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# Metal-Organic Framework functionalized bipolar electrodes for bulk electroenzymatic synthesis

Chunhua Zhang,<sup>1</sup> Xiaoyu Zhang,<sup>1</sup> Yuheng Fu,<sup>1</sup> Lin Zhang,<sup>1\*</sup> Alexander Kuhn<sup>1,2\*</sup>

<sup>1</sup>Engineering Research Center for Nanomaterials, Henan University, Kaifeng, China

<sup>2</sup>University Bordeaux, CNRS, Bordeaux INP, ISM UMR 5255, Pessac, France

\* kuhn@enscbp.fr

## Abstract

Electrochemical regeneration of the cofactor nicotinamide adenine dinucleotide (NADH) is crucial for the enzymatic synthesis of a variety of valuable chemicals.  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  has been reported as one of the most efficient non-enzymatic catalysts for electrochemical regeneration of NADH, but the efficiency of the existing systems is still far from satisfying. Here we propose to suspend metal-organic framework functionalized glassy carbon beads in a bipolar electrochemical cell under mechanical stirring for bulk electroenzymatic synthesis. The beads are modified with a metal-organic framework NU-1000 shell, in which  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  catalyst is incorporated via the solvent-assisted ligand incorporation approach. Enzymatically active 1,4-NADH is electrochemically regenerated, and finally bipolar electrochemical bulk conversion of pyruvate into L-lactate is performed in the presence of NADH dependent L-lactate dehydrogenase as a model system. The very high turnover frequency (TOF) of  $3100 \pm 106 \text{ h}^{-1}$  opens up promising perspectives for employing this concept also for various other electroenzymatic bulk processes.

Keywords: NADH regeneration, bipolar electrochemistry, metal organic frameworks, electrocatalysis, electroenzymatic synthesis

## Introduction

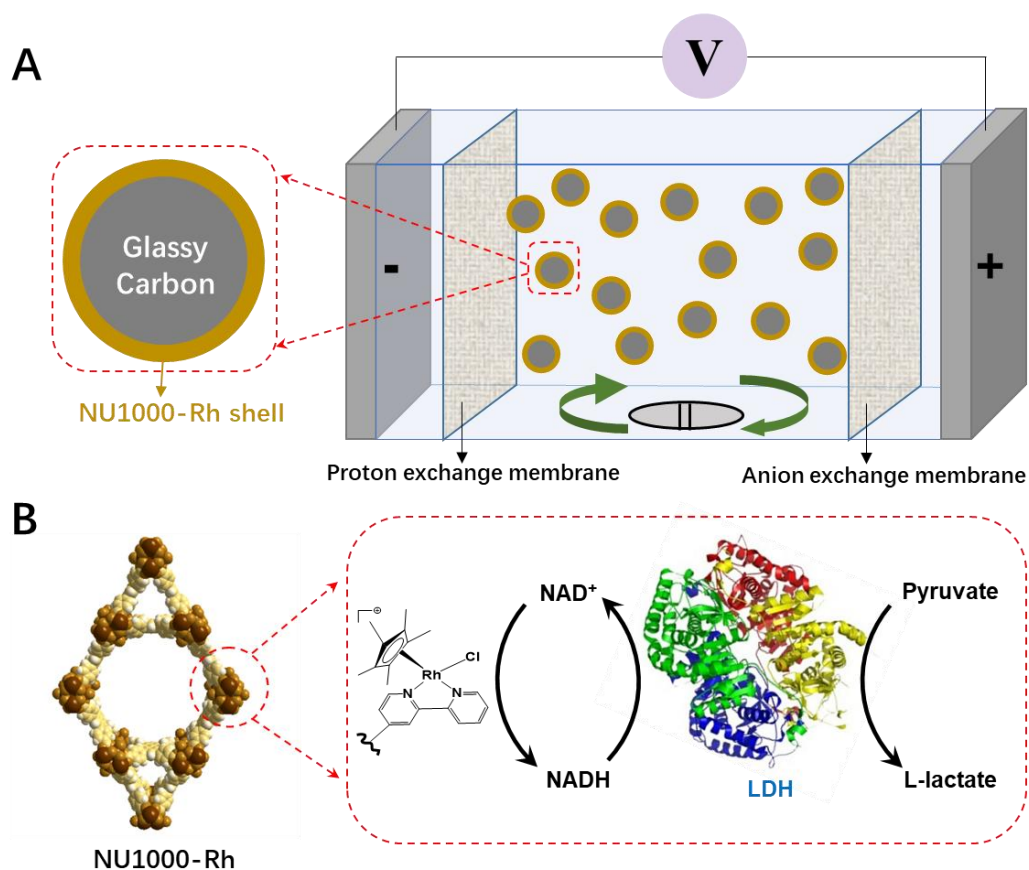
Enzymes are biocatalysts with high specificity and catalytic efficiency, among which NADH-dependent oxidoreductases constitute one of the largest class of enzymes for the synthesis of many valuable chiral products[1,2]. In this context, the regeneration of the cofactor 1,4-NADH is essential for large scale enzymatic synthesis, since 1,4-NADH is always required as an electron donor for the oxidoreductase to catalyze the enzymatic reaction. However, the high price of NADH imposes a recycling of the oxidized form NAD<sup>+</sup> back to NADH[3,4]. Among different regeneration methods, the electrochemical regeneration of 1,4-NADH in combination with the enzymatic reactions has been considered as a green and sustainable approach, and different electroenzymatic systems have been developed for the synthesis of a variety of chiral products in recent years[5–10]. Since the direct electrochemical reduction of NAD<sup>+</sup> requires high overpotentials, enzymatically inactive side products such as NAD<sub>2</sub> dimers and other isomers of NADH are formed. Thus, it is necessary to use a catalyst for the regeneration to obtain selectively enzymatically active 1,4-NADH[5,11]. Up to now, in addition to the possibility to use an enzymatic catalyst (eg. diaphorase) [7,12], [Rh(Cp\*)(bpy)Cl]<sup>+</sup> and its derivatives are reported to be among the most efficient and robust non-enzymatic catalysts for NADH regeneration[13,14]. The classical strategy to achieve efficient electrochemical regeneration of NADH is to immobilize the [Rh(Cp\*)(bpy)Cl]<sup>+</sup> catalyst on porous electrodes, in order to increase the loading of catalyst. Different porous electrodes (carbon fibers[15,16], carbon nanotubes[17,18], metal organic frameworks[19]) were functionalized with [Rh(Cp\*)(bpy)Cl]<sup>+</sup> catalyst and applied for NADH regeneration. In a recently reported work, a NU-1000 metal-organic framework film was used as supporting matrices on a working electrode, and [Rh(Cp\*)(bpy)Cl]<sup>+</sup> entities were regularly positioned on the Zr nodes of NU-1000. The so-obtained electrode has been shown to be very efficient for NADH regeneration with a high TOF of 1400 h<sup>-1</sup> for 1,4-NADH regeneration, indicating that the metal-organic framework film is an interesting support for designing efficient electrocatalytic systems[19].

In addition to normal electrochemistry, bipolar electrochemistry provides the possibility to further improve the efficiency of the system. According to the principles of bipolar electrochemistry, multiple electrodes can be placed between two feeder electrodes and polarized under the influence of an electric field. In this case, electrochemical reactions will be triggered at the two extremities (polarized anode  $\delta^+$  and cathode  $\delta^-$ ) of each individual electrode, if the electric field exceeds a certain threshold value[20–24]. In our previous work, several [Rh(Cp\*)(bpy)Cl]<sup>+</sup> functionalized carbon beads were suspended in the reaction compartment of a bipolar electrochemical cell with the help of viscous cellulose as electrolyte. The 3D assembly of these electrodes in the bulk solution allows a fine-tuning of the efficiency of the electrochemical regeneration of 1,4-NADH[25]. However, if this bipolar electrochemical regeneration system is coupled with an enzymatic follow-up reaction, there are still two important problems which need to be solved. First, as the electron transfer between the enzymes and the Rh catalyst is achieved by shuttling 1,4-NADH, the diffusion of this

cofactor can become the rate limiting step in a viscous electrolyte. Second, it has been reported that some functional groups (eg. -SH, -NH<sub>2</sub>), present at the surface of enzymes, can react with the metal catalyst, leading to a degradation of the Rh complex activity[26]. Thus a physical separation between the Rh complex and the enzymes is necessary during long-term electroenzymatic synthesis[27].

Taking into account the above mentioned two limitations, we propose in this work a bipolar electroenzymatic system based on multiple glassy carbon beads, functionalized with a NU-1000 shell containing [Rh(Cp\*)(bpy)Cl]<sup>+</sup> catalyst, incorporated at its Zr nodes. On the one hand, the NU-1000 layer is expected to increase the local concentration of Rh catalyst on the surface of the beads, leading to an improvement of the bipolar electrocatalytic efficiency for NADH regeneration. On the other hand, the NU-1000 shell can be used as a physical separation matrix between the Rh catalyst and the enzymes. The pore size of NU-1000 being 3.4 nm, the large NADH-dependent dehydrogenases will be kept outside the pores of NU-1000, while the NADH molecules (1.3 × 1.1 × 1.9 nm) are small enough to diffuse into the pores and shuttle electrons between the Rh catalyst and the enzymes. Thus, the degradation of the Rh catalyst and enzyme during long-term electrochemical synthesis can be avoided.

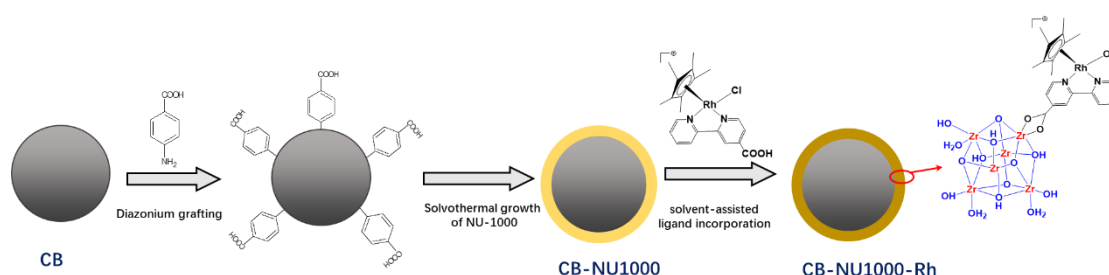
The general concept of this work is shown in Scheme 1. In order to enhance mass transport, the beads are kept in suspension in the reaction compartment of a bipolar cell by simple mechanical stirring. The bipolar electrocatalytic NADH regeneration, catalyzed by immobilized Rh catalyst, is achieved at the cathodically polarized extremity (δ<sup>-</sup>), and the sacrificial electrooxidation of ascorbic acid occurs at the anodically polarized side (δ<sup>+</sup>). The efficiency of the bipolar electrocatalytic NADH regeneration scales with the number of suspended beads. Finally, L-lactate dehydrogenase (with dimensions of 13.6 × 8.6 × 6.0 nm[28]) is added to the solution, and the bipolar electrochemical bulk conversion of pyruvate into L-lactate is carried out under stirring with this model system.



Scheme 1. Illustration of (A) the experimental set-up for the bulk bipolar electroenzymatic synthesis and (B) the catalytic cascade with NU-1000-Rh for the bipolar electroenzymatic synthesis of lactate in the presence of NADH-dependent l-lactate dehydrogenase.

## Results and discussion

The glassy carbon beads with a NU-1000 shell are prepared following the process illustrated in Scheme 2. The glassy carbon beads (CB) are first polished with sand paper in order to obtain a rough surface, then the beads are functionalized with carboxyl groups via chemical diazonium grafting. The NU-1000 shell is grown on the surface of CB via a solvothermal synthesis step, followed by a solvent-assisted ligand incorporation process for the  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  immobilization at the nodes of the NU-1000 shell[19]. The crystal structure and the chemical analysis of the obtained CB-NU1000-Rh are supported by XRD (Figure S1) and XPS (Figure S2) measurements. Furthermore, EDS measurements (Figure S2D) indicate an atom% of Zr and Rh of 85.6% and 14.4%, respectively. This corresponds to an atomic ratio of 5.94 for Zr to Rh, which means that, within error bars, there is statistically one Rh catalyst per Zr<sub>6</sub> node.



Scheme 2. Elaboration process of a  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  functionalized NU-1000 layer on a glassy carbon bead. The process includes a chemical diazonium grafting step for carboxyl group functionalization, a solvothermal step for the growth of NU-1000, and a solvent-assisted ligand incorporation step for  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  immobilization.

The morphology of the active layer at different magnifications is shown in Figure 1, indicating a homogeneous growth of NU-1000 on the beads. Elemental mapping of Zr and Rh illustrates a homogeneous incorporation of  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  into the NU-1000 crystalline rods.

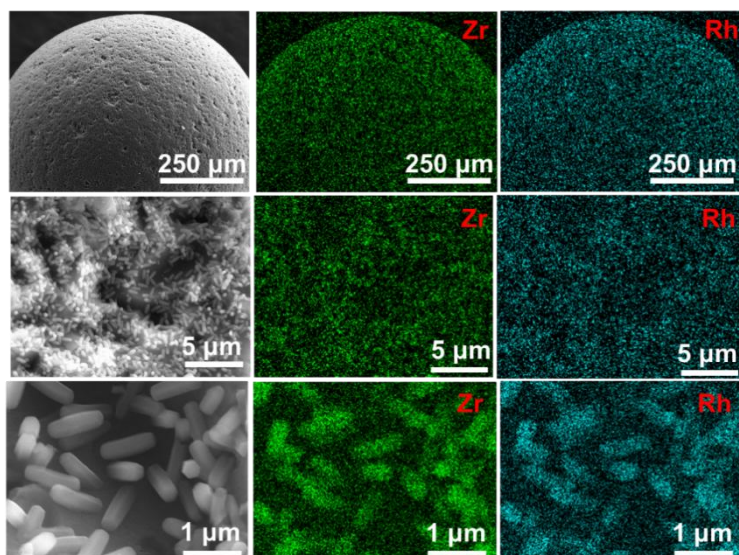


Figure 1. SEM images of a CB-NU1000-Rh electrode recorded with different magnifications and the corresponding elemental mapping.

The CB-NU1000-Rh catalyst particles are then placed in the reaction compartment for bipolar electrochemical NADH regeneration, and the amount of generated enzymatically active 1,4-NADH is measured. In this work we employed a combination of UV-vis spectroscopy and biological assays in order to correctly measure the produced 1,4-NADH. UV/Vis spectroscopy for characterizing 1,4-NADH has been already reported in the literature. 1,4-NADH has a typical absorption peak at 340 nm, whereas possible side products such as NAD<sub>2</sub>, 1,2-NADH, and 1,6-NADH have different characteristic absorption peaks. To further verify that the absorption peak at 340 nm is originating from the enzymatically active 1,4-NADH, rather than inactive 1,6-NADH or NAD<sub>2</sub>, we used a direct enzymatic assay, which consists of the conversion of acetaldehyde by an alcohol dehydrogenase (ADH). This reaction can only occur in the presence of enzymatically active 1,4-NADH. Thus, the complete disappearance of the absorption peak at 340 nm indicates that the formed product is 1,4-NADH (details are described in the experimental part). First, forty CB-NU1000-Rh beads are placed at the bottom of the bipolar reaction compartment (assembled in a 2D array), and electric fields with different strengths are applied to trigger the bipolar electrochemical reduction of NAD<sup>+</sup> at the  $\delta$ -extremity of each bead. The product generated after 1.5 h is characterized by UV/vis spectroscopy (Figure S3). As shown in Figure 2A, the concentration of produced 1,4-NADH is almost zero for an electric field of 10 V/cm, however the concentration of 1,4-NADH starts to be measurable for an electric field of 12 V/cm and further increases for 12.5 V/cm, whereas a decrease is observed for even higher electric fields (13.5 V/cm). This trend can be understood by calculating for the different electric fields the fractional surface area of the carbon beads where the bipolar electrochemical reaction can take place due to a sufficient polarization (see details in supporting information). The conversion barely starts at an electric field of 10 V/cm with a very small cathodically polarized surface area, and gradually increases when the electric field is raised to 12.5 V/cm. For even higher fields (13.5 V/cm) the direct non-catalytic electrochemical reduction of NAD<sup>+</sup> starts, leading to the partial formation of enzymatically inactive side product. Since the polarization potential difference is proportional to the effective length of bipolar electrodes, 12.5 V/cm has been chosen as the optimum electric field for the electrochemical regeneration of 1,4-NADH for beads with a characteristic size of  $960 \pm 20 \mu\text{m}$ . A long-term bipolar electrochemical transformation experiment has been carried out as shown in Figure 2B. The typical absorption peak of 1,4-NADH at 340 nm is

increasing as a function of time, corresponding to a linear increase in concentration up to 6 h (the inset of Figure 2B). The same peak completely disappears during the enzymatic test (Figure S4A) indicating the high selectivity of this system during long-term bipolar electrochemical synthesis. A blank experiment with forty glassy carbon beads, covalently modified by diazonium grafting with Rh catalyst was carried out under the same experimental conditions (Figure S4B). UV/Vis spectra were used to quantify the bipolar electrochemical product in both cases, and a roughly two times higher amount of 1,4-NADH was obtained in the presence of the NU-1000 shell on the beads, indicating that the NU-1000 matrix is playing a significant role for improving the electrocatalytic efficiency for NADH regeneration.

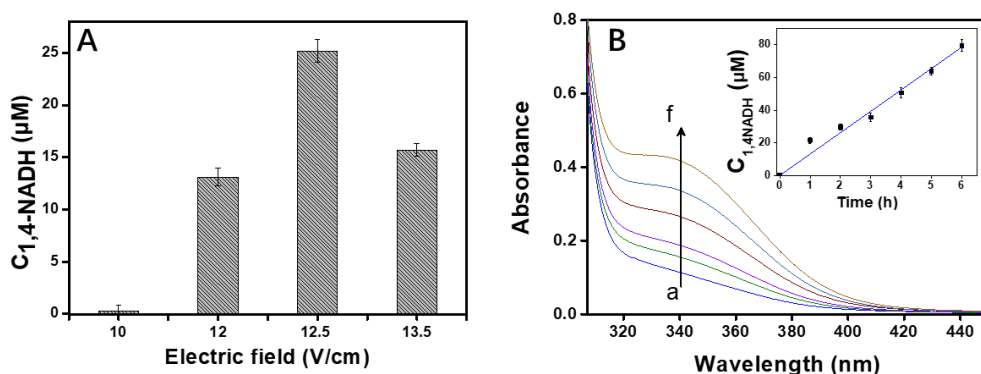


Figure 2. (A) Bipolar electrochemically produced enzymatically active 1,4-NADH concentration as a function of the applied electric field after 1.5 h; (B) UV-Vis absorption spectra of the bipolar electrochemical product obtained with an electric field of 12.5 V/cm after (a) 1 h, (b) 2 h, (c) 3 h, (d) 4 h, (e) 5 h, and (f) 6 h. The inset indicates the corresponding concentration of 1,4-NADH as a function of time. Experiments were carried out with 40 CB-NU1000-Rh electrodes ( $960 \pm 20 \mu\text{m}$ ) placed at the bottom of the bipolar cell containing 10 mL Tris buffer at pH 7.2 in the presence of 5 mM ascorbic acid and 1 mM NAD<sup>+</sup> under nitrogen atmosphere. Error bars refer to the standard deviations of three measurements.

Before combining the bipolar NADH regeneration system with enzymatic reactions, we first tested the possibility to suspend the beads in the bulk solution by mechanical stirring. This can be achieved by stirring with a magnetic bar at 1400 rpm (Video S1). The bipolar electrochemical regeneration of NADH is carried out with forty CB-NU1000-Rh microelectrodes in the 3D stirring mode, and the obtained 1,4-NADH is characterized by UV-Vis spectra. As shown in Figure 3A, the absorption peak at 340 nm increases from 0.2 to 0.6, almost three times more compared to the previous experiment with stationary beads in the bipolar cell. In the latter mode, the beads sink to the bottom, where only a limited number (maximum sixty) can be placed without mechanical or electrical interference. A physical interaction between beads should be avoided, because a contact between them will increase their effective length, leading to the formation of side products caused by a higher polarization potential difference. In contrast, under stirring, over one hundred CB-NU1000-Rh beads can be accommodated in the 3D reaction space before physical interactions become statistically significant.

The bipolar electrochemical regeneration of NADH with different numbers of CB-NU1000-Rh electrodes was performed both in the 2D and 3D mode, and the efficiency of both systems was



evaluated by characterizing the concentration of 1,4-NADH after 1.5 h. Figure 3B reports the concentration of produced 1,4-NADH as a function of the number of CB-NU1000-Rh electrodes for the 2D and 3D system. The enzymatic activity of 1,4-NADH was again confirmed by addition of alcohol dehydrogenase and acetaldehyde as shown in Figure S5 and Figure S6. For the 2D system, a linear increase of 1,4-NADH concentration is observed up to 40 beads, which is expected due to the increase of total active surface area of the electrodes in the cell. Then a slight decrease is observed with 60 microbeads, which can be explained by diffusion restrictions due to smaller distance between the individual electrodes. Similarly, a linear increase is also observed with the stirred system, and the saturation occurs for around 80 beads, followed by a gradual decrease with an increasing number of electrodes. The slope of the linear range for the 3D system is higher than for the 2D system, indicating a faster reaction kinetics, which can be attributed to an improved mass transport. In addition, the reaction is also promoted by the movement and rotation of the CB-NU1000-Rh beads. When the beads rotate during the bipolar electrochemical process, the effective length of the beads with respect to the electric field remains constant due to their isotropic shape, but the region on their surface where the reduction takes place changes continuously. Thus, the educt and the product have enough time to diffuse in/out of the catalytic layer during the rotation to a new position where fresh catalyst surface becomes activated by the electric field. Compared to our previously reported NADH regeneration system with a gellified medium [25], that we used to maintain bipolar beads suspended in the 3D reaction space, the here employed liquid medium allows continuous stirring and therefore a considerably improved mass transport. This gives the possibility to couple this coenzyme regeneration system with an electroenzymatic follow-up reaction in order to perform the conversion of a substrate with high efficiency.

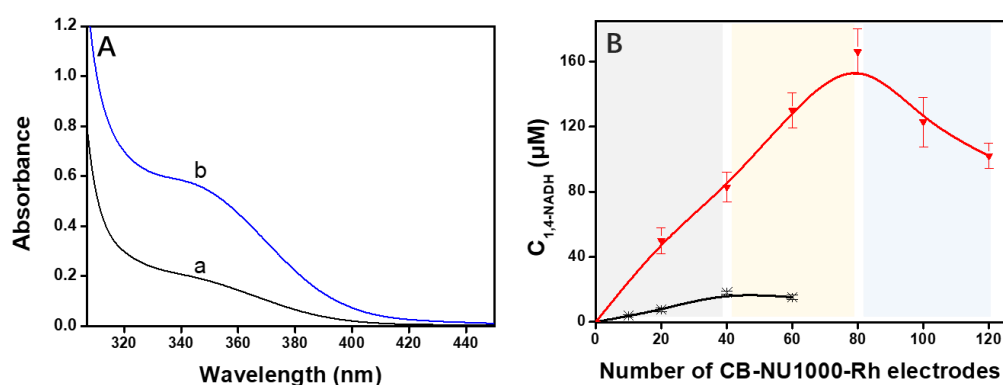


Figure 3. (A) UV-Vis absorption spectra of the bipolar electrochemical product obtained with fourty CB-NU1000-Rh electrodes (a) placed at the bottom of the bipolar cell (b) suspended in the bulk phase of the bipolar cell under stirring (1400 rpm). (B) Bipolar electrochemically produced 1,4-NADH concentration as a function of the number of CB-NU1000-Rh beads in the 2D mode (black) and in the 3D mode (red). Experiments were carried out with an electric field of 12.5 V/cm for 1.5 h in 5 mL Tris pH 7.2 in the presence of 5 mM ascorbic acid and 1 mM NAD<sup>+</sup> under nitrogen atmosphere. Error bars refer to the standard deviations of three measurements.

Finally, 100U L-lactate dehydrogenase (dimensions of 13.6 × 8.6 × 6.0 nm), 2 mM pyruvate, and 0.5 mM NAD<sup>+</sup> have been added to the solution for the bipolar electroenzymatic bulk conversion from pyruvate to L-lactate as a model reaction. 5 mM ascorbic acid has also been added as a sacrificial reactant for the electrooxidation at the anodically polarized face of the beads. The bipolar electrochemical bulk conversion is carried out under stirring with eighty CB-NU1000-Rh beads with



an electric field of 12.5 V/cm for 4 h, and the product is analyzed by HPLC (Figure S7). According to the calibration curve of L-lactate,  $(2.23 \pm 0.08) \times 10^{-6}$  mol L-lactate is produced, the quantity of  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  catalysts on each CB-NU1000-Rh microbead is estimated to be  $2.25 \times 10^{-12}$  mol, and thus the corresponding turnover frequency (TOF) for  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  can be calculated to be  $3100 \pm 106$  h<sup>-1</sup> (see calculation details in supporting information). To the best of our knowledge, this is the highest TOF value reported so far for a non-enzymatic catalyst in electroenzymatic synthesis, very close to what has been obtained when using an enzyme as a catalyst for NADH regeneration (3680 h<sup>-1</sup> with diaphorase[29], and 3600 h<sup>-1</sup> with MeFDH1- $\beta$  [30]). Last-but-not-least, the catalytic layer on the beads seems to be rather stable, as indicated by XRD and EDX measurements performed after the catalysis (Figure S1).

## Conclusions

In conclusion, we propose a new strategy for electroenzymatic synthesis, based on bulk bipolar electrochemical NADH regeneration, carried out with suspended functionalized carbon beads. A MOF shell (NU-1000), grown on the surface of glassy carbon microbeads, is post-functionalized with  $[\text{Rh}(\text{Cp}^*)(\text{bpy})\text{Cl}]^+$  at the zirconium nodes. The resulting beads are then exposed in solution to an electric field, and their cathodically polarized face  $\delta^-$  can be used for triggering the electrocatalytic regeneration of 1,4-NADH catalyzed by the immobilized Rh catalyst. Electrochemical bulk regeneration of NADH is achieved when suspending the CB-NU1000-Rh beads by stirring, and the efficiency of the system as a function of the number of beads is evaluated. The proposed approach shows a much higher efficiency compared to other systems (2D ensembles of beads or their suspension in a viscous medium). Coupling this NADH regeneration strategy with L-lactate dehydrogenase as an enzymatic model system to transform in situ pyruvate into L-lactate, reveals a very high TOF of  $3100 \pm 106$  h<sup>-1</sup>, thus making this approach very attractive for the bulk electroenzymatic synthesis of other high added-value products.

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