

A Novel Approach for Multiplex Imaging of Protein Abundances in Tissues and Organs: Initial Application to the Mouse Brain

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Modeling and predicting the behavior of biological systems requires consideration of not only temporal changes in biomolecule abundance levels, but also consideration of their spatial distribution. One of the most evident examples of such a biological system is the mammalian brain, where 3D relationships between cells and molecular networks must be accounted for to understand how it functions. Before mathematical models can be developed and refined to accurately describe how the networks in the brain function, experimental approaches are needed that can provide comprehensive information in terms of dynamic changes in abundances and spatial distributions of biomolecules such as mRNA, proteins, and metabolites. Although routine analytical approaches exist (e.g., histochemical and confocal microscopy) for a single type of biomolecule, multiplexed characterization at the genome level is a daunting task. In particular, effective genome-scale measurements of protein spatial distributions are challenged by the availability of antibodies and automation of high-throughput immunohistochemical processes, among other technological challenges.

We have developed an alternative antibody-free approach based on voxelation integrated with automated microscale sample processing and high-throughput reversed phase liquid chromatography coupled on-line with high-resolution mass spectrometry (RPLC-MS), using either Fourier transform ion cyclotron resonance or LTQ-Orbitrap instruments [1]. We initially applied this approach to spatially map protein abundances in a mouse brain. The brain was dissected into ~700 1-mm³ cubes called voxels and then extracted proteins were tryptically digested into peptides prior to RPLC-MS analysis. Confident peptide identifications were obtained by matching observed RPLC-MS accurate mass and elution time features to mass and elution time information contained in a reference database composed of confidently identified peptides previously determined from multiple LC-MS/MS experiments in which peptide amino acid sequences were identified by their fragmentation patterns (**Fig. 1**). The false discovery rate of peptide identification was estimated to be below 5%. In turn, the confidently identified peptides allowed for reconstruction of both the protein content and protein abundances for each voxel sample.

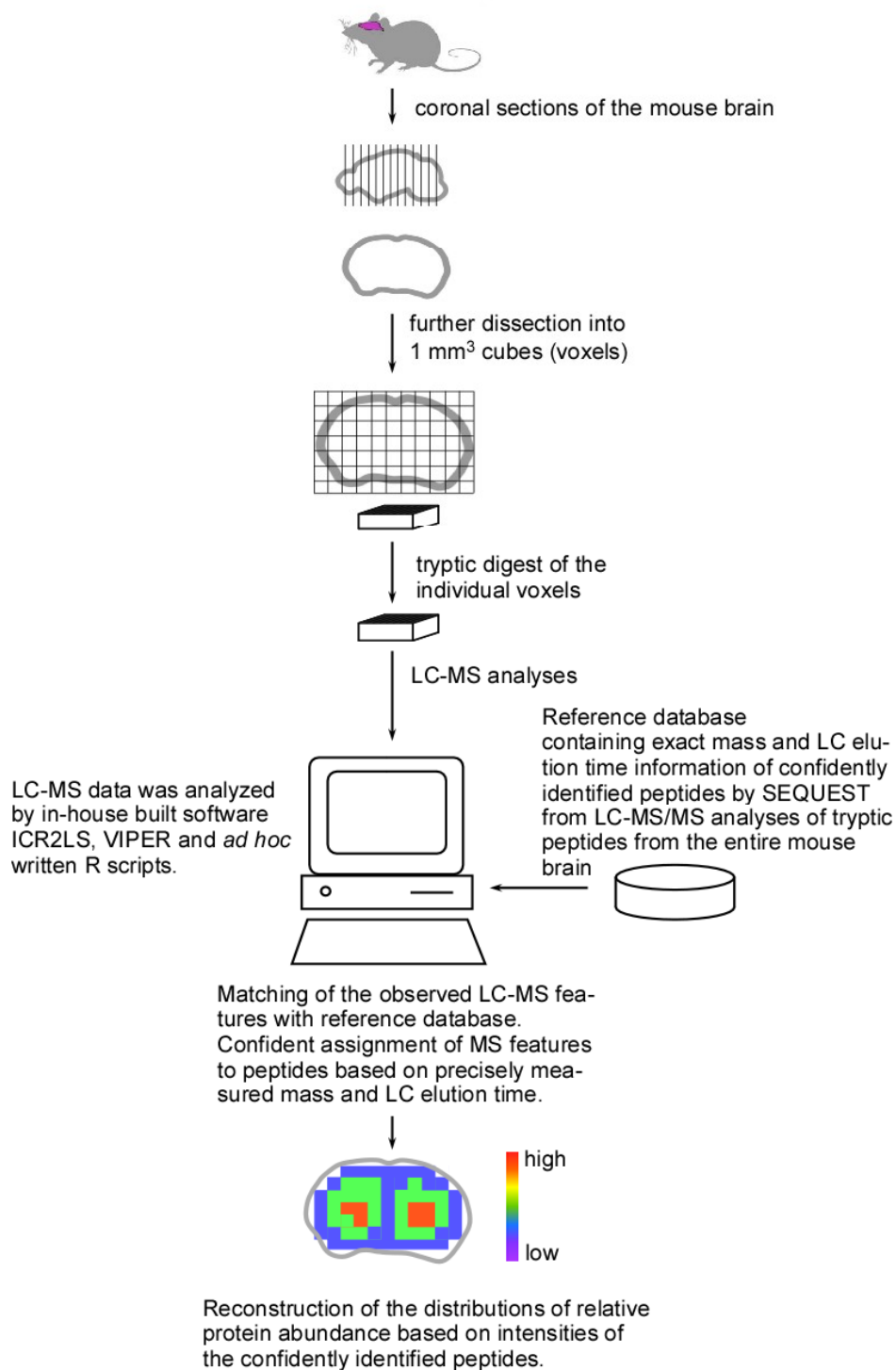


Figure 1. Strategy for spatial mapping of protein abundance patterns in the mouse brain. The brain is dissected into voxels (1 mm³ cubes) followed by tryptic digestion of each voxel sample. Observed LC-MS features are matched against a reference database that contains theoretical masses and observed elution times of peptides previously identified from LC-MS/MS analyses.

By effectively multiplexing peptide detection with RPLC-MS, up to a 1000 proteins were observed in a single sample analysis. 3D protein abundance patterns were constructed by arranging the voxels according to their spatial coordinates (**Fig. 2**), which enabled determination of protein distributions for >1000 proteins from a normal mouse brain. The 1-mm spatial resolution proved sufficient for distinguishing major structural components of the brain, i.e., cerebral cortex, striatum, diencephalon, white matter, cerebellum, and olfactory bulb. Overall we found significant agreement of our results with corresponding mRNA and protein abundance patterns available in public databases and the literature. This concordance supports the validity of the described methodology, opening new opportunities for obtaining experimental data needed to model the spatially complex molecular networks of the brain or other organs.

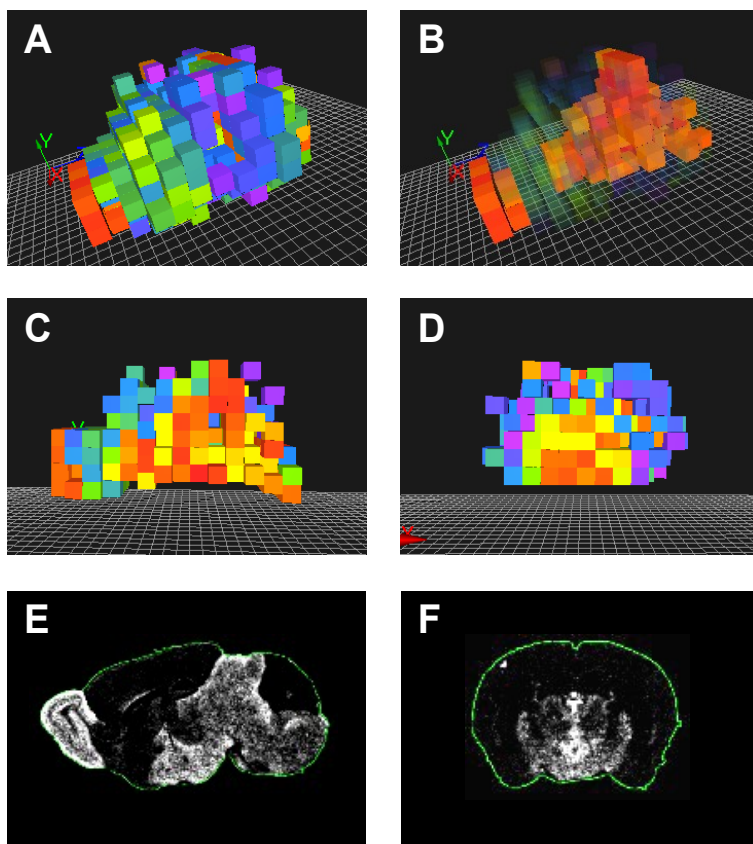


Figure 2. An example of spatial distribution for GABA transporter 4 protein (*Slc6a11* gene) depicted as **A**) general 3D view, **B**) view with decreased transparency for voxels with lower protein abundances, **C**) sagittal section view, **D**) coronal section view. The protein distribution agrees well with mRNA abundance distribution retrieved from Allen Brain Atlas (www.brain-map.org) shown as **E**) sagittal and **F**) coronal sections views.

1. Petyuk, V. A., Qian, W. J., Chin, M. H., Wang, H., Livesay, E. A., Monroe, M. E., Adkins, J. N., Jaitly, N., Anderson, D. J., Camp, D. G., 2nd, Smith, D. J., and Smith, R. D. (2007) *Genome Res* 17(3), 328-336