Supporting Information

Development of enzyme-inorganic hybrid nanoflower-modified electrodes and a smartphone-controlled electrochemical analyzer for point-of-care testing of salivary amylase in saliva

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1. Supplementary Figures

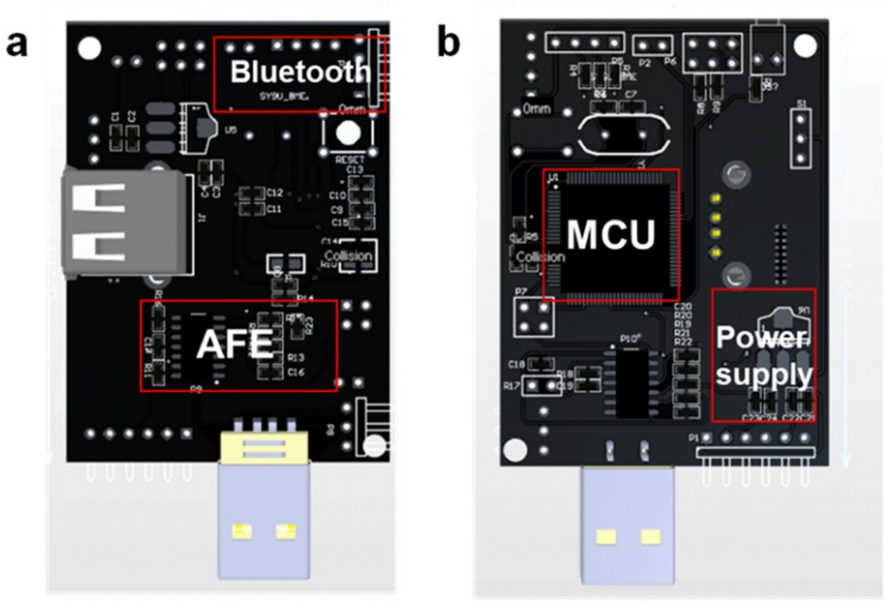

Fig. S1. System schematic diagram of the smartphone-controlled electrochemical analyzer.

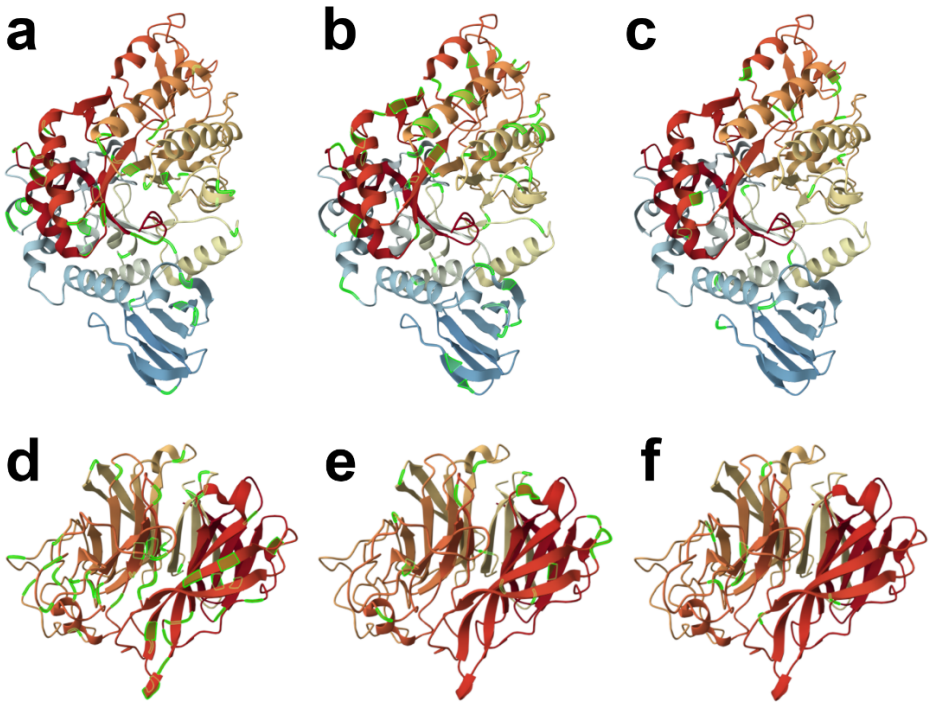

Fig. S2. Crystal structure of α-glucosidase (a-c) (Image copied from PDB DOI: https://doi.org/10.2210/pdb3WY2/pdb, amide group: total 626, dissociated 89; histidine:15)[12] and glucose dehydrogenase (d-f) (Image copied from PDB DOI: https://doi.org/10.2210/pdb7CDY/pdb, amide group: total 416, dissociated 62; histidine: 7). Residues highlighted in bright green: Glycine (a, d), Asparagine (b, e), and Histidine (c, f).

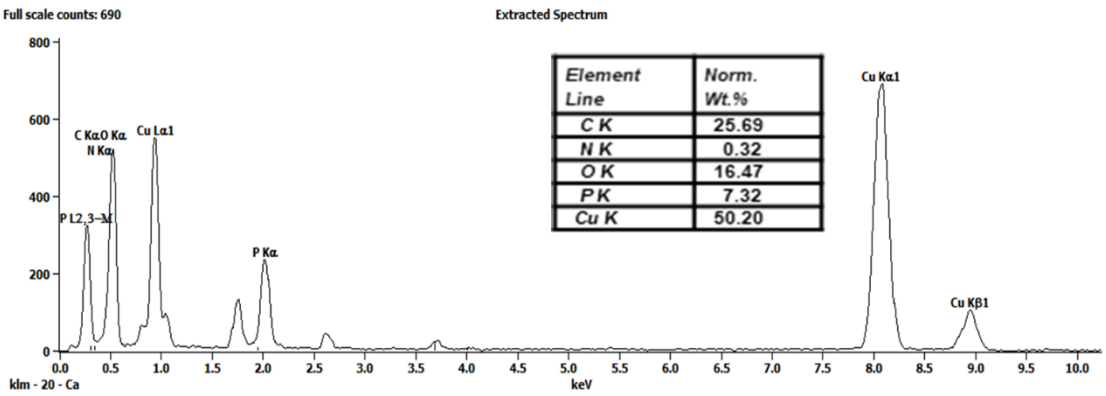

Fig. S3. The EDX characterization results of the NFs.

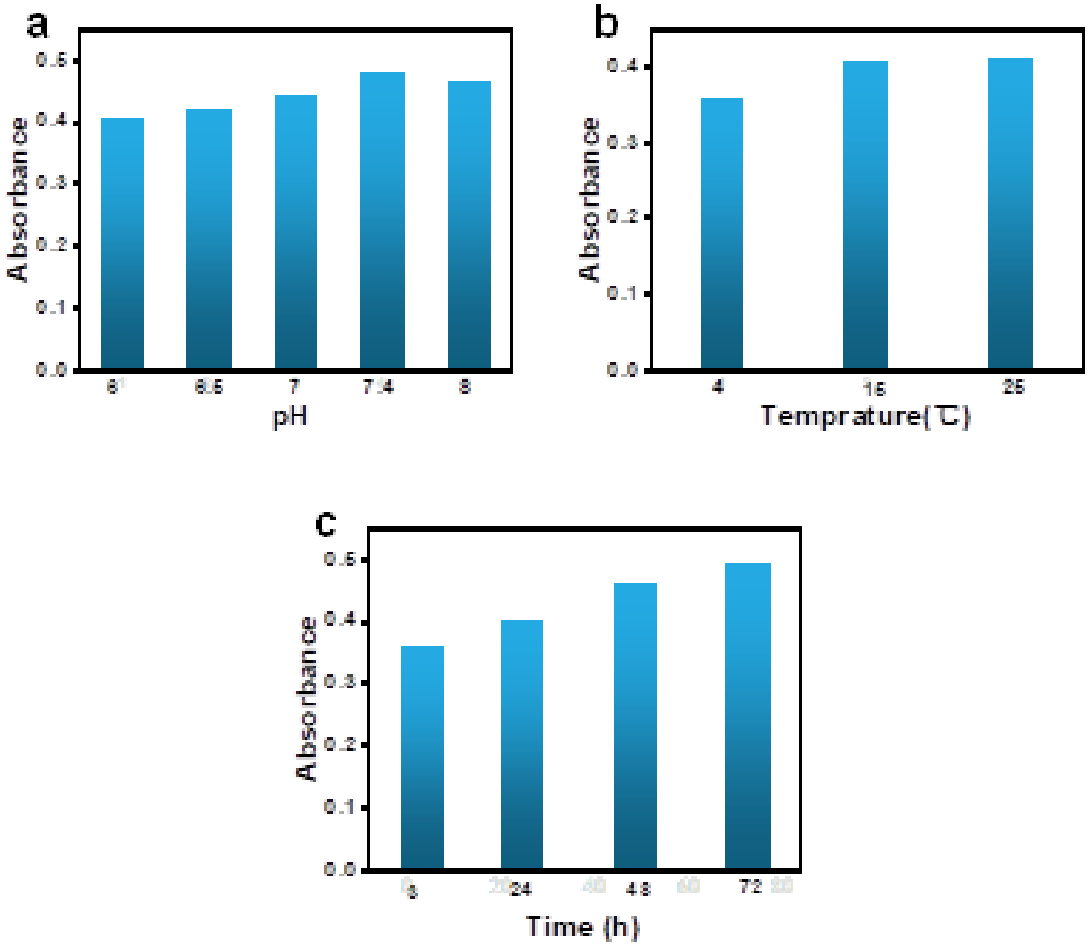

Fig. S4. NF synthesis optimization using activity of enzymes loaded on the NFs as the critical parameter. (a) pH; (b) temperature; (c) Time.

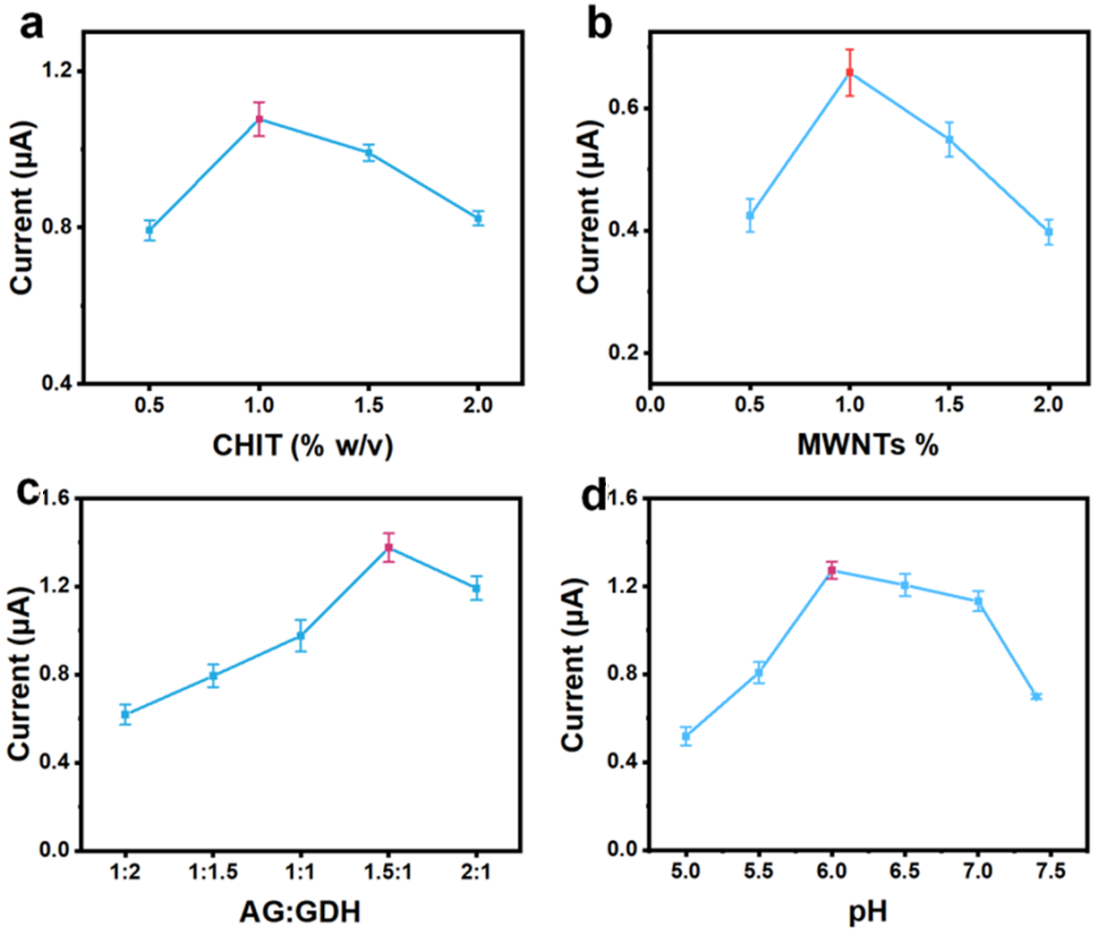

Fig. S5. Optimization for maltose sensing assay. (a) Current response of the NFs/CHIT-MWNTs /SPCE with different CHIT concentrations in acetate buffer (pH 6.0) containing 1.0 mM NAD^+ and 5.0 mM maltose; (b) Current response of the NFs/CHIT-MWNTs/SPCE with different MWNT concentrations in acetate buffer (pH 6.0) containing 1.0 mM NAD^+ and 5.0 mM maltose; (c) Current response of the NFs/CHIT-MWNTs/SPCE in acetate buffer (pH 5.0-6.5) and phosphate buffer (pH 7.0 -7.5) containing 1.0 mM NAD^+ and 5.0 mM maltose; (d) Current response of the NFs/CHIT-MWNTs/SPCE with different AG/GDH ratios in acetate buffer (pH 6.0) containing 1.0 mM NAD^+ and 5.0 mM maltose

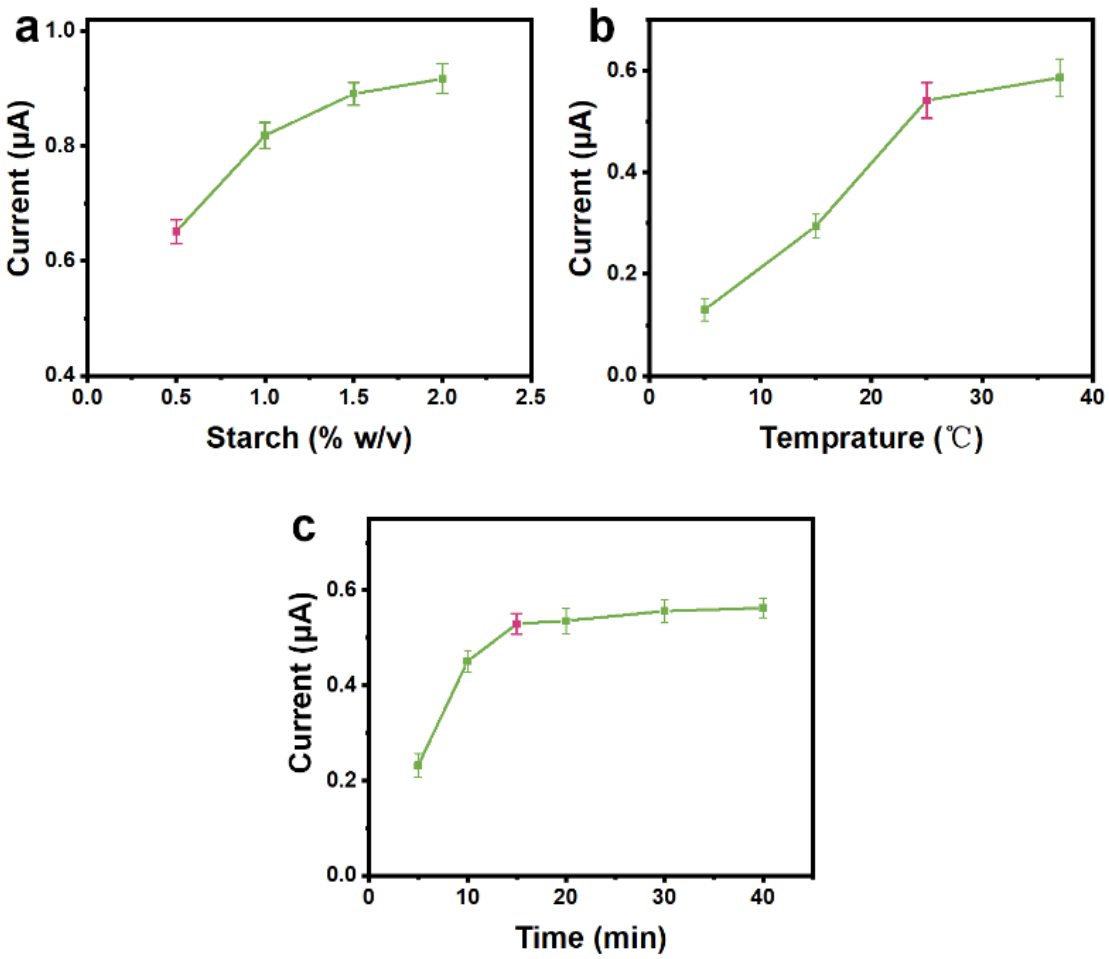

Fig. S6. Optimization for sAA sensing assay. (a) Current response of the NFs/CHIT-MWNTs/SPCE in the solution containing 600 U/mL sAA in the presence of different concentrations of starch at room temperature; (b) Current response of the NFs/CHIT-MWNTs/SPCE in the solution containing 600 U/mL sAA and 0.5% starch at different incubation temperatures; (c) Current response of the NFs/CHIT-MWNTs/SPCE *versus* time in the presence of 600 U/mL sAA and 0.5% starch at room temperatures.

2. Supplementary Tables

Table S1. Comparison of different methods for sAA quantitation.

Sensor	Method	Linear range(U/mL)	LOD (U/mL)	Assay time (min)	Samples type	POCT	Ref
Paper-based strip	colorimetry	20–500	8	20	Saliva	N	[1]
Colour reader	colorimetry	10–230	/	1	Saliva	N	[2]
paper-sensor	colorimetry	0.01-0.11	/	37	Serum	N	[3]
Immunosensor	QCM	3.09 -47.08	0.077	/	Saliva	N	[4]
Hydrogel films	Impedance spectroscopy and QCM	0.08-8	0.008	20	/	N	[5]
SPCE	Amperometry	100-1200	1.1	20	Saliva	N	[6]
SPE	Potentiometry	200-800	0.12	5	Saliva	Y	[7]
CuO/Cu	Amperometry	/	0.05	1	Saliva	N	[8]
Test-strip	Glucometer	200-1000	20	37	Serum Urine	Y	[9]
Modified SPE	Amperometry	5-250	5	10	Artificial Saliva	N	[10]
GOD/AG/ SPE	Amperometry	60-840	17	30	Saliva	N	[11]
NFs/CHIT-MWNTs/SPCE	Amperometry	100-2000	5.02	20	Saliva	Y	This Work

Table S2. Quantitation of sAA activity in clinical saliva samples.

Sample	Methods		
	This Work(U/mL)	Bernfeld method(U/mL)	Deviation rate (%)
1	11452.82±978.417	10619.06	7.851537
2	9767.802±198.957	9967.7545	-2.00599
3	5119.211±235.387	5291.9604	-3.26436
4	9217.277±203.231	9910.6104	3.465001
5	9217.277±240.533	9148.5499	0.751237
6	4692.169±180.078	4519.3779	3.823343
7	5548.827±32.157	5531.076	0.320931
8	8985.748±324.172	8980.5425	0.057966
9	3158.932±142.478	2986.5766	5.770996
10	3526.806±247.623	3633.9238	-2.94772
11	7918.142±187.796	7386.5947	7.196101
12	8872.556±102.902	8357.4915	6.162908
13	4362.883±276.549	3760.5392	16.01749

Table S3. BMI, HbA1c level and sAA activity of 20 obese volunteers.

Samples	Gender	BMI	[HbA1c]%	[sAA] U/mL
1	M	35.00	9.90	5522.84±218.81
2	M	29.90	11.50	2787.38±252.65
3	M	41.00	6.30	801.42±516.39
4	F	35.70	6.20	714.06±142.34
5	M	34.40	4.70	1296.55±90.99
6	F	26.60	4.66	1554.38±112.14
7	M	37.90	6.00	1106.30±217.20
8	M	35.00	6.60	1011.40±283.91
9	M	27.80	7.70	3934.22±139.79
10	M	33.90	11.70	1264.32±287.20
11	F	32.00	7.20	2438.71±234.09
12	M	37.00	5.60	2354.34±179.99
13	M	38.70	6.80	1693.54±152.20
14	M	29.50	8.20	3903.59±234.09
15	M	31.80	12.80	7343.84±372.80
16	M	34.90	14.80	7791.56±257.80
17	M	/	6.80	1525.53±260.84
18	M	25.60	25.60	6612.02±149.18
19	M	36.80	5.80	1456.22±97.62
20	F	26.60	5.10	1754.78±219.97

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