Supplemental material

Designing a cross-linked redox network for a mediated enzyme-based electrode

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Materials

FAD-GDH from *Aspergillus terreus* and glucose oxidase from *Aspergillus* species were obtained from Ikeda Tohka Industries Co., Ltd. and Toyobo Japan. The concentration of FAD-GDH and GOx was 25.2 mg mL^{-1}. Poly (ethylene glycol) diglycidyl ether (PEGDGE, Mn 500), and azure A chloride was purchased from Sigma-Aldrich Co. Methylene blue hydrate and Toluidine blue (Basic blue 17) were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Thionine acetate, D(+)-glucose, potassium dihydrogen phosphate (KH_2PO_4), and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Fujifilm Wako Pure Chemical Corporation, Japan. A phosphate buffer (100 mM (M = mol dm^{-3}), pH 7.0) was prepared by mixing KH_2PO_4 and K_2HPO_4. A glucose solution was prepared using the phosphate buffer (100 mM, pH 7.0) and kept overnight at 4 °C to achieve mutarotation equilibrium.

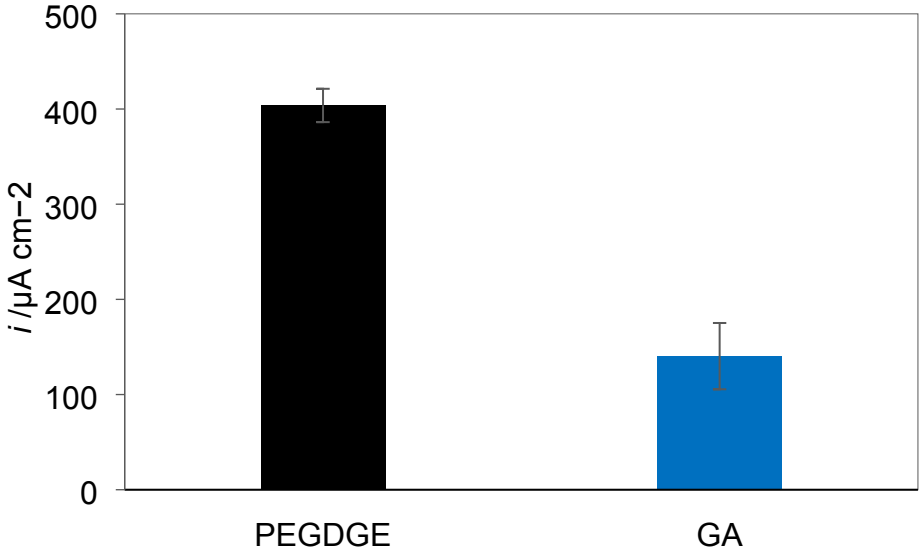


Figure S1 Amperometric glucose oxidation current at 500 s, at 0.2 V vs. Ag|AgCl (KCl sat.) on FAD-GDH/PEGDGE/TH electrode (black bar) and FAD-GDH/Glutaraldehyde(GA)/TH electrode (blue bar). 200 mM of glucose in 100 mM phosphate buffer (pH 7.0), wt% ratio of FAD-GDH:PEGDGE(or GA):TH = 45:25:30, loading 635 µg/cm^2, curing time 30 hours for FAD-GDH/PEGDGE/TH and 10 hours for FAD-GDH/GA/TH .

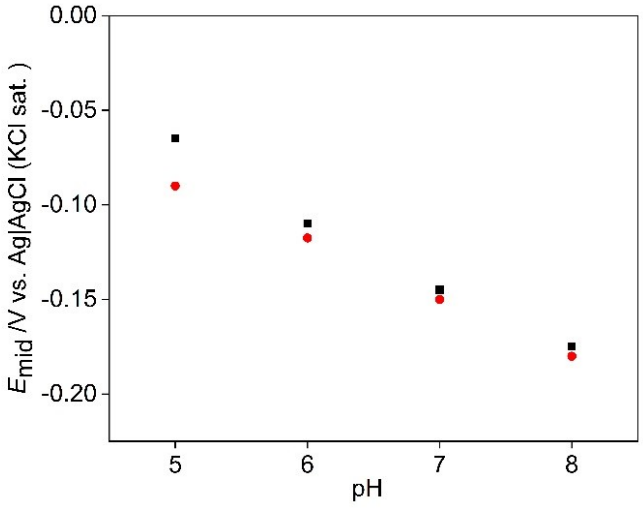


Figure S2 Midpoint potential vs. pH of cross-linked TH on FAD-GDH/PEGDGE/TH electrode (red circle) and free TH in solution (black square).

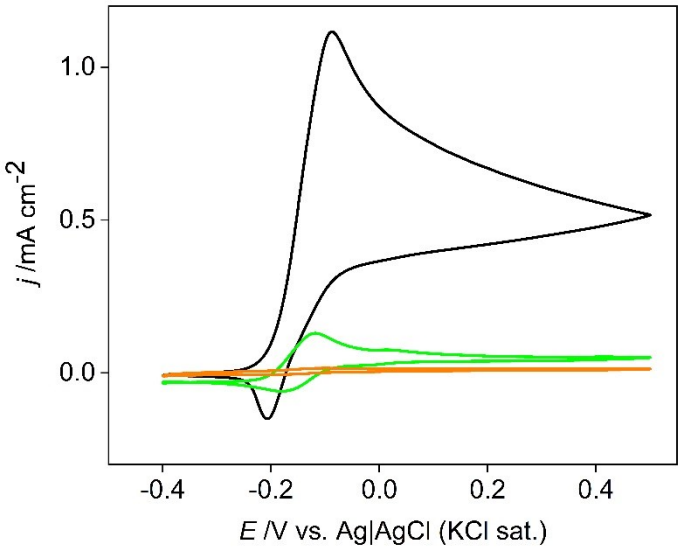


Figure S3 Cyclic voltammograms of FAD-GDH/PEGDGE/TH (black curve), GOx/PEGDGE/TH (green curve) and FAD-GDH/TH (orange curve) electrode. 200 mM glucose in 100 mM phosphate buffer, scan rate 5 mV s^{-1}, and 25 ºC.

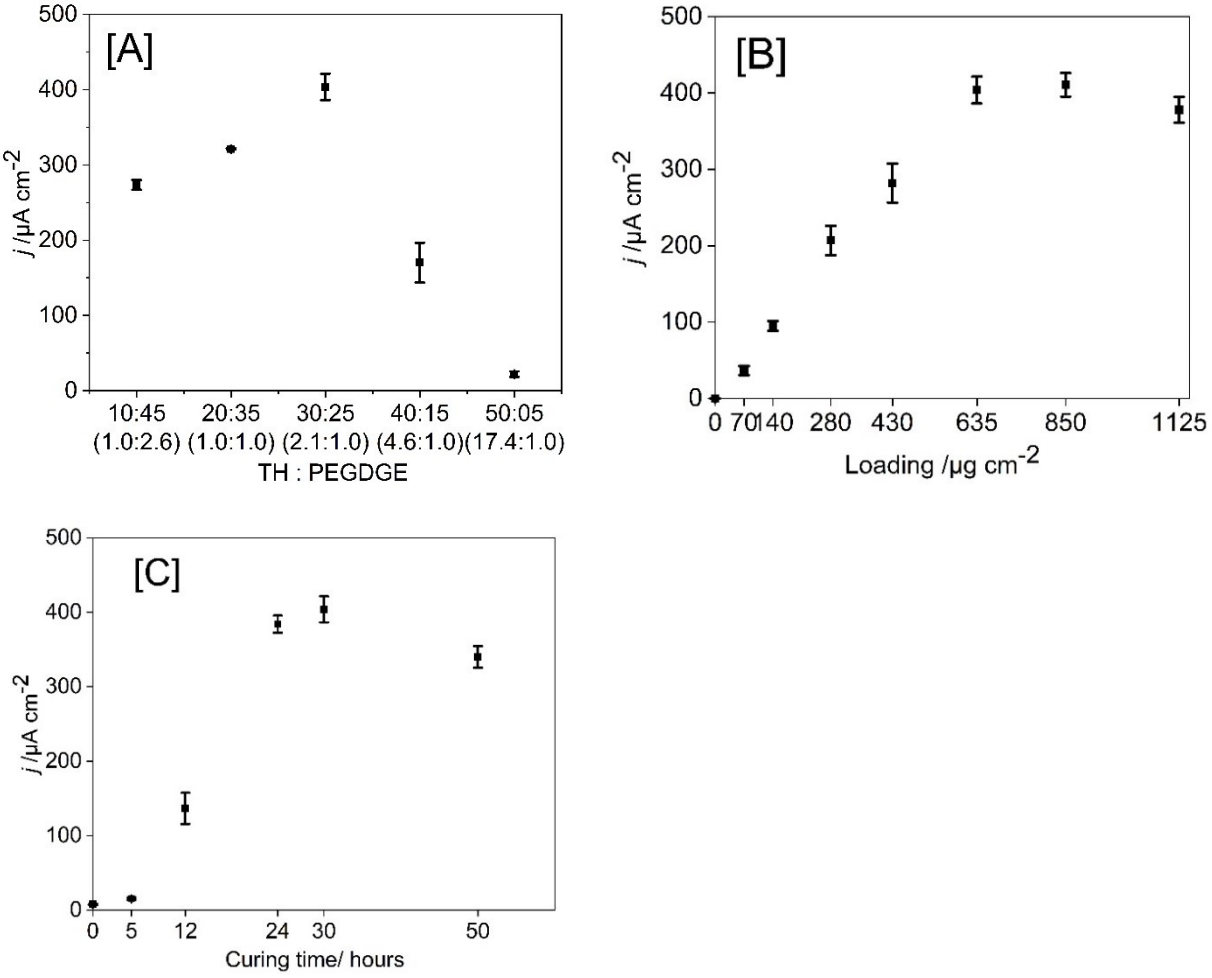


Figure S4 Dependence of [A] wt% ratio (molar ratio) of TH and PEGDGE, [B] loading amount, and [C] curing time on glucose oxidation current density of the FAD-GDH/PEGDGE/TH electrode at 0.2 V vs. Ag|AgCl (KCl sat.), 200 mM glucose in 100 mM phosphate buffer, pH 7.0 and 25 ºC.

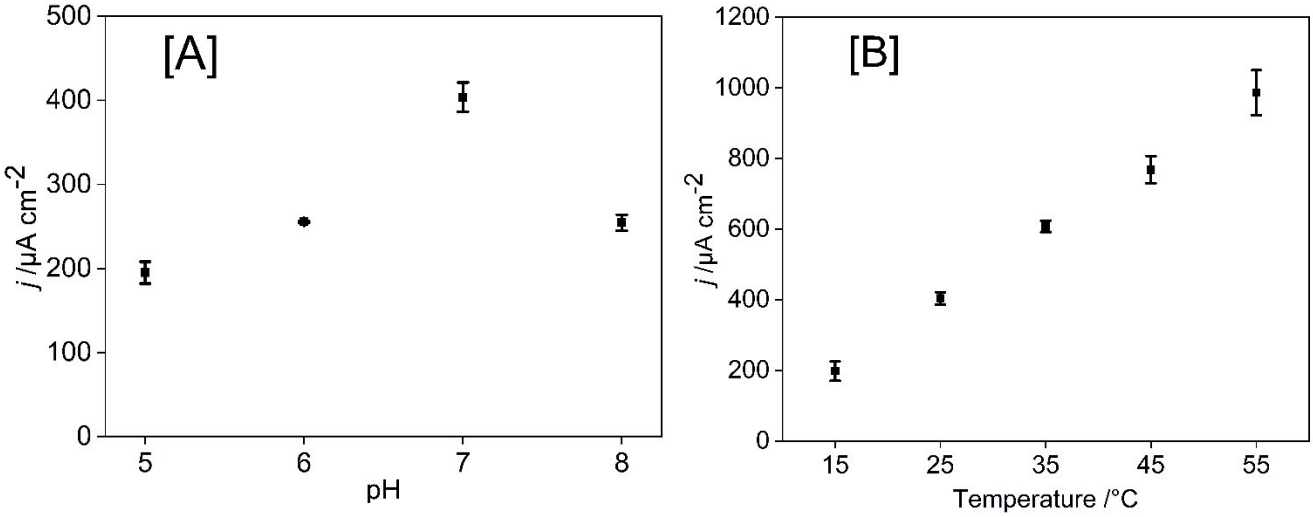


Figure S5 FAD-GDH/PEGDGE/TH-immobilized electrode performance at various (A) pHs (under 25 ºC) and (B) temperatures (pH 7.0). Amperometric glucose oxidation current density at 0.2 V vs. Ag|AgCl (KCl sat.). 200 mM glucose. Wt% ratio of FAD-GDH:PEGDGE:TH = 45:25:30, 30 hours curing time, loading 635 μg/cm^2.

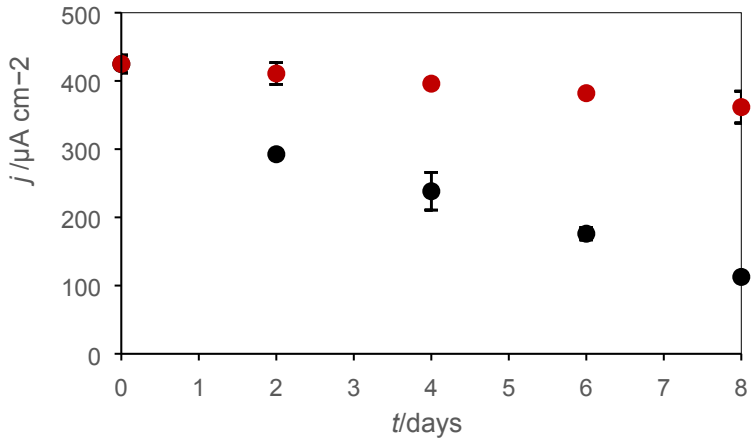
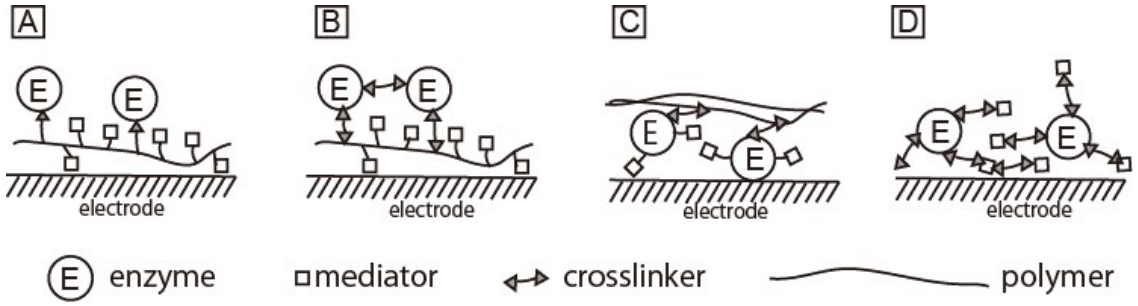


Figure S6 Storage stability test of FAD-GDH/PEGDGE/TH-modified electrodes stored at 4 (red) and at 25 °C (black). Amperometric glucose oxidation current at 500 s, at 0.2 V vs. Ag|AgCl (KCl sat.), 200 mM of glucose in 100 mM phosphate buffer (pH 7.0), wt% ratio of FAD-GDH:PEGDGE :TH = 45:25:30, loading 635 µg/cm^2, and 25°C.

Table S1: Covalent-immobilisation of organic redox mediator and FAD-GDH

Immobilization type*	Enzyme	Mediator	Electrode	Enzyme Loading (μg cm^{-2})	Operational Conditions: pH/glucose conc./mM	Current Density (mA cm^{-2})	Current Production Efficiency (μA $μg^{-1}$)	Reference
A	FAD-CDH	Toluidine blue	Graphite	307	7.8/120	0.056	0.18	1
A	FAD-GDH	Thionine	Buckypaper with MWCNT	353	7/50	3.7	10.48	2
B	FAD-GDH	2-Carboxyethyl-1,4-NQ	GC	1126	6.0/50	1.2	1.07	3
B	FAD-GDH	1,2-NQ-4-glycidyl	GC with MWCNT	367	6.5/22	0.8	2.18	4
C	FAD-GDH	1,2-NQ-4-glycidyl		1980	6.5/210	0.6	0.30	
D	FAD-GDH	Thionine	GC	282	7.0/200	0.4	1.42	This work

* immobilization type



References:

1 Poller et al., *Electrochim. Acta*, 2013, **110**, 152– 158.

2 Fritea et al., *J. Mater. Chem. A*, 2019, 7, 1447-1450.

3 Hou et al., *Electrochim. Acta*, 2016, **211**, 663–670.

4 Milton et al., *Chem. Sci.*, 2015, **6**, 4867-4875.