AstraZeneca

The Role of Arsenic Methyl Transferase

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AstraZeneca IMED Neuroscience

IASOC, Ischia, 2018

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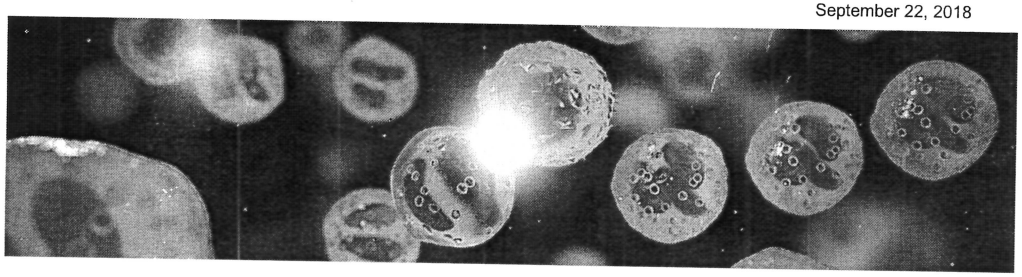


Outline

- AstraZeneca Neuroscience collaboration with the Lieber Institute for Brain Development
- Genetic & disease association of Arsenic methyl transferase (AS3MT)
- Known function of AS3MT & identification of tool inhibitors
- Identification of interaction partner

The Lieber Institute for Brain Development

The Institute

- **Affiliate of Johns Hopkins**
- **Led by Daniel Weinberger**
  Ron McKay
  Sol Snyder

- **Mission**
  identify basic mechanisms of psychiatric disease and contribute to identification of drugs to treat illnesses of the brain

- **Collaborative culture**

Capabilities

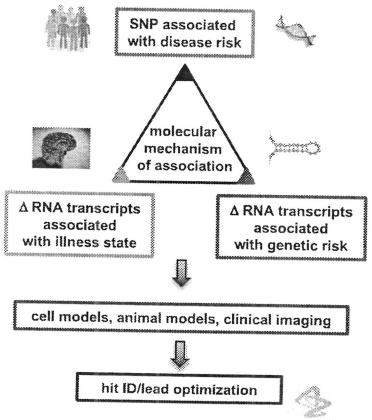
- **Patient cells & tissues**
  Extensive post-mortem brain bank (>2800 with diagnosis)

- **iPS cell technology**
  Extensive iPSCs collection, leading iPS technology

- **Transcriptomics/Informatics**
  Established expertise in transcriptomics & human genetic analysis

- **Identified disease-relevant targets/pathways**

Road map: Genes to Drugs

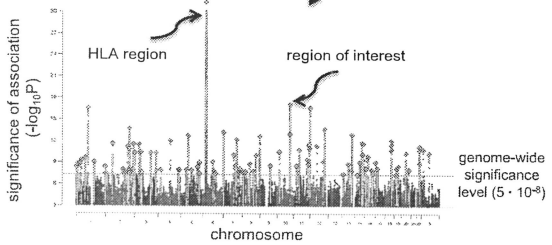


Genetics & Disease Association

GWAS identified 108 genetic loci associated with Schizophrenia

Schizophrenia ⟷ (*GWAS*) **SNPs** point to gene loci associated with disease ⟷ (?) **risk genes** impact of SNP on transcriptome

Ripke et al. *Nature*, 2014, 511, 421.



Manhattan plot showing 108 schizophrenia associations

- 150,000 subjects analyzed
- identified 108 independent genomic loci with genome wide significant statistical association

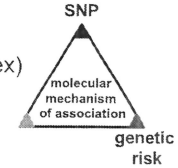
The next challenge

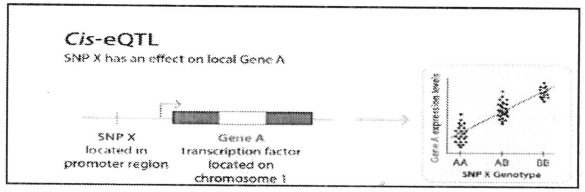
- How to define a risk gene from a genetic loci?
- What is the disease relevance of variations in non-coding regions?

HLA: human leukocyte antigen

Identifying molecular mechanisms for Schizophrenia

- ***The problem***
  - 108 genetic loci span ~21Mb and contain ~500 genes across ~2000 transcripts
  - How do we "translate" these statistical associations into discovery of a drug target?
- ***The approach***
  - Use RNAseq (postmortem brain samples from dorsolateral prefrontal cortex) to characterize relationship between risk SNP and RNA transcripts associated with genetic risk (eQTLs)

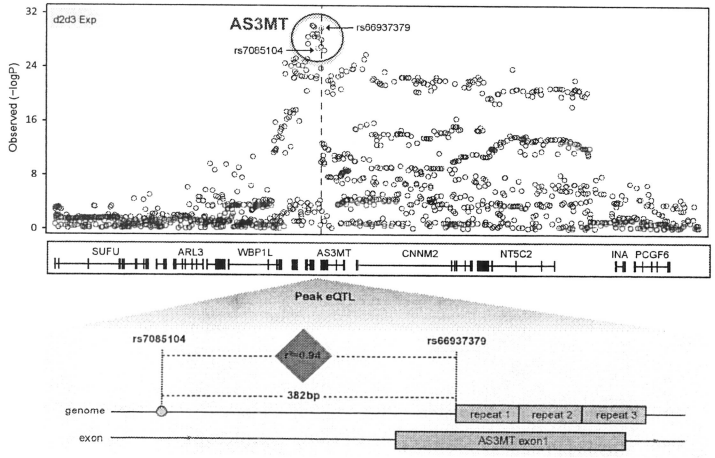


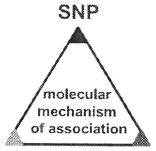


Westra et al. *Biochim. Biophys. Acta*, 2014, 1896.

Jaffe et al. *Nat. Neurosci.* 2018, 21, 1117.

SNPs in AS3MT region associated with risk for Schizophrenia



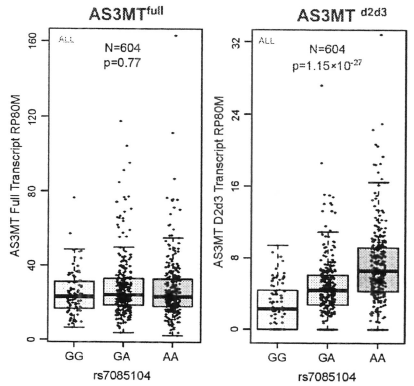


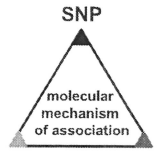
- PGC2 GWAS: association signal in 10q24.32 region (150,000 subjects)
- SNPs are associated with human-unique VNTR in 5'-untranslated region of AS3MT

SNP: single nucleotide polymorphism
VNTR: variable number of tandem repeat

 Li et al. *Nat. Med.* 2016, 22, 649.

Risk associated SNP (G→A) elevates expression of AS3MT^{d2d3} (not AS3MT^{full})



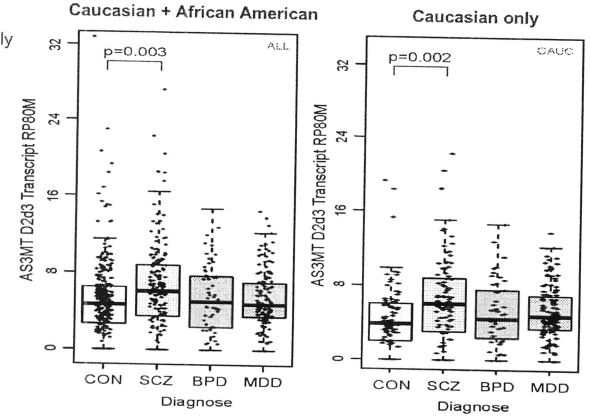


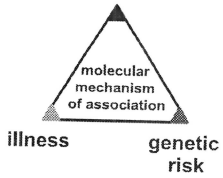
- SNP associated with human-unique VNTR in 5'-untranslated region of AS3MT
- VNTR affects AS3MT^{d2d3} expression (VNTR number ↑ then expression ↑, data not shown)

 Li et al. *Nat. Med.* 2016, 22, 649.

Relative changes in AS3MT^{d2d3} transcription

p < 0.05 statistically significant



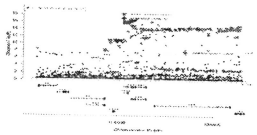


control (CON)
schizophrenia (SCZ)
bipolar (BPD)
depression (MDD)
African American (AA)
Caucasian (CAUC)

Increased expression of AS3MT^{d2d3} transcript in SCZ, not BPD or MDD
No significant increase of AS3MT^{full} in SCZ compared to CON

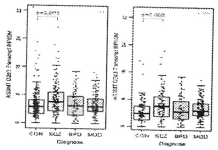
9 Li et al. *Nat. Med.* 2016, 22, 649.

Molecular mechanism of association for AS3MT^{d2d3}



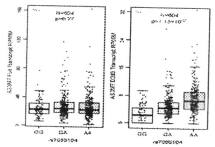
SNP in gene associated with disease risk (G/A)

molecular mechanism of association



Δ RNA transcripts associated with illness state (↑ RNA ~ SCZ)

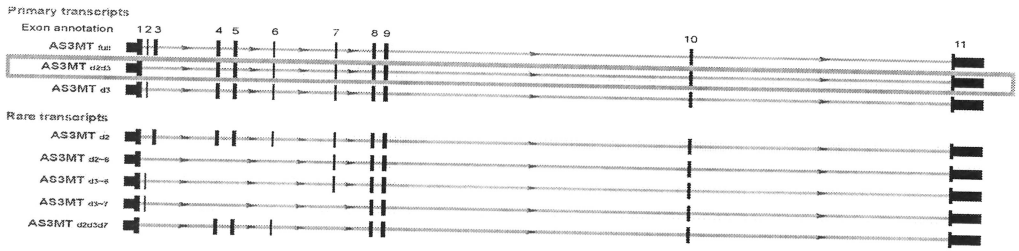
Δ RNA transcripts associated with genetic risk (A, ↑ RNA)



same directionality

10 Li et al. *Nat. Med.* 2016, 22, 649.

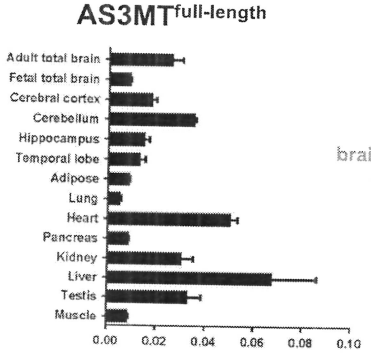
AS3MT has multiple transcripts



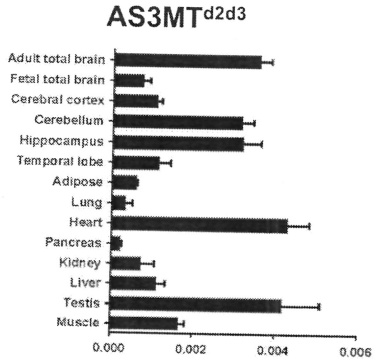
11 Li et al. *Nat. Med.* 2016, 22, 649.

Expression of AS3MT^{full} and AS3MT^{d2d3} in different tissues

qPCR analysis in tissues of non-affected humans
AS3MT^{full}: highest expression in liver, heart
AS3MT^{d2d3}: highest expression in brain, heart, testis



brain/liver ratio changes



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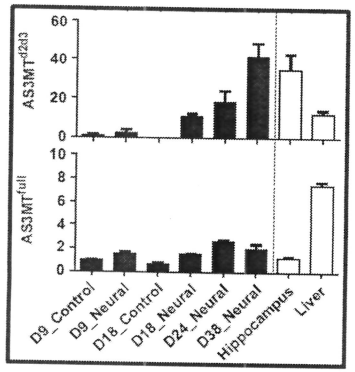
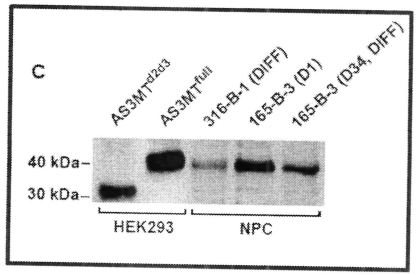
Expression of AS3MT^{full} and AS3MT^{d2d3} in iPS cells



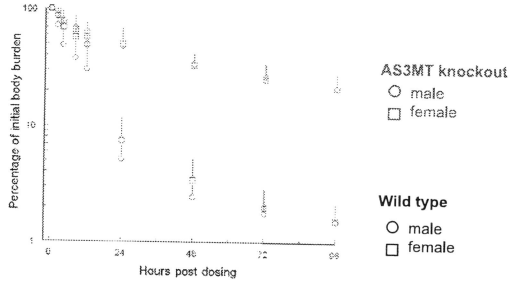


- AS3MT^{d2d3} mRNA dramatically upregulated early during human stem cell differentiation which specify neuronal fates (proteins of appropriate M_w also observed)

AS3MT knockout mice mainly studied in context of arsenic metabolism (literature)

- Inorganic arsenic: potent carcinogen, hypertension, atherosclerosis
- AS3MT detoxifies As^{3+} by mono-, di-, and trimethylation (elimination via urine)

Whole body retention of [^{73}As]



Drobna et al. *Chem. Res. Toxicol.* 2009, 22, 1713.

Working hypothesis

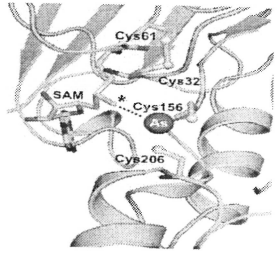
Beyond detoxification, AS3MT may exhibit other functions, methylate substrates relevant to neuro-development, and lead to susceptibility to psychiatric illnesses

- Multiple forms of AS3MT exist
- Why are they expressed in the brain?
- Are they catalytically active? Do they exhibit unknown functions?
- How do we go about this?
  - we are interested in CNS function full-length and truncated forms
  - assay for arsenic methylation
  - new substrates, interaction partners?
  - identification of tool inhibitors?
  - subcellular localization?

Known Function of Arsenic Methyl Transferase
&
Identification of Tool Inhibitors

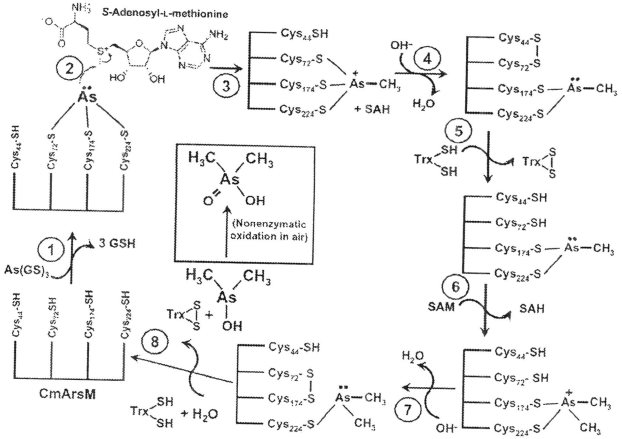
Arsenic methyl transferase (AS3MT)

- AS3MT^{full} detoxifies As^{3+} by mono-, di-, and trimethylation
- X-ray structures known
- Conserved Cys residues bind As^{3+} to present to to cofactor for methylation
- In the absence of As^{3+}, Cys residues can form S-S bridges
- AS3MT$^{-/-}$ mice: viable, fertile, but phenotype not well characterized
  (mainly studied in context of arsenic metabolism: Drobna et al. *Chem. Res. Toxicol.* 2009, 1713.)



Dheeman et al. *Chem. Res. Tox.* 2014, 27, 1979.

Putative mechanism of As(III) methylation (AS3MT^{full})



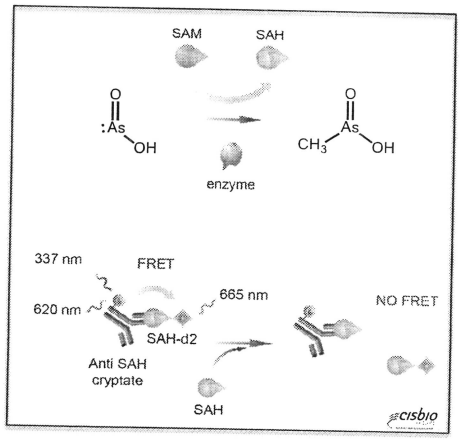
Marapakala et al. *Acta Cryst.* 2015, D71, 505.

- 3 Cysteines 'clamp' As^{3+}
- 4th Cys and external SH (Trx, Grx or DHLA) assist in liberation of methylated As^{3+} species
- AS3MT^{d2d3} is missing two Cys in proximity of the SAM binding pocket!

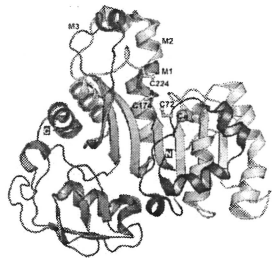
thioredoxin (Trx)
glutaredoxin (Grx)
dihydrolipoic acid (DHLA)

Biochemical activity of AS3MT

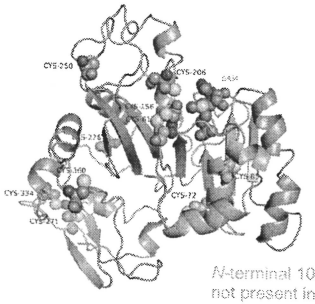
- AS3MT^{full} and AS3MT^{d2d3} were purified
- Assay to follow conversion of SAM to SAH
- **AS3MT^{full} displayed robust activity**
- **No significant activity observed for AS3MT^{d2d3} under these conditions (probe As^{3+} species)**
- A few other *potential* substrates were tested but no activity observed



The homology modeling



Rosen and coworkers, *Biochemistry* **2012**, *51*, 5476.

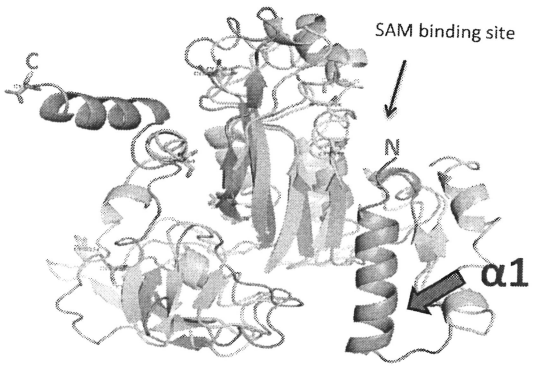


- Thermophilic eukaryotic alga (4FR0)
- Sequence similarity to human 42.4%
- 10 models evaluated
- Preservation of catalytic Cys (C72, C174, C224)

- 10 models generated
- Assessed based on preservation of cat. site & plausibility of loop structures
- Visualization of portion not found in AS3MT^{d2d3}
- 2 Cys proximal to cat. site absent in AS3MT^{d2d3}

Molecular dynamics simulations suggest conformational change
(100 ns simulation)

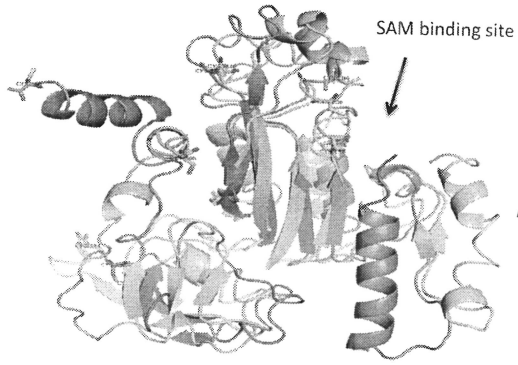
AS3MT^{d2d3}: before simulation (light green)
after simulation (dark green)

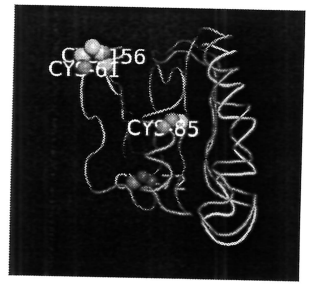


- AS3MT^{d2d3} and *N*-terminus remain folded

Molecular dynamics simulations suggest conformational change
(100 ns simulation)

AS3MT^{d2d3}: before simulation (light green)
after simulation (dark green)





AS3MT^{d2d3}
AS3MT^{full}

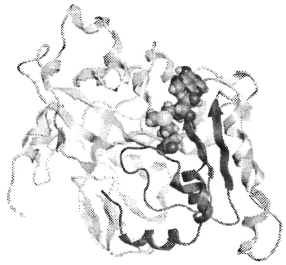
- AS3MT^{d2d3} and *N*-terminus remain folded
- helix α1 shows significant movement toward globular structure and occupies position of *N*-terminal helix in AS3MT^{full}

Tool inhibitors of AS3MT identified by a virtual screen

- Virtual screen of AZ v-library based on:

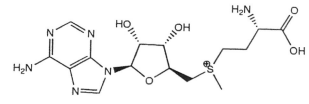
  a) homology model of AS3MT

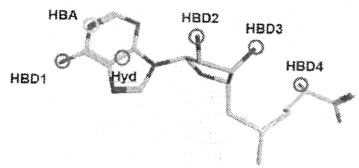
  b) putative binding conformation of cofactor (SAM) in AS3MT



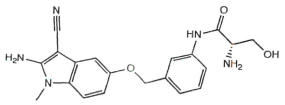
The SAM pharmacophore model

- Six virtual atoms defined to represent SAM:
  4 HBDs, 1 HBA,1 aromatic ring center
- For hits from docking: okay if distance between pharmacophore and docked atom <0.6Å
- ~4200 top-scored poses satisfying ≥2 of the 6 pharmacophore elements
- → 'manual' inspection: 426 compounds tested for inhibitory activity of AS3MT (10 µM)

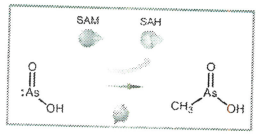




Most promising initial hit

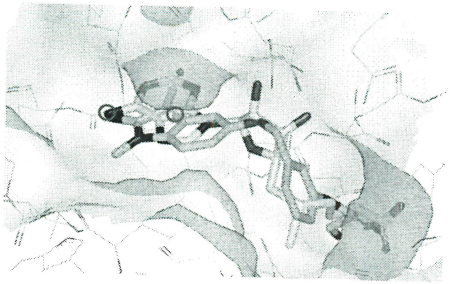


AS3MT (IC_{50}) 2.9 µM

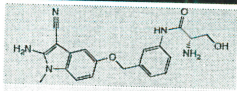
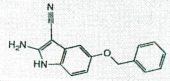
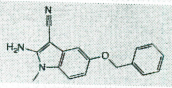
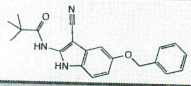
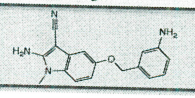


SAM

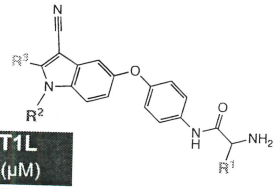
docking poses of initial hit and SAM

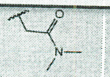


Initial structure activity relationship

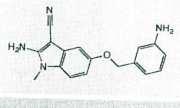
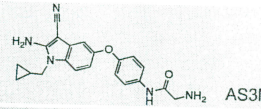
structure	AS3MT IC_{50} (µM)	NSD2 IC_{50} (µM)	DOT1L IC_{50} (µM)
	2.9	n.d.	n.d.
	3.6	49	>100
	13.6	>100	>100
	>100	n.d.	n.d.
	1.4	>100	>100

Initial structure activity relationship



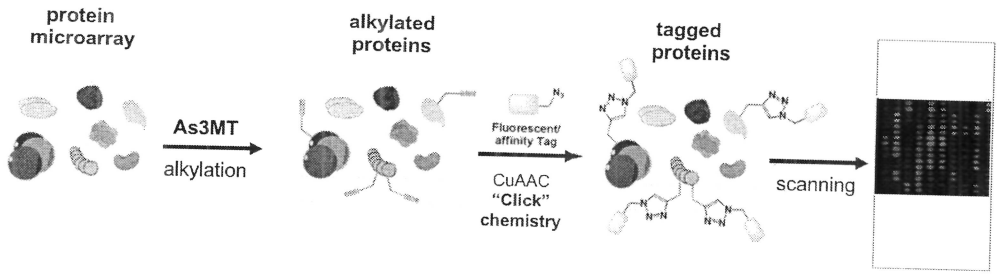
R^1	R^2	R^3	AS3MT IC_{50} (µM)	NSD2 IC_{50} (µM)	DOT1L IC_{50} (µM)
H	CH_3	NH_2	3.3	47	>100
H	CH_2CH_3	H	>100	n.d.	n.d.
H	CH_2CH_3	NH_2	1.8	12	99
H		NH_2	1.5	5.8	96
H		NH_2	8.3	n.d.	n.d.
(*R*)-CH_2OH	H	NH_2	5	9.1	>100

Off target activities of selected hits

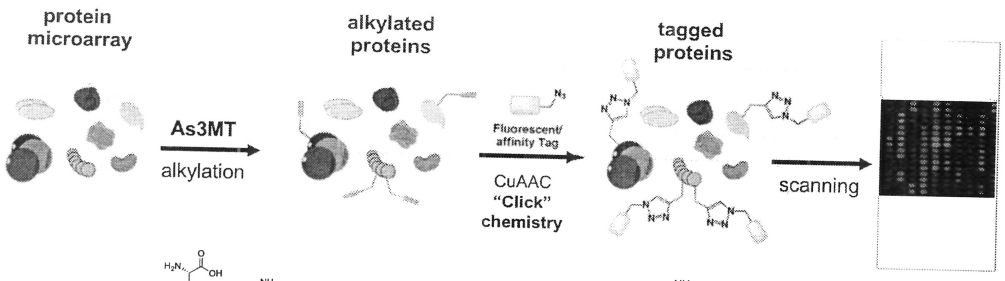
	 AS3MT: 1.4 µM	 AS3MT: 1.5 µM
# of targets tested	92	97
# targets with K_i > 10 µM	85	65
# targets with $K_i \leq$ 10 µM	7	32
Specific targets with K_i > 10 µM	NET (4.54), GABAA (1.51), Sigma 1 (0.66), PPARγ (1.70), AT Gpig (0.60) TSPO (3.80), 5HT2B (7.40)	α1A (5.08), α1B (1.48), α1C (3.22), α2A (7.07), D1 (3.24), D2 (4.1), D3 (3.58), DAT (0.6), GR (4.34), CB1 (0.37), GlyR (1.8), 5HT1D (6.63), 5HT7 (5.17), 5HT4 (8.35), 5HT2B (8.62), 5HT1B (7.75), GABA (4.34), M2 (9.44), NachA1 (9.78), NET (3.22), MR2 (6.18), NK1 (0.84), Sigma 1 (0.20), PPARγ (4.47), Ghre (7.93), AT Gpig (1.27), CCKA (4.94), CaV-L (7.97), PDE4D (4.07), COX1 (2.13), COX2 (4.76), TXA2 (0.028)

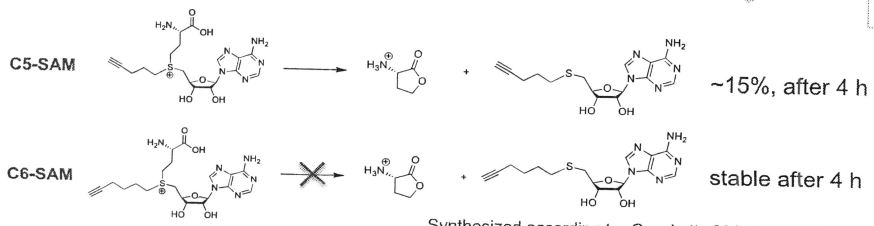
Identification of Interaction Partners

1. Approach: Click chemistry to identify AS3MT$^{full\text{-}length}$ substrates using protein microarrays



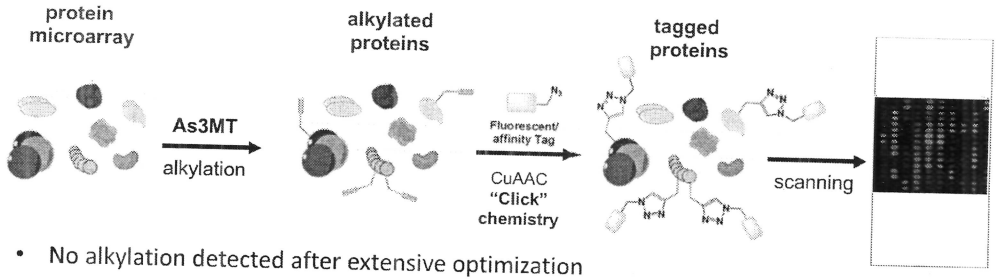
The Click chemistry approach





Synthesized according to: *Org. Lett.*, 2014, 16, 3056.

Limitations of the chemistry approach



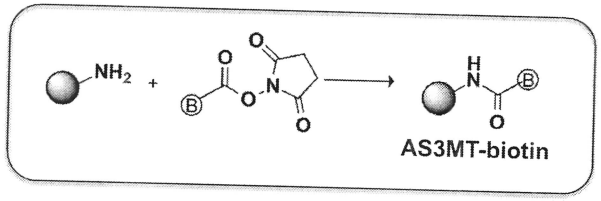
- No alkylation detected after extensive optimization

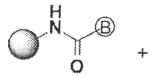
Limitations

- Toxicity of copper salt precluded application of click chemistry in living cells
- C-6 SAM not an ideal alkyl donor (strained alkyne ?)

Alternative: study direct binding of AS3MT to proteins on chip

2. *Approach*: Identification of interactors using biotinylated AS3MT and a protein microarray chip





protein micro-array chip (~19,000 proteins printed in pairs)

1. wash
2. Cy5-Streptavidin
3. wash

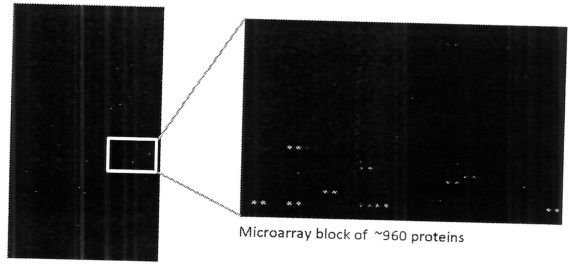
AS3MT interactor proteins visible on chip

Analysis of the microarray study

Protein microarray

~ 19,000 proteins (present in CNS)

1. **AS3MT-biotin** or buffer
2. **Cy5-Streptavidin**
3. buffer



Analysis

- Align array grid & quantify signal
- Average dual-spot signal
- Ratio of foreground signal/background (f/b)
- Select proteins with signal above 5x SD
- Select reproducible hits in 3 experiments
- Verify signal on protein chip
- Annotate function

Results

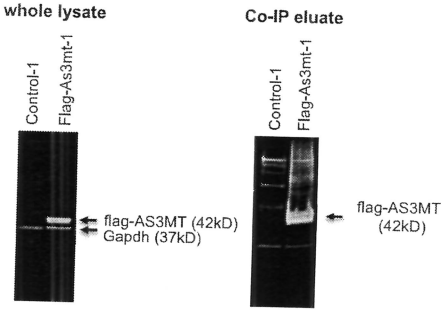
13 novel candidate interactors identified as hits from the chip

Novel candidate AS3MT interactors microarray chip study

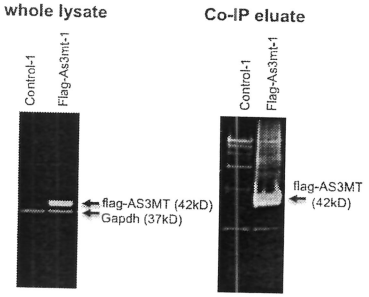
	Protein	Name	Function
Trafficking transport	GORASP1	Golgi reassembly stacking -1	Interacts with syntaxins and golga2, protein sorting
	RAB5A	Ras-related protein- Rab-5A	Early to late endosome transport, regulation of synaptic vesicles
	GORASP2	Golgi reassembly stacking -2	Plays a role in assembly and membrane stacking of the Golgi cisternae
	ASNA1	ATPase ASNA1	Post-translational delivery of tail-anchored proteins from cytosol to ER
	GDI1	Rab GDP dissociation inh. α	Protein binding, RAB GDP-dissociation inhibitor
	ATCAY	Caytaxin	Neurotransmission-regulates glutaminase
Cell stress response	HSPA6	HSP70 protein 6	Response to unfolded proteins
	PPM1G	Protein phosphatase 1G	Neg. regulator of cell stress response pathways, regulation of USP7
	PSMA3	Proteasome subunit α 3	Protein polyubiquitination
	USP7	Ubiquitin specific peptidase 7	Deubiquitination/stabilization/ubiquitin., maintenance of DNA methylation
Development plasticity	PICK1	PRKCA-binding protein	Regulates trafficking and internalization of AMPA receptors
	HOMER3	Homer protein homolog 3	Post-synaptic density scaffolding protein-maintaining plasticity of glutamatergic synapses
	CHGA	Chromogranin A	negative regulator of neuroendocrine system

3. *Approach*: Identification of AS3MT interactors using co-immuno-precipitation and mass spectrometry

- Flag-AS3MT (or empty plasmid control) overexpressed in rat neuroblastoma cells
- Immunoprecipitation with flag antibody → NH_4OH elution → ITRAQ labeling → LC/MS



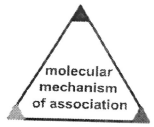
AS3MT interactors using co-IP and mass spectrometry



Protein	Function
Drebrin isoform X3	Cell migration/extension of neuronal processes
Plectin isoform X1	Structural constituent of muscles
Drebrin isoform X2	Sell migration/extension of neuronal processes
Alpha-actinin-1	F-actin crosslinking protein
Proliferation-assoc. protein 2G4	Regulates cell proliferation and survival
Voltage-dep. anion channel prot. 1	Regulation of cell volume and apoptosis
Protein phosphatase IG	Control of chromatin structure
Golgi apparatus protein 1 prec.	Golgi structure maintenance

- 8 Proteins were identified (reproducible in at least 2 of the 3 experiments)
- Voltage-dependent anion channel protein 1 (VDAC1) and AS3MT-GFP are localized in mitochondria
- Validation pending- specific antibody for AS3MT

Where are we?



- GWAS: AS3MT^{d2d3} high risk association for schizophrenia from PGC2 study
- Methyl transferase function confirmed for AS3MT$^{full\text{-}length}$ but not for AS3MT^{d2d3}
- Study function of both proteins important for target validation & therapeutic strategy (initial focus on AS3MT$^{full\text{-}length}$)
- Enzymatic assay, homology model, virtual screen yielded hits (low µM)
- Various approaches to identify interactors of AS3MT explored:
  - derivatized alkynyl-SAM: no new substrates found
  - biotinylated AS3MT – microarray & Co-IP/MS identified potential protein interactors

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Alan Cross	(Neuroscience)
Steve Wesolowski	(Neuroscience)
Ian Hardern	(protein production)
Jan Schülke	(Co-IP, MS)
Hongming	Chen (virtual screen)
Bojana Popovic	(molecular dynamics)