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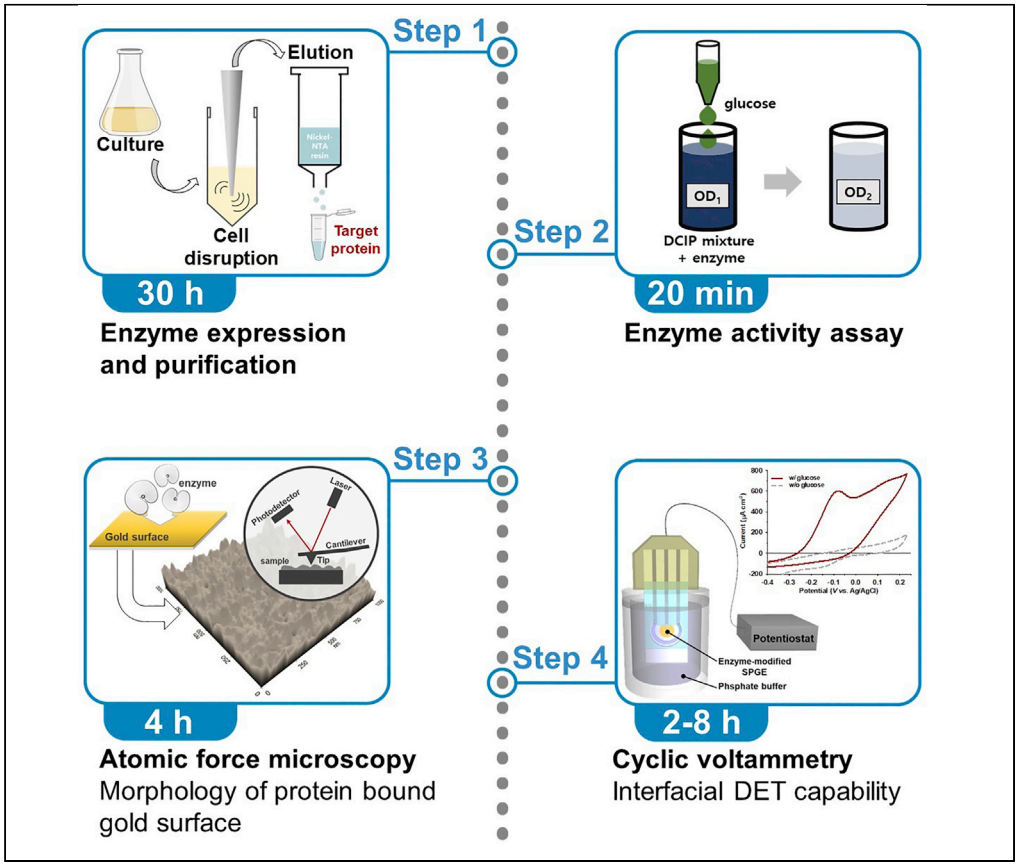
Protocol

Protocol for construction and characterization of direct electron transfer-based enzyme-electrode using gold binding peptide as molecular binder



Here, we present a protocol for constructing direct electron transfer (DET)-based enzyme-electrodes using gold-binding peptide (GBP). We describe fusion of four GBPs to flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase gamma-alpha complex (GDHγα), as model oxidoreductase, to generate four GDHγα variants. We then detail the measurements of catalytic and bioelectrochemical properties of these GDHγα variants on electrode together with surface morphology of GDHγα variants immobilized on gold surface. This protocol is useful for construction and validation of enzyme-based electrocatalytic system.

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Highlights

GBP fusion technique to regulate enzymatic surface-orientation

Simple genetic modification to tailor synthetic enzyme on electrode

Procedures to verify catalytic or gold-binding ability

Electrochemical assay to identify interfacial DET of enzyme-electrode

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Protocol for construction and characterization of direct electron transfer-based enzyme-electrode using gold binding peptide as molecular binder

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SUMMARY

Here, we present a protocol for constructing direct electron transfer (DET)-based enzyme-electrodes using gold-binding peptide (GBP). We describe fusion of four GBPs to flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenase gamma-alpha complex (GDHγα), as model oxidoreductase, to generate four GDHγα variants. We then detail the measurements of catalytic and bio-electrochemical properties of these GDHγα variants on electrode together with surface morphology of GDHγα variants immobilized on gold surface. This protocol is useful for construction and validation of enzyme-based electrocatalytic system.

For complete details on the use and execution of this protocol, please refer to Lee et al. (2021).

BEFORE YOU BEGIN

Plasmid construction for GBP-expressing enzymes

Timing: 3–4 days

Construct plasmid expressing wild-type and GBP-fused GDHγα; steps 1 and 2 describe plasmid modification for constructing expression vector for wild-type and GBP-fused GDHγα.

1. Acquire pET21a (+) plasmids with insert encoding FAD-dependent glucose dehydrogenase gamma-alpha complex (GDHγα) of *Burkholderia lata* (GenBank ID: α subunit of FAD-GDH: Bcep18194_B1293, γ subunit of FAD-GDH: Bcep18194_B1292) as template.

2. Insert the gene encoding gold-binding peptides that includes L_{GBP} (LKAHLPPSRLPS), M_{GBP} (MHGKTQATSGTIQS), T_{GBP} (TGTSVLIATPGV), or V_{GBP} (VSGSSPDS), at each C-terminus of catalytic α subunits using whole plasmid PCR mutagenesis.

   Note: The sequence and fusion site of GBPs could be varied depending on type of target proteins or research objectives.

   a. Prepare the PCR reaction mixture using Q5® Hot Start High-Fidelity 2× Master Mix (NEB) and set PCR cycles using corresponding primer sets as indicated in materials and equipment.
   b. Purify the fragment DNA from PCR reaction mixtures using PCR purification kit (GeneAll).

c. Ligate the DNA fragments using KLD enzyme mix (NEB) for 5 min at room temperature (25°C).
d. Transform DH5α Chemically Competent *E. coli* with 1 μL of the above ligation mixture by performing the heat-shock in a water bath at 42°C for 30 s followed by incubation on ice for 2 min and incubate at 37°C for 1 h after adding 400 μL SOC medium (NEB).
e. Transfer 50 μL of the cultures to Luria-Berani (LB) agar plates containing 100 μg/mL Ampicillin and incubate for 14–16 h at 37°C on rotary shaker of 180 rpm.
f. Pick 8 single colonies or more, for each construct and inoculate with 4 mL LB medium containing 100 μg/mL of Ampicillin and incubate for 16 h at 37°C with shaking at 180 rpm. Then, extract plasmids DNA using Exprep™ Plasmid SV (GeneAll).
g. Sequence the purified plasmids.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
DH5α Chemically Competent *E. coli*	(Enzynomics, Daejeon, Republic of Korea)	CP010
Rosetta (DE3) Chemically Competent *E. coli*	(Enzynomics, Daejeon, Republic of Korea)	CP1010
Recombinant DNA		
pET21a (+) plasmid encoding GDHγα of *Burkholderia lata*	(Lee et al., 2021)	(Available upon request)
Chemicals, peptides, and recombinant proteins		
Q5® Hot Start High-Fidelity 2× Master Mix	New England Biolabs (Ipswich, MA, USA)	M0494S
KLD enzyme mix	New England Biolabs (Ipswich, MA, USA)	M0554S
Expin™ PCR SV	GeneAll (Seoul, South Korea)	103-150
Tryptone	Sigma-Aldrich (St. Louis, MO)	T9410
Yeast extract	BD Difco, USA	212750
SOC medium	New England Biolabs (Ipswich, MA, USA)	B9020
2,6-dichloroindophenol	Sigma-Aldrich (St. Louis, MO)	D1878
Phenazine methosulfate	Sigma-Aldrich (St. Louis, MO)	P9625
TEV protease and 10× TEV Protease Reaction Buffer	New England Biolabs (Ipswich, MA, USA)	P8112S
Ni-NTA Agarose	QIAGEN (Hilden, Germany)	30210
Ampicillin sodium salt	Sigma-Aldrich (St. Louis, MO)	A9518-5G
IPTG	GoldBio	367-93-1
Gold-coated silicon wafer	Sigma-Aldrich (St. Louis, MO)	643262
Hydrogen Peroxide Solution 30%	Sigma-Aldrich (St. Louis, MO)	7722-84-1
Sulfuric acid	Sigma-Aldrich (St. Louis, MO)	258105-2.5L
Urea	Junsei, Japan	57-13-6
Imidazole	Sigma-Aldrich (St. Louis, MO)	56750
99.9% nitrogen gas	Sinil Gas, Korea	N/A
Oligonucleotides		
L_{GBP}_Forward: GCCGCCGTCTCGTCTGCCGTCTTGATTTTAGGGGCGCAAAGGAGAAG	This study	N/A
L_{GBP}_Reverse: AGGTGAGCTTTCAGGCCGCCACCACCGCCGACTT	This study	N/A
M_{GBP}_Forward CAACCTCGGGCACGATCCAAAGTTGACCATGGCCAACACTTGT	This study	N/A
M_{GBP}_Reverse: CTTGTGTCTTACCGTGCATCCCGCCCCCCCGCCGACTT	This study	N/A
T_{GBP}_Forward: ATTGCTACTCCGGGTGTGTAGCTGAAAGCTCA	This study	N/A
T_{GBP}_Reverse: CAGTACGGACGTACCAGTGCCGCCACCACCGCCGACTTCCTT	This study	N/A
V_{GBP}_Forward: TCCCCGGATAGCTAGCTGAAAG	This study	N/A
V_{GBP}_Reverse: GCTACCGCTTACGCCGCCACCACCGCCGACTTCCTT	This study	N/A

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
XEI software	Park Systems	https://www.parksystems.com/kr/
Nova 1.11	Metrohm Autolab	https://www.metrohm-autolab.com/Products/Echem/Software/Nova.html
Other		
0.45-μm syringe filter	ADVANTEC, Japan	25cs045as
Amicon Ultra-15 30K filter	Millipore, USA	UFC903024
96-well plate	SPL Life Sciences, Korea	30096
Screen-printed gold electrode	DropSens, Spain	250AT
125-μm Si/Al-coated cantilevers, PPP-NCHR 10 M	Park Systems, Korea	610-1051
AFM instrument, XE-100	Park Systems, Langen, Germany	N/A
Cuvette	ratiolab, Dreieich, Germany	2712120
potentiostat	Metrohm Autolab BV, Netherlands	N/A

MATERIALS AND EQUIPMENT

Set of primers used for genetic GBP linkage

Type of GBPs	Primer	Sequence (5′– 3′)
L_{GBP}	L_{GBP}_Forward	GCCGCCGTCTCGTCTGCCGTCTTGATTTTAGGGGCGCAAAGGAGAAG
	L_{GBP}_Reverse	AGGTGAGCTTTCAGGCCGCCACCACCGCCGACTT
M_{GBP}	M_{GBP}_Forward	CAACCTCGGGCACGATCCAAAGTTGACCATGGCCAACACTTGT
	M_{GBP}_Reverse	CTTGTGTCTTACCGTGCATCCCGCCCCCCCCGCCGACTT
T_{GBP}	T_{GBP}_Forward	ATTGCTACTCCGGGTGTGTAGCTGAAAGCTCA
	T_{GBP}_Reverse	CAGTACGGACGTACCAGTGCCGCCACCACCGCCGACTTCCTT
V_{GBP}	V_{GBP}_Forward	TCCCCGGATAGCTAGCTGAAAG
	V_{GBP}_Reverse	GCTACCGCTTACGCCGCCACCACCGCCGACTTCCTT

PCR reaction master mix

Reagent	Amount
Q5 High-Fidelity 2× Master Mix	12.5 μL
10 μM Forward Primer	1.25 μL
10 μM Reverse Primer	1.25 μL
Template DNA	1 μL (30 ng)
Nuclease-Free Water	9 μL
Total	**25 μL**

Store the reagents at 4°C, for 1 year.

PCR cycling conditions

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	30 s	1
Denaturation	98°C	10 s	27 cycles
Annealing	75°C	30 s	
Extension	72°C	4 min 30 s	
Final extension	72°C	2 min	1
Hold	4°C	forever	

KLD enzyme mix reaction	
Reagent	Amount
PCR Product	1 μL (100 ng)
KLD Reaction Buffer (2×)	5 μL
KLD Enzyme Mix (10×)	1 μL
Nuclease-Free Water	3 μL
Total	**10 μL**
Store the reagents at 4°C, for 1 year.	

LB medium	
Reagent	Amount
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
H_2O	~ 1 L
Total	**1 L**
Store the solution at room temperature after sterilization. Use it within 1 year.	

TB medium	
Reagent	Amount
Tryptone	20 g
Yeast extract	24 g
Glycerol	4 mL
1 M Phosphate buffer (0.17 M KH_2PO_4, 0.72 M K_2HPO_4)	100 mL
H_2O	~ 1 L
Total	**1 L**
Store the solution at room temperature after sterilization. Use it within 1 year.	

1 M phosphate buffer		
Reagent	Amount	Final concentration
K_2HPO_4 (174.18 g/mol)	125.41 g	0.72 M
KH_2PO_4 (136.09 g/mol)	23.14 g	0.17 M
H_2O	~ 1 L	N/A
Total	**1 L**	N/A
Store the solution at room temperature after sterilization. Use it within 1 year.		

Buffer A, pH 7.4		
Reagent	Amount	Final concentration
Na_2HPO_4 (141.96 g/mol)	2.14 g	15.08 mM
NaH2PO_4 (119.98 g/mol)	0.59 g	4.92 mM
NaCl (58.44 g/mol)	29.22 g	500 mM
H_2O	~ 1 L	N/A
Total	**1 L**	N/A
Store the solution at room temperature after sterilization. Use it within 1 year.		

Note: Adjust pH with concentrated HCl or NaOH when the pH could be different after making mixture.

Buffer A500, pH 7.4		
Reagent	Amount	Final concentration
Na_2HPO_4 (141.96 g/mol)	2.14 g	15.08 mM
NaH2PO_4 (119.98 g/mol)	0.59 g	4.92 mM
NaCl (58.44 g/mol)	29.22 g	500 mM
Imidazole (68.08 g/mol)	34.04	500 mM
H_2O	~ 1 L	N/A
Total	**1 L**	N/A
Store the solution at room temperature after sterilization. Use it within 1 year.		

Note: Adjust pH with concentrated HCl or NaOH when the pH could be different after making mixture.

Buffer An (n=imidazole content in mM), pH 7.4			
	Volume for buffer An		
Solution	n=50	n=70	n=350
Buffer A	90 mL	86 mL	30 mL
Buffer A500	10 mL	14 mL	70 mL
Total	**100 mL**	**100 mL**	**100 mL**
Store the prepared mixture at 4°C. Use it within 1 week.			

Phosphate buffered saline (PBS), pH 7.4		
Reagent	Amount	Final concentration
Na_2HPO_4 (141.96 g/mol)	1.42 g	0.01 M
KH_2PO_4 (136.09 g/mol)	0.245 g	0.0018 M
NaCl (58.44 g/mol)	8 g	0.137 M
KCl	0.2 g	0.0027 M
H_2O	~ 1 L	N/A
Total	**1 L**	N/A
Store the solution at room temperature after sterilization. Use it within 1 year.		

10 mM potassium phosphate buffer, pH 7.4		
Reagent	Amount	Final concentration
K_2HPO_4 (174.18 g/mol)	1.212 g	6.958 mM
KH_2PO_4 (136.09 g/mol)	0.414 g	3.042 mM
H_2O	~ 1 L	N/A
Total	**1 L**	N/A
Store the solution at room temperature after sterilization. Use it within 1 year.		

DCIP mixture	
Reagent	Amount
50 mM Dichlorophenolindophenol (DCIP)	2 μL
60 mM Phenazine methosulfate (PMS)	20 μL
100 mM potassium phosphate buffer (pH 7.4)	20 μL
H_2O	18 μL
Total	**60 μL**

Note: The DCIP and PMS stocks are dissolved in H_2O, and they are highly sensitive to light.

Store the reagents at 4°C. Use it within 1 h.

STEP-BY-STEP METHOD DETAILS

Starter culture

Timing: 24 h

This section describes transforming the the expression hosts and prepare start cultures.

1. Transform expression plasmid into *Escherichia coli* BL21-Rosetta (DE3) with heat shock method.
2. Plate on LB agar plates containing 100 μg/mL of ampicillin and incubate 14–16 h Tat 37°C.
3. Pick a single colony from LB-ampicillin agar plate that has been incubated 14–16 h and inoculate a starter culture of 5 mL LB-ampicillin medium and incubate at 37°C for 8 h.
4. Inoculate Terrific Broth (TB) medium containing 100 μg/mL ampicillin with the starter culture at a 1:50 dilution.

Enzyme expression and purification

Timing: 30 h

This section describes the cultivation of expression hosts and performing the extraction and purification of target proteins.

5. Culture the *Escherichia coli* BL21-Rosetta (DE3) harboring target gene containing plasmid in 500 mL TB medium containing 100 μg/mL ampicillin in 1 L flask at 37°C. Let the cells grow for 2–3 h until OD_{600} reaches to 0.6.
6. Add IPTG to a final concentration of 0.2 mM and further incubate the cell culture at 16°C for 18 h.
7. Harvest the cells by centrifugation at 3,500 g and 4°C for 20 min.
8. Mix harvested cells with adding 15 mL Buffer A50 (pH 7.4).
9. Disrupt the resuspended cells by sonication (VCX 500, Sonics & Materials) using 150 cycles of 2 s ON and 4 s OFF at 40% amplitude (500 Watts and 20 kHz frequency) to assure complete cell lysis.
10. Centrifuge the lysates at 13,000 g and 4°C for 30 min.
11. Collect the supernatant and filter it through 0.45-μm syringe filter (ADVANTEC) to remove remaining cell debris and inclusion bodies.

Note: This step is essential to increase protein purity.

12. Equilibrate the filtered soluble fractions (~15 mL) with Ni-NTA agarose resin (4 mL) with end-to-end rotation (40 rpm).
    a. The nickel affinity agarose resin is priorly equilibrated with Buffer A50 (pH 7.4).
13. Wash the column with Buffer A50 (pH 7.4) of 10 column volumes.
14. Pass the two column volumes of Buffer A70, Buffer A350, and Buffer A500 (pH 7.4) through nickel column, sequentially.
15. Exchange the protein-containing buffer with PBS (pH 7.4) using a Amicon Ultra-15 30K filter (Millipore) centrifugal filter.
    a. Select a molecular weight cut-off (MWCO) close to one-third the molecular weight of the target protein size.
16. Remove the his-tag by cleaving the TEV protease recognition site with the treatment of TEV protease. (NEB).
    a. Estimate the protein concentration by Bradford assay (Bradford, 1976).

b. Adjust the protein concentration to be 0.33 μg/μL in 45 μL total volume.
c. Add 5 μL of TEV Protease Reaction Buffer (10×) (NEB).
d. Add 1 μL of TEV Protease (10 U) (NEB).
e. Incubate at 30°C for 2 h.
f. Conform the cleavage of his-tag on 10% SDS-PAGE gel.
g. To remove or minimize cleaved his-tag and TEV protease (~28 kDa) contents, filter the protein mixture through Amicon Ultra-15 30K filter (Millipore).

Note: The retention of protein structure after cleavage of his-tag could be confirmed with comparison of catalytic activities of protein before/after TEV protease treatment.

Note: The obtained proteins is recommended to be stored at 4°C and utilized within a week to avoid possible protein structural change which can lead to variation in biochemical and electrochemical results.

Note: The obtained protein concentration could be largely varied depending on protein types.

Enzyme activity assay

Timing: 20 min

This section describes the measurements of the enzyme activity for glucose oxidation by DCIP assay.

17. Prepare 60 μL DCIP mixture in 96-well plate (SPL) at 25°C as indicated in materials and equipment section (Inose et al., 2003).
18. Add 20 μL purified enzyme (0.05 mg/mL) and incubate at 25°C for 3 min without stirring.
19. Add 20 μL of 2 M glucose to initiate glucose oxidation, or 20 μL of deionized water to perform blank at OD_{600}.
20. After 1 min, stop the reaction by adding 100 μL of 6 M Urea and measure adsorption at OD_{600}.
21. Calculate enzyme activity based on the Beer-Lambert Law (Swinehart, 1962).

Note: Enzyme activity (U) = $(A_{glucose} - A_{blank}) / [\epsilon \times \text{path length (cm)} \times \text{incubation time (min)}]$, where A is adsorption at OD_{600} and ϵ is molar absorption coefficient of DCIP (16.3 mM/cm at pH 7.0) (Inose et al., 2003).

Note: In the case of GDH$\gamma\alpha$, approximately 12 U/mg of specific activity was obtained.

Atomic force microscopy (AFM)

Timing: 4 h

This section describes the binding morphology of surface-immobilized gold binding peptide-fused enzyme could be examined *via* observing biofilm morphology on a gold substrate.

22. To remove the dust or organic contaminants on gold coated silicon wafer (e.g., 5 mm × 5 mm), prepare 100 mL of 1:3 (v/v) of 30% H_2O_2/H_2SO_4 (piranha solution) in 500 mL beaker.
23. Incubate the gold substrate in piranha solution for 10 min, at 25°C.
24. Rinse the gold substrate with deionized water and dry it with pressurized nitrogen gas.
25. Add 500 μL of 0.1 μM target enzyme dissolved in PBS (pH 7.4) into 1.5 mL microtube.
26. Insert the surface-cleaned gold substrate in the enzyme solution containing microtube.
27. Incubate the gold substrate in the enzyme solution-containing microtube, on a rotary shaker of 200 rpm, for 2 h (25°C).

28. Remove weakly bound proteins on the gold surface by sonication (30 Hz) in enzyme-free PBS for 30 s.
29. To remove salts on the surface, wash off the surface with stream of deionized water for 30 s.
30. Dry the protein-bound gold surface with 99.9% nitrogen gas (Sinil Gas, Korea).
31. Measure the surface morphology of protein-bound gold surface using AFM in non-contact mode.
    a. Fix the protein-bound gold substrate on sample stage of AFM instrument (XE-100; Park Systems, Langen, Germany).
    b. Install the 125-μm Si/Al-coated cantilevers (PPP-NCHR 10 M; Park Systems) with 200–400-kHz resonance frequency and 42 N/m spring constant.
    c. Align the cantilever on the center of sample surface.
    d. Image the surface morphology in 1 μm × 1 μm scan fields, at 1 Hz scan rate.
    e. After Acquiring raw AFM image, process the image flattening to remove the undesired artifacts such as slope and curvature, with XEI software (Park Systems).

Note: In this study, the 1st order fitting curve was utilized during image flattening.

⚠ CRITICAL: The enzyme-bound specimen must be fully dried since remained water molecules could cause vertical drift in AFM imaging.

Cyclic voltammetry (CV)

Timing: 2–8 h

This section describes cyclic voltammetry (CV) that is used to measure interfacial electron transfer rate between the enzymatic cofactor and electrode surface. By analyzing the onset potential, the presence of a direct electron transfer signal is identified, and the peak current in the cyclic voltammogram can provide evidence of interfacial direct electron transfer efficiency (Lee et al., 2020).

Note: In the case of FAD-dependent enzyme, it is known that -0.46 V (vs. Ag/AgCl) is ideal onset potential.

32. Prepare screen-printed gold electrode (SPGE) (DropSens) that consists of Au working electrode (4 mm in diameter), Pt auxiliary electrode, and Ag pseudo-reference electrode (Lee et al., 2020).

Note: Before enzyme immobilization, the SPGE should be verified not to have interfering electrochemical signal by conducting CV with bare SPGE.

33. Add 800 μL of 0.5 μM target enzyme dissolved in PBS buffer (pH 7.4) into a disposable cuvette.
34. Immerse the SPGE into the enzyme solution containing cuvette and incubate it on the rotary shaker of 200 rpm, for 2 h at 25°C.
35. Wash off the excess proteins on the gold working electrode surfaces with a stream of PBS, for 30 s.
36. Connect the enzyme-modified SPGE to potentiostat (Autolab, Metrohm) *via* a connector.
37. Immerse the enzyme-modified SPGE in the reactor (working volume: 10 mL) with 8 mL of 10 mM potassium phosphate buffer (pH 7.4).

Note: To detect glucose-led oxidative current, add 2 M glucose stock dissolved in 10 mM potassium-based phosphate buffer (pH 7.4) for glucose content to be 0–100 mM.

38. Set the scan rate to be 100 mV/s and perform the CV in the potential range of -500 mV–400 mV, using Nova 1.11 program.

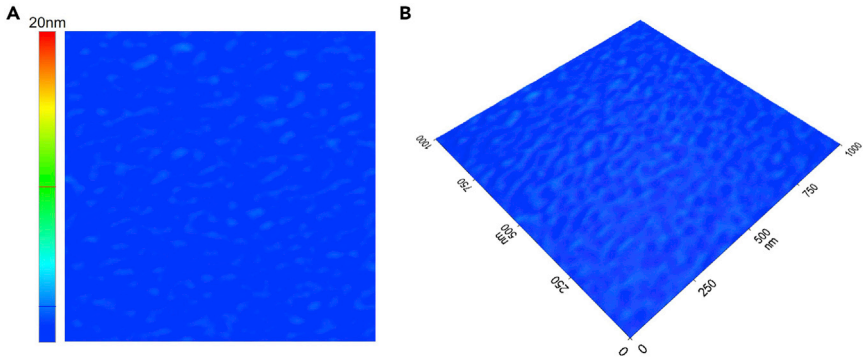

Figure 1. AFM images for bare Au surface used for enzyme binding studies
(A and B) (A) Image in 2-D topography; (B) image in 3-D topography; scanned area is 1 μm × 1 μm.

Note: The number of cycles was determined until the signal is stabilized. In this case, the signal was shown to be stabilized after individual 20–30 cycles. Also, when the scan direction is changed to reverse (compared with original direction), we did not observe large difference.

EXPECTED OUTCOMES

Surface morphology

This protocol provides methods to validate the relative binding affinity of fusion proteins toward gold material compared with the wild-type protein. The fusion proteins with GBP binder will be densely packed on the gold surfaces as GBP tag is specifically attached the gold material. The data below is the case of native and GBP-fused GDHγα which has dimension of 52.2 Å × 91.4 Å × 61.9 Å (Figures 1 and 2). As fusion GDHγα is densely crowded on the surface, the average height in the cross-sectional analysis was shown to be similar with molecular size. In contrast, the wild-type protein would be sparsely populated on the same surface, due to the non-specificity of wild-type protein toward Au surface. Further, the partial protein agglomeration in wild-type protein-bound surfaces could be observed (Figure 2).

CV analysis

From CV analysis of enzyme-modified SPGE, the interfacial DET occurrence and DET-based oxidative current could be evaluated. In the case of wild-type enzyme on SPGE, the weak signal of oxidative current will appear since unfavorable ET conditions were created by the uncontrolled orientation of the native enzyme on the electrode surface due to non-specific interaction of the enzyme with the inorganic surface. In the fusion protein modified SPGE, the controlled orientation of fusion protein on inorganic surface could present onset potential close to the standard potential of cofactor (electron donor) and clear oxidation wave, given that the binding conformation of the fusion protein on the surface is optimized for cofactor-surface interface is in close-proximity (Figure 3).

Note: Depending on fusion site of GBP or fused GBP type, the interfacial DET capability between redox cofactor and electrode surface could be highly varied. According to Figure 3, GDHγα-T_{GBP}/SPGE shows sluggish oxidative current while GDHγα-M_{GBP}/SPGE exhibit highly efficient DET-based electrocatalytic current.

LIMITATIONS

For in-depth analysis of electrochemical phenomenon, the peptide-surface interactions and the binding conformations of fusion proteins on the gold surface are needed to be computationally simulated. If the molecular dynamics (MD) simulation could be performed, the more precise information about structural and electrochemical conditions between enzymatic cofactor and electrode can be achieved.

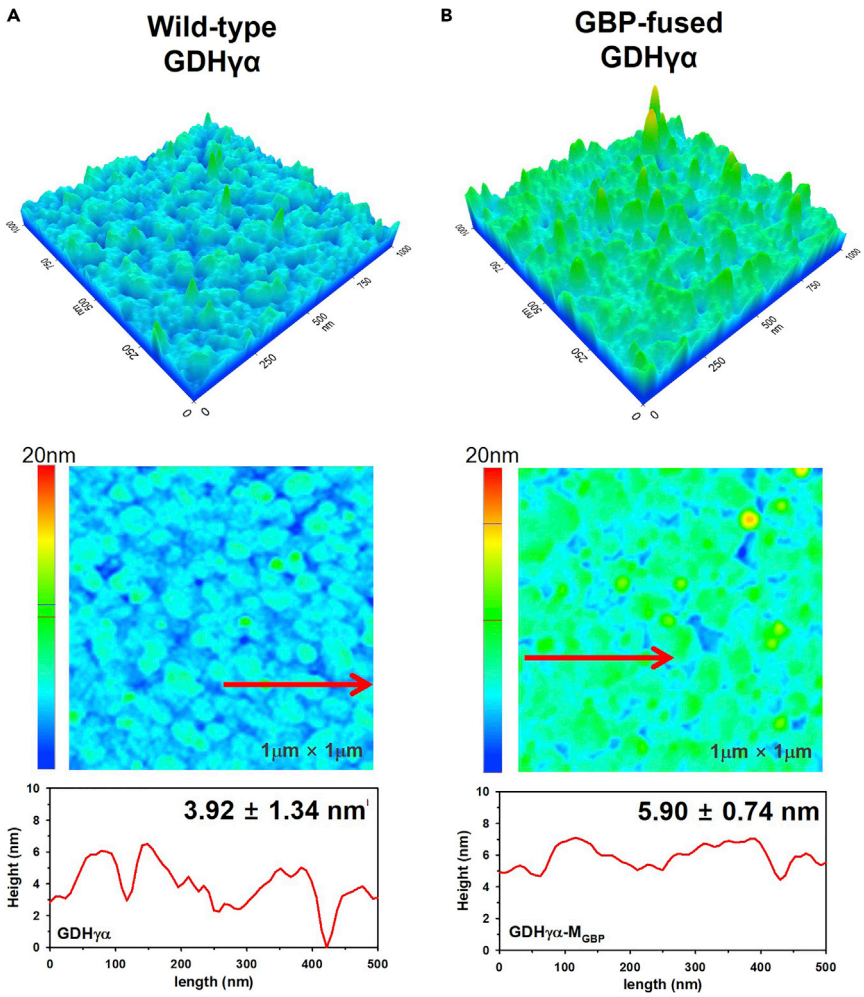

Figure 2. AFM images of enzyme-immobilized gold surface

(A and B) (A) wild-type GDHγα, and (B) GBP-fused GDHγα immobilized on gold substrates. 5 mm × 5 mm of gold substrate were immersed in 0.1 μM of each protein for 2 h, for formation of enzymatic biofilm; scanned area is 1 μm × 1 μm: (upper panel) 3-D topography; (middle panel) 2-D topography; (lower panel) cross-sectional profile along the arrow in middle panel.

TROUBLESHOOTING

Problem 1

The solubility is lowered after modifying plasmid constructs (step 8).

Potential solution

Add additives (e.g., 0.1%–0.5% Triton-X 100, 0.1%–0.5% Tween20, etc.) in the buffer A.

> *Note:* Avoid using β-mercaptoethanol as it breaks disulfide bond which is required for GDHα and GDHγ subunits to be combined.

Add glycerol in the buffer A to stabilize the proteins.

Problem 2

The cleavage efficiency of his-tag from enzyme is highly low (step 16).

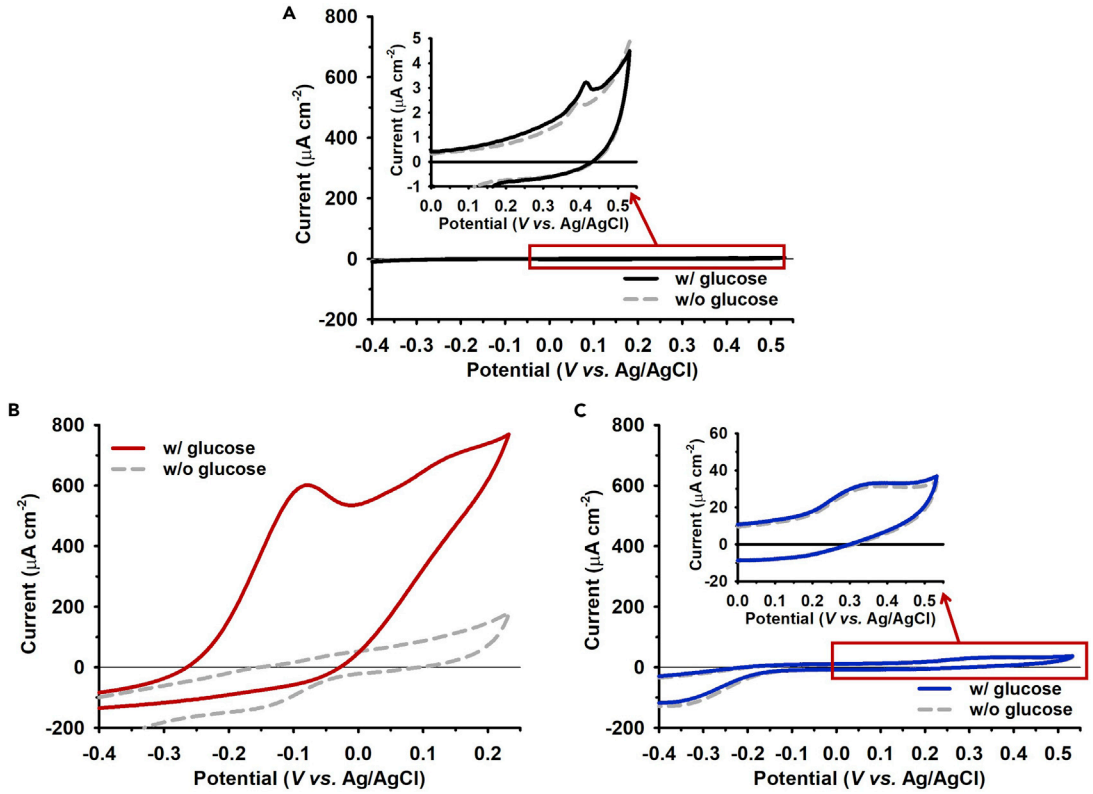

Figure 3. CV profiles of enzyme-modified SPGEs

(A–C) CV profiles at (A) wild-type GDHγα/SPGE, (B) M_{GBP}-fused GDHγα/SPGE, and (C) T_{GBP}-fused GDHγα/SPGE in the absence and presence of 100 mM glucose in PBS buffer (pH 7.4) (scan rate: 100 mV/s); inserted graph in (A): CV graphs of the selected region in the figures.

Potential solution

Extend the incubation time to 3 h for 30°C.

Use freshly purified enzyme.

Problem 3

The fusion protein is not densely packed on target inorganic surface during AFM measurement (step 28).

Potential solution

Extend the enzyme immobilization time up to 12 h.

Find optimum pH (6–9) of binding buffer possessing target enzyme by confirming adsorption of wild-type enzymes on Au surface.

Problem 4

Non-specific binding of wild-type protein is severely observed (step 29).

Potential solution

Add the non-ionic detergent (e.g., 0.1%–0.5% Tween-20, 0.1%–0.5% Triton X-100, etc.) in the protein solution.

Adjust to optimum pH (6–9) or NaCl content (0–500 mM) of PBS (pH7.4).

Problem 5

During AFM measurement, the surface topography is severely drifted (step 32).

Potential solution

Confirm cantilever contamination.

Lower the scan rate to ~0.3 Hz.

Dry the sample in the desiccator over 12 h.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, In Seop Chang (ischang@gist.ac.kr).

Materials availability

This study did not generate new materials.

Data and code availability

This study did not generate any unique code or datasets. All data supporting the finding of this study are available within the paper. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request. All software's used in this study are commercially available.

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AUTHOR CONTRIBUTIONS

H.L.: Conceptualization, Methodology, Investigation, Writing-Original Draft, Writing-Review & Editing, Visualization; E.M.L.: Investigation; S.S.R.: Writing-Review & Editing; I.S.C.: Writing-Review & Editing, Supervision, Project Administration, Funding Acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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