

Microfluidic Chip Analysis of Protein Complexes

Commercial instrumentation based on microfluidic chip technology developed at Oak Ridge National Laboratory (ORNL) (Fig. 1) is currently being used for high-throughput analysis of protein complexes for the ORNL Genomes-to-Life project. The proteins are fluorescently labeled on-chip and electrophoretically separated for detection by laser-induced fluorescence (LIF). Comparisons with known marker proteins allow estimates of the size (kDaltons) and concentration of the complex subunits. These assays are used to determine the quality and yield of isolated microbial complexes prior to their digestion and peptide analysis by mass spectrometry and provide quantitative data for the intact proteins.

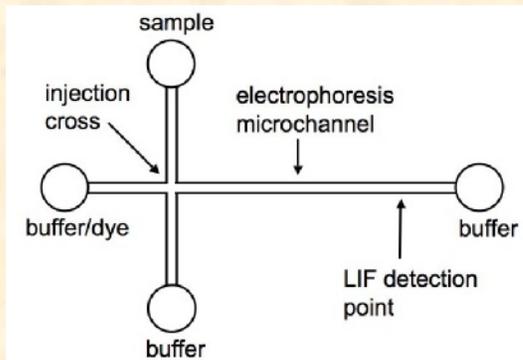


Fig. 1. Basic design of microfluidic chip for electrophoretic analysis. Enclosed microchannels in glass or polymer substrates are connected to fluid reservoirs (circles) containing sample, buffers and dye. Sample staining, injection and electrophoresis steps are performed by applying appropriate voltages to each solution.

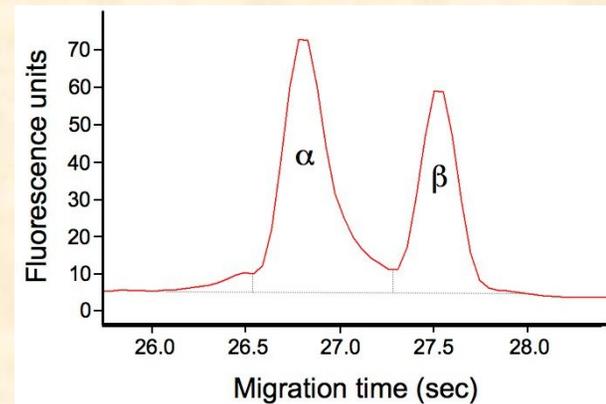


Fig. 2. Microfluidic chip analysis of nitrogenase protein complex from *Rhodopseudomonas palustris*, showing separation of the putative α (55 kDa) and β (58 kDa) subunits.

- **>400 microbial protein complex preparations from *Rhodopseudomonas palustris* and *Shewanella oneidensis* have been analyzed on microfluidic chips.**
- **Microfluidic assay data has been correlated with mass spectrometry data for ~200 samples.**
- **Quantitative data from microfluidic assays can be used to distinguish potential complex proteins from minor contaminants that are non-quantitatively detected by mass spectrometry .**
- **The microfluidic quantitation data can also allow predictions of possible subunit stoichiometries in the complex.**