Supplementary Information

Three‑dimensional Nitrogen-doped Graphene Structure: a High Efficient carrier of Enzyme for Biosensors

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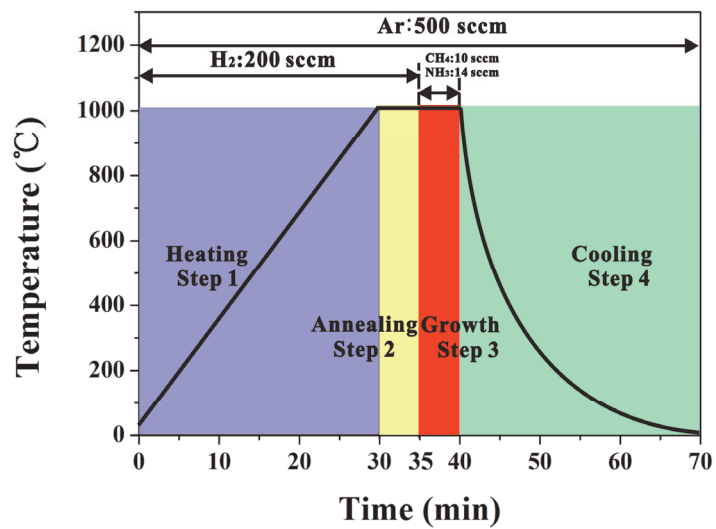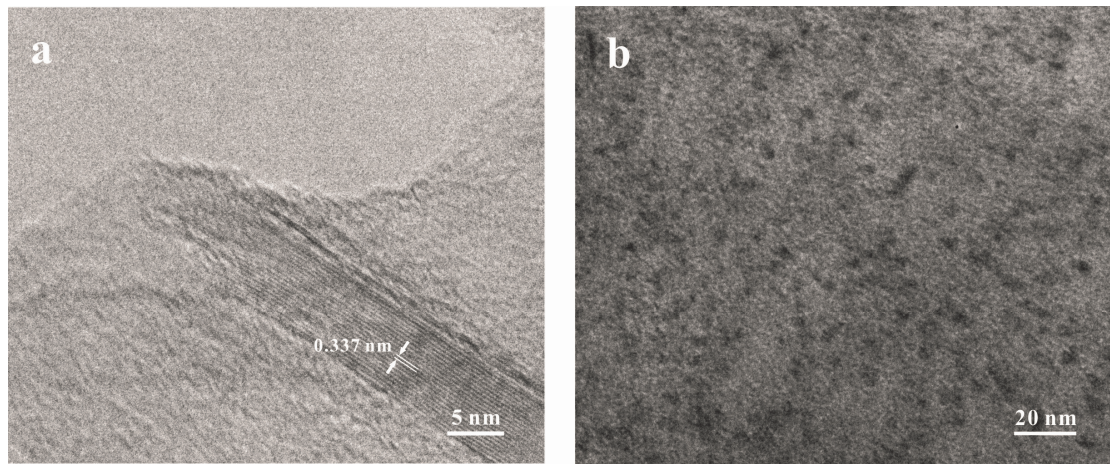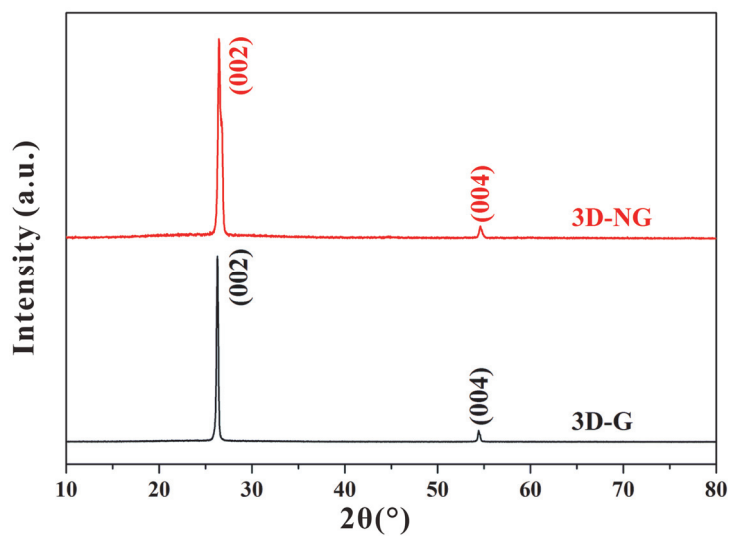## 1. The 3D–NG growth procedure.

Fig. S1. Procedures for CVD growth of 3D–NG.

2. The transmission electron microscopy (TEM) of 3D–NG/CS/GOx.

Fig. S2. TEM images of 3D–NG/CS/GOx. (a) The folded edge of the 3D–NG; (b) Many particles (~ 3 nm) were observed on the surface of 3D–NG, which should be the molecules of GOx.

3. The characterizations of X-ray diffraction (XRD) of 3D–G and 3D–NG.

Fig. S3. XRD profiles of 3D–G and 3D–NG.

For both 3D–G and 3D–NG, two typical diffraction peaks at 26.5° and 54.6° are attributed to the (002) and (004) reflections of graphitic carbon, respectively (JCPDS card 75-1621). The results indicate a high crystalline degree for 3D–G and 3D–NG.

4. The immobilization stability of 3D–NG/CS/GOx biosensor.
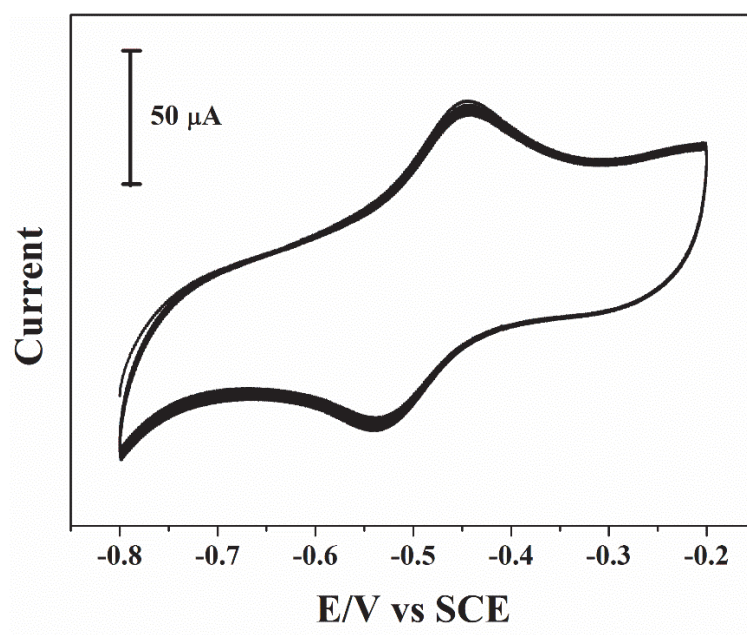
Fig. S4. Cyclic voltammograms of 3D–NG/CS/GOx in N_2-saturated 0.1 M PBS (pH = 7.40) with 21 cycles. The scan rate was 0.1 V·s^{-1}.

5. The materials facilitating the immobilization of enzymes and loaded enzymes are not unique.

In CS molecules, there are many amino groups ($-NH_2$) on the sides. These $-NH_2$ should be positively charged in pH 7.4 solution and may facilitate the adsorption of GOx (the isoelectric point of GOx is 4.2, so it should be negatively charged in the solution) on N–doped graphene via electrostatic interactions. Controlled experiments are performed to study the adsorption of GOx on 3D–NG using other charged polymers, i.e., polymine (PEI) and polyacrylic acid (PAA). PEI also has amino groups, which may have the same enhanced adsorption effect as CS. On the contrary, PAA with carboxyl groups ($-COOH$) should inhibit the adsorption of GOx by electrostatic repulsion. In Figure S5, it is clear that only CS (red curve) and PEI composite (blue curve) have obvious redox peaks. Therefore, positive charge absorption from $-NH_3^+$ is helpful to the adsorption of GOx. At the same time, the results indicate that the material facilitating the immobilization of enzymes is not unique.
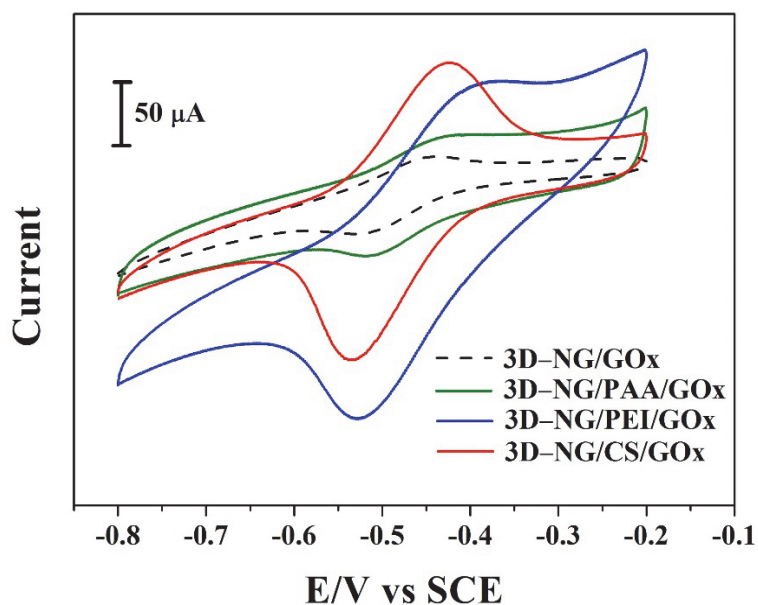
Fig. S5. Cyclic voltammograms of 3D–NG/GOx (black curve), 3D–NG/PAA/GOx (green curve), 3D–NG/PEI/GOx (blue curve) and 3D–NG/CS/GOx (red curve) in N_2-saturated 0.1 M PBS (pH = 7.40). The scan rate was 0.1 V·s^{-1}.

Other kinds of enzyme can also easily be loaded on 3D–NG with the help of

suitable materials. Based on the similar strategy, we successfully constructed 3D–NG based myoglobin (Mb) composite electrode, and studied its direct electrochemistry (Figure S6). The formal potential of the well-defined quasi-reversible redox peaks was –0.420V, which coincides with the direct electrochemistry of heme Fe(III)/Fe(II) couples in Mb molecule.[10] This characteristic indeed illustrates that 3D–NG is a universal enzyme carrier.
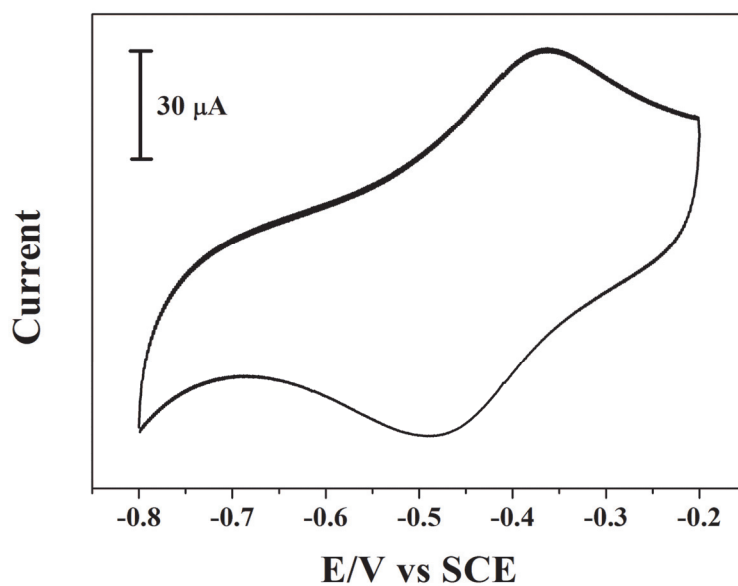
Fig. S6. Cyclic voltammograms of 3D–NG/PEI/Mb in N_2-saturated 0.05 M PBS (pH = 7.40). The scan rate was 0.1 $V \cdot s^{-1}$.

**6. Comparison of surface coverage concentration (Γ) and sensitivity between various graphene/GOx based glucose biosensors.**
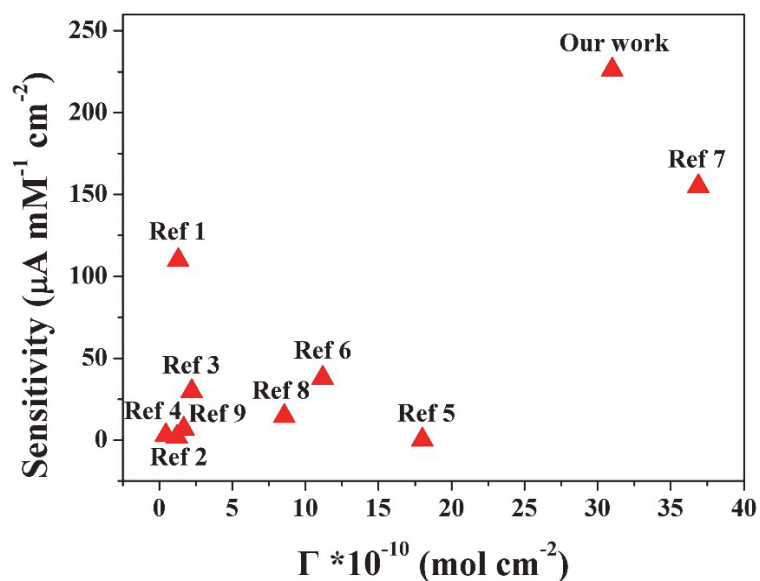
Fig. S7. Comparison of surface coverage concentration (Γ) and sensitivity between various graphene/GOx based glucose biosensors.

Table S1. Comparison of electrode materials between these graphene–based glucose biosensors.

Electrode materials	Ref.
3D–NG/CS/GOx	This work
GOx/graphene	1
GCE/RGO/GOx	2
GOx/AuNPs/G/MWCNTs	3
Nafion–MGF–GOx	4
GOx/GQD/CCE	5
GOx–graphene–CS	6
GOx/PDDA–G	7
SGN/Au/GOx	8
CS–GOx–ERO/GCE	9

7. The selectivity of 3D–NG/CS/GOx biosensor.
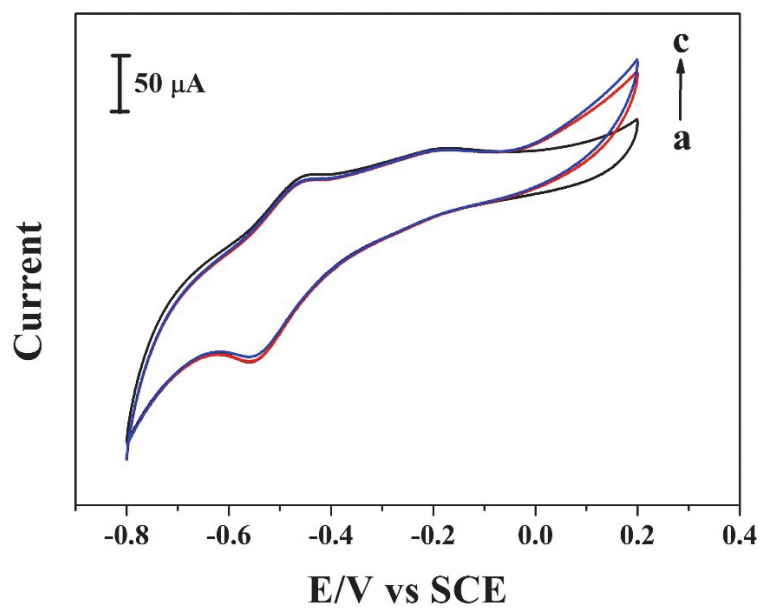
Fig. S8. Cyclic voltammograms of 3D–NG/CS/GOx biosensor in 0.1 M PBS (O_2-saturated, pH = 7.40) when added 0.1 mM glucose (curve a) and added 1.0 mM AA (curve b) and 1.0 mM UA (curve c). The scan rate was 0.1 V·s^{-1}.

References

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