Electronic Supplementary Information

A high performance metal-free hydrogen-evolution reaction electrocatalyst from bacterium derived carbon

Li Wei,[†] Huseyin Enis Karahan,[†] Kunli Goh,[†] Wenchao Jiang,[†] Dingshan Yu,[†] Özgür Birer [‡,#] Rongrong Jiang,[†] and Yuan Chen *,[†]

[†]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459, Singapore.

[‡]Chemistry Department, Koç University, Rumelifeneri Yolu, Sarıyer, 34450, Istanbul, Turkey

[#]KUYTAM Surface Science and Technology Center, Koç University, Rumelifeneri Yolu, Sarıyer, 34450, Istanbul, Turkey

*To whom correspondence should be addressed.

Tel.: +65 63168939. E-mail: chenyuan@ntu.edu.sg (Yuan Chen)

1. Synthesis of carbon catalysts

Staphylococcus aureus (*S. aureus,* ATCC® 6538^{TM}) cells were obtained from American Type Culture Collection. They were cultured in lysogeny broth (LB) media at 37 ℃ for 10 h. Cell concentration was monitored by the optical density of cell aqueous suspension at 600 nm (OD_{600}) using an absorption spectrometer (Cary 5000, Varian). The cultured cells were collected at their plateau growth stage by centrifugation (Kubota, Model 2420, 2,600 *g*, 10 min). The collected cell pellets were washed with 0.1 wt% saline solution 5 times and then deionized (DI) water 3 times to remove remaining growth media. Afterwards, they were dried by lyophilization (Sigma, Model Alpha 2-4 LSC).

The freeze-dried cells (100 mg) were loaded in a ceramic boat in a quartz tube reactor. They were carbonized at 900 ℃ in flowing Ar (100 sccm, 99.99%, Soxal) for 3 h. The resulting material was bathed in HCl solution (1 M) for 2 h to remove possible metal residues (such as Na, K and Ca), and then recovered by centrifugation (2600 *g*). Afterwards, it was washed with DI water several times before dried in a vacuum oven for 8 h. The obtained material was about 20 mg (about 20% production yield from the freeze-dried cells). This sample was denoted as SA900.

In order to generate mesopores in carbon materials, the freeze-dried cells were mixed with $ZnCl_2$ (99.99% metal basis, Alfa-Aesar) at the weight ratio of 1:4. The mixture was then carbonized at 900 ℃ in flowing Ar (100 sccm, 99.99%, Soxal) for 3 h. The resulting material was bathed in HCl solution (1 M) for 2 h, and further washed by DI water. Afterwards, it was dried in a vacuum oven. This material was denoted as SA900Z.

The cathodic activation was performed by continuously discharging a working electrode loaded with SA900Z at -2 V *vs.* saturated calomel electrode (SCE) for 4 h in H_2SO_4 (0.1 M). A Pt wire or a graphite rod was used as the counter electrode and the HER performance of carbon catalysts resulted from both counter electrodes did not show much difference. The resulting material was denoted as SA900ZC. The electrode was immersed in DI water (50 mL) with stirring for 3 times to clean the material before it was used for HER performance testing and other characterization. After the cathodic activation, the H_2SO_4 electrolyte solution was also centrifuged at 50,000 *g* for 30 min. No solid residues were found. Thus we assumed the weight loading of SA900ZC on the electrode was similar to that of the SA900Z electrode.

Furthermore, reduced graphene oxide (rGO) and nitrogen doped graphene (NG) were also prepared from graphene oxide (GO) as reference catalysts. GO was synthesized following a modified Hummers' method reported elsewhere.[1] GO was carefully dialyzed to remove metal ion contamination before use. rGO was obtained by first hydrothermal treatment of GO solution (1 mg/mL) at 180 ℃ for 12 h. The resulting rGO hydrogel was then freeze-dried, and further thermally reduced at 900 ℃ in flowing Ar (100 sccm, 99.99%) for 3h. NG were prepared by a similar procedure as that used for rGO, except that urea (500:1 in weight ratio over GO) was added in the hydrothermal treatment.

2. Physicochemical characterization of carbon catalysts

Physicochemical properties of the synthesized carbon materials were characterized by a comprehensive set of techniques. The surface morphology was examined by field emission scanning electron microscope (FE-SEM, Jeol, JSM-6700F). Elemental analysis

was carried out by Energy-dispersive X-ray spectroscopy (EDX) using the same FE-SEM. Their specific surface areas were measured using a surface area analyzer (QuantaChrome, Autosorb-6B) and calculated by the Brunauer–Emmett–Teller (BET) method. Pore diameter distribution was calculated from the N_2 physisorption isotherms using the Barrett-Joyner-Halenda (BJH) method. Raman spectra were recorded on a Raman microscope (Renishaw inVia) in the backscattering configuration under a 633 nm (1.96 eV) laser. The chemical composition of the material surfaces and heteroatoms was analyzed by X-ray photoelectron spectroscopy (XPS, Thermo Scientific, K-Alpha spectrometer) equipped with an Al-Kα (1486.3 eV) radiation source.

3. Cell fixation for SEM

Cell fixation was performed by drop casting a drop of bacteria saline suspension on a cleaned Si wafer. After drying, small amount of DI water was applied to remove salts. The wafer was then immersed in 2.5% glutardehyde water solution for 60 min at room temperature. After washing with DI water and drying, cells were dehydrated by 5 min washing using 50, 60, 70, 80 and 90 wt% ethanol water solution consecutively and finally immerse in absolute ethanol for 10 min. After drying under ambient environment, the fixed cell was sputtered with Pt for SEM observation. *S. aureus* cells are spherical in shape with an average diameter ~0.5 μm.

4. Preparation of Electrodes

Carbon materials were firstly bath sonicated in ethanol solution for 30 min to make a homogenous dispersion at a concentration of 1 mg/mL (weighted by a precision balance,

XPE205, Mettler Toledo). 30 μL of the dispersion was drop-casted on a pre-polished glassy carbon (GC) rotary disk electrode (RDE, d_t = 5 mm, area = 0.19625 cm^2). A similar mass loading (~0.030 mg or a mass density of ~0.152 mg/cm^2) was used for every carbon catalyst. After drying in an ambient environment, a drop of Nafion ethanol/water solution (0.5 wt%) was added. Afterwards, dried electrodes were used for electrochemical performance tests. Commercial 20 wt% Pt/C catalyst (Aldrich) was also loaded on GC electrode at similar mass density as a reference sample. At least five electrodes were prepared and tested for each carbon catalysts to ensure the reproducibility of results.

5. Electrochemical measurements

All electrochemical measurements were carried out using a potentiostat (CHI 660D) in a three-electrode configuration, with a graphite rod as the counter electrode, a SCE as the reference electrode and a carbon catalyst loaded GC electrode as the working electrode. An electrode rotator (MSRX, Pine Instrument) was used for the rotating disk electrode measurement. HER experiments were carried out in an acidic medium (0.5 M H$_2$SO$_4$). The electrolyte was purged by N$_2$ gas (99.99%, Alphagaz One, Soxal) for 60 min to remove dissolved O$_2$ before each test. The linear sweep voltammetry (LSV) of each electrode was recorded at a scan rate of 2 mV/s at 1600 rpm to remove gas (H$_2$) bubbles generated during the scan. Durability test was performed by cyclic voltammetry (CV) scanning of the electrode from 0 to -0.8 V *vs.* SCE at a scan rate of 50 mV/s for 5000 cycles. The LSV of the electrode before and after the CV scan was recorded for comparison. Electrochemical impedance spectroscopy (EIS) of each carbon catalysts was

recorded on a potentiostat (VersaSTAT 3, Princeton Applied Research) from 10^6 to 0.1

Hz with the amplitude of 0.02 V. All reported potential was against a reversible hydrogen

electrode (RHE) by adding 0.2444 + 0.0591×pH to the potential measured against the

SCE.

6. Exchange current density calculation

The exchange current density (j_0) was obtained from extrapolation of the Tafel plots

by fitting the linear part of the Tafel plot using the following equation:

$$\eta = b \log j + a$$

Where b is the Tafel slope and a is the intercept on the y-axis. The obtained Tafel

equations for the carbon catalysts are listed below:

SA900: $\quad \eta = 0.0884 \log j + 0.309$

SA900Z: $\quad \eta = 0.0802 \log j + 0.262$

SA900ZC: $\quad \eta = 0.0584 \log j + 0.103$

The value of j_0 is determined when $\eta = 0$ V.

7. Normalization of j_0 for comparison

In order to compare j_0 of different catalysts more accurately, both the mass loading of

catalysts and their effective electrochemical surface area should be considered. HER is a

typical surface area, thus the effective electrochemical surface area could be evaluated by

measuring the electrochemical double layer capacitance (C_{dl}) of a catalyst. C_{dl} (mF/cm^2)

was measured by the CV scan method. [2,3] The CV curves were recorded at different scan

rates in the potential window of 0.15-0.25 V *vs* RHE in a Na_2SO_4 electrolyte (0.5 M). The

narrow potential window avoids generating Faradic currents. The difference between

forward and backward CV scans ($\Delta j = |j_a - j_c|$) at 0.2 V *vs*. RHE was plotted against the CV scan rate, and the data were fitted linearly by the equation $\Delta j = a \cdot Rate_{CV} + b$, while the value of the slope (a) is twice of the C_{dl} value. (see Figure S1).

The value of C_{dl} was normalized to mass by dividing it using the mass loading of each catalyst (μg/cm^2). The mass C_{dl} value of SA900ZC (0.085 mF/μg) was set as the standard to divide other catalysts to obtain the normalized mass C_{dl}. j_0 normalized by mass and area was then obtained by dividing the j_0 (by area) with the normalized mass C_{dl} for each catalyst. The results are listed in Table S3. This method of normalizing j_0 has been used in several previous studies.[2,3]
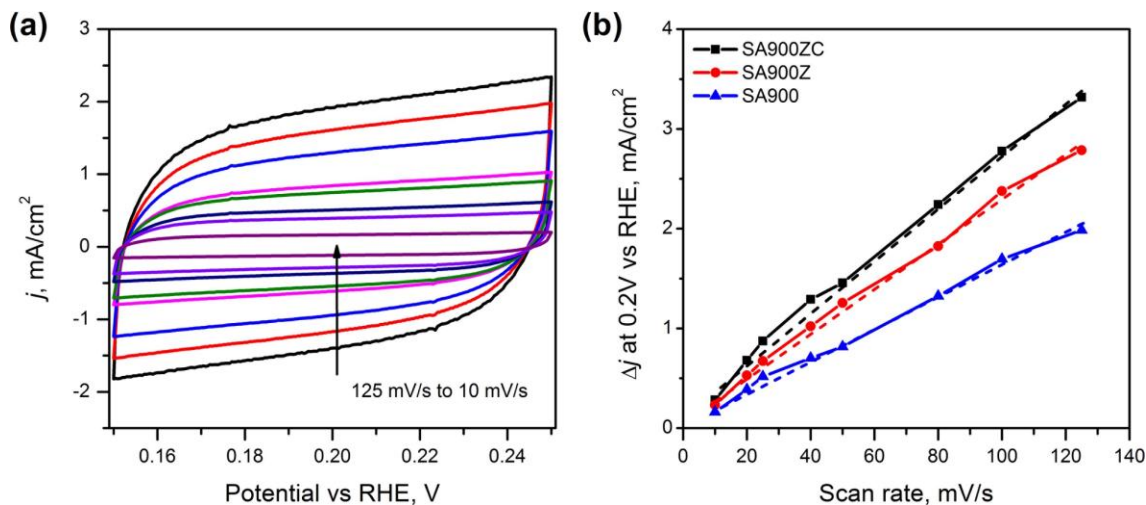
Figure S1. (a) CV scans of SA900ZC at different scan rates between 0.15 to 0.25 V (*vs* RHE). (b) The correction between Δj at 0.2 V *vs*. RHE and CV scan rate and their linear fittings.
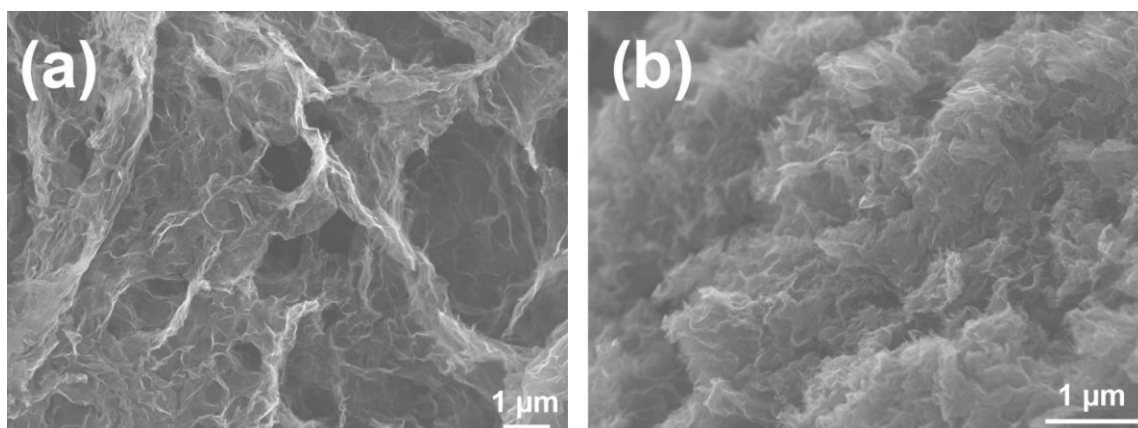
Figure S2. SEM images of (a) rGO and (b) NG. Both samples have porous stacked graphene layer structures.

Table S1. Physiochemical properties of the bacterium (*S. aureus*) derived carbon materials.

|	BET surface area, m^2/g	Pore volume, cm^3/g	Raman I_D/I_G ratio	C, at%	O, at%	N, at%	P, at%
SA900	341.4	0.303	1.056	81.62	9.27	4.88	4.23
SA900Z	816.4	1.043	1.064	81.46	10.66	3.97	3.91
SA900ZC	-	-	1.142	79.3	14.41	3.22	3.07
rGO	338.6	0.115	1.196	88.76	11.24	-	-
NG	274.6	0.097	1.379	85.36	9.19	5.45	-

Table S2. HER performances of the carbon catalysts reported in this study and other metal-free catalysts in the literatures.

Carbon catalyst	Onset μ, mV [a]	Tafel slope, mV/dec	Geometric current density at specific overpotential	j_0, $\times 10^{-3}$ mA/cm^2	Ref.
N-graphene	330	116	10 mA/cm^2 at 490	0.0704	3
P-graphene	370	133	10 mA/cm^2 at 553	0.00897	3
N,P-graphene	~260	91.0	10 mA/cm^2 at 420 mV	0.24	3
N,S-graphene	130	81	10 mA/cm^2 at 276 mV	8.4	4
C$_3$N$_4$@NG	~160	51.5	10 mA/cm^2 at 240 mV	0.35	5
C$_3$N$_4$@G	80	54	10 mA/cm^2 at 207 mV	39.8	6
p-MWCNT-ao-cp	100	71.3	2 mA/cm^2 at 280 mV	16	7
144 h-activated SWCNT	30	N/A	30 mA/cm^2 at 200 mV	N/A	8
C$_{60}$(OH)$_8$	110	78	2 mA/cm^2 at 280 mV	0.7	9
rGO	480	N/A	N/A	0.003	This work
NG	316	506	10 mA/cm^2 at 506 mV	0.08	This work
SA900	254	88.4	10 mA/cm^2 at 419 mV	0.32	This work
SA900Z	236	80.2	10 mA/cm^2 at 387 mV	0.54	This work
SA900ZC	76	58.4	10 mA/cm^2 at 204 mV	17.23	This work

Table S3. Normalized exchange current densities to catalyst electrochemical active surface area on various nanostructured catalysts.

Catalyst	Loading, μg/cm^2	j_0, mA/cm^2	C_{dl}, by geometric mF/cm^2	C_{dl}, by mass, mF/μg	Normalized C_{dl} by mass*	Normalized j_0, by mass and area, mA/cm^2	Ref
N,P-graphene	200	2.4×10^{-4}	10.6	0.053	0.63	3.83×10^{-4}	3
N,S-graphene	N/A	8.4×10^{-3}	N/A	N/A	N/A	N/A	4
C$_3$N$_4$@NG	100	3.5×10^{-4}	5.0	0.050	0.59	5.92×10^{-4}	5
C$_3$N$_4$@G	143	3.98×10^{-2}	13	0.091	1.06	3.70×10^{-2}	6
C$_{60}$(OH)$_8$	2	7×10^{-4}	N/A	N/A	N/A	N/A	9
MoS$_2$ nanosheet	285	1.26×10^{-2}	33.7	0.118	1.40	9.01×10^{-3}	10
MoS$_2$/graphene	210	3.0×10^{-3}	10.4	0.050	0.59	5.121×10^{-3}	11
Nanostructured MoS$_2$	60	6.9×10^{-4}	4.8	0.080	0.95	7.29×10^{-4}	12
SA900	152	3.2×10^{-4}	7.26	0.048	0.56	5.67×10^{-4}	This work
SA900Z	152	5.4×10^{-4}	11.03	0.073	0.86	6.29×10^{-4}	This work
SA900ZC	152	1.72×10^{-2}	12.85	0.085	1	1.72×10^{-2}	This work

*Experimental procedure session for the detailed calculation method.
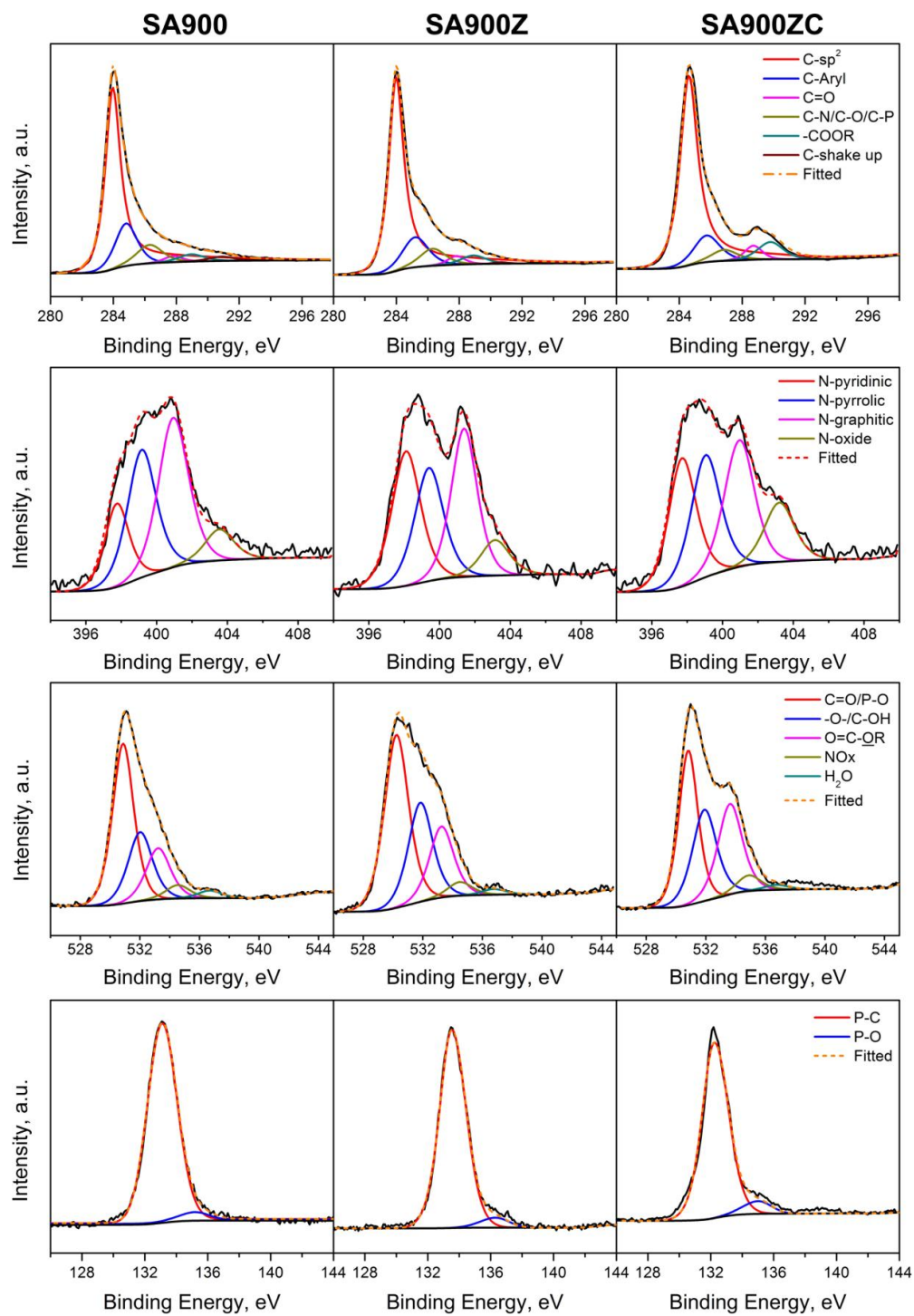
Figure S3. High-resolution XPS spectra of C, O, N and P in (a) SA900, (b) SA900Z and (c) SA900ZC.

Table S4. Elemental composition and heteroatom bonding status of the different carbon samples (XPS)

		B.E., eV	Relative abundance, %			
			SA900	SA900Z	SA900ZC	NG
N	Pyridinic-	398.3 [a]	18.30	32.25	28.07	35.43
	Pyrrolic-	399.8 [a]	33.21	27.14	28.24	29.01
	Graphitic-	401.3 [a]	40.39	32.1	29.74	27.44
	Oxidized-	403.1 [a]	8.10	8.52	13.95	8.13
O	-C=O/P-O	530.8 [b]	49.73	47.70	37.69	-
	C-O/-O-	532.1 [b]	24.52	27.36	28.09	-
	O=C-<u>O</u>R	533.3 [b]	18.22	19.58	27.85	-
	NO_x	534.6 [b]	4.81	3.78	4.82	-
	Chemisorbed H_2O	536.7 [b]	2.72	1.57	1.54	-
P	P-C	132.9 [c]	96.01	94.94	92.85	-
	P-O	135.2 [c]	3.99	5.06	7.15	-

a. Ref [13,14]; b. Ref [15,16]; c. Ref [17]
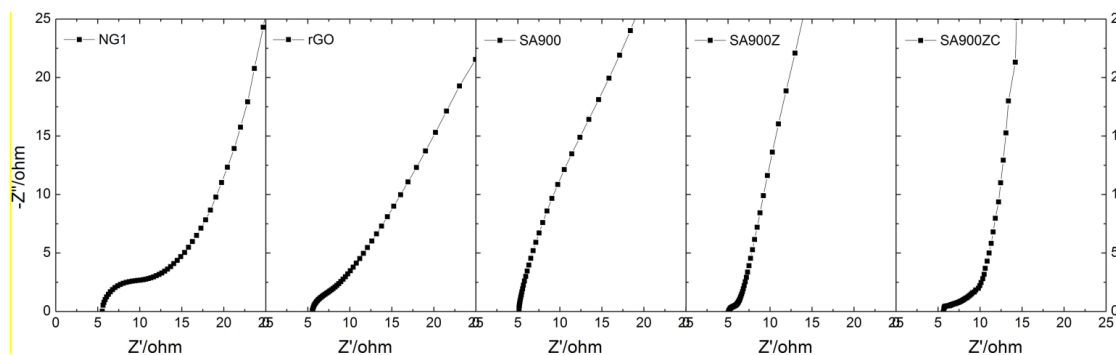
Figure S4. EIS of the samples prepared here.

References

(1) Hummers, W. S.; Offeman, R. E. *J. Am. Chem. Soc.* **1958**, *80*, 1339.

(2) Lukowski, M. A.; Daniel, A. S.; Meng, F.; Forticaux, A.; Li, L.; Jin, S. *J. Am. Chem. Soc.* **2013**, *135*, 10274.

(3) Zheng, Y.; Jiao, Y.; Li, L. H.; Xing, T.; Chen, Y.; Jaroniec, M.; Qiao, S. Z. *ACS Nano* **2014**, *8*, 5290.

(4) Faber, M. S.; Dziedzic, R.; Lukowski, M. A.; Kaiser, N. S.; Ding, Q.; Jin, S. *J. Am. Chem. Soc.* **2014**, *136*, 10053.

(5) Zheng, Y.; Jiao, Y.; Zhu, Y.; Li, L. H.; Han, Y.; Chen, Y.; Du, A.; Jaroniec, M.; Qiao, S. Z. *Nat Commun* **2014**, *5*, 3783.

(6) Zhao, Y.; Zhao, F.; Wang, X.; Xu, C.; Zhang, Z.; Shi, G.; Qu, L. *Angew. Chem. Int. Ed.* **2014**, n/a.

(7) Cui, W.; Liu, Q.; Cheng, N.; Asiri, A. M.; Sun, X. *Chem Commun (Camb)* **2014**, *50*, 9340.

(8) Das, R. K.; Wang, Y.; Vasilyeva, S. V.; Donoghue, E.; Pucher, I.; Kamenov, G.; Cheng, H.-P.; Rinzler, A. G. *ACS Nano* **2014**, *8*, 8447.

(9) Zhuo, J.; Wang, T.; Zhang, G.; Liu, L.; Gan, L.; Li, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 10867.

(10)   Xie, J.; Zhang, J.; Li, S.; Grote, F.; Zhang, X.; Zhang, H.; Wang, R.; Lei, Y.; Pan, B.; Xie, Y. *J. Am. Chem. Soc.* **2013**, *135*, 17881.

(11) Liao, L.; Zhu, J.; Bian, X.; Zhu, L.; Scanlon, M. D.; Girault, H. H.; Liu, B. *Adv. Funct. Mater.* **2013**, *23*, 5326.

(12) Kibsgaard, J.; Chen, Z.; Reinecke, B. N.; Jaramillo, T. F. *Nat Mater* **2012**, *11*, 963.

(13) Liu, R.; Wu, D.; Feng, X.; Müllen, K. *Angew. Chem. Int. Ed.* **2010**, *49*, 2565.

(14) Gong, K.; Du, F.; Xia, Z.; Durstock, M.; Dai, L. *Science* **2009**, *323*, 760.

(15) Pei, S.; Cheng, H.-M. *Carbon* **2012**, *50*, 3210.

(16) Puziy, A. M.; Poddubnaya, O. I.; Socha, R. P.; Gurgul, J.; Wisniewski, M. *Carbon* **2008**, *46*, 2113.

(17) Hart, J. N.; May, P. W.; Allan, N. L.; Hallam, K. R.; Claeyssens, F.; Fuge, G. M.; Ruda, M.; Heard, P. J. *J. Solid State Chem.* **2013**, *198*, 466.