

Supplementary Information

**An impedimetric determination of alkaline phosphatase activity
based on the oxidation reaction mediated by Cu²⁺ bound to poly-
thymine DNA**

*Joon Young Lee,^a Jun Ki Ahn,^a Ki Soo Park^b and Hyun Gyu Park^{*a}*

^a Department of Chemical and Biomolecular Engineering (BK21+ program), Korea Advanced Institute of Science and Technology (KAIST), 291, Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

^b Department of Biological Engineering, College of Engineering, Konkuk University, Seoul 05029, Republic of Korea

*Corresponding author. Tel.: +82 42 350 3932; Fax: +82 42 350 3910; E-mail: hgpark@kaist.ac.kr

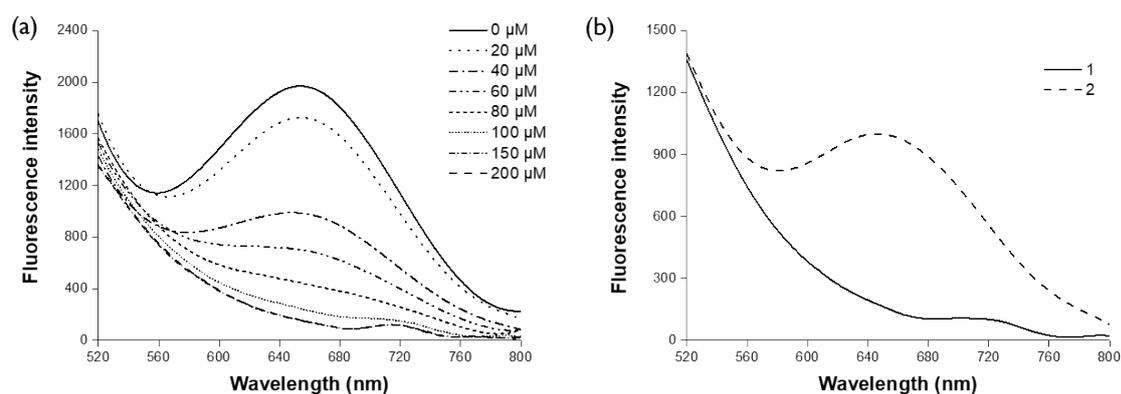


Fig. S1 Fluorescence spectra obtained from CuNP formed by the reduction of Cu^{2+} . (a) Fluorescence spectra from CuNP formed through treating Cu^{2+} with ascorbic acid in the presence of PPI at varying concentrations. (b) Fluorescence spectra obtained from solutions containing $100 \mu\text{M}$ Cu^{2+} , 2 mM ascorbic acid, $1 \mu\text{M}$ poly-thymine DNA probe and $100 \mu\text{M}$ PPI (1), or $100 \mu\text{M}$ PPI previously treated with 1 nM ALP (2).

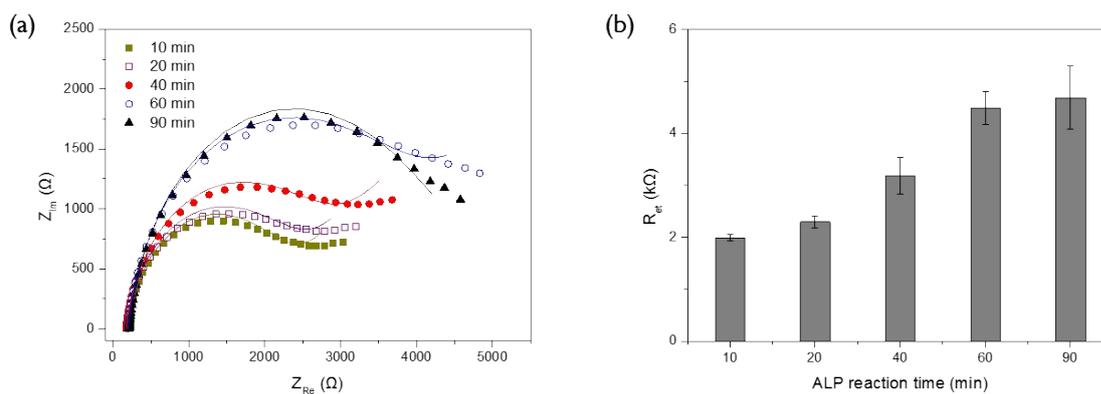


Fig. S2 Optimization of the reaction time for ALP reaction. (a) Nyquist plots of the impedance spectra and (b) electron transfer resistance (R_{et}) obtained from the corresponding impedance spectra at different ALP reaction times. The final concentrations of ALP, PPI, Cu^{2+} , and ascorbic acid are 1 nM, 100 μ M, 100 μ M, and 2 mM, respectively.

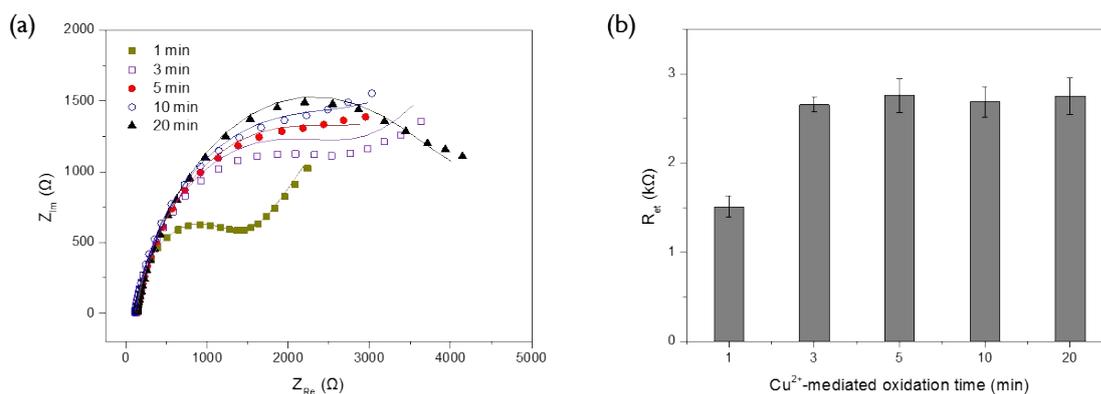


Fig. S3 Optimization of the reaction time for Cu^{2+} -mediated oxidation of ascorbic acid on the poly-thymine DNA-modified electrode. (a) Nyquist plots of the impedance spectra and (b) electron transfer resistance (R_{et}) obtained from the corresponding impedance spectra at different Cu^{2+} -mediated oxidation times. The final concentrations of ALP, PPI, Cu^{2+} , and ascorbic acid are 1 nM, 100 μ M, 100 μ M, and 2 mM, respectively.

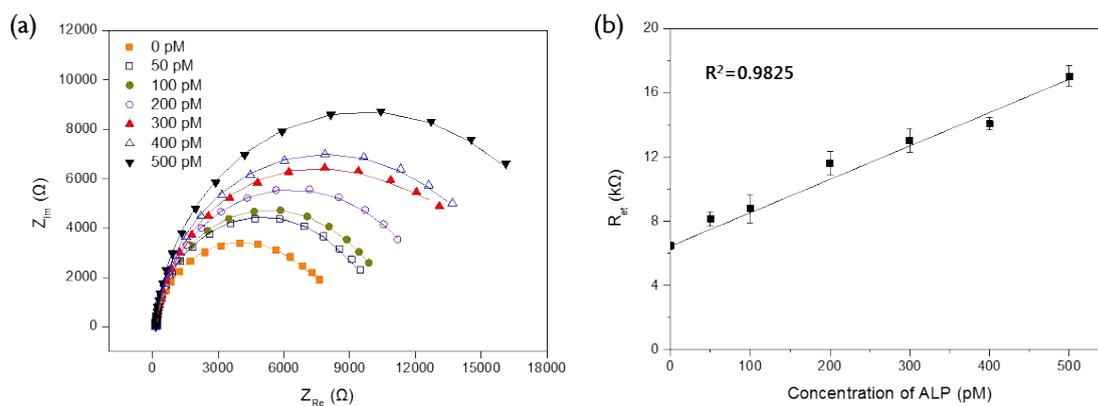


Fig. S4 ALP assay in human serum samples. (a) Nyquist plots of the impedance spectra and (b) electron transfer resistance (R_{et}) obtained from the corresponding impedance spectra upon Cu^{2+} -mediated oxidation in the presence of diluted human serum spiked with varying concentrations of ALP.

Table S1 Comparison of this method with previous electrochemical methods.

Key material/method	Detection limit (U/L)	Linear range (U/L)	Limitations	Reference
Nitrophenylphosphate plastic membrane sensor	30	30 - 3400	- Time-consuming preparation of membrane sensor (28 hr) - Low sensitivity	(Hassan et al. 2009)
λ Exonuclease-mediated signal amplification	100	1000 - 20000	- Requirement of additional enzyme - Low sensitivity	(Miao et al. 2011)
Nanoceria particle-mediated signal amplification	20	5000 - 640000	- Low sensitivity	(Hayat et al. 2013)
Ferrocene-derived substrate	0.4	1 - 250	- Complex synthesis of organic substrate	(Goggins et al. 2015)
Cu ²⁺ -mediated oxidation	7.2	22 - 565	-	This work

References

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