

A Centrifugal Microfluidic Platform Using SLM Extraction for combined sample clean-up and enrichment of trace analytes

Andreasen, Sune Zoëga; Burger, Robert; Emnéus, Jenny; Boisen, Anja

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A Centrifugal Microfluidic Platform Using SLM Extraction

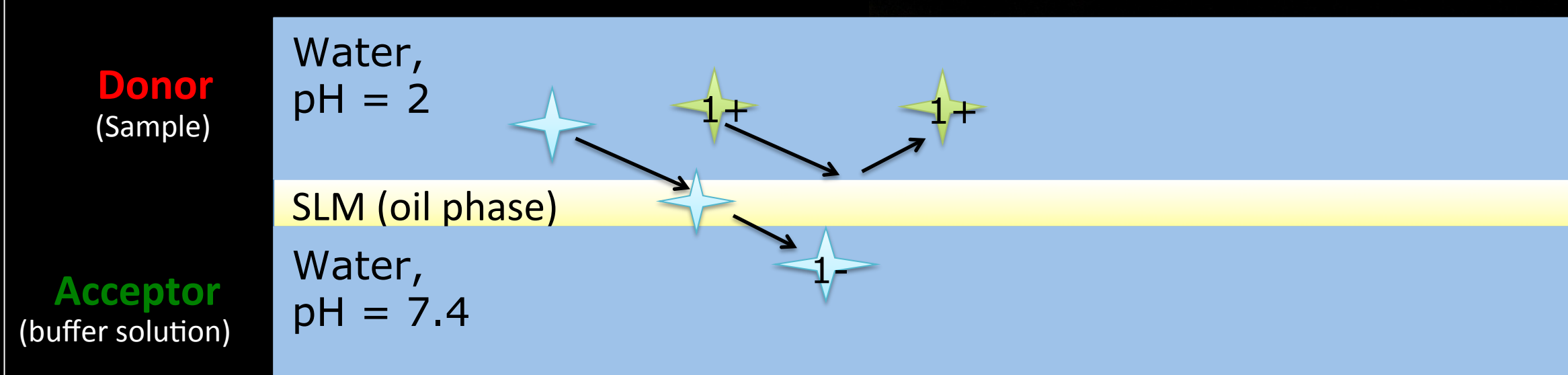
- for combined sample clean-up and enrichment of trace analytes

Sune Z. Andreasen, Robert Burger, Jenny Emneus & Anja Boisen. DTU Nanotech, Technical University of Denmark

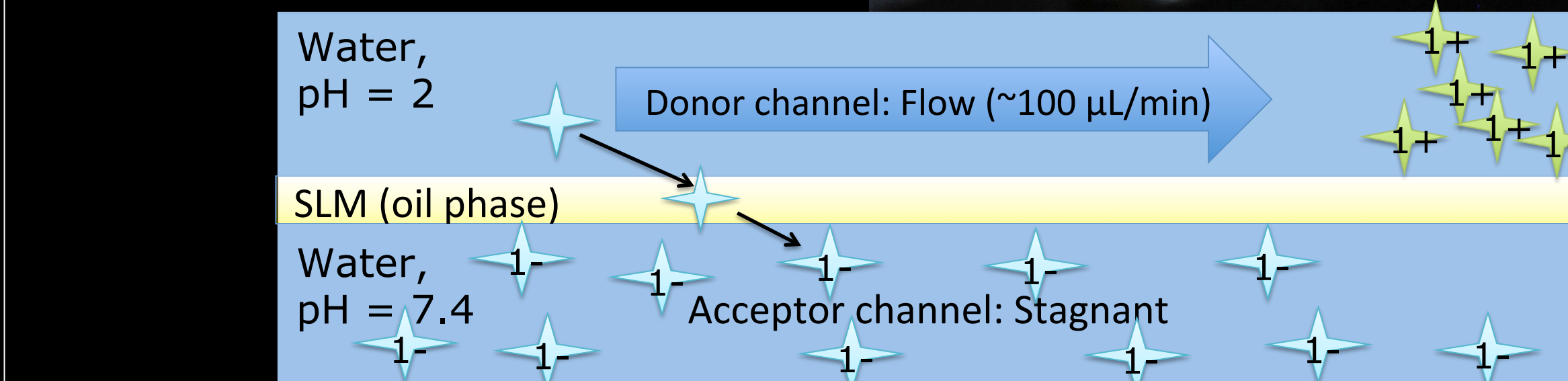
Here we present a pump-less microfluidic platform which performs sample clean-up and enrichment in a single step, by integrating Supported Liquid Membrane (SLM) extraction. Our platform offers a simple, yet very efficient, method for achieving sample pre-treatment and enrichment of rare analytes, in an easy to use and highly efficient device.

Working principle: Separating & trapping of weak acids (or bases)

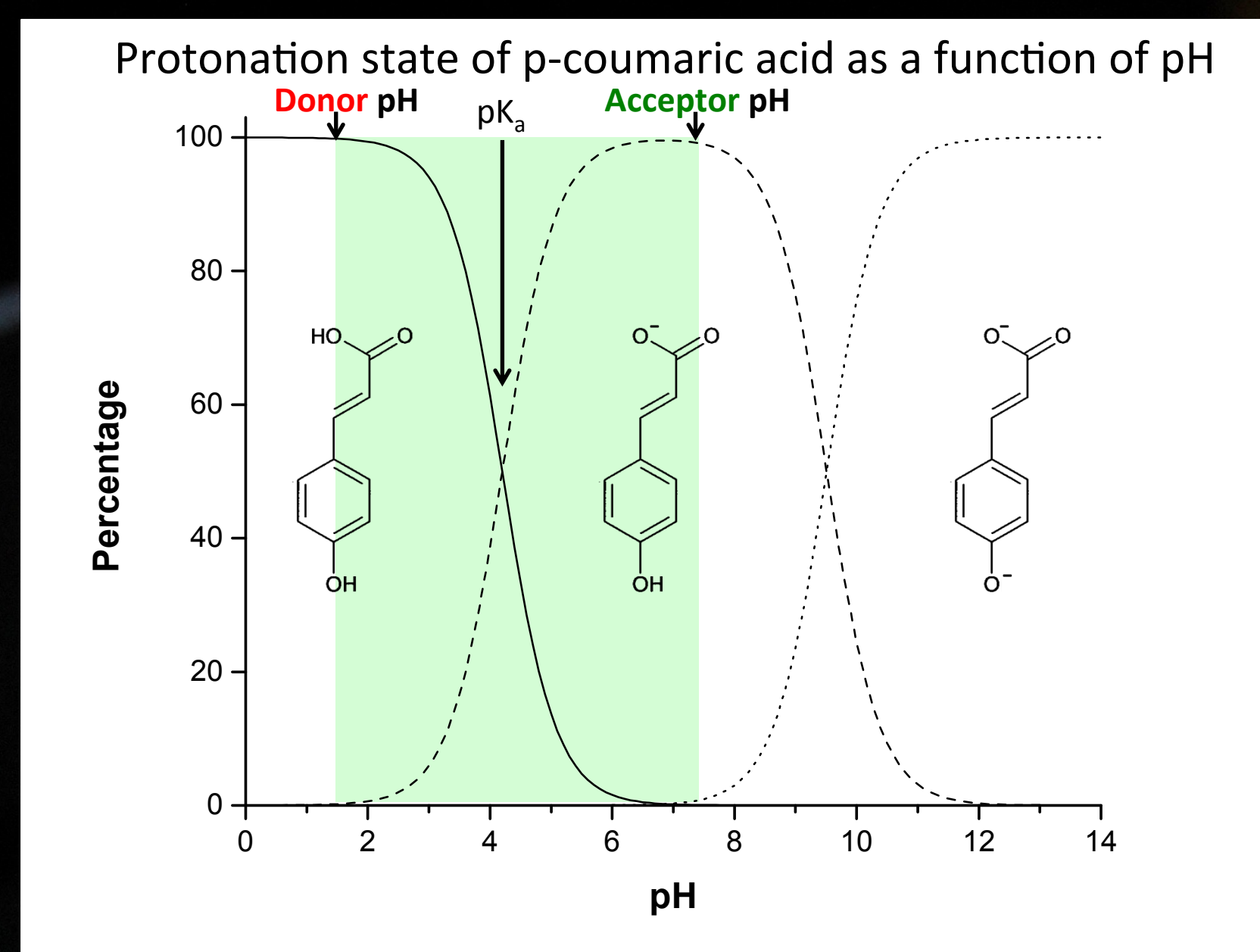
EXPERIMENT **START**: Non-porous extraction of analyte; only small molecules, on their neutral form, can pass through the oil film, separating the donor flow from a stagnant acceptor buffer. The oil is kept in place by a 25 μm thick nanoporous polypropylene membrane.



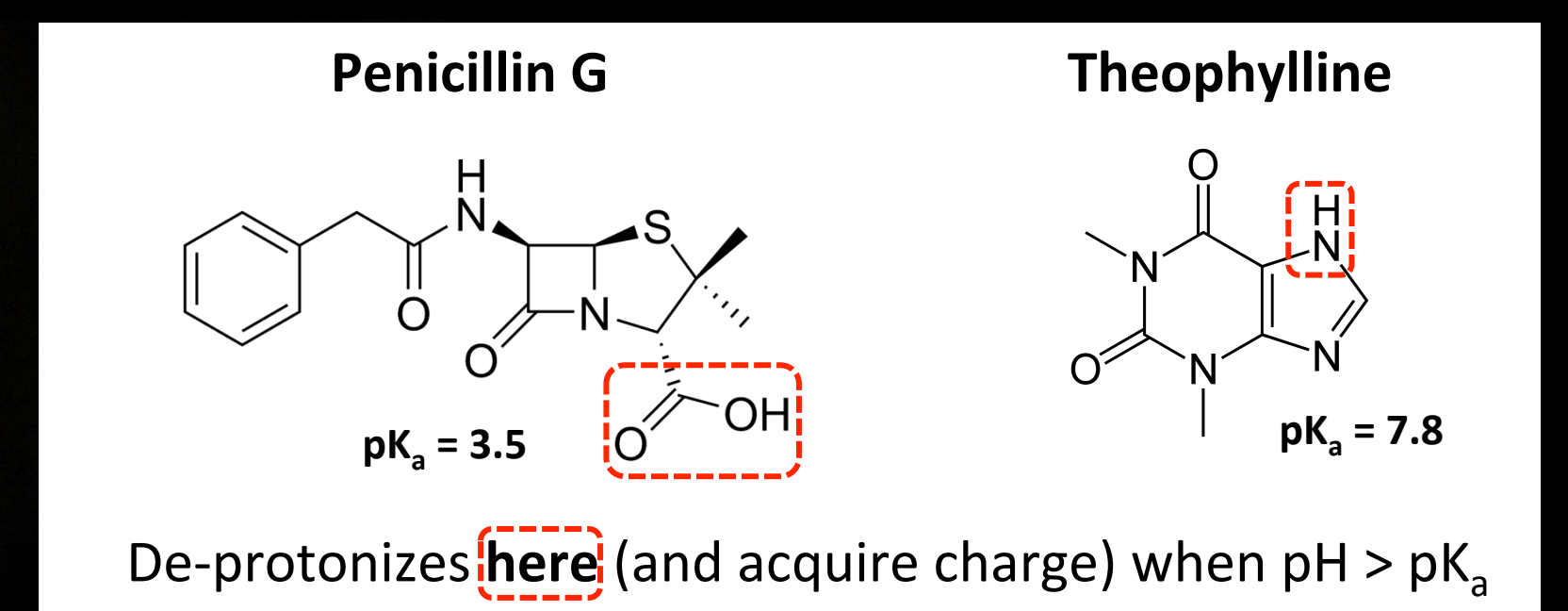
EXPERIMENT **ONGOING**: By choosing a pH above (below) the pK_a of the acid (base) in the acceptor buffer, the analyte is trapped. While continuing to replenish the donor, both separation and enrichment of the target analyte is possible.



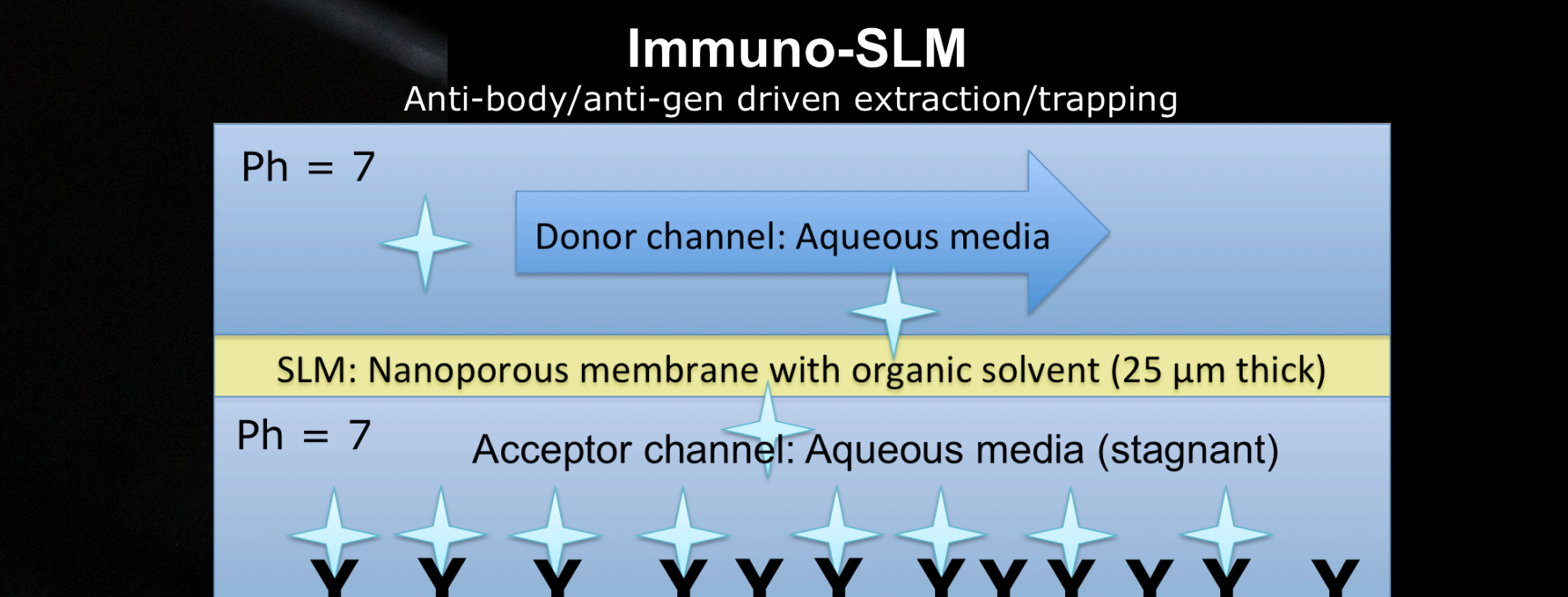
Example: pH trapping of p-coumaric acid



Other small molecules tested and used for SLM extraction (Neutral (no charge) at low pH):

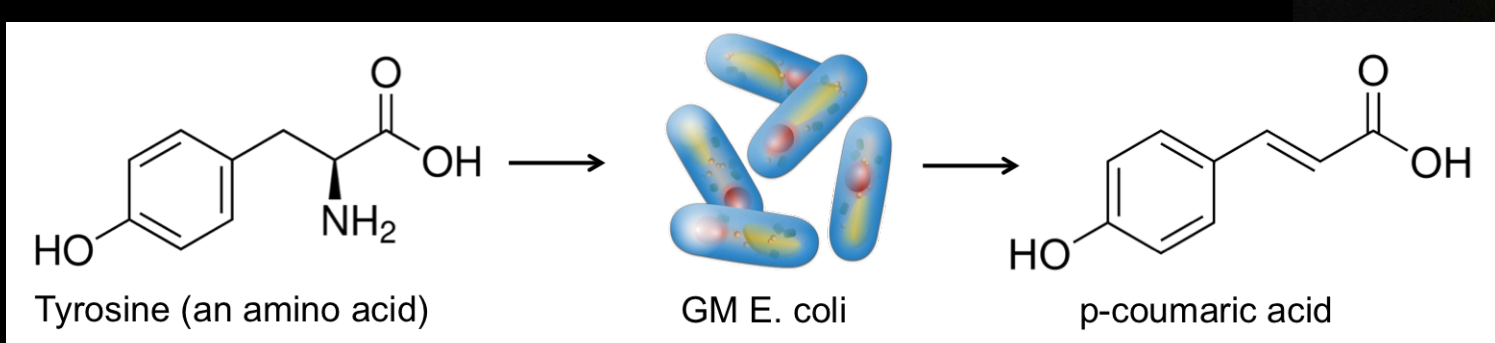


Alternative tactics for achieving even more specific trapping:

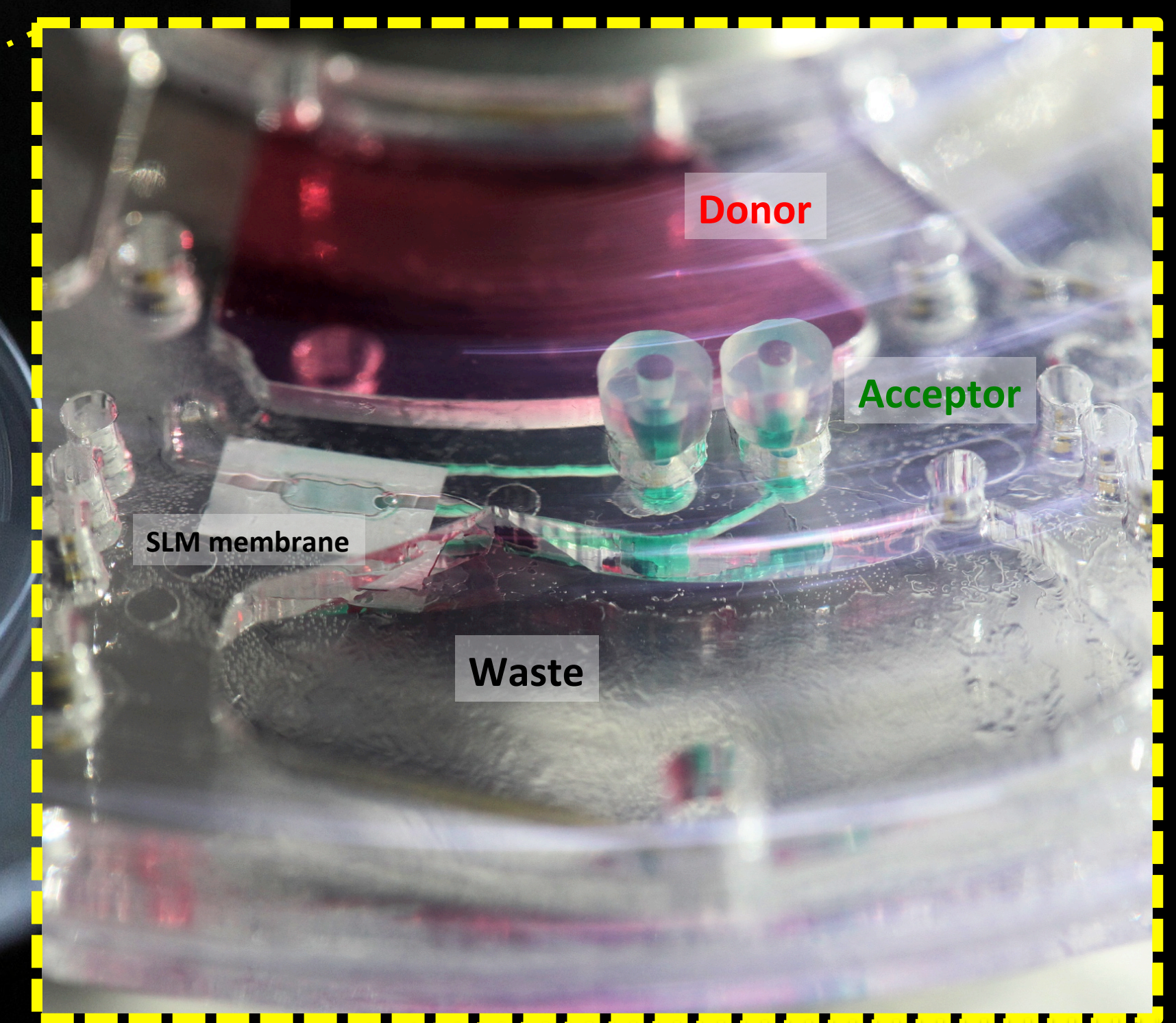
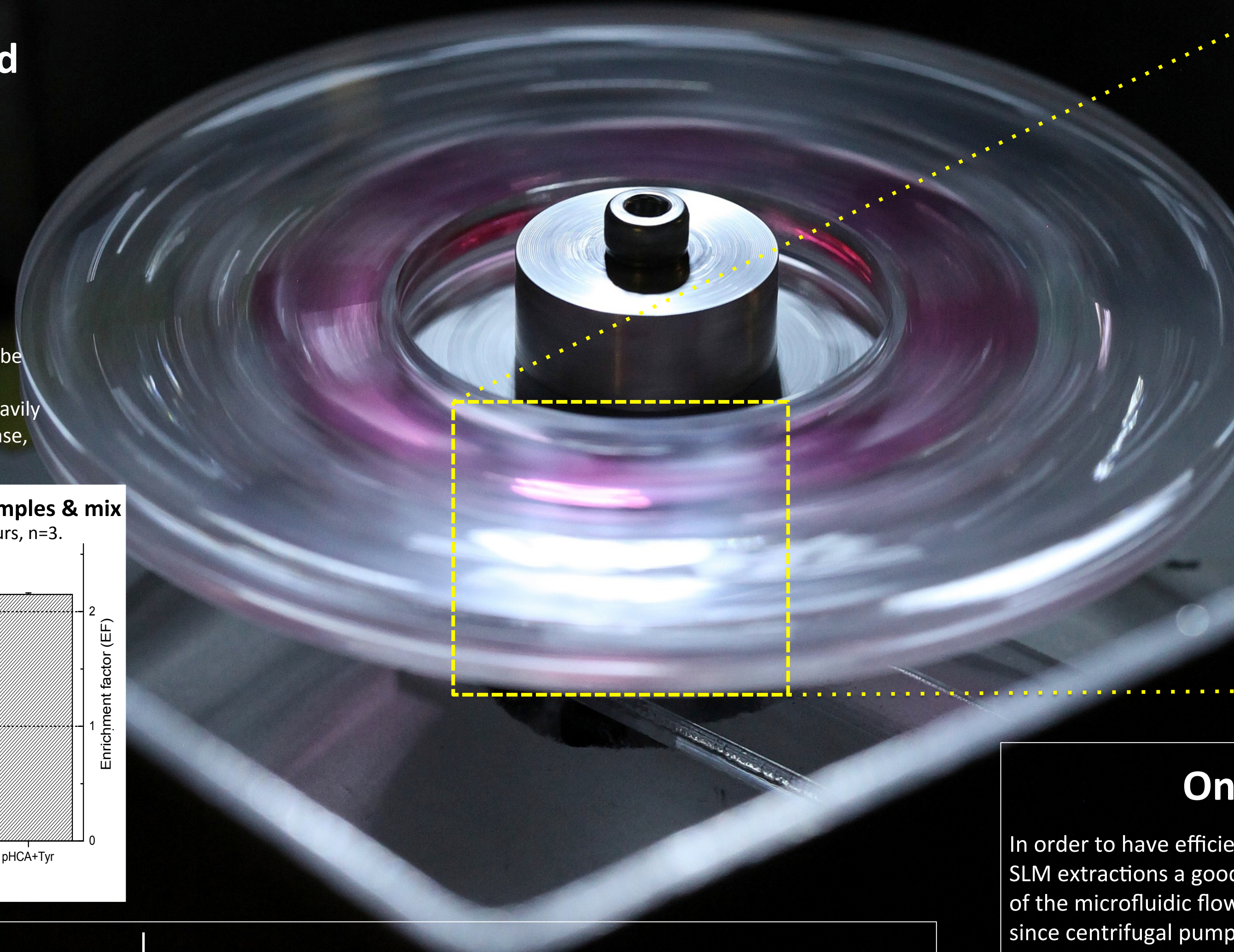
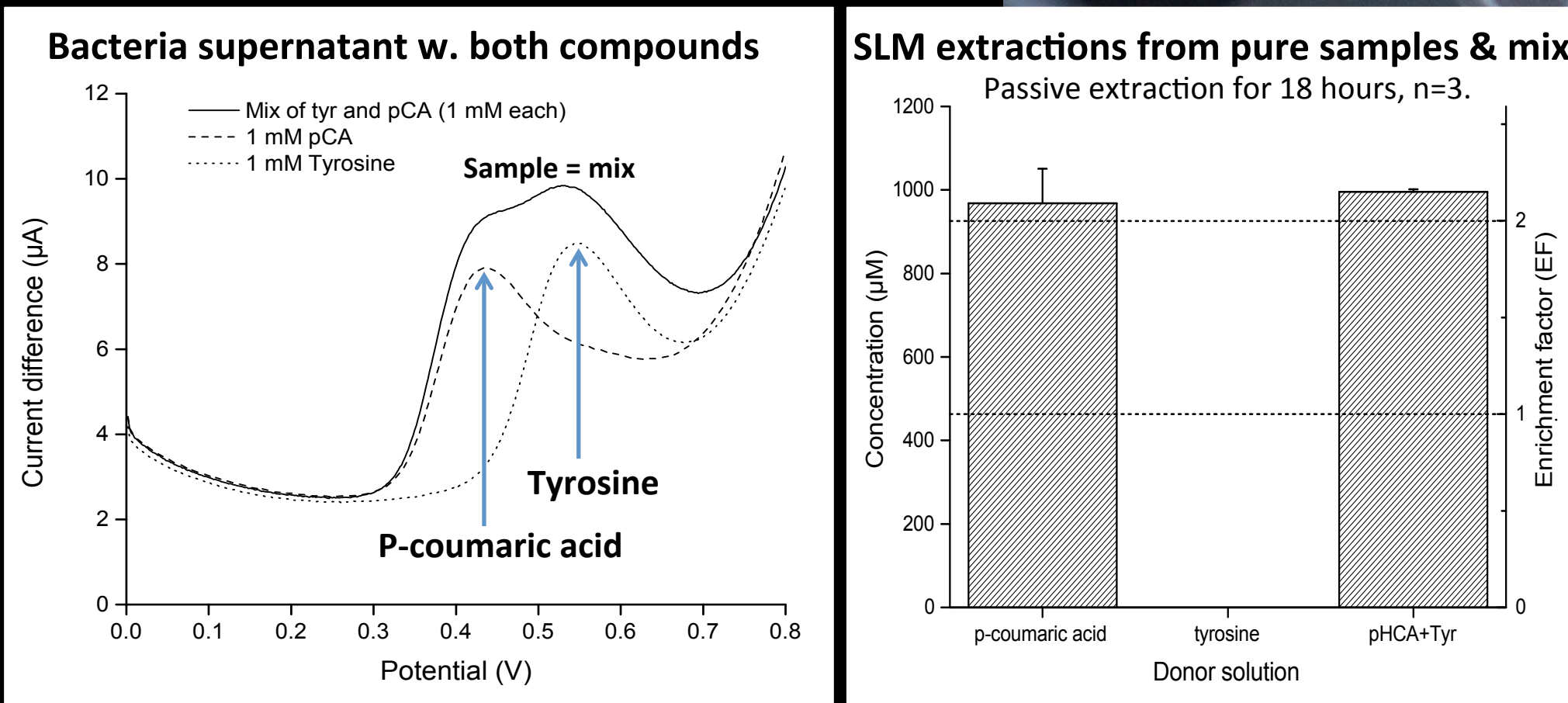


Real application:

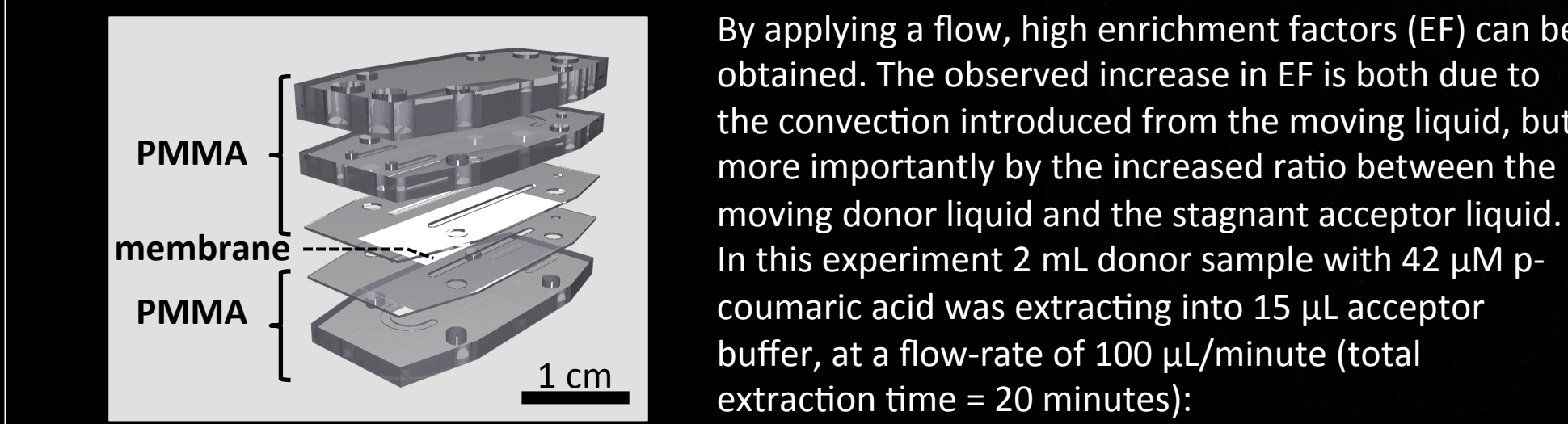
Screening production of genetically modified bacteria strains:



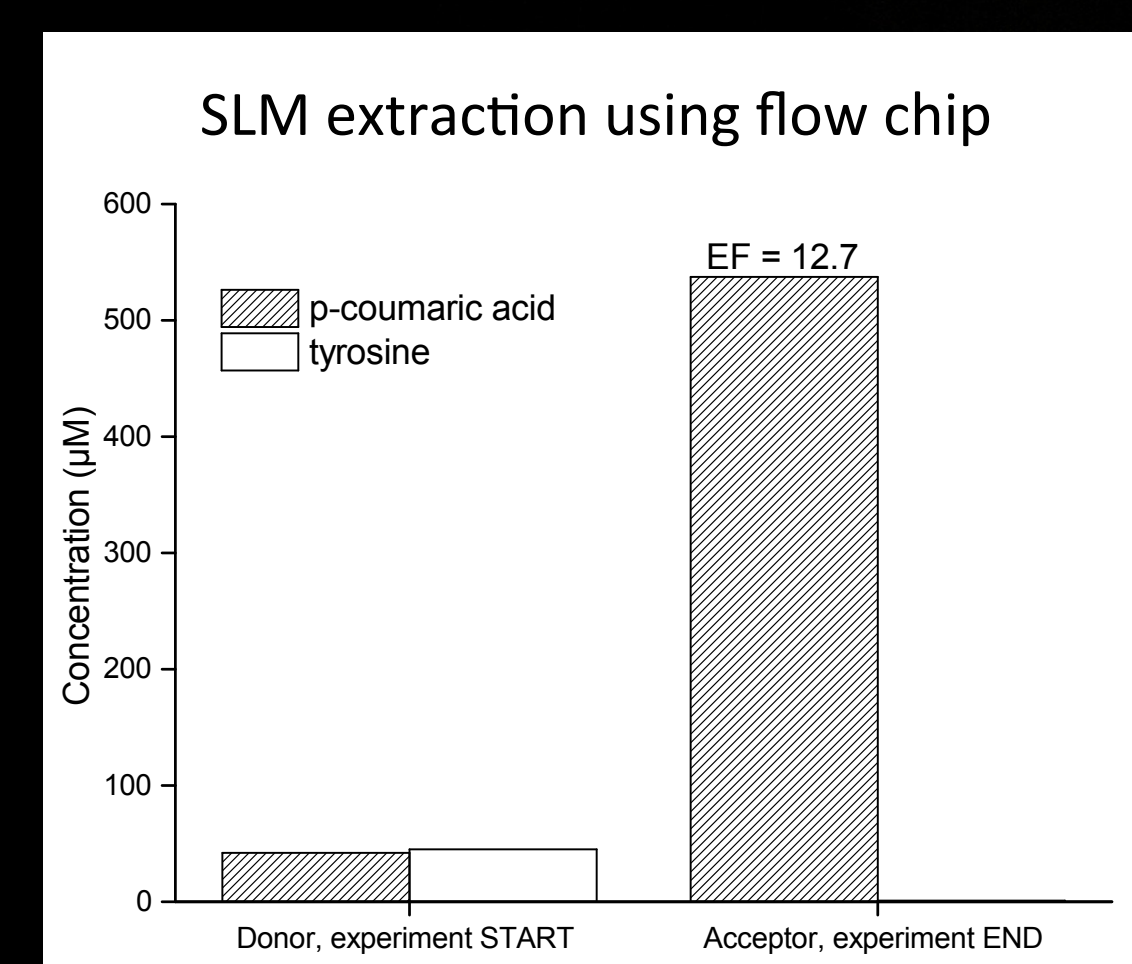
E. coli bacteria genetically modified to convert tyrosine to p-coumaric acid (an important precursor for a number of drugs). To choose the best producing strains it is imperative to be able to distinguish between the two compounds. Both compounds can be detected by electrochemistry, but unfortunately the oxidation potential of the two compounds are heavily overlapping (see figure below, left), making quantitative measurements difficult. In this case, SLM extraction is a very powerful method to separate the two (figure below, right).



Flow chips for testing extraction during flow:

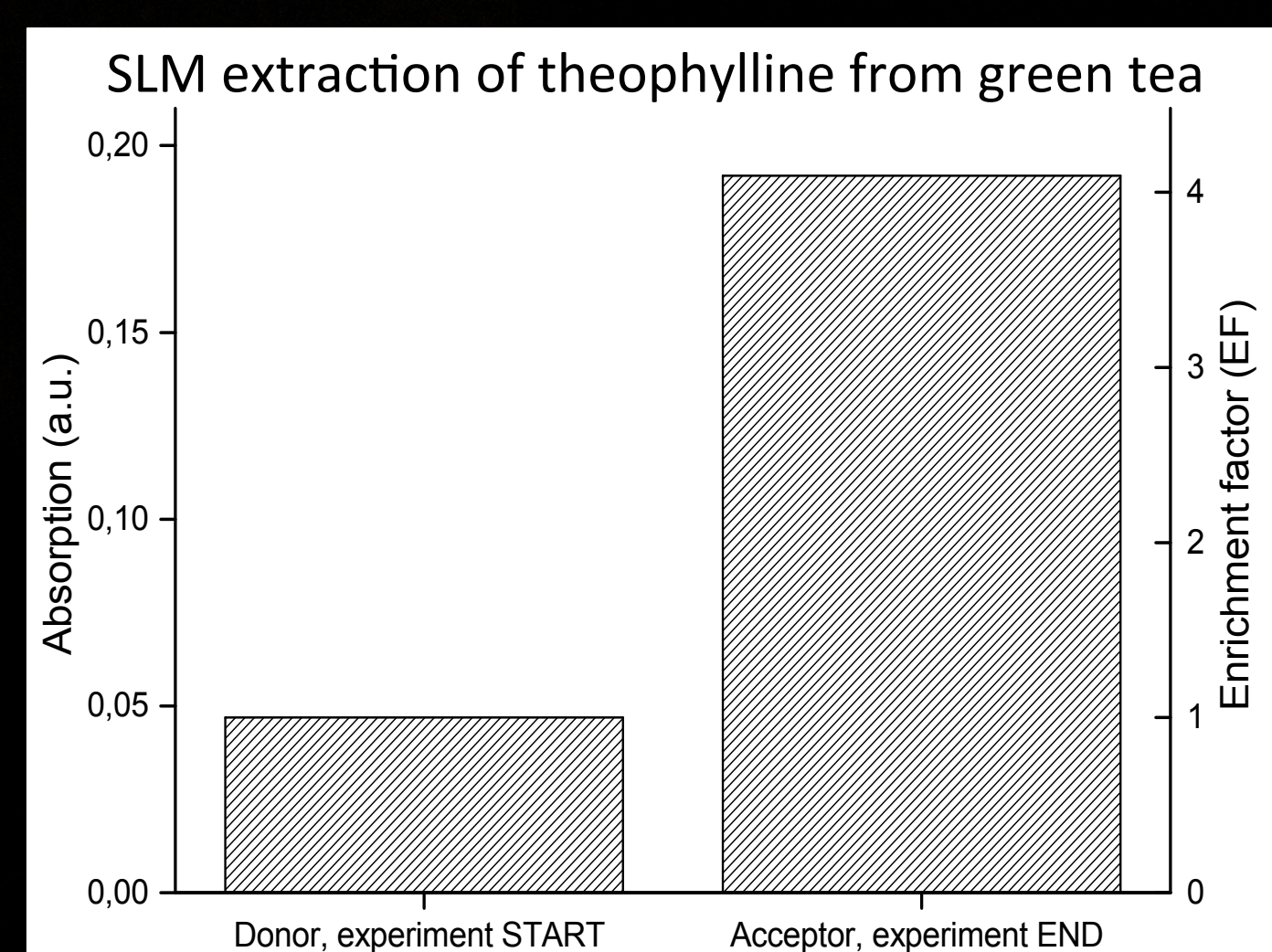


By applying a flow, high enrichment factors (EF) can be obtained. The observed increase in EF is both due to the convection introduced from the moving liquid, but more importantly by the increased ratio between the moving donor liquid and the stagnant acceptor liquid. In this experiment 2 mL donor sample with 42 μM p-coumaric acid was extracting into 15 μL acceptor buffer, at a flow-rate of 100 $\mu\text{L}/\text{minute}$ (total extraction time = 20 minutes).



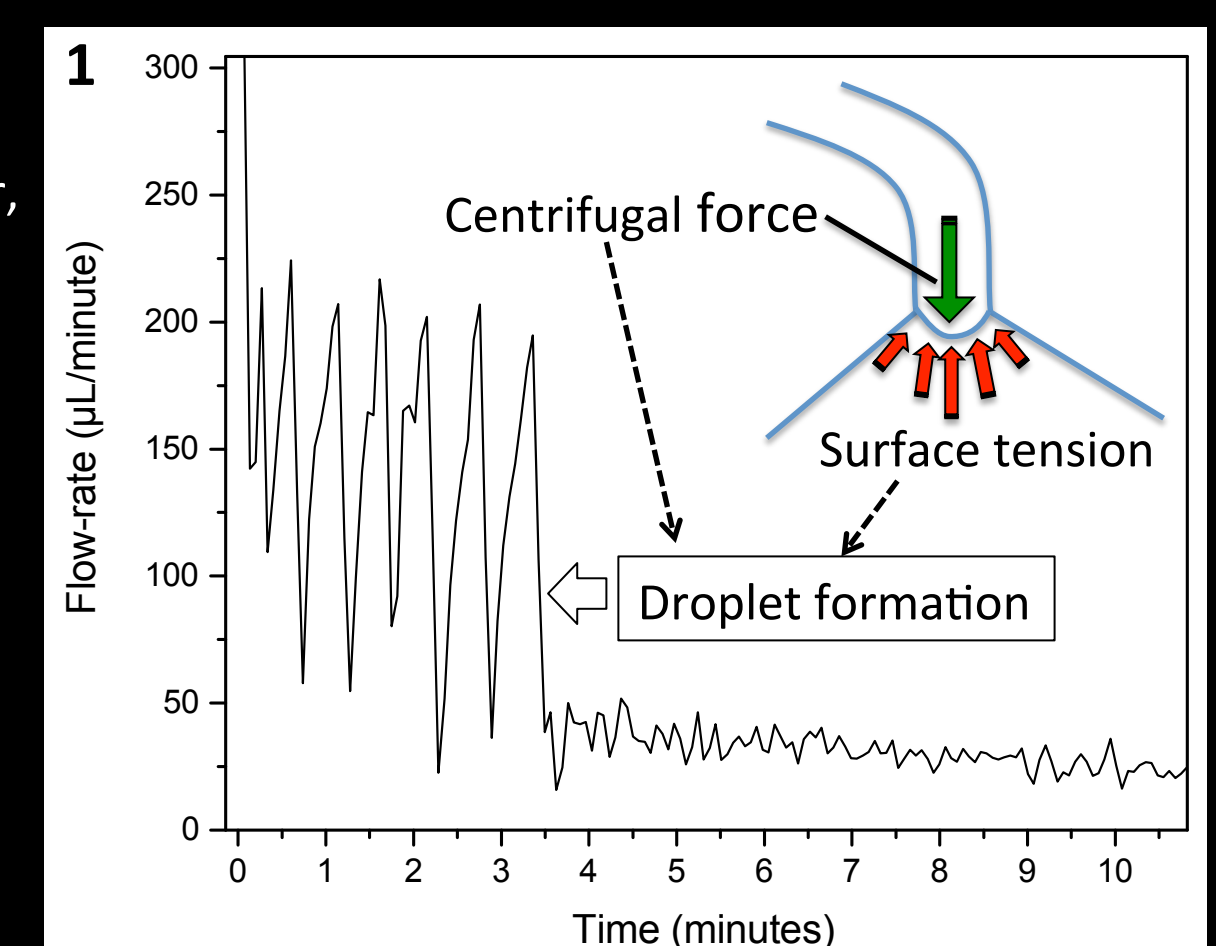
On-disc extraction w. enrichment:

As mentioned elsewhere on this poster, one of the main challenges of integrating SLM extraction on a disc is to achieve reproducible, but slow, flow-rates. A slow flow-rate is important for two reasons: 1) The mass transport from the donor to the acceptor side, through the oil membrane, is diffusion controlled and thus fairly slow. 2) The amount of donor liquid volume that can be put on a 10-12 centimeter diameter disc is limited. However, with our discs we have been able to fit volumes around 0.5-1 mL, and achieved enrichment factors of ~ 4 (as well as the purification of the sample).



On-disc flow control: A challenge!

In order to have efficient and reproducible SLM extractions a good and reliable control of the microfluidic flow is needed. However, since centrifugal pumping cannot be controlled directly, but only indirectly, through the rotational speed of the disc, several factors are affecting the observed flow-rate. For instance, the centrifugally induced pressure needs to overcome the surface tension of the liquid. This is an unstable scenario, where a certain centrifugal speed is necessary to start a flow, but as soon as the flow has finally started the flow should be as slow as possible (see figure 1).



Also, since the hydrostatic resistance of the channels are very sensitive to the channel dimensions, slight changes in this during fabrication can lead to rather large differences in observed flow speeds (see figure 2). One way to remedy these inherent challenges of the platform would be to use injection molding so that all disc are fabricated from the same mold. Another exciting method that we are working on currently is to use pneumatic flow control in combination with the spinning to pump the fluid back and forth over the membrane continuously.

