Electronic supplementary Information (ESI†)

Hemoproteins-nickel foam hybrid as effective supercapacitors

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Experimental section

Reagents

All materials were used without further purification. Cytochrome C (CytC, 12,384 kDa, 3.0 nm), Myoglobin (Mb, 16,950 kDa, 4.0 nm), and Hemoglobin (Hb, 68,000 kDa, 7.0 nm) were obtained from Sigma-Adrich Company Ltd., USA. The sodium hydroxide is obtained from Wako Company Ltd. Osaka, Japan. All protein solutions were prepared by dissolving an appreciate amount of protein in water.

Electrochemical experiment

A three-electrode cell system was used to evaluate the electrochemical performance by CV and galvanostatic charge-discharge techniques by using a Zennium/ZAHNER-Elektrik GmbH & CoKG, which was controlled by the Thales Z 2.0 software at room temperature. Ni foam (1 cm×2 cm, Nilaco Co.) was used as the working electrode and substrate for hemeprotein immobilization. A platinum sheet and an Ag/AgCl/3 M NaCl electrode were used as counter and reference electrodes, respectively. NF was carefully cleaned with a concentrated HCl solution (2 M) in an ultrasound bath for 5 min to remove the surface NiO layer. Deionized water and absolute ethanol were then used for 5 min each to ensure that the surface of the Ni foam was completely clean. A specific amount of hemeprotein was dropped onto the NF electrode and dried at 60 °C prior to the electrochemical analysis. A freshly prepared 6 M NaOH solution used as the supporting electrolyte. The specific capacitance (C_s) was derived from the CV measurements by using the following equation:[1]

$$C = \frac{1}{\nu m(Vc - Va)} \int_{Va}^{Vc} i(V)dV \qquad (1),$$

where C_s is the specific capacitance in Fg^{-1}, ν is the voltammetric sweep rate in Vs^{-1}, V(V) is the potential window (V_a to V_c) in V, and m is the total mass of the electrode (Ni foam and protein) in grams. The specific capacitance was also calculated from the charge/discharge curves by using the following equation:[1]

$$C_s = \frac{I \times \Delta t}{m \times \Delta V} \qquad (2)$$

The energy density (*E*) and power density (*P*) of the supercapacitor were derived from the galvanostatic charge/discharge curves at different current densities. The energy density was calculated using the following equation:

$$E = \frac{1}{2} C_s (\Delta V)^2 \qquad (3)$$

The specific power density was calculated using the following equation:

$$P = \frac{E}{\Delta t} \qquad (4)$$

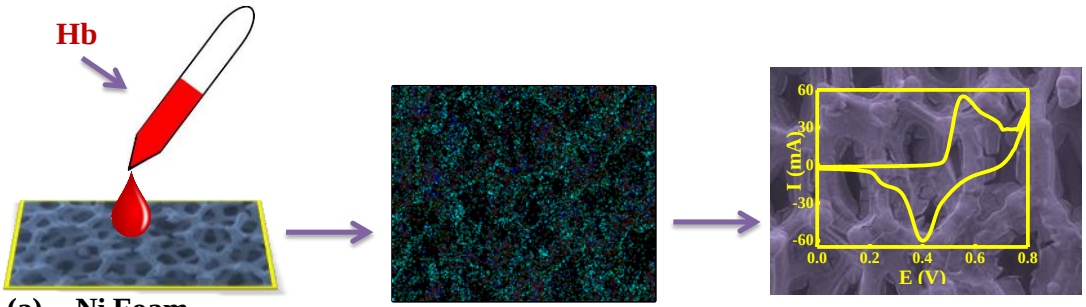
Where, ΔV is the potential at the end of charge, t is the time consuming at different scan rate.

Characterization of supercapacitor electrode.

FESEM images were measured by a field-emission scanning electron microscopy (JEOL model 6500). The SEM micrographs were operated at 15keV to better record the SEM images of Ni foam samples. Before insertion into the chamber the Ni foam was fixed on a SEM stage using carbon double side tapes. The Pt films were deposited on adsorbent substrates at room temperature by using a fine coat ion-sputter (JEOL model JFC-11000). The distance between the target and the adsorbent substrate was 5.0 cm. The sputtering deposition system used for the experiments consists of a stainless steel chamber, which was evacuated down to 8×10^{-5} Pa with a turbomolecular pump backed up by a rotary pump. Before sputtering deposition, the Pt target (10 nm diameter, purity 99.95%) was sputter cleaned in pure Ar. The Ar working pressure (2.8×10^{-1} Pa), the power supply (100 W) and the deposition rate were kept constant throughout these investigations. Moreover, the elemental analyses were done by energy-dispersive X-ray micro-analyzers (EDX) using an x-ray micro-analyzer integrated in the FE-SEM instrument.

Supplementary S1

The fabrication of the bio-supercapacitor electrode was carried out by dropping a specific concentration of proteins onto cleaned and dried Ni foam surface (Fig. S1a). Then, the electrode was dried at 60 ^{o}C for overnight. Before electrochemical measurements, the electrode was stabilized for 50 cycles in alkaline media prior to the final measurement.In this process, the hemeproteins were directly used without conductive carbon additives or even a polymeric binder. These results suggest that our Hb film-based supercapacitor maintains a high electrochemical performance level and high stability without sacrificing much specific capacitance.



(a) Ni Foam **(b) Hb-film** **(c) CV measurement on Hb biofilm**

Figure S1: Simple immobilization of Hb onto Ni foam electrode, and EDX image of Hb film.

Supplementary S2

The heme unit consists of a extrovert porphyrin ring, which is surrounded the iron atoms. The iron atom in heme unit binds to the 4 nitrogen atoms at the centre of the porphyrin ring; however, this binding usually leaves two free bonding sites for the iron in proteins (a &b). These two bonding sites are loacted on either side of the heme plane. One of the free bonding sites of iron is joined to one of the histidines, leaving the final bonding site on the other side of the ring available to bon with oxygen (c &d). The oxygenated heme groups in alkaline medium leads to formation of superoxides(e). These superoxide radicals can accumulate at the electrode surfaces where the metal atoms are under coordinated and they can react further to form surface adsorbed $HO^{\cdot}$ or O_2^{-} . Due to the accumulation of the oxygen or peroxide radicals at the electrode surface, in addition to the faradic reaction of heme group that enhance the capacitance values, some assumptions should be taken in considerations to calculate the theoretical capacitance of heme group, as follows.[2]

1- In our system, the heme group with similar molecular stuructre and wiegt of M.wt =616 mol/g is recognized as the active species of all proteins.
2- The redex reaction of iron atom in heme group is stable in 6 M NaOH.
3- The diffusion process of hydroxide ions is fast enough and has no effect on the faradic reactions.
4- The heme group has high conductivity under various conditions.

Thus, the theoretical capacitance of heme group can be calculated according to this equation[2]:

$$C_s = \frac{nF}{\Delta V * M}$$

Cs is the specific capacitance on F/g, n is a number of moles of the reactive heme group, F is faraday constant (96485.3 C/mol), M is the molecular weight, ΔV is the potential range.
Accordingly, for example, 1 mole of hemoglobin consists of 4 moles of heme group, therefore, the calculated specific capacitance related to active site of the heme group of proteins is 895 F/g.

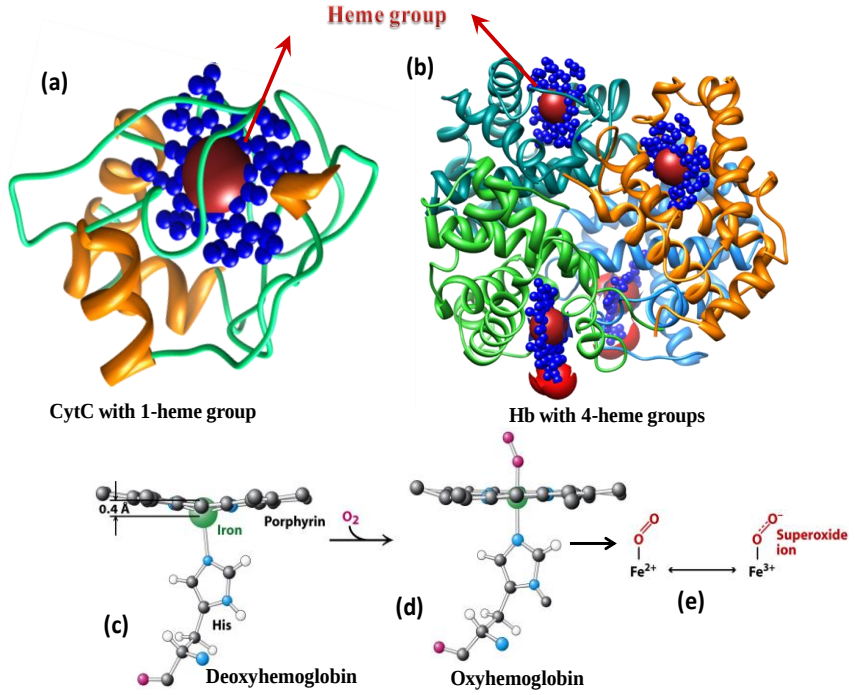


Figure S2 Molecular structures of hemeproteins (a) Cytochrome C, (b) Hemoglobin, (c, and d) are the deoxygenated and oxygenated forms hemoglobin molecules, and enlarge image of the oxygen molecules interacted with one side of heme group and formation of superoxide.

Supplementary S3

Figure S3 shows a typical SEM micrograph of an NF and an NF functionalized with Hb. The micrographs reveal a 3D cross-linked grid structure that contains small and large open pores ranging from 50 μm to 500 μm. The large pores dominate the 3D structure, whereas the small pores were mostly observed in the regions where grains interconnect. The higher-magnification SEM image shows a compact structure of the interconnected grain-like vertebrae with narrow grain boundaries. The vertebrae exhibit 10 μm spherical shapes. When Hb was dropped on the NF surface, it formed a fairly uniform film over the external surface of the porous network backbone with a thickness of ~ 80 nm. The uniform distribution of Hb is due to its high binding affinity with the Ni materials.[15] Such 3D networks of NF provide an interconnected, electrolyte-filled porous network that guarantees high accessibility and enables rapid ion transport.

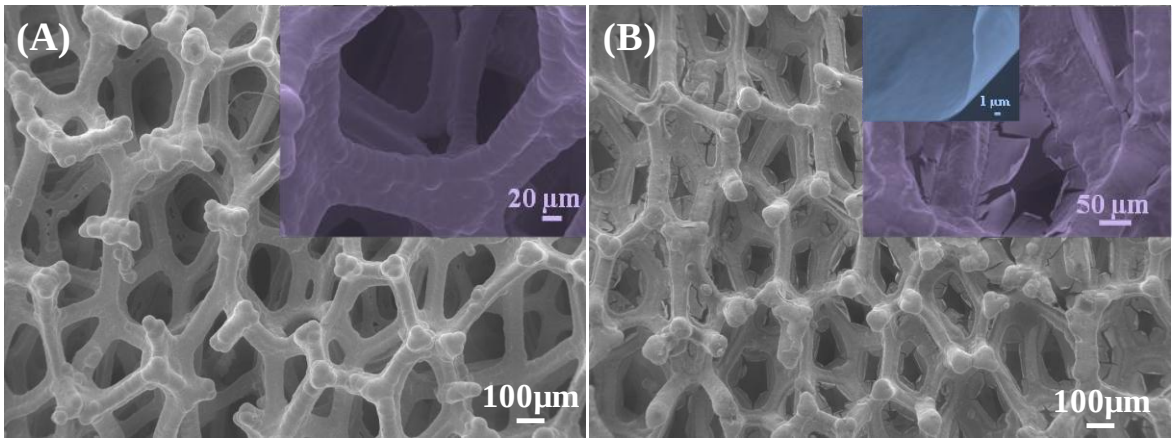
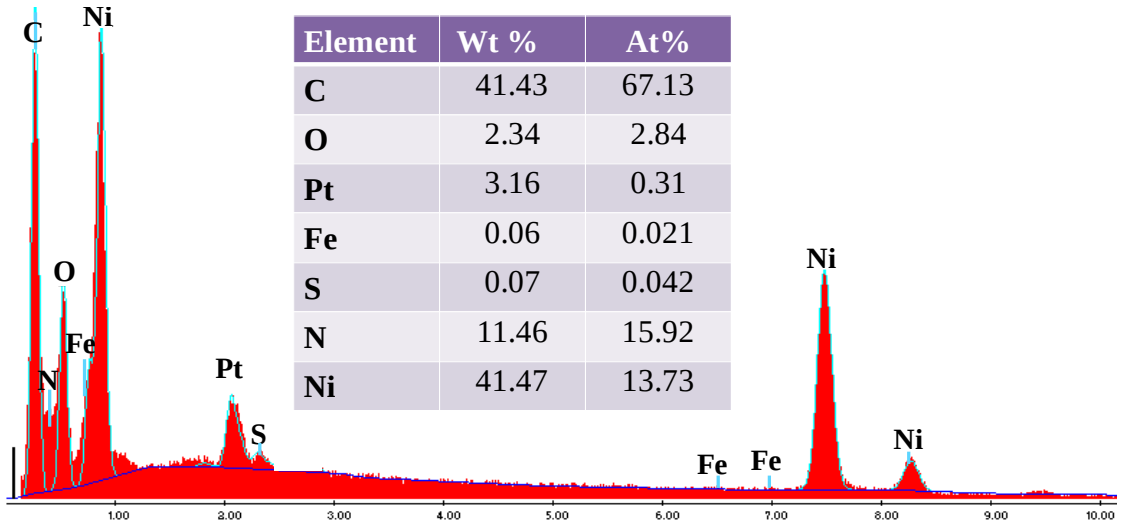


Figure S3. SEM images of (A) Ni-foam and (B) Ni -foam functionalised with Hb.

Supplementary S4



Element	Wt %	At%
C	41.43	67.13
O	2.34	2.84
Pt	3.16	0.31
Fe	0.06	0.021
S	0.07	0.042
N	11.46	15.92
Ni	41.47	13.73

Figure S4: Elemental composition of the Ni foam functionalized by Hemoglobin molecules

Supplementary S5

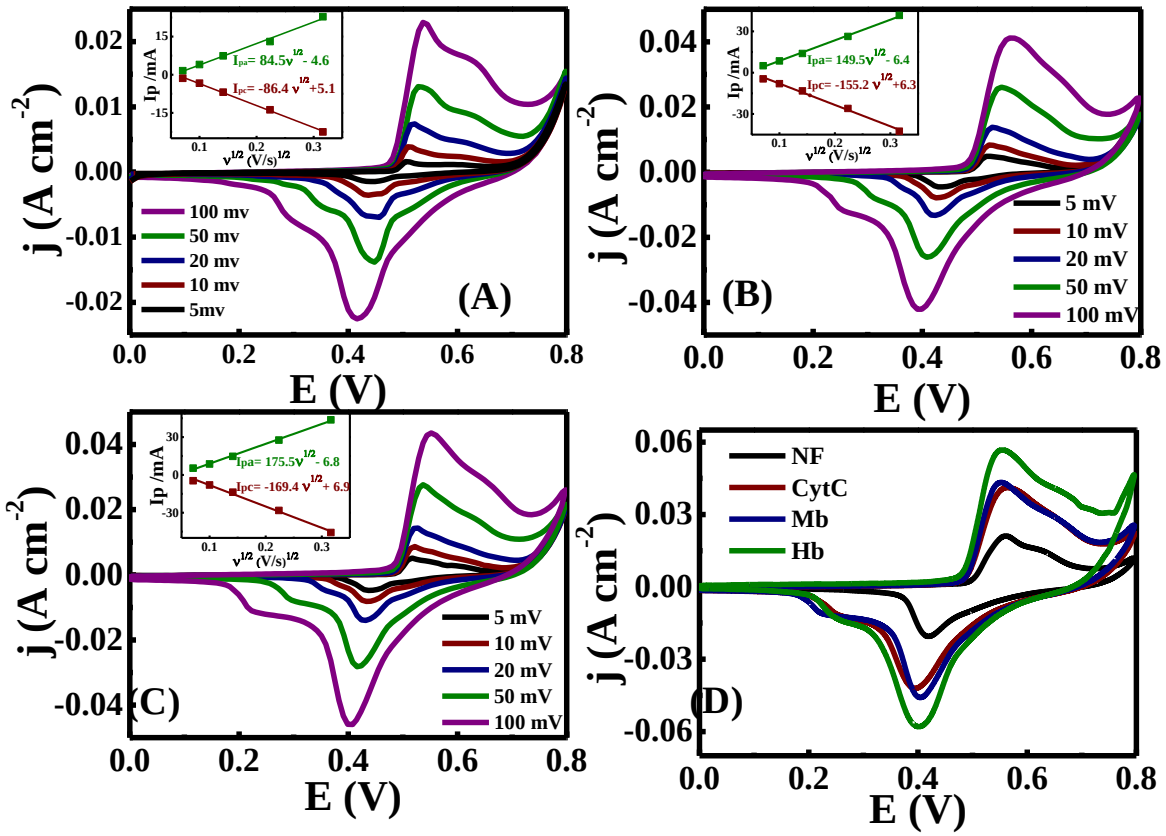


Figure S5: Typical CV curves of Ni foam electrode (A) and the protein films (B and C) for Mb and CytC respectively in 6 M NaOH. D) CV of NF and Hemeproteins electrodes at scan rate 100 mV/s.

Supplementary S6

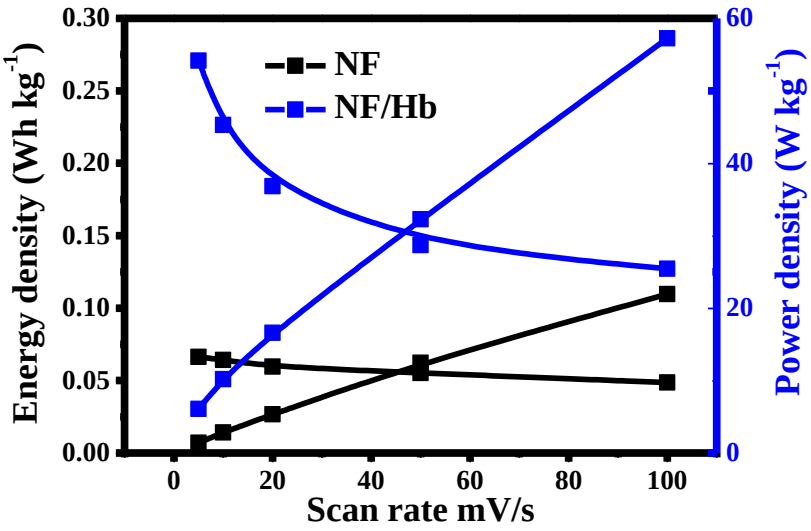


Figure S6: Derived energy and power densities as a function of the scan rate based on supercapacitor cell. [Not that the E and P values were calculated based on total mass of the electrodes (100.68 mg)].

Supplementary S7

Regardless of the applied discharge current value, nonlinear curves were observed for all porous protein films. The Hb film exhibited a longer discharge time than Mb and CytC. This result indicates that the Hb film has enhanced electrochemical reactivity with lower polarization. This finding was confirmed previously by the CV measurements (Fig. 2).These results indicate the satisfactory capability of NF protein film electrodes in addition to their high specific capacitance and rate capacitance.

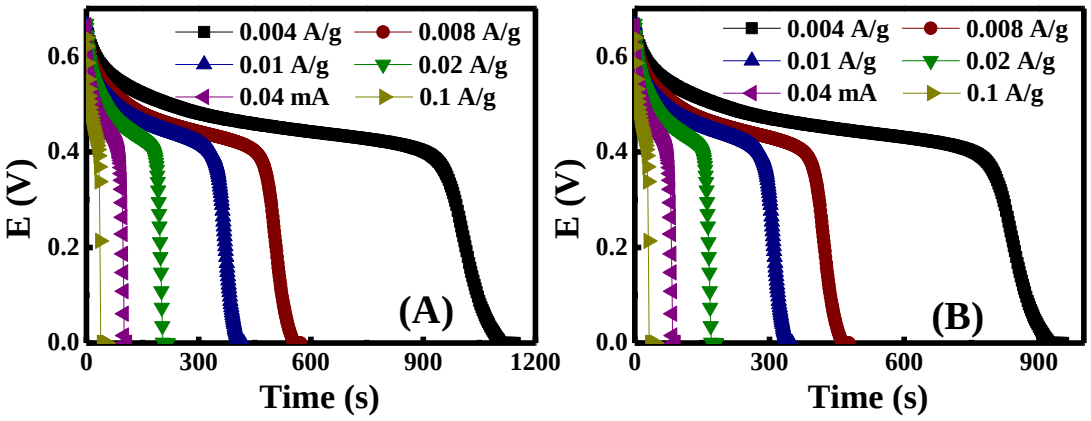


Figure S7: Galvanostatic discharge curve at different discharge current 0.004 – 0.1 A/g in 6 M NaOH for Mb (A) and CytC films (B). The weight of the protein is 0.68 mg immobilized on to Ni foam.

Supplementary S8

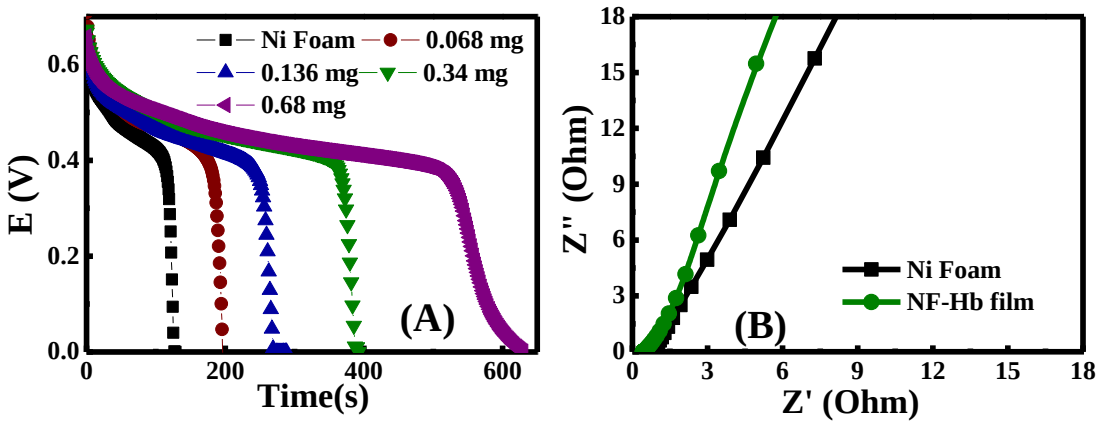


Figure S8. A) Galvanostatic discharge curves of different masses of Hb immobilized on an Ni foam electrode in 6 M NaOH at a discharge current of 0.1 A/g. B) the electrochemical impedance spectroscopy (Nyquist diagram) of porous Ni foam and Hb film at 0.55 V vs. Ag/AgCl in the frequency range between 100 kHz and 10m Hz; the excitation voltage was 5 mV.

Supplementary S9

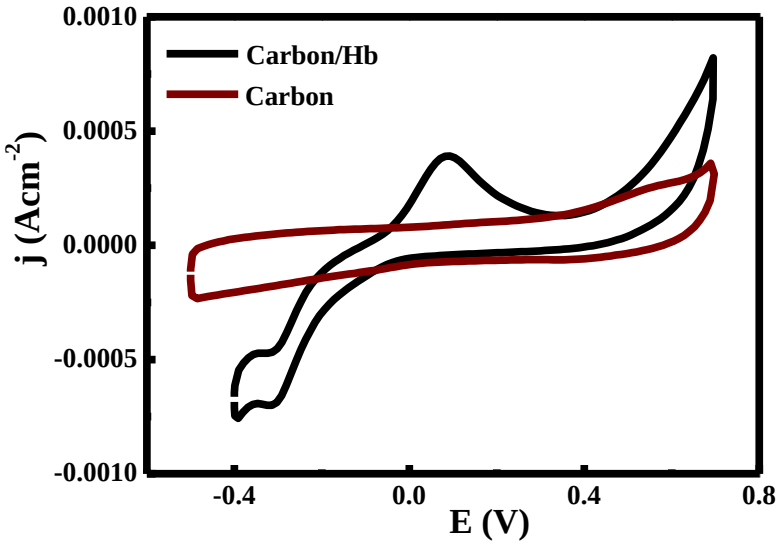


Figure S9. CV profiles of carbon and 0.68 mg of Hb modified carbon electrodes in 6 M NaOH at 50 mV/s. The hemoglobin film shows faradic redox peak around 0.1 V (vs. Ag/AgCl /3M NaCl) due to the heme active sites. The immobilization of Hb onto carbon electrode enhances the oxygen evaluation reaction in alkaline medium. However, in presence of 3D Ni foam electrode in basic medium; this subsequent oxygen evaluation reaction leads to formation of superoxide radicals that enhance the capacitive behavior of Ni foam.

Supplementary S10

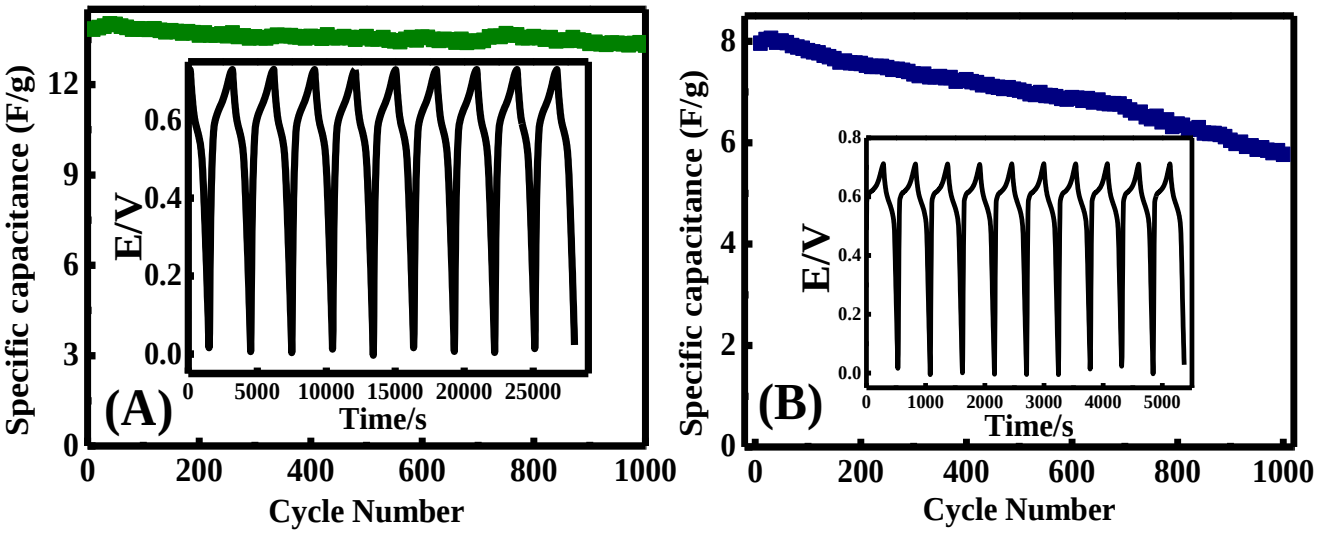


Figure S10. Applicability and cycling performance of Hb and Mb film onto 3D porous Ni foam electrode in 6 M NaOH at 0.004 A/g.

1 - H. B. Li, M. H. Yu, F. X. Wang, P. Liu, Y. Liang, J. Xiao, C. X. Wang, Y. X. Tong, G. W. Yang, *Nature Commun*. **2013**, *4*, 1894; C. Yuan , J. Li , L. Hou , X. Zhang , L. Shen , X. W. (David) Lou, *Adv. Funct. Mater*. **2012**, *22*, 4592–4597; J. Yan, E. Khoo, A. Sumboja, P. S. Lee, *ACS Nano*, **2010**, *4*, 7, 4247–4255.

2- H. Lia, J. Wang, Q. Chu, Z. Wang, F. Zhang, S. Wang, *J. Power Sources*, **2009**, 190, 578–586; C. Peng, D.Hu, G.Z.Chen, *Chem.comm.* **2011**, 47, 4105-4107