#### Electronic Supplementary Material (ESI) for Nanoscale. This journal is © The Royal Society of Chemistry 2015

# Supplementary Information

## Detailed explanation for calculation of the BL\_force signal denoted by F<sub>BL</sub>

The force signal in approach (extend) mode is linearly fitted by two segments (see figure SI01): the first fit is obtained along a piezo-extension of 10nm at point of maximum force (~6nN). The second comes from the linear fit along a segment, 200nm in length, starting from the most distant point in the extend curve. The value, along the "piezo-extension" axis, of the intersection between these two segments is the lower bound for the calculation of the average value of  $F_{BL}$ , the BL\_Force signal. The upper bound is the most distant point in the extend curve. It must be noted that the absolute value of the slope for the best linear fit of the constant portion of F(d) curve is lower than 30pN/micron whatever the pixel.

## Study of intermixing of "fast" and "slow" effects

Figure SI06 shows variations of raw BL\_Force signal ( $F_{BL}$ ) along the fast axis for the *same* four locations as in figure 3. No temporal filter is applied and fast variations of the raw signal can be detected as for figure 3. Two successive fastscan lines are shown: the first in magenta, the second in red. Equivalent results to those for the low digitization rate are clearly visible on the first two images (figures SI06.a-b) i.e. before AFM tip reaches the first third of the bacterial complex. The  $F_{BL}$  signal reveals as for 64 pixels case a quasi-linear variation of signal (figure SI06a) with a slope of roughly -15pN/s as in figure 3. We can again attribute it as for the case of 64 pixels digitization rate to an uncompensated thermal drift. The so-called "fast" regime is still present in  $F_{BL}(t)$  curves as  $\delta F_{BL}$ , as defined in case of a digitization rate of 64 pixels, still has a negative value upon the bacteria and comes back to zero away from them. This explains again the observed contrast in reconstituted images based on this  $\delta F_{BL}$  signal (figure 4.c). The new and important point is that when AFM tip starts to scan over the bacterial aggregate we observe that raw BL\_Force signal,  $F_{BL}$ , is strongly perturbed: the slope of  $F_{BL}(t)$  curve becomes positive leading to a correlative increase of  $F_{BL}$ versus time as observed in figure 5.c. This effect ("slow" one) occurs at a time scale higher than a threshold we can roughly estimate to be equal to the duration of one scan line with 64 pixels, ~800ms.

#### Figure SI01:

This figure, plotting the interaction force between the AFM apex and the sample versus the elongation of Z-piezo illustrates how the BL\_Force signal,  $F_{BL}$ , is calculated. The force vs elongation curve in extend mode is fitted by two straight segments: the first one (left dashed line) is obtained from a linear fit of the repulsive region starting from point of maximum force (~6nN) and over a 10nm range. The second (right dashed line) comes from a linear fit, parallel to the piezo extension axis, along a segment, 200nm in length, starting from the most distant point in the extend curve. The  $F_{BL}$  signal is the mean value of the interaction force calculated along the constant part of the F(d) curve between the two following bounds : the intersection between the two former linear fits and the most distant point in the extend curve (domain of calculation is indicated by horizontal arrows in Fig. Sl01). The red curve is typical to almost every data point of the AFM image except those at the edges of bacteria. In this case a typical F(d) plot is drawn with the blue line. The bump visible at the foot of the repulsive domain is due to increase lateral interaction between the tip and the edge of bacteria; it explains the presence of spurious increase of BL\_Force signal  $(F_{BL})$ , as calculated by the automatic procedure, along edges of bacteria as visible in figures 3 and Sl06.

### Figure SI02:

Variations of current versus voltage as measured during AFM acquisition in a small zone (10nm square) of the two bacteria consortium of figures 2.g-h. Same kind of voltammogram was obtained for a very large panel for conditions of measurement: see text for more details.

#### Figure SI03:

Images calculated from the  $\delta F_{BL}$  signal are plotted in figures SI03.a-c and SI03.d-e for the two bacteria (case of V = 0mV)and three bacteria consortia (case of no electrical connexion) respectively. The scale bar represents 1µm. These images were taken under following conditions:

fig. SI03.a: (5μm/64pixels)<sup>2</sup>; fig. SI03.b and SI03.d: (4μm/64pixels)<sup>2</sup>; fig. SI03.c and SI03.e: (4μm/128pixels)<sup>2</sup>.

#### Figure SI04:

In figure SI04.a is described the temporal succession of images before the AFM scanning of image in figure 2.g.  $1^{st}$ : yellow line; scan size:  $10\mu$ m.  $2^{nd}$ : blue line; scan size:  $20\mu$ m.  $3^{rd}$  : red line; scan size:  $10\mu$ m.  $4^{th}$  : green line; scan size:  $5\mu$ m.  $5^{th}$ : magenta line; scan size:  $4\mu$ m. Then a last image (see figure 2.g) was taken. All these images except that plotted in figure 2.g were scanned at a digitization rate of  $(64pixels)^2$ . Figure SI04.b: time variation of current intensity (orange line), raw BL\_Force ( $F_{BL}$ ) signal (the color curves near the black dashed line) and AFM height data (the upper colored curves) for successive AFM images according to the sequence described in figure SI04.a with the same color code. AFM data in violet corresponds to figure 2.g.

#### Figure SI05:

Variations of  $\delta F_{BL}$  signal (black lines) and height signals versus time for two AFM (4µm)<sup>2</sup> images of the bacterial consortium shown in figure 2.a-c with different digitization rates: fig. SI05.a: (64pixels)<sup>2</sup>; fig. SI05.b: (128pixels)<sup>2</sup>. In this case electrodes were not connected to the potentiostat (what we called the open circuit –O.C.- conditions).

#### Figure SI06:

Spatial variations of the raw BL\_Force ( $F_{BL}$ ) along two *successive* horizontal scan lines at four different positions over the two-bacteria consortium as indexed in inserts. The first acquired line is plotted in magenta, the second in red. The AFM data corresponds to the image shown in inserts and acquisition conditions are: scanned area ( $4\mu$ m)<sup>2</sup>; digitization rate: (128pixels)<sup>2</sup>. The black dashed lines are the best linear fits of red profiles as determined in the portions without bacteria (left and right sides).

## Figure SI07:

Variations of  $\delta F_{BL}$  signal (black lines) and height (blue lines) signals (figure SI07.c-d) versus time related to the two AFM images (figure SI07.a-b) of non-living *Rhodococcus wratislaviensis* bacteria: they were studied by AFM five hours after the AFM electro-chemical cell has been filled with a pure NaCl solution (0.15M), i.e. without any nutriment. Scan size:  $(7.9\mu m)^2$ . Digitization rates: fig. SI07.a and SI07.c:  $(64pixels)^2$ ; fig. SI07.b and SI07.d:  $(128pixels)^2$ .





Figure SI03.b



## Figure SI04.a



Figure SI04.b





Figure SI05.b





Figure SI07.a

Figure SI07.b



Figure SI07.c

Figure SI07.d

