Supplementary Materials

Designed Mini Protein 20 Mimicking Uricase Encapsulated in ZIF-8 as Nanozyme Biosensor for Uric Acid Detection

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Table S1. Central composite design (CCD) for the optimization of uric acid detection by mp20@ZIF-8/rGO/SPCE using the RSM method and results of the current response.

Standard	A: pH	B: Deposition Potential (V)	C: Deposition Time (s)	Peak Current (µA)	
				Experimental	Predicted
1	5.4	-0.50	30.00	4.87	4.84
2	9.4	-0.50	30.00	3.62	3.63
3	5.4	-0.10	30.00	4.72	4.74
4	9.4	-0.10	30.00	3.22	3.20
5	5.4	-0.50	210.00	3.32	3.34
6	9.4	-0.50	210.00	4.01	3.99
7	5.4	-0.10	210.00	3.06	3.05
8	9.4	-0.10	210.00	3.34	3.37
11	7.4	-0.50	120.00	5.34	5.37
12	7.4	-0.10	120.00	5.04	5.01
13	7.4	-0.30	30.00	4.70	4.71
14	7.4	-0.30	210.00	4.06	4.05
15	7.4	-0.30	120.00	5.51	5.50
16	7.4	-0.30	120.00	5.44	5.50
17	7.4	-0.30	120.00	5.48	5.50
18	7.4	-0.30	120.00	5.52	5.50
19	7.4	-0.30	120.00	5.54	5.50
20	7.4	-0.30	120.00	5.51	5.50

Table S2. ANOVA table of the response surface quadratic model.

Source	Sum of Squares	Degree of Freedom	Mean Square	F - value	Prob > F	Significant
Model	14.78	9	1.64	1108.75	< 0.0001	Significant
A	0.40	1	0.40	267.38	< 0.0001	Significant
B	0.32	1	0.32	213.90	< 0.0001	Significant
C	1.12	1	1.12	753.12	< 0.0001	Significant
A^2	0.070	1	0.070	47.04	0.0001	Significant
B^2	0.14	1	0.14	97.32	< 0.0001	Significant
C^2	1.88	1	1.88	1270.28	< 0.0001	Significant
AB	0.054	1	0.054	36.76	0.0003	Significant
AC	1.73	1	1.73	1167.80	< 0.0001	Significant
BC	0.018	1	0.018	12.19	0.0082	Significant
Residual	0.012	8	0.00148			
Lack of Fit	0.00565	3	0.00188	1.52	0.3180	Not significant
Pure Error	0.00620	5	0.00124			
Corrected Total	14.79	17				

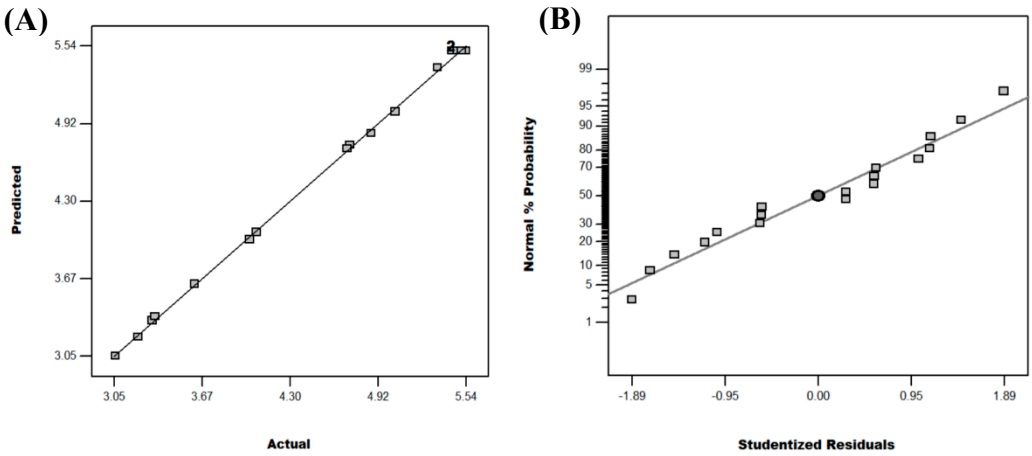


Figure S1. (A) Plot of the predicted versus actual correlation of the two values and (B) Normal probability plot of the residual for peak current magnitude of uric acid detection by mp20@ZIF-8/rGO/SPCE.

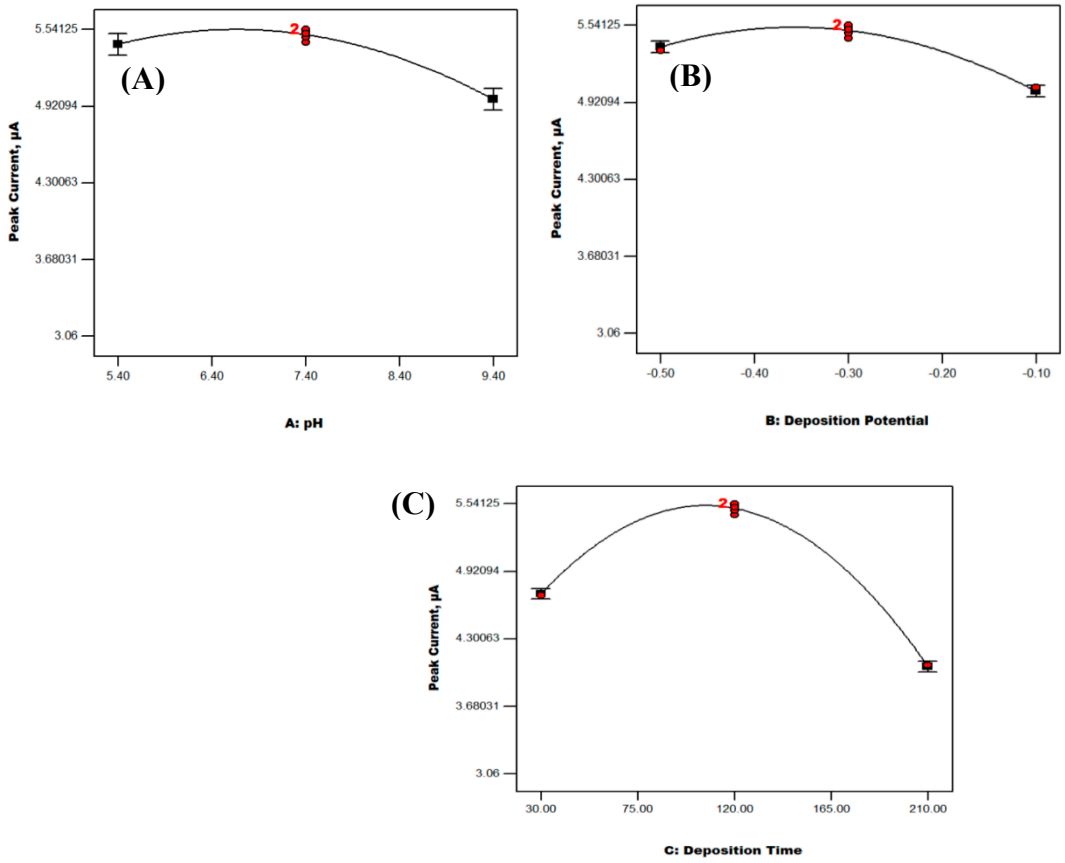
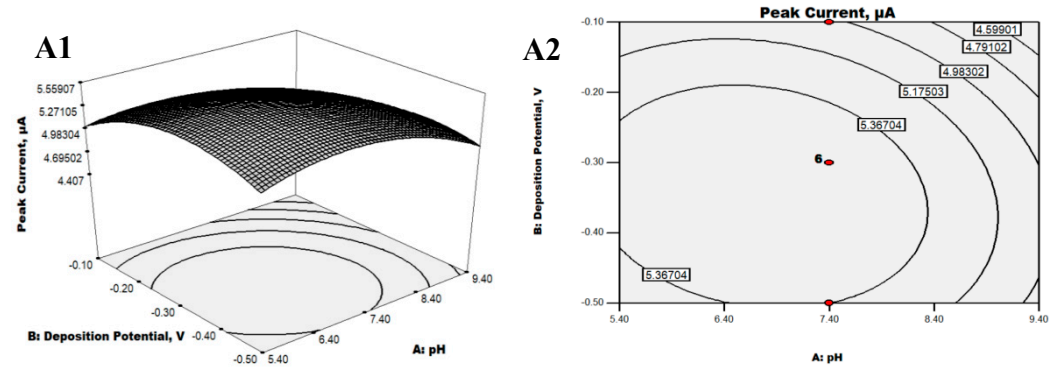
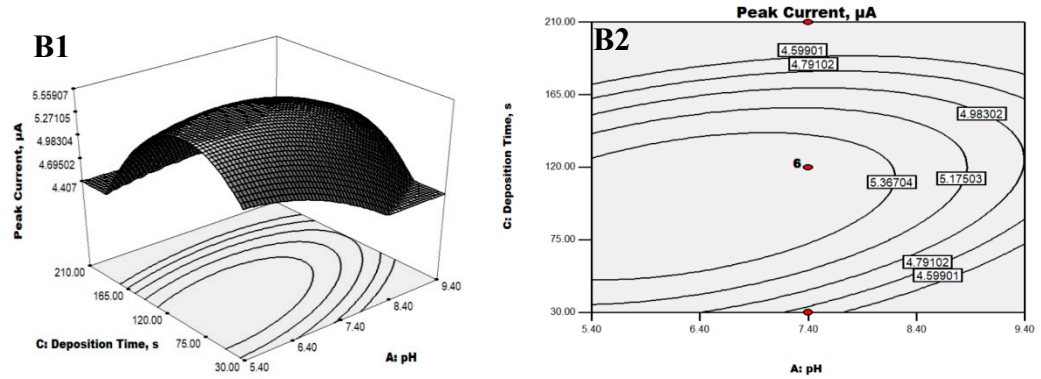


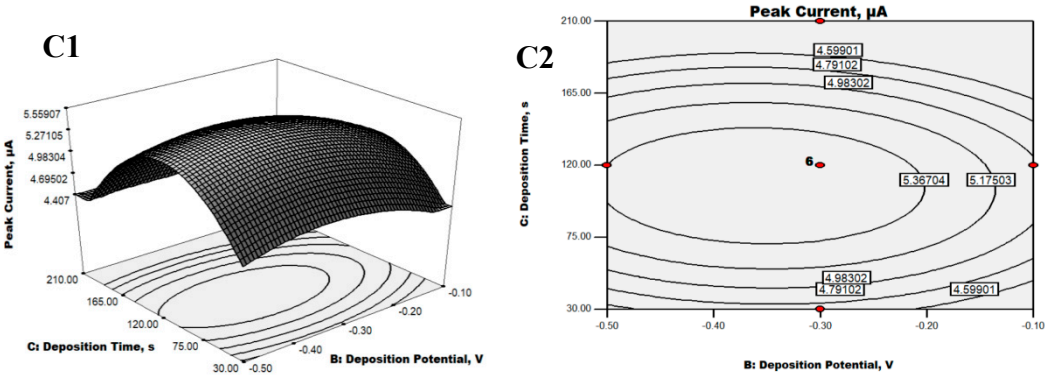
Figure S2. One factor plot of peak current magnitude as a function of (A) pH (at deposition potential = –0.3 V; deposition potential = 120 s); (B) deposition potential of uric acid (pH = 7.4; deposition time = 120 s); and (C) deposition time of uric acid (pH = 7.4; deposition potential = –0.30 V).



(A)



(B)



(C)

Figure S3. 3D surface and 2D contour plots of peak current as a function of (A) pH and deposition potential, (B) pH and deposition time, and (C) deposition potential and deposition time.

Table S3. Constraints applied for the optimization of the peak current of uric acid detection by mp20@ZIF-8/rGO/SPCE

Variables	Goal	Limit	
		Lower	Upper
pH	is target = 7.4	5.4	9.4
Deposition Potential , V	is in range	–0.50	–0.10
Deposition Time, s	minimize	30.00	210.00
Peak Current, µA	maximize	3.06	5.54

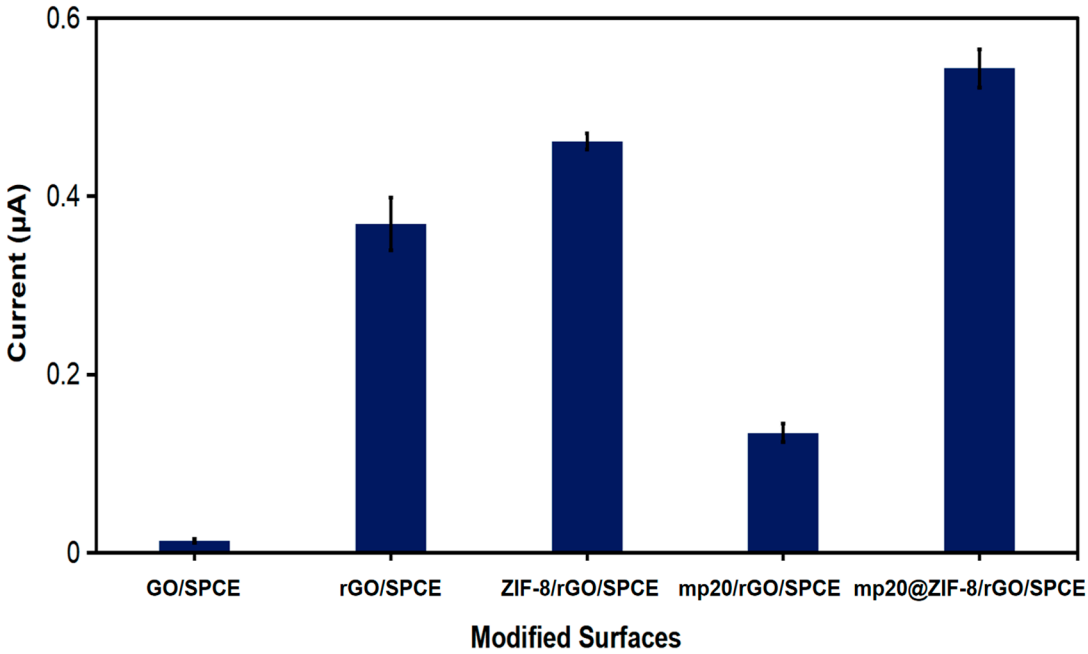
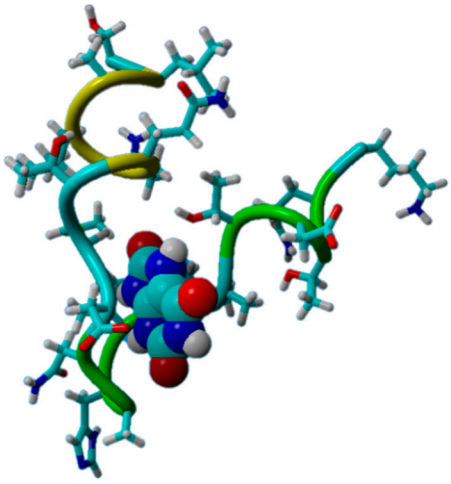


Figure S4. Comparative study of different modified surfaces towards the detection of 1 µM uric acid in 0.1 M PBS solution at pH 7.4.



Peptide	*Contacting Residue*
mp20	Val13,Val14,Ala15**, Thr18, Gln19**

Figure S5. Predicted interaction between mp20 with target analyte uric acid (colored balls) and contacting residues of peptide involved. Residues that form hydrogen bonds with uric acid are indicated in bold.

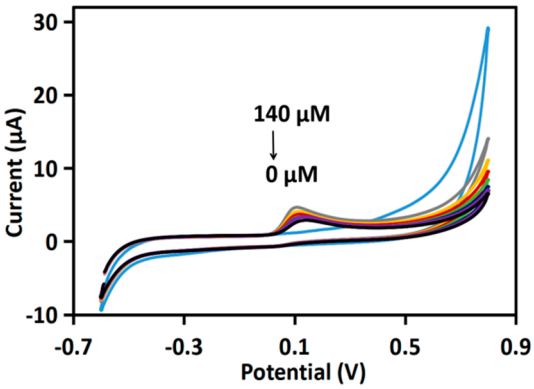


Figure S6. CV curves of the mp20@ZIF-8/rGO/SPCE biosensor after spiking of different uric acid concentrations in 0.1 M PBS (pH 7.4) at scan rate of 100 mV/s.

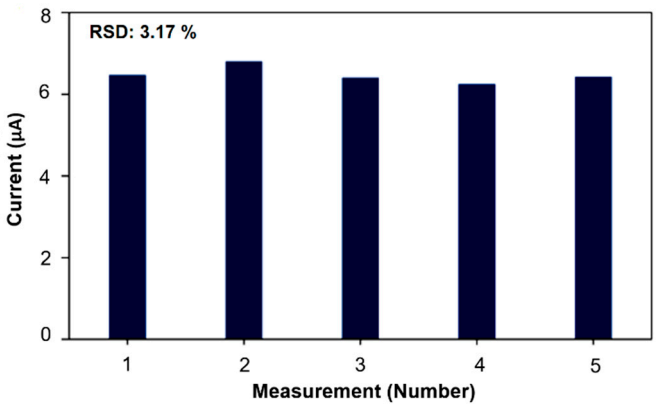


Figure S7. The reproducibility study of mp20@ZIF-8/rGO/SPCE in the detection of 26 μM uric acid.