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ELSEVIER

## Review Article

## Beyond the hype surrounding biofuel cells: What's the future of enzymatic fuel cells?

Serge Cosnier\*, Andrew J. Gross, Fabien Giroud and Michael Holzinger

1 After a short comparison of biofuel cells based on enzymes  
2 and microorganisms, several important developments and  
3 applications of enzymatic fuel cells (EFCs) are discussed. This  
4 discussion emphasizes how to evaluate the performance of  
5 EFCs, and highlights the influence of temperature and how it  
6 must be carefully considered for practical use of EFCs as  
7 power sources. Some of the latest and most important  
8 innovations in EFC design using buckypapers and redox  
9 nanoparticles are briefly reviewed.

## 10 Address

11 Département de Chimie Moléculaire, CNRS-University of Grenoble  
12 Alpes, 38041, France

13 \*Corresponding author: Cosnier, Serge  
14 ([serge.cosnier@univ-grenoble-alpes.fr](mailto:serge.cosnier@univ-grenoble-alpes.fr))

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## 15 Introduction

16 The development of sustainable and renewable energy  
17 sources with greater respect for the environment is a cru-  
18 cial challenge for the 21st century. Among various alterna-  
19 tive energy sources, fuel cells have generated great inter-  
20 est for the production of clean electrical energy. Fuel cells  
21 generate power through electrochemical reactions via the  
22 oxidation of fuels such as hydrogen and alcohols at the an-  
23 ode, and the reduction of oxidants, typically oxygen, at the  
24 cathode. Fuel cells will produce electricity with heat and  
25 water as by-products providing that fuel and oxidant are  
26 available. Enzymatic fuel cells (EFCs) and microbial fuel  
27 cells (MFCs) are sub-categories of fuel cells that share  
28 similar operational principles for energy production [1,2].  
29 However, biological fuel cells are not intended to over-

come the depletion of fossil fuels and enter the category  
of renewable energy resources such as hydroelectric, solar,  
and wind energy. The scope of applications for EFCs and  
MFCs is restricted, at least for now, to electricity genera-  
tion for powering microelectronic systems such as actua-  
tors and sensors [3,4,5,6] and for use in environmental  
remediation and sanitation systems [7,8].

Enzymatic and microbial biofuel cells differ vastly in  
terms of their volumetric size ( $\text{cm}^3$  to  $\text{m}^3$ ), power density  
output ( $\mu\text{W}$  to  $\text{mW}$ ) and their targeted applications (small-  
to large-scale). Another important consideration lies in the  
cost of the biocatalyst, which ranges from low-cost bacte-  
ria or yeast cells, for MFCs, to the higher cost of puri-  
fied wild-type and engineered enzymes for EFCs. MFCs  
have been successfully employed as long-term power-  
generating systems, for example, for self-powered sen-  
sors placed in "hostile" environments or remote locations  
[9]. Although MFCs have successfully powered different  
generations of robots, this approach remains at the aca-  
demic level because of limitations such as the large vol-  
ume of MFCs required and their long amortization cost  
[8]. Nevertheless, MFCs constitute an original and eco-  
friendly system for producing electrical energy, e.g. from  
waste, and have become an attractive bioreactor concept  
for purification of wastewaters. EFCs on the other hand,  
because of their superior power density and compactness,  
are better suited to miniaturization and use as portable  
power sources for wearable and implantable electronics  
devices. EFCs appear to carry greater promise as battery-  
free power solutions, but it is necessary to define if this is  
a simple scientific curiosity or if their potentialities really  
do have conceivable real-world applications. This opin-  
ion article attempts to shed light on some hitherto ne-  
glected facets of EFCs, to briefly revisit their possible  
applications, and to reveal new insights concerning fu-  
ture directions. It is emphasized that this review does  
not cover all of the exciting facets and developments in  
EFC research. Recent reviews are available for further  
information on important topics such as the evolution of  
bioinspired strategies for the electrical wiring of enzymes  
and the development of high surface area bioelectrodes  
[10,11]. Notable innovations on high surface area elec-  
trodes include the hierarchically porous MgO-templated  
carbon and carbon nanotube (CNT) yarn textile elec-  
trodes [12,13].

## 2 Bioelectrochem

At the very beginning, EFCs were developed to generate electrical energy mainly through the oxidation of saccharides at the anode coupled with the reduction of oxygen at the cathode [4,14]. In addition to glucose, which is by far the most exploited fuel at the anode, other fuels that have been explored include fructose, pyruvate, hydrogen, lactate, methanol and ethanol [3,4,6]. Since glucose is an essential and relatively abundant source of energy in living organisms, special attention has been given to the development of implantable EFCs for powering implanted medical devices from physiological fluids [15]. In parallel, EFCs are considered for powering portable electronic devices such as mobile phones, sensors, digital music players, laptops, and GPS systems. A fascinating flagship application, proposed by many authors for more than a decade, is the use of EFCs as a lifetime power supply for cardiac pacemakers. However, despite all of the promises, there are many hurdles and roadblocks to overcome. It is now time to recognize that the pacemaker application is unrealistic. Besides sterilization and biocompatibility problems, the major issue with implanted EFCs is their operational stability that ranges from a few hours to one year compared to 5–8 years for sealed batteries [16].

A recent and promising generation of biofuel cells concerns  $H_2/O_2$  EFCs [17\*,18\*\*]. The adsorption, entrapment and chemical immobilization of NiFe and FeFe hydrogenases, and their “electrical wiring”, is at the origin of bioanodes for electrocatalytic interconversion of protons and hydrogen [19–23]. Hydrogenases entrapped in redox polymers are particularly active for bioelectrocatalytic hydrogen oxidation [24\*,25,26]. Recent hydrogenase electrodes exhibit characteristics that are competitive to those of Pt-based electrodes such as long-term stability, mW power densities, insensitivity to fuel impurities such as CO and sulfides, and tolerance to  $O_2$ . EFCs with hydrogenase bioanodes thus show great promise as noble metal-free hydrogen fuel cells [27–31]. Compared with glucose EFCs, a major advantage of hydrogen EFCs lies in the size of the fuel. Hydrogen is markedly smaller than glucose or various redox mediators used for electrical enzyme wiring. The small size can thus be exploited, via careful electrode design with permselective membranes, to prevent the leakage of redox mediators whilst allowing  $H_2$  to efficiently diffuse into the cell. Hydrogen EFCs have been successfully developed with gas-diffusion electrodes, like air-breathing cathodes or  $H_2$ -breathing anodes, which make it possible to increase the availability of dissolved  $O_2$  and  $H_2$  in quiescent solutions. This is an essential development for portable EFCs used outside of the body. Owing to electrode nanostructuring, and improvements in enzyme orientation and electrical wiring, great improvements in power densities have been achieved, which can match, or even surpass, the performance of glucose EFCs. However, the use of hydrogen for EFCs confronts us with problems of  $H_2$  storage and transport. This is in contrast to the use of glucose, which

is a safe, abundant and easy-to-handle energy compound. Hydrogen can be physically stored as either a gas or a liquid but generally requires high pressure and large volume tanks. Practical high density hydrogen storage remains a major challenge for EFCs dedicated to portable applications.

### How to define EFC power?

One of the problematic points for the comparison and continuous optimization of EFC performance concerns their power output and how it is defined. As already pointed out by Katz and coworkers, the power density of an EFC is an important parameter that must be associated with the real power generated by the EFC in order to determine which applications can actually be considered [32]. There are a multitude of electronic and wireless electrical products that consume microwatt to milliwatt powers during operation. Many electronic devices have multiple power modes such as a “standby” mode, where low power is consumed, and “active modes”, for example, for data transmission. High milliwatt powers, for example, may therefore only be required for very short periods of peak activity. Future advances in ultra-low power and low voltage electronics in the next decade are expected which will further valorise energy-harvesting devices with  $\mu W$  to mW outputs. For instance, an ultra-low power 3.5  $\mu W$ -consuming cell phone without a battery is under development [33].

In the research field of biological fuel cells, power densities can be misleading. For example, microwatt power densities obtained using microelectrodes seem extremely attractive, but in reality, are almost useless when the actual power output is on the order of nanowatts. Another issue for bioelectrodes is that the power does not evolve proportionally to the surface area owing to the electrode morphology that plays, for example, on the continuous supply and removal of reactants and products. The power is highly dependent on electrode morphology, including the structure and thickness of the adsorbed enzymes, and the crucial mass transport and charge propagation processes that occur inside and outside of the biocatalyst layer(s) [34].

Regarding the electrode architecture, which ranges from simple planar electrodes with monolayers of enzymes to 3D-structured mesoporous carbon, CNT pellets, and CNT yarns with embedded enzymes, the electroactive surfaces and enzyme loadings can be vastly different [12\*,13]. Despite this, the power densities of biofuel cells are conventionally calculated only with respect to the geometric surface of the electrodes. So what about the fact that most bioelectrodes are now based on 3D architectures with huge differences in electrode thickness and surface area? Another difficulty in defining power also lies sometimes in the design of EFCs that exploit anodes and cathodes with different sizes. For example, a  $H_2/O_2$

185 EFC combines a  $6\text{ cm}^2$  bilirubin oxidase-modified cathode  
186 with a  $1.2\text{ cm}^2$  hydrogenase-modified anode to balance  
187 the catalytic performance as a function of the  $\text{H}_2$ -air  
188 mixture used [18\*\*]. The raw power output must surely  
189 now be reported in all cases? It now seems valuable to define  
190 the volumetric power of bioelectrodes in  $\text{mW cm}^{-3}$ ,  
191 especially if 3D electrodes with thicknesses ranging from  
192 micrometres and centimetres are being used. Indeed, we  
193 have recently reported the raw power together with the  
194 current density of an EFC in both  $\text{cm}^2$  and  $\text{cm}^3$  [35\*].  
195 A crucial power definition for the future, which will become  
196 more relevant as EFCs emerge for practical device  
197 powering, is the volumetric power density of the single  
198 or stacked EFC in  $\text{cm}^3$ , taking into account the space between  
199 the anode(s) and the cathode(s) and the entire cell.  
200 For a single EFC, the optimisation of distance between  
201 the bioelectrodes, which also plays on the resistance, substrate  
202 supply, and by-product formation, will become a key  
203 consideration for the power/size ratio.

#### 204 Influence of temperature on the operation of EFCs

205 Besides the difficulty of designing EFCs whose enzymatic  
206 reactions at the anode and the cathode must operate at the  
207 same pH, another major problem for EFCs concerns the  
208 influence of temperature on the rate and stability of enzyme-  
209 catalyzed reactions, and hence on EFC performance. This  
210 key parameter has often been underestimated or neglected,  
211 yet can drastically change the perspectives of EFCs for  
212 different applications. Depending on the origin of the enzyme,  
213 the temperature/activity profile may show optima ranging from  
214 low temperatures ( $3\text{--}5\text{ }^\circ\text{C}$ ) to high temperatures ( $50\text{--}80\text{ }^\circ\text{C}$ ).  
215 Regarding the more common glucose/ $\text{O}_2$  EFCs, cathodes are  
216 usually based on immobilized bilirubin oxidase or laccase for  
217 the reduction of oxygen. Laccase from *Trametes versicolor*  
218 exhibits its greatest stability at  $30\text{ }^\circ\text{C}$  and undergoes a  
219 thermal deactivation above  $50\text{ }^\circ\text{C}$ , which for example, leads  
220 to a loss in activity of 53% and 90% after 6 and 48 hrs,  
221 respectively [36]. In contrast, bilirubin oxidase from  
222 *Myrothecium verrucaria* is considered relatively stable in  
223 the range of  $40\text{--}60\text{ }^\circ\text{C}$  but loses 40% of its activity when  
224 the temperature decreases from  $40$  to  $25\text{ }^\circ\text{C}$  [37]. At the  
225 anode, the oxidation of glucose is mainly catalyzed by  
226 glucose oxidase. The latter has a maximum activity at  
227  $20\text{--}35\text{ }^\circ\text{C}$  but retains only 65% of its activity at  $45\text{ }^\circ\text{C}$ ,  
228 and exhibits total thermal inactivation at  $60\text{ }^\circ\text{C}$  [38–40].

230 The different thermal behavior of oxidoreductase enzymes  
231 is a real problem for the performance and stability of EFCs,  
232 especially considering that an EFC must employ two very  
233 different enzymes at the anode and cathode. The influence  
234 of temperature may call into question the use of EFCs for  
235 powering portable electronics exposed to certain environmental  
236 conditions with wide variations in temperature. This problem  
237 actually becomes an attractive point for implanted biofuel  
238 cells, of which one of the main features is their exposure,  
239 by definition, to a constant

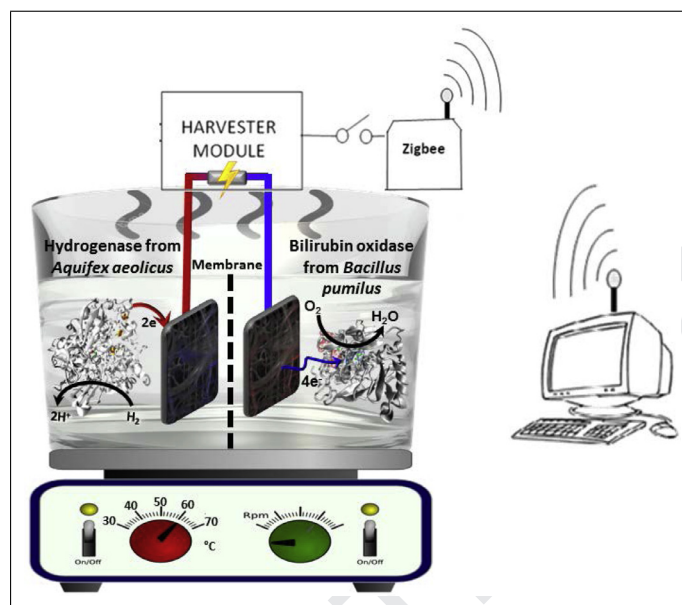
240 physiological temperature of  $37\text{ }^\circ\text{C}$ . Nevertheless, this  
241 does not mean that relevant enzymes are unsuitable for  
242 operation at elevated temperatures. In fact, it appears that  
243 the power of EFCs can be increased by increasing the  
244 temperature, as demonstrated by Lojou and coworkers for  
245  $\text{H}_2/\text{O}_2$  EFCs using thermostable enzymes such as hydrogenase  
246 from *Aquifex aeolicus* and bilirubin oxidase from *Bacillus*  
247 *pumilus* [17\*,41]. High power densities have been obtained  
248 under these (relatively) mild conditions, making it possible  
249 to power a wireless transmission system [42] as illustrated  
250 in Figure 1. However, to accurately evaluate the performance  
251 and practicality of such hydrogen EFCs, it is necessary to  
252 also consider the power required to maintain the EFC at  
253  $60\text{ }^\circ\text{C}$ . Rather than the possibility for improved EFC power  
254 output, the main advantage of this approach lies in the use  
255 of thermostable enzymes that allow the EFC to withstand  
256 significant temperature variations (e.g. from  $30\text{--}80\text{ }^\circ\text{C}$ )  
257 without denaturation.

258 As well as temperature considerations, the type of solution  
259 used in the biofuel cell is another critical but often overlooked  
260 factor in EFC research. Until now, relatively few studies have  
261 investigated EFC performance in matrices for practical  
262 applications such as biological fluids, environmental waters  
263 or wastewater. The vast majority of experiments have  
264 simply been performed in aqueous buffer solutions. Towards  
265 human body-integrated applications, recent studies have  
266 started to address bioelectrode and EFC performance  
267 directly in human serum, urine, saliva and blood [43–47].  
268 These works have highlighted complications that reduce  
269 EFC power output and stability due to low substrate  
270 concentrations and interferences from ascorbic acid,  
271 chloride, uric acid and biophysical matter. Reported  
272 strategies to address interferences include the use of a  
273 dialysis bag as a protective membrane, a strategy first  
274 reported by our group in 2010 for an EFC implanted in  
275 a rat [48].

#### 276 Buckypaper-based EFCs for wearables and implantable devices

277 Carbon nanotubes are a widely used material in bioelectrode  
278 design [6\*]. In the last 5–7 years, free-standing thin films  
279 of CNTs, called buckypapers, have emerged for use in  
280 biofuel cells [49\*]. Buckypapers are in fact at the origin  
281 of remarkable advances in the field of EFCs [50–53],  
282 particularly concerning implanted biofuel cells [51\*\*,54\*\*].  
283 Buckypaper films are self-supporting macroscopic sheets  
284 of entangled CNTs held together by  $\pi$ - $\pi$  stacking interactions  
285 with an average thickness of  $5\text{--}200\text{ }\mu\text{m}$ . Buckypapers  
286 are typically obtained either from a commercial source  
287 (e.g. NanoTechLabs Inc.) via roll-to-roll processing,  
288 or via conventional vacuum filtration of a dispersion  
289 of CNTs in organic or aqueous solution. Compared  
290 with classical electrodes based on glassy carbon, buckypapers  
291 constitute a break of concept since they are the electrode  
292 itself. Furthermore, these electrodes are lighter, more  
293 compact and flexible, and more easily processed into  
294

Figure 1



Schematic presentation of a  $\text{H}_2/\text{O}_2$  biofuel cell setup with thermophilic biocatalysts producing sufficient power at elevated temperature for supplying a wireless transmission system.

295 different shapes and sizes compared to electrodes based  
 296 on glassy carbon. Until now, buckypapers have mainly  
 297 been modified via  $\pi$ -stacking interactions with pyrene  
 298 derivatives, which are used for covalent or non-covalent  
 299 binding of enzymes and/or mediators, and have led to  
 300 high performance bioelectrodes for *in-vivo* biofuel cells.  
 301 In particular, Katz and coworkers have highlighted the  
 302 practical advantages of buckypaper for implantation com-  
 303 pared to traditional bulk electrodes (e.g. glassy carbon),  
 304 such as their ideal microscale thickness and tunable di-  
 305 mensions [54\*\*,55]. Owing to attractive properties such  
 306 as their flexibility, Minteer and coworkers developed a  
 307 miniaturized buckypaper-based lactate/ $\text{O}_2$  EFC embed-  
 308 ded in a contact lens for operation in human tears [56].  
 309 Besides increasing the length and alignment of CNTs,  
 310 another possibility to improve the strength and flexibil-  
 311 ity of buckypapers lies with the incorporation of an or-  
 312 ganic crosslinker. An elegant approach is to “molecularly”  
 313 cross-link the CNTs of buckypaper with bis-pyrene-2,2’-  
 314 azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (bis-  
 315 pyrene-ABTS), which enhances the mechanical stability  
 316 of the buckypaper and gives it redox properties for wiring  
 317 multicopper enzymes [57,58]. Furthermore, Cosnier and  
 318 coworkers reported a new approach for fabricating robust  
 319 and flexible buckypaper bioelectrodes based on the in-  
 320 timate association of CNTs and linear polynorbornene  
 321 polymers [59,60]. These polymers were functionalized  
 322 with pyrene groups, for cross-linking, and activated esters,  
 323 for covalent attachment of enzymes and/or molecules for  
 324 electrical enzyme wiring. Besides improved flexibility and  
 325 handleability, enzyme-modified buckypapers are proving

to be effective bioelectrodes for EFCs and will certainly  
 have an important role to play in future implantable or  
 wearable EFCs. To improve the limited performance of  
 biocathodes, which commonly suffer from oxygen limi-  
 tations, we note the cathode design from Atanassov and  
 his colleagues that combined a gas-diffusion layer with a  
 catalytic buckypaper such that the individual benefits of  
 both materials could be exploited [61]. Some examples of  
 buckypaper interfaces and their applications are sketched  
 in Figure 2.

### Perspectives on nanoparticle-based rechargeable EFCs

EFCs are faced with two major technological bottle-  
 necks that currently block their development, namely  
 their short lifetime, and to a lesser degree, their weak  
 power output. The low stability of EFCs is largely re-  
 lated to the deactivation of immobilized enzymes, the  
 intrinsic catalytic component, and this seems somewhat  
 ineluctable. In particular, enzymes have a limited stabil-  
 ity that depends on the environment in which they are  
 used (temperature, pH, inhibitors...). Weak power out-  
 put is related to factors including the sub-optimal fixa-  
 tion of enzymes and redox mediators to electrodes, which  
 reduces markedly their mobility and flexibility, and in  
 some cases, fixes enzymes in poor orientations for elec-  
 tron transfer. An attractive solution lies in the devel-  
 opment of bioelectrodes based on confined solutions of  
 enzymes and supramolecular assemblies of redox medi-  
 ators or electron transfer-promoting molecules. The elec-  
 trical wiring of enzymes in solution with electrodes form-  
 ing a conductive chamber only permeable to the enzyme

Figure 2

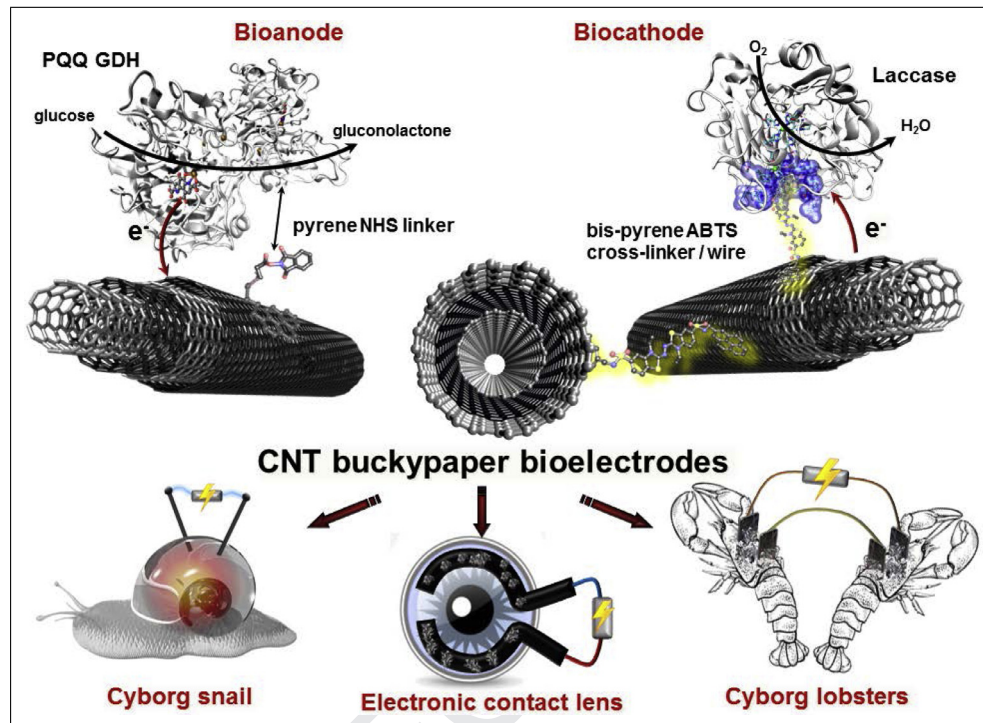
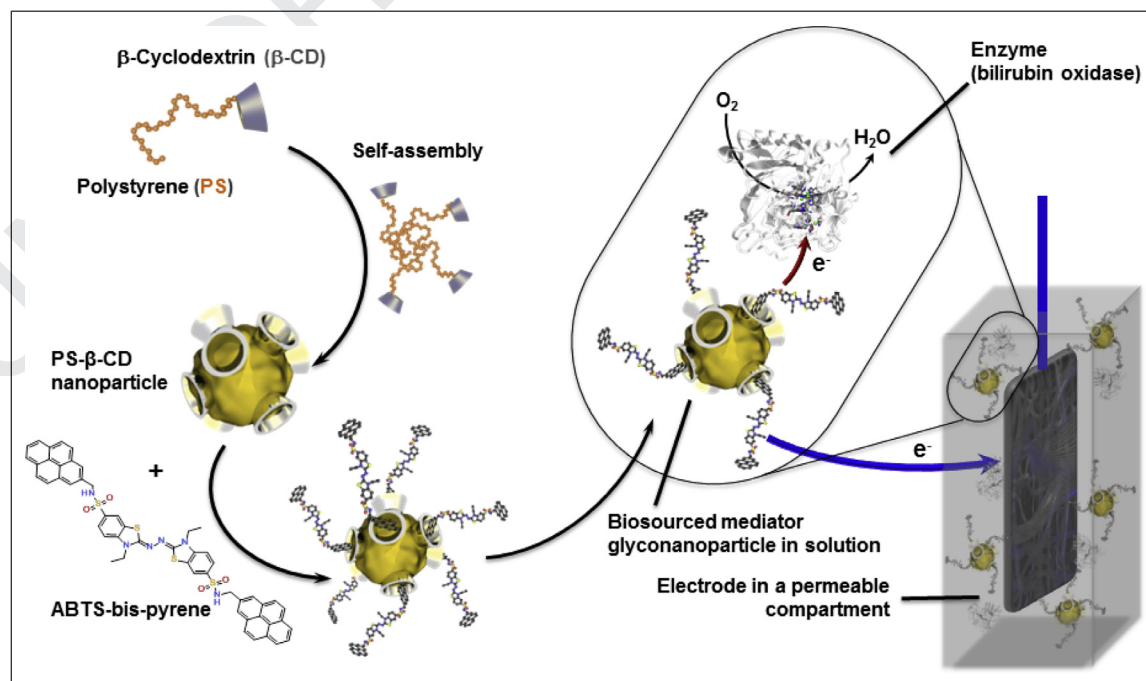


Illustration of buckypaper-based bioelectrodes with various immobilization and enzyme wiring functions (pyrroloquinoline quinone glucose dehydrogenase (PQQ GDH) and laccase) for efficient energy harvesting wearable and implantable biofuel cells.

Figure 3



Scheme illustrating the formation and modification of redox-active glyconanoparticles used for construction of an "all in solution" biocathode.

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substrates is envisaged. For enzymes that require or benefit from the use of a redox mediator, bioelectrocatalysis can be ensured using supramolecular assemblies of redox mediator freely diffusing in this chamber. This strategy will overcome the low operational stability of EFCs by allowing the renewal of solubilized enzymes and redox mediators, leading thus to rechargeable EFCs. This concept can also enhance the electrical wiring process, for example, via rotation and redox-hopping effects, and should lead to increased fuel cell performance. Recently, the first step of this unconventional approach was conceived with biosourced nanoparticles functionalized with  $\beta$ -cyclodextrin groups [62]. For construction of a biocathode, the nanoparticles were modified via host-guest interactions with bis-pyrene-ABTS and used as electron shuttles to facilitate electron transfer between the electrode and bilirubin oxidase in solution (Figure 3). The redox nanoparticles led to enhanced catalytic currents and improved stability for O<sub>2</sub> reduction compared to the same electro-enzymatic system with ABTS in solution [63\*].

## Conclusion

A significant number of emerging directions for EFCs are now under exploration, such as metabolon systems for cascade reactions of sequential enzymes [64,65\*\*], hybrid fuel cells that seek to combine the advantages of metals and enzymes [66], engineered enzymes with enhanced thermal stability [67], the use of molecular catalysts that mimic enzyme activity with very large turnover frequencies and high stability [68\*], and the integration of supercapacitors into fuel cell devices [69]. Another key emerging area concerns biofuel cell power management systems and the incorporation of energy storage systems such as capacitors to prolong biofuel cell lifetime for practical applications [70,53]. The promises and challenges of this exciting research field now require truly interdisciplinary research efforts between chemists, surface scientists, bio-scientists, electronic engineers and materials scientists. Despite the presence of technological obstacles to circumvent, there are real potential applications for EFCs ranging from implantable EFCs, for periodic monitoring of diseases during short periods (1–3 months), to totally biodegradable EFCs for disposable medical devices.

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## Declarations

None.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- Paper of special interest
- Paper of outstanding interest.

1. Zhao C, Gai P, Song R, Chen Y, Zhang J, Zhu J-J: **Nanostructured material-based biofuel cells: recent advances and future prospects.** *Chem Soc Rev* 2017, **46**:1545–1564. 408–410
2. Santoro C, Arbizzani C, Erable B, Ieropoulos I: **Microbial fuel cells: from fundamentals to applications. A review.** *J Power Sources* 2017, **356**:225–244. 411–413
3. Bandothkar AJ: **Review—wearable biofuel cells: Past, present and future.** *J Electrochem Soc* 2017, **164**:H3007–H3014. 414–415
4. Rasmussen M, Abdellaoui S, Minteer SD: **Enzymatic biofuel cells: 30 years of critical advancements.** *Biosens Bioelectron* 2016, **76**:91–102. 416–418
5. Bandothkar AJ, You J-M, Kim N-H, Gu Y, Kumar R, Mohan AMV, Kurniawan J, Imani S, Nakagawa T, Parish B, Parthasarathy M, Mercier PP, Xu S, Wang J: **Soft, stretchable, high power density electronic skin-based biofuel cells for scavenging energy from human sweat.** *Energy Environ Sci* 2017, **10**:1581–1589. 419–423  
 The highest power density wearable EFC cell to date and the first example for powering a bluetooth low energy radio. The biofuel cell device with integrated DC-DC converter can withstand repeated mechanical strains and was demonstrated for actual in-vivo energy harvesting from the sweat of human subjects 424–428
6. Cosnier S, Gross AJ, Goff AL, Holzinger M: **Recent advances on enzymatic glucose/oxygen and hydrogen/oxygen biofuel cells: achievements and limitations.** *J. Power Sources* 2016, **325**:252–263. 429–432  
 This paper presents an overview of recent advances in the fields of both glucose and hydrogen biofuel cells, and highlights the impact of new nanostructured electrode materials for enzyme wiring 433–435
7. Ieropoulos IA, Stinchcombe A, Gajda I, Forbes S, Merino-Jimenez I, Pasternak G, Sanchez-Herranz D, Greenman J: **Pee power urinal – Microbial fuel cell technology field trials in the context of sanitation.** *Environ. Sci. Water Res. Technol.* 2016, **2**:336–343. 436–440  
 This paper demonstrates the feasibility of generating several hundreds of mWs from urine using modular MFCs for internal lighting. This is the first time that urinal field trials were used in a practical setting in the context of sanitation and public health 441–444
8. Tommasi T, Lombardelli G: **Energy sustainability of microbial fuel cell (MFC): a case study.** *J Power Sources* 2017, **356**:438–447. 445–446
9. Pasternak G, Greenman J, Ieropoulos IA: **Self-powered, autonomous biological oxygen demand biosensor for online water quality monitoring.** *Sens Actuators B Chem* 2017, **244**:815–822. 447–450
10. Mazurenko I, de Poulpique A, Lojou E: **Recent developments in high surface area bioelectrodes for enzymatic fuel cells.** *Curr Opin Electrochem* 2017, **5**:74–84. 451–453
11. Saboe PO, Conte E, Farrell M, Bazan GC, Kumar M: **Biomimetic and bioinspired approaches for wiring enzymes to electrode interfaces.** *Energy Environ Sci* 2017, **10**:14–42. 454–456
12. Tsujimura S, Murata K, Akatsuka W: **Exceptionally high glucose current on a hierarchically structured porous carbon electrode with “Wired” flavin adenine dinucleotide-dependent glucose dehydrogenase.** *J Am Chem Soc* 2014, **136**:14432–14437. 457–459  
 This paper demonstrates exceptionally high catalytic currents at templated mesoporous carbons using the FAD-GDH enzyme, and brings forward the important concept of pore size engineering for bioelectrocatalysis 460–464
13. Kwon CH, Lee S-H, Choi Y-B, Lee JA, Kim SH, Kim H-H, Spinks GM, Wallace GG, Lima MD, Kozlov ME, Baughman RH, Kim SJ: *Nat Commun* 2014, **5**:3928. 465–467
14. Mano N: **A. de Poulpique, <sup>O2</sup> reduction in enzymatic biofuel cells.** *Chem Rev* 2017, **5**:2392–2468. 468–469
15. Cosnier S, Goff AL, Holzinger M: **Towards glucose biofuel cells implanted in human body for powering artificial organs: review.** *Electrochem Commun* 2014, **38**:19–23. 470–472
16. Reuillard B, Abreu C, Lalaoui N, Le Goff A, Holzinger M, Ondel O, Buret F, Cosnier S: **One-year stability for a glucose/oxygen biofuel cell combined with pH reactivation of the laccase/carbon nanotube biocathode.** *Bioelectrochemistry* 2015, **106**:73–76. 473–476

- 478 17. Mazurenko I, Wang X, de Poulpique A, Lojou E: **H<sub>2</sub>/O<sub>2</sub> enzymatic**  
479 **• fuel cells: from proof-of-concept to powerful devices.** *Sustain*  
480 *Energy Fuels* 2017, **1**:1475–1501.
- 481 This review summarizes the latest advances in the field of H<sub>2</sub>/O<sub>2</sub> EFCs  
482 in terms of hydrogenase immobilization, hydrogenase structure,  
483 electrode nanostructuring, enzyme engineering, and the use of  
484 thermostable enzymes
- 485 18. Xu L, Armstrong FA: **Pushing the limits for enzyme-based**  
486 **• membrane-less hydrogen fuel cells – achieving useful power**  
487 **and stability.** *RSC Adv* 2014, **5**:3649–3656.
- 488 This paper describes “3D” porous hydrogenase electrodes combined in  
489 an EFC stack design that operates in parallel or in series
- 490 19. Jones AK, Sillery E, Albracht SPJ, Armstrong FA: **Direct**  
491 **comparison of the electrocatalytic oxidation of hydrogen by an**  
492 **enzyme and a platinum catalyst.** *Chem Commun* 2002,  
493 **0**:866–867.
- 494 20. Alonso-Lomillo MA, Rüdiger O, Maroto-Valiente A, Velez M,  
495 Rodriguez-Ramos I, Muñoz FJ, Fernández VM, De Lacey AL:  
496 **Hydrogenase-coated carbon nanotubes for efficient H<sub>2</sub>**  
497 **oxidation.** *Nano Lett* 2007, **7**:1603–1608.
- 498 21. Ciaccafava A, Infossi P, Ilbert M, Guiral M, Lecomte S,  
499 Giudici-Ortoni MT, Lojou E: **Electrochemistry, AFM, and**  
500 **PM-IRRA spectroscopy of immobilized hydrogenase: Role of a**  
501 **hydrophobic helix in enzyme orientation for efficient H<sub>2</sub>**  
502 **oxidation.** *Angew Chem Int Ed* 2012, **51**:953–956.
- 503 22. Liu J, Wu W-J, Fang F, Zorin NA, Chen M, Qian D-J:  
504 **Immobilization of hydrogenase on carbon nanotube**  
505 **polyelectrolytes as heterogeneous catalysts for**  
506 **electrocatalytic interconversion of protons and hydrogen.** *J*  
507 *Nanoparticle Res* 2016, **18**:220.
- 508 23. Karyakin AA, Morozov SV, Karyakina EE, Varfolomeyev SD,  
509 Zorin NA, Cosnier S: **Hydrogen fuel electrode based on**  
510 **bioelectrocatalysis by the enzyme hydrogenase.** *Electrochem*  
511 *Commun* 2002, **4**:417–420.
- 512 24. Morozov SV, Vignais PM, Cournac L, Zorin NA, Karyakina EE,  
513 Karyakin AA, Cosnier S: **Bioelectrocatalytic hydrogen**  
514 **production by hydrogenase electrodes.** *Int J Hydrog Energy*  
515 2002, **27**:1501–1505.
- 516 This is the first report of a redox polypyrrole-hydrogenase electrode.  
517 This paper also demonstrates the production of hydrogen by mass  
518 spectrometry and electrochemistry
- 519 25. Morozov SV, Voronin OG, Karyakina EE, Zorin NA, Cosnier S,  
520 Karyakin AA: **Tolerance to oxygen of hydrogen enzyme**  
521 **electrodes.** *Electrochem Commun* 2006, **8**:851–854.
- 522 26. Karyakin AA, Morozov SV, Voronin OG, Zorin NA, Karyakina EE,  
523 Fateyev VN, Cosnier S: **The limiting performance characteristics**  
524 **in bioelectrocatalysis of hydrogenase enzymes.** *Angew Chem*  
525 *Int Ed* 2007, **46**:7244–7246.
- 526 27. Pandelia M-E, Fourmond V, Tron-Infossi P, Lojou E, Bertrand P,  
527 Léger C, Giudici-Ortoni M-T, Lubitz W: **Membrane-bound**  
528 **hydrogenase I from the hyperthermophilic bacterium aquifex**  
529 **aeolicus: enzyme activation, redox intermediates and oxygen**  
530 **tolerance.** *J Am Chem Soc* 2010, **132**:6991–7004.
- 531 28. Vincent KA, Cracknell JA, Lenz O, Zebger I, Friedrich B,  
532 Armstrong FA: **Electrocatalytic hydrogen oxidation by an**  
533 **enzyme at high carbon monoxide or oxygen levels.** *Proc Natl*  
534 *Acad Sci* 2005, **102**:16951–16954.
- 535 29. Xu L, Armstrong FA: **Optimizing the power of enzyme-based**  
536 **membrane-less hydrogen fuel cells for hydrogen-rich H<sub>2</sub>-air**  
537 **mixtures.** *Energy Environ Sci* 2013, **6**:2166–2171.
- 538 30. Plumeré N, Rüdiger O, Oughli AA, Williams R, Vivekananthan J,  
539 Pöller S, Schuhmann W, Lubitz W: **A redox hydrogel protects**  
540 **hydrogenase from high-potential deactivation and oxygen**  
541 **damage.** *Nat Chem*, vol 6 2014.
- 542 31. Fourmond V, Stapf S, Li H, Buesen D, Birrell J, Rüdiger O,  
543 Lubitz W, Schuhmann W, Plumeré N, Léger C: **Mechanism of**  
544 **protection of catalysts supported in redox hydrogel films.** *J Am*  
545 *Chem Soc* 2015, **137**:5494–5505.
- 546 32. Gamella M, Koushanpour A, Katz E: **Biofuel cells – activation of**  
547 **micro- and macro-electronic devices.** *Bioelectrochemistry* 2018,  
548 **119**:33–42.
- 549 33. Talla V, Kellogg B, Gollakota S, Smith JR: **Battery-Free**  
550 **Cellphone.** *Proc ACM Interact Mob Wearable Ubiquitous Technol*,  
551 vol 1 2017 25:1–25:20.
- 552 34. Rajendran L, Kirthiga M, Laborda E: **Mathematical modeling of**  
553 **nonlinear reaction–diffusion processes in enzymatic biofuel**  
554 **cells.** *Curr Opin Electrochem* 2017, **1**:121–132.
- 555 35. Gross AJ, Chen X, Giroud F, Abreu C, Le Goff A, Holzinger M,  
556 Cosnier S: **A high power buckypaper biofuel cell: exploiting**  
557 **1,10-phenanthroline-5,6-dione with FAD-dependent**  
558 **dehydrogenase for catalytically-powerful glucose oxidation.**  
559 *ACS Catal* 2017, **7**:4408–4416.
- 560 This report demonstrates the preparation of lab-made buckypaper  
561 electrodes which exhibit excellent voltammetry and their use for the  
562 construction of a high power glucose/O<sub>2</sub> biofuel cell with the emerging  
563 FAD-GDH enzyme at the anode
- 564 36. Stoilova I, Krastanov A, Stanchev V: **Properties of crude laccase**  
565 **from *Trametes versicolor* produced by solid-substrate**  
566 **fermentation.** *Adv Biosci Biotechnol* 2010, **01**:208.
- 567 37. Tasca F, Farias D, Castro C, Acuna-Rougier C, Antiochia R:  
568 **Bilirubin oxidase from *Myrothecium verrucaria* physically**  
569 **adsorbed on graphite electrodes. Insights into the alternative**  
570 **resting form and the sources of activity loss.** *PLoS One*, vol 10  
571 2015 e0132181.
- 572 38. Gouda MD, Singh SA, Rao AGA, Thakur MS, Karanth NG: **Thermal**  
573 **inactivation of glucose oxidase: mechanism and stabilization**  
574 **using additives.** *J Biol Chem* 2003, **278**:24324–24333.
- 575 39. El-Sherbeny GA, Shindia AA, Sheriff YMMM: **Optimization of**  
576 **various factors affecting glucose oxidase activity produced by**  
577 ***Aspergillus niger*.** *Int J Agric Biol* 2005, **7**:953–958.
- 578 40. Singh J, Verma N: **Glucose oxidase from *Aspergillus niger*:**  
579 **Production, characterization and immobilization for glucose**  
580 **oxidation.** *Adv Appl Sci Res* 2013, **4**:250–257.
- 581 41. de Poulpique A, Ciaccafava A, Gadiou R, Gounel S,  
582 Giudici-Ortoni MT, Mano N, Lojou E: **Design of a H<sub>2</sub>/O<sub>2</sub> biofuel**  
583 **cell based on thermostable enzymes.** *Electrochem Commun*  
584 2014, **42**:72–74.
- 585 42. Monsalve K, Mazurenko I, Lalaoui N, Le Goff A, Holzinger M,  
586 Infossi P, Nitsche S, Lojou JY, Giudici-Ortoni MT, Cosnier S,  
587 Lojou E: **A H<sub>2</sub>/O<sub>2</sub> enzymatic fuel cell as a sustainable power for**  
588 **a wireless device.** *Electrochem Commun* 2015, **60**:216–220.
- 589 43. Cadet M, Gounel S, S-Chaumeil C, Brilland X, Rouhana J,  
590 Louerat F, Mano N: **An enzymatic glucose/O<sub>2</sub> biofuel**  
591 **cell operating in human blood.** *Biosens Bioelectron* 2016,  
592 **83**:60–67.
- 593 44. Kwon CH, Lee JA, Choi Y-B, Kim H-H, Spinks GM, Lima MD,  
594 Baughman RH, Kim SJ: **Stability of carbon nanotube yarn**  
595 **biofuel cell in human body fluid.** *J Power Sources* 2015,  
596 **286**:103–108.
- 597 45. Milton RD, Lim K, Hickey DP, Minter SD: **Employing**  
598 **FAD-dependent glucose dehydrogenase within a**  
599 **glucose/oxygen enzymatic fuel cell operating in human serum.**  
600 *Bioelectrochemistry* 2015, **106**:56–63.
- 601 46. Ó Conghaile P, Falk M, MacAodha D, Yakovleva ME, Gonaus C,  
602 Peterbauer CK, Gorton L, Shleev S, Leech D: **A fully enzymatic**  
603 **membrane-less glucose/oxygen fuel cell provides 0.275 mA**  
604 **cm<sup>-2</sup> in 5 mM glucose operates in human physiological**  
605 **solutions and powers transmission of sensing data.** *Anal Chem*  
606 2016, **88**:2156–2163.
- 607 47. Gobel G, Beltran ML, Mundhenk J, Heinlein T, Schneider J,  
608 Lisdat F: **Operation of a carbon nanotube-based**  
609 **glucose/oxygen biofuel cell in human body**  
610 **liquids-performance factors and characteristics.** *Electrochim*  
611 *Acta* 2016, **218**:278–284.
- 612 48. Cinquin P, Gondran C, Giroud F, Mazabrard S, Pellissier A,  
613 Boucher F, Alcaraz J-P, Gorgy K, Lenouvel F, Mathé S, Porcu P,



## 8 Bioelectrochem

- 614 Cosnier S: **A glucose bioFuel cell implanted in rats.** *PLOS ONE* 2010, **5**:e10476. 668
- 615 60. Gross AJ, Robin MP, Nedellec Y, O'Reilly RK, Shan D, Cosnier S: **Robust bifunctional buckypapers from carbon nanotubes and polynorborene copolymers for flexible engineering of enzymatic bioelectrodes.** *Carbon* 2016, **107**:542–547. 669
- 616 49. Gross A, Holzinger M, Cosnier S: **Buckypaper bioelectrodes: 670**
- 617 • **Emerging materials for implantable and wearable biofuel cells.** 671
- 618 *Energy Environ Sci* 2018. <https://doi.org/10.1039/C8EE00330K>. 672
- 619 This paper is the first review on buckypapers in the field of 673
- 620 (bio)electrochemistry and biofuel cell research for body-integrated 674
- 621 applications 675
- 622 50. Villarrubia CWN, Soavi F, Santoro C, Arbizzani C, Serov A, 676
- 623 Rojas-Carbonell S, Gupta G, Atanassov P: **Self-feeding paper 677**
- 624 **based biofuel cell/self-powered hybrid  $\mu$ -supercapacitor 678**
- 625 **integrated system.** *Biosens Bioelectron* 2016, **86**:459–465. 679
- 626 51. Halámková L, Halámek J, Bocharova V, Szczupak A, Alfonta L, 680
- 627 •• Katz E: **Implanted biofuel cell operating in a living snail.** *J Am 681*
- 628 *Chem Soc* 2012, **134**:5040–5043. 682
- 629 This was the first example of an EFC based on commercial buckypaper 683
- 630 implanted in a live animal in a natural environment. The power was 684
- 631 demonstrated continuously over 2 weeks and could be metabolically 685
- 632 recharged upon feeding or resting of the snail 686
- 633 52. Fokina O, Eipper J, Winandy L, Kerzenmacher S, Fischer R: 687
- 634 **Improving the performance of a biofuel cell cathode with 688**
- 635 **laccase-containing culture supernatant from pycnopus 689**
- 636 **sanguineus.** *Bioresour Technol* 2015, **175**:445–453. 690
- 637 53. Hou C, Liu A: **An integrated device of enzymatic biofuel cells 691**
- 638 **and supercapacitor for both efficient electric energy 692**
- 639 **conversion and storage.** *Electrochim Acta* 2017, **245**:303–308. 693
- 640 54. MacVittie K, Halámek J, Halámková L, Southcott M, Jemison WD, 694
- 641 •• Lobel R, Katz E: **From “cyborg” lobsters to a pacemaker 695**
- 642 **powered by implantable biofuel cells.** *Energy Environ Sci* 2013, 696
- 643 **6**:81–86. 697
- 644 The first paper to address the problem of interfacing electronics with 698
- 645 implantable glucose/O<sub>2</sub> EFCs. This paper highlighted the unfortunate 699
- 646 issue of tissue conductivity which prevented biofuel cell operation in 700
- 647 series in the same organism. Successful activation of a watch was 701
- 648 nevertheless demonstrated from in-vivo fuel cells in multiple organisms 702
- 649 55. MacVittie K, Conlon T, Katz E: **A wireless transmission system 703**
- 650 **powered by an enzyme biofuel cell implanted in an orange.** 704
- 651 *Bioelectrochemistry* 2015, **106**:28–33. 705
- 652 56. Reid RC, Minter SD, Gale BK: **Contact lens biofuel cell tested in 706**
- 653 **a synthetic tear solution.** *Biosens Bioelectron* 2015, **68**:142–148. 707
- 654 57. Bourourou M, Elouarzaki K, Holzinger M, Agnès C, Le Goff A, 708
- 655 Reverdy-Bruas N, Chaussy D, Party M, Maaref A, Cosnier S: 709
- 656 **Freestanding redox buckypaper electrodes from multi-wall 710**
- 657 **carbon nanotubes for bioelectrocatalytic oxygen reduction via 711**
- 658 **mediated electron transfer.** *Chem Sci* 2014, **5**:2885–2888. 712
- 659 58. Elouarzaki K, Bourourou M, Holzinger M, Goff AL, Marks RS, 713
- 660 Cosnier S: **Freestanding HRP-GOx redox buckypaper as an 714**
- 661 **oxygen-reducing biocathode for biofuel cell applications.** 715
- 662 *Energy Environ Sci* 2015, **8**:2069–2074. 716
- 663 59. Cosnier S, Haddad R, Moatsou D, O'Reilly RK: 717
- 664 **Biofunctionalizable flexible bucky paper by combination of 718**
- 665 **multi-walled carbon nanotubes and polynorborene-pyrene - 719**
- 666 **Application to the bioelectrocatalytic reduction of oxygen.** 720
- 667 *Carbon* 2015, **93**:713–718. 721
60. Gross AJ, Robin MP, Nedellec Y, O'Reilly RK, Shan D, Cosnier S: 668
- 609 **Robust bifunctional buckypapers from carbon nanotubes and 669**
- 610 **polynorborene copolymers for flexible engineering of 670**
- 611 **enzymatic bioelectrodes.** *Carbon* 2016, **107**:542–547. 671
61. Ciniciato GPMK, Lau C, Cochrane A, Sibbett SS, Gonzalez ER, 672
- Atanassov P: *Electrochim Acta* 2012, **82**:208–213. 673
62. Gross AJ, Haddad R, Travelet C, Reynaud E, Audebert P, Borsali R, 674
- Cosnier S: **Redox-active carbohydrate-coated nanoparticles: 675**
- 626 **self-assembly of a cyclodextrin-polystyrene glycopolymer with 676**
- 627 **tetrazine-naphthalimide.** *Langmuir* 2016, **32**:11939–11945. 677
63. Gross AJ, Chen X, Giroud F, Travelet C, Borsali R, Cosnier S: 678
- **Redox-active glyconanoparticles as electron shuttles for 679**
- 630 **mediated electron transfer with bilirubin oxidase in solution.** *J 680*
- 631 *Am Chem Soc* 2017, **139**:16076–16079. 681
- The first enzyme wiring by organic redox nanoparticles is described and 682
- 632 demonstrated for O<sub>2</sub> reduction via mediated electron transfer. This 683
- 633 paper reports the self-assembly, characterization and 684
- 634 bioelectrocatalysis of  $\beta$ -cyclodextrin-based nanoparticles with enzyme 685
- 635 in solution 686
64. Liu Y, Hickey DP, Guo J-Y, Earl E, Abdellaoui S, Milton RD, 687
- Sigman MS, Minter SD, Calabrese Barton S: **Substrate 688**
- 640 **channeling in an artificial metabolon: a molecular dynamics 689**
- 641 **blueprint for an experimental peptide bridge.** *ACS Catal* 2017, 690
- 642 **7**:2486–2493. 691
65. Wheeldon I, Minter SD, Banta S, Barton SC, Atanassov P, 692
- Sigman M: **Substrate channeling as an approach to cascade 693**
- 650 **reactions.** *Nat Chem* 2016, **8**:299–309. 694
- This work describes different natural and non-natural approaches for 695
- 651 channeling substrates between enzyme active sites for cascade 696
- 652 reactions for catalyzing sequential reactions 697
66. Majdecka D, Draminska S, Janusek D, Krysinski P, Bilewicz R: **A 698**
- 660 **self-powered biosensing device with an integrated hybrid 699**
- 661 **biofuel cell for intermittent monitoring of analytes.** *Biosens 700*
- 662 *Bioelectron* 2018, **102**:383–388. 701
67. K. Sode, K. Mori, Glucose dehydrogenase, US Pat., 8999691B2, 702
2015. 703
68. Gentil S, Lalaoui N, Dutta A, Nedellec Y, Cosnier S, Shaw WJ, 704
- Artero V, Le Goff A: **Carbon-nanotube-supported bio-inspired 705**
- 680 **nickel catalyst and its integration in hybrid hydrogen/air fuel 706**
- 681 **cells.** *Angew Chem Int Ed* 2017, **56**:1845–1849. 707
- The most efficient example of a hybrid H<sub>2</sub>/O<sub>2</sub> EFC to date which exploits 708
- 682 a bioinspired complex at the anode with an enzymatic biocathode 709
69. Xiao X, Conghaile PÓ, Leech D, Ludwig R, Magner E: **A symmetric 710**
- 691 **supercapacitor/biofuel cell hybrid device based on 711**
- 692 **enzyme-modified nanoporous gold: an autonomous pulse 712**
- 693 **generator.** *Biosens. Bioelectron* 2017, **90**:96–102. 713
70. Abreu C, Nedellec Y, Gross AJ, Ondel O, Buret F, Le Goff A, 714
- Holzinger M, Cosnier S: **Assembly and stacking of flow-through 715**
- 700 **enzymatic bioelectrodes for high power glucose fuel cells.** 716
- 701 *ACS Appl Mater Interfaces* 2017, **9**:23836–23842. 717