A Database of S-nitrosylation Sites identified in Human, Mouse and Rat

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S-nitrosylation

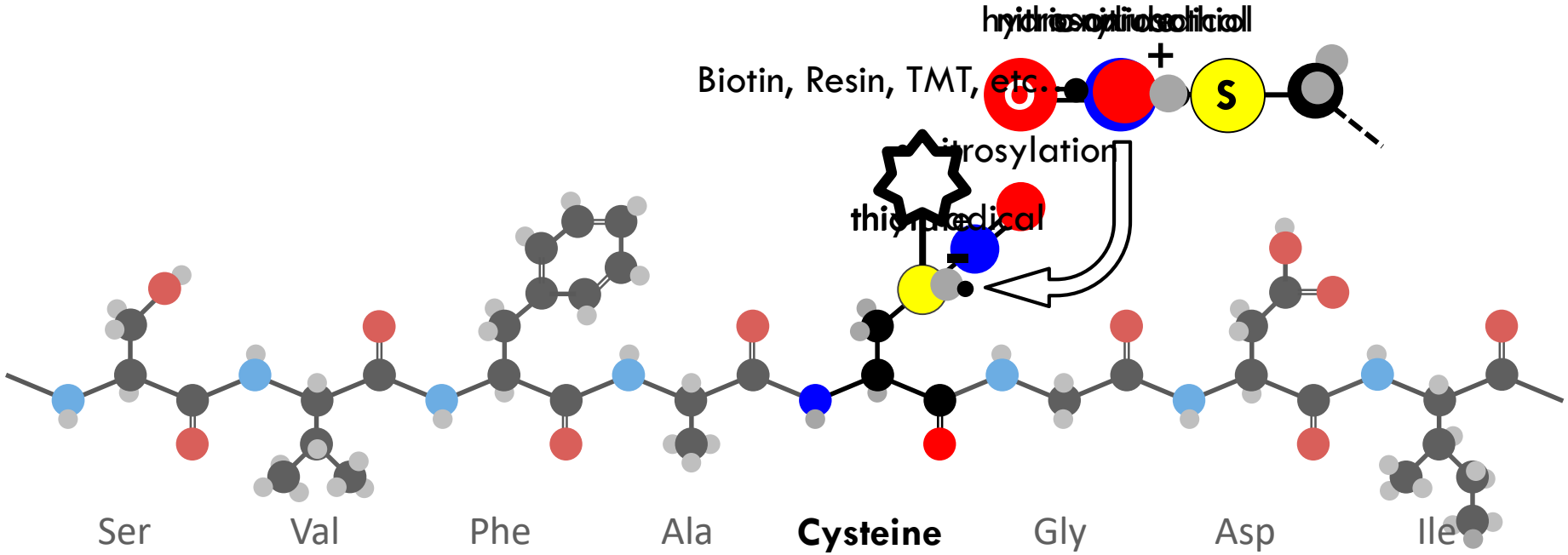
Post-translational modification - Covalent addition of NO group to a cysteine thiol

Ubiquitous mechanism, signaling changes in cell's redox balance

Readily reversible, can alter proteins structure/function relationship to regulate activity, localization

Protective capacity – occupy and protect critical Cys residues from other oxidative radicles (preconditioning)

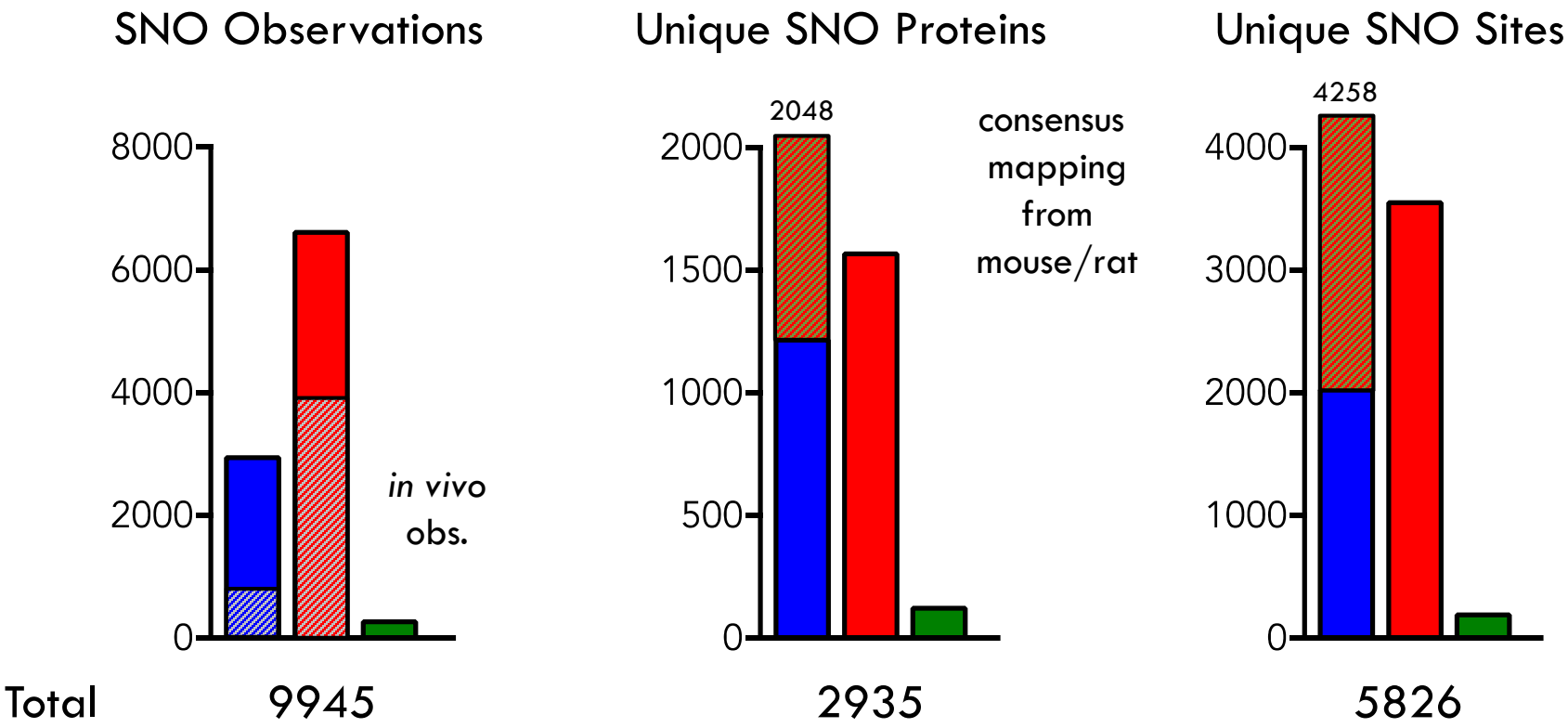
Very labile – requires 'switch' with more stable group to enrich and identify



Database of SNO modifications

Sites of SNO modification were manually compiled from the literature and a previously assembled database (dbSNO2.0 – last updated in 2014)

Focused on 3 species; human, mouse and rat



Uniprot: 72 human, 64 mouse and 51 Rat SNO proteins (reviewed)

Annotations in our SNO Data Base

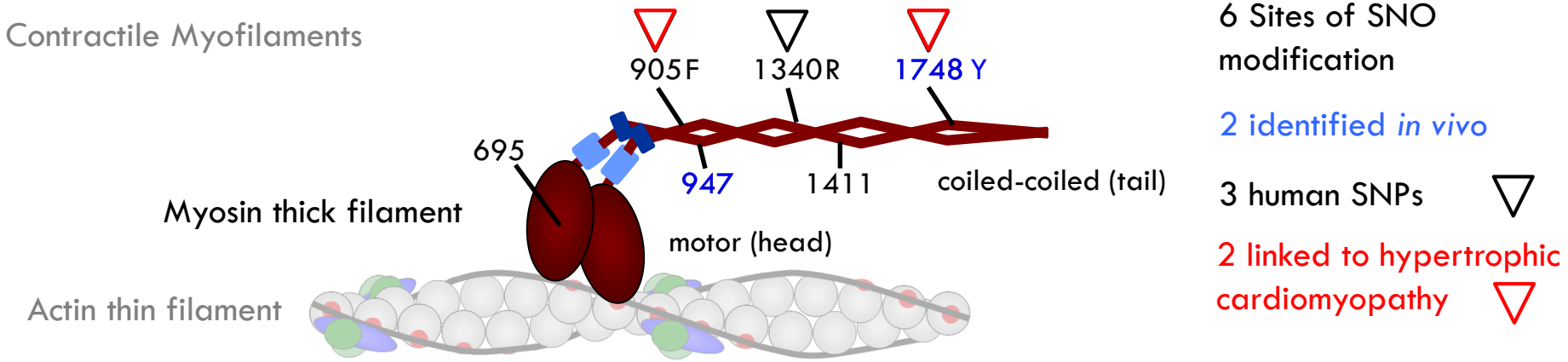
Experimental Designation of the site ID (*in vivo*, *in vitro* or mapped by consensus from mouse or rat)

Popularity rank for each modified protein across 6 organ systems (obtained from the PubPular app)

Subcellular localization and pathway designation for the protein (Gene Ontology)

SNPs or disease associated with the modified Cys (999 human SNPs)

Example: Cardiac Myosin Heavy Chain



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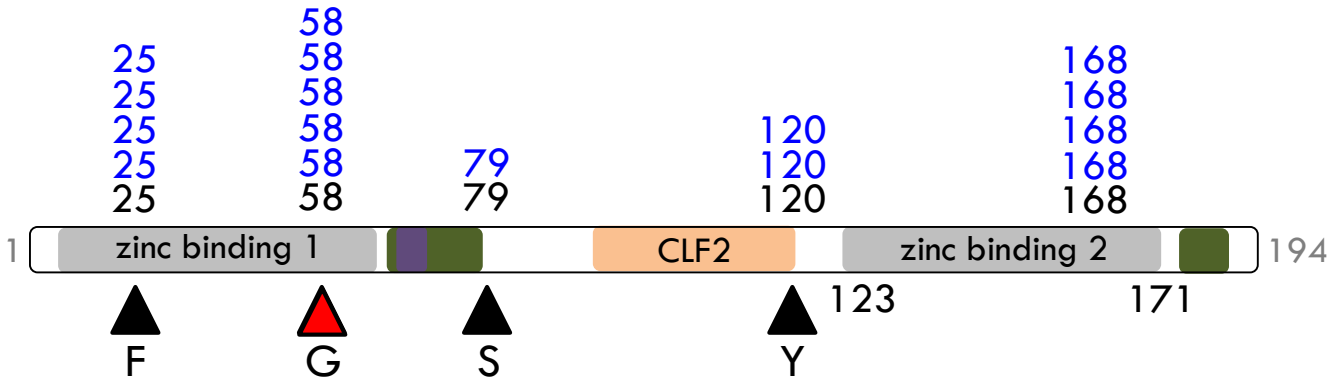
Example: Muscle LIM protein (MLP – P50461; CSRP3)



7 Sites of SNO
(23 observations)

5 identified *in vivo*
(16 observations)

4 human SNPs

1 linked to hypertrophic cardiomyopathy

SNO Modified Proteins are Popular Proteins

Cerebral (1893; 3674)

5.	BDNF/NT-3 growth factors receptor	4 sites
8.	Isocitrate dehydrog. [NADP] cyto.	4 sites
11.	Glutamate receptor zeta-1	744,798
13.	Glial fibrillary acidic protein	294
14.	Glutamate receptor epsilon-1	3 sites

Cardiovascular (1645; 3340)

8.	Troponin I, cardiac muscle	80,97
11.	Nitric oxide synthase, endo.	13 sites
14.	Myosin-7	6 sites
15.	Myosin-binding protein C	651
16.	KCNQ1 channel	445

Pulmonary (1339; 2777)

1.	Epidermal growth factor receptor	5 sites
2.	Pulmonary surfactant-associated D	35,40
14.	GTPase KRas	80
16.	Alpha-1-antitrypsin	256
18.	Major vault protein	59

Hepatic (1616; 3092)

6.	Alanine aminotransferase 1	3 sites
10.	Fatty acid-binding protein, liver	69
18.	Serine--pyruvate aminotransferase	387
20.	Asialoglycoprotein receptor 1	36,139
38.	Regucalcin	4 sites

Intestinal (694; 1566)

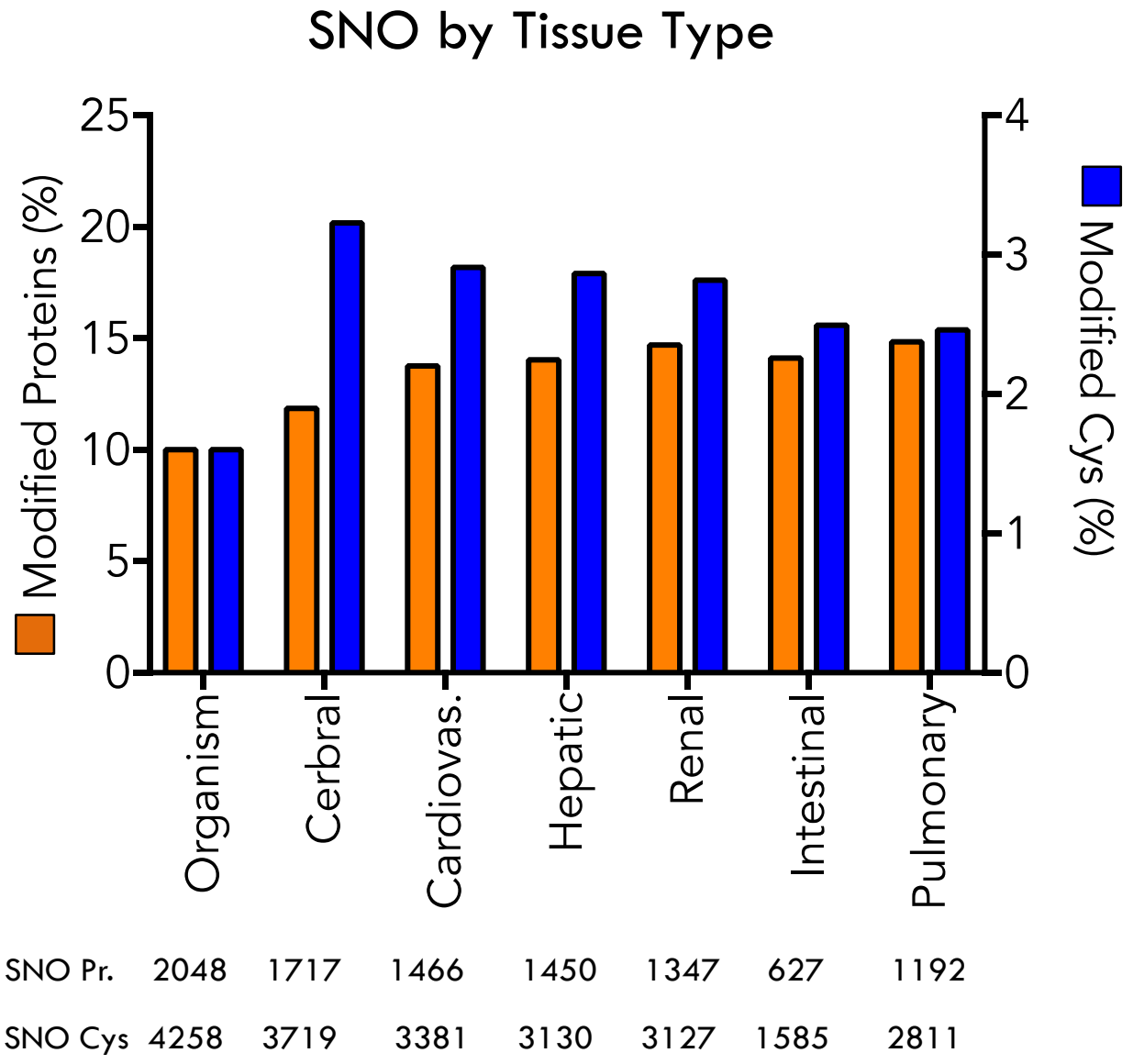
5.	Mucin-2	263
110.	UDP-glucuronosyltransferase 1-8	277,380
113.	Long-chain-FA--CoA ligase 5	3 sites
125.	GTP-binding protein SAR1b	178
145.	Fatty acid-binding protein, liver	69

Renal (1504; 3090)

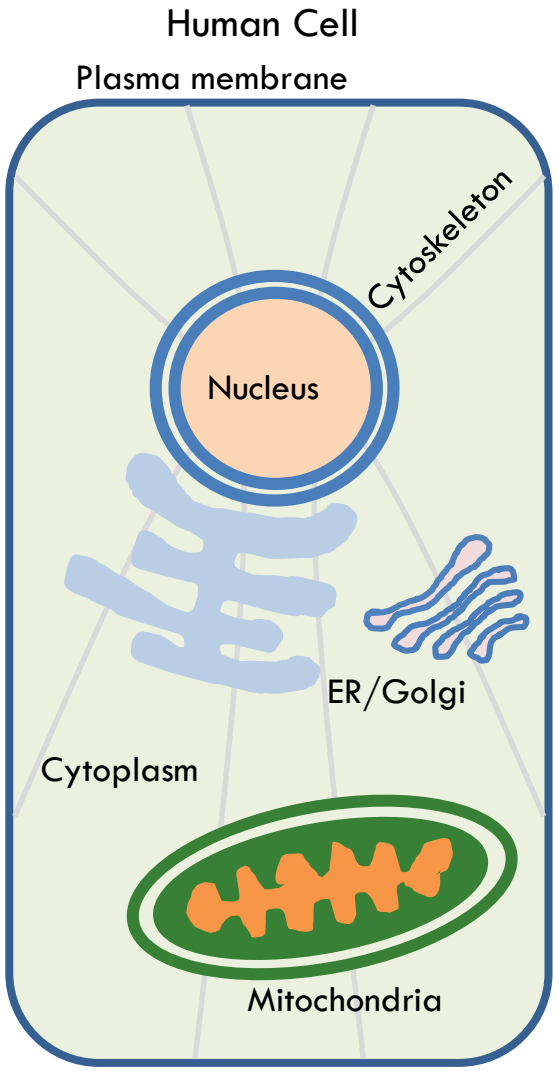
38.	Fumarate hydratase, mito	333,434
105.	Putative 2-oxo-4-decarboxylase	66
116.	Serpin B7	117
133.	Serine--pyruvate aminotransferase	387
138.	Band 3 anion transport protein	201,317

(Total SNO proteins; total SNO sites for each tissue)
Red text indicates the sites annotated in Uniprot

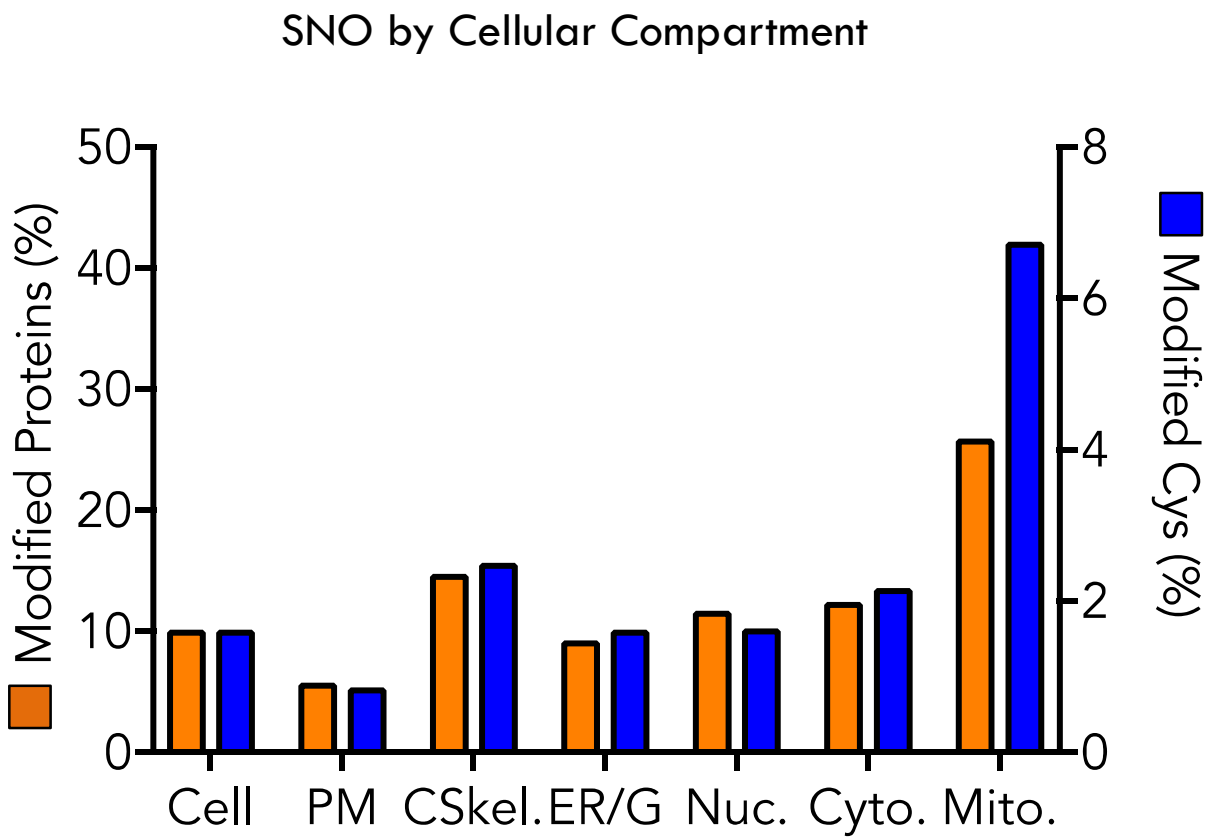
The Brain has the highest proportion of identified SNO sites



Mitochondria has the highest proportion of identified SNO sites



20k proteins; 260k Cys

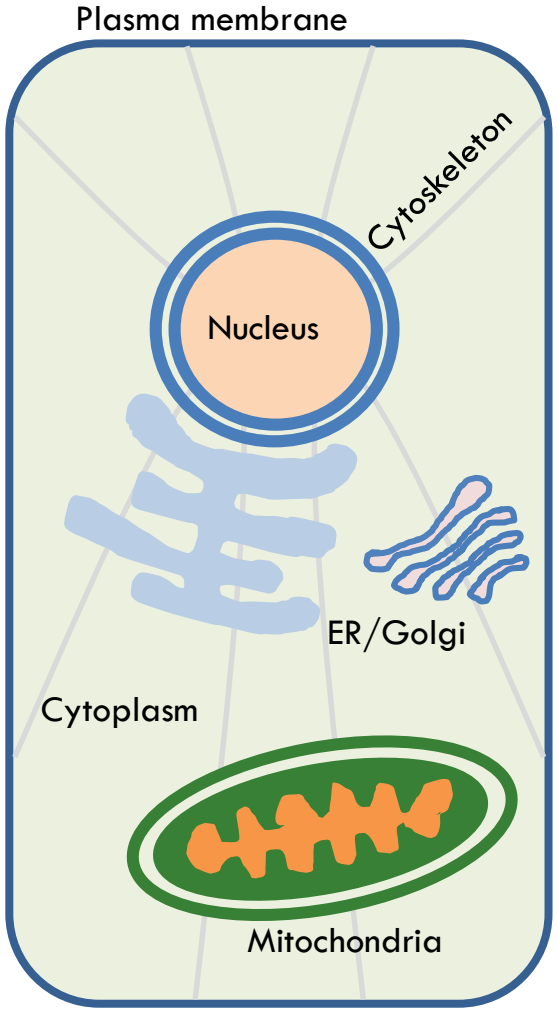


	Cell	PM	CSkel.	ER/G	Nuc.	Cyto.	Mito.
SNO Pr.	2048	190	175	168	647	901	385
SNO Cys	4258	412	427	327	1286	2035	918

S-nitrosylation Modifies Metabolic Pathways

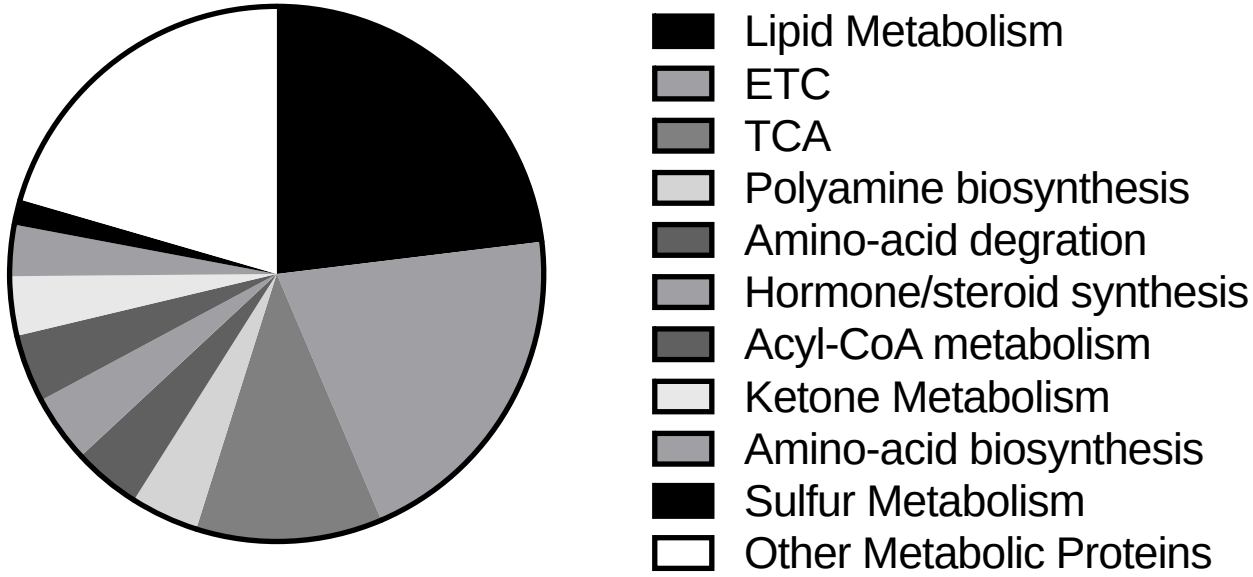
SNO was found to 1403 Cys on 576 proteins involved in metabolic pathways, including a disproportional number of mitochondrial sites.

Human Cell

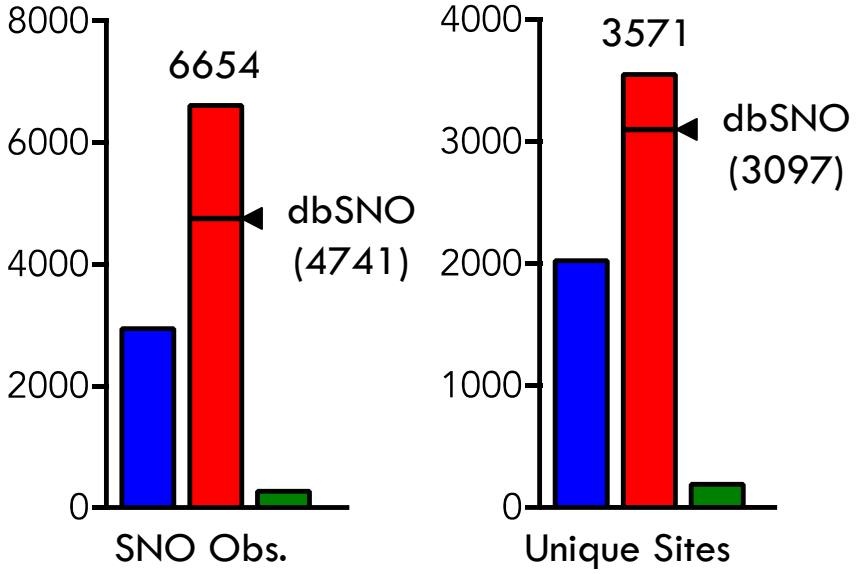


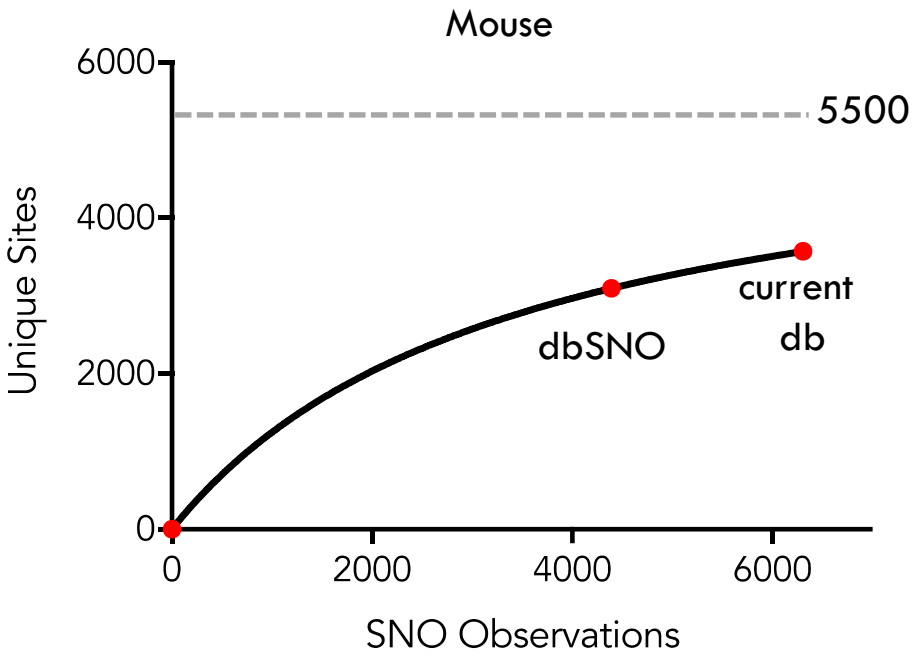
Metab. 2.3k proteins; 28k Cys

SNO Mitochondrial Metabolic Pathways



Estimation of a “complete” SNO database





5500 is likely an upper limit for discovery using standard derivatives of the biotin switch assay

Chung *et al.* (2015) found that using a different thiol reactive chemistry for the label resulted in a ~40% increase in identified SNO sites within that data set (mouse).

Identification of SNO sites beyond this threshold will require the development of more sensitive detection assays

Acknowledgements

Christopher Murray

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Top 5 most popular SNO proteins for each tissue and the modification sites

Cerebral (1375; 3057)

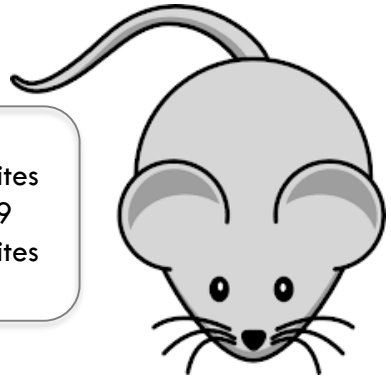
6.	Glial fibrillary acidic protein	291
8.	Calbindin	100
13.	Tubulin beta-3 chain	12,354
20.	Microtubule-associated protein tau	614
22.	BDNF/NT-3 growth factors receptor	4 sites

Cardiovascular (976; 2417)

9.	Actin, aortic smooth muscle	259
10.	Atrial natriuretic factor	129,145
15.	Myosin-6	9 sites
17.	Troponin I, cardiac muscle	81,98
20.	Myosin light chain 2, atrial	3 sites

Pulmonary (594; 1424)

6.	GTPase KRas	80
22.	Cytochrome P450 2F2	3 sites
33.	Actin, aortic smooth muscle	259
44.	Tubulin beta-4 chain	3 sites
96.	Fibulin-5	40



Hepatic (1224; 2771)

9.	Cytochrome P450 2E1	4 sites
17.	Serum albumin	22 sites
21.	Fatty acid-binding protein, liver	69
25.	Fumarylacetoacetase	5 sites
38.	Peroxisomal acyl-coA oxidase 1	4 sites

Intestinal (399; 984)

55.	Fatty acid-binding protein, liver	69
143.	Na(+)/H(+) exchange cofactor	455
154.	Acetyl-CoA acetyltransferase,	65,360
197.	Cytochrome P450 2C54	372,435
219.	ADP-ribosyl cyclase 2	123

Renal (1472; 3136)

139.	Na(+)/H(+) exchange cofactor	201
145.	Estradiol 17-beta-dehydrog. 8	39,192
148.	Laminin subunit alpha-5	3 sites
152.	Alpha-actinin-4	352,794
155.	Solute carrier family 13 member 1	328

(Total SNO proteins; total SNO sites for each tissue)

Number of modified Cys within the top 100 most popular SNO modified proteins for each tissue type