

## Supporting Information

**Title: Electropolymerization conditions of methylene green used as an electron transfer mediator for coenzyme-dependent oxidoreductases**

Tomoe NAKAGAWA,<sup>a,b,†,§</sup> Tomoko GESSEI,<sup>a</sup> Akira MONKAWA,<sup>a</sup> and Nobuhumi NAKAMURA<sup>b,\*††,§§</sup>

<sup>a</sup> *Tokyo Metropolitan Industrial Technology Research Institute, 2-4-10 Aomi, Koto-ku, Tokyo 135-0064, Japan*

<sup>b</sup> *Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan*

\* *Corresponding author:* nobu1@cc.tuat.ac.jp

† A part of this paper has been presented in the 91th ECSJ Meeting in 2024  
(Presentation S5\_3\_10)

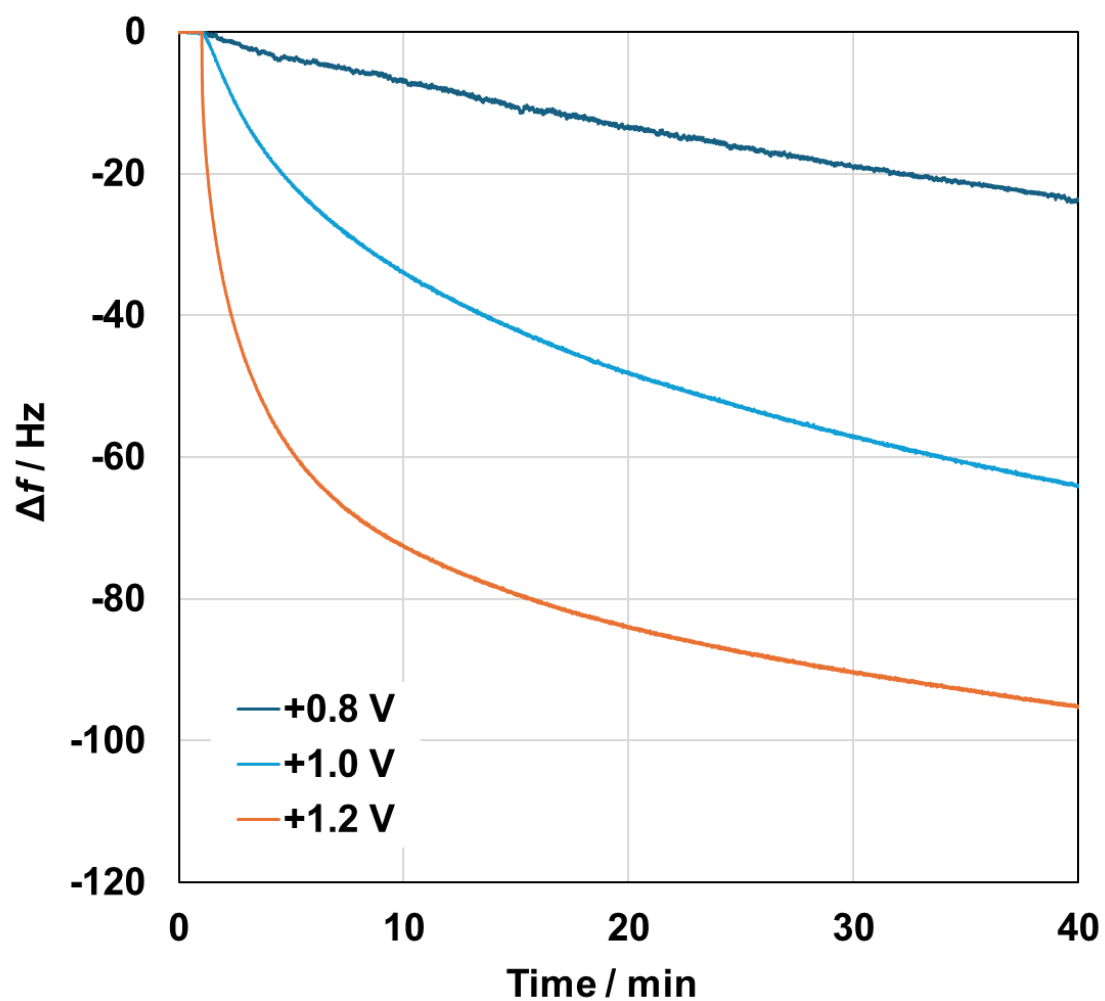
†† The content of this paper has been published as a preprint in SSRN. URL:  
<http://dx.doi.org/10.2139/ssrn.4996366>

§ ECSJ Active Member

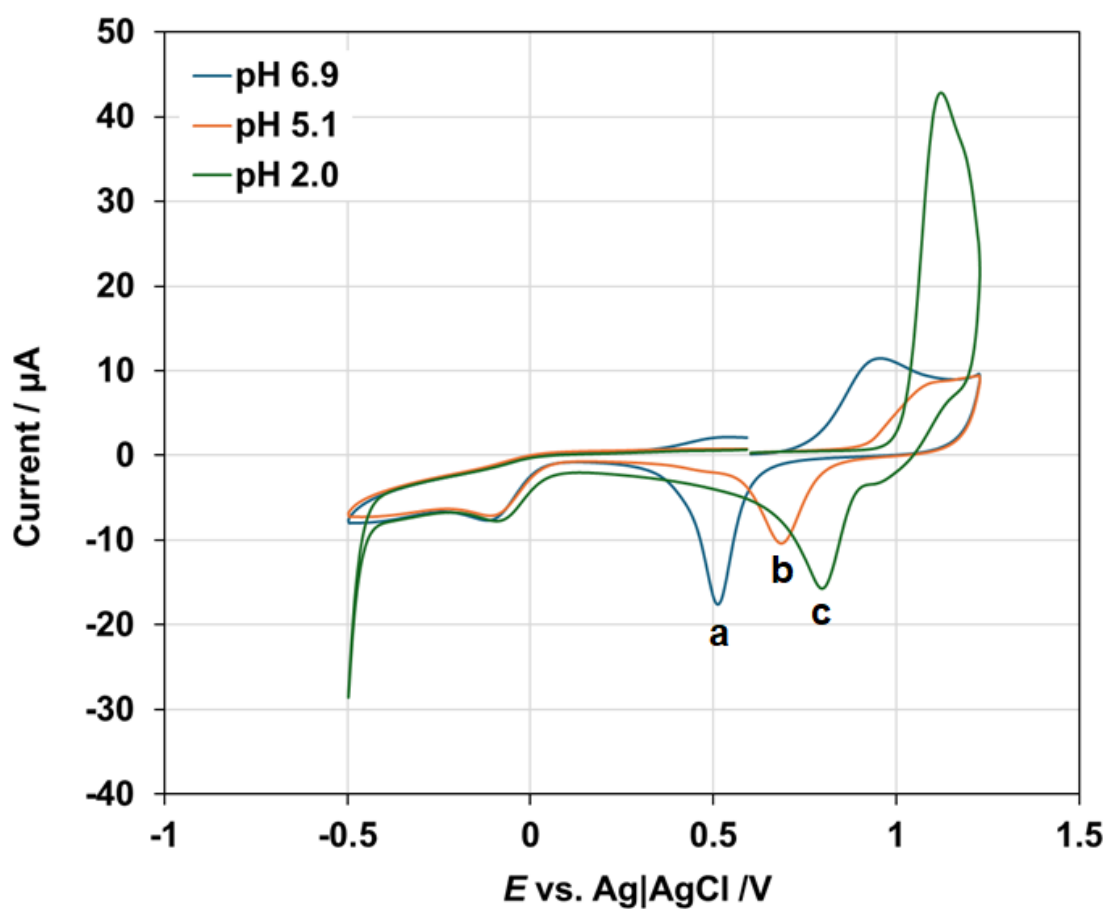
§ § ECSJ Fellow

ORCID:

Nobuhumi Nakamura: 0000-0002-5373-2972

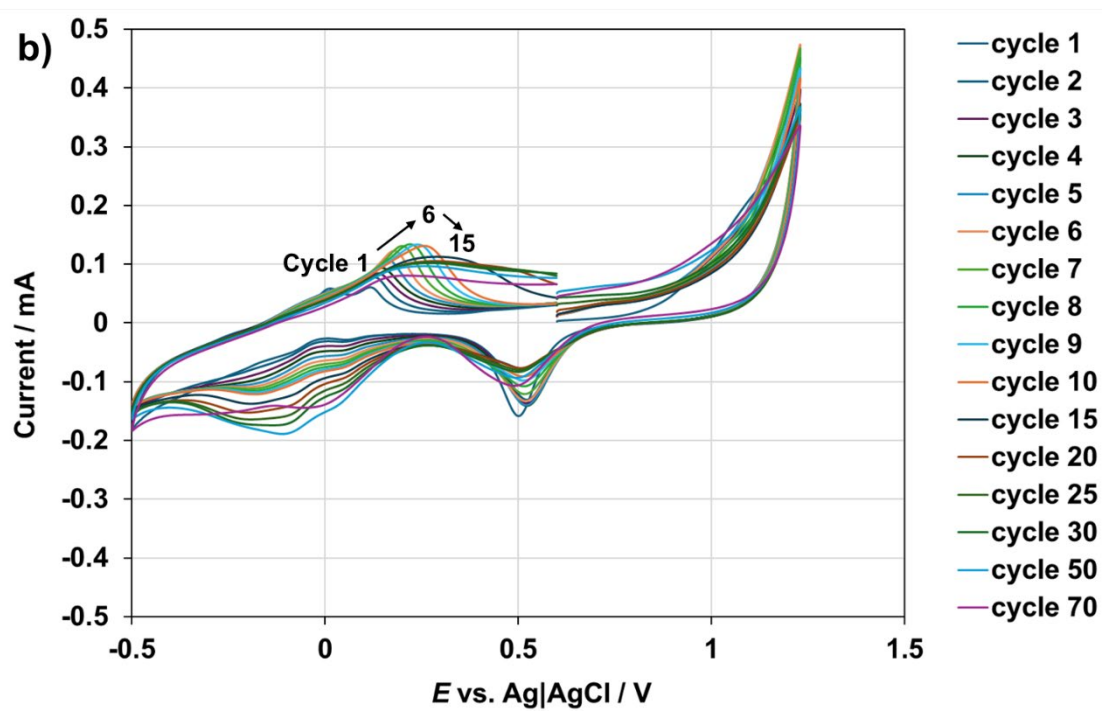
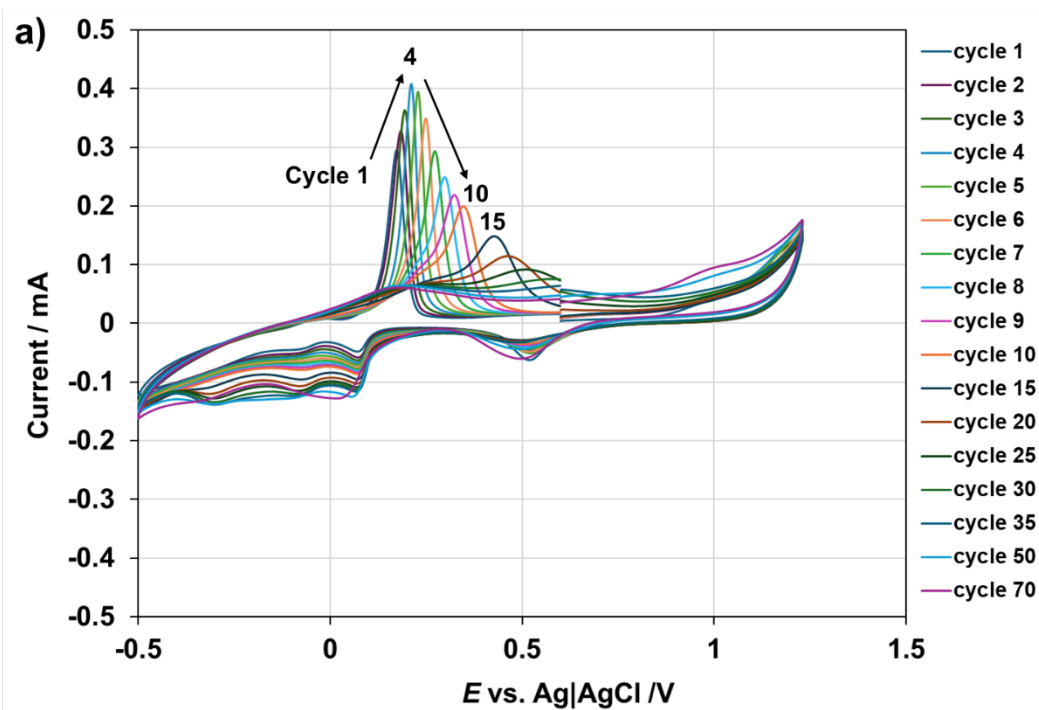


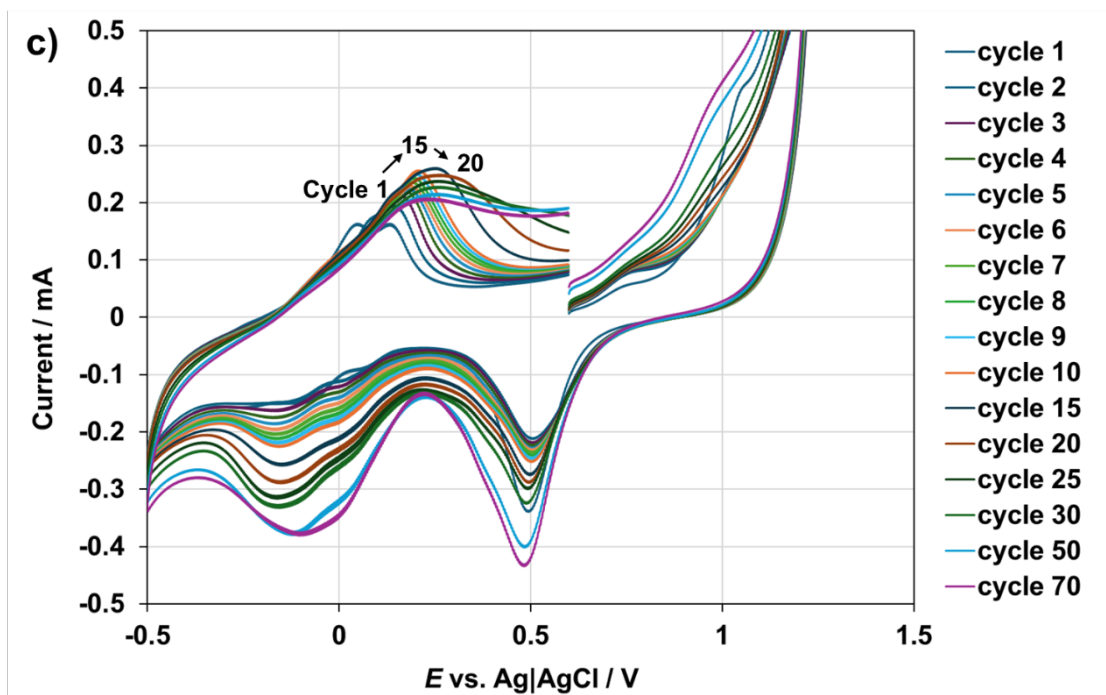
**Figure S1.** Frequency shift ( $\Delta f$ ) recorded during the electropolymerization of MG at different potentials. The electropolymerization was carried out in 0.5 mol dm<sup>-3</sup> phosphate buffer solution containing 0.5 mmol dm<sup>-3</sup> MG on AuQCM sensors.



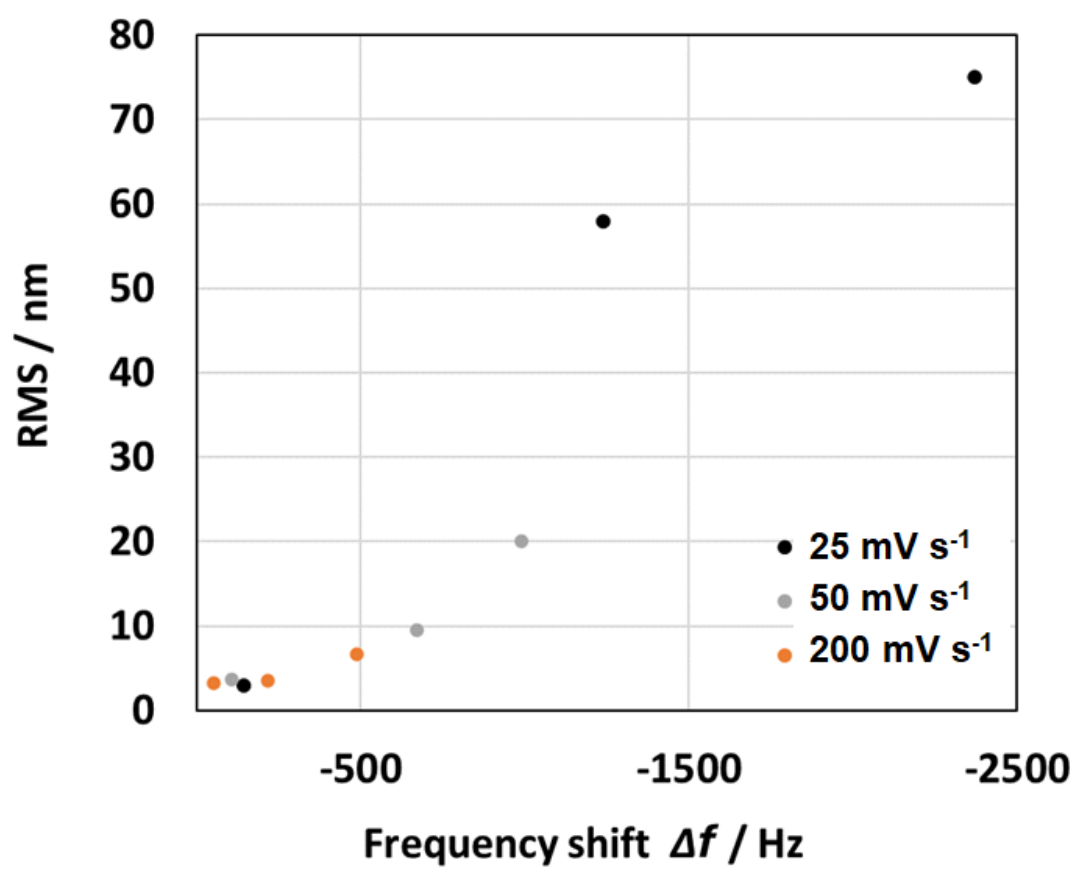
**Figure S2.** Cyclic voltammograms of nonmodified Au electrodes in 0.5 M phosphate buffer solutions prepared at different pH values: (a) 6.9, (b) 5.1, and (c) 2.0, using  $\text{H}_2\text{SO}_4$ .

The scan rate was  $50 \text{ mV s}^{-1}$ . As the pH decreased, the reduction peak shifted to a lower potential, indicating that the peak was derived from the Au oxide film.

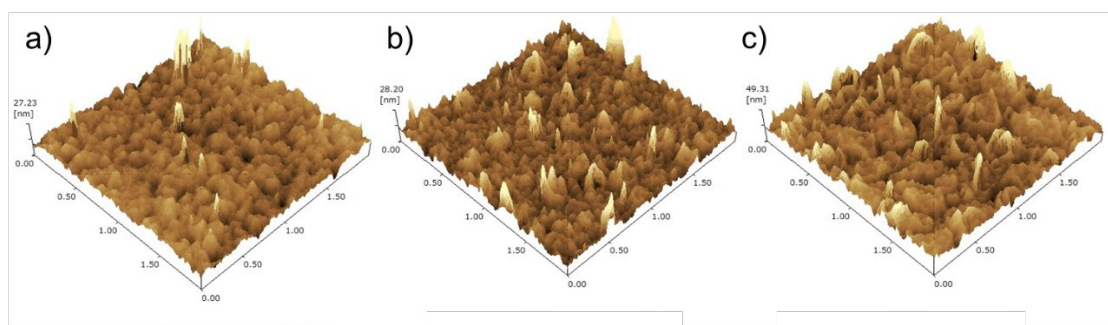




**Figure S3.** Cyclic voltammograms of the electropolymerization of MG on the AuQCM sensor in  $0.5 \text{ mol dm}^{-3}$  phosphate buffer solution containing  $0.5 \text{ mmol dm}^{-3}$  MG. The scan rate was a)  $25 \text{ mV s}^{-1}$ , b)  $50 \text{ mV s}^{-1}$ , c)  $200 \text{ mV s}^{-1}$ .

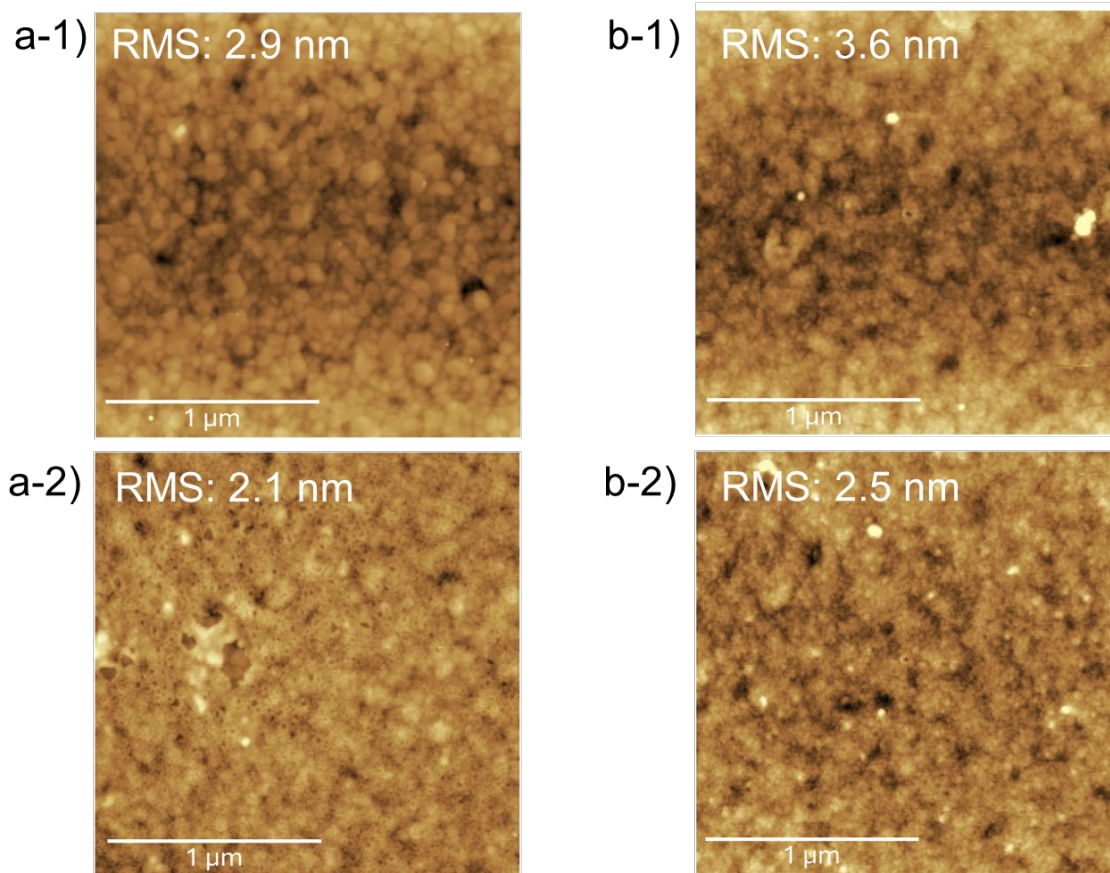


**Figure S4.** Correlation between the frequency shift ( $\Delta f$ ) during electropolymerization and the root-mean-square roughness (RMS) of the formed polyMG film.



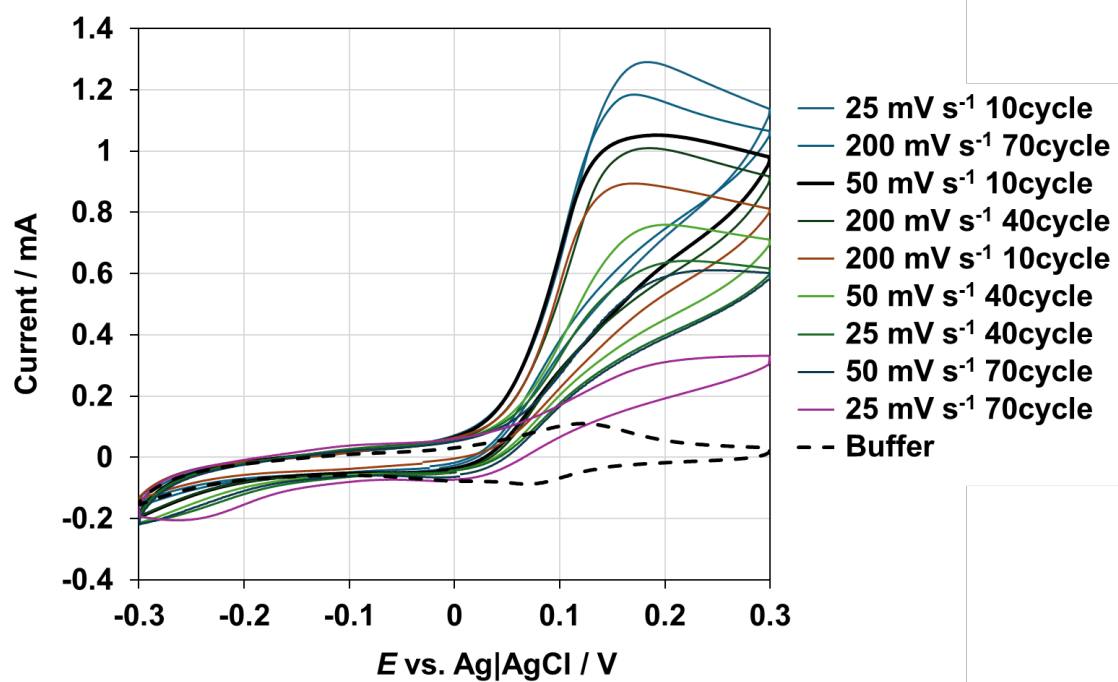
**Figure S5.** 3D AFM images of polyMG films electropolymerized at  $200 \text{ mV s}^{-1}$  for a) 10 cycles, b) 40 cycles and c) 70 cycles on the AuQCM sensor.



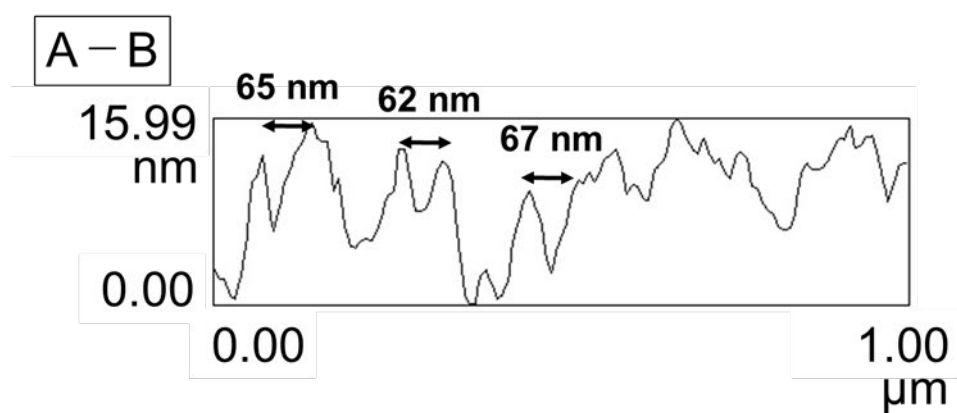
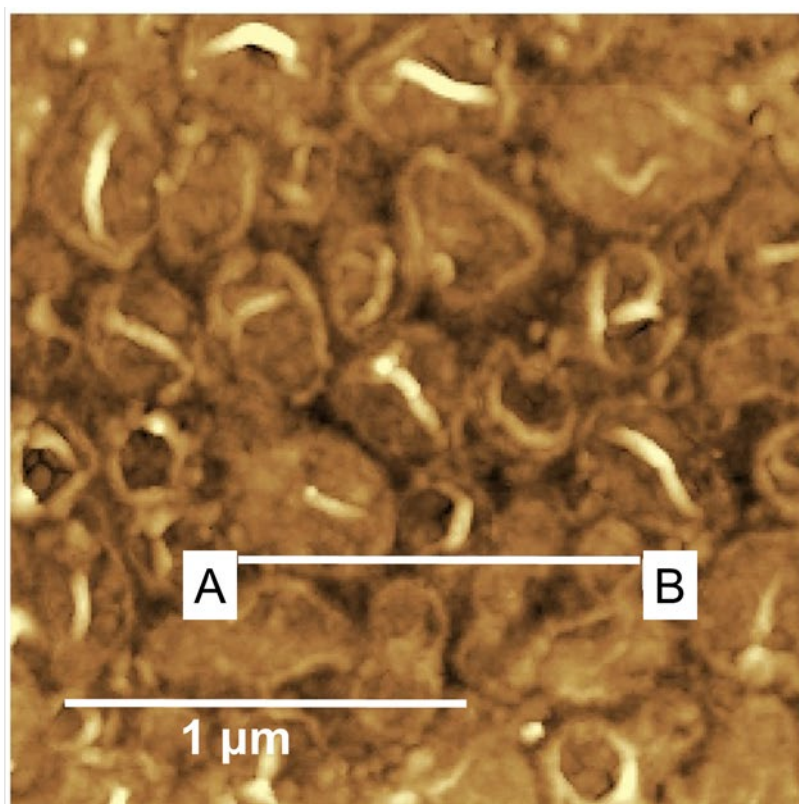


**Figure S6.** AFM images before and after enzyme adsorption onto the AuQCM sensor.

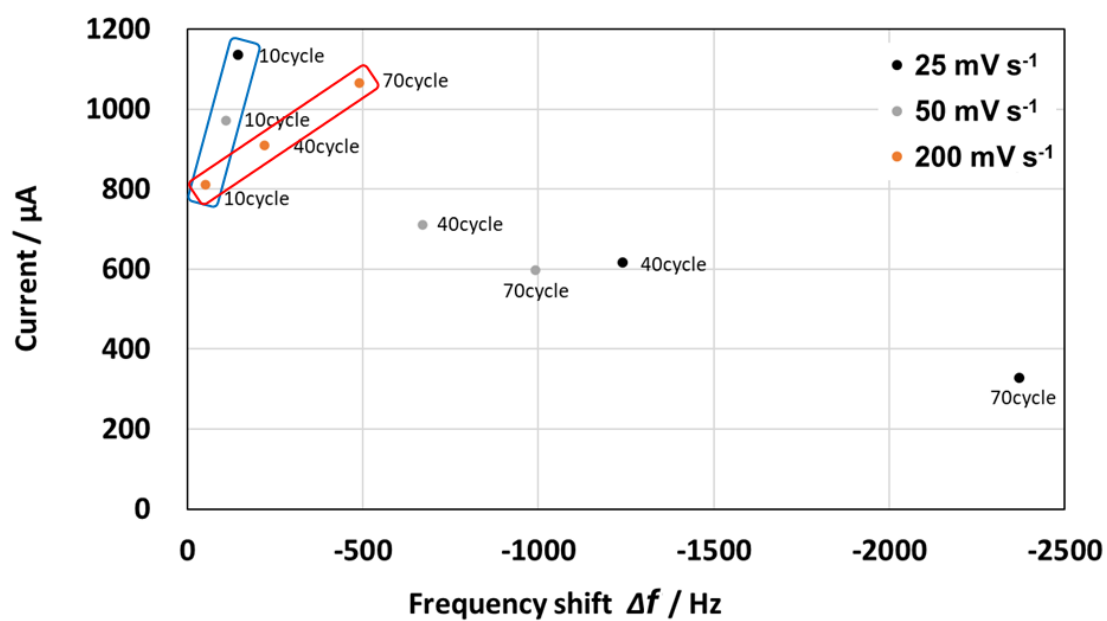
Surface of bare gold sensor without electropolymerization (a-1) and that with enzyme adsorbed on it (a-2). Surface of polyMG film obtained by electrochemical polymerization at  $25 \text{ mV s}^{-1}$  for 10 cycles (b-1) and that with enzyme adsorbed on it (b-2).



**Figure S7.** Cyclic voltammograms of polyMG and PQQ-GDH-modified CF electrodes in 0.1 mol dm<sup>-3</sup> glucose. The scan rate was 2 mV s<sup>-1</sup>.



**Figure S8.** 2D image and cross-sectional analysis of polyMG films electropolymerized at  $200 \text{ mV s}^{-1}$  for 70 cycles on the AuQCM sensor.



**Figure S9.** Relationship between the frequency shift ( $\Delta f$ ) during electrochemical polymerization on the gold surface and the catalytic current value at the carbon felt electrode.