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Poster

# PROXIMITY LIGATION ASSAY FOR HIGH CONTENT PROFILING OF CELL SIGNALING PATHWAYS ON A MICROFLUIDIC CHIP

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## Summary
We present the full integration of the **proximity ligation assay** (PLA) on a microfluidic chip for **systematic cell signaling** studies. The PLA is an *in situ* immuno-DNA based protein analytical technology [1]. Advances of the PLA, in particular a high sensitivity through transfer of the readout signal from protein to DNA level has been inaccessible for **large-scale approaches.** This is mainly due to long cell sample preparation times and high material costs. Performance advances on our **integrated polydimethylsiloxane (PDMS)** chip are achieved, including **full automation** of the assay, temporal and chemical control over 128 cell cultures with time resolution of tens of seconds, protein target multiplexing, and reduction of antibody consumptions by two orders of magnitudes compared to the standard bench procedure. We demonstrate the use of the combined cell-culture and **protein analytical** chip by characterizing the kinetics of a signal transduction event within the Akt pathway upon PDGF stimulation.

## Introduction
Signal transduction from the extracellular microenvironment to the inner compartments of the cell combines protein interactions, post-translational modifications, and translocation. The PLA exhibits the same analytical workflow for detecting *in situ* all different protein events occurring during a signal transduction [1]. The simplest PLA setup is shown in figure 1A. In short the principle of the PLA combines two antibodies labeled with different oligonucleotide strands. Upon binding in close proximity of both antibodies the oligonucleotide sequences can be complemented, ligated, amplified by rolling circle amplification (RCA) and detected with a fluorescence probe. Integration and multiplexing of the PLA provides cell signaling research a new analytical tool for systems biology approaches.

## Methods and Experimental Results
The large-scale fluidic design [2] of the PDMS chip is shown in figure 1B. Cell culture chambers are arranged in a matrix configuration. Figure 1C illustrates the addressability of the single cultures on chip. With the 2 dimensional approach of the matrix it is possible to multiplex two assay parameters as for example the time intervals after chemical stimulation in the row and different primary antibodies for the PLA in the column direction [3]. The working principle of the chip is demonstrated by stimulating NIH 3T3 fibroblast cells with the growth factor PDGF in 15 time intervals between 2 to 120 minutes. Example images of cells stained with the PLA probes on chip are shown figure 2. Time traces of 6 phosphorylation sites obtained after cell stimulation with PDGF are given in figure 3. Phosphorylation dynamics of Akt, GSK3ß, p706SK, Erk and S6 ribosomal protein are in agreement with literature reports [4] but the time resolution obtained here is higher than in any other published Western blot or immunofluorescence experiment.

## Conclusion
The presented integrated chip combines a high sensitive protein analytical assay with a microfluidic cell-culturing platform. Temporal control over the 128 cell cultures and their microenvironments has been resolved successfully during signal transduction within the Akt pathway. The PLA chip

technology will allow in future application high content analysis of protein interactions, modifications and translocations in signaling events on systems level.

**Word Count: 498**

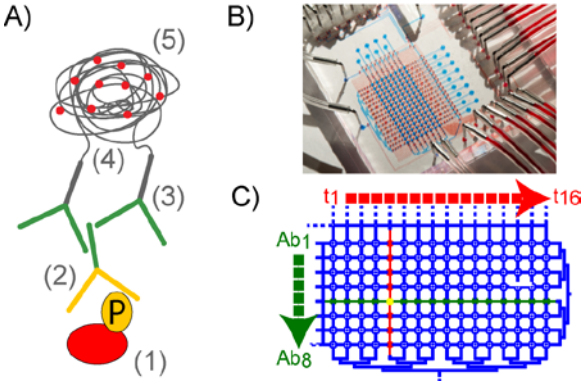


*Figure 1. A) Working principle of the PLA. Primary (2) and secondary antibody pairs (3) bind to target proteins or phosphorylation sites (1). Oligonucleotide labels on the secondary antibodies can hybridize if both are in close proximity. With help of a DNA probe a close DNA circle is formed (4). The DNA circle is amplified by rolling circle amplification and stained with a fluorescence dye. B) Large-scale microfluidic chip platform with integrated cell culturing systems and PLA. C) 2 dimensional flow logics allow to multiplex two assay parameters.*

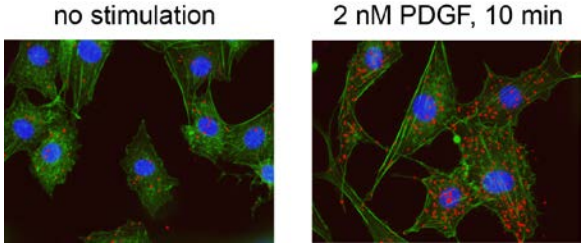


*Figure 2. Example images of biological cells from either a control or 2nM PDGF stimulated cell culture on chip. Cells were processed with the PLA program and an anti-Akt antibody (S473). Positive PLA events indicating the phosphorylated Akt protein result in localized DNA polymers with a hydrodynamic diameter in the range of 1 µm, which can be detected with a complementary DNA probe (red dots).*

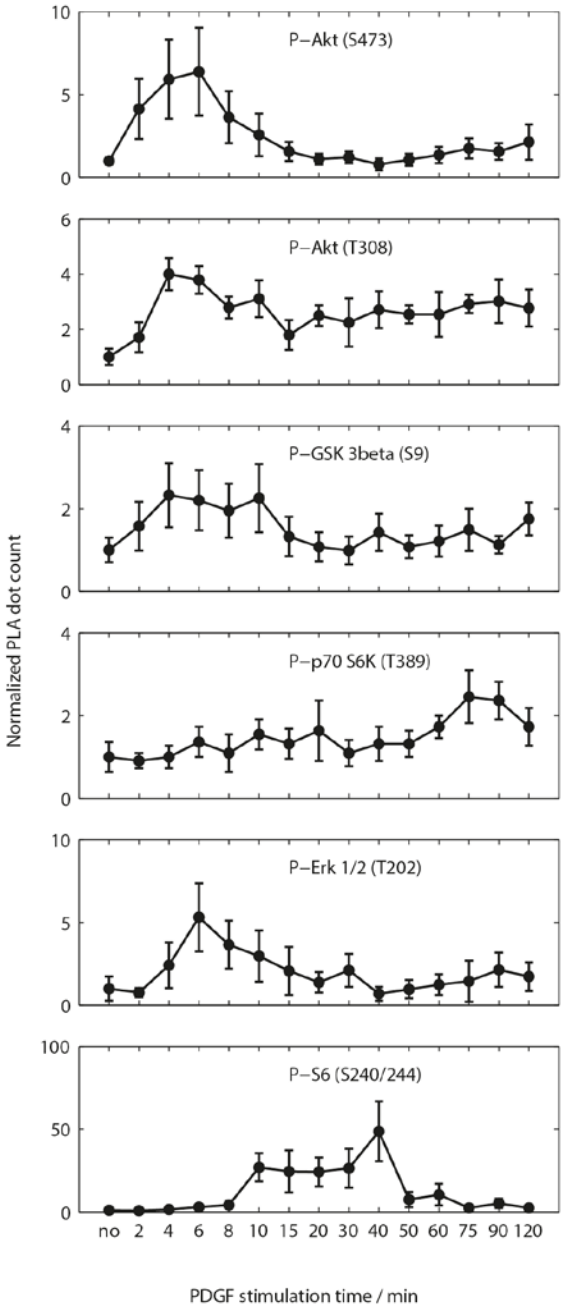


*Figure 3. Time traces of 6 phosphorylation sites activated during PDGF signal transduction detected with the on chip PLA and specific antibodies.*

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