Electronic Supplementary Material (ESI) for Chemical Communications

Rapid electrochemical screening of NAD-dependant dehydrogenase in a 96-well format

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Material and Methods

Chemicals

Unless otherwise indicated, Except noticed, all chemicals are from Sigma-Aldrich (St-Quentin-Fallavier, France) or from BDH Prolabo (VWR, Fontenay-sous-Bois, France). Commercial formate dehydrogenase from *Candida Boidinii* (E.C. 1.2.1.2, 200 U ml^{-1}, batch-No.: H62411.01) is from Jülich Fine Chemicals GmBH (Jülich, Germany).

Electrochemical screening system

The electrochemical assays were performed with an AndCare 9600 sensor (Alderon Biosciences Inc., Beaufort, NC, USA). The screen-printing of the 96 two-electrodes systems is directly performed onto tailored Printed Circuit Board (PCB) (CirLy SA, Brignais, France) displaying 192 copper connectors. The PCB is used as an electrical connector between the Andcare 9600 sensor and the screen-printed electrodes as well as support for the electrodes (Fig. S1). Screen-printing is performed with a DEK 248 device and appropriate templates. Inks are from Acheson (Carbon paste is Electrodag® PF-407 C and Ag/AgCl paste is Electrodag® 6038 SS, Acheson, Scheemda, The Netherlands). The working electrode is made of carbon while the counter/ref is of Ag/AgCl. After printing, electrodes are cured one hour at 100°C. The wells (6 mm diameter) are obtained by sticking perforated adhesive plastic foil (2.5 mm thickness, 3M, Cergy-Pontoise, France) prepared with a craft ROBO (Silhouette America, Inc., Orem, UT, USA). The electrode surface is covered with a drop of 50 µL of electrolyte solution. Measurements were performed simultaneously on 96 electrodes in a few seconds by intermittent pulse amperometry (IPA): series of millisecond pulses of potentials were applied to the working electrode, separated by longer periods when the electrode is disconnected from the potentiostat (f = 1 Hz, pulse width 41 ms, measurement time 1 min, 60 data points). The last 10 measurements were averaged and used as representative current intensity on the electrode. Intensity is usually stable during the last 30 seconds.

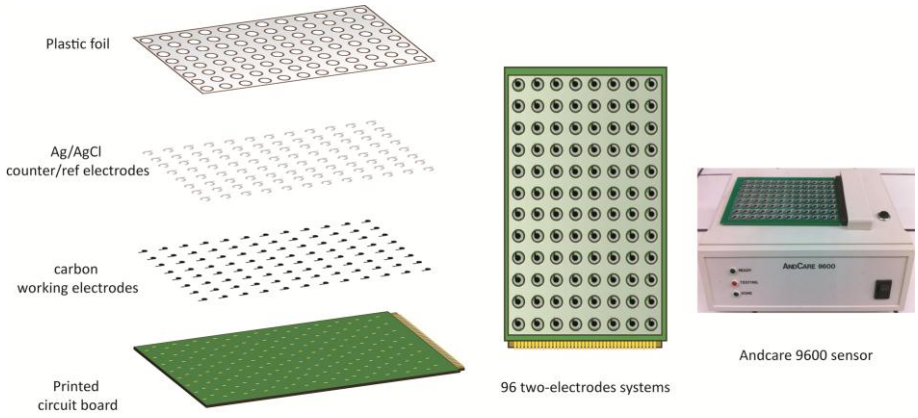

Fig. S1: Exploded view of the 96-well screen-printed PCB (left), top view of the PCB (middle) and picture of Andcare 9600 sensor (right).

Diazonium Electrografting

The diazonium derivatives of five phenazines (Toluidine Blue O, Neutral Red, Azure A, Brilliant Cresyl Blue and Thionine Acetate) were formed as follow, according to classical procedure[1]: 40 µL of the phenazine (10 mM solution in ethanol) is mixed with 340 µL of 1 M HCl at 4°C. Then, 20 µL of a freshly prepared $NaNO_2$ solution (20 mM in ice-cold water) is slowly added to the phenazine solution. Reaction takes place at 4°C during 20 minutes. To obtain screen-printed carbon electrodes modified by immobilization of phenazines (spCPs), the resulting diazonium solution (10 µL) is added to 40 µL of 1 M HCl onto the electrode surface. The spCPs are obtained by electrochemical reduction of the diazonium at the electrode surface by applying pulses at a potential of -1.5 V *vs.* Ag/AgCl during 5 minutes with a frequency of 0.5 Hz and a pulse width of 164 ms. Electrodes are extensively rinsed with water before use. Control experiment is performed without phenazine and $NaNO_2$.

Cloning and expression of FDH mutants library.

The CbFDH gene (AJ245935, 1095 base pairs) was commercially synthesized (GenScript, Piscataway, New Jersey, USA) with additional sequences 5'-ggatccataaaaggagatatacc-3' and 5'-ctcgagtctaga-3' at the 5' and 3' extremity of the gene sequence, respectively. The sequence introduced at the 5'-extremity sequentially harbours a BamHI restriction site (G/GATCC), a stop codon, the Shine-Dalgarno sequence and seven extra nucleotides before the FDH open

reading frame. The sequence introduced at the 3'-extremity harbours restriction sites for XhoI (C/TCGAG) and XbaI (T/CTAGA). For simplicity this construct was named pUC57-FDH. After re-isolation and amplification of the pUC57-FDH plasmid, the FDH gene was subcloned into the pET-28b expression vector using the BamHI and XhoI sites. The construct pET-28B-FDH was then sequenced to check for integrity, and used to transform *E. coli* BL-21(DE-3). After induction by IPTG (1 mM), the wild-type FDH was shown to be expressed and functional.

FDH mutant library was created as following: Error-prone PCR was performed using 250 µM Mn^{2+}, non-biased concentration of dNTP (2.5 mM) and the pUC57-FDH as template. PCR product was then purified by the QIAquick gel extraction kit (Qiagen, Courtaboeuf, France) and cloned into the pET-28b. Transformants were first selected on LB-Agar-kanamycine (100 $\mu g.mL^{-1}$), and further replicated and induced overnight on LB-Agar containing kanamycine (100 $\mu g.mL^{-1}$) and IPTG (1 mM). The functional screening was adapted from the one described by Ansorge-Schumacher et al.[2]: Agar solution (1.6% w:v) in phosphate buffer [0.1 M, pH 7.4, 0.2% Triton X-100 (v/v), 10 mM EDTA] and cooled down below 70 °C was overlaid on the replicates to fix them. Cells were permeabilized with 5 mL of phosphate buffer [0.1 M, pH 7.4, 0.1% Triton X-100 (v: v), 200 mM EDTA] under stirring during 10-15 minutes. This operation is repeated three times. Cells were then washed three times with phosphate buffer (0.1 M, pH 7.4) and incubated with 3.5 mL of the substrates solution (1.25 M sodium formate, 5 mM NAD^+) under stirring for 5 minutes. Actives clones were identified by revelation with 120 µL of phenazine ethosulfate (60 mM) and 200 µL of nitrosium blue tetrazolium (6 mM) in the dark under stirring for 20-30 minutes. Cells were then washed with water. Actives colonies appear as large dark purple spots.

A library replicate was made in a new 96-well plate containing 1 mL of LB and grown at 37°C until an optical density of 1 is reached. After induction by IPTG (1mM), cells were

grown for additional 5 hours at 37°C, harvested by centrifugation at 2 000 x g during 15 min at 4°C, re-suspended and lysed in 400 µL of a potassium 0.1 M phosphate buffer, pH 7.4 containing 0.1 % of Triton X-100 and 200 mM of EDTA. The plate was centrifuged again at 2000 x g (15 min., 4°C). Crude cell lysates (20 µL) were used without additional purification step for the screening assay.

Formate dehydrogenase activity assay in solution

Crude cell lysate is used for the FDH activity screening in solution as following: Reaction media is composed of 40 µL of crude cell lysate, 20 µL of substrate solution (50 mM NAD^+, 1.5 M sodium formate), 10 µL of phenazine ethosulphate (PES) and nitroblue tetrazolium (NBT) solution (160 µM and 1mM respectively). Final volume is adjusted to 200 µL with phosphate buffer saline (pH 7). The formation of formazan blue precipitate is measured at 560 nm after 5 min.

Electrochemical measurements

Each spCP is covered with a drop of 40 µL of phosphate buffer (pH 7.0, 100 mM, 100 mM KCl). The surfaces of spCPs are oxidized by applying a potential of 600 mV *vs.* Ag/AgCl (1 minute, f= 1 Hz, pulse width 41 ms). Measurement is performed before and after the addition of 10 µL of NADH (0-1 mM) at 0 mV *vs.* Ag/AgCl (1 minute, f= 1 Hz, pulse width 41 ms).

FDH activity is detected with spCPs with TBO. The FDH (10 µL of solution presenting an activity of 0-21 $\mu M.min^{-1}$) is added to 40 µL of substrate solution in phosphate buffer (pH 7.0, 100 mM, 100 mM KCl, 300 mM sodium formate, 5 mM NAD^+). Electrochemical response is measured as above. The same procedure is used for the measurement of the FDH mutants library. In this case, 20µL of the crude cell lysates are added to 30 µL of substrate solution.

1. S. Abdellaoui, B. C. Corgier, C. A. Mandon, B. Doumèche, C. A. Marquette and L. J. Blum, *Electroanalysis*, 2013, **25**, 671.
2. M. B. Ansorge-Schumacher, H. Slusarczyk, J. Schuemers and D. Hirtz, *Febs Journal*, 2006, **273**, 3938-3945.