NEUROTROPHIC FACTORS

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What is a growth factor ?
What is a trophic factor ?

DEFINITION

Growth factors include substances that stimulate cells to divide (hyperplasia) or increase in size (hypertrophy). Many growth factors are now known to exist

Trophic factors include those substances that have effects on cell differentiation, cell survival, expression of a specific cellular phenotype (e.g. a cell becomes an inhibitory or an excitatory neuron), cellular morphological plasticity, as well as cell hypertrophy including, for example, the induction of neurite extention (also considered a "trophic" action). Importantly, some growth factors may also act as trophic factors and viceversa and each growth/trophic factor may have a specific combination of cellular effects.

Neurotrophic factors

Neurotrophic factors are endogenous soluble proteins regulating survival, growth, morphological and synaptic* plasticity, or synthesis of proteins for differentiated functions of neurons or glial cells.

(* synaptic plasticity = regulation of the transmission activity at the level of the synapse)

List of characterized proteins exhibiting neurotrophic activities

Growth factor	References
Nerve growth factor (NGF)	Thoenen et al., 1987
	Whittemore and Sciger, 1987
	Hefti et al., 1989
Brain-derived neurotrophic factor (BDNF)	Barde et al., 1982
	Leibrock et al., 1989
Neurotrophin-3 (NT-3)	Ernfors et al., 1990
	Hohn et al., 1990
	Maisonpierre et al., 1990
	Rosenthal et al., 1990
Neurotrophin-4 (NT-4)	Hallbrook et al., 1991
Neurotrophin-5 (NT-S)	Berkerneier et al., 1991
Ciliary neurotrophic factor (CNTF)	Lin et al., 1989
	Stóckli et al., 1989
Heparin-binding neurotrophic factor (HBNF)	Kovesdi et al., 1990
Growth factors vith neurotrophic activity	
Basic fibroblast growth factor (bFGF)	Morrison et al., 1986
	Walicke, 1988
Acidic fibroblast growth factor (aFGF)	Walicke, 1988
Insulin-like growth factors (IGFs), insulin	Aizenman et al., 1986
	Baskin et al., 1987
Epidermal growth factor (EGF)	Fallon et al., 1984
	Morrison et al., 1987
Transforming growth factor cc (TGFcc)	Deryncl,, 1988
	Fallon et al., 1990
Interleukin 1	Spranger et al., 1990
Interleukin 3	Kamegai, 1990
Interleukin 6	Harna et al., 1989
Protease nexin 1 and II	Monard, 1987
	Oltersdorf et al., 1989
	Whitson et al., 1989
Cholinergic neuronal differentiation factor	Yarnarnori et al., 1989

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	Whitson et al., 1989
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EARLY DAYS OF THE NERVE GROWTH FACTOR

THE NEUROTROPHIN PROTOTYPE: NGF – NERVE GROWTH

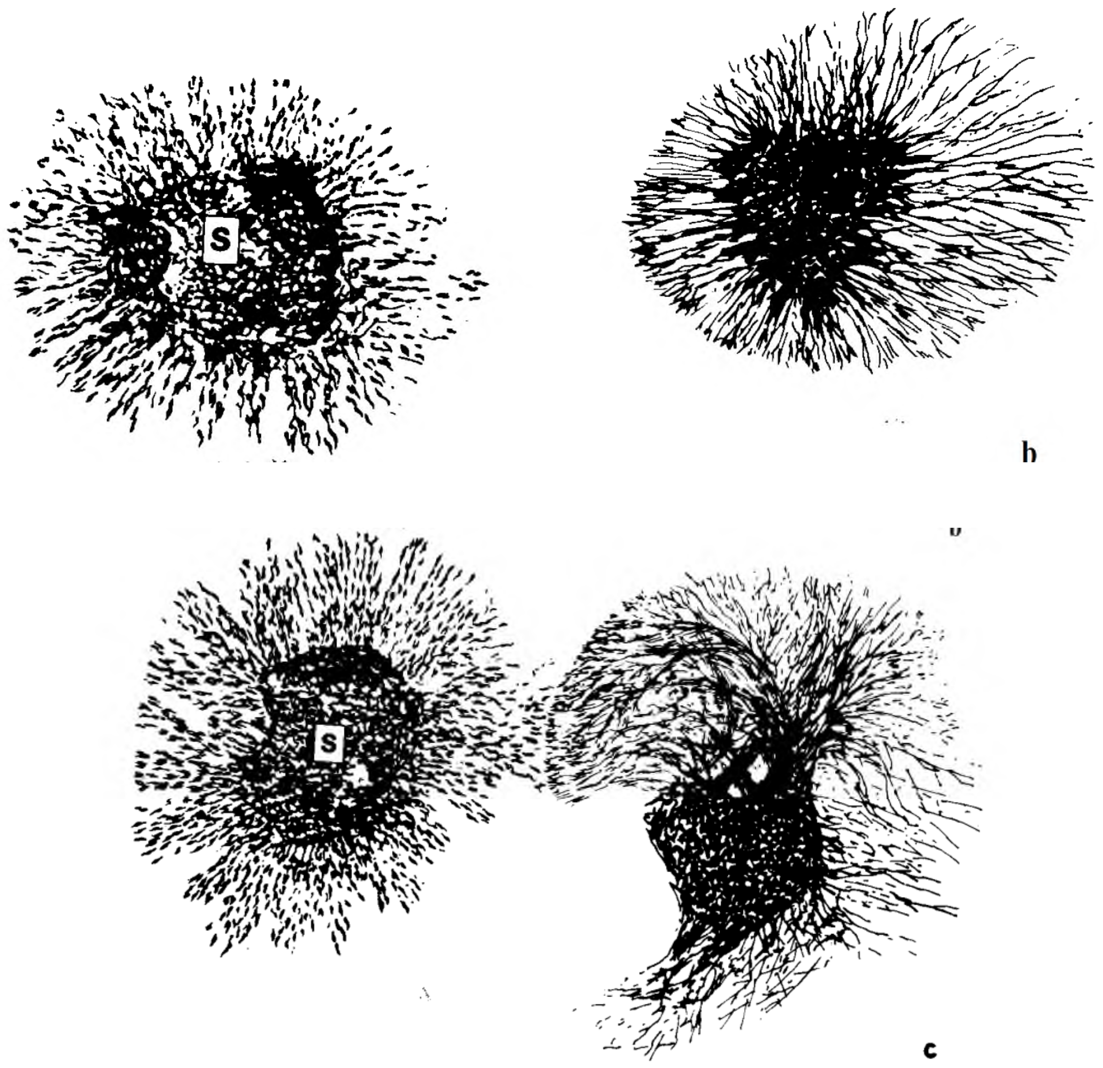
Rita Levi-Montalcini (1909 – 2012)

The history of NGF is more like a detective story than a scientific enterprise, which usually unfolds according to well-defined rules along a route paved by previous findings.

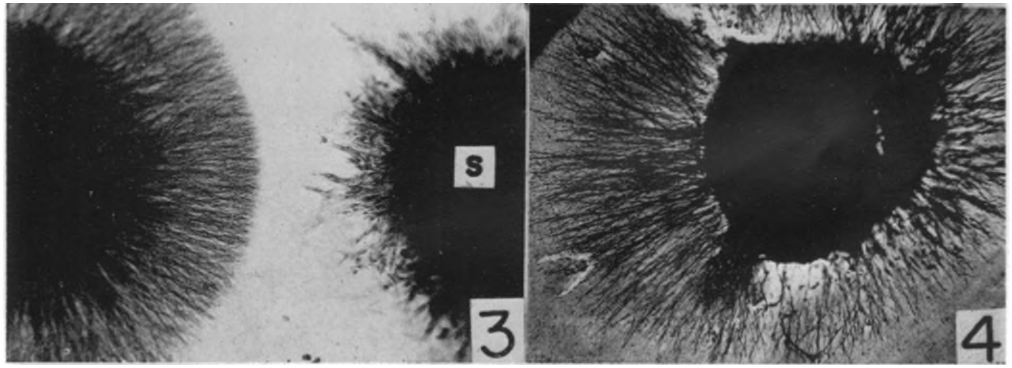
Rita Levi-Montalcini
(The Saga of the Nerve Growth Factor)

Few words on Nerve Growth Factor discovery

In December 1952, working on the identification of the development of the nervous system at embryonic stage in chick embryos, Rita Levi-Montalcini identified a «diffusible factor» able to induce nerve fibres sprouting.



NGF IDENTIFICATION: SERENDIPITY



PNAS, 1956

Chance, rather than calculated search, signed a new, most fortunate turn of events. In order to degrade the nucleic acids present in this active fraction, Stan (Stanley Cohen) made use of snake venom which contains, among other enzymes, also the nucleic acid degrading enzyme, phosphodiesterase. (…) The startling result was **a marked increase in the density of the fibrillar halo around the ganglia** incubated in the presence of the tumoral fraction treated with snake venom.

(…) **From the latter** *[mouse submandibulary glands]* **he isolated, after several purification steps, a non-dialyzable, heat-labile substance endowed with nerve growth promoting activity, identified as a protein molecule with a molecular weight in the order of 20,000**.

Stanley Cohen and Rita Levi-Montalcini in Campidoglio in Roma, on the occasion of Rita's 100th birthday (22.04.2009).

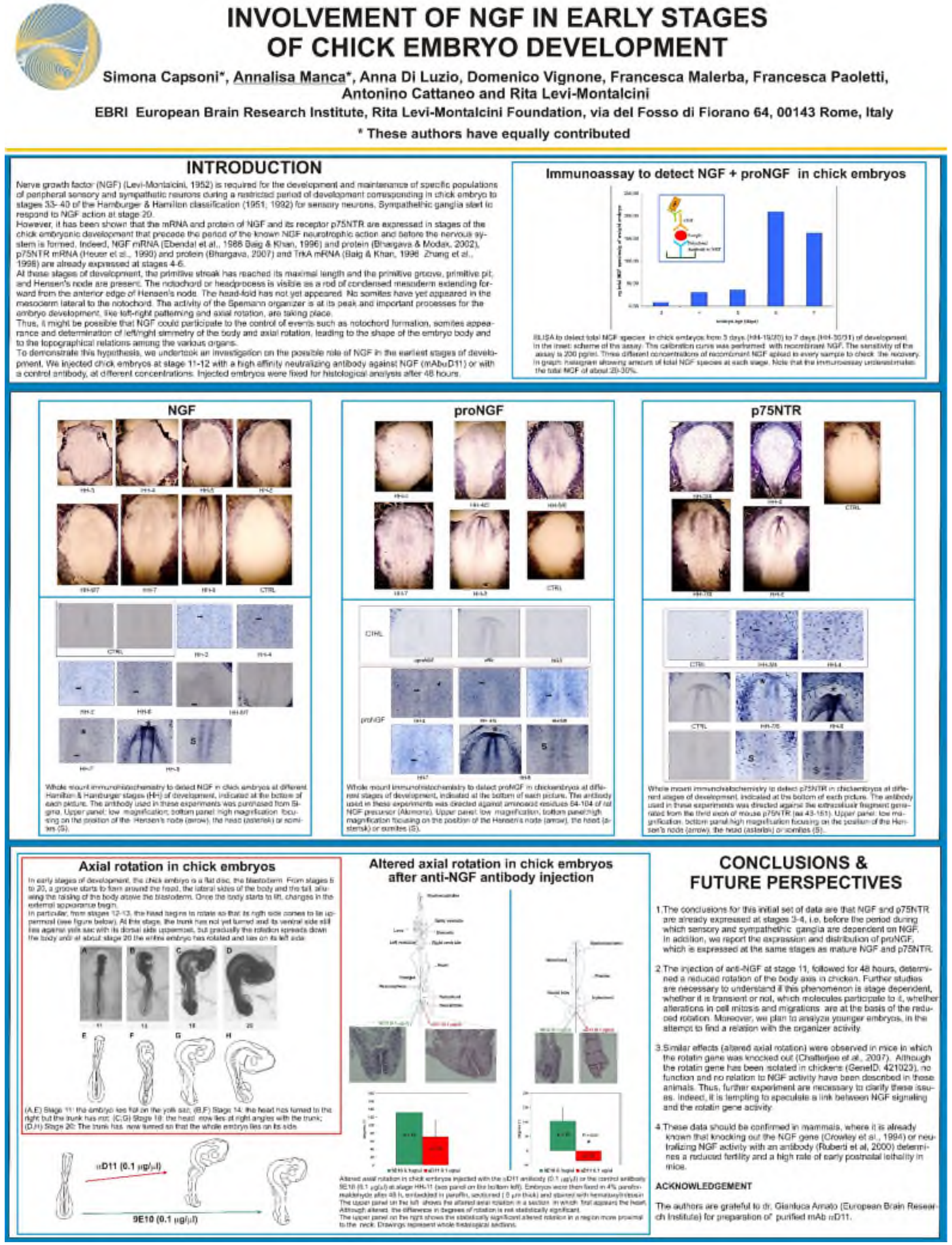
In spite of, or perhaps because of its most unusual and almost extravagant deeds in living organisms and in-vitro systems, NGF did not at first find enthusiastic reception by the scientific community, as also indicated by the reluctance of other investigators to engage in this line of research. The finding that a protein molecule from such diverse and unrelated sources as mouse sarcomas, snake venom and mouse salivary glands, elicited such a potent and disrupting action on normal neurogenetic processes, **did not fit into any conceptual preexisting schemes, nor did it seem to bear any relationship to normal control mechanisms at work during ontogenesis**. It was in this skeptical atmosphere that NGF asserted, in a most forceful way, **its vital role in the life of its target cells**.

The **main causes of unpredictability of the findings, reside in the intricacy of the new surroundings where NGF is moving** - the CNS and the immune system-rather than in NGF itself.

Table 1. NGF TARGET CELLS

NEURAL CREST DERIVATIVES	*Sympathoadrenal*	*Long sympathetic neurons*	
		Short sympathetic neurons	
		Cells of *paraganglia* (carotid & abdominal paraganglia)	
		SIF (small, intensely fluorescent) cells	
		Chromaffin cells	normal
			neoplastic (PC12)
	Sensory neurons		
CENTRAL NERVOUS SYSTEM	*Cholinergic neurons:*	corpus striatum, basal forebrain, septum,	
	Adrenergic, indoleaminergic,	nucleus diagonal band of Broca	
	Peptidergic neurons	Xenopus laevis tadpoles	
NON NEURONAL ORIGIN	*Mast cells*		

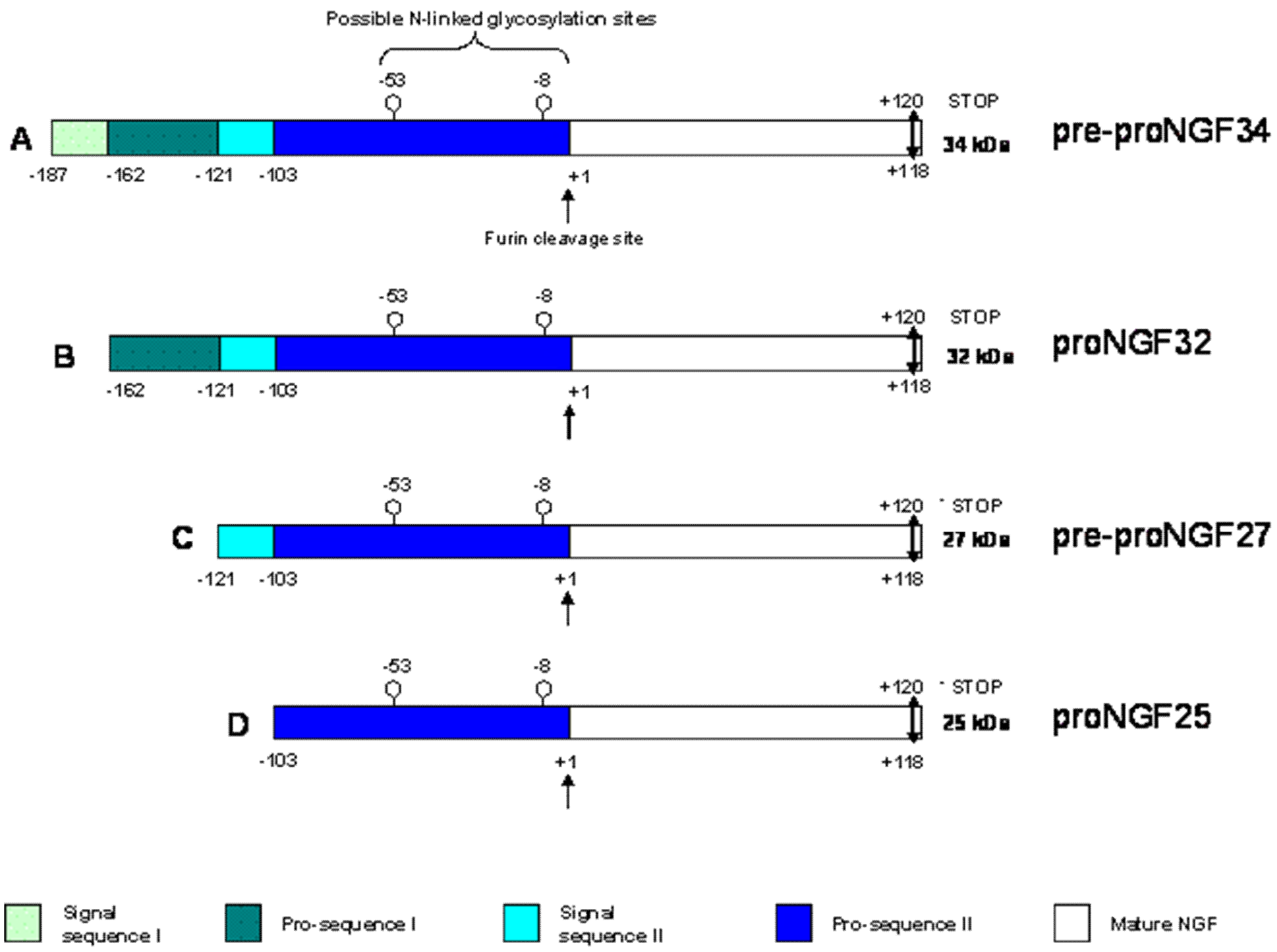
The subsequent demonstration that **labelled NGF is taken up by the nerve endings of sympathetic or sensory fibers and is retrogradely transported to the cell perikarya**, lent strong support to the concept of NGF as a **trophic messenger**, conveyed through nerve fibers from peripheral cells to the innervating neurons.
(…)
Another important property of NGF - its ability to direct growing or regenerating axons of sensory and sympathetic fibers along its concentration gradient (**neutrotropism**).



Rita's last scientific enterprise

NGF Meeting - September 2008

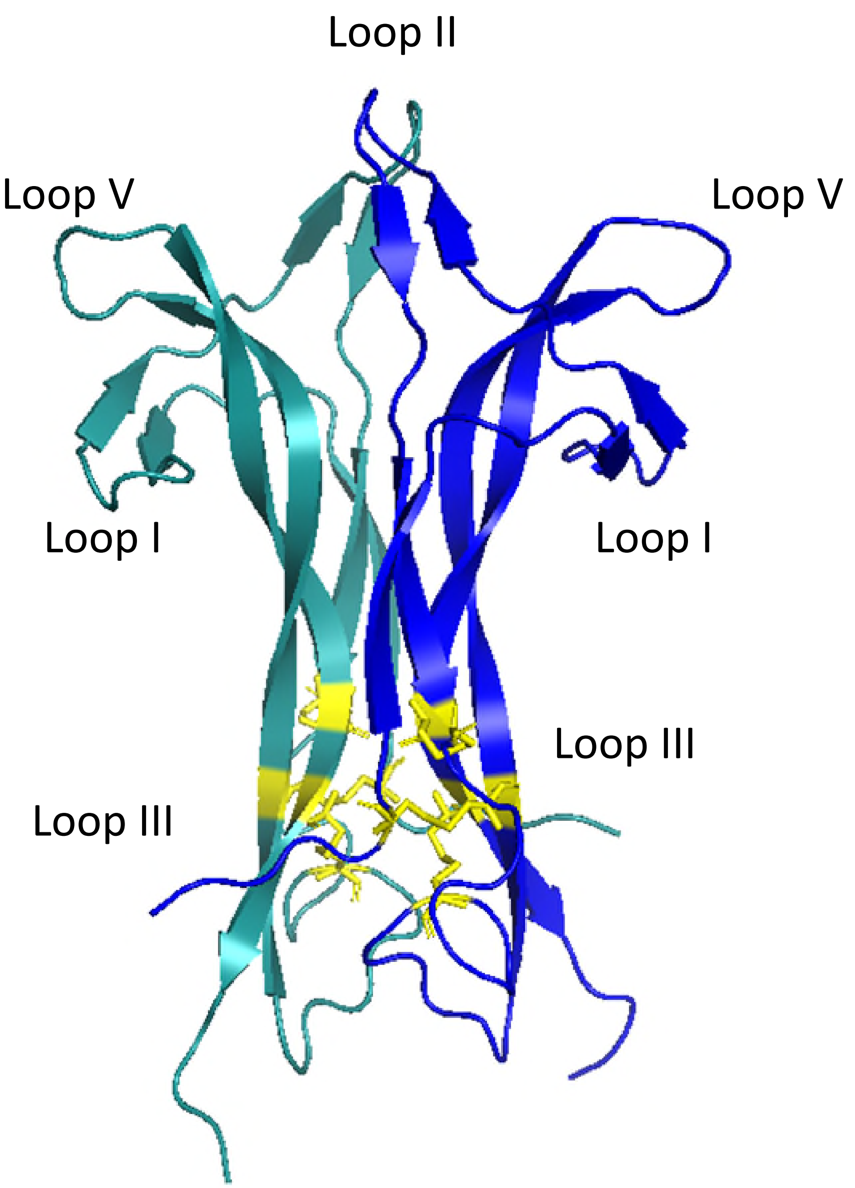
MOLECULAR DESCRIPTION OF THE NEUROTROPHIN FAMILY



Furin cleavage site

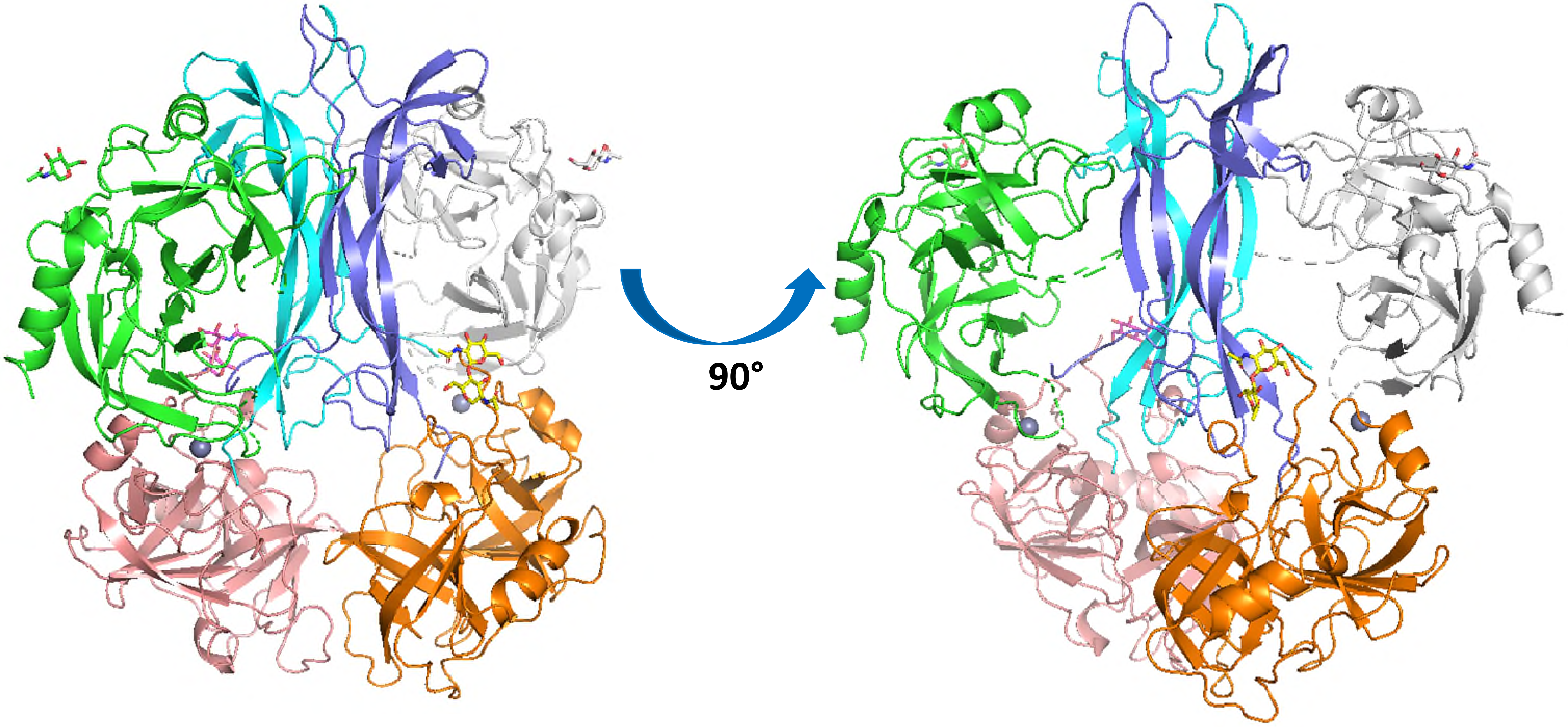
RTHRSKR SS

NGF is a dimer in solution (Angeletti ***et** al.,* 1971), and this dimerization has an unusually **high affinity constant** such that at physiologically active concentrations NGF is a dimer (Bothwell and Shooter, 1977).
The recent structure of murine βNGF obtained by x-ray crystallography (McDonald ***et** al.,* 1991) revealed the existence of a **hydrophobic face involved in dimerization and also a secondary structure rich in β-sheet but containing no α-helix**. The conserved hydrophobic regions of the protein could be involved in this contact site.



Nahri et al, JBC, 1993

IN THE MOUSE SUBMANDIBULARY GLANDS…7S COMPLEX



THE NEUROTROPHINS FAMILY, besides NGF

BDNF -brain-derived neurotrophic factor (BDNF), isolated by Barde et al. (1982). BDNF is 55% homologous to NGF

NT3 – Discovered by primer sequences constructed from sequences in the conserved regions (Maisonpierre et al., 1990; Hohn et al., 1990; Rosenthal et al., 1990; Ernfors et al., 1990; Jones and Reichardt,1990). 58% homologous to BDNF and 57% homologous to NGF.

NT4/5 – Firstly identified in Xenopus ovary (Hallböök et al, 1991) and later on found by PCR in mammals (Ip et al, 1992).

The influence of neurotrophins spans from developmental neurobiology to neurodegenerative and psychiatric disorders. In addition to their classic effects on neuronal cell survival, neurotrophins can also regulate axonal and dendritic growth and guidance, synaptic structure and connections, neurotransmitter release, LONG-TERM POTENTIATION (LTP) and synaptic plasticity

Chao, Nat Rev Neurosci, 2003

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sp|P23560|BDNF_HUMAN      MTILFLTMVISYFGCMKAAPMKEANIRGQGGLAYPGVRTHGTLES---------------
sp|P34130|NTF4_HUMAN      -----------------MLPLPSCSLPILLLFLLPSVPIESQPPP---------------
sp|P20783|NTF3_HUMAN      MSILFYVIFLAYLRGIQGNNMDQRSLPEDSLNSLIIKLIQADILKNKLSKQMVDVKENYQ
sp|P01138|NGF_HUMAN       MSMLFYTLITAFLIGIQAEPHSESNVPAGHTIPQAHWTKLQHSLD---------------
                                                .  .:

sp|P23560|BDNF_HUMAN      VNGPKAGSRGLTSLADTFEHVIEELLDEDQKVRPNEENNKDADLYTSRVMLSSQVPLEPP
sp|P34130|NTF4_HUMAN      STLPPFLAP-------------------------------EWDLLSPRVVLSRGAPAGPP
sp|P20783|NTF3_HUMAN      STLPKAEAPREPERGGPAKSAFQPVIAMDTELLR-----QQRRYNSPRVLLSDSTPLEPP
sp|P01138|NGF_HUMAN       TALRRARSAPAAAIAARVAGQTRNITVDPRLFKK-------RRLRSPRVLFSTQPPREAA
                                 :                                     :.**::*   *  ..

sp|P23560|BDNF_HUMAN      LLFLLEEYKNYLDAANMSMRVRR----HSDPARRGELSVCDSISEWVTAADKKTAVDMSG
sp|P34130|NTF4_HUMAN      LLFLLEAGAFRESAGAPANRSRRGVSETAPASRRGELAVCDAVSGWVT--DRRTAVDLRG
sp|P20783|NTF3_HUMAN      PLYLMEDYVGSPVVANRTSRRKR---YAEHKSHRGEYSVCDSESLWVT--DKSSAIDIRG
sp|P01138|NGF_HUMAN       DTQDLDFEVGGAAPFNRTHRSKR--SSSHPIFHRGEFSVCDSVSVWVG--DKTTATDIKG
                              ::           : * :*         :*** :***: * **   *: :* *: *

sp|P23560|BDNF_HUMAN      GTVTVLEKVPVSKGQ-LKQYFYETKCNPMGYTKEG-------CRGIDKRHWNSQCRTTQS
sp|P34130|NTF4_HUMAN      REVEVLGEVPAAGGSPLRQYFFETRCKADNAEEGGPGAGGGGCRGVDRRHWVSECKAKQS
sp|P20783|NTF3_HUMAN      HQVTVLGEIKT-GNSPVKQYFYETRCKEARPVKNG-------CRGIDDKHWNSQCKTSQT
sp|P01138|NGF_HUMAN       KEVMVLGEVNI-NNSVFKQYFFETKCRDPNPVDSG-------CRGIDSKHWNSYCTTTHT
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sp|P23560|BDNF_HUMAN      YVRALTMDSKKRIGWRFIRIDTSCVCTLTIKRGR--
sp|P34130|NTF4_HUMAN      YVRALTADAQGRVGWRWIRIDTACVCTLLSRTGRA-
sp|P20783|NTF3_HUMAN      YVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT-
sp|P01138|NGF_HUMAN       FVKALTMDG-KQAAWRFIRIDTACVCVLSRKAVRRA
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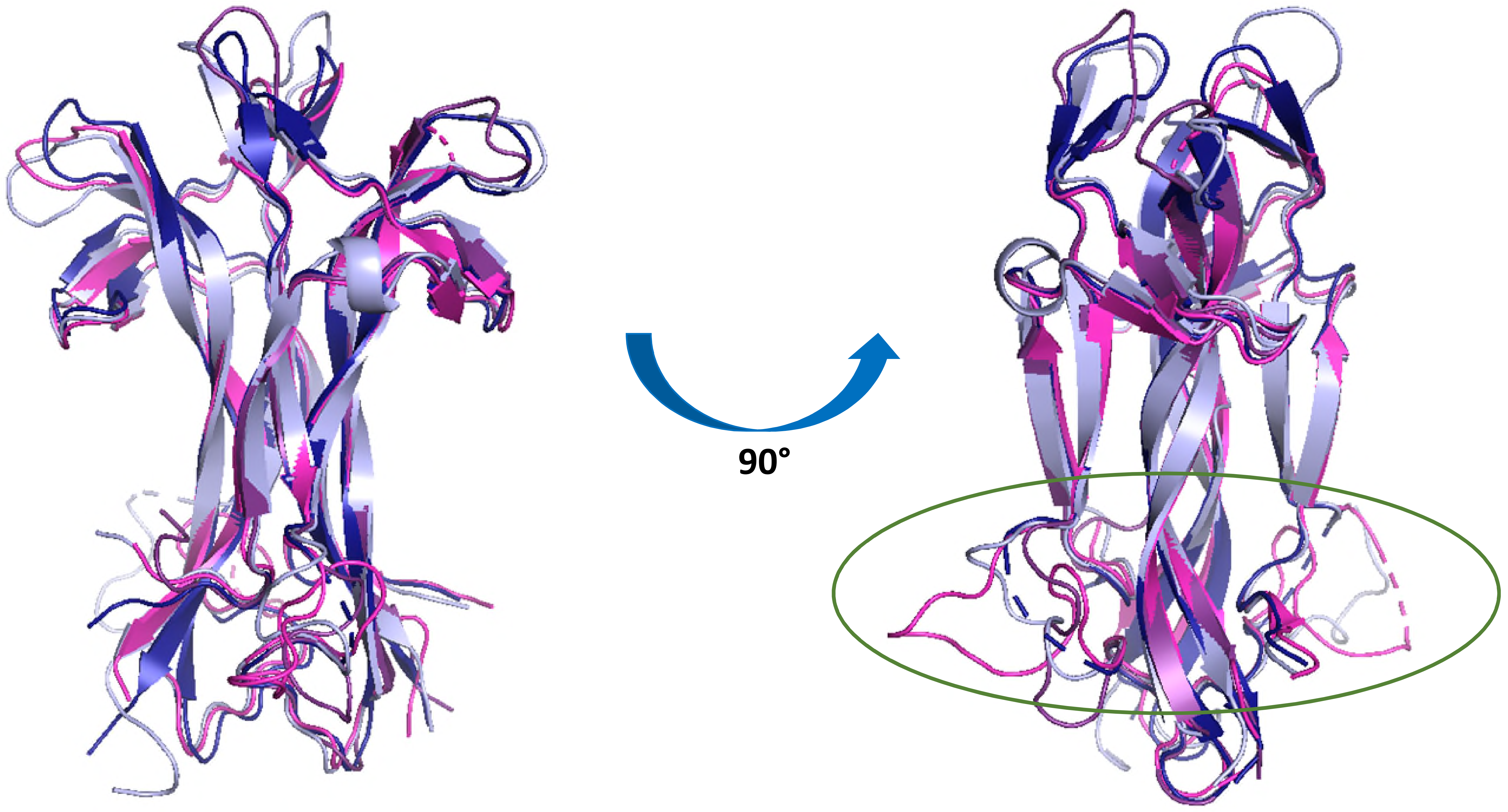
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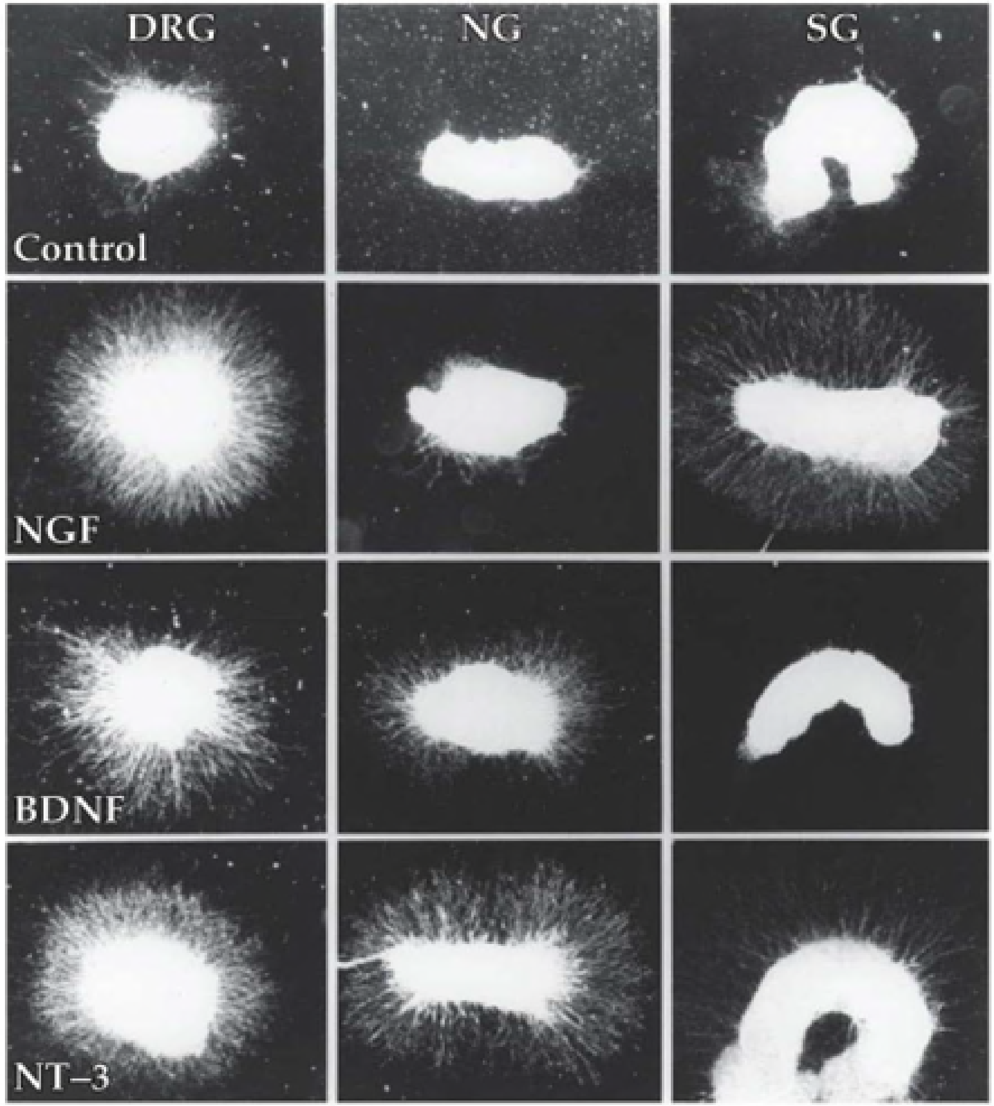
Table 1
Neurotrophins that have been detected in various organisms with its role and corresponding receptors.

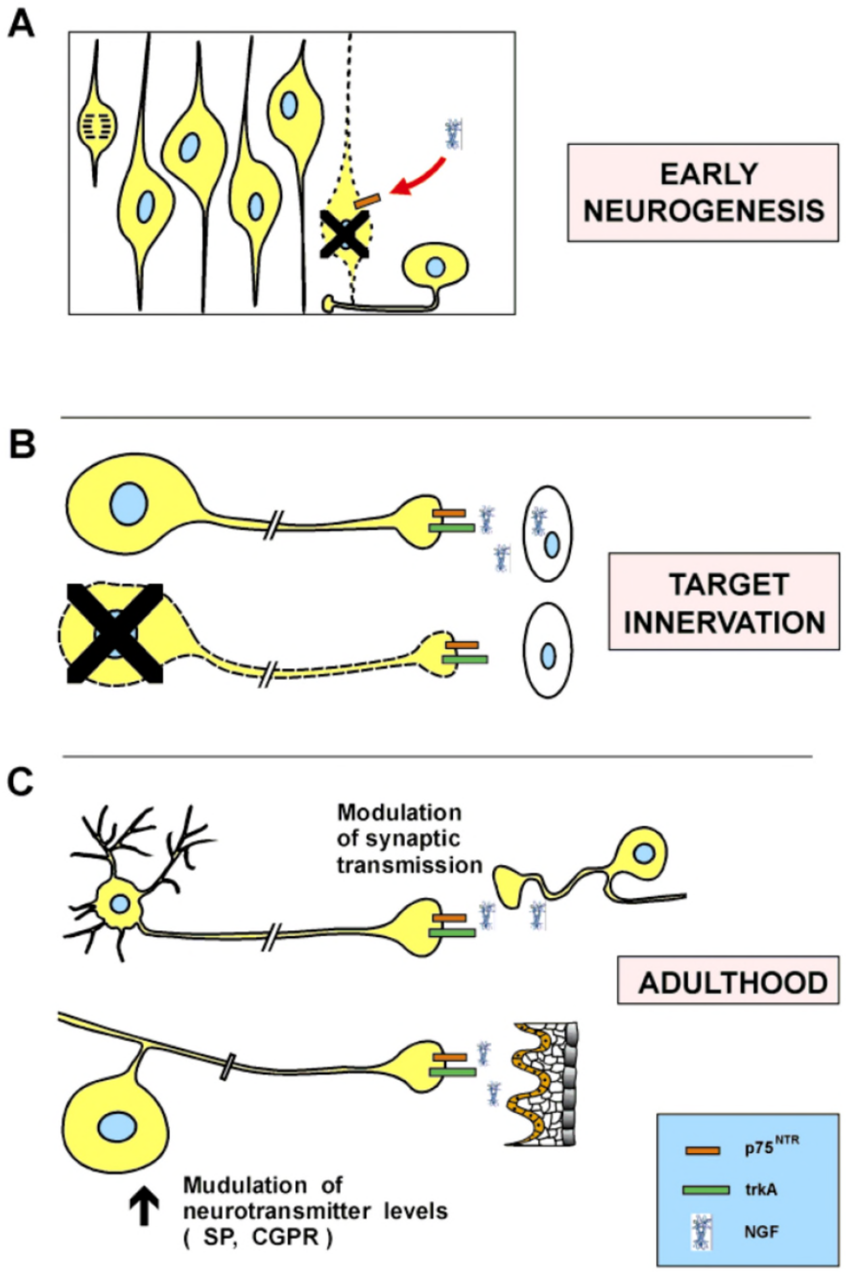
NT[a]	Year	General role	Location	Receptor	Study
NGF	1953	Differentiation and survival of sensory and sympathetic nervous system (cholinergic neurons). Important for attention, arousal, motivation, memory and consciousness.	Neocortex, olfactory bulb, hippocampus, pituitary gland. Also found in basal ganglia, thalamus, spinal cord and retina.	TrkA $P75^{NTR}$ A9β1 integrin	Cohen et al. (1954); Staniszewska et al. (2008); Bhardwaj and Deshmukh (2018)
BDNF	1982	Growth and survival of dorsal root ganglion neurons, hippocampal and cortical neurons. More widespread neurotrophic effects influencing non-cholinergic system including dopamine within substantia nigra. Prevents degeneration and increase functional activity of dopaminergic neuron. Supports learning and memory.	Dorsal root ganglion, hippocampus, cerebral cortex.	TrkB $P75^{NTR}$ A9β1 integrin	Barde et al. (1982); Staniszewska et al. (2008); Bhardwaj and Deshmukh (2018)
NT3	1990	Neuronal survival and differentiation. Amounts are high during fetal development	Dorsal rot ganglia, nodose ganglion, sympathetic ganglia, trigeminal mesencephalic nucleus, hippocampus, cerebellum, dopaminergic and GABAergic cells in ventral mesencephalon, motor neurons in spinal cord.	TrkC $P75^{NTR}$ A9β1 integrin	Maisonpierre et al. (1990); Holtzman and Mobley (1994); Staniszewska et al. (2008)
NT4	1991	Neuronal proliferation and differentiation. Studies has shown its potential in repairing peripheral nerve injury and spinal cord transection.	Trigeminal, dorsal root ganglion, jugular, sympathetic, nodose ganglion neurons, hippocampus, dopaminergic, GABAergic neurons in ventral mesencephalon, medulla, hypothalamus, thalamus, cerebellum.	TrkB $P75^{NTR}$	Hallbook et al. (1991); Holtzman and Mobley (1994)
NT6	1994	Found in platyfish *Xiphqphorus maculatus*. It promotes the survival of sympathetic and sensory dorsal root ganglion.	Mature neurons of the forebrain, midbrain and hindbrain. It is also expressed in adult gill, liver and eye with weak expression in skin, spleen, heart and skeletal muscle.	Not documented	Gotz et al. (1994); Leggieri et al. (2019)
NT7	1998	Found in zebrafish *Danio rerio*. Closely related to NGF and NT6. Demonstrated by its ability to promote neurite outgrowth and neuronal survival of chick dorsal root ganglia.	Eye, gills, skin and heart. There is also weak expression in brain and intestine.	TrkA P75	Lai et al. (1998); Nilsson et al. (1998)

[a] NT, neurotrophin; BDNF, brain-derived neurotrophic factor; NGF, neuronal growth factor; NT3, neurotrophin 3; NT4, neurotrophin 4; NT6, neurotrophin 6; NT7, neurotrophin 7; p75NTR, p75 pan neurotrophin factor; Trk, tropomyosin related kinase.

Different Neurons have Different Requirements for Neurotrophic Factors



DRG = Dorsal Root Granglion, NG = Nodose Ganglion, SG = Sympatethic ganglion



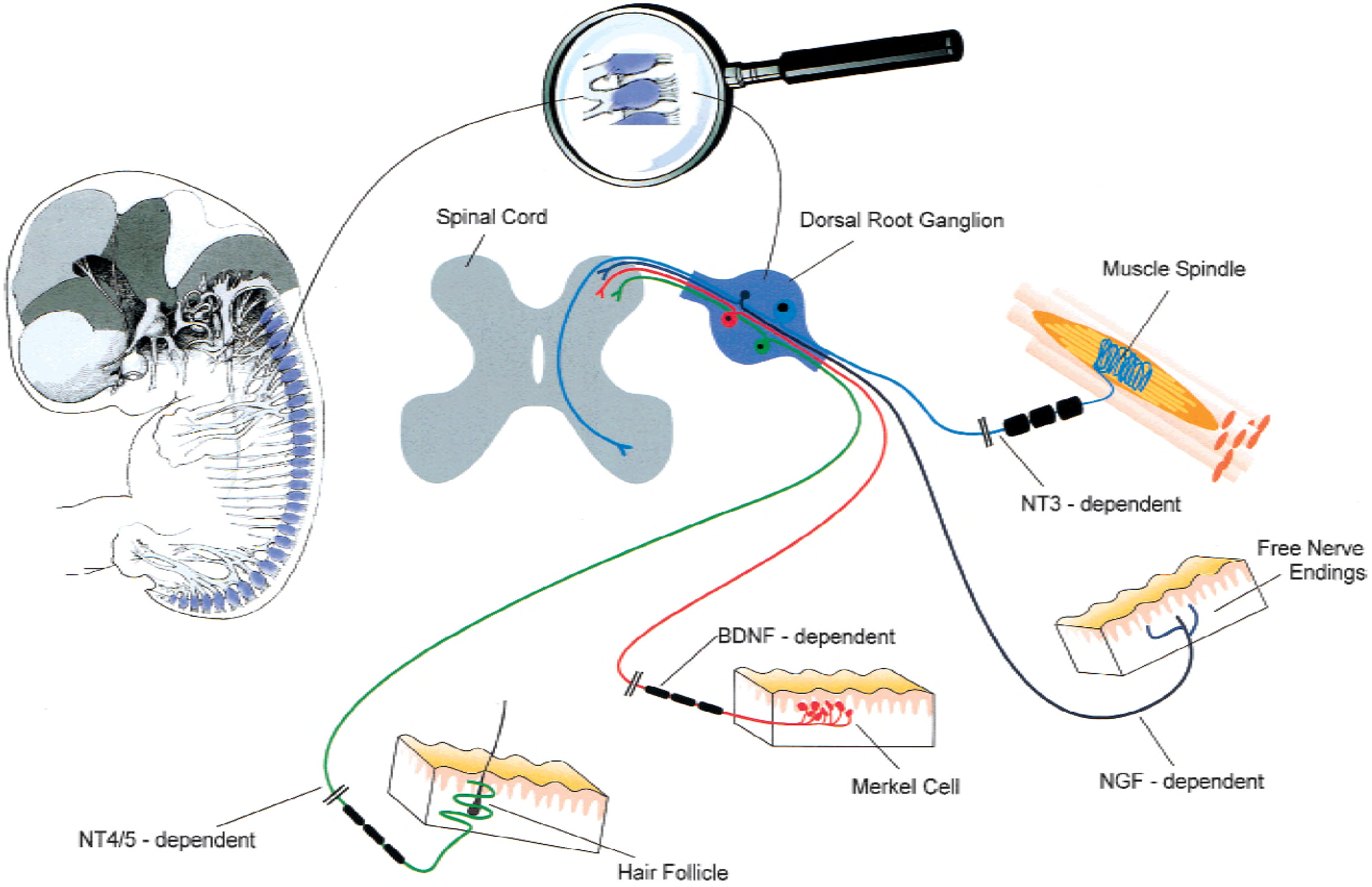
Biological actions of NGF in the developing and adult nervous system.

A: During early neurogenesis, NGF triggers the elimination of newly born neurones expressing $p75_{NTR}$ and presumably in competition for space with other neurones.

B: At later developmental stages, during the period of target innervation, NGF (and other neurotrophins) support the survival of some neurones expressing the appropriate trk receptors. The limited quantities of secreted neurotrophins do not allow the survival of all neurones, which typically can be rescued by the administration of exogenous neurotrophins.

C: In the adult, NGF, as well as the other neurotrophins, modulate neuronal plasticity (top), reflected by the modulation of synaptic transmission and the complexity of dendritic arborization. In the CNS, this latter property is best documented for non-NGF neurotrophins. In addition, NGF is also involved in the regulation of hyperalgesia during inflammation (bottom) by increasing the levels of neurotransmitters expressed by neurones concerned with the detection of potentially painful stimuli.

Frade & Barde, Bioessays, 1998



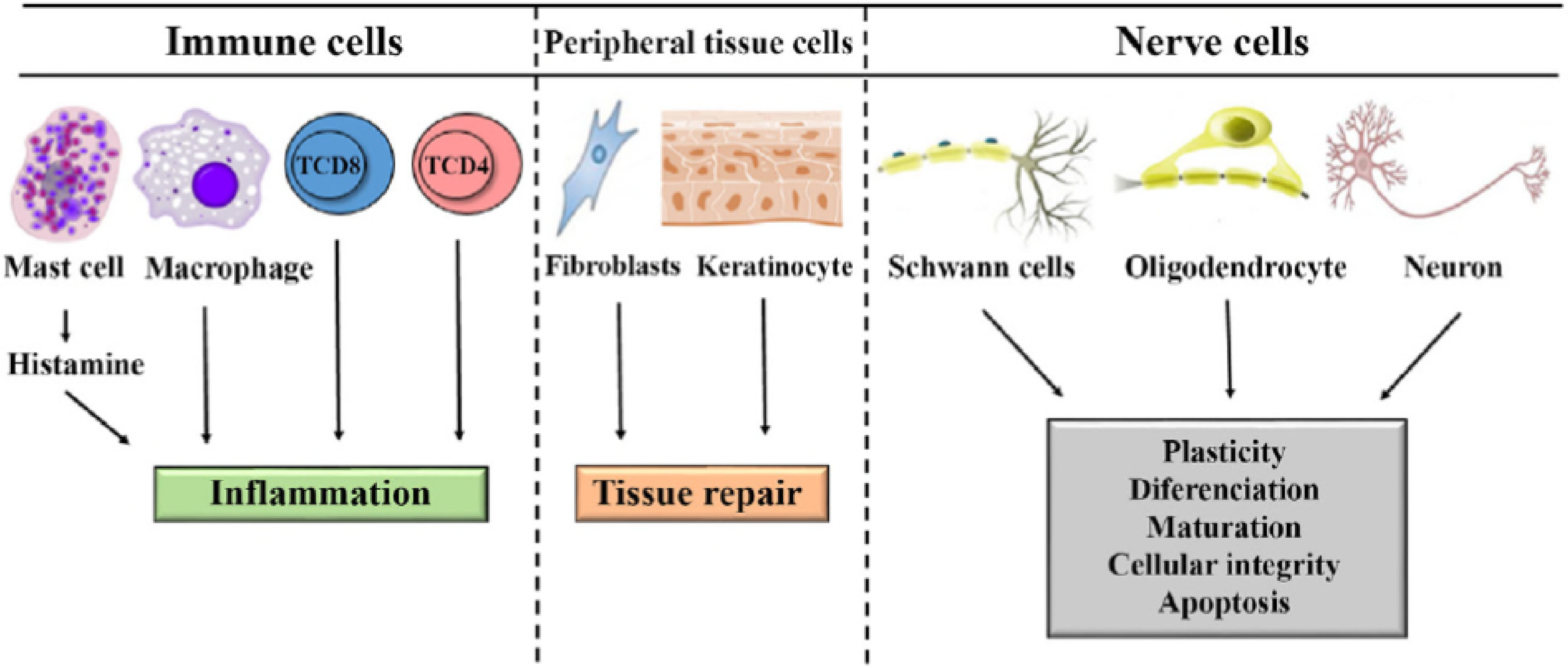
Neurotrophins control the development of specific subpopulations of sensory neurons. Sensory neurons of the dorsal root ganglia (DRGs) have specific patterns of projection both in the spinal cord and in the periphery, and they also transmit specific sensory modalities.

Bibel, Barde. Genes&Devlop, 2000

At the beginning: a secretory protein was shown to be essential for the survival of neurons during the development of the nervous system.

NGF was later shown to be synthesized in limiting amounts by tissues innervated by NGF-dependent neurons thereby allowing target tissues to dictate the density of their own innervation (Korsching and Thoenen, 1983; Edwards et al., 1989)

S. Ateaque, S. Merkouris and Y.A. Barde, *Front. Mol. Neurosci.* 16:1225373.



Frontiers in Immunology (2018) *Aarão TLdS et al*

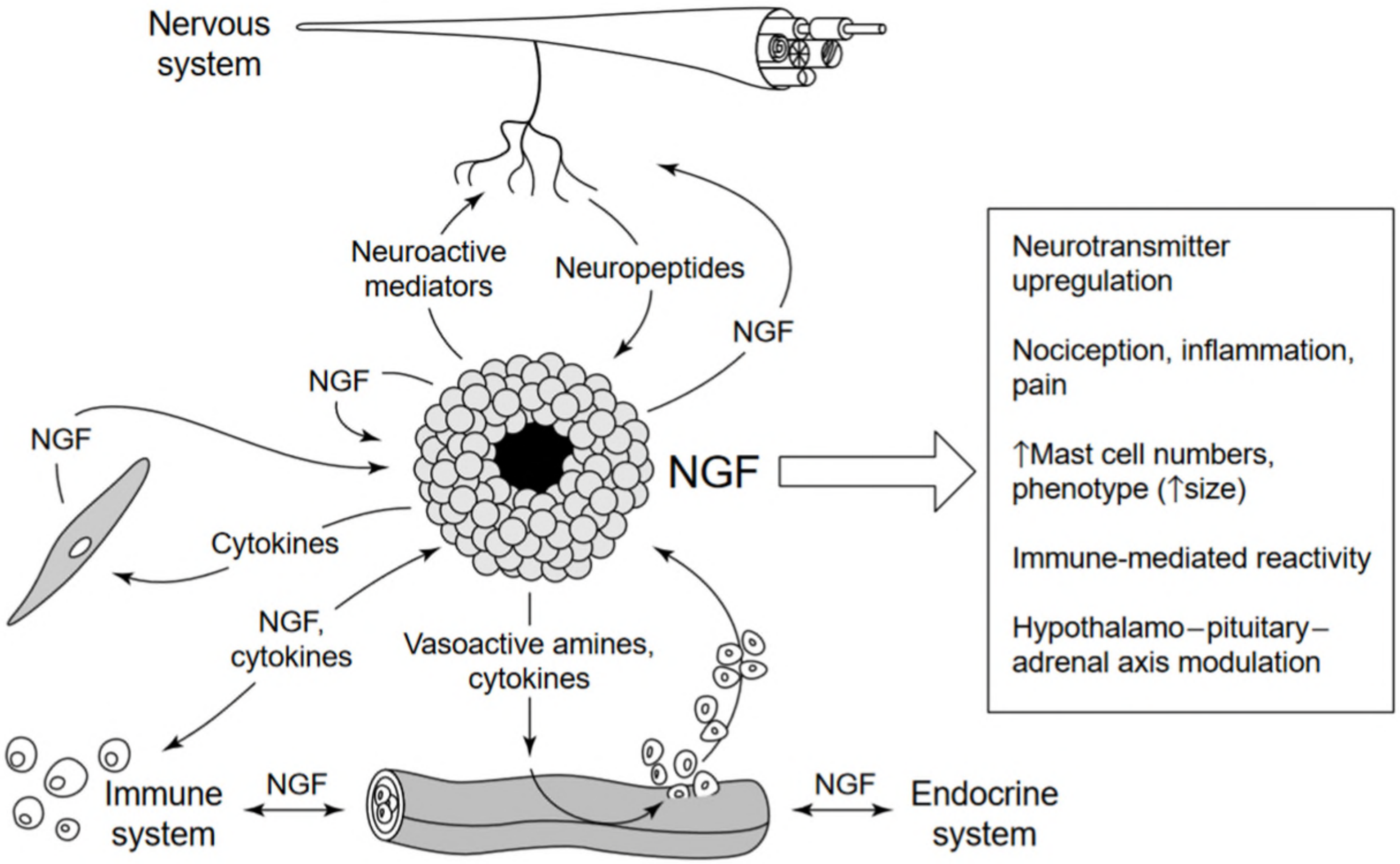
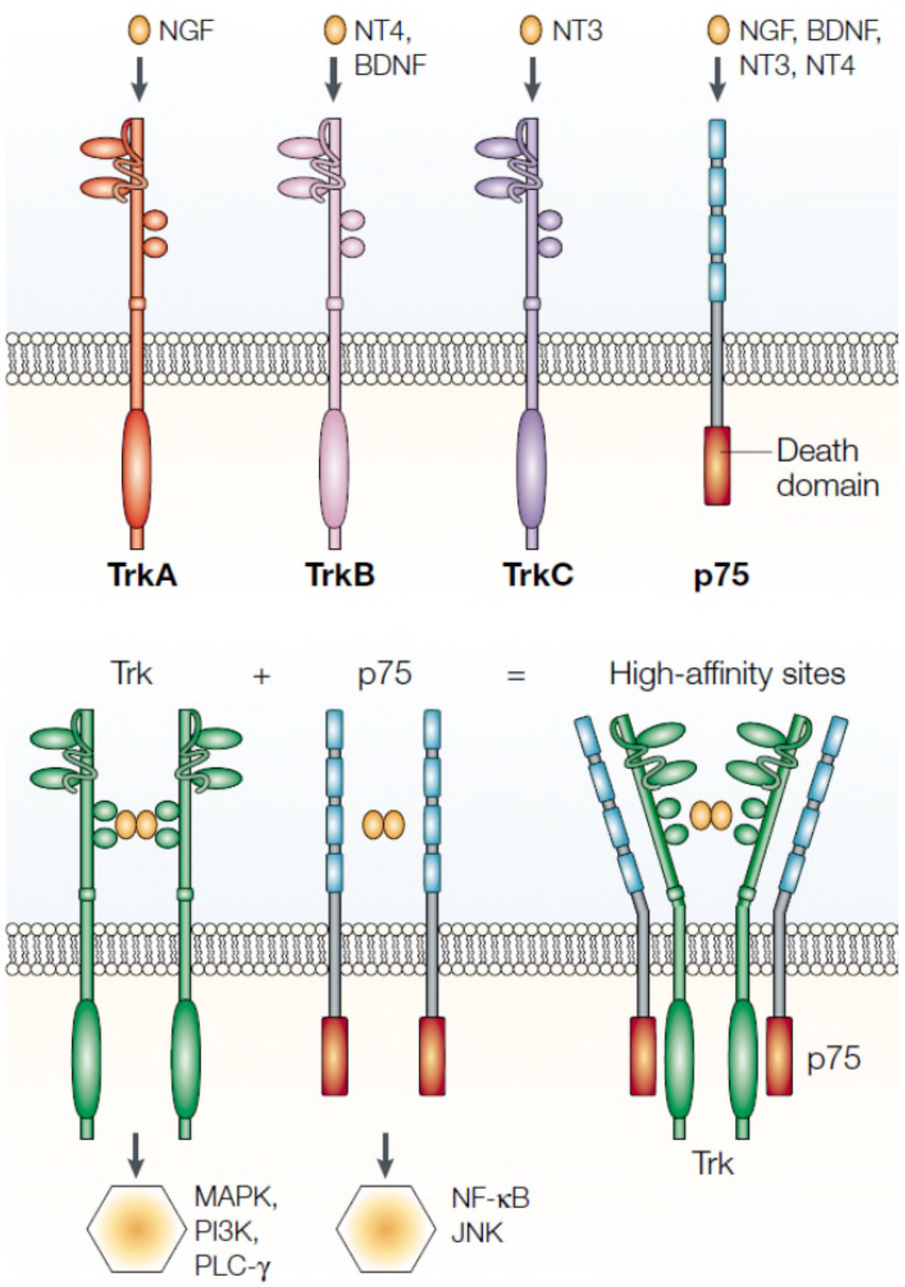
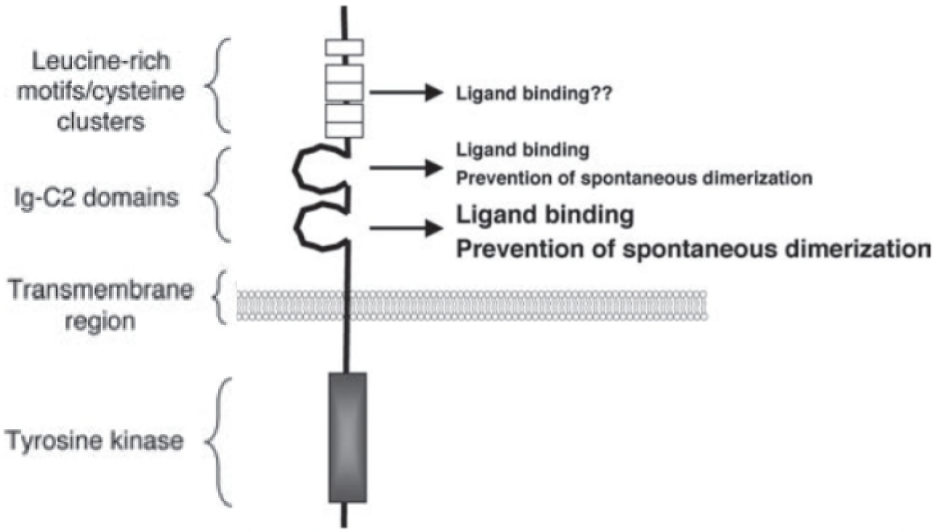


Fig. 3. ***Scheme showing how NGF might modulate neuro–endocrino–immune interactions.*** *Nerve growth factor released from tissue mast cells as a consequence of nervous, immune or endocrine system inputs can, in turn, influence the same systems (locally or via the circulation). Nerve growth factor released from mast cells could also function in an autocrine manner. Possible actions of NGF derived from mast cells are listed in the box.*

Rita Levi-Montalcini, Stephen D. Skaper, Roberto Dal Toso, Lucia Petrelli and Alberta Leon, Trends Neurosci. (1996) 19, 514–520



Models of Trk and p75 receptor activation.
Neurotrophin binding results in dimerization of each receptor.
The extracellular portion of p75 contains four cysteine-rich repeats, and the intracellular part contains a death domain. Neurotrophin binding to the p75 receptor mediates survival, cell migration and myelination through several signalling pathways.
Interactions between Trk and p75 receptors can lead to changes in the binding affinity for neurotrophins.
BDNF, brain-derived neurotrophic factor; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NT, neurotrophin; PI3K, phosphatidylinositol 3-kinase; PLC-γ, phospholipase Cγ.

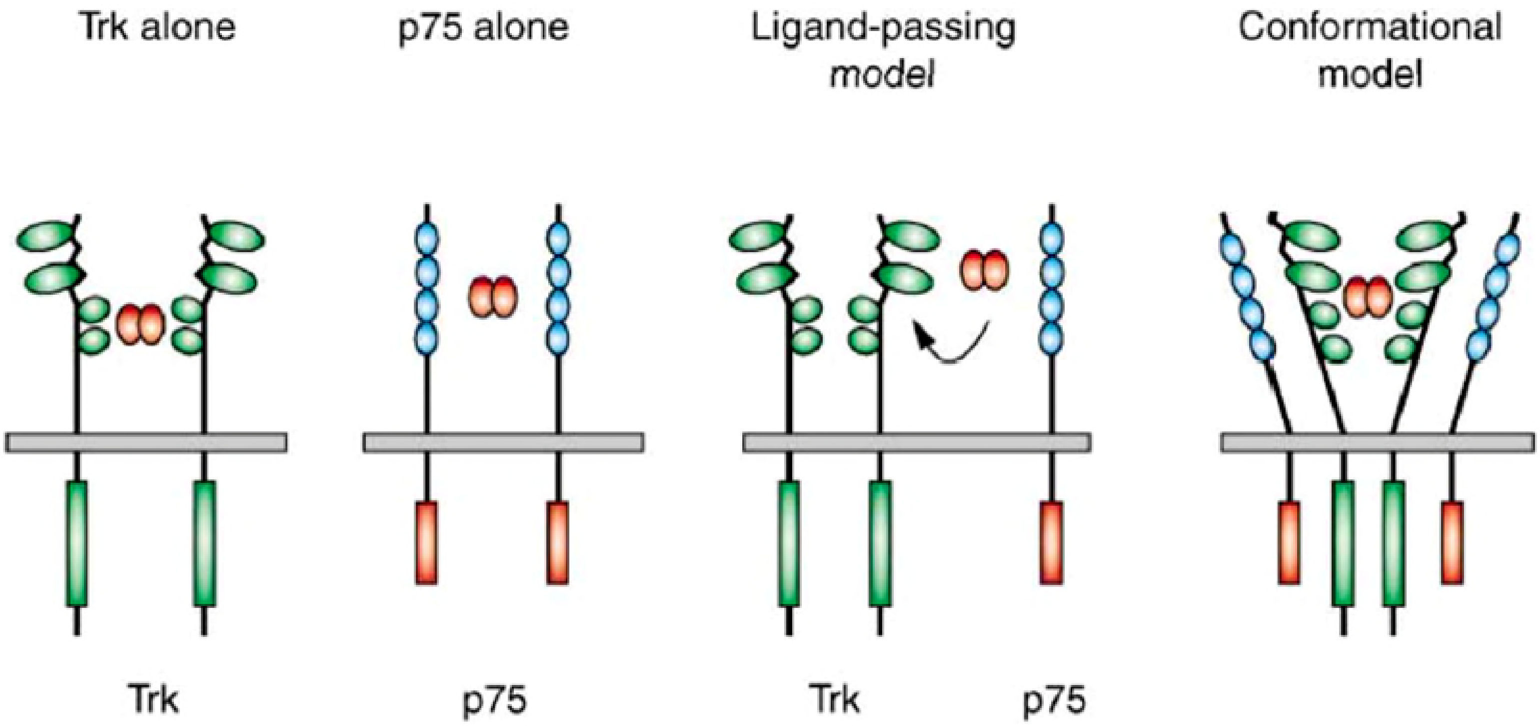


Chao, Nat Rev Neurosci, 2003

Arevalo & Wu, Cell Mol Life Sci, 2006

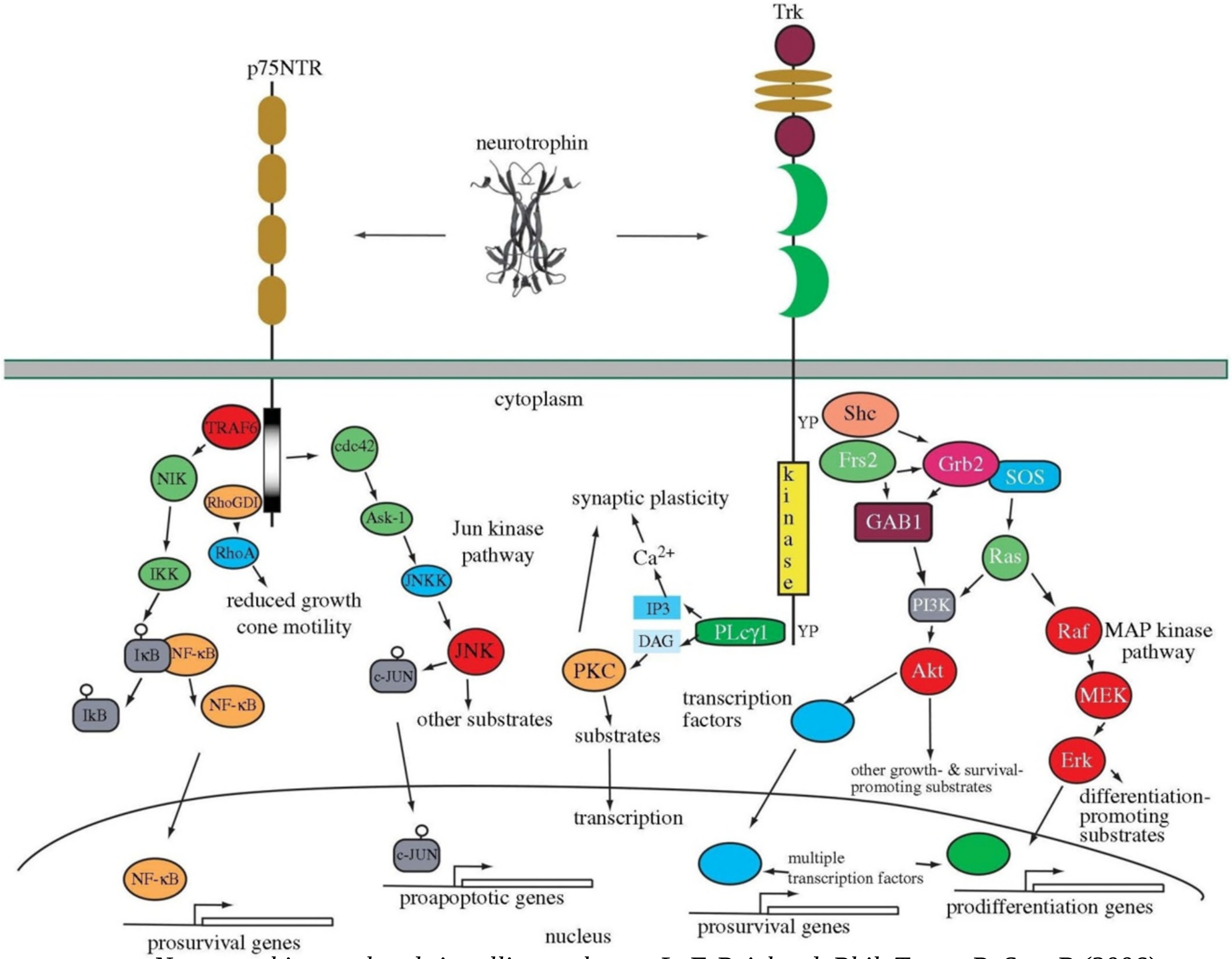
Models for Trk and p75^{NTR} interaction

The p75 receptor can bind to each NT, and also acts as a co-receptor for Trk receptors



Chao and Bothwell (2002) Neuron

A comparison of p75 and Trk signaling

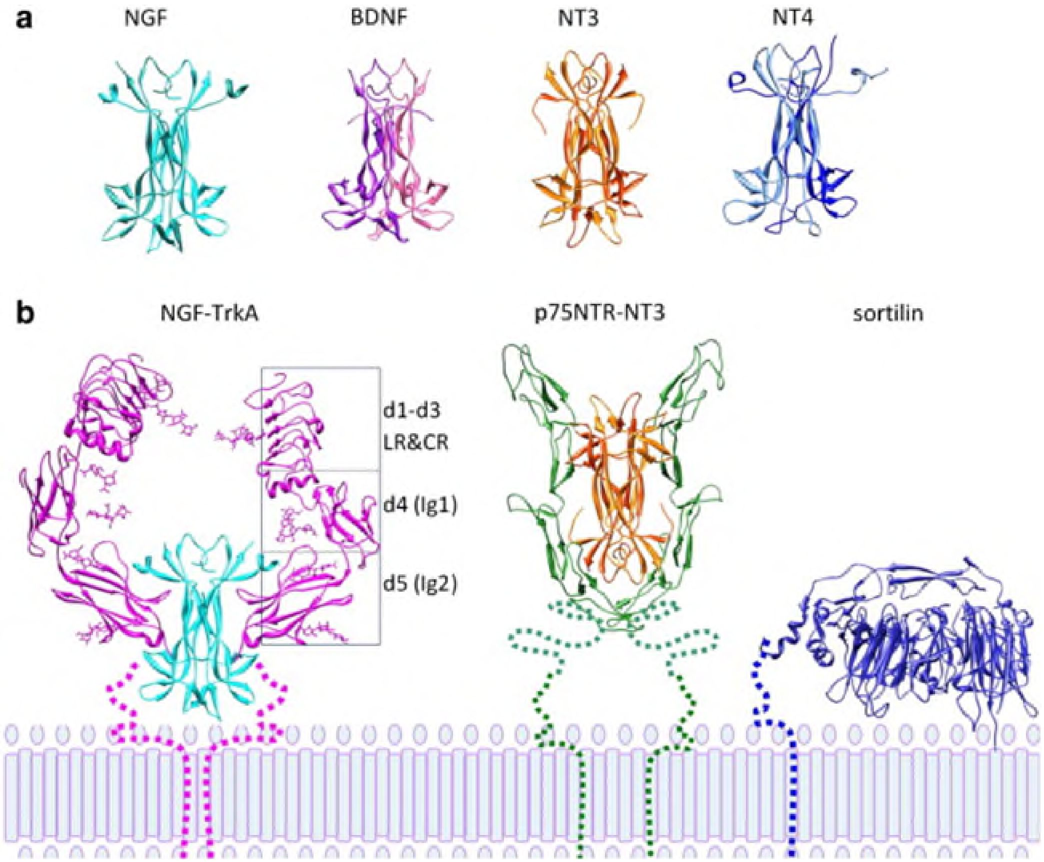


Neurotrophin-regulated signalling pathways L. F. Reichardt Phil. Trans. R. Soc. B (2006)

NT signalling through Trk and p75 receptors

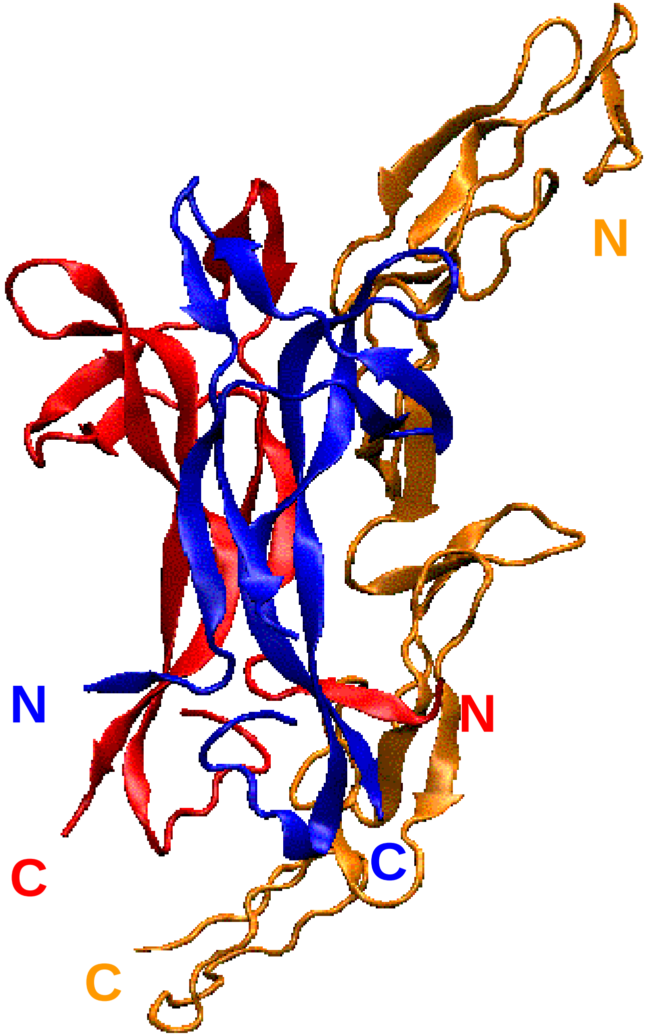
Pro and mature-NT bind as dimers to p75- and Trk-receptors.

Trk receptor dimerization leads to trans-autophosphorylation and to the activation of intracellular signalling cascades mediating differentiation and survival.



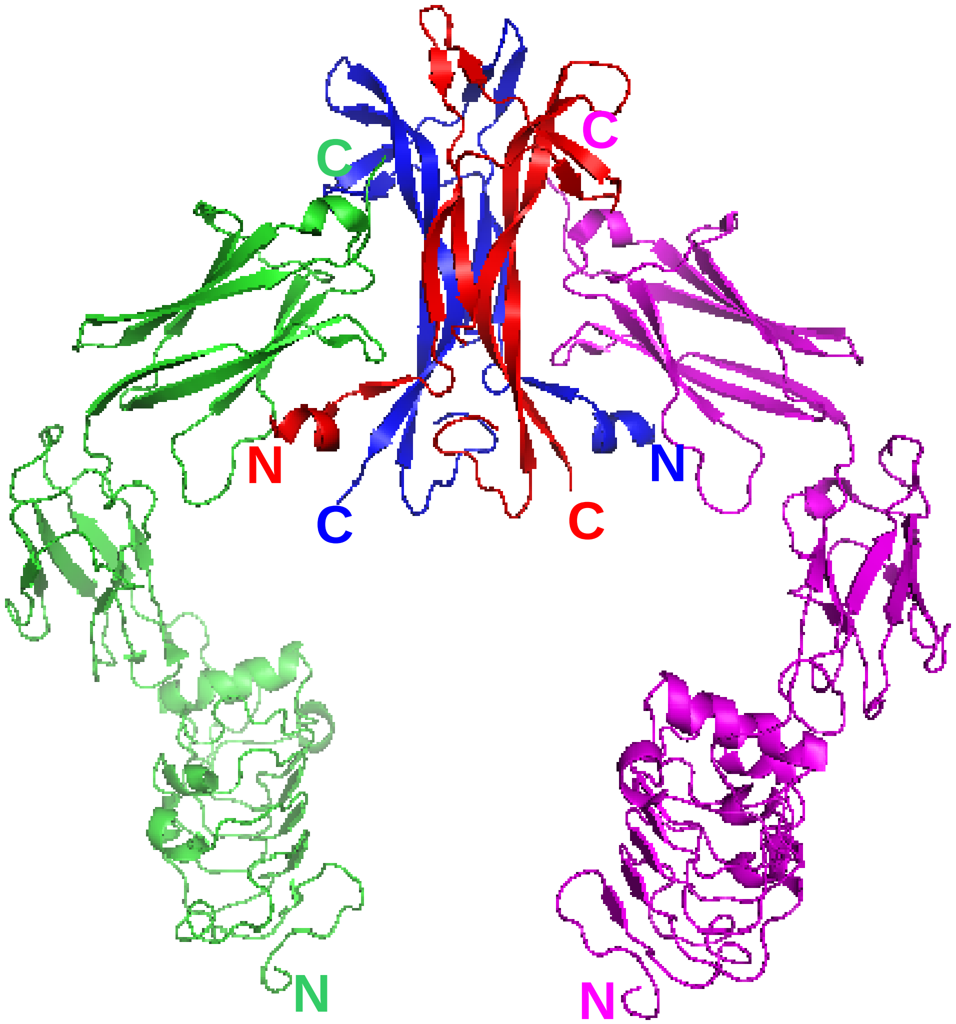
Complex NGF + $p75^{NTR}$ extracellular domain

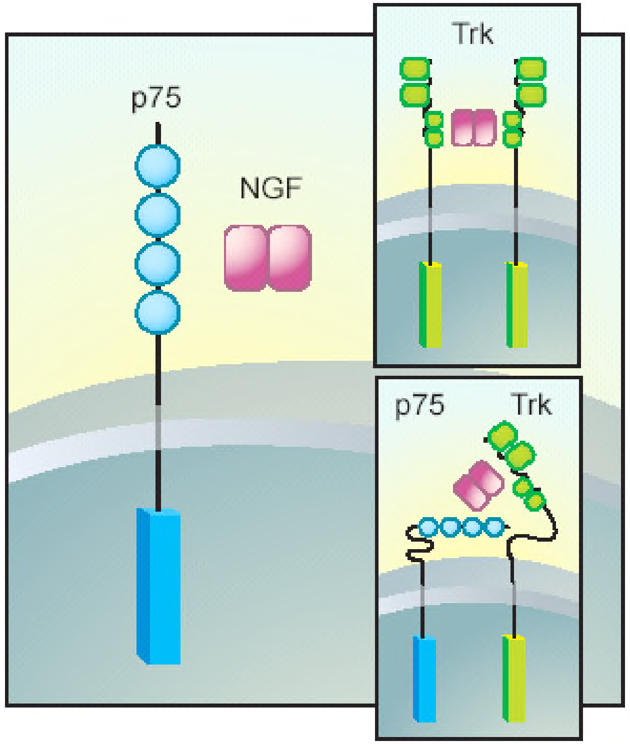
(PDB:1SG1; He and Garcia, Science, 2004)



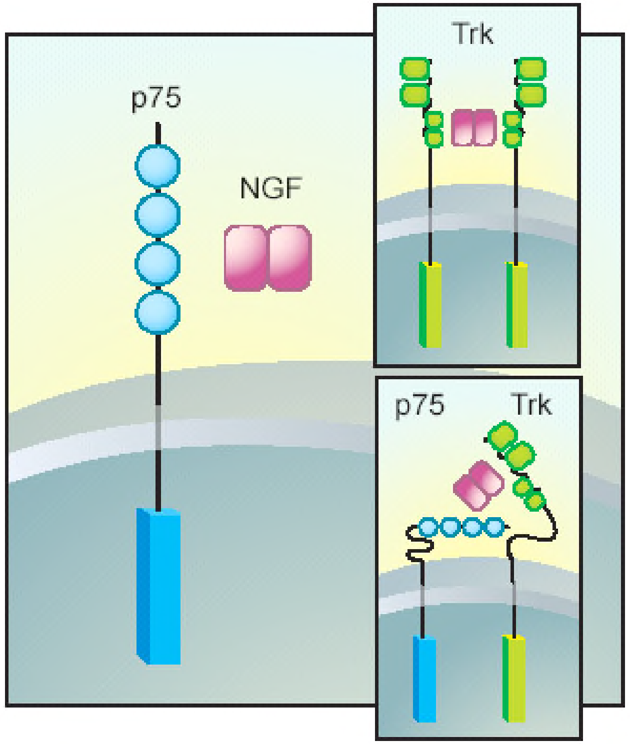
Complex NGF+ TrkA extracellular domain

(PDB: 2IFG; Wehrman et al, Neuron, 2007)





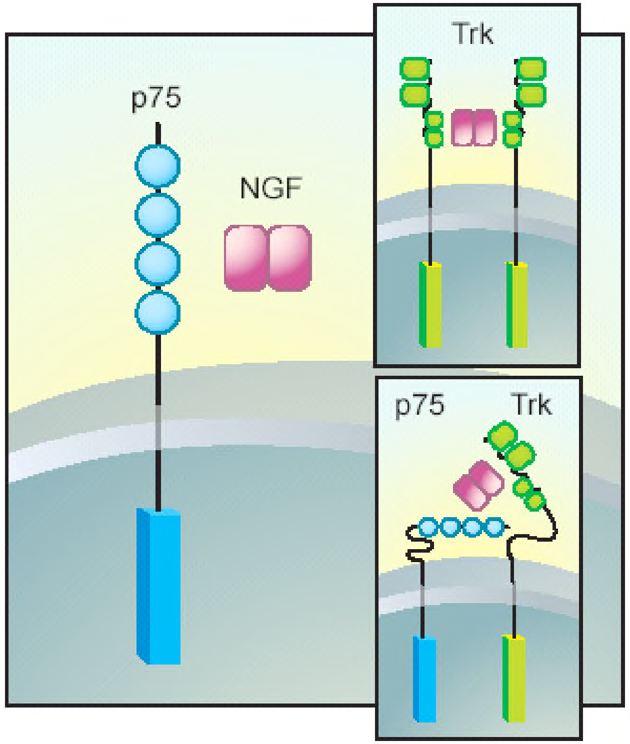
Zampieri and Chao, Science 2004.



Zampieri and Chao, Science 2004.

Dimer of dimers

Covaceuszach et al, Biophys J, 2015



Zampieri and Chao, Science 2004.

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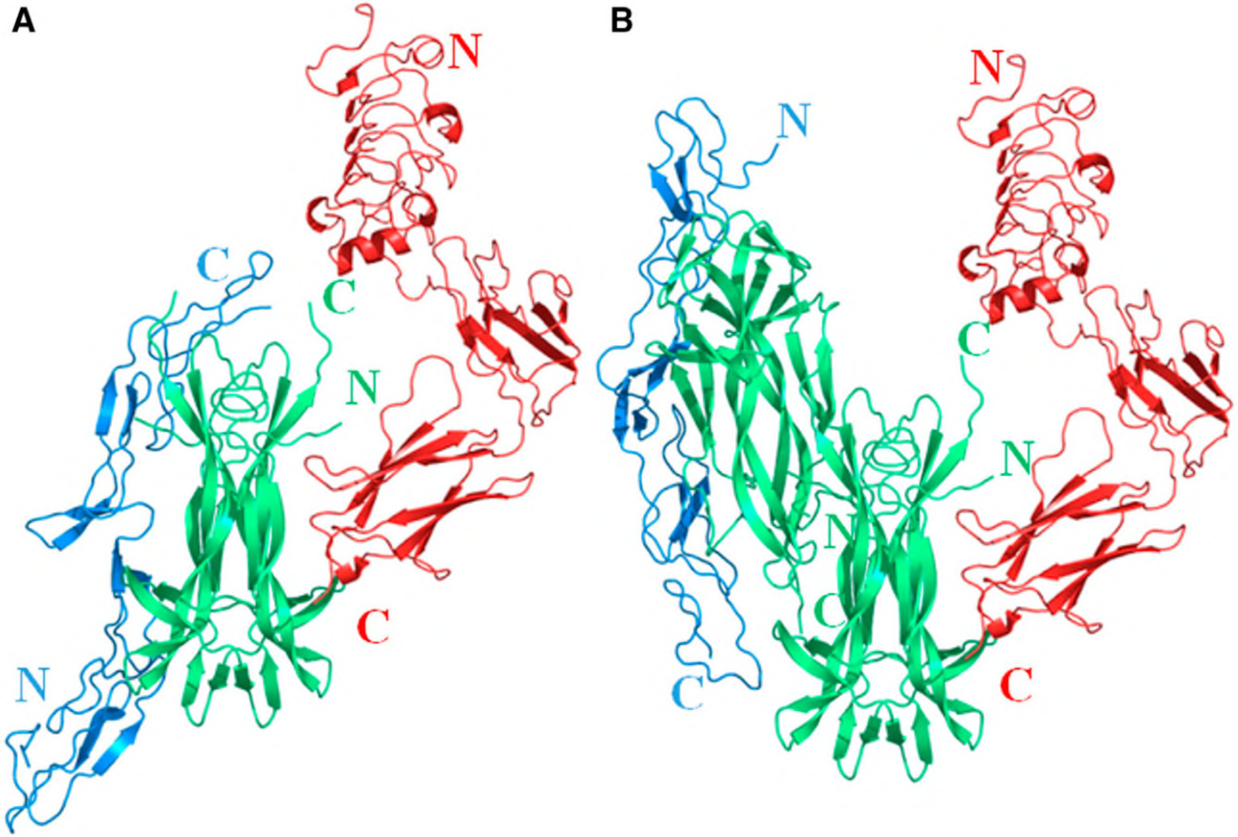
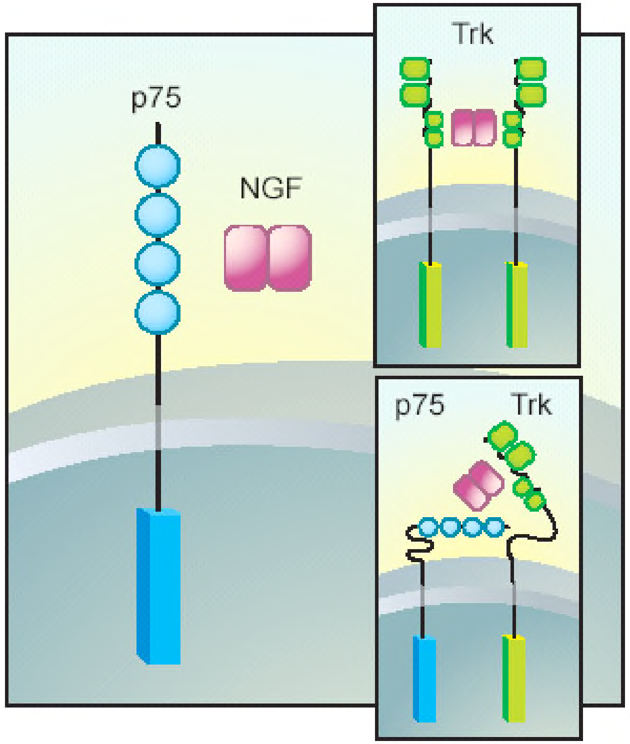
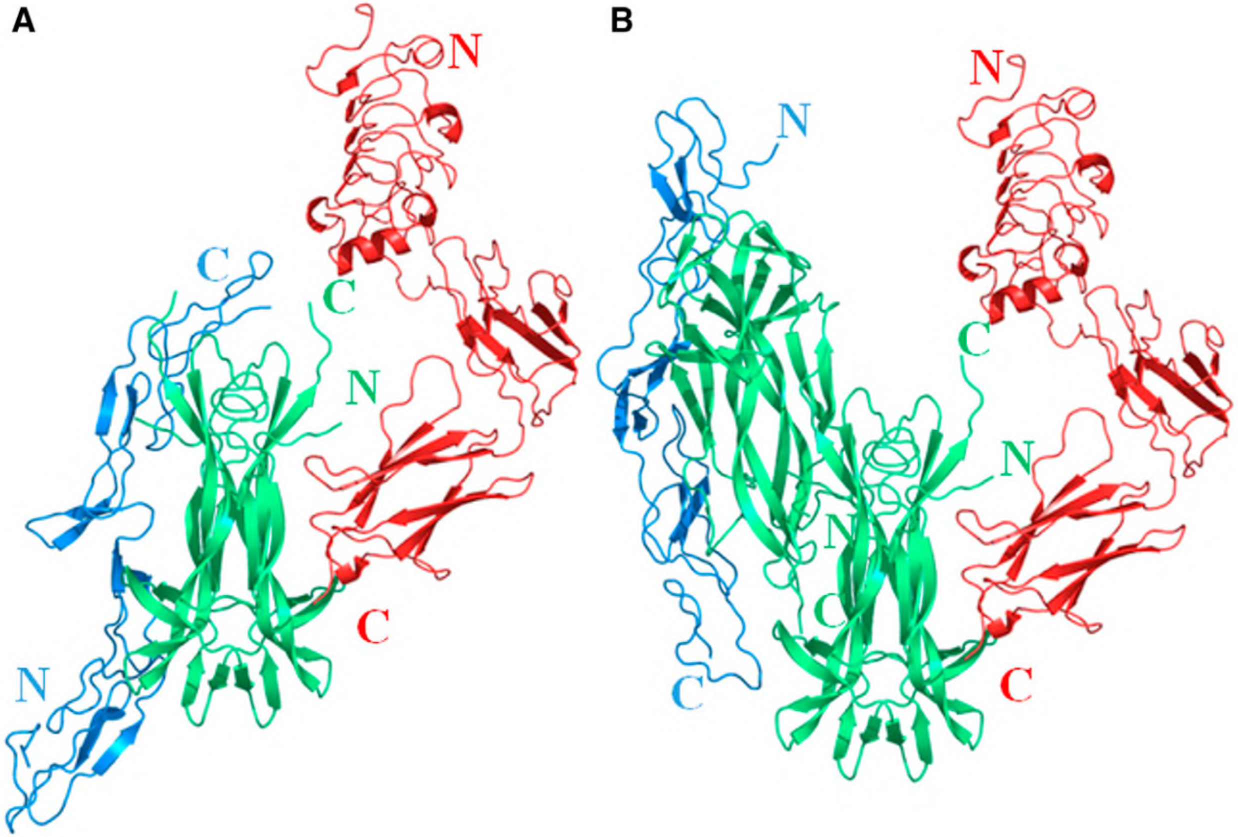


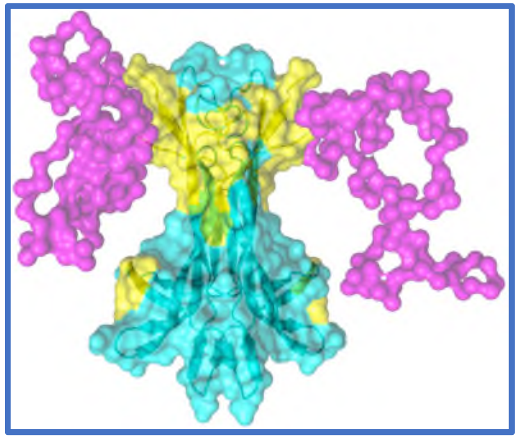
Covaceuszach et al, Biophys J, 2015



Zampieri and Chao, Science 2004.

Dimer of dimers





Covaceuszach et al, Biophys J, 2015

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May 2015, Volume 10, Issue 5
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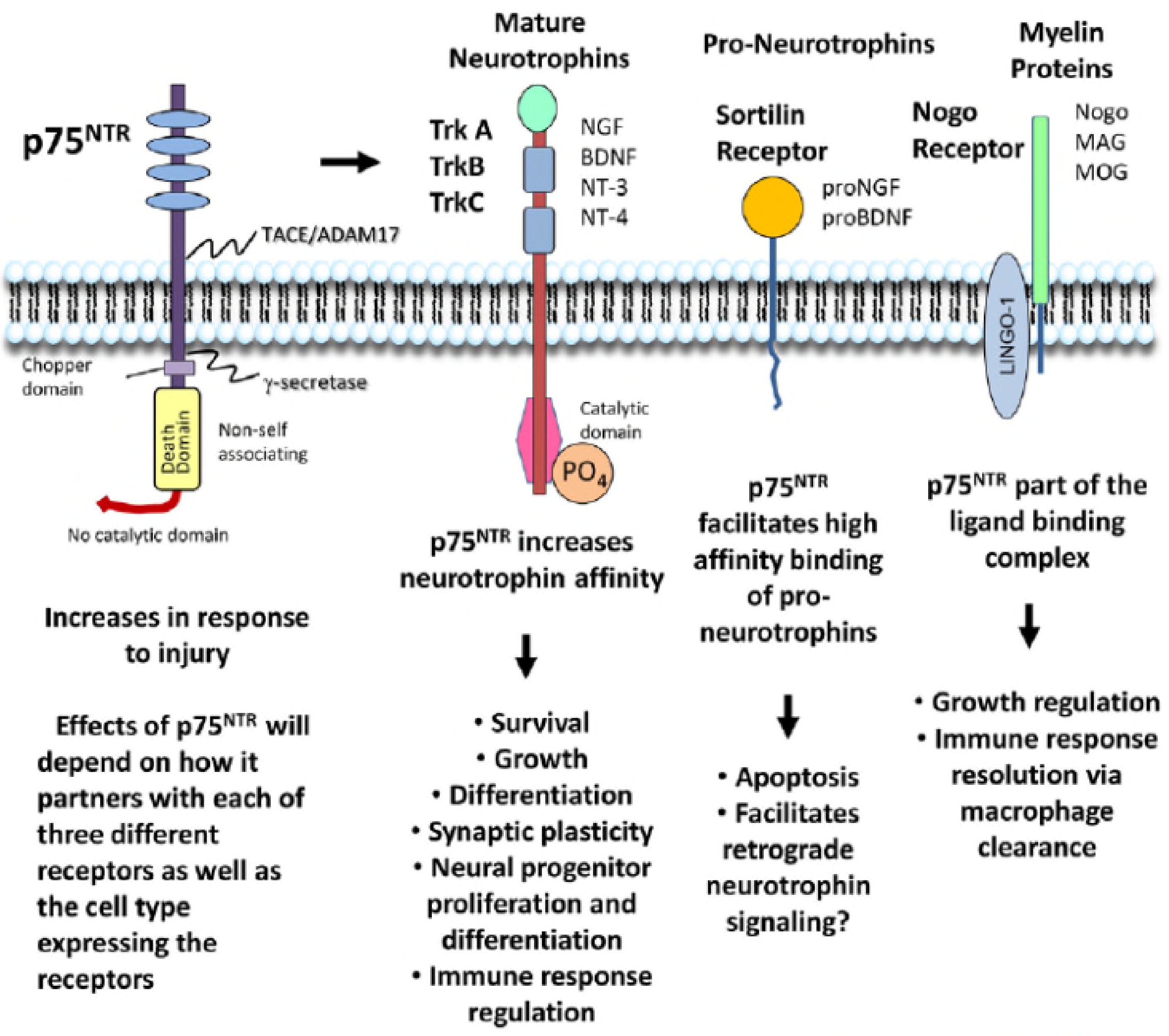
● INVITED REVIEW

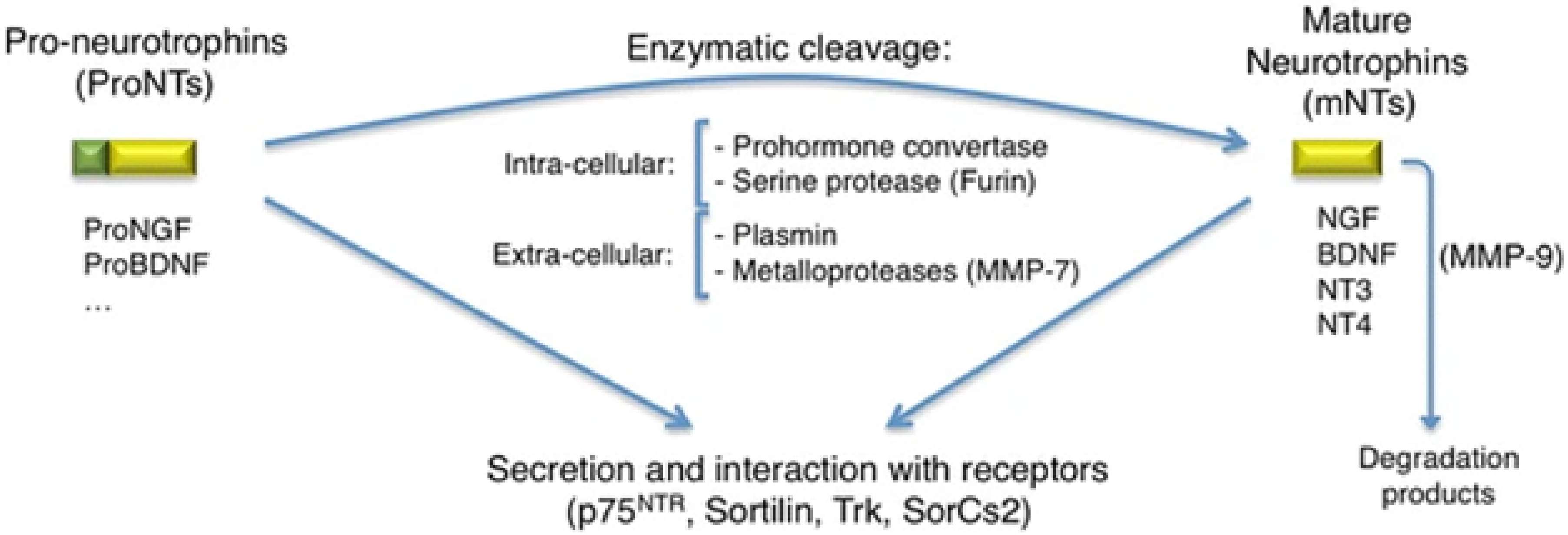
The p75 neurotrophin receptor: at the crossroad of neural repair and death

Rick B. Meeker[1,*], Kimberly S. Williams[2]



Proneurotrophins

Expressed as precursors with IUD (Intrinsically Unstructured Domain) pro-peptides and with different biological functions.



Costa et al, Mol Neurobiol, 2018

THE INTERACTION BETWEEN SORTILIN AND THE PRO-NEUROTROPHINS

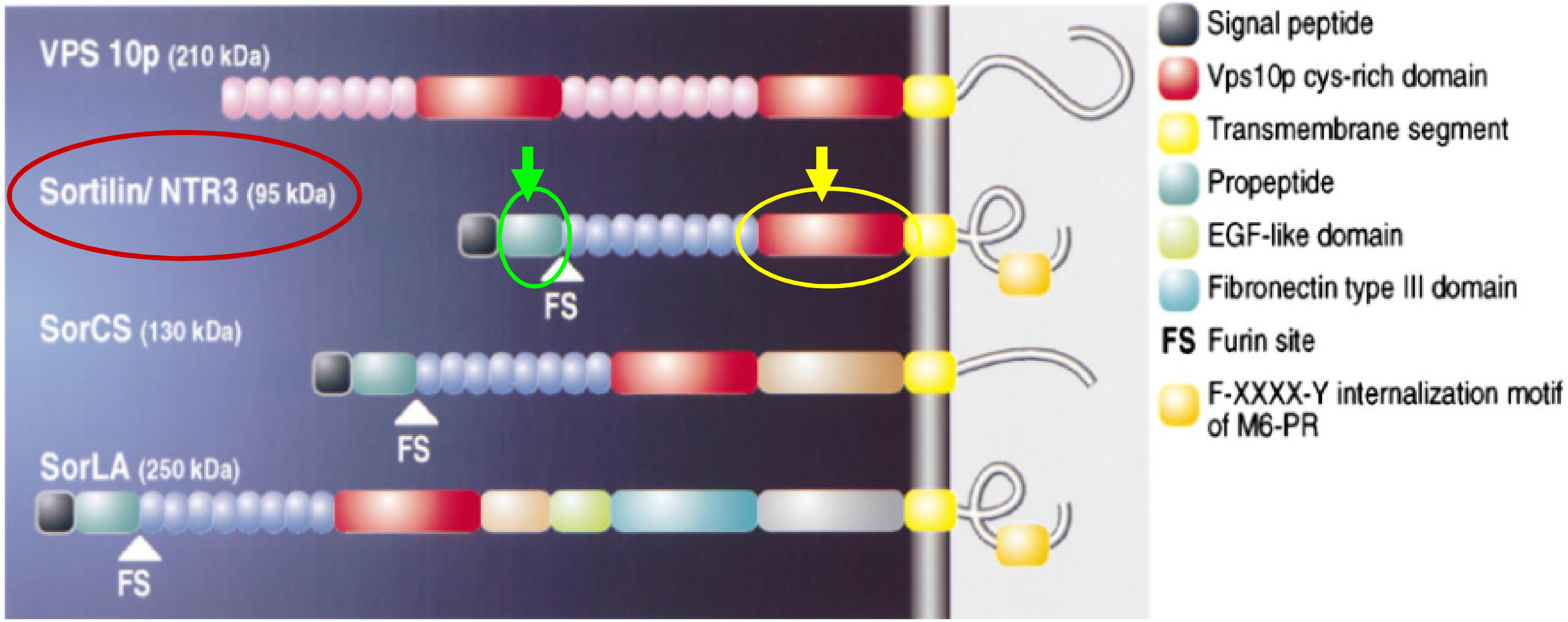


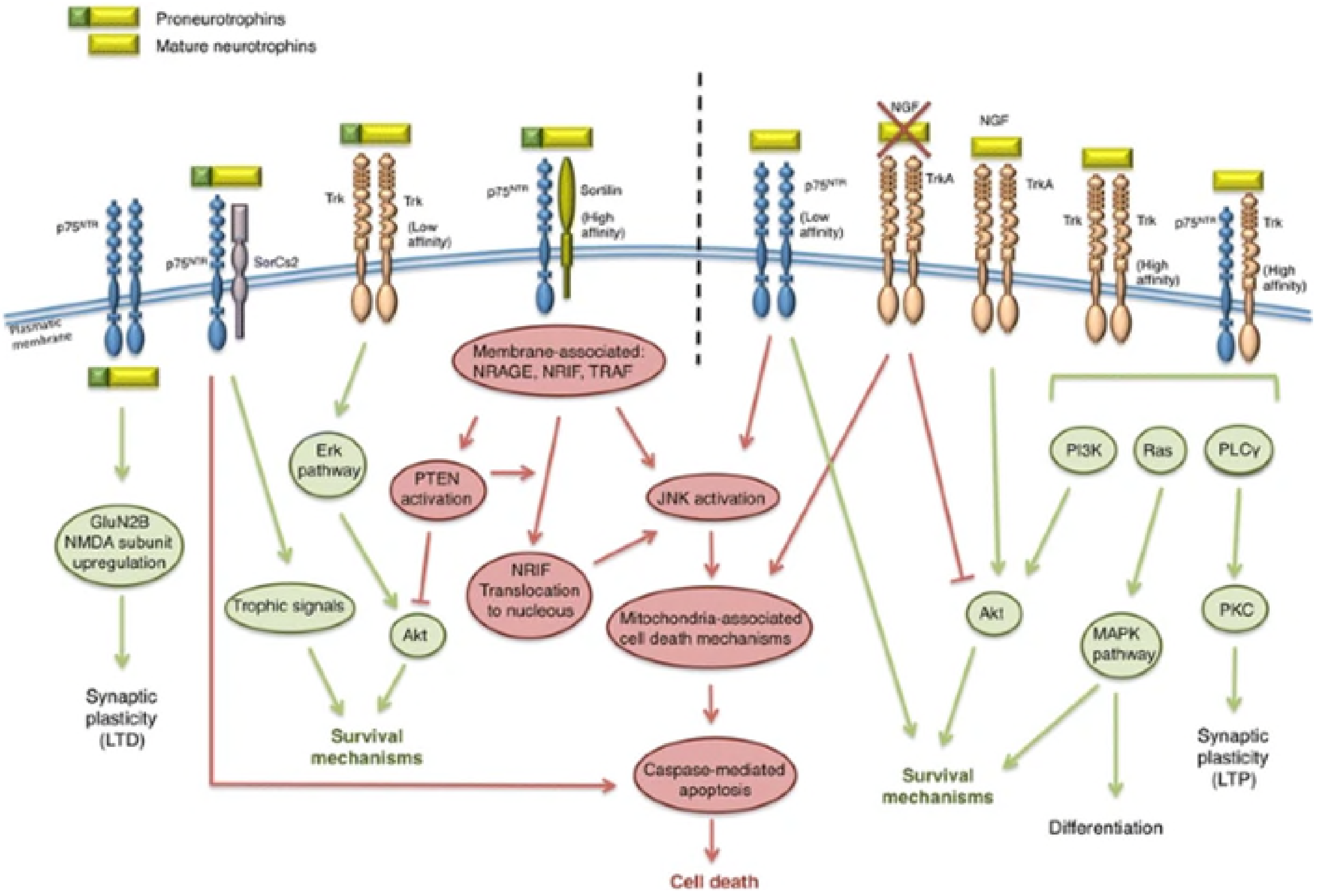
Fig. 1. The Vps10p domain receptor family. Members of the Vps10p containing domain receptor family (SorLA, Sortilin, and SorCS) share common motifs, including the 10CC-rich domain, a single transmembrane segment, a signal peptide (SP), at least one furin site (FS), and a short cytoplasmic tail. SorLA and sortilin bear the consensus hexapeptide internalization motif (F/Y)ENTL(F/Y).

Mazella, Cell.Signal., 2001

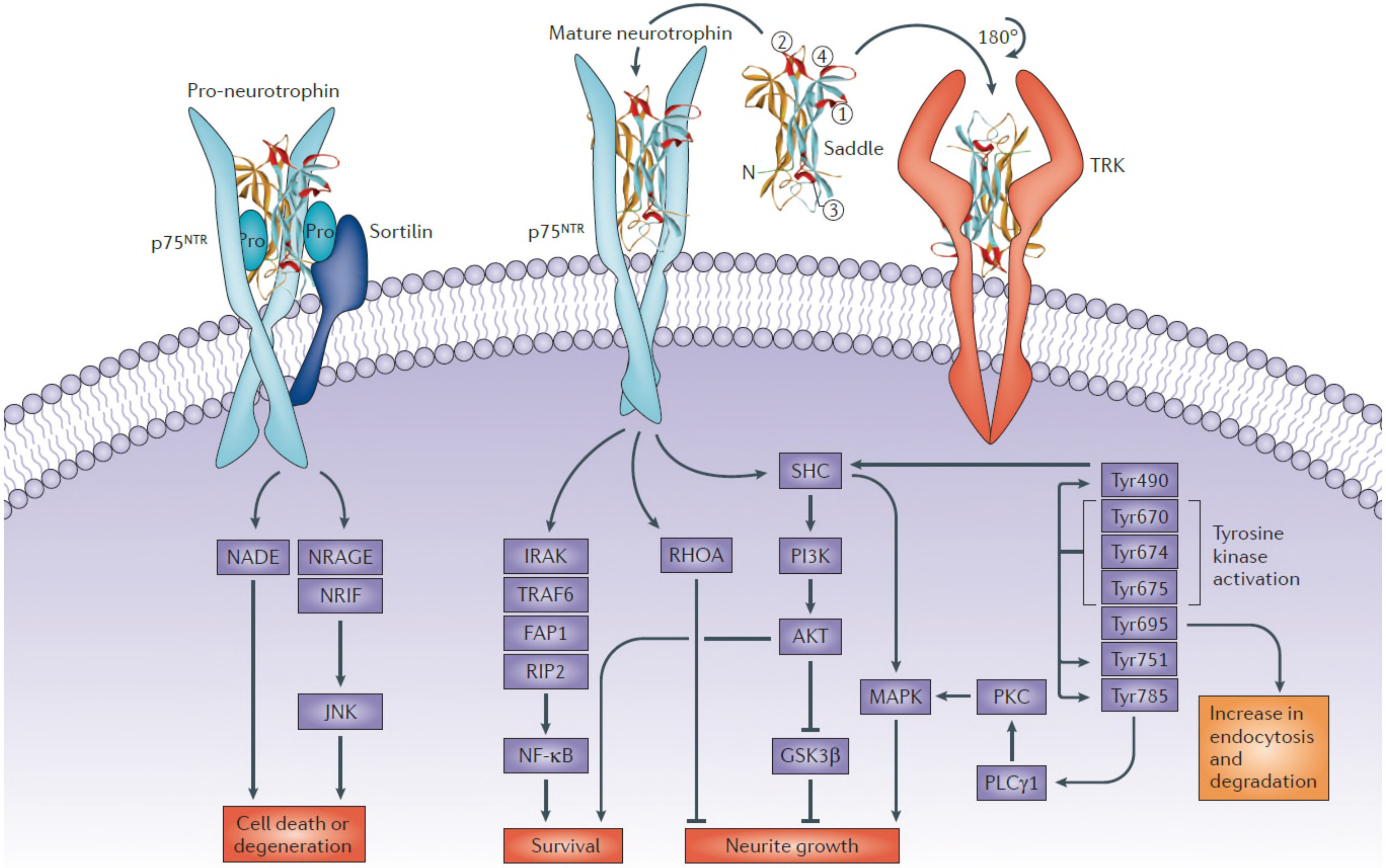
- Role of propeptide: functions as a safeguard that protects the cells against formation of death-signaling intracellular complexes
- Role of 10CC module in Vps10p: ligand binding region

Westergaard et al, JBC, 2004

Expressed as precursors with IUD (Intrinsically Unstructured Domain) pro-peptides and with different biological functions.



Costa et al, Mol Neurobiol, 2018

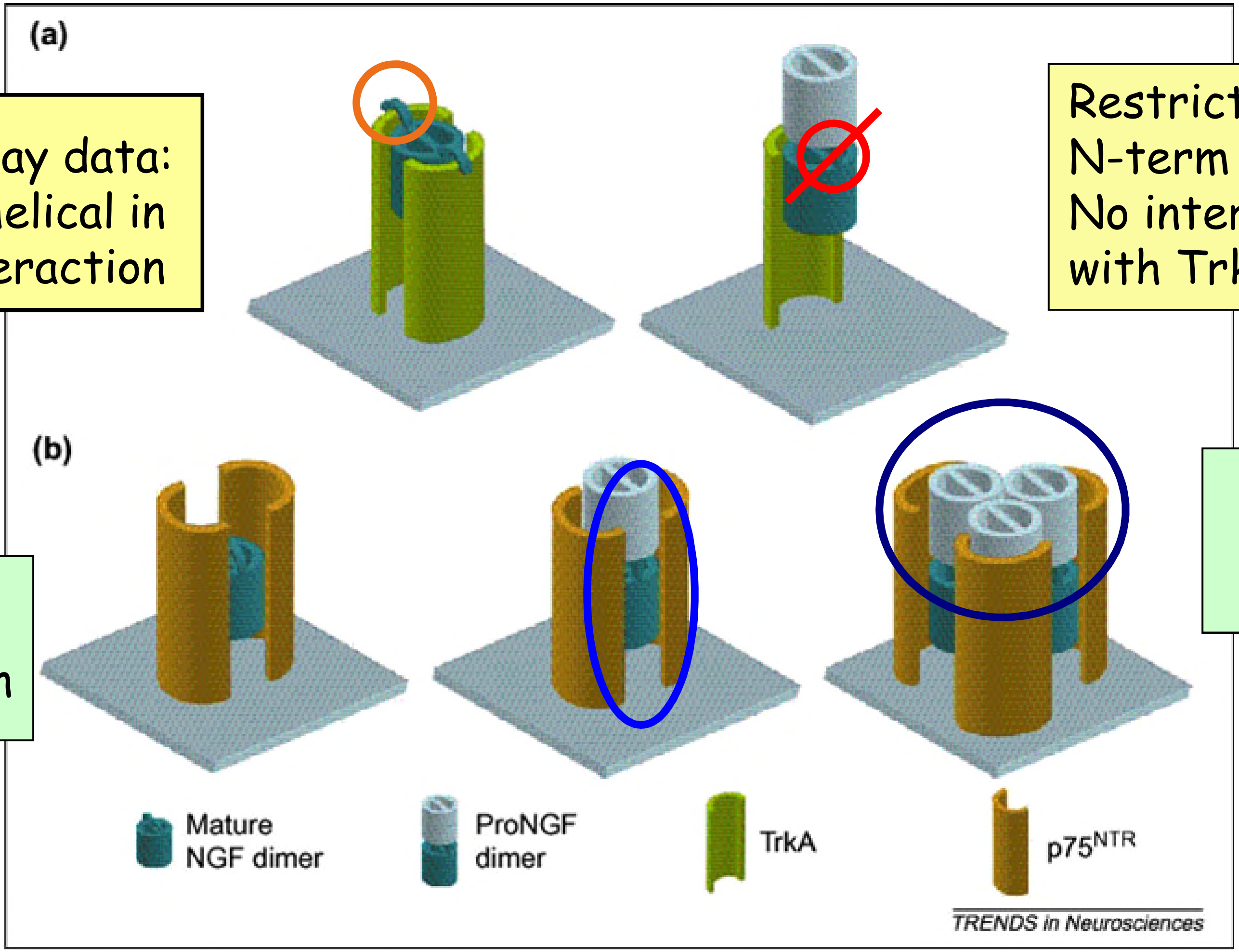


Longo, Massa, Nat Rev Drug Disc, 2013

Model for receptor discrimination by mature NGF and pro-NGF

From X-ray data: N-term helical in TrkA interaction

Restriction on N-term residues. No interaction with TrkA

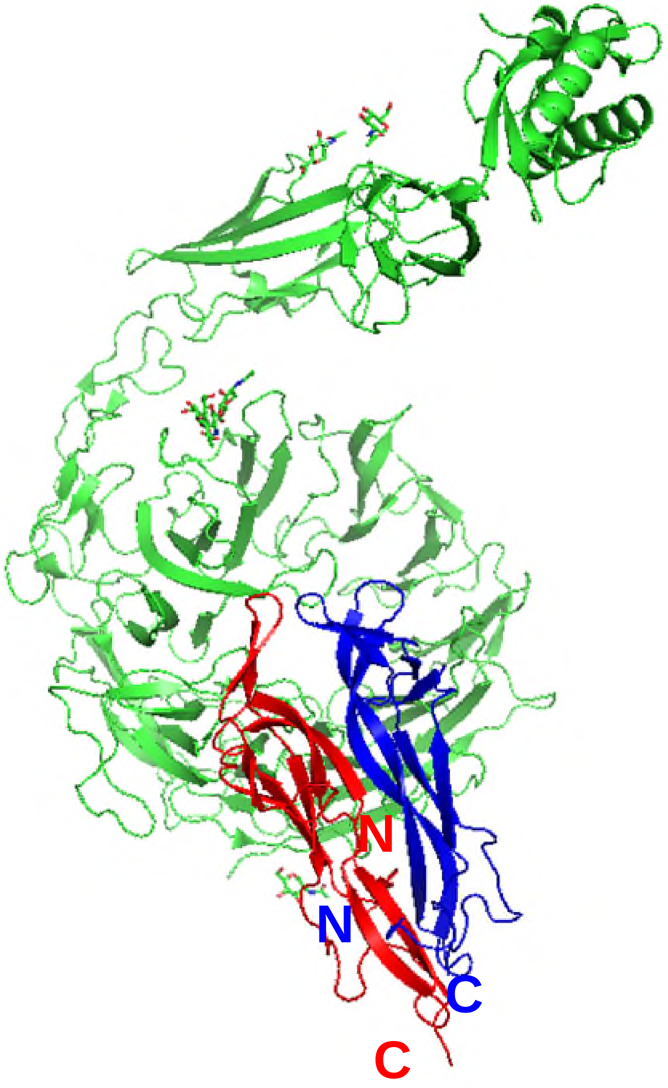


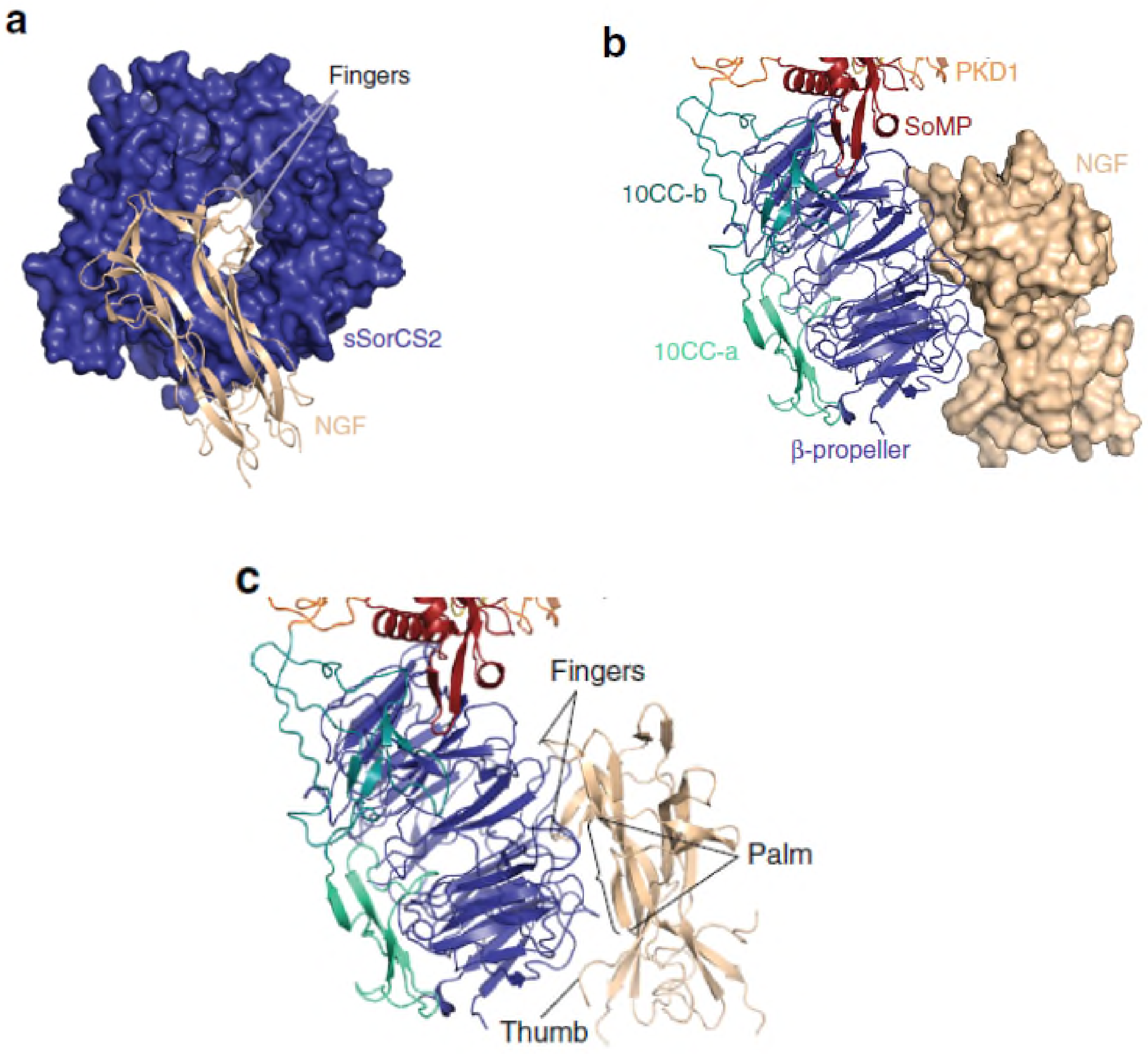
Direct interaction

Additional contacts

Ibanez, 2002, 25(6): 284

SorCS2-NGF complex
(PDB: 6YYF; Leloup et al, Nat Comm, 2018)





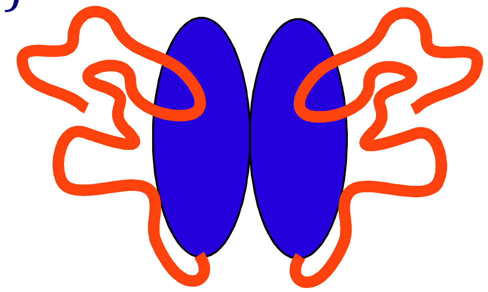
The NGF precursor: proNGF

Promotes protein folding (Rattenholl et al., 2001; Ibanez, 2002)

Regulates protein-protein interaction (Lee et al., 2001)

Regulates neurotrophin secretion (Suter et al., 1991)

NGF: homodimer, 26kDa



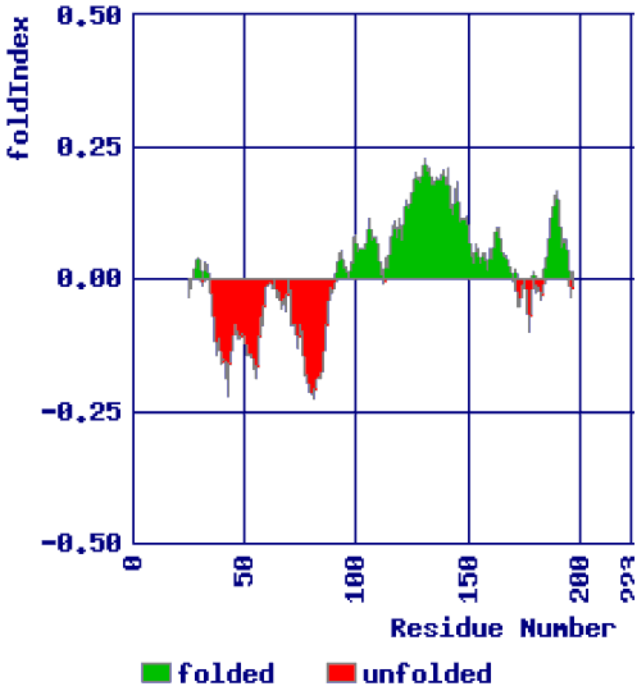
ProNGF: homodimer, 50 kDa

proNGF

- *Fahnestock et al. (2001):* proNGF is the predominant form of NGF in brain tissue. Twofold increase in AD parietal cortex
- *Lee et al. (2001):* proNGF high-affinity ligand for p75 and induces p75-dependent apoptosis in neurons.

The proNGF pro-domain is functionally independent since it induces growth cone collapse (Yan et al, 2018, Structure)

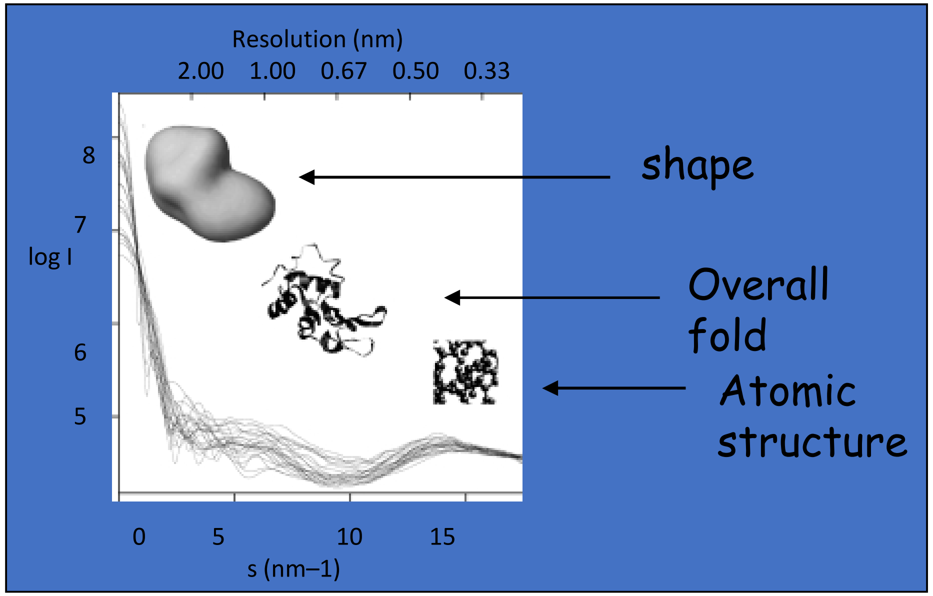
STRUCTURAL INSIGHTS INTO rm-proNGF



FoldIndex© Plot for the prediction of the folding propensity of rm-proNGF.
The protein proNGF is basically unfolded in its pro-peptide

Crystallography is not the technique of choice of structural determination

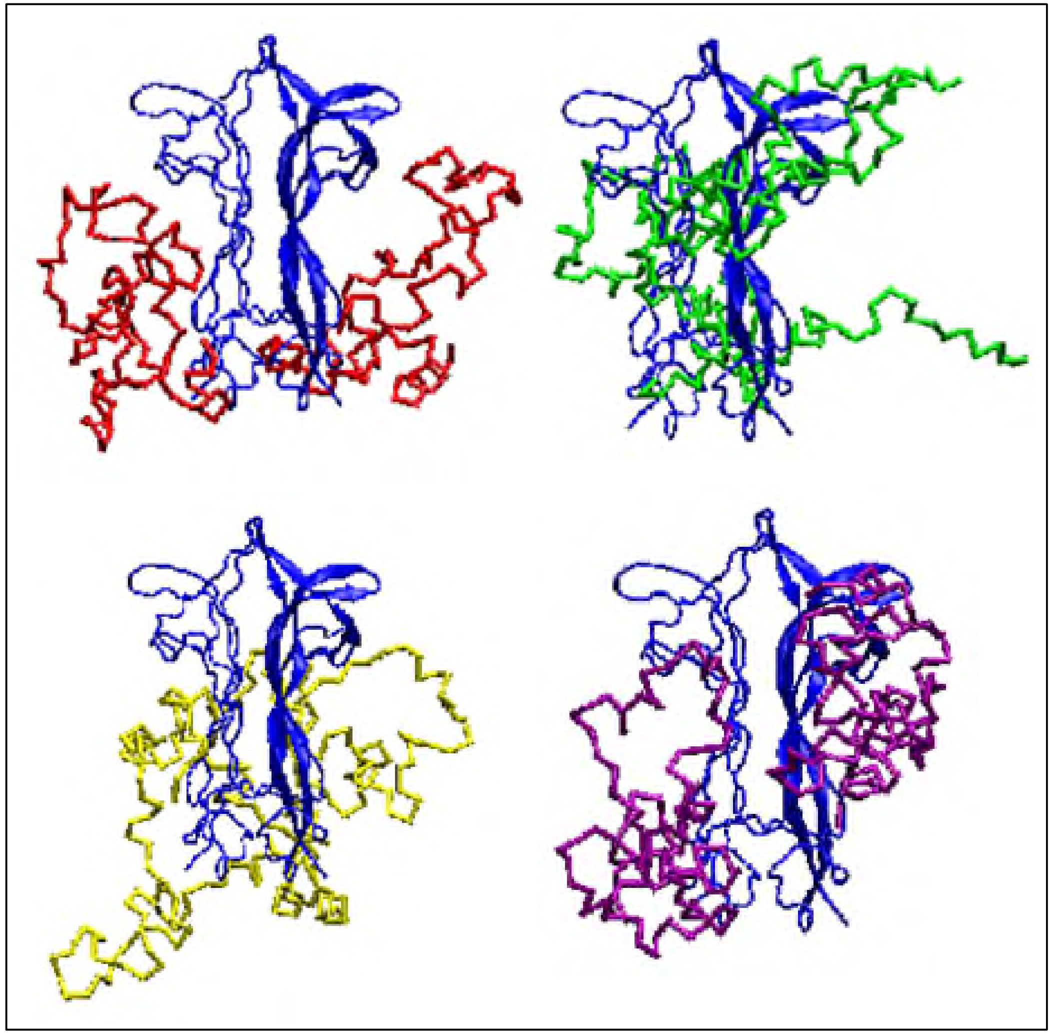
Use SAXS:
Small Angle X-ray Scattering



Adapted from: Svergun and Koch (Curr. Op. Str. Biol, 2002).

We obtained the first available structure of proNGF, although at low resolution.

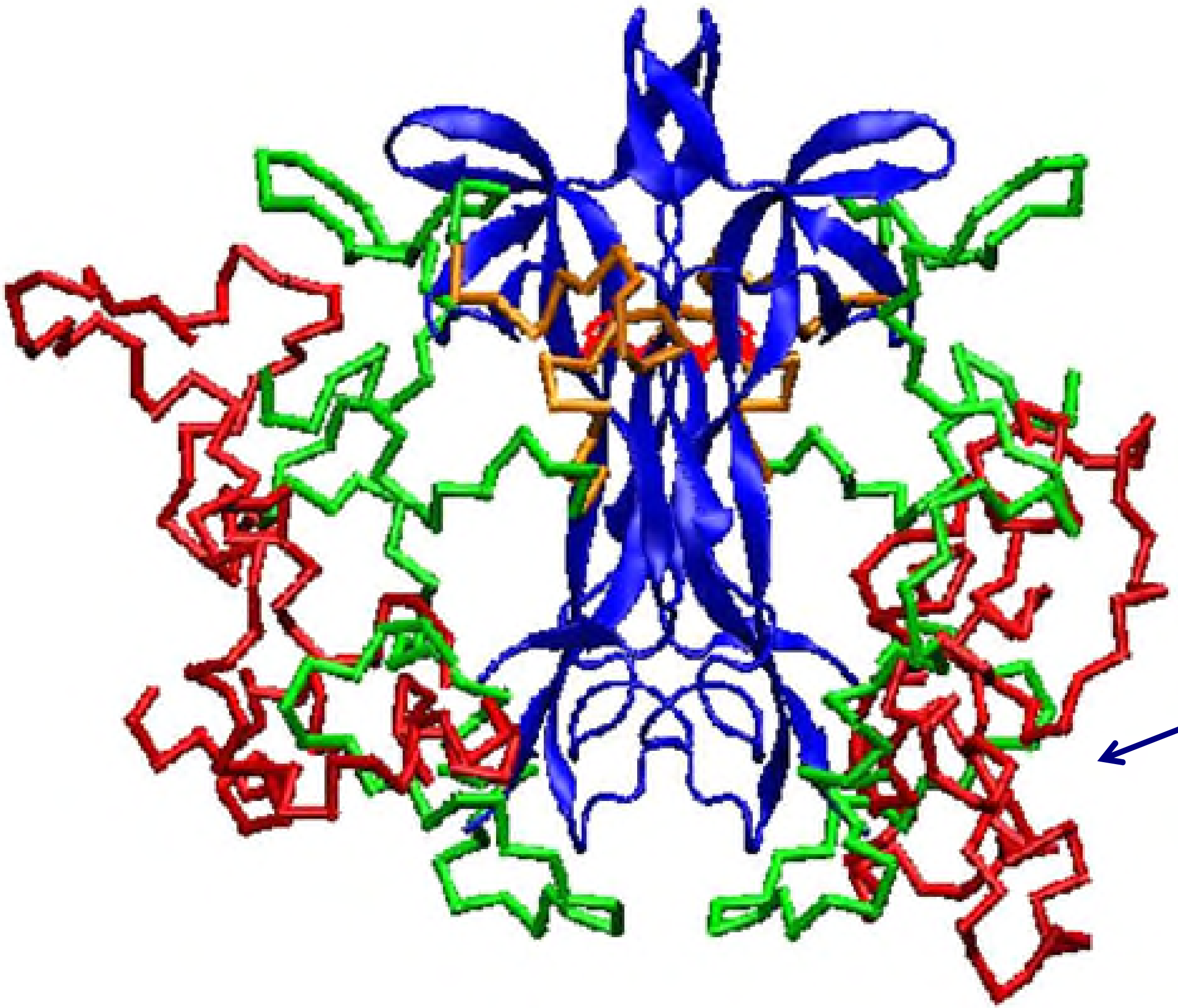
Analysis with SAXS method
Ensemble
Optimization
Method for
disordered proteins.
The compact model is the predominant one.



Paoletti et al., Proteins, 2009

proNGF is a IUP:

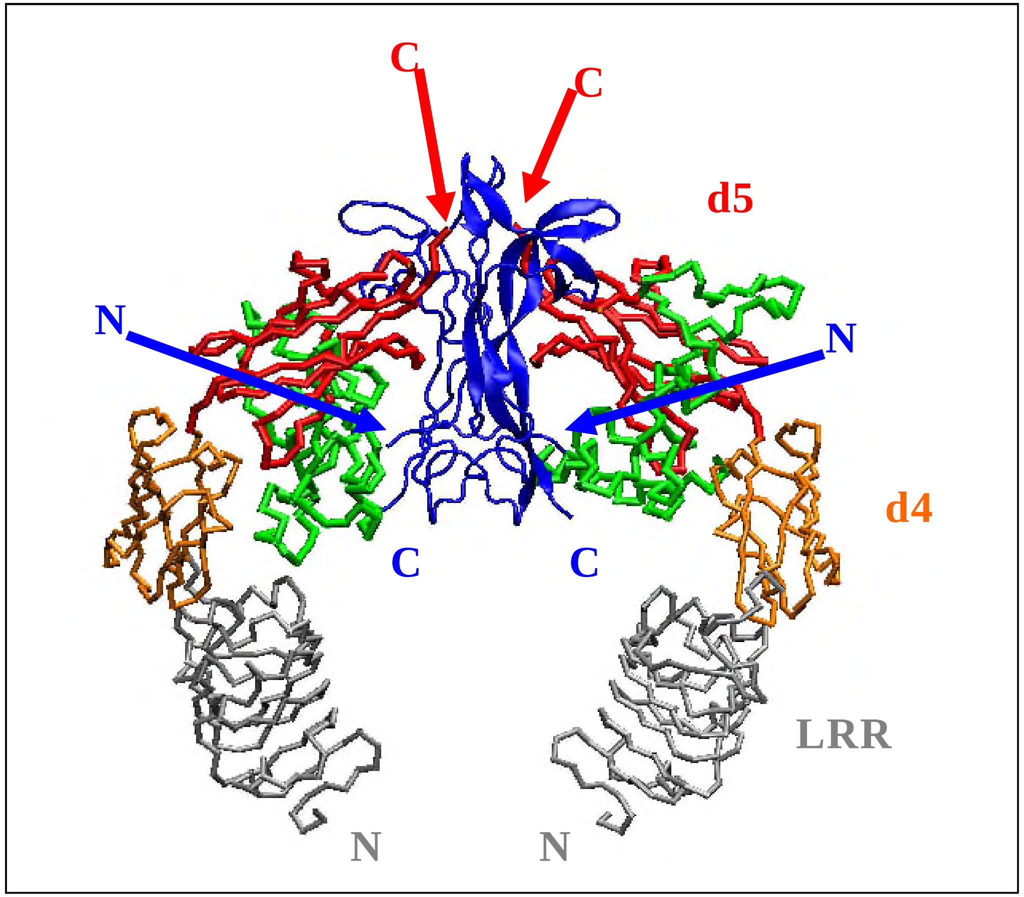
SAXS analysis in presence of ammonium sulphate (AMS) as a chemical stabilizer

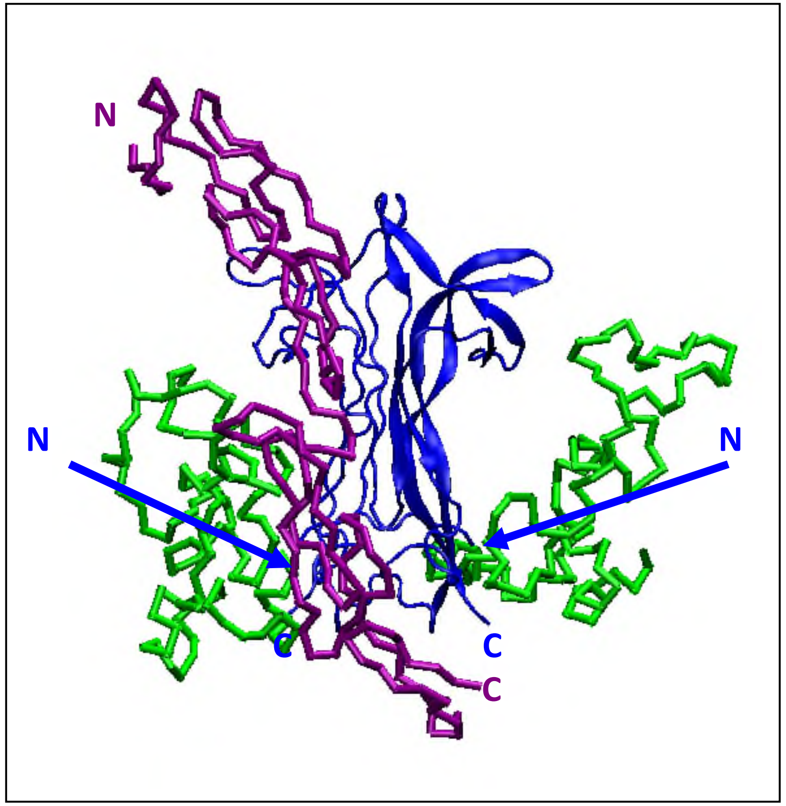


Comparison of proNGF with (**green**) and without (**red**) AMS: increased compactness

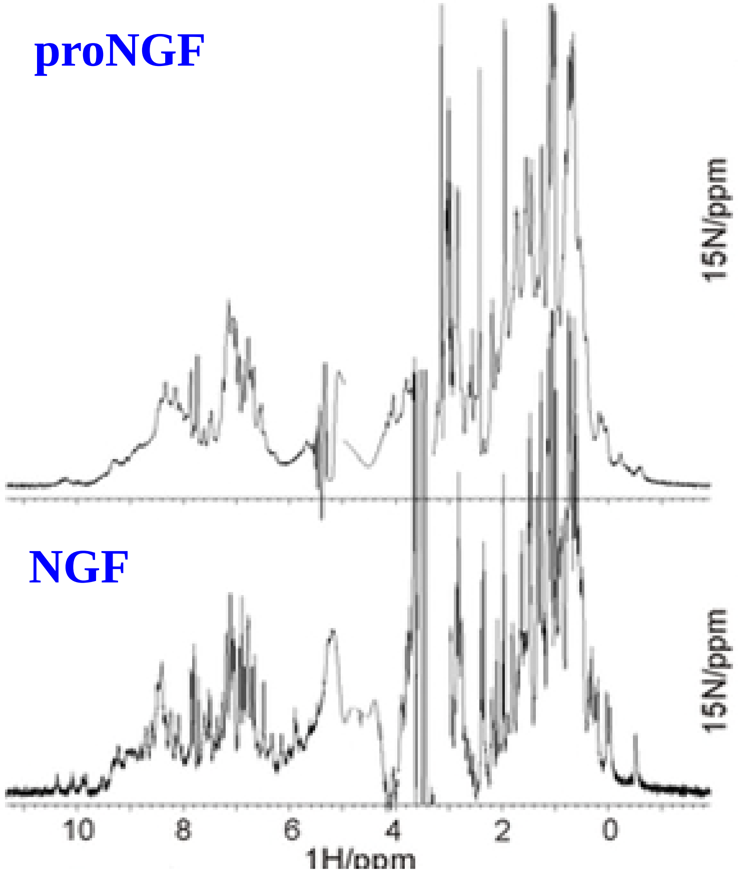
Paoletti et al., Proteins, 2009

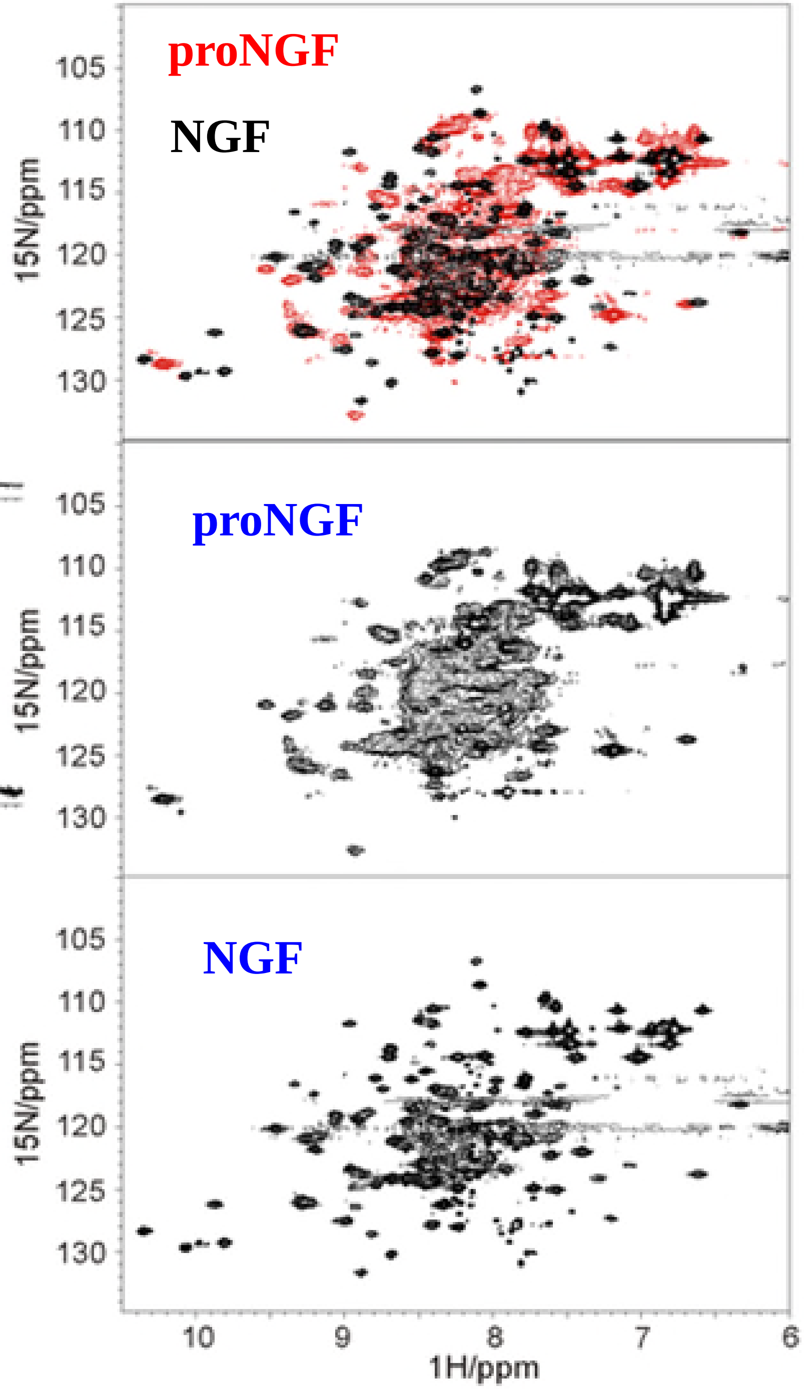
Proposed interaction of proNGF with the TrkA receptor
(crystallographic structure of the human NGF/TrkA extracellular domain - PDB code 2IFG)





Proposed interaction of proNGF with the p75^{NTR} receptor (crystallographic structure of the human NGF/p75NTR receptor - PDB code 1SG1)



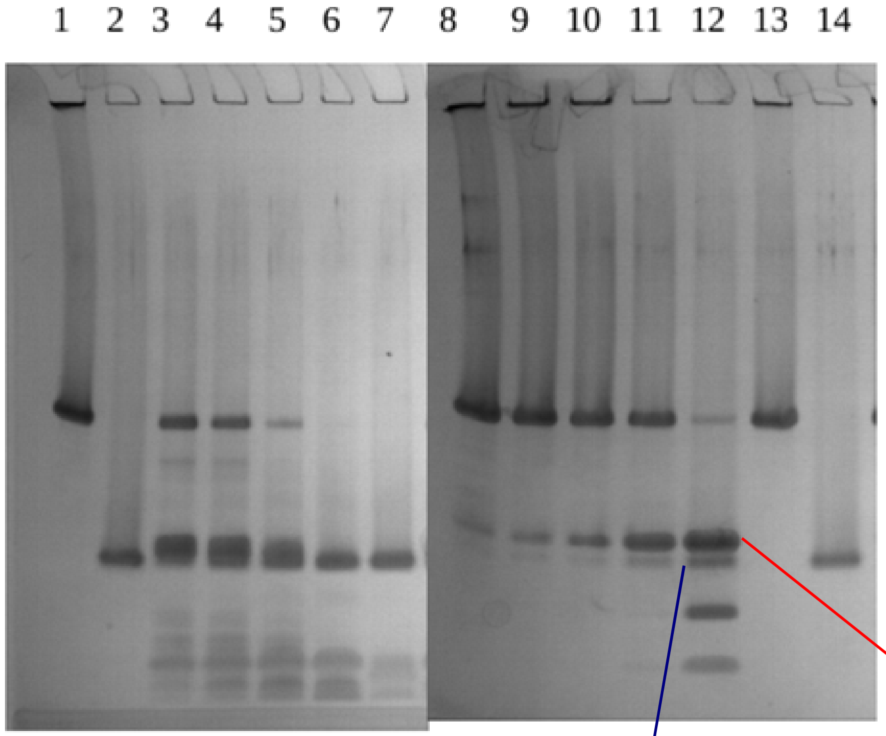


Towards a high resolution structure of proNGF: NMR studies

Paoletti et al., PloS One, 2011

NMR in-tube proteolytic digestion of proNGF to NGF

- ✓ *With trypsin*: pro-peptide of proNGF completely digested (ESI-MS), 1D spectra change gradually from proNGF to NGF
- ✓ *With furin*: slow kinetic, no quantitative digestion



1	rm-proNGF
2	rm-NGF
3	trypsin,4°C, 15'
4	trypsin,4°C, 30'
5	trypsin,4°C, 1h
6	trypsin,4°C, 2h
7	trypsin,4°C, 18h
8	furin, HEPES, 15'
9	furin, HEPES, 30'
10	furin, HEPES, 1h
11	furin, HEPES, 3h
12	furin, HEPES, 18h
13	rm-proNGF
14	rm-NGF

Pro-peptide of proNGF

NGF

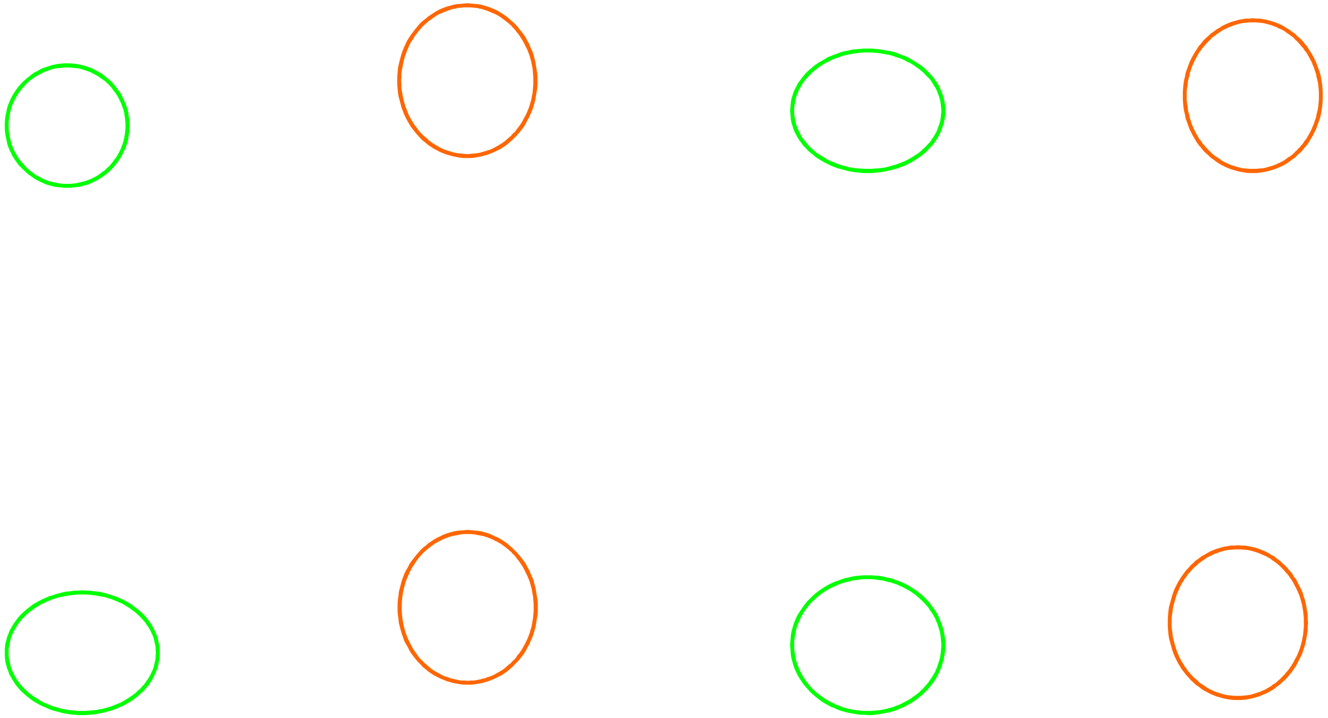
Looking into intramolecular interactions of proNGF domains

Cleavage in tube on labelled samples:

✓*With trypsin*: monitor the change in the peaks shape and chemical shift, to isolate those peaks changing significantly.

✓*With furin*: once digestion complete, in the tube = NGF + pro-peptide of proNGF. Is the spectrum of proNGF different from the one of the pro+NGF? Are there residual contacts left? Is the pro-peptide becoming a random coil upon cleavage?

2D HSQC of rm-proNGF cut with furin – time zero



Paoletti et al., PloS One, 2011

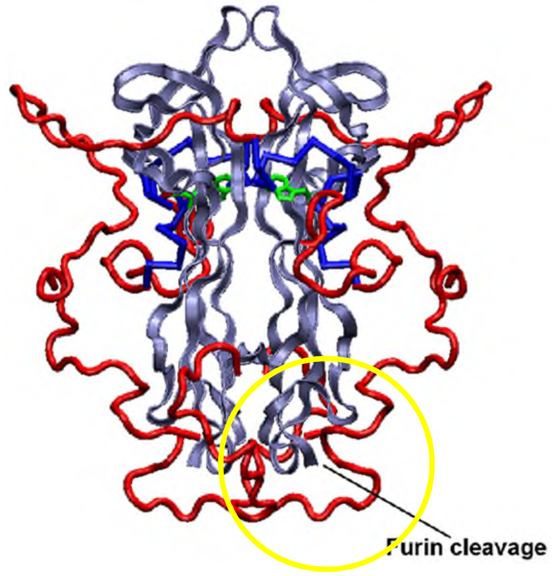
2D HSQC of rm-proNGF cut with furin – after cleavage

The cleavage with furin produces a spectrum which does not reproduce all the peaks of NGF, even if the digestion is complete

Are there residual contact between the pro-peptide and NGF?

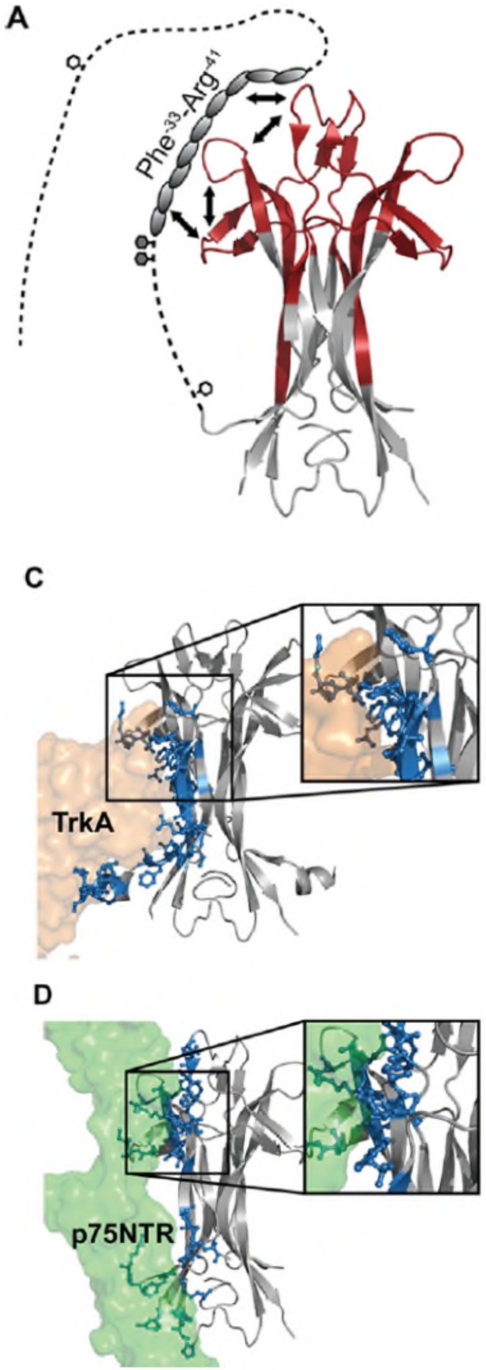
↓

Add trypsin to the furin-digested sample:
- no NGF degradation (SDS-PAGE and ESI-MS)
- no more pro-peptide (SDS-PAGE)
- peaks of the HSQC of NGF



Confirmed by Yan et al, 2018, Structure:

NMR titration of pro-peptide over mature NGF. Paramagnetic relaxation enhancement (PRE) restraints were used in combination with fully atomistic unrestrained molecular dynamics (MD) simulations that were validated against previous SAXS data. We show that proNGF forms a dimer in which the pro-domain collapses on NGF and forms local transient and dynamics tertiary interactions of a hydrophobic nature, rather than gaining a stable tertiary structure



The structural stabilization of the three loops in the upper part of the mature part of proNGF is caused by a direct molecular interaction between the pro-part and the mature part.
Not possible to discern, if it is only intramolecular nature or also intramolecular (more stable proNGF *vs* NGF).

The detected local conformational stabilization of the mature part by the pro-part may directly explain the decreased affinity of proNGF for TrkA and $p75^{NTR}$ compared with NGF.
But in the $p75^{NTR}$ binding there is a region of NGF not affected by the pro-peptide, still able to engage in binding to $p75^{NTR}$.

Trabjerg et al, JBC, 2017

BDNF & proBDNF

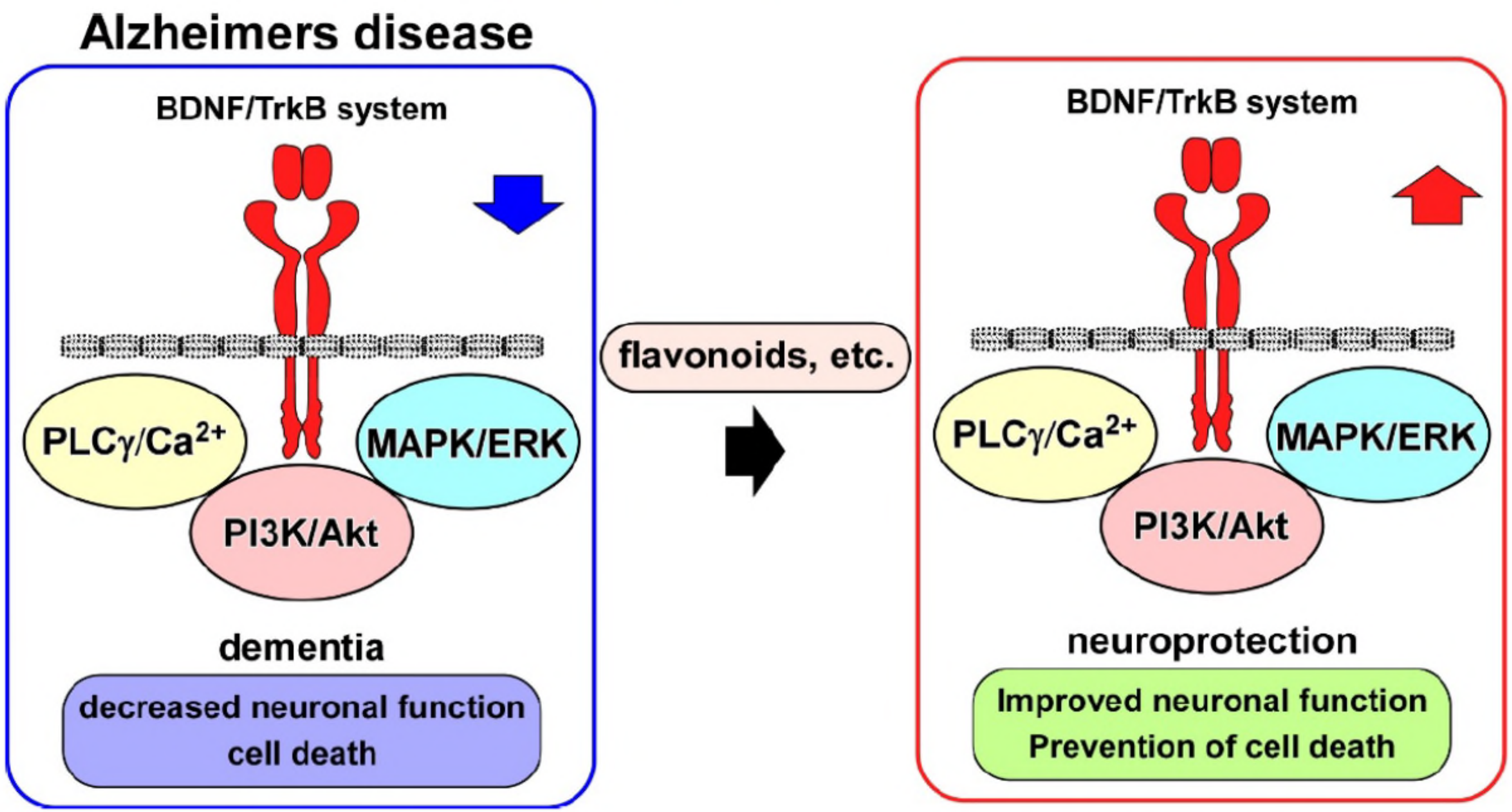
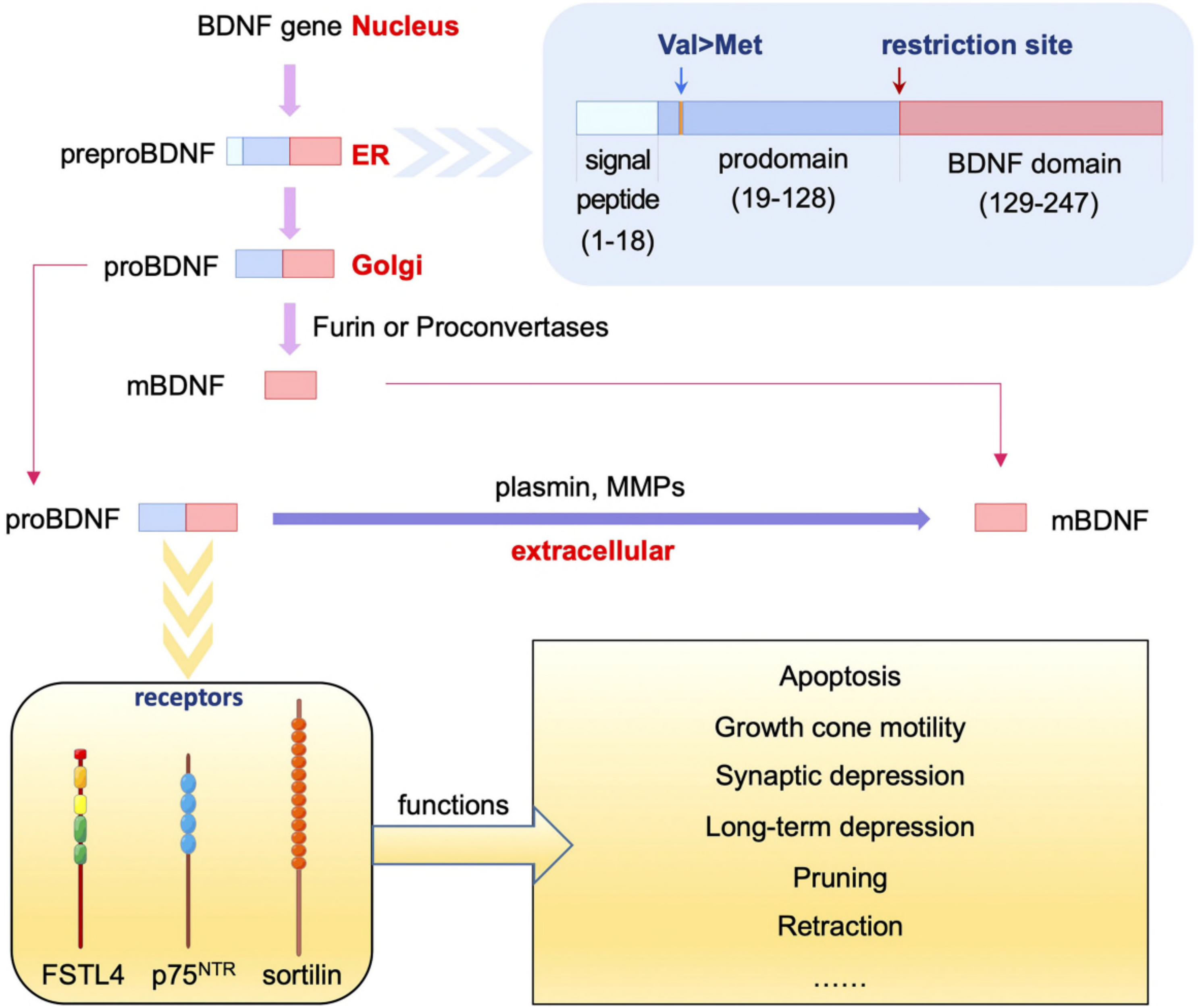


Figure 2. Downregulation of BDNF/TrkB system in Alzheimer's disease (AD), and its upregulation by drug candidates. Blue and red arrows indicate downregulation and upregulation, respectively.

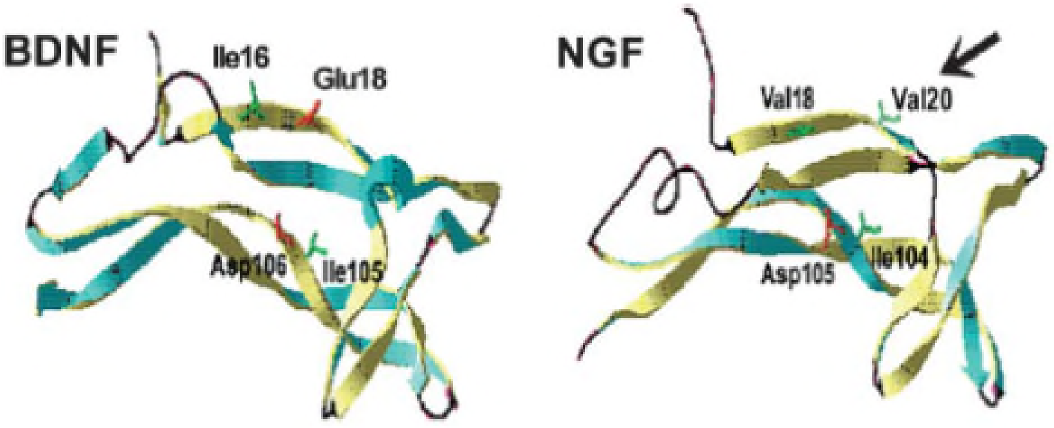


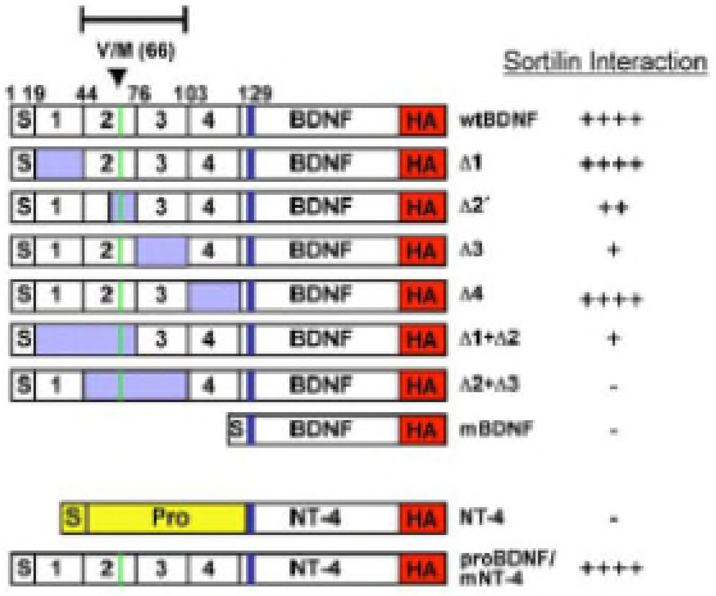
Processing and function of proBDNF and its receptors. The BDNF gene produces preproBDNF protein in the ER, which is processed to proBDNF in
Golgi. ProBDNF may be cleaved into mBDNF intracellularly by furin or proconvertases, or extracellularly by plasmin or MMPs. ProBDNF binds to p75NTR, sortilin or FSTL4 to exert different effects including apoptosis, growth cone motility, synaptic depression, long-term depression, pruning,
retraction and other functions.

Li Q, Hu Y-Z, Gao S, Wang P-F, Hu Z-L and
Dai R-P (2023) ProBDNF and its receptors
in immune-mediated inflammatory diseases: novel insights into the regulation
of metabolism and mitochondria.
Front. Immunol. 14:1155333.

SORTILIN & SORTING OF PRO-NEUROTROPHINS

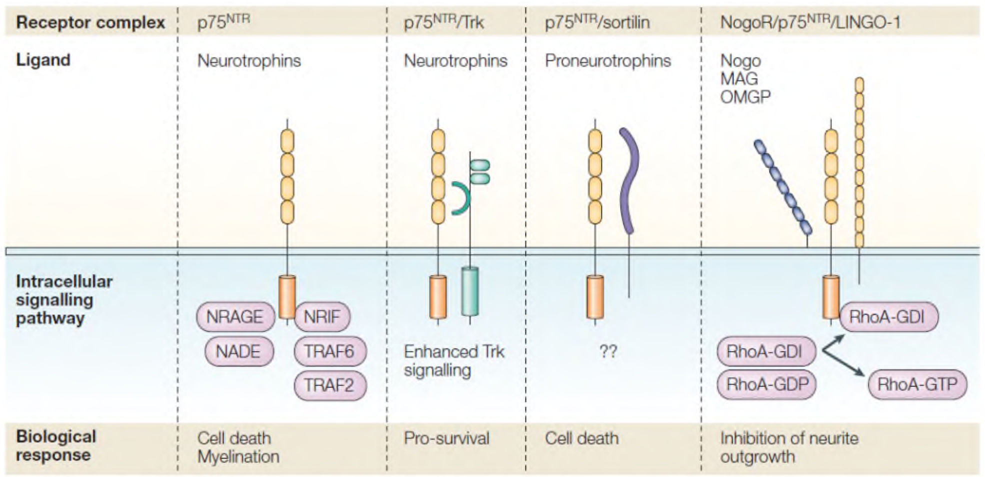
- sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor Carboxypeptidase E. Val66Met in proBDNF alters trafficking *(Lou et al, Neuron, 2005)*





- sortilin controls intracellular sorting of BDNF to the regulated secretory pathway (truncated sortilin w/o cytoplasmic tail – post-Golgi trafficking domain - alters BDNF trafficking) *(Chen et al, J Neurosc, 2005)*

Box 1 | Multiple partners of p75NTR



Historically, the p75 neurotrophin receptor (p75NTR) has been referred to as a 'low-affinity' neurotrophin receptor, but this definition should be avoided because proNGF binds p75NTR with an affinity similar to that of nerve growth factor (NGF) binding to TrkA. Although it lacks a kinase domain, p75NTR can cooperate with many different protein partners and form multimeric receptor complexes to produce a number of cellular responses, including apoptosis, neurite outgrowth and myelination[111–113]. So far, sortilin[61], LINGO-1, Nogo-66 (NgR)[114] and Trk receptors[115] have been identified as co-receptors. In addition to extracellular interactions that yield multimeric receptor complexes, the intracellular domain of p75NTR can also interact with many different adaptor and signalling proteins. These include neurotrophin-receptor-interacting MAGE (melanoma-associated antigen) homologue (NRAGE)[116], neurotrophin-associated cell death executor (NADE)[117], TNF (tumour necrosis factor)-receptor-associated factors 2 and 6 (TRAF2 and TRAF6)[118,119], and neurotrophin-receptor-interacting factor (NRIF)[120,121]. GDI, guanine-nucleotide dissociation inhibitor; MAG, myelin-associated glycoprotein; OMGP, oligodendrocyte myelin glycoprotein; RhoA, small G protein.

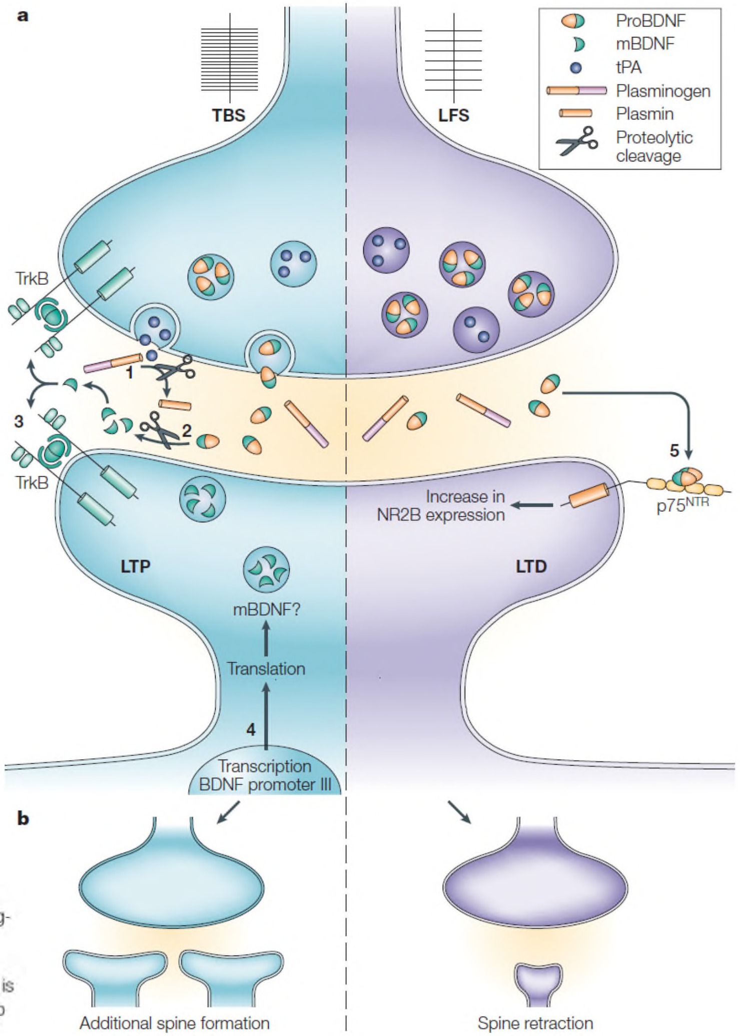


Figure 3 | **The yin and yang of long-term synaptic regulation by pro- and mature BDNF.** **a** | Molecular cascade of brain-derived neurotrophic factor (BDNF) processing in late-phase long-term potentiation (L-LTP). In response to theta-burst stimulation (TBS), tissue plasminogen activator (tPA) is secreted into the synaptic cleft and cleaves the extracellular protease plasminogen to yield plasmin (1). Plasmin then cleaves proBDNF (the precursor of BDNF, which is released in an activity-dependent manner), yielding mature BDNF (mBDNF) (2). mBDNF binds to TrkB and triggers a series of downstream signalling pathways to induce LTP (3). During the maintenance stage of LTP, mBDNF might be generated by intracellular cleavage after postsynaptic transcription and translation (4). By contrast, proBDNF secreted extracellularly remains uncleaved after low-frequency stimulation (LFS). Uncleaved proBDNF binds to the p75 neurotrophin receptor (p75NTR) (5) to facilitate the induction of long-term depression (LTD), possibly through the regulation of NMDA (*N*-methyl-D-aspartate) receptor NR2B subunit expression. **b** | Morphological alterations in synapses induced by pro- and mature BDNF. Left, BDNF–Trk signalling might be an active mechanism that converts activity-induced molecular signals into structural plasticity, contributing to synapse formation. Right, proBDNF–p75NTR signalling might be important in translating activity-dependent signals into negative modulation of structural plasticity, contributing to synapse retraction.

THE YIN AND YANG OF NEUROTROPHIN ACTION

Bai Lu, Petti T. Pang and Newton H. Woo

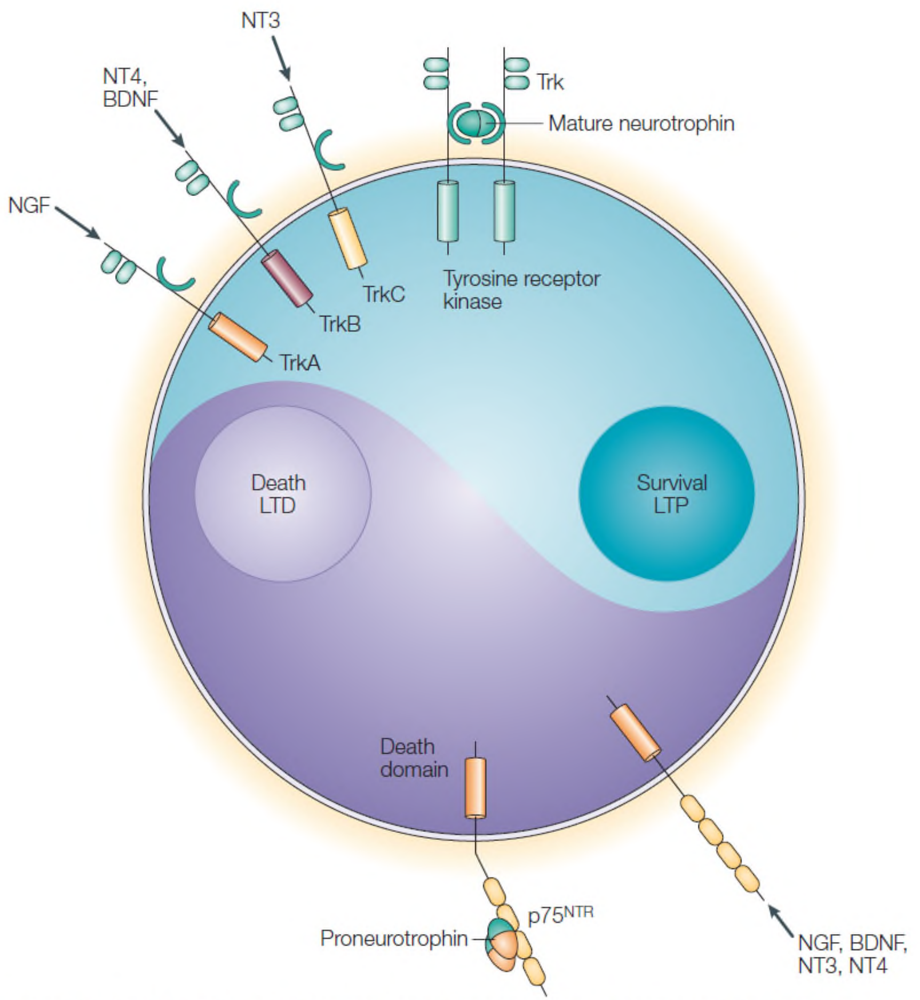


Figure 1 | **The yin and yang of neurotrophin receptors and neurotrophin function.** The actions of neurotrophins are mediated by two principal transmembrane-receptor signalling systems. Each neurotrophin receptor — TrkA, TrkB, TrkC and the p75 neurotrophin receptor ($p75^{NTR}$) — is characterized by specific affinities for the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and NT4. An emerging concept is that the two distinct receptor classes, Trk (top) and $p75^{NTR}$ (bottom), preferentially bind mature and proneurotrophins (neurotrophin precursors), respectively, to elicit opposing biological responses. LTD, long-term depression; LTP, long-term potentiation.

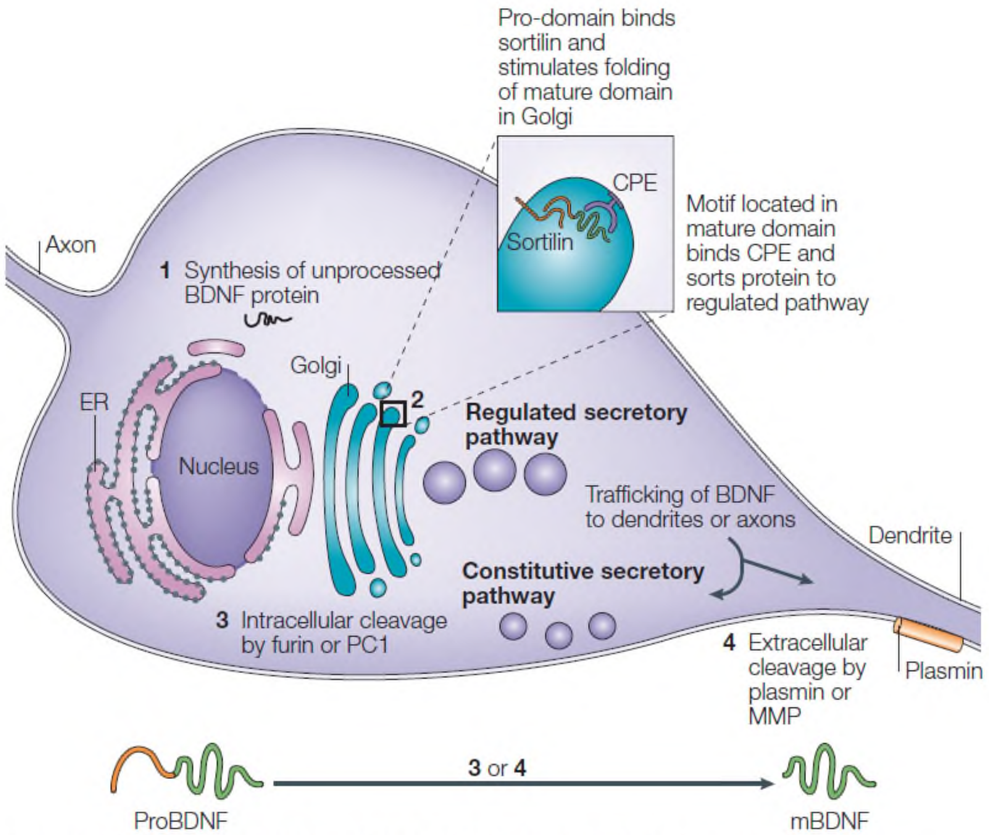


Figure 2 | **The synthesis and sorting of BDNF.** A schematic showing the synthesis and sorting of brain-derived neurotrophic factor (BDNF) in a typical neuron. First synthesized in the endoplasmic reticulum (ER) (1), proBDNF (precursor of BDNF) binds to intracellular sortilin in the Golgi to facilitate proper folding of the mature domain (2). A motif in the mature domain of BDNF binds to carboxypeptidase E (CPE), an interaction that sorts BDNF into large dense core vesicles, which are a component of the regulated secretory pathway. In the absence of this motif, BDNF is sorted into the constitutive pathway. After the binary decision of sorting, BDNF is transported to the appropriate site of release, either in dendrites or in axons. Because, in some cases, the pro-domain is not cleaved intracellularly by furin or protein convertases (such as protein convertase 1, PC1) (3), proBDNF can be released by neurons. Extracellular proteases, such as metalloproteinases and plasmin, can subsequently cleave the pro-region to yield mature BDNF (mBDNF) (4). MMP, matrix metalloproteinase.

WHY ARE NEUROTROPHINS STILL INTERESTING?

Involvement in an a big number of pathways/cellular conditions/pathologies. Pharmacological interest.

Research Article

MOLECULAR PAIN

Molecular Pain
Volume 14: 1–11

DOI: 10.1177/1744806918809223
journals.sagepub.com/home/mpx
SAGE

A third HSAN5 mutation disrupts the nerve growth factor furin cleavage site

Samiha S Shaikh[1], Michael S Nahorski[1], and C Geoffrey Woods[1,2]

Mol Neurobiol (2018) 55:2934–2951
DOI 10.1007/s12035-017-0505-7

Joint Bone Spine xxx (2018) xxx–xxx

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PROneurotrophins and CONSequences

Rui O. Costa[1,2] · Tânia Perestrelo[1,2,3] · Ramiro D. Almeida[1,2,4]

Editorial

Targeting nerve growth factor to relieve pain from osteoarthritis: What can we expect?

KEMIJSKI INŠTITUT

Received: 20 April 2017 | Revised: 22 January 2018 | Accepted: 31 January 2018
DOI: 10.1002/glia.23312
GLIA
WILEY

RESEARCH ARTICLE

NGF steers microglia toward a neuroprotective phenotype

Caterina Rizzi[1] | Alexia Tiberi[1] | Michela Giustizieri[2] | Maria Cristina Marrone[2] | Francesco Gobbo[1] | Nicola Maria Carucci[1] | Giovanni Meli[2] | Ivan Arisi[2] | Mara D'Onofrio[2] | Silvia Marinelli[2] | Simona Capsoni[1,3] | Antonino Cattaneo[1,2]

Differentiation by nerve growth factor (NGF) involves mechanisms of crosstalk between energy homeostasis and mitochondrial remodeling

Francesca Martorana, Daniela Gaglio, Maria Rosaria Bianco, Federica Aprea, Assunta Virtuoso, Marcella Bonanomi, Lilia Alberghina, Michele Papa & Anna Maria Colangelo

Cell Death & Disease **9**, Article number: 391 (2018)
Received: 14 December 2017
ruary 2018

Prolyl isomerase Pin1 and neurotrophins: a loop that may determine the fate of cells in cancer and neurodegeneration

Francesco Angelucci and Jakub Hort

Ther Adv Med Oncol
2017, Vol. 9(1) 59–62
DOI: 10.1177/1758834016665776

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journal homepage: www.elsevier.com/locate/yphrs
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Invited Review

Neurotrophin receptors in the pathogenesis, diagnosis and therapy of neurodegenerative diseases

Jacopo Meldolesi
Department of Neuroscience, Vita-Salute San Raffaele University and Scientific Institute San Raffaele, via Olgettina 58, 20132 Milan, Italy

KEMIJSKI INŠTITUT

Mol Neurobiol (2017) 54:7401–7459
DOI 10.1007/s12035-016-0214-7

Neurotrophin Signaling and Stem Cells—Implications for Neurodegenerative Diseases and Stem Cell Therapy

Subrata Pramanik[1] · Yanuar Alan Sulistio[1] · Klaus Heese[1]

International Journal of *Molecular Sciences*

Review

The Role of Nerve Growth Factor (NGF) and Its Precursor Forms in Oral Wound Healing

Karl Schenck[1,*], Olav Schreurs[1], Katsuhiko Hayashi[1,2] and Kristen Helgeland[1]

International Journal of *Molecular Sciences*

Review

NGF and Its Receptors in the Regulation of Inflammatory Response

Gaetana Minnone[1], Fabrizio De Benedetti[1] and Luisa Bracci-Laudiero[1,2,*]

International Journal of *Molecular Sciences*

MDPI

Article

ProNGF, but Not NGF, Switches from Neurotrophic to Apoptotic Activity in Response to Reductions in TrkA Receptor Levels

Maria S. Ioannou[†] and Margaret Fahnestock[*]

Dev Neurobiol. 2017 April ; 77(4): 405–418. doi:10.1002/dneu.22427.

The Neurotrophin Receptor Signaling Endosome: Where Trafficking Meets Signaling

Kelly Barford[1], Christopher Deppmann[2], and Bettina Winckler[1]

International Journal of *Molecular Sciences*

Review

A Review on Ubiquitination of Neurotrophin Receptors: Facts and Perspectives

Julia Sánchez-Sánchez [1] and Juan Carlos Arévalo [2,*]

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/freeradbiomed

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Review Article

Dysregulation of neurotrophin signaling in the pathogenesis of Alzheimer disease and of Alzheimer disease in Down syndrome☆

Xu-Qiao Chen*, Mariko Sawa, William C. Mobley*

Cell. Mol. Life Sci. (2016) 73:1859–1870
DOI 10.1007/s00018-016-2156-7

Cellular and Molecular Life Sciences

REVIEW

CrossMark

Neurotrophin signaling in cancer stem cells

Valérie Chopin[1,2] · Chann Lagadec[1] · Robert-Alain Toillon[1] · Xuefen Le Bourhis[1]

Journal of Medicinal Chemistry

Perspective

pubs.acs.org/jmc

Targeting the Nerve Growth Factor (NGF) Pathway in Drug Discovery. Potential Applications to New Therapies for Chronic Pain

Bryan H. Norman*,† and Jeff S. McDermott‡

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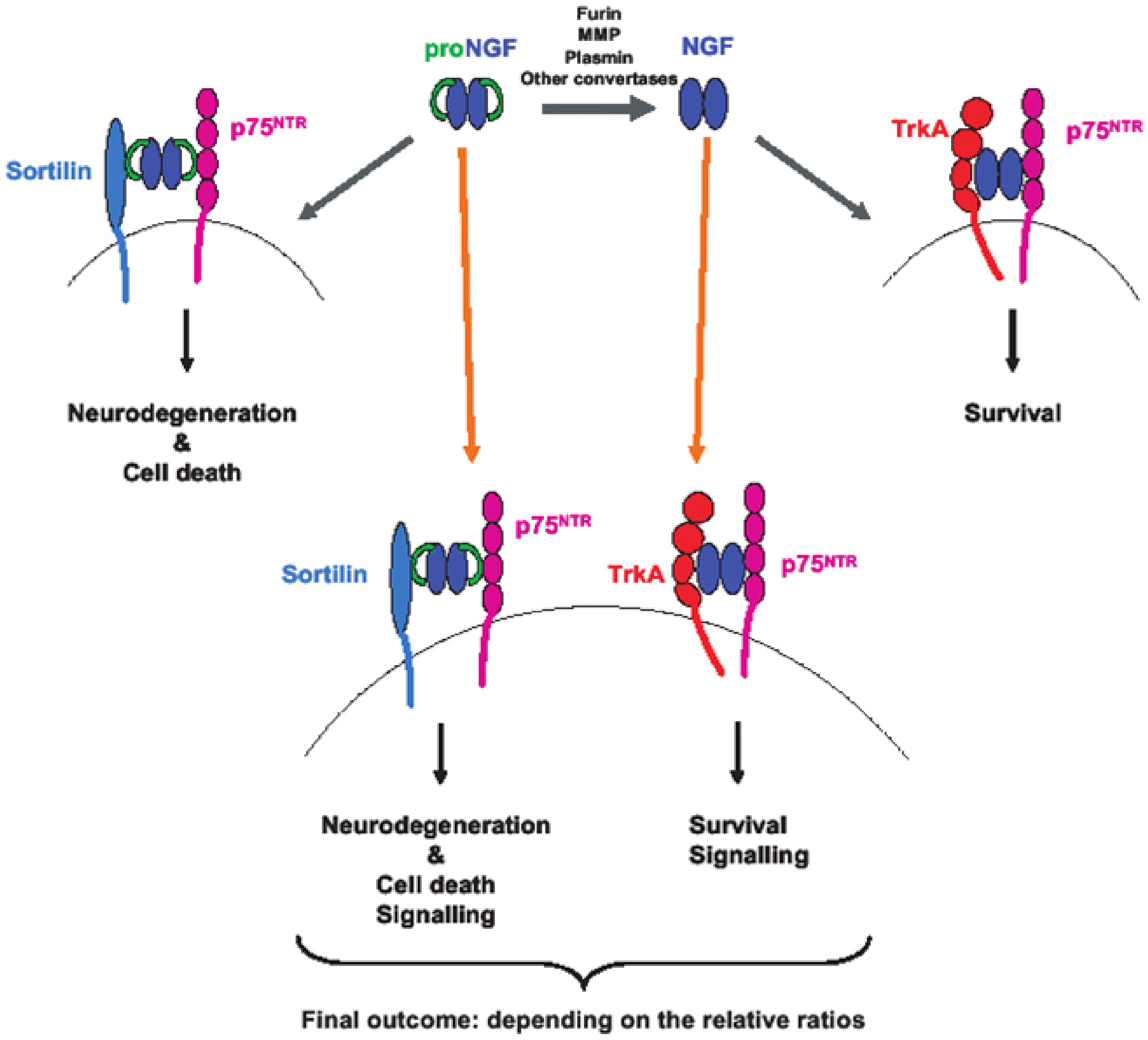
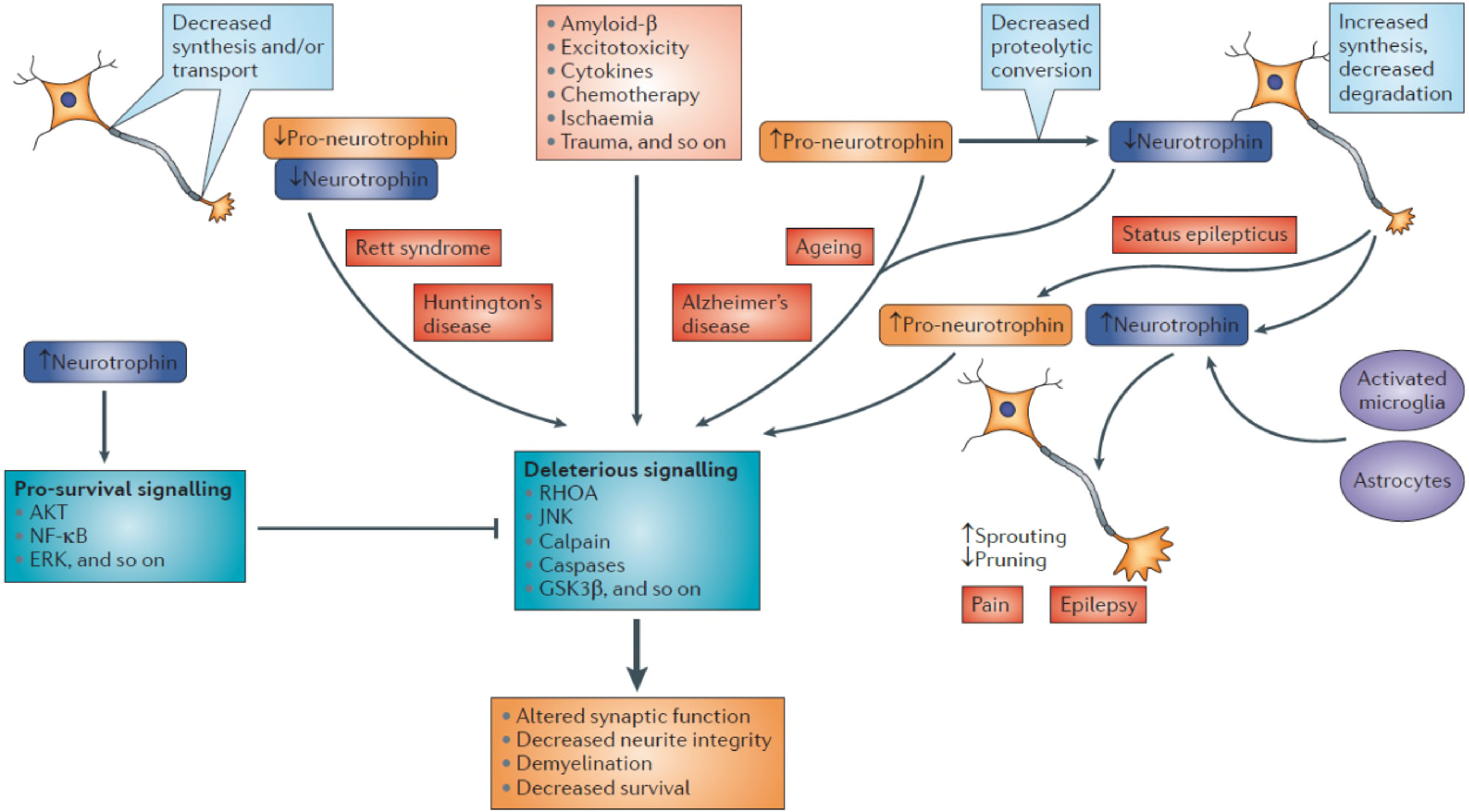
NGF & proNGF balance and disregulation

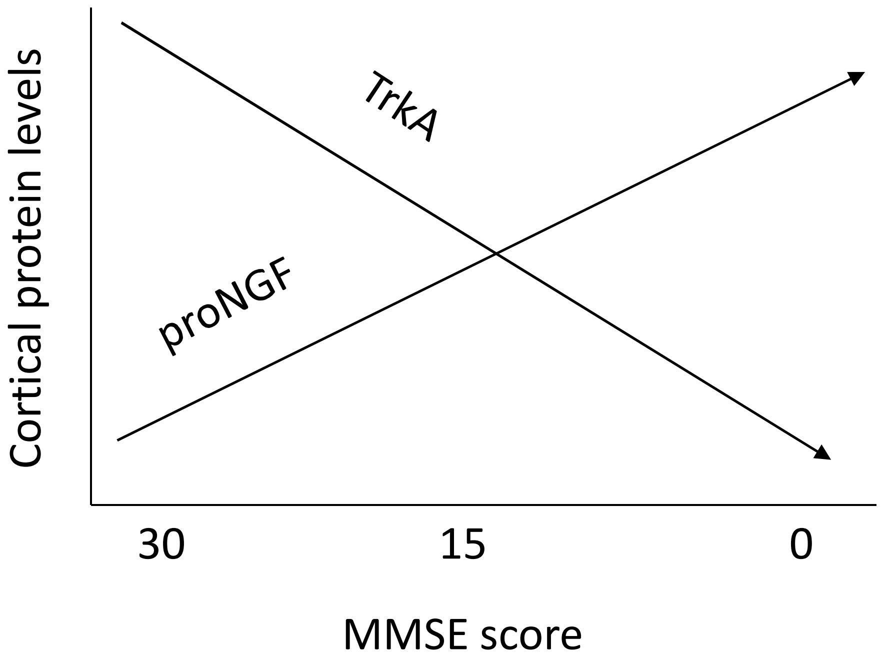
NGF & proNGF in neurodegeneration





Longo, Massa, Nat Rev Drug Disc, 2013

Experimental evidences for the effects of increased proNGF levels



Schematic diagram of the relationship between cortical levels of TrkA and Mini Mental State Exam (MMSE) scores.

Counts and Mufson, J Neuropathol Exp Neurol, 2005

THE ROLE OF proNGF IN NEURODEGENERATION:
AN EMERGING ACTOR - 1

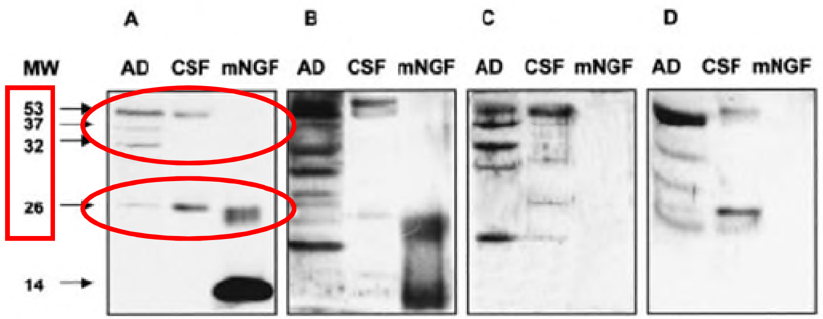


Figure 1. The different antibodies anti-pro-NGF and anti-mNGF recognize high molecular weight forms of pro-NGF in human brain tissue and cerebrospinal fluid. Human samples from AD brain tissue or cerebrospinal fluid (CSF) (30 μg per lane) were analyzed by Western blot using two different antibodies directed against either the pro-domain of pro-NGF (**C:** anti-pre-pro-NGF, Prohormone Sciences; **D:** anti-pro-NGF, see Materials and Methods) or the mature part of the molecule (**A:** H20, Santa Cruz; **B:** anti-mNGF, Cederlane Labs.).

- proNGF is present in human brain cortex and increases in AD *(Carlos E. Pedraza et al., Am J Path, 2005)*

- proNGF isolated from the human brain affected by AD induces neuronal apoptosis mediated by $p75^{NTR}$ *(Carlos E. Pedraza et al., Am J Path, 2005)*

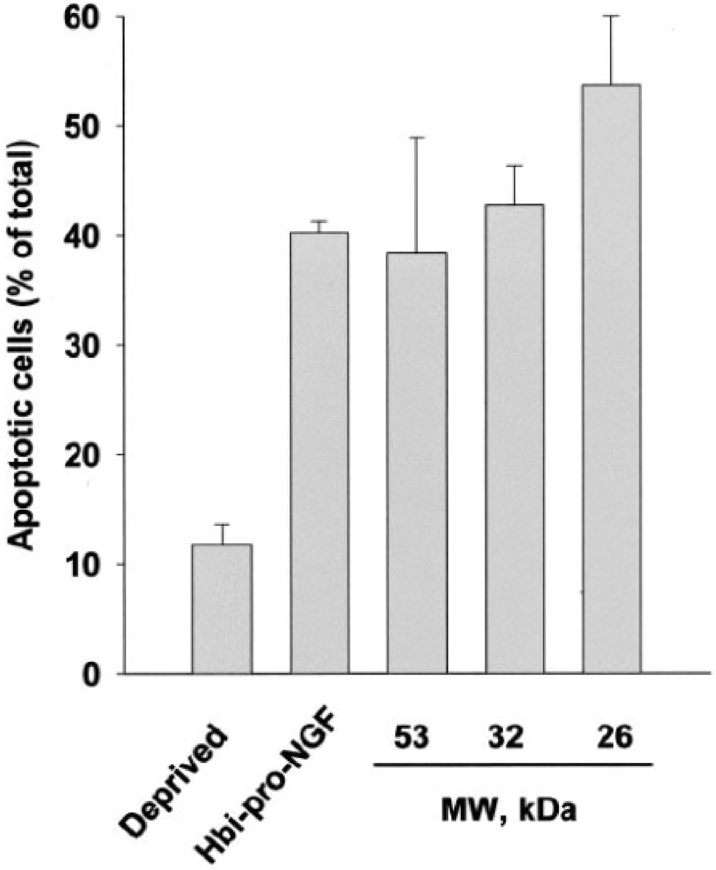


Figure 8. Hbi-pro-NGF forms separated by gel filtration chromatography induce cell death in 3T3-p75st cells. Three fractions of gel filtration chromatography of hbi-pro-NGF (8 μg total protein of each fraction) containing pro-NGF forms of 54, 32, and 26 kd, were added to 3T3-p75st for 30 hours. Apoptotic nuclei morphology was determined by Hoechst staining. Three μg of Hbi-pro-NGF was used as control of death induction in deprived cells. Apoptotic cell nuclei were counted as percentage of the total cells in each treatment. **Bars** are median ± SD of triplicates.

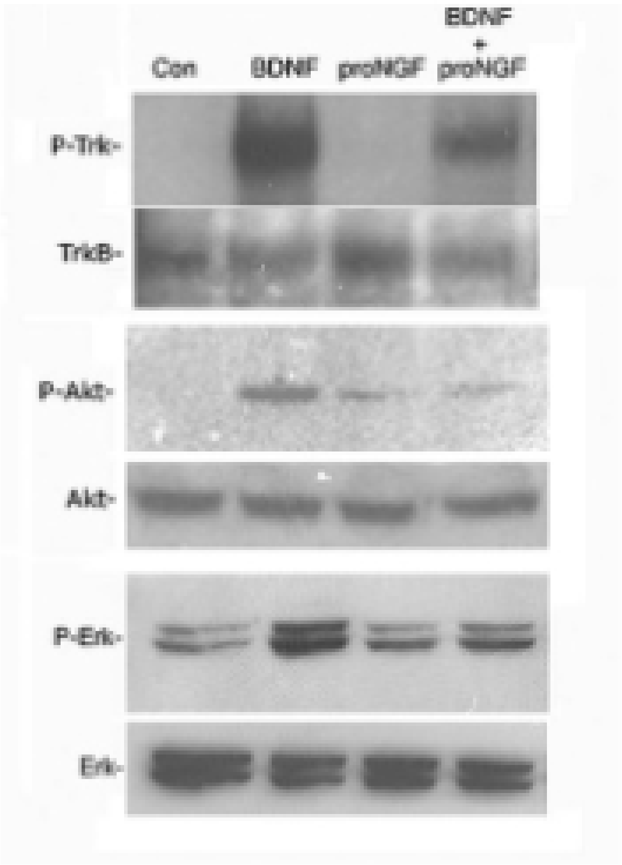
THE ROLE OF proNGF IN NEURODEGENERATION:
AN EMERGING ACTOR - 2

• proNGF elicits apoptosis of central BF neurons even in presence of mature NT and phosphorylation of Trk Receptors: novel checkpoint in survival versus apoptosis signal downstream Trk activation *(Volosin et al., J Neurosc, 2006)*

• Sortilin is required in complex with p75 to mediate apoptosis by proNGF *(Volosin et al., J Neurosc, 2006)*

• The coexpression of the two receptors is not sufficient for apoptosis: additional receptor component needed? *(Volosin et al., J Neurosc, 2006)*

• proNGF signalling through p75 could contribute to neuronal loss in AD, given that in AD there is a loss of TrkA but maintenance level of p75 *(Volosin et al., J Neurosc, 2006)*



THE ROLE OF proNGF IN NEURODEGENERATION:
AN EMERGING ACTOR - 3

- p75 can be cleaved by α- and γ-secretase releasing respectively a C-term fragment (CTF) and the intracellular domain (ICD)

- In AD p75-ICD is increased *(Podlesniy et al, Am J Pathol, 2006)*

- proNGF isolated from AD-affected brains differes functionally from control brains *(Podlesniy et al, Am J Pathol, 2006)*

- AD-proNGF stimulates processing of p75 with the secretase; this process is needed for apoptosis caused by AD-proNGF binding to p75. Control proNGF doeas not induce apoptosis *(Podlesniy et al, Am J Pathol, 2006)*

- Possible difference in the two proNGF might be in the post-translational modification, especially glycosylation *(Podlesniy et al, Am J Pathol, 2006)*

THE ROLE OF proNGF IN NEURODEGENERATION:
AN EMERGING ACTOR - 4

- Sympathetic neurons synthesize and secrete pro-NGF protein *(Hasan et al., 2003)*

- Mature NGF is a minor species in most peripheral tissues *(Bierl et al., Neurosc.Letters, 2005)*

- Increased NGF proforms in agend sympathetic neurons and their targets *(Bierl & Isaacson, Neurobiol. Aging, 2005)*

- In old-age, increased expression of sortilin, and the sortilin ligand proNGF contribute to neuronal atrophy and loss of vulnerable NGF-responsive neurons of the CNS and PNS.
(Al-Shawi, Eur J Neurosci, 2008)

- Causal link between proNGF and Abeta *(Cuello, J Mol Neurosci, 2010)*

- *Hippocampal NGF signaling abnormalities play a pervasive and key role in cognitive impairment during the onset of AD and represent drug targets for the treatment of dementia.*
(Mufson, JneuropatholExpNeurol, 2012)

proNGF PROCESSING: A POSSIBLE MECHANISM

Activity-dependent release of precursor NGF, conversion to mature NGF, and its degradation by a protease cascade *(Bruno & Cuello, PNAS, 2006)*:

- direct evidence of an activity-dependent release of the components of the proteolytic cascade responsible for the conversion of proNGF to NGF in CNS
- demonstrate the mechanism leading to in vivo enzymatic degradation of mNGF within extracellular space
- deregulation of the protease cascade controlling proNGF conversion and NGF degradation important for the pathological alterations of the CNS (from AD to inflammatory diseases)
- it would be possible to manipulate the ratio of proNGF to mature NGF in adult CNS

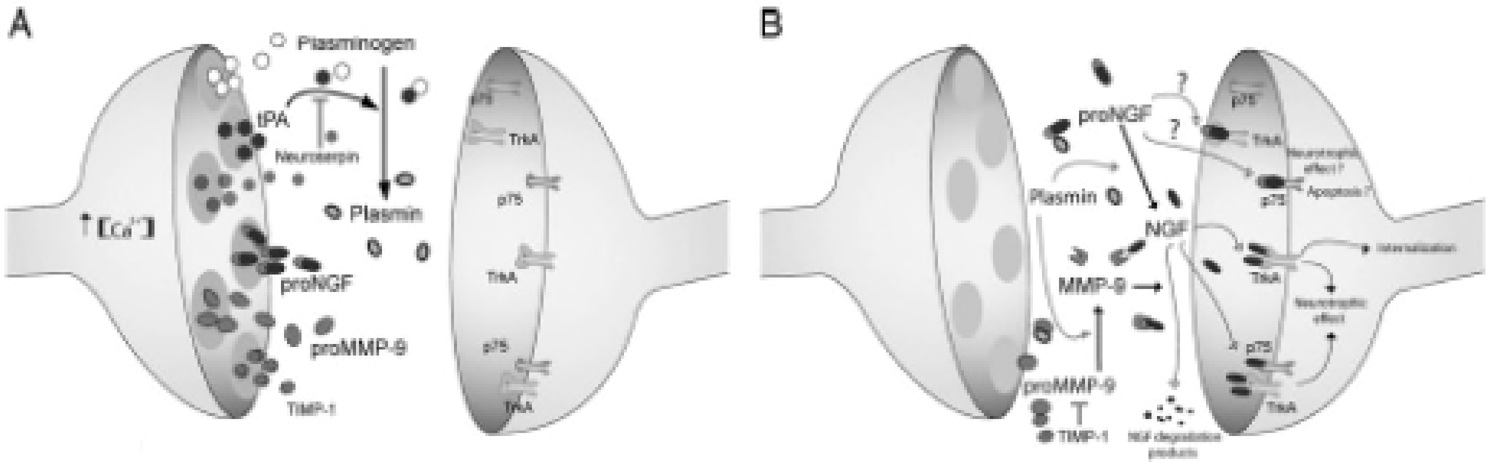
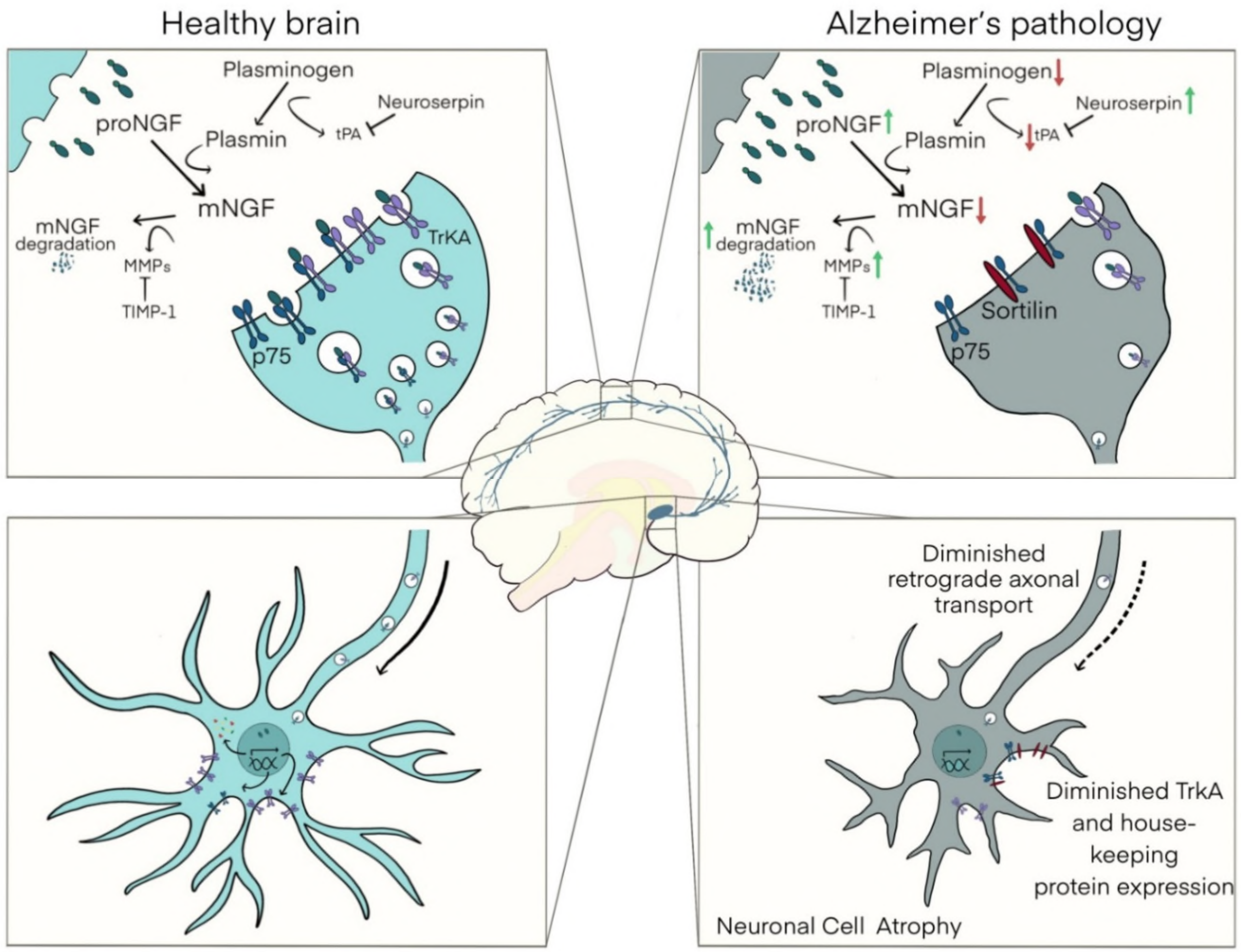
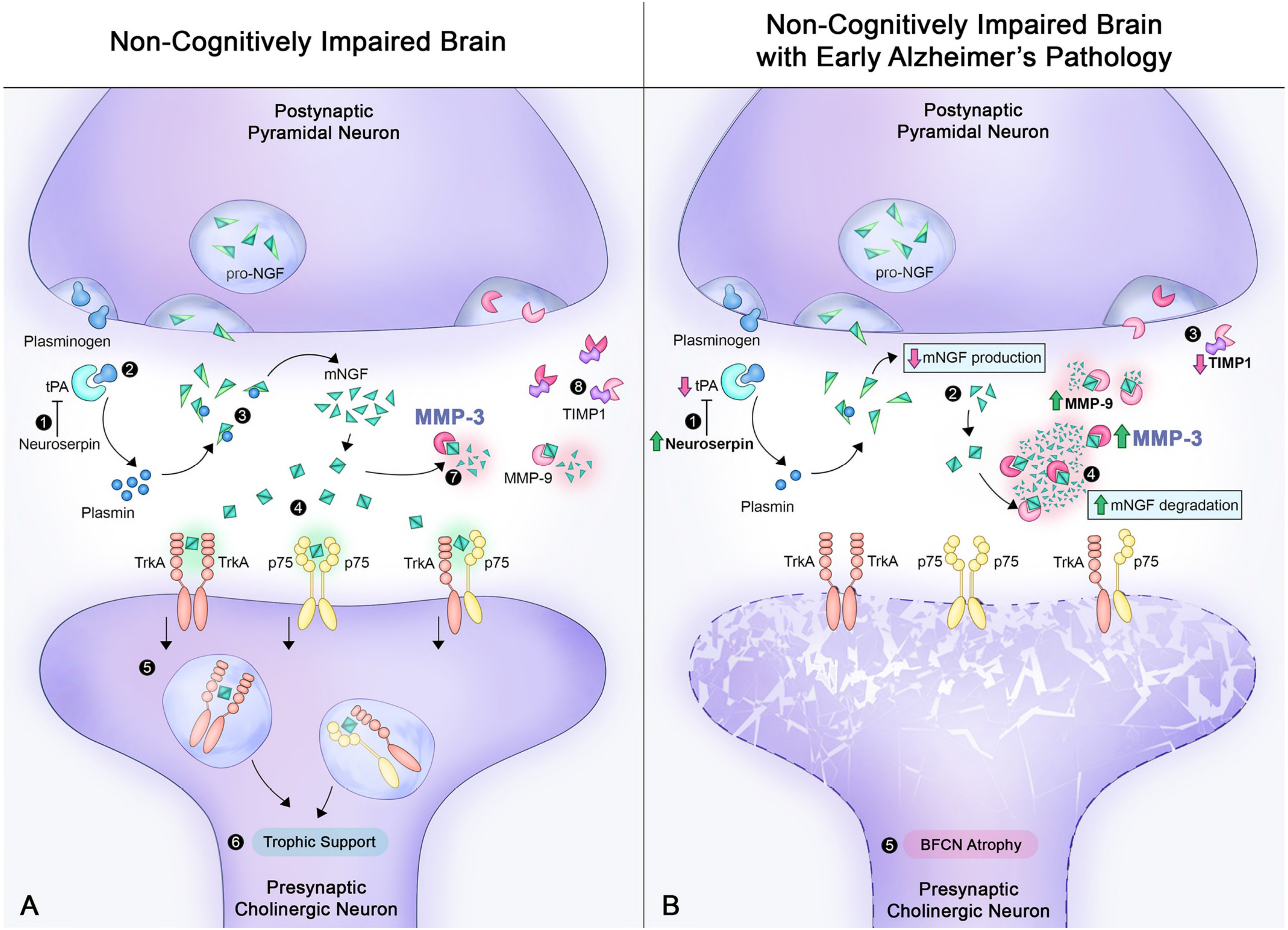


Fig. 7. Schematic representations of events leading to proNGF conversion into mNGF and its degradation. Neuronally stored proNGF, plasminogen, tPA, neuroserpin, proMMP-9, and TIMP-1 would be released into the extracellular space upon neuronal stimulation. Released tPA would induce the conversion of plasminogen to plasmin, where its activity is tightly regulated by secreted neuroserpin. The generated plasmin would convert proNGF into mature NGF and activate proMMP-9 into active MMP-9. Mature NGF would interact with its cognate receptors (TrkA and p75 neurotrophin receptor) or suffer degradation by activated MMP-9.

The NGF pathway and its dysregulation in Alzheimer's and Down syndrome pathology

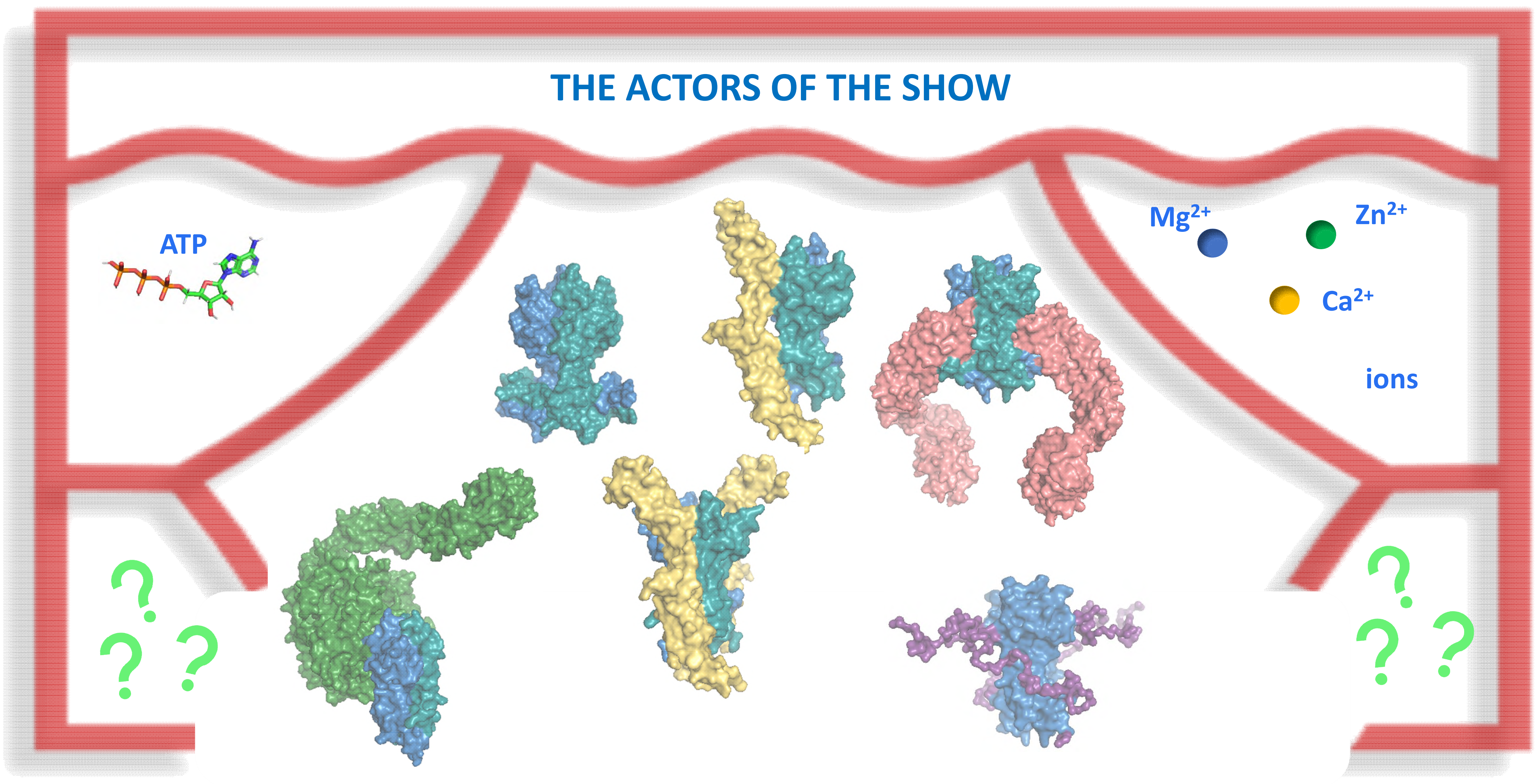


Do Carmo et al, Cells, 2022



Pentz et al, Neurobiol Dis, 2021

ONE RECENT DEVELOPMENT


THE ACTORS OF THE SHOW
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Zn2+
Ca2+
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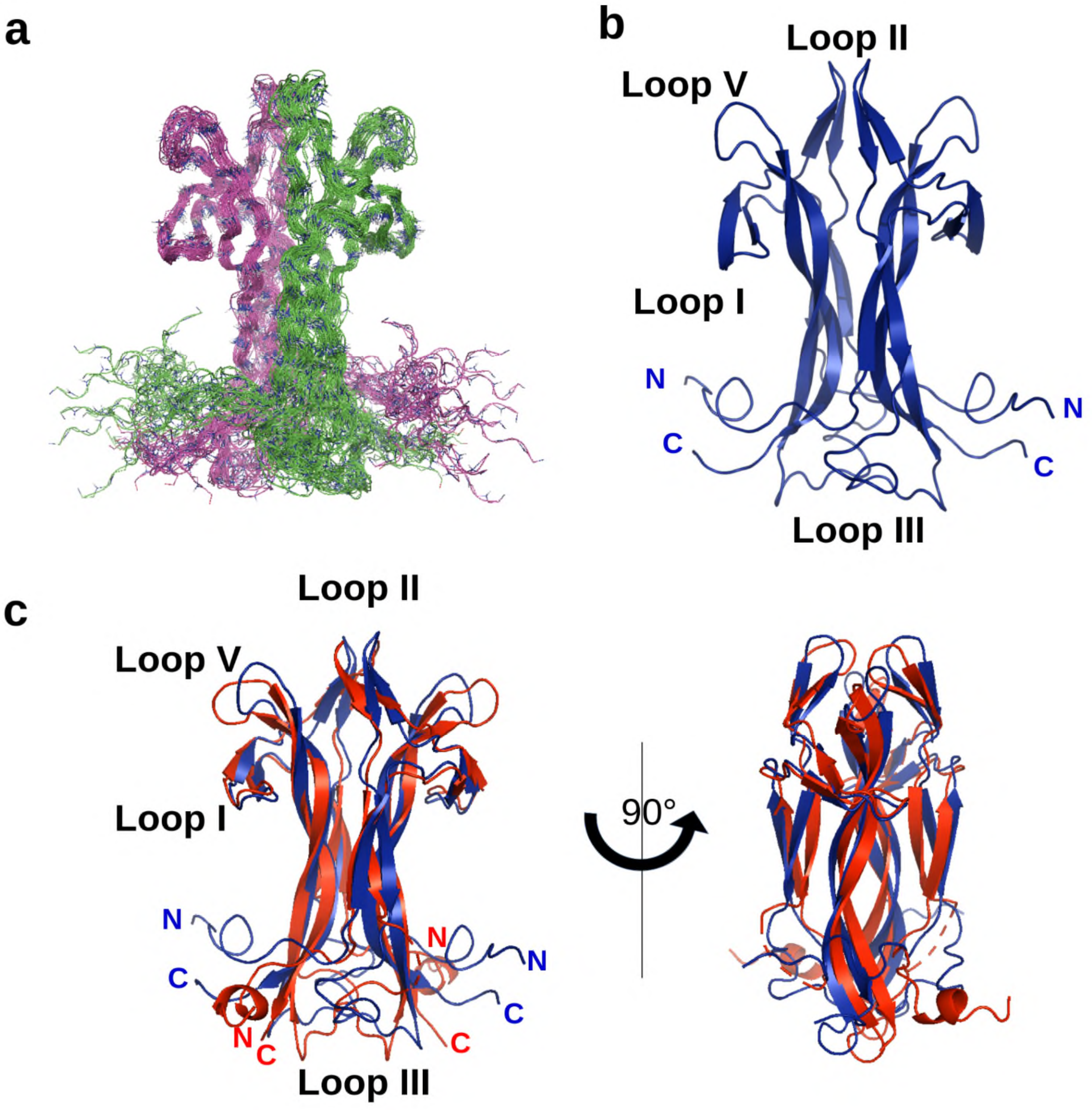
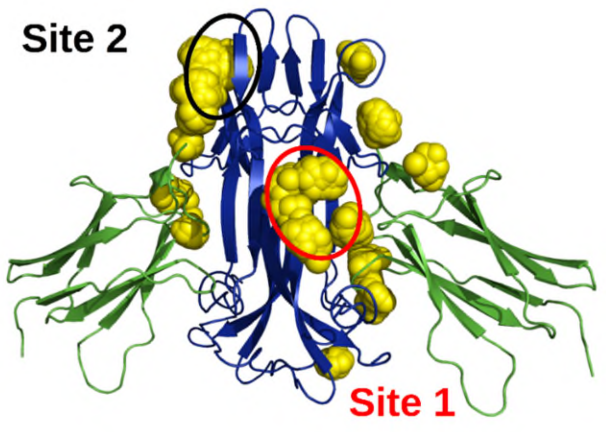


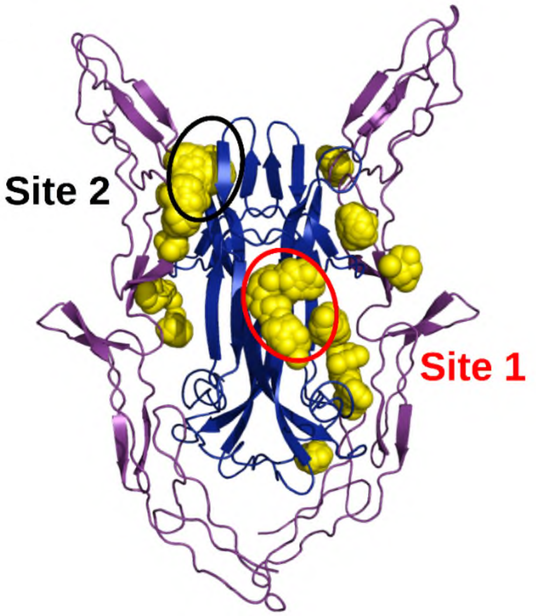
First step: determine the 3D structure of rhNGF in solution by NMR
(PDB ID: 6YW8)

Solution **NMR** structure of rhNGF
(**PDB: 6YW8**)
versus
X-ray crystal structure of rhNGF
(**PDB ID: 1WWW**)

Binding orientation of ATP on rhNGF – interaction with the receptors

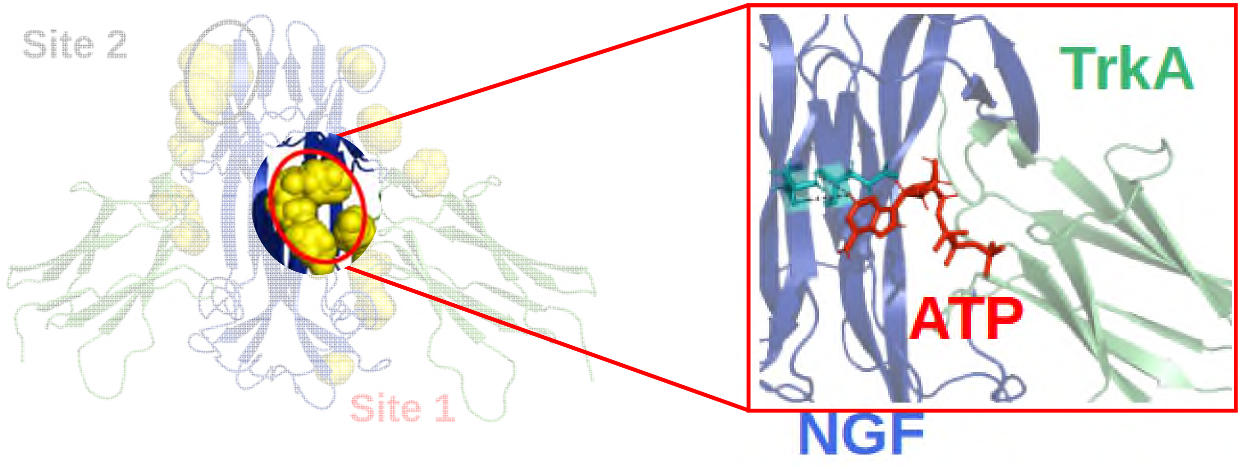


Site 1 -
Interference with TrkA
Green: TrkA d5 (PDB: 1WWW)

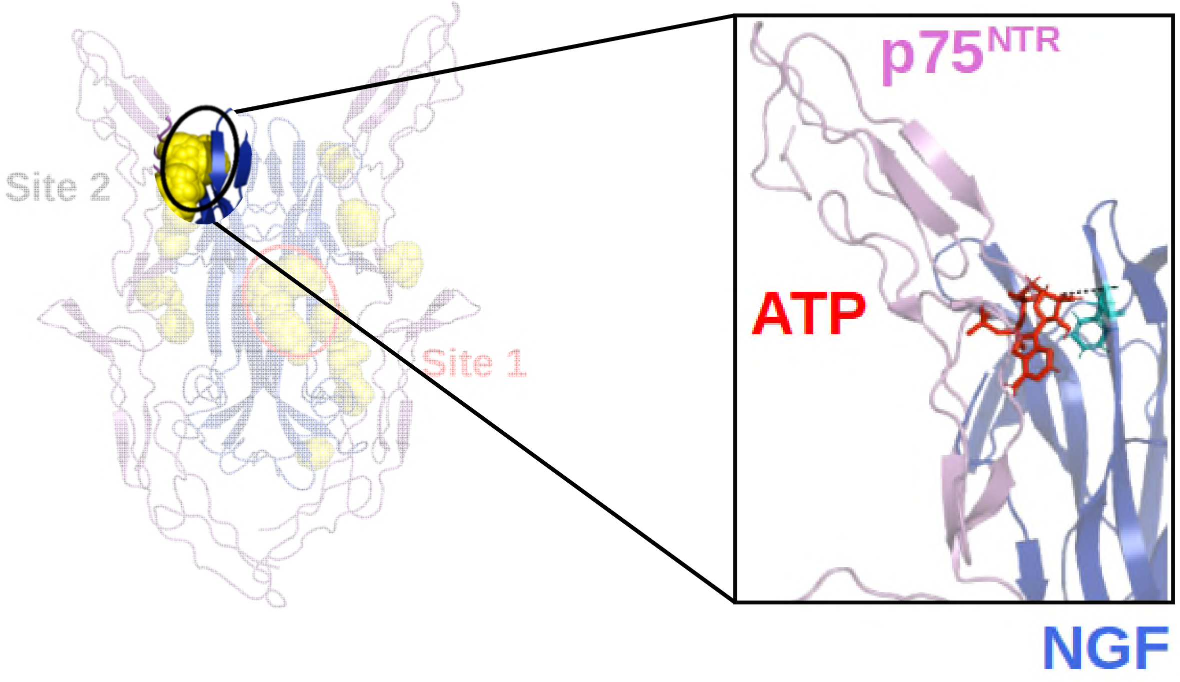


Site 2 -
Interference with p75^{NTR}
Magenta: p75^{NTR} extracellular domain (PDB: 1SG1)

Binding orientation of ATP on rhNGF – interaction with the receptors

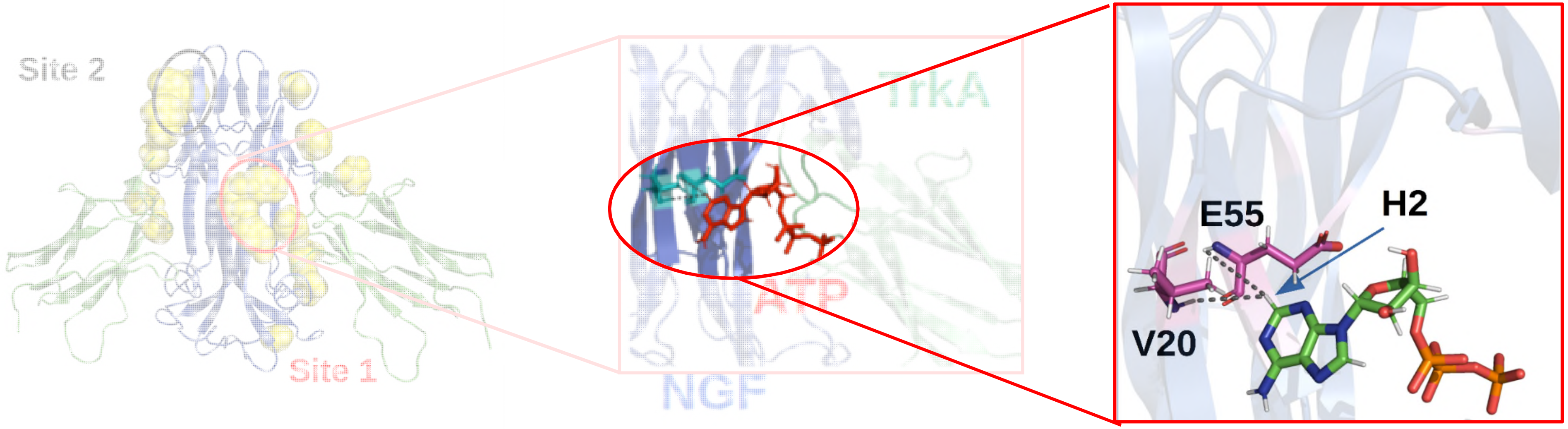


Site 1 -
Interference
with TrkA
Green: TrkA d5
(PDB: 1WWW)

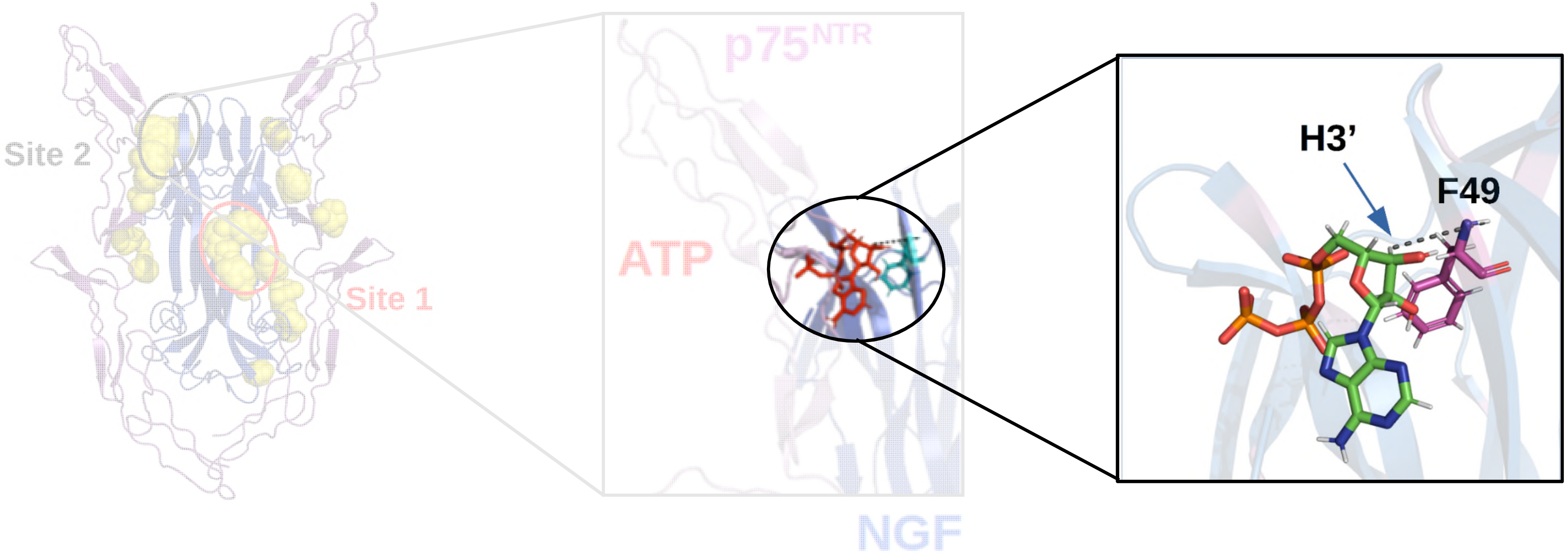


Site 2 -
Interference
with $p75^{NTR}$
Magenta:
$p75^{NTR}$
extracellular
domain
(PDB: 1SG1)

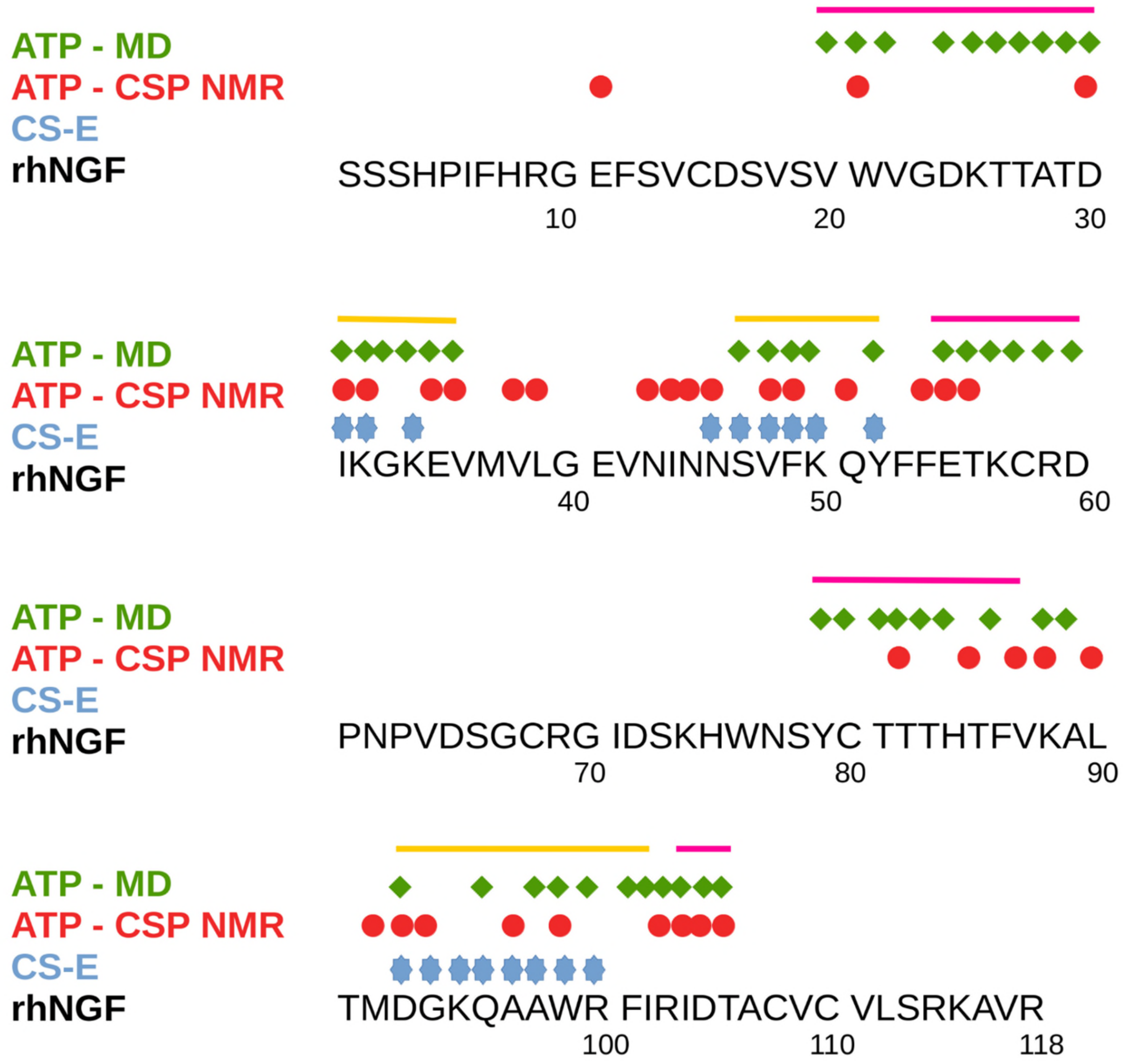
Binding orientation of ATP on rhNGF – interaction with the receptors



Site 1 -
Interference with TrkA
Green: TrkA d5 (PDB: 1WWW)



Site 2 -
Interference with $p75^{NTR}$
Magenta: $p75^{NTR}$ extracellular domain (PDB: 1SG1)



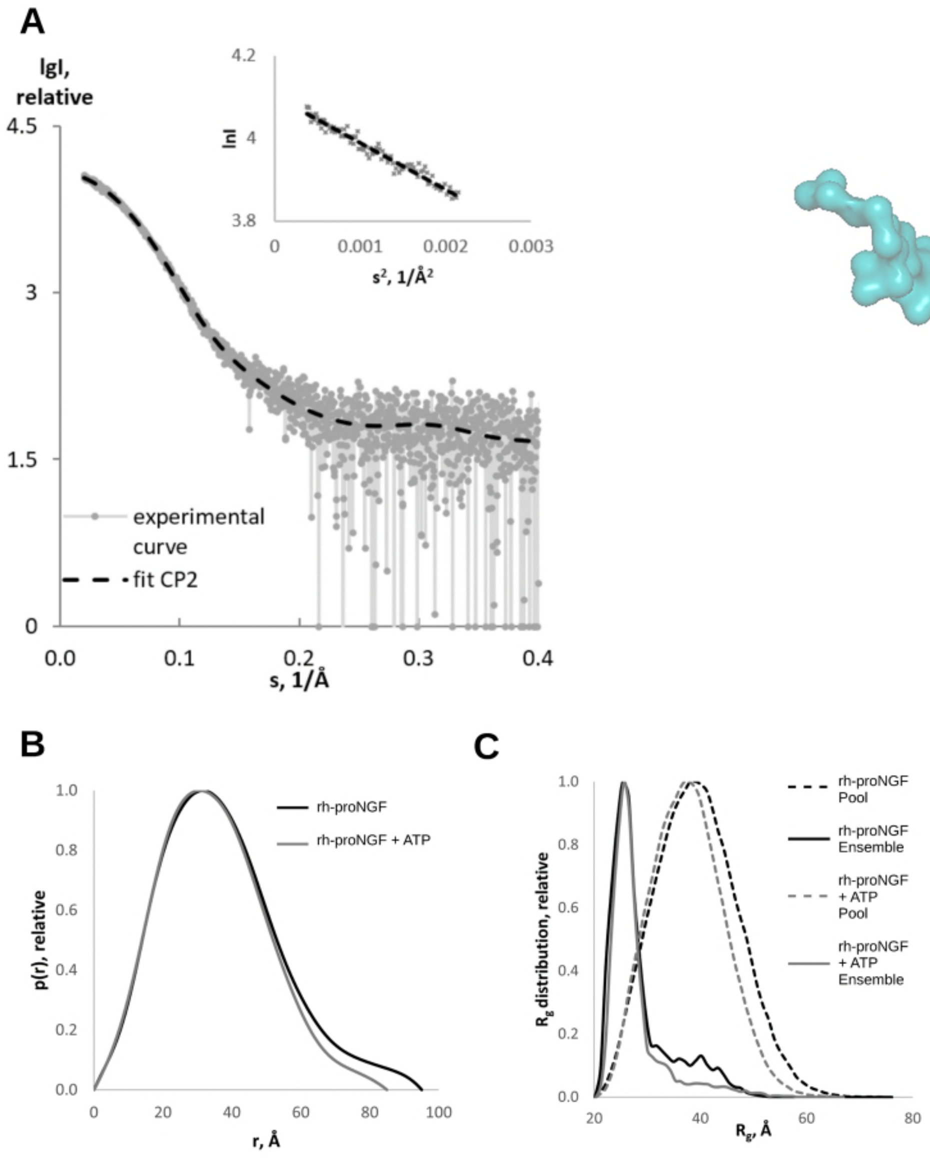
rhNGF ligands binding sites specificity

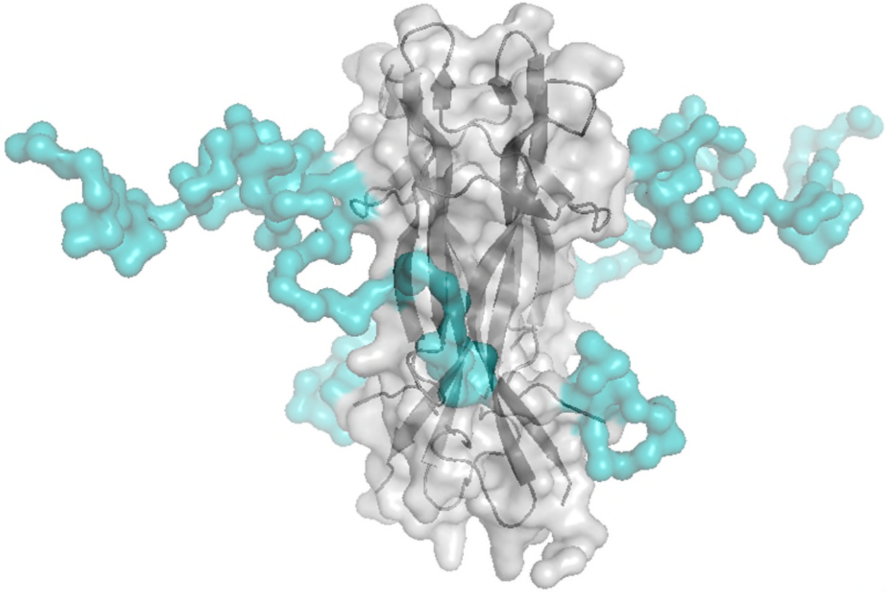
Site 1: specific ATP-rhNGF binding site

Site 2: promiscuous rhNGF binding site for endogenous ligands such as ATP, glycosaminoglycans and lipids

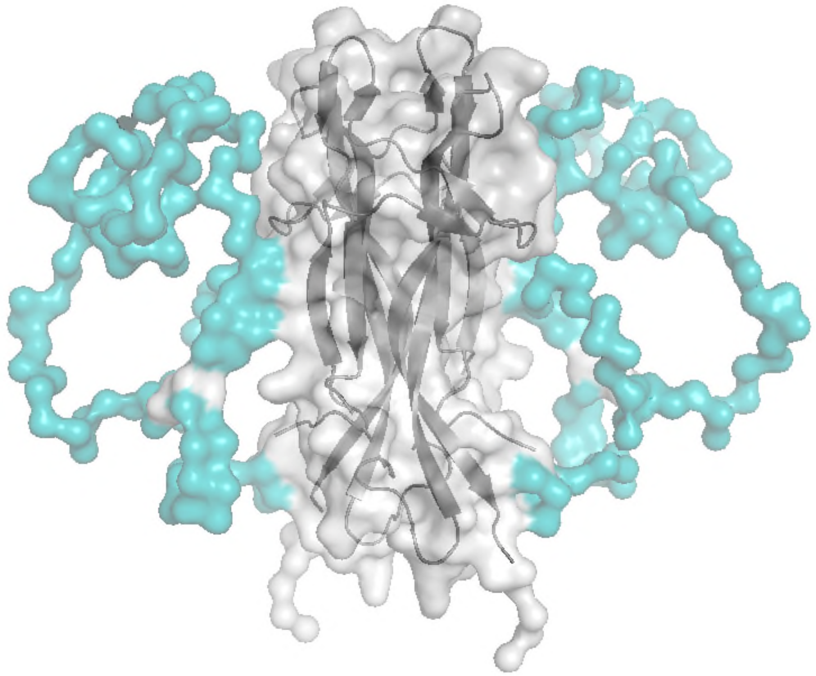
The relative binding enthalpy of ATP for Site 1 is indeed -12.2 kcal/mol lower than for Site 2.

ATP binding induces a conformational change into the IDR pro-peptide region (SAXS)



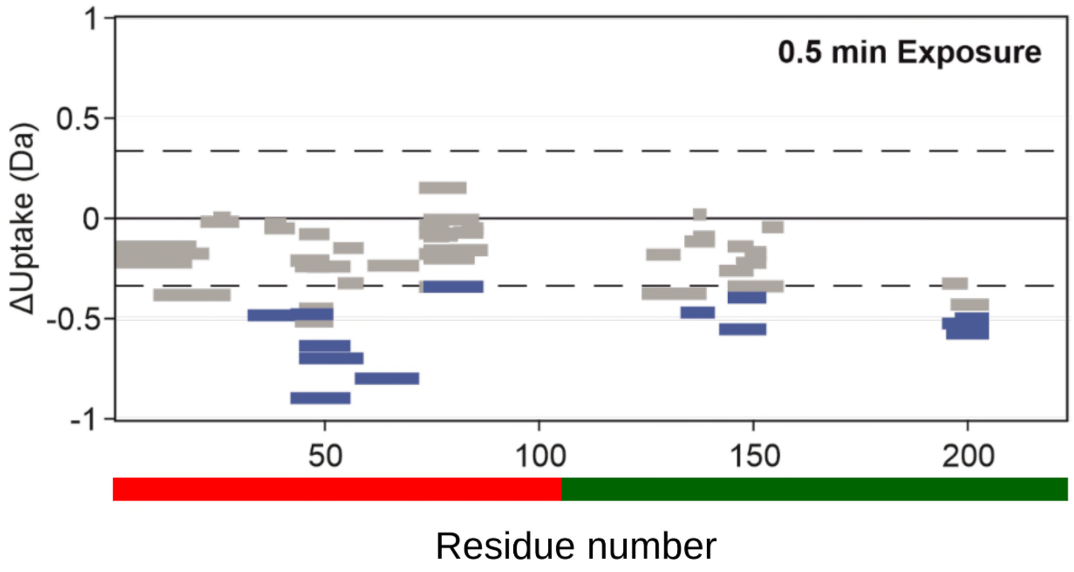


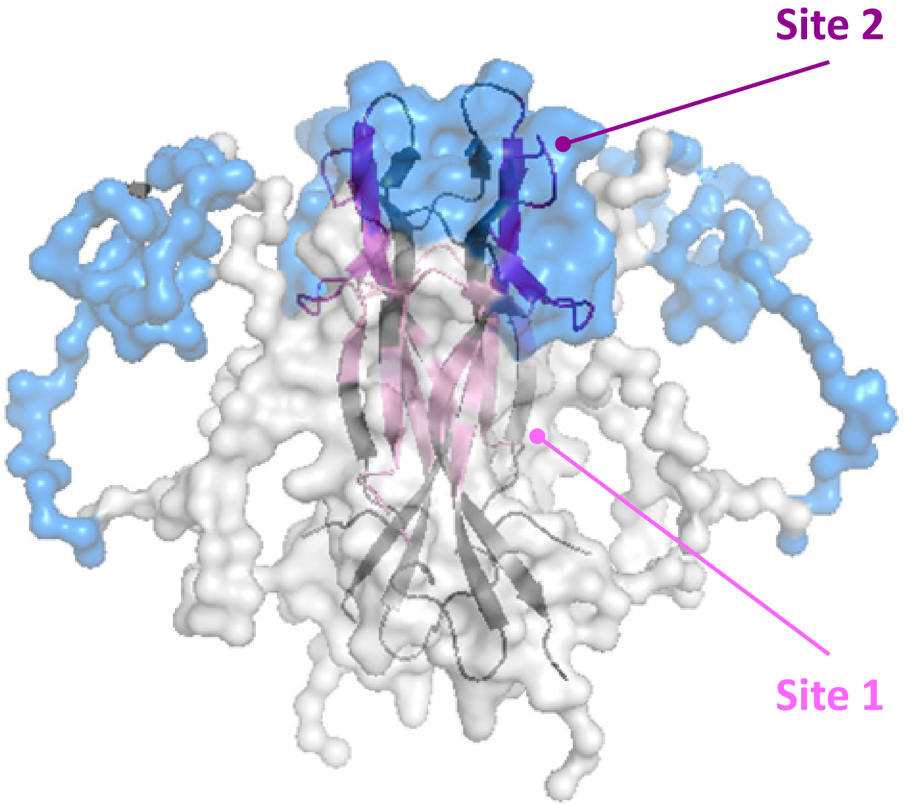
rh-proNGF

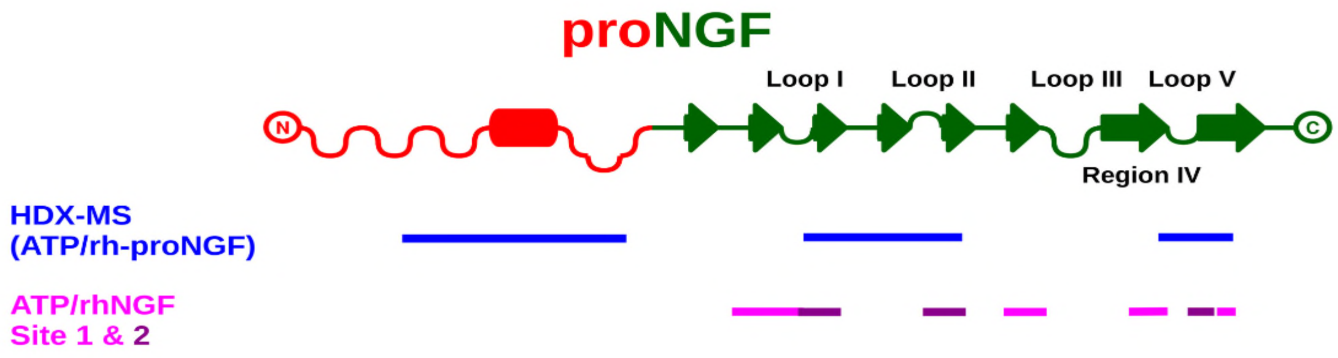


rh-proNGF + ATP

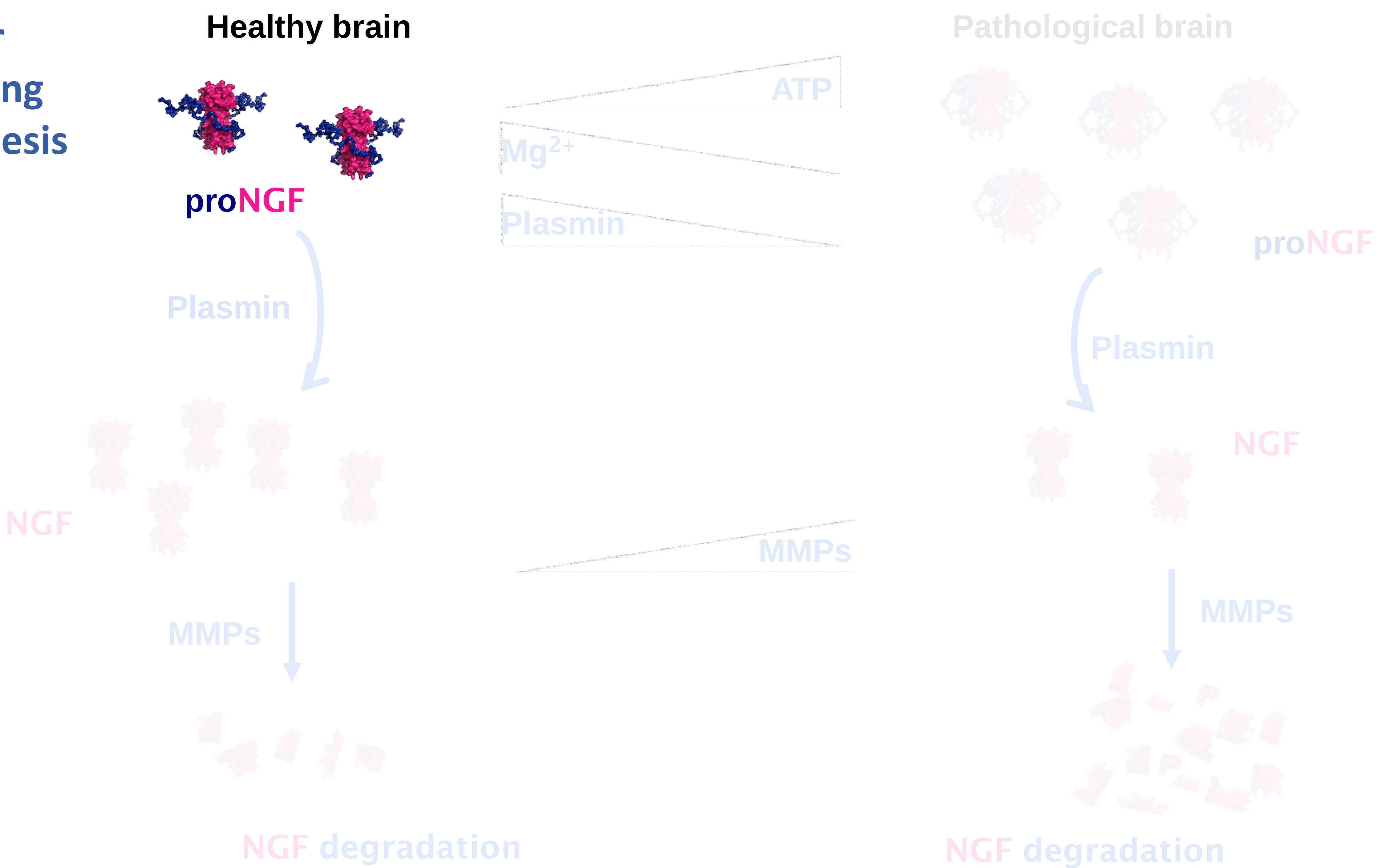
Identification of the regions involved in the conformational rearrangement: HDX-MS







Our working hypothesis



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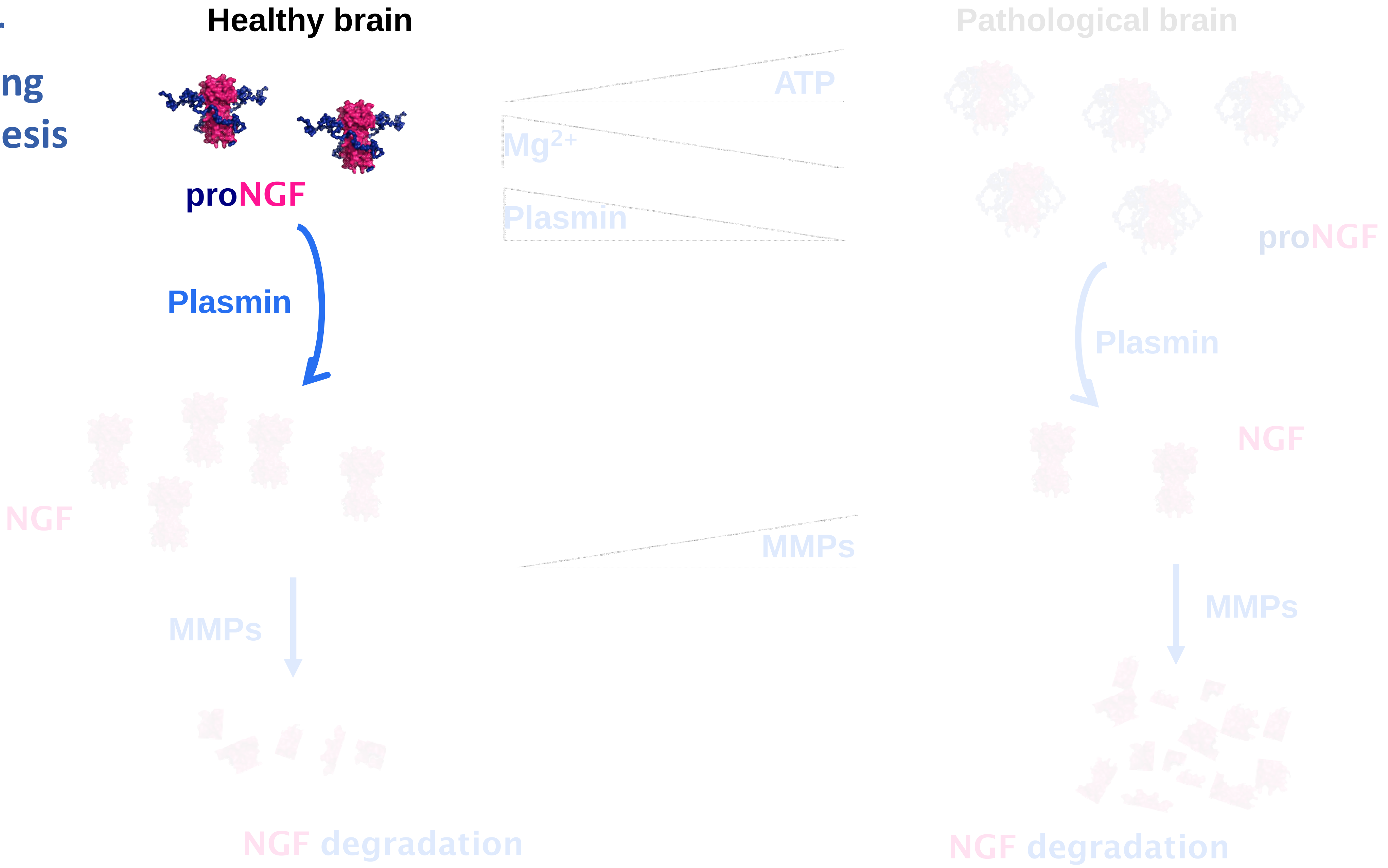

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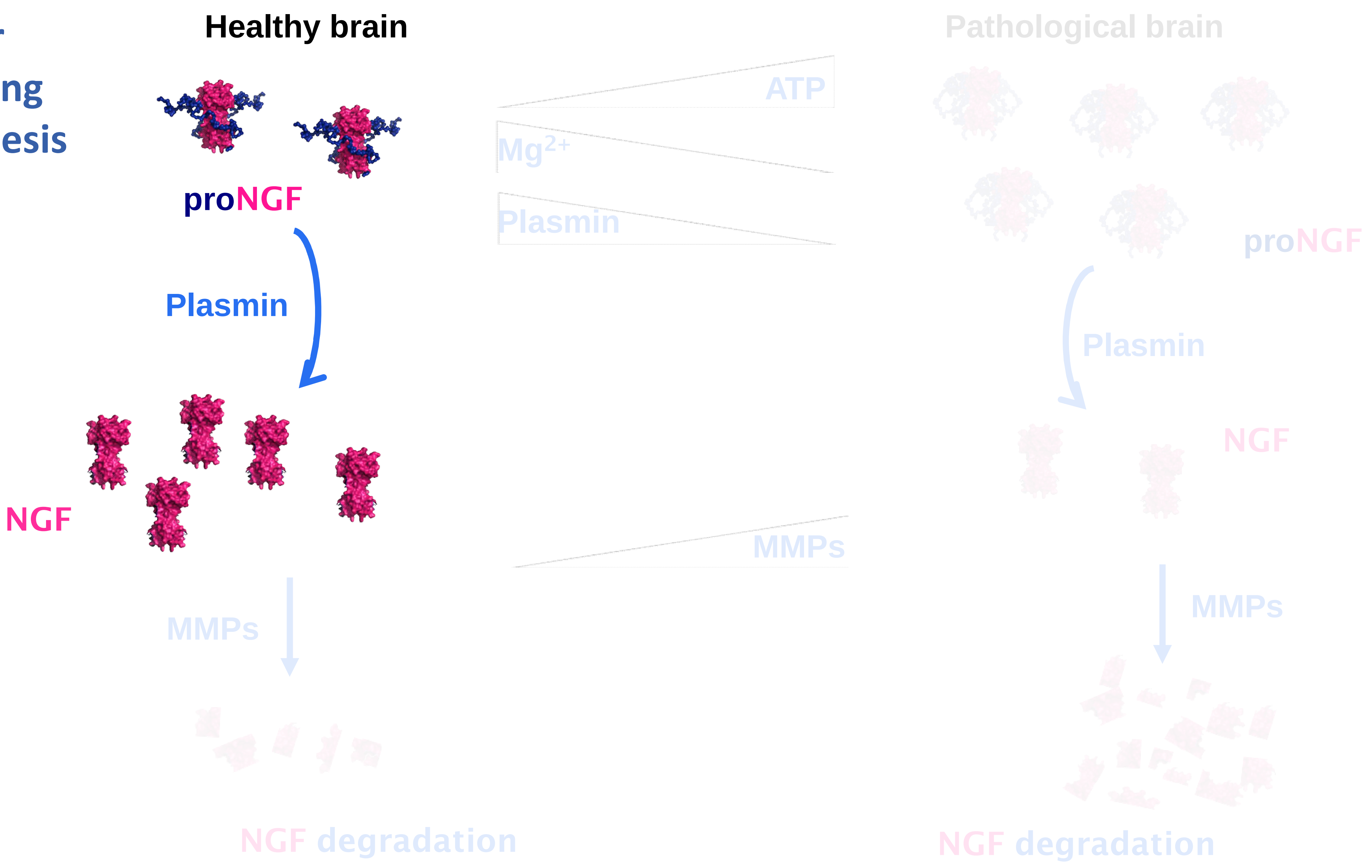

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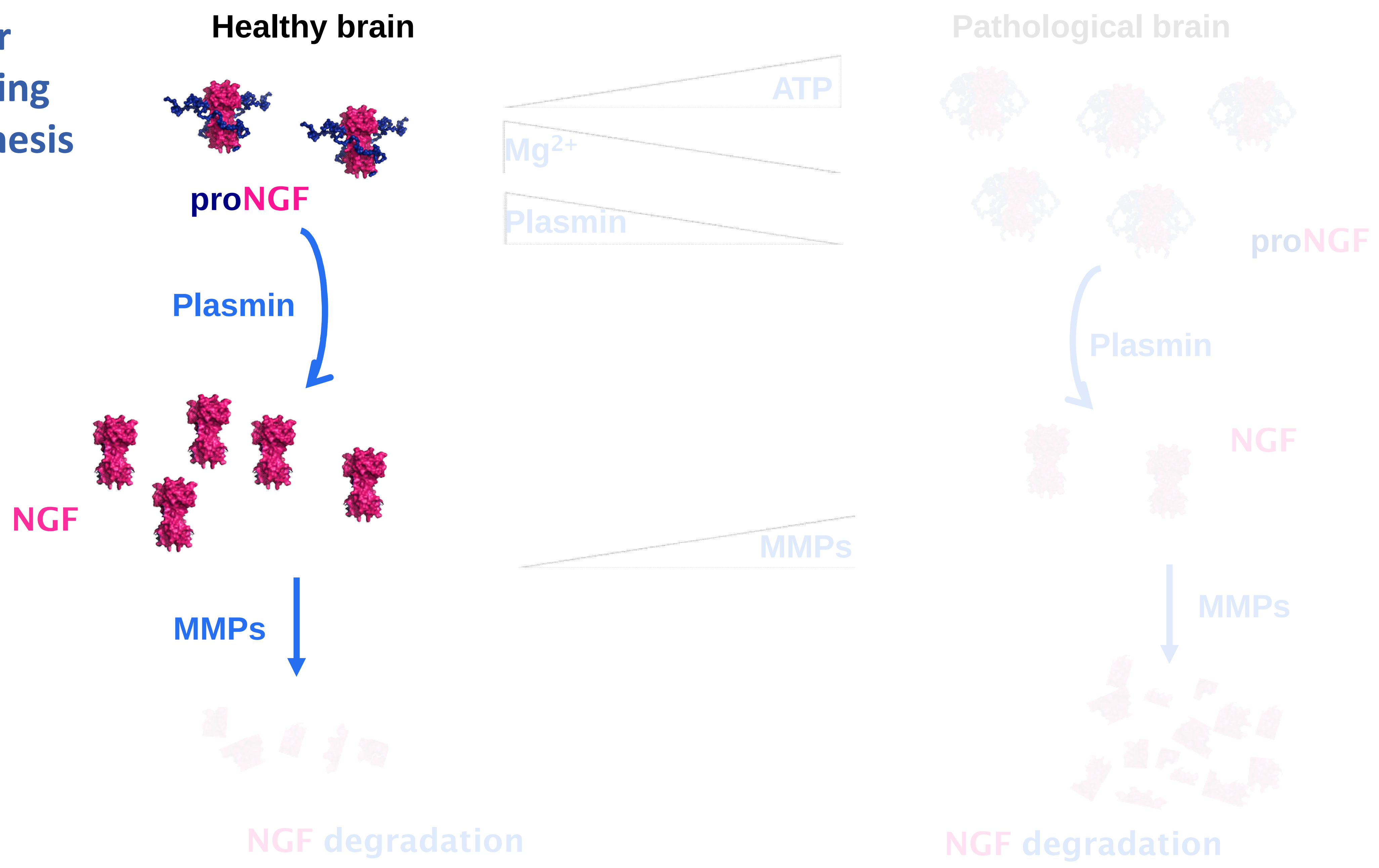

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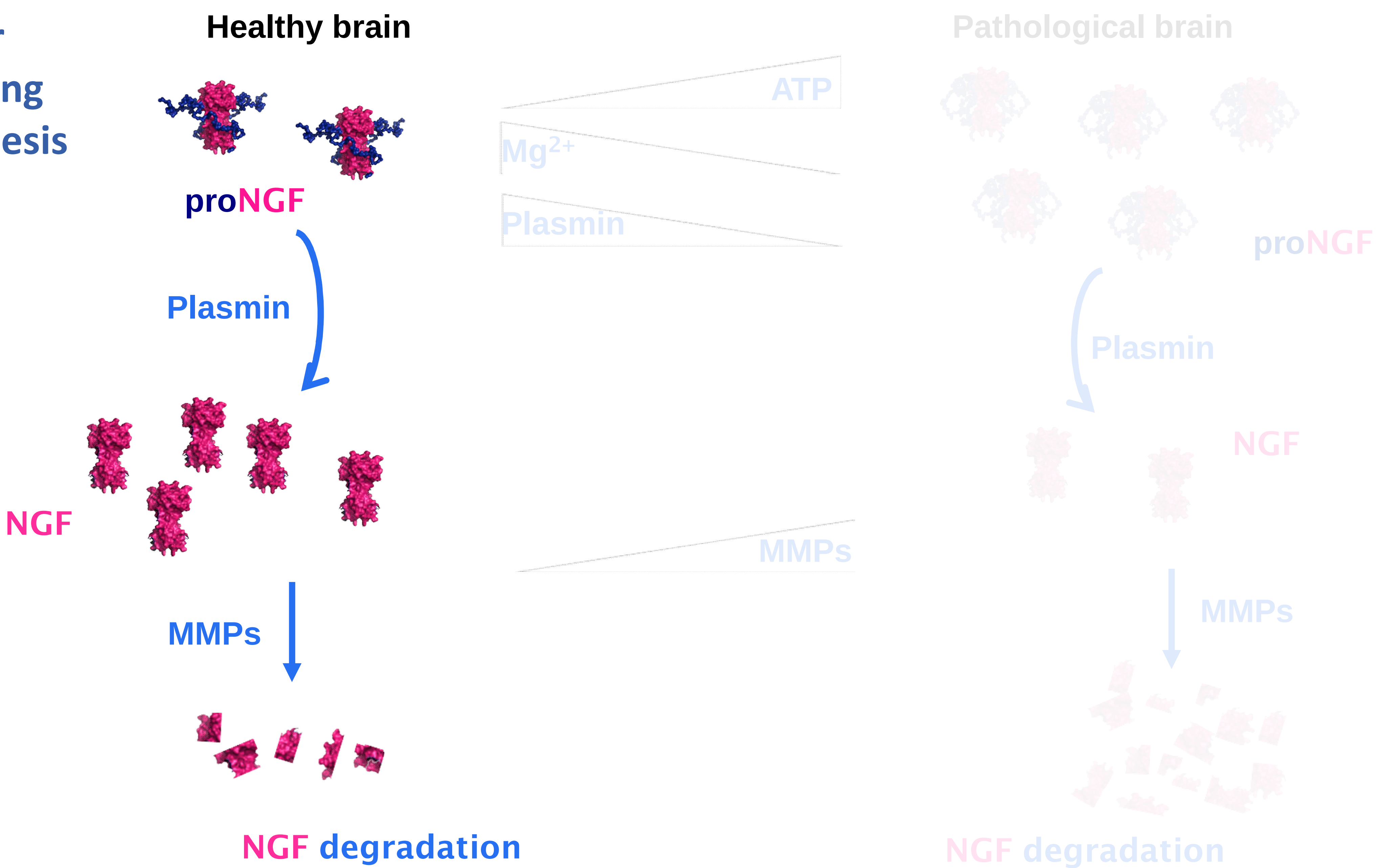

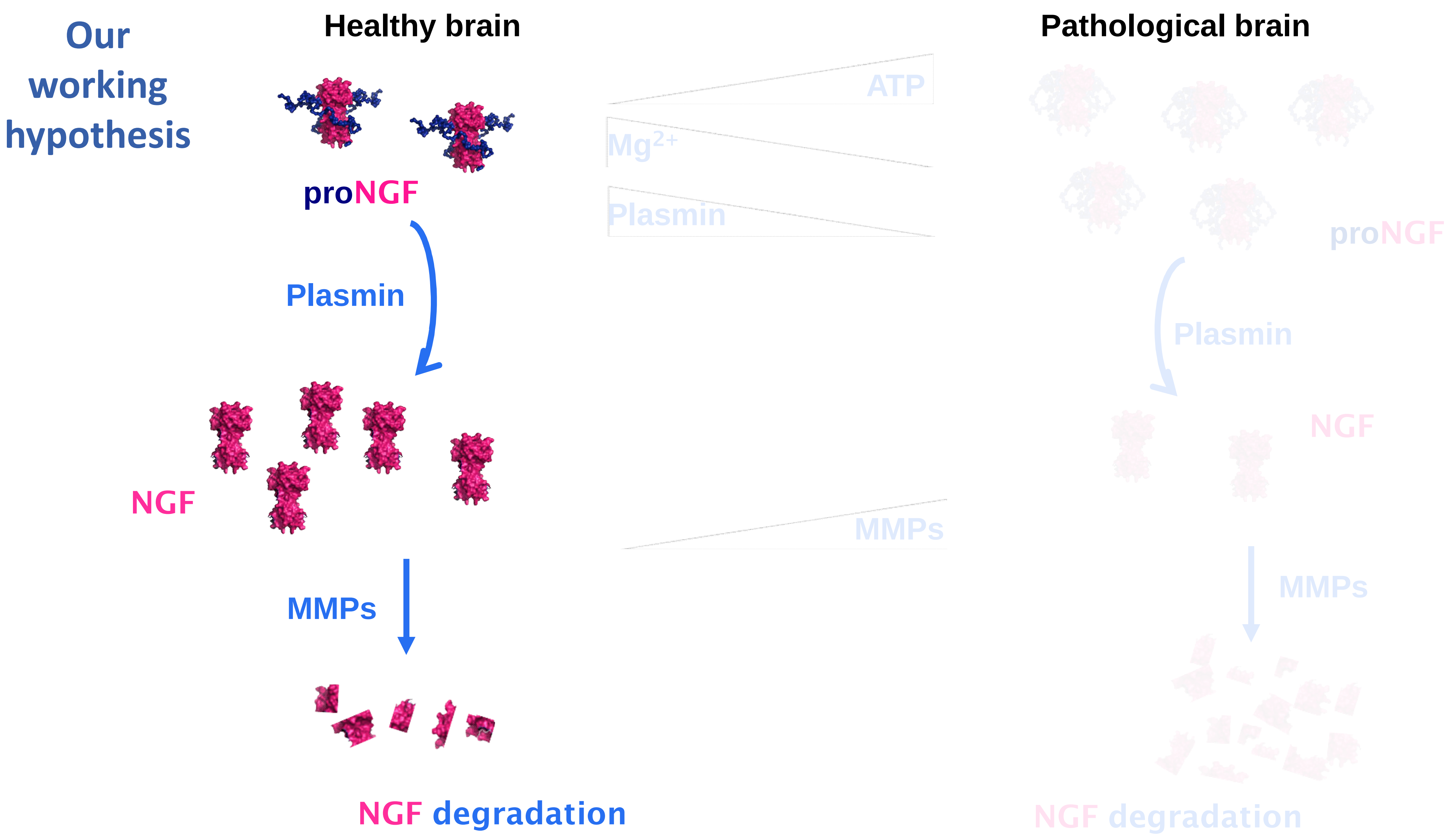
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Healthy brain
Pathological brain
proNGF
Plasmin
NGF
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NGF degradation
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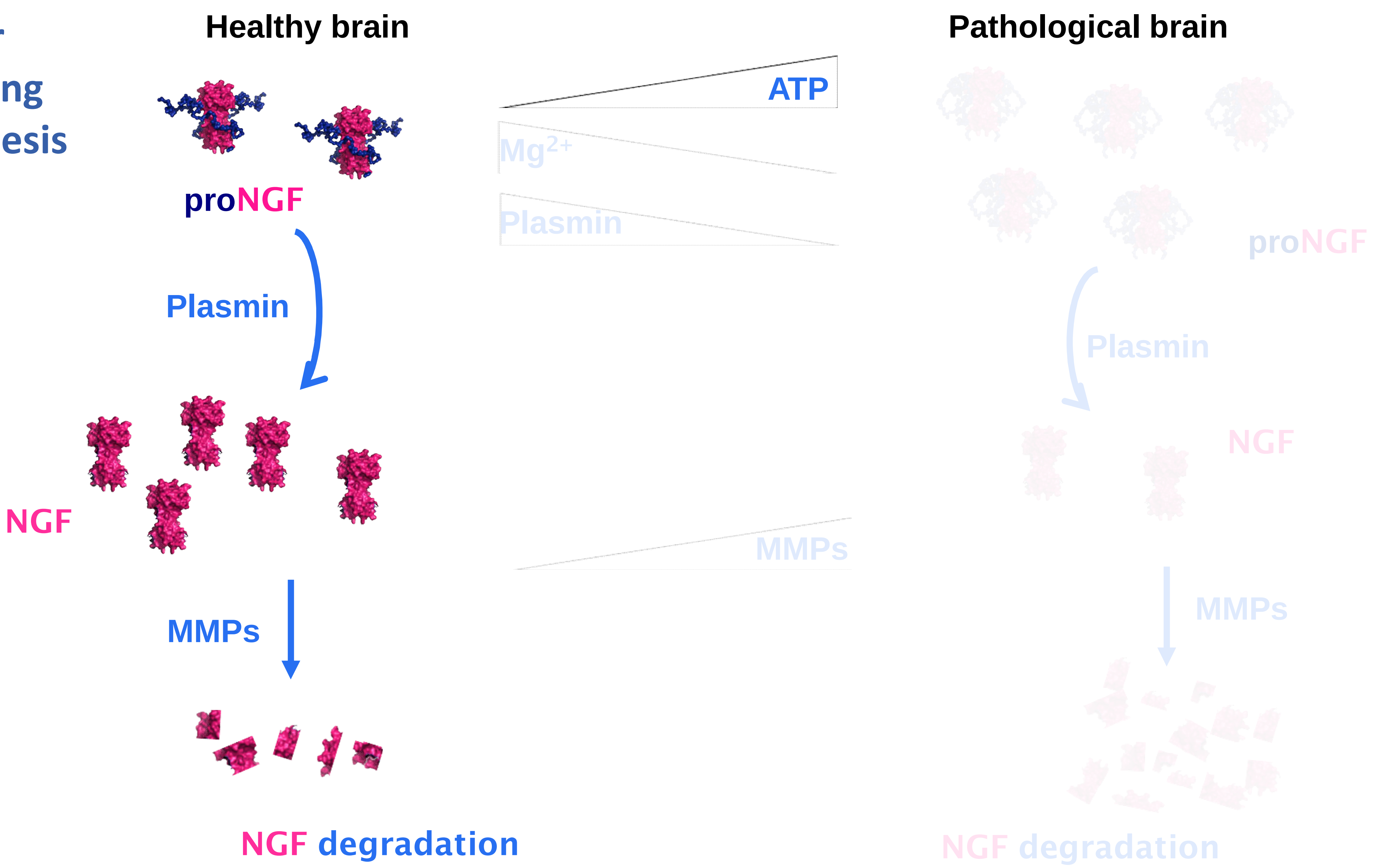

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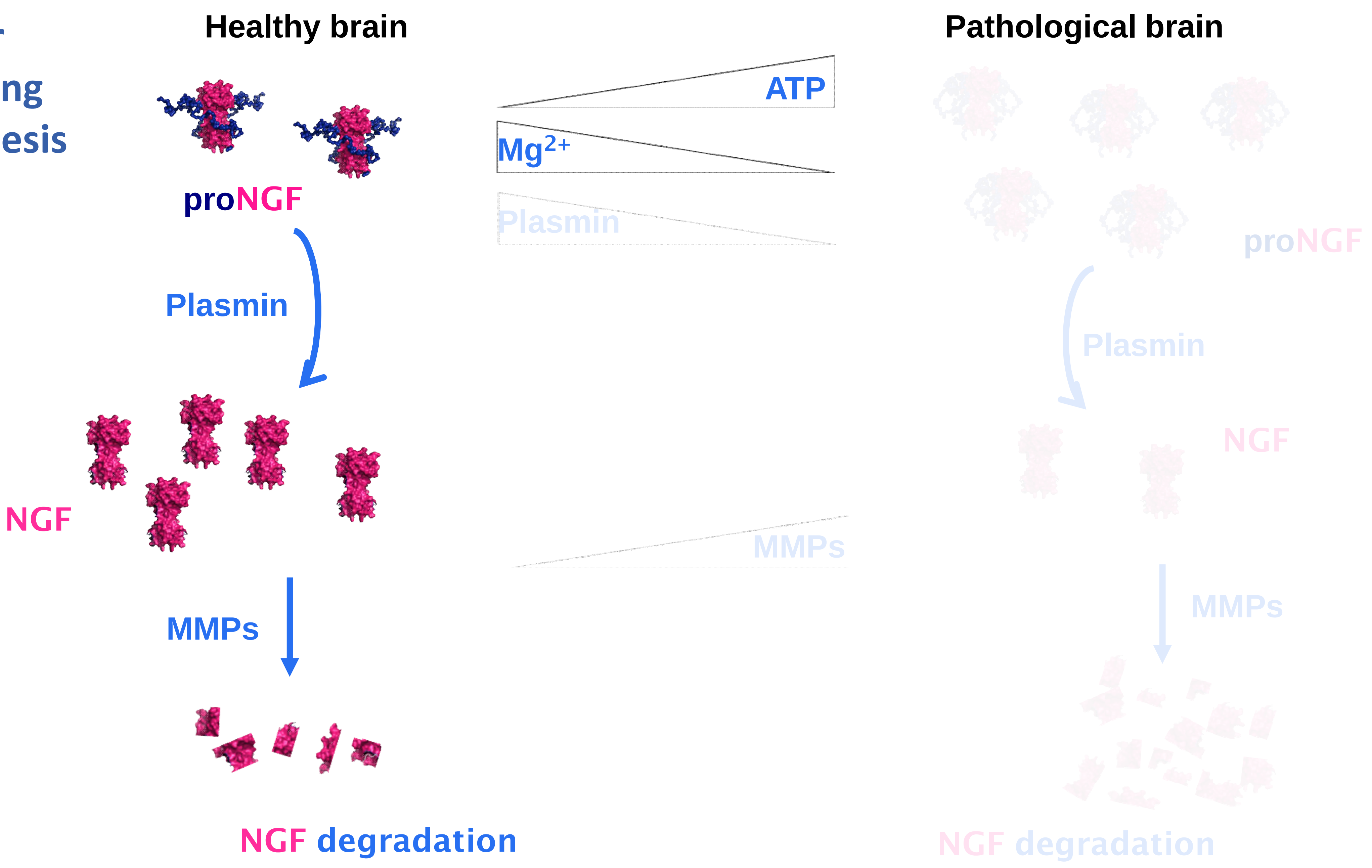

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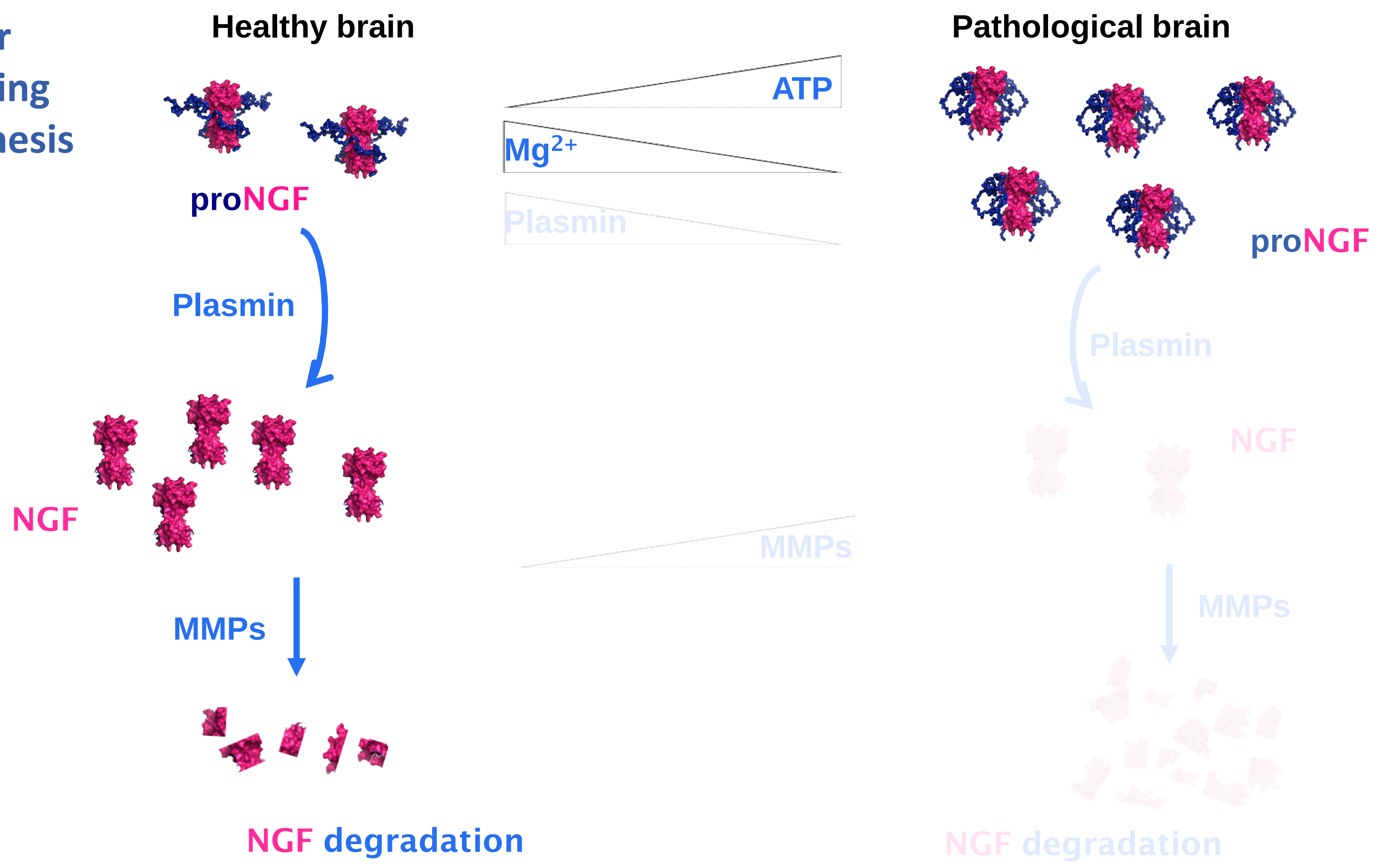

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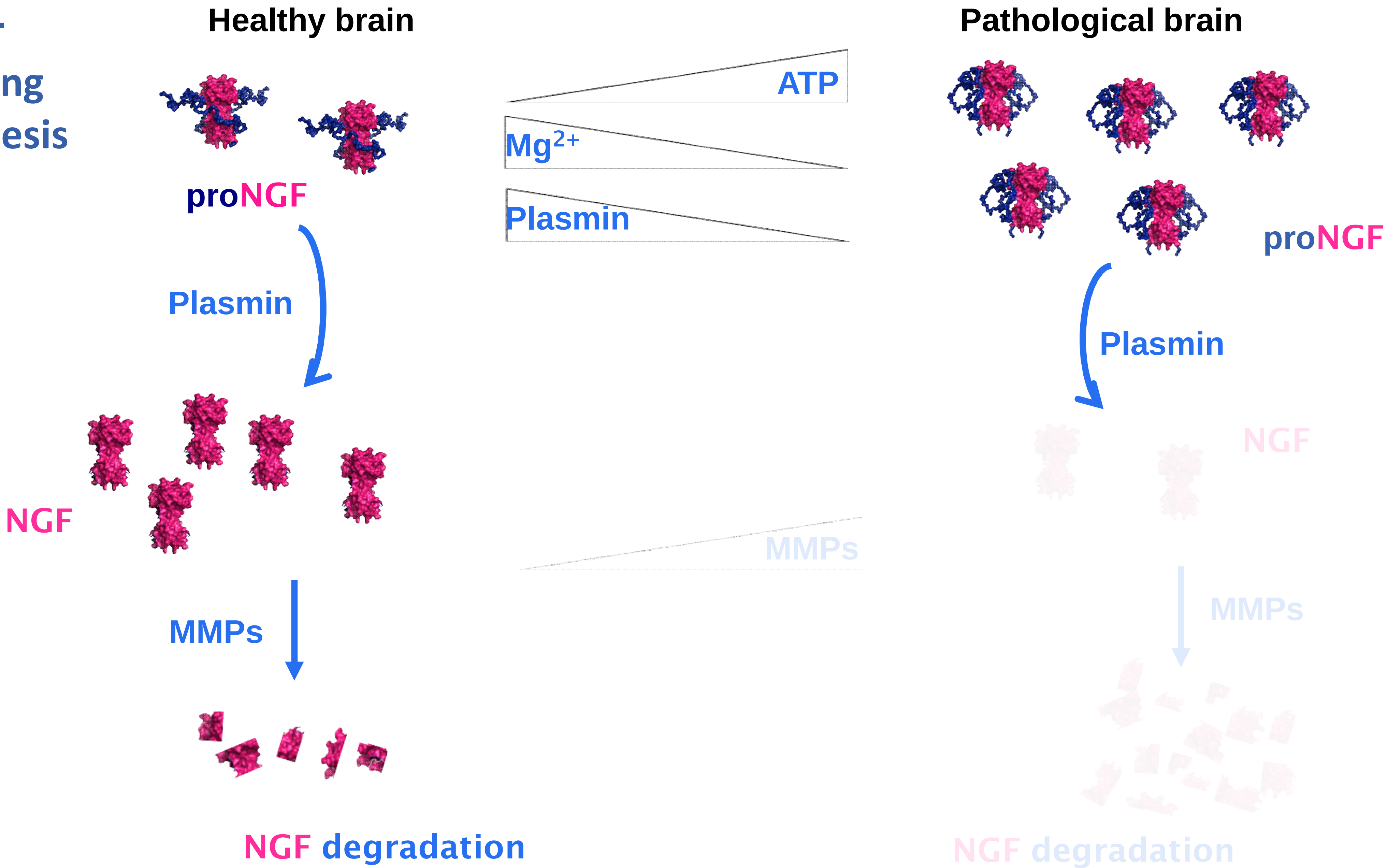

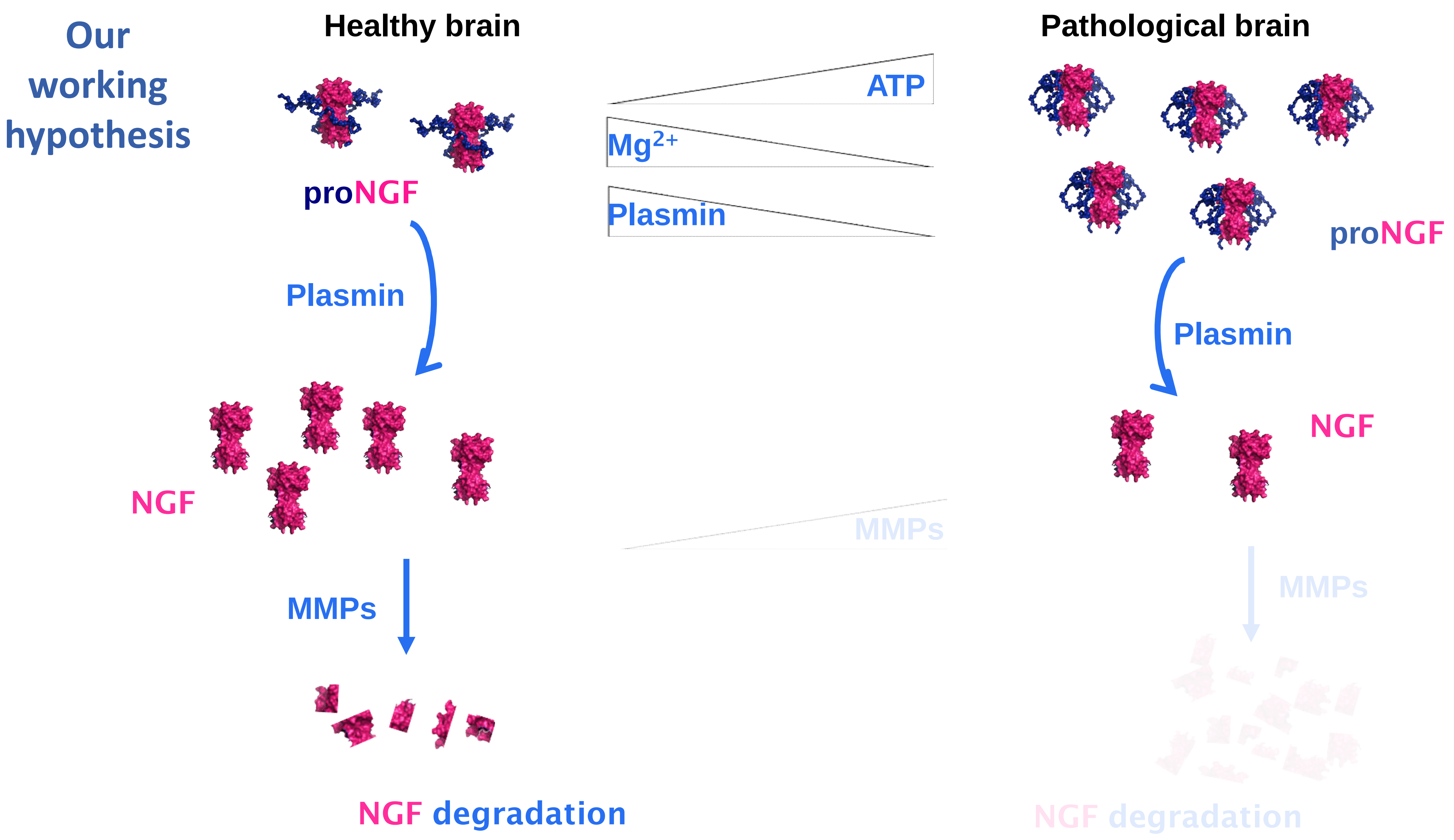
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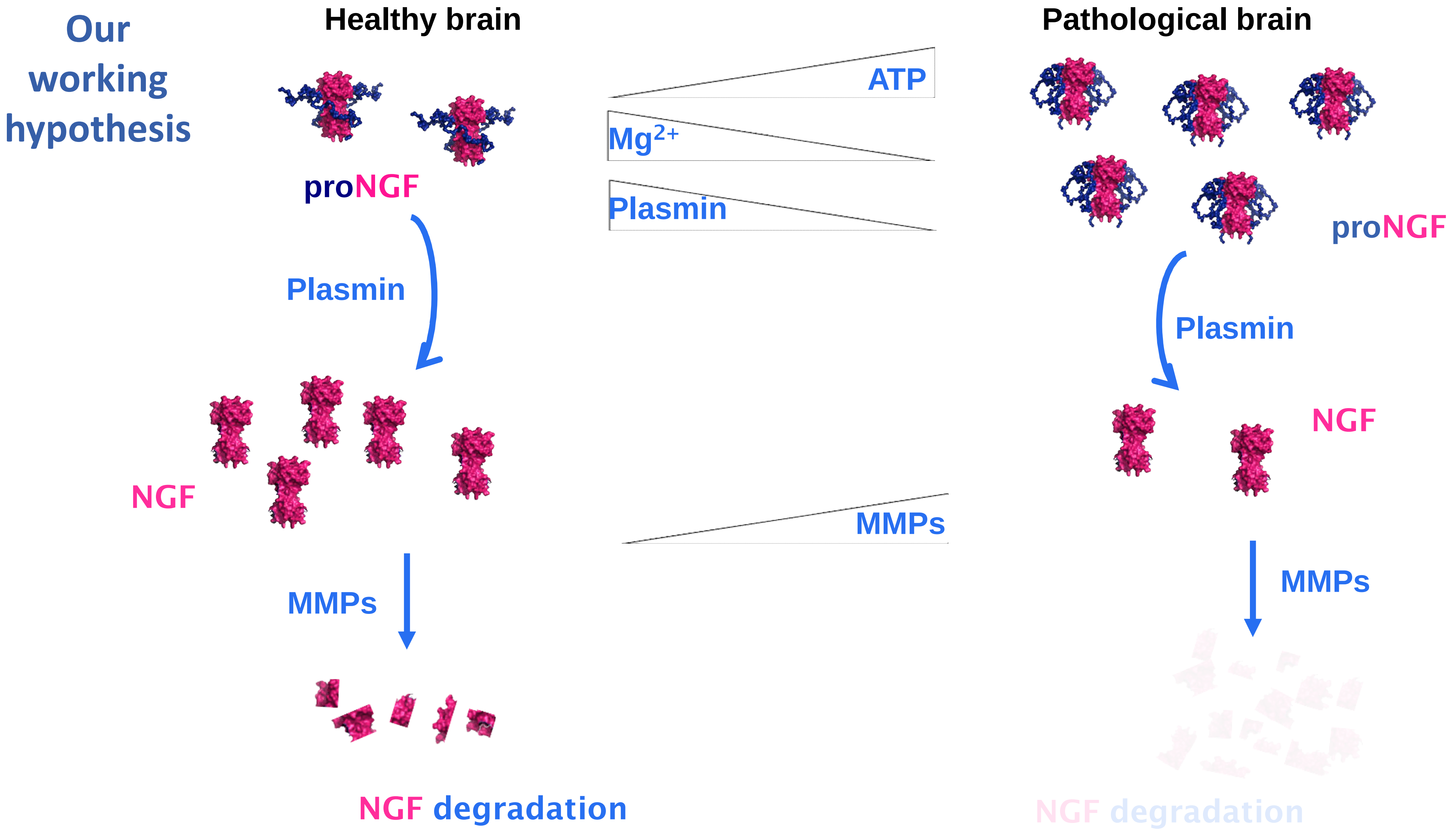
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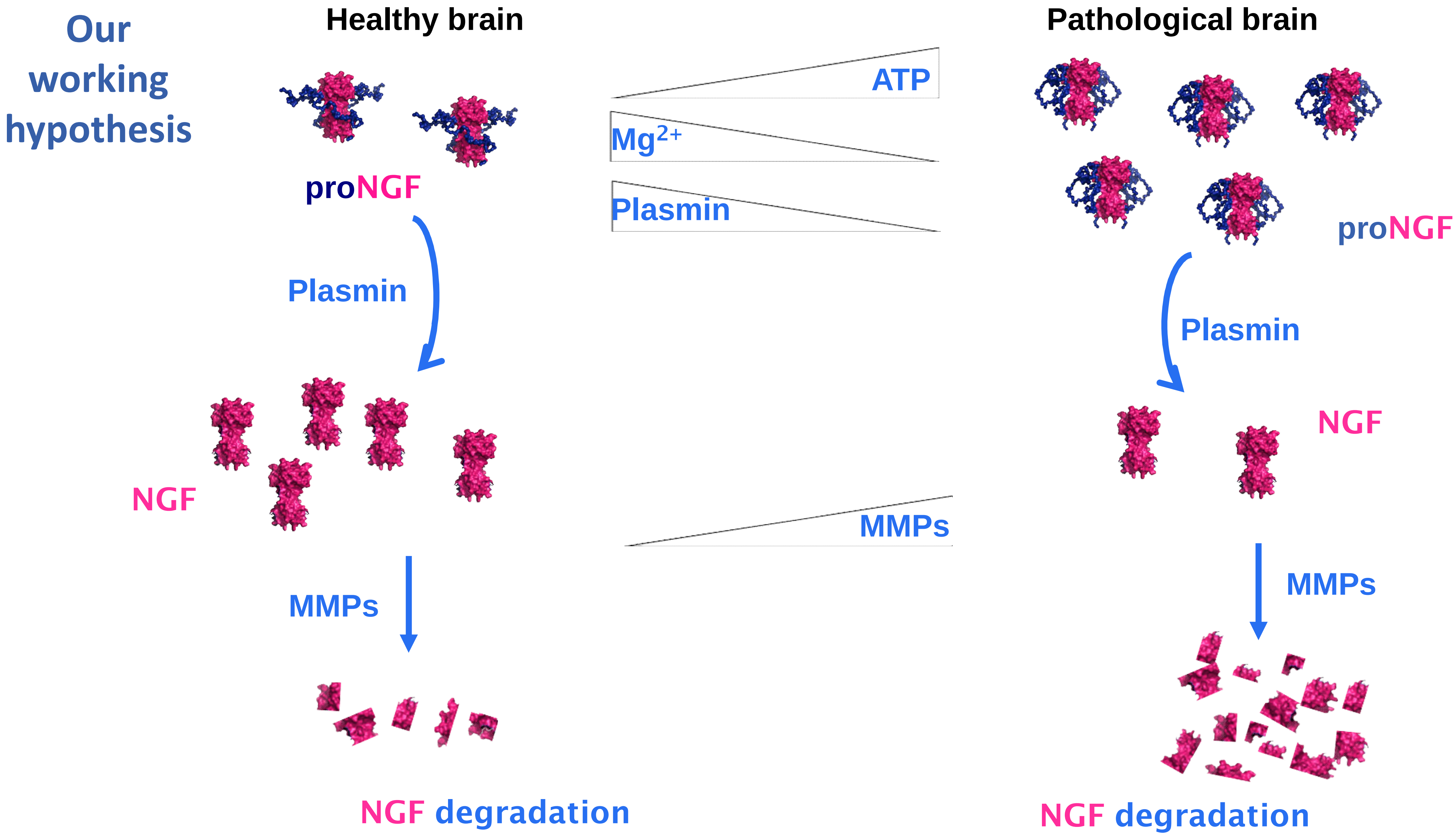
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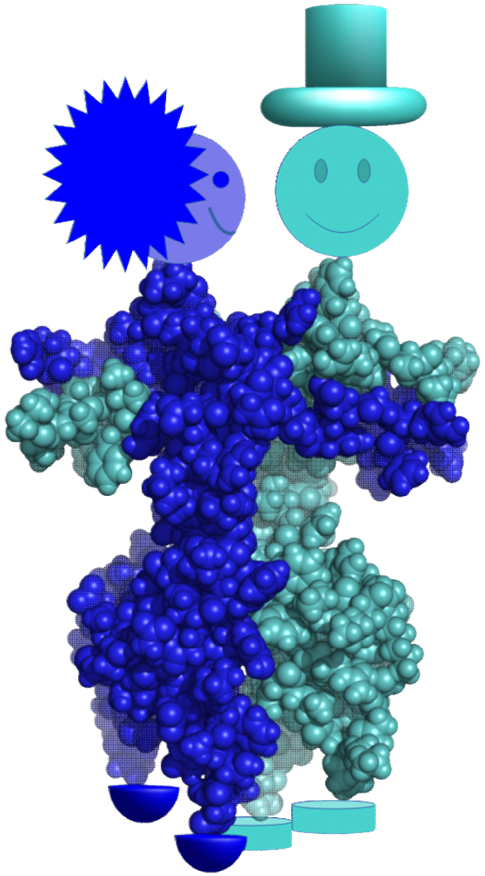

The Nerve Growth Factor at its early in-vitro and in-vivo debut

'The tumor had given a first hint of its existence in St. Louis, but it was in Rio de Janeiro that it revealed itself, and it did so in a theatrical and grand way, as if spurred by the bright atmosphere of that explosive and exuberant manifestation of life that is the Carnival in Rio" [14].