

Supporting Information for

**Interparticle Mesoporous Silica as an Effective
Support for Enzyme Immobilization**

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Additional Data:

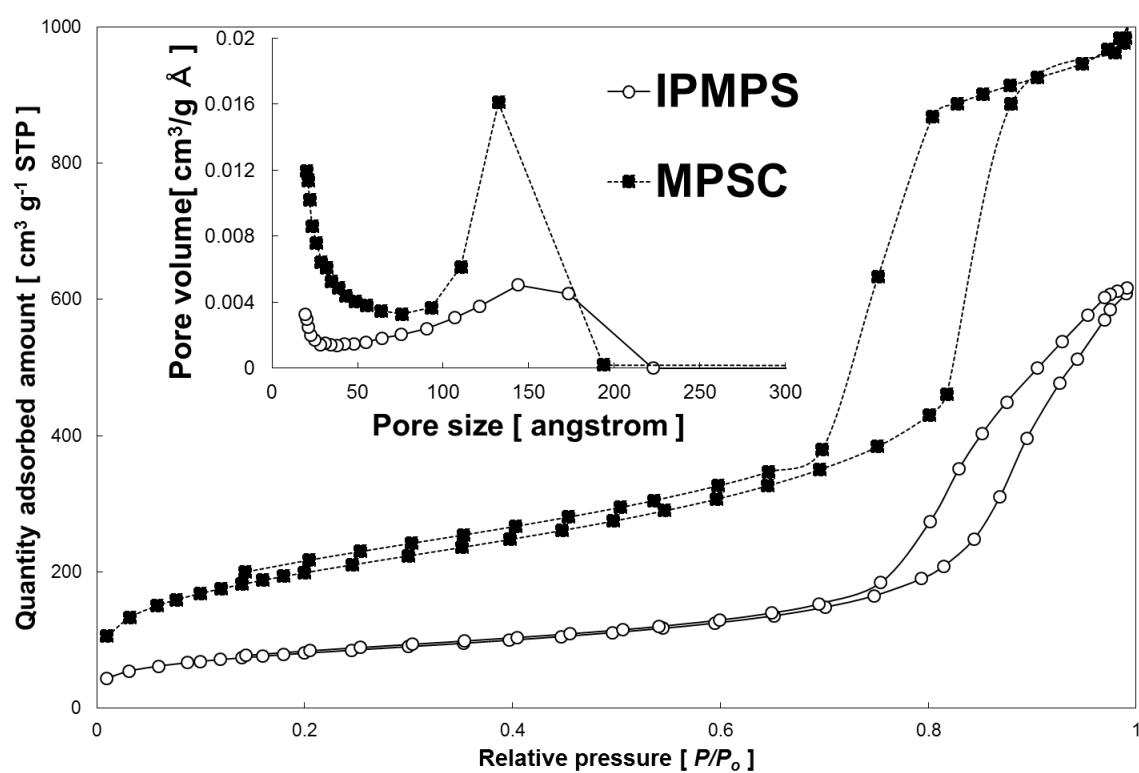


Fig. S1. Nitrogen adsorption–desorption isotherms and BJH analysis (*inset*) for MPS (squares) and IPMPS (circles).

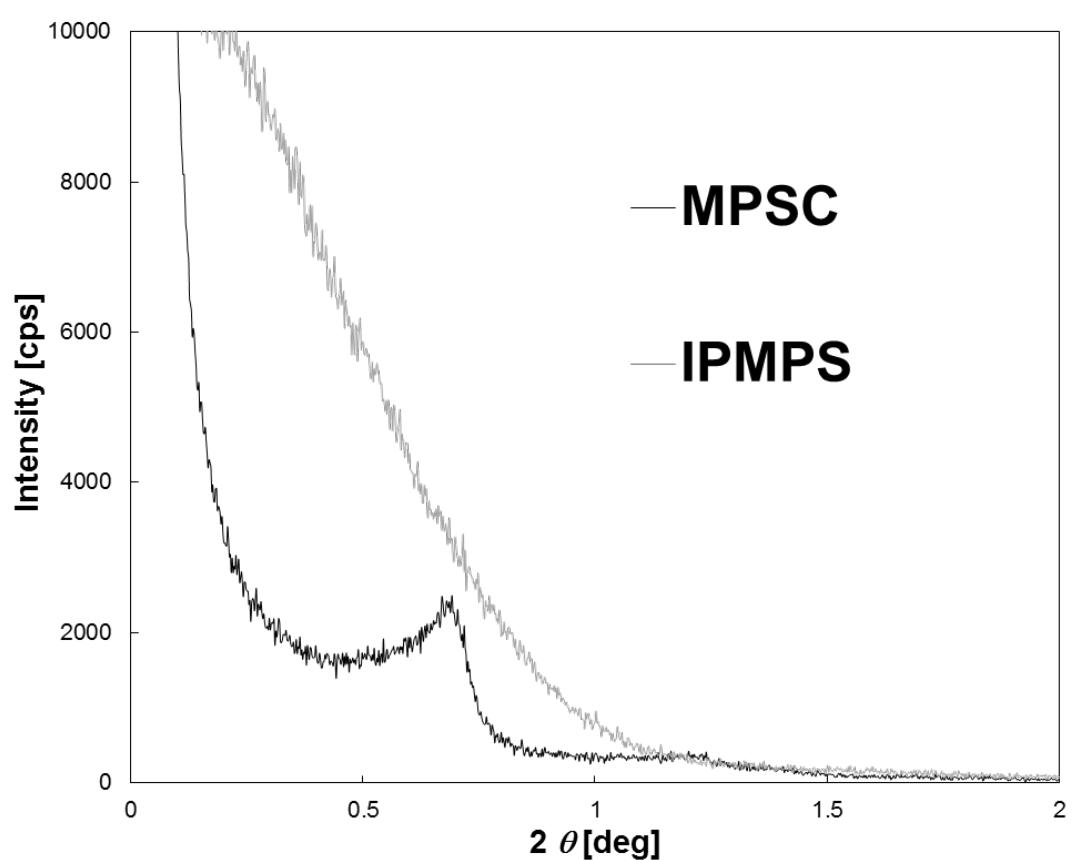


Fig. S2. Small-angle XRD patterns of MPS and IPMPS.

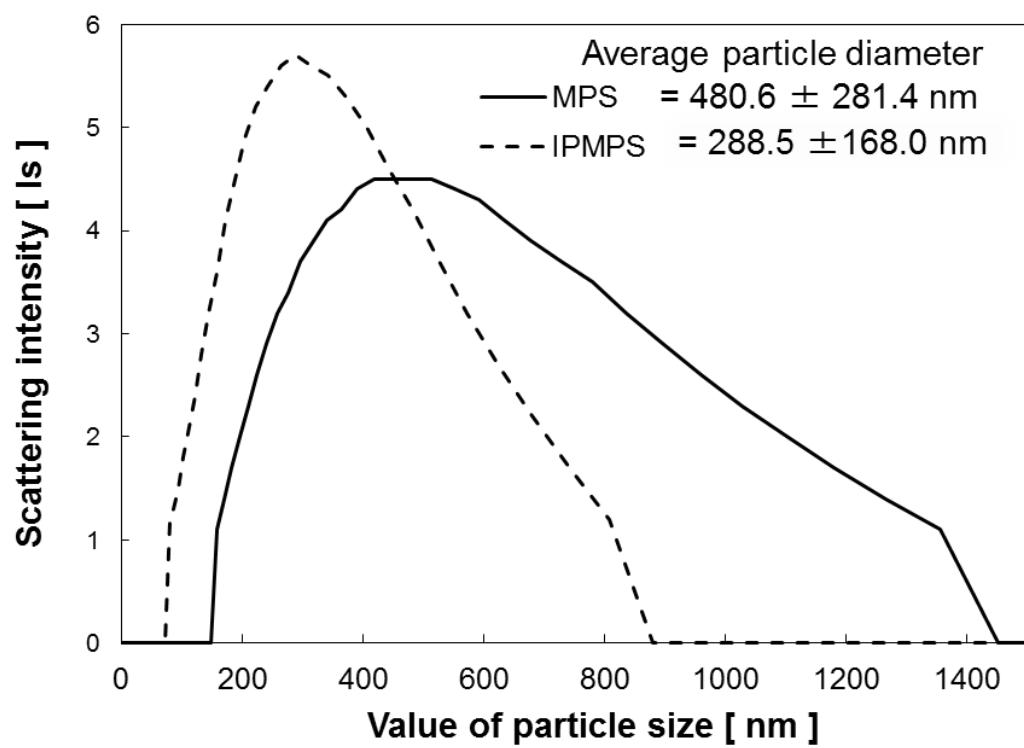


Fig. S3. Average diameters of porous silica materials.

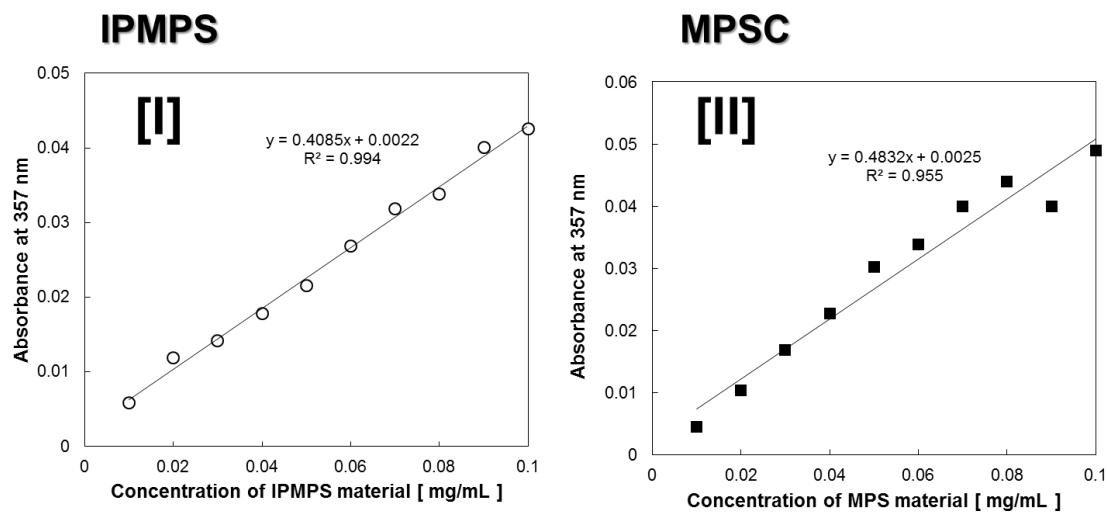


Fig. S4. Correlation of absorbance at 357 nm with concentration of [I] IPMPS and [II] MPS.

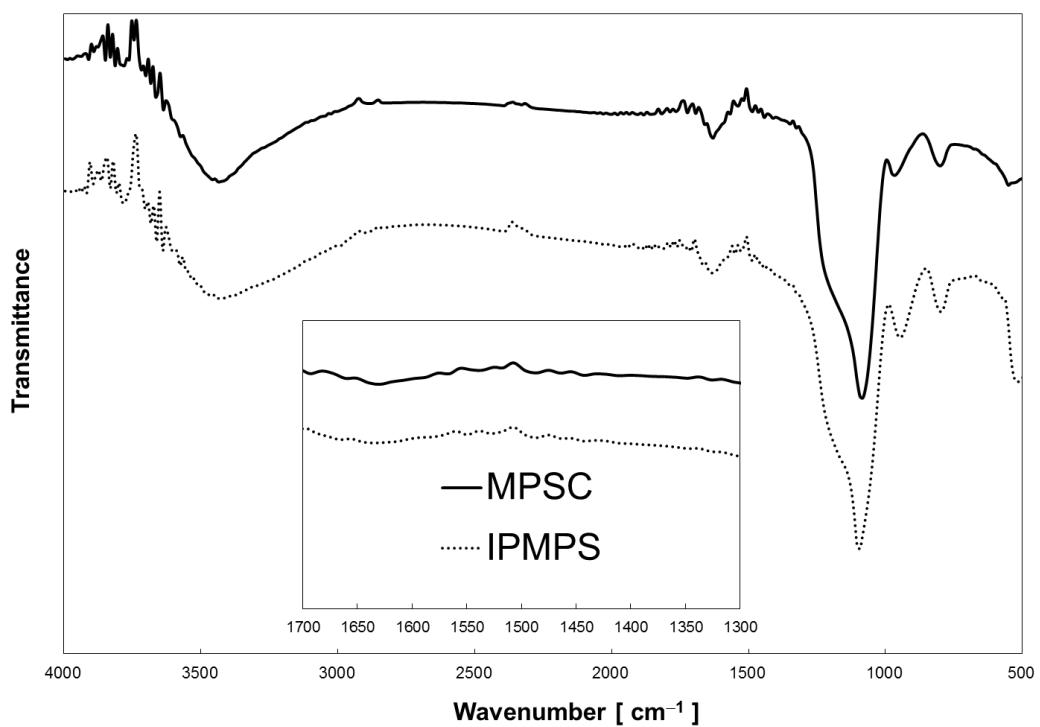


Fig. S5. FTIR spectra of MPS (*solid line*) and IPMPS (*dotted line*).

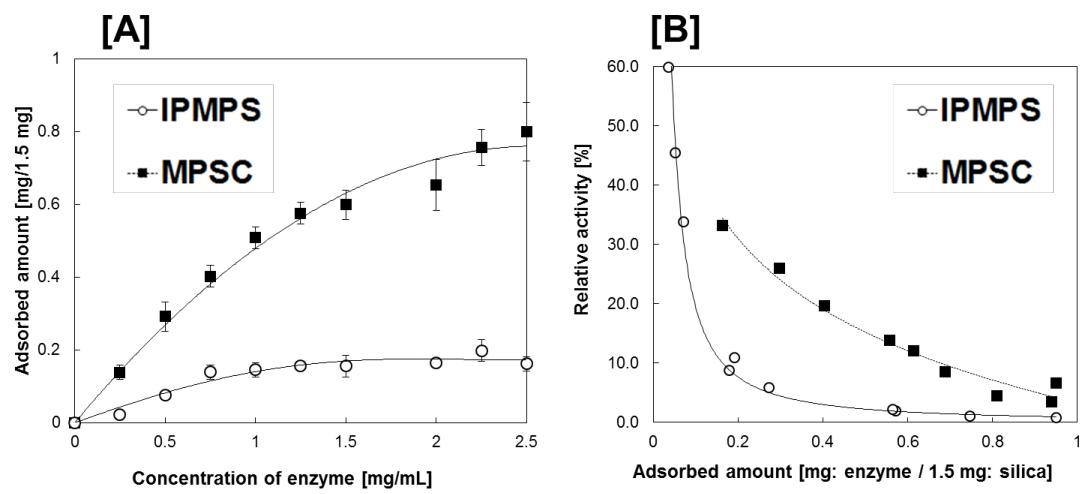


Fig. S6. [A] Isotherms of FDH adsorption versus different immobilization concentrations: MPS (squares), IPMPS (circles). [B] Relative activities of FDH immobilized on each mesoporous material: MPS (squares) and IPMPS (circles).

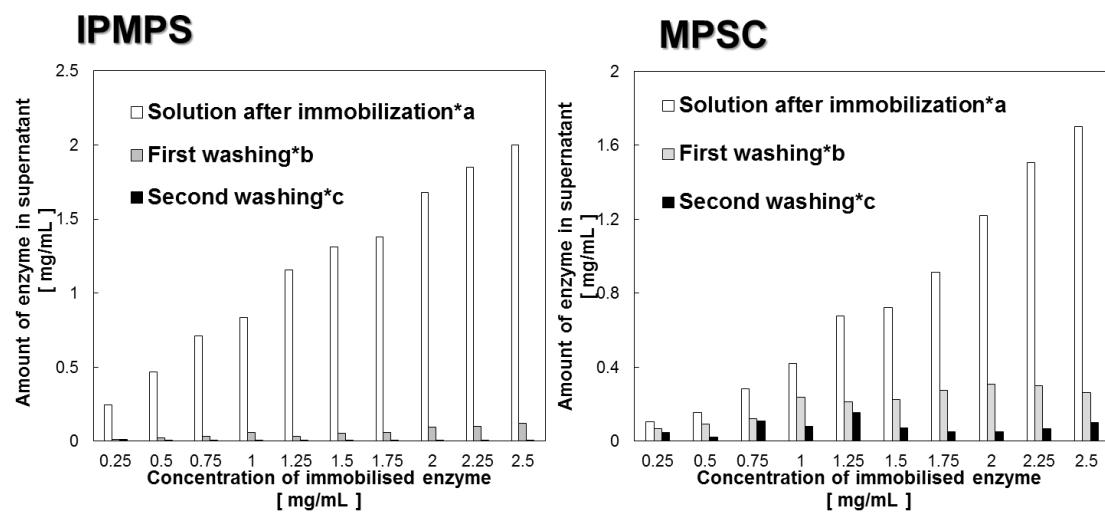


Fig. S7. Amount of enzyme in the supernatant after immobilization and washing with aqueous solution.

*^a Porous silica material (1.5 mg) was added to an FDH solution, and the mixture was stirred overnight at 4 °C. The precipitate was separated by centrifugation (12,000 rpm) at 4 °C for 10 min. The amount of enzyme contained in the supernatant solution is shown in Figure S1. *^b Solid materials obtained in *^a were dispersed in an aqueous solution. After centrifugation, the amount of enzyme in the supernatant solution was measured.

*^c The materials were dispersed and washed again, and the amount of protein was determined in the supernatant.

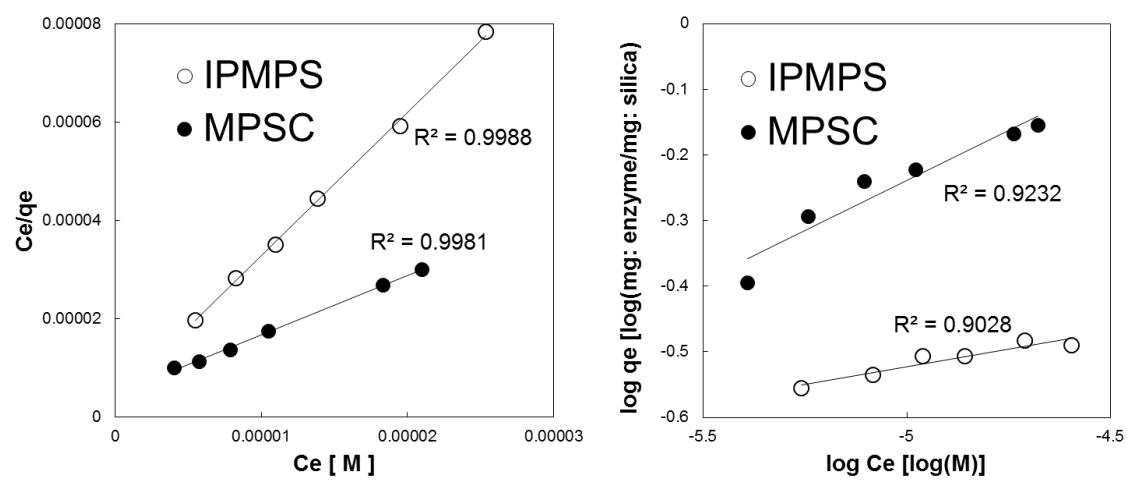


Fig. S8. Langmuir (**A**) and Freundlich (**B**) plots of enzyme adsorption onto porous silica materials.

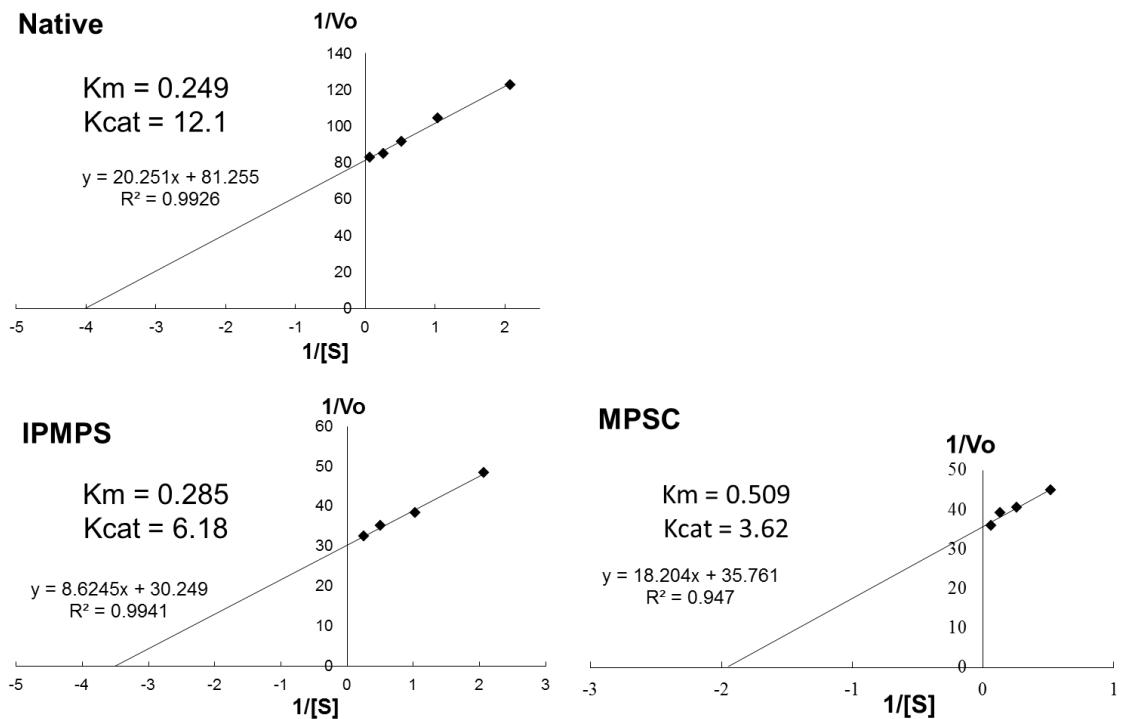


Fig. S9. Kinetic parameters of immobilized FDHs on each MPS material.

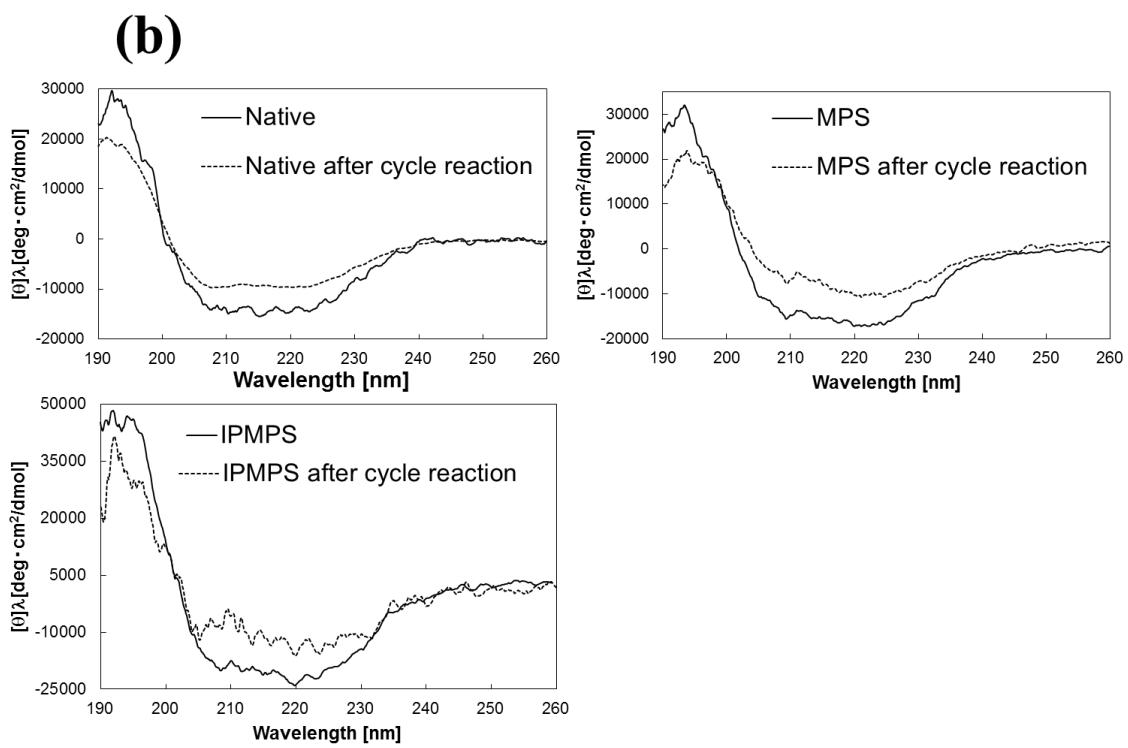
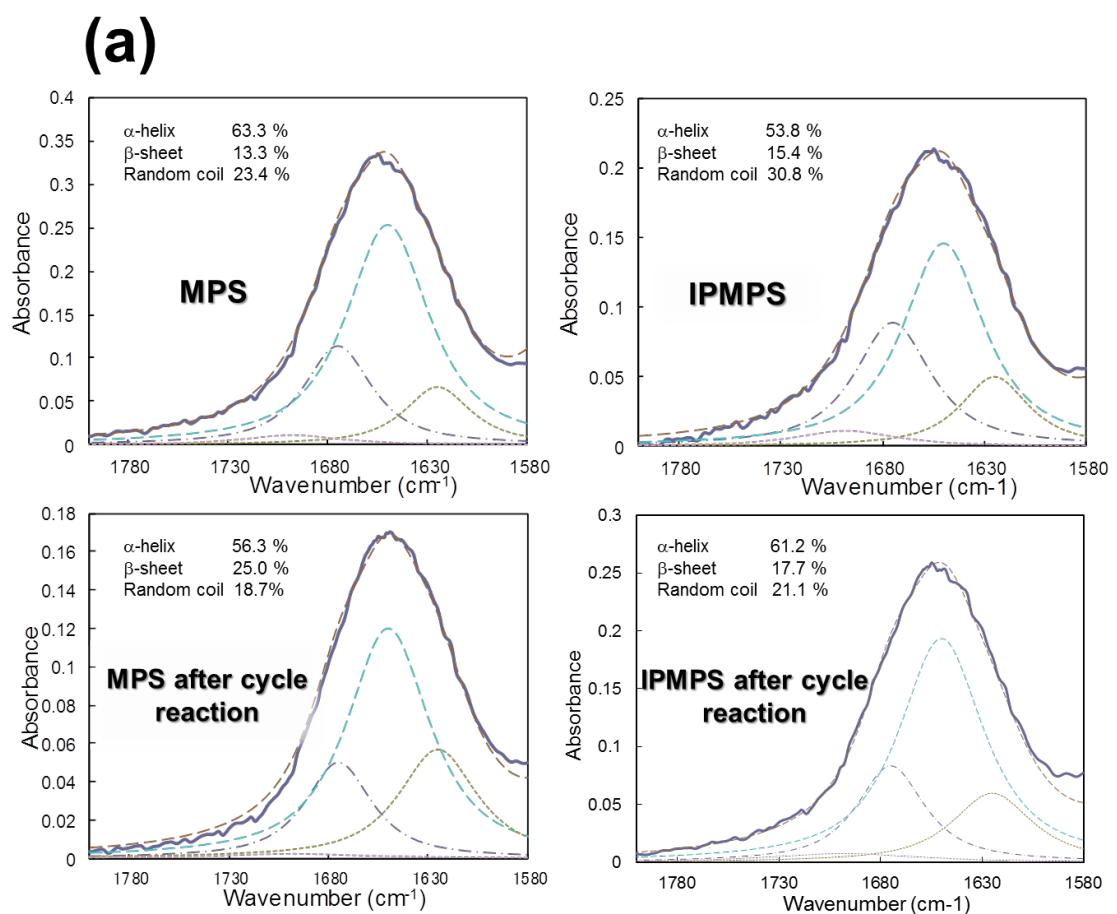


Fig. S10. Analysis of changes in the secondary structure of native and immobilized FDHs on various MPSs after 10 cycle reactions. (a) FTIR and (b) CD spectra.

Table S1. Structural properties of MPS materials

| Sample name | Pore size ^a [nm] | Surface area ^b [m ² /g] | Pore volume ^a [cm ³ /g] | Pore structures ^c | Average particle diameter ^d [nm] | Zeta potential ^d [mV] |
|-------------|----------------------------------|--|--|------------------------------|--|---------------------------------------|
| MPSC | 13.2 | 723.7 | 1.8 | 2D-hexagonal | 480.6 ± 281.4 | -13.8 |
| IPMPS | 14.4 | 291.8 | 0.9 | disordered | 288.5 ± 168.0 | -30.9 |

^aCalculated according to BJH analysis (adsorption branch between 1.7 and 300 nm diameter).

^bSpecific surface area according to BET.

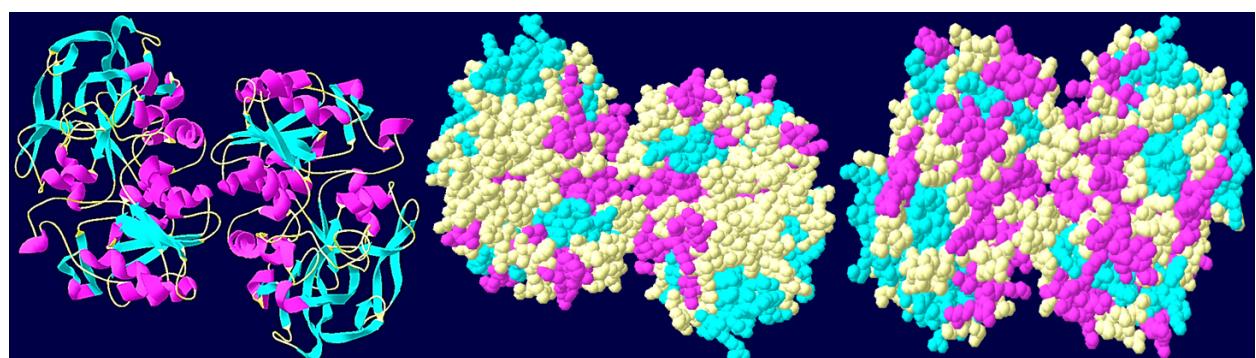
^cPore structure was obtained from small-angle X-ray diffraction spectra.

^dCalculated value was obtained from zeta potential and particle size distribution measurements.

Table S2. Enzyme release amount by the washing of immobilized FDH on each MPS material

| | Amount of enzyme adsorbed [mg] | Amount of released enzyme [mg] ^a | Ratio [%] |
|-------|----------------------------------|---|-------------|
| MPSC | 0.291 ± 0.029 | 0.020 ± 0.001 | 6.78 ± 0.37 |
| IPMPS | 0.120 ± 0.041 | 0.021 ± 0.003 | 18.9 ± 3.80 |

^aAmount of enzyme released after 10 reaction cycles with washing.



Scheme S1. α -Helix (*red*) and β -sheet (*blue*) in FDH. The structural data were obtained from the Protein Data Bank (1KOL).