

Supporting information

for

Enzyme-inorganic hybrid nanoflowers based immobilized enzyme reactor with enhanced enzymatic activity

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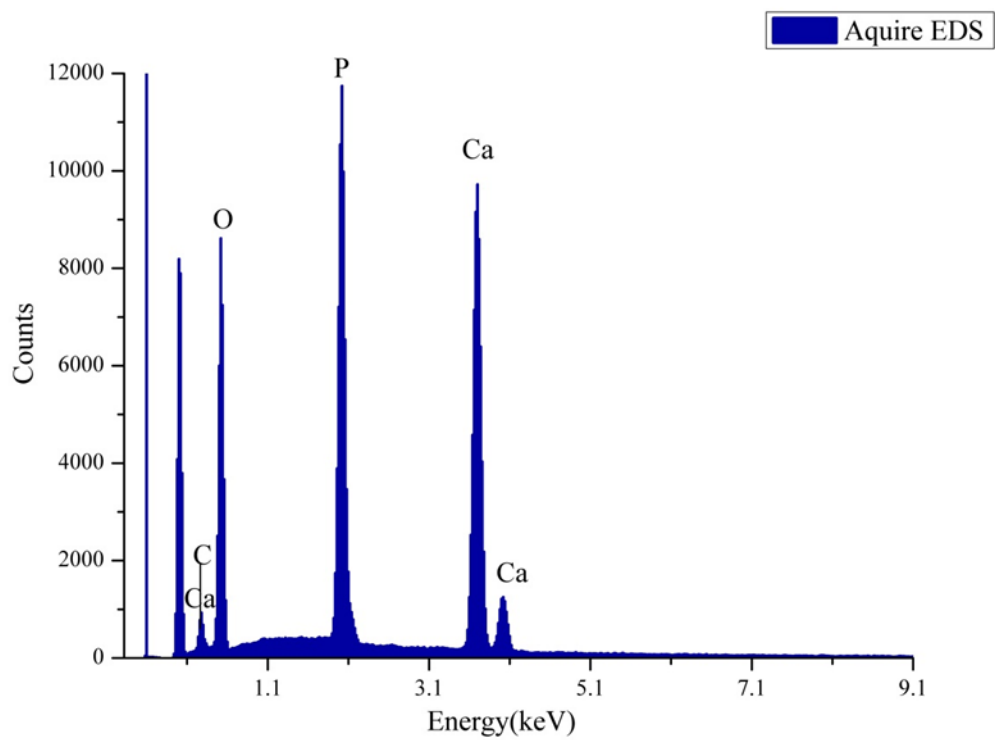


Fig.S1 EDS pattern of the hybrid $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$ - chymotrypsin nanoflowers

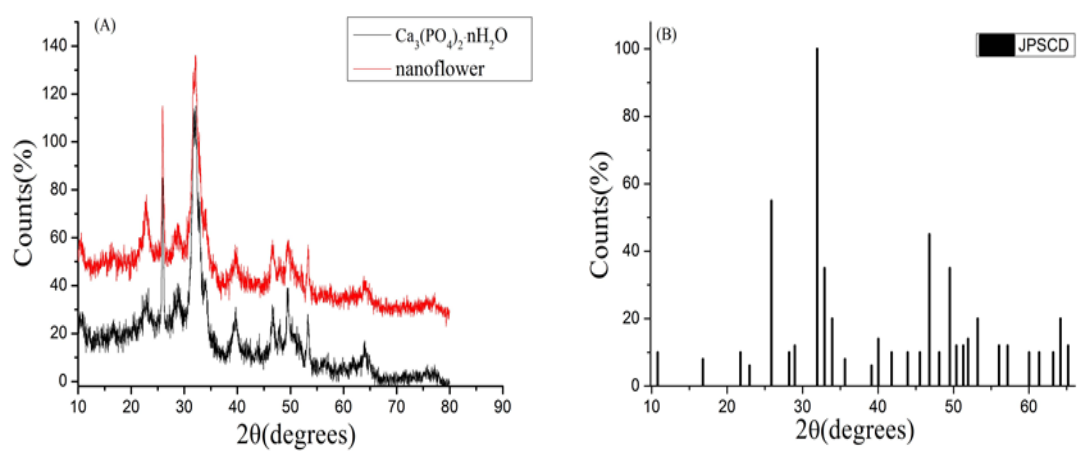


Fig.S2 XRD patterns of (A) hybrid nanoflowers and $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$; (B) peak of $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$ (JCPDS 00-018-0303)

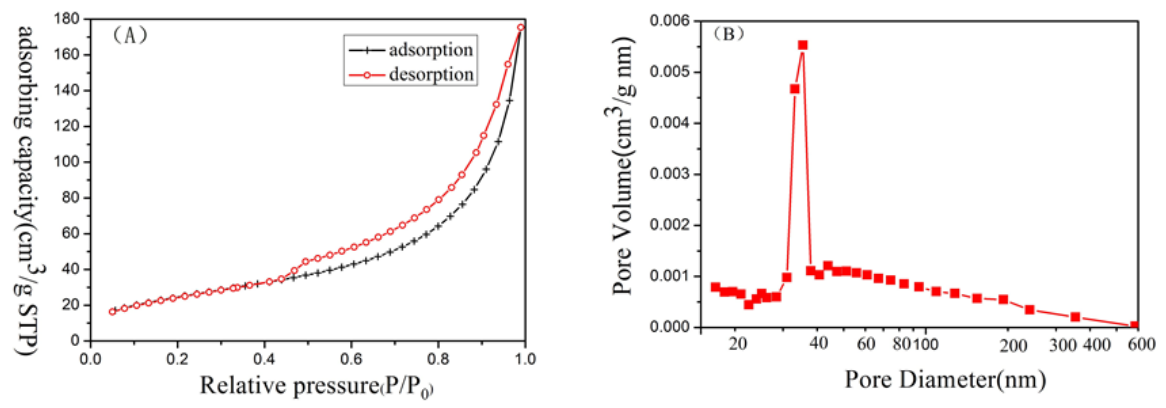


Fig.S3 Adsorption of N₂ nanoflowers desorption isotherm (A) and the pore size distribution (B)

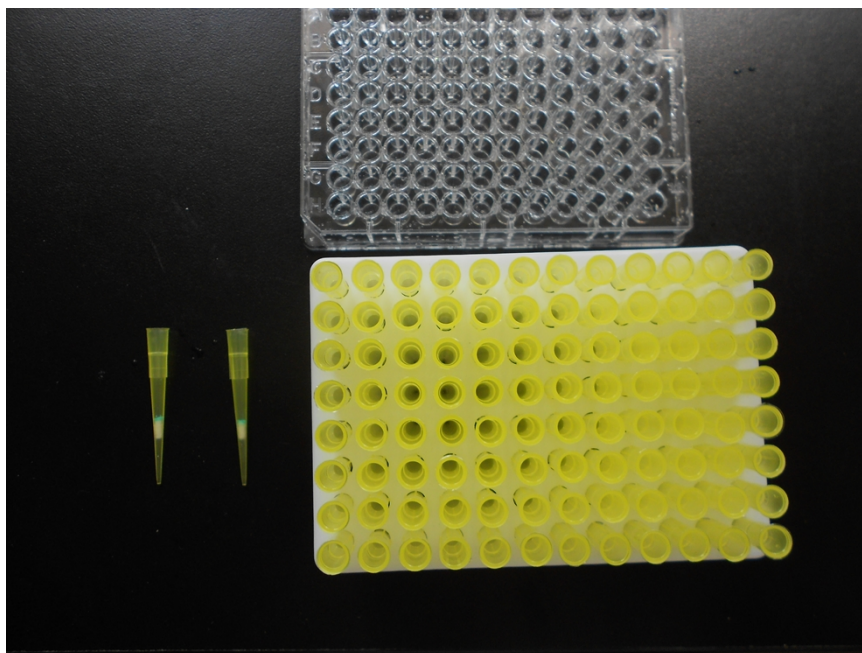


Fig. S4. Photograph of a home-made IMER devices

Table S1. The actual encapsulation yield ChT in the nanoflowers was further determined by UV/Vis methods).

theoretical concentration (mg mL ⁻¹)	Actual concentration (mg mL ⁻¹)	Absorbance (253 nm)	The supernatant concentration (mg mL ⁻¹)	The supernatant absorbance (253 nm)	The weight percentage (%)	Encapsulation yield (%)
0.05	0.05059	0.098	0.01244	0.01862	8.0292	75.4101
0.1	0.10007	0.181	0.07934	0.13801	10.6986	20.3125
0.2	0.19644	0.332	0.16636	0.29330	21.7391	15.3124
0.5	0.56844	0.904	0.49539	0.88047	26.7442	13.1728
1.0	1.08409	1.786	1.00858	1.79628	8.03	7.01421

(a) Regression equations: $Y = 1.78455X - 0.00358$ ($r = 0.9996$) (X: chymotrypsin concentration, Y: absorbance)

Table S2. Comparison of the proteolytic efficiency of chymotrypsin-immobilized nanoflower and free chymotrypsin for BSA digestion (for 12h incubation, BSA: SwissProt Accession: P02769)

	Position	Mass	Peptide sequence	Free	Nanoflower ²	Nanoflower ³
				chymotrypsin ¹		
Incubation time				12h	2min	2min
Matched peptides				36	39	32
Sequence coverage, %				41	48	43
1	1-12	1340.671	(-)DTHKSEIAHRF(K)	✓	✓	✓
2	12-19	974.458	(F)KDLGEEHF(K)	✓	✓	✓
3	12-22	1272.658	(F)KDLGEEHFKGL(V)	✓	*	✓
4	27-36	1171.509	(F)SQYLQQCPF(D)	✓	✓	✓
5	31-36	793.355	(Y)LQQCPF(D)	✓	✓	✓
6	31-42	1514.731	(Y)LQQCPFDEHVKL(V)	*	*	✓
7	32-42	1401.647	(L)QQCPFDEHVKL(V)	*	✓	✓
8	37-42	740.394	(F)DEHVKL(V)	✓	✓	✓
9	37-49	1572.79	(F)DEHVKLVNELTEF(A)	*	✓	*
10	43-49	851.414	(L)VNELTEF(A)	*	✓	✓
11	71-80	1092.524	(F)GDELCKVASL(R)	*	✓	✓
12	85-102	2277.804	(Y)GDMADCCEKQEPERNECF(L)	✓	✓	✓
13	85-103	2390.888	(Y)GDMADCCEKQEPERNECF(L)	✓	*	✓
14	127-134	1051.557	(F)KADEKKFW(G)	✓	✓	✓
15	138-147	1317.706	(Y)LYEIAARRHPY(F)	*	✓	*
16	139-147	1204.622	(L)YEIAARRHPY(F)	*	✓	✓
17	156-160	658.319	(Y)YANKY(N)	✓	✓	✓
18	156-163	865.478	(F)KADEKKF(W)	✓	✓	✓
19	161-177	1988.767	(Y)NGVFQECQAEDKGACL(L)	✓	*	✓
20	198-205	1010.509	(L)RCASIQKF(G)	*	✓	*
21	198-213	1922.007	(L)RCASIQKFGERALKAW(S)	*	*	*
22	206-213	930.516	(F)GERALKAW(S)	✓	✓	✓
23	214-218	545.341	(W)SVARL(S)	✓	✓	*
24	214-227	1607.89	(W)SVARLSQKFPKAEF(V)	*	*	*
25	219-227	1081.568	(L)SQKFPKAEF(V)	✓	*	*
26	251-259	1065.415	(L)ECADDRADL(A)	✓	*	*
27	263-274	1394.647	(Y)ICDNQDTISSKL(K)	✓	✓	✓
28	283-309	2937.455	(L)LEKSHCIAEVEKDAIPENLPPLTADF(A)	✓	✓	✓
29	318-325	808.384	(Y)QEAKDAF(L)	✓	✓	*
30	319-326	921.468	(Y)QEAKDAFL(G)	*	✓	*
31	319-329	1212.59	(Y)QEAKDAFLGSF(L)	✓	✓	✓
32	334-345	1413.76	(Y)SRRHPEYAVSVL(L)	*	✓	*

33	334-346	1526.844	(Y)SRRHPEYAVSVLL(R)	*	✓	✓
34	347-352	779.441	(L)RLAKEY(E)	✓	✓	*
35	353-369	2071.757	(Y)EATLECCAKDDPHACY(S)	✓	✓	✓
36	374-386	1548.838	(F)DKLKHLVDEPQNL(I)	*	*	*
37	377-386	1192.632	(L)KHLVDEPQNL(I)	*	*	*
38	387-394	1053.467	(L)IKQNCDQF(E)	✓	✓	✓
39	387-397	1423.689	(L)IKQNCDQFEKL(G)	✓	*	*
40	387-400	1772.816	(L)IKQNCDQFEKLGEY(G)	✓	✓	✓
41	401-406	649.33	(Y)GFQNAL(I)	*	✓	*
42	401-410	1180.647	(Y)GFQNALIVRY(T)	✓	*	*
43	403-410	976.557	(F)QNALIVRY(T)	*	*	*
44	406-410	550.335	(L)IVRY(T)	✓	✓	✓
45	411-429	2097.203	(Y)TRKVPQVSTPTLVEVSRL(G)	*	*	*
46	423-428	688.424	(F)VEVTKL(V)	*	✓	*
47	455-459	628.414	(L)ILNRL(C)	✓	✓	✓
48	455-462	1001.581	(L)ILNRLCVL(H)	✓	✓	*
49	457-462	775.413	(L)NRLCVL(H)	✓	✓	✓
50	481-496	1926.901	(L)VNRRPCFSALTPDETY(V)	✓	*	*
51	488-496	996.452	(F)SALTPDETY(V)	✓	✓	✓
52	502-506	651.335	(F)DEKLF(T)	✓	✓	✓
53	533-550	2158.133	(L)KHKPKATEEQLKTMENF(V)	*	*	*
54	533-552	2475.306	(L)KHKPKATEEQLKTMENFVAF(V)	*	*	*
55	544-550	868.423	(L)KTMENF(V)	*	*	*
56	544-553	1185.597	(L)KTMENFVAF(V)	✓	*	*
57	551-567	2008.797	(F)VAFVDKCCAADDKEACF(A)	✓	✓	✓
58	554-567	1691.623	(F)VDKCCAADDKEACF(A)	✓	*	*
59	568-603	1583.9	(F)AVEGPKLVVSTQTALA(-)	*	*	*

¹Free ChT with 12 h of digestion at 37°C.

²Nanoflowers within 2 min of digestion at 37°C.

³Nanoflowers were stored at -20°C for 30 days.

Table S3. Comparison of the proteolytic efficiency of chymotrypsin-immobilized nanoflower and free chymotrypsin for HSA digestion (for 12h incubation, HSA: SwissProt Accession: P02768)

	Position	Mass	Peptide sequence	Free chymotrypsin ¹	Nanoflower ²
	Incubation time			12h	2min
	Matched peptides			27	33
	Sequence coverage, %			32	34
1	36-43	951.442	(F)KDLGEEHF(K)	*	✓
2	55-60	793.355	(Y)LQQCPF(E)	✓	✓
3	61-66	740.394	(F)EDHVKL(V)	✓	*
4	74-90	1899.795	(F)AKTCVADESAENCDKSL(H)	✓	✓
5	74-94	2398.054	(F)AKTCVADESAENCDKSLHTLF(G)	✓	✓
6	105-126	2783.069	(L)RETYGEMADCCAKQEPERNECF(L)	✓	*
7	109-126	2233.814	(Y)GEMADCCAKQEPERNECF(L)	*	✓
8	152-159	1004.432	(F)HDNEETFL(K)	✓	✓
9	159-162	551.355	(F)LKKY(L)	*	✓
10	164-174	1514.731	(Y)LQQCPFEDHVKL(V)	✓	✓
11	239-243	529.346	(W)AVARL(S)	✓	✓
12	244-252	1109.574	(L)SQRFPKAEF(A)	✓	*
13	253-258	646.377	(F)AEVSKL(V)	✓	✓
14	288-299	1394.647	(Y)ICENQDSISSKL(K)	✓	✓
15	344-350	779.393	(Y)AEAKDVF(L)	✓	✓
16	344-351	892.477	(Y)AEAKDVF(L)	✓	✓
17	378-394	2057.777	(Y)ETTLKCCAAADPHECY(A)	✓	✓
18	395-401	855.425	(Y)AKVFDEF(K)	*	✓
19	395-404	1193.657	(Y)AKVFDEFKPL(V)	✓	✓
20	399-404	748.388	(F)DEFKPL(V)	✓	✓
21	399-411	1557.780	(F)DEFKPLVEEPQNL(I)	✓	✓
22	402-411	1166.642	(F)KPLVEEPQNL(I)	*	✓
23	405-411	828.410	(L)VEEPQNL(I)	*	✓
24	412-419	1052.508	(L)IKQNCEL(E)	✓	✓
25	412-422	1422.693	(L)IKQNCELFEQL(G)	✓	✓
26	432-435	550.335	(L)LVRY(T)	✓	*
27	506-512	949.467	(L)VNRPCF(S)	✓	*
28	506-521	1956.912	(L)VNRPCFSALEVDETY(V)	*	✓
29	513-521	1026.463	(F)SALEVDETY(V)	✓	✓
30	513-526	1626.790	(F)SALEVDETYVPKEF(N)	✓	✓
31	516-526	1355.637	(L)EVDETYVPKEF(N)	✓	✓
32	522-526	619.345	(Y)VPKEF(N)	✓	✓
33	532-540	1078.487	(F)TFHADICTL(S)	✓	✓
34	569-578	1114.524	(L)KAVMDDFAAF(V)	*	✓

35 579-592	1792.707	(F)VEKCCKADDKETCF(A)	✓	✓
36 579-599	2548.125	(F)VEKCCKADDKETCFAEEGKKL(V)	*	✓
37 593-599	774.436	(F)AEEGKKL(V)	*	✓

¹Free ChT with 12 h of digestion at 37°C.

²Nanoflowers within 2 min of digestion at 37°C.

Table S4. Identification of proteins from human serum digested by ChT-embedded nanoflowers. (n=3)

Group (#)	Distinct Peptides(#)	Distinct Summed MS/MS Search Score	% AA Coverage	Protein Name
1	5	56.24	19	Ig alpha-1 chain C region
1	3	31.43	16	Ig alpha-2 chain C region
2	4	56.02	38	Ig lambda chain C regions
3	2	22.00	6	Immunoglobulin lambda-like polypeptide 1 precursor
3	3	55.52	33	Ig kappa chain C region
4	4	50.02	21	Alpha-1B-glycoprotein precursor
5	3	39.11	6	Haptoglobin precursor [Contains: Haptoglobin alpha chain; Haptoglobin beta chain]
6	3	38.83	6	Serum albumin precursor
7	3	33.02	14	Beta-2-glycoprotein 1 precursor
8	2	26.40	9	Alpha-2-HS-glycoprotein precursor
9	2	23.29	12	Ig gamma-4 chain C region

Table S5. Identification of proteins from human serum digested by free ChT. (n=3)

Group (#)	Distinct Peptides(#)	Distinct Summed MS/MS Search Score	% Coverage	AA	Protein Name
1	10	148.58	16		Serum albumin precursor
2	4	67.18	2		Complement C3 precursor [Contains: Complement C3 beta chain; Complement C3 alpha chain; C3a anaphylatoxin; Complement C3b alpha' chain; Complement C3c alpha' chain fragment 1; Complement C3dg fragment; Complemen...
3	3	49.56	2		Alpha-2-macroglobulin precursor
4	4	48.39	4		Serotransferrin precursor
5	4	48.03	14		Ig gamma-2 chain C region
5	3	37.85	9		Ig gamma-1 chain C region
5	3	37.85	9		Ig gamma-4 chain C region
6	1	25.13	4		Ig mu chain C region
7	2	24.73	30		Ig kappa chain C region
8	2	21.89	4		Haptoglobin precursor [Contains: Haptoglobin alpha chain; Haptoglobin beta chain]