Supporting information

to

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

by

Hien Thi Phan, Yumi Hosoe, Maureen Guex, Masayoshi Tomoi,

Hiroya Tomita, Manfred Zinn, and Ken'ichiro Matsumoto

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

msqpsygplfealahyndkllamakaqtertaqallqtnlddlgqvleqgsqqpwqliqaqmnwwqdqlklmqhtllksagqps epvitpersdrrfkaeawseqpiydylkqsylltarhllasvdalegvpqksrerlrfftrqyvnamapsNFLATNPEAQRLLIE SGGESLRAGVRNMMEDLTRGKISQTDESAFEVGRNVAVTEGAVVFENEYFQLLQYKPL TDKVHARPLLMVPPCINKYYILDLQPESSLVRHVVEQGHTVFLVSWRNPDASMAGSTW DDYIEHAAIRAIEVARDISGQDKINVLGFCVGGTIVSTALAVLAARGEHPAASVTLLTTLL DFADTGILDVFVDEGHVQLREATLGGGAGAPCALLRGLELANTFSFLRPNDLVWNYVV DNYLKGNTPVPFDLLFWNGDATNLPGPWYCWYLRHTYLQNELKVPGKLTVCGVPVDL ASIDVPTYIYGSREDHIVPWTAAYASTALLANKLRFVLGASGHIAGVINPPAKNKRSHWT NDALPESPQQWLAGAIEHHGSWWPDWTAWLAGQAGAKRAAPANYGNARYRAIEPAP 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 $PhaC_{AR}F314H$.

Based on ^{13}C NMR of P(3HB-*co*-3HHx) produced by $PhaC_{AR}F314H$ (Fig. S3), D value was calculated as follows. From the signals of carbonyl carbon at δ ~ 169-170, the relative resonance intensities ascribed to each dyad sequence were determined.

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

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

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

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

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

This suggests that the copolymer has a random sequence.

D ~ 0: alternative copolymer
D ~ 1: random copolymer
D >> 1: block copolymer

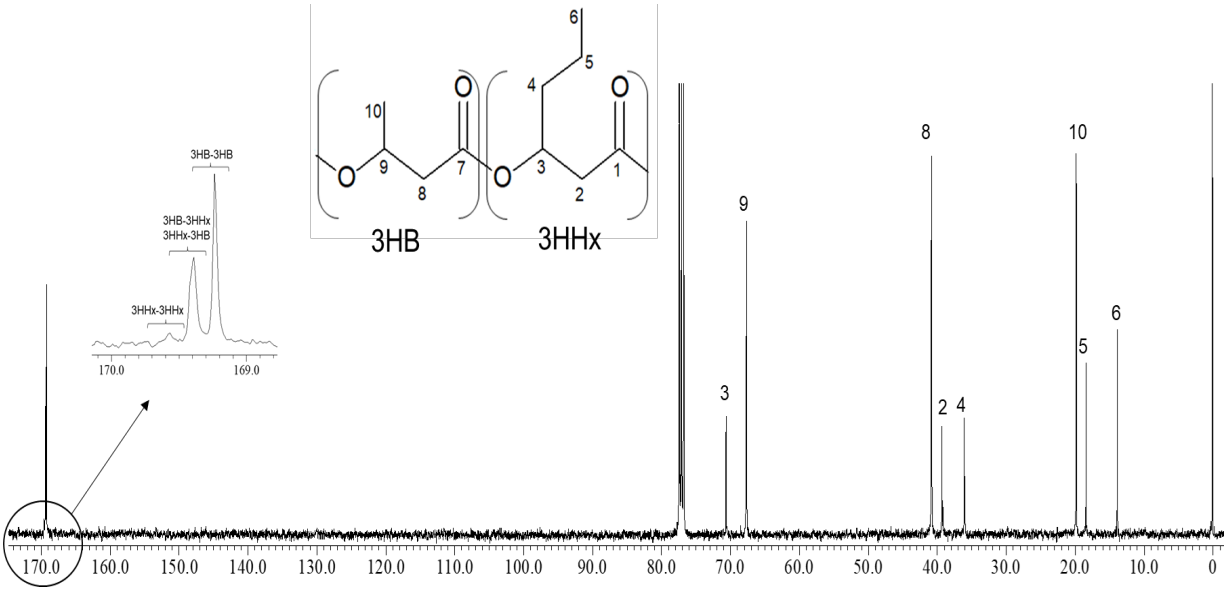


Figure S1. ^{13}C NMR of P(3HB-*co*-3HHx) produced by parent $PhaC_{AR}$.

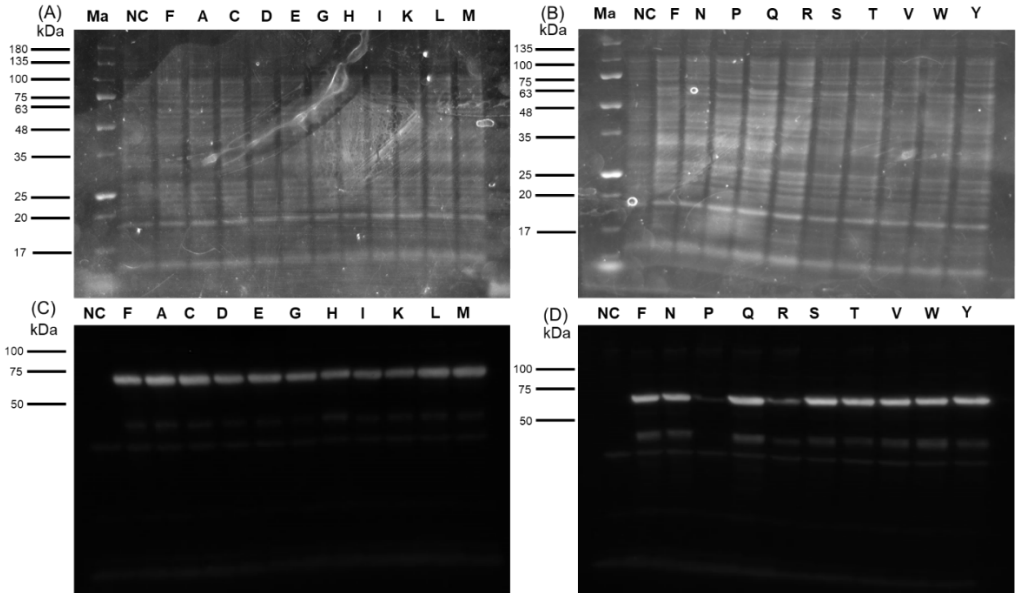


Figure S2. Immunoblot analysis of saturation mutations at position 314 in $PhaC_{AR}$ using crude cell extracts and the anti-$PhaC_{Re}$ antibody. Ma, size maker (invisible in chemiluminescence). NC, negative control (the crude extract of *E. coli* harboring the empty plasmid, pUC18); single letters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

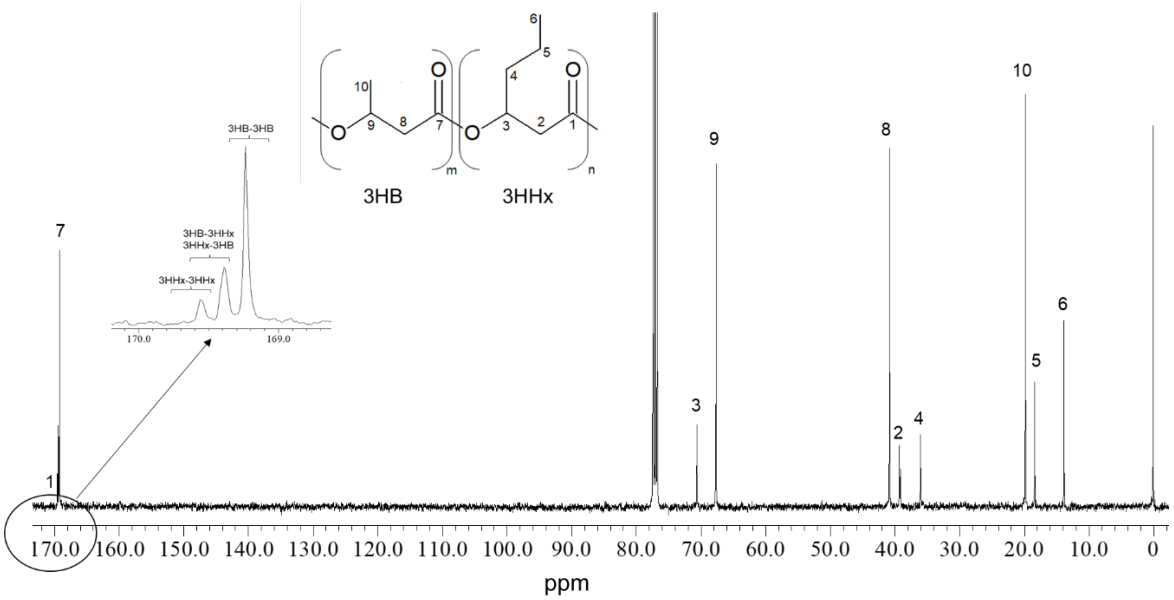


Figure S3. ^{13}C NMR spectrum of P(3HB-*co*-3HHx) produced by PhaC$_{AR}$F314H.

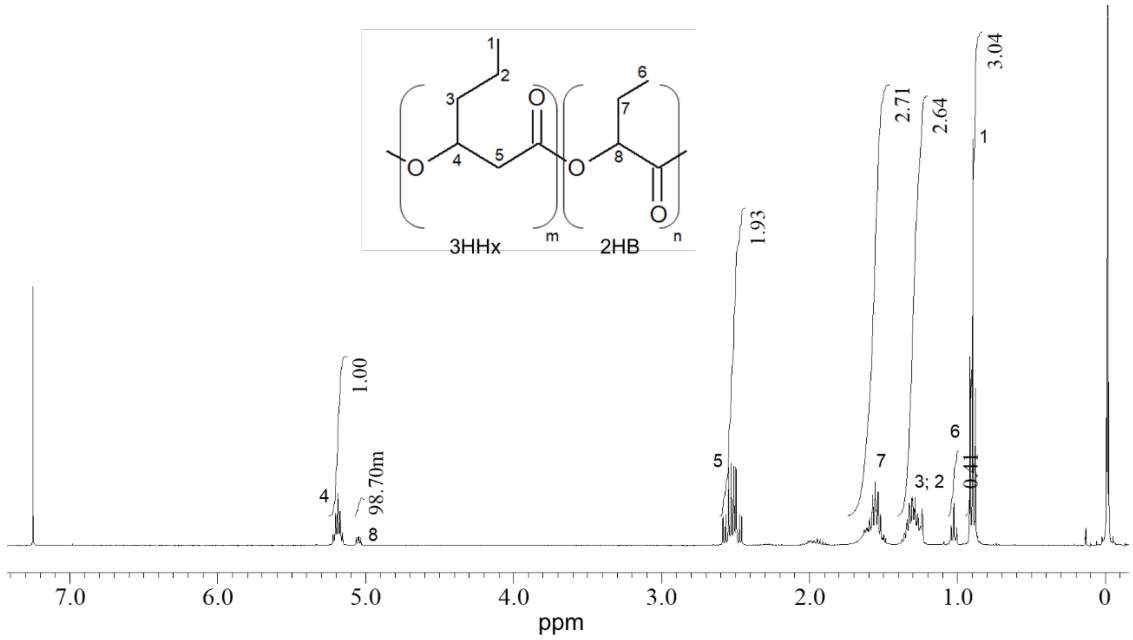
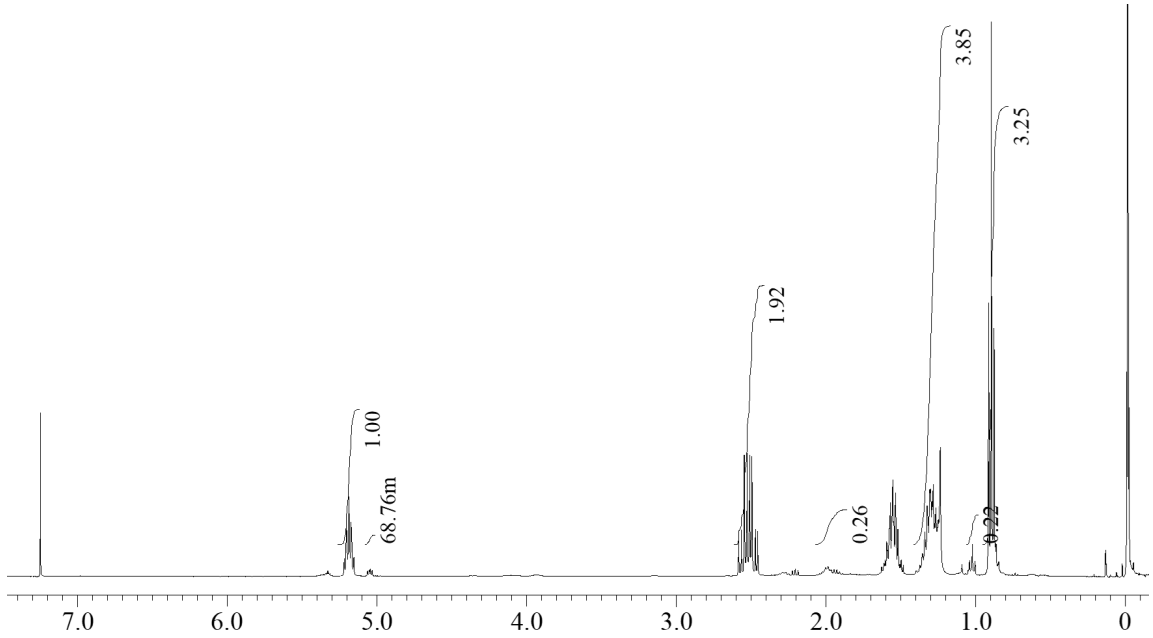
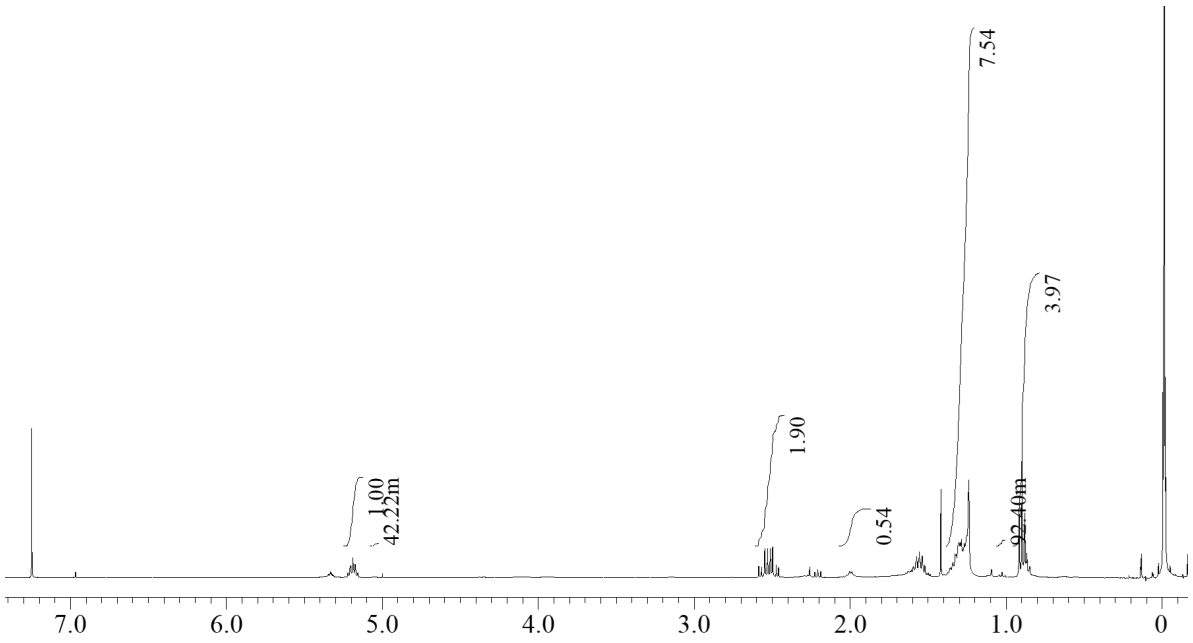


Figure S4. ^{1}H NMR spectrum of P(3HHx)-*b*-(2HB) produced by PhaC$_{AR}$F314H.

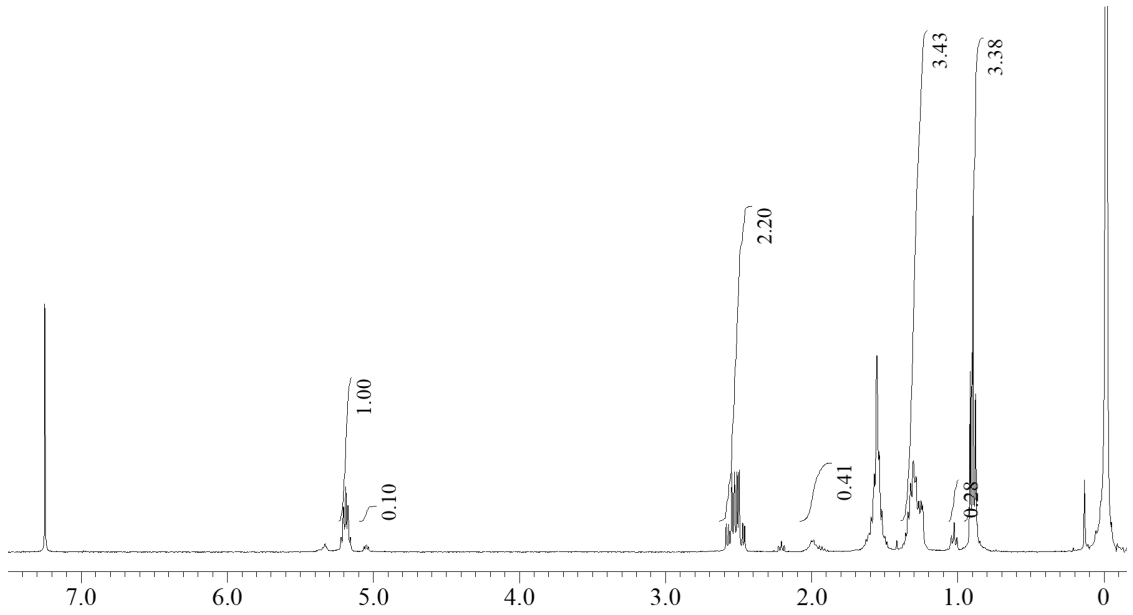
(A) Original P(3HHx)-*b*-(2HB) before fractionation



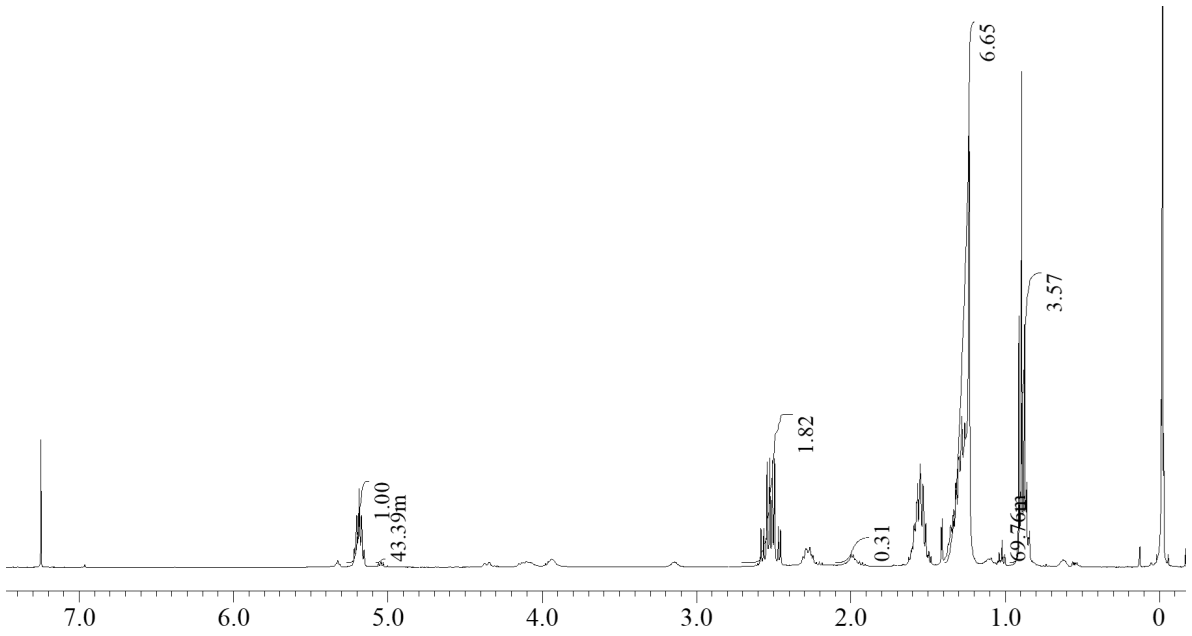
(B) P(3HHx)-*b*-(2HB) in the cyclohexane-soluble fraction



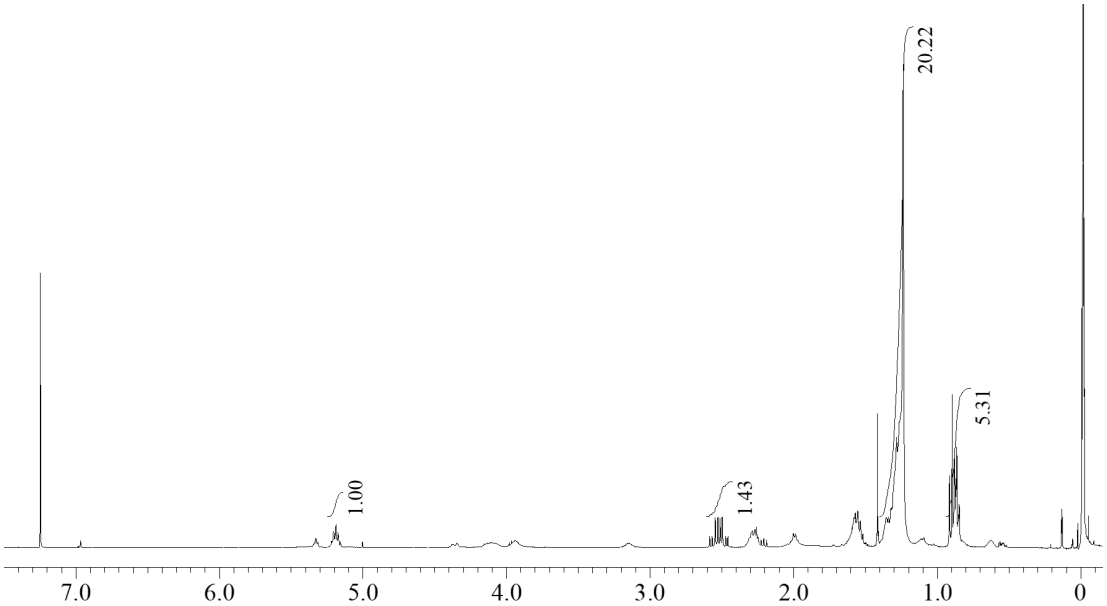
(C) P(3HHx)-*b*-(2HB) in the cyclohexane-insoluble fraction



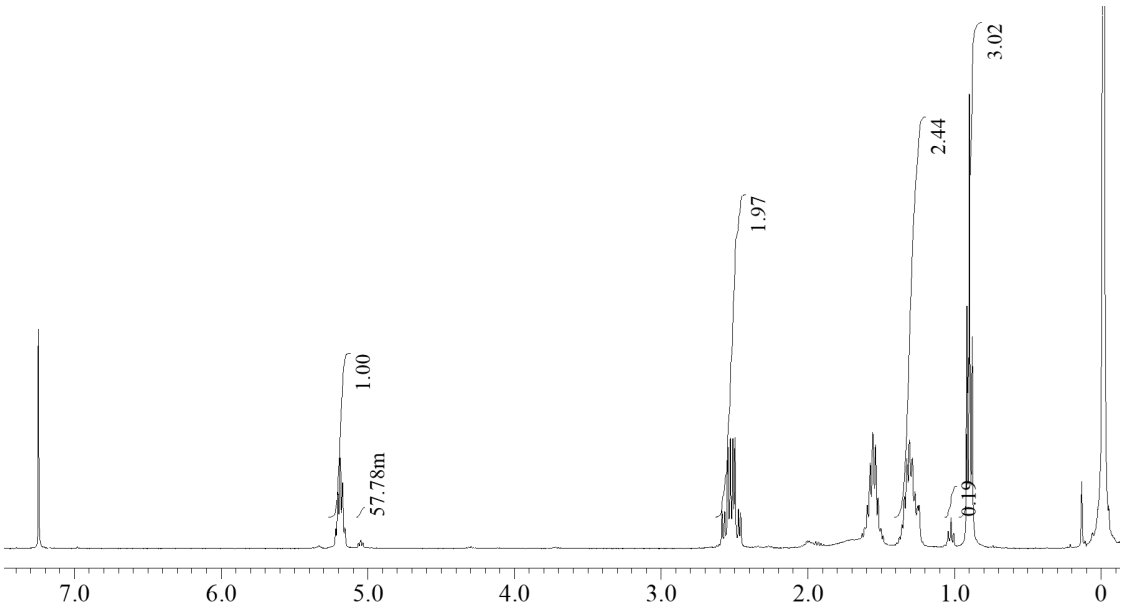
(D) Blend of P(3HHx) and P(2HB) before fractionation



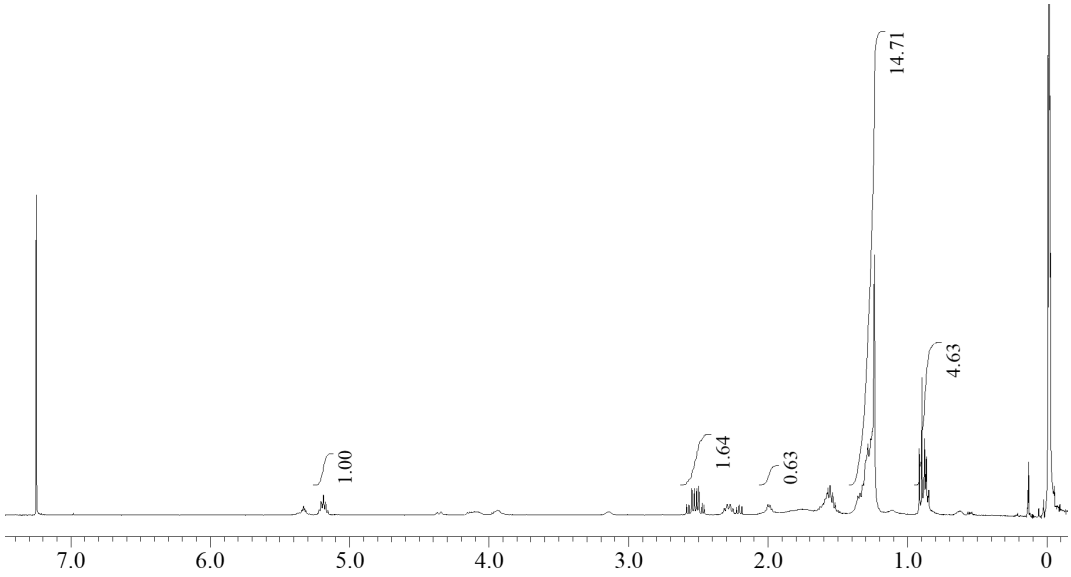
(E) Cyclohexane-soluble fraction of the blend



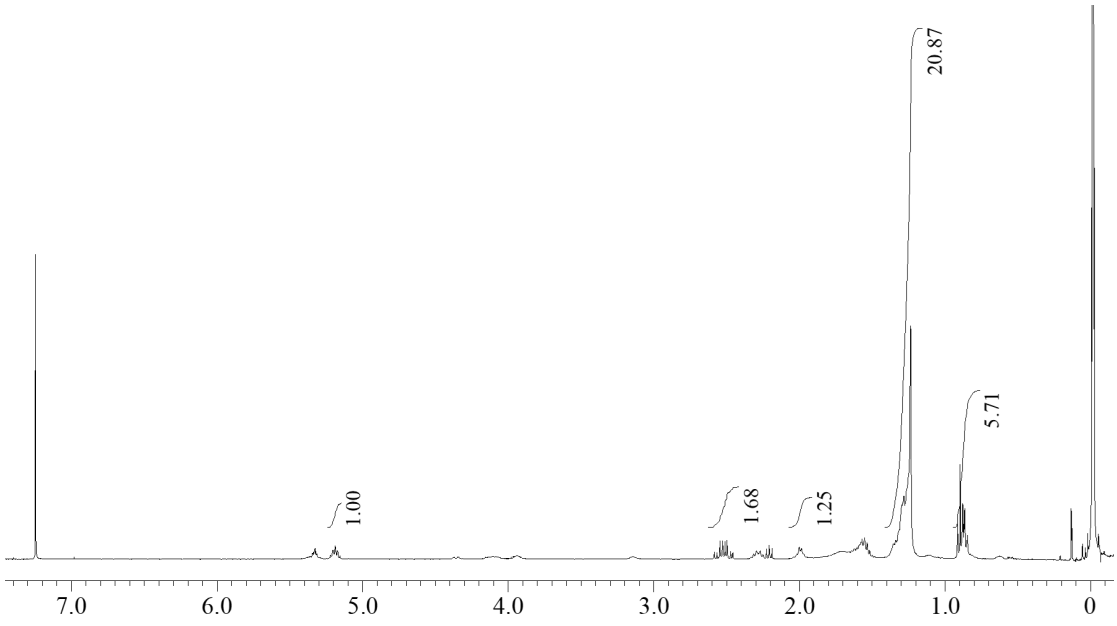
(F) Cyclohexane-insoluble fraction of the blend



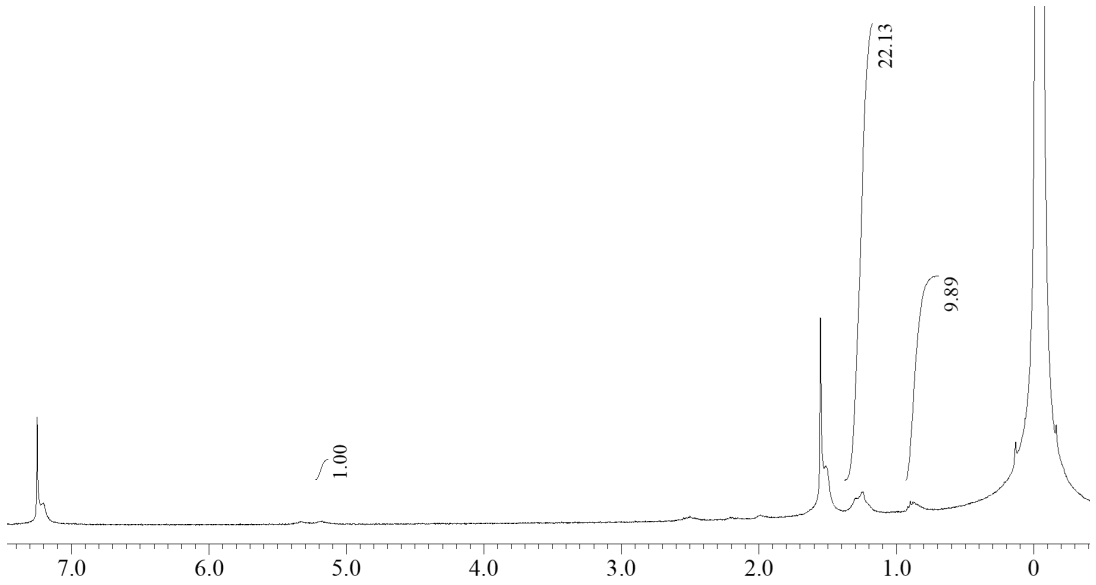
(G) P(3HHx) before fractionation



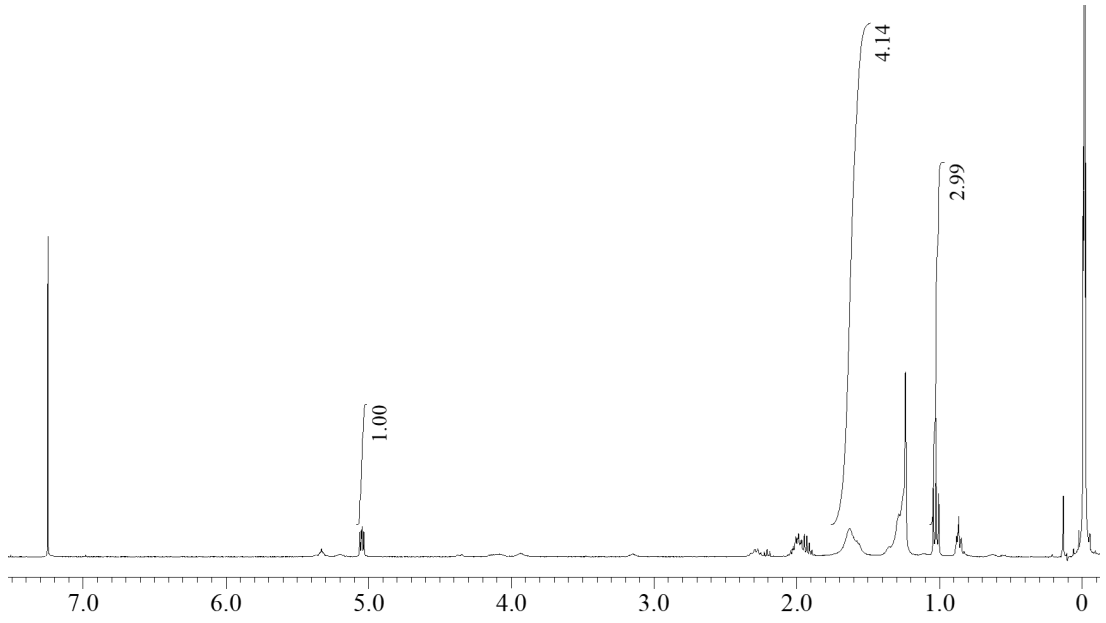
(H) P(3HHx) in the cyclohexane-soluble fraction



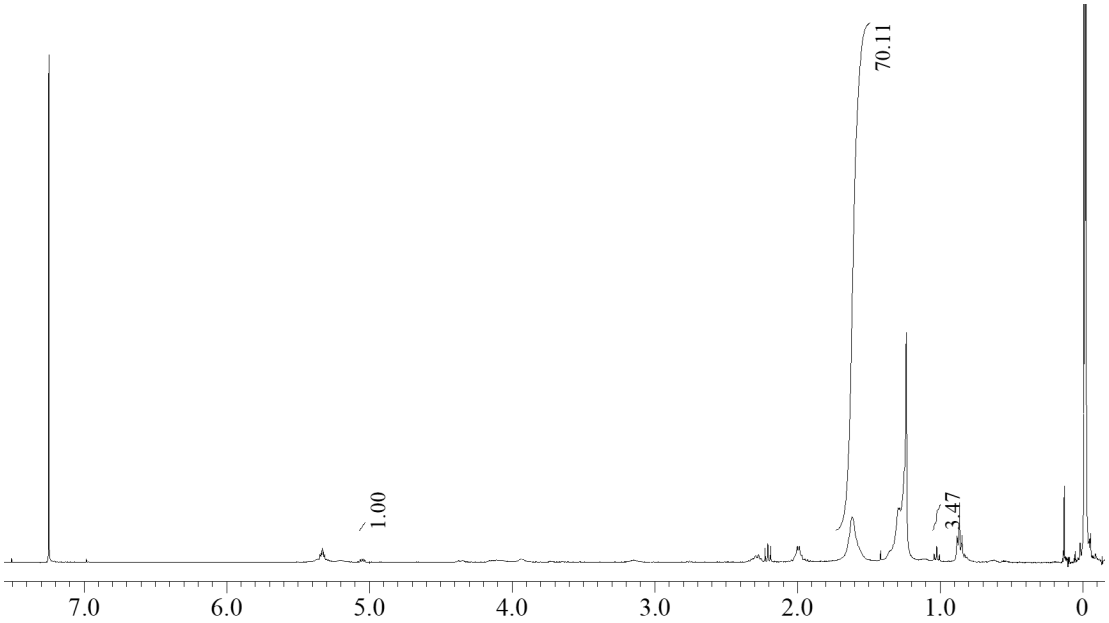
(I) P(3HHx) in the cyclohexane-insoluble fraction



(J) P(2HB) before fractionation



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



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

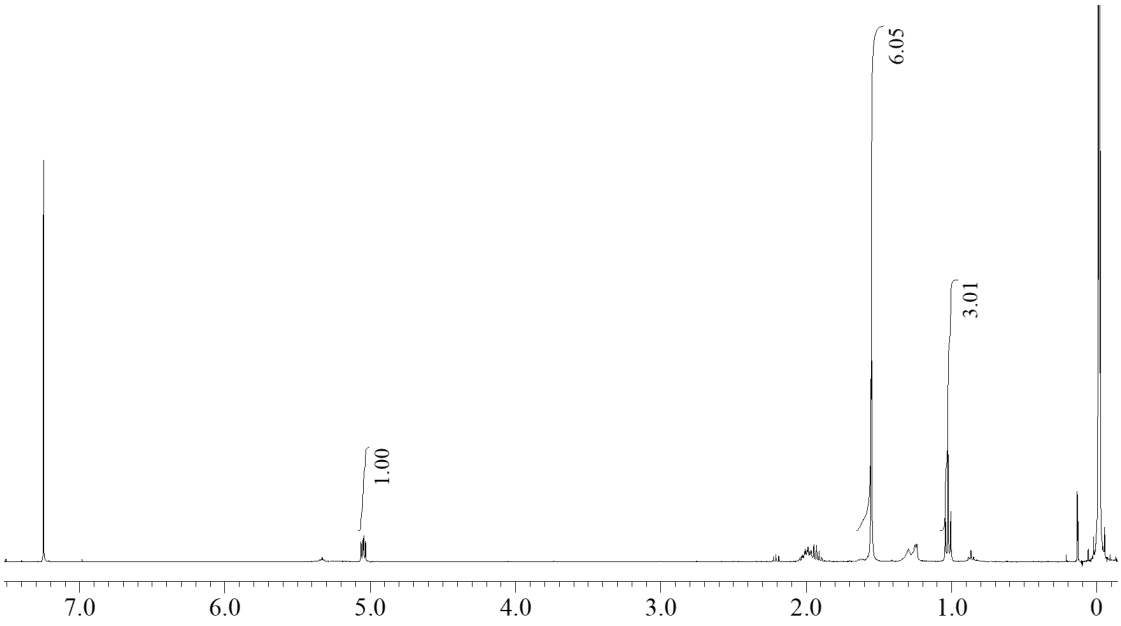


Figure S5. ^{1}H NMR spectra of the produced polymers. Block copolymer before solvent fractionation (A), in the cyclohexane-soluble fraction (B), and in the cyclohexane-insoluble fraction (C). Blend of P(2HB) and P(3HHx) before fractionation (D), in the cyclohexane-soluble

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).

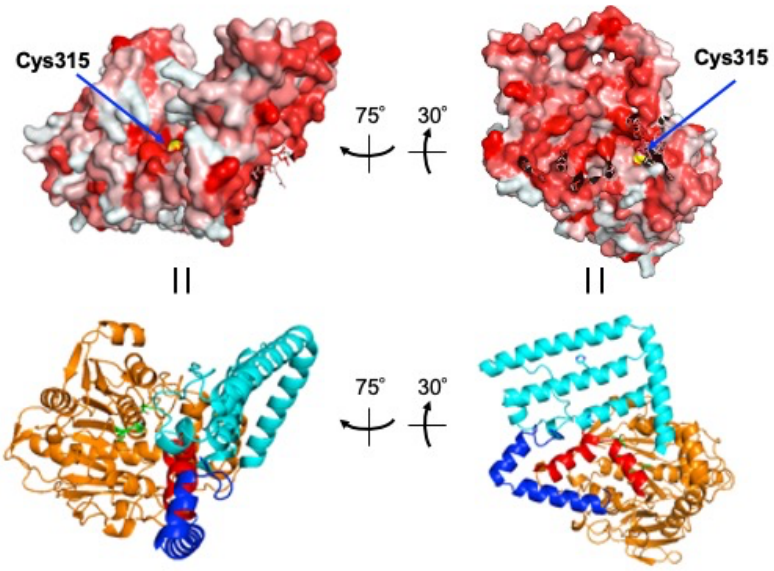


Figure S6. (Upper) Calculated surface hydrophobicity of the predicted structure of $PhaC_{AR}$. Red: hydrophobic, white: hydrophilic. N-terminal 30 residues are not shown because of their low fidelity of the prediction. The yellow residue is the catalytic center Cys315. (Lower) The corresponding cartoon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