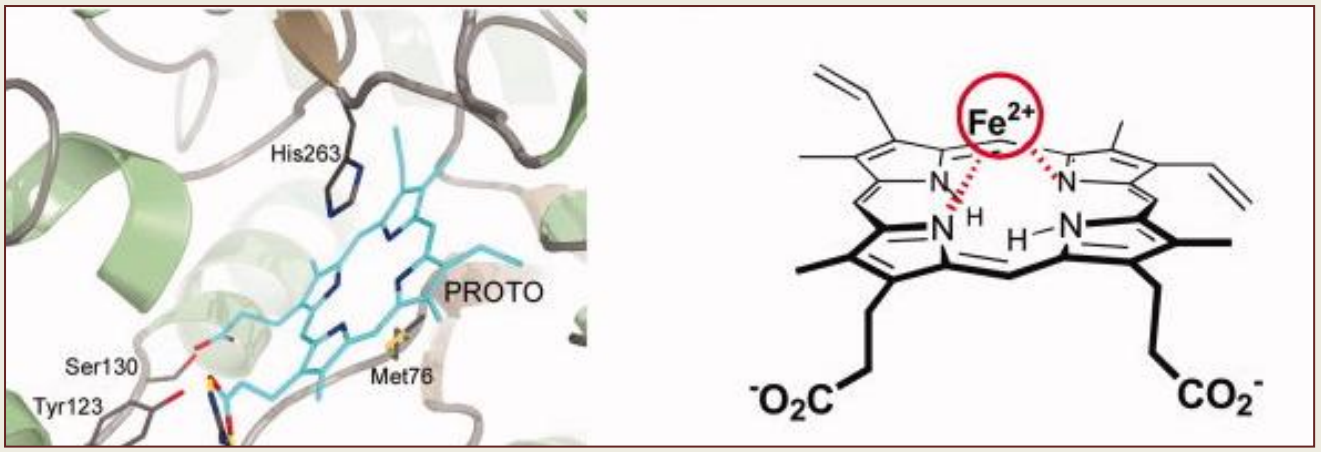

MOLECULAR DOCKING ANALYSIS OF HEME BINDING TO MAPK SIGNALING CASCADE MEMBERS INVOLVED IN NEURONS DEVELOPMENT AND SURVIVAL

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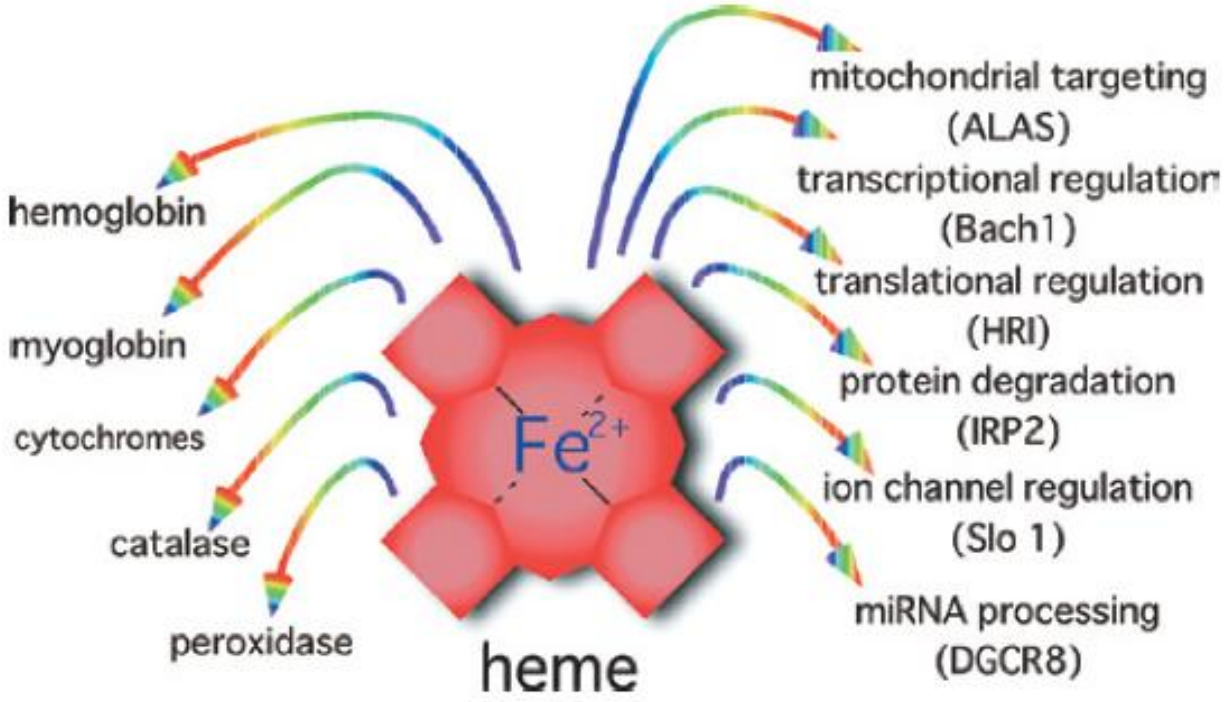
BACKGROUND 1: heme effects on signaling cascades



Conventional and newly identified roles of heme in higher eukaryote.
Some of representative proteins, which require heme as a prosthetic group, are listed at left side of this figure. Recently identified biological events, in which heme is involved, and the names of the target proteins (within parentheses) are listed at right side. Note that many heme-containing enzymes, such as nitric oxide synthase and cyclooxygenase, are not shown.

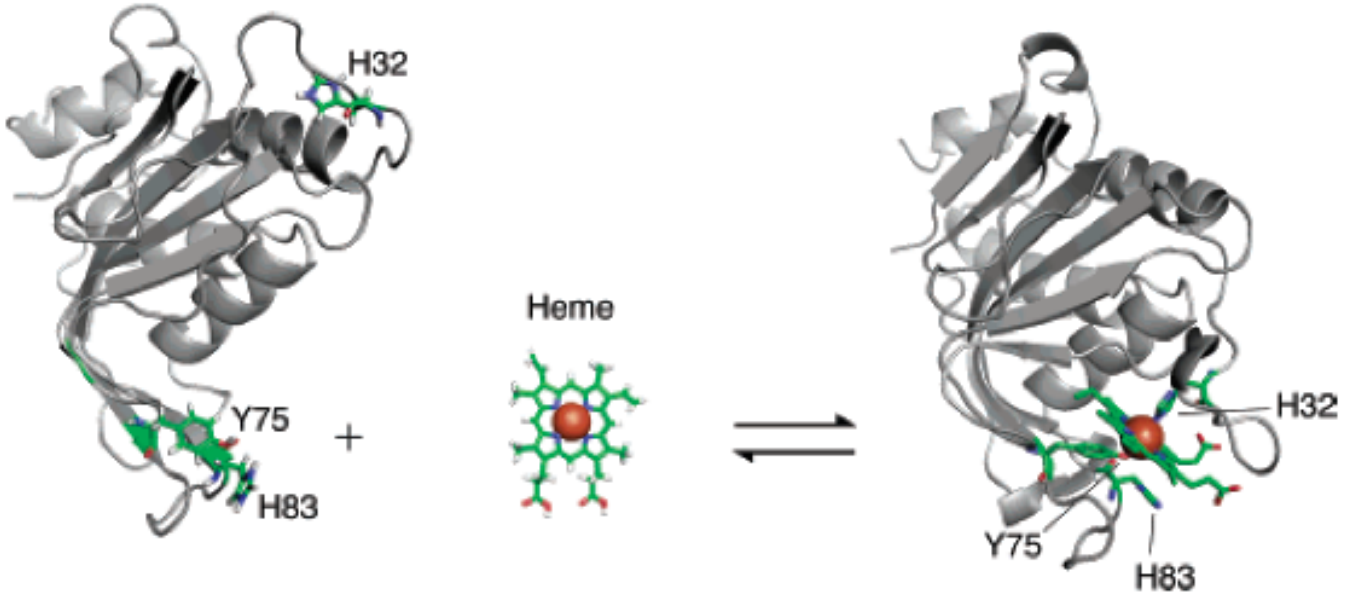
Furuyama K., Kaneko K., Vargas P.D.

Heme as a magnificent molecule with multiple missions: heme determines its own fate and governs cellular homeostasis//Tohoku J Exp Med. -2007.- V.213(1):1-16.

Putative mechanisms for heme effects on signaling

Direct effect on signaling cascade members:

- Heme binding to receptors
- Heme binding to signaling kinases
- Heme binding to transcription regulators
- Heme binding to channels and membrane transporters



Reversible binding of heme to HasA. Structures of apoHasA characterized with NMR spectroscopy (1YBJ) (left)[21] and the heme-bound HasA determined with X-ray crystallography (1B2V) (right).[20] The numbering is according to 1B2V.

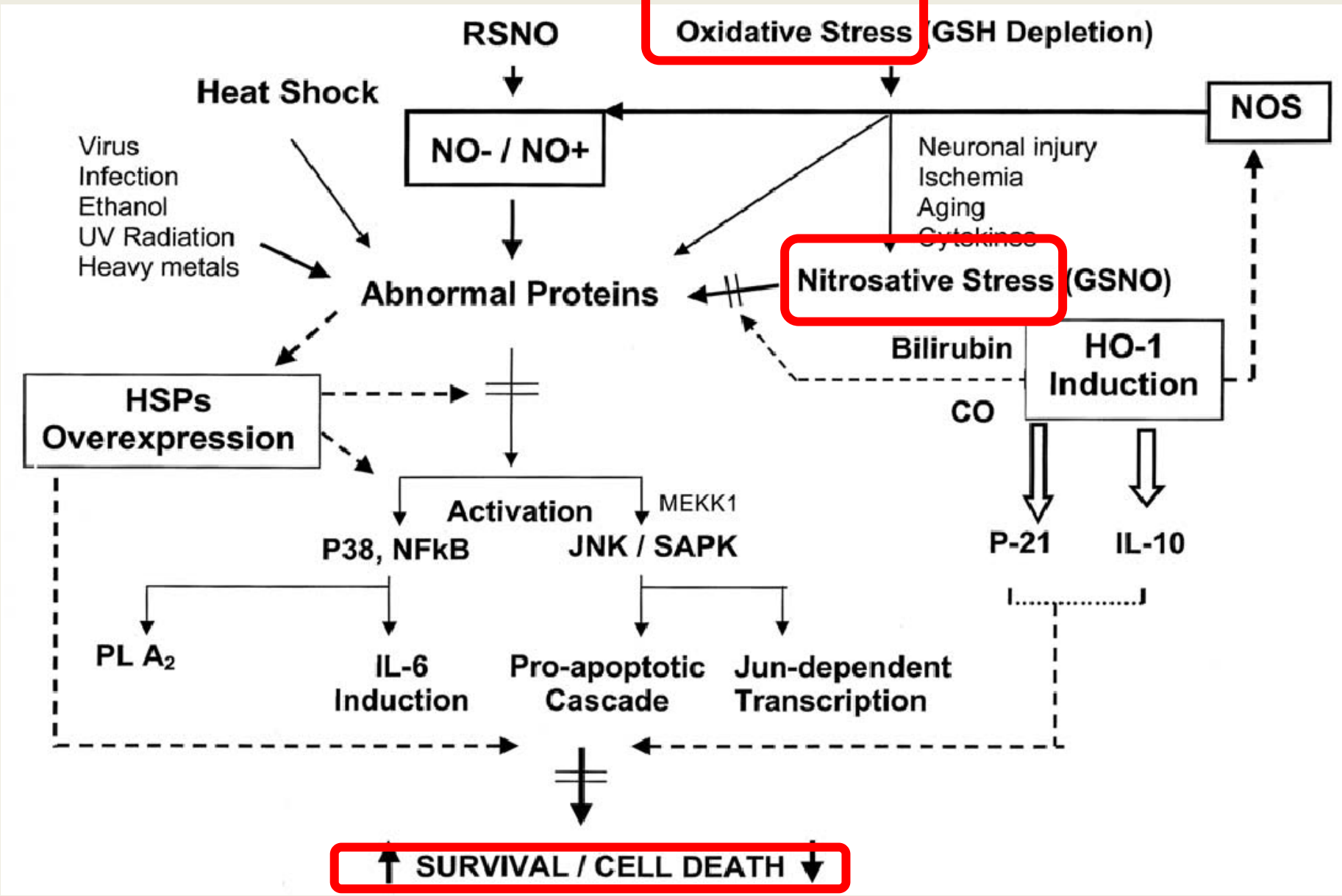
Recent results, however, show that the functions of selected proteins are acutely modulated by reversible binding of heme; thus heme acts as a cellular signaling messenger.

Hou S, Reynolds MF, Horrigan FT, Heinemann SH, Hoshi T.
Reversible binding of heme to proteins in cellular signal transduction//
Acc Chem Res. 2006 Dec;39(12):918-24.

Putative mechanisms for heme effects on signaling

Indirect effects:

- Heme binding to signaling gases (NO, CO)
- Heme oxygenase reaction (→ lower level of heme, higher level of CO and Fe3+)



Calabrese V, Boyd-Kimball D, Scapagnini G, Butterfield DA. **Nitric oxide and cellular stress response in brain aging and neurodegenerative disorders: the role of vitagenes**// In Vivo.- 2004.- V.18(3):245-67.

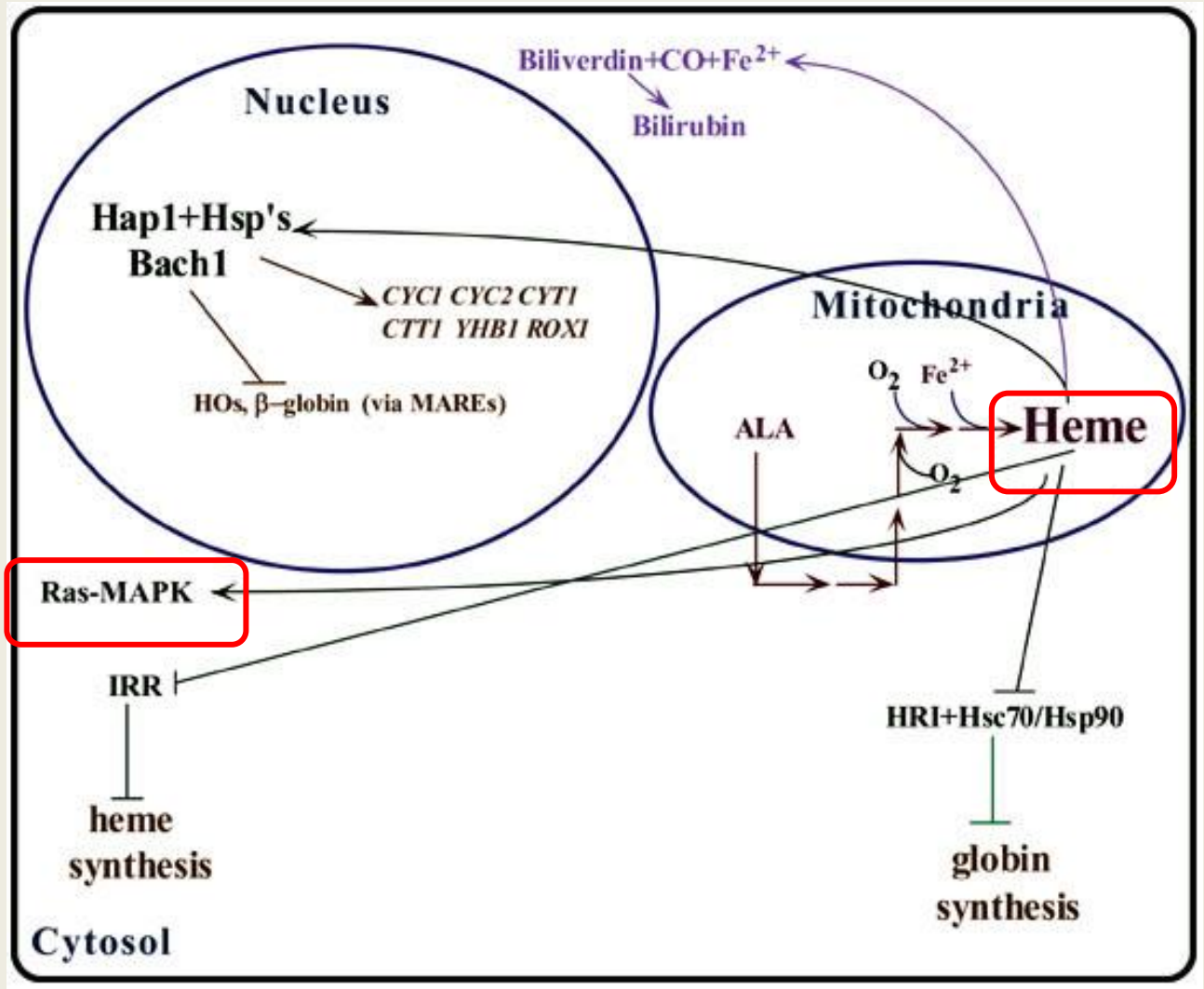
Yao X, Balamurugan P, Arvey A, Leslie C, Zhang L.
Heme controls the regulation of protein tyrosine kinases Jak2 and Src//
Biochem Biophys Res Commun.-2010.- V.403(1):30-35.

Using a *computational search*, authors found that a group of non-membrane spanning tyrosine kinases contains one or more **CysPro motifs** that can potentially bind to heme and mediate heme regulation.

By *experimental approaches* it was found that**:**

- **heme addition to cells (10 microM) results in phosphorylation of key Tyr** in Src (inhibition of activity *soon* after) and
  Jak2 (activation but only *12 hrs* after addition)
- **heme actively interacts with Jak2 and Src and alters their conformation;** the presence of heme **increases sensitivity to trypsin digestion** (for Src – more; for JAK2 - less). Full-length Src and the truncated Jak2 fragment (808-1132) can bind to heme directly (hemin-agarose).
- It was showed that **ERK1/2 were phosphorylated/activated soon after the addition of heme**.

BACKGROUND 2: heme as the regulator of Ras-MAPK signaling



Mense S.M., Zhang L.

Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases// Cell Res. -2006.- V.16(8):681-92.

Ye W, Zhang L.
Heme controls the expression of cell cycle regulators and cell growth in HeLa cells//Biochem Biophys Res Commun. 2004.- V.12;315(3):546-54.

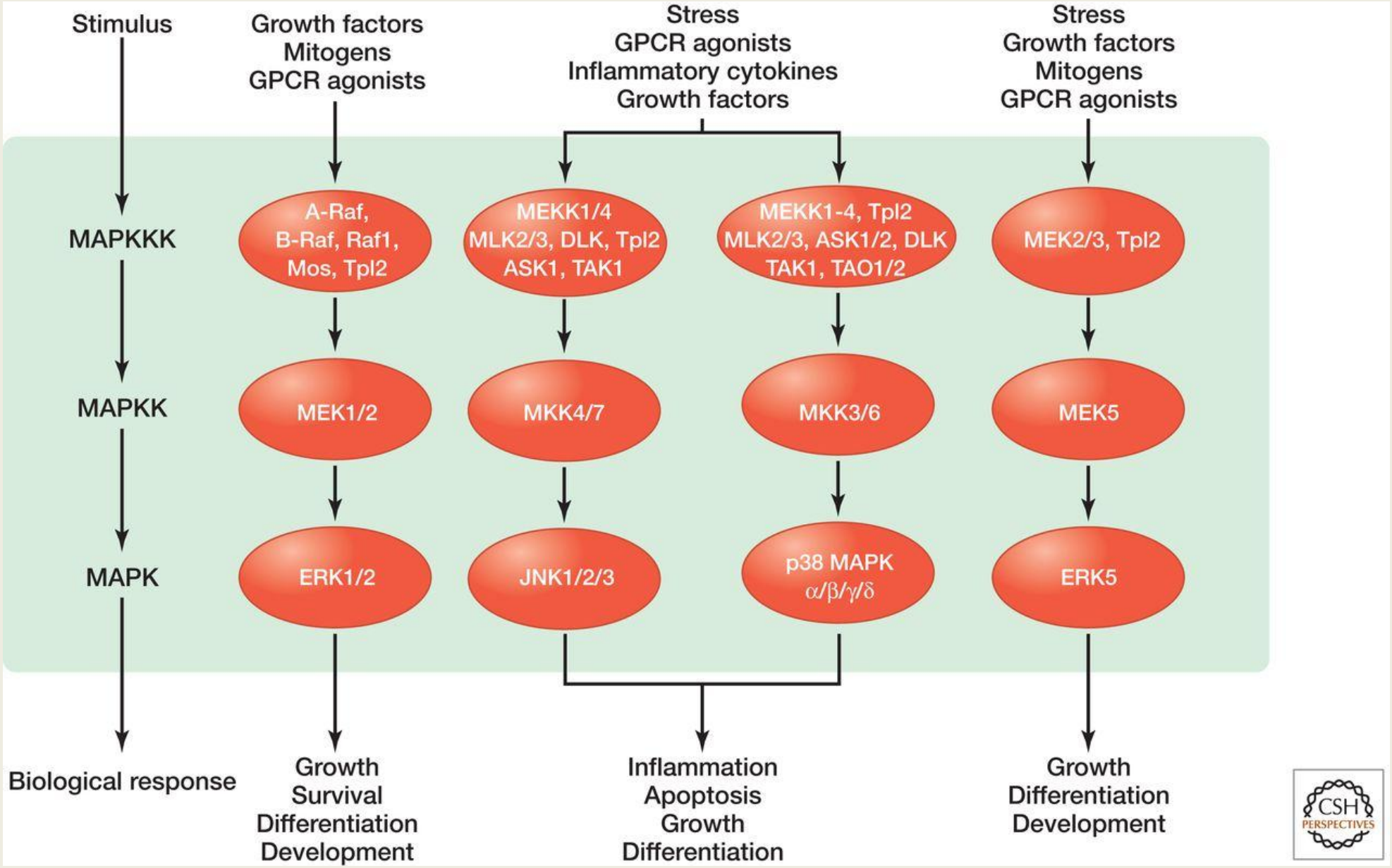
Inhibition of heme synthesis by succinyl acetone greatly diminishes the activity of components of the MAPK signaling pathway, greatly reduced the activation/ phosphorylation of both MEK (MAPK kinase) 1/2 and ERK (extracellular signal regulated kinase) 1/2 but *did not considerably affect the total protein levels* of MEK1/2 and ERK1/2.

Heme deficiency diminishes the activation of the MAPK signaling pathway by NGF in the neuronal PC12 cells and **interferes with neuronal differentiation**, and ultimately causes apoptosis.

These results suggest that **heme acts directly** on the MAPK signaling pathway.

The activation of MEK1/2 and ERK1/2 is suppressed by succinyl acetone, and this suppression is reversed by addition of heme.

MAPK pathways



Morrison D K MAP Kinase Pathways //Cold Spring Harb Perspect Biol 2012;4:a011254

BACKGROUND 3: heme as regulator of neurons fate

Smith A.G., Raven E.L. and Chernova T. **The regulatory role of heme in neurons**// Metallomics, 2011, V.3, P.955–962

There is increasing evidence for heme involvement at the focal points of neurodegenerative changes, and… it is likely that the ***neuroprotective, neurotrophic and signalling roles of heme*** will open new avenues in the treatment of age related neuronal decay.

One possible mechanism of heme … control may be ***through modulation of kinase activity*****.** This would be consistent with the emerging idea that heme may affect or regulate kinase activity in other systems.

BACKGROUND 3: heme as regulator of neurons fate

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One possible mechanism of heme … control may be ***through modulation of kinase activity***. This would be consistent with the emerging idea that heme may affect or regulate kinase activity in other systems.

Zhu Y1, Hon T, Ye W, Zhang L. **Heme deficiency interferes with the Ras-mitogen-activated protein kinase signaling pathway and expression of a subset of neuronal genes**// Cell Growth Differ. 2002 Sep;13(9):431-9.

The Ras-MAPK signaling pathway mediates both NGF-dependent neuronal differentiation and survival. The inhibition of heme synthesis affected only a few of NGF-affected genes, which suggests that heme plays a specific and selective role in NGF signaling and neuronal gene expression.

Thus, **the effect of heme deficiency on the Ras-MAPK signaling pathway not only may interfere with the initiation of neuronal differentiation but may also impact on neuronal maintenance and survival**.

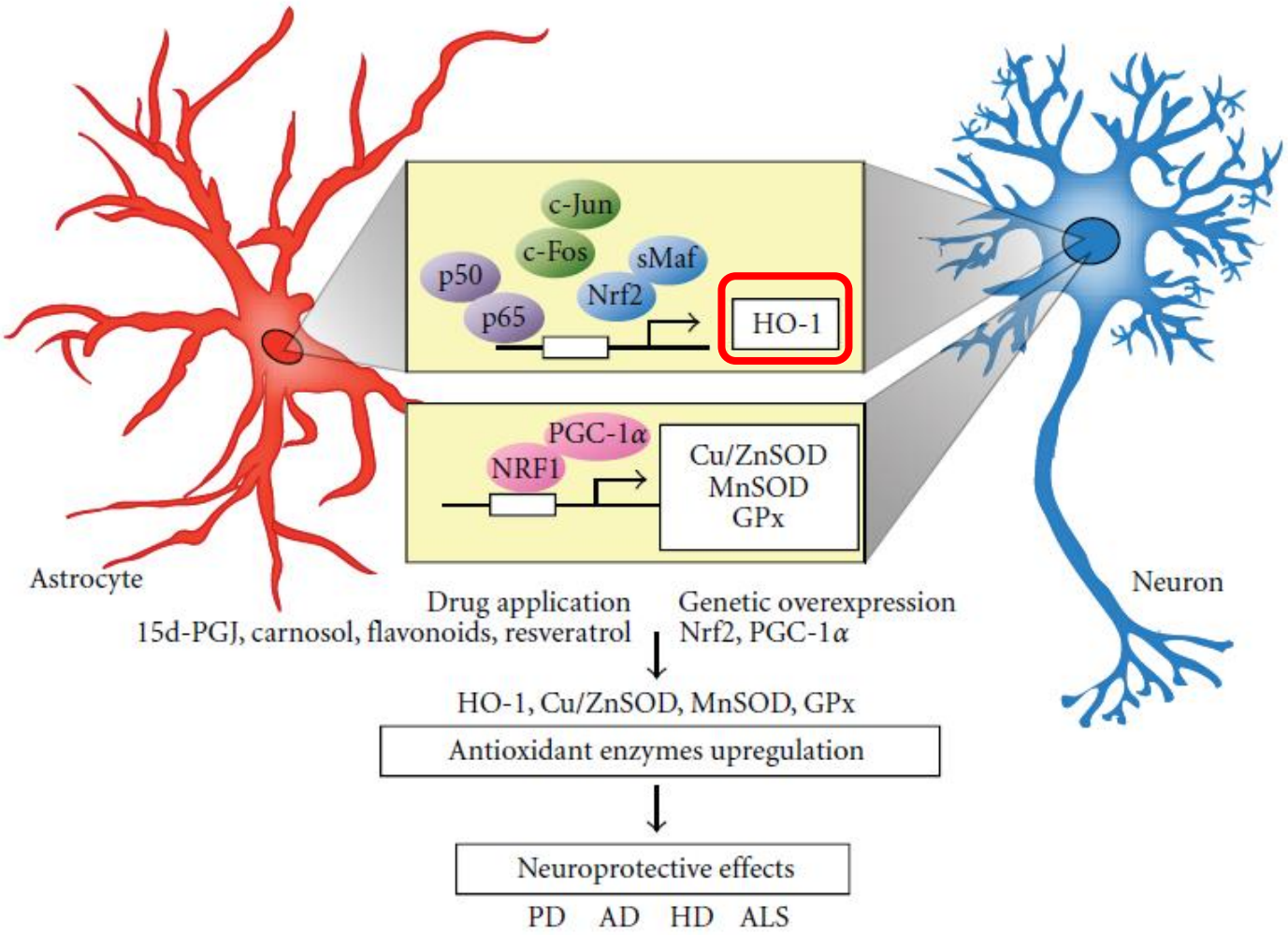


FIGURE 1: The transcriptional upregulation of antioxidant enzymes in neurodegenerative diseases. Both neurons and astrocytes can increase several antioxidant enzymes including heme oxygenase-1 (HO-1), copper and zinc-containing SOD (Cu/ZnSOD), manganese-containing SOD (MnSOD), and glutathione peroxidase (GPx). By drug application or genetic overexpression of transcription factor, the transcriptional responses via NF-κB (p50/p65), AP-1 (c-Jun/c-Fos), Nrf2/sMaf, and NRF1/PGC-1α in response to oxidative stress and related neurodegenerative disease are activated.

Heme effects on neurons: experiment and theory

Heme deficiency, (porphyria, low heme synthesis)	Heme at normal physiological level, ≤ 100 nM	Free intracellular heme accumulation (hemolysis, hemorrhage, trauma)
Lack of heme as regulator and cofactor	Saturation of specific heme-binding sites	Heme binding to sites with low-affinity (non-specific)
Oxidative stress, mitochondrial failure	Pro-antioxidant balance	Oxidative stress; disruption of NO signaling; increased heme degradation by HO → accumulation of Fe^{3+} and CO
Disruption of NGF signaling (low activity of ERK1/2)	Activation of MAPK signaling (ERK1/2)	?
Pro-apoptotic action	Anti-apoptotic action, neurotrophic effects, differentiation	?
Dysfunction of neurons, neurodegeneration, AD, neuropathy	Neuronal survival, neuroprotection	?

Study Design

AIM:

to compare potential affinity for heme of MAPK cascade members involved in neuron development and survival;

SELECTION OF PROTEINS FOR ANALYSIS:

Among more than 50 kinases of MAPK signaling cascades and their partners acting in human about 20 are linked *to neurotrophin signaling and/or regulation of apoptosis (by Gene Ontology annotation)*.

Information on signaling pathways was selected from KEGG Pathway (http://www.genome.jp/) and Reactome (http://www.reactome.org/) databases.

Sequence annotations and gene ontology terms for human MAPK cascade members were loaded from UniProt server (http://www.uniprot.org/).

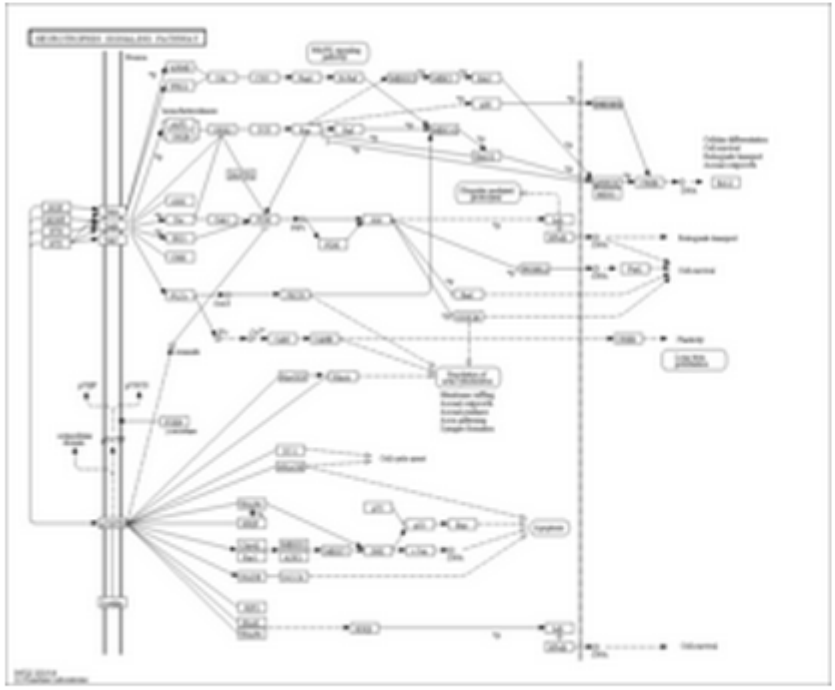
Only proteins with **known 3D-structure (PDB)** were selected for docking analysis and included protein kinases MAPK1/3/7/8/9/11, MAP2K1/2/5, inhibitor protein MAPKAP1 and kinases MAPKAPK2/3.

http://www.genome.jp/kegg/pathway.html

Pathway Text Search

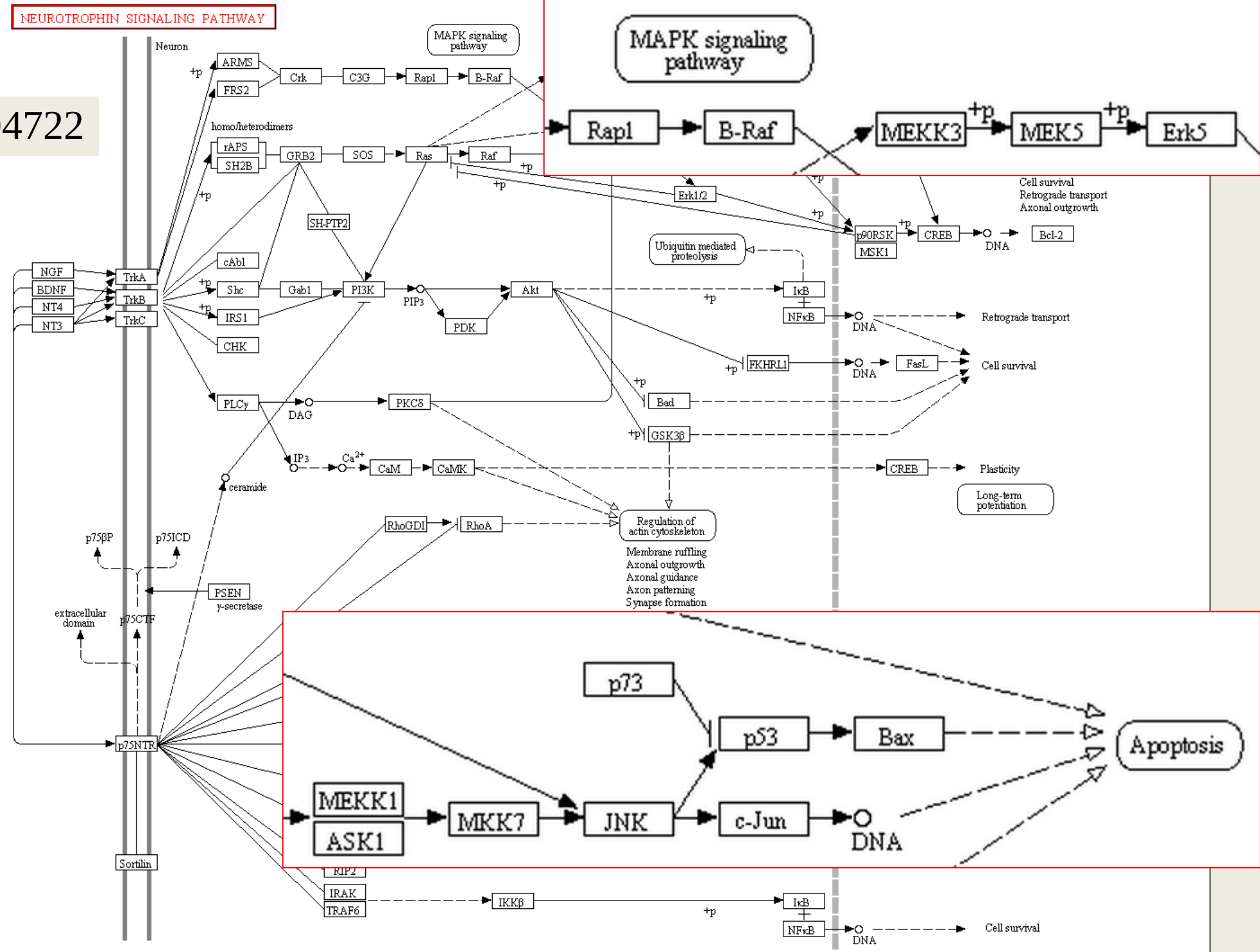
Number of entries in a page 20 ▾ Hide thumbnail

Items : 1 - 1 of 1

Entry	Thumbnail Image	Name	Description
map04722		Neurotrophin signaling pathway	Neurotrophins are a family of trophic factors involved in differentiation and survival of neural cel...

Items : 1 - 1 of 1

map04722


NEUROTROPHIN SIGNALING PATHWAY
Neuron
MAPK signaling pathway
ARMS
FRS2
Crk
C3G
Rap1
B-Raf
homo/heterodimers
rAPS
SH2B
GRB2
SOS
Ras
Raf
MEKK3
MEK5
Erk5
Erk1/2
SH-PTP2
Cell survival
Retrograde transport
Axonal outgrowth
p90RSK
MSK1
CREB
DNA
Bcl-2
Ubiquitin mediated proteolysis
NGF
BDNF
NT4
NT3
TrkA
TrkB
TrkC
cAbl
Shc
Gab1
PI3K
PIP3
PDK
Akt
IκB
NFκB
Retrograde transport
FKHRL1
FasL
Cell survival
CHK
IRS1
PLCγ
DAG
PKCδ
Bad
GSK3β
IP3
Ca2+
CaM
CaMK
CREB
Plasticity
Long-term potentiation
ceramide
RhoGDI
RhoA
Regulation of actin cytoskeleton
Membrane ruffling
Axonal outgrowth
Axonal guidance
Axon patterning
Synapse formation
p75βP
p75ICD
PSEN
γ-secretase
extracellular domain
p75CTF
p75NTR
p73
p53
Bax
Apoptosis
MEKK1
ASK1
MKK7
JNK
c-Jun
DNA
Sortilin
RIP2
IRAK
TRAF6
IKKβ
IκB
NFκB
Cell survival
04722 2/21/14
(c) Kanehisa Laboratories

Methods of investigation

Molecular docking studies were performed using HEX server http://hexserver.loria.fr/

Total energy of interactions (Etotal) was calculated based on shape and electrostatics, and the range angle of 90^{o}; other parameters were set to default values.

HEX citation: Ritchie DW, Venkatraman V. **Ultra-fast FFT protein docking on graphics processors**// Bioinformatics. 2010;26:2398–405.

Structural files in *.pdb format for proteins with their annotations were loaded from Protein Data Bank (PDB, http://www.rcsb.org/). Pdb-file for heme b was loaded from PubeChem (http://www.ebi.ac.uk/pdbe-srv/pdbechem/).

Structure visualization and analysis were performed by Swiss-PdbViewer 4.1.0.

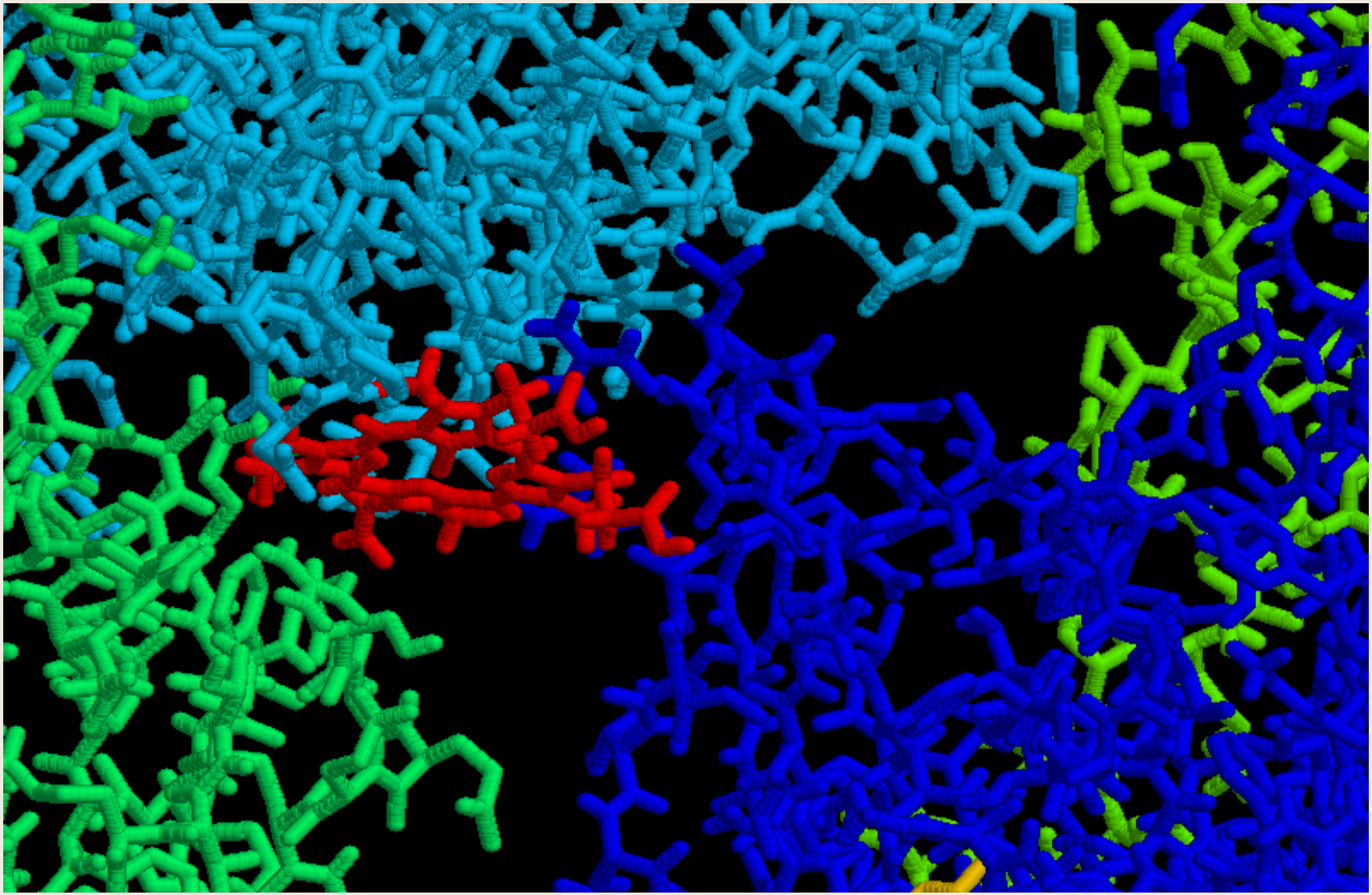
Selected results for MAPK docking to heme (by HEX)

Cascade member	PDB	Chains	Interactions in model (crystallography)	Etotal, kJ/mol	Chains bound to heme	CP/PC
MAPK1	4H3P	A,D	with peptide	-349.27	A	
	3SA0	A	with inhibitor	-227.49	A	
MAPK3	2ZOQ	A, B		-333.27	A	
MAPK7	4IC7	A,B,D,E	with MAP2K5	-369.76	A,B,D	
MAPK8	3O17	A/B	chain A/B	-324.79	B, multiple sites	
	3V3V	A	With inhibitor	-244.14	A	
MAPK9	3E7O	A		-371.14	A, multiple sites	
	3NPC	A	8 mutations, include cys	-353.64	A	
MAPK11	3GC8	A, B		-337.63	A	
	3GC9	A, B	2 mutations C119S, C162S, with inhibitor	-341.97	A, multiple sites	
	3GP0	A	with Nilotinib	-321.09	A	
MAPK12	1CM8	A,B		-325.47	A	
MAPKAPK2	2OZA	A,B	in complex with MAPK14	-424.74	A,B	
MAPK2K1	1S9J	A	monomer+Mg	-44.78	A	
	3VVH	A,B,C	ABC-chains	-397.79	A,B	

Selected results for MAPK Cys-Pro motifs

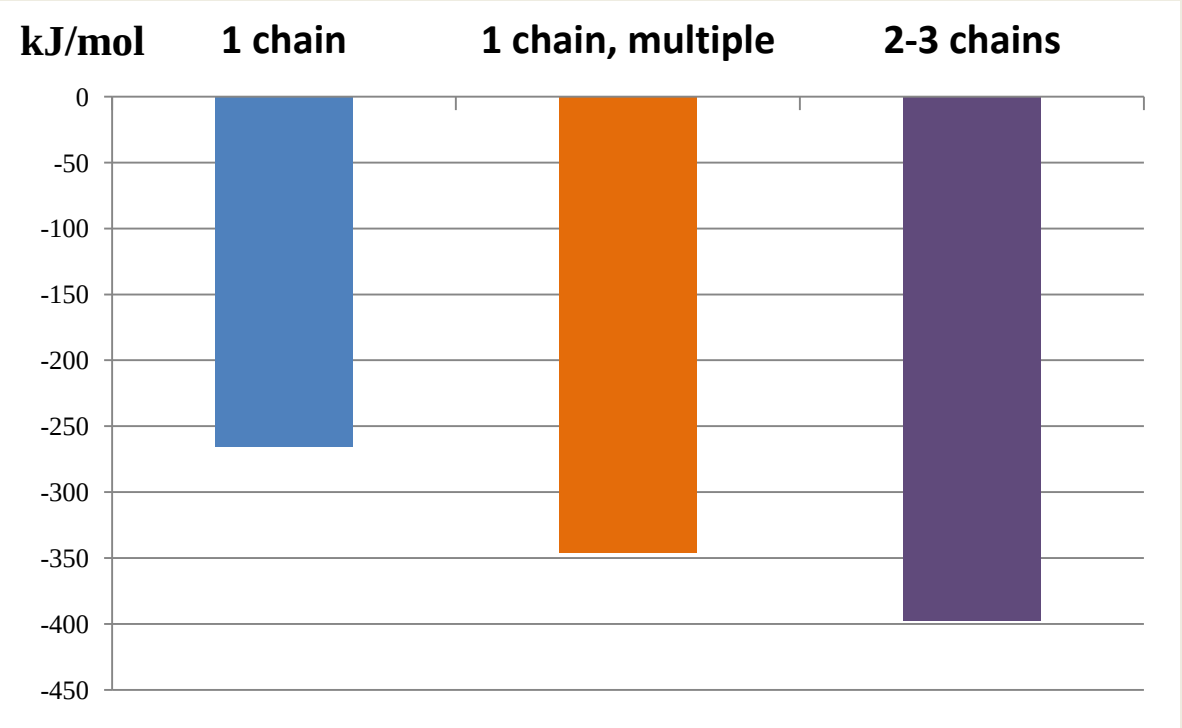
Cascade member	PDB	Chains	Interactions in model (crystallography)	Etotal, kJ/mol	Chains bound to heme	CP/PC
MAPK1	4H3P	A,D	with peptide	-349.27	A	-/-
	3SA0	A	with inhibitor	-227.49	A	
MAPK3	2ZOQ	A, B		-333.27	A	-/-
MAPK7	4IC7	A,B,D,E	with MAP2K5	-369.76	A,B,D	++/+ (PCP)
MAPK8	3O17	A/B	chain A/B	-324.79	B, multiple sites	+/-
	3V3V	A	With inhibitor	-244.14	A	
MAPK9	3E7O	A		-371.14	A, multiple sites	-/-
	3NPC	A	8 mutations, include cys	-353.64	A	
MAPK11	3GC8	A, B		-337.63	A	-/-
	3GC9	A, B	2 mutations C119S, C162S, with inhibitor	-341.97	A, multiple sites	
	3GP0	A	with Nilotinib	-321.09	A	
MAPK12	1CM8	A,B		-325.47	A	-/-
MAPKAPK2	2OZA	A,B	in complex with MAPK14	-424.74	A,B	++/-
MAPK2K1	1S9J	A	monomer+Mg	-44.78	A	-/-
	3VVH	A,B,C	ABC-chains	-397.79	A,B	

Heme binding to several protein chains in complex



Heme binding to protein kinases (Etotal) by HEX

MAPK

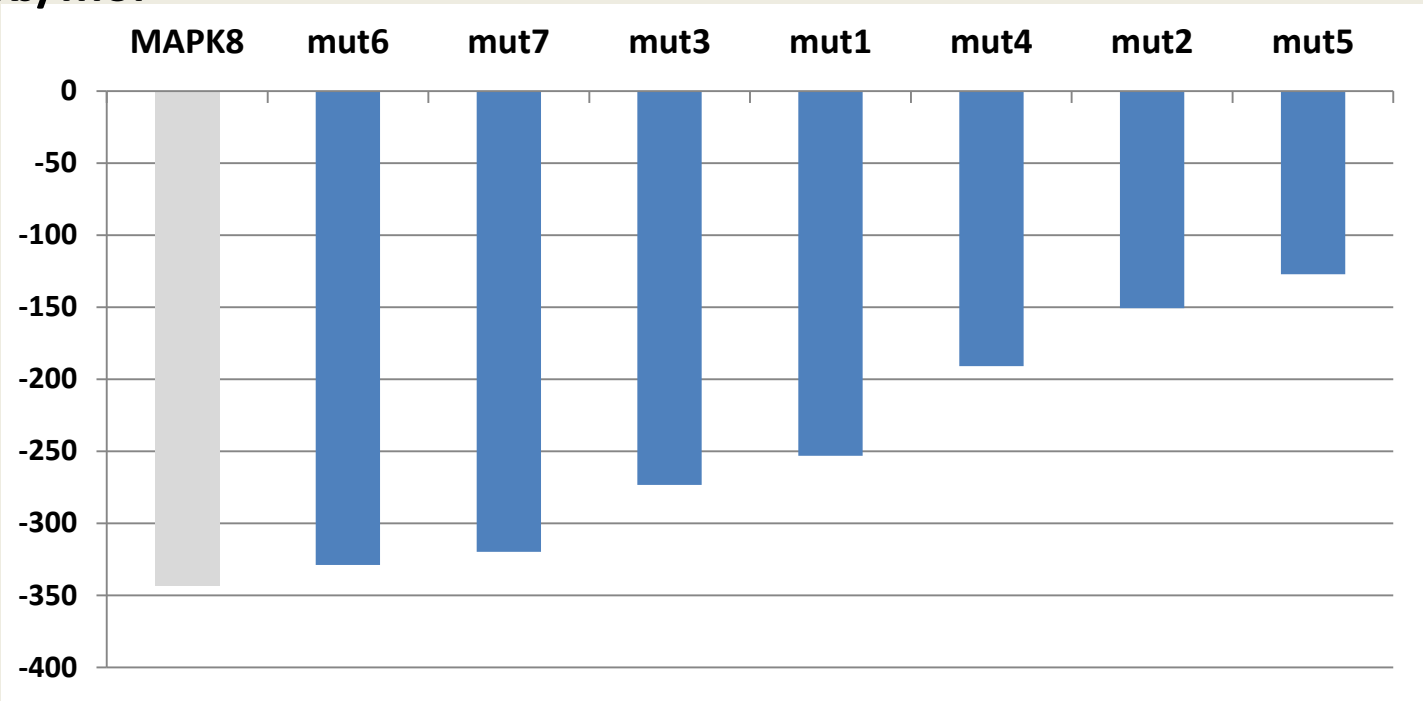


Src and JAK2

Src	Src-Nend	1M61		Etotal: -320.30
	Src-Cend	1U59		Etotal: -181.06
JAK2	840-1132	2B7A	A/B	Etotal: -381.51
	835-1132	2W1I	A/B	Etotal: -368.24
	835-1132	2XA4	A/B	Etotal: -355.06

Heme binding to mutant protein chains of MAPK8 (JNK1)

KJ/mol

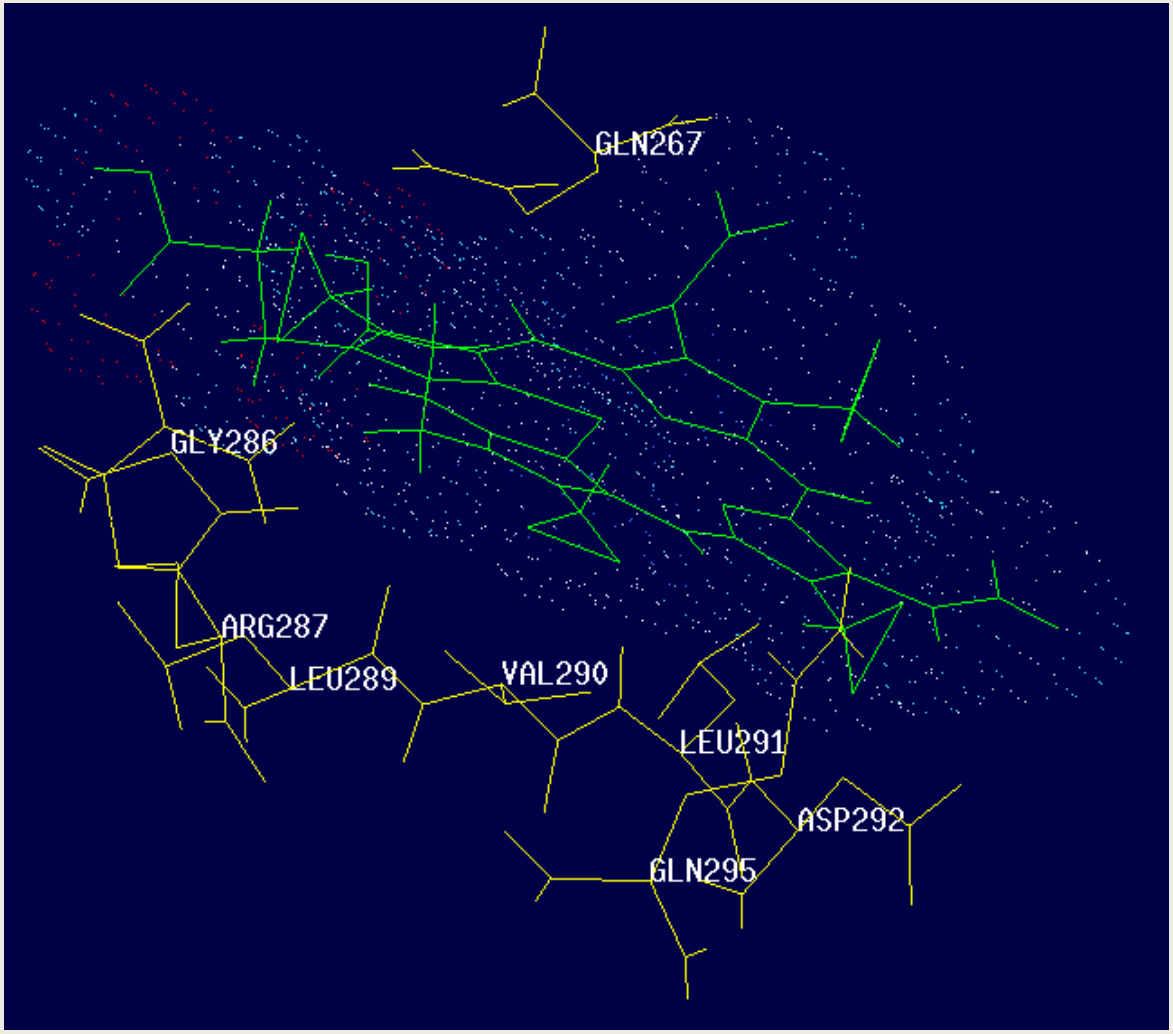


mut6	3VUL	-329,06
mut7	3VUM	-319,93
mut3	3VUH	-273,43
mut1	3VUD	-253,11
mut4	3VUI	-190,95
mut2	3VUG	-150,82
mut5	3VUK	-127,16

3VUD	-253.11	3VUD: C245S
3VUG	-150.82	3VUG: C245S, C116S
3VUH	-273.43	3VUH: C245S, C116S, C163A
3VUI	-190.95	3VUI: C245S, C116S, C163A, C79V
3VUK	-127.16	3VUK: C245S, C116S, C163A, C79V, C137V
3VUL	-329.06	3VUL: C245S, C116S, C163A, C79V, C137V, C213V
3VUM	-319.93	3VUM: C245S, C116S, C163A, C79V, C137V, C213V, C41V

C116	surface
C245	near surface, partly exposed
C79	near surface, partly exposed
C137	no access (burried)
C213	no access (burried)
C41	no access (burried)
C163	no access (burried)

Common neighbor residues (within 3.5 Å from Fe)

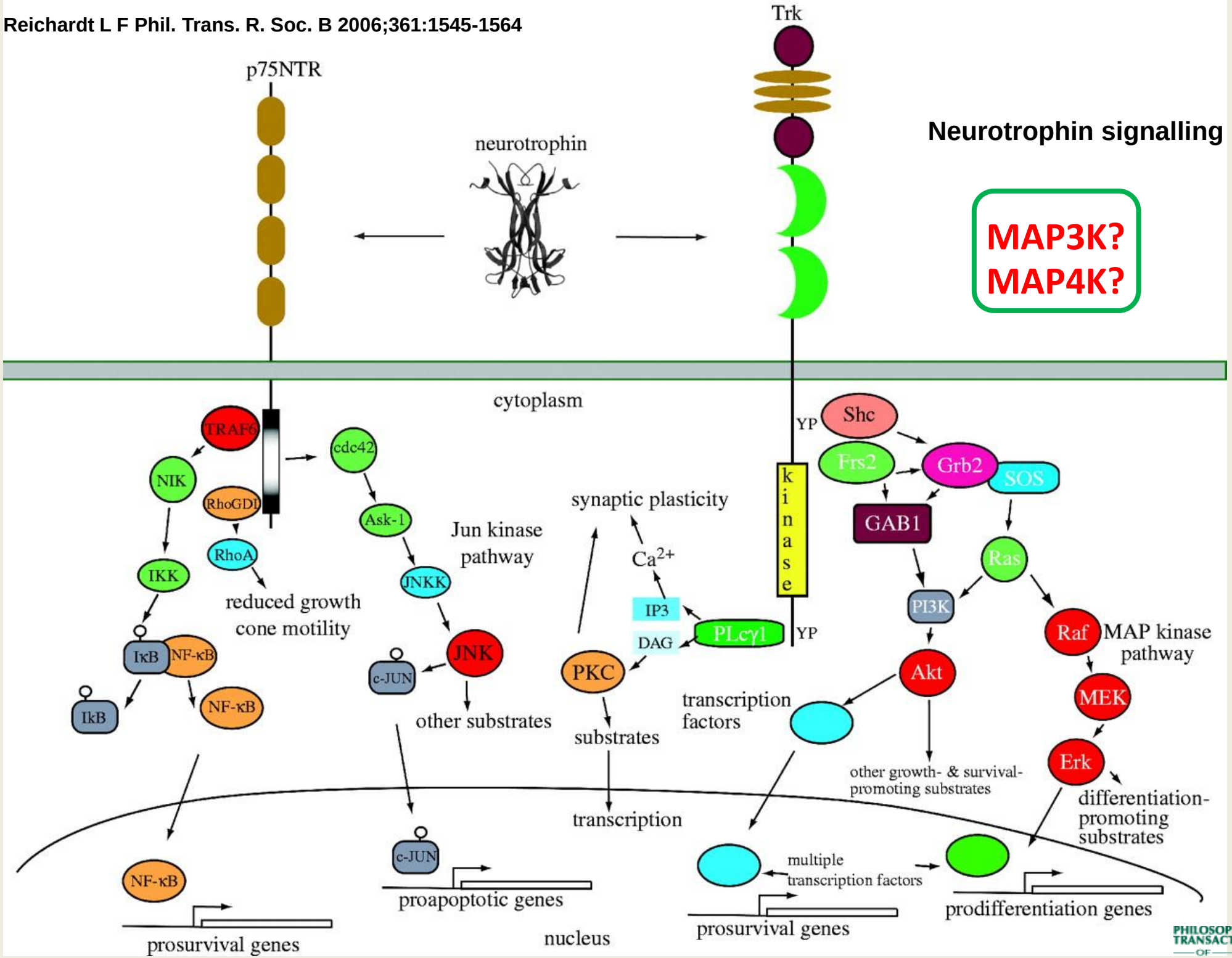


Lys	Arg	Asp	Glu	Asn	Gln	Cys	Tyr	His	Pro	Val	Ile
+		-		polar, neutral			aromatic		nonpolar		

Cys-Pro motifs in MAPK cascade members in human

Cascade member	N in Uniprot	CP	PC	CP+PC	PDB
MAPK	13 (1-15)	MAPK4/8/10	MAPK6	MAPK7	+
MAP2K	7	MAP2K4/6/7	-	-	+
MAP3K	16 (1-15;19)	MAP3K2/4/10/14	-	MAP3K1/5-7/9/ 11-13/15/19	-/+
MAP4K	5	MAP4K1/2/5	-	MAP4k3	-/+
MAPKAP	1	+	-		+
MAPKBP	1	-	+		-

Reichardt L F Phil. Trans. R. Soc. B 2006;361:1545-1564



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Conclusions

- So MAPK signaling members exhibit **different affinity to heme that is dependent mostly on proteins association and cysteines important for conformation**
- The main **cross-talk and competition for heme binding** under neurotrophin signaling could be between MAPK7 (=ERK5→ CREB→survival, differentiation) and MAPK8 (=JNK1→ p53→apoptosis).

→**The oscillations of cellular heme level may alter protein-binding and catalytic activity of MAPK-signaling members that could be one of the factors affecting neurons development and survival**

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