SETAC North America 40th Annual Meeting
3–7 November 2019 | Toronto, ON, CA

Using a multiomic approach to unravel the mecanisms of acrylamide neurotoxicity

Benjamin Piña[a], Demetrio Raldua[a], Eva Prats[b], Marta Casado[a], Melissa Faria[a], Francesc Puig-Castellví[a], Yolanda Pérez[c], Ignacio Alfonso[c], Natalia Garcia-Reyero[d], Mark Arick II[e], Arie Admon[f], Tamar Ziv[f], Chuan-Yu Hsu[e], Shani Ben-Lulu[f]

a) Institute for Environmental Assessment and Water Research (IDAEA-CSIC). Jordi Girona, 18. 08034 Barcelona, Spain
b) Research and Development Center (CID-CSIC). Jordi Girona, 18. 08034 Barcelona, Spain
c) Department of Biological Chemistry, Institute of Advanced Chemistry of Catalonia (IQAC-CSIC). Jordi Girona, 18. 08034 Barcelona, Spain
d) Environmental Laboratory, US Army Engineer Research and Development Center, Vicksburg, MS, USA
e) Institute for Genomics, Biocomputing & Biotechnology (IGBB), Mississippi State University, Starkville, MS, USA.
f) Faculty of Biology, Technion-Israel Institute of Technology Haifa 32000, Israel

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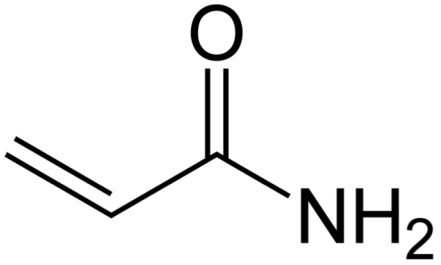
PUBLIC HEALTH STATEMENT
Acrylamide
CAS # 79-06-1

ATSDR
AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY

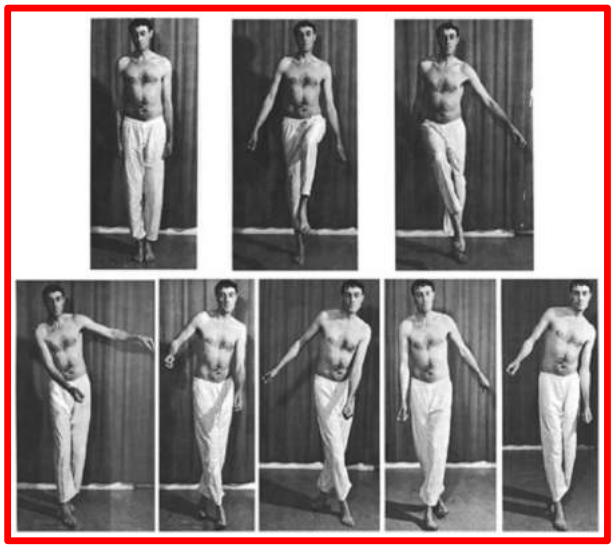
Nervous system effects	Nervous system effects such as muscle weakness, numbness in hands and feet, sweating, unsteadiness, and clumsiness were reported in some acrylamide workers. However, most people are not exposed to acrylamide levels high enough to cause these effects.
Reproductive effects	Acrylamide reduces the ability of male animals to produce offspring and could cause similar effects in humans, but not likely at exposure levels experienced by most people.
Cancer	Acrylamide has caused several types of cancer in animals. We do not know whether acrylamide causes cancer in humans. The EPA, International Agency for Research on Cancer (IARC), National Toxicology Program (NTP), and the Department of Health and Human Services have concluded that acrylamide is likely to be carcinogenic to humans.



Human

Gait abnormalities

Muscle weakness



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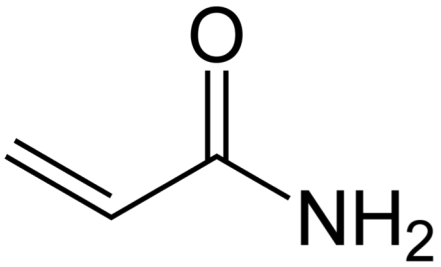

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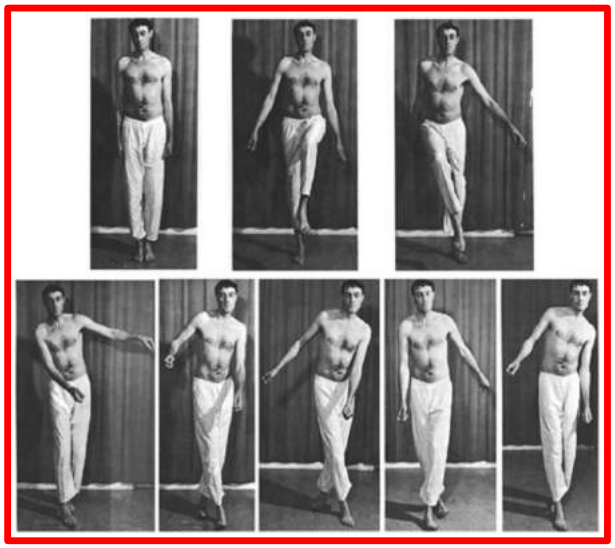
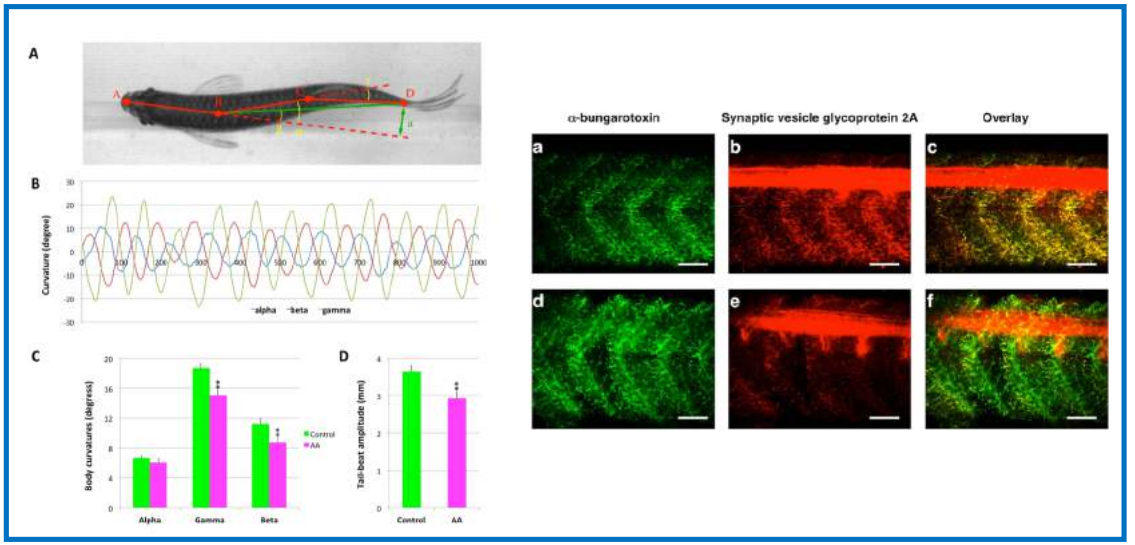


Zebrafish



Faria et al., (2019) srep 9:7075; Prats et al., (2017) srep 7: 13952

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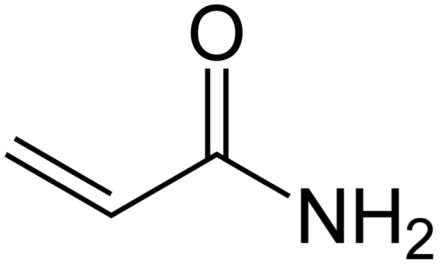
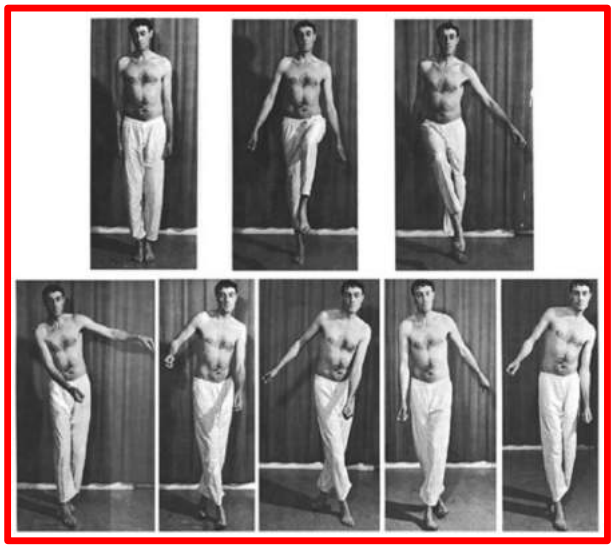


Objective: Use the zebrafish model to study the molecular neurotoxic mechanisms of acrylamide

Human

Gait abnormalities

Muscle weakness



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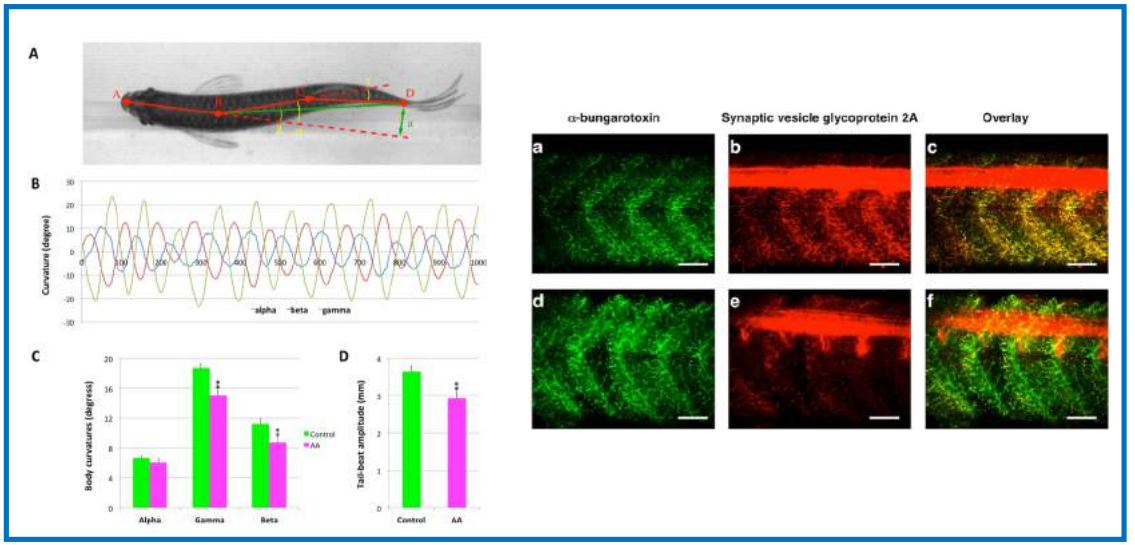

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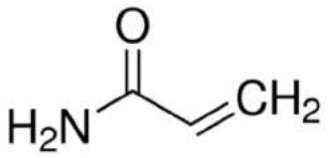
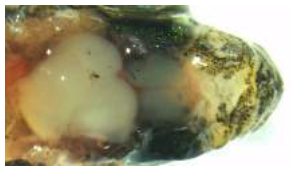
Methodological approach



Acrylamide (AA)

0.75 mM AA
72h (in water)
4 brains/sample
50% sex ratio



Metabolism

^{1}H-NMR
Neurotransmitter analysis (HPLC-MS)
Biochemical assays

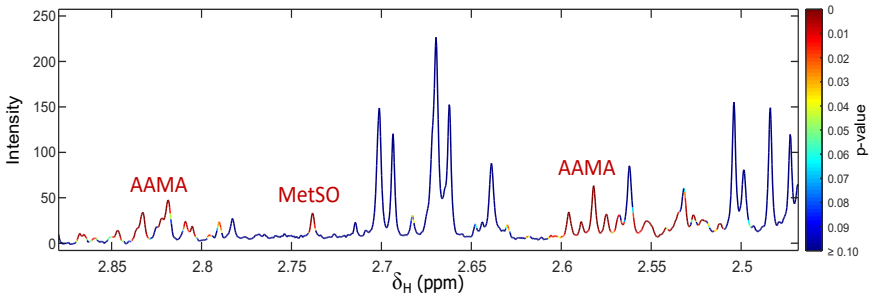
Proteome

Proteome profiles
Protein adduct analyses (MALDI-TOF)

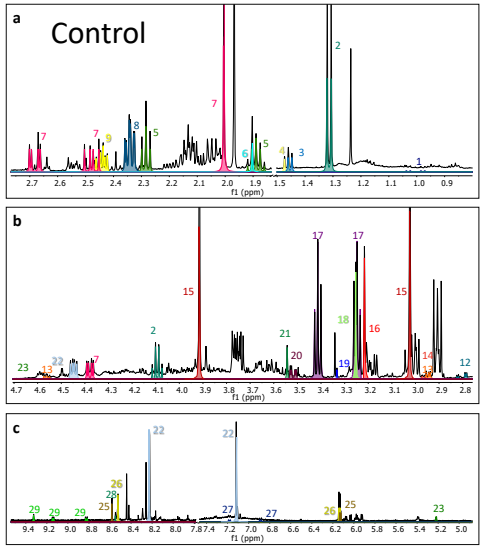
Transcriptome

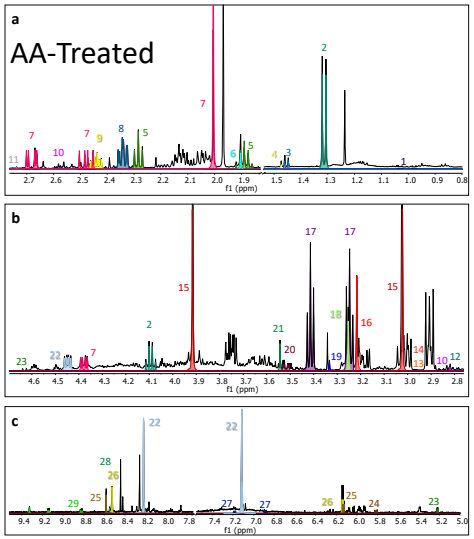
RNA iSeq

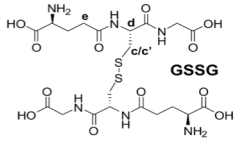
Non-Target Metabolomic Analysis: NMR

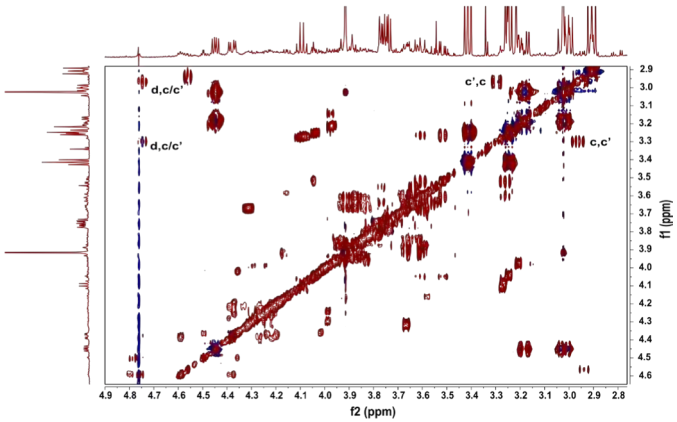


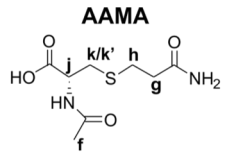
Compound	Fold Change	Sig.[a]
AAMA	7.43	***
Acrylamide	5.77	***
MetSO	3.59	**
L-Alanine	0.85	*
L-Glutamic acid	0.84	*
NAD	0.79	**
Carnosine	0.77	*
L-Aspartic acid	0.73	***
Betaine	0.68	*
GSSG	0.58	**
GSH	0.20	***



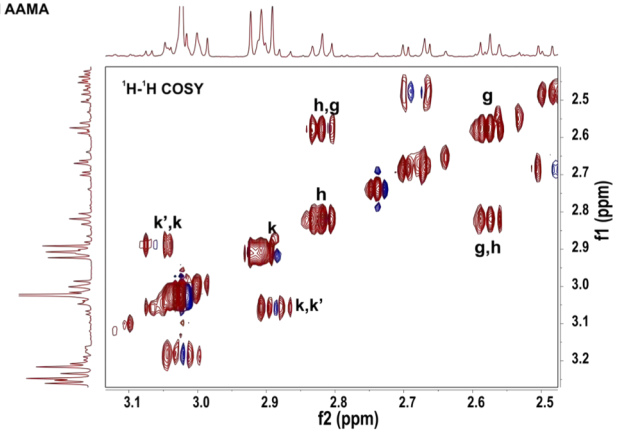


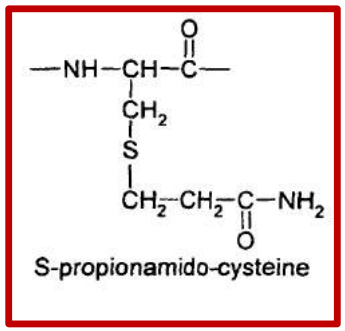




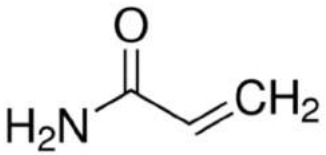


AA treated Brain ZF extract
+ 500 μM AAMA



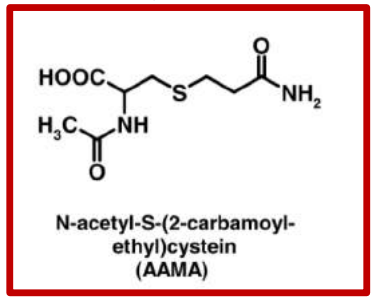


Protein-Cys

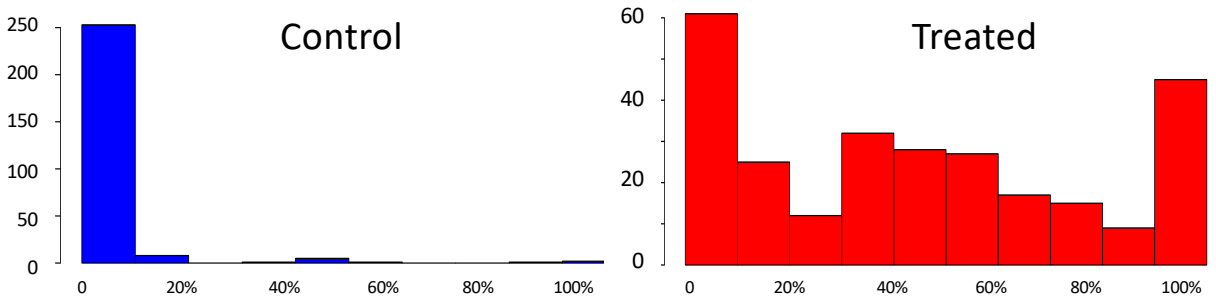


Acrylamide

GSH



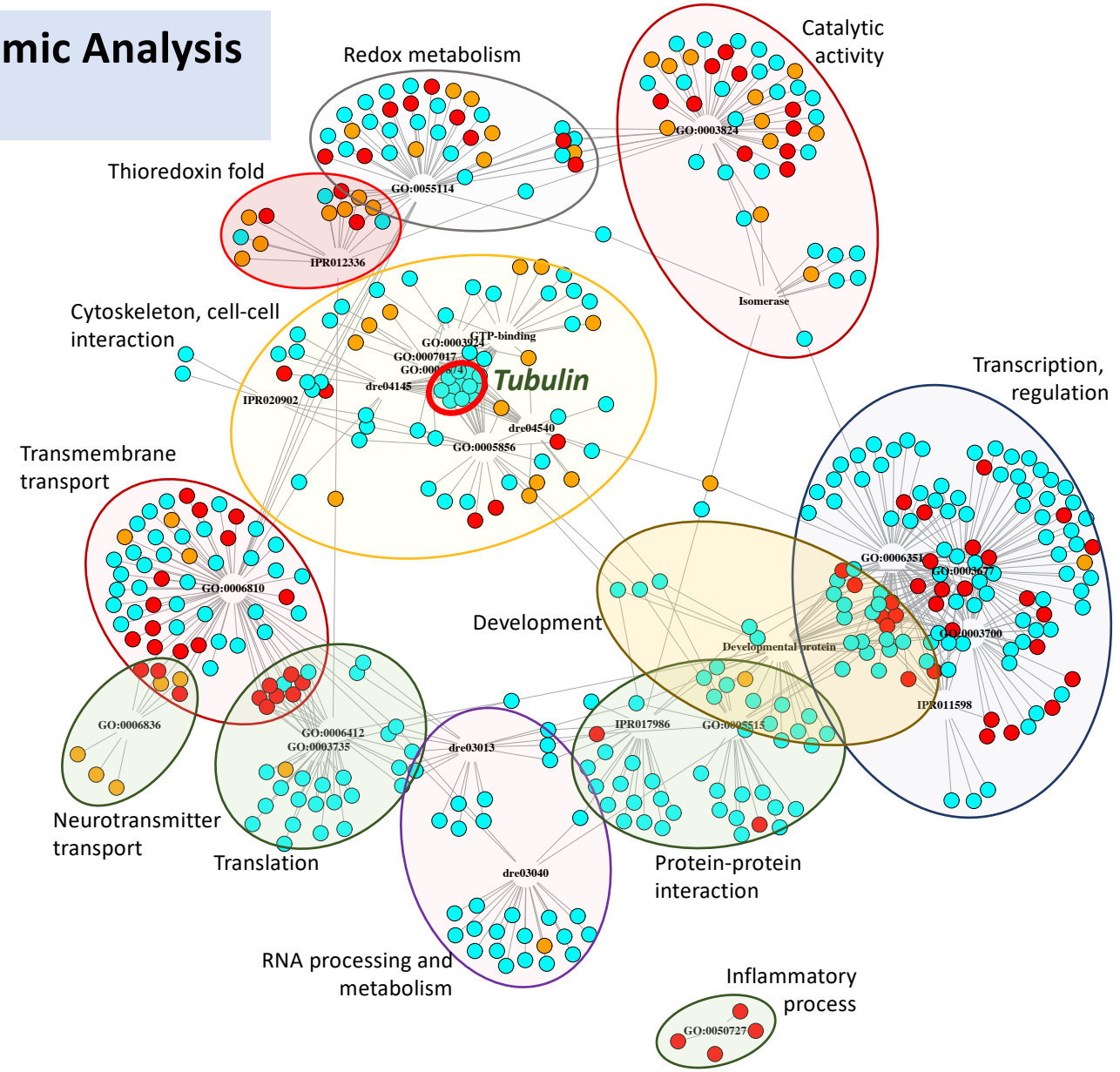
Proteomic Analysis of Cys adducts (MALDI-TOF)



Fraction of Cysteine residues as adducts

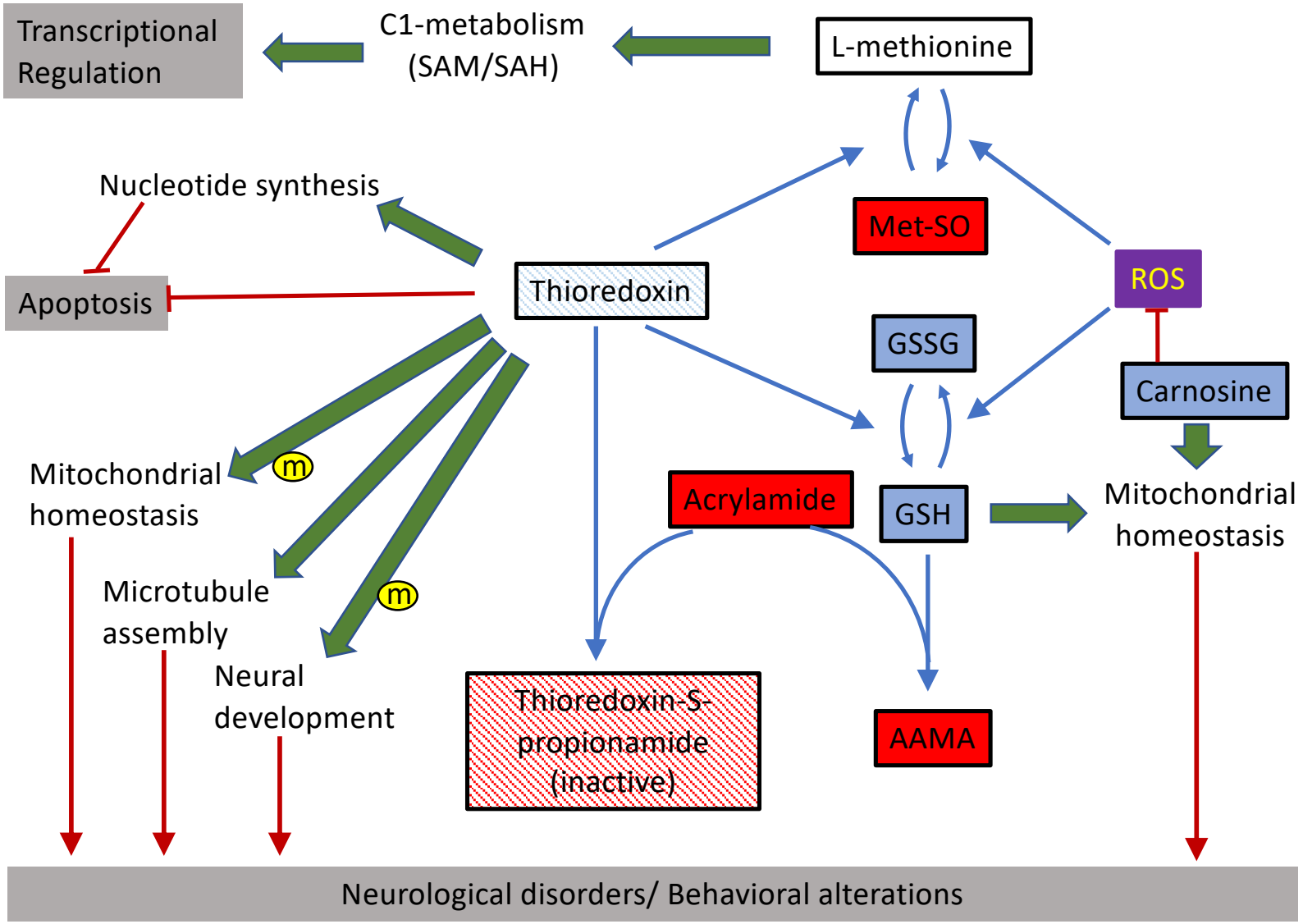
Term	Count	Fold Enrichment	PValue	FDR (%)	Genes
IPR012336:Thioredoxin-like fold	8	10.7	1.0E-05	0.01	ZGC:56493, TXNDC17, TXN2, PRDX6, TXN, EEF1G, GSTP2, GSTP1
GO:0016671~oxidoreductase activity, acting on a sulfur group of donors, disulfide as acceptor	4	61.2	3.2E-05	0.04	ZGC:56493, TXN2, TXN, MSRB2
GO:0006836~neurotransmitter transport	5	18.0	1.6E-04	0.20	SLC17A6B, SLC17A6A, CPLX2, LOC563082, CPLX2L
IPR005746:Thioredoxin	3	138.9	1.7E-04	0.22	ZGC:56493, TXN2, TXN
GO:0005882~intermediate filament	5	16.1	2.5E-04	0.24	NEFMB, NEFMA, LMNB1, LMNB2, LMNA
GO:0003824~catalytic activity	11	4.1	2.9E-04	0.34	PPM1G, GAD1A, ALDOCA, GAD2, GMPR2, ALDOCB, CKMT1, MTAP, SYN2B, PGAM1A, PCCA
IPR026074:Microtubule associated protein 1	3	111.1	2.8E-04	0.36	MAP1AB, MAP1AA, MAP1B
IPR004142:Ndr	3	92.6	4.2E-04	0.54	NDRG4, NDRG3A, NDRG2
GO:0016829~lyase activity	5	10.2	1.4E-03	1.63	GAD1A, ALDOCA, GAD2, ALDOCB, GLO1
GO:0055114~oxidation-reduction process	11	3.3	1.6E-03	2.00	ZGC:56493, OGDHA, GMPR2, TXN2, PRDX6, TXN, PNPO, ALDH9A1A.1, ALDH9A1A.2, MSRB2, SOD2

Transcriptomic Analysis (iSeq)

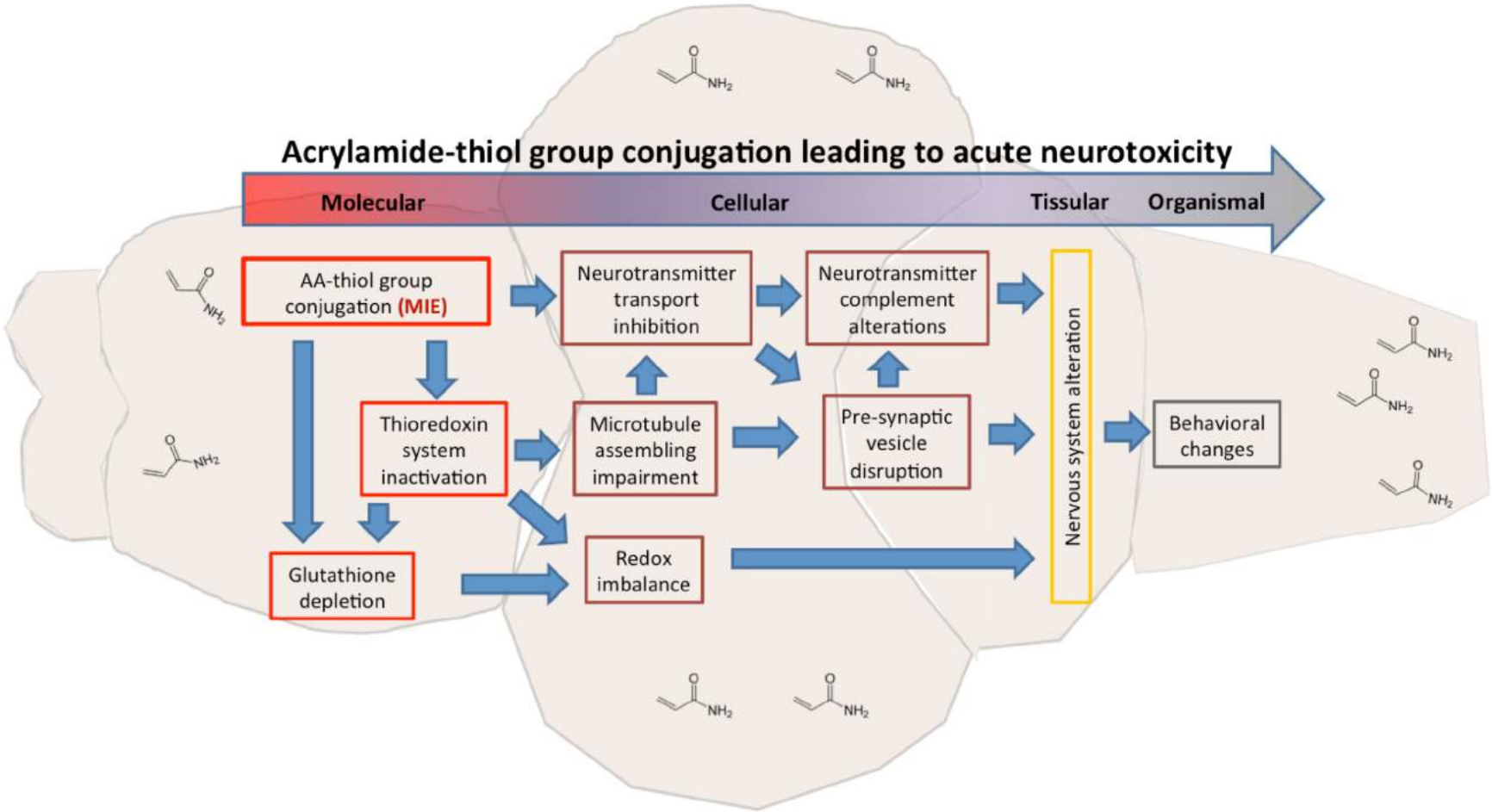


Transcriptome		Proteome	
3	0	5	GO:0006836~neurotransmitter transport
4	0	0	GO:0050727~inflammatory response
12	24	11	GO:0055114~oxidation-reduction
12	24	11	GO:0003824~catalytic activity
3	4	8	IPR012336:Thioredoxin-like fold
23	43	5	GO:0006810~transport
20	26	0	GO:0003700~transcription factor
4	12	0	IPR011598:Myc-type, bHLH domain
7	14	1	GO:0003735~ribosome structure
7	20	2	GO:0006412~translation
18	49	1	GO:0006351~transcription,
21	67	1	GO:0003677~DNA binding
6	30	1	Developmental protein
3	27	2	GO:0005856~cytoskeleton
0	8	2	Isomerase
0	5	0	IPR020902:Actin/actin-like
2	20	0	GO:0005515~protein binding
1	17	0	dre03013:RNA transport
1	18	0	dre04145:Phagosome
0	22	4	GTP-binding
0	15	4	dre04540:Gap junction
0	17	4	GO:0005874~microtubule
0	19	1	GO:0003924~GTPase activity
0	21	1	dre03040:Spliceosome
0	17	0	GO:0007017~microtubule
0	22	0	IPR017986:WD40-repeat

Up
Down
AA-Adducts


Transcriptional Regulation
C1-metabolism (SAM/SAH)
L-methionine
Nucleotide synthesis
Met-SO
Apoptosis
Thioredoxin
ROS
GSSG
Carnosine
Mitochondrial homeostasis
m
Acrylamide
GSH
Mitochondrial homeostasis
Microtubule assembly
m
Neural development
Thioredoxin-S-propionamide (inactive)
AAMA
Neurological disorders/ Behavioral alterations

Conclusion: A perfect storm. Disruption of metabolites AND enzymatic activities AND structural proteins



We think a similar mechanism of toxic action may apply to other neurotoxicants, like methyl mercury

Thank you

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NATO SfP project MD.SFPP 984777; Advanced Grant ERC-2012-AdG-320737; CTM2017-83242-R Spanish Government; I-CORE, The Israel Science Foundation (1775/12)

725- Acrylamide is a recognized carcinogen that has strong neurotoxic effects in humans and in experimental animals, although the molecular mechanisms underlying these neurotoxic effects are not completely understood. We studied acrylamide neurotoxicity in the brain of adult zebrafish using an integrated approach that included biochemical, transcriptomic (RNAseq), proteomic (MALDI-TOF mass spectrometry) and metabolomic (proton-NMR)data. We detected the formation of acrylamide adducts with thiol groups in the brain metabolome, and the accumulation of acrylamide conjugates and propionamide adducts in Cys residues of proteins. These combined effects resulted in a quasi-complete depletion of glutathione and to the inactivation of different components of the thioredoxin system. Multi-omic functional analyses identified microtubules, thioredoxin-related proteins, transmembrane transport, redox metabolism and catalytic activity, as the cellular functions significantly altered by acrylamide in the fish brain. We propose that the combined loss-of-function of both redox metabolism-related systems configure a perfect storm that explains most, if not all, observed acrylamide neurotoxic effects. We derived an Adverse Outcome Pathway for acrylamide neurotoxicity at different levels of organization, from molecular interactions to behavioral changes. We think our mechanistic approach may be applied to other neurotoxicants that may share its toxic mode of action.