Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Chain Dynamics and Crystallinite Network Structure of Poly[R-3-hydroxybutyrate-co-4-hydroxybutyrate] as revealed by Solid-state NMR

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The *in-situ* WAXS data of annealing experiment for P(3HB-co-4HB)

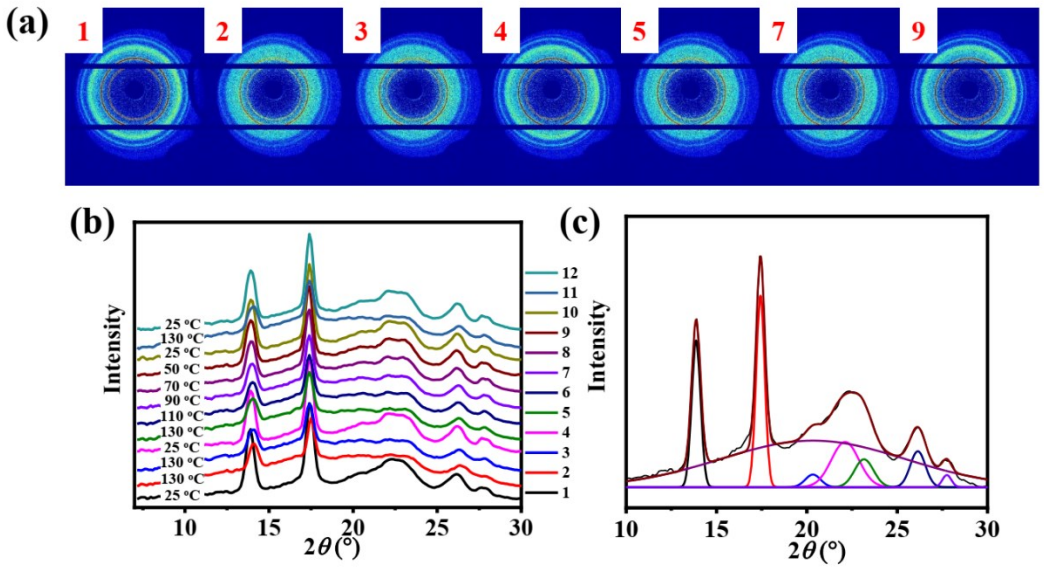

Figure S1. (a) The WAXS pattern of P(3HB-co-4HB) during the annealing experiments; the top number is the experimental sequence; (b) the 1D integrated WAXS intensity curves; the temperature is shown in the left; (c) the multi-peak deconvolution of 1D integrated WAXS curve.

The annealing experiments of P(3HB-co-4HB) films were *in-situ* tracked by WAXS. The 2D-WAXS pattern was shown in Figure S1a. The 1D integrated WAXS intensity curves were summarized in Figure S1b. In order to determine the crystallinity of P(3HB-co-4HB) by WAXS, the relative crystallinity χ_c can be quantitatively analyzed by the multi-peak fitting of 1D integrated WAXS curves as shown in Figure S1c and the expression is described as follows:

$$\chi_c = \frac{A_c}{A_c + A_{amor}} \times 100\% \quad (S1)$$

where A_c represents the sum of the peak area of the crystalline domain, A_{amor} represents the peak area of the amorphous domain.

The SAXS data of heating process for P(3HB-co-4HB)

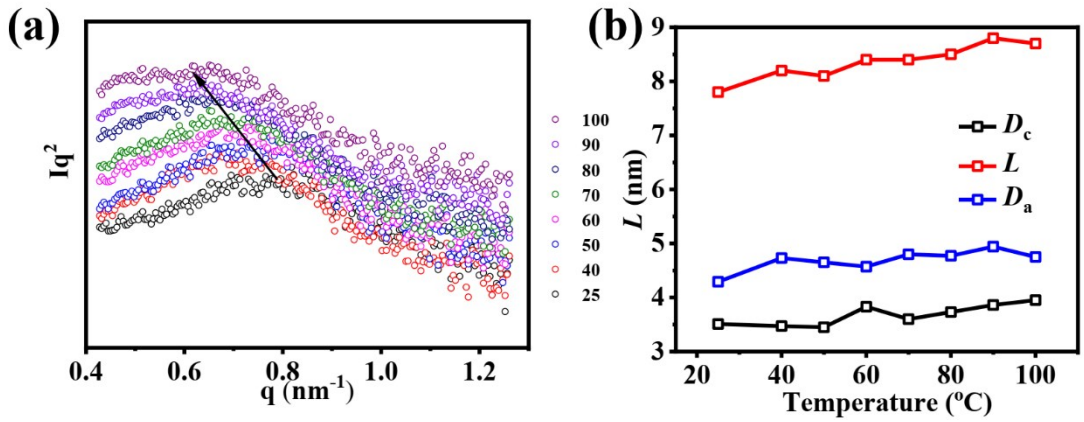

Figure S2. (a) The relative integrated 1D SAXS intensity (Iq^2) curves for different temperature; the temperature unit is celsius (°C); (b)the long period(L), lamellae thickness (D_c) and the distance between the lamellae (D_a)as a function of temperature.

The heating process of P(3HB-co-4HB) films were tracked by SAXS. The wavelength of synchrotron X-ray is 0.124 nm. The SAXS scattering profiles are obtained as a function of

$$q = \frac{4\pi \sin(\theta)}{\lambda} \quad (S2)$$

where q is the module of the scattering vector, λ is the X-ray wavelength and θ is the scattering angle.

The long period is calculated by Bragg's law:

$$L = \frac{2\pi}{q_{max}} \quad (S3)$$

where q_{max} is peak position of the relative integrated 1D SAXS intensity (Iq^2) curves. The lamellar thickness (D_c) and spacing amphous distance between two lamellae (D_a) are calculated by a correlation function obtained from the equation as follows:

$$\gamma(r) = \frac{\int_0^{\infty} I(q)\cos(qr)dq}{\int_0^{\infty} I(q)dq} \quad (S4)$$

The relative integrated 1D SAXS intensity (Iq^2) curves calculated by eq. S2 are shown in Figure S2a. The long period(L), lamellae thickness (D_c) and the distance

between the lamellae (D_a) calculated by eq. S3 and eq. S4 is shown in Figure S2b.

Chemical sequence analysis of P(3HB-co-4HB)

Table S1. Chemical Shifts and Relative Intensities of ^{13}C Resonances in P(3HB-co-4HB) Samples

	Chemical shift	Sequence	Area	Area/%	Sum of area	Unit Contents/%
$CH_2(2)$	40.94	43*34	2.2	3	75.97	86
	40.88	43*33	6.34	8		
	40.83	33*34	13.57	18		
	40.78	33*33	45.44	60		
	40.63	43*4	0.91	1		
	40.52	33*4	7.51	10		
$CH_2(6)$	30.56	34*4	1.03	8	12.47	14
	30.61	44*4	0.33	3		
	30.77	34*33	7.79	62		
	30.8	34*33	1.71	14		
	30.82	44*33	1.61	13		
	30.88	44*34	0	0		
C=O(1)	169.12	33*3	32.01	77	41.47	85
	169.21	43*3	4.85	12		
	169.97	33*4	4.21	10		
	170.05	43*4	0.4	1		
C=O(5)	171.81	4*3	3.57	49	7.26	15
	171.85	4*3	2.5	34		
	172.59	34*4	1.19	16		
Probabilities/%						
	P_3	P_4	P_{33}	P_{34}	P_{43}	P_{44}
$CH_2(2)$	86	□	76.4	9.5		
$CH_2(6)$	□	14	□	□	12.6	1.2
C=O(1)	85	□	75.6	9.5		
C=O(5)	□	15	□	□	12.5	2.4
averag	85.5□	14.5□	76	9.5	12.5	1.8

The chemical shift of P(3HB-co-4HB) is determined by ^{13}C NMR in the solution

state and the result is shown in Figure S3a. The different resonances are assigned to specific carbon sequences in 3HB and 4HB units according to the ref 2.[1] In order to analyze the chemical sequence between 3HB and 4HB units quantitatively, the expanded ^{13}C NMR spectra for the different group is fitted by Lorenz function and results are shown in Figure S3b-d. The quantitative data of probabilities for different carbon sequences is shown in Table S1. If the polymer is a statistically random copolymer described by the Bernoulli statistics. To determine whether a polymer is a random copolymer or not, parameter *D* is defined as follows:

$$D = \frac{P_{33} * P_{44}}{P_{34} * P_{43}} \quad (S5)$$

where P_{33}, P_{44}, P_{34}, and P_{43} represent the probability calculated by the ratio of characteristic peak area for carbon sequence of 3HB*3HB, 4HB*4HB, 3HB*4HB, and 4HB*3HB respectively and sum area. In this work, the *D* value is calculated about 1.15, which indicates that the sequence distribution of 3HB and 4HB units is close to a statistically random distribution.

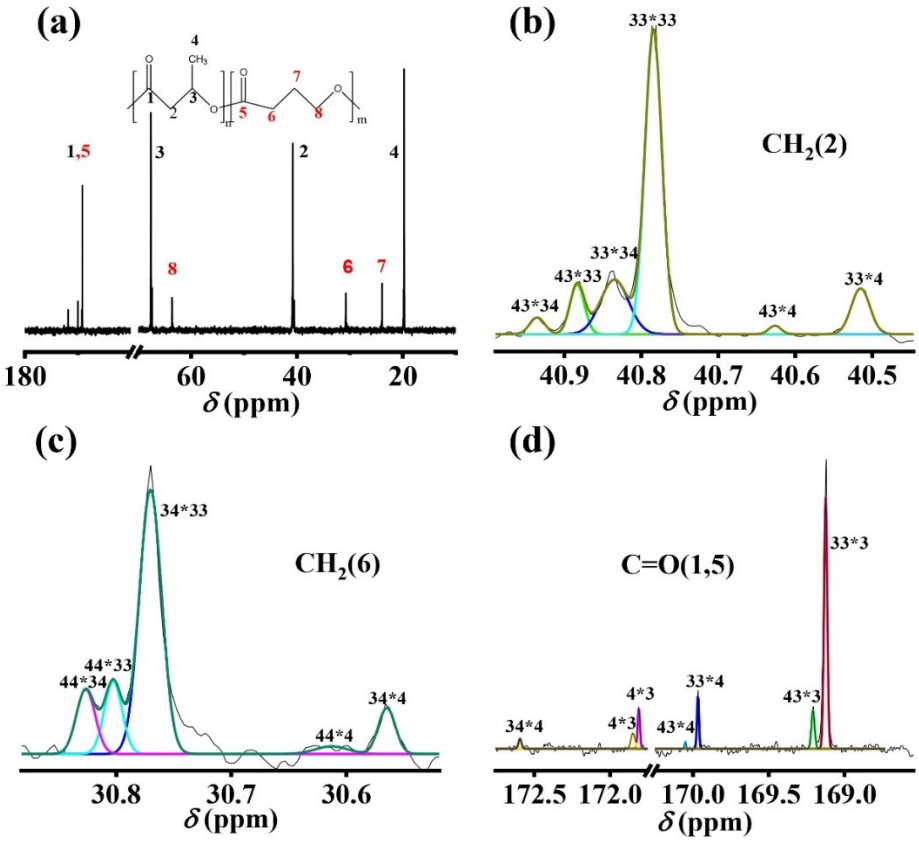

Figure S3. (a) ^{13}C NMR spectra of P(3HB-co-4HB) in solution state; multi-peak

deconvolution of expanded ^{13}C NMR spectra for different group:(b) CH_2(2), (c) CH_2(2) and (d) C=O(1,5); The ^{13}C chemical shift assignments are given in Table S1.

^1H-^1H NOESY contour plots at different mixing time

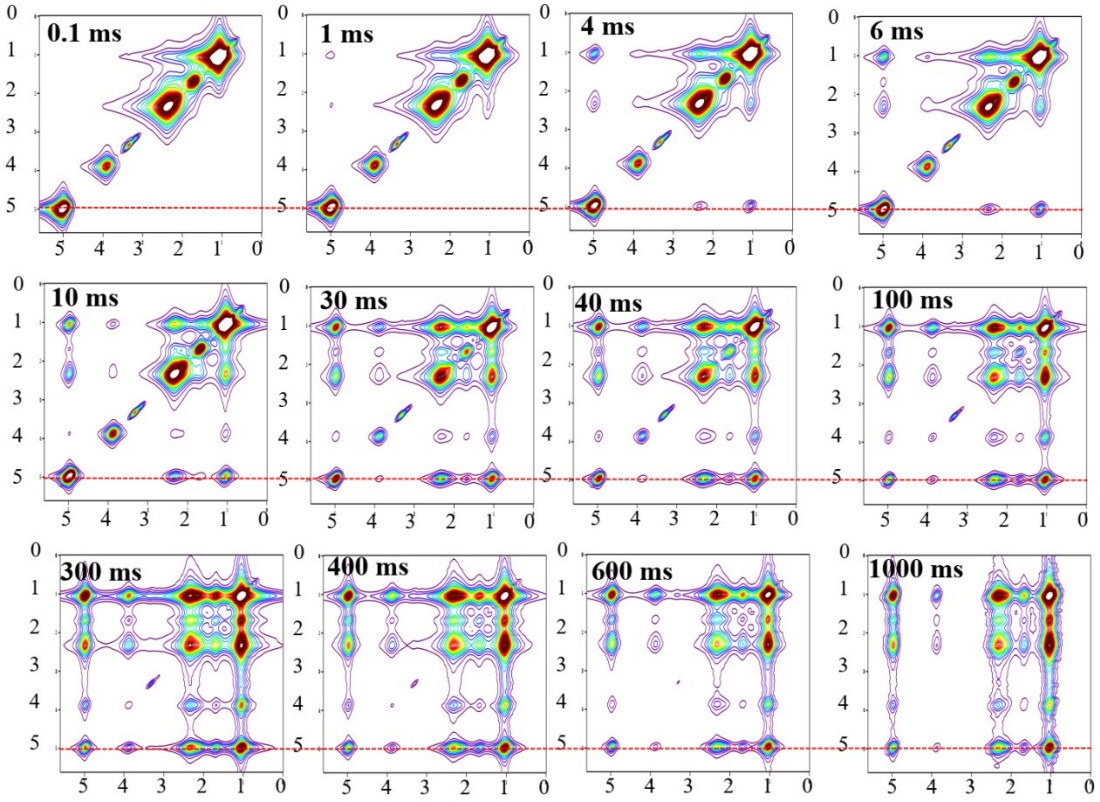

Figure S4. ^1H-^1H NOESY 2D spectra of P(3HB-co-4HB) of different mixing time(t_{mix}) respectively to 0.1、1、4、6、10、30、40、100、300、400、600 and 1000 ms.

The selected MSE-FID curves at different temperatures.

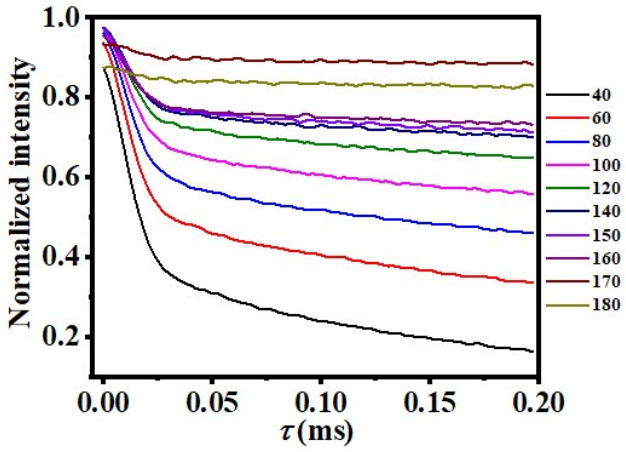

Figure S5. The selected MSE-FID curves at different temperatures;the temperature

unit is celsius (°C).

The ^{13}C CODEX-NMR results.

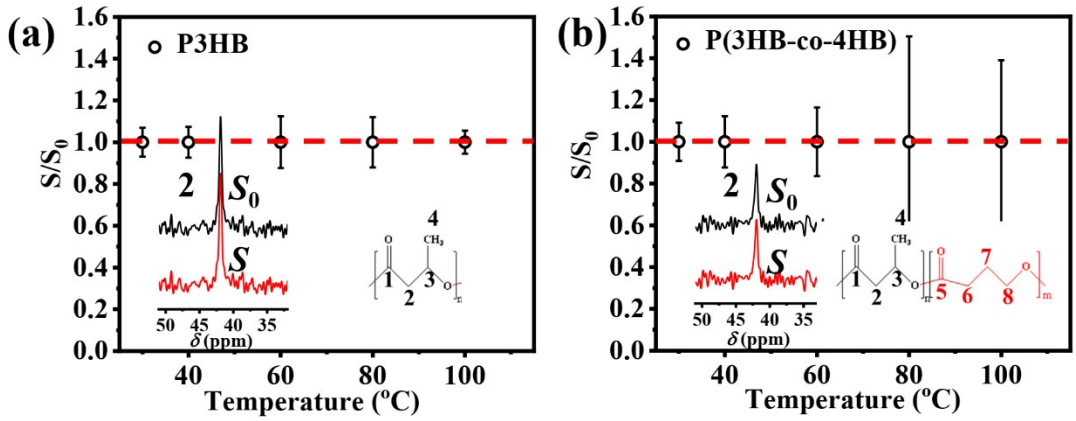

Figure S6. CODEX (S/S_0) decay curves of the CH_2 (2) carbon with evolution of temperature: (a) P3HB; (b) P(3HB-co-4HB); the CODEX S and S_0 spectra at 303 K with t_{mix}=500 ms is shown in bottom left.

The MAPE-FID curves at different MAPE filter time (τ_{MAPE}).

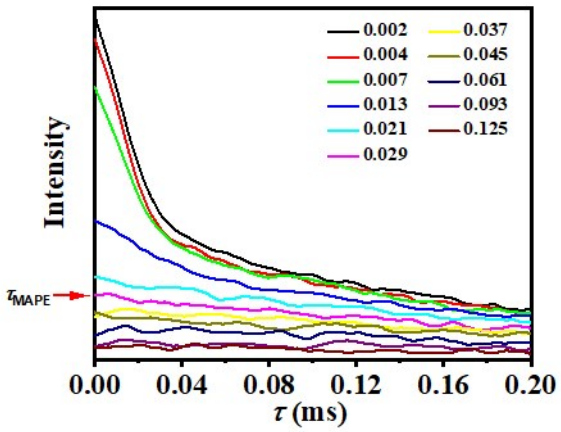

Figure S7. The MAPE-FID curves at different MAPE filter time (τ_{MAPE});the unit of filter time is millisecond (ms).

References:

(1) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. Nuclear Magnetic Resonance Studies on Unusual Bacterial Copolyesters of 3-Hydroxybutyrate and 4-Hydroxy Butyrate. *Macromolecules* **1988**, *21* (9), 2722–2727.