



Title	Directed Evolution of Sequence-Regulating Polyhydroxyalkanoate Synthase to Synthesize a Medium-Chain-Length-Short-Chain-Length (MCL-SCL) Block Copolymer
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Supporting information

to

Directed evolution of sequence-regulating polyhydroxyalkanoate synthase to synthesize medium-chain-length-short-chain-length (MCL-SCL) block copolymer

by

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Appendix 1. Amino acid sequence of *phaC_{AR}* with the codon-optimized *phaC_{Re}* region

msqpsygplfealahyndkllamakaqtertaqallqtnlddlgqvleqgsqqpwqliqaqmnwwqdqklmqhtllksagqps
epvitpersdrffkaeawseqpiydylkqsylltarhllasvdalegvpqksrerlrftrqyvnamapsNFLATNPEAQRLIE
SGGESLRAGVRNMEDLTRGKISQTDESAFEVGRNVAVTEGAVVFENEYFOLLOQYKPL
TDKVHARPLLMVPPCINKYYILDLOPESSLVRHVVEQGHTVFLVSWRNPDASMAGSTW
DDYIEHAAIRAIEVARDISGQDKINVLGFCVGGTIVSTALAVLAARGEHPAASVTLLTLL
DFADTGILDVVFVDEGHVQLREATLGGGAGAPCALLRGLELANTFSFLRPNDLVWNYVV
DNYLKGNTVPFDLLFWNGDATNLPGPWYCWYLRHTYLQNELKVP GKLTVC GVPVDL
ASIDVPTYIYGSREDHIVPWTAAYASTALLANKLRFVLGASGHIAGVINPPAKNKRSHWT
NDALPESPOQWLAGAIEHHGSWWPDWTAWLAGQAGAKRAAPANYGNARYRAIEPAP
GRYVKAKA

Lowercase and uppercase letters indicate the *PhaC_{Ac}* and *PhaC_{Re}* regions, respectively. The codons of the underlined amino acid residues were optimized for *E. coli*.

Appendix 2. Calculation of D value of P(3HB-*co*-3HHx) produced by PhaCARF314H.

Based on ^{13}C NMR of P(3HB-*co*-3HHx) produced by PhaCARF314H (Fig. S3), D value was calculated as follows. From the signals of carbonyl carbon at $\delta \sim 169$ -170, the relative resonance intensities ascribed to each dyad sequence were determined.

$$F_{3\text{HB-3HB}} = 0.50$$

$$F_{3\text{HB-3HHx}} + F_{3\text{HHx-3HB}} = 0.24$$

$$F_{3\text{HHx-3HHx}} = 0.12$$

Here, F_{X-Y} is a relative resonance intensity corresponding to X-Y dyad. From these values, D value was calculated as follows.

$$D = (F_{3\text{HB-3HB}} \times F_{3\text{HHx-3HHx}}) / (F_{3\text{HB-3HHx}} \times F_{3\text{HHx-3HB}}) = 4.2$$

This suggests that the copolymer has a random sequence.

D ~ 0: alternative copolymer

D ~ 1: random copolymer

D >> 1: block copolymer

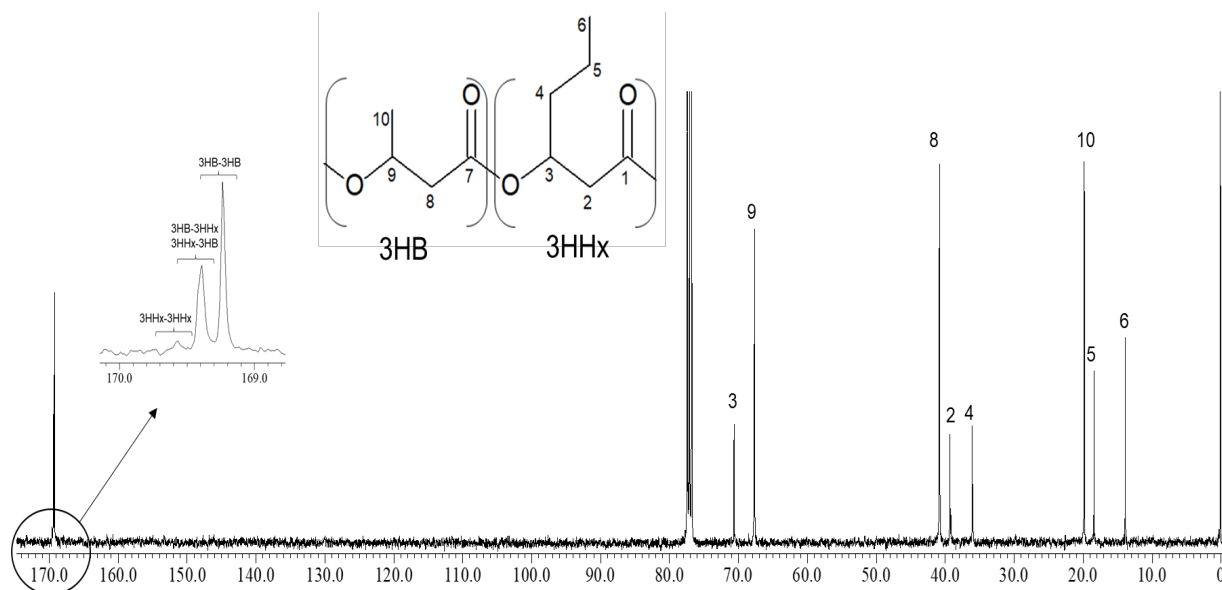


Figure S1. ^{13}C NMR of P(3HB-*co*-3HHx) produced by parent PhaCAR.

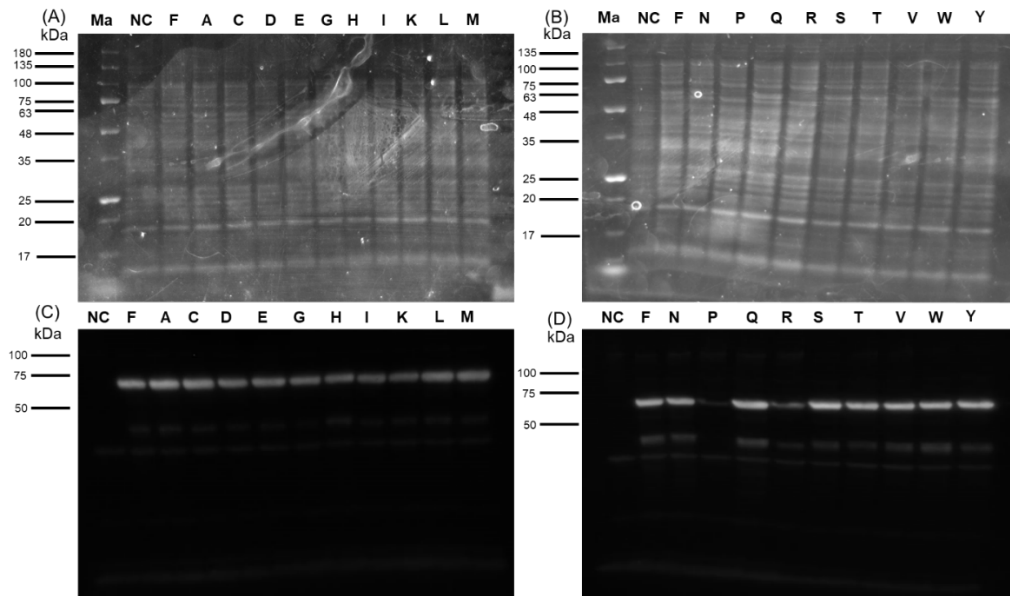


Figure S2. Immunoblot analysis of saturation mutations at position 314 in PhaC_{AR} using crude cell extracts and the anti-PhaC_{Re} antibody. Ma, size maker (invisible in chemiluminescence). NC, negative control (the crude extract of *E. coli* harboring the empty plasmid, pUC18); single letters indicate F314X substitutions.

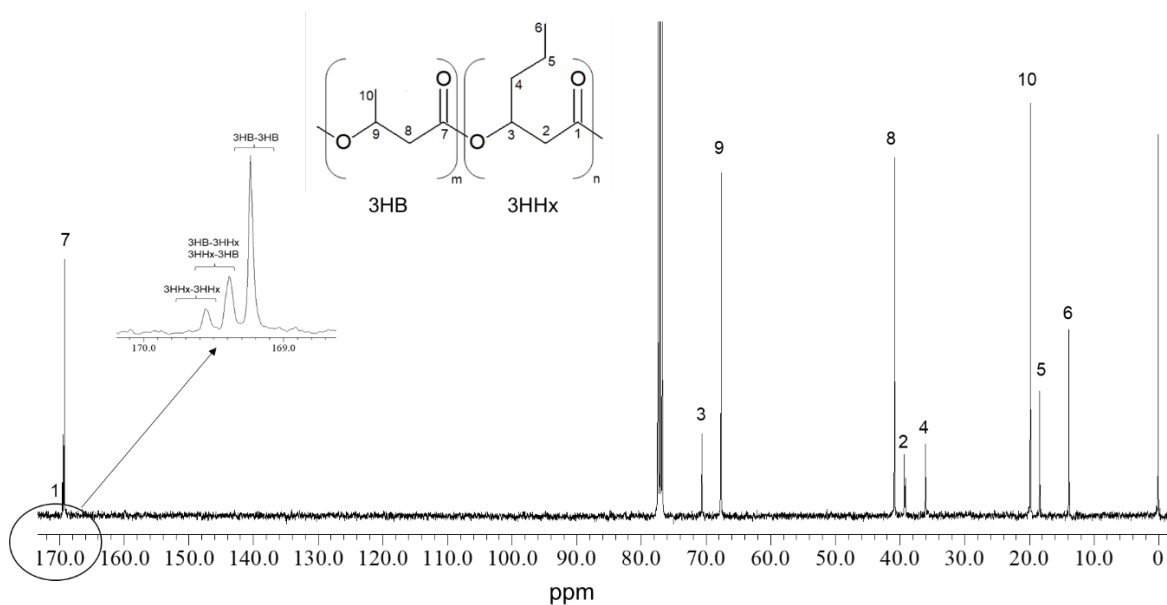


Figure S3. ¹³C NMR spectrum of P(3HB-*co*-3HHx) produced by PhaC_{AR}F314H.

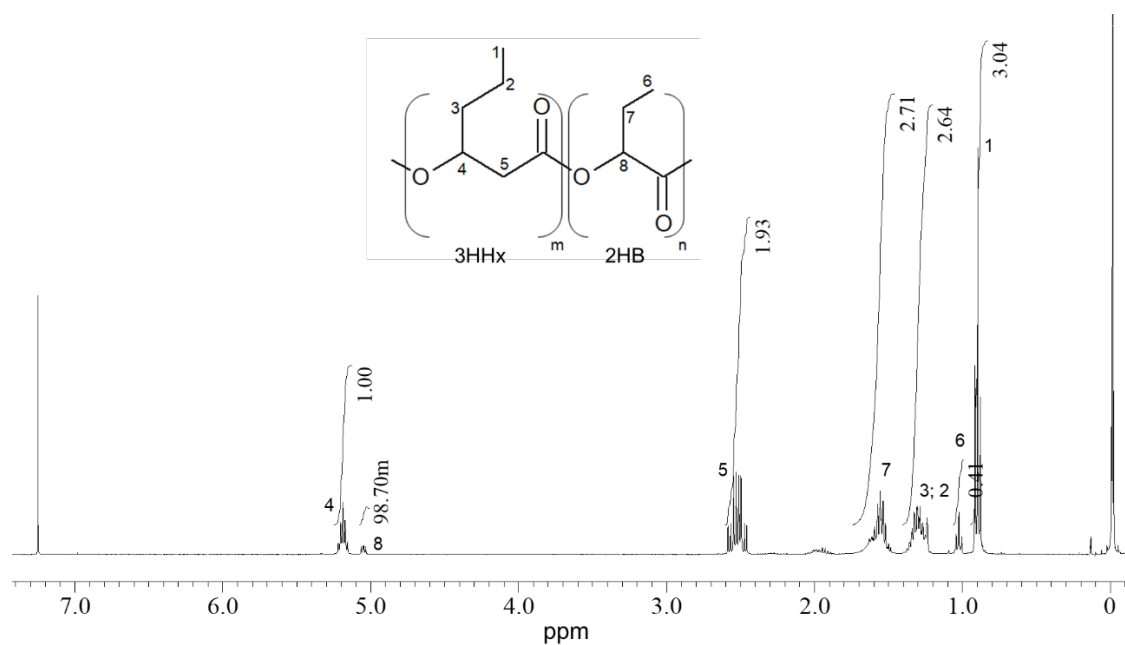
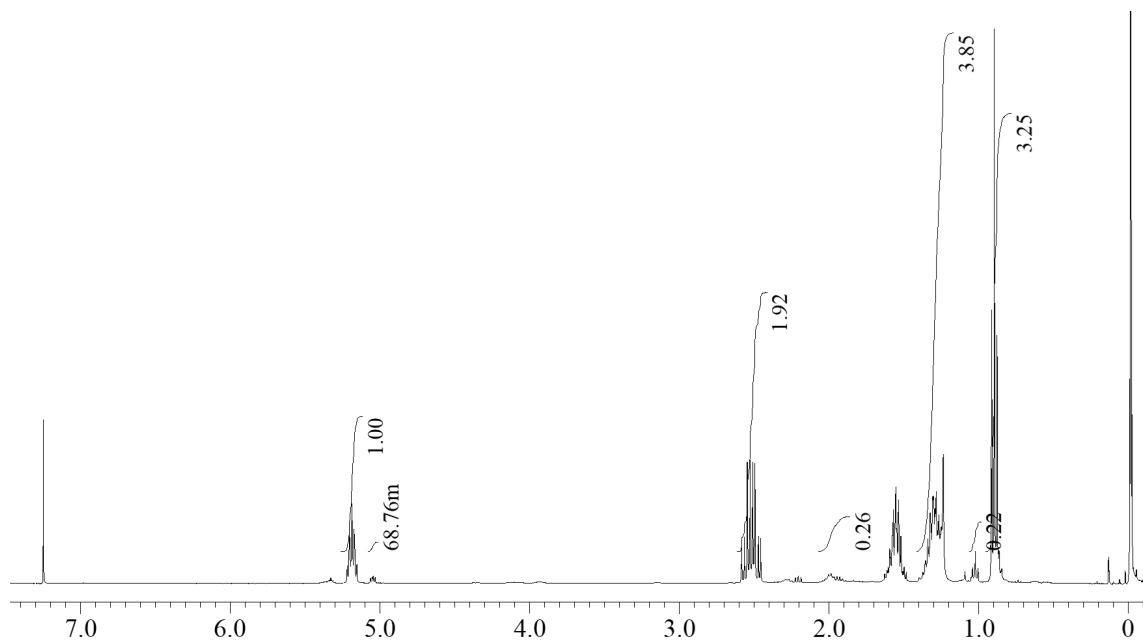
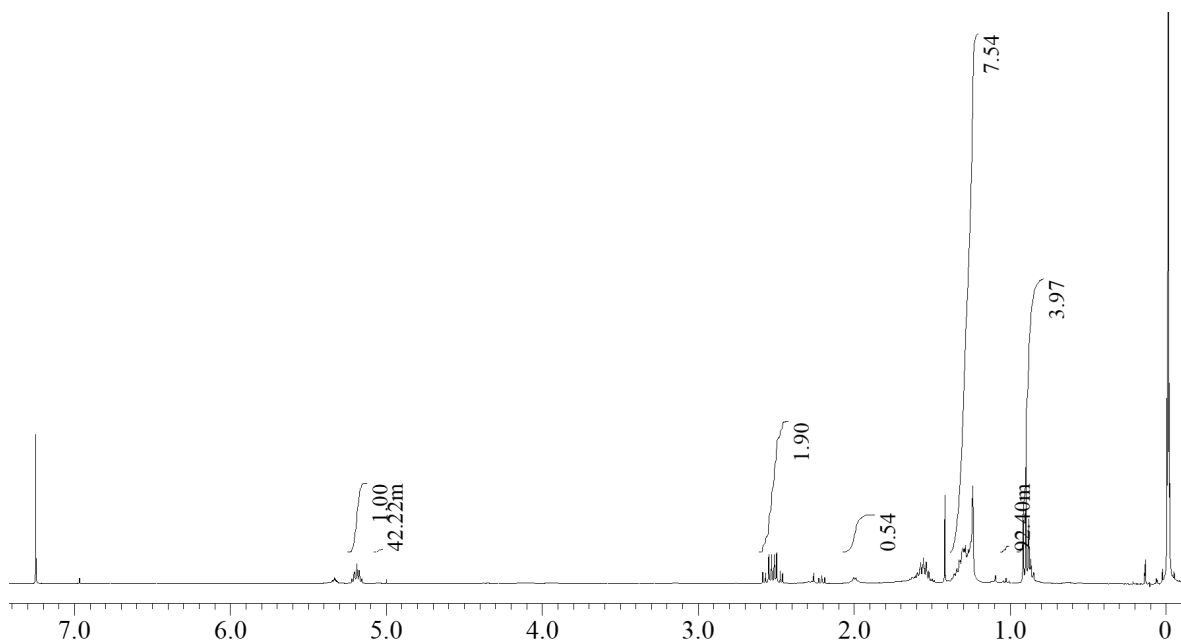


Figure S4. ¹H NMR spectrum of P(3HHx)-*b*-(2HB) produced by PhaC_{AR}F314H.

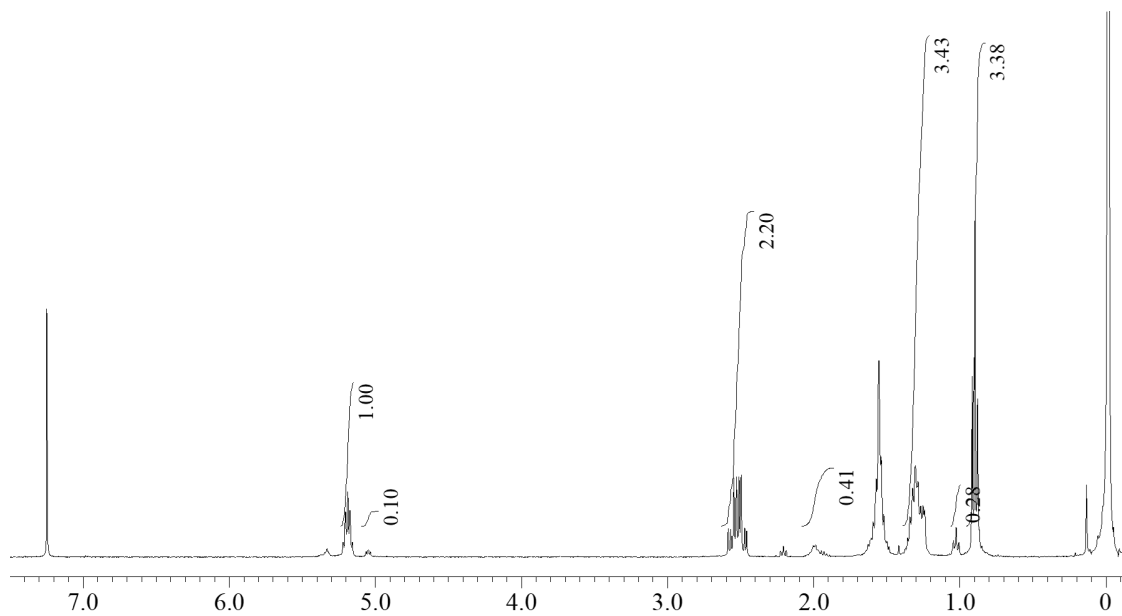
(A) Original P(3HHx)-*b*-(2HB) before fractionation



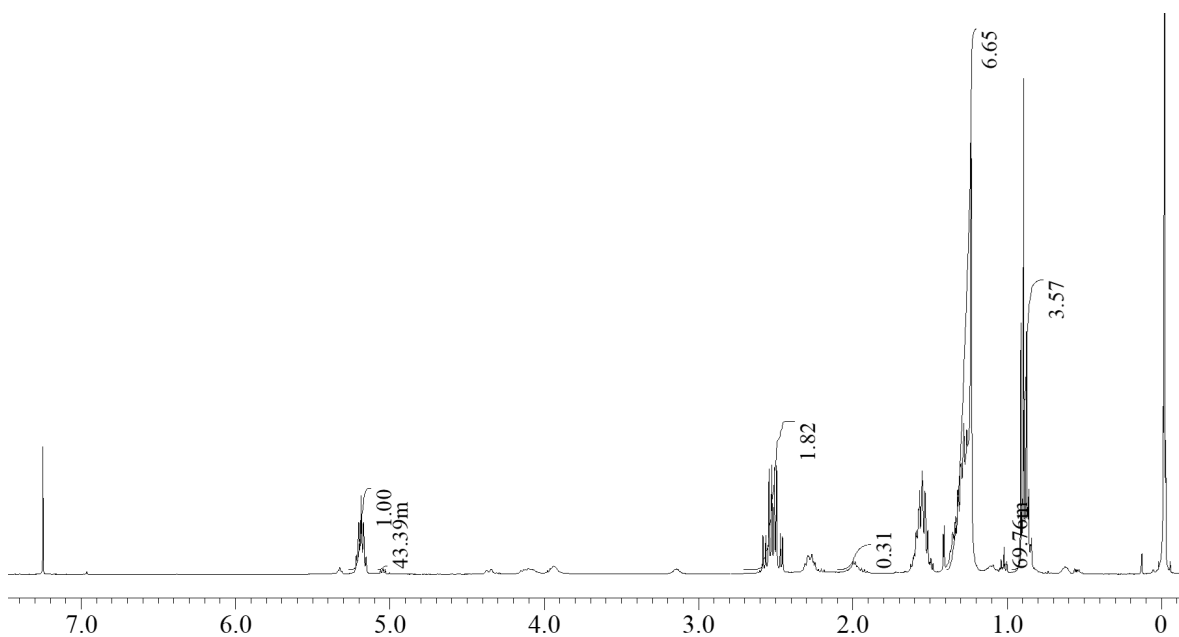
(B) P(3HHx)-*b*-(2HB) in the cyclohexane-soluble fraction



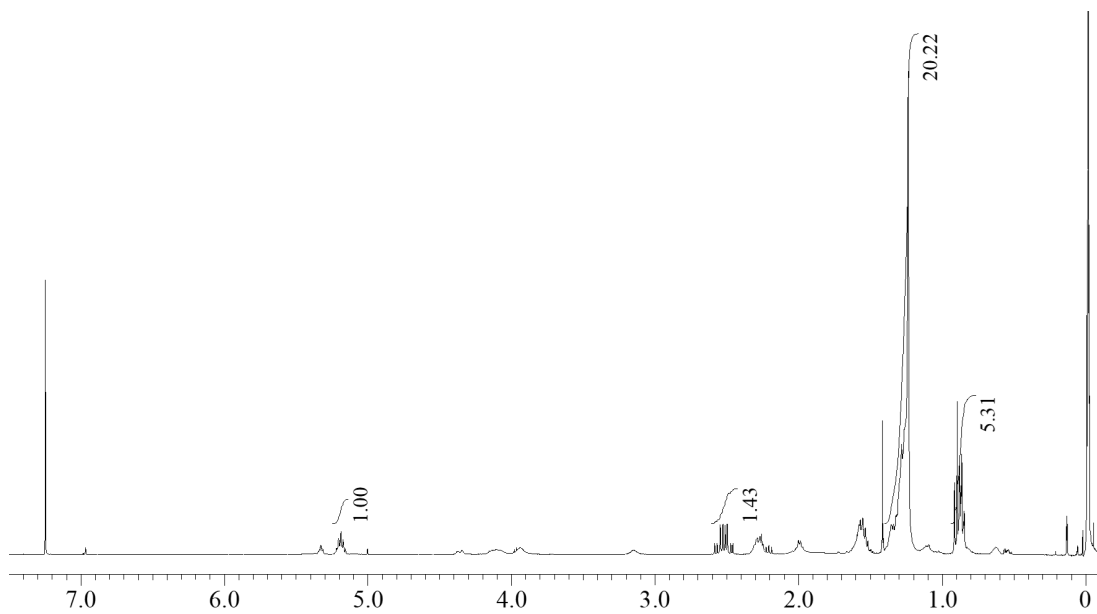
(C) P(3HHx)-*b*-(2HB) in the cyclohexane-insoluble fraction



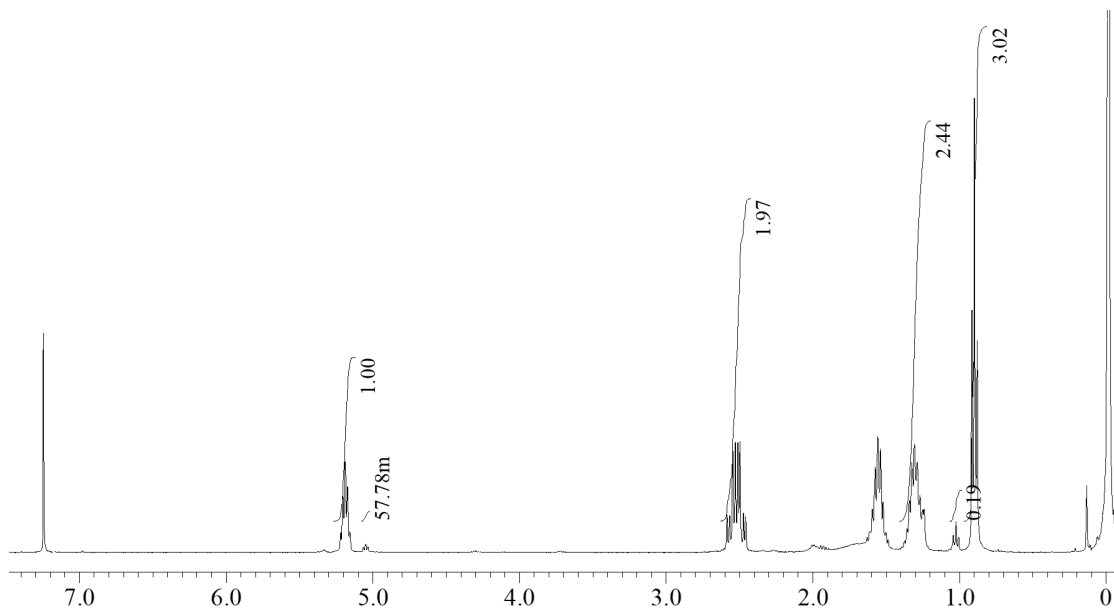
(D) Blend of P(3HHx) and P(2HB) before fractionation



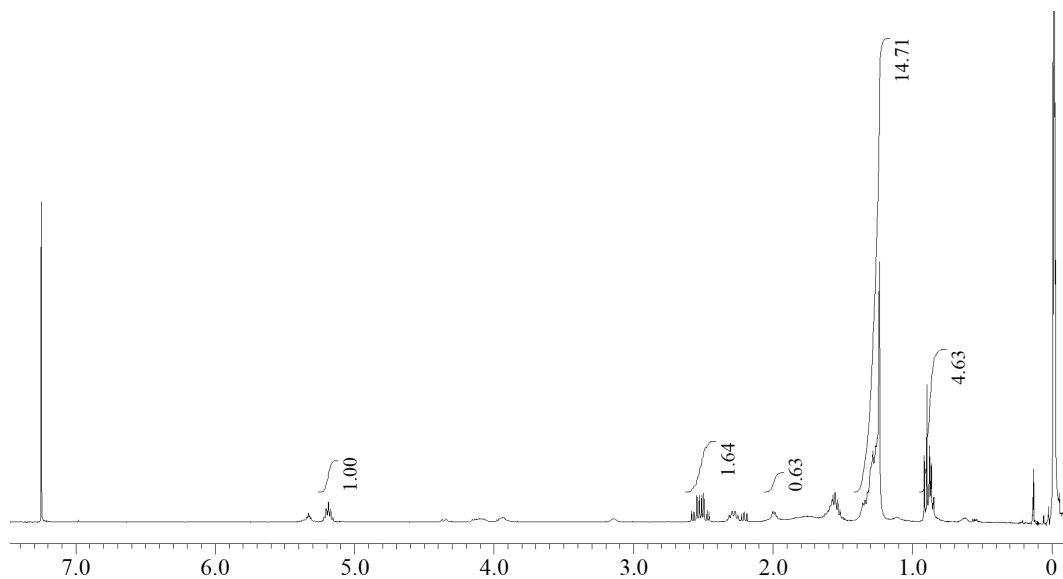
(E) Cyclohexane-soluble fraction of the blend



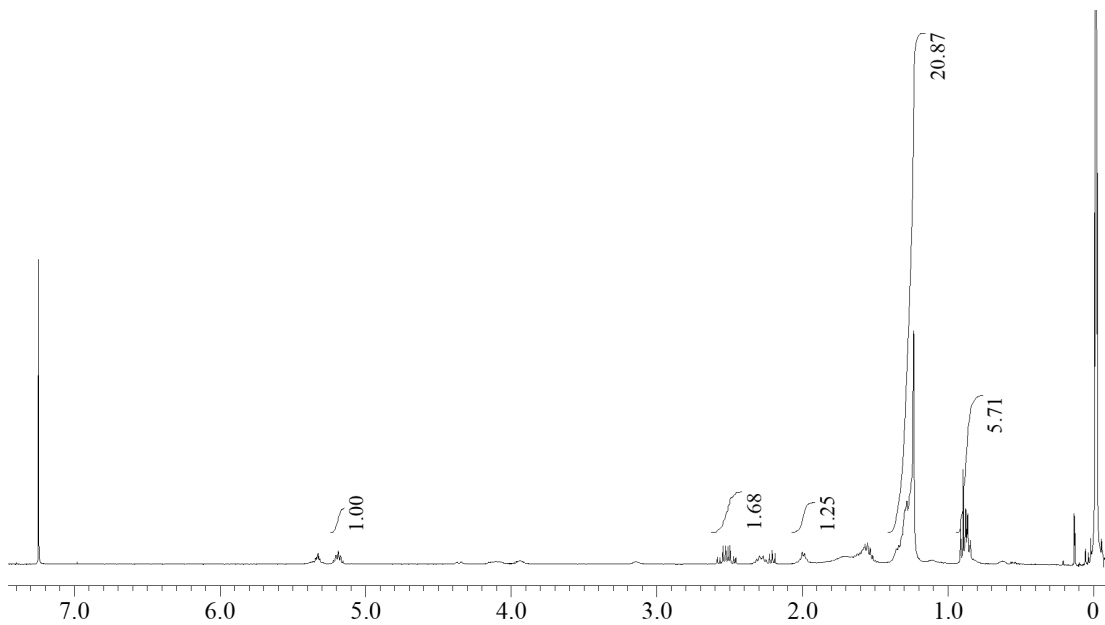
(F) Cyclohexane-insoluble fraction of the blend



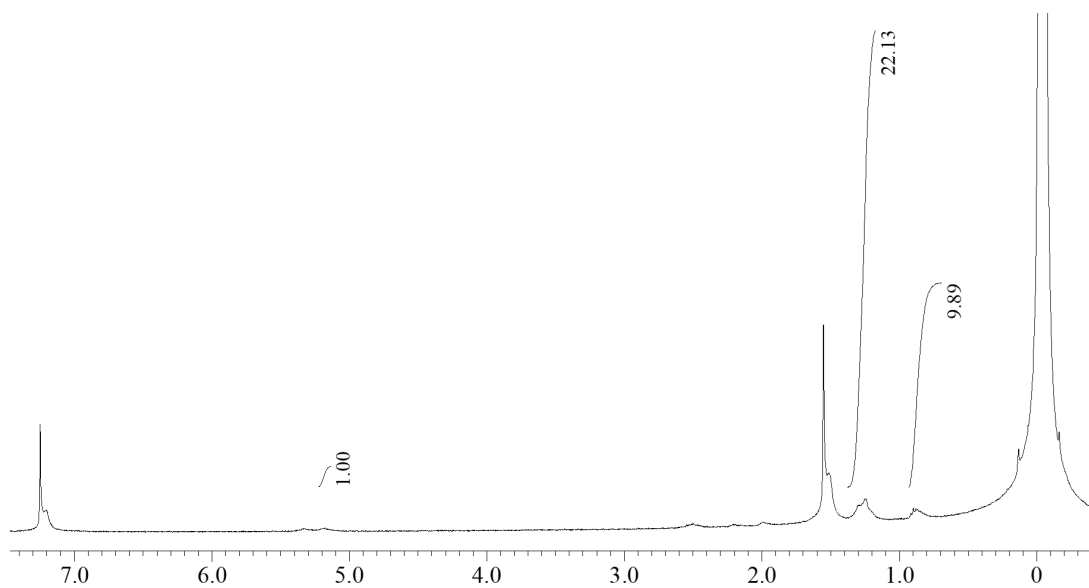
(G) P(3HHx) before fractionation



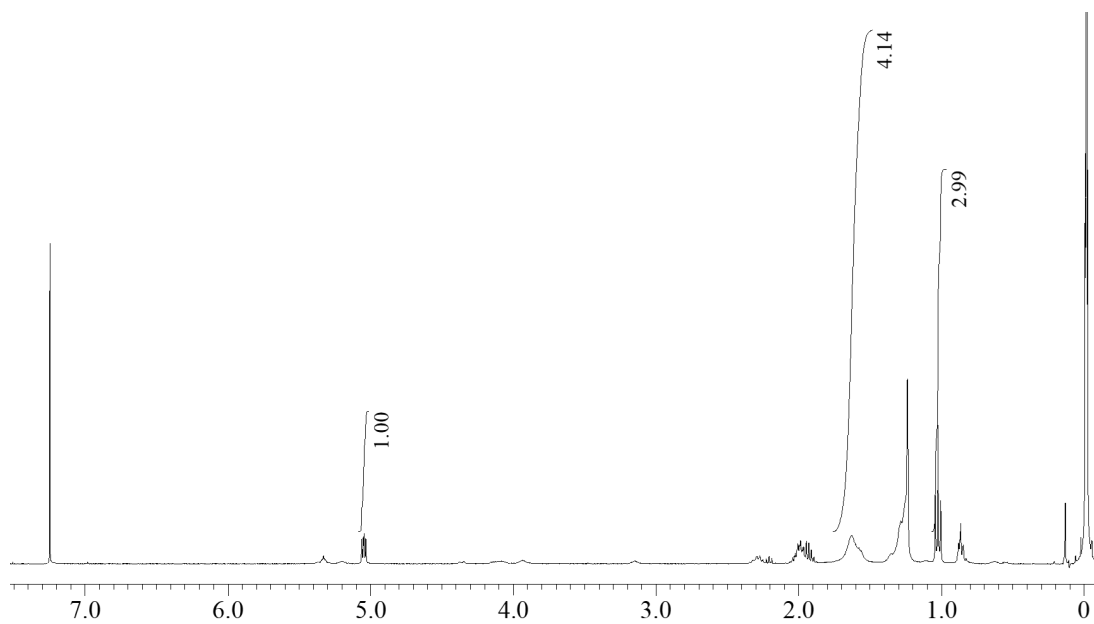
(H) P(3HHx) in the cyclohexane-soluble fraction



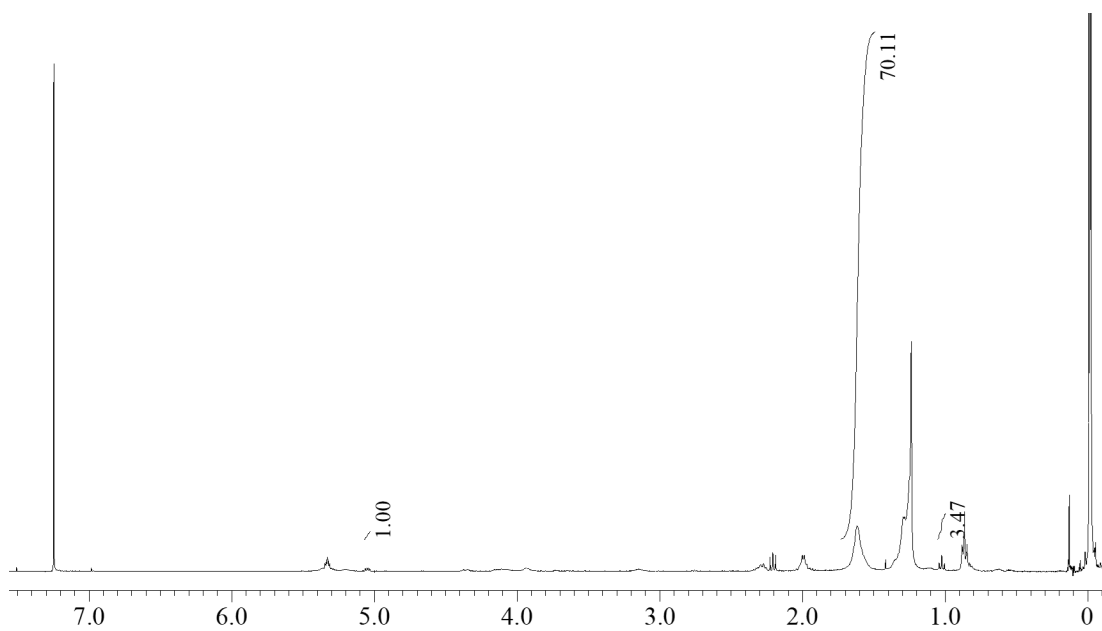
(I) P(3HHx) in the cyclohexane-insoluble fraction



(J) P(2HB) before fractionation



(K) P(2HB) in the cyclohexane-soluble fraction



(L) P(2HB) in the cyclohexane-insoluble fraction

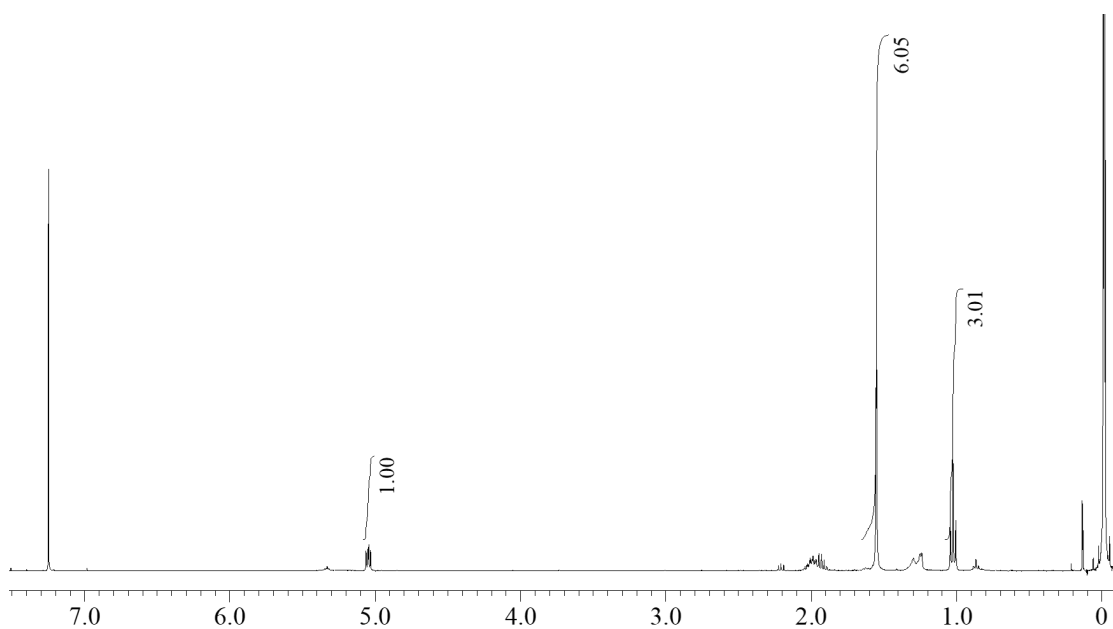


Figure S5. ¹H NMR spectra of the produced polymers. Block copolymer before solvent fractionation (A), in the cyclohexane-soluble fraction (B), and in the cyclohexane-insoluble fraction (C). Blend of P(2HB) and P(3HHx) before fractionation (D), in the cyclohexane-soluble

fraction (E), and in the cyclohexane-insoluble fraction (F). P(3HHx) before solvent fractionation (G), in the cyclohexane-soluble fraction (H), and in the cyclohexane-insoluble fraction (I). P(2HB) before fractionation (J), in the cyclohexane-soluble fraction (K), and in the cyclohexane-insoluble fraction (L).

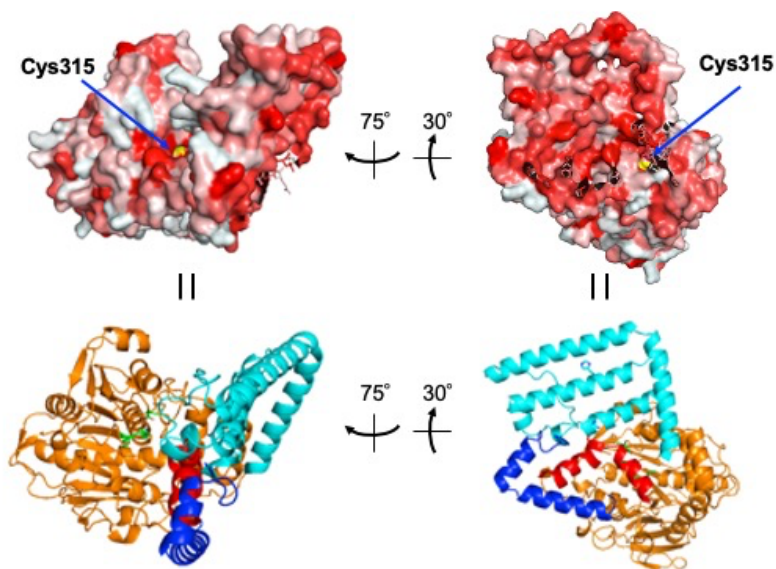


Figure S6. (Upper) Calculated surface hydrophobicity of the predicted structure of PhaC_{AR}. Red: hydrophobic, white: hydrophilic. N-terminal 30 residues are not shown because of their low fidelity of the prediction. The yellow residue is the catalytic center Cys315. (Lower) The corresponding cartoon images. The colors are same as Figure 6.