Proteomic Assays of Neuronal Protein Palmitoylation

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Fatty acylation of Proteins: A set of protein post-translational modifications of increasing importance

Only cytoplasmic proteins and transmembrane proteins in either their transmembrane or cytoplasmic domains are acylated.

Type I: Static

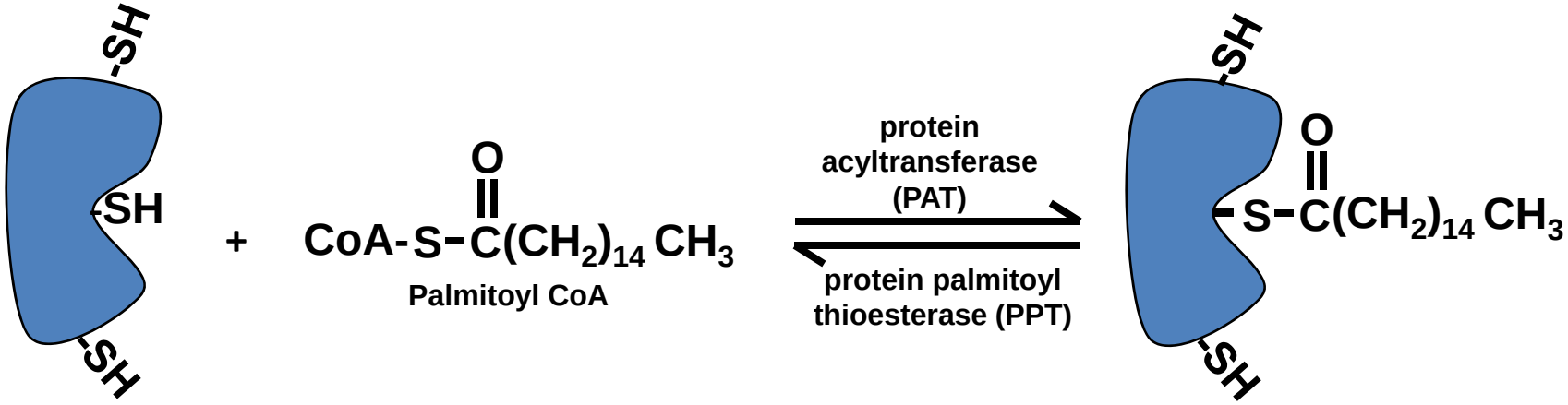
- **irreversible with set consensus amino acid sequences:**
  1. **N-myristoylation (GXXXS motif) at the N-terminus.**
  2. **S-prenylation (CAAX farnesylation, CCXX geranylgeranylation)**

Type II: Dynamic

- **reversible with no consensus sequences:**

S-palmitoylation: Currently exponential growth of studies of protein palmitoylation (e.g. recent science paper on ras trafficking) and new proteins identified as palmitoylated.

Mechanism of Protein Palmitoylation



Types of Palmitoylated Proteins

- **Soluble proteins: occurs at cysteines throughout the protein**

Examples:

1. **N-terminal; PSD-95, GAP43, Fyn**
2. **Internal; SNAP25, synaptotagmin, synaptobrevin**
3. **C-terminal; H-Ras, RhoB, paralemmin**

- **Membrane proteins: occurs at cysteines within transmembrane and cytoplasmic domains**

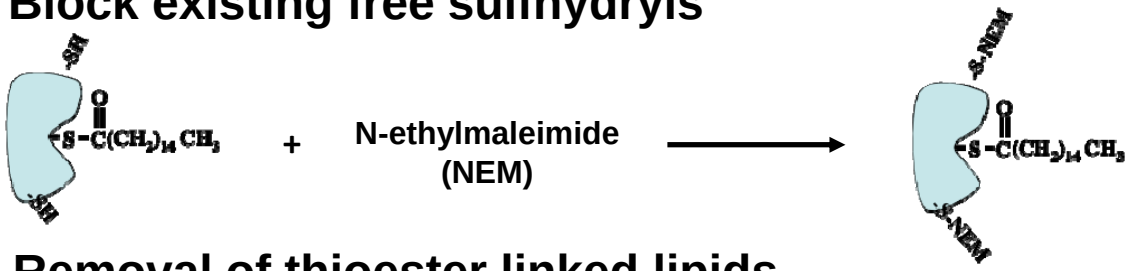
Examples:
Identified sites: HA, rhodopsin, dopamine D1R, GluR6,
Unidentified sites: nAChR subunits, Na channel alpha subunits

The importance of protein palmitoylation in neurons and at synapses

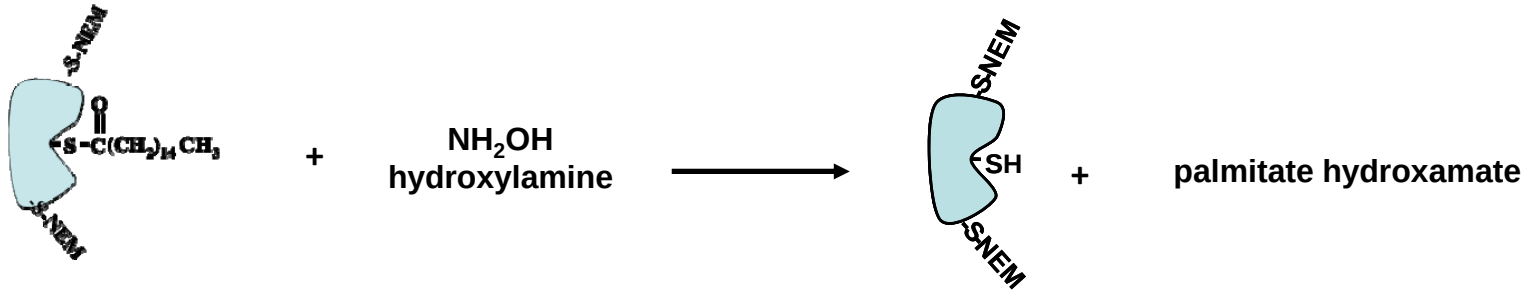
- 1) Activity dependent palmitoylation of PSD-95 at synapses (El-Husseini et al. 2002).
- 2) Trafficking of AMPARs into synapses (Hayashi et al. 2005)
- 3) Assembly of α7 nicotinic receptors (Drisdel et al. 2005).
- 4) Palmitoylation of CDC42 and spine formation (Kang et al. 2008).

New Method for Measuring Protein Palmitoylation (Acyl-Biotin Exchange or ABE)

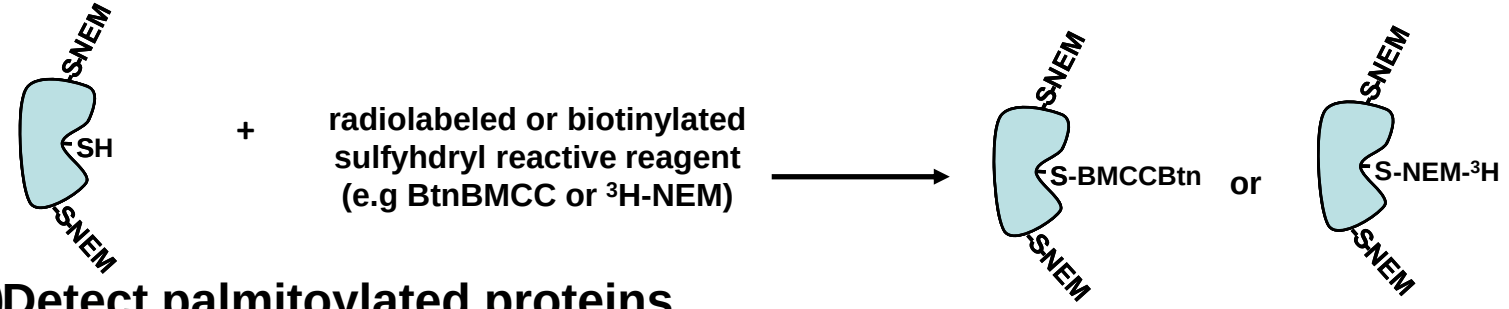
Step 1) Block existing free sulfhydryls



Step 2) Removal of thioester linked lipids



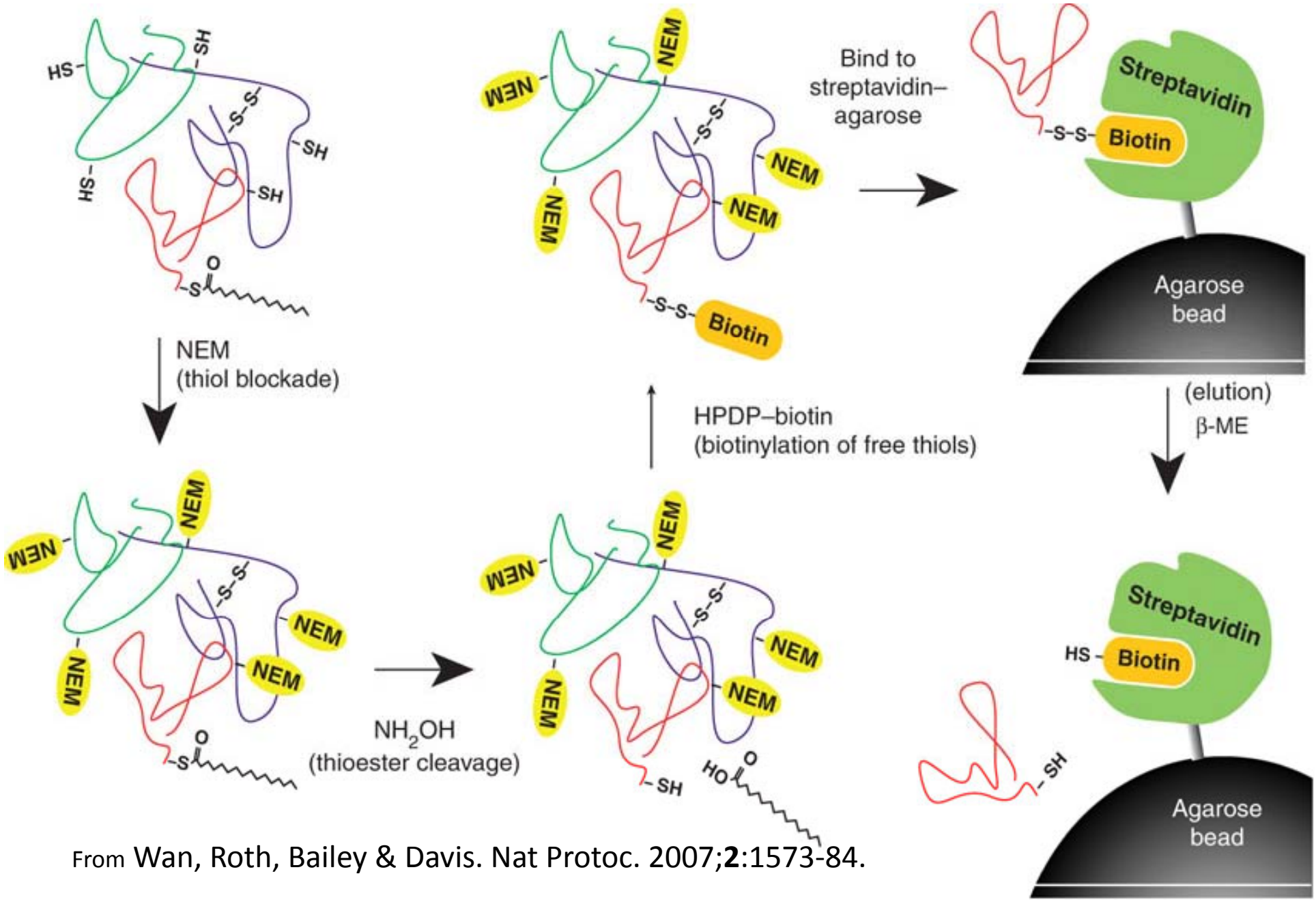
Step 3) Label free sulfhydryls generated by removal of lipid



Step 4) Detect palmitoylated proteins

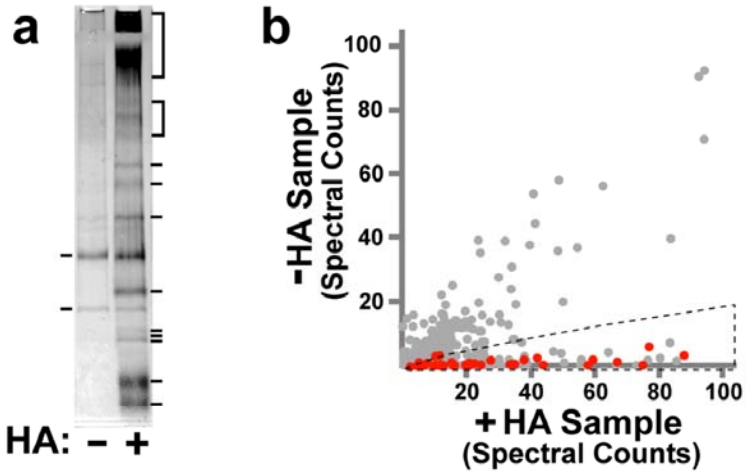
SDS-PAGE → electrophoretic transfer → Chemiluminscence assay
o
Phosphor imager

Modification of ABE for Proteomic Analysis (Nick Davis)



From Wan, Roth, Bailey & Davis. Nat Protoc. 2007;**2**:1573-84.

Neuronal Palmitoyl-Proteomics



Receptors and Channels
- Grin2a(NR2A) • Grin2b(NR2B)
- Scn (Voltage-gated Na channel,typeI, II, III, IX)
- Ttyh1, Ttyh3(tweety Cl channel)
- Ptgfm (Prostaglandin receptor negative regulator)
- Htr3c (ionotropic 5-HT receptor subunit)
- Kcnma1(BK channel)

Scaffolding proteins
- • Akap5(AKAP79/150)
- Dlgap2(PSD95 associated protein)
- • Gphn(Gephyrin)

Membrane trafficking
- Stx•1a, •1b2, • 6, 7, 8, •12, • 19 (Syntaxin)
- Sybl1 (synaptobrevin-like 1)
- Vamp1,4
- Scamp •1,3, •5
- Rab1b, 3, 5, 7,10,14
- Sort1 (Sortilin)
- Rtn1, -3 (Reticulon)

Cell Adhesion
- Mcam(M-CAM)
- Epha5(Eph receptorA5) Epha3(Eph receptorA3)
- Ephb2(Eph receptor B2)Efnb3(Ephrin B3; ligand)
- • Igsf4C (SynCAM4; Necl4)
- Pcdh • 1, 8, 9,10,17 (Protocadherin)
- • Ctnnd2 (δ-catenin-2)
- Cxadr (coxsackie and adenovirus receptor)
- Astn1(Astrontactin 1)
- • Cntn1 (Contactin)
- Jam3 (junctional CAM-3)
- Igsf8 (PGRL; EWI-2)
- Pkp4 (plakophilin4)
- Sema4d (Semaphorin 4D)
- Lphn1 (Latrophilin 1)
- Plxnb2 (Plexin B2)
- Neo1 (Neogenin 1)

Myelin associated
- Mbp (MBP and Golli-MBP)
- Mobp (myelin-associated oligodendrocyte basic protein)
- mog (myelin oligodendrocyte glycoprotein)
- • M6a

Transporters
- Slc 1a1,2,3,4 (• GluT1;Glutamate transporter)
- Slc 6a1,32a1 (vesicular GABA transporters)
- Atp2b1,b3,c1 (Ca transporters)
- Slc8a1,a2 (Na/Ca exchangers)
- Slc3a2 (amino acid transporter)
- Slc4a2 (choline transporter)

GTPases and signaling proteins
- • Cdc42, Rac1
- R-Ras(R-Ras) Rras2 (R-Ras2)
- Rala,Ralb(Ral-A, Ral-B)
- Sept3, -6, -8 (Septins)
- • Gprin1, Gprin3 (Gα-interacting)
- Arhgdia (Rho-GDIα)
- Spred1, Spred2 (Sprouty related)
- * Adcy1,5,6,9 (Adenylyl cyclase)
- Inpp5a (inositol phosphatase)
- Zfyve28 (FYVE PI-binding)
- Pde10a (cAMP/cGMP dual phosphodiesterase)

Cytoskeletal proteins
- Ablim2 (actin-binding LIM protein 2)
- • Dync1i1 (DIC; dynein intermediate chain)

Chaperones
- • Canx (calnexin)
- Ppib (peptidylprolyl isomerase B)

Metabolism
- Zdhhc5 (DHHC palmitoylation enzyme)
- Agpat1 (lipid biosynthesis)
- Capn5 (calpain 5)
- Ggtl3 (gamma-glutamyl transpeptidase)
- Cyb5r3 (Cytochrome B5 reductase)
- Mpst (mercaptopyruvate sulfotransferase)

Mitochondrial
- Cox6c (respiratory chain)
- Vdac1,2,3 (outer membrane porins)
- Aldh6a1

From R. Kang, J. Wan, P. Arstikaitis, H. Takahashi, K. Huang, A. O. Bailey, J. X. Thompson, A. F. Roth, R. C. Drisdel, R. Mastro, W. N. Green, J. R. Yates, N. G. Davis & A. El-Husseini. 2008. Nature (in press).

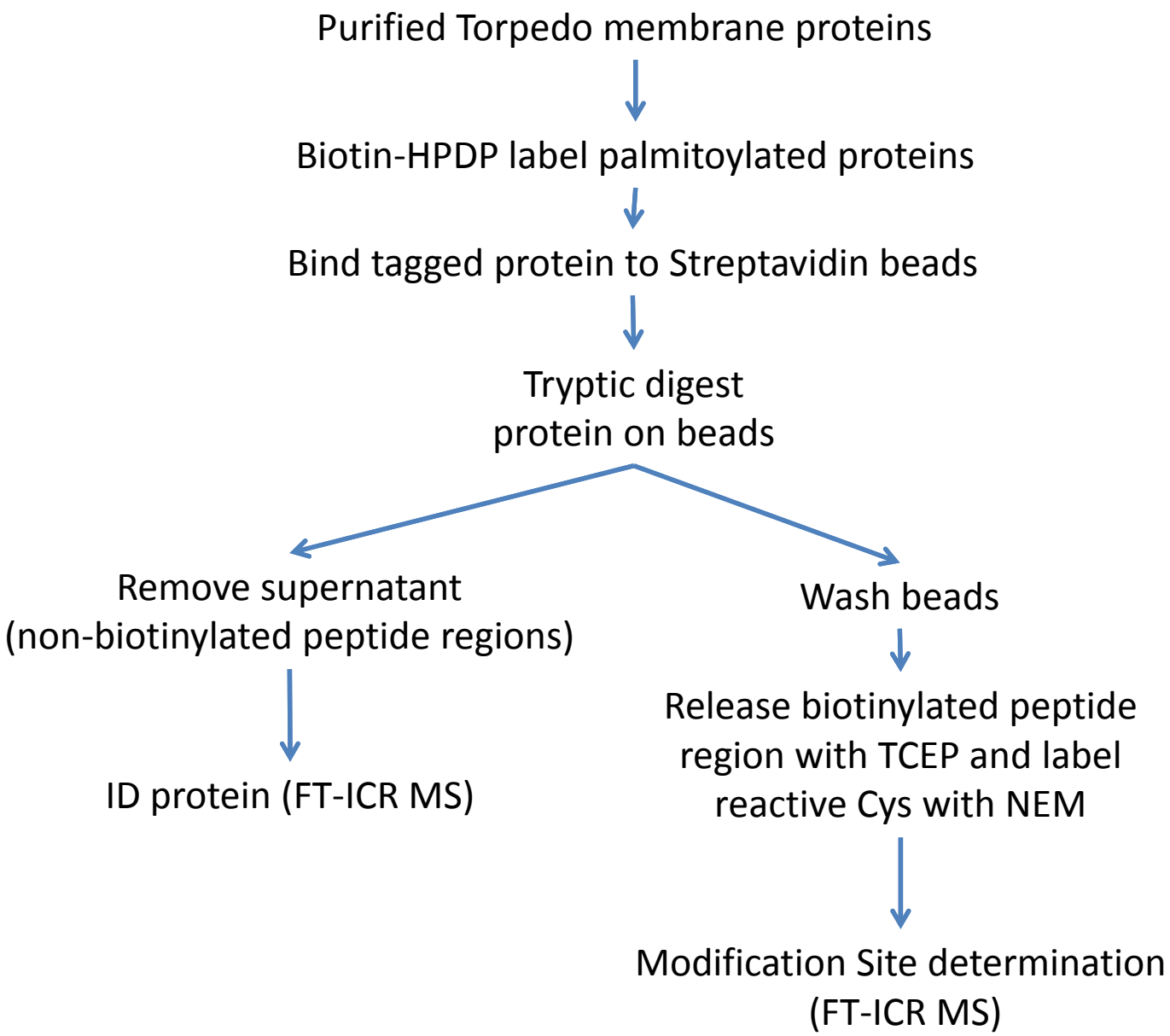
Aim 1: To develop a proteomic approach to identify sites of palmitoylation labeled using the ABE method on purified ionotropic neurotransmitter receptors.

To begin, we will use highly purified nicotinic receptors from *Torpedo californica* electric organ and human $\alpha4\beta2$ receptors expressed in HEK cells. We will also assay SNAP-25, which is a highly palmitoylated membrane-associated protein and not an integral membrane protein like the other two proteins.

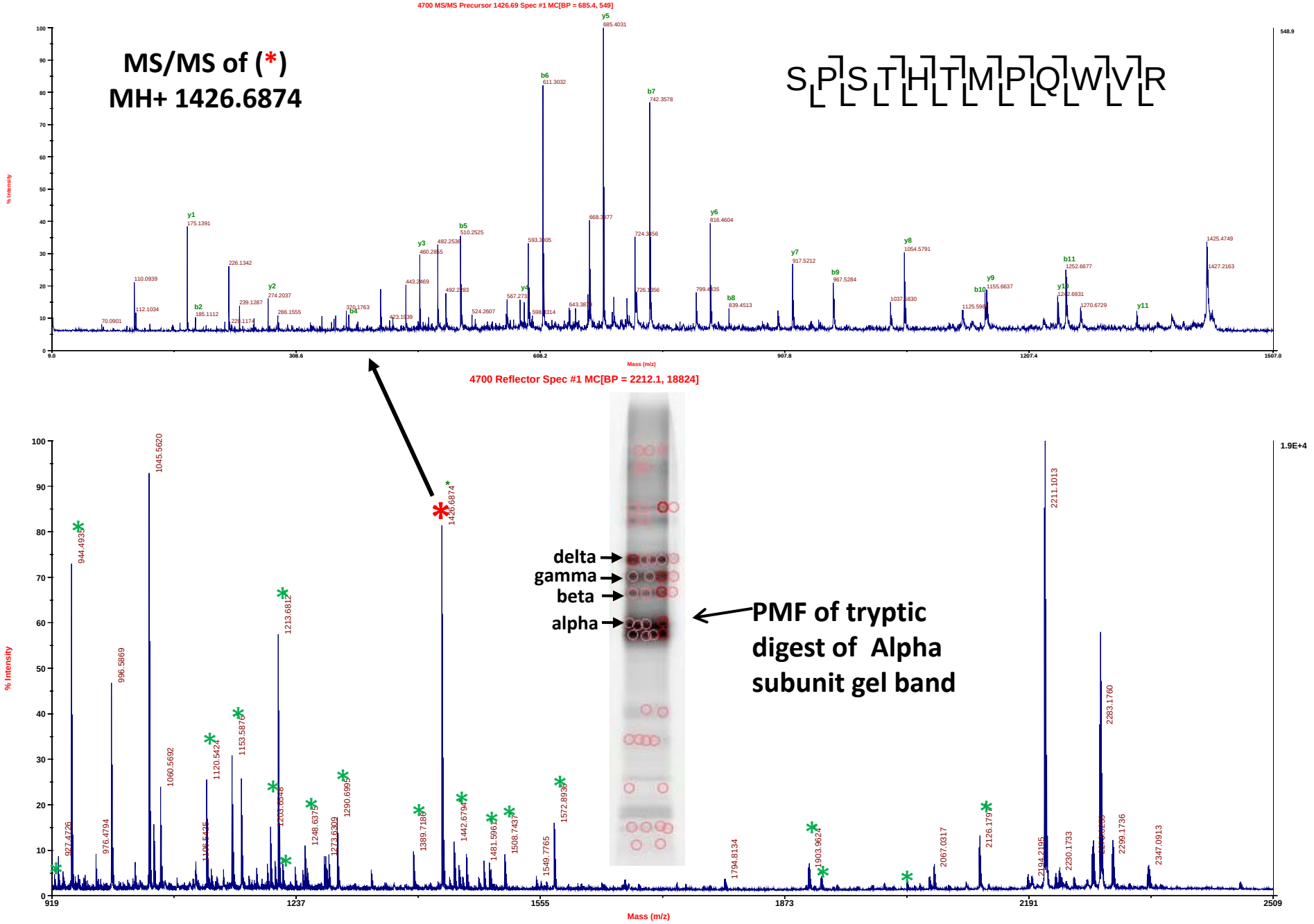
Aim 2: Identify palmitoylated proteins from rat nucleus accumbens and test for changes in their palmitoylation with amphetamine-induced sensitization.

In this aim we will use large-scale screening mass-spec techniques (MudPIT) in order to identify palmitoylated proteins that change during drug-induced sensitization in rats.

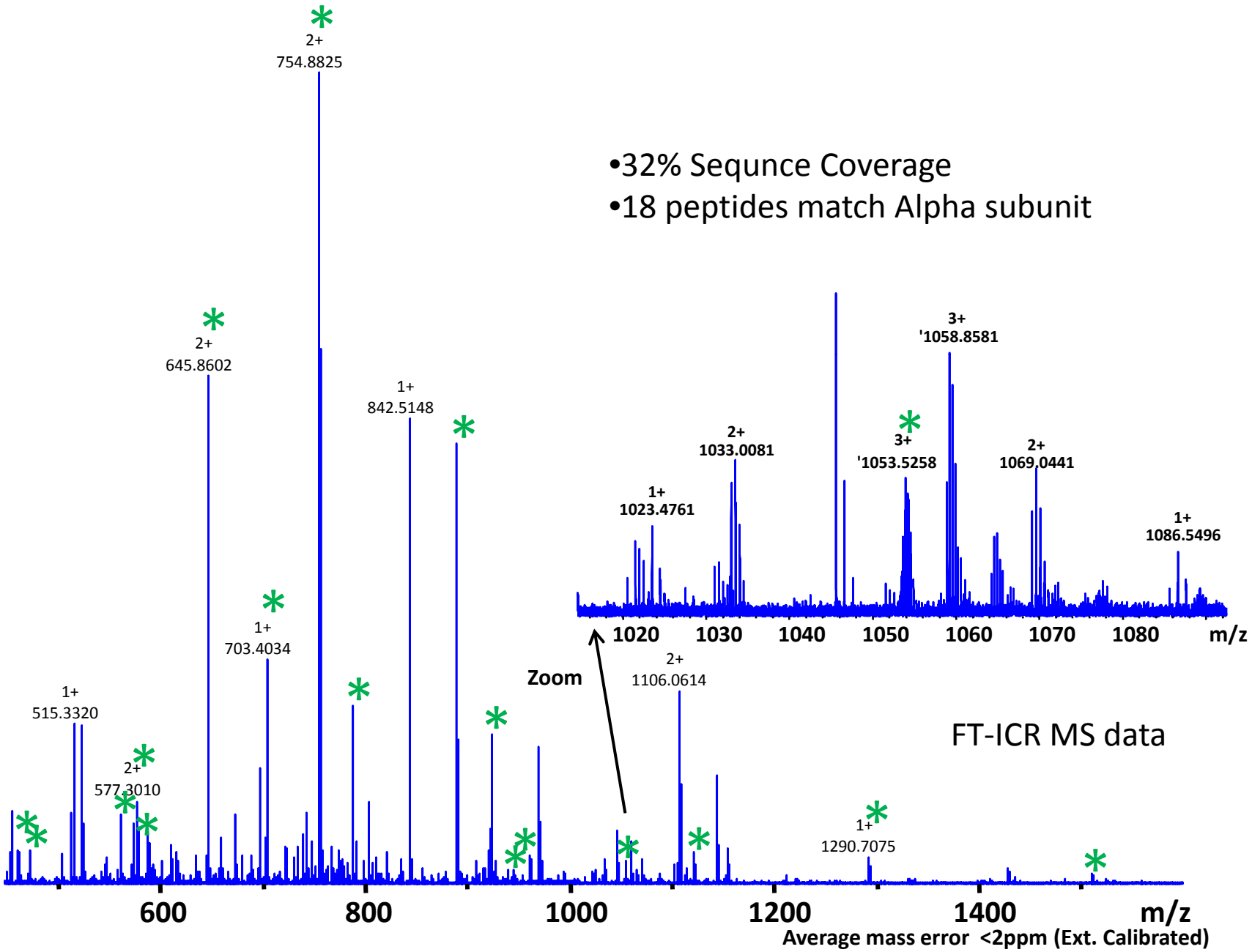
Methodology (Site of Palmitoylation)



Aim 1: 4800 MALDI Tof/Tof Identification of Torpedo protein/subunits



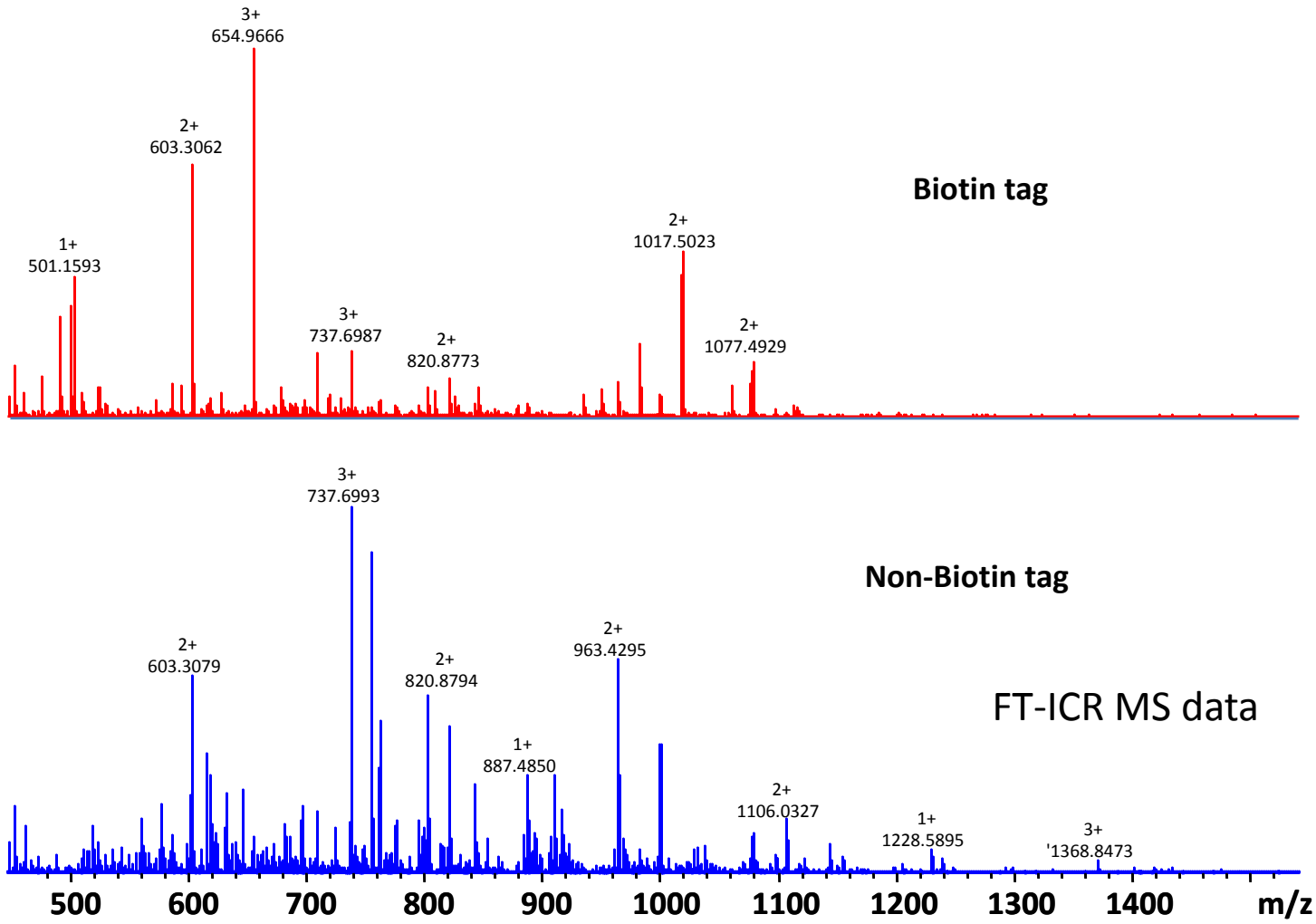
Identification of Acetylcholine receptor subunit Alpha subunit



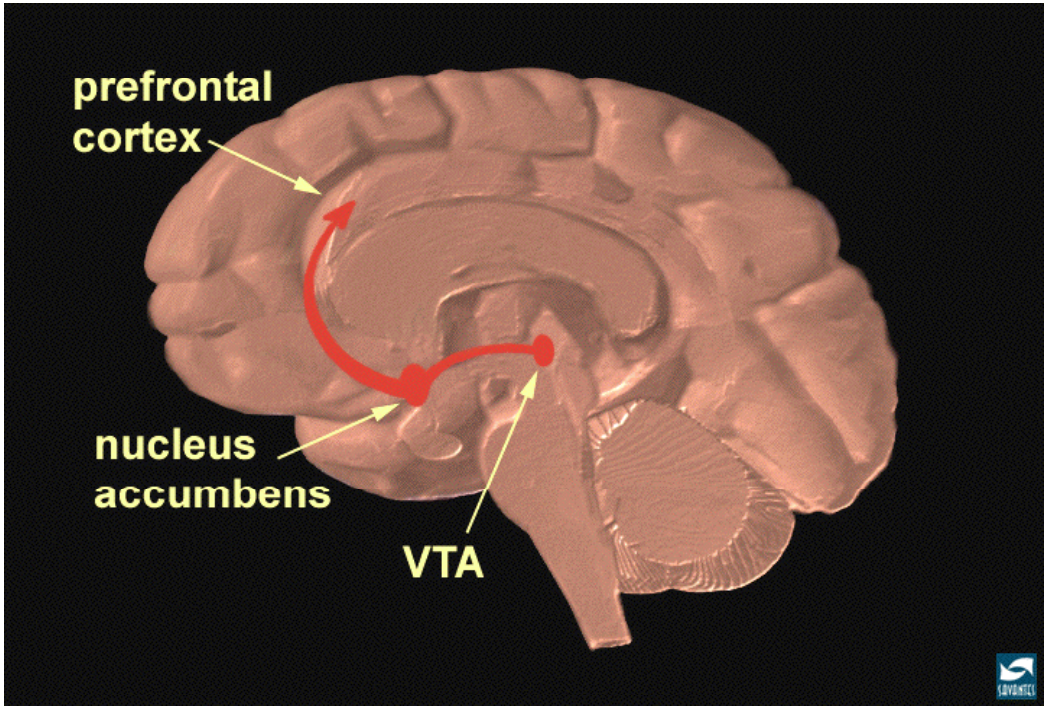
- 32% Sequnce Coverage
- 18 peptides match Alpha subunit

FT-ICR MS data

Spectra peptides from Non-Biotin and Biotin tagged proteins on Streptavidin beads

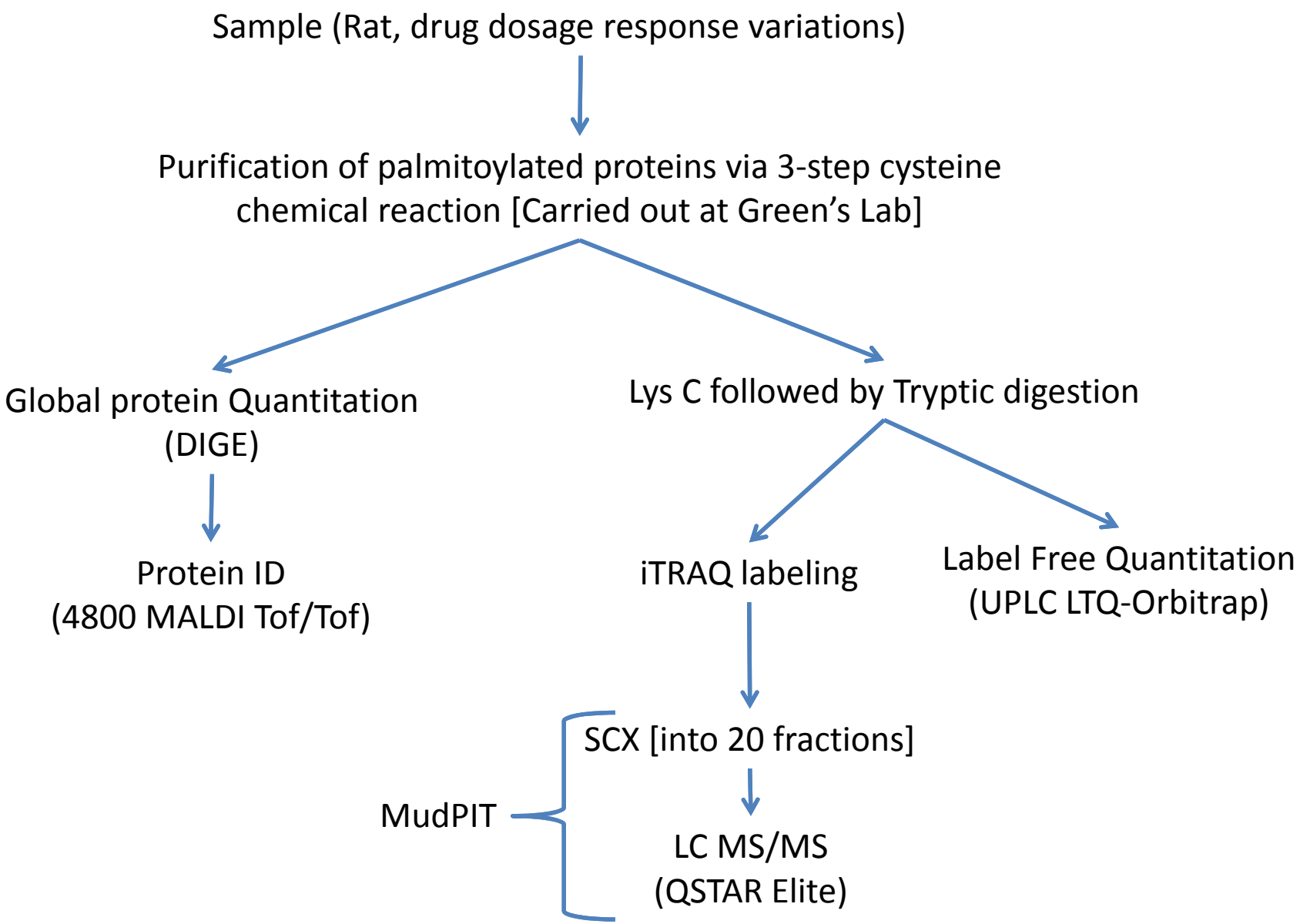


Aim 2: The Reward Pathway in the Brain



Rats will be injected with amphetamine or saline to induce behavioral and biochemical sensitization. We will dissect out the VTA, NA, PFC and striatum for analysis.

Methodology (Global Palmitoylation changes in drug dosed rat)



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