

Supporting Material

A. Evaluation of local and nonlocal contributions

1. Derivation and analysis of Eqn. 6.

Substitution of (4) into (3), (3) into (1) and (2) by taking into account (5) and the Fresnel and local field factors for the sum frequency radiation lead to the general expression of Eqn.6:

$$\chi_{eff,ijk}^{(2)} = L_{ii}(\omega_{SF})e_i(\omega_{SF}) \cdot L_{jj}(\omega_{VIS})e_j(\omega_{VIS}) \cdot L_{kk}(\omega_{IR})e_k(\omega_{IR}) \times \chi_{eff,ijk}^{(2)} \quad (\text{A.1a})$$

$$\begin{aligned} \chi_{eff,ijk}^{(2)} = & \int_0^d f_i(\omega_{SF}, z) \chi_{ijk}^D(z) f_j(\omega_{VIS}, z) f_k(\omega_{IR}, z) dz + \int_0^d f_i(\omega_{SF}, z) \chi_{ijkz}^{P_1}(z) \left[\frac{\partial}{\partial z} f_j(\omega_{VIS}, z) \right] f_k(\omega_{IR}, z) dz \\ & + \int_0^d f_i(\omega_{SF}, z) \chi_{ijkz}^{P_2}(z) f_j(\omega_{VIS}, z) \left[\frac{\partial}{\partial z} f_k(\omega_{IR}, z) \right] dz - \int_0^d f_i(\omega_{SF}, z) \frac{\partial}{\partial z} \left[\chi_{ijkz}^Q(z) f_j(\omega_{VIS}, z) f_k(\omega_{IR}, z) \right] dz \\ & + \dots \end{aligned} \quad (\text{A.1b})$$

where $e_m(\omega_n)$ is the m -th component of the polarization unit vector of the n -th electric field in medium 2. A partial integration in the fourth integral leads to the result

$$\begin{aligned} \chi_{eff,ijk}^{(2)} = & \int_0^d f_i(\omega_{SF}, z) \chi_{ijk}^D(z) f_j(\omega_{VIS}, z) f_k(\omega_{IR}, z) dz + \int_0^d f_i(\omega_{SF}, z) \chi_{ijkz}^{P_1}(z) \left[\frac{\partial}{\partial z} f_j(\omega_{VIS}, z) \right] f_k(\omega_{IR}, z) dz \\ & + \int_0^d f_i(\omega_{SF}, z) \chi_{ijkz}^{P_2}(z) f_j(\omega_{VIS}, z) \left[\frac{\partial}{\partial z} f_k(\omega_{IR}, z) \right] dz + \int_0^d \left[\frac{\partial}{\partial z} f_i(\omega_{SF}, z) \right] \chi_{ijkz}^Q(z) f_j(\omega_{VIS}, z) f_k(\omega_{IR}, z) dz \\ & - \left\{ f_i(\omega_{SF}, d) \chi_{ijkz}^Q(d) f_j(\omega_{VIS}, d) f_k(\omega_{IR}, d) - f_i(\omega_{SF}, 0) \chi_{ijkz}^Q(0) f_j(\omega_{VIS}, 0) f_k(\omega_{IR}, 0) \right\} + \dots \end{aligned} \quad (\text{A.1c})$$

We note that an analogous formula was obtained by Shen in Ref.21, although in that work the local field corrections were not explicitly considered.

The first integral relates to the dipolar (local) contribution and was discussed in detail in Section 3.2. As it was already noted in Ref.21, in the case of SFG (or SHG) from a semi-infinite medium Eqn.A.1c gives two kinds of nonlocal contributions. The first, described by the second,

third and fourth integrals, is due to the rapid field variation in the interfacial region and can be suppressed if the dielectric functions of the ambient and the substrate are matched.⁴¹ The second, given by the bracketed ($\{ \}$) expression in Eqn.A.1c, describes a contribution induced by structural variation in the interfacial region. We note that the susceptibilities as material parameters are expected to be proportional to the local density of the nonlinear medium. Since we can choose the boundaries of the thin layer in such a way that the susceptibilities are negligible at both boundaries, we can see that this expression in Eqn.A.1c vanishes in the thin film model, thus leading to the final expression of Eqn. 6.

2. Estimation of the second- and higher-order hyperpolarizabilities

We want to evaluate the hyperpolarizabilities according to the following formulas (Eqn. 9):

$$\begin{aligned}\beta_{\alpha\beta\gamma} &\approx -\frac{1}{2\epsilon_0\omega_s} \frac{\partial a_{\alpha\beta}}{\partial q_s} \frac{\partial \mu_\gamma}{\partial q_s} \frac{1}{\omega - \omega_s + i\Gamma_s} \\ \beta_{\alpha\beta\gamma\delta}^{P_1} &\approx -\frac{1}{2\epsilon_0\omega_s} \frac{\partial A_{\alpha\beta\gamma}}{\partial q_s} \frac{\partial \mu_\delta}{\partial q_s} \frac{1}{\omega - \omega_s + i\Gamma_s} \\ \beta_{\alpha\beta\gamma\delta}^{P_2} &\approx -\frac{1}{2\epsilon_0\omega_s} \frac{\partial a_{\alpha\beta}}{\partial q_s} \frac{\partial Q_{\gamma\delta}}{\partial q_s} \frac{1}{\omega - \omega_s + i\Gamma_s} \\ \beta_{\alpha\beta\gamma\delta}^Q &= \beta_{\beta\alpha\gamma\delta}^{P_1}\end{aligned}\tag{A.2}$$

where μ , \vec{a} , \vec{A} , \vec{Q} are the dipole moment, polarizability, electric-dipole-quadrupole polarizability and quadrupole moment, respectively. The derivatives are taken with respect to the s -th normal coordinate, while ω_s and Γ_s denote the frequency and width of the s -th normal vibration.

Using a bond additivity model^a we can deduce bond polarizability derivatives taken with respect to the bond length such as $\frac{\partial a_{\zeta\zeta}}{\partial r}$ or $\frac{\partial A_{\zeta\zeta\zeta}}{\partial r}$ (ζ is the direction of the bond) from the published polarizability data. For example, the value for $\frac{\partial a_{\zeta\zeta}}{\partial r}$ of a C-H bond from the data of Ref.b is quite close to the value (3×10^{-30} Cm/V) usually used in the SFG literature.²² Unfortunately, we were unable to find a value for $\frac{\partial A_{\zeta\zeta\zeta}}{\partial r}$ of a C-H bond. In the literature, we found the electric-dipole-quadrupole polarizability functions for CF₄ and HCl.^{c,d} From these data the bond additivity model yields $\frac{\partial A_{\zeta\zeta\zeta}}{\partial r} = 12 \times 10^{-40}$ Cm²/V for a C-F bond and 4×10^{-40} Cm²/V for a Cl-H bond. Here we approximate $\frac{\partial A_{\zeta\zeta\zeta}}{\partial r}$ of the C-H bond to be 10×10^{-40} Cm²/V. Assuming C_{3v} symmetry for the methyl group and using the value $\frac{\partial \mu_{\zeta}}{\partial r} = -2.9 \times 10^{-20}$ C,²² the bond additivity model gives the following nonzero elements of the $B_{\alpha\beta\gamma\delta}^{P_1,s}$ tensor for the symmetric methyl stretch:

$$\begin{aligned}
 B_{\xi\xi\xi\xi}^{P_1,sym} &= -B_{\xi\eta\eta\xi}^{P_1,sym} = -B_{\eta\xi\xi\eta}^{P_1,sym} = -B_{\eta\eta\xi\xi}^{P_1,sym} = -3.9 \times 10^{-37} \frac{m^5}{Vs} \\
 B_{\xi\xi\xi\xi}^{P_1,sym} &= B_{\eta\eta\xi\xi}^{P_1,sym} = B_{\xi\xi\xi\xi}^{P_1,sym} = B_{\eta\xi\xi\eta}^{P_1,sym} = B_{\xi\xi\xi\xi}^{P_1,sym} = B_{\xi\eta\eta\xi}^{P_1,sym} = 2.8 \times 10^{-37} \frac{m^5}{Vs} \\
 B_{\xi\xi\xi\xi}^{P_1,sym} &= 6.9 \times 10^{-38} \frac{m^5}{Vs}
 \end{aligned} \tag{A.3}$$

Although it is difficult to judge how reasonable is the approximation of the electric-dipole-quadrupole derivative of a C-H bond, we note that the resulting hyperpolarizability is of the same order of magnitude as the product of the dipole hyperpolarizability and the bond length, as

expected.²¹ Therefore, we believe the above hyperpolarizabilities are suitable for our order of magnitude estimation purposes.

With these values and assuming azimuthal isotropy for the thin layer, we can evaluate the nonlocal susceptibility $\chi_{ijkl}^{P_1}$ for the symmetric methyl stretch. Although we were unable to find values for $\frac{\partial Q_{\gamma\delta}}{\partial q_s}$, we expect that the elements of $\chi_{ijkl}^{P_2}$ are in the same order of magnitude.^{21,44}

Furthermore, we assume that the approximate relation $\chi_{ijkl}^O \approx \chi_{jikl}^{P_1}$ is also valid.⁴⁴

We note that as far as the methyl groups can be approximated as harmonic oscillators, their higher order hyperpolarizabilities are connected in a simple way to their linear polarizability, as described in Refs.e and f. The nonlocal hyperpolarizabilities obtained from such an estimation are of the same order of magnitude as the ones derived above. Thus we believe that our approximations above are reasonable.

3. Estimation of the nonlocal contributions

As Eqn.6 indicates, the nonlinear response from the thin film is determined by both its optical and structural properties. Therefore, in order to evaluate the nonlocal contributions, we have to use a structural and optical model for the film. As the properties of such a layer are largely unknown, for the purpose of our model calculation we have to assume some specific forms for the optical functions $f_i(\omega, z)$ and the distribution $G(\theta, z)$.

We understand that the problem of optical functions such as local fields is extremely complicated. The Lorentz model is one of the traditional ways to deal with the local field correction problem within the bulk of a material. Nevertheless, several calculations indicate that even at a surface it can give reasonable estimations.⁴⁵ In addition it is often found that if the local fields are evaluated by planewise summation (as in Ref. 46) in a multilayered system, the result

depends mostly on the properties of the actual layer and is only slightly influenced by the adjacent layers.^h Again this means that the local fields may not differ too strongly from their bulk value even in the case of the loss of centrosymmetry at a surface. Also, the protein film we considered here is several nm thick, thus it is more like a bulk than an infinitesimally sharp interface for the calculation of local field correction factors. We believe that it is a reasonable approach to use the local field correction factor from the Lorentz model:^{i,j}

$$f_i(\omega, z) = \left(\frac{n_2(\omega)^2}{\epsilon(\omega, z)} \right)^{\epsilon_{iz}} \cdot \frac{\epsilon(\omega, z) + 2}{3} \quad (\text{A.4})$$

where $\epsilon(\omega, z) \equiv n(\omega, z)^2$ is the local dielectric function and we assume that the latter is a linear function of the local density $N(z)$. Furthermore, we may neglect the frequency dependence of the local optical properties:

$$\epsilon(z) = 1 + \rho \cdot N(z) \quad (\text{A.5})$$

where ρ is an empirical parameter.

As far as the distribution function is concerned, let us assume that we have a monolayer of BSA adsorbed on a fused silica substrate. We assume that the nonlinearly polarizable groups are the methyl groups, which are randomly and uniformly distributed across the film. From previous studies we can assume that the thickness of an adsorbed BSA monolayer is around 4 nm. The average density of the CH_3 groups corresponds to that expected for an adsorbed BSA monolayer (which contains 2×10^{12} molecule/cm², with 300 methyl groups on each molecule). The ambient in the positive half-space is air with dielectric function $\epsilon_l = 1$, while the substrate is fused silica with $\epsilon_2 = 2.1$ ($n_2 = 1.45$) at all relevant wavelengths. The refractive index inside the protein layer is assumed to be 1.4-1.6.^k We used 1.4 here, but 1.6 has also been found not to

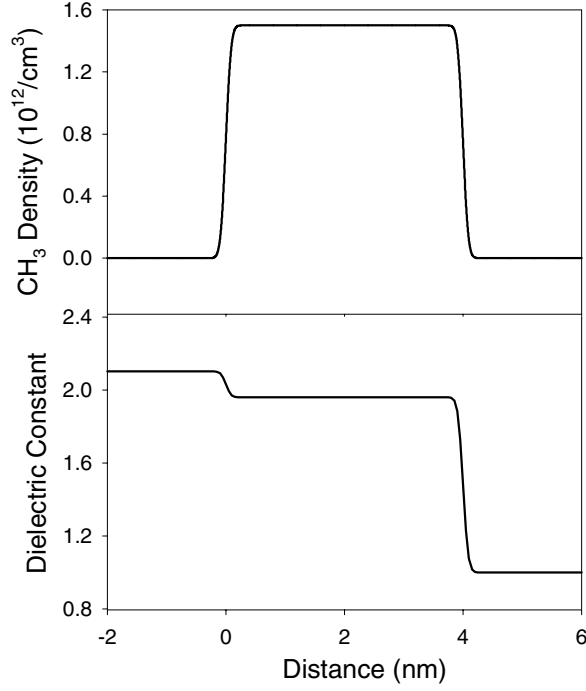


Fig. A The density and dielectric function profiles of the adsorbed protein layer used in our calculation on nonlocal contributions.

change the conclusion. The density and dielectric function profiles used in our calculation are shown in Fig.A.

Furthermore, we assume that the distribution function $G(\theta, z)$ can be written as a product of a depth-dependent density and a depth-independent orientation distribution $g(\theta)$ (i.e. the orientation of the methyl groups is independent of their location):

$$G(\theta, z) = \frac{N(z)}{N_0} \cdot g(\theta) \quad (\text{A.6})$$

and we assume that the orientation distribution is a random distribution, for which the local contribution vanishes.

B. Further discussions about the local field corrections

We used the local refractive index term $n(z)$ instead of the average n' which is used in the infinitesimally thin film layer model, and ignored the phase variation of the optical field (protein monolayer adsorption). The calculated results of F_{ijk} as a function of $n(z)$ by using the Lorentz model and Eqn. A4 and Eqn. 10 are shown in Fig. B. Please note that according to the definition

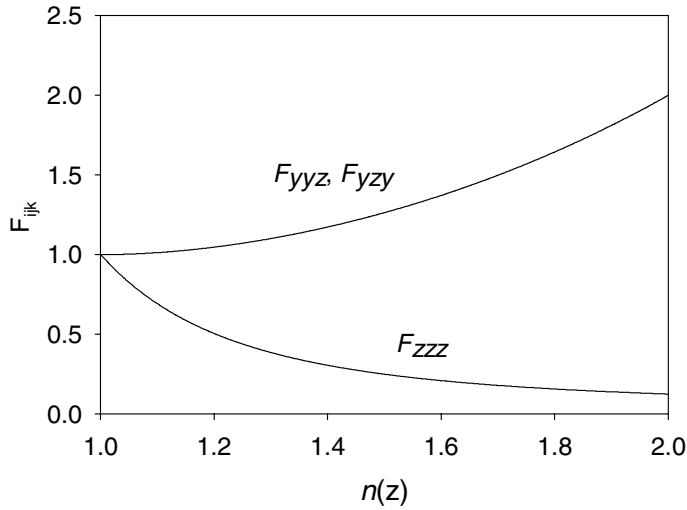


Fig. B Calculated F_{ijk} as a function of local refractive index by using Lorentz model ($n_2=1$)

in Eq.5, F_{ijk} includes both the local

refractive index term $\left(\frac{n_2^2}{n^2(z)} \right)$ and the

local optical field correction factor. With

this approximation, the ssp and sps

polarization combinations have no local

field correction factors and no $\left(\frac{1}{n^2(z)} \right)$

terms in the calculation of the local optical

field, which is quite different from the

common infinitesimally thin film model. Our

model also indicates that the quantitative

interpretation of ppp spectra collected from interfacial proteins may need more in-depth analysis.

C. Discussion of the effect of the protein film thickness

We emphasized in our paper that our treatment is suitable for thin protein films (e.g. monolayer adsorption). Under this condition, the protein film is thin enough to ignore the slow

phase shift due to propagation of the light beams across the film (equation 4 in the paper). In order to provide more insights into this assumption, we will use two examples to evaluate the effect of the slow phase shift. We consider two extreme conditions in which the chemical groups we are measuring are separated into two parts by a distance d (Figure C). In case *a*, all the groups orient along the film surface normal in the same direction, while case *b* has a symmetric film structure. The calculated SFG intensities with and without considering the slow phase shifts are shown in Figure D. The calculation method can be found in Ref. 42. Our calculations are based on our SFG geometry indicated in this paper and we assume the refractive indices of the films are 1.45.

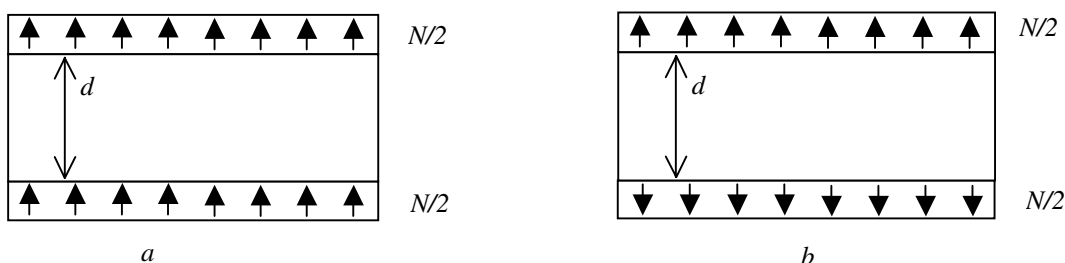


Fig. C Model films used to evaluate the slow phase shift effect.

From the calculated results we can see that if the protein film is thick, the slow phase shift should be considered in the calculation. The calculation of the thick film requires an additional knowledge of the depth dependent density of chemical groups, which is usually unknown. If the protein film is very thin (<10 nm), ignoring the phase shift will not generate substantial errors and can simplify the calculation. Usually, the thickness of an adsorbed protein monolayer is only several nanometers. Therefore, our treatments in the paper are suitable for most conditions.

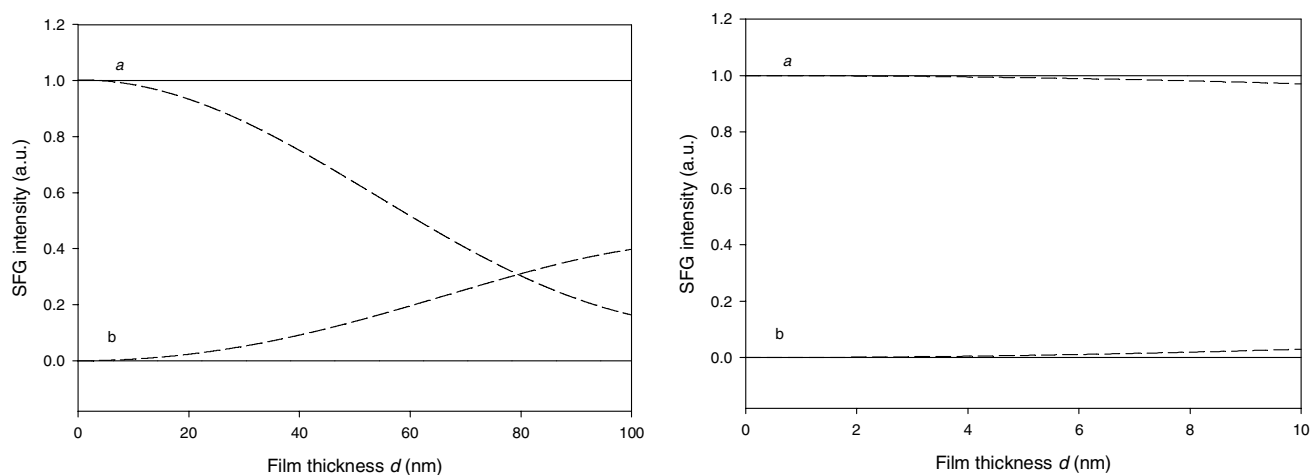


Fig. D Calculated SFG intensity without considering the slow phase shift (solid line); considering the slow phase shift (dash); a: calculated results for Figure Ca; b: calculated results for Figure Cb;

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