Molecular Dynamics at the Synapse in Response to Seizure

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Using multidimensional LC and TiO$_2$ enrichment, we identify:

- 20,000 non-redundant peptide spectra
- 2,000 unique proteins
- 3,000 unique phosphopeptides
- 850 phosphorylated proteins

On average, at least one site of phosphorylation was found on $\approx 30\%$ of identified proteins.
iTRAQ-type experiments that rely on MS/MS for quantification have limitations.

Phosphopeptides quantified in six replicates

6 of 6 = 34
5 of 6 = 198
4 of 6 = 278
3 of 6 = 457
2 of 6 = 604
1 of 6 = 1157
Total = 2728

To consistently profile phosphopeptides, we developed 3000 selective reaction monitoring assays.
Using actual MS/MS spectra as a guide for choosing transitions, five SRMs for each peptide were validated.

Experimental Quantification Phase

Retention time guided scheduled SRMs are conducted on a QTRAP 5500.

We chose to monitor three SRMs per peptide. This allows us to quantify over 800 peptides per run.

A single sample can be profiled in six hours.
The phosphopeptide HASEPQPGPR from DLAP3 has a greater than three fold increase in response to seizure.

Using an established model of epilepsy, we analyzed the temporal dynamics of proteins and phosphorylation sites.

Seizures were induced by IP injection of the muscarinic acetylcholine receptor agonist pilocarpine.

Proper electrical homeostasis in the CNS is maintained by a balance of excitatory and inhibitor activity.
Protein functional classes display distinct post-seizure dynamics

Hierarchical clustering can be used to group proteins that show similar activity-dependent dynamics.
The degree of correlated protein regulation can be used to construct “co-regulation” maps. In over 50 instances, phosphorylation sites displayed greater than a two-fold change in response to seizure.
Changes in phosphorylation stoichiometry mirror changes in protein localization

What have we learned from analyzing mouse models of seizure?

Synaptic machinery can be divided into functional units as determined by coordinated responses to neuronal stimulation.

Protein-level changes were on the order of 10-15%.

Changes in phosphorylation levels were much more pronounced, with a subset of phosphorylation sites displaying greater than a two-fold change.
Implications and Future Directions

Phosphorylation changes often occur in the same direction as protein levels, with phosphorylation changes being several fold higher in magnitude.

This subset of phosphorylation sites may be involved in protein-protein interactions and hence control protein synaptic localization.

Experiments with finer temporal resolution will shed light on these issues.

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