Supplementary Information

Functionalized hydrogel microparticles prepared by microfluidics and their interaction with specific tumor marker carbonic anhydrase IX

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Fabrication process of the microfluidic chip

The microfluidic chip was made by a soft lithography technique. The master with a designed structured of the chip layout was fabricated from a silicon wafer. The microfluidic chip was prepared from the PDMS upper part to the thin layer of PDMS on a glass slide (Fig. SI 1).

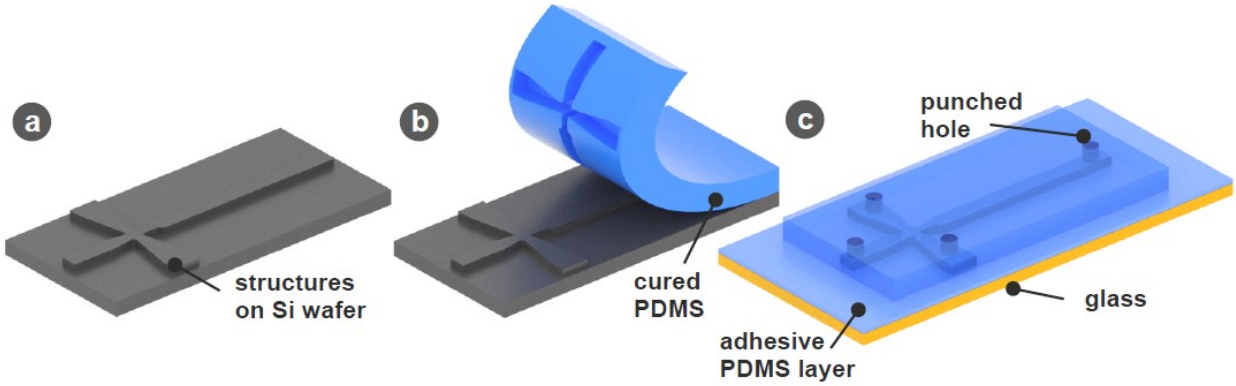

Fig. SI 1: Fabrication process of the microfluidic chip: a) silicon master with designed structures, b) cured PDMS replica, c) assembled microfluidic chip

Fluorescent labeling and characterization of Carbonic Anhydrase IX

For the labeling,*N*-(5-Fluoresceinyl)maleimide (FLM) from Sigma-Aldrich was used, CAS 75350-46-8, with λ_{ex} 490 nm; λ_{em} 518 nm in 0.1 M Tris pH 8.0.

The absorption spectrum (Fig. SI 2) was measured in a 1cm cuvette using Specord 205 (Analytik Jena). The CA IX-F concentration was calculated according to the absorbance at 280 nm using extinction coefficient $\varepsilon_{CA\ IX}$= 0.892 after subtraction of a corresponding intensity of FLM at this wavelength.

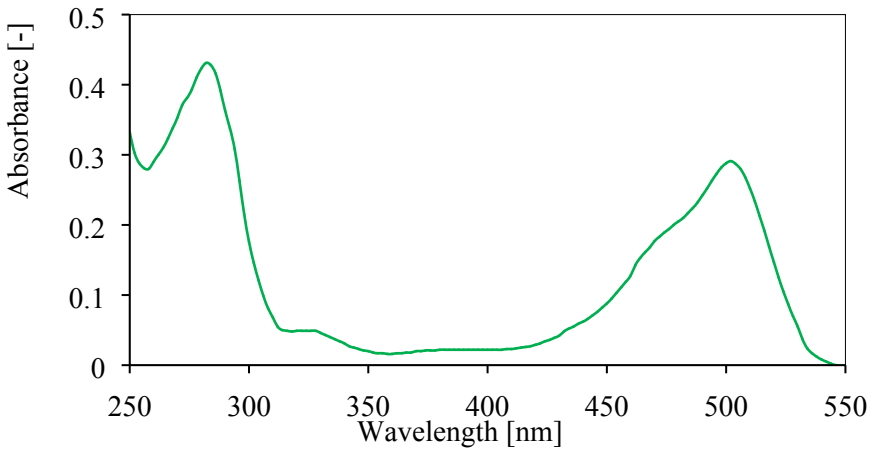

Fig. SI 2: Absorption spectrum of CA IX-F with concentration 0.4 mg/mL.

The fluorescence emission spectrum of CA IX-F (Fig. SI 3) was measured using Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies). The labelled protein exhibited fluorescence maximum at 525 nm (excitation 488 nm).

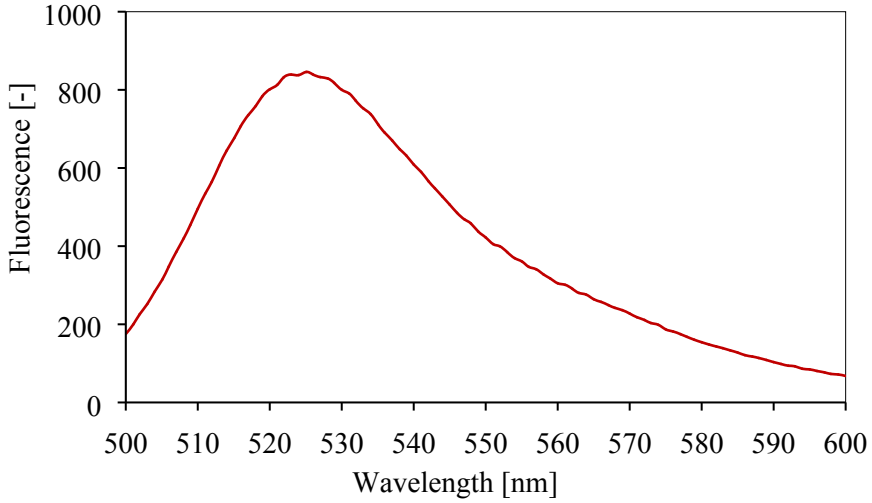

Fig. SI 3: Fluorescence emission spectrum of CA IX-F, excitation 488 nm.

Microfluidic chip layout

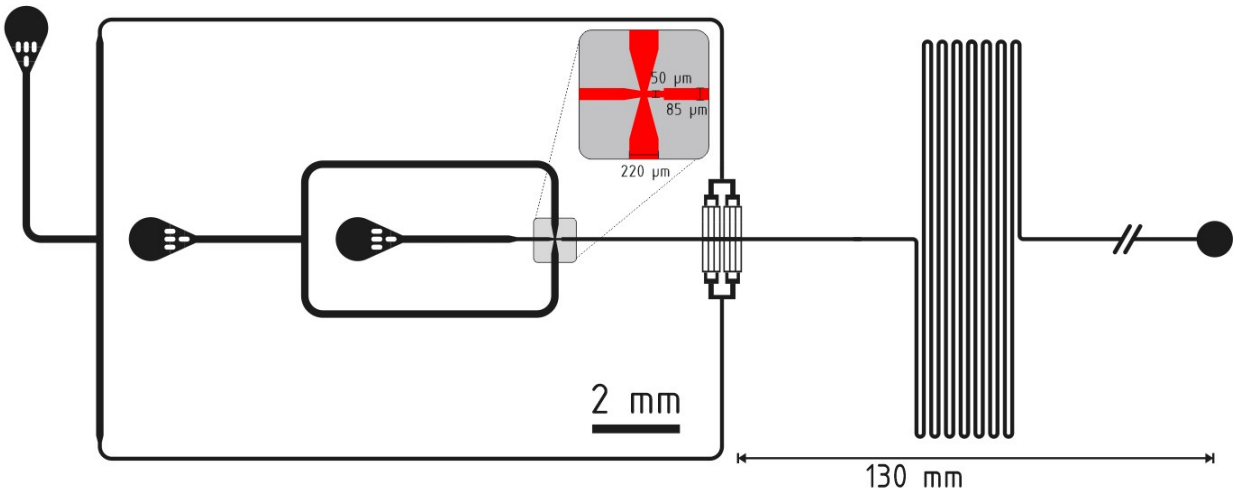

Fig. SI 4: The layout of the 1st generation chip with a combined gelation and extraction part

References

1. Zavadova Z, Zavada J. Carbonic anhydrase IX (CA IX) mediates tumor cell interactions with microenvironment. Oncology reports. 2005 May 1;13(5):977-82.
2. Ivanov SV, Kuzmin I, Wei MH, Pack S, Geil L, Johnson BE, Stanbridge EJ, Lerman MI. Down-regulation of transmembrane carbonic anhydrases in renal cell carcinoma cell lines by wild-type von Hippel-Lindau transgenes. Proceedings of the National Academy of Sciences. 1998 Oct 13;95(21):12596-601.