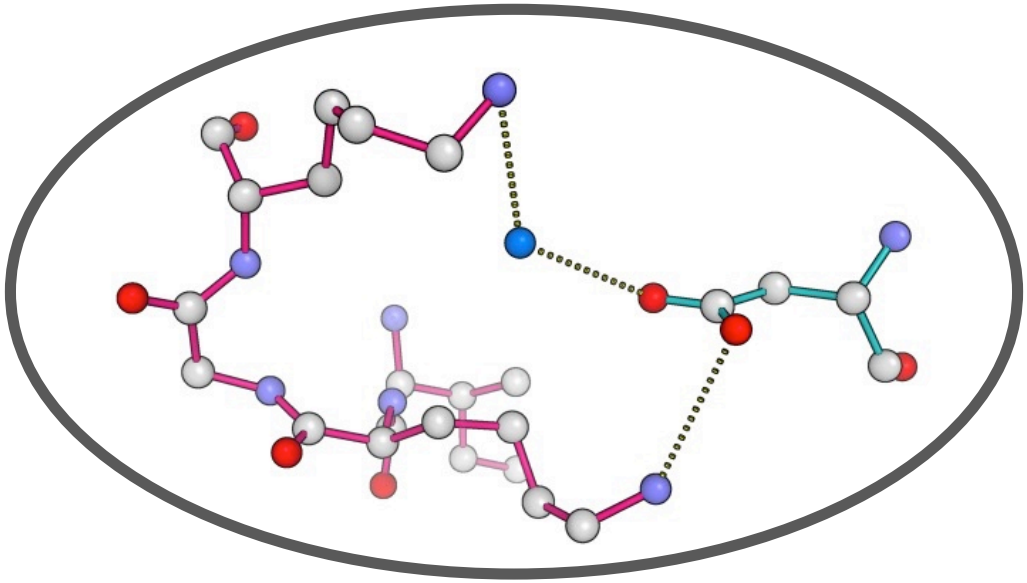
Synthetic Efforts towards the Development of p75 Neurotrophin Receptor Inhibitors



Evan Carder
Research Topic Seminar
Wipf Group
June 04, 2016

Presentation Outline

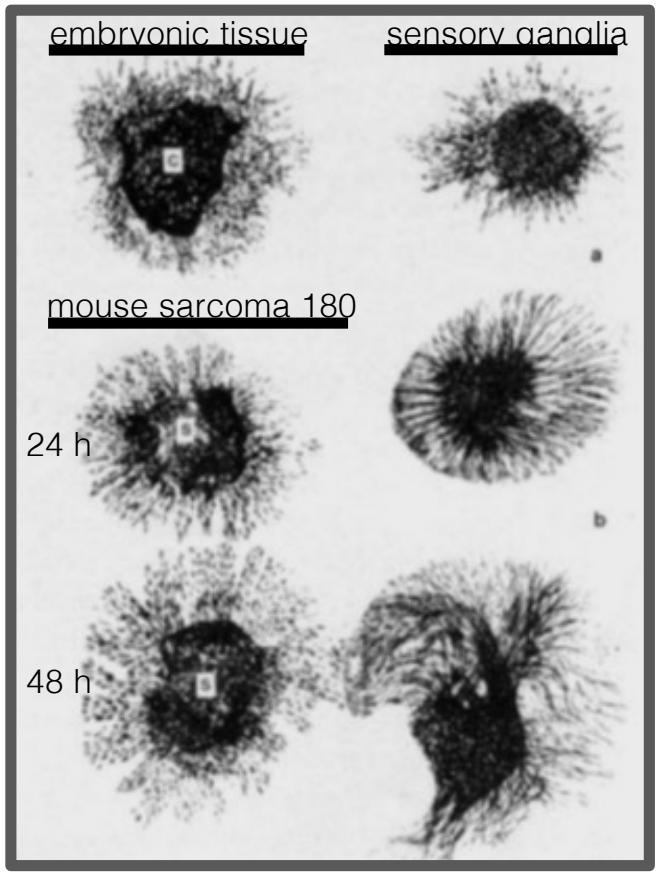
Summary and Future Outlook

Nerve Growth Factor (NGF)

Stanley Cohen

Rita Levi-Montalcini

- NGF was discovered by Dr. Rita Levi-Montalcini, MD and Dr. Stanley Cohen, PhD in the early 1950's.
- In an important experiment, Levi-Montalcini demonstrated the first evidence of a neurotrophic-induced 'halo' effect.
- Cohen isolated significant amounts of NGF from mice submaxillary salivary glands. NGF amino acid sequenced was established in 1971 and the crystal structure was elucidated in 1991.
- NGF was the first characterized growth factor and the most studied neurotrophin family member.
- NGF has been shown to be important in the development and maintenance of the sympathetic and sensory nervous systems.



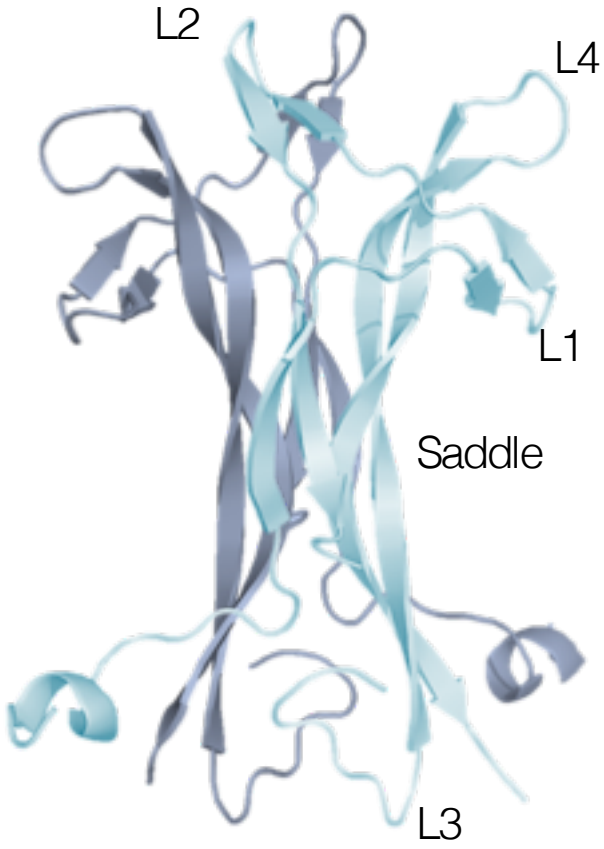
Trends Cell Biol. **2004**, 7, 395.
Angewandte Chemie **1987**, 26, 707.
Proc. Natl. Acad. Sci. **1971**, 68, 2417.
Nature **1991**, 354, 411.

Neurotrophins

NGF BDNF NT-3 NT-4

- A family of secreted growth factors that regulate neuronal cell survival, development, and function.
- Four characterized mammalian neurotrophins:

  nerve growth factor (NGF)
  brain-derived neurotrophic factor (BDNF)
  neurotrophin-3 (NT-3)
  neurotrophin-4 (NT-4).
- Derived from a common ancestral gene and portray similar sequence and structure, functioning as noncovalently associated homodimers.
- These closely related molecules act by binding to two distinct classes of transmembrane receptors



PDB:1WWW

Nat. Rev. Neuroscience **2005**, 6, 603.
Cell. Mol. Life Sci. **2001**, 58, 748.
J. Biol. Chem. **1995**, 270, 19669.

Neurotrophin receptors

- Neurotrophins bind to two distinct classes of transmembrane receptors

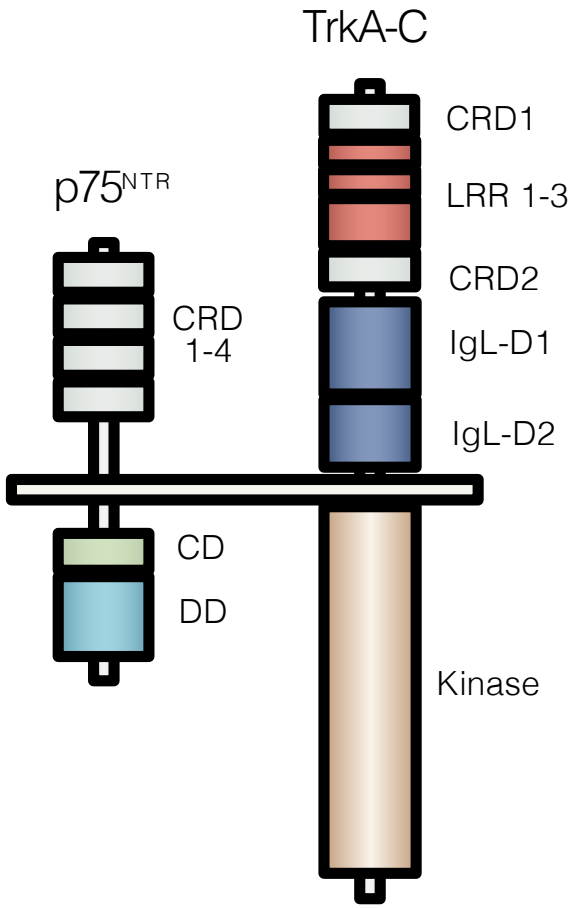
  p75 Neurotrophin receptor ($p75^{NTR}$)
  Tropomyosin receptor kinase (TrK)

- p75 Neurotrophin receptor

  - Member of the tumor necrosis factor receptor superfamily
  - Nonselective, low affinity neurotrophin binding
  - Lacks intrinsic enzymatic activity
  - Contains an intracellular death domain

- Tropomyosin receptor kinase

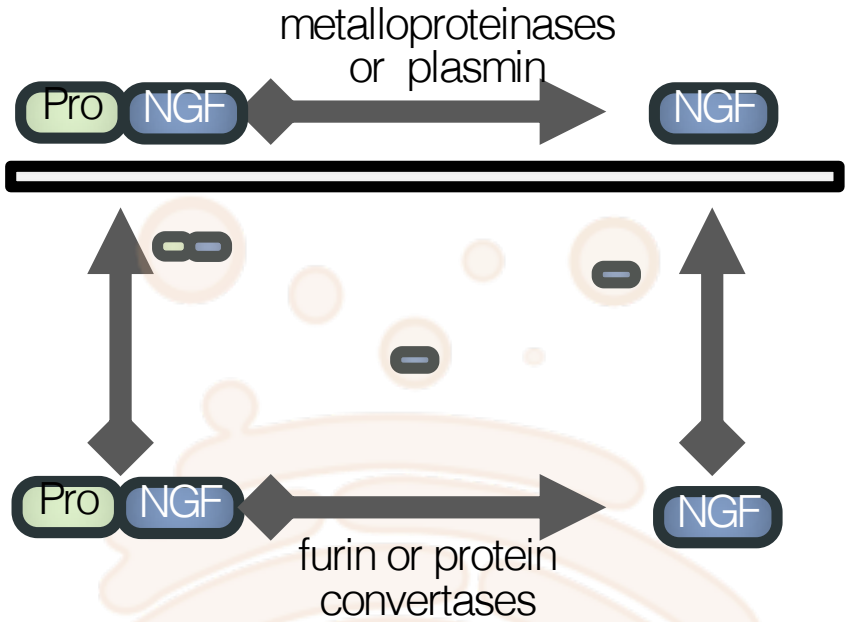
  - Receptor tyrosine kinases
  - Three family members: TrkA, TrkB, TrkC
  - Neurotrophin binding specificity with high affinity



Nat. Rev. Neuroscience **2003**, 4, 299.
Annu. Rev. Neurosci. **2001**, 24, 677.
Nat. Rev. Drug Disc. **2013**, 12, 507.

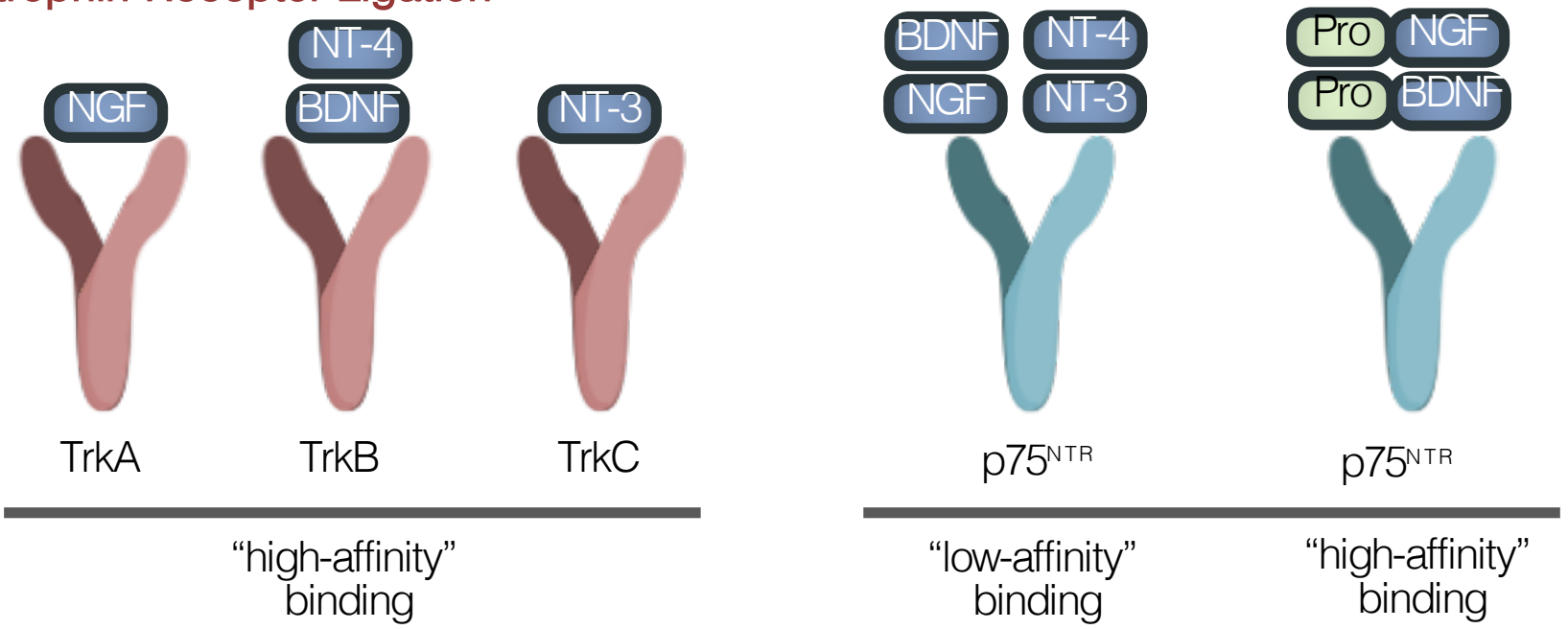
Neurotrophin Processing: Pro- and Mature-Neurotrophins

- Neurotrophins are initially synthesized as precursors (pro-neurtrophins); however, they can be cleaved to produce mature proteins.
- Intracellular pro-neurotrophins undergo three fates:
  1. Intracellular cleavage to mature protein and secretion
  2. Secretion followed by extracellular cleavage
  3. Secretion without subsequent cleavage
- Interestingly, pro-neurotrophins often have biological effects that oppose those of mature neurotrophins
- The proteolytic cleavage of pro-neurotrophins represents a mechanism that controls the direction of action of neurotrophins.



Nat. Rev. Neuroscience **2003**, 4, 299.
Annu. Rev. Neurosci. **2001**, 24, 677.
Nat. Rev. Drug Disc. **2013**, 12, 507.

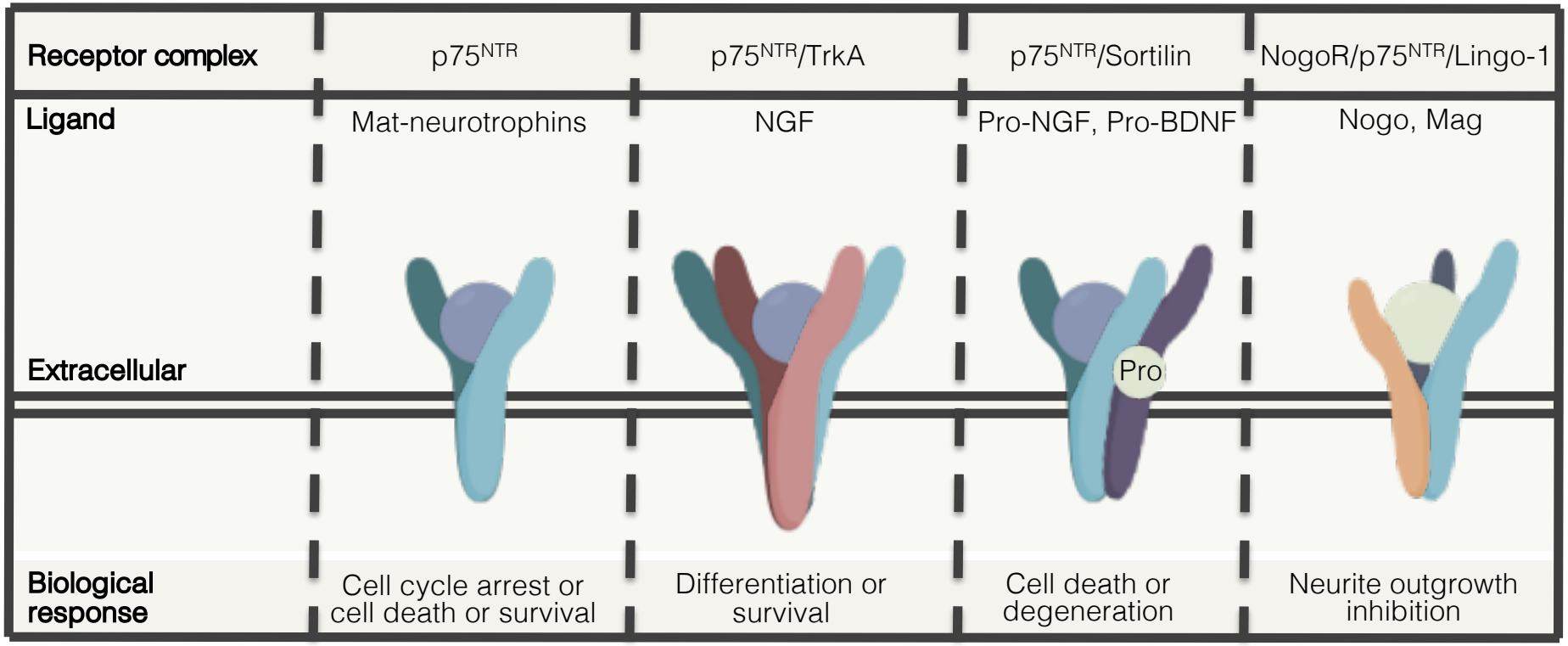
Neurotrophin-Receptor Ligation



- NGF is specific for TrkA; BDNF and NT-4 are specific for TrkB; NT-3 activates TrkC. The binding of neurotrophins induces receptor dimerization, resulting in the activation of the tyrosine kinase present in the cytoplasmic domain.
- $p75^{NTR}$ unselectively binds to all mature neurotrophins albeit with "low-affinity'.
- Contrary to early notions of neurotrophin signaling, pro-neurotrophins can serve as signaling molecules by interacting with $p75^{NTR}$ with high-affinity.
- The biological outcome depends on the cellular context of the receptors and the selective secretion of pro- or mature-neurotrophins. For example, interaction of mature neurotrophins with Trk receptors leads to cell survival, whereas binding of proNGF to $p75^{NTR}$ can lead to apoptosis.

Science **2001**, 294, 1945. Nat. *Rev. Neuroscience* **2003**, 4, 299.; *Annu. Rev. Neurosci.* **2001**, 24, 677.; *Nat. Rev. Drug Disc.* **2013**, 12, 507.

p75^{NTR} Receptor Complexes



Neuron **2007**, 53, 25.
Curr. Opin. Neurobiol. **2005**, 15, 49.
Trends in Neuroscience **2012**, 35

proNGF/p75NTR: Disease and Injury Association

Postnatal expression of $p75^{NTR}$ is downregulated in adult organisms. However, $p75^{NTR}$ can be upregulated during various pathological events.

The ratio of pro-NGF/mNGF is critical in regulating the balance between cell survival and death.

Pro-NGF has been shown to be the predominant form of this neurotrophin in human brain.

The pro-NGF and $p75^{NTR}$ is upregulatation:

Neurodegenerative Disease

- Alzheimer's disease
- Parkinson's disease

Injury

- Traumatic brain injury
- Spinal cord injury
- Chemotherapeutic and radiation induced neuropathy

Pro-NGF in Alzheimer's Disease Progression

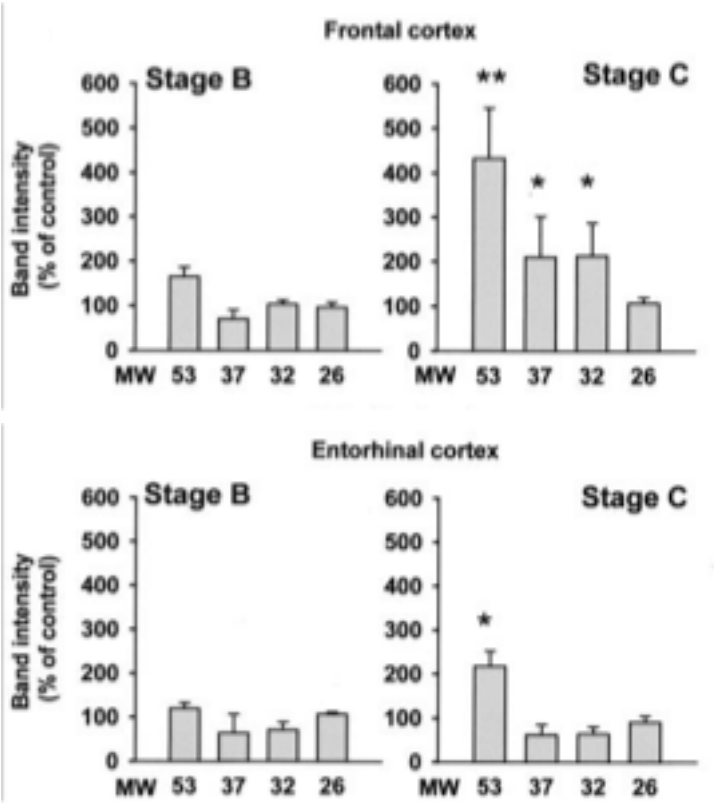
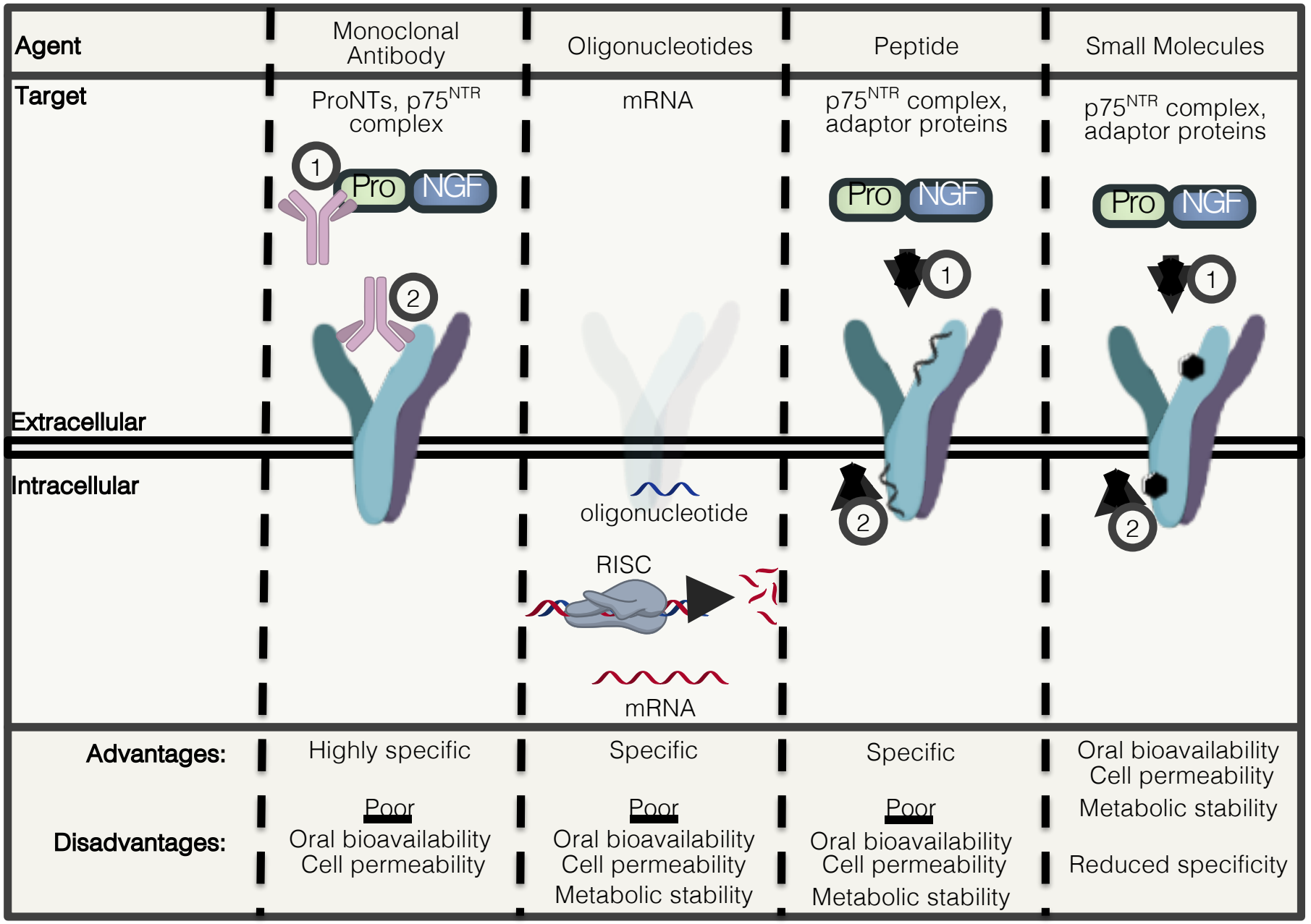


Figure from: Am. J. Pathol. **2005**, 166, 533.

Am. J. Pathol. **2005**, 166, 533.*; J. Neuropathol. Exp. Neurol.* **2004**, 63, 641.
Mol. Cell Neurosci. **2001**, 18, 210.; *Int. J. Mol. Sci.* **2013**, 14, 14085.
Neuron **2002**, 36, 375.; *PNAS.* **2004**, 101, 6226.

Presentation Outline

Therapeutic modalities

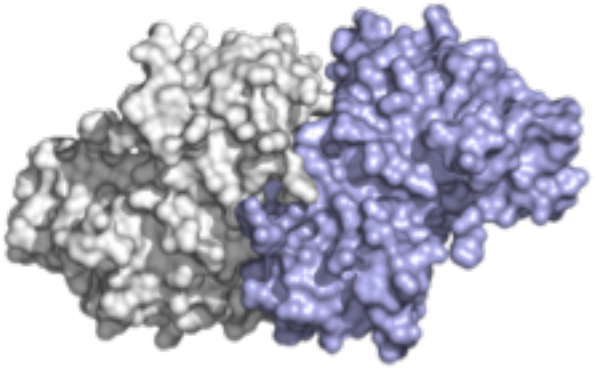


Nat. Rev. Drug Disc. **2010**, 9, 767.*;* Drug Disc. Today **2015**, 20, 1.;
Nat. Rev. Drug. Disc. **21012**, 11, 125.; *Nat. Rev. Cancer* **2006**, 9, 714.

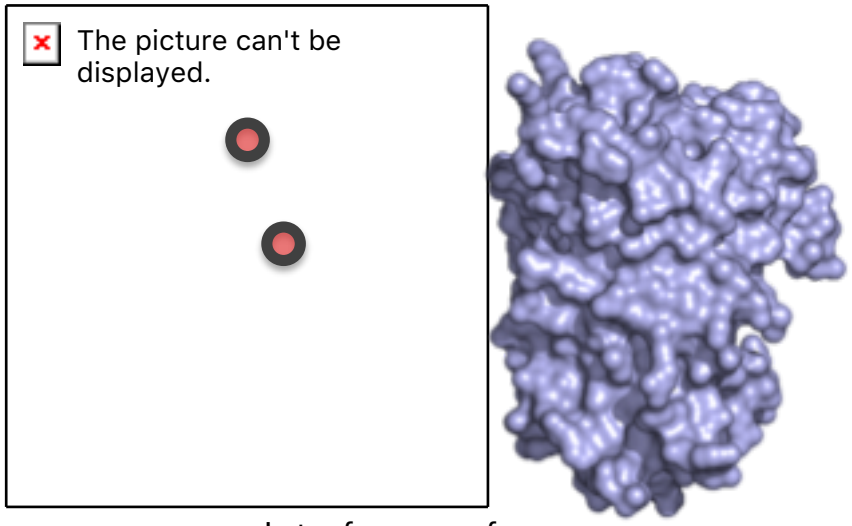
Protein-Protein Interactions (PPI)

- There is an estimated 130,000 – 650,000 types of PPI in the human interactome.
- Protein-protein interfaces tend to be flat with large surface area (1,500 – 3,000 A^2) and dominated by hydrophobic and complementary charge interactions.
- Not all residues at the protein-protein interface contribute equally toward binding. Only a small subset of contact residues contribute toward the binding free energy.
- "Hot spot" residues or regions are significantly responsible for the majority of the PPI binding free energy.
- Hot spot identification

  1. Alanine scanning mutagenesis
  2. Nuclear magnetic resonance
  3. X-ray crystallography



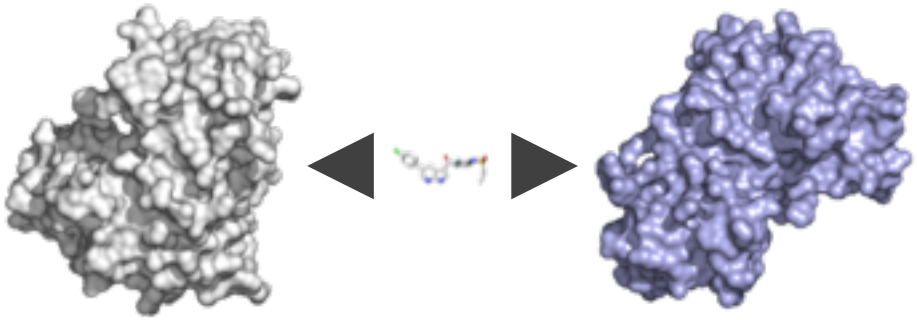
Side-view



Interface surface

Nat. Rev. Drug. Disc. **2004**, 3, 301.
Annu. Rev. Pharmacol, Toxicol. **2014**, 54, 435.
Chem. Soc. Rev. **2015**, 44, 8238.
Chem. Biol. **2014**, 9, 1102.
Bioorg. Med. Lett. **2014**, 24, 2546.

Small Molecule Protein-Protein Interactions Inhibitor (smPPII) Identification



- smPPII mechanism of Inhibition
  1. Orthosteric inhibition
  2. Allosteric regulation
  3. Interfacial binding/stabilization

- Screening strategies – discover smPPII from known compounds
  1. High throughput screening
  2. Fragment screening
  3. Virtual screening

- Designing strategies – novel assembly of chemotypes that mimic or target hot spot residues
  1. Small molecule mimetics
  2. Anchor-based PPI inhibitor design

- Synthetic strategies – expanding and/or enhancing the chemical space for smPPII screening
  1. Diversity-oriented synthesis
  2. Biology-oriented synthesis
  3. Multi-component reactions

Chem. Soc. Rev. **2015**, 44, 8238.

Presentation Outline

NGF-p75^{NTR} Molecular Interactions

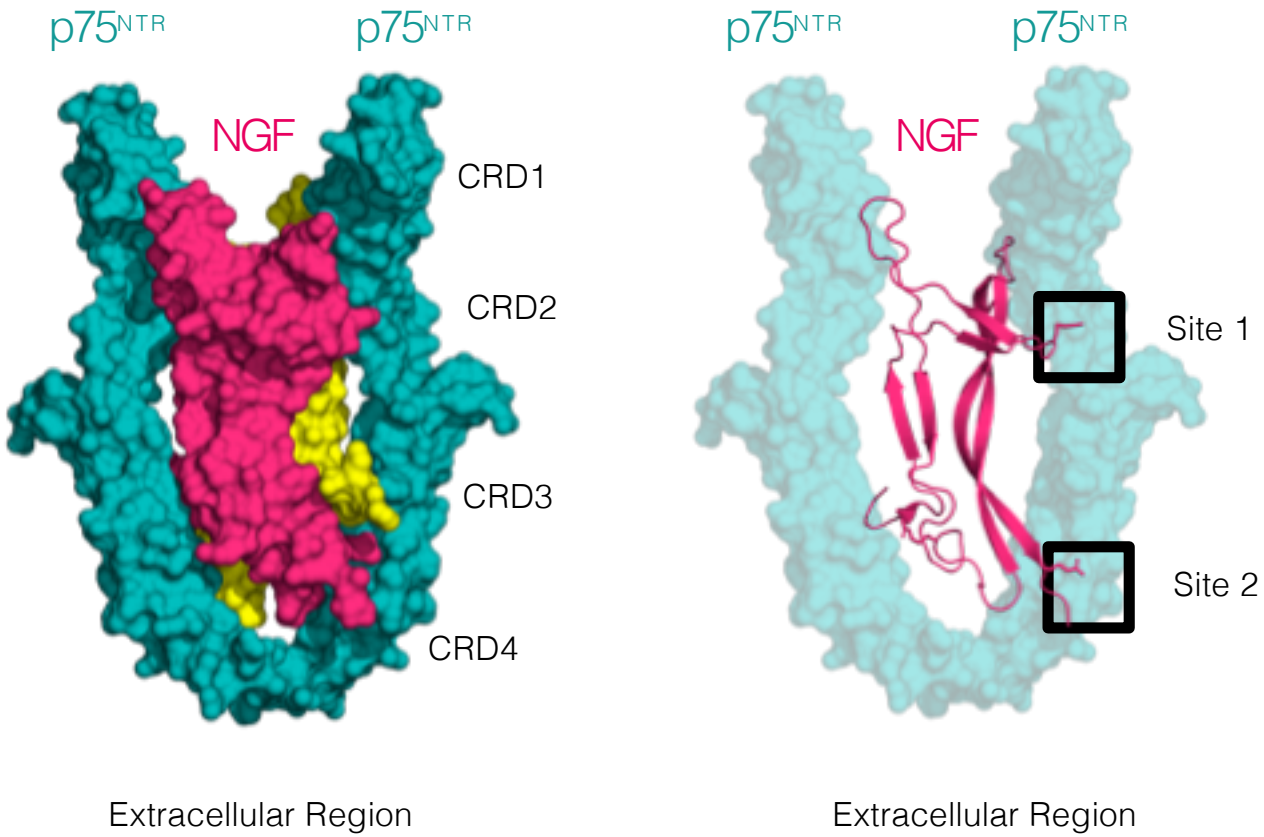


Figure. Key interactions between NGF and p75^{NTR}; PDB: 1S1G. **A.** NGF-p75^{NTR} interface at site 1. One NGF monomer is shown in red, p75^{NTR} is shown in teal, and water molecule is shown in blue. **B.** NGF-p75^{NTR} interface at site 2.

NGF-p75^{NTR} Interface: Site 1

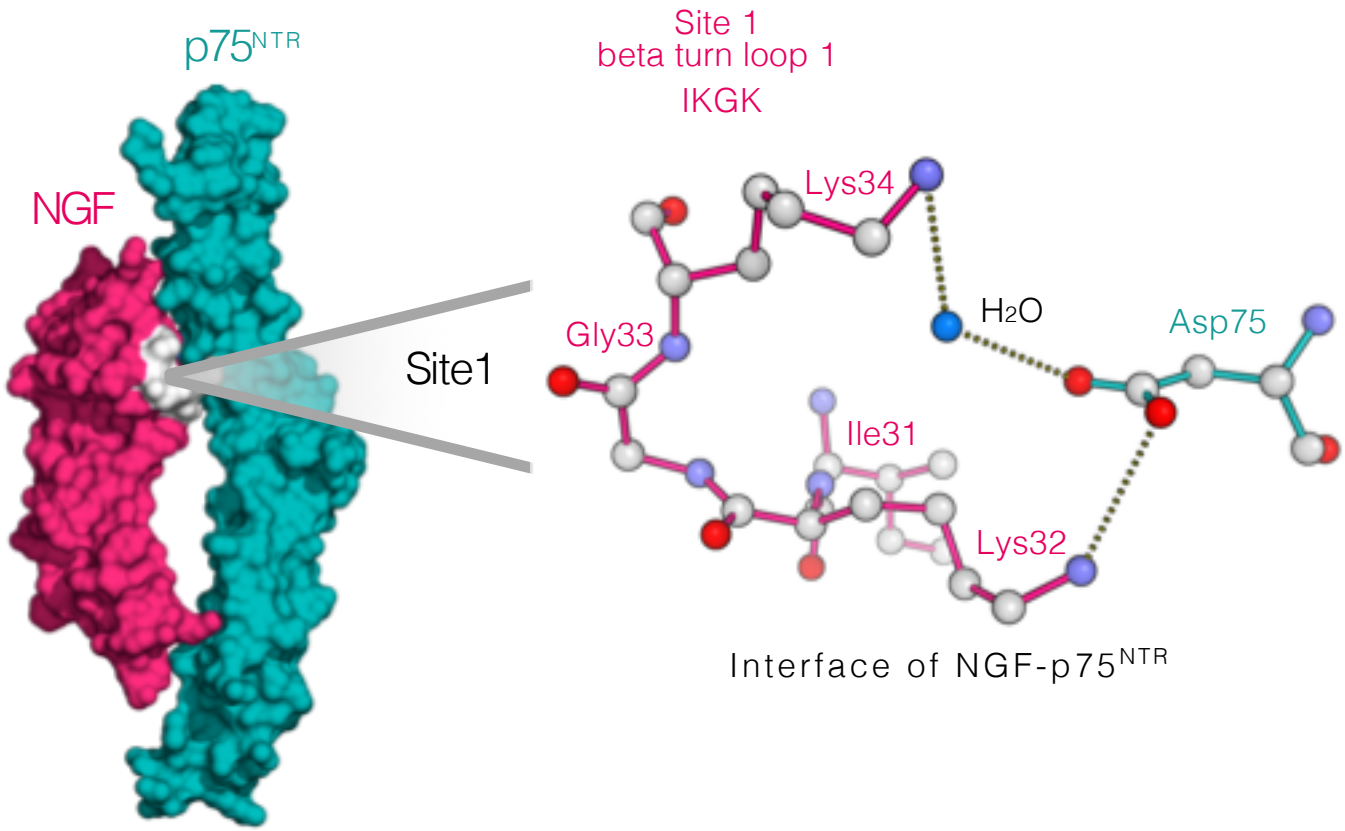
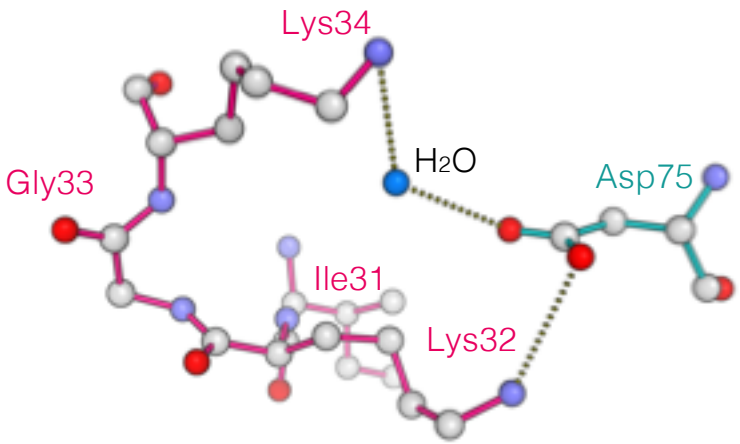


Figure. NGF beta turn loop 1 amino acids IKGK at the interface of NGF-p75^{NTR} site 1; PDB: 1S1G. One NGF monomer is shown in red, p75^{NTR} is shown in teal, and NGF beta turn loop 1 is highlighted in white. NGF beta turn loop 1 – IKGK coordinates with p75^{NTR} Asp75 with a network of hydrogen bonding with water (shown in blue), Lys34, and Lys 32.

PDB: 1SG

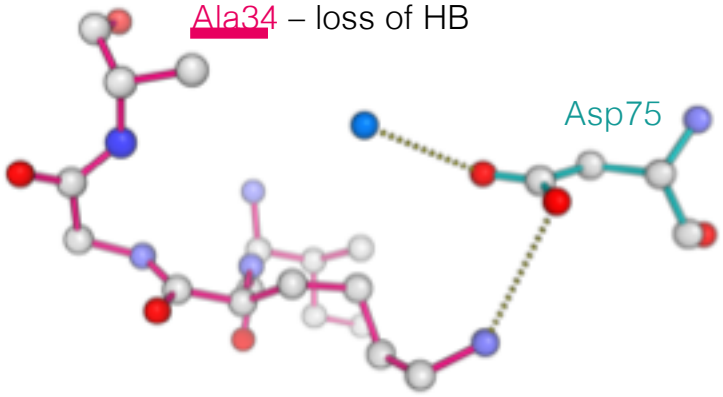
Hot spot identification – alanine scanning mutagenesis



IKGK Wild Type
100% receptor binding[1]

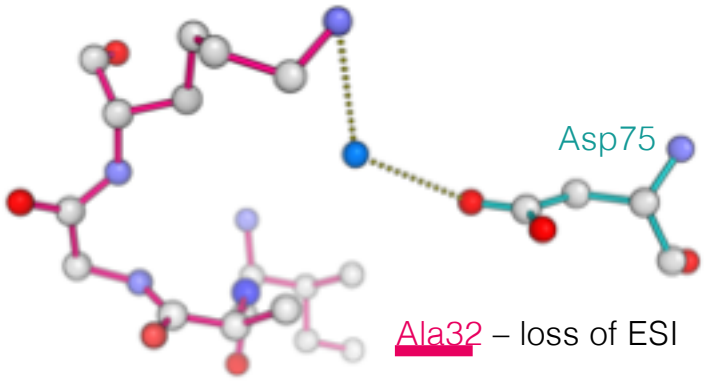
Site-directed mutagenesis

K34A Mutant



IAGK Mutant
50% receptor binding[1]

K32A Mutant



IKGA Mutant
16% receptor binding[1]

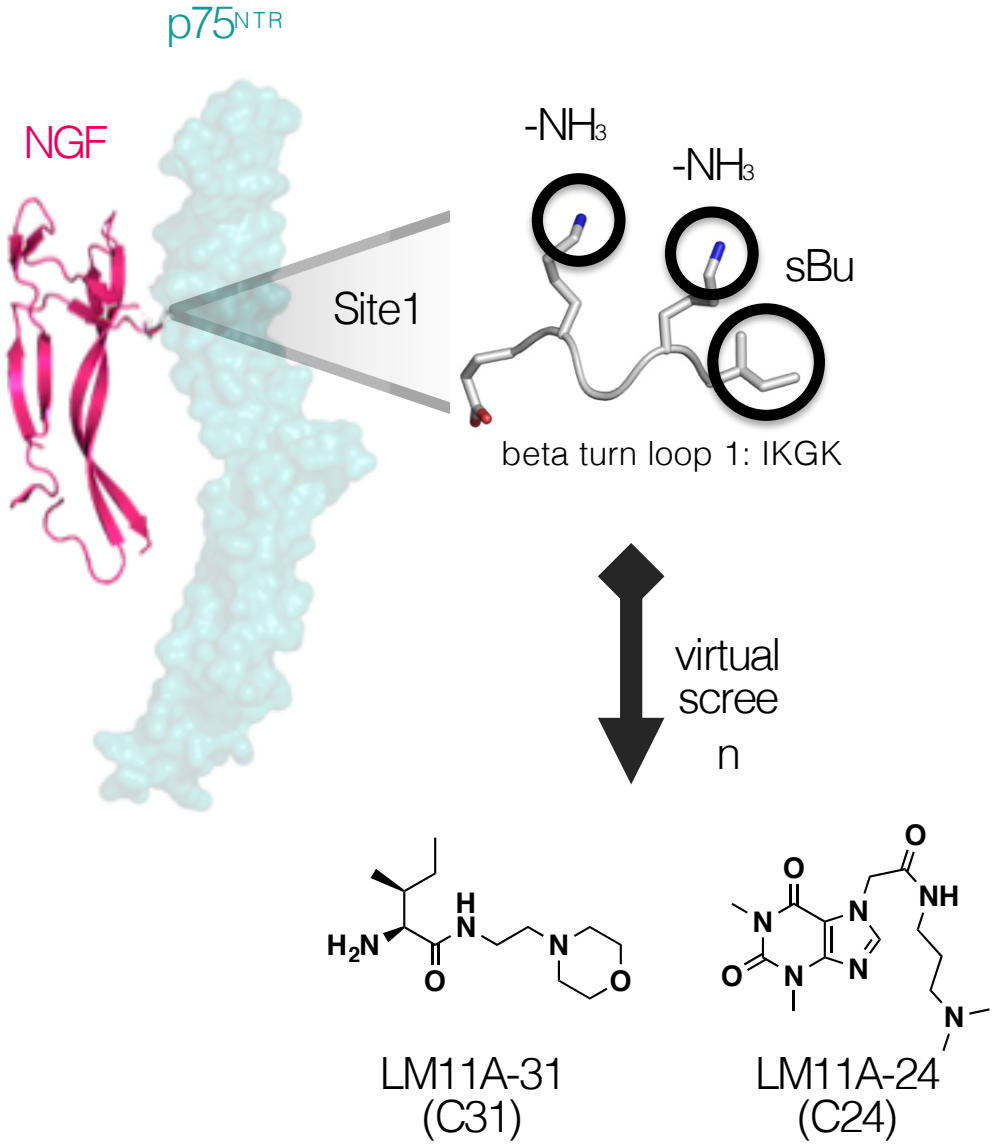
Cell **1992**, 69, 329.

LM11A-31 (C31): Discovery

- Developed by Dr. Frank Longo, MD, PhD – Professor and Chairman, Stanford Medicine Department of Neurology & Neurological Sciences

In silico and in vitro screening

- Alanine scanning mutagenesis and peptide screening studies confirmed the biological significance of NGF beta turn loop 1 for receptor binding.
- Employed X-ray crystal structure of human NGF. Pharmacophore screen of β-turn loops 1 residues IKGK.
- Virtual screening of >800,000 compounds against the chemical and structural features of β-turn loops 1
- Virtual hits were evaluated in neuronal cell culture.



J. Neur. **2006**, 26, 5288.

PDB: 1SG

LM11A-31 (C31): Background

- Biological Profile:
  - Water soluble
  - Pico - nanomolar activity in cell culture
  - Orally bioavailable: Not reported
  - Penetrates the BBB; brain half-life 3-4 h
  - No overt toxicity
  - CEREP panel screen: alternative drug targets – negative
  - AMES/hERG/CYP tests: pass

PharmatrophiX

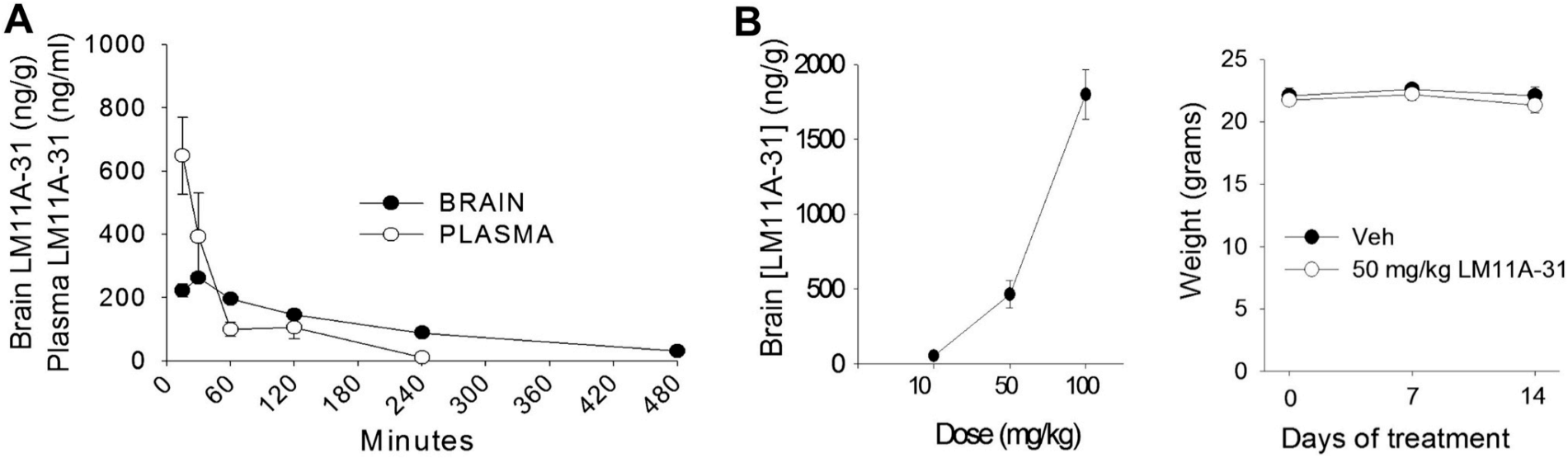
- Previous status: Clinical Trials – Phase I (Passed)
  - Safety, dosing, and pharmacokinetic profile
    Tested on 68 healthy subjects
    No significant drug-related adverse events
    Maximum tolerated dose (MTD): 2000 mg/Kg

- Current status: Clinical Trials – Phase 2a
  - Focus: Patients with mild to moderate
  - Endpoints: Delay onset/inhibit progression of AD
  - Sponsor: Alzheimer's Drug Discovery Foundation
    Provided $500,000

- LM11A-31 and its biological impact were featured on the cover of Time magazine.

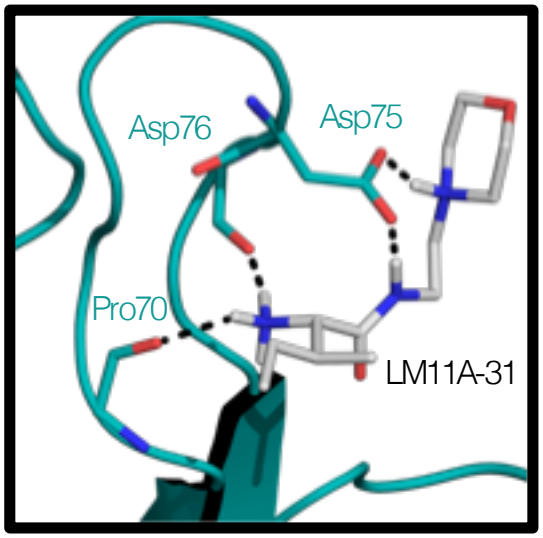
Neurobiology of Aging **2013**, 34, 2052.
Time (cover story): "Alzheimer's from a New Angle," published February 2016

LM11A-31 (C31): Basic pharmacokinetic profile

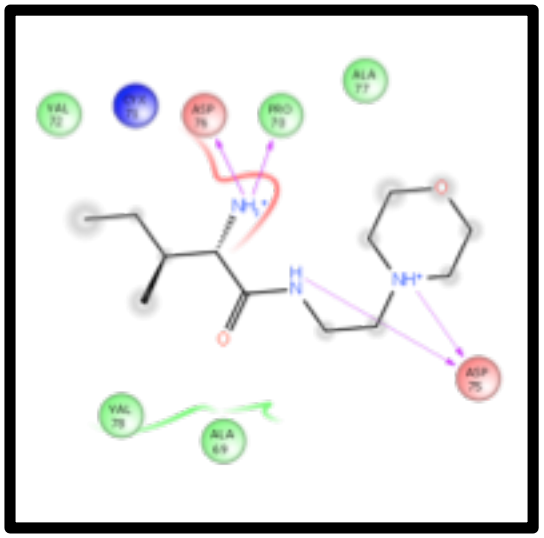


Neurobiology of Aging **2013**, 34, 2052.

Molecular docking: Putative binding mode of p75^{NTR}-bound LM11A-31 at Site 1



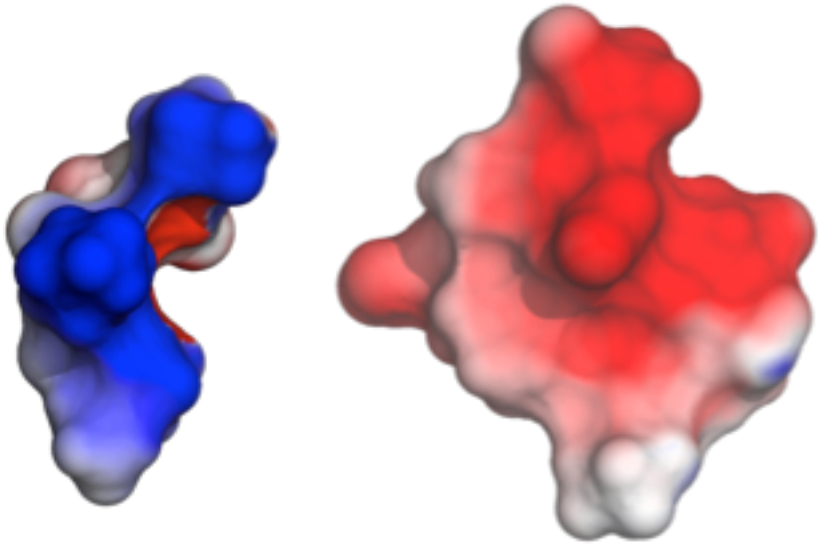
3D - model



2D - model

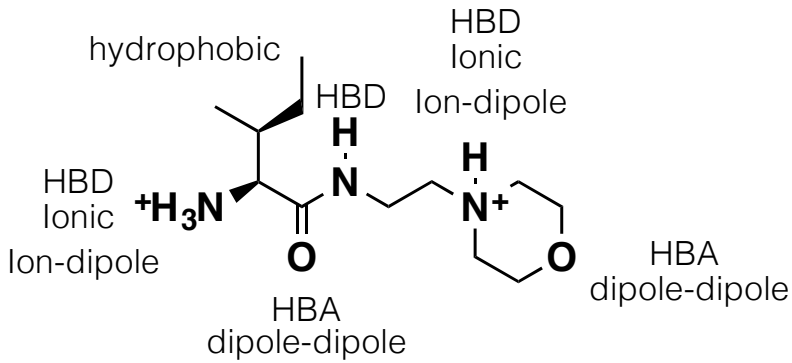
Figure. Putative binding mode of LM11A-31 at site 1 of p75^{NTR}

LM11A-31 Analysis: potential intermolecular interactions



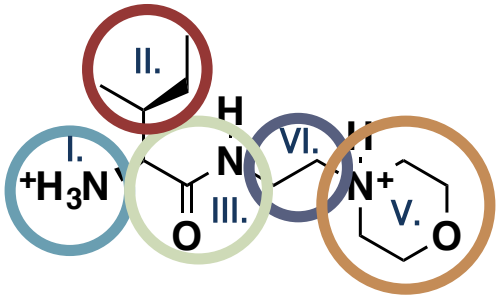
NGF p75^{NTR}

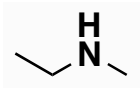
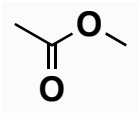
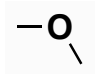
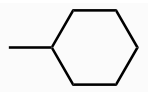
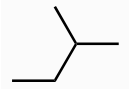
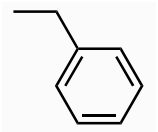
Electrostatic potential map
Interface residues at Site 1



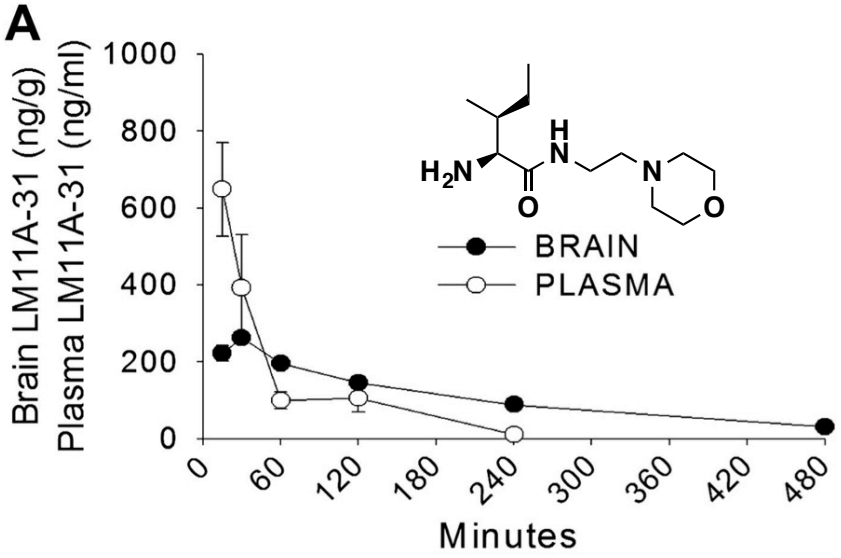
Molecular analysis of LM11A-31

Focused structure-activity relationship (SAR) of LM11A-31



Zone I	Zone II	Zone III	Zone IV	Zone V
—H	—H		n = 3	
—Br	—Me			
				
				
				

LM11A-31 (C31): Basic pharmacokinetic profile



Goals

- Develop novel $p75^{NTR}$ inhibitors
- Conserve potency
- Modify physiochemical properties

  Solubility
  Permeability
  Chemical stability

- Improve pharmacokinetic profile

  Improve percent bioavailability (%F)
  Exposure (AUC)
  Half-life ($t_{1/2}$)
  Time of maximum drug concentration

Neurobiology of Aging **2013**, 34, 2052.

Closing Remark

"Medicinal chemists today live in exciting times. They are key participants in the effort to produce more selective, more effective and safer medicines to treat the diseases of mankind. Their work can have a beneficial effect on millions of suffering patients — surely an important motivating factor for any scientist."

Nature Rev Drug Disc. **2004,** 3, 853.

Acknowledgements

Dr. Peter Wipf, PhD
Wipf group members

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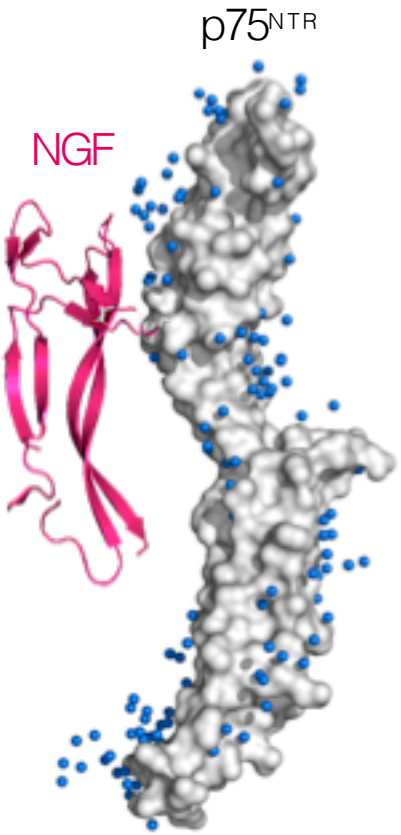
Dr. Anthony Kanai, PhD
Dr. Irina Zabbarova, PhD

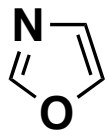
Funding
Funding agencies
-American taxpayers

Back-up Slides

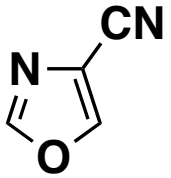
p75^{NTR}- NGF Interface: water molecules



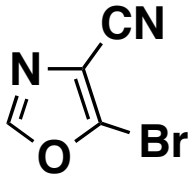
NMR Analysis



2: 7.95, 150.6 ppm
4: 7.09, 125.4 ppm
5: 7.69, 138.1 ppm

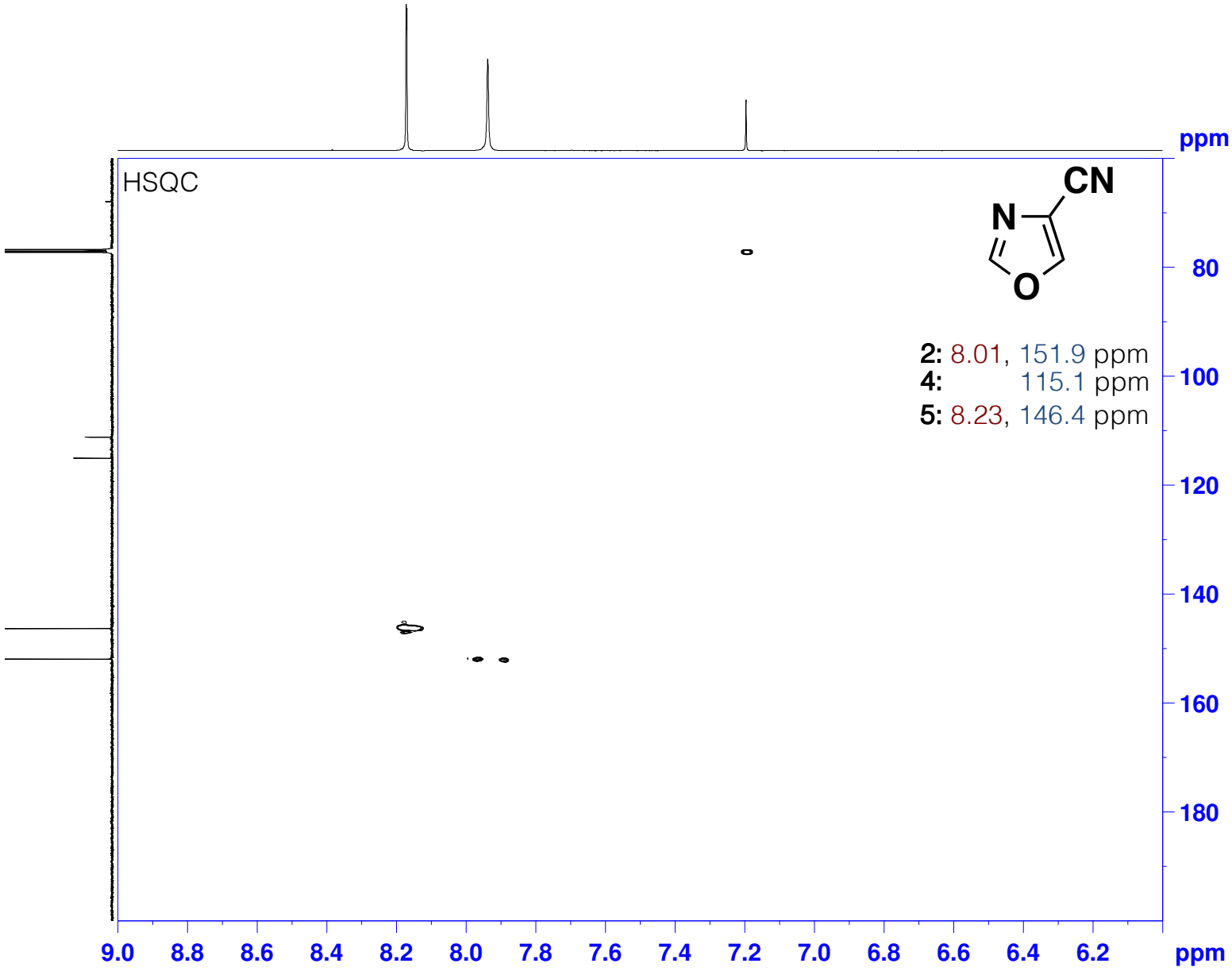


2: 8.01, 151.9 ppm
4: 115.1 ppm
5: 8.23, 146.4 ppm

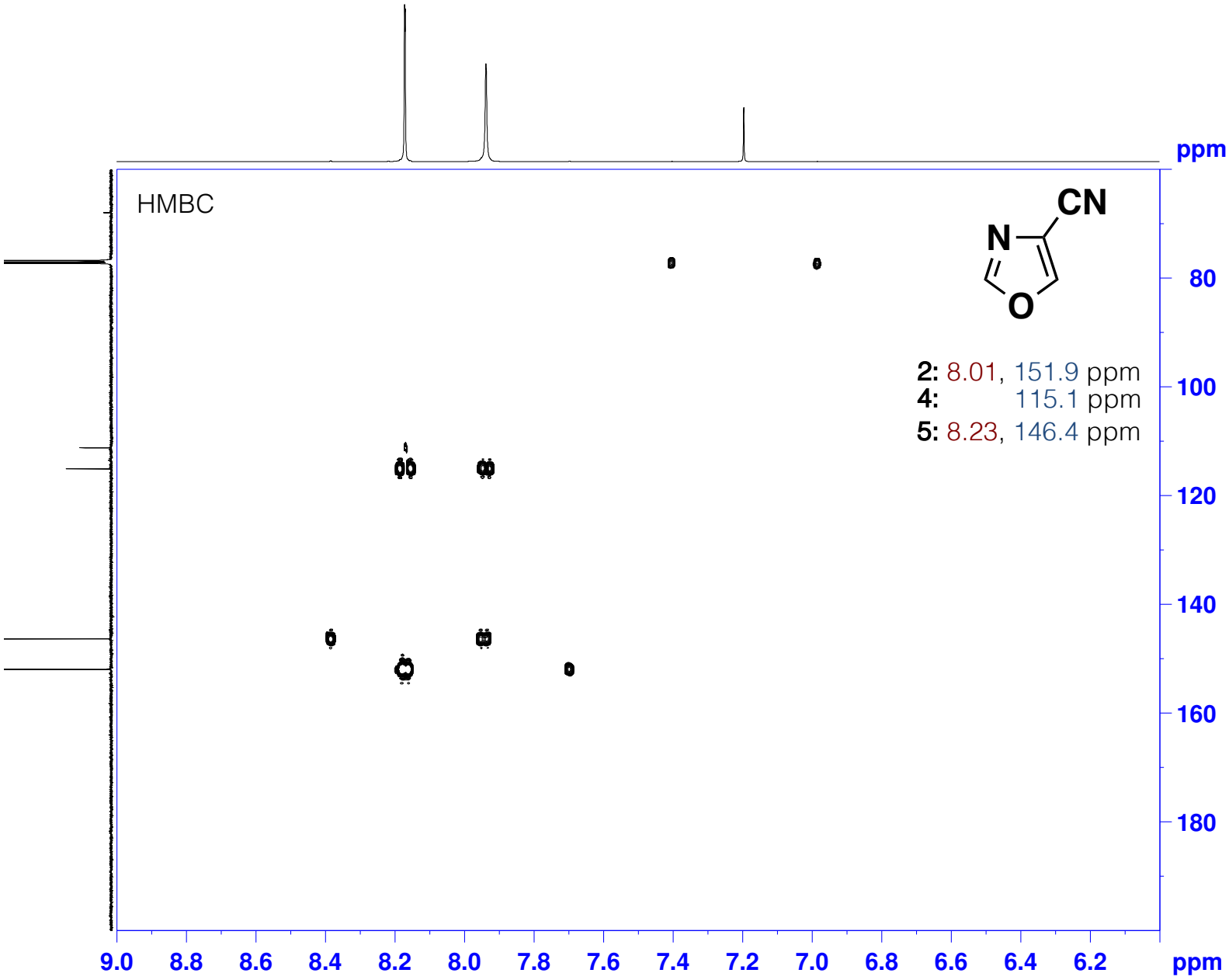


2: 8.21, 149.3 ppm
4: 117.6 ppm
5: 136.0 ppm

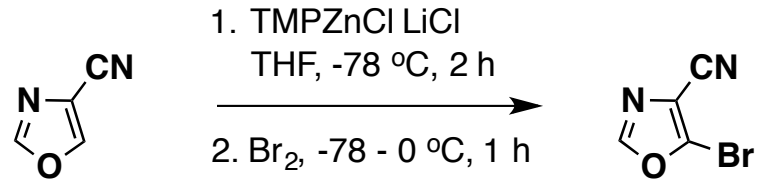
NMR Analysis

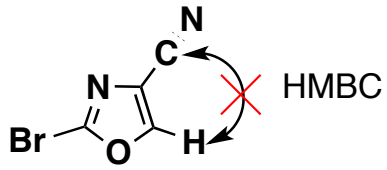


NMR Analysis



Oxazole Analog series



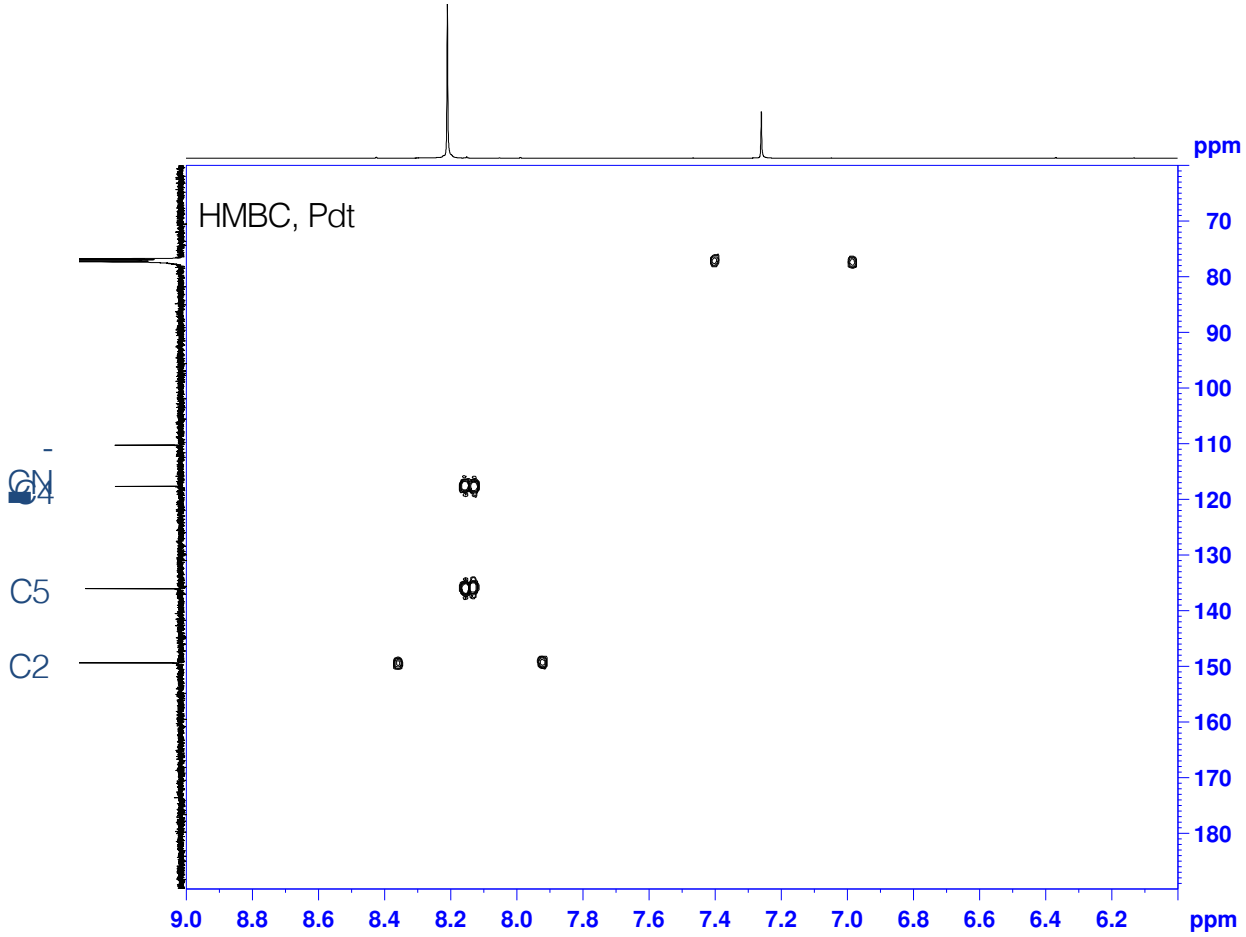


H^1 NMR, C^{13} NMR

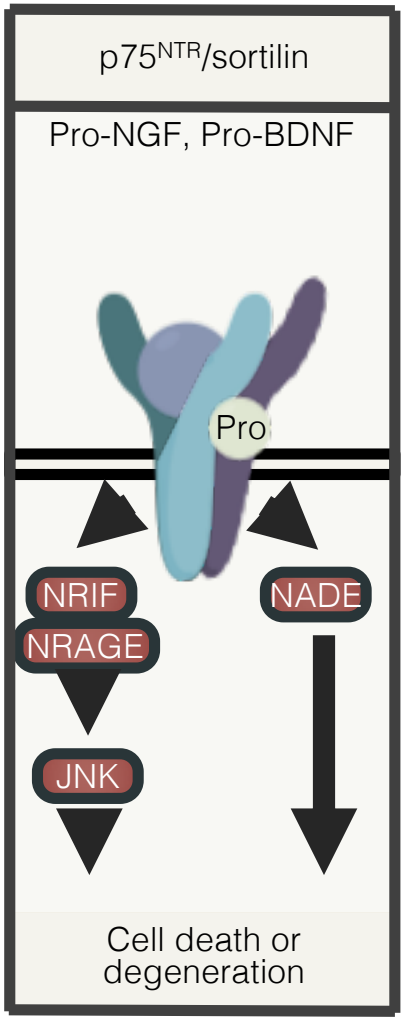
2: 8.01, 151.9 ppm
4: 115.1 ppm
5: 8.23, 146.4 ppm

H^1 NMR, C^{13} NMR

2: 8.21, 149.3 ppm
4: 117.6 ppm
5: 136.0 ppm



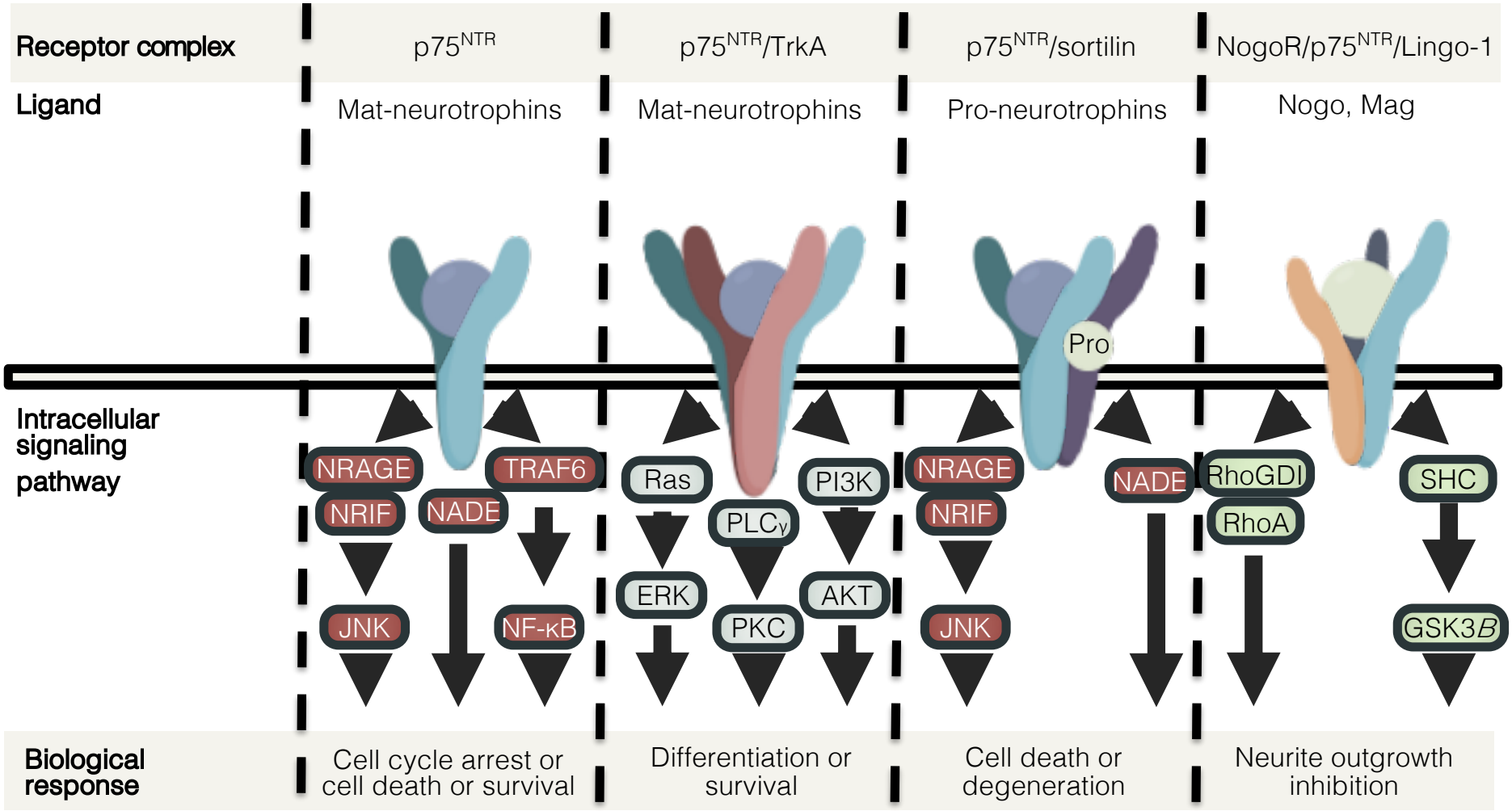
proNT/p75^{NTR}/Sortilin Complex: Cellular Signaling



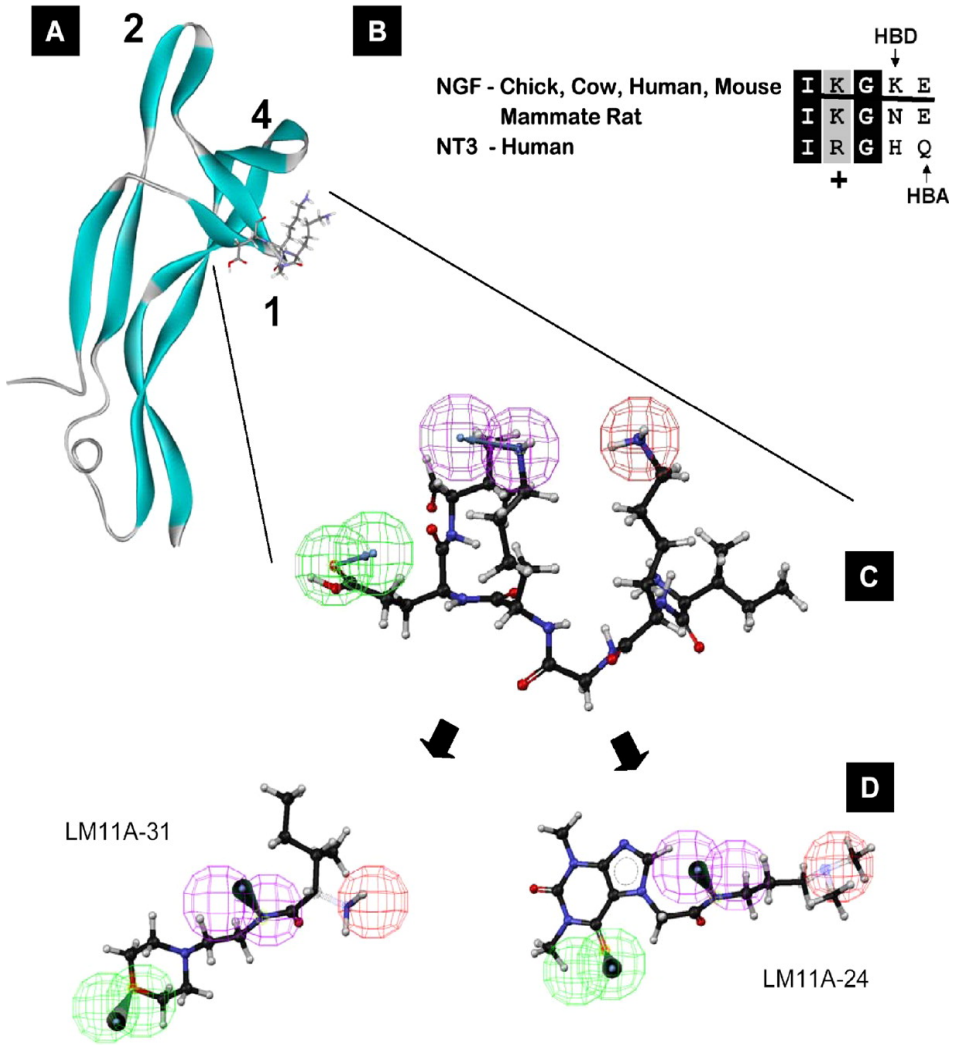
- Sortilin specifically binds the pro-domain of NGF or BDNF and serves as a co-receptor with p75^{NTR} in mediating cell death
- NRIF is a widely expressed Zn-finger-containing protein that interacts with both the juxtamembrane and death domains of p75NTR.
- NRAGE prevents the association of p75^{NTR} with TrkA, and overexpression of NRAGE promotes NGF-stimulated, p75NTR-dependent cell cycle arrest and death by activating JNK and caspase 3.
- The p75^{NTR} associated cell death executor (NADE) has been reported to bind to the p75^{NTR} death domain and induce caspase activation and death within primary cortical neurons.
- Continued efforts are required to fully characterize the complex expression patterns and signaling mechanisms in neurons.

Nature **2004**, 427, 843.
PNAS. **2010**, 107, 3817.
Trends in Neuroscience **2012**, 35

p75^{NTR} Receptor complex

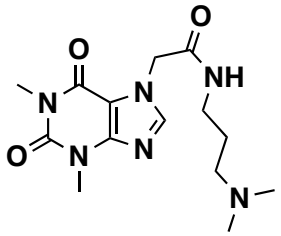


LMA11-31 (C31) Discovery

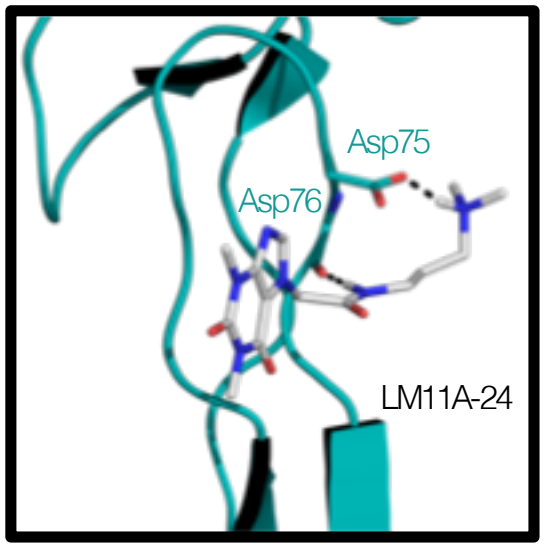


S. M. Massa *et. al. J. Neur.* **2006**, 26

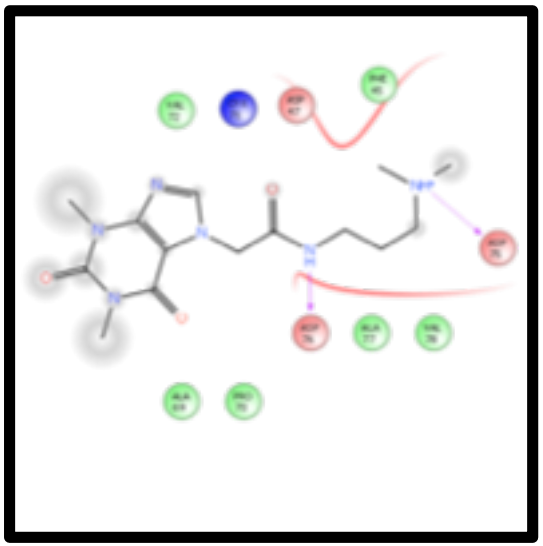
Molecular docking of LM11A-24



LM11A-24



3D - model



2D - model

Figure. Putative binding mode of LM11A-24 at site 1 of $p75^{NTR}$

p75NTR- NGF Interface