Laboratoire d'Analyse et d'Architecture des Systèmes

THERMORESPONSIVE POLYMER-BASED MICRODEVICE FOR NANO-LIQUID CHROMATOGRAPHY

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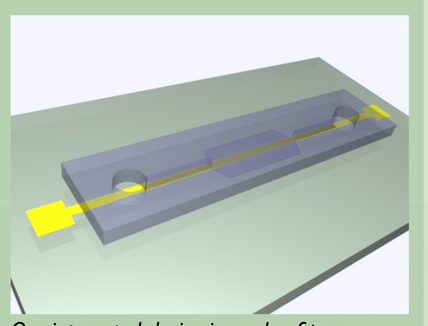
Sample preparation for nanoLC

Goal & principle

We report on a novel approach for desalting and concentrating samples for nanoLC / ESI-MS, relying on reversible adsorption of peptids on microbeads caused by on-demand surface actuation.

Proteins are usually fractionated and digested into peptides that need to be desalted and preconcentrated before being analysed. Our device allows to reversibly trap them during these steps.

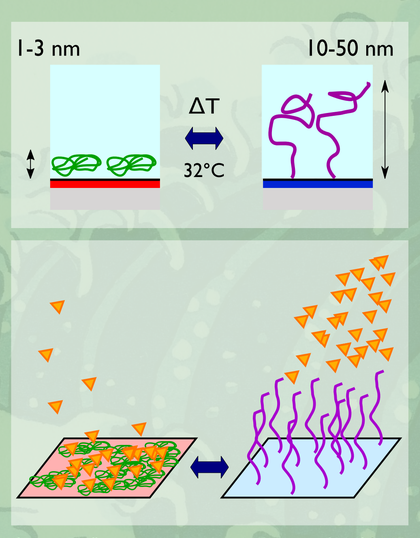
Spatio-temporal control of surfaces properties is achieved through switchable thermo-sensitive polymers grafted on surfaces and activated by integrated microheaters.



Our integrated device is made of pyrex, Ti/Au heater, PECVD SiO2 and a molded PDMS channel with a geometry designed to trap microbeads.

Thermoresponsive polymers

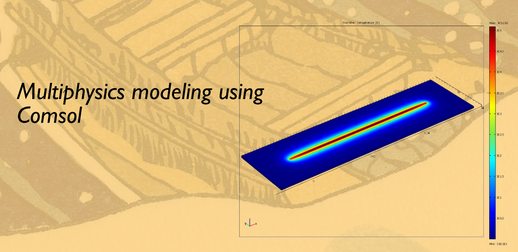
- Surface chemistry: poly(N-isopropylacrylamide) (PNIPAM) grafted via a silane layer (3-trimethoxysilyl propylmethacrylate)
- Reversible switch around 32°C (LCST, lower critical solution temperature) from a hydrophilic, swollen & non-fouling state to a hydrophobic, collapsed & protein-adsorbing state.
- Application to controlled adsorption and release of peptides to desalt and preconcentrate samples for nano-LC / ESI-MS analysis.



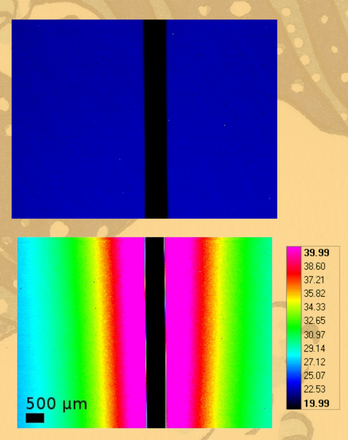
Proteins & peptides adsorb on hot PNIPAM and are then released when it cools down.

Integrated resistive heaters

- 500µm- and 100µm-wide lines
- Ti 1000 Å / Au 8000 Å on pyrex substrates
- PECVD SiO2 as grafting & isolating layer
- LCST reached with a few volts



Multiphysics modeling using Comsol

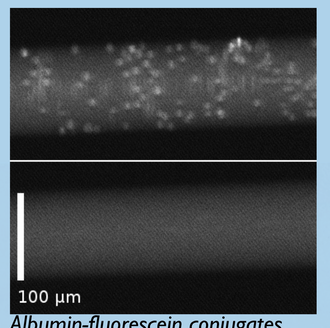


Infrared thermal imaging. Top: 0V, bottom: 4V, 51°C

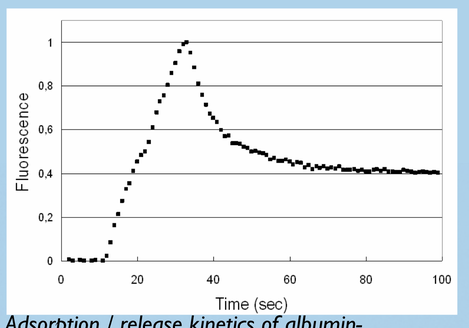
Adsorption / release of proteins

Beads with PNIPAM

Preliminary tests in capillaries with PNIPAM-grafted microbeads validated the adsorption and release of BSA-fluorescein conjugates upon external thermal actuation, though not fully reversibly.



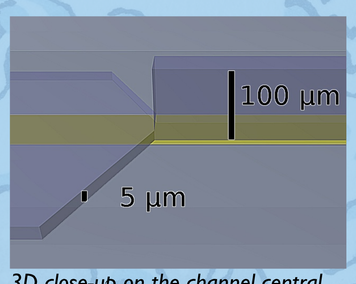
Albumin-fluorescein conjugates adsorbed on 5 µm beads (top, T > LCST) and then released (bottom, T < LCST)



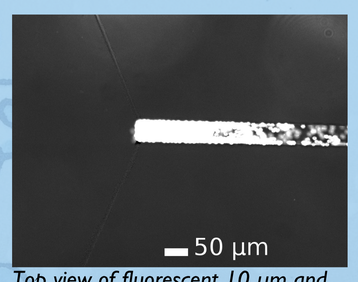
Adsorption / release kinetics of albumin-fluorescein conjugates (1mg/ml in PBS) from glass beads functionalized with PNIPAM (normalized units)

Beads in microchannels

The injection and behaviour of beads injected in our device was studied and their size was optimized to be trapped and serve as stationary phase. Injection of 5µm beads grafted with PNIPAM and adsorption/release of fluorescent proteins in our device are currently being done.



3D close-up on the channel central zone where beads are trapped

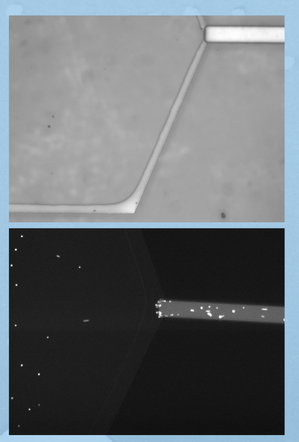


Top view of fluorescent 10 µm and 5 µm beads trapped at the entry of the central part

Improvements to our device

The thin, central part of the channel sometimes collapses and sticks to the substrate, due to the elasticity of PDMS. For the same reason, some 5µm beads manage to enter the 4µm central part. The channel geometry must be adapted, for instance by adding pillars to consolidate the structure.

The thickness of the PDMS channel prevents the use of zooms > 5x (short DOF). An inverted microscope can be used to monitor fluorescence from the backside, but that requires a modification of the opaque heater then hiding the channel.



(top) Channel whose central part stuck to the substrate. (bottom) Beads that entered the central part.

References

[1] Aebersold et al, Mass spectrometry-based proteomics, Nature, 422, p.198 (2003)

[2] Huber et al., Programmed adsorption and release of proteins in a microfluidic device, Science, 301, p.352 (2002)

Perspectives

- Screening of proteins (size, isoelectric point)
- Comparing the microdevice's performances with usual methods of sample preparation
- Developing hybrid PNIPAM-based microsystems for biochemichal analyses.