### **Supporting Information**

# Probing the Enzymatic Activity of Individual Biocatalytic *fd*-Viral Particles by Electrochemical-Atomic Force Microscopy

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(I) Step by step assembly of a random array of Fc-PEG / PQQ-GDH immunodecorated *fd* on SiO<sub>2</sub>. Evidencing the homogenous decoration of the viral particles with antibodies.

#### (a) Immunological assembly process and AFM imaging of the resulting fd nanoarray



**Figure S1**. Assembly steps of the *fd*-scaffolded ferrocene-PEG-(Fc-PEG) / PQQ-GDH integrated system on the surface of an ultra-flat  $SiO_2/Si$  chip. Right: in-situ tapping mode AFM topography image of a random array of *fd* particles recorded after step (4) of the assembly process. Commercial MSNL-10 tip. Imaging buffer Tris pH 7.5.

#### (b) Apparent height of the decorated fd-particles as a function of the decoration steps

The height of the decorated fd particles was measured from in situ tapping mode AFM images of the virus nanoarray, such as the one presented in Figure S1. Images were recorded after each step following the assembly of the first antibody, the anti fd-antibody. We did not measure the height of bare fd adsorbed onto the aminated SiO<sub>2</sub> surface (i.e. before decoration), because high electrostatic interactions between the amino-silane layer and the AFM tip made imaging very difficult. The height of the fd-particle immediately after BSA backfilling could not be confidently measured either, since bare fd particles were partially embedded by the surrounding BSA molecules. The height values of several fd particles, measured after immobilization of the anti-fd IgG, the IgG-GDH conjugate, the anti-GDH antibody and finally the IgG-PEG-Fc, are plotted in Figure S2.



**Figure S2**. Apparent height of the immuno-decorated *fd* particles, as measured from in situ tapping mode AFM images recorded immediately following the assembly of : the anti( $\alpha$ )-*fd* IgG, IgG-GDH conjugate,  $\alpha$ -GDH IgG and finally IgG-PEG-Fc (steps 4-7 in Figure S1, respectively). Note that the height was measured versus the BSA background. Open symbols are individual measurements, filled symbols are average values, box height is standard deviation. Commercial MSNL-10 tip. Imaging buffer Tris pH 7.5.

An increase of the apparent height of the decorated particle is noted following each incubation step of the surface with the antibodies, confirming the effective immobilization of a new antibody "layer" on the *fd*-scaffold at each step. The height increments corresponding to the sequential immobilization steps were of about 1.7 nm, 3.7 nm, 4 nm and 2 nm, respectively. These values are compatible with, albeit lower than, the size of an IgG (15 nm x 5 nm x 5 nm),<sup>1</sup> and of GDH (10 nm x 5nm x 5 nm).<sup>2</sup> This apparent discrepancy is to be attributed to the well documented artifact of AFM, that tends to underestimate the height of biological objects, primarily due to compression of the biomolecules by the tip. In the present case, beyond the intrinsic compressibility of the proteins, Fab and Fc (Fragment crystallizable) domains of the antibodies can undergo hinge motions with respect to each other, which makes the multi-layered IgG assembly particularly flexible and soft, and explains why it is imperfectly sensed by the incoming AFM tip. This is also most likely the reason why, in literature, it was concluded that individual IgGs were not clearly identifiable by AFM when bound to viral particle.<sup>3</sup>

However, the mere fact that the apparent height of the decorated fd particle increased with the number of IgG "layers" (Figure S2) shows that topographic tapping mode AFM can be used to image the antibody coating of the fd-particles, albeit with reduced height sensitivity.

#### (c) High resolution tapping mode AFM imaging of fully decorated fd-particles

In order to assess the spatial homogeneity of the decoration of the fd particles by the antibody construct, fd-particles bearing the (Fc-PEG) / PQQ-GDH integrated system were imaged in situ in AFM tapping mode. For this we used particularly sharp home-made tips (radius of curvature ~20 nm),

prepared from 25  $\mu$ m diameter gold wires. In our experience these tips are much less prone to contaminations than commercial Si or Si<sub>3</sub>N<sub>4</sub> based tips. As can be seen from Figure S3 we imaged individual decorated *fd* particles with very high resolution. In these images, and from the corresponding cross sections, the particles appear as strings of very closely spaced bead-like objects, that we assign to individual antibodies (or small groups thereof). This result ascertains that, within the resolution of achieved here (a few tens of nanometers) the antibody coating the individual *fd* particles is homogenously distributed along the contour length of the *fd* particles.



**Figure S3.** Evidencing the homogenous decoration of the viral particles with the antibodies. (a) In-situ high resolution topography AFM image of three *fd*-particles decorated by the Fc-PEG / PQQ-GDH integrated system. (b) Height profiles of the particles seen in (a), taken along the red lines shown in the image. Profiles taken in the image background are drawn in black. Home-made tip prepared from a 25  $\mu$ m diameter etched and flattened gold wire, spring constant ~ 0.1 N/m. Imaging buffer Tris pH 7.5.

#### (II) Dependence of the particle-averaged catalytic tip current on the tip potential, $E_{tip}$ .

Mt/AFM-SECM images of a SiO<sub>2</sub> surface bearing a random array of *fd*-particles decorated by the Fc-PEG/PQQ-GDH integrated system were acquired in Tris buffer containing 10 mM glucose, with the tip potential,  $E_{tip}$ , set to various values. The catalytic tip current,  $i_{max}$ , was measured for ~10 individual particles and averaged. The resulting particle-averaged catalytic tip current,  $i_{max}^{av}$ , is plotted as a function of the tip potential,  $E_{tip}$ , in Figure S4.



**Figure S4**. Dependence of the particle-averaged catalytic tip current,  $i_{max}^{av}$ , on the tip potential  $E_{tip}$ . The red line is a guide to the eye. The mid-height potential of the "tip voltammogram" thus obtained is ~ 0.14 V/SCE. Tris buffer pH 7.5 containing 10 mM glucose.

### (III) Dependence of the catalytic tip current of the *fd*-decorated particles on their curvature.



**Figure S5**. Dependence of the catalytic tip current,  $i_{max}$ , of individual *fd*-decorated particles on their curvature. Particles were sorted qualitatively into three groups depending on their curvature, as assessed from the Mt/AFM-SECM topography images: Nearly straight, slightly curved and sharply curved particles. Open dots correspond to individual particles, filled symbols are average values, box height is standard deviation. Imaging buffer Tris pH 7.5 containing 10 mM glucose.

### (IV) Effect of tip convolution on the topography and current Mt/AFM-SECM images of decorated *fd*-particles.

#### Tip convolution effect on the apparent particle width

The apparent width of the decorated *fd*-particles, as seen in the topography and current Mt/AFM-SECM images, is expected to depend on the actual tip radius as illustrated in Figure S6A and S6B, respectively.



**Figure S6.** Schematics showing how the tip-object convolution effect enlarges the apparent width of the decorated *fd*-particles as seen in topography, (A), or in current, (B), images. The schemes are drawn to scale, with a tip ~100 nm in radius, and considering IgGs and GDH as spheres, 15 nm in diameter, as depicted in (B). It is assumed the Fc<sup>+</sup> heads can explore a space region (colored in a light blue gradient) extending ~10 nm around the PEGylated IgG. The *fd*-particles are predicted to display apparent widths of  $2\Delta X_T$  and  $2\Delta X_i$ , in the topography and current images, respectively.

In topography, the tip-particle convolution yields an apparent particle width  $2\Delta X_T$ , related to the apparent particle height,  $h_{app}$ , and tip radius,  $R_{tip}$ , by the equation reproduced in Figure S6A. Taking  $h_{app}$  = 12 nm (as derived from the actual topography images given in the main text), and taking  $R_{tip} \sim 100$  nm, yields an apparent particle width of ~100 nm, in reasonable agreement with the particle width values measured from the topography images reproduced in the text (~100-150 nm).

In current, the tip-particle convolution results in an apparent particle width  $2\Delta X_i$ , related to the tip radius and the effective height of the immune-assembly,  $\varepsilon$ , as shown in Figure S6B. The  $\varepsilon$  value can be coarsely estimated by considering: (i) IgGs and GDH as spheres, 15 nm in diameter, and (ii) that the Fc<sup>+</sup> heads can efficiently explore a space region extending ~10 nm around the PEGylated IgG.<sup>4</sup> This yields  $\varepsilon$  = 90 nm, and an apparent "electrochemical" width of the particle of ~ 320 nm, which is comparable with the width of the particles observed in current images reproduced in the main text (~300-400 nm).

Not surprisingly the apparent width of the decorated-fd particles is much larger in current than in topography, since, upon approaching the decorated particle laterally, the tip electrochemically senses the Fc<sup>+</sup> heads much before it can actually touch the fd-particle itself.

#### Tip convolution effect on the topographic and current profiles taken along the decorated fdparticles

The "travel" of the Mt/AFM-SECM tip along the contour length of a decorated *fd*-particle is represented schematically in Figure S7. IgG's, GDH and the Fc<sup>+</sup>- accessible region are color-coded as in Figure S6, the tip radius is ~100 nm and the scheme is drawn to scale. Also reproduced in Figure S7 are the set of superimposed topography and current profiles presented in Figure 3C of the main text. One can see that, since the virus length is much larger than the tip radius, convolution effects do not affect significantly the topography profile. It can also be seen that convolution affects the current profiles in regions located around each of the extremities of the decorated *fd*-particles (x/L < 0 and x/L > 1). The

simple geometric representation shown in Figure S7 satisfyingly accounts for the observed  $\sim 100 - 200$  nm width of these regions.



**Figure S7.** (Top): Schematic representation of the decorated *fd*-particle, showing the molecular components of the integrated system (IgGs, GDH,  $Fc^+$  accessible area) and a 100 nm radius tip. The scheme is drawn to scale, using the color code introduced in Figure S6. (Bottom): Sets of superimposed topography and current profiles presented in Figure 3 of the main text, scaled to the scheme of the *fd*-decorated particle and tip.

### (V) Extra zoomed-in Mt/AFM-SECM images of individual decorated *fd*-particles evidencing a "catalytic hot spot" in their central region.

Close examination of high resolution current images of the *fd*-decorated particles, recorded in the presence of 10 mM glucose, enabled us to reveal the presence of a "catalytic hot spot" (i.e. a zone of enhanced current) in the central region of the particles, as exemplified in Figure 3C of the main text, and in Figure S8 below.



**Figures S8.** High resolution Mt/AFM-SECM images of five individual Fc-PEG/ PQQ-GDH decorated *fd*-particles. Imaging buffer. Tris buffer pH 7.5 containing 10 mM glucose. Tip potential  $E_{tip} = +$  0.3 V/SCE.

## (VI) Exact derivation of the catalytic tip current profile considering the complete rate law of GDH kinetics – Comparison with the first order analytical approximation.

The reaction/diffusion profile of the  $Fc^+$  heads along the viral particle is given by the differential Equation 7 of the main text:

$$D_e \frac{\partial^2 \gamma}{\partial x^2} - \frac{2k_{ox}\gamma_E \gamma}{(1+K_i C_g^0) + \gamma \left[\frac{k_{ox}}{k_{cat}} \left(1 + \frac{K_M}{k_{cat} C_g^0}\right)\right]} = 0$$

The kinetic term appearing in this equation is the complete rate law for GDH-catalyzed oxidation of glucose, using  $Fc^+$  as a co-substrate.

Introducing the following dimensionless kinetic parameters:

$$\lambda^* = \frac{2k_{ox}L^2\gamma_E}{[D_e(1+K_iC_g^0)]} \text{ and } \sigma = \frac{k_{ox}\gamma_0(1+K_M/C_g^0)}{[k_{cat}(1+K_iC_g^0)]}$$

The differential equation can be re-written as:

$$\frac{\partial^2 q}{\partial y^2} - \frac{\lambda^* q}{1 + \sigma \gamma} = 0 \tag{S1}$$

with  $q = \gamma / \gamma_0$  and y = x/L

This equation was solved numerically using the finite element differential equation solver FlexPDE, for sets of  $\lambda^*$  and  $\sigma$  values. The exact catalytic tip current,  $i_{max}^{exact}$ , was derived from the resulting calculated Fc<sup>+</sup> profiles, and compared to the current calculated using the analytical Equation 4 given in the main text:

$$i_{max} = 2i^*\sqrt{\lambda} \tanh(\sqrt{\lambda}/2)$$

taking  $\lambda = \lambda^*/(1+\sigma)$ .

Figure S9 presents the  $i_{max}$  / $i_{max}^{exact}$  ratio, calculated for various  $\lambda^*$  and  $\sigma$  values.



**Figure S9.** Ratio of the catalytic tip current values calculated either using Equation 4 of the main text,  $i_{max}$ , or by solving Equation S1 numerically,  $i_{max}^{exact}$ , for several values of the  $\lambda^*$  and  $\sigma$  parameters as indicated.

We observe that, within the range of  $\lambda^* = [1...30]$  and  $\sigma = [0...10]$ , corresponding to the experimental conditions explored here, agreement between the exact and approximate current values is better than ~20 %.

#### (VII) Extra data: Lessons from fragmented particles. Catalytic activity mapping.

In rare occasions Mt/AFM-SECM images revealed decorated *fd*-particles that did not seem intact, but that rather consisted in a few aligned fragments, as if they had been damaged following surface adsorption. One of such particle is shown in Figure S10, where three segments can be identified in the topography image, their separations being indicated by white arrows. The upper segment seems detached from the rest of the particle, whereas the two lower segments appear still connected to each other, being only separated by a zone of reduced height (see the topography cross section).



**Figure S10.** In-situ tapping-mode Mt/AFM-SECM imaging of a segmented Fc-PEG / PQQ-GDH immunodecorated *fd*-particle. Simultaneous topography and tip current images are shown, together with profiles taken along the particle contour. The squared regions in the images and cross-sections enclose a segment that seems detached from the rest of the particle. The arrows designate the location of height valleys between the segments. Imaging buffer. Tip potential  $E_{tip} = + 0.3$  V/SCE. Tris buffer pH 7.5 containing 10 mM glucose.

Most interestingly it can be seen in the current image that, unlike what we observed for the "intact" particles, no catalytic hot spot can be found at the overall center of the segmented particle. Indeed, the current profile taken along the particle axis is not bell-shaped but shows two large peaks separated by a valley. Moreover, comparison of the topographic and current images shows that the detached fragment of the decorated fd particle is not associated with a current spot. At the opposite, the two longer, still connected, lower segments are each associated with a current spot, but separated by a lower current region.

These remarkable features can be fully explained by the occurrence of electron diffusion along the *fd* scaffold, coupled to the enzymatic reaction, as follows.

We notice that the maximum height of the three fragments ( $\sim 10-12$  nm) is compatible with the height we measured for intact *fd*-particles (see Figure 2c), indicating that each fragment is properly decorated by the Fc-PEG / PQQ-GDH system. Yet only the fully detached fragment does not yield a current spot intense enought to be measurable over the background current. This can be explained by the fact that for such an isolated fragment the path of electron propagation is limited to the segment length, so only the limited number of GDH molecules it bears can be addressed by the tip. This confirms the role of electron propagation, coupled to GDH activity, in the catalytic tip current generation.

Considering now the two longer fragments, we note that they are connected via a low height valley region, which most likely correspond to a location where decoration by the antibodies is incomplete,

possibly because the *fd*-scaffold itself is disrupted (since both the antibodies and the *fd*-scaffold contribute to the height contrast). In this region the local concentrations in Fc and in GDH are expected to be low, implying slower electron propagation and/or lower local enzymatic activity. We believe that this is precisely what is reflected by the decrease in tip current recorded at this location (see current cross-section in Figure S10).

The above results demonstrate the ability of Mt/AFM-SECM to differentiate high and low activity regions along *fd*-particles, i.e. to actually *map* the local activity of the *fd*-supported integrated system. The resolution achieved is better than the ~150 nm wide (uncorrected from tip convolution) transition region separating the two longer fragments seen in Figure S10.

The fact that the vast majority of the intact decorated *fd*-particles displayed a single hot spot (i.e. were characterized by a bell-shaped current profile) can thus be safely interpreted as the catalytic activity of the decorated *fd*-particle being homogenously distributed along the particles.

### (VIII) Extra data: tip potential dependence of the tip catalytic current recorded by recursively imaging individual decorated *fd*-particles.

The exact same location of a SiO<sub>2</sub> surface, bearing a random array of *fd*-particles decorated by the Fc-PEG/PQQ-GDH integrated system, was recursively imaged by Mt/AFM-SECM, in Tris buffer containing 10 mM glucose, while changing the tip potential,  $E_{tip}$ , between each image scan. A selection of the resulting set of images is reproduced in Figure S11.



**Figure S11.** (A) Mt/AFM-SECM images of individual Fc-PEG/ PQQ-GDH decorated *fd*-particles recorded at various tip potential values ( $E_{tip}$ , indicated on top of the images). The very same particles were imaged recursively after incrementing the tip potential. Four particles, labelled 1 to 4, can be clearly identified in all of the images. (B) Tip CV plots showing the variation of the catalytic tip current,  $i_{max}$ , as a function of  $E_{tip}$  for each of the four indexed particles. Imaging buffer: Tris buffer pH 7.5 containing 10 mM glucose. Note that, for clarity, only 6 out of a total of 8 topography-current image pairs, corresponding to differing  $E_{tip}$  values, are presented.

The sequence of current images shows that the intensities of the current spots increases progressively as the tip potential is raised, but tends to level off at high enough potential values. The intensities of the catalytic tip current,  $i_{max}$ , measured for 4 of the particles visualized on each of the images, is plotted in Figure S11, part (B). The resulting individual "tip CVs", display the expected S-shape, and are all centered around a potential region of  $0.14 \pm 0.01$  V/SCE, coinciding with the standard potential of the Fc heads:  $E^{\circ} = 0.15$  V/SCE.

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