Supplementary Information (SI) for Analytical Methods.

supporting information

Paper-based colorimetric sensor using single-atom nanozyme for the ultrasensitive detection of Cr(VI) in Short Necked Clam

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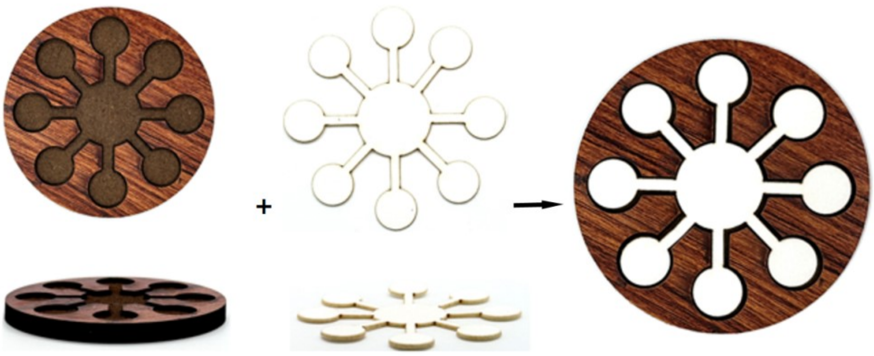

Figure S1. Schematic of PAD fabrication.

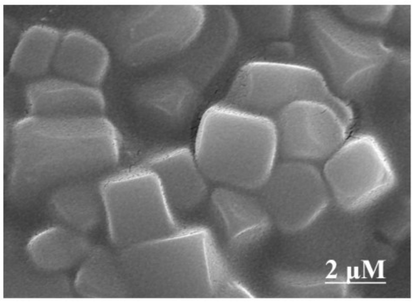

Figure S2. SEM of lyophilized sample before the pyrolysis.

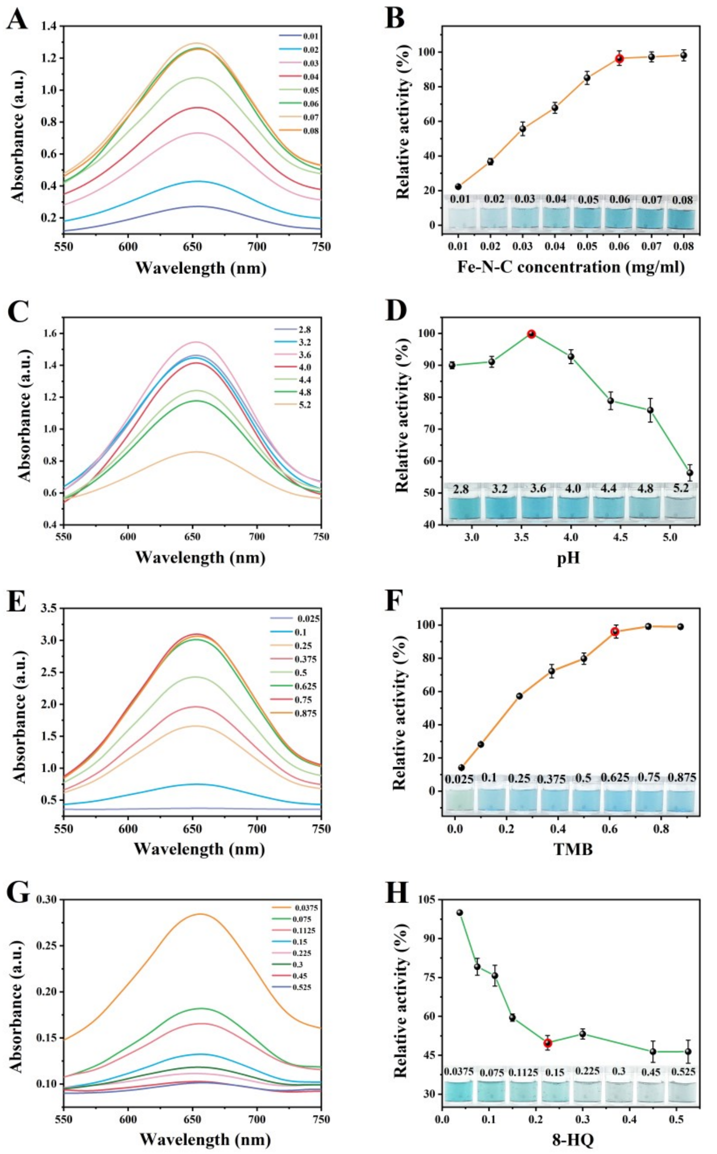

Figure S3. Optimization of experimental parameters. Fe-N-C concentration (A, B); pH (C, D); TMB (E.F); 8-HQ (G,H).

Table S1 Compared the apparent Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) of Fe-C-N with those of previously reported SAzymes.

SAzymes	substrate	Km (mM)	Vm ($\times10^{-8}Ms^{-1}$)	Reference
FeCu-NC		0.05	7.88	[1]
Fe-N/S-C		0.453	9.497	[2]
FeBi-NC		0.21	7.0	[3]
P-N-C	TMB	0.696	10.215	[4]
SA-Fe/NG		0.1772	4.3	[5]
Co-N-C		0.1496	1.073	[6]
Fe-N-C		0.3589	7.819	This work

Table S2 Comparison of the method developed in this work with other published methods for Cr(VI) detection.

Methods	Sensing probe	Linear range (μM)	LOD (μM)	Reference
Colorimetric	H-Fe-POP	2-130 μM	0.23 μM	[7]
Colorimetric	Fe-N-C	0.03-3 μM	3 nM	[5]
Fluorescence	CDs	1-400 μM	0.24 μM	[8]
fluorescence analysis	B, N-CDs	0.3-500 μM	0.24 μM	[9]
Fluorescent Test Papers	CD	0-0.1M	1 μM	[10]
Colorimetric	Fe-N-C	4-1300 μM	1.11 μM	This work
Paper chip	Fe-N-C	5-1000 μM	1.19 μM	This work

Table S3 The UV-vis absorption spectra of TMB + Fe-N-C (a), Fe-N-C+TMB+8-HQ (b) and TMB + Fe-N-C+8-HQ+Cr (VI) (c).

Wavelength (nm)	a.TMB	b.Fe-N-C	c.TMB+Fe-N-C
550	0.0351	0.2709	0.5055
560	0.0351	0.2707	0.5353
570	0.0351	0.271	0.5701
580	0.035	0.2699	0.6263
590	0.0352	0.2708	0.7034
600	0.0355	0.2707	0.7794
610	0.0355	0.2707	0.8514
620	0.0353	0.2707	0.9307
630	0.0352	0.2702	1.0175
640	0.0351	0.27	1.0805
650	0.0351	0.2698	1.1112
660	0.0351	0.2695	1.1122
670	0.0351	0.2695	1.0753
680	0.0351	0.2696	1.0008
690	0.0353	0.2695	0.9112
700	0.0353	0.2693	0.8231
710	0.0361	0.2704	0.73
720	0.0373	0.2712	0.6534
730	0.0394	0.2731	0.6041
740	0.0412	0.2749	0.5772
750	0.0415	0.275	0.5644

Table S4 The UV-vis absorption spectra of TMB + Fe-N-C (a), TMB + Fe-N-C+8-HQ (b), TMB + Fe-N-C+8-HQ+Cr(VI) (c).

Wavelength (nm)	a.Fe-N-C+TMB	b.Fe-N-C+TMB+8-HQ	c.Fe-N-C+TMB+8-HQ+Cr
550	0.1556	0.1141	0.3647
560	0.1674	0.1149	0.3948
570	0.1815	0.1165	0.4301
580	0.1973	0.1201	0.4782
590	0.2175	0.1242	0.5403
600	0.2402	0.1286	0.6028
610	0.2604	0.1335	0.665
620	0.2832	0.1386	0.7317
630	0.3084	0.1442	0.8042
640	0.3253	0.1473	0.8593
650	0.3345	0.1487	0.8887
660	0.3344	0.149	0.891
670	0.3229	0.1462	0.8596
680	0.2998	0.1413	0.79
690	0.2734	0.1351	0.7077
700	0.2476	0.1283	0.625
710	0.221	0.1243	0.5348
720	0.2004	0.1202	0.4631
730	0.1868	0.1177	0.4092
740	0.1803	0.1169	0.3814
750	0.177	0.1171	0.3673

Table S5 Optimization of experimental parameters (TMB).

Wavelength (nm)	0.025	0.1	0.25	0.375	0.5	0.625	0.75	0.875
550	0.3572	0.4331	0.6154	0.6589	0.7744	0.8631	0.8712	0.8439
560	0.3555	0.4505	0.6834	0.7403	0.8806	1.0005	1.0064	0.9816
570	0.3546	0.4718	0.7611	0.8495	1.0044	1.1595	1.1891	1.1424
580	0.3554	0.5021	0.8671	0.9697	1.1716	1.3755	1.393	1.3587
590	0.3577	0.5418	1.0033	1.1306	1.3835	1.6524	1.6614	1.6376
600	0.3613	0.5818	1.1384	1.3084	1.5964	1.9279	1.9657	1.9165
610	0.3639	0.6209	1.2692	1.4631	1.7965	2.1985	2.2296	2.1902
620	0.3667	0.6622	1.4034	1.6314	2.0105	2.4803	2.5167	2.477
630	0.3697	0.705	1.5381	1.8094	2.2292	2.7549	2.8165	2.7666
640	0.3718	0.736	1.6288	1.9212	2.3796	2.9475	3.0248	2.9807
650	0.3733	0.7502	1.6664	1.968	2.4368	3.0245	3.1069	3.0742
660	0.3732	0.7479	1.654	1.9529	2.4175	3.0021	3.0928	3.0636
670	0.3718	0.7242	1.5787	1.8611	2.2977	2.8713	2.9522	2.9543
680	0.3688	0.6771	1.4425	1.6933	2.0834	2.5879	2.6754	2.6682
690	0.3657	0.6267	1.289	1.5039	1.8441	2.2859	2.3658	2.3604
700	0.3624	0.576	1.1327	1.3076	1.61	1.9672	2.0344	2.0332
710	0.3603	0.5296	0.9889	1.1304	1.3589	1.6719	1.7271	1.7231
720	0.3583	0.4854	0.8513	0.9578	1.149	1.3845	1.4278	1.4205
730	0.3582	0.4557	0.7575	0.8394	1.0062	1.186	1.2203	1.2092
740	0.3586	0.4407	0.7068	0.7755	0.926	1.0793	1.1091	1.0946
750	0.3582	0.4323	0.6807	0.7437	0.8841	1.025	1.0509	1.0349

Table S6 Optimization of experimental parameters (8-HQ).

Wavelength (nm)	0.0375	0.075	0.1125	0.15	0.225	0.3	0.45	0.525
550	0.1476	0.1075	0.1076	0.0961	0.0962	0.095	0.0938	0.0901
560	0.1548	0.1117	0.1112	0.0979	0.0968	0.0961	0.0929	0.09
570	0.164	0.1169	0.1134	0.1002	0.0982	0.0982	0.0925	0.0906
580	0.1764	0.1229	0.1184	0.1032	0.0994	0.1001	0.0935	0.091
590	0.1925	0.1319	0.1251	0.1073	0.1008	0.1026	0.0949	0.0918
600	0.2084	0.1403	0.1321	0.1115	0.1028	0.1059	0.0961	0.0931
610	0.225	0.1489	0.1392	0.1162	0.1048	0.1092	0.0978	0.0945
620	0.2424	0.1584	0.1468	0.1209	0.1073	0.1119	0.0991	0.0965
630	0.2621	0.1696	0.1555	0.1265	0.1094	0.1148	0.1009	0.0982
640	0.2766	0.1776	0.1617	0.1301	0.1109	0.1171	0.1021	0.1
650	0.2841	0.1816	0.1653	0.1322	0.1116	0.1184	0.1027	0.1012
660	0.2852	0.1825	0.1661	0.1326	0.1112	0.1183	0.1027	0.1016
670	0.2777	0.1787	0.163	0.1303	0.1101	0.1167	0.1014	0.1008
680	0.2614	0.1698	0.156	0.1255	0.1079	0.1137	0.0993	0.0991
690	0.2415	0.1589	0.1476	0.1203	0.1051	0.1101	0.0972	0.0976
700	0.221	0.1473	0.1392	0.1146	0.1021	0.1063	0.0952	0.0951
710	0.199	0.135	0.1302	0.1089	0.0993	0.1024	0.0929	0.0933
720	0.1808	0.1251	0.1232	0.1045	0.0981	0.0999	0.0916	0.0929
730	0.1694	0.1196	0.1193	0.1024	0.0981	0.0989	0.0914	0.0932
740	0.1637	0.1191	0.1167	0.1022	0.0992	0.0993	0.0921	0.0944
750	0.1605	0.1185	0.1157	0.102	0.0991	0.0991	0.0921	0.0942

Table S7 Optimization of experimental parameters (pH).

Wavelength (nm)	2.8	3.2	3.6	4.0	4.4	4.8	5.2
550	0.616	0.639	0.616	0.54	0.57	0.56	0.559
560	0.67	0.691	0.675	0.592	0.611	0.599	0.577
570	0.741	0.759	0.752	0.667	0.667	0.65	0.602
580	0.824	0.842	0.844	0.751	0.734	0.711	0.632
590	0.922	0.938	0.952	0.854	0.811	0.781	0.666
600	1.028	1.042	1.07	0.962	0.895	0.858	0.702
610	1.143	1.153	1.196	1.081	0.985	0.941	0.742
620	1.259	1.261	1.322	1.204	1.076	1.026	0.781
630	1.358	1.353	1.43	1.303	1.155	1.098	0.817
640	1.431	1.421	1.512	1.38	1.214	1.154	0.845
650	1.468	1.454	1.552	1.421	1.246	1.181	0.859
660	1.458	1.439	1.541	1.41	1.239	1.175	0.856
670	1.396	1.372	1.47	1.345	1.191	1.13	0.833
680	1.29	1.262	1.352	1.232	1.107	1.053	0.793
690	1.162	1.131	1.21	1.098	1.007	0.96	0.746
700	1.027	0.993	1.06	0.96	0.902	0.861	0.696
710	0.91	0.864	0.924	0.832	0.801	0.774	0.653
720	0.809	0.768	0.814	0.726	0.728	0.703	0.616
730	0.735	0.698	0.739	0.657	0.675	0.65	0.588
740	0.692	0.652	0.691	0.612	0.642	0.62	0.574
750	0.672	0.626	0.667	0.591	0.625	0.605	0.566

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