Supporting information

Dynamic changes of intracellular monomer levels regulate block sequence of polyhydroxyalkanoates in engineered *Escherichia coli*

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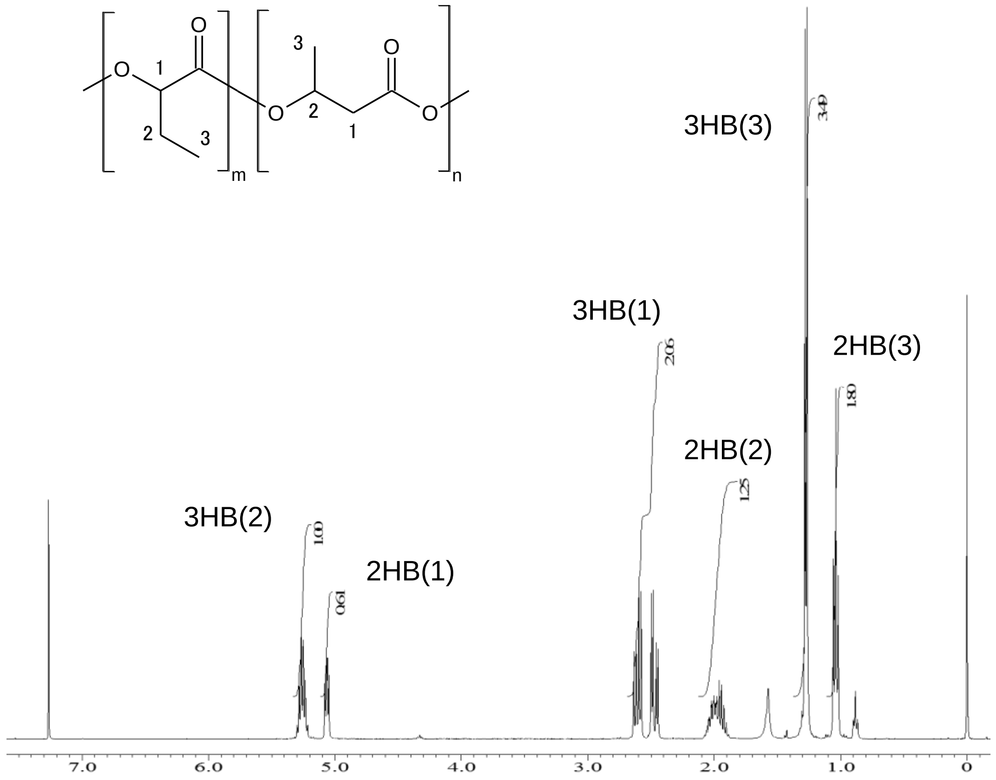
Plasmid construction. The *phb* operon promoter region of *R. eutropha* was amplified by PCR using a pair of primers; AAGGTACCCGGGCAAGTACCTTGCCGACAT and TCTCTCGAGTCACTATTCGAACCGGCTCCG. The *Kpn*I/*Xho*I fragment was inserted into the *Kpn*I/*Xho*I sites of pBluescript KS^{+} to yield pBSP$_{Re}$. The propionyl-CoA transferase (*pct*) gene from *Megasphaera elsdenii* was amplified from pTV118NpctC1STQKAB using a pair of primers; AGATCTAGGAGGTAAACAATGAGAAAAGTAGAAATCA and GAGCTCTGCAGGTTATTTTTTCAGTC. The *Bgl*II/*Sac*I fragment was inserted into the *Bam*HI/*Sac*I sites of pBSP$_{Re}$ to yield pBSP$_{Re}$pct. The chimeric PHA synthase gene, which is composed of PhaCs from *Aeromonas caviae* and *Ralstonia eutropha*, designated as PhaC$_{AR}$, was amplified from pGEMC$_{AcRe12}$AB[23] using a pair of primers; CGGTTCGAATAGTGACTCGAGCCGGTTCGAATCTAGAAAT and CGATACCGTCGACCTCGACAATGGAAACGGGAGGGAAC. The amplified fragment was inserted into the *Xho*I site of pBSP$_{Re}$pct using an In-Fusion dry-down PCR cloning kit (Clontech) to yield pBSP$_{Re}$phaC$_{AR}$pct.

Table S1. P(2HB-*co*-3HB) production in recombinant *E. coli* expressing PhaC1$_{Ps}$STQK.

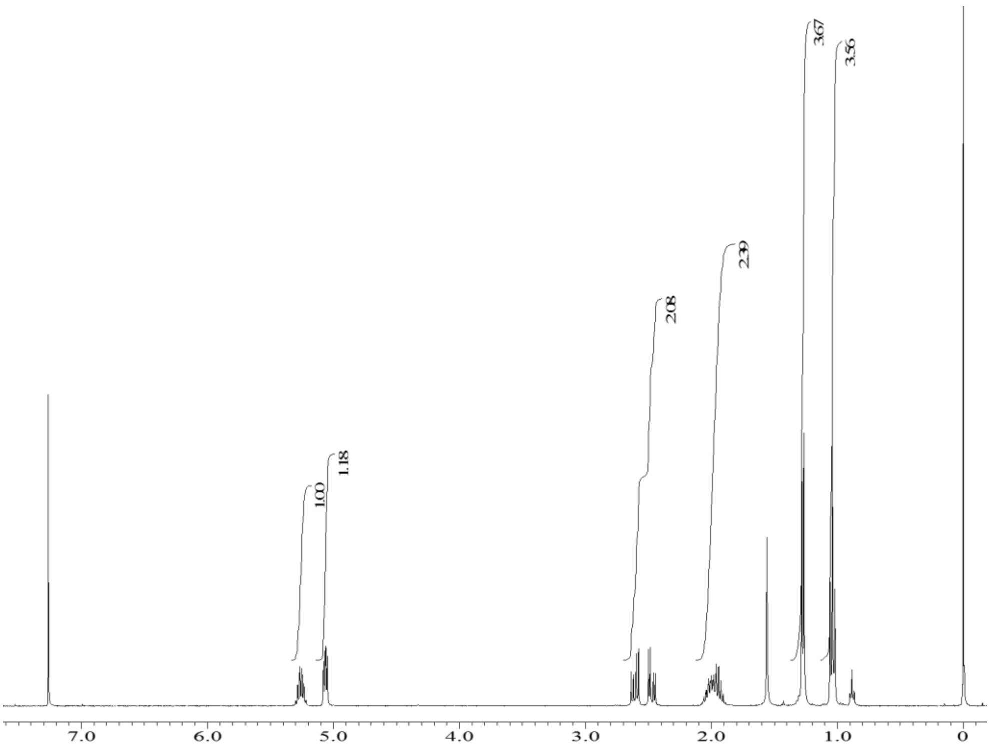
PhaC	Sodium 3HB (g/L)	Sodium 2HB (g/L)	Cell dry weight (g/L)	Polymer production (g/L)	2HB fraction (mol%)	M_w ($\times 10^{-4}$)	PDI[a]
STQK	5	5	2.2±0.24	0.11±0.01	51.5±3.6	5.9	1.6
STQK	5	10	0.66±0.23	0.12±0.01	88.4±0.5	5.7	2.2

[a]polydispersity index

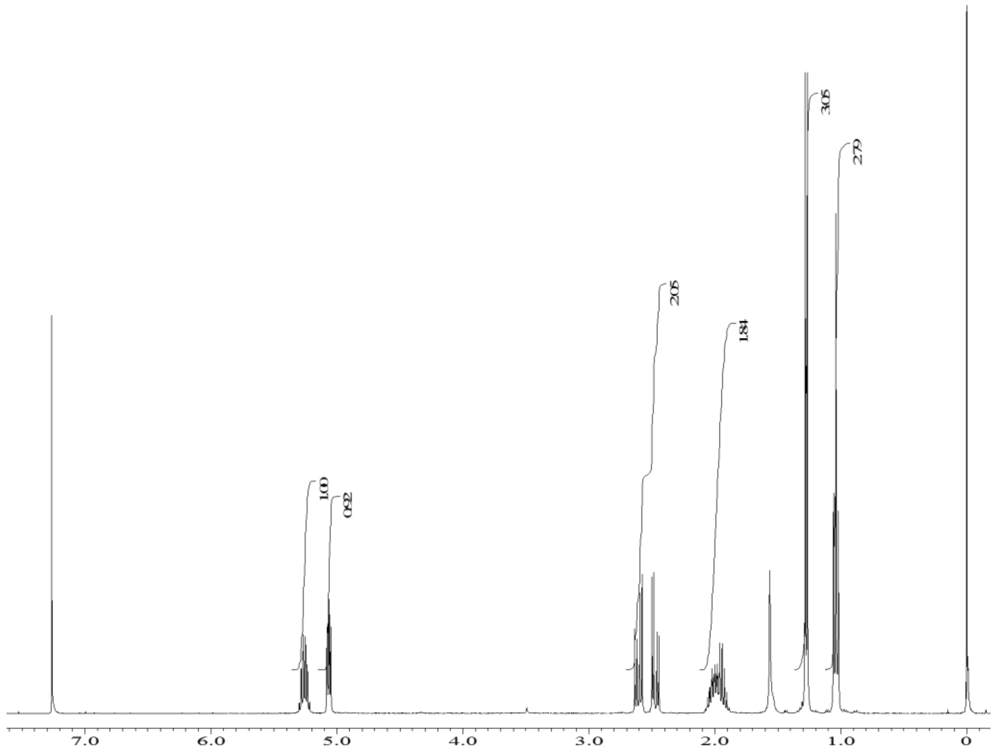
(a) P(2HB-*b*-3HB) at 12 h



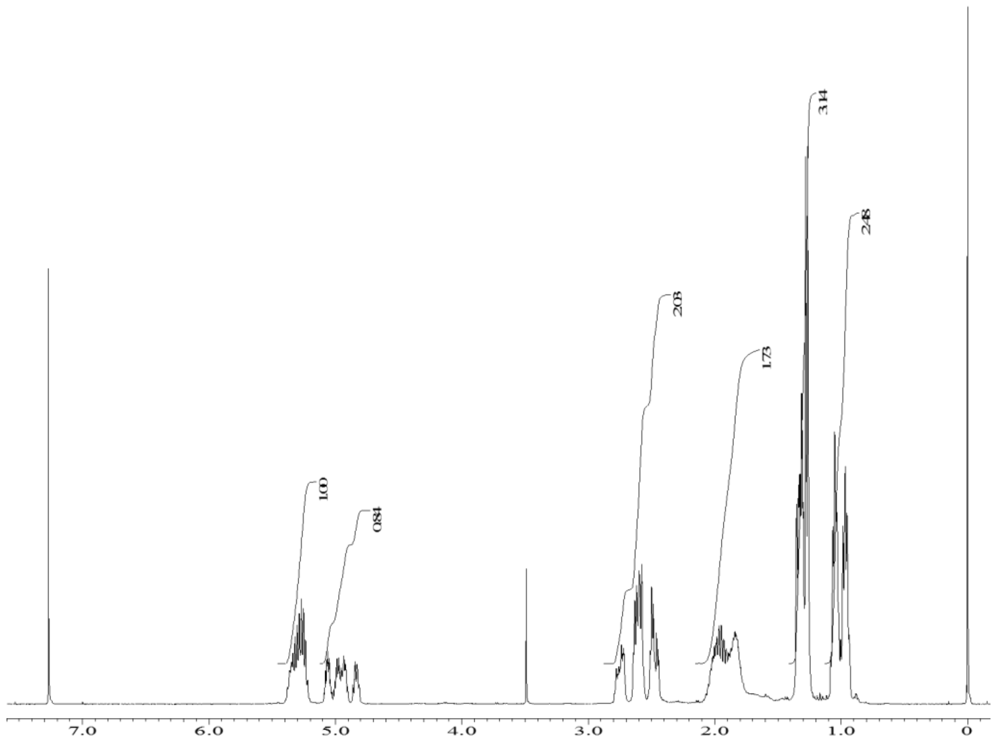
(b) P(2HB-*b*-3HB) at 18 h



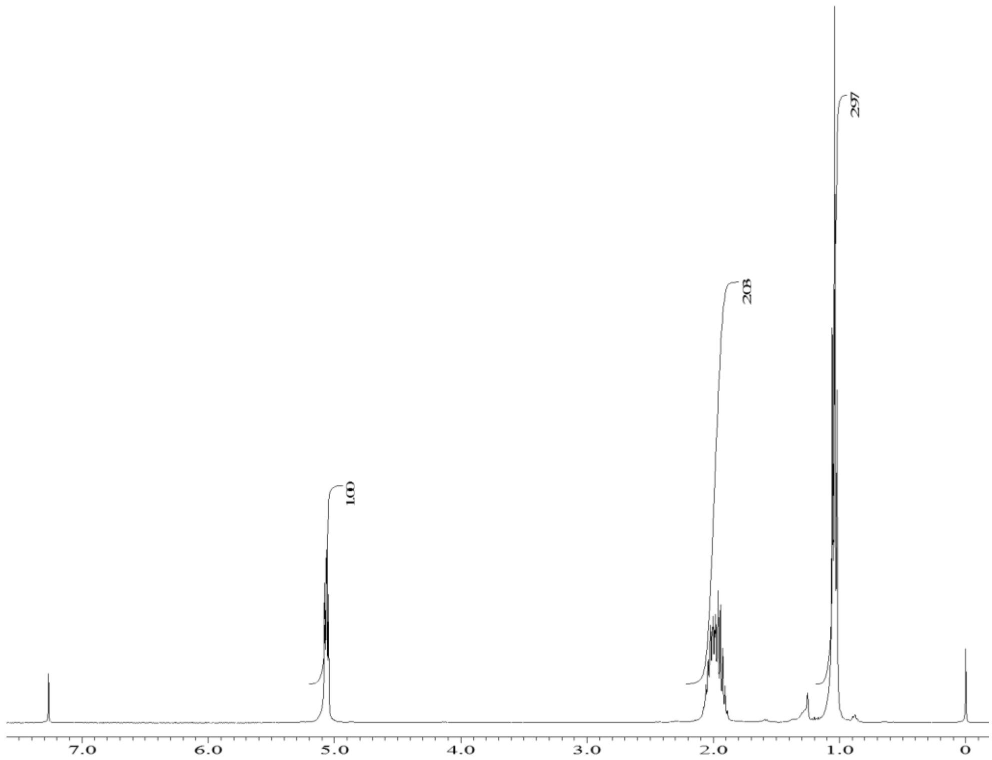
(c) P(2HB-*b*-3HB) at 24 h



(d) P(2HB-*ran*-3HB)



(e) P(2HB)



(f) P(3HB)

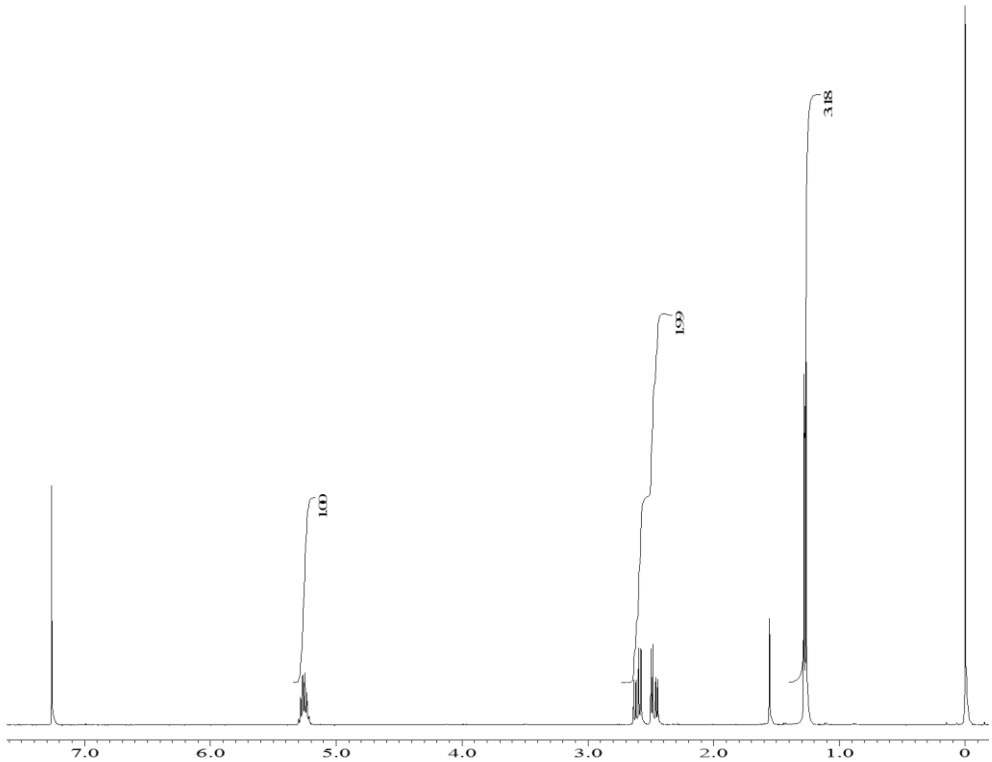
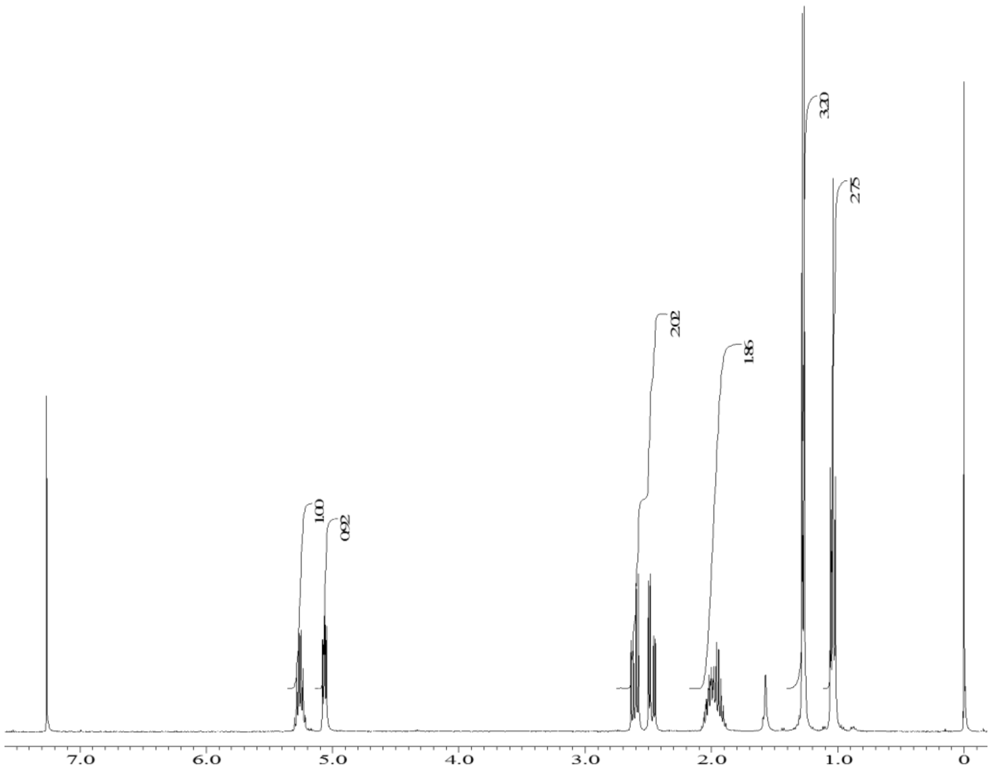
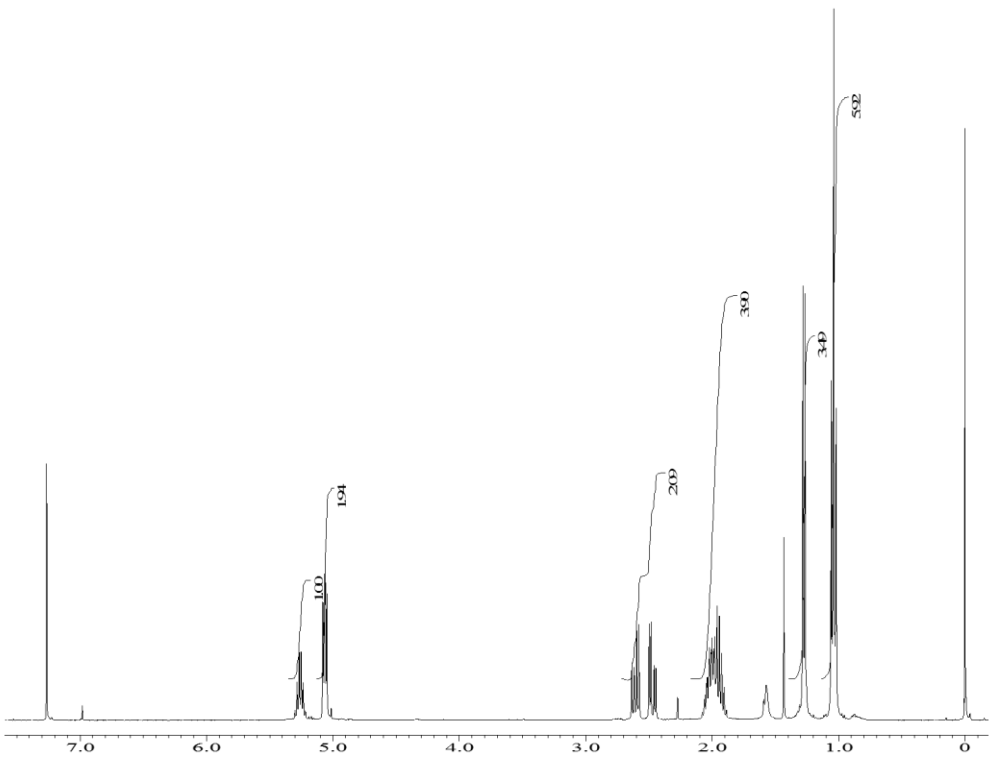


Figure S1. [1]H NMR of polyesters synthesized in *E. coli*. P(2HB-*b*-3HB) at 12 h (a), 18 h (b) and 24 h (c), P(2HB-*ran*-3HB) (d), P(2HB) (e) and P(3HB) (f).

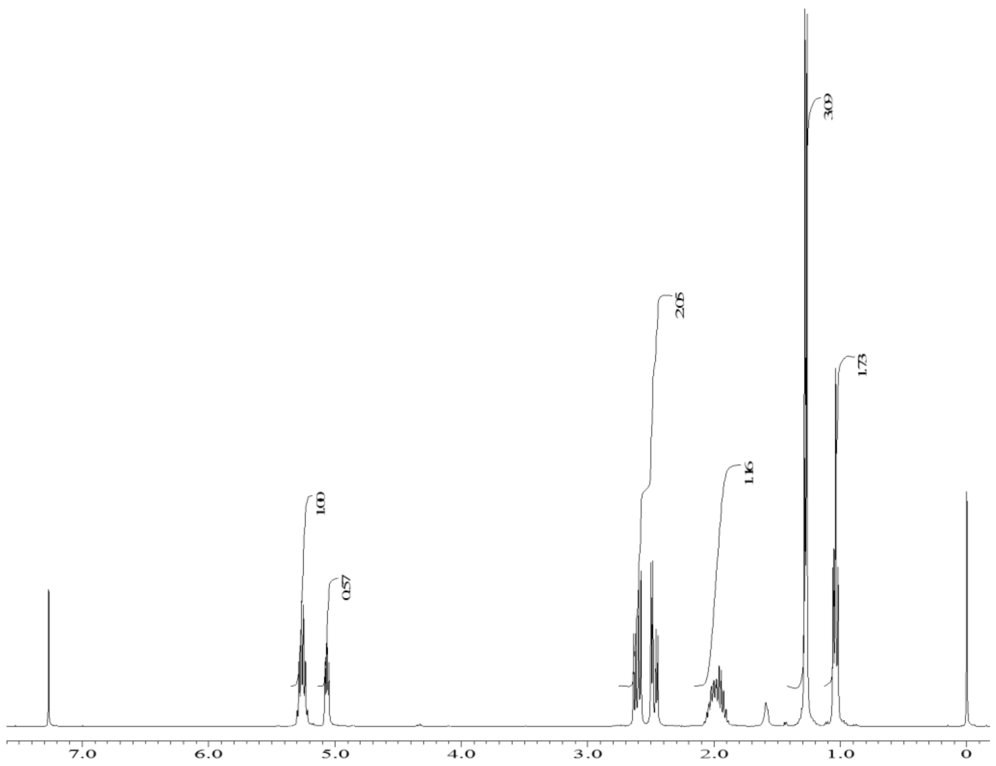
(a) P(2HB-*b*-3HB) before fractionation



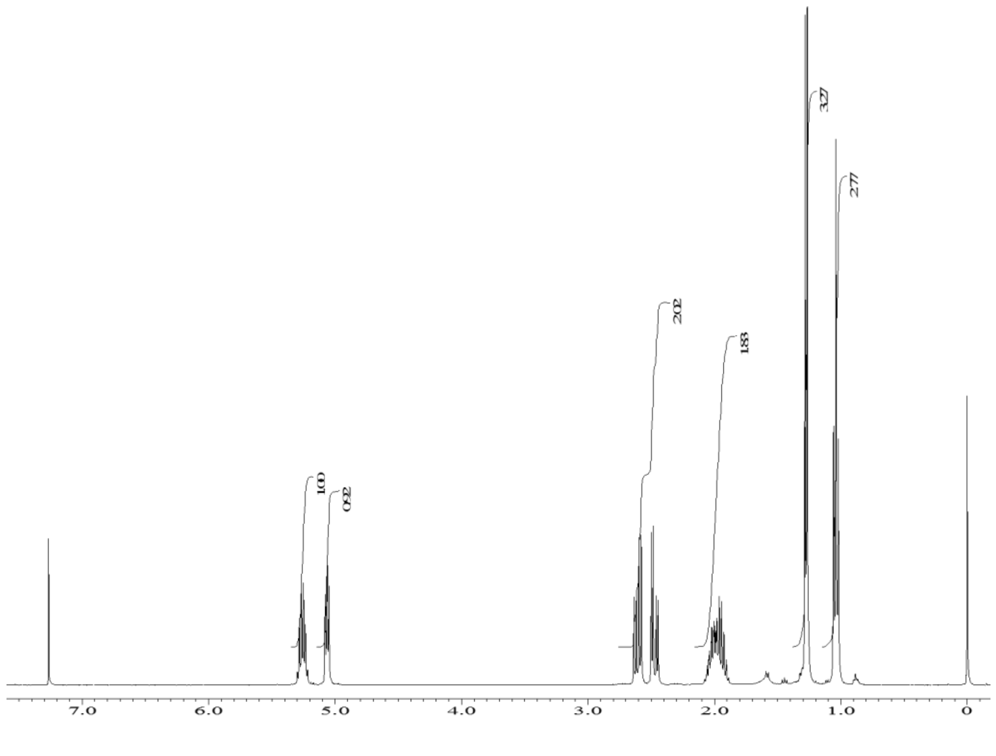
(b) THF-soluble fraction of P(2HB-*b*-3HB)



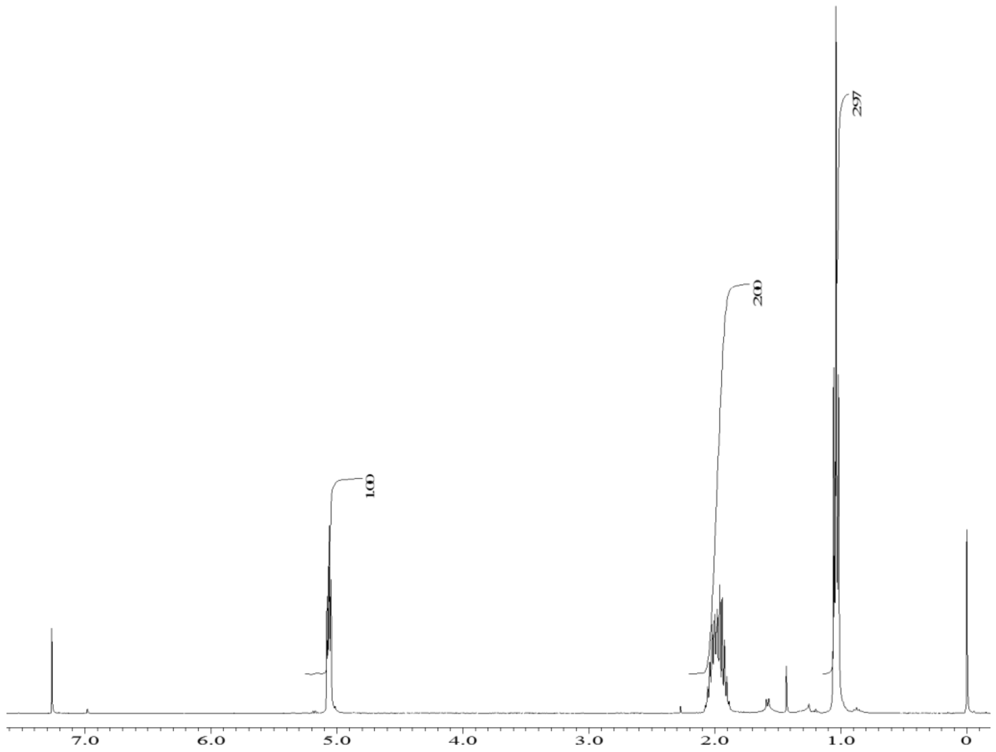
(c) THF-insoluble fraction of P(2HB-*b*-3HB)



(d) a blend of P(2HB) and P(3HB)



(e) THF-soluble fraction of the blend



(f) THF-insoluble fraction of the blend

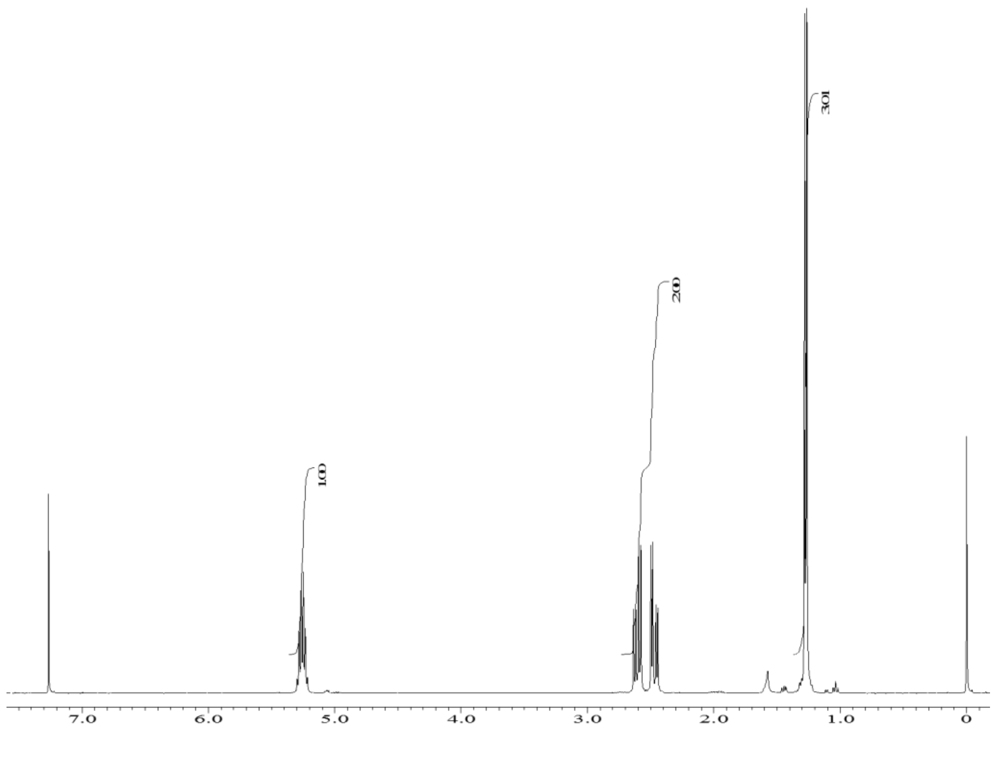


Figure S2. ^{1}H NMR analysis of solvent-fractionated samples of P(2HB-*b*-3HB) and blend of P(2HB) and P(3HB). (a) P(2HB-*b*-3HB) before fractionation, (b) tetrahydrofuran (THF)-soluble fraction of P(2HB-*b*-3HB), (c) THF-insoluble fraction of P(2HB-*b*-3HB), (d) a blend of P(2HB) and P(3HB). (e) THF-soluble fraction of the blend, and (f) THF-insoluble fraction of the blend.

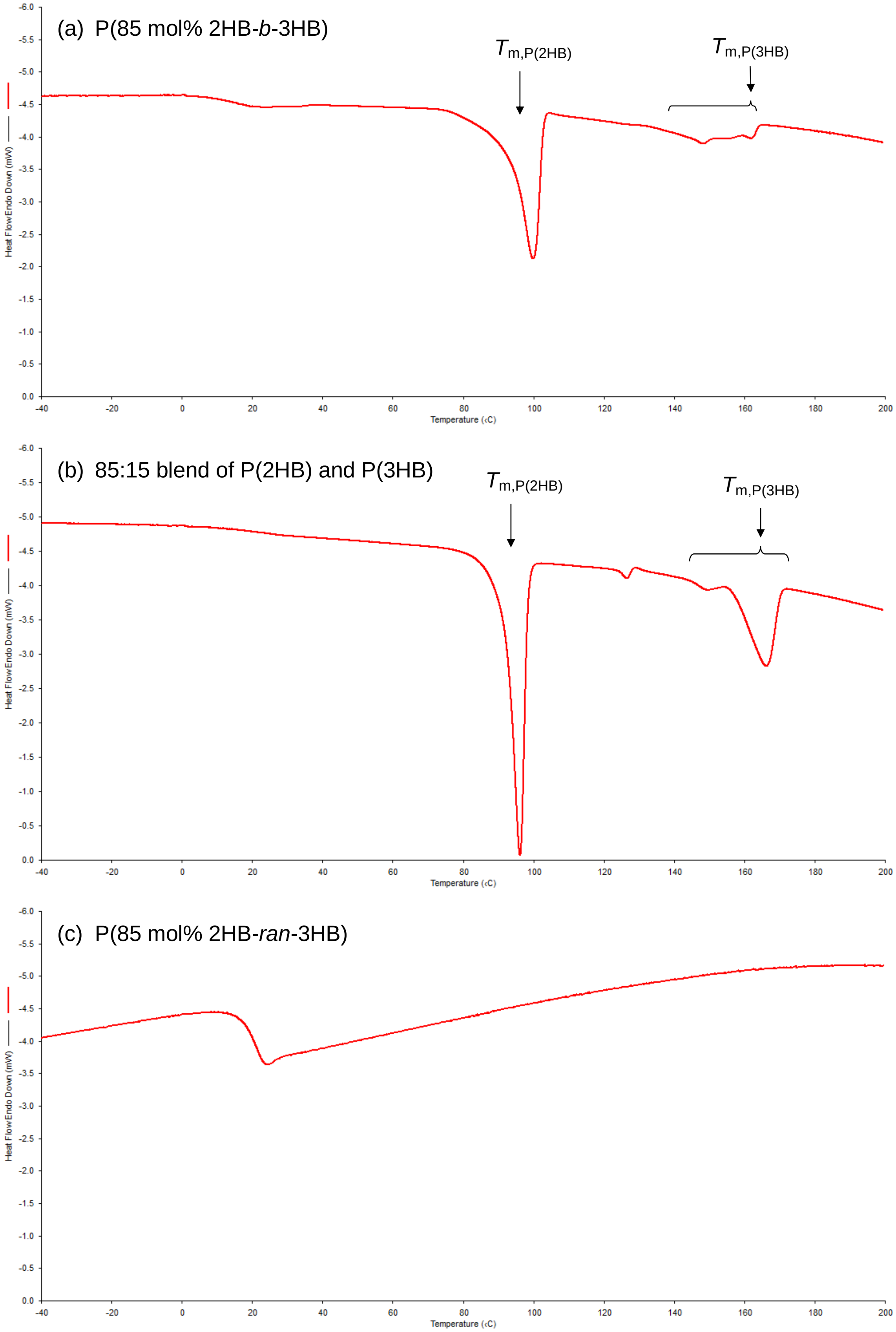
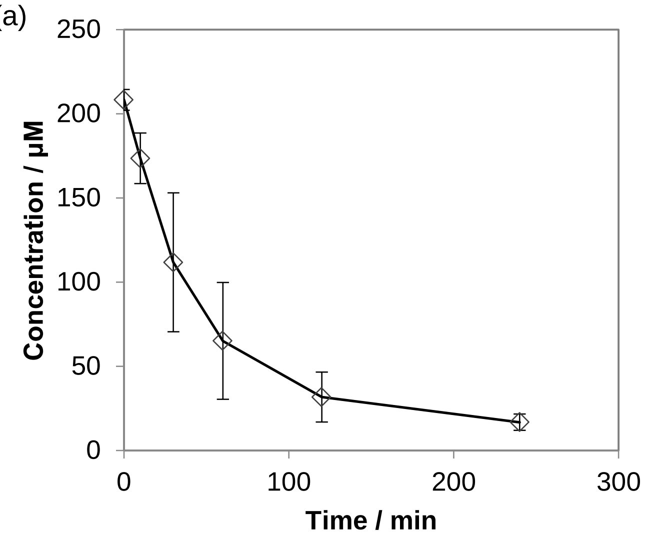

(a) P(85 mol% 2HB-b-3HB)
Tm,P(2HB)
Tm,P(3HB)
Heat Flow Endo Down (mW)
Temperature (‹C)
(b) 85:15 blend of P(2HB) and P(3HB)
Tm,P(2HB)
Tm,P(3HB)
Heat Flow Endo Down (mW)
Temperature (‹C)
(c) P(85 mol% 2HB-ran-3HB)
Heat Flow Endo Down (mW)
Temperature (‹C)

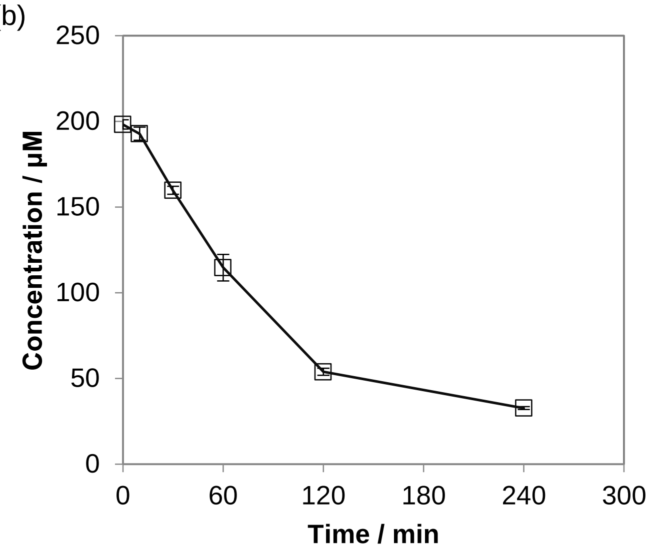
Figure S3. DSC thermograms of the polymers composed of 2HB and 3HB. (a) P(87 mol% 2HB-*b*-3HB), (b) a 85:15 blend of P(2HB) and P(3HB), and (c) P(85 mol% 2HB-*b*-3HB). The samples were melted at 200 ˚C for 3 min, rapidly cooled to 70 ˚C and annealed at 70 ˚C for 6 h for promoting crystallization. The sample was cooled to -70 ˚C and heated to 200 ˚C at heating rate of 10 ˚C/min. The thermogram was reported during the heating scan.

(a)



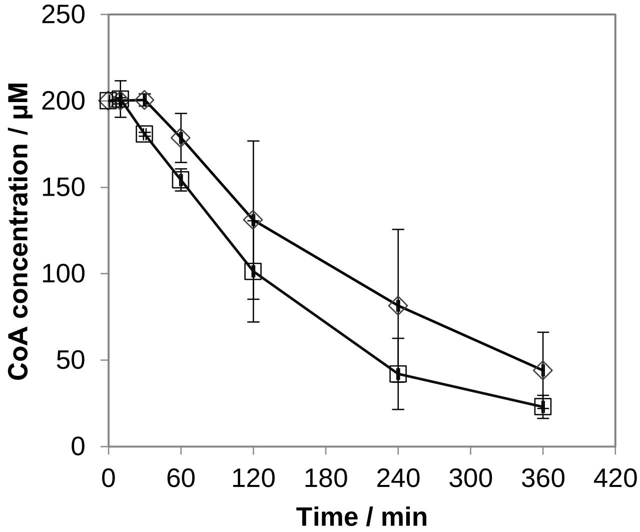
250
200
150
100
50
0
Concentration / µM
0
100
200
300
Time / min

(b)



250
200
150
100
50
0
Concentration / µM
0
60
120
180
240
300
Time / min

(c)



250
200
150
100
50
0
CoA concentration / µM
0
60
120
180
240
300
360
420
Time / min

Figure S4. Reaction of PhaC1$_{Ps}$STQK with 2HB-CoA and 3HB-CoA. (a) 3HB-CoA was used as a substrate, (b) 2HB-CoA was used as a substrate and (c) 2HB-CoA and 3HB-CoA were used as substrates. Square: 2HB-CoA, and diamond: 3HB-CoA.