

## Expanding the toolbox for *in vivo* monitoring

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### Introduction

The information gleaned and experiments that can be performed using microdialysis sampling experiments are limited by the spatial and temporal resolution obtainable. Resolution in turn is limited by the analytical methods coupled to the probes, the fluidics of the system, and the size of the sampling probe itself. We have been investigating methods of improving all aspects of *in vivo* monitoring sampling and analysis systems for a variety of neurotransmitters and metabolites. This talk will survey these recent improvements and describe applications of the more mature technologies.

To improve temporal resolution for monitoring low molecular weight, relatively abundant amine transmitters such as glutamate and dopamine, coupling of microdialysis and capillary electrophoresis has proven useful. This method has allowed 10-90 s temporal resolution for multiple neurotransmitters simultaneously [1,2]. The improvement in temporal resolution was made possible by the extremely high sensitivity. This temporal resolution has proven useful for tracking relatively fast concentration changes that occur during behavior and electrical stimulation.

Further improvements in temporal resolution have been limited by the dispersion of analyte zones moving from the sampling system to the fraction collector or analytical system. We have explored the concept of partitioning dialysate fractions as aqueous plugs in an immiscible (oil) carrier phase at the point of sampling to prevent analyte dispersion during transport [3].

Improvements in temporal resolution of neuropeptide monitoring have been more challenging. Neuropeptides are typically present at lower concentrations making it difficult to achieve resolution better than 30 min. We have explored the use of capillary liquid chromatography coupled to mass spectrometry (cLC-MS) for determination of peptides in dialysate [4]. In recent work we have shown detection of several endogenous opioid peptides using this method. Initial studies suggest that temporal resolution of 1-5 min is possible. This method has been used to examine the effect of drugs of abuse on enkephalin levels in the brain.

Spatial resolution of the dialysis sampling method precludes use in many smaller brain nuclei in rats and all but the largest brain regions in mice. Decreasing the size of the sampling probes requires high sensitivity methods and new approaches to sample handling. Low flow push-pull sampling is a recently

developed method that offers high spatial resolution, but generates sample streams in the nanoliter/min range. We have developed systems for automated push-pull sampling and analysis that appear effective at making measurements from 100 to 500-fold smaller areas than typical dialysis probes.

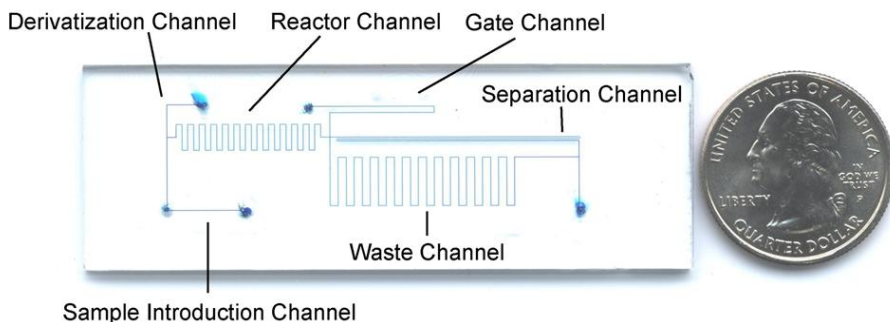
## Methods

*Microfabricated analytical systems.* Microfluidic analytical devices were fabricated using a previously described methods.[5] Briefly, Borofloat glass coated with chrome and positive photoresist were used as photomask blanks. A photomask was placed on top of the photomask blanks and exposed to collimated UV light. The exposed blanks were developed for 30 s photoresist developer followed by 30 s in chrome etchant. The exposed glass was etched in HF solution for ~40 min resulting in an etch depth of 50  $\mu\text{m}$ . The etched glass and was sealed with another glass slide in a furnace. For fluidic reservoirs, NanoPorts from Upchurch Scientific were attached to the device as per manufacturer's instructions. Detection was accomplished using a epifluorescence system.

*Capillary LC-MS for neuropeptide measurements.* Capillary chromatography columns with integrated electrospray emitters were prepared as previously described [4]. Briefly, 500  $\mu\text{m}$  long frits were formed within 20 cm lengths of 25 to 75  $\mu\text{m}$  i.d. x 360  $\mu\text{m}$  o.d. fused silica capillary. A 5.0 – 5.5 cm length of the fritted capillary was slurry-packed with HPLC particles. The outlet of the capillary was then pulled to a fine tip on a pipette puller so that the i.d. at the tip was ~3  $\mu\text{m}$ . The chromatography column was coupled to a mass spectrometer (LCQ Deca, Thermo Electron Corporation) using electrospray ionization (ESI). The emitter tip was positioned ~0.5 mm from the heated capillary of the mass spectrometer using a micromanipulator operated in x, y, and z directions.

## Results and Discussion

*Amine Neurotransmitters.* The figure below illustrates a microfluidic “chip” that has been fabricated in our laboratory for coupling to microdialysis. Dialysate is pulled into the chip, derivatized, and then separated by electrophoresis. Separations can be performed at 10-90 s intervals depending on the analytes of interest.



These chips, and similar devices, have been used to monitor amino acid transmitters during fear conditioning, during electrical stimulation for examining regulation of glutamate in Huntington's disease, and for monitoring dopamine regulation by leptin. These examples serve to show their potential utility for in vivo monitoring. Preliminary experiments have shown that segmented flows on such chips can be used to improve temporal resolution[3]. Preliminary experiments have also shown that similar chips with built in pumping systems may be coupled to push-pull sampling for improved spatial resolution.

Ease of use of these methods remains a significant concern. One route to dissemination of this technology is through an NIH resource center. As a research project of the Center for Neural Communication Technology, we are refining these microfluidic devices to the point that they will be easily transferrable among labs making use of these approaches more available.

*Neuropeptides.* We have developed cLC-MS methods to measure met-enkephalin, leu-enkephalin, dynorphin A, and  $\beta$ -endorphin in dialysate. The method has been used to determine the dose dependence of amphetamine and nicotine effects on enkephalins in the globus pallidus illustrating the utility in pharmacological studies. Preliminary data suggests that the method can be extended to neurotensin and oxytocin. Other peptides remain to be tested. These methods may also be extended to identification of novel peptides in the brain.

## References

1. Parrot S, Sauvinet V, Riban V, Depaulis A, Renaud B, Denoroy L (2004) High temporal resolution for in vivo monitoring of neurotransmitters in awake epileptic rats using brain microdialysis and capillary electrophoresis with laser-induced fluorescence detection. *J Neurosci Methods* **140**:29-38.
2. Shou M, Robinson TE, Ferraro CM, Kennedy RT (2006) In vivo measurement of dopamine by microdialysis with on-line capillary electrophoresis. *Anal Chem* **78**:6717-6725.
3. Wang M, Roman GT, Schultz KN, Jennings C, Kennedy RT (2008) Improving temporal resolution in microdialysis sampling using segmented flow. *Anal Chem* **80**: in press.
4. Haskins WE, Wang ZQ, Watson CJ, Rostand RR, Witowski SR, Powell DH, Kennedy RT (2001) Capillary LC-MS2 at the attomole level for monitoring and discovering endogenous peptides in microdialysis samples collected in vivo. *Anal Chem* **73**: 5005-5014.
5. Sandlin ZD, Shou MS, Shackman JG, Kennedy RT (2005) Microfluidic electrophoresis chip coupled to microdialysis for in vivo monitoring of amino acid neurotransmitters. *Anal Chem* **77**:7702-7708.