

Supplementary Materials

Development of a Versatile Method to Construct Direct Electron Transfer-Type Enzyme Complexes Employing SpyCatcher/SpyTag System

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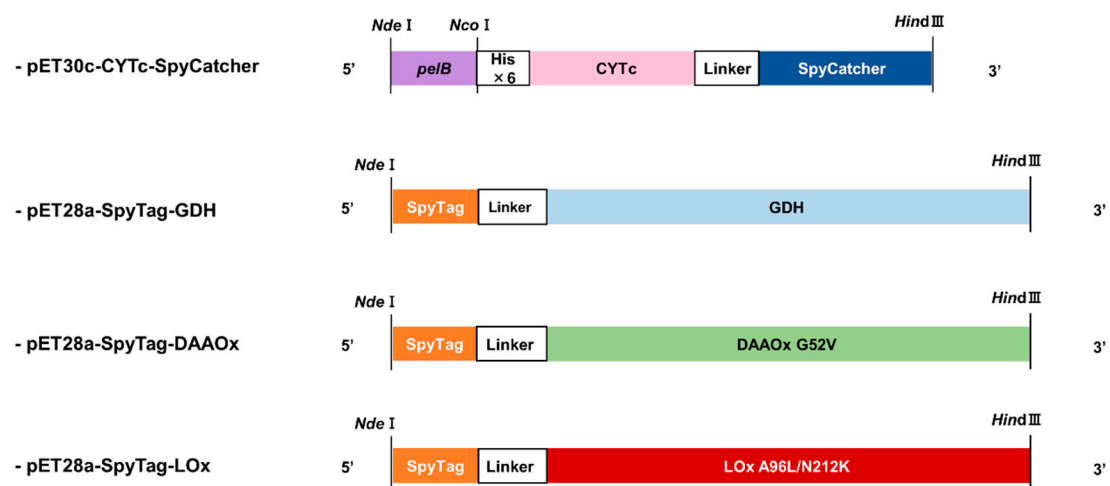


Figure S1. Inserted sequence of constructed expression vectors (pET30c-CYTc-SpyCatcher, pET28a-SpyTag-GDH, pET28a-SpyTag-LOx, and pET28a-SpyTag-DAAOx)
All linker regions were composed of 3 amino acids (-Gly-Thr-Gly-).

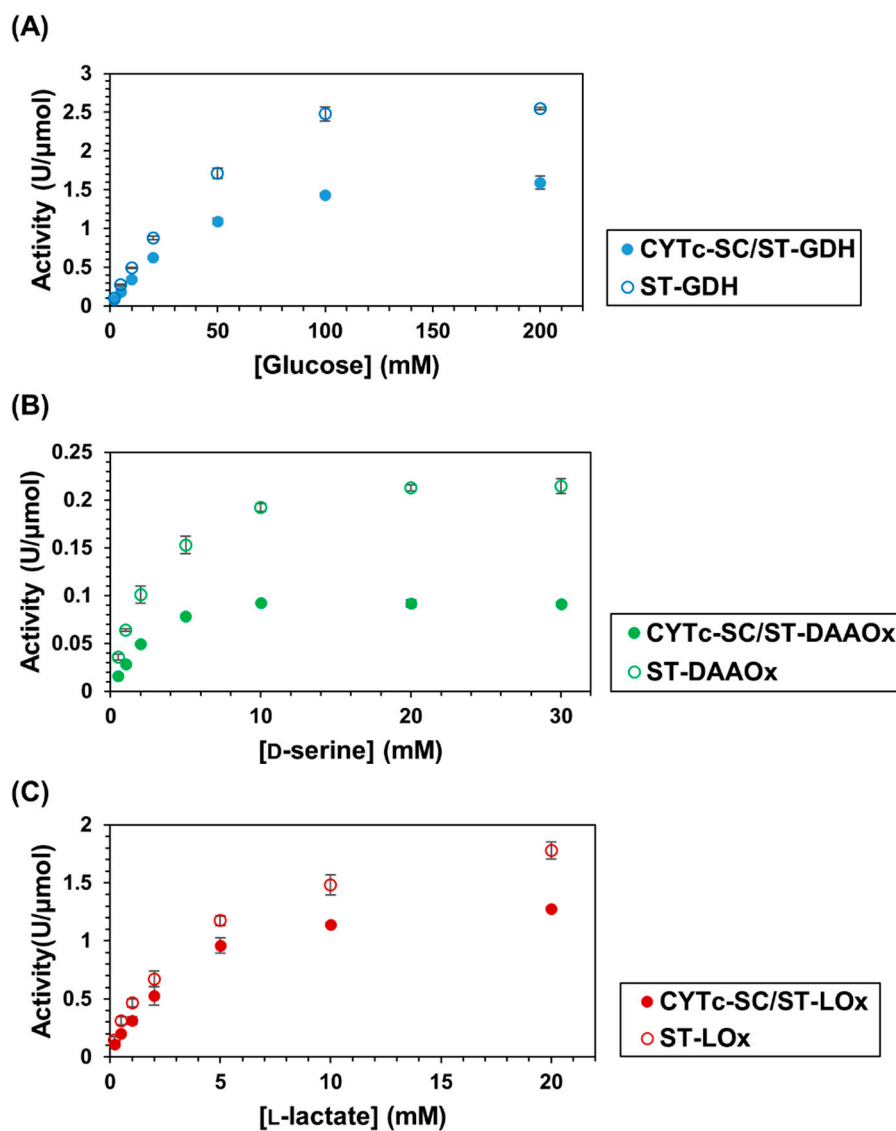
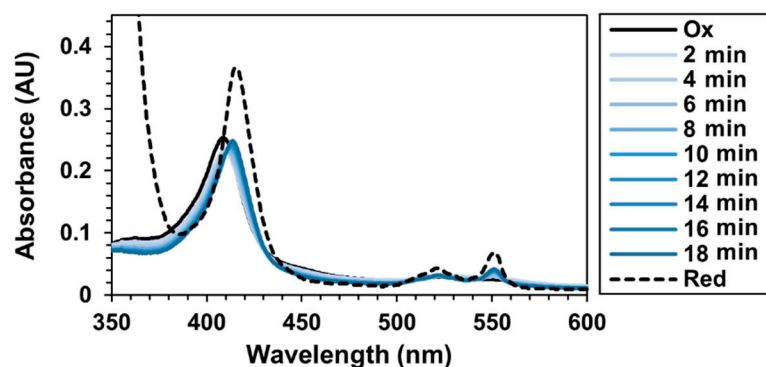


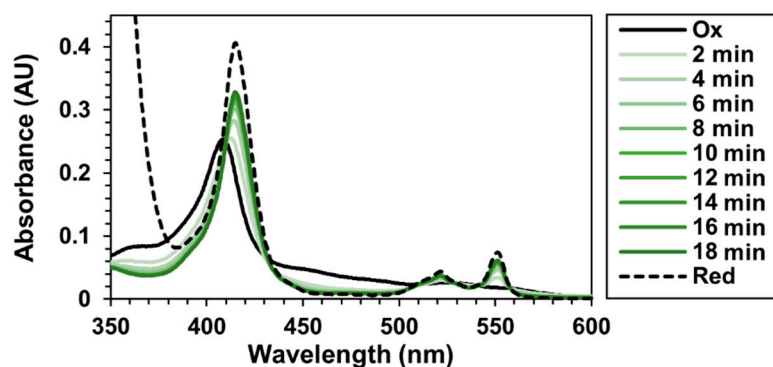
Figure S2. Dehydrogenase activity of each enzyme complex

The specific dehydrogenase activity of CYTc-SC/ST-Enzyme complexes or ST-Enzymes toward each substrate concentration was plotted as (A) CYTc-SC/ST-GDH, (B) CYTc-SC/ST-DAAOx, and (C) CYTc-SC/ST-LOx. Each dehydrogenase activity was determined using PMS/DCIP as the primary electron acceptor and redox dye. All experiments were performed in triplicate. Each error bars represents the standard deviation for triplicated experiment.

(A)



(B)



(C)

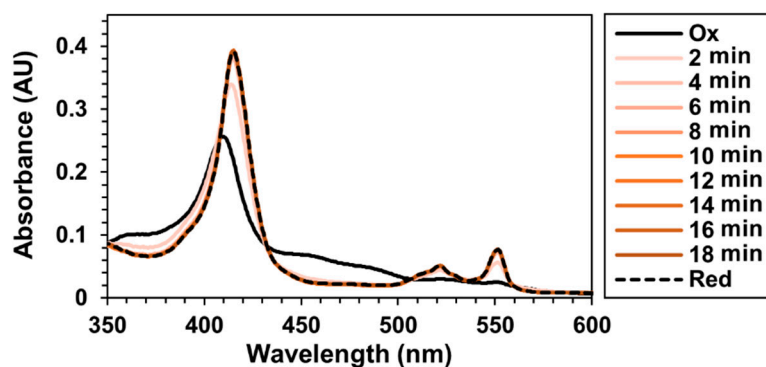


Figure S3. Time-dependent spectroscopic analysis of CYTc-SC/ST-Enzyme complexes

Time-dependent absorbance spectrum changes of CYTc-SC/ST-Enzyme complexes in the presence of substrate. Absorbance spectra between 350 and 600 nm were recorded, and at every minute after the addition of substrate solution to the (A) CYTc-SC/ST-GDH, (B) CYTc-SC/ST-DAAOx, and (C) CYTc-SC/ST-LOx are shown. Black-solid line (Ox) and black-dashed line (Red) indicate each CYTc-SC/ST Enzyme complex sample in oxidized state and reduced state treated with sodium dithionite. Recorded spectrum in each minute was superimposed to observe time-dependent spectral change after the addition of substrate.