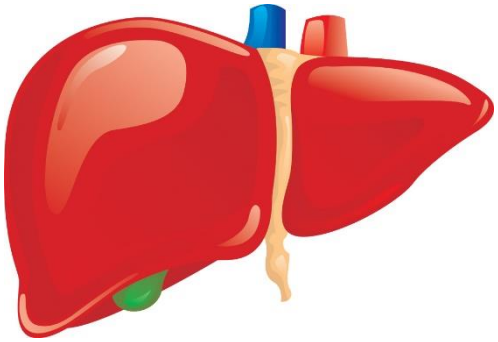
Zellweger Syndrome Spectrum and *PEX1*

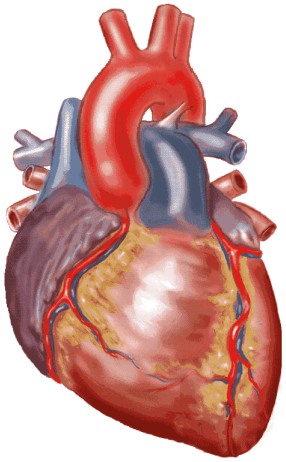
Gina Castelvecchi

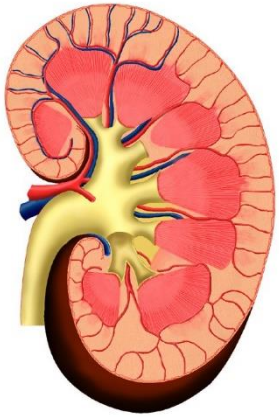
?

What is Zellweger Syndrome Spectrum (ZSS)?

What are the symptoms of ZSS?



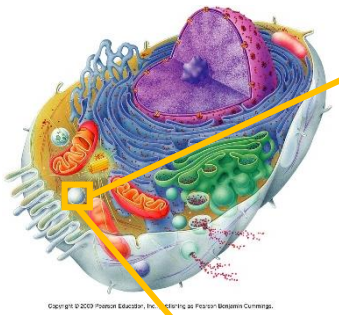




↑ long chain fatty acids

↓ plasmalogens

What are peroxisomes?



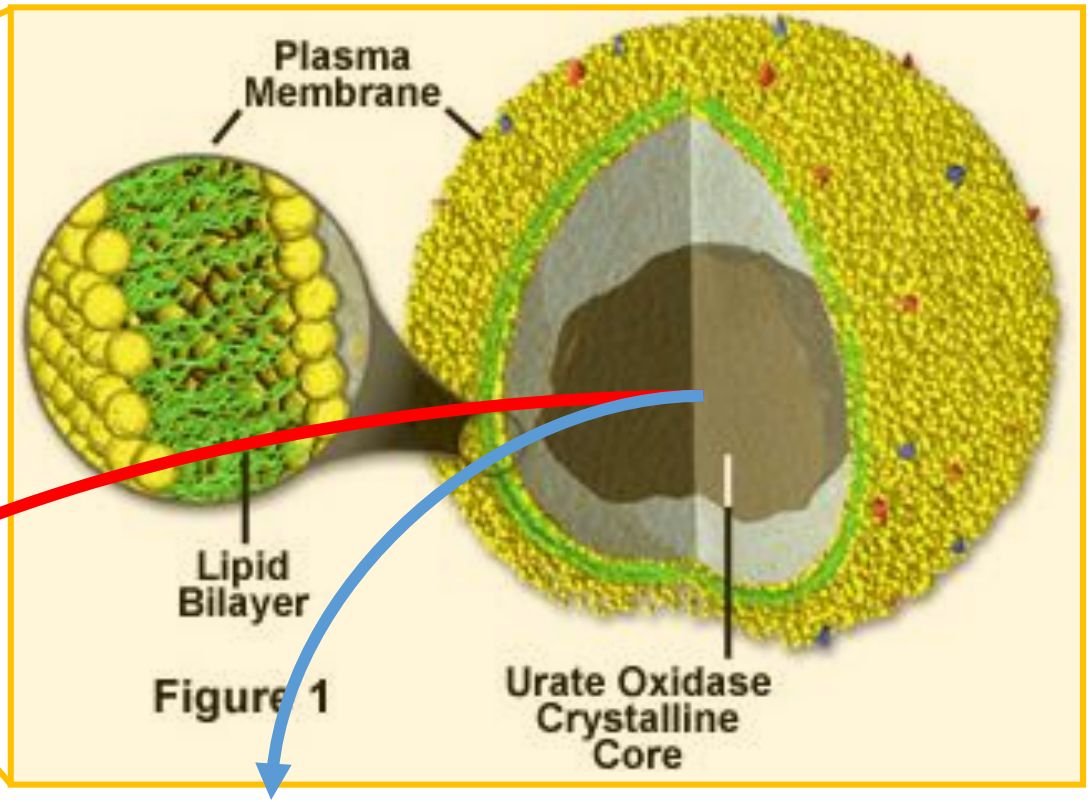
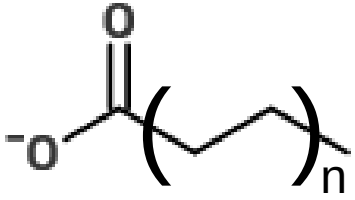


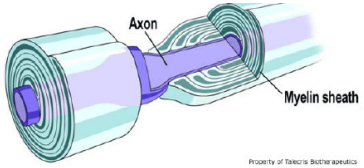
Figure 1

Breakdown:

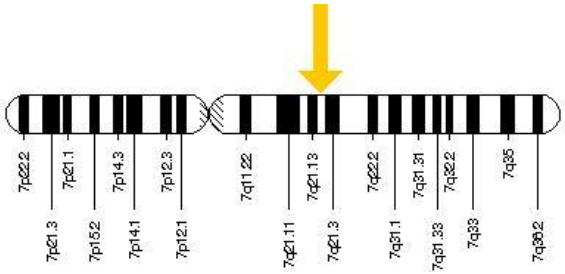


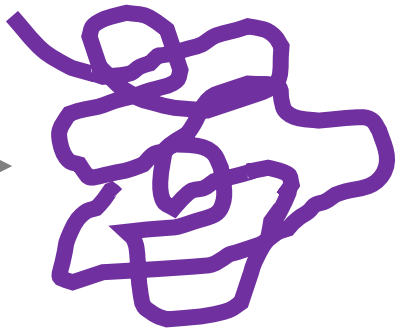
Synthesis:

plasmalogens:



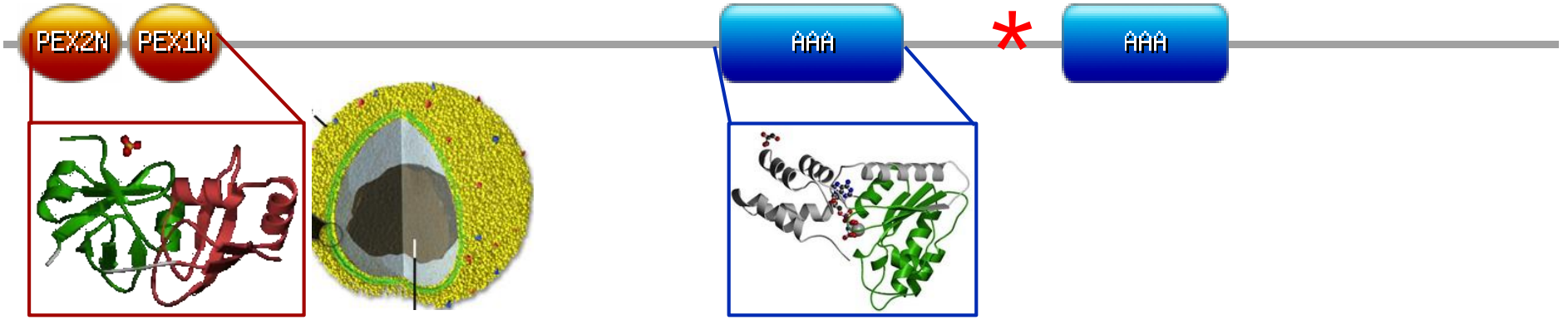
What is the genetic basis for ZSS?





PEX1: C→A

PEX1: G843D
(misfolded)



PEX2N/PEX1N domain

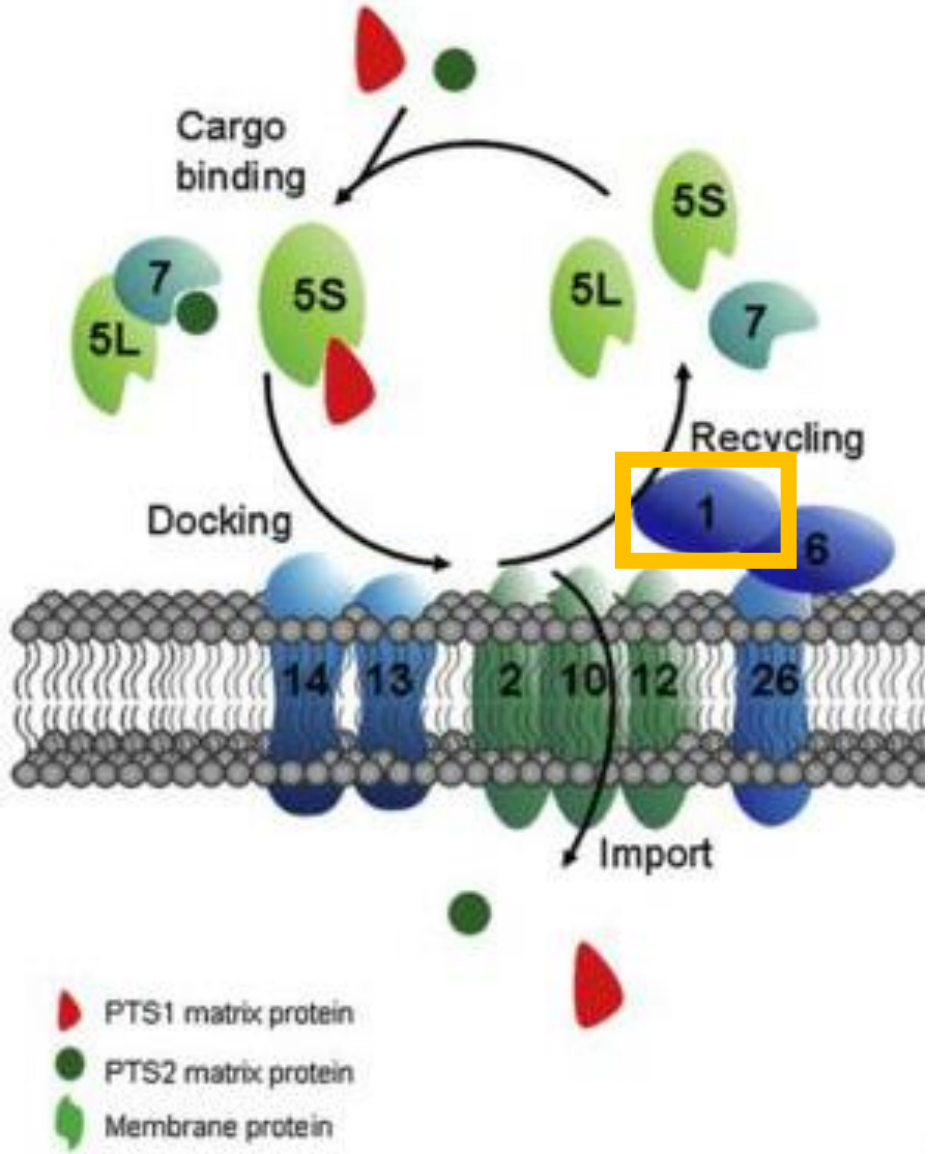
Phospholipid binding

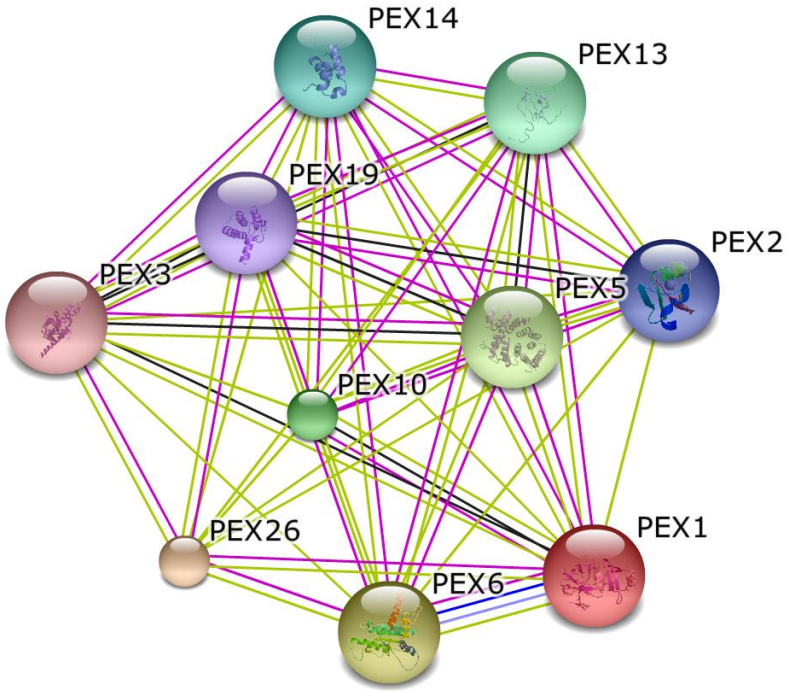
AAA ATPase domains

ATP hydrolysis

Protein-protein interaction

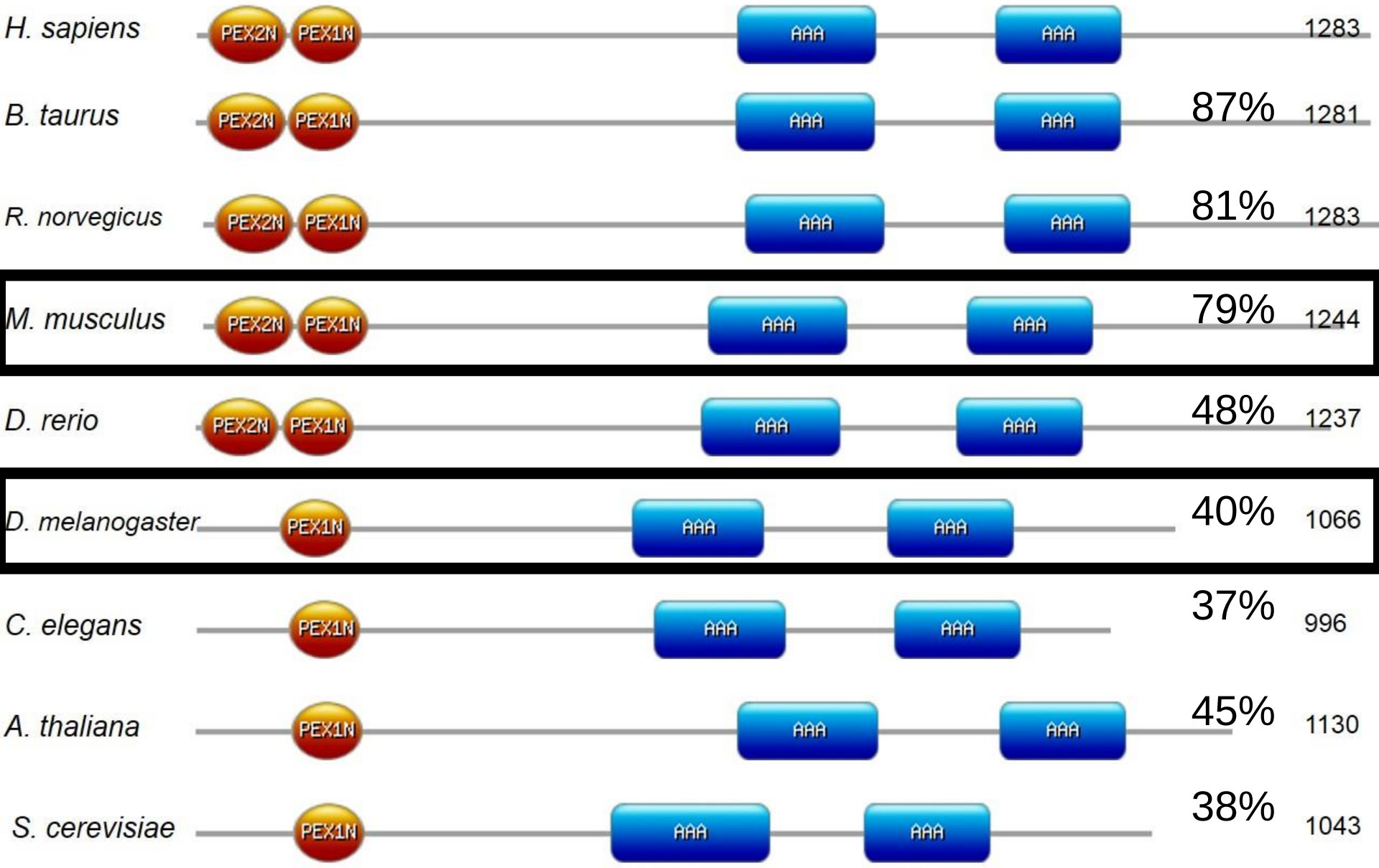
What is the role of *PEX1*?



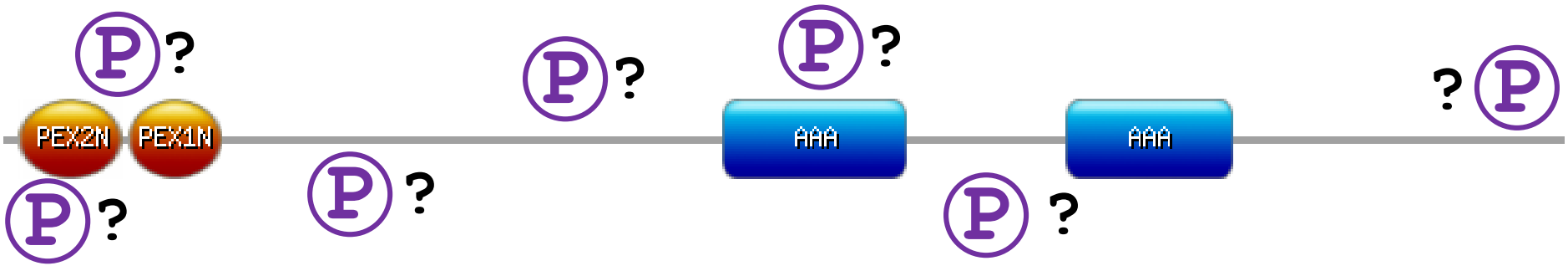


STRING

How well-conserved is PEX1?



Overall goal: to understand how phosphorylation affects PEX1 structure and function

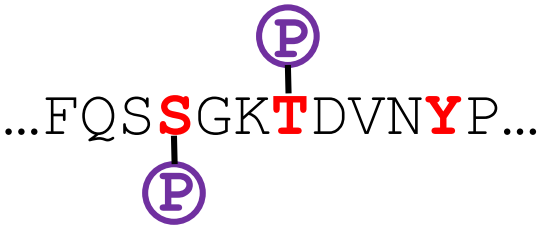


Hypothesis:
Phosphorylation of PEX1 is required for proper tertiary structure and function

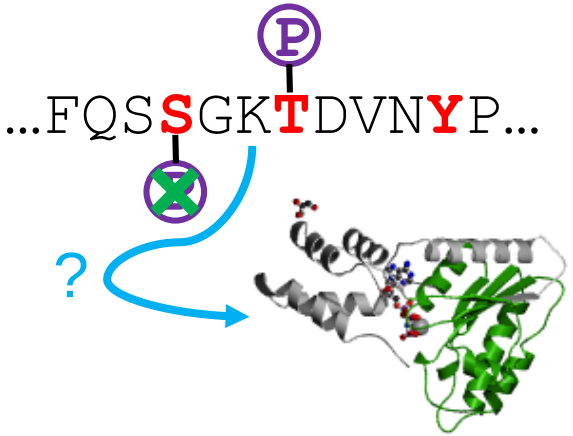
Aim 1:
Predict conserved phosphorylation sites

...FQ**S**S**S**GK**T**DVN**Y**P...
...FQ**S**S**S**GK**T**DVN**Y**P...
...FQL**S**GK**T**DVN**Y**P...

Aim 2:
Determine phosphorylated sites *in vivo*

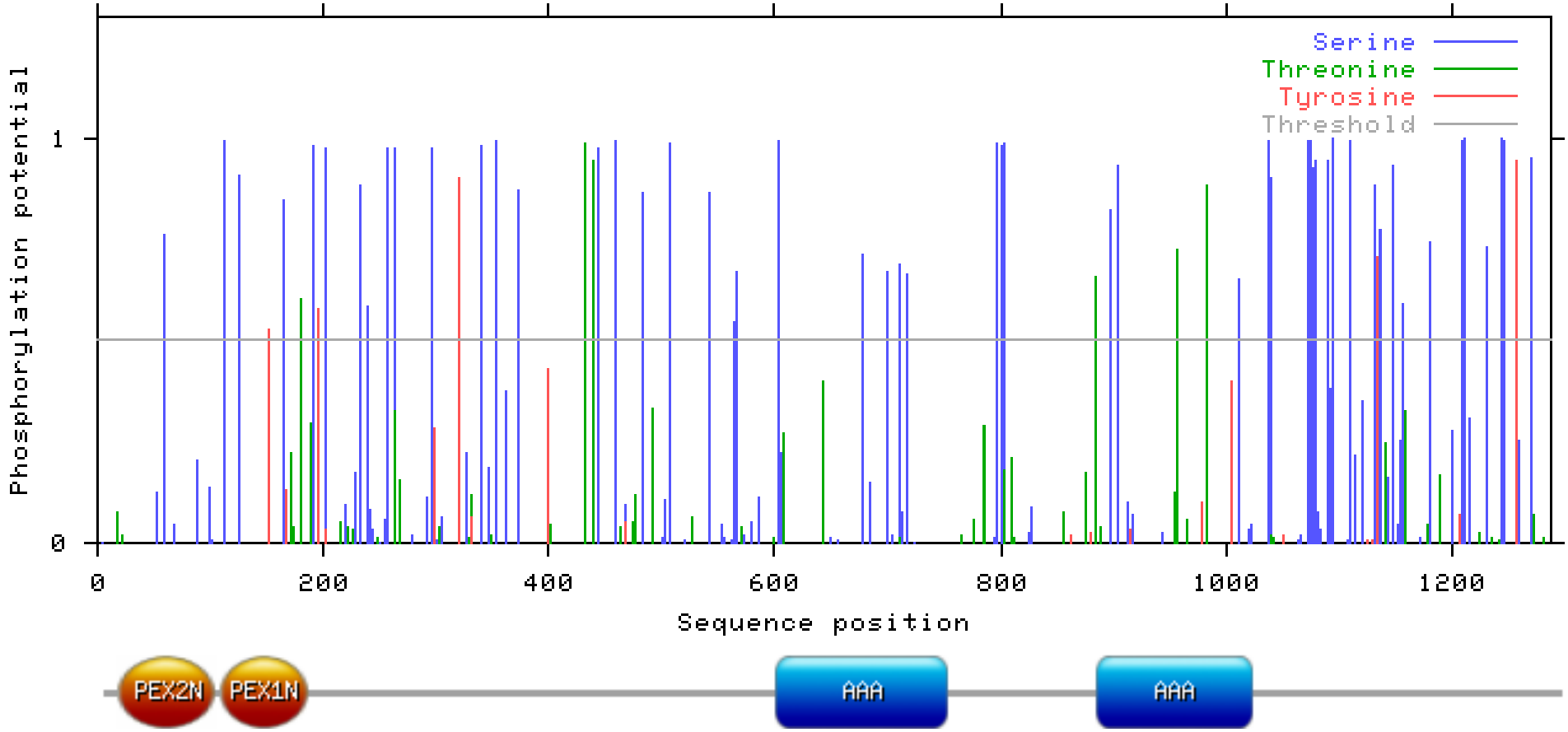


Aim 3:
Determine which phosphorylation sites are required



Aim 1: To identify predicted phosphorylation sites

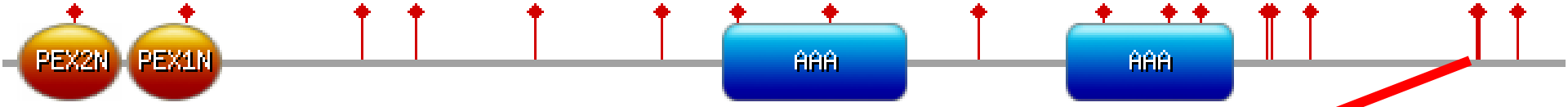
Predicted phosphorylation sites in human PEX1



Data generated with NetPhos 2.0 from Tech U. of Denmark

Aim 1: To identify which predicted phosphorylation sites are conserved

Identify candidate sites with NetPhos 2.0 → Align sites with Clustal Omega → Select sites with high score and conservation



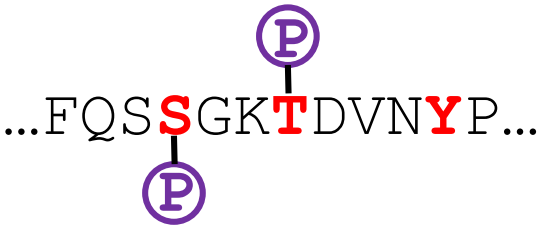
H. sapiens:	GRYR**S**Q**S**GE
B. taurus:	GRYR**S**Q**T**GE
R. norvegicus:	GRYQ**S**Q**S**GE
M. musculus:	GRYR**S**Q**S**GE
D. rerio:	TTDE**S**S**S**SL
D. melanogaster:	TTRP**S**L**S**AS
C. elegans:	IDQA**S**IESV
A. thaliana:	KTKP**S**V**S**ET
S. cerevisiae:	ETKP**S**I**S**TS

Hypothesis:
Phosphorylation of PEX1 is required for proper tertiary structure and function

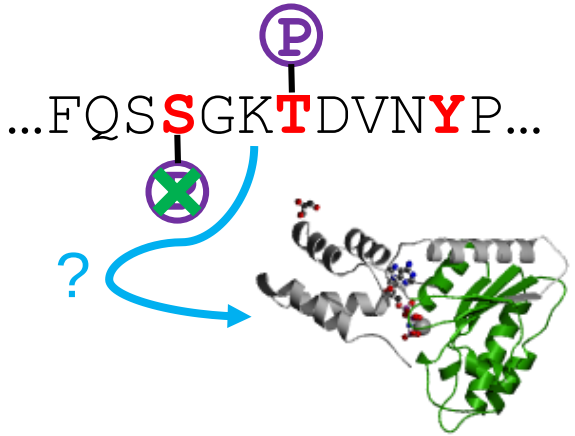
Aim 1:
Predict conserved phosphorylation sites

...FQ**S**S**S**GK**T**DVN**Y**P...
...FQ**S**S**S**GK**T**DVN**Y**P...
...FQL**S**GK**T**DVN**Y**P...

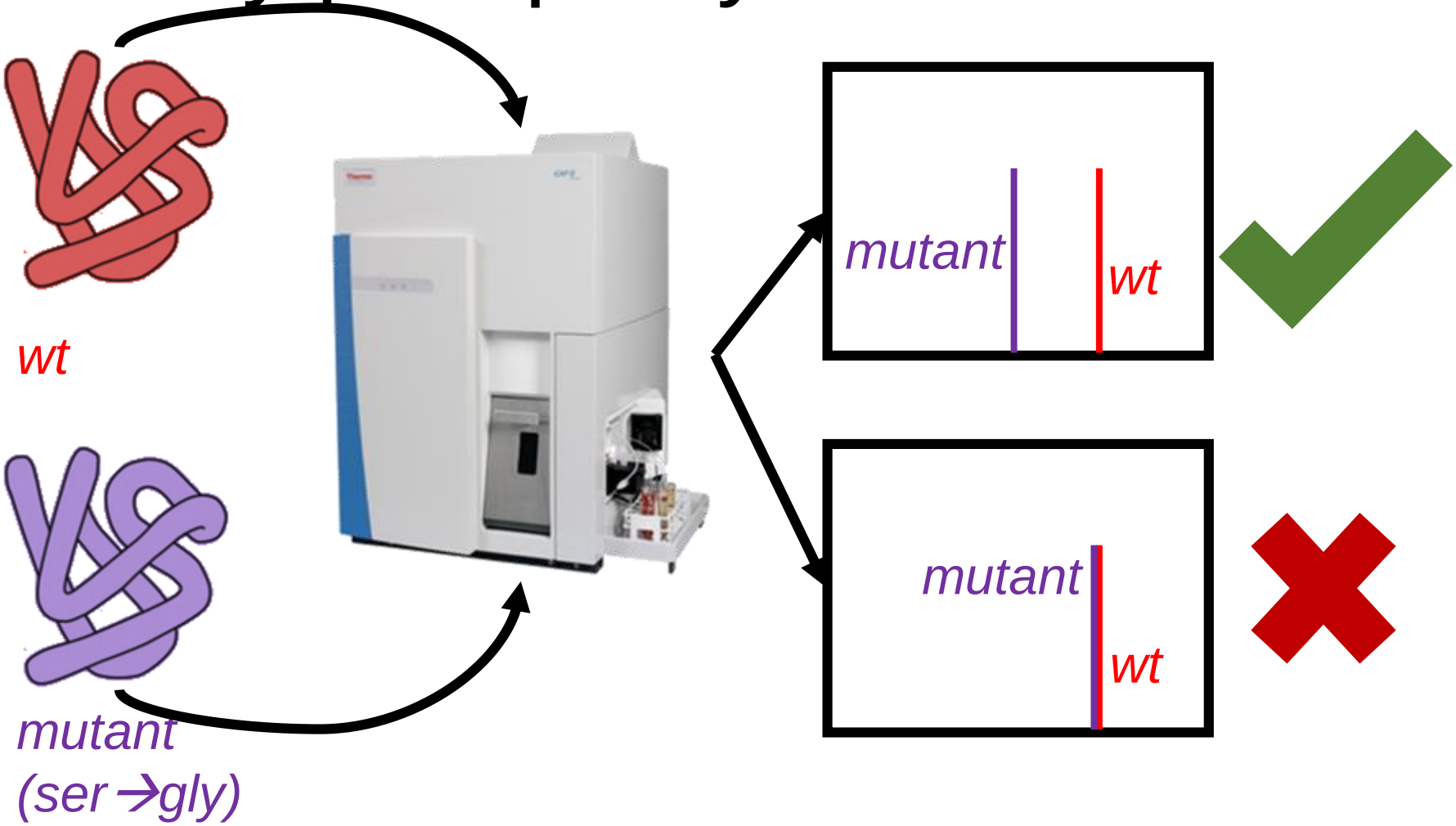
Aim 2:
Determine phosphorylated sites *in vivo*



Aim 3:
Determine which phosphorylation sites are required



Aim 2: To determine which sites are actually phosphorylated *in vivo*

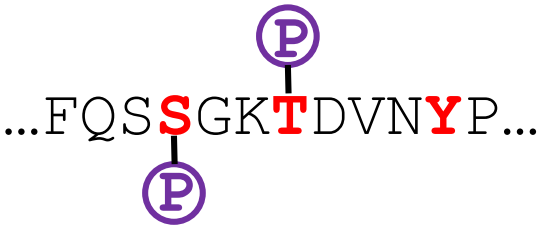


Hypothesis:
Phosphorylation of PEX1 is required for proper tertiary structure and function

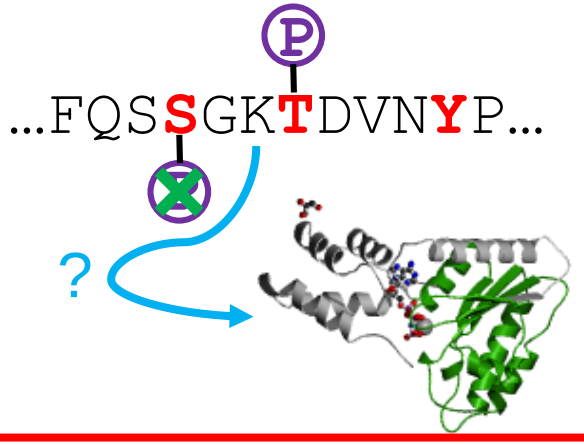
Aim 1:
Predict conserved phosphorylation sites

...FQ**S**S**S**GK**T**DVN**Y**P...
...FQ**S**S**S**GK**T**DVN**Y**P...
...FQL**S**GK**T**DVN**Y**P...

Aim 2:
Determine phosphorylated sites *in vivo*



Aim 3:
Determine which phosphorylation sites are required

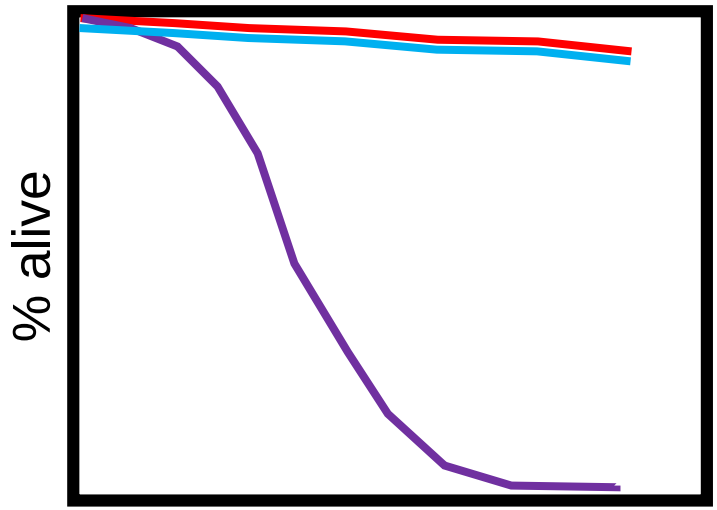


Aim 3: To determine which phosphorylation sites are required for PEX1 function

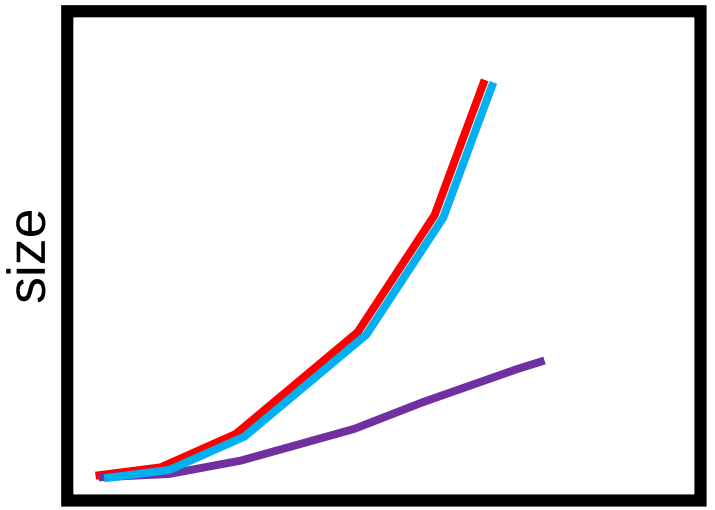
phosphorylation mutants

wt *m/+* *m/m*

Survival

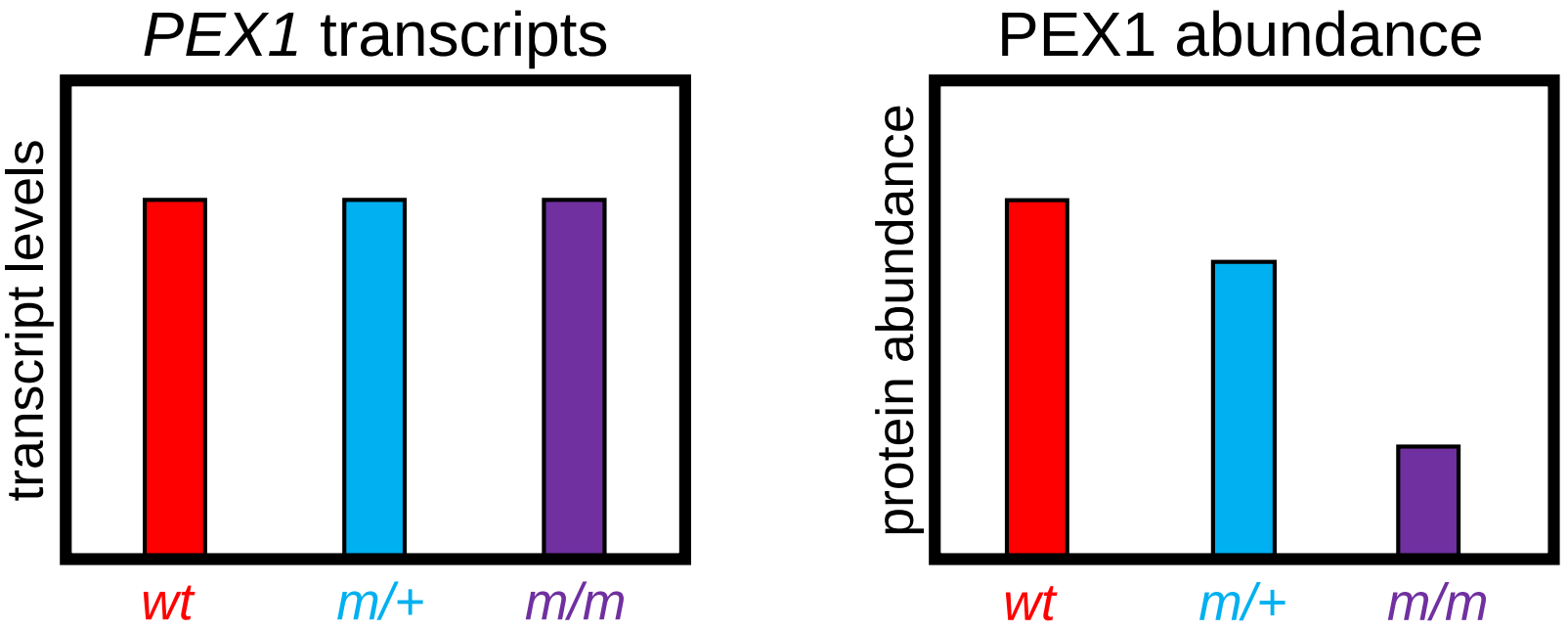


Growth



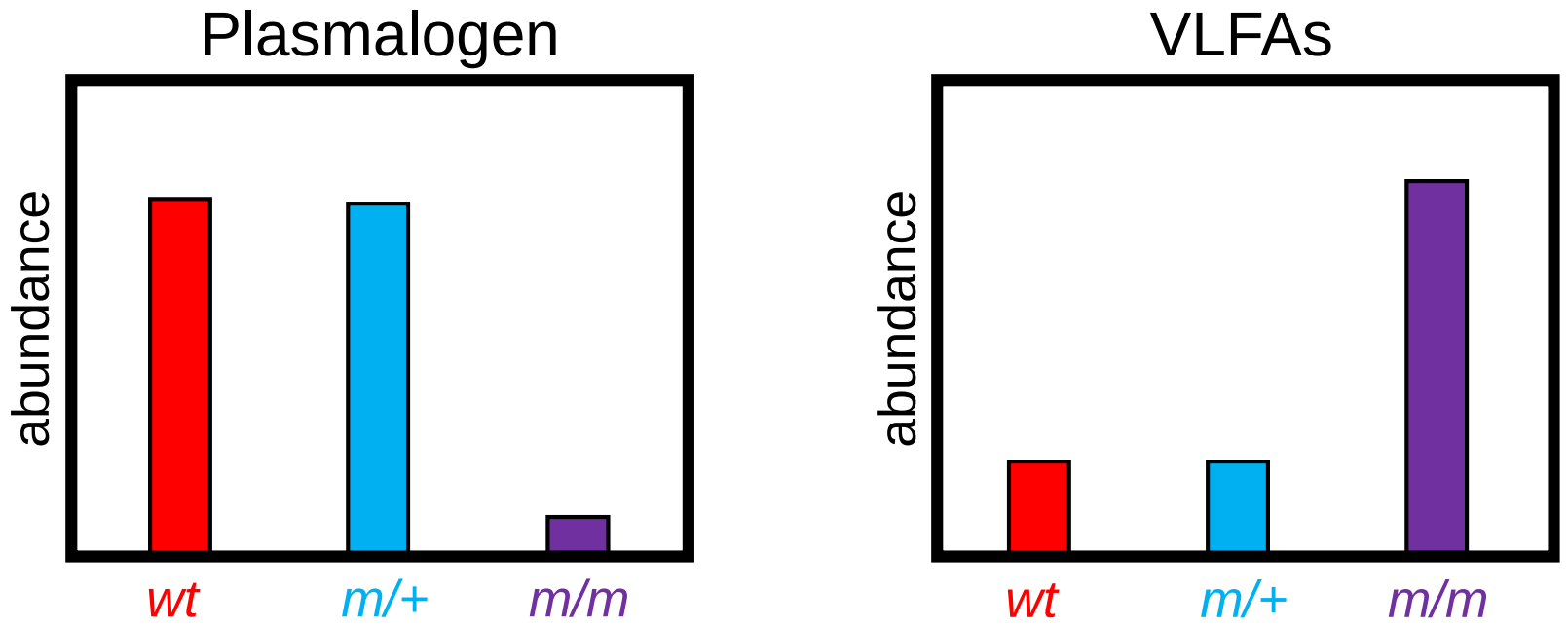
Aim 3: To determine which phosphorylation sites are required for PEX1 function

phosphorylation mutants



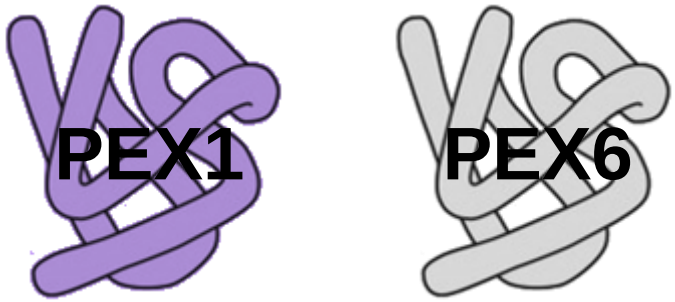
Aim 3: To determine which phosphorylation sites are required for PEX1 function

phosphorylation mutants



Future Directions

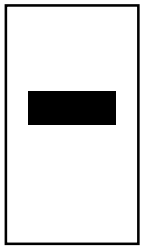
wt



mutant

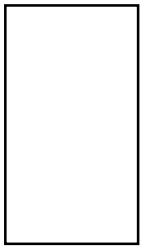
Co-IP: pull down PEX1

lysate, α-PEX6

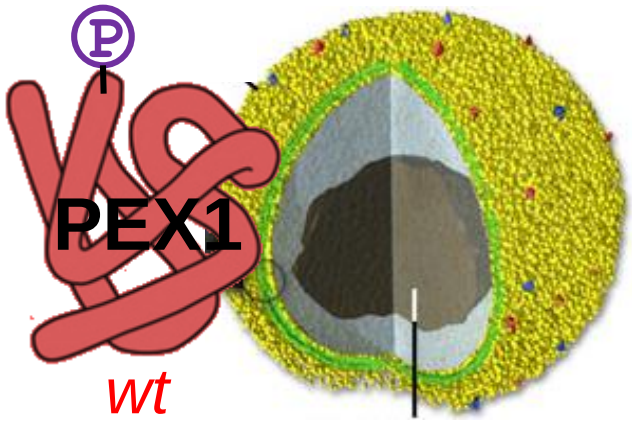


Co-IP: pull down PEX1

lysate, α-PEX6

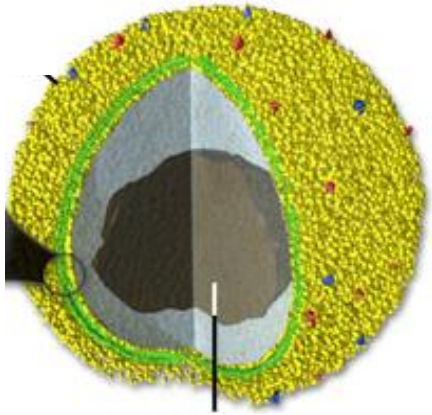


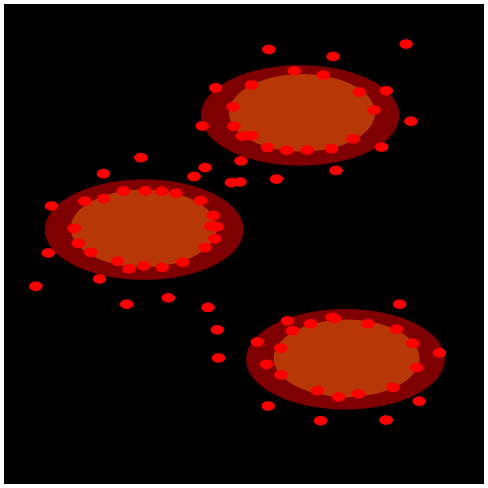
Future Directions



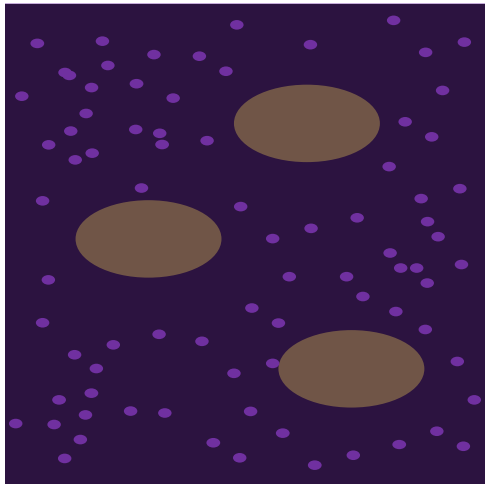
wt

mutant





peroxisome
α-PEX1 (*wt*)



peroxisome
α-PEX1 (*mutant*)

References

Images

Slide 1

Background: http://www.iatsmartdial.com/2013/10/25/compliant-dialing-its-in-our-dna-how-about-yours/

Slide 2

Renee Zellweger: http://www.philly.com/philly/blogs/phillygossip/Renee_Zellweger_spends_St_Patricks_Day_in_Jenkintown.html

Babies: faceinhole.com

Slide 3

ZSS Children: http://thejourneyweareon.blogspot.com/2012/05/faces-of-peroxisomal-biogenesis.html

Slide 4

Liver: http://www.hairlossrevolution.com/liver/

Heart: http://www.tooloop.com/realistic-heart-drawing/realistic-heart-drawing/

Kidney: http://www.sclerodermasociety.co.uk/Theheartandscleroderma1.php

Brain: http://www.clipartsfree.net/vector/small/brain_in_profile_Clipart_Free.png

Slide 5

Cell: http://thefollyofhumanconceits.wordpress.com/tag/science/

Peroxisome: http://micro.magnet.fsu.edu/cells/peroxisomes/peroxisomes.html

Myelin sheath: http://www.babymassagecentre.co.uk/apps/blog/

Slide 6

N-terminal tertiary structure: http://pfam.sanger.ac.uk/structure/1WLF

AAA ATPase tertiary structure: http://pfam.sanger.ac.uk/structure/1D2N

Chromosomal location: http://ghr.nlm.nih.gov/gene/PEX1

Domain builder: http://prosite.expasy.org/cgi-bin/prosite/mydomains/

Peroxisome: http://micro.magnet.fsu.edu/cells/peroxisomes/peroxisomes.html

Slide 7

Peroxisome import: http://www.sciencedirect.com/science/article/pii/S0925443912000932

Interaction network: http://string-db.org/newstring_cgi/show_network_section.pl

References

Slide 8

Domain builder: http://prosite.expasy.org/cgi-bin/prosite/mydomains/

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Domain builder: http://prosite.expasy.org/cgi-bin/prosite/mydomains/

Slide 10

AAA ATPase tertiary structure: http://pfam.sanger.ac.uk/structure/1D2N

Slide 11

Phosphorylation site graph: http://www.cbs.dtu.dk/services/NetPhos/

Domain builder: http://prosite.expasy.org/cgi-bin/prosite/mydomains/

Slide 12

Renee: http://www.salon.com/2013/02/12/is_there_such_a_thing_as_an_oscar_curse/

Cow: http://commons.wikimedia.org/wiki/File:Bos_taurus_taurus_frontalview.JPG

Rat: http://genome-mirror.duhs.duke.edu/cgi-bin/hgGateway?hgsid=3253225&clade=other&org=Rat&db=0

Mouse: http://bioweb.uwlax.edu/bio203/s2009/smith_meg2/

Zebrafish: http://www.watergardenersinternational.org/fish/danio_rerio.html

Fly: http://en.wikipedia.org/wiki/File:Drosophila_melanogaster_-_front_(aka).jpg

Nematode: http://www.mun.ca/biology/scarr/4241_Devo_Germ_Celegans.html

Thale cress: ihttp://blogs.scientificamerican.com/observations/2010/01/01/good-mutations-stalking-evolution-through-genetic-mutation-in-plants/

Yeast: http://earthemphasis.com/key-research-articles/a-new-biological-test-utilising-the-yeast-saccharomyces-cerevisiae-for-the-rapid-detection-of-toxic-substances-in-water/

Slide 13

AAA ATPase tertiary structure: http://pfam.sanger.ac.uk/structure/1D2N

Slide 14

Protein: http://www.ib.bioninja.com.au/higher-level/topic-7-nucleic-acids-and/75-proteins.html

Spectrometer: http://www.tcd.ie/Geology/news/articles/2012/mass-spec.php

Slide 15

AAA ATPase tertiary structure: http://pfam.sanger.ac.uk/structure/1D2N

References

Slide 16-18

Mouse: http://bioweb.uwlax.edu/bio203/s2009/smith_meg2/

Fly: http://de.wikipedia.org/wiki/Drosophila_melanogaster

Slide 19-20

Peroxisome: http://micro.magnet.fsu.edu/cells/peroxisomes/peroxisomes.html

Protein: http://www.ib.bioninja.com.au/higher-level/topic-7-nucleic-acids-and/75-proteins.html

Literature referenced in talk:

[1] "NINDS Zellweger Syndrome Information Page." National Institute of Neurological Disorders and Stroke. National Institutes of Health. U.S. Department of Health and Human Services, 22 Oct. 2012. Web. 24 Jan. 2014. <http://www.ninds.nih.gov/disorders/zellweger/zellweger.htm>.

[2] S.J. Huybrechts, P.P. Van Veldhoven, C. Brees, G.P. Mannaerts, G.V. Los, M. Fransen, Peroxisome dynamics in cultured mammalian cells, Traffic 10 (2009) 1722–1733. PMID: 19719477.

[3] Cooper GM. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. Peroxisomes. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK9930>.

[4] "Myelin." National Multiple Sclerosis Society. Web. 24 Jan. 2014. <http://www.nationalmssociety.org/about-multiple-sclerosis/what-we-know-about-ms/what-is-ms/myelin/index.aspx>.

[5] Hans R. Waterham, Merel S. Ebberink, Genetics and molecular basis of human peroxisome biogenesis disorders, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Volume 1822, Issue 9, September 2012, Pages 1430-1441. PMID: 22871920.

[6] Zhang R, Chen L, Jiralerspong S, Snowden A, Steinberg S, Braverman N, Recovery of PEX1-Gly843Asp peroxisome dysfunction by small-molecule compounds, PNAS 107(2010) 5569-5574.

[7] Hiebler S, Masuda T, Hacia J, Moser A, Faust P, Liu A, Chowdhury N, Huang N, Lauer A, Bennett J, Watkins P, Zack D, Braverman N, Raymond G, Steinberg S, The Pex1-G844D mouse: A model for mild human Zellweger spectrum disorder, Mol Genet Metab. 14 (2014) epub. PMID: 24503136

[8] Chen H, Liu Z, Huang X, *Drosophila* models of peroxisomal biogenesis disorder: peroxins are required for spermatogenesis and very-long-chain fatty acid metabolism, Human Mol Genet. 19 (2010) 494-505. PMID: 19933170

[9] Mast F, Li J, Virk M, Hughes S, Simmonds A, Rachubinski, A *Drosophila* model for the Zellweger spectrum of peroxisome biogenesis disorders, Dis Model Mech. 4 (2011) 659-672. PMID: 21669930