

# SINGLE CELL ELISA

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## ABSTRACT

We present a microfluidic device that allows parallel single cell trapping and isolation in microchambers, repeated treatment and washing steps, subsequent lysis and analysis by enzyme linked immunosorbent assays (ELISA).

## KEYWORDS

Single cell analysis, ELISA, chemical analysis, intracellular, cell lysis, cell trapping

## INTRODUCTION

Cell-to-cell differences play a key role in the ability of cell populations to adapt and evolve under varying conditions, and are considered to impact on the development of several diseases [1,2]. New microfluidic-based analytical methods developed recently have allowed studying the response of living cells to variations in chemical, physical or mechanical parameters [3-5]. However, the quantitative chemical analysis of single cell lysates remained difficult. Here, we combine a microfluidic device for single cell studies with the analytical power of enzyme linked immunosorbent assays (ELISA) to reliably quantify intracellular proteins and second messengers.

## EXPERIMENT

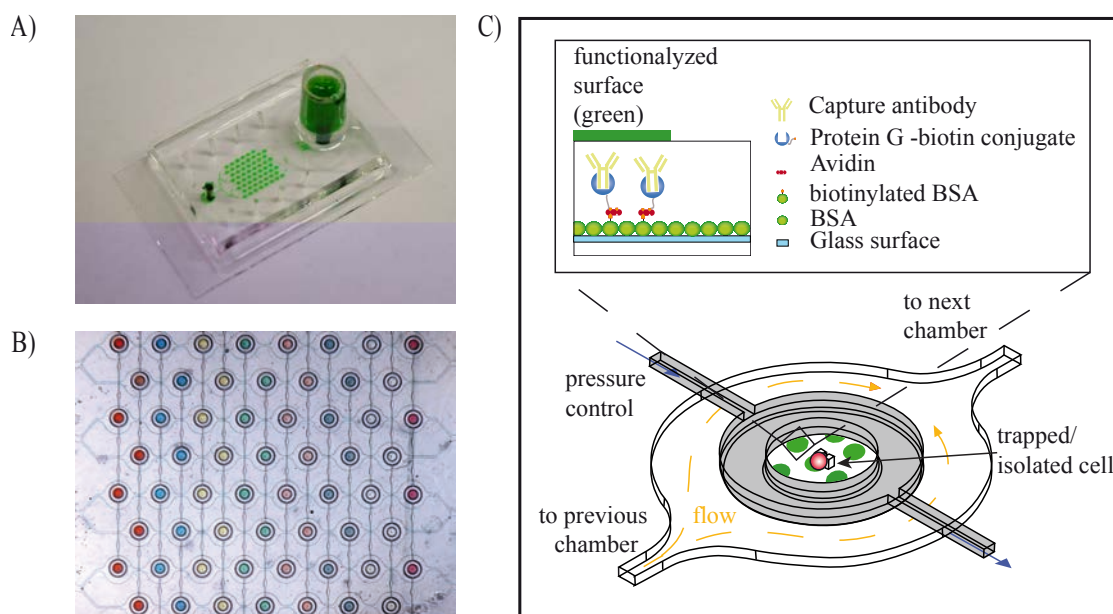
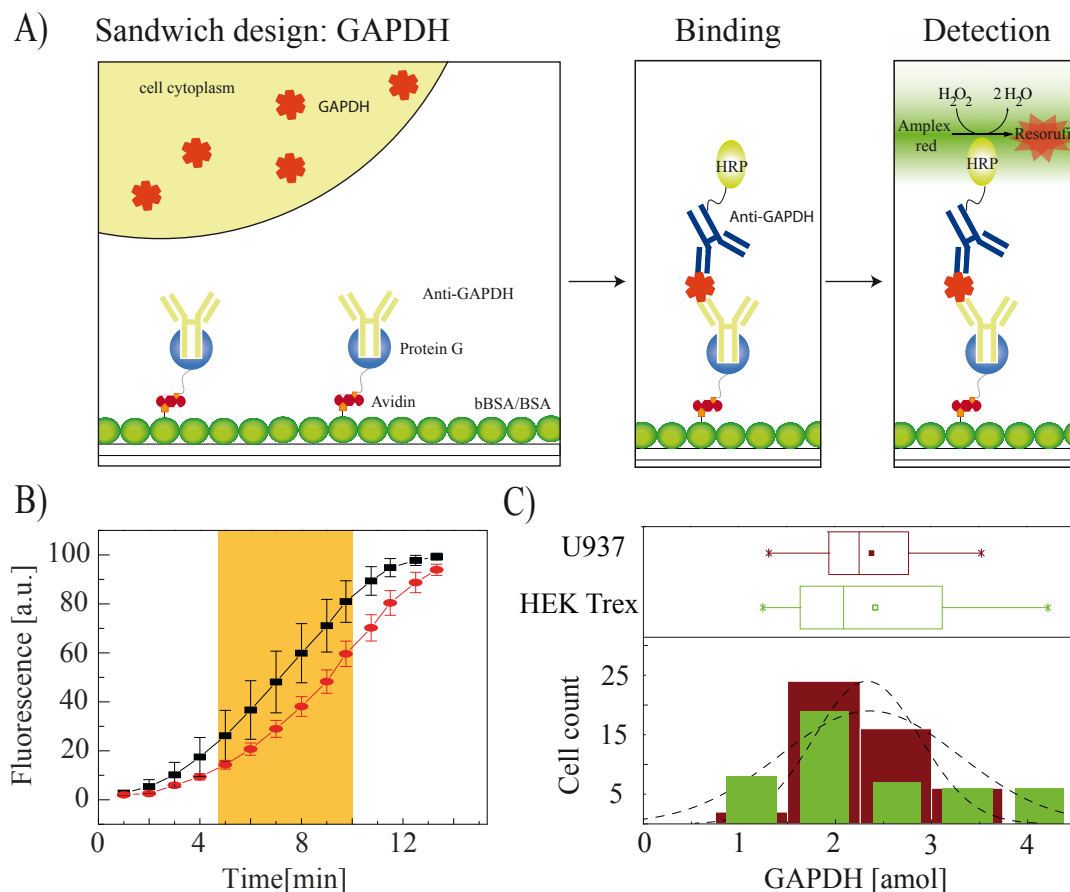


Fig. 1: A) Photograph of the device, showing the fluid reservoir for the application of different reagents. Colored with green food dye. B) Picture of the array. In total, the device consists of 60 traps and chambers. As visible by the differently trapped food colors, the chambers of one row can be actuated differently, therefore avoiding cross-contamination. C) Schematic of a microchamber. Fluid flow, here from left to right, is used to introduce and trap a cell inside the microhurdle and to deliver compounds to the cell. The microhurdle can be isolated from the environment by hydraulic actuation of the microchamber (grey color). Upon pressurizing the control channels, a thin PDMS membrane with the doughnut-like design is pressed onto the bottom of the channel. The glass surface of the PDMS chip is prepared by microcontact printing (green area) to bind capture antibodies for the immobilization of the targeted antigen as depicted in the inset.

The two-layer microfluidic device consists of 60 circular microchambers (volume 625 pL), each featuring a central cell trap (Fig. 1). Within a few hundred milliseconds, the content of the microchamber can be fully exchanged, while the cells remain trapped. In their closed state, the microchambers fully isolate the cells from surrounding solution [4]. Prior to assembling the chip, the bottom glass slide of the device is patterned by microcontact printing with binding spots as required for the ELISA (see scheme in Fig. 1C). In the experimental procedure, adherent or suspension cells are immobilized at the traps and the microchambers are closed afterwards to wash out the residual cell suspension. Next, lysis buffer is introduced and the chambers are shortly opened for 500 ms and

closed again. The trapped cells are lysed within seconds, and the released target molecules bind to the antibodies on the surface. After 15 min incubation time, the chamber is flushed with buffer to remove residual cell lysate and the enzyme (horse-radish peroxidase)-linked detection agent is added to the bound antigen. Due to the consecutive opening and closing of the microchambers, cross contamination is successfully avoided. Next, the substrate (Amplex Red) is introduced, and the emergence of the fluorescent product (resorufin) is detected in the closed microchamber by fluorescence microscopy (Fig. 2A).



*Figure 2. Direct ELISA for GAPDH. A) Schematic drawing of the assay. Antibodies against GAPDH are immobilized on the surface. Cells with intracellular GAPDH are lysed and GAPDH is detected afterwards using a secondary antibody tagged with horseradish peroxidase (HRP), which converts non-fluorescent Amplex red into fluorescent resorufin. B) Typical measured curves for no cell (red) and one cell (black). The slopes of the curves in the orange indicated time are analyzed, which correspond to the concentration of captured GAPDH. C) The histograms and the box plots show the concentration distribution of GAPDH in U937 (red) and HEK cells (green).*

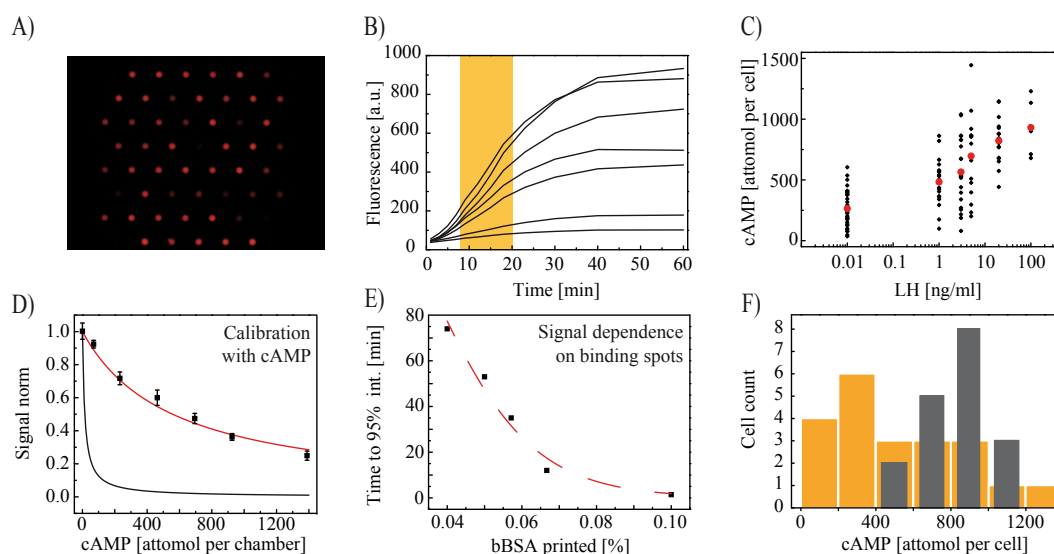
## RESULTS

The performance of the device is demonstrated in a number of studies on adherent and suspension cells (i) by an immunoassay directly imaging the green fluorescent protein (GFP) expressed in human embryonic kidney (HEK 293) cells (data not shown here). This assay was performed to confirm that intracellular expressed proteins bind to the surface via antibodies.

Furthermore, a direct sandwich ELISA analyzing the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in histiocytic lymphoma (U937) cells was performed (Fig. 2). In this assay, the antibody against GAPDH was immobilized on the surface; the cells were lysed to release the enzyme GAPDH (here the antigen). The antigen was detected afterwards via a secondary antibody labeled with an enzyme (Fig. 2A). In this assay, the detection limit is currently as low as 1.29 amol GAPDH (calibration curve not shown here). As visible in figure 2C, the expression of the enzyme varies only within a small range from cell to cell, which is expected due to the essential function of the enzyme in the sugar metabolism.

As a third assay, we performed a competitive ELISA format for the determination of the second messenger cyclic adenosine monophosphate (cAMP) in MLTC cells. In this experiment, we analyzed the cellular response to the luteinizing hormone in murine Leydig tumor cells (MLTC)(Fig. 3). Our results prove that the device enables the determination of cell-to-cell variations in the cAMP levels of cells stimulated with the hormone and non-stimulated

cells. Further experiments involved the addition of 3-isobutyl-1-methylxanthine (IBMX) that inhibits the conversion of cAMP to AMP, resulting in an elevated intracellular cAMP level (Fig. 3F).



**Figure 3. Competitive ELISA to determine cAMP amounts in single cells.** A) Fluorescent micrograph after running the full procedure. During an experiment, micrographs of the microchamber are taken in a constant time sequence. B) Data analysis. Curves are generated by analyzing the micrographs from A, and the linear part is fitted. C) Stimulation curve for MLTC cells and different LH concentrations ( $n=37$  for 0.01 n/ml,  $n=21$  for 1 ng/ml,  $n=22$  for 3 ng/ml,  $n=15$  for 5 ng/ml,  $n=18$  for 20 ng/ml and  $n=5$  for 100ng/ml). Determined EC 50: 3.2 ng/ml. Red dots indicate mean values. D) Calibration curve. Correlation between cAMP content of the chamber and fitted linear slope. Curves show theoretical calculations for the binding events when assuming equal ligand concentrations and binding strengths (black) and an adapted situation (red). E) Dependence of time to 95% signal intensity versus binding sites. F) Results from stimulated cells (20 ng/ml LH) with (grey) and without (orange) 0.5mM IBMX.

## CONCLUSION

Due to the great specificity of ELISA and the large number of antibodies available, we believe that our device provides a new, powerful means for single cell proteomics and metabolomics.

## ACKNOWLEDGMENTS

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