

Short Communication

## Electrochemical behaviour of chloroplast, thylakoid, and PSII on the chitosan modified glassy carbon electrode

Jinglun Zhao<sup>1,\*</sup>, Suqing Zhu<sup>2,\*</sup>, Lingjuan Tang<sup>3</sup>, Hao Huang<sup>1</sup>, Mengru Ming<sup>2</sup>, Wenping Huang<sup>2</sup>, Haiying Gu<sup>1,\*</sup>, Lijun Sun<sup>2,\*</sup>

<sup>1</sup> School of Public Health, Nantong University, Nantong, Jiangsu, 226019, China.

<sup>2</sup> School of Life Sciences, Nantong University, Nantong, Jiangsu, 226019, China.

<sup>3</sup> Analysis and Testing Center, Nantong University, Nantong 226019, Jiangsu, China

\*E-mail: [Hygu@ntu.edu.cn](mailto:Hygu@ntu.edu.cn) (H.-Y. Gu), [slj.1226@163.com](mailto:slj.1226@163.com) (L.-J. Sun).

Received: 8 June 2020 / Accepted: 30 July 2020 / Published: 31 August 2020

One primary and critical issue in a photosynthesis process is the absorption, transfer, and conversion of light energy in chloroplast. Although previous studies have shown that the absorption, transfer and conversion of light energy are involved in a series of ordered pigment protein complexes, there is still lack of direct evidence on the electron transfer mechanism within a chloroplast. In this study, chloroplast, thylakoid, and photosystem II (PSII) were assembled on the chitosan modified glassy carbon electrodes to explore the corresponding photosynthetic properties. The scanning electron microscopic and UV-visible spectral analysis showed that the chloroplast, thylakoid, and PSII were successfully modified on the electrode surfaces with their photosynthetic activity. The electrochemical features of chloroplast, thylakoid, and PSII were investigated through cyclic voltammetry. The results showed that only chloroplast modified electrodes didn't present any electrochemical activity, while the chloroplast modified electrodes soaked in water (hypotonic treatment) for 1 to 3 hours presented a couple of quasi-reversible redox peaks. Both thylakoid and PSII modified electrodes without hypotonic treatment showed similar redox peaks as that of chloroplast modified one, suggesting that the electrochemical signal sources for chloroplast, and thylakoid are out of PSII. In summary, this study not only explained the direct electrochemical signal source of the chloroplast, but also provided a new strategy to study the electron transfer process inside an organelle or cell.

**Keywords:** Direct electrochemistry; Modified electrode; Chloroplast; Thylakoid; Photosystem II

### 1. INTRODUCTION

Photosynthesis is the process that green plants convert solar energy to chemical energy by synthesizing CO<sub>2</sub> and H<sub>2</sub>O into organic compounds and releasing O<sub>2</sub>. It is the largest energy and material conversion process on earth [1]. Chloroplast is an organelle responsible for photosynthesis and CO<sub>2</sub> consumption in plants and algal cells. One primary and critical issue in a photosynthesis process is the

absorption, transmission, and conversion of light energy in a chloroplast. Although previous studies have shown that the absorption, transfer and conversion of light energy are involved in a series of ordered pigment protein complexes, there is still lack of direct evidence on the electron transfer mechanism within a chloroplast [2-4].

Electrochemical methods are useful tools for revealing the corresponding nature of life processes [5-8]. In our previous studies, the haemoglobins modified with different nanoparticles were assembled on the electrodes to study their oxygen carrying characters [9,10]. The chloroplast and its protein complexes were modified on the electrodes for solar energy study [11-13]. For example, the thylakoid membrane was directly modified on the surface of a nanostructured titanium dioxide electrode for photo-electrochemical cell [11]. In addition, the chloroplast and thylakoid membranes were also used to fabricate the electrochemical sensors [14,15]. Previous studies on pigment protein complexes were more concentrated on solar cells or electrochemical sensors. There still remain certain unclear issues about the electrochemical properties of these complexes in these complexes.

In order to study the electrochemical character of chloroplast and its compositions, the chloroplast, thylakoid, and photosystem II (PSII) were independently assembled on the chitosan modified glassy carbon electrodes. The electrochemical features of chloroplast, thylakoid and PSII modified electrodes were studied through electrochemical methods. The results showed that chloroplast (with hypotonic treatment), thylakoid, and PSII have similar redox peaks, which can further be analyzed to trace the sources of the corresponding electrochemical signals.

## 2. EXPERIMENTAL

### 2.1 Reagents and materials

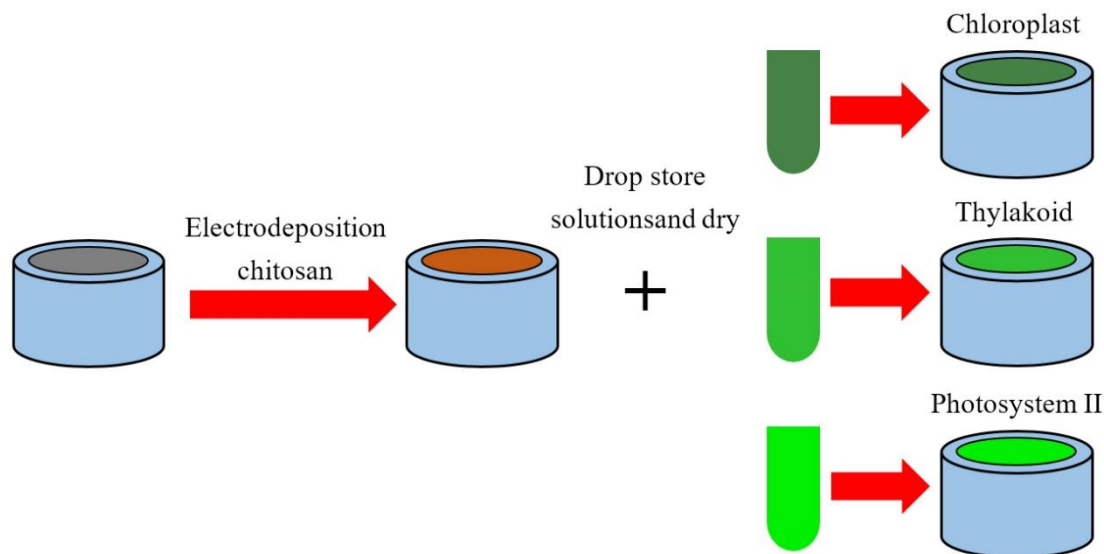
Chitosan (90% deacetylation, CS) was purchased from Shanghai Yuanye Biotech Company. Chloroplast extraction kit (BB-3622-2) was purchased from BestBio Company. The spinach was purchased at the local supermarket. Phosphate buffered saline (PBS) was prepared by mixing  $\text{Na}_2\text{HPO}_4$  and  $\text{Na}_2\text{HPO}_4$  with the pH 7.0 solution. All solutions were prepared with the quartz sub-boiling water and stored at 4°C.

### 2.2 Extraction of chloroplast, thylakoid and PSII

Chloroplast was isolated according to the method described in the chloroplast extraction kit (BestBio Co. Ltd., BB-3622-2). Thylakoid was isolated according to Chen's method with the necessary modification [16]. PSII was isolated according to the BBY method [17] with the necessary modification. All detailed procedures were put into the Supporting Information. After the extraction process, the chlorophyll concentrations in the collected chloroplast, thylakoid, and PSII sample solutions were measured with a UV-vis absorption spectrophotometer (Shimadzu, UV-2450). Afterwards, the chlorophyll concentrations were adjusted to be 2 mg/ml for further experiments.

### 2.3 The Modification of Electrodes

Bare glassy carbon electrode was firstly polished with abrasive paper and then with alumina slurry (1.0, 0.30 and 0.05  $\mu\text{m}$ ), followed by ultrasonical cleaning in ethanol and water.



**Figure 1.** Schematic diagram of electrode modification.

As shown in Fig. 1, the chitosan thin film was prepared on the polished glassy carbon electrode surface by electrodeposition in 2.0 mg/mL chitosan solution at -2.0V (vs. SCE). Before the electrode modification process of the chloroplast, thylakoid, and PSII, the chlorophyll concentrations of chloroplast, thylakoid, and PSII were measured by the UV-vis absorption spectrophotometer and all the chlorophyll concentrations were adjusted to be 2mg/ml. The chloroplast, thylakoid and PSII modified electrodes were prepared by dropping the 10  $\mu\text{L}$  store solution on the chitosan modified electrodes and dried in dark. The final electrodes were denoted as chloroplast-chitosan modified on glass carbon electrode (chloroplast/CS/GCE), thylakoid-chitosan modified glass carbon electrode (thylakoid/CS/GCE) surface, and PSII-chitosan modified glass carbon electrode (PSII/CS/GCE), respectively.

### 2.4 The Morphological Characterization of chloroplast, thylakoid, PSII, and their Modified Electrodes

The morphologies of chloroplast, thylakoid, PSII, and their modified electrode surfaces were studied by ZEISS Gemini SEM 300 field emission scanning electron microscope at Nantong university analysis and testing centre. The store solutions of chloroplast, thylakoid, and PSII were dried on the monocrystalline silicon wafers and the modified electrodes tips were placed in the cavity of the electron microscope for morphological characterization.

### 2.5 Spectral Characterization of chloroplast, thylakoid, PSII, and their Modified Electrodes

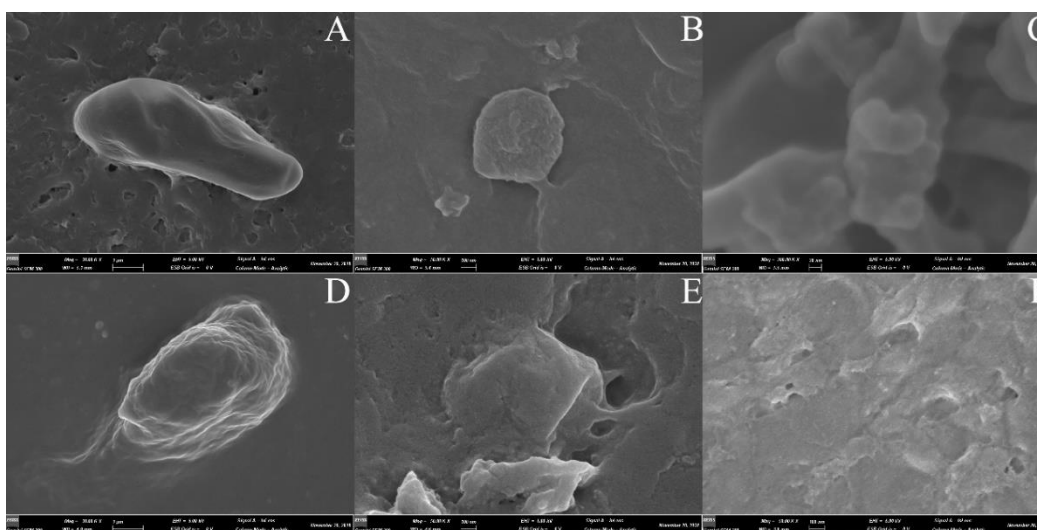
The UV-visible spectral analysis was performed with a UV-2450 SHIMADZU spectrophotometer. The spectra were recorded as a function of reaction time with a UV spectrophotometer at a resolution of 0.1 nm in the 350-750 nm range. The solutions of chloroplast, thylakoid, and PSII were placed in standard 10-mm cuvettes for UV-visible spectral analysis. The solutions of chloroplast, thylakoid, and PSII were dropped and dried on chitosan modified ITO glass for spectral UV-visible spectral analysis.

### 2.6 Electrochemical Experiment of Chloroplast, Thylakoid, and PSII

The electrochemical experiments were performed with a CHI660E electrochemical working station (CH Instruments Co., USA). The three-electrode system was composed of a modified glassy carbon electrode (diameter = 3mm), a saturated calomel electrode (SCE) as a reference electrode, and a platinum wire as an auxiliary electrode. All electrochemical experiments were carried out in a cell containing 5.0 ml of 0.1M phosphate buffer solution. Electrolytic solutions were purged with highly purified nitrogen for at least 15 min prior to the experiments and maintained in the nitrogen atmosphere. The scanning range was -0.8 V ~0.6 V (vs. SCE) and the scan rate was 0.1 V/s.

## 3. RESULTS AND DISCUSSION

### 3.1 Morphological Characterization of chloroplast, thylakoid, PSII, and their Modified Electrodes



**Figure 2.** SEM image of (A) chloroplast in the stock solution; (B) thylakoid in the stock solution; (C) PSII in the stock solution; (D) chloroplast/CS/GCE surface; (E) thylakoid/CS/GCE surface; (F) PSII/CS/GCE surface.

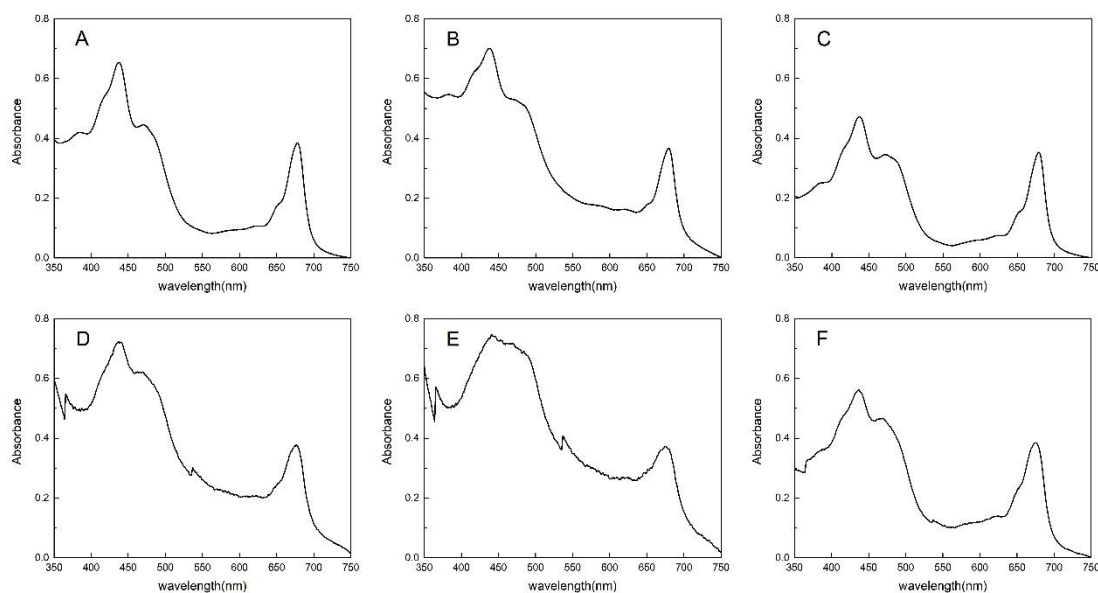
Fig. 2 showed the surface morphologies of chloroplast, thylakoid, PSII, and their modified electrodes. There was a convex lens shape object with 1~2 $\mu$ m thick and roughly 5 $\mu$ m diameter, which was consistent with description of chloroplasts in size and shape (Fig. 2A). Fig. 2D showed the

morphology of chloroplast modified electrode surface. It was observed there was an object embedded on the electrode surface.

The observed size and shape of the object were matched with those of the chloroplast in the solution and a previous research [18], which indicated the chloroplast was successfully modified on the electrode surface. In Fig. 2B and Fig 2E, the flat disc shaped objects with diameters of  $\sim 700$  nm in the thylakoid store solution and on thylakoid modified electrode surface were observed, consistent with a previous research [19]. This indicates that the thylakoid was also successfully modified on the electrode surface. Objects with sizes of 20 nm were observed for PSII modified electrode, as shown in Fig 2C. As shown Fig. 2F, on the chitosan modified electrodes where PSII was deposited, there still existed holes filled with PSII.

### 3.2 Spectral Characterization of Chloroplast, Thylakoid, PSII, and their Modified Electrodes

The spectral properties of chloroplast, thylakoid, PSII, and their modified electrodes were studied, as shown in the UV-visible spectra in Fig. 3. The results showed that the absorption wavelength ranges for chloroplast, thylakoid, and PSII (Fig. 3A to Fig 3C) were consistent with those of the previous studies [17,20,21]. As shown in Fig. 3D, Fig. 3E and Fig 3F, similar absorption peaks were observed for chloroplast, thylakoid, and PSII on the modified electrode surfaces (Fig. 3A to Fig 3C).



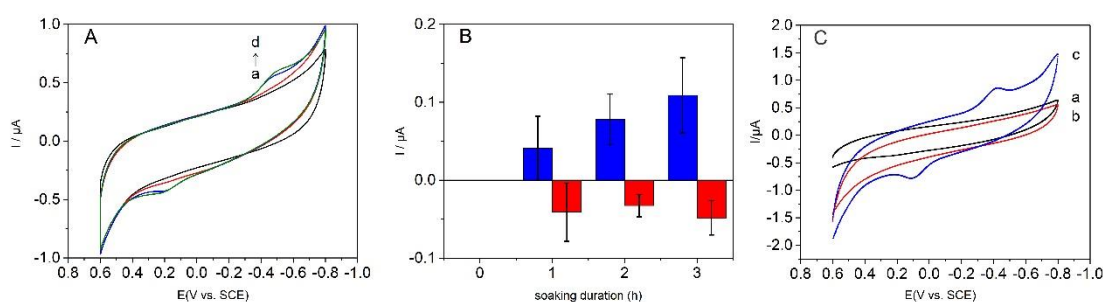
**Figure 3.** The UV-Vis absorption spectrum of (A) chloroplast solution; (B) thylakoid solution; (C) chloroplast solution; (D) chloroplast-chitosan modified ITO electrode surface; (E) thylakoid-chitosan modified ITO electrode surface; (F) thylakoid-chitosan modified ITO electrode surface.

For chloroplast modified electrode, the absorption peaks at 437 nm, 469 nm, and 680 nm were observed. For thylakoid modified electrode, the absorption peaks at 437nm, and 680nm were observed. The similar absorption peaks were also observed for PSII. All these results indicated that chloroplast,

thylakoid, and PSII may keep their biological activities on their modified electrodes, judged from their spectral characters.

### 3.3 Electrochemical Characterization of Chloroplast

Cyclic voltammetry was applied to study the electrochemical signal in PBS buffers with pH 7.0. The interesting thing was that the chloroplast modified electrode didn't show any current peak in the initial stage. After such a chloroplast modified electrode was soaked in water for 1 to 3 hours (hypotonic treatment), a couple of quasi-reversible redox peaks at -0.46 V and 0.18 V were observed, as shown in Fig 4A. As shown in Fig 4B, when the soaking time was 2 h, the peak current looked more stable. For bare GCE and CS/GCE, no redox peak could be observed under the same condition (Fig 4C).

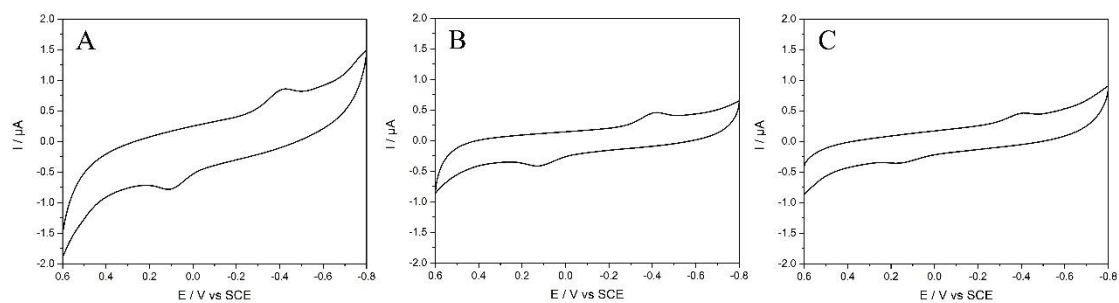


**Figure 4.** (A) Cyclic voltammograms of chloroplast/CS/GCE in pH7.0 PBS at different soaking times (a-d):0h, 1h, 2h, 3h at 0.1V/s scan rate; (B) peak current histogram of chloroplast/CS/GCE in pH7.0 PBS at different soaking times at 0.1V/s scan rate (n=3). (C)Cyclic voltammograms of (a)GCE, (b)CS/GCE, (c) chloroplast/CS/GCE in pH 7.0 PBS at 0.1V/s scan rate

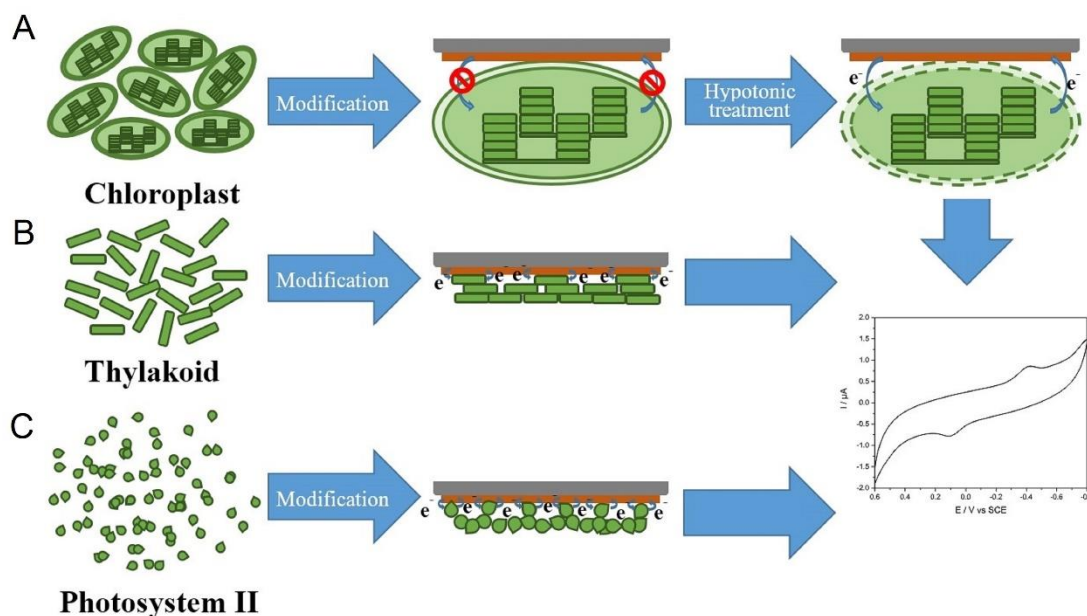
As is known, it is difficult to directly acquire the electrochemical response from chloroplast. Considering the structure of chloroplast [22] and the water soaking process, we suspected that the envelop membrane of chloroplast maybe block the electron transfer from inside to outside. However, the conductivity of the envelop membrane of chloroplast was improved after hypotonic treatment so that the electron transfer feature could be captured on the electrode surface.

### 3.4 Direct Electrochemical Signal Source in Chloroplast

So the question arises, where was the electron of the chloroplast from? Since thylakoid is the key part of chloroplast in photosynthesis and PSII is the key composition of thylakoid, thylakoid and PSII of chloroplast were selected for the further study.



**Figure 5.** Cyclic voltammograms of (A) chloroplast/CS/GCE, (B) thylakoid/CS/GCE, (C) PSII/CS/GCE, in pH7.0 PBS at 0.1V/s scan rate



**Figure 6.** Schematic diagram of electron transfer on the chloroplast/CS/GCE, thylakoid/CS/GCE and PSII/CS/GCE

As showed in Fig 5, a pair of similar irreversible redox peaks in cyclic voltammetric curves appeared in the chloroplast modified electrode with hypotonic treatment, and thylakoid/PSII modified electrodes without hypotonic treatment, respectively. In previous studies, lots of materials were modified on electrodes which can improved the electron transfer ability, such as polymer [23,24] and nanomaterials [25]. Such modifications can achieve better reversibility, but may cause structural and functional deterioration of chloroplast, thylakoid and PSII. Because chitosan has excellent biocompatibility [26], the experimental result with respect to our modification may be more related to chloroplast's nature status. It was observed that the maximum for the reduction peaks was about -0.40 V, and the maximum for the oxidation peaks was about 0.10 V. The results showed that the electrochemical properties of chloroplast, thylakoid, and PSII have inheritance and the electrochemical signal source of chloroplast and thylakoid was from PSII. In previous studies, the cyclic voltammetric results of cytochrome  $c_6$ , photosystem I, plastoquinone, and plastocyanin were very different from those

of our chloroplast, thylakoid, and PSII [27-30], which indicated that only the key of the electrochemical activity should be PSII in our experiment.

#### 4. CONCLUSIONS

In this study, chloroplast, thylakoid, and PSII were assembled on the chitosan modified glassy carbon electrodes with photosynthetic activity. The electrochemical features of chloroplast, thylakoid, and PSII were studied through cyclic voltammetry. The results showed that the chloroplast didn't have any electrochemical activity, while hypotonic chloroplast showed the similar electrochemical activity as thylakoid and PSII. It is well known that phospholipid membrane is not a good conductive structure. The envelope membrane of chloroplast included two layers of phospholipid membranes, which blocked the electron transfer from inside to outside (Fig 6A). The hypotonic process improved the envelope membrane conductivity of chloroplast and accomplished the electron transfer from inside to outside. The electron transfer for thylakoid from inside to outside could be obtained directly when thylakoid was modified on the electrode because there was only a layer of the thylakoid membrane (Fig 6B). There was barely no membrane structure to undermine electron transfer when PSII was modified on the electrode (Fig 6C). Our results suggested that the electrochemical signal source of hypotonic chloroplast, and thylakoid was from PSII. In summary, this study not only explored direct electrochemical signal source of the chloroplast, but also provided a new strategy to study the electron transfer process inside an organelle or cell.

#### SUPPLEMENTARY INFORMATION

The extraction of chloroplast, thylakoid, and photosystem II

Chloroplast was isolated according to the method described in the chloroplast extraction kit (BestBio Co. Ltd., BB-3622-2). Briefly, the spinach leaves were washed with doubly distilled water and cut into small fragments. The small fragments were ground into the paste and mixed with the PBS solution (4 °C, pH 7.0) for further pestling until there were no visible solid ingredients in the mortar. After pestling, the mixture was put in a refrigerator (4°C) for 2 h. The supernatant was then centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was discarded and the remaining precipitates were re-suspended in the extraction solution A (BestBio Co. Ltd., BB-3622-2). The solution was again centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was then discarded. The remaining precipitates were re-suspended with the extraction solution B (BestBio Co. Ltd., BB-3622-2) and shaken for 20 min. The obtained solution was centrifuged at 200 rpm for 2 min at 4 °C and the precipitates were discarded. Finally, the supernatant was centrifuged at 3000 rpm for 10 min at 4 °C. The precipitates were collected and dispersed in the stock solution for the further experiment.

Thylakoid were isolated according to Chen's method with small modification [1]. Briefly, 1 g of spinach leaves was washed with doubly distilled water, scraped and ground into the paste. The paste was mixed with 5 ml of the pre-cooled grinding buffer solution and ground for 30 min in an ice-cold box. After that, 5 ml of the pre-cooled grinding buffer solution was added. The mixture was further ground



for 20 min and then filtered with two layers of nylon mesh (20  $\mu\text{m}$  pore size) and centrifuged at 1000 rpm at 4 °C for 5 min. The precipitates were re-suspended in the grinding buffer solution and centrifuged again at 1000 rpm for 5 min at 4 °C to obtain the precipitates. The precipitates were resuspended in 4 ml of shock buffer and centrifuged at 5000 rpm for 5 min at 4 °C. The precipitates were then resuspended in 2 ml of stock buffer and centrifuged at 5000 rpm for 5 min at 4 °C. The same procedure was repeated for twice. The finally collected precipitates were re-suspended in 1 ml of the stock buffer solution and stored at 4 °C for the further experiment.

PSII was isolated according to the BBY method [2] with small modification. Briefly, 50 g of spinach leaves were washed with doubly distilled water and put in the refrigerator (4 °C) for 30 min. The cooled spinach leaves were mixed with 150 ml of the BBY-1 solution and ground into the paste in a pre-cooled blender (Maierkang Environmental Technology Co., Ltd., Aoke-A300). The homogenates were filtered through screen mesh and centrifuged at 6000 rpm for 10 min at 4 °C. The precipitates were resuspended in 40 ml of the BBY-1 solution and centrifuged at 400 g for 1 min at 4 °C. After that, the supernatant was centrifuged at 35000 rpm for 7 min at 4 °C. The precipitates were re-suspended with 20 ml BBY-2 solution and centrifuged at 35000 rpm for 7 min at 4 °C to obtain the precipitates. The precipitates were re-suspended in 3.5 ml of BBY-3 solution and the mixture was measured by spectrometer for determining the chlorophyll concentration. After that, the BBY-3 solution was added to adjust the chlorophyll concentration to be 2.5 mg/ml. Afterwards, 1 ml of BBY-3T solution was slowly added into 4 ml of BBY-3 and stirred for 10 min. The mixture was centrifuged at 35000 rpm for 15 min at 4 °C to obtain the precipitates. The precipitates were re-suspended in 20 ml of BBY-3 solution and centrifuged at 3000 rpm for 3 min at 4 °C. After that, the supernatant was centrifuged at 35000 rpm for 20 min at 4 °C. After centrifuging, if the supernatant was green, the pellets were re-suspended in 20 ml of BBY-3 solution and centrifuged at 35000 rpm for 20 min at 4 °C again. Otherwise the precipitates were re-suspended in 5 ml of BBY-4 solution. The mixture was frozen in liquid nitrogen for 1 min and store at -80 °C for the further experiment. Because the sucrose and ethylene glycol in BBY-4 solution will obstruct the electrode modification process, 0.2 ml of PSII store solution was centrifuged at 35000 rpm for 20 min at 4 °C to obtain the precipitates and then re-suspended with 0.2 ml of BBY-4E (BBY-4 without sucrose and ethylene glycol) before each PSII modification process.

#### ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Nos:21874077, 31770399 and 21475070), Key R&D projects of Jiangsu Province (No: BE 2019690), the Science and Technology R&D Fund of Nantong City (XG202008-02), Six talent peaks project in Jiangsu Province (No: SWYY-061), Jiangsu Government Scholarship for Overseas studies (No:JS-2018-205).

#### References

1. M. F. Hohmann-Marriott, R. E. Blankenship, *Annu. Rev. Plant Biol.*, 62 (2011) 515.
2. M. Iwai, K. Takizawa, R. Tokutsu, A. Okamuro, Y. Takahashi, J. Minagawa, *Nature*, 464 (2010) 1210.
3. H. Takahashi, S. Clouez, F.A. Wollman, O. Vallon, F. Rappaport, *Nat. Commun.*, 9 (2013) 1954.
4. N. Nelson, A. Ben-Shem, *Nat. Rev. Mol. Cell Biol.*, 12 (2004) 971.
5. V. Fourmond, C. Léger, *Adv. Biochem. Eng./Biotechnol.*, 158 (2016) 1.
6. T. Sierra, A.G., Crevillen A, *Electrophoresis*, 38 (2017) 2695.

7. Y. Zang, J. Fan, Y. Ju, H. Xue, H. Pang, *Chemistry*, 24 (2018) 14010.
8. J. Ihssen, A. Braun, G. Faccio, K. Gajda-Schranz, L. Thöny-Meyer, *Curr. Protein Pept. Sci.*, 15 (2014) 374.
9. L. Sun, X. Liu, Q. Sun, M. Cai, J. Zhou, Z. Dong, N. Bao, H. Gu, H. He, *Appl. Surf. Sci.*, 363 (2016) 566.
10. L. Sun, Y. Lu, Z. Pan, T. Wu, X. Liu, N. Bao, C. Yu, H. He, H. Gu, *RSC Adv.*, 6 (2016) 59984.
11. S. Kavadiya, T.S. Chadha, H. Liu, V.B. Shah, R.E. Blankenship, P. Biswas, *Nanoscale*, 8 (2016) 1868.
12. G. LeBlanc, E. Gizzie, S. Yang, D.E. Cliffler, G.K. Jennings, *Langmuir*, 37 (2014) 10990.
13. W. Wang, Z. Wang, Q. Zhu, G. Han, C. Ding, J. Chen, J. Shen, C. Li, *Chem. Commun.*, 51 (2015) 16952.
14. Y. Zhang, G. Gao, Q. Qian, D. Cui, *Nanoscale Res. Lett.*, 7 (2012) 475.
15. F. Bettazzi, S. Laschi, M. Mascini, *Anal. Chim. Acta.*, 589 (2007) 14.
16. Y.E. Chen, S. Yuan, W.P. Schröder, *Physiol. Plant.*, 156 (2016) 3.
17. D.A. Berthold, G.T. Babcock, C.F. Yocum, *FEBS Lett.*, 134 (1981) 231.
18. Douglas C. Dahn, Katrina Cake, Lawrence R. Hale, *Ultramicroscopy*, 3 (1992) 1222.
19. D.H. Ryu, Y.J. Kim, S.I. Kim, H. Hong, H.S. Ahn, K. Kim, W.H. Ryu, *Nanomaterials*, 8 (2018) 189.
20. M.N. Merzlyak, O.B. Chivkunova, T.V. Zhigalova, K.R. Naqvi, *Photosynth. Res.*, 102 (2009) 31.
21. R. Goss, C. Wilhelm, G. Garab, *Biochim. Biophys. Acta.*, 1457 (2000) 190
22. A. Mechela, S. Schwenkert, J. Soll, *Open Biol.*, 1 (2019) 180237.
23. H. Hamidi, K. Hasan, S. C. Emek, Y. Dilgin, H.E. Åkerlund, P.Å. Albertsson, D. Leech, L. Gorton, *ChemSusChem*, 8 (2015) 990-993.
24. E. Çevi, M. Titiz, M. Şenel, *Electrochim. Acta*, 291 (2018) 41.
25. J. O. Calkins, Y. Umasankar, H. O'Neill, R. P. Ramasamy, *Energy Environ. Sci.*, 6 (2013) 1891.
26. C.K.S. Pillai, W.Paul, C. P. Sharma, *Prog. Polym. Sci.*, 34 (2009) 641.
27. K. Hasan, Y. Dilgin, S. Emek, M. Tavahodi, H. Åkerlund, P. Albertsson, L. Gorton, *ChemElectroChem*, 1 (2014) 131.
28. V. Proux-Delrouyre, C. Demaille, W. Leibl, P. Sétif, H. Bottin, C. Bourdillon, *J. Am. Chem. Soc.*, 125 (2003) 13686.
29. D. Marchal, W. Boireau, JM. Laval, J. Moiroux, C. Bourdillon, *Biophys. J.*, 72 (1997) 2679.
30. J. Olloqui-Sariego, A. Díaz-Quintana, M. Rosa, J. Calvente, I. Márquez, I. Díaz-Moreno, R. Andreu, *Bioelectrochemistry*, 124 (2018) 127.