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Macrocellular Beads to the Assembly of Microbial Consortia

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Experimental Procedures

Chemicals.

Tetraethyl-orthosilane (TEOS), tetradecyltrimethylammonium bromide (TTAB), dodecane and sodium silicate solution ($\text{SiO}_2 \geq 27\%$, $\text{NaOH} \geq 10\%$) are purchased from Sigma-Aldrich. Amplex UltraRed is bought from Thermo Fischer, and "Amplex Red Glucose/Glucose Oxidase Assay Kit" from Invitrogen. All other chemicals and reagents used in this study are of analytical grade and are used without any further purification.

Synthesis of Si(HIPE) beads.

Typically, TEOS (5.02 g) is added to 16.02 g of an aqueous solution of tetradecyltrimethylammonium bromide (TTAB) (35% w/w). Then, concentrated hydrochloric acid solution (37%) is added (5.88g). The aqueous phase is left under stirring for 5 minutes in order to perform TEOS hydrolysis. The emulsion is prepared by hand in a mortar by adding 35 g of dodecane drop-by-drop in the aqueous phase. With a pipette (7 mL), the emulsion is transferred drop-by-drop to viscous polydimethylsiloxane oil to form droplets and left to age for 3 days at room temperature in order to get the Si(HIPE) beads. The resulting beads are washed 3 times with THF/Acetone (50/50), dried in air, and then calcinated at 650°C for 6h (heating rate of 2°C/min with a first step at 180°C for 2h, the cooling temperature is driven by the oven inertia).

Preparation of Si(HIPE) materials for bacterial colonization.

Si(HIPE) beads (**Figure S1**) are sterilized at 180°C for 1h30. They are transferred to an Erlenmeyer flask containing sterilized M9 medium consisting of Na_2HPO_4 (6.78 g.L^{-1}), KH_2PO_4 (3 g.L^{-1}), NH_4Cl (1 g.L^{-1}), NaCl (0.5 g.L^{-1}), MgSO_4 (2 mM), glucose (2 mM) and casamino acids solution (0,5% w/w). The flask is placed under dynamic vacuum at 200mbar. When the effervescence from the air leaving the Si(HIPE) beads stops, the flask is left under a static vacuum for 3 days.

Colonization of Si(HIPE) beads by bacteria.

Bacteria used in this work are an *E. coli* strain expressing L-asparaginase (EcASNase-2 in pAPEx4b for anchored periplasmic expression) and displaying resistance to chloramphenicol. First, the strain is isolated from an LB agar plate containing chloramphenicol (25 $\mu\text{g.mL}^{-1}$). After 24h at 37°C, one colony is inoculated into an Erlenmeyer flask containing M9 medium and chloramphenicol (25 $\mu\text{g.mL}^{-1}$). The flask is placed in an incubator overnight at 37°C, with agitation at 220 rpm. After one night, sub-culturing at $\text{OD}_{600\text{ nm}} = 0.1$ is performed. Cultures are incubated at 37°C until an $\text{OD}_{600\text{ nm}} = 0.6$ is reached (exponential growth phase). IPTG (0.1mM, Isopropyl β -D-1-thiogalactopyranoside) is added for inducing overexpression of L-asparaginase. At that time, Si(HIPE) beads are put into the culture medium for bacterial colonization. The incubation is continued overnight at 25°C. (**Figure S1**)

Sodium silicate shell around Si(HIPE) beads.

In order to confine bacteria inside the Si(HIPE) beads, but still allow the diffusion of molecules between the beads and the medium, a sodium silicate shell is produced. A solution is prepared with 1X PBS and sodium silicate (200 mM) and acidified with hydrochloric acid until the pH is brought down to 7. The bacteria-loaded beads are rinsed with PBS buffer (pH 7.4) and added to this solution. The solution is agitated until gelation occurred. This manipulation is repeated five times in order to produce a homogeneous shell.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM).

The *E. coli*-loaded beads are rinsed in 1X PBS and treated in a 5% (v/v) aqueous solution of glutaraldehyde for 24h at 4°C. Samples are dehydrated in successive ethanol baths (20%, 40%, 60%, 80% and 96% (v/v) ethanol in water) for one hour for each bath. The samples are dried in hexamethyldisilazane-ethanol baths (1:2 ratio for 20 min; then 2:1 ratio for 20 min, and finally 1:0 (pure hexamethyldisilazane) for 20 min.) and then dried at room temperature for 24h. SEM is performed with HITACHI TM-1000 and HITACHI S2500 microscopes operating at 15 and 25 kV, respectively. TEM is performed with a HITACHI-H600 microscope operating at 75 kV. The silica shell is ripped off the glass, crashed to powder, dispersed in ethanol, spread over formvar/carbon TEM grids, and left to dry in air before observation (**Figures S2-3**).

Porosimetry analysis.

Nitrogen adsorption-desorption isotherms are determined by using a TriStar 3000 (Micrometrics). Samples are outgassed at 200°C overnight and weighed before sorption experiments. The surface area is determined by applying the multipoint Brunauer-Emmett-Teller (BET) model to describe the adsorption isotherm in a relative pressure range (p/p_0) from 0 to 1. The macroporous structure of the final beads is investigated by using intrusion/extrusion mercury experiments with an AutoPore IV 9500 porosimeter from Micrometrics (**Figures S2-3**).

Bacterial strains for enzyme production and purification

Asparaginase is produced by *E. coli* C41(DE3) containing the EcASNase-2 coding sequence in pAPEx4b (for Anchored Periplasmic Expression). Aspartate oxidase (AspOx) is overproduced by the same *E. coli* strain harboring the L-AspOx coding sequence (*E. coli nadB* gene) in pET14b- Δ thrombin-SUMO (cytoplasmic expression)[36]. The histidine-tagged enzyme is purified via nickel-agarose affinity chromatography (**Figure S9**).

Stability of bacterial encapsulation

An ampicillin-resistant *E. coli* strain (RP437, pZE1R-mCherry) expressing the red fluorescent protein mCherry is cultivated in 20mL LB at 37°C and 200rpm. Bacterial growth is monitored by optical density measurements until the bacteria reached the exponential growth phase ($OD_{600\text{ nm}} = 0.5$ corresponding to a concentration of about $5 \cdot 10^7$ cells/mL), 200 μ L are transferred into an Erlenmeyer containing the beads and incubated at 37°C, 200rpm. Fluorescence stereomicroscopy on whole samples is performed with a Zeiss Axiozoom V16 equipped with a fluorescence module. Confocal microscopy was done on a Leica DMIRE2 confocal microscope (**Figure S3**). Three-dimensional reconstructions are obtained with the imagery software Image Surfer.

Establishment of a relation between $OD_{600\text{ nm}}$ measurements and cell concentration.

E. coli C41(DE3) harboring EcASNase-2 in pAPEx4b is grown in LB medium with chloramphenicol (25 μ g.mL⁻¹). After overnight incubation at 37°C, the bacterial culture are diluted to OD_{600nm} = 1. Serial dilution is performed in the range 10⁻¹ to 10⁻⁸. 100 μ L of each dilution is spread on an agar plate before overnight incubation at 37°C. The numbers of bacterial colonies are 11 and 104 for the dilutions of 10⁻⁶ and 10⁻⁵, respectively (other dilutions lead to too few or too large numbers of colonies on the plate for a reliable counting). OD_{600 nm} = 1 corresponds to 10⁸ bacteria per mL.

Cloning of periplasmic L-AspOx.

The plasmid pET14b containing the *E. coli* gene *nadB* coding for the L-AspOx is digested using NdeI and BamHI restriction enzymes. The digestion product is agarose gel purified and ligated at 16°C during 3h into the pAPEx4b vector using T4 DNA ligase. The ligation product is transformed into *E. coli* XL-Blue competent cells. Positive clones are identified by PCR using the following primers: Ec_AspOx_NdeI5'_Forward GGAATTCCATATGAATACTCTCCCTGAACATTC and Ec_AspOx_BamHI3'_Reverse 5'CGCGGATCCTTATCTGTTTATGTAATGATTGC. Finally, plasmid DNA isolated from single bacterial colonies is digested with the same restriction enzymes to check the correct size of the cloned insert before sequencing (Genewiz, Takeley, UK). Vector maps are shown in **Figure S8**.

Glucose consumption assay.

Two *E. coli*@Si(HIPE) beads are placed into 2mL-culture media containing different concentrations of glucose (2 mM, 1 mM, 500 μ M and 100 μ M). The beads are incubated at 37°C during 24h. Glucose level and glucose consumption are determined using two different kits: the Amplex® Red Glucose/Glucose Oxidase Assay Kit from Molecular Probes, and the 2,2'-azinodi-[3-ethylbenzthiazoline-6-sulfonic acid] (ABTS) dependent fluorogenic assay. The Amplex® Red Glucose/Glucose Oxidase Assay Kit is used according to the manufacturer's instructions. For each measurement, 50 μ L of supernatant is mixed with 50 μ L of the reaction mixture containing 10mM Amplex Ultra Red (AUR). The oxidized Amplex Ultra Red (AUR) is determined using a Spectramax Plate Reader (Molecular Devices). In the glucose oxidase/horseradish peroxidase system, glucose oxidase (GOx; 100 U.mL⁻¹ final concentration) catalyzes the oxidation of glucose by molecular oxygen to generate gluconolactone and hydrogen peroxide. This hydrogen peroxide is responsible for the oxidation of the reducing agent ABTS (100 mM) via horseradish peroxidase (HRP; 10 U.mL⁻¹). For each measurement, 10 μ L of supernatant (in 50 mM sodium phosphate buffer, pH = 5,1) is mixed with 100 μ L of the reaction mixture. After 15min incubation, the oxidized ABTS was quantified by UV-Vis measurements at 420 nm (**Figure S7**)

Asparaginase Assay.

A fluorescence-based assay is used to monitor L-asparaginase activity from *E. coli*. [36] In this enzymatic cascade, bacterial L-asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonium. L-aspartate is then oxidized by aspartate oxidase and the coenzyme flavin adenine dinucleotide (FAD) to form oxaloacetate and hydrogen peroxide. This hydrogen peroxide is finally used by horseradish peroxidase to oxidize Amplex Ultra Red (AUR) to fluorescent resorufin. For the preparation of the positive control, 10 ml of *E. coli* culture were centrifuged (4200g, 15min, 4°C). The cell pellets are resuspended in PBS and centrifuged again.

Washed cells are resuspended in 1 mL of PBS, and cell concentrations are determined. Bacterial suspension is diluted until $OD_{600\text{ nm}} = 0.1$ (10^7 cells per mL). The fluorescence assay is performed in 96-wells plates: one silica bead colonized by *E. coli* is added using a small autoclave sterilized spatula to 100 μ L of reaction mix in PBS (L-asparagine (250 μ M), L-aspartate oxidase (700 μ g.mL⁻¹), HRP (0.2 U.mL⁻¹), FAD (20 μ M), AUR (12.5 μ M)). The positive control is performed with 100 μ L of *E. coli* suspension. Fluorescence is measured every 20 sec during three hours ($\lambda_{\text{excitation}} = 532\text{nm}$, $\lambda_{\text{emission}} = 581\text{nm}$) with a Spectramax Plate Reader (Molecular Devices).

Asparaginase activity in cell suspensions

A single colony of *E. coli* C41DE3 cells expressing L-asparaginase is isolated and inoculated in an Erlenmeyer flask containing 2mL of Lysogeny Broth (LB) medium with chloramphenicol (25 μ g.mL⁻¹). After 4-5h at 37°C, sub-culturing at $OD_{600\text{ nm}} = 0.1$ is performed. *E. coli* cells are incubated at 37°C until an $OD_{600\text{ nm}} = 0.6$ is reached, IPTG (0.1mM) is added for overexpression of L-asparaginase, and incubation is continued overnight at 25°C. The *E. coli* cells are centrifuged at 4200g and rinsed in PBS buffer. This step is repeated twice. The cells are finally diluted until $OD_{600\text{ nm}} = 0.00125, 0.0025, 0.005, 0.1,$ and 0.2 for the asparaginase assay. 100 μ L of each bacterial suspension is mixed with 100 μ L of the reaction mixture. The oxidized AUR is measured every 20 sec during 2h ($\lambda_{\text{excitation}} = 532\text{nm}$, $\lambda_{\text{emission}} = 581\text{nm}$) with a Spectramax Plate Reader (Molecular Devices) (**Figure S9**)

Bacteria entrapment and viability within a semi-permeable silica-based membrane.

E. coli C41(DE3) + *EcASNase-2* is inoculated in M9 medium containing chloramphenicol (25 μ g.mL⁻¹) and several Si(HIPE) beads. After overnight incubation at 37°C, silica shell is produced using the same protocol as described above. One bead colonized is introduced in 4 wells of a microtiter plate containing 200 μ L of M9 medium and chloramphenicol (25 μ g.mL⁻¹). Each day, 5 μ L of medium is spread on a LB agar plate before incubation at 37°C during 16h. The same experiment is realized with colonized beads without silica shell (**Figure S4**). Each plate is divided in four quadrants, and each replicate was plated on a quarter of the plate. Finally, to show cells viability within the Si-HIPE after one week, beads are manually crushed and inoculated in LB medium containing chloramphenicol (25 μ g.mL⁻¹), and then incubated during four hours at 37°C before spreading on LB agar plate (**Figure S5**)

Influence of the Si(HIPE) beads size on the reaction kinetics.

One colony of *E. coli* expressing asparaginase is inoculated into 20mL of M9 medium and chloramphenicol (25 μ g.mL⁻¹) and incubated overnight at 37°C. The next morning, a sub-culturing was realized. The culture is incubated at 37°C until an $OD_{600\text{ nm}} = 0.6$ is reached. At this time, Si(HIPE) beads of various sizes (0.8 to 2.2mm) and 0.1mM of IPTG are added to the medium. After overnight incubation at 25°C, silica shell is produced. Finally, the asparaginase assay is performed using the same protocol as described previously.

Supplementary Results and Discussion

Preparation of the beads and encapsulation of bacteria

In order to create a stand-alone compartment, we develop a system based on Silica High Internal Phase Emulsion (Si(HIPE)) [30-32]. We first generate beads Si(HIPE) beads (**Figure S1a-b**) using the protocol described in the **methods section** [32]. The pore sizes are distributed over a wide range of sizes up to several micrometers (**Figure S2**). After production of the inorganic foam, the beads are incubated in a standard LB culture medium containing *E. coli* cells. The bacteria infiltrate the pores of the beads (**Figure S1c**). In order to sequester the bacteria inside the Si(HIPE) and guarantee the compartmentalization of the bacteria over long periods of time – typically several days – we promote heterogeneous nucleation and growth of a silica membrane (**Figure S1c,d**) using a silicate sol as sol-gel precursor while performing the membrane poly-condensation at pH 7 (**Figure S3**). The beads are then resuspended in bacteria-free cell culture medium and the trapped bacteria grown inside the pores of the beads (**Figure S1e**). We have previously shown that the growth reaches cell densities far above the ones reached in standard culture, suggesting a real synergistic effect between the hosted bacterial colonies [32]. Our final material is an assembly of synthetic core-shell particles (**Figure S1f-h, Figure S2**): *E. coli* colonies are sequestered within a Si(HIPE) core, surrounded by a semi-permeable silica-based membrane (**Figure S1i-k**). These living core-shell bio-reactors are stable for several days as revealed by the absence of leaching of cells into the continuous phase (**Figure S4**). Our goal is to use these living hybrid systems as micro-reactors for complex chemical syntheses.

Glucose consumption

First, we perform the control glucose consumption assay of free *E. coli* cells in different proliferation phases in bulk (**Figure S5**). In this case, the optical density at 600 nm provides a measure of the corresponding cell density (**Main Figure 1f**). For $OD_{600\text{nm}} = 0.1$, 70% of glucose is consumed in 3 hours, while the same consumption takes 6 hours for bacterial colonies at $OD_{600\text{nm}} = 0.002$. Quantitatively, the glucose concentration kinetics is expected to decrease as a function of the number of cells in the medium, and this number is itself expected to follow an exponential increase as a function of time. Therefore all kinetic data are fitted with a model equation of the form:

$$C(t) = C_0 - \lambda N_0 \exp(t/\tau) \quad (\text{Eq. 1})$$

where C_0 is the initial glucose concentration (fixed to 0.5 mM) and N_0 the initial number of cells in the medium (proportional to the $OD_{600\text{nm}}$ values). λ is a constant with the dimension of a concentration, and τ is the cell doubling-time scale in the exponential phase, expected to be in the range of 40 – 60 min for *E. coli* in liquid medium at 37 °C. The fit parameters C_0 , λN_0 and τ behave as expected, and the parameter λN_0 is a linear function of the initial cell density, confirming that λ is indeed a constant of the experimental conditions (**Figure S6**).

We then measure the glucose consumption for the bacteria in the compartment in two steps (**Main Figure 1g**). First, two beads loaded with bacteria are incubated for 24 hours in 2 mL of medium. At the end of the incubation, glucose is added to the medium to a concentration of 0.5 mM, and the concentration of glucose in the supernatant is measured over 6 hours. The glucose consumption is complete after 6 hours showing that the bacteria were metabolically active. The data are consistent with the model of **Eq. 1**. The time-scale for proliferation is found to be twice as long (1.25 hr) as for the free cells, and the prefactor $\lambda N_0 = 0.0046$, corresponding

to free bacteria at $OD_{600\text{ nm}} \sim 0.025$. Accounting for the relationship between $OD_{600\text{ nm}}$ and number of bacteria, we estimate the population density as 2.5×10^6 bacteria per bead after 24 hours of incubation. The beads are then re-suspended in culture medium for another 24 hours. After this second incubation, glucose is again added to the medium to 0.5 mM, and the glucose concentration is again measured over time. After 3 hours, all the glucose is consumed. The kinetics is consistent with Eq. 1 and leads to a similar proliferation time (1.3 hr), but the prefactor $\lambda N_0 = 0.043$ corresponds to free bacteria at $OD_{600\text{ nm}} \sim 0.1$. We therefore reach a density of 10^7 bacteria per bead. It is important to note that the proliferation occurs during the first glucose feeding. During the 6 hours of the first glucose consumption, the bacteria doubles within 1.25 hours corresponding to a total of five doublings. The total number of cells in the bead would be at maximum ~ 30 times larger than the initial cell number. At the start of the second glucose feeding, the number of cells is only four times larger than at the start of the first glucose feed, which indicates that a fraction of the cells probably died between the two glucose feeds, as is seen also in standard cell cultures. We notice that the volume of one single bead is of the order of 30 μL . After 24 hours of incubation, a single bead contains 2.5×10^6 cells, corresponding to a density of bacteria of $\sim 8 \times 10^7$ cell/mL ($OD_{600\text{ nm}} \sim 0.8$), and after 48 hours, the density reaches $OD_{600\text{ nm}} \sim 3.2$. These results indicate that the density of cells in the beads is large, thus providing extremely active systems. The high densities reached here might also explain why the proliferation rate is slowed down compared to free bacteria. The difference in kinetics shows that the permeability of the membrane is not the limiting factor in the kinetics of glucose consumption. From a back-of-the-envelope calculation, the diffusion length for sugar over a time-scale of three hours is of the order of two millimeters (using the diffusion constant $D = 6 \times 10^{-10}$ m²/s), the typical size of the capsule. The glucose concentration in the capsule is therefore expected to be uniform at the time-scale of the assay. Our results show that glucose is effectively transported through the silica-based membrane and that the activity of the bacteria inside the capsule is increasing over time as a result of proliferation.

Finally, for the sake of completeness, we estimate the glucose consumption for bacteria in capsules incubated at 37°C during 24h (**Main Figure 1h**) in the presence of increasing glucose concentrations ranging from 100 μM to 2 mM. After 24 hours, the glucose concentration is measured using a fluorogenic assay. For all concentrations smaller than 2 mM, the glucose signal is at the level of the background. For the 2 mM case, we find a residual glucose concentration in the range of 100 to 500 μM (**Main Figure 1f**). The initial 2 mM glucose concentration is sufficiently high such that all the glucose is not completely consumed anymore. This result is consistent with the data of **Figure 1e** in which 0.5 mM of glucose is consumed in 6 hours by capsules incubated for 24 hours. Our experiments thus show that the bacteria inside the core-shell particles are alive and functional and that their activity is controllable – here through the number of bacteria inside the bead.

Supporting Figures

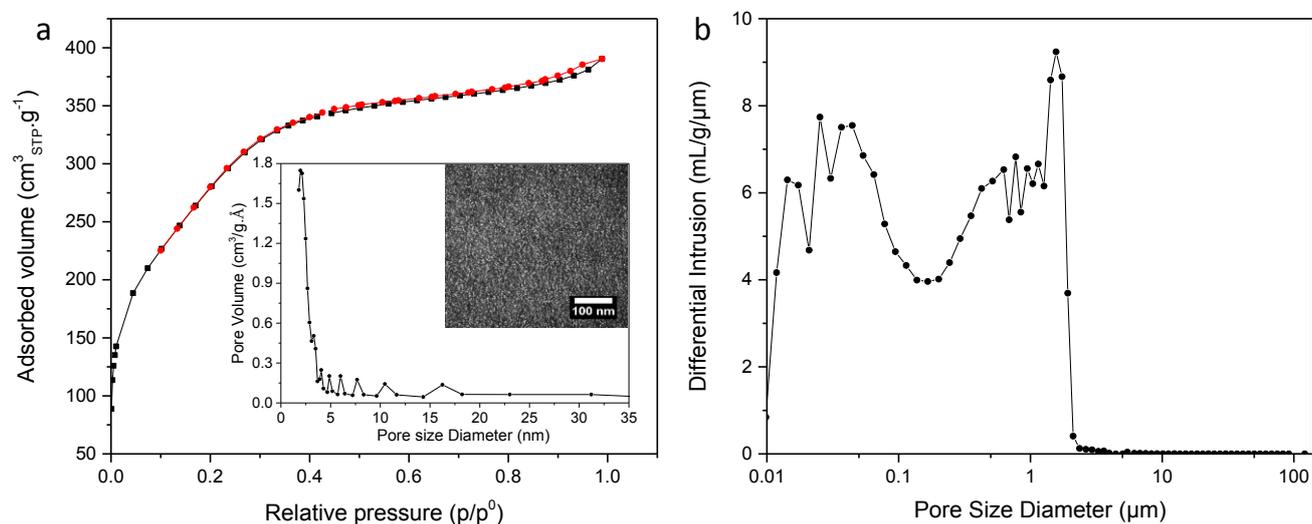


FIGURE S1: **a.** Nitrogen adsorption/desorption isotherms (black, adsorption; red, desorption curves) and distribution of pore sizes calculated by the Barrett-Joyner-Halenda method from the desorption curve. Embedded TEM micrograph of the Si(HIPE) depicting the vermicular-type mesoporosity. The BET (including the microporosity and mesoporosity) surface area is typical for Si(HIPE) and found to be around 800-1000 m²·g⁻¹, (essentially microporous, where only 90 m²·g⁻¹ are associated to the mesoporosity). Calculation (pore sizes above 1.5 Å) [1] **b.** Distribution of the Si(HIPE) macropore sizes obtained by mercury porosimetry. We underline that the pore size distribution relies on the voids that limit the mercury infiltration within the Si(HIPE), i.e. the connecting windows being internal or external to the macroscopic cells observed in Figure 1d,e, f. Here again, we found the typical bimodal character of internal and external windows connecting adjacent macroscopic cells. The pore volume assessed by mercury porosimetry is 93%, while the bulk and skeletal densities are found at 1 g·cm⁻³ and 0.07 g·cm⁻¹, respectively, being again in total agreement with classical Si(HIPE). [1]

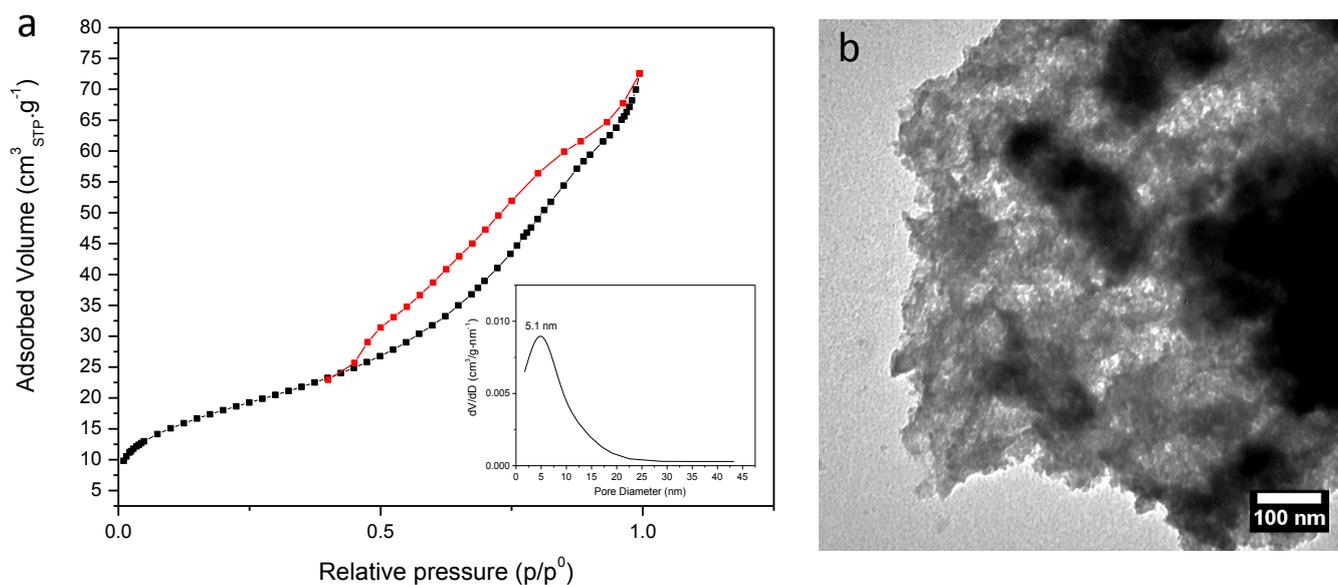


Figure S2: **a.** Capsules silica shell nitrogen adsorption/desorption isotherms (adsorption curves in black, desorption curves in red), and the pore size distribution calculated by BJH from the desorption curve, embedded. The size of the mesopores centers around 5 nm corresponding to a BJH specific volume of $0.09 \text{ cm}^3/\text{g}$, while the BET analysis provided a specific surface area of $65 \text{ m}^2 \text{ g}^{-1}$ (BJH $63 \text{ m}^2 \text{ g}^{-1}$). **b.** TEM of the silica shell. To obtain the silica shell exclusively, a specific surface area of the gel employed to generate the shell of the *E. coli*@Si(HIPE) beads was transferred onto a microscope glass coverslip instead of the Si(HIPE) beads. Finally, the gel was ripped off the glass coverslip as a powder.

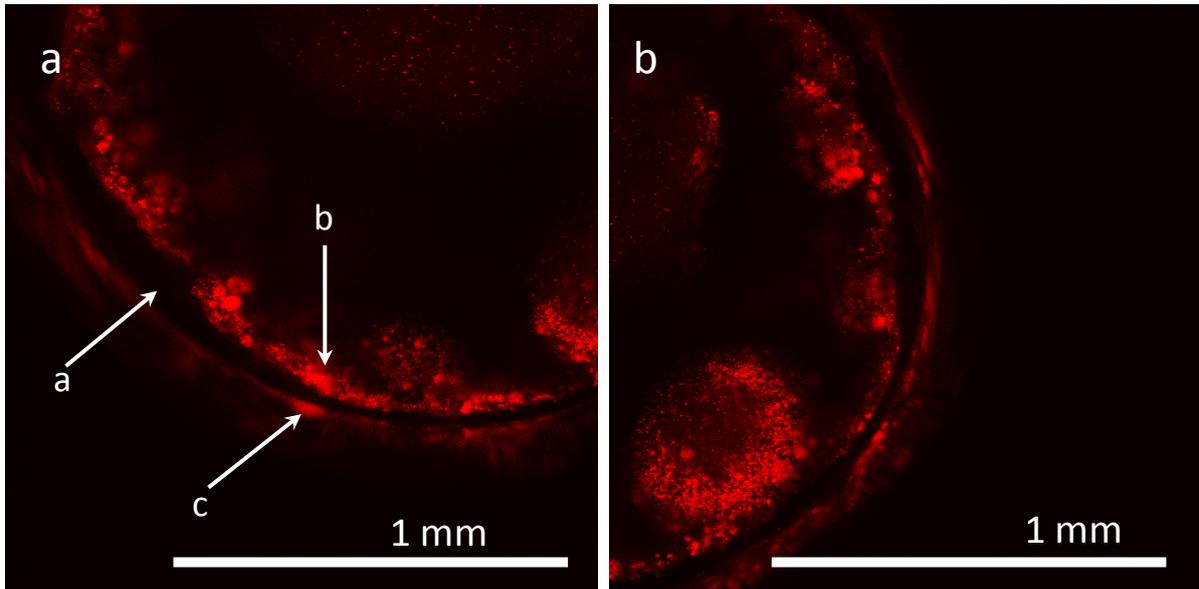


Figure S3. Confocal microscopy of a silica bead inoculated with *E. coli* RP437 carrying pZE1R-mCherry after one week of bacterial growth. The arrow "a" indicates the external silica membrane, the arrow "b" indicates a bacterial colony, while the arrow "c" shows the reflected image of the bacteria at the surface of the outer silica membrane.

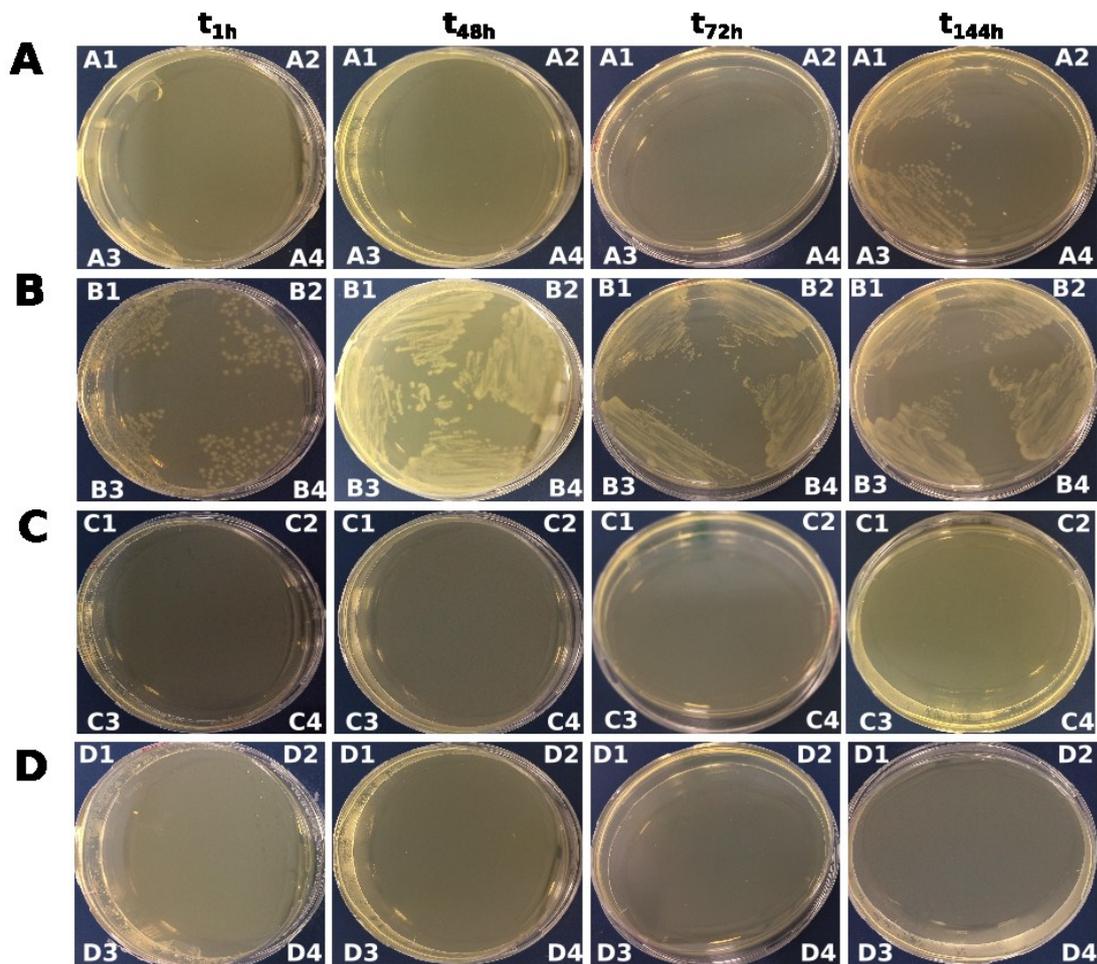


Figure S4. Bacteria entrapment within a semi-permeable silica-based membrane. Ability of the silica shell to entrap *E.coli* cells within Si-HIPE bead (A). One bead is introduced into a well to obtain four replicates (A1 to A4) in a microtiter plate containing 200 μ L of M9 medium and chloramphenicol (25 μ g.mL⁻¹). Each day, 5 μ L of the medium is spread on a LB agar plates and then incubated at 37°C during 16h. After three days (t_{72h}), 7 colonies are found in the well A1, whereas the wells A2 to A4 remain sterile. After six days (t_{144h}), two wells are still sterile (A2 and A4). The same experiment is realized with colonized beads without silica shell (B1 to B4). In the absence of silica shell, bacteria colonize the culture medium within one hour. C and D represent the controls using uncolonized beads with (C1 to C4) or without silica shell (D1 to D4) showing that the medium remains sterile.

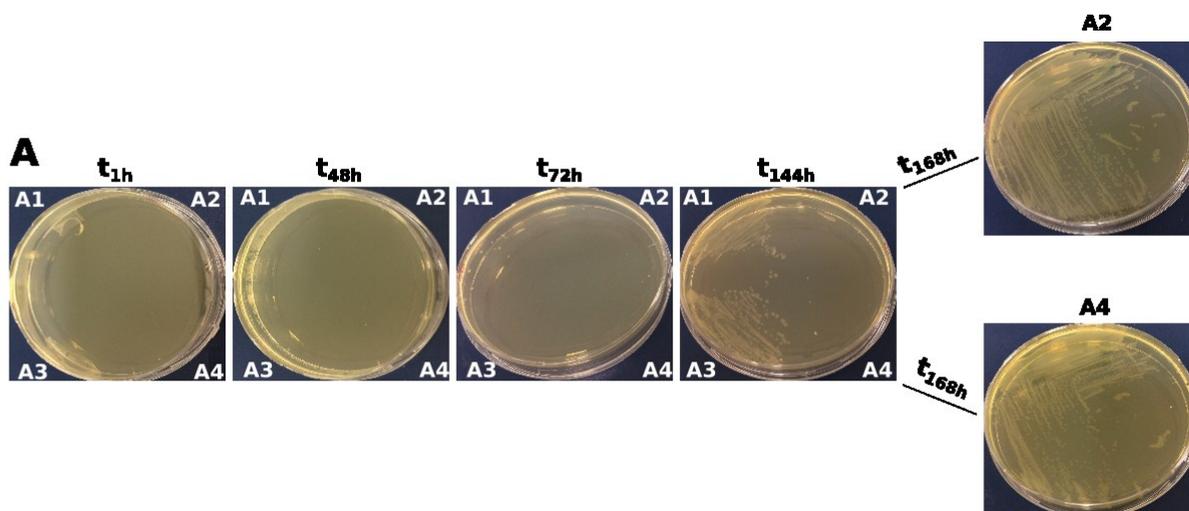


Figure S5. To show the viability of cells residing in the Si-HIPE after one week (t168h), the beads A2 and A4 are destroyed and inoculated in LB medium containing chloramphenicol ($25\mu\text{g}\cdot\text{mL}^{-1}$) and incubated during 4h at 37°C before spreading on LB agar plates. Both plates reveal colonies showing that the bacteria are alive after one week in the beads.

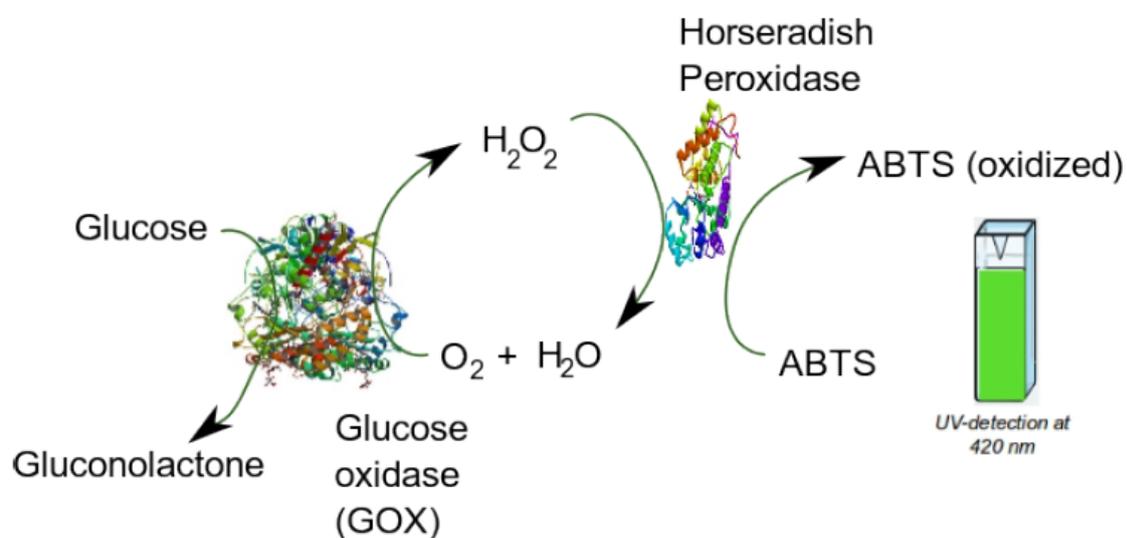


Figure S6. Scheme of the reaction cascade for glucose detection using glucose oxidase (GOx) as a hydrogen peroxide producer. The hydrogen peroxide is used by the enzyme horseradish peroxidase to oxidize the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS)).

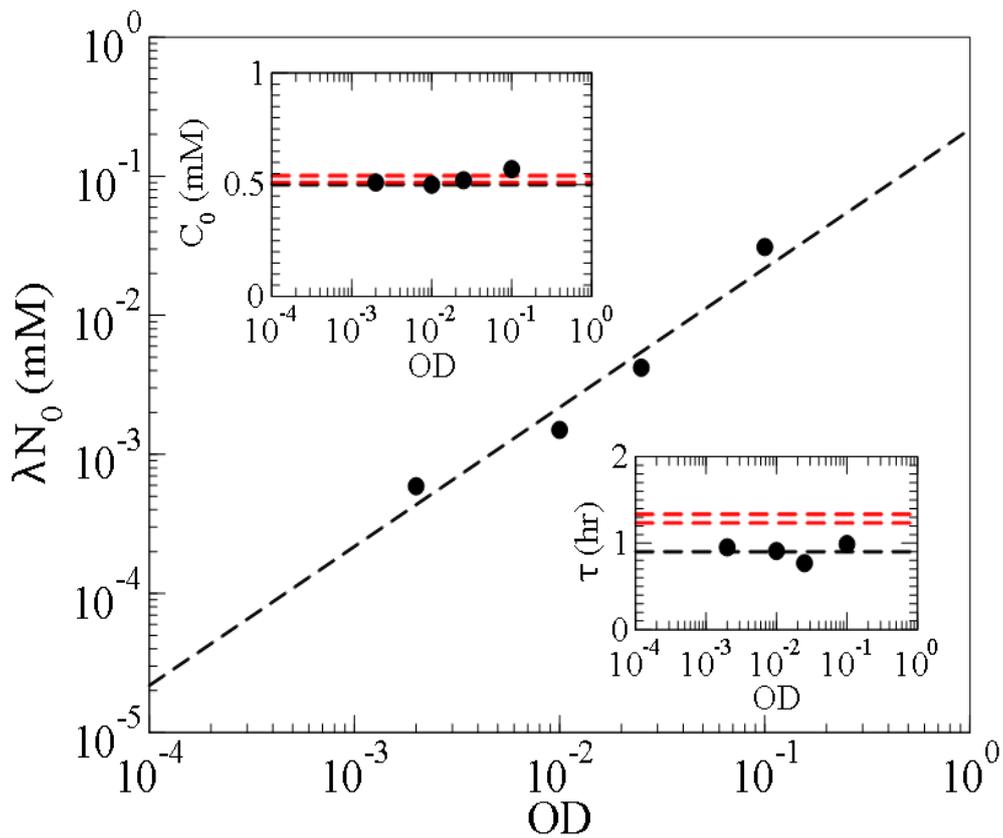


Figure S7. Fit parameters of the glucose consumption assay (Fig. 2) using Eq. 1. The dots correspond to free bacteria in suspension, the red lines to the values extracted for the bacteria-loaded beads. The initial glucose concentration C_0 corresponds in all cases to the 0.5 mM of the prepared solution. The cell doubling time of the exponential term (Eq.1) is the same for all free bacteria and slightly larger for the bacteria in beads after incubation. The prefactor λN_0 is proportional to the optical density of the starting solution as expected.

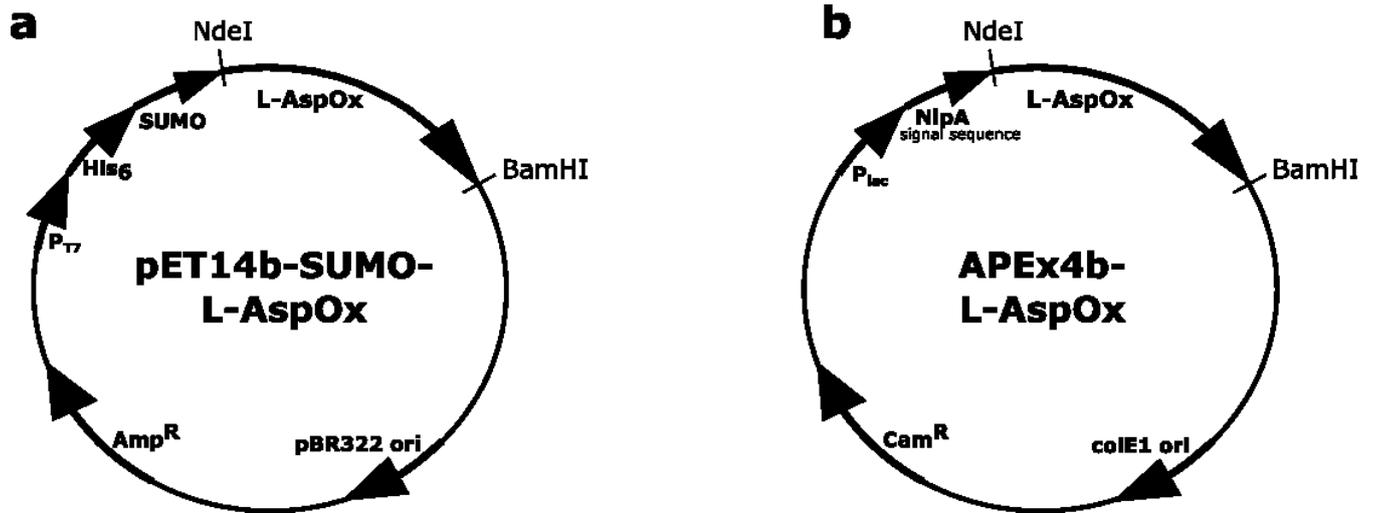


Figure S8. Vectors used in this study for the expression of L-aspartate oxidase (L-AspOx). **a.** pET14b vector containing the *E. coli* gene *nadB* coding for L-AspOx for cytoplasmic expression. **b.** APEX4b vector for Anchored Periplasmic Expression.

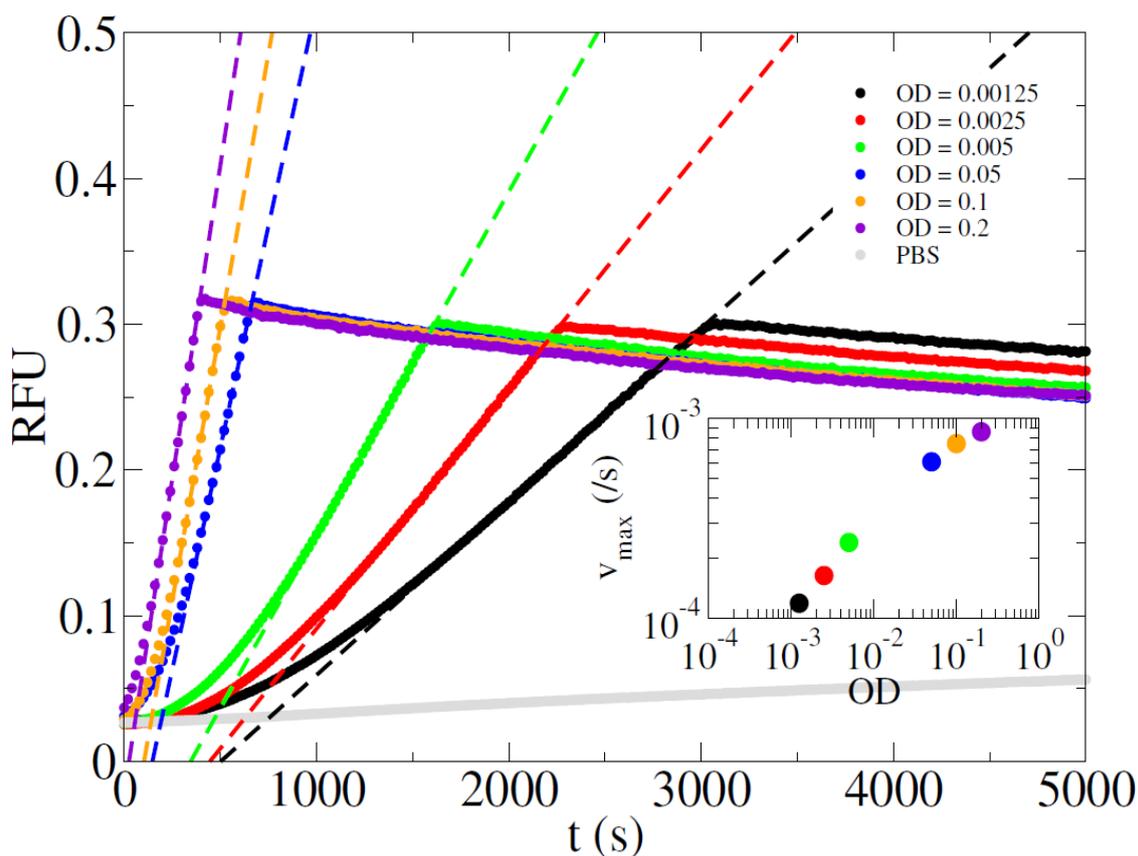


Figure S9. Characterization of the bacterial L-asparaginase activity. The maximum velocity of the reaction is measured at different cell densities ($OD_{600\text{ nm}}$ values). We obtain a direct link between the velocity and the optical density (inset) usable to characterize the cell density by measurement of the enzymatic activity (Fig. 3, main text).

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Author Contributions

J.-C.B. & R.B. conceived and designed the experiments and analyzed the data, A.R, M.M, M.D. & D.P performed the experiments and analyzed the data. M.K. & M.M. contributed to the biochemical methods and measurements, JLB & VS contributed to the materials synthesis method. JCB, RB, AR, MM & DP wrote the manuscript, and all authors discussed the results and commented on the manuscript.