Supporting information DOI:

**Biomimetic cell-mediated three-dimensional assembly of halloysite nanotubes**


**Materials and methods**

*Chemicals and cells.* Halloysite nanotubes were obtained from Applied Minerals, Inc., USA. All other chemicals were received from Sigma-Aldrich and used without further purification. MilliQ water (resistivity >18.2 Ohm) was used throughout the study. Yeast cells *Saccharomyces cerevisiae* were taken from the collection of the Department of Microbiology of Kazan Federal University.

**Halloysite samples preparation.** Prior to deposition onto yeast, halloysite nanotubes were rinsed with saline (0.9% NaCl in water), then centrifuged, redispersed in water, sonicated for 20 min using a Bandelin Sonopuls HD 2070 (Germany) sonifier and then washed with pure ethanol and saline. FITC-PAH-coated halloysite nanotubes were prepared via the layer-by-layer assembly of FITC-PAH and PSS using 1 mg ml⁻¹ aqueous solutions of polyelectrolytes.

**Halloysite sample loading with glucose.** Glucose as a model nutrient was loaded to halloysite by vacuum puling from 20 wt % aqueous solution. The clay nanotubes were washed after loading to remove externally attached glucose. Glucose concentration was determined with discoloration of the potassium dichromate solution by glycerol. K₂Cr₂O₇ solution was mixed with the collected supernatants at certain volume ratio. After mixing, the samples were kept for 12 hours to complete reaction. The concentrations of glucose in the supernatants were calculated based on the reduction of the intensity of the potassium dichromate absorbance at 350 nm as compared to its signal in the control samples.

**Yeast cell encapsulation.** Yeast cells were coated as described earlier¹ using PAH and PSS 1 mg ml⁻¹ aqueous solution. The cells (5×10⁸ cells in saline) were carefully introduced into the appropriate suspensions of polyelectrolytes (or halloysite nanotubes) in centrifuge tubes, incubated for 15 min, then centrifuged and washed with saline. The final architecture was PAH/halloysite/PAH/PSS.

**Viability Tests.** Membrane integrity and enzymatyc activity of cells was tested using FDA and propidium iodide as described elsewhere.² Fluorescent microscopy was used to estimate the viability in the stained samples. Automated cell growth monitoring was performed using a
Tecan Infinite F200 PRO (Switzerland) microplate reader. Cells were loaded into the plastic plates supplemented with the growth media and the optical density at 550 nm was automatically recorded for 20 hours.

**Charaterisation Techniques**

\(\zeta\)-potentials of cells and halloysite nanotubes were determined using a Malvern Zetasizer Nano ZS equipped with standard disposable cells.

*Flow cytometry* was performed using a BD FACS Canto II (USA) flow cytometer. Data was obtained using Cell Quest Pro software.

**Microscopy.**

*Optical and fluorescence microscopy* images were obtained using a Carl Zeiss AxioScope A1 microscope equipped with an AxioCam MRc5 CCD camera.

*TEM images* were obtained using a Jeol 1200 EX microscope operating at 80 kV. The cells were fixed with 2.5% glutaraldehyde, gradually dehydrated using a series of ethanol solutions (30, 60, 70, 80, and 100%), embedded into Epon resin and then thin sections were cut using a LKB ultramicrotome equipped with a diamond knife and mounted on copper grids. The samples were stained with 2% aqueous uranyl acetate and lead citrate.

*SEM images and EDX spectra* and mapping images were obtained using a Merlin (Carl Zeiss) instrument equipped with Inca Energy 350 X-Max (Oxford Instruments) spectrometer. Cells were dehydrated as described above and placed on glass stubs. Samples were sputter-coated with Au (60%) and Pd (40%) alloy using a Q150R (Quorum Technologies) instrument. Images were obtained at 3\times10^{-4} \text{ Pa} working pressure and 15 keV accelerating voltage using InLens detection mode (2 mm working distance).

**References:**


Additional Figures and Table

Fig. S1. SEM image of yeast cells used in this study (scale bar 5 µm).

Fig. S2. TEM (left) and SEM (right) images of halloysite nanotubes used in this study (scale bar 100 nm).

Fig. S3. Zeta-potential of halloysite nanotubes during the coating with FITC-PAH and PSS.
Fig. S4. Upper row: optical microscopy of bare (left) and PAH/HNTs/PAH/PSS-coated cells. Lower row: fluorescence microscopy images of yeast cells coated with PAH/HNTs/PAH/PSS (HNTs are labelled with FITC-PAH and coated with PSS) (left) and polarized microscopy of PAH/HNTs/PAH/PSS-coated cells.

Fig. S5. A) – a larger magnification image of a HNTs/PEs coated yeast cell preserving its overall ellipsoid geometry; B) – a close-up SEM image from a squared area in A, demonstrating the random orientation of HNTs on cells.
Fig. S6. SEM images of a single hollow yeast-shaped halloysite microparticle produced after decomposition of the cell templates. Note the focus at the top (left) and the bottom (right) demonstrating the hollow structure of the microparticle.

Fig. S7. Release profile of nutrient (glucose) loaded to halloysite from the HNTs/PEs-coated cells.

Fig. S8. Fluorescence microscopy images demonstrating the typical distribution of viable (green FDA-stained) and non-viable (red, propidium iodide-stained): a) – intact cells, b) PEs-coated cells, c) – HNTs/PEs-coated cells, d) cells mixed with HNTs.
Table S1. Size distribution of yeast cells, yeast@HNTs/PEs and HNTs capsules.

<table>
<thead>
<tr>
<th>Size</th>
<th>Bare yeast</th>
<th>Yeast@HNTs/PEs</th>
<th>HNTs capsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>4.1±0.6 µm</td>
<td>4.8±0.8 µm</td>
<td>3.7±0.4 µm</td>
</tr>
<tr>
<td>Width</td>
<td>3.5±0.5 µm</td>
<td>4.0±0.5 µm</td>
<td>2.7±0.3 µm</td>
</tr>
<tr>
<td>Length/width</td>
<td>1.17</td>
<td>1.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>