

Delocalized Surface Modes Reveal Three-Dimensional Structures of Complex Biomolecules

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Introduction

- VSFG is employed to probe vibrational modes in the high-frequency region (1500-4000 cm^{-1}) and low-frequency region (below 1500 cm^{-1}) of the IR spectrum.

❖ high-frequency localized (non-skeletal)modes:

- composition of the movement of a single chemical group
- localized nature

❖ low-frequency (skeletal) modes :

- composition of the movement of several chemical groups
- delocalized nature,
- To sensitive the 3D structure of molecules.

-To report femtosecond VSFG spectroscopy in the fingerprint region for [poly(lactic acid) (PLA)].

-To study the 3D surface structure of amorphous L-PLA (LA), crystalline L-PLA (L-C), and racemic D/L-PLA (R).

Sum-frequency generation- second-order nonlinear optical process

The SF intensity :

$$I_{\text{SFG}}(\omega) \propto \left| E_{\text{IR}}(\omega) \sum_n \int_{-\infty}^{\infty} \chi^{(2)}(\omega') E_{\text{VIS}}(\omega' - \omega) d\omega' \right|^2$$

$$\begin{aligned} \chi^{(2)}(\omega) &= \chi_{\text{NR}}^{(2)} + \sum_n \chi_n^{(2)}(\omega) \\ &= A_{\text{NR}} e^{i\Delta\phi} + \sum_n \frac{A_n}{(\omega - \omega_{0n}) + i\Gamma_n} \end{aligned}$$

E_{IR} : the envelope of the IR spectrum

E_{VIS} the envelope of the visible spectrum

A_n : the amplitude

ω : the IR frequency

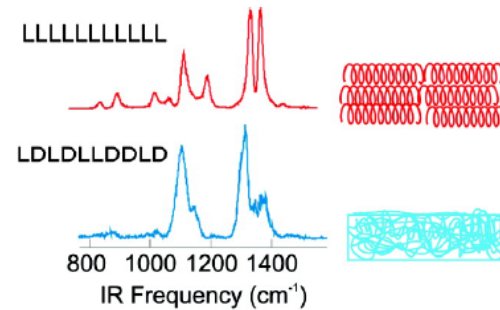
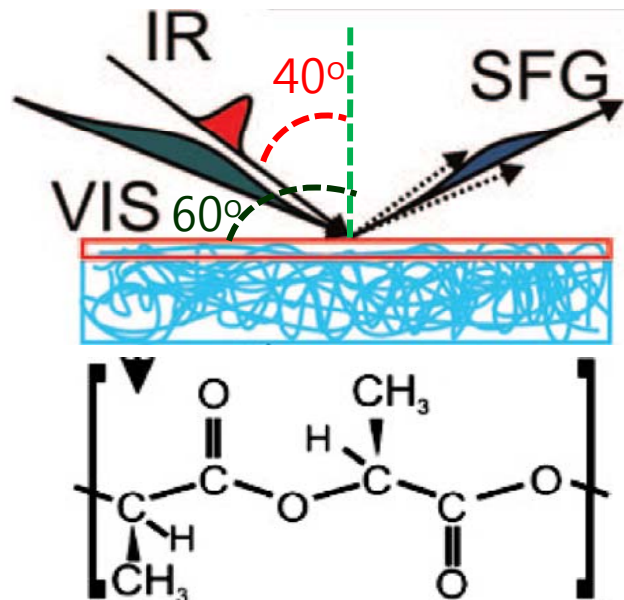
ω_{0n} : the peak centers

Γ_n : the damping constants of the vibrational mode n.

$\Delta\phi$: the relative phase difference between the resonant and the non-resonant fields

VSFG Experiment

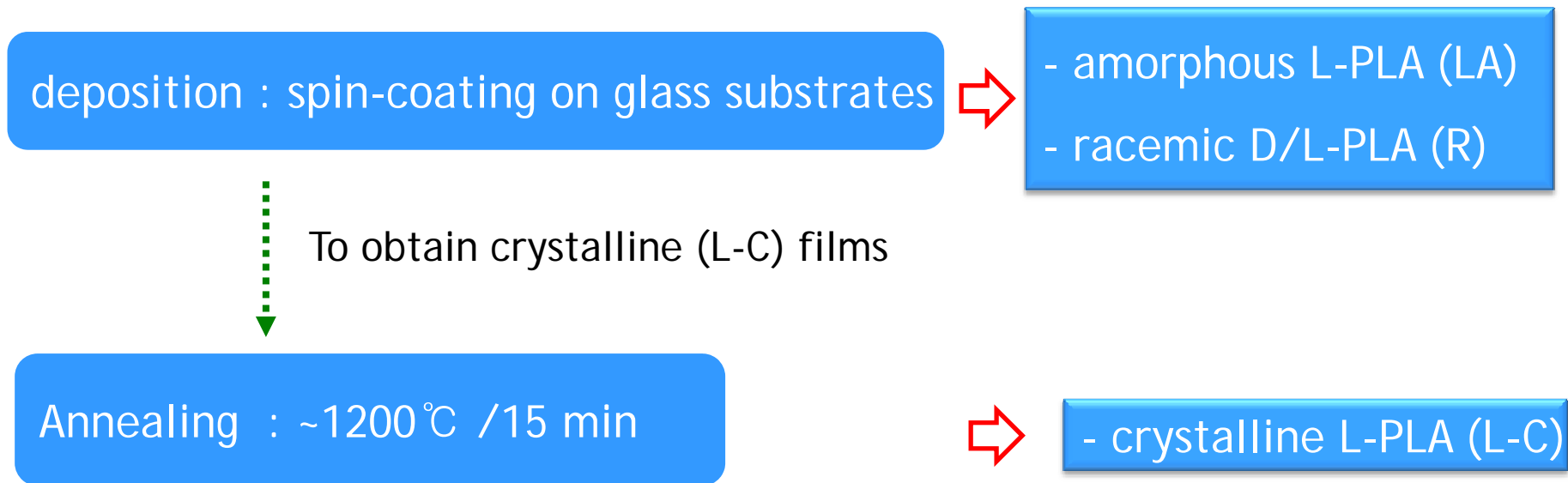
laser system : 1 kHz broad-band high-power Ti:Sapphire laser



- p-polarized IR pulses : 5 μJ energy
150-250 fs (pulse durations)
- s-polarized visible pulses (800 nm) : 3 μJ energy
5 cm⁻¹ FWHM
0.75 mm focus diameter

The sample preparation

2.5 wt% solutions : polymer powder (L-PLA or PLA polymerized from a 1:1 mixture of L-lactide and D-lactide) in chloroform solvent



Results and Discussion

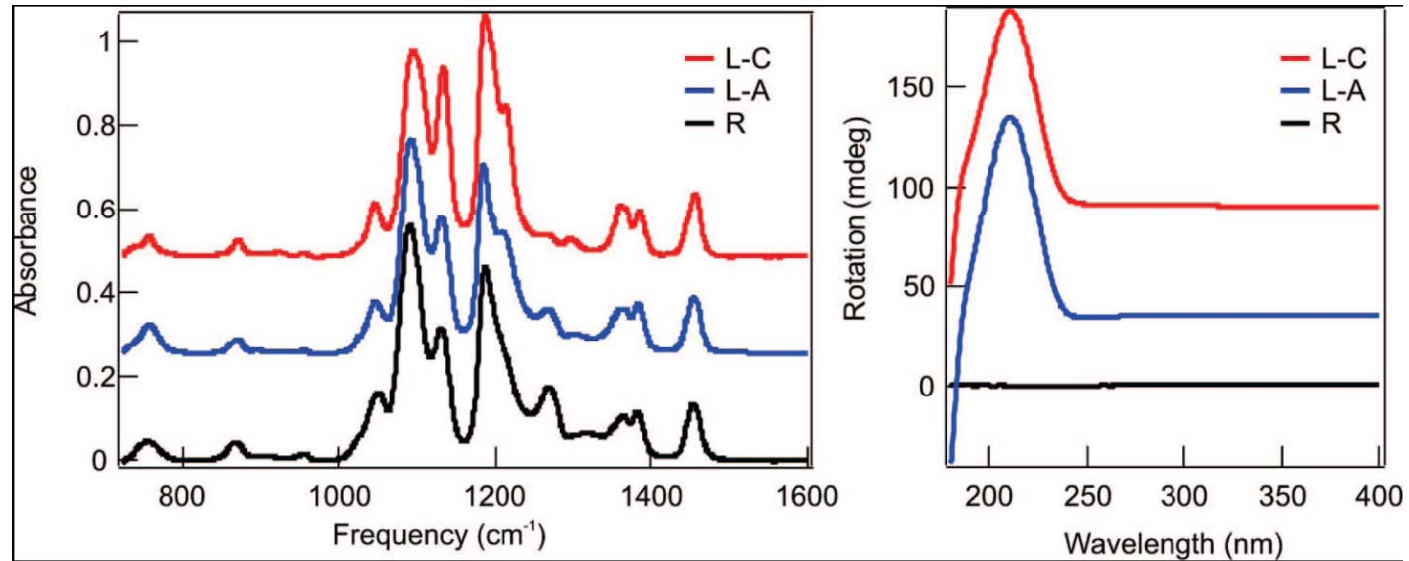


Figure 1. Left: IR spectra (left) and CD measurements (right) of L-crystalline (L-C), L-amorphous (L-A), and racemic (R) PLA films. The IR spectra display the average bulk structure of the films, and since they are chemically identical, only small differences are observed. The CD spectra show that the L-C and L-A films consist of helices, while the R film displays no rotation since it is racemic.

- A IR spectra show some changes in the fingerprint region
- A circular dichroism (CD) measurements : the bulk of the L-C and the L-A film have structure of helices

At lower frequency area

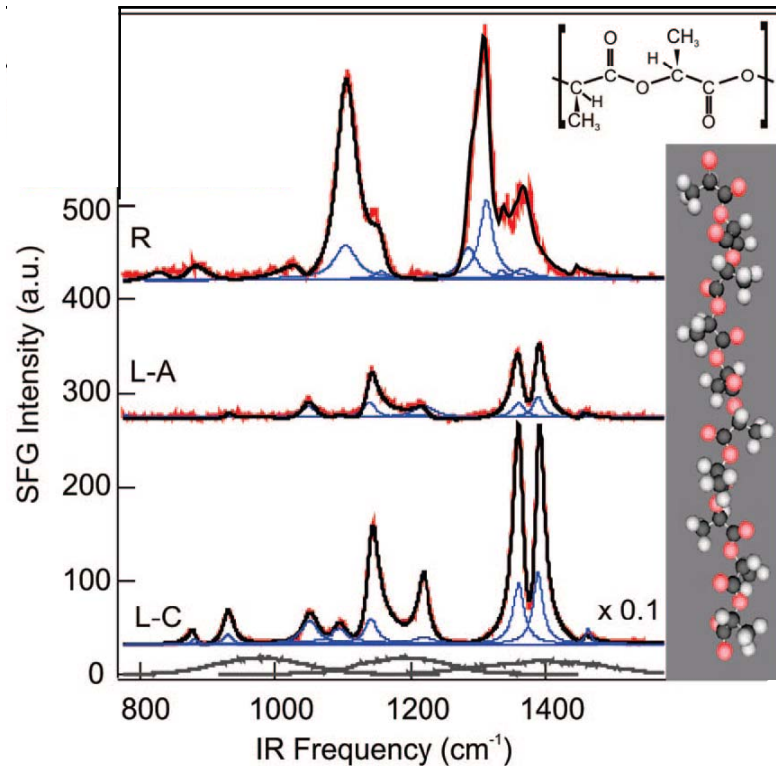


Figure 2. VSG spectra of the delocalized modes of L-crystalline (LC), L-amorphous (L-A), and racemic (R) PLA films, taken with three different IR pulses, which are displayed in the bottom. The black lines are fits to the data in which all contributions to the reflected electrical sum frequency field are added. The blue Lorentzians display the most prominent vibrational modes. The chemical repeat unit of L-PLA is also shown, as well as a molecular model of a 10_3 helix.

The spectra reflects the secondary and tertiary structure of PLA films

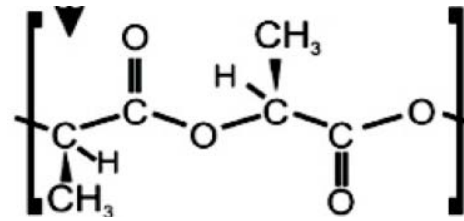
Global Fitting Parameters

TABLE 1: Mode Assignments, Resonance Frequencies (ω_{0n}), Relative Amplitudes (A_n), and Spectral Half-Width at Half-Maximum (Γ_n) Used in the Global Fitting Procedures (Parameters Given in the Order L-C/L-A/R)

mode assignment	ω_{0n} (cm ⁻¹)	A_n	Γ_n (cm ⁻¹)
C-COO stretching	-/-/832	-/-/-0.15	-/-/20
C-COO + C-CH ₃ + O-CO stretching	878/878/881	0.22/-0.04/-0.15	6.7/15.5/20
C-CH ₃ rocking + O-CH stretching	929/929/-	0.39/0.02/-	9.2/9.3/-
C-CH ₃ rocking + C-H bending	1051/1049/1050	0.87/0.18/0.02	7.0/12.5/12
C-CH ₃ stretching + C-CH ₃ rocking	1093/1093/1104	0.31/0.04/-0.52	12.7/12.7/18
C-H bending	1141/1139/1157	0.70/0.14/0.14	10.7/10.6/11
C-COO + O-CH stretching + C-CH ₃ rocking + C-H bending	1187/-/-	-0.40/-/-	17.1/-/-
C-H bending + O-CH stretching	1216/1220/1216	0.66/-0.07/-0.02	10.6/12.6/20
C-CH ₃ rocking	-/-/1286	-/-/0.37	-/-/14.4
C-H bending	-/-/1312	-/-/-0.48	-/-/11.6
O-CO stretching	1360/1359/1335	1.1/0.17/-0.08	9.2/10.1/6.3
sym. CH ₃ bending	1388/1387/1367	0.96/0.16/0.24	9.6/9.2/16.4
asym. CH ₃ bending	1454/1453/1443	0.22/0.038/0.05	9.0/7.7/6.0

For the L-C spectra

skeletal mode



delocalized vibrational modes :

- 878 cm⁻¹ (C-COO + C-CH₃ + O-CO stretch modes)
- 1187 cm⁻¹ (C-COO + O-CH stretching + CH₃ rocking + C-H bending modes)
- 1216 cm⁻¹ (C-H bending + O-CH stretching modes)

slightly more localized combination modes :

- 1093 cm⁻¹ (C-CH₃ stretch mode + C-CH₃ rocking mode)
- 1360 cm⁻¹ (O-CO stretching mode)

Non-skeletal mode

localized modes :

- 929 and 1051 cm⁻¹ (mainly CH₃ rocking mode)
- 1141 cm⁻¹ (C-H bending mode)
- 1388 cm⁻¹ (sym. CH₃ bending mode)
- 1454 cm⁻¹ (asym. CH₃ bending mode)

we can for the first time selectively and directly probe the helical motion at the surface.

For the L-A film (compared with L-C film)

Above 1300 cm^{-1} IR frequencies : intensity different in 1360, 1388 cm^{-1}

→ A reflection of the disordered nature of the L-A film

Mode disappearance : 878, 1187, 929, and 1093 cm^{-1}

→ A distortion of the skeletal geometry

The relative intensities have not changed at 1139 cm^{-1} (C-H bending mode), 1220 cm^{-1} (skeletal combination mode), and 1359 cm^{-1} (O-CO stretching modes)

→ A reflection of helically shaped

The packing and orientation different

→ the rocking modes changes (at 929, 1051, and 1093 cm^{-1})

the R film (Compared with these L-C and L-A spectra)

Mode disappearance : 929 cm⁻¹ (CH₃ rocking)

1220 cm⁻¹ (delocalized skeletal mode)

Shifts appearance : C-CH₃ stretch modes (from 1093 to 1104 cm⁻¹),

C-H bending mode (from 1141 to 1157 cm⁻¹),

C—COO stretch mode (from 878 to 881 cm⁻¹).

New mode appearance : 832, 1286, 1312, and 1335 cm⁻¹.

- 832 cm⁻¹ coincides with the C-C stretch frequency of an unbound carboxylic acid group.

- 1286 cm⁻¹ has never been reported for racemic PLA but has been calculated to be typical of a helical structure

The chain geometry of the R polymer film is totally different
compare with the L-A and L-C films.



Indication of a more loosely connected monomer-like structure

At high-frequency region

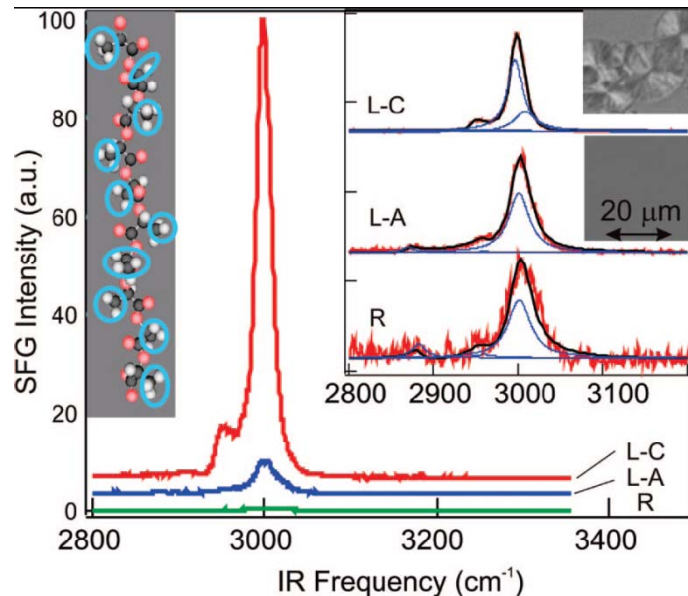


Figure 3. VSGF spectra of L-crystalline (L-C), L-amorphous (L-A), and racemic (R) PLA films, probing the localized methyl stretch modes. The inset shows the scaled spectra together with the fits (black lines). The blue Lorentzians display the vibrational modes. Crossed-polarized microscopy images of the L-C and L-A films reflect the difference in the state of the films. The image of the R film was featureless (just like the one from the L-A film) and has therefore been omitted.

The R and L-A spectra : two modes (2947 and 2997 cm⁻¹)
(symmetrical and antisymmetrical stretch modes of the CH₃ groups)

The L-C spectrum : two modes (2965 and 3007 cm⁻¹)
(the intensities difference reflects surface order difference)

The limited amount of knowledge obtainable from probing localized surface modes
→ into the 3D arrangement of the biopolymer cannot be obtained
versus A large amount of knowledge obtainable from probing delocalized modes

Conclusions

- The interface of biopolymers in the fingerprint region reveals enormous differences in the 3D structure of the complex chains.
- Vibrational modes are delocalized over a number of groups on a single chain.
- Delocalized vibrational modes are sensitive to secondary and tertiary structural elements.
- This ability to follow in detail 3D structures of large (bio)molecules at interfaces should be of enormous value in many fields of research.