

Supporting Information

Factors influencing the detergent-free membrane protein isolation using synthetic nanodisc-forming polymers

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Amino acid sequences of FBD and Cyt-b5

Rat flavin mononucleotide binding domain (FBD) of cytochrome P450 reductase

MGDSHEDTSATMPEAVAAEEVSLFSTTDMVLFSLIVGVLT^YWFI^{FRKKKEE}IPEFSKIQT^TAPPV
KES^SFVEKMKKTGRNII^VFYGSQTGTAE^EFANRLSKDAHRYGMRGMSADPEEYDLADLSSLPEI
DKSLVVFCMATYGE^GDPTDNAQDFYDWLQETD^VDLTG^VKFAVFGLGNKTYEHFNAMGKYVDQRL
EQLGAQRI FELGLGDDDDGNLEEDFITWREQFWPAVCEFFGVEATGEEHHHHHH

Rabbit cytochrome-b5

GHHHHHAAQSDKDVKYYTLEEIKKH^NHSKSTWLILHHKVYDLTKFLEEHPGGEEVLR^EQAGGD
ATENFEDVGHSTDARELSKTFIIGELHPDDRSKLSKPMETLITTVDSNSSWWTNWVIPAISALI
VALMYRLYMADD

The transmembrane domains are underlined.

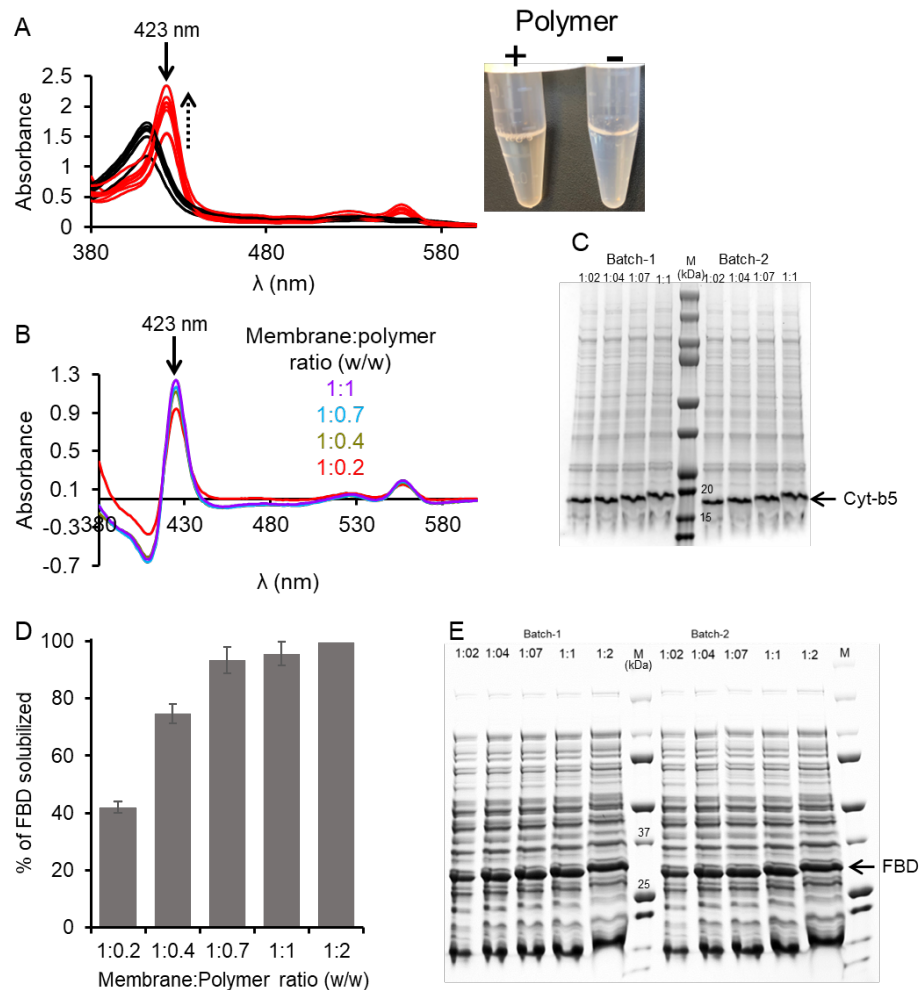


Figure S1. (A) Absorbance spectra of oxidized (black) and sodium dithionate reduced Cyt-b5 in *E. coli* lipid-pentyl-inulin nanodiscs. The samples were made using 4 different membrane:polymer (w/w) ratios as indicated. The absorbance peak intensity change at 423 nm is indicated with an upside dotted arrow. *Picture:* The light-red colored (left-side) and colorless (right-side) solutions in tubes are supernatants after centrifugation of Cyt-b5-enriched cell membranes with and without the polymer, respectively. (B) Difference absorbance spectra (reduced minus oxidized) of pentyl-inulin-solubilized *E. coli* membranes enriched with a ~15.7-kDa rabbit cytochrome-b5 showing the maximal absorbance differences at 409, 423, 526 and 556 nm. The data were collected on the solubilization samples prepared using 4 different polymer concentrations as indicated. (C) SDS-PAGE analysis of pentyl-inulin-solubilized Cyt-b5-rich *E. coli* cell membranes. (D) Bar plot depicting the percentage of 27.8-kDa FBD protein band intensities at 5 different membrane:polymer ratios (w/w) as indicated. (E) SDS-PAGE analysis of pentyl-inulin-solubilized FBD-rich *E. coli* cell membranes. Both Cyt-b5 and FBD are anchored to the lipid membrane via a transmembrane helical domain. The solubilization experiments were performed in duplicates (Batches 1 and 2). Cyt-b5 and FBD proteins have been directly isolated using SMA-EA and pentyl-inulin polymers, respectively [1-4].

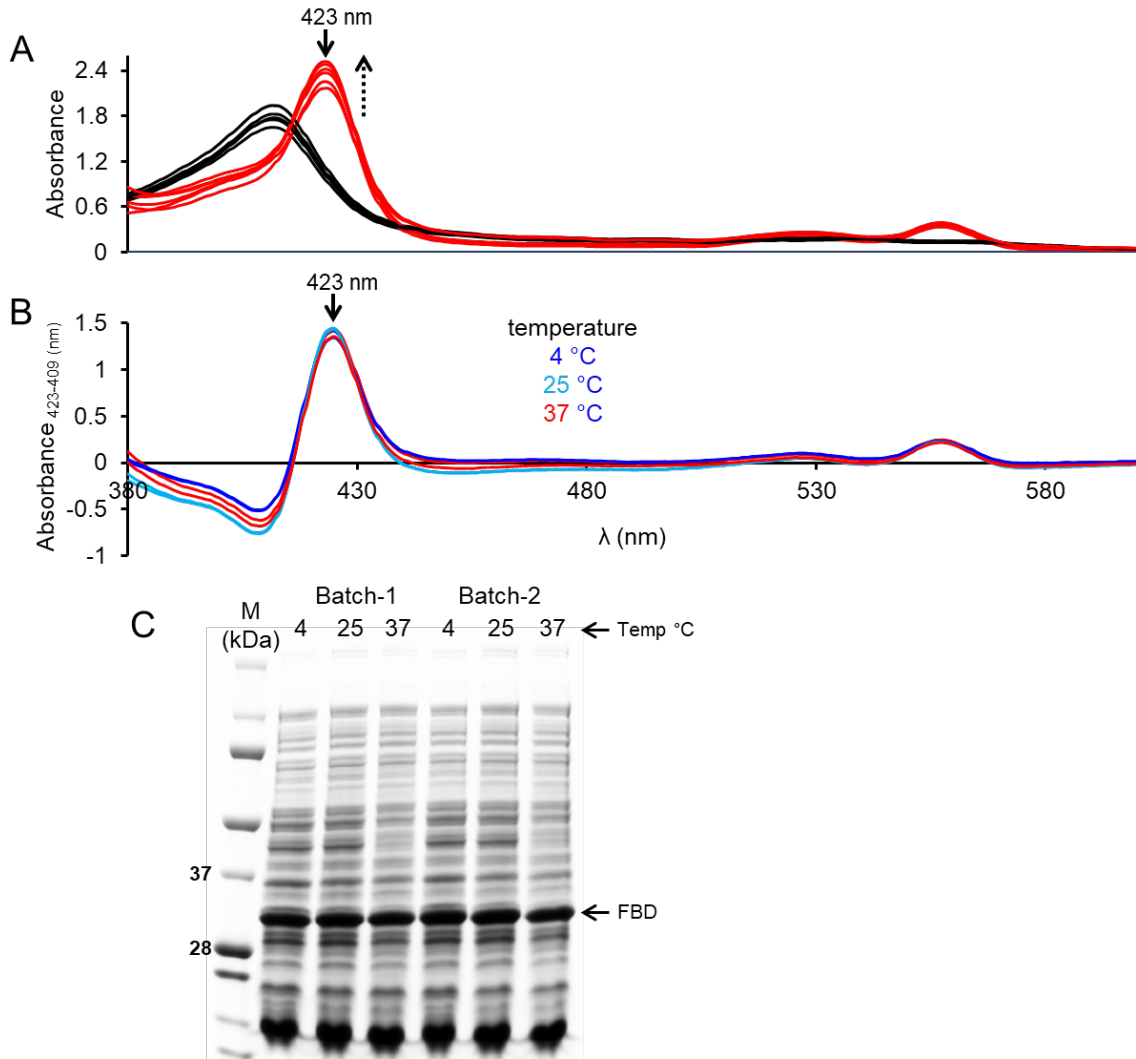


Figure S2. (A) Absorbance spectra of oxidized (black) and sodium dithionite reduced Cyt-b5 in *E. coli* lipid-pentyl-inulin nanodiscs. The samples were made using 1:1 membrane:polymer (w/w) ratio and solubilization was performed at 3 different temperatures as indicated. The absorbance peak intensity change at 423 nm is indicated with upside dotted arrow. **(B)** Difference absorbance spectra (reduced minus oxidized) of pentyl-inulin-solubilized *E.coli* membranes enriched with a ~15.7-kDa rabbit cytochrome-b5 showing the maximal absorbance differences at 409 and 423 nm. The data were collected on the solubilization samples prepared at 3 different temperature conditions as indicated. **(C)** SDS-PAGE analysis of pentyl-inulin-solubilized 27.8-kDa FBD-rich *E. coli* cell membranes obtained at different temperature conditions. M denotes the protein marker. The solubilization experiments were performed in duplicates (Batches 1 and 2).

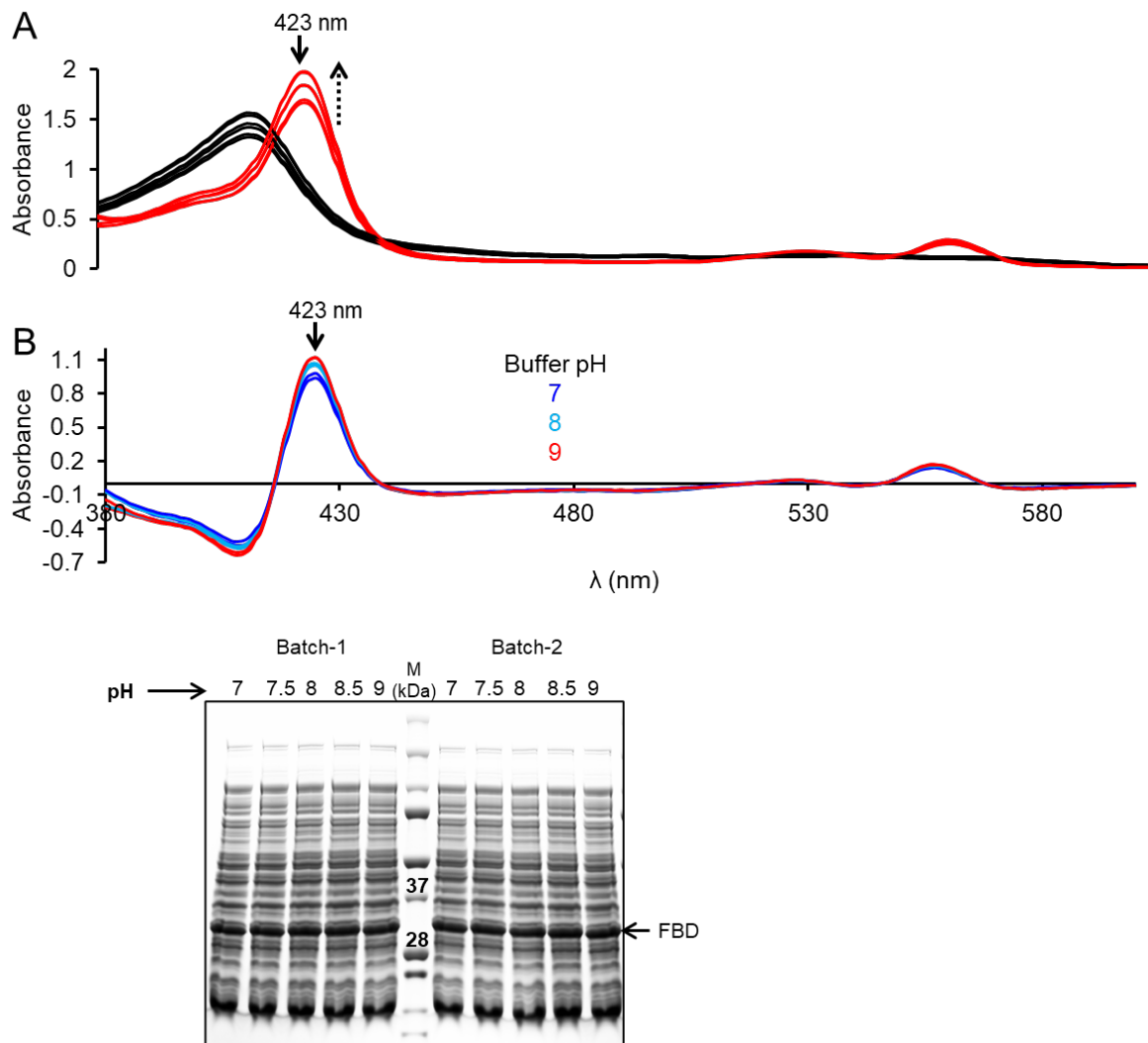


Figure S3. (A) Absorbance spectra of oxidized (black) and sodium dithionite reduced Cyt-b5 in *E. coli* lipid-pentyl-inulin nanodiscs. The samples were made using 1:1 membrane:polymer (w/w) ratio and solubilization was performed at 3 different pH conditions as indicated. The absorbance peak intensity change at 423 nm is indicated with an upside dotted arrow. (B) Difference absorbance spectra (reduced minus oxidized) of pentyl-inulin-solubilized *E. coli* membranes enriched with a ~15.7-kDa rabbit cytochrome-b5 showing the maximal absorbance differences at 409 and 423 nm. The data were collected on the solubilization samples prepared at 3 different pH conditions as indicated. (C) SDS-PAGE analysis of pentyl-inulin-solubilized 27.8-kDa FBD-rich *E. coli* cell membranes at different pH conditions. M denoted the protein marker. The solubilization experiments were performed in duplicates (Batches 1 and 2).

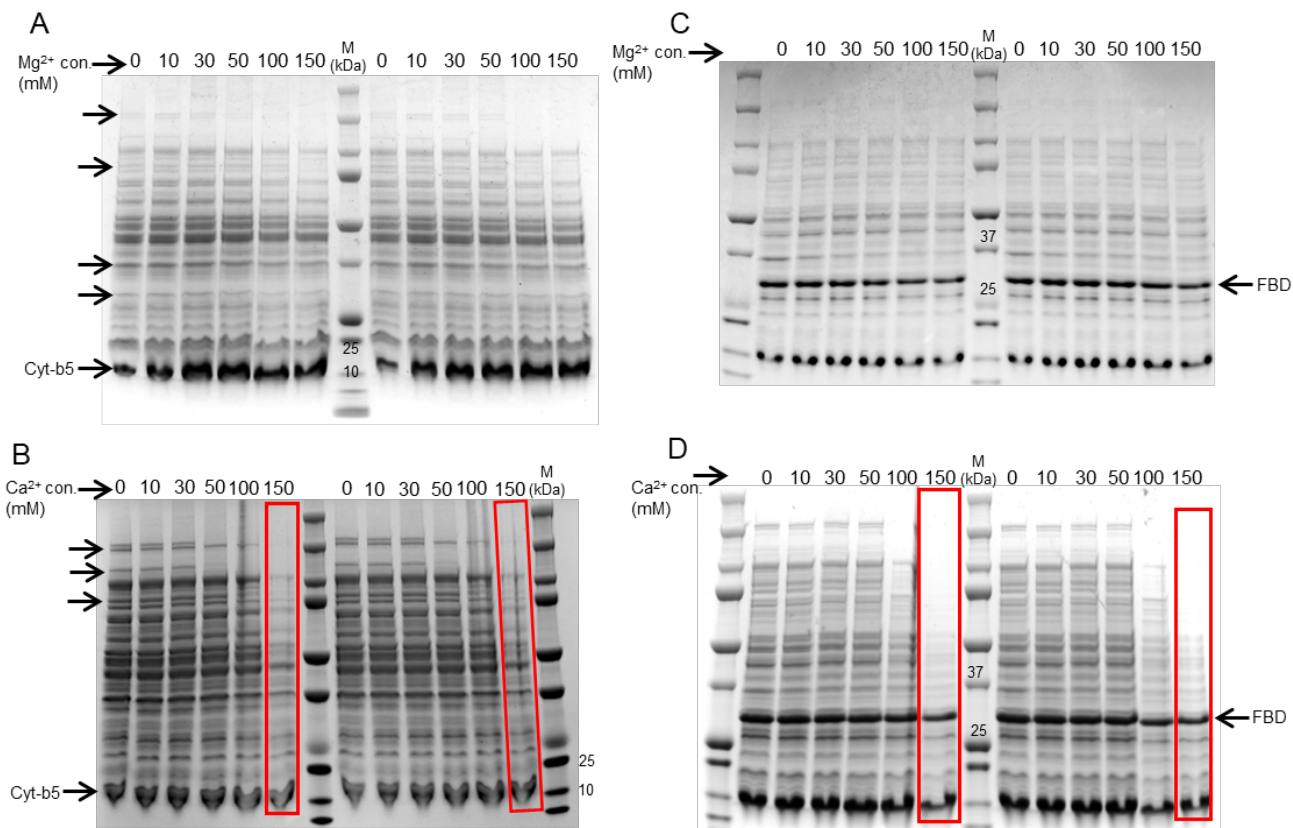


Figure S4. SDS-PAGE analysis of Cyt-b5 (A, B) and FBD-enriched (C, D) *E. coli* membranes that were solubilized using pentyl-inulin at the indicated concentrations of divalent metal ions. A and B are Cyt-b5 samples in the presence of MgCl₂ and CaCl₂, respectively. C and D are FBD samples in the presence of MgCl₂ and CaCl₂, respectively. The protein bands corresponding to Cyt-b5 and FBD are labelled. The metal ion concentrations are indicated. The lanes highlighted in (B) and (D) with red boxes indicate a significant decrease in the intensity of protein bands at higher concentrations of divalent metal ions. The solubilization experiments were performed in duplicates.

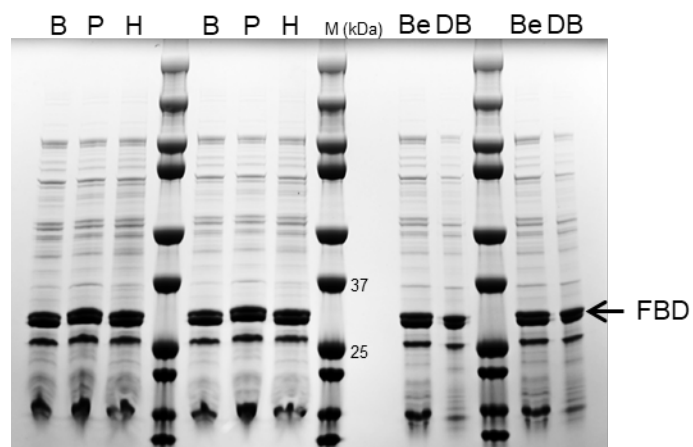


Figure S5. SDS-PAGE analysis of FBD-enriched *E. coli* membranes that were solubilized using inulin based polymers functionalized with butyl (B), pentyl (P), hexyl (H), benzyl (Be) or di-benzyl (DB) hydrophobic groups. The solubilization experiments were performed in duplicates.

References

- [1] B. Krishnarjuna, A. Ramamoorthy, Detergent-free isolation of membrane proteins and strategies to study them in a near-native membrane environment, *Biomolecules*, 12 (2022) 1076.
- [2] B. Krishnarjuna, T. Ravula, A. Ramamoorthy, Detergent-free extraction, reconstitution and characterization of membrane-anchored cytochrome-b5 in native lipids, *ChemComm*, 56 (2020) 6511-6514.
- [3] T. Ravula, A. Ramamoorthy, Synthesis, characterization, and nanodisc formation of non-ionic polymers, *Angew. Chem. Int. Ed.*, 60 (2021) 16885-16888.
- [4] T. Ravula, S.K. Ramadugu, G. Di Mauro, A. Ramamoorthy, Bioinspired, size-tunable self-assembly of polymer-lipid bilayer nanodiscs, *Angew. Chem. Int. Ed.*, 56 (2017) 11466-11470.