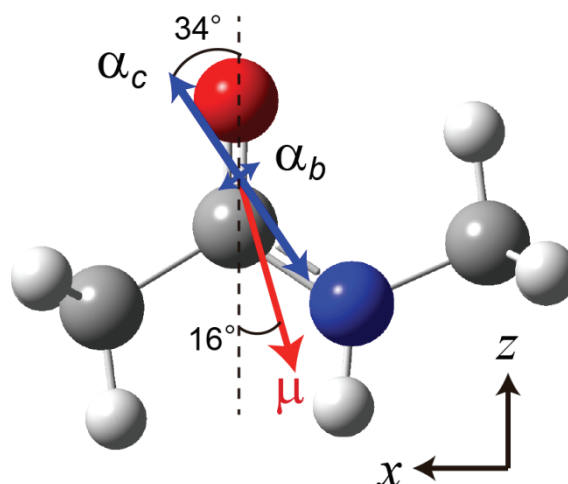


Figure S4 Ball and stick representation of NMA and transition dipole moment (red arrow) and Raman tensor (blue arrows) of the amide I band.

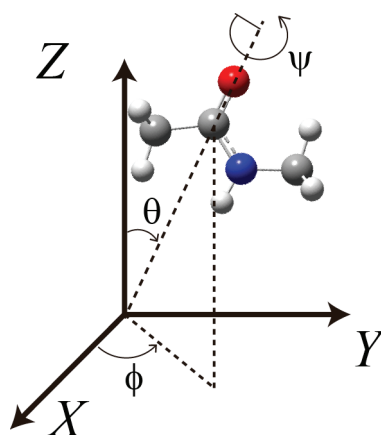


Figure S5 Definition of Euler angles (θ, ϕ, ψ) and laboratory axes (X, Y, Z).

REFERENCES

- (1) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Gaussian 09 (Revision B. 01) ed.; Gaussian, Inc.: Wallingford CT, 2009.
- (2) Perry, J. M.; Moad, A. J.; Begue, N. J.; Wampler, R. D.; Simpson, G. J. *J. Phys. Chem. B*

2005, *109*, 20009.

(3) Laaser, J. E.; Skoff, D. R.; Ho, J. J.; Joo, Y.; Serrano, A. L.; Steinkruger, J. D.; Gopalan, P.; Gellman, S. H.; Zanni, M. T. *J. Am. Chem. Soc.* **2014**, *136*, 956.

(4) Roeters, S. J.; van Dijk, C. N.; Torres-Knoop, A.; Backus, E. H. G.; Campen, R. K.; Bonn, M.; Woutersen, S. *J. Phys. Chem. A* **2013**, *117*, 6311.