# Fast, sensitive bioassays with microfluidics preconcentration of samples

Researchers in Prof. Juan Santiago's laboratory have developed a technique to rapidly preconcentrate and capture biological targets with high specificity and efficiency. The process can be used to reduce reaction times for microarray analyses and affinity chromatography. The protocol includes isotachophoresis (ITP) to selectively focus target molecules as well as other strategies to achieve high efficiency capture of target. Specifically, the liquid phase is quickly displaced using gas flow to preserve the high capture rate achieved during a brief ITP reaction. The process increases sensitivity of assays and reduces the total ligand needed for detection and purification, thereby decreasing the cost of the assay. This microfluidics invention could be coupled with a variety of assays for rapidly purifying, detecting and analyzing proteins or nucleic acids for SNP analyses, biomarker discovery, or clinical diagnostics.



**ITP-Affinity Chromatography schematic and results.** (a) Illustration of assay for ITP-aided affinity chromatography. The target sample is loaded into the TE reservoir of the column and ITP focuses the target before it migrates into the affinity region to be captured by the probe and then eluted. (b) Experimentally measured target concentration showing dynamics of a typical ITP-AC binding

#### experiment.

#### Stage of Research

The inventors have demonstrated ITP preconcentration with affinity chromatography by purifying a 25nt target DNA from 10,000-fold higher abundance background in less than 10 minutes. In an ITP-microarray experiment, the inventors showed 8.2fold higher signal in a 30 minute assay (compared to conventional overnight hybridization) with rapid and quantitative detection over 4 orders of magnitude dynamic range.

### Applications

- **Microfluidics** protein or nucleic acid detection, purification and analysis with assays such as:
  - microarrays
  - affinity chromatography

#### Advantages

- **Fast** overcomes slow reaction kinetics for microarray assays or affinity purification in minutes (100 -1000x faster than conventional techniques)
- **Sensitive** concentrating the target molecules improves sensitivity 10 fold without increasing non-specific signal
- **Reduced cost** concentrated sample requires 100 1000x less ligand than standard affinity purification, thereby reducing reagent costs
- High dynamic range demonstrated targeted concentration over 4 orders of magnitude (1 pg/μL through 100 pg/μL)
- Multiplexed analysis

### **Publications**

 Viktor Shkolnikov and Juan G. Santiago, <u>"Coupling Isotachophoresis with Affinity</u> <u>Chromatography for Rapid and Selective Purification with High Column</u> <u>Utilization, Part 1: Theory,</u> *Analytical Chemistry*.

- Viktor Shkolnikov and Juan G. Santiago, <u>"Coupling Isotachophoresis with Affinity</u> <u>Chromatography for Rapid and Selective Purification with High Column</u> Utilization, Part 2: Experimental Study," *Analytical Chemistry*.
- Crystal M. Han, Evaldas Katiliusb and Juan G. Santiago, <u>"Increasing</u> <u>hybridization rate and sensitivity of DNA microarrays using isotachophoresis."</u> *Royal Society of Chemistry.*

#### Patents

- Published Application: 20160177382
- Published Application: 20170306394
- Issued: <u>9,574,232 (USA)</u>
- Issued: <u>10,392,653 (USA)</u>

#### Innovators

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