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Mitochondrial targets for multi-target ligand design

Rona R. Ramsay,

Amelia Hunter, Gayle H. Doherty

Biomedical Sciences Research Complex, University of St Andrews, North Haugh, St Andrews KY16 9ST, UK

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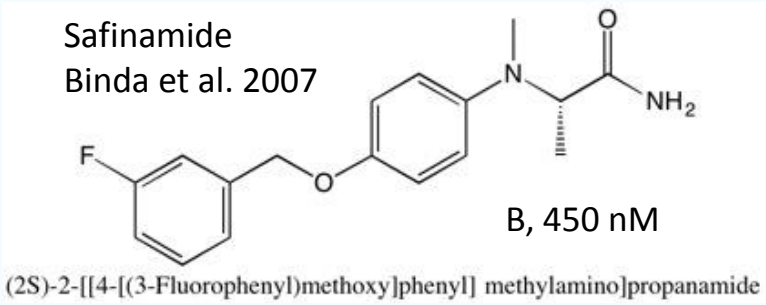
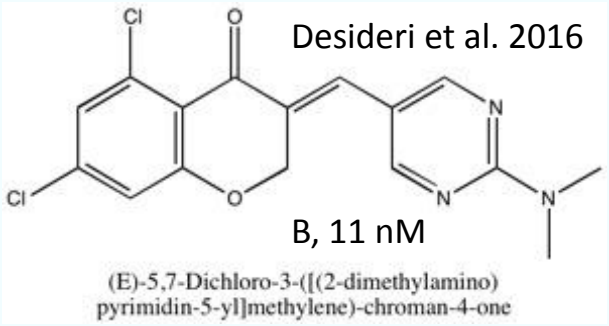
Outline

Why we look for targets to protect mitochondrial function

- Mitochondrial enzymes such as MAO are targets for treatment of neuropathology
- Some MAOI protect mitochondrial function
- Mitochondria interact with the rest of the cell and movement, fusion, and fission are vital to cell survival
- Compromised mitochondrial function leads to cell death, e.g., permeant cations are accumulated – can interfere with ATP production

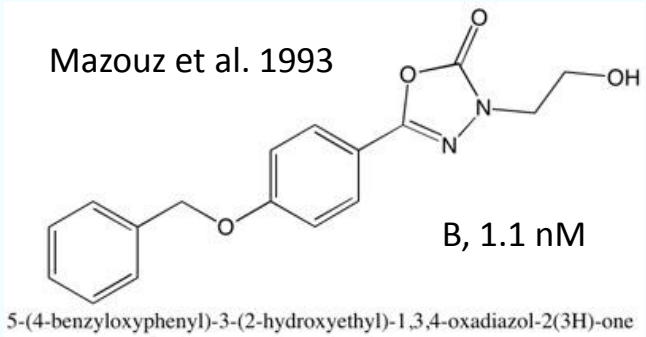
Many structures inhibit MAO B reversibly



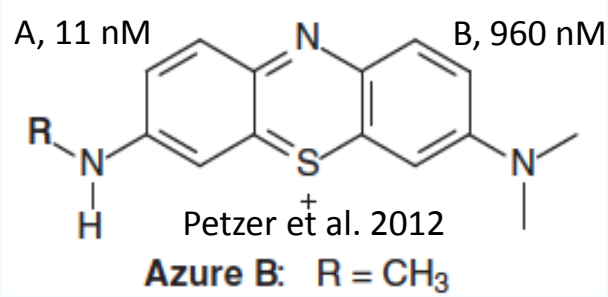


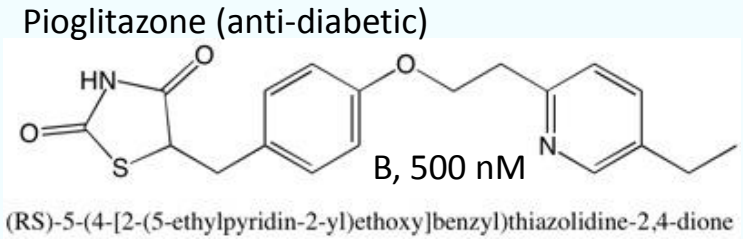
Designed inhibition

OR

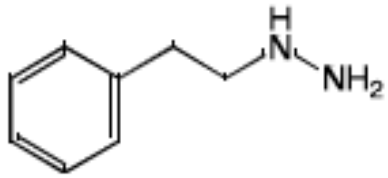
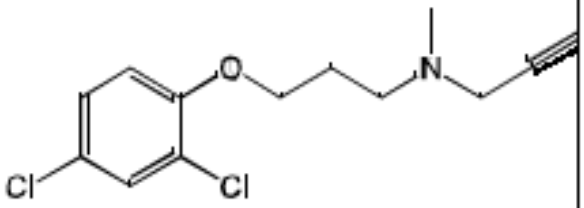
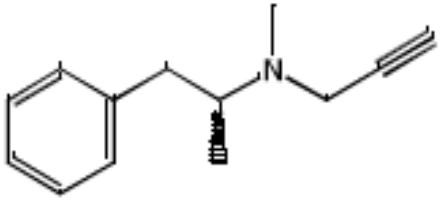
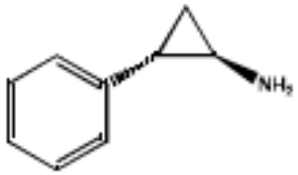


Unintended inhibition



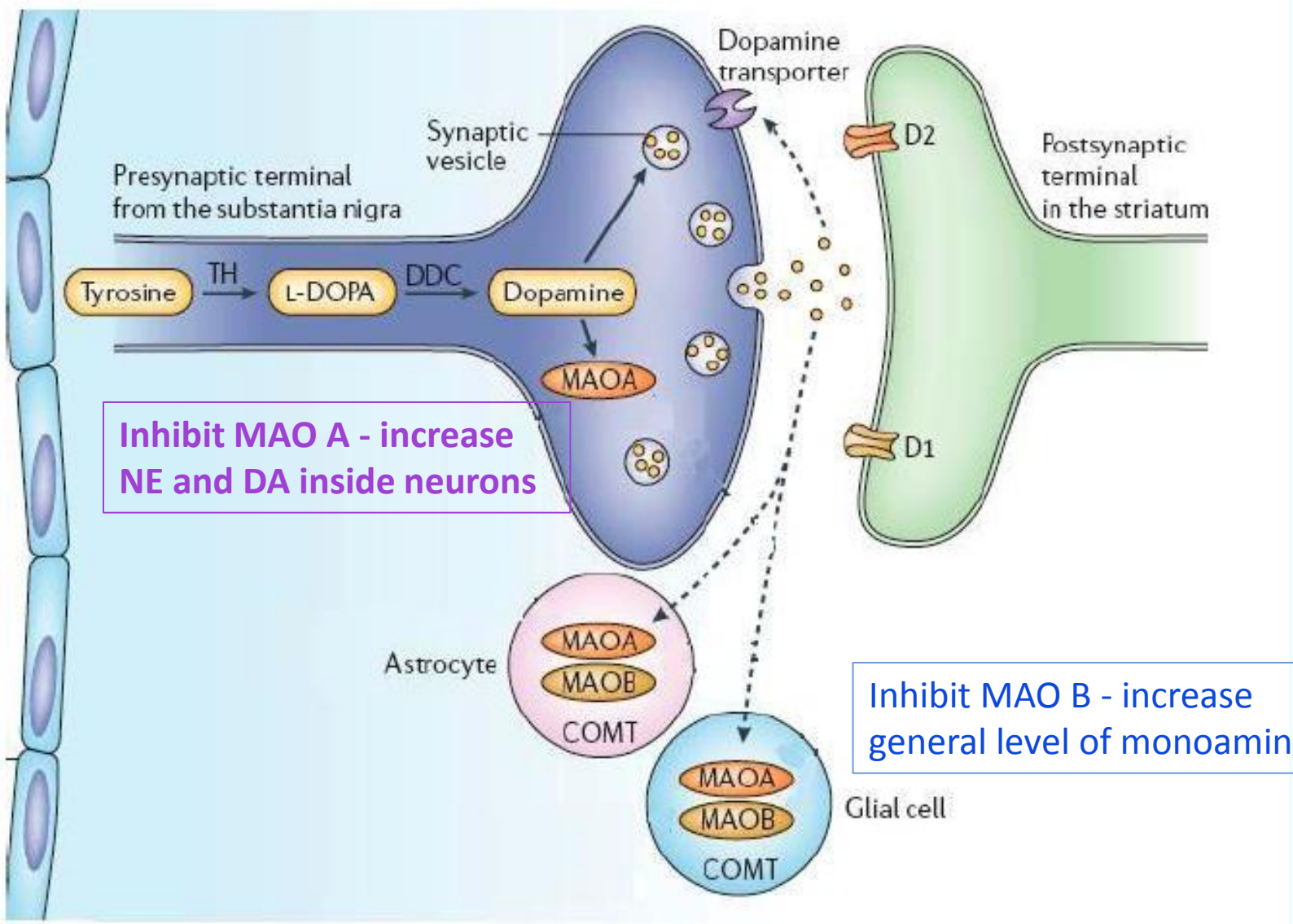


Successful MAO drugs inhibit irreversibly

Chemical type	Example	Structure	Drug name	Selectivity	Inhibition type
Hydrazine	**Phenylzine** 2-phenylethylhydrazine		Nardil	both	Irreversible *flavin N5*
Acetylenic	**Clorgyline** N-(3-(2,4-dichlorophenoxy)propyl)-N-methylprop-2-yn-1-amine		Clorgyline	A	Irreversible *flavin N5*
Acetylenic	**Deprenyl** (R)-N-methyl-N-(1-phenylpropan-2-yl)prop-2-yn-1-amine		Selegiline	B	Irreversible *flavin N5*
Cyclopropylamine	**Tranylcypromine** (1R,2S)-2-phenylcyclopropanamine		Parnate	B>A	Irreversible *flavin C4a*

Why inhibit MAO, and which form ?

MAO oxidises neurotransmitters and scavenges biogenic amines



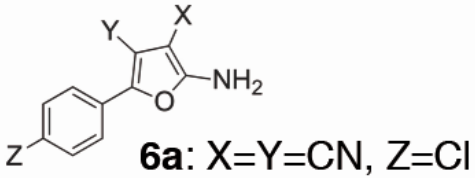
Assessing MAO inhibition – caution !

- Enzyme: rat and human MAO differ
- Substrates: MAO A and B have different Km values for all the common substrates
- IC_{50} value varies with the substrate concentration
- In the coupled assay, beware inhibition of horseradish peroxidase
- There are two forms of the enzyme that can bind inhibitor: oxidized and reduced. The two forms have different affinities for ligands
- Definitive parameter for reversible inhibition is K_i , BUT for irreversible inhibition there is a time factor, so need K_I and k_{inact}

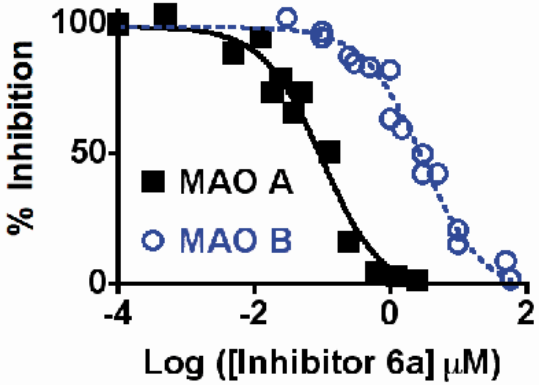
Reversible inhibition e.g. 2

Kinetics, computation and spectroscopy show origin of specificity

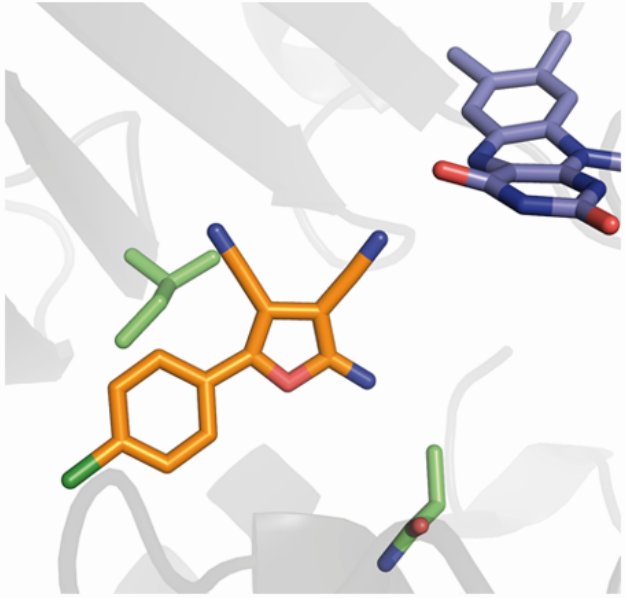
Selectivity



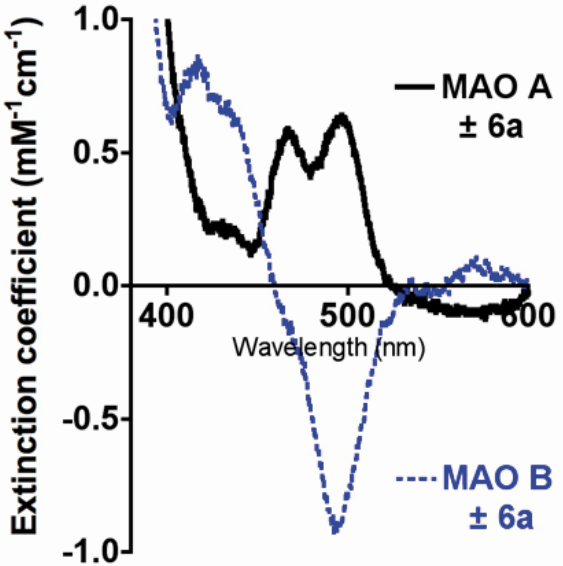
6a: X=Y=CN, Z=Cl



Binding Mode

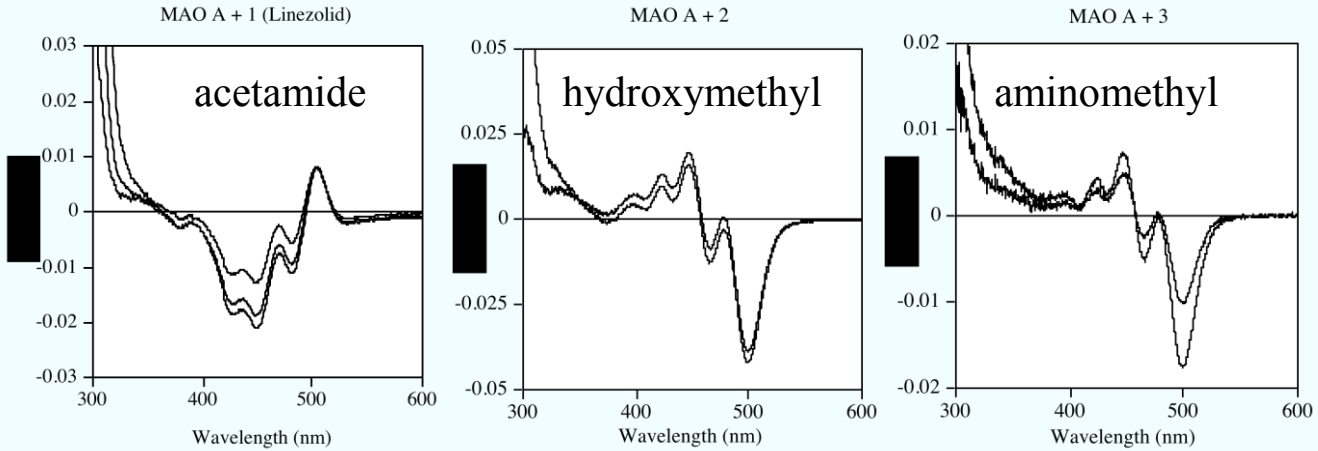


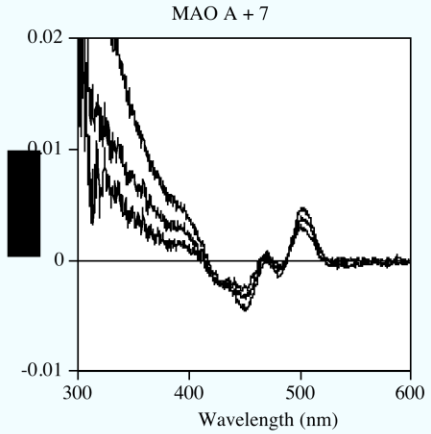
Spectral Changes

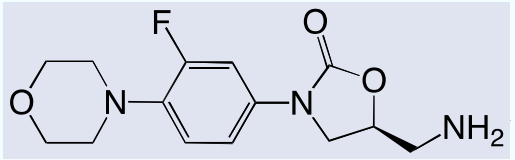


Juárez-Jiménez et al **(2014) Biochim. Biophys. Acta 1844: 389–397** Exploring the structural basis of the selective inhibition of monoamine oxidase A by dicarbonitrile aminoheterocycles: Role of Asn181 and Ile335 validated by spectroscopic and computational studies.

Type of spectral change depends on the end group and this permits determination of the orientation of oxazolidinones in MAO A



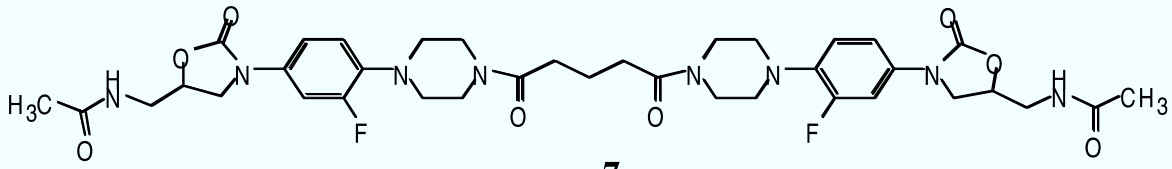




1 -NHCOCH$_3$

2 -OH

3 -NH$_2$



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Jones et al. (2005) Biochemical Pharmacology 70, 407-416

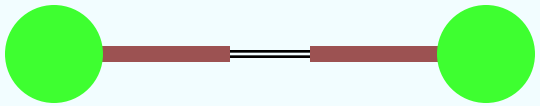
Orientation of **oxazolidinones** in MAO A

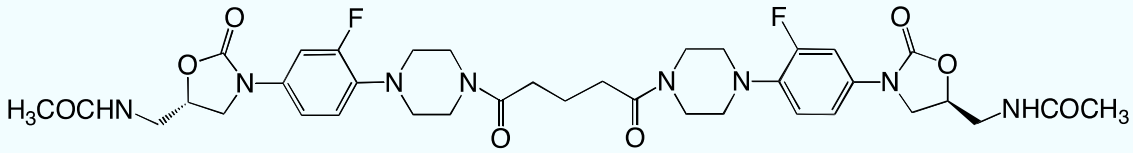
Jones et al., 2005

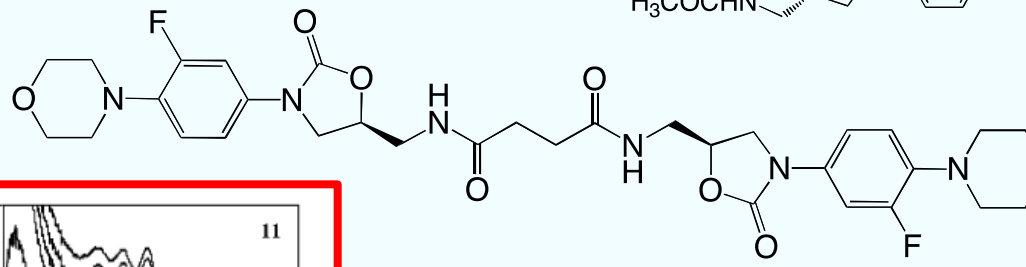
Oxa molecules with small terminal groups can orient in either direction:

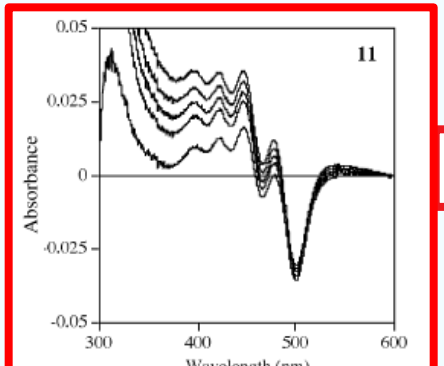
or

So test inhibition by double-ended molecules:

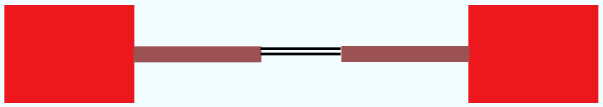




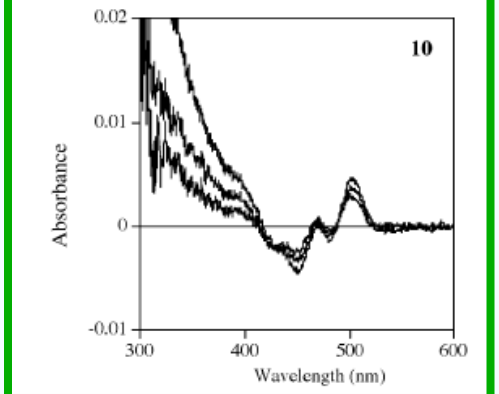




IC_{50} = 90 uM



IC_{50} = 0.5 uM



Compare reversible binding with inactivation

µM **nM**

	Reversible	Irreversible		
	K_i (µM)	IC_{50} (nM)		MAO A Selectivity
	MAO A	MAO A	MAO B	IC_{50} B/ IC_{50} A
ASS234	0.053 ± 0.013	0.17 ± 0.03	15830 ± 1040	93118
Clorgyline	0.014 ± 0.001	0.42 ± 0.08	10660 ± 953	25380
PF9601N	25 ± 5	790 ± 105	11 ± 2	0.0139
L-Deprenyl	75 ± 11	630 ± 86	3.0 ± 0.9	0.0048
Tranylcypromine	6.7 ± 0.5	237 ± 61	73.5 ± 4.9	3.2

K_i : Reversible inhibition

IC_{50}: Activity was measured after 30 minutes incubation with the inhibitor using 1 mM tyramine as substrate for both MAO A and MAO B.

Outline

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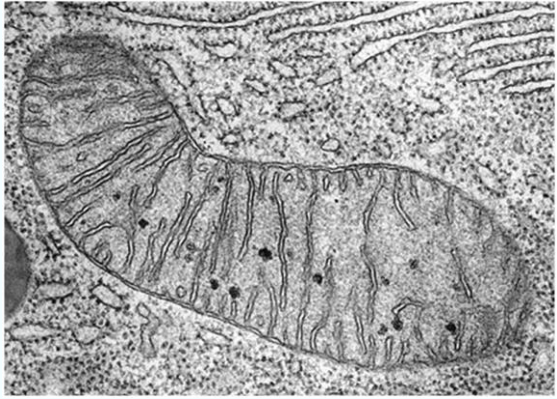

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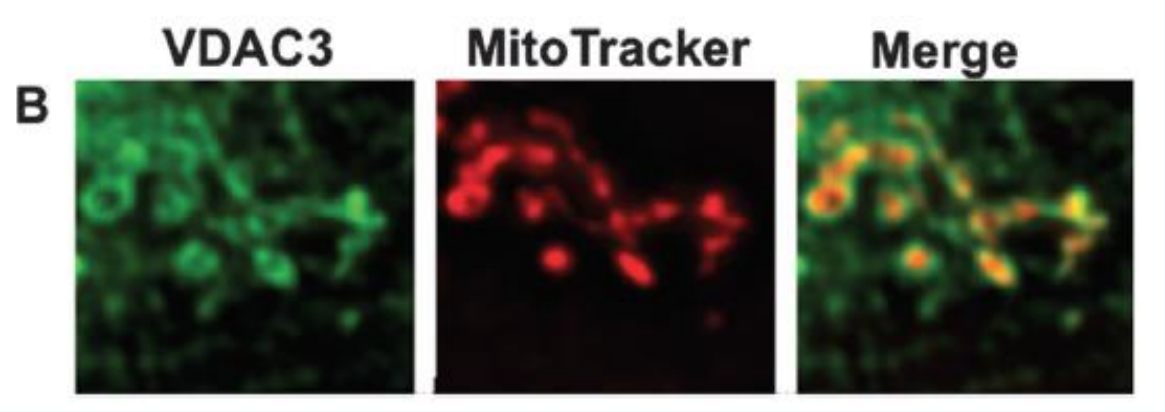


(Richard Clark)

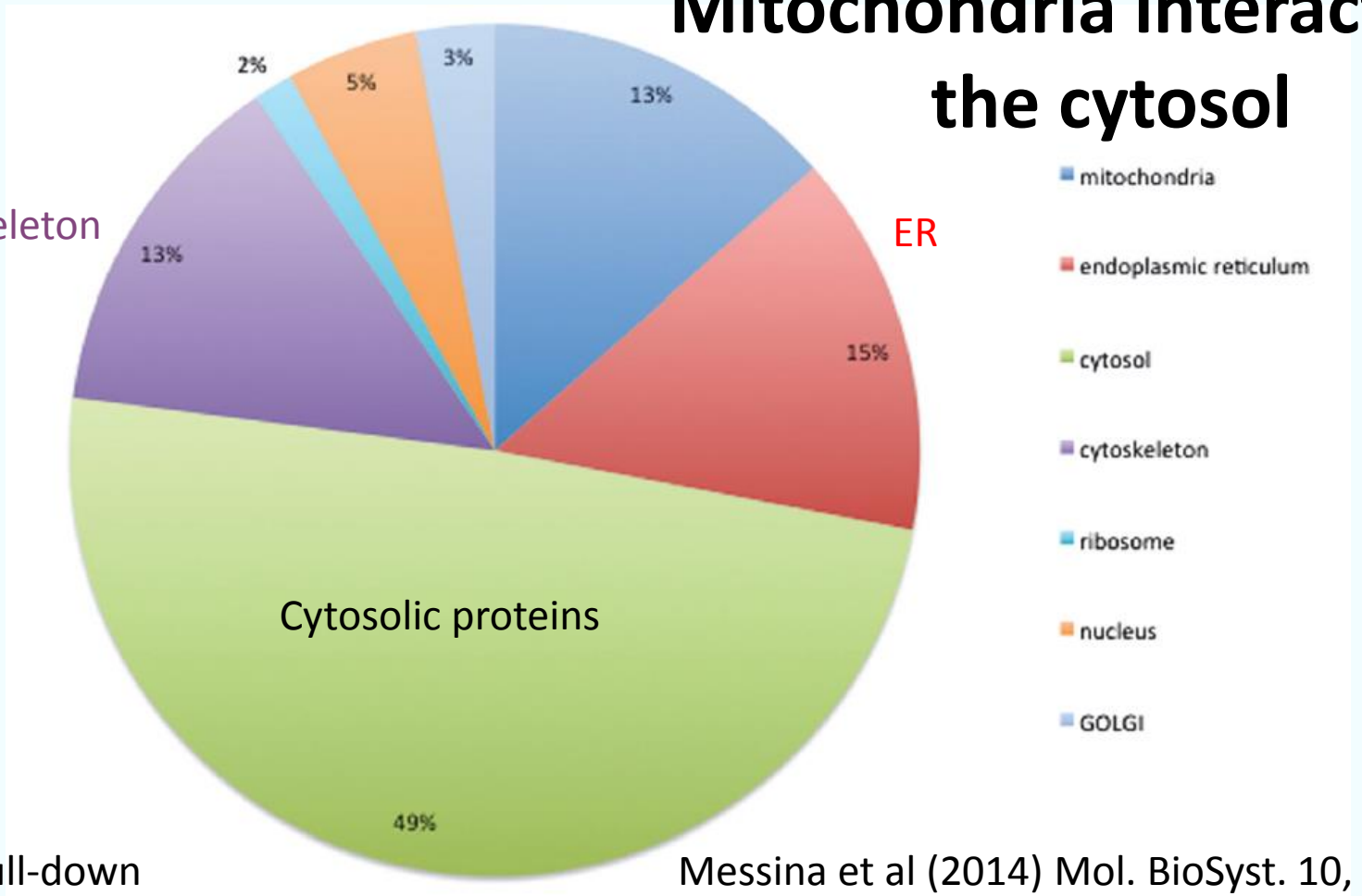
Mito: 0.2 of liver cell volume
(0.5 of heart cell volume)

Williams et al. PNAS 110, 10479–10486

Mitochondria interact with the cytosol

cytoskeleton

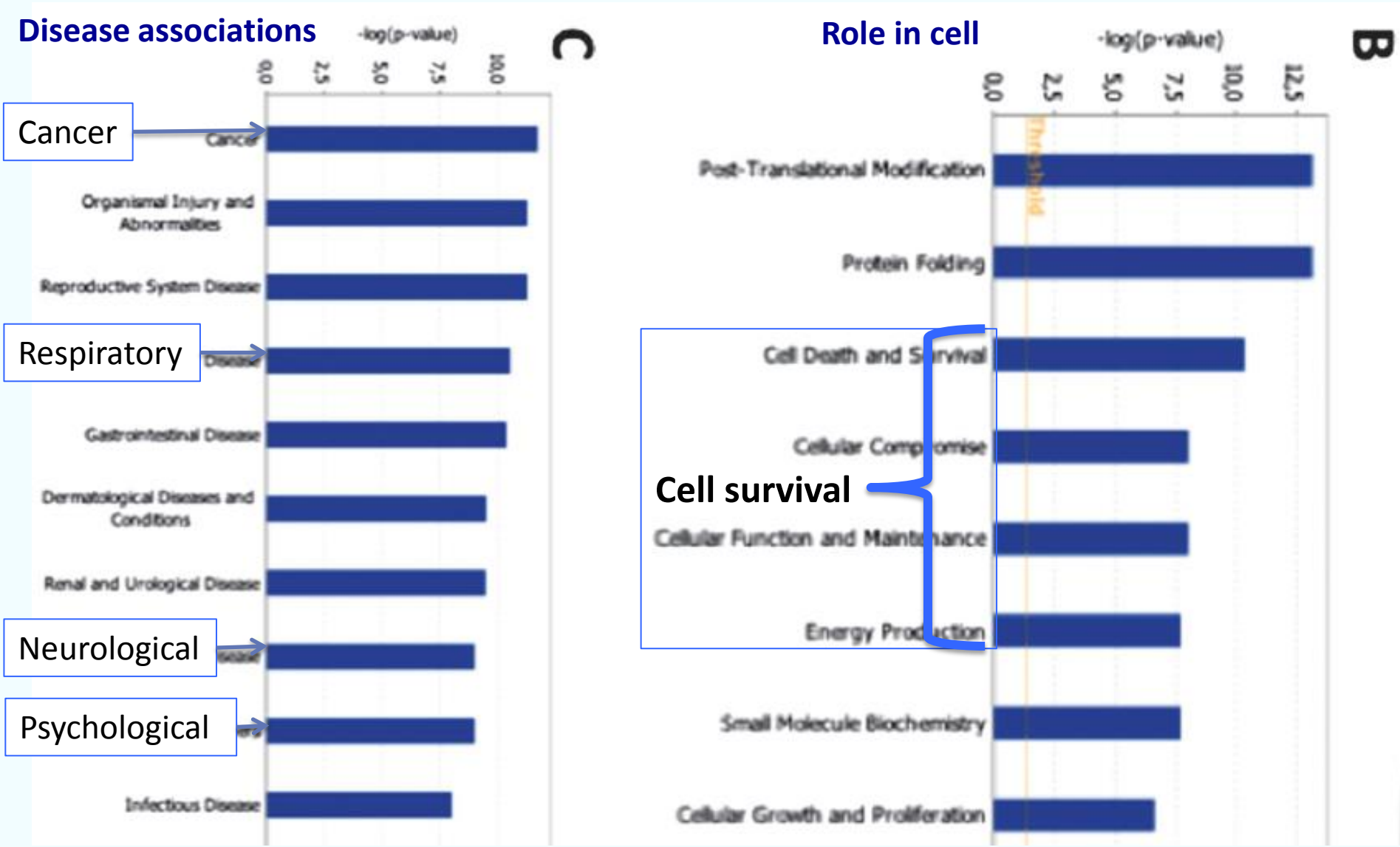
ER



VDAC pull-down

Messina et al (2014) Mol. BioSyst. 10, 2134--2145

VDAC interactome: pathways and diseases



Messina et al (2014) Mol. BioSyst. 10, 2134--2145

New experiments
(not published so data deleted)

- Seahorse Analyser

  Measured oxygen consumption and ATP generation in SY5Y cells before and after full differentiation into neuronal cells: MAOI protect against loss of bioenergetic function. (Mechanism unrelated to MAO inhibition?)

- HCA stress

  decreased cell viability, increased ROS production, increased stressed morphology, increased MFN1 and FIS1: clorgyline and tranylcypromine effects differ

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