# Phosphoproteome of synaptoneurosomes from cocaine treated rats

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## Introduction

- The aim of this work was to find an optimized method for phosphopeptide enrichment from synaptoneurosomes in studies of drugs of abuse.
- Synaptoneurosomes are closed, post-synaptic compart-ments that form as the membrane reseals following homogenization of brain tissue.
- In addition to offering con-siderably reduced sample complexity, which in itself is crucial in neuroproteomics, synaptoneurosomes also consti-tute a metabolically active compartment that can respond to external stimuli. As such, synaptoneurosome fractions themselves can serve as "control" versus "experimental" samples, and potentially provide valuable information on the proteins involved in signalling cascades in neurons.
- In this study, commercially available ($TiO_2$, $ZrO_2$, and IMAC materials) as well as metal oxides that have not been reported previously used for phospho-peptide enrichment ($Fe_2TiO_5$, $FeTiO_3$) were tested for their potential in enrichment of phosphoproteins or phospho-peptides from synapto-neurosome samples.

## References

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## Materials and Methods

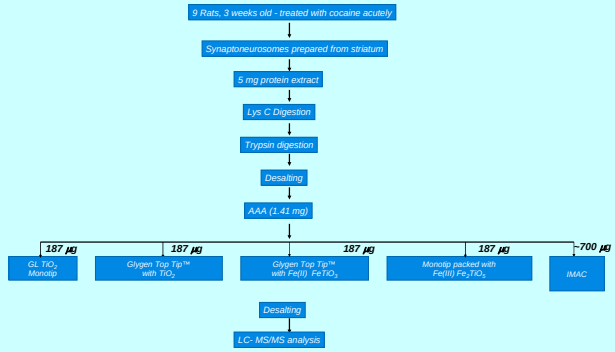


**Figure 1.** Phosphopeptide enrichment by $TiO_2$, metal oxides or IMAC. Following an initial comparison of a series of $TiO_2$, metal oxide and IMAC materials using α- and β-casein digests as sample, 5 methods were selected for further comparison of their potential for phosphopeptide enrichment from synaptoneurosome protein digest.

## Results

1. Low complexity samples:

- Analysis by LC-MS/MS (Q-TOF) showed that all of the resins tested provided a quick and robust method for enriching phosphopeptides from samples of low/moderate complexity (mix of α- and β-casein digests).
- The materials giving the best relative enrichment in our initial test were $TiO_2$ Monotip (GL Sciences), $TiO_2$ TopTip (Glygen), $FeTiO_3$ (Alfa Aesar) loaded in an empty TopTip, and $TiO_2$ Monotip with $Fe_2TiO_5$ (Alfa Aesar) loaded on top of the $TiO_2$ monolith crystal provided with these tips.

2. High complexity samples:

- The four top-performing materials from the initial comparison were selected for further testing with a synaptoneurosomal protein digest. Samples were split in four (187 µg each), and phosphopeptides were enriched using one of the four metal oxide materials. Results are summarized in Fig. 2.

***Phosphopeptide enrichment comparison by metal oxides***

***Scale-up, cellular proteins***

| Phosphopeptide Enrichment Method | Number of ID'd Phosphopeptides (>90% conf) | Number of all ID'd peptides (>90% conf) | % ratio of Phosphopeptides to all peptides (>90% conf) | Number of ID'd Proteins (>90% conf) | Number of ID'd Phosphoproteins (>90% conf) |
|---|---|---|---|---|---|
| $Fe_2TiO_5$ Fe(III) | - | - | - | - | - |
| $FeTiO_3$ Fe(II) | - | 13 | - | 9 | - |
| GL $TiO_2$ monotip | 4 | 108 | 4% | 52 | 3 |
| Glygen $TiO_2$ TopTip | 33 | 50 | 66% | 35 | 23 |

**Figure 2.** Performance comparison of methods for phosphopeptide enrichment from synaptoneurosome samples (rat striatum) by the four top performing resins from our initial tests.

## Results (cont.)

- The results showed that with the synaptoneurosomal protein digest, the $TiO_2$ affinity materials generally outperformed the other metal oxide materials.
- An IMAC phosphopeptide enrichment strategy was also compared against the best performing metal oxide resin (Glygen TopTip $TiO_2$) using ~700 ug of synaptoneurosomal proteins as starting sample. Despite the larger amount of starting material, phosphopeptide enrichment using IMAC resulted in very similar number of phosphopeptides than with Glygen TopTip $TiO_2$.
- We next performed a comparison of two groups of rats, one of which received an acute cocaine injection, the other received saline vehicle. Following phosphopeptide enrichment using IMAC or $TiO_2$, peptides were labeled with iTRAQ reagents and quantified by LC-MS/MS (QStar):
- 35 Phosphopeptides Were Identified (MASCOT >90% confidence)
- 102 Proteins Identified (MASCOT Score>90% confidence)
- 37 Phosphoproteins Identified (MASCOT>90% protein confidence with any phosphopeptide), 21 of which had phosphopeptide scores>90% confidence

***Regulation of protein phosphorylation following cocaine treatment***

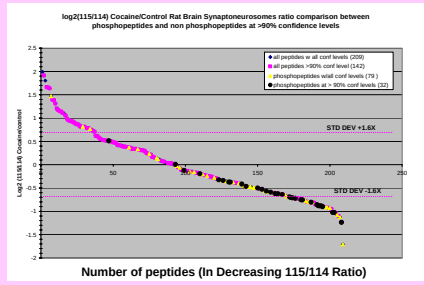


**Figure 3.** iTRAQ quantification of synaptoneurosome phosphopeptides enriched by IMAC. Digests were prepared according to the scheme in Fig. 1, from striatum of rats that had been given an injection of cocaine or saline vehicle.

## Concluding remarks

- Our results demonstrate that synaptoneurosomes are a valuable tool for studying cellular signaling cascades and actions of drugs of abuse by phospho-proteomics. To increase the number of identified phosphopeptides after enrichment and iTRAQ labeling, starting amounts of proteins should be scaled up from the experiments presented here.
- $TiO_2$ (with almost 4 times less material) and IMAC yielded a similar number of phosphopeptide identifications; however, IMAC enriched phosphopeptides represented only 10% of all detected peptides compared to 66% detected by TiO2 enrichment.