金离子生物矿化研究

Detecting Gold Biomineralization by Delftia acidovorans Biofilms on QCM-D

使用耗散型石英晶体微天平分析仪 **QCM-D** 仪器，利用石英晶片上的代尔夫特食酸菌检测研究生物矿化。

使用 **QCM-D** 研究生物矿化，这种方法可用于重金属探测，金恢复，工业废水处理。

下图：生物矿化过程。右图 24 小时生物膜 AFM 图片

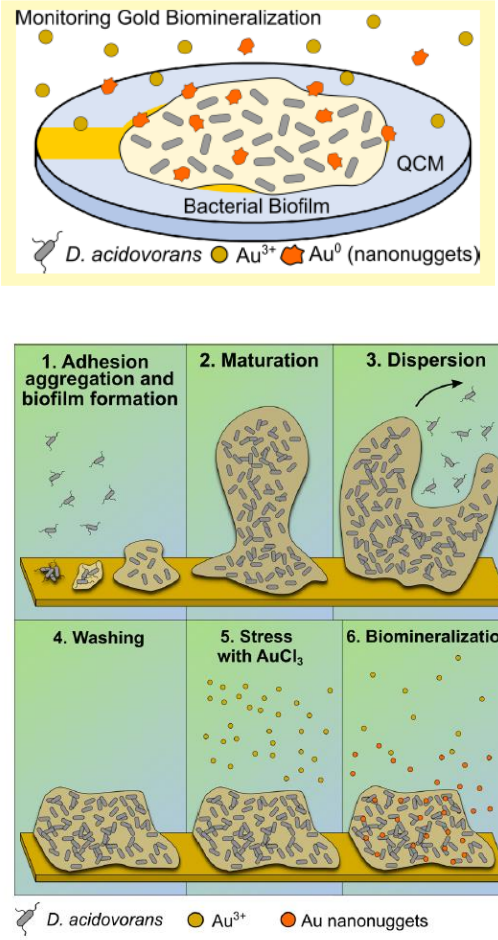

Figure 1. Stages of *D. acidovorans* biofilm development and gold biomineralization. The bacteria first grow for 24 h on the gold-coated quartz sensor surface of the QCM (from step 1 to 3), and then the biofilm is washed with PBS (step 4) and stressed with $AuCl_3$ for about 14 h (step 5). The exposure to soluble Au^{3+} ions induces the production of the nonribosomal peptide delftibactin, which neutralizes the ionic gold, creating the so-called nanonuggets (step 6).

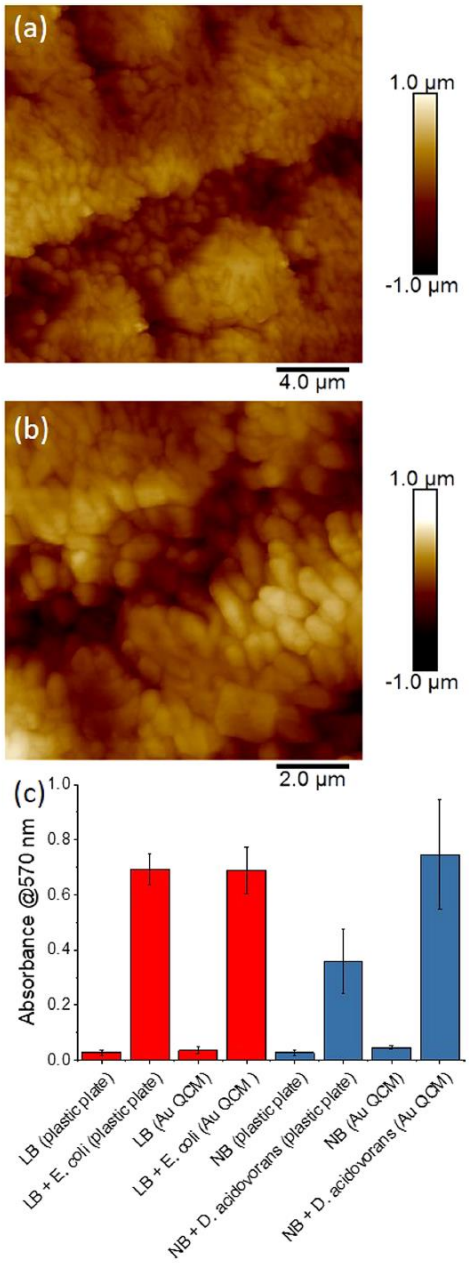

Figure 3. High-resolution AFM images of a 24 h *D. acidovorans* biofilm on a QCM gold surface. (a) 20 × 20 μm^2 and (b) 10 × 10 μm^2 scans. Stable and compact structures of bacteria and EPSs are observed. *D. acidovorans* biofilm formation has also been characterized using the conventional crystal violet assay, (c) while *E. coli* is used as a reference microorganism. These experiments have been performed on both plastic (well plate) and gold (QCM) surfaces. Both bacteria are grown for 24 h before staining. While *E. coli* (red data) reaches the same biomass on both plastic and gold substrates, *D. acidovorans* (blue data) produces a more massive biofilm on gold. The absorbance values reported in the histogram correspond to three independent experiments, with the error bars denoting the standard deviation.

下图：QCM 检测到的 24 小时生物膜生长过程，结果与 AFM 图片一致

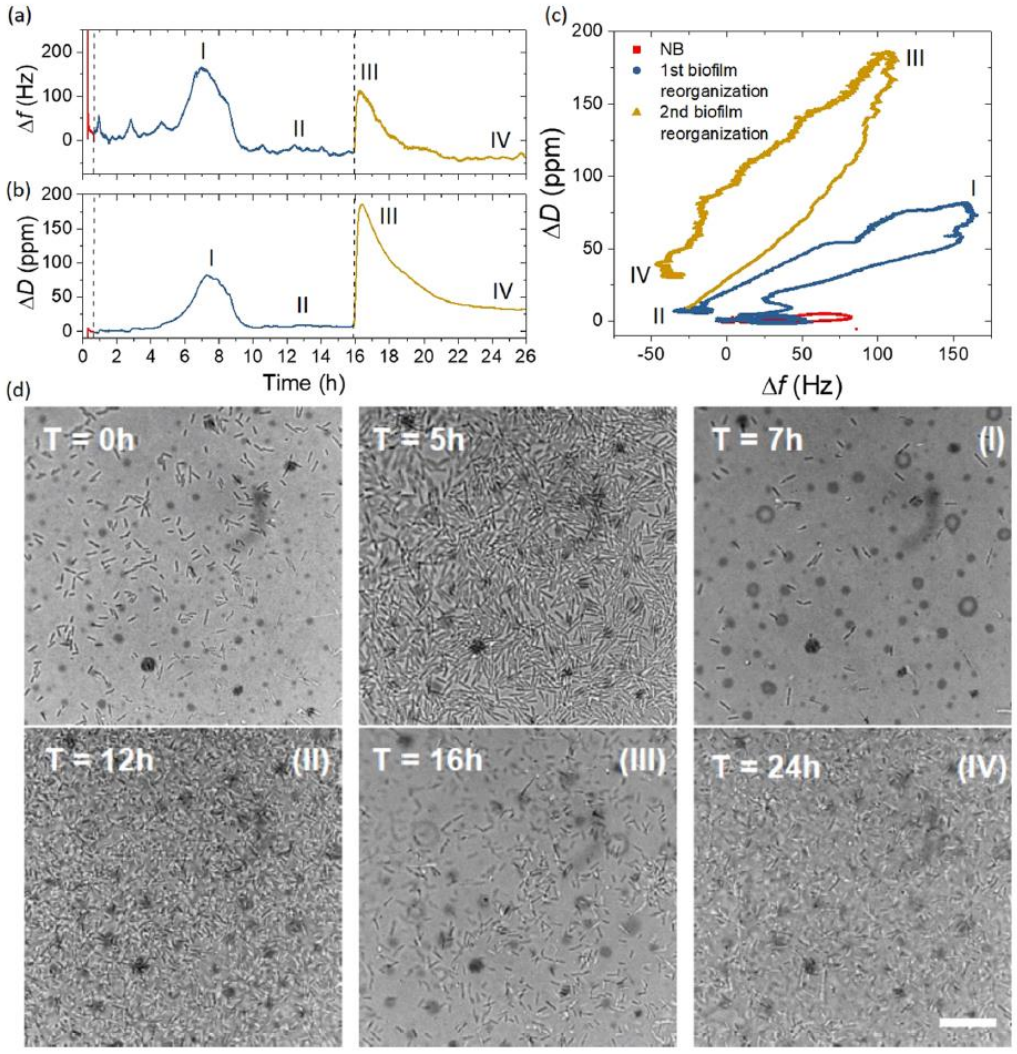

Figure 2. QCM sensorgrams of *D. acidovorans* growth on gold and optical images of the biofilm structure. Panels (a) and (b) show frequency and dissipation responses versus time, respectively. An initial stabilization in NB (red data), corresponding to protein adsorption on the gold sensor surface, is followed by a gradual increase in both frequency and dissipation (blue data). This peak (I) is a signature of bacterial reorganization on the gold sensor surface. Next, bond maturation of the cells onto the gold surface results in another stabilization regime for both frequency and dissipation (II). A second increase in both signals can be observed at about 16 h (yellow data, III), followed by a final stabilization (IV). While the first peak can be attributed to a rearrangement of the cell layer directly adhering to the gold surface, the second one is related to reorganization of the external part of the biofilm. These stages can be captured in the *Df*-plot (c), where the dissipation shift is plotted against the frequency change, and further validated in a sequence of images extracted from a time-lapse series of *D. acidovorans* grown on a 15 nm gold layer evaporated on a glass cover slip (d). Images of bacterial growth are captured every 2 min over ≈ 24 h at 25 °C (see SI video). In these conditions, biofilm reorganization occurs around 7 and 16 h. The scale bar represents 20 μm.

下图：14小时生物矿化过称中AuCl浓度变化、活细胞与死

细胞数量变化

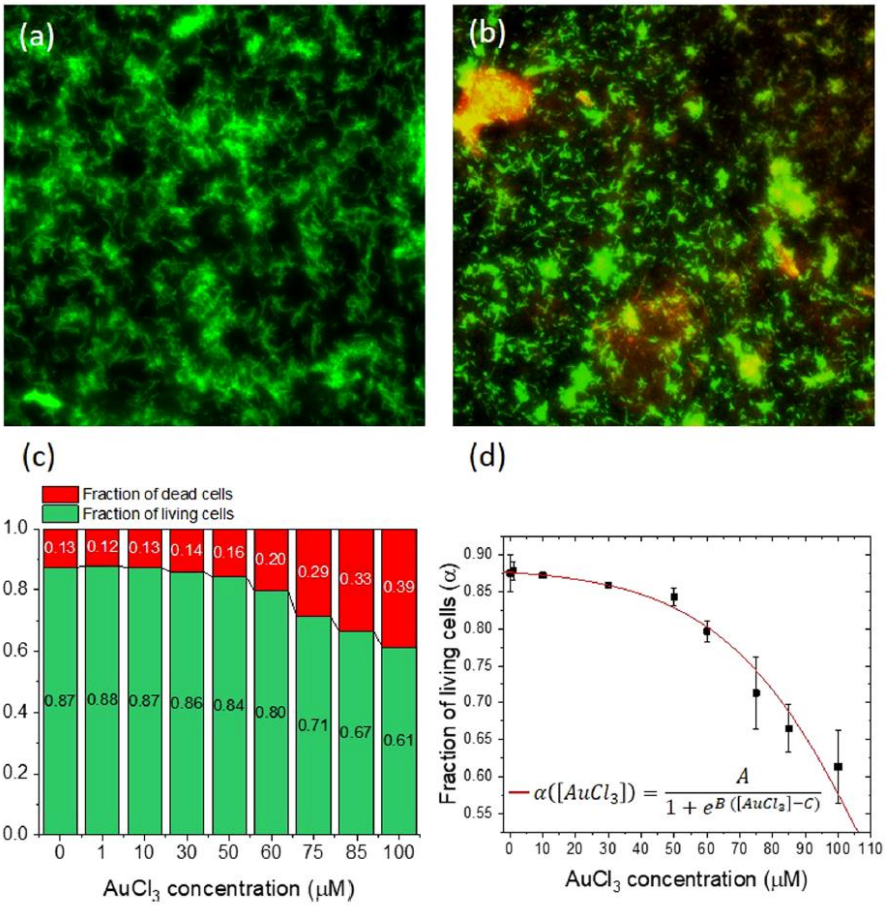

Figure 4. Viability staining for *D. acidovorans* stressed for 14 h with different concentrations of $AuCl_3$. The experiment is conducted using two different fluorescent dyes (SYTO 9 and propidium iodide). These emit in the green and red spectral regions and are specific for living and dead cells, respectively. (a) Control experiment performed by exposing the cells to PBS and (b) bacterial biofilm stressed with 100 μM of $AuCl_3$. *D. acidovorans* is able to survive by neutralizing the gold ions; however, the lack of nutrients (the measurement is performed in PBS) and the increasing concentration of Au result in a progressive decrease in cell vitality, as shown in (c). These experimental data are shown as black squares, with the best curve fit using a logistic-type relationship shown as a red solid curve (d). Each data point results from the analysis of three independent areas, with the error bars denoting the standard deviation.

左图：

a,虚线后为矿化沉积后频率下降，耗散下降（刚性增加）

b，使用QCM-D检测不同浓度AuCl产生的频率变化。

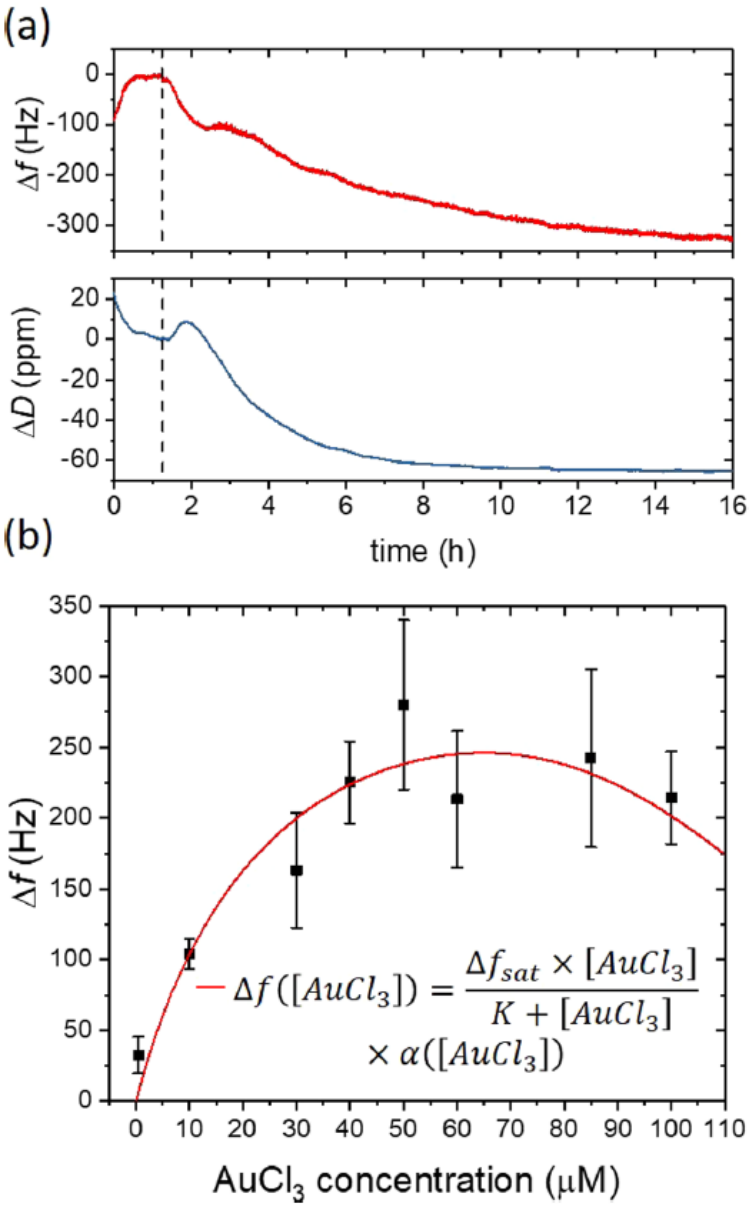

Figure 5. *D. acidovorans* response at several concentrations of $AuCl_3$ monitored using QCM. (a) The bacterial biofilm is first washed with PBS until the QCM resonance frequency stabilizes. The micro-organisms are then stressed with soluble gold (50 μM $AuCl_3$), and both frequency and dissipation are monitored for more than 14 h. The vertical dashed line highlights the instant when 50 μM $AuCl_3$ is added to the system. The gold biomineralization and the subsequent gold nanonuggets production yield a progressive decrease in the resonant frequency of the gold coated quartz substrate. This is due to the deposition of gold complexes on the QCM sensor, functionalized by the bacterial biofilm. A decrease in the dissipation signal is also observed, which indicates a gradual stiffening of the material that is in contact with the QCM sensor surface due to the gold nanonugget formation. (b) Frequency response of the biofilm-covered QCM sensor surface at different concentrations of the soluble gold. Each data point corresponds to the average frequency shift from at least three independent experiments, with the error bars denoting the standard deviation. A modified Hill model incorporating the viability staining data is shown as a red solid curve.

下图：TMB分析结果与QCM测量结果一致

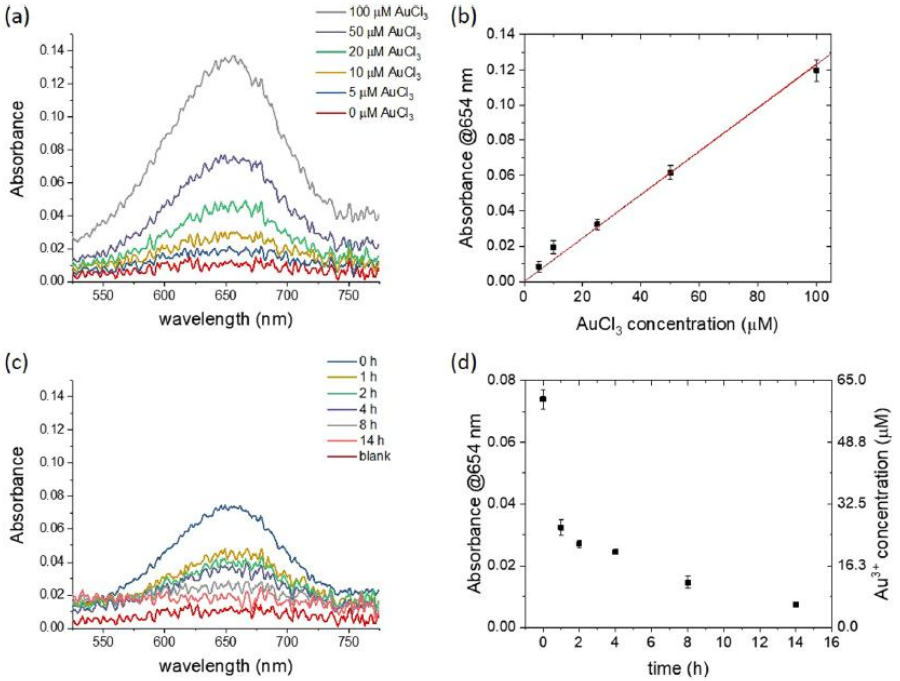

Figure 6. TMB assay to verify the gold biomineralized by *D. acidovorans* biofilm for 14 h. (a) Assay calibration performed on $AuCl_3$ samples in PBS. The reaction between gold ions and TMB molecules produces an increase in the absorbance at 654 nm, which can be used to quantify the concentration of the gold ions. (b) TMB assay calibration. (c) The *D. acidovorans* biofilm is stressed with 60 μM of $AuCl_3$ and samples of 10 μL are collected from the solution in the QCM cell. These are stored at 4 °C until the last volume is acquired at 14 h. Then, 2 μL of TMB solution is added to each sample and the absorbance spectrum in the visible range is recorded after 10 min. (d) The progressive decrease in absorbance at 654 nm documents the progressive depletion of the free ionic gold, neutralized by delftibactin in the form of nanonuggets. Each data point corresponds to the average of the absorbance of three different samples, while the error bars denote the standard deviation.