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# Integration of single cell traps, chemical gradient generator and photosensors in a microfluidic platform for the study of alpha-synuclein toxicity in yeast

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**Abstract**

Alpha-synuclein (aSyn) is a key player in Parkinson's disease. Genetically engineered yeast cells producing aSyn fused with GFP (aSyn-GFP) have been used to study this protein. In this work, we present a microfluidic platform with integrated photosensors that captures single yeast cells in arrays of hydrodynamic traps and exposes them to a chemical gradient of precise composition. This platform enables the study of the effects of aSyn expression level and aggregation in genetically modified yeast cells by chemical stimulation. The photosensors allow the detection of cells in the traps by measuring the variations in light transmission or of the fluorescence produced by aSyn-GFP for real-time signal acquisition.


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*Keywords:* cell chip; microfluidics; gradient generator; cell trapping; yeast; alpha-synuclein; Parkinson's disease; photodiodes

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## 1. Introduction

Alpha-synuclein (aSyn) is the main component of Lewy bodies, protein aggregates that are the hallmark of Parkinson's disease (PD). Although aSyn has an important role in PD, the mechanisms behind its aggregation and toxicity are not yet well understood and yeast cell models have been used to study the biological effects of normal and misfolded aSyn [1]. *S. cerevisiae* (Sc) was chosen since it is a well known organism, and it is easily manipulated into producing and correctly folding human proteins. To study the effects of aSyn expression and aggregation, Sc cells were genetically engineered to express aSyn fused with GFP (aSyn-GFP) under the control of a galactose-induced promotor [1].

Classical cell culture offers tools that study yeast cell populations by exposing them to chemical stimuli, however it is almost impossible to achieve single-cell resolution, real time monitoring and dynamic microenvironment control in these classical systems. Microfluidic technology, which has been widely used for biological applications [2], can potentially address these challenges through the use of single-cell capturing techniques [3] and gradient generators [4]. The combination of these two strategies allows the precise control of a cell's microenvironment and the tracking of its behaviour.

## 2. Device description

### *2.1. Microfluidic device*

To be able to track hundreds of single cells and study their behaviour when exposed to chemical stimuli, we developed a PDMS-based microfluidic device that combines a chemical gradient generator (Fig. 1) **[4]** with hydrodynamic single cell traps (Fig. 2). The chemical gradient generator is composed of a network of microfluidic channels that allow the diffusive mixing of three initial solutions with different chemical compositions. This network is coupled to 9 chambers, each of which have an array of hundreds of hydrodynamic traps with dimensions designed to hold Sc cells. These traps are distributed along the streamlines of cells in suspension inserted into the device, and are able to capture individual cells. The cell traps are semi-circular structures with a gap in the middle: fluid is able to flow through the trap when it is empty, but the gap becomes blocked when the trap is occupied, rerouting incoming cells to other traps.

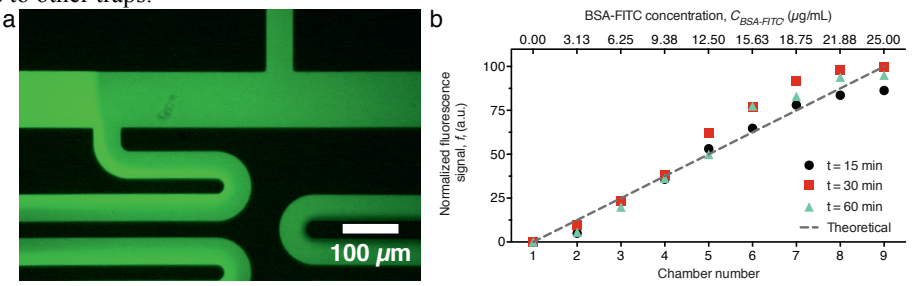


Fig. 1. (a) Gradient generator. Fluorescence micrograph of two fluids with different concentrations of FITC splitting and mixing by diffusion at the start of a new level of the network.; (b) Linearity and stability of the chemical gradient generator. Normalized fluorescence signals of BSA-FITC gradients generated by the microfluidic device with three initial solutions with concentrations of 0, 12.5 and 25 μg.mL$^{-1}$.

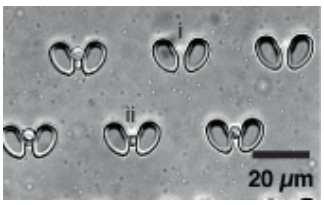


Fig. 2. Hydrodynamic cell traps. Bright field optical microscopy image of hydrodynamic traps in a chessboard configuration. The traps have a circular cell receptacle with 8 μm in diameter and a 4 μm gap. They can be empty (i) or occupied with cells (ii).