Supplementary Data for

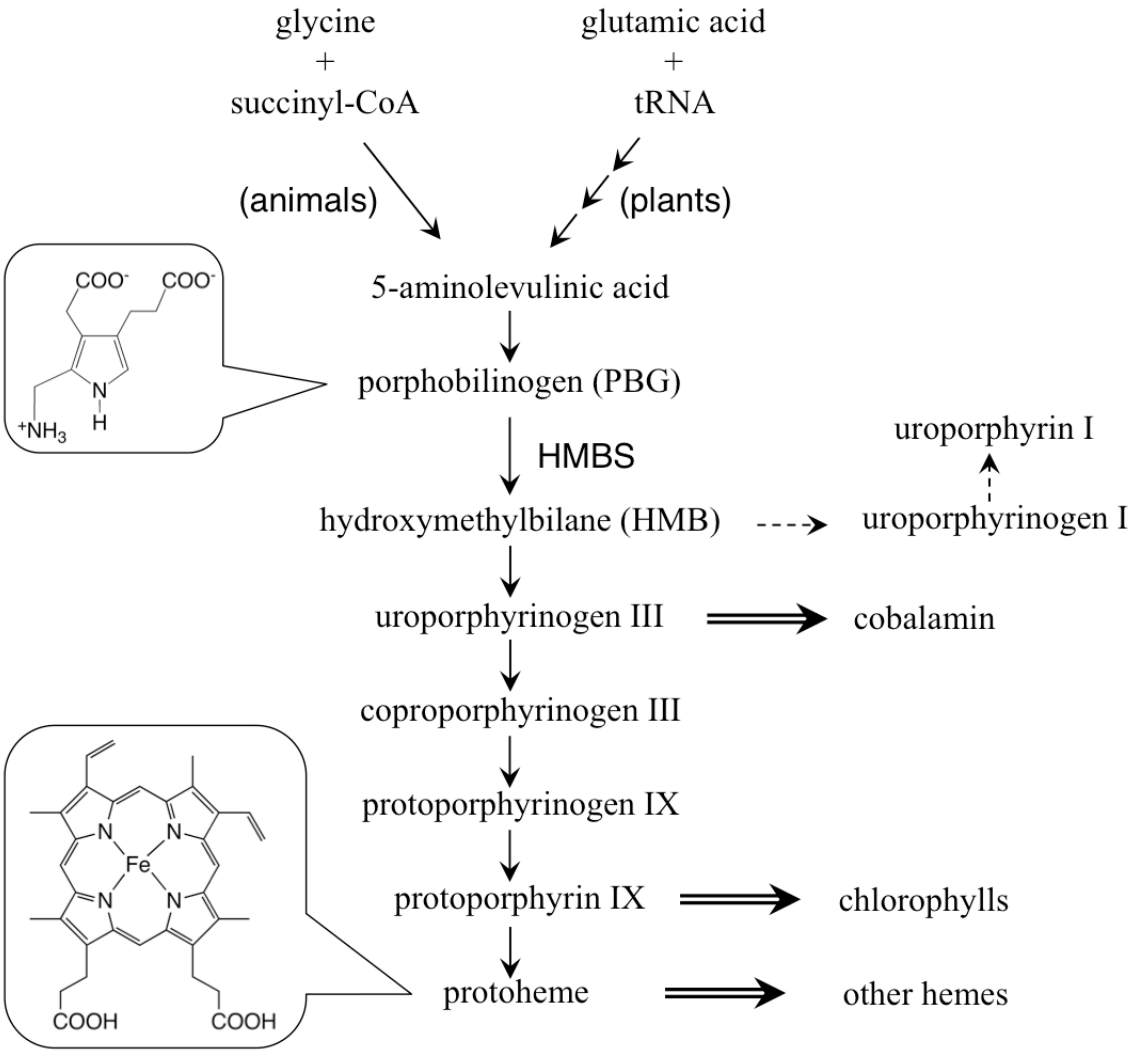


Figure S1. Porphyrin biosynthesis pathway.

Solid and dashed arrows indicate enzymatic and non-enzymatic reactions, respectively. Each double arrow shows several steps of enzymatic reactions. HMBS is the third enzyme in the heme biosynthesis pathway in animals and forms one molecule of HMB from four molecules of PBG.

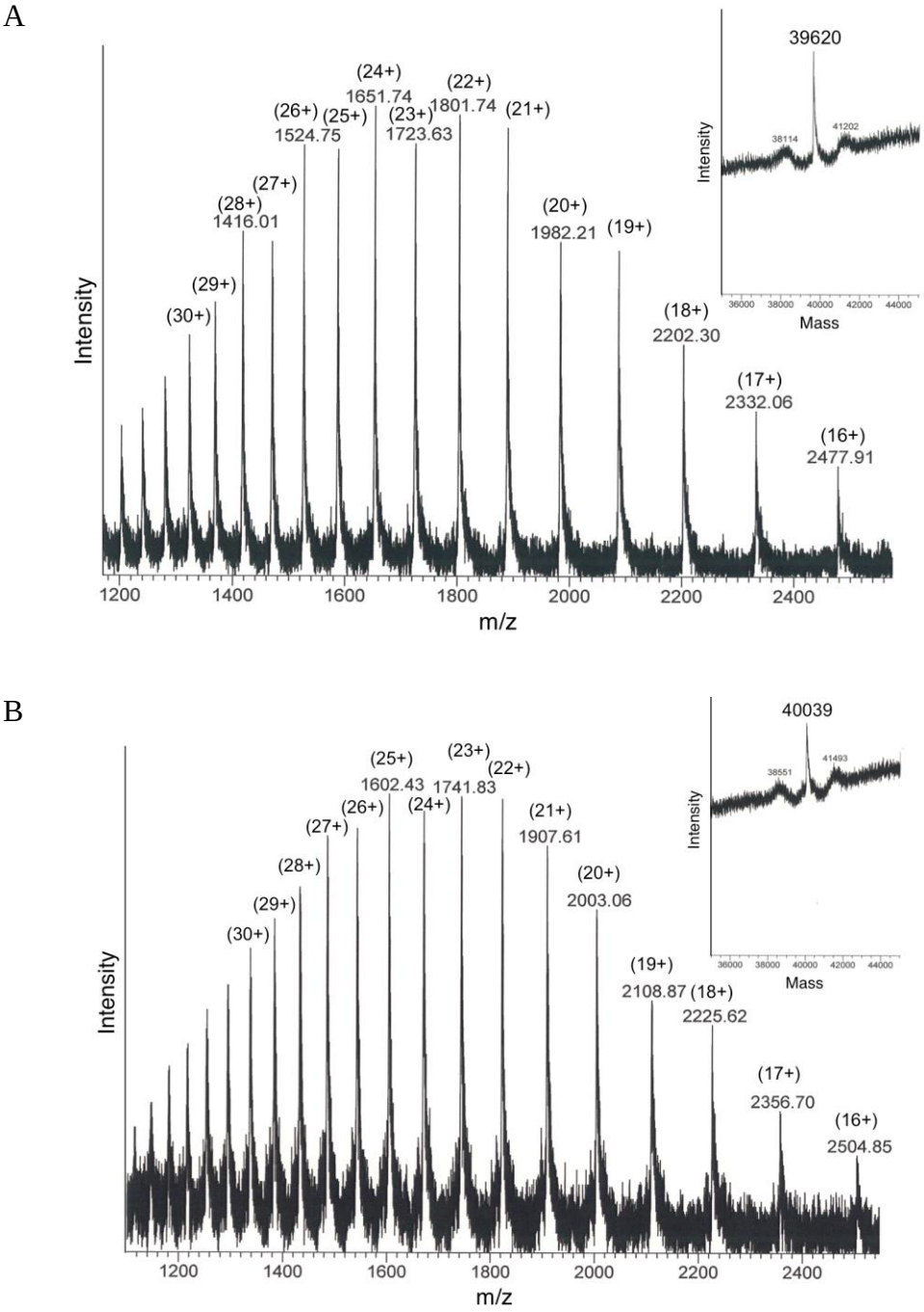
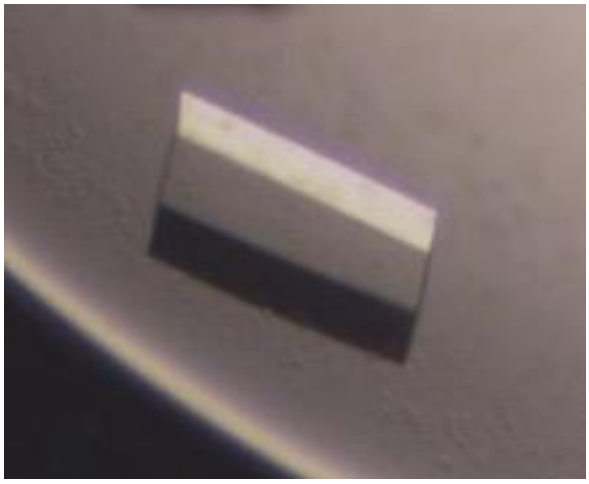


Figure S2. Electrospray ionization time-of-flight mass spectra of purified HMBS.

The values in parentheses denote the charge states. Insets show spectra after deconvolution. (**A**) Substrate-free holo-HMBS fraction. The deconvoluted spectrum indicates the molecular mass is 39620, which corresponds to the molecular weight of DPM cofactor-bound human HMBS (calcd 39617). (**B**) A reaction intermediate fraction of HMBS. After deconvolution, the molecular mass is 40039, which corresponds to the molecular weight of the ES_2 intermediate of human HMBS (calcd 40036).

A



B

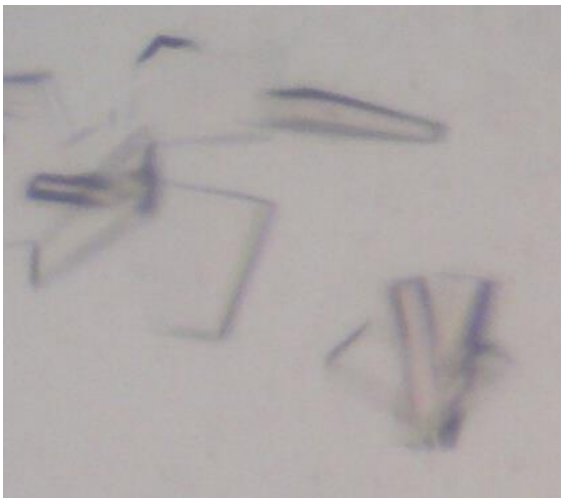


Figure S3. Typical crystals of human HMBS.

(**A**) Inhibitor-free holo form. Size: ca. 0.2 x 0.2 x 0.02 mm. (**B**) Inhibitor-free ES_2 intermediate. Size: ca. 0.1 x 0.1 x 0.01 mm.

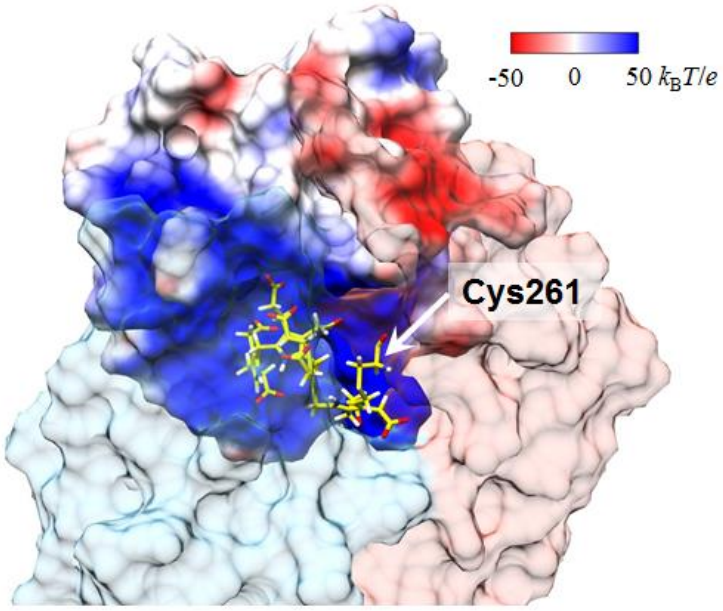
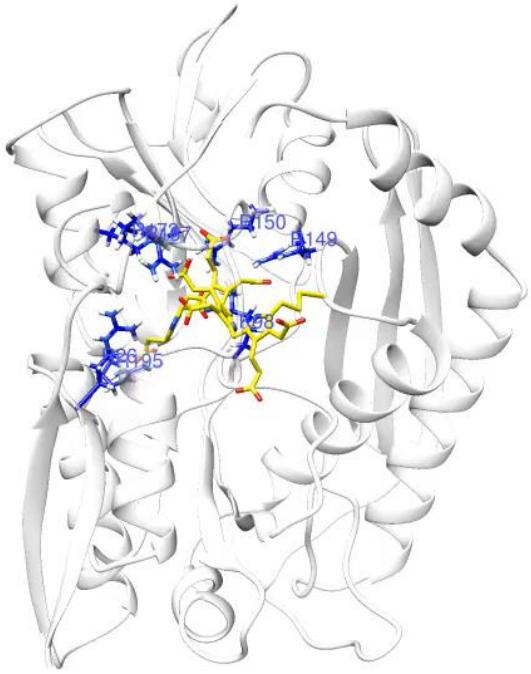


Figure S4. Electrostatic potential in the PBG-binding region of ES_2 intermediate.

Electrostatic potential due to the residues in domain 2 is shown on the surface of domain 2. The electrostatic potential was calculated by APBS [S1]. The tetrapyrrole chain (DPM and two PBGs) covalently bound to Cys261 is shown by yellow sticks. For visibility of the PBG-binding region, domains 1 (cyan) and 3 (magenta) on the near side are made transparent.

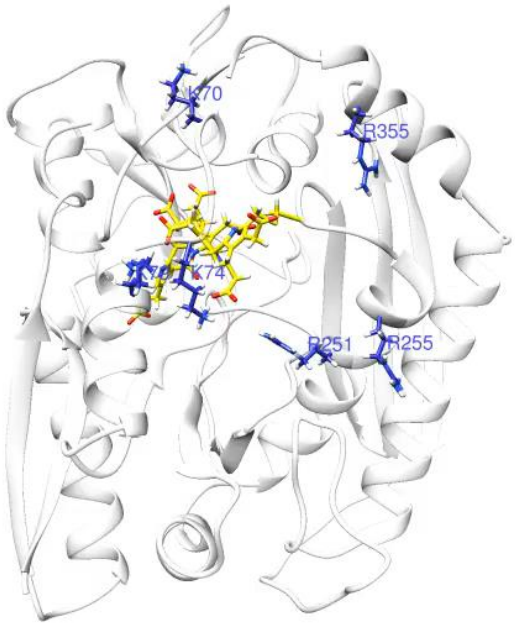
Reference

[S1] Baker, N. A., Sept, D., Joseph, S., Holst, M. J. and McCammon, J. A. (2001) Electrostatics of nanosystems: Application to microtubules and the ribosome. *Proc. Natl. Acad. Sci. U. S. A.*, **98**, 10037−10041 https://doi.org/10.1073/pnas.181342398



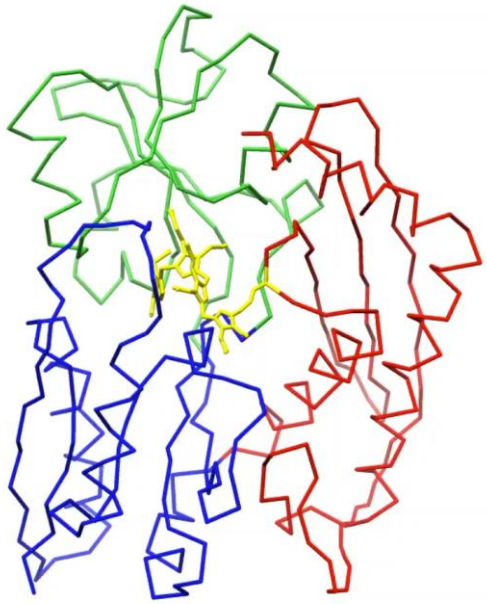
Movie S1. Molecular dynamics of ES_2 intermediate and extensive electrostatic interactions involving PBGs.

MD trajectory of the ES_2 intermediate is shown at 0.1 μs interval (total 11.2 μs) where sixteen 0.7-μs-long MD trajectories are combined into a single movie. The tetrapyrrole chain (DPM and two PBGs) covalently bonded to Cys261 is shown by yellow sticks. Note that while the pyrrole rings of DPM are mobile, those of PBGs (particularly ring A) are almost immobilized due to extensive electrostatic interactions between the acetate/propionate groups of PBGs and surrounding basic residues. The side chains of those residues (Arg26, Lys98, Arg149, Arg150, Arg167, Arg173, and Arg195) are shown by blue sticks.



Movie S2. Molecular dynamics of ES_2 intermediate and intermittent electrostatic interactions involving DPM.

This movie is the same as Movie S1 except that basic residues that form intermittent electrostatic interactions with the acetate/propionate groups of DPM are highlighted. The side chains of those residues (Lys70, Lys74, Lys79, Arg251, Arg255, and Arg355) are shown by blue sticks.



Movie S3. Principal mode of the ES_2 intermediate.

Principal component analysis was conducted for the thermal fluctuation of the ES_2 intermediate, and the second largest principal mode (eigenvector) is shown. For visibility, the amplitude is magnified by a factor of 2. The movement of the cofactor-binding loop is most prominently seen in this mode. Cys261 and covalently bound tetrapyrrole chain are colored in yellow, and domains 1, 2, and 3 are colored in blue, green, and red, respectively. Note that the lid loop in domain 1, the cofactor-binding loop (and the C-terminal helix), and the insertion region in domain 3 fluctuate largely in a collective manner.