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SURFACE ENHANCED RAMAN SPECTROSCOPY DETECTION OF p-COUMARIC ACID FROM CELL SUPERNATANT USING GOLD-CAPPED SILICON NANOPILLAR SUBSTRATES

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Aim of the Project

The purpose of the project is to use Surface Enhanced Raman Spectroscopy (SERS) to discriminate between two different bacterial populations, based on their p-coumaric acid (pHCA) production. The pHCA concentration is measured in a droplet of diluted supernatant dried on SERS substrates, using a Raman microscope. By analyzing the SERS signal of pHCA from the supernatant, considering the peak height at the characteristic frequency (1169 cm^{-1}) **it is possible to distinguish between a producing and control strain**, as also confirmed by HPLC analysis.

SERS: fabrication and working principle
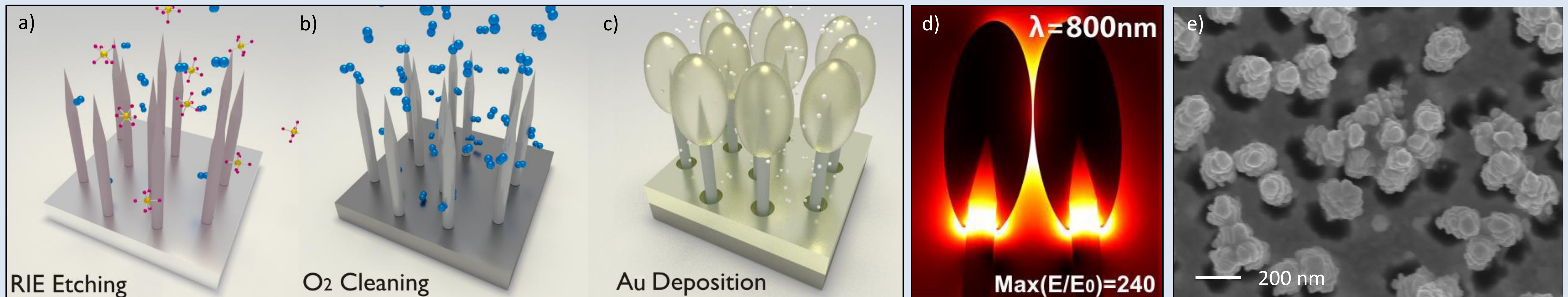
Fig.1: Process steps for Au capped nanopillars (NPs) fabrication (courtesy of Kaiyu Wu) [1]: **(a)** silicon NPs are fabricated by maskless reactive ion etching (RIE); **(b)** O$_2$ cleaning removes the Si RIE contaminants from the Si surface; **(c)** a Au metal film is deposited by e-beam evaporation. **(d)** When a droplet of solution is dried on the substrate, the surface tension tends to pull the Au NPs together, forming irreversible clusters and trapping the analyte molecules between the NPs. Furthermore, E-field hotspots are created when two NPs lean close to each other. **(e)** SEM picture of leaning pillars.

## Bacterial cultures and measurements
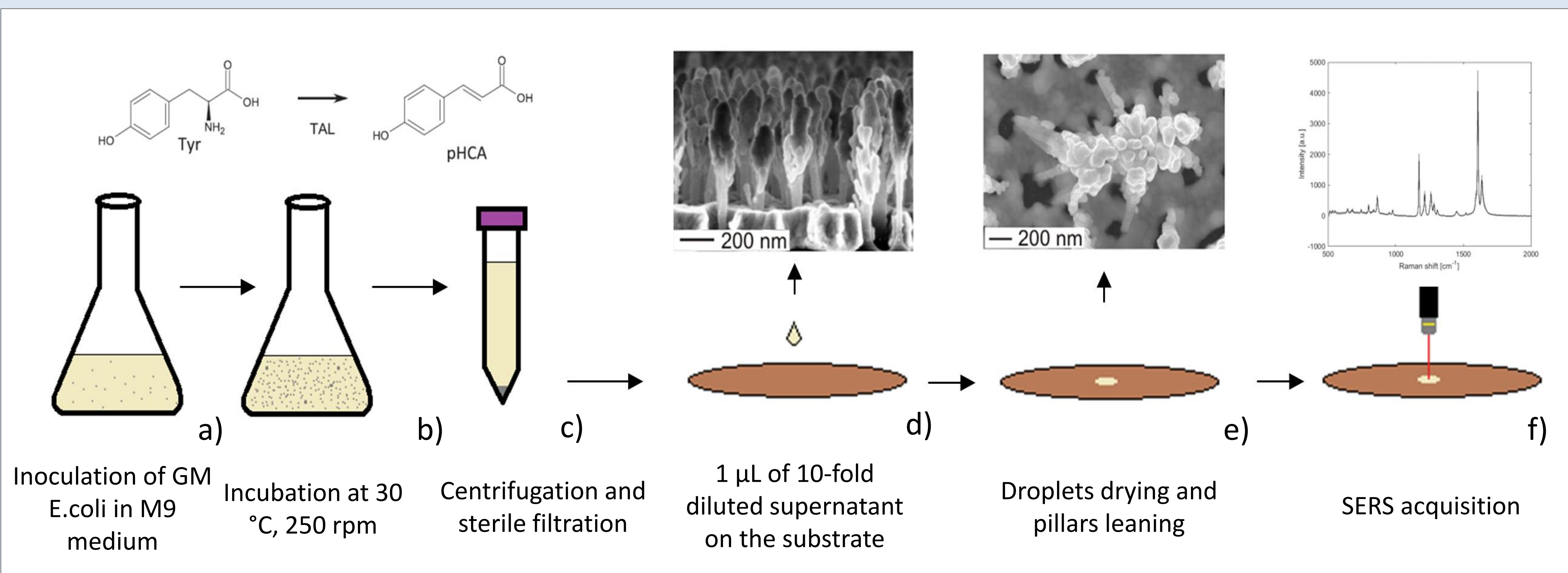
Fig.2: Bacterial culture, supernatant extraction and SERS evaluation: **(a)** and **(b)** genetically modified E.coli are cultured in triplicates [2]; **(c)** aliquots of bacterial solution are extracted at 0, 3, 24 and 48 h, centrifuged and sterile filtered to extract cell supernatant; **(d)** and **(e)** 1 µL of supernatant diluted 10-fold with water is dried on SERS substrates. The SEM pictures show that the pillars stand vertically before wetting, and they lean towards each other after drying. **(f)** 5x5 maps are acquired on the droplet area with a DXR Raman microscope (Thermo Fisher Scientific Inc.) at 780 nm, 1 mW, 10x objective, 25 µm slit.

Salt dilution
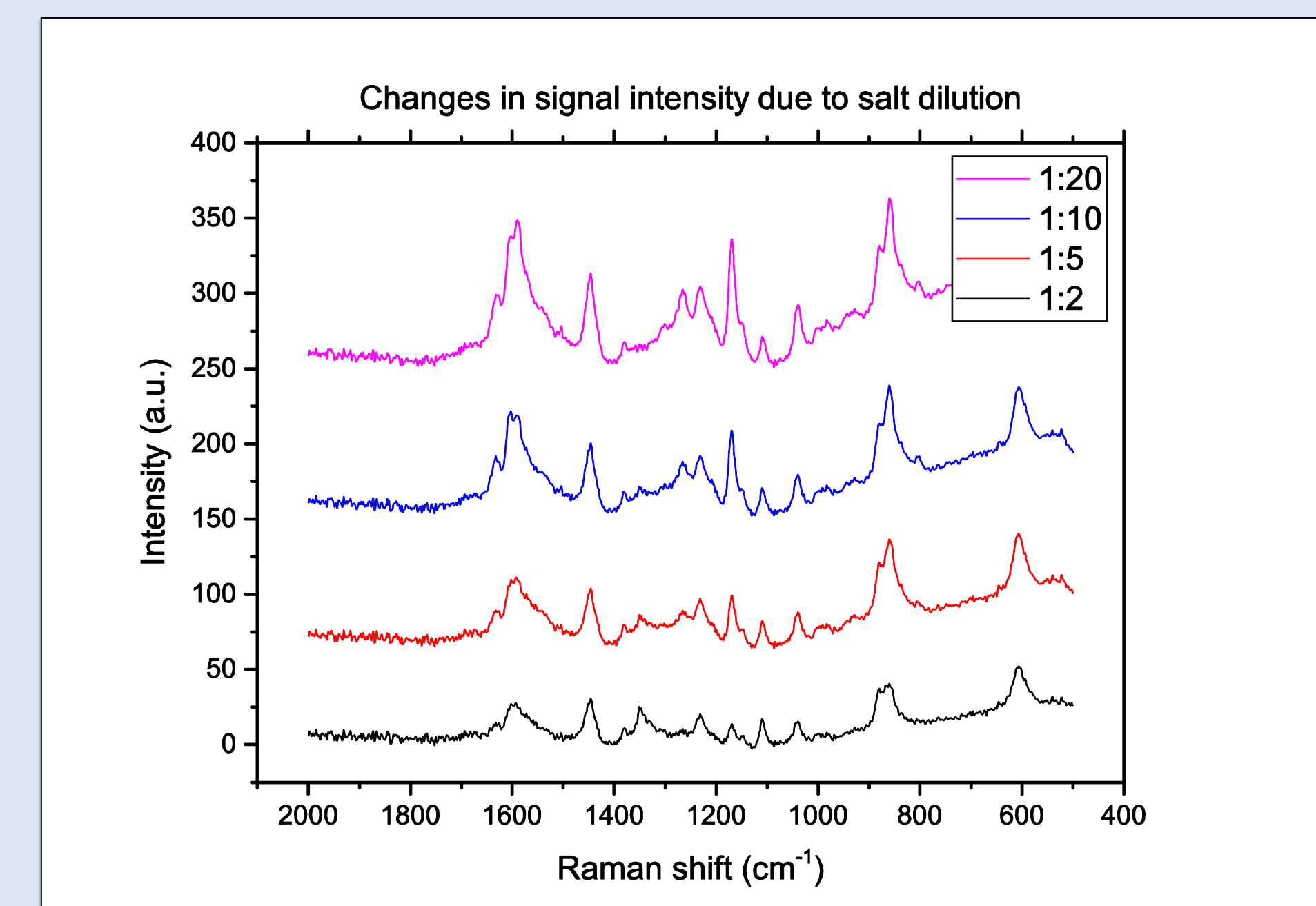
Fig.3: 100 µM pHCA spiked in culture medium diluted with MilliQ water in different ratios (1:2, 1:5, 1:10, 1:20). The signal increases with medium dilution, as higher salt concentration clogs up the active surface and decreases the signal. 1:10 dilution was chosen as a compromise between signal intensity and dilution for measurements in the supernatant.

Validation with HPLC
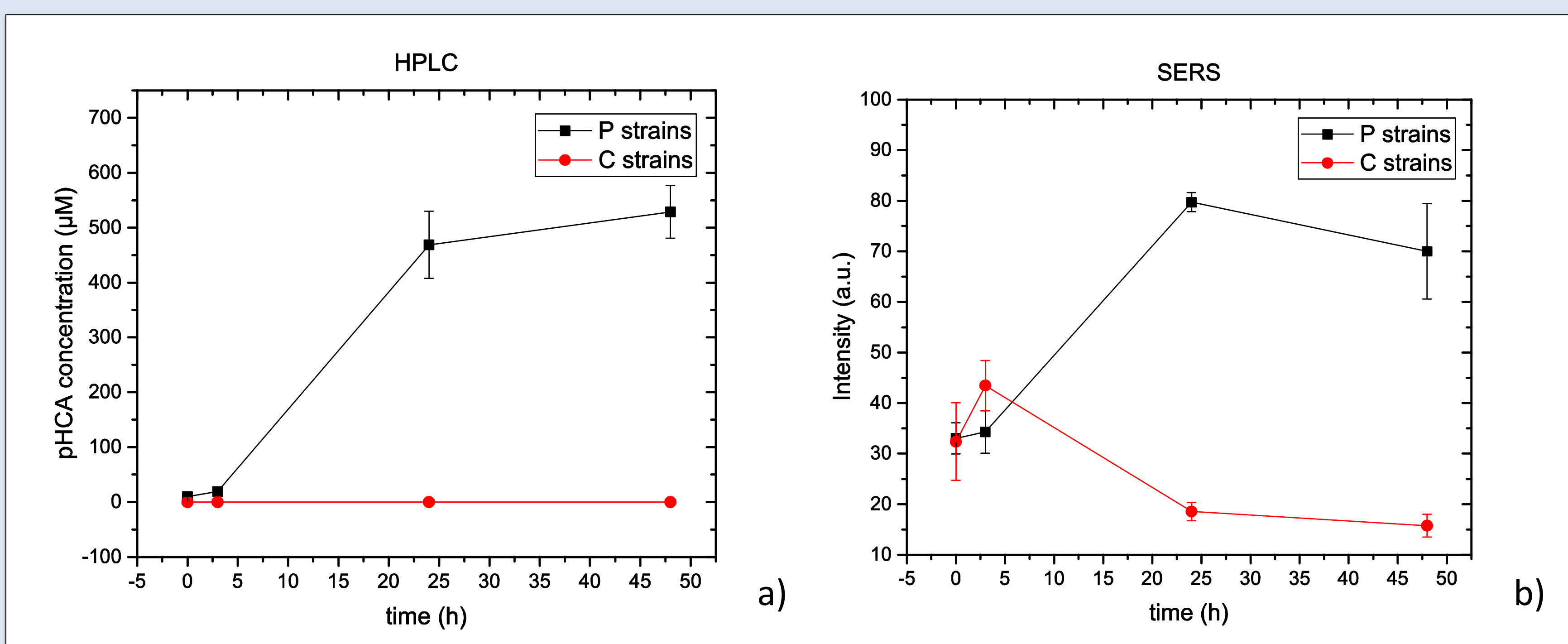
Fig.4: **(a)** Concentration of pHCA in cell supernatant for producing (P, black) and control (C, red) strains measured with HPLC. Each point is the average of 3 measurements, each one obtained from one strain. **(b)** SERS signal at 1169 cm^{-1} after baseline correction. Each point in the graph is the average of 3 maps of 25 points, whereas the error bars represent the standard error of the mean, calculated on the 3 average values.

Outlook and conclusions

In this work we demonstrated that SERS is a rapid and **effective tool for qualitative screening** of bacterial strains, based on the amount of synthetized secondary metabolites (e.g. pHCA). These results open up new **possibilities for high-throughput quantitative analysis**. Currently we are focusing on improving sensitivity by extracting pHCA in organic solvent and on integration of the assays on automated and high-throughput microfluidic platforms, such as lab-on-a-discs.

References

[1] K. Wu, T. Rindzevicius, M. S. Schmidt, K. B. Mogensen, A. Hakonen, and A. Boisen, *J. Phys. Chem. C*, vol. 119, no. 4, pp. 2053–2062, 2015.
[2] C. B. Jendresen, S. G. Stahlhut, M. Li et al., Appl. Environ. Microbiol., vol. 81, no. 13, pp. AEM.00405–15, 2015.

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