

Supporting Information

Mechanisms of interaction between colloids and bacteria as evidenced by tailored silica-lysozyme composites

Luciane França de Oliveira, Kaliandra de Almeida Gonçalves, Fábio Henrique Boreli, Jörg Kobarg, Mateus Borba Cardoso

SAXS data analysis

SAXS data analysis was carried out using the Irena evaluation routine¹ implemented in commercially available Igor Pro Software (WaveMetrics, Portland, USA).² A multi-level unified fit was used to describe the three levels of structural organization evident in the scattering data.^{3,4} In this method, the scattering provided by each structural level is the sum of a Guinier exponential-form and a structurally limited Power-law tail. A generalized equation, representing any number of spherical levels, is written as:^{3,4}

$$I(q) = \sum_{i=1}^n G_i \exp\left(\frac{-q^2 R_{gi}^2}{3}\right) + B_i \exp\left(\frac{-q^2 R_{g(i+1)}^2}{3}\right) \left[\frac{(\text{erf}(qR_{gi}/\sqrt{6}))^3}{q}\right]^{P_i} \quad (1)$$

where n is the number of structural levels observed, G is the Guinier prefactor, R_g is the radius of gyration and B is a prefactor specific to the Power-law scattering which is specified as the decay of the exponent P.

References

1. Ilavsky, J.; Jemian, P. R., Irena: tool suite for modeling and analysis of small-angle scattering. *J. Appl. Crystallogr.* **2009**, *42* (2), 347-353.
2. Kline, S., Reduction and analysis of SANS and USANS data using IGOR Pro. *J. Appl. Crystallogr.* **2006**, *39* (6), 895-900.
3. Beaucage, G., Approximations Leading to a Unified Exponential/Power-Law Approach to Small-Angle Scattering. *J. Appl. Crystallogr.* **1995**, *28* (6), 717-728.
4. Beaucage, G., Small-Angle Scattering from Polymeric Mass Fractals of Arbitrary Mass-Fractal Dimension. *Journal of Applied Crystallography* **1996**, *29* (2), 134-146.

Details about the theoretical silica to lysozyme stoichiometric calculation

The TEOS to silica (SiO₂) reaction can be expressed by Equation S1 assuming 100 % of conversion yield.



Along the process, it was used 210 mg (1 mmol) of TEOS which would form 60 mg (1 mmol) of SiO₂, if the reaction was allowed to go to completion. The amount of lysozyme used during the process was 60 mg which makes 1:1 (w/w) silica to lysozyme ratio.

Selective precipitation of composite in presence of bacteria

An assay was performed to demonstrate that the composite resides next to the bacteria cell wall. A single colony of bacteria (either *E. coli* or *S. Aureus*) was taken and transferred into 250 mL of LB broth. The mixture was incubated overnight at 37 °C under vigorous shaking. This solution was diluted with 1 L of LB broth and incubated at 37 °C in an orbital shaker. The evolution of the bacteria growth was determined through the increase of the optical density (OD) at 600 nm until 1. The bacteria suspension was centrifuged at 8000 rpm (Thermo Scientific- Sorvall RC⁺) for 10 minutes at 4 °C and the obtained pellet was resuspended in 10 mL of LB broth. Then, 1 mL of this bacteria suspension was added to a flask containing 10 mg of silica-lysozyme composite. The solution was mixed in vortex mixer and allowed to precipitate for 10 min. The supernatant was removed, another 1 mL of bacteria suspension was added and the solution was mixed in vortex mixer and allowed to precipitate for 10 min. This procedure was repeated 10 times. At the end of this process, the resulting composite was washed 5 times with 1 mL of LB broth to remove bacteria from the medium. Then,

1 ml of LB broth was added to the precipitate and the optical density (OD) at 600 nm was determined. The experiments were conducted in triplicate, performed simultaneously and the average values were reported. This same procedure was conducted simultaneously with a suspension of composite in LB broth without bacteria.

S. aureus Composite in LB broth – OD = 0.361 ± 0.012

Composite in bacteria suspension – OD = 0.217 ± 0.030

E. coli Composite in LB broth – OD = 0.396 ± 0.039

Composite in bacteria suspension – OD = 0.283 ± 0.029

Circular dichroism (CD) measurements

CD spectra were made on a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at LEC laboratory (LNBio – CNPEM) using a 1 mm cell. Bandwidth was 1 nm and the scanning speed was 50 nm/min measured from 190 to 260 nm. Measurements were performed on freshly prepared lysozyme solution (15 μM) and lysozyme solution kept under stirring for 18h at 37 °C in an orbital shaker.

FIGURE S1

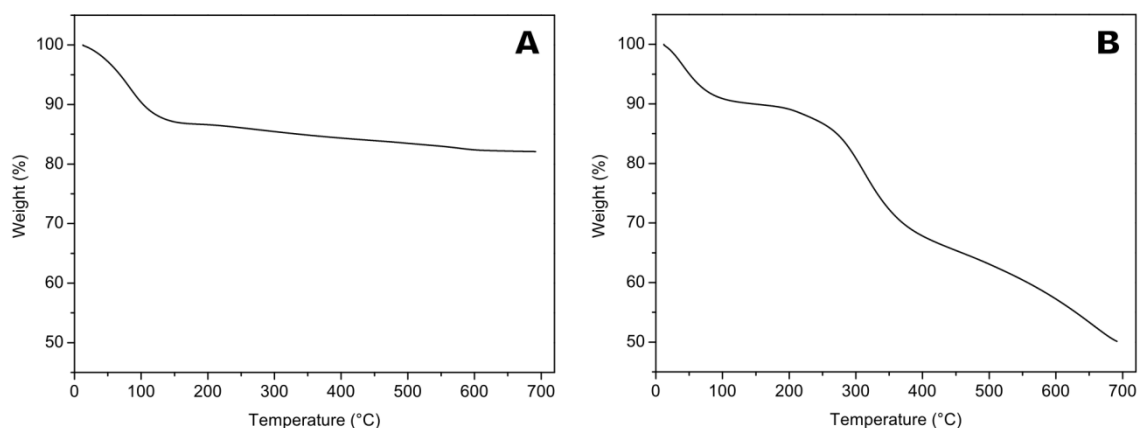


Figure S1. TGA curve of (A) bare silica and (B) silica-lysozyme composites.

FIGURE S2

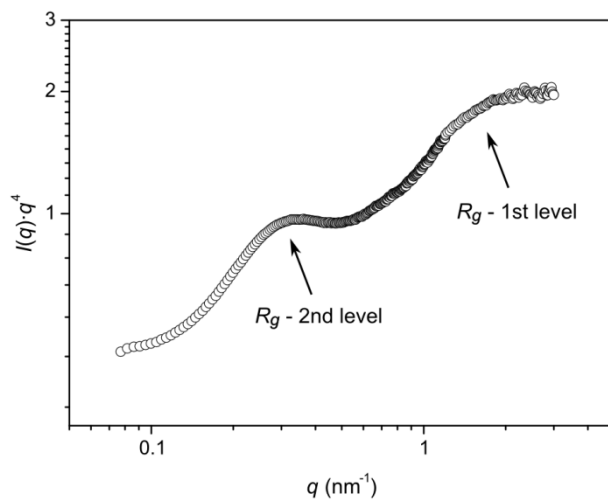


Figure S2. $I(q) \cdot q^4$ SAXS plot evidencing the two R_g structural levels of silica-lysozyme composite.

FIGURE S3

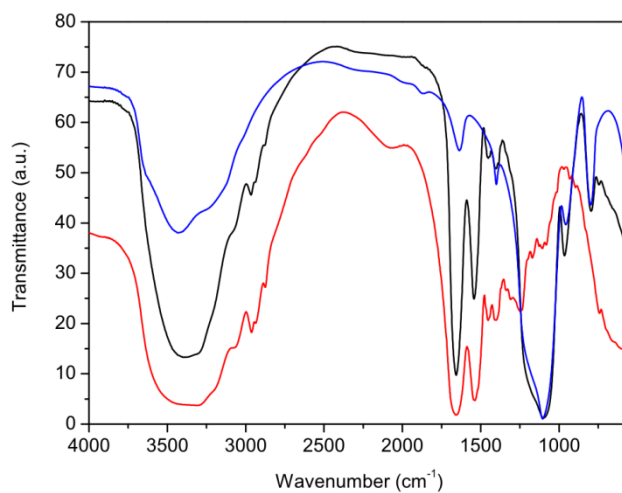


Figure S3. FTIR spectra of native lysozyme (red), silica-lysozyme composite (black) and bare silica (blue).

FIGURE S4

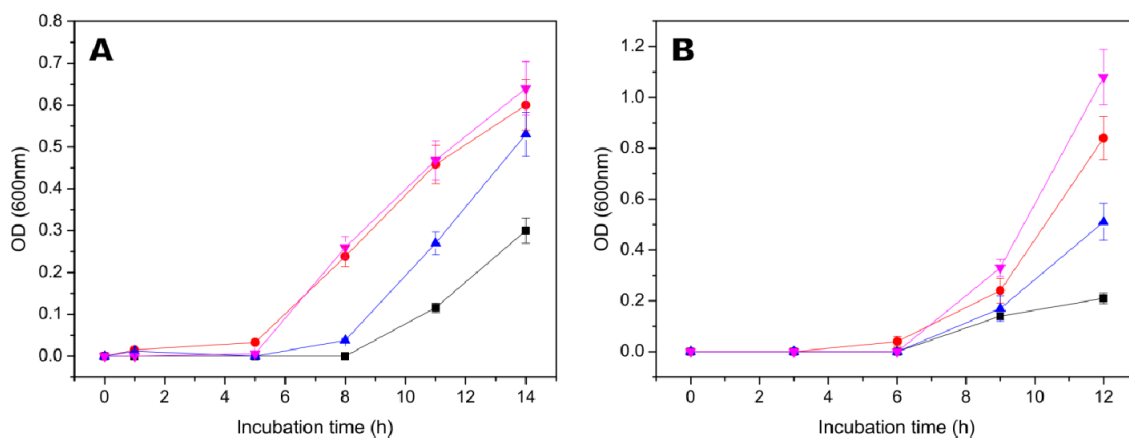


Figure S4. Bacterial growth curve in LB media. The growth of the (A) *E. coli* and (B) *S. aureus* was investigated in the presence of (■) silica-lysozyme composites, (▲) lysozyme and (●) silica. (▼) Bacterial growth in absence of silica and lysozyme was measured for comparison.

FIGURE S5

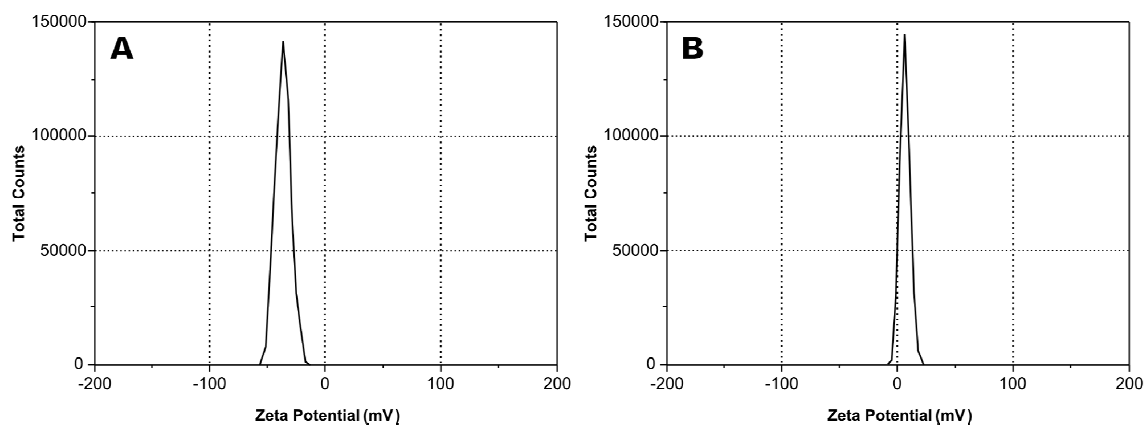


Figure S5. Zeta potential of (A) bare silica and (B) silica-lysozyme composite.

FIGURE S6

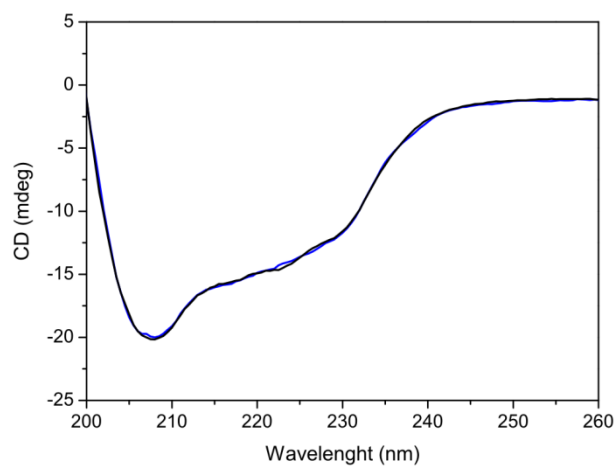


Figure S6. CD spectra of freshly prepared lysozyme solution (black line) and lysozyme solution kept at 37 °C for 18h (blue line).