Supporting Information

Single layer of Prussian blue grid as a versatile enzyme trap for low-potential biosensors

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Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

Supplement 1 (S1). The surface image of PB grid under low magnification

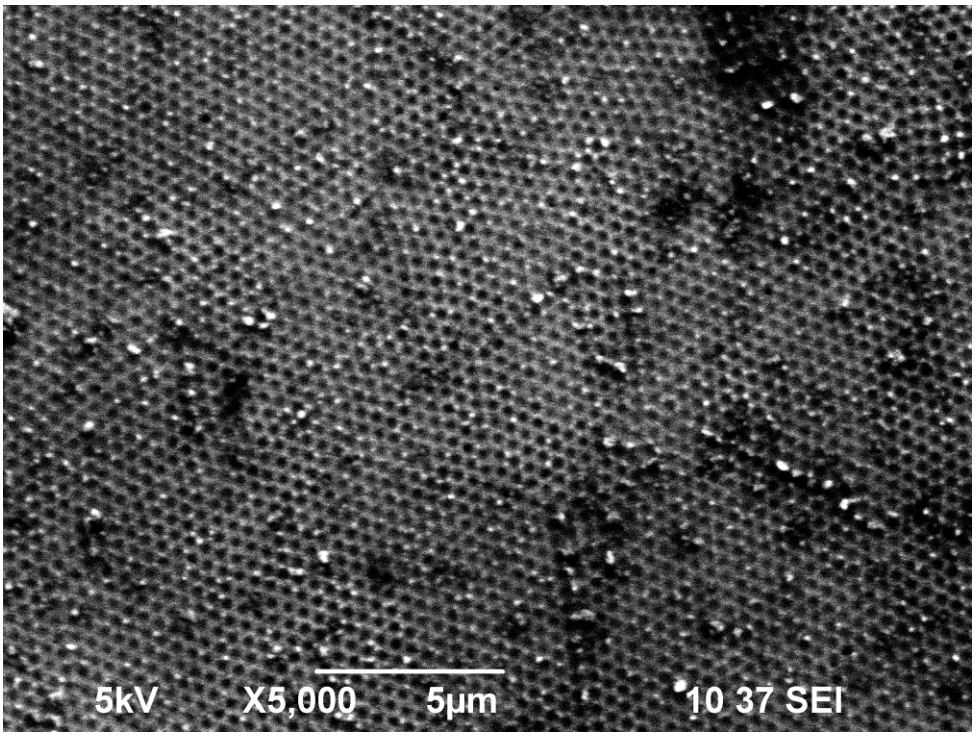

Figure S1. SEM image of PB grid which was prepared by 40 cycles of self-assembly. The magnification is ×5000.

Often in the case of polystyrene bead templates it is difficult to create a uniform film on the whole support surface due to the uncontrollable distribution of the bead droplet following addition of surfactant. However, according to the SEM image taken under low magnification, it was found that the so-prepared film can maintain a continuous and uniform nature over this area.

Supplement 2 (S2). Enzyme immobilization process by GS silane

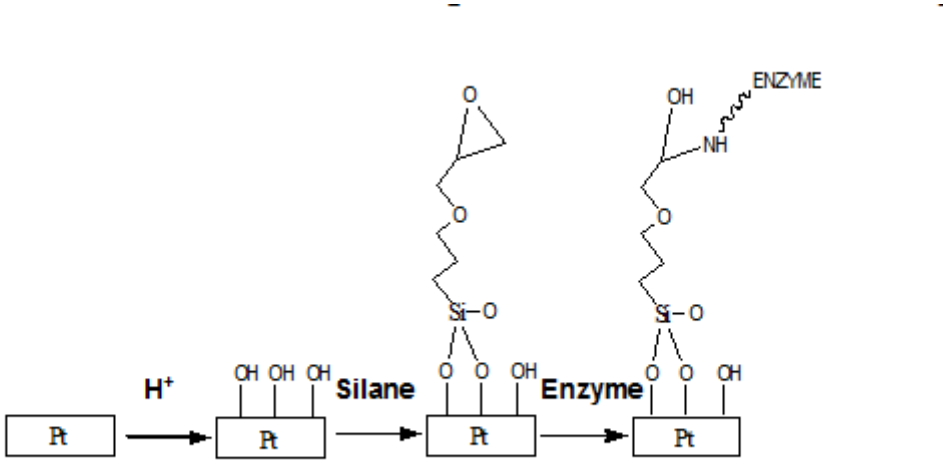

Figure S2. Schematic for the enzyme loading approach using GS silane coupling agent.

Supplement 3 (S3). Simulative results of bond populations and adsorption energy

Table S3 (a). The calculated bond population of model for GS adsorption on PB surface

Bond		Population	Length (Å)				
H 001 --	O 003	0.56	0.97422	H 002 --	C 009	0.82	1.1038
H 011 --	C 014	0.8	1.09434	H 003 --	C 009	0.82	1.10421
H 012 --	C 014	0.82	1.09434	H 008 --	C 012	0.82	1.1071
H 010 --	C 013	0.81	1.09692	H 006 --	C 011	0.83	1.10913
H 004 --	C 010	0.83	1.09933	H 009 --	C 012	0.81	1.10925
H 005 --	C 010	0.86	1.1005	H 007 --	C 011	0.83	1.11019
				C 008 --	N 008	1.76	1.17801

C 007 --	N 007	1.75	1.17801	N 005 --	Fe 003	0.37	1.93708
C 006 --	N 006	1.77	1.17806	N 006 --	Fe 003	0.29	1.93708
C 005 --	N 005	1.63	1.17806	C 002 --	Fe 001	0.32	1.93737
C 001 --	N 001	1.74	1.17936	C 004 --	Fe 001	0.33	1.93737
C 003 --	N 003	1.74	1.17936	C 003 --	Fe 001	0.33	1.93744
C 004 --	N 004	1.74	1.17937	C 001 --	Fe 001	0.33	1.93744
Bond		Population	Length (Å)	N 003 --	Fe 002	0.3	1.95475
C 002 --	N 002	1.74	1.17937	N 001 --	Fe 002	0.3	1.95475
C 012 --	O 004	0.52	1.42473	N 002 --	Fe 003	0.3	1.95477
C 011 --	O 004	0.53	1.42851	N 004 --	Fe 003	0.3	1.95477
C 013 --	O 005	0.33	1.44561	C 006 --	Fe 004	0.29	1.95641
C 014 --	O 005	0.3	1.44802	C 005 --	Fe 004	0.02	1.95641
C 013 --	C 014	0.49	1.46832	C 007 --	Fe 004	0.34	1.95646
C 012 --	C 013	0.71	1.50447	C 008 --	Fe 004	0.3	1.95646
C 010 --	C 011	0.71	1.51802	H 007 --	H 009	0.01	2.33493
C 009 --	C 010	0.66	1.53058	H 006 --	H 008	0.01	2.47366
C 005 --	O 001	0.37	1.55946	H 002 --	H 006	0	2.61515
O 002 --	Si 001	0.79	1.56237	H 010 --	O 004	0	2.65873
O 003 --	Si 001	0.44	1.64368	H 008 --	O 005	0	2.69512
O 001 --	Si 001	0.26	1.81638	C 001 --	C 002	0.01	2.7399
O 001 --	Fe 004	0.12	1.82902	C 003 --	C 004	0.01	2.7399
C 009 --	Si 001	0.58	1.87991	C 002 --	C 003	0.01	2.7399
N 007 --	Fe 002	0.3	1.93704	C 001 --	C 004	0.01	2.7399
N 008 --	Fe 002	0.29	1.93704	C 006 --	C 007	0.01	2.76682

C 006 --	C 008	0.01	2.76682	H 001 --	H 003	0	2.93529
C 005 --	C 008	0.01	2.76682	H 002 --	H 007	0	2.98642
C 005 --	C 007	0	2.76682	C 007 --	O 002	0	2.9992
H 001 --	H 002	0	2.87487				

Table S3 (b). The calculated bond population of model for GS adsorption on Pt

Bond		Population	Length (Å)	C 005 --	C 006	0.53	1.46993
H 001 --	O 003	0.61	0.99471	C 004 --	C 005	0.72	1.50339
H 012 --	C 006	0.88	1.09468	C 002 --	C 003	0.71	1.5272
H 010 --	C 005	0.85	1.09675	C 001 --	C 002	0.67	1.52998
H 004 --	C 002	0.83	1.10041	O 002 --	Si 001	0.66	1.63126
H 005 --	C 002	0.84	1.10136	O 001 --	Si 001	0.61	1.63283
H 011 --	C 006	0.94	1.10258	O 003 --	Si 001	0.41	1.72213
Bond		Population	Length (Å)	C 001 --	Si 001	0.59	1.87525
H 003 --	C 001	0.84	1.10517	O 001 --	Pt 002	0.45	2.03141
H 008 --	C 004	0.82	1.10737	H 001 --	O 002	0.06	2.04042
H 002 --	C 001	0.83	1.10826	O 002 --	Pt 003	0.37	2.13062
H 006 --	C 003	0.83	1.11012	O 003 --	Pt 004	0.18	2.16811
H 007 --	C 003	0.85	1.111	H 007 --	H 009	0	2.19399
H 009 --	C 004	0.82	1.11128	H 006 --	H 008	0.01	2.43374
C 003 --	O 004	0.54	1.42826	H 010 --	O 004	0	2.66991
C 004 --	O 004	0.52	1.42851	H 008 --	O 005	0	2.69322
C 006 --	O 005	0.33	1.44146	H 001 --	H 003	0	2.73519
C 005 --	O 005	0.33	1.45126	H 005 --	O 004	0	2.82144

Pt 002 --	Pt 003	0.24	2.82857	Pt 002 --	Pt 004	0.27	2.82857
Pt 001 --	Pt 003	0.25	2.82857	Pt 001 --	Pt 002	0.26	2.82857
Pt 003 --	Pt 004	0.31	2.82857	H 003 --	H 005	0.03	2.90965

According to the above tables, we can see that only one O atom of GS can make the adsorption function to PB, instead, whereas 3 O atoms can combine with Pt atoms on Pt (110) plane. The population value of a bond can represent the covalent or ionic interaction. A high value of bond population indicates a covalent bond, and a low value shows an ionic interaction. Through the comparison, the interactions between O and metal atoms tend to ionic bonds.

The adsorption energy can be calculated using the following equation:

$$E_{ad} = E_{to} - \sum_{1}^{n} E_i \qquad \text{Eq. S3}$$

Where E_{ad} is the adsorption energy; E_{to} the total energy of whole system after adsorption; $\sum_{1}^{n} E_i$ the sum energy of all single molecules before adsorption.

The calculated results were listed as follows:

	GS adsorption on PB	GS adsorption on Pt
E_{to} (eV)	-10267.4464	-6279.7985
$\sum_{1}^{n} E_i$ (eV)	-6856.4584 (PB) + -3408.1078 (GS)	-2867.5252 (Pt) + -3408.3015 (GS)
E_{ad} (eV)	-2.8802	-3.9718

These results can illustrate that the adsorption of GS on Pt surface will release more energy to make the system reach a lower energy state and accordingly, GS will prefer to combine with Pt surface.

Supplement 4 (S4). EDX analysis of PB film loaded by GOD

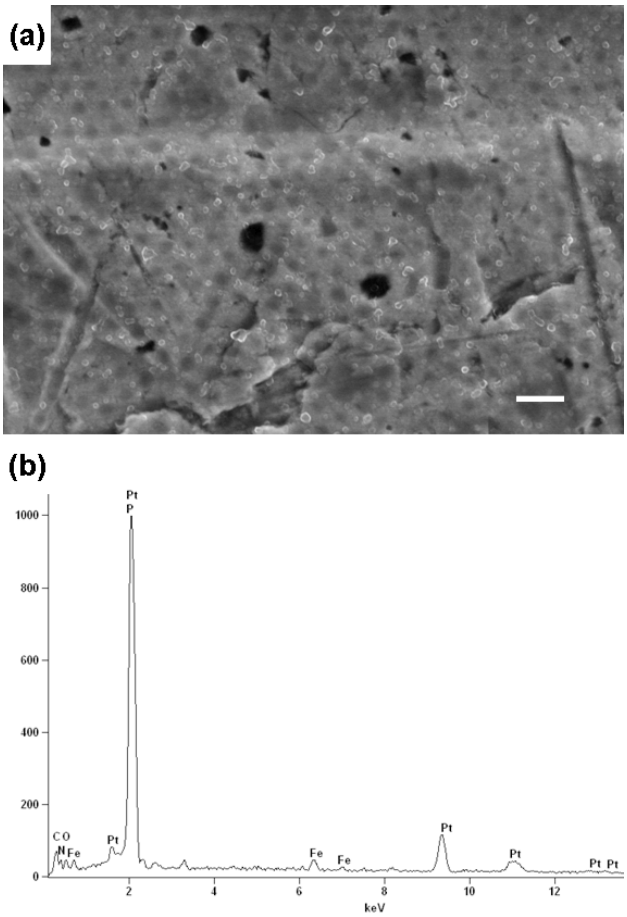

Figure S4. (a) SEM image of the film area for EDX characterization. Scale bar is 1μm. (b) Elemental analysis report of selected film surface.

We chose a large area of the prepared film (Fig. S4 (a)) which was already loaded with GOD for EDX characterization. According to the elemental analysis report (Fig. S4(b)), we can find the existence of P content. It should be noticed that there is not any P in the structures of PB and GS and therefore P atoms are from GOD which coats the surface under these conditions.

Supplement 5 (S5). The efficiency demonstration of enzyme immobilization method for prepared biosensor.

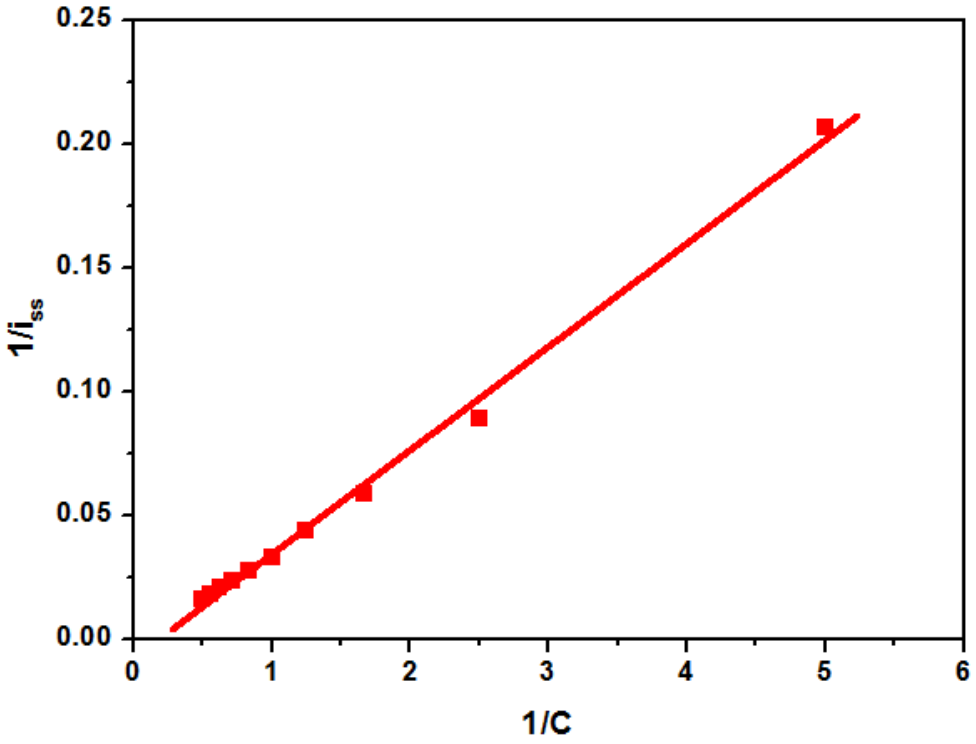

Figure S5. The diagram of fitted Lineweaver–Burk equation by using prepared glucose biosensor as a sample.

In order to demonstrate whether GS silane modification method is an effective means of enzyme loading, we introduced a constant: Michaelis-Menten constant K_M. This value represents the substrate concentration at which the reaction rate is at half-maximum, and is an inverse measure of the substrate's affinity for the enzyme. The low value shows the high affinity. We used the data from the glucose biosensor to calculate our K_M. The equation of Lineweaver–Burk (Eq. S1) should be used according to the steady state hydrodynamic amperometric results.

$$\frac{1}{i_{ss}} = \frac{1}{i_{\max}} + \frac{K_M}{i_{\max}} \frac{1}{C} \qquad \text{(Eq. S1)}$$

Fig. S5 is the fitted diagram of $\frac{1}{i_{ss}}$ vs. $\frac{1}{C}$. According to the linear data, we can obtain a K_M value of our prepared glucose biosensor of 5.28 mM.