The Crystal Structures of TrkA and TrkB Suggest Key Regions for Achieving Selective Inhibition

Thomas Bertrand – PSDI 2012
**Trk** (tropomyosin related kinases*) family:

3 receptor tyrosine kinases

**TrkA, TrkB, and TrkC**

MW 140-145 kD

LLR: Leucine-rich motif
C: Cystein cluster
Ig: Immunoglobulin-like domain

~70% aa identity in Trks kinase domain

---

* name derived from oncogene, isolated from a human colon carcinoma, activated by a somatic rearrangement that fused a non-muscle tropomyosin gene to the kinase domain of a novel tyrosine kinase receptor. Proto-oncogene was named tropomyosin-related kinase (Trk), now commonly referred as TrkA
Rationale in CNS disorders and Oncology

**CNS disorders:**

- **TrkA agonists**
  - Alzheimer
  - Peripheral neuropathies
  - …

- **TrkB agonists**
  - Alzheimer
  - Parkinson
  - …

- **TrkB antagonists**
  - Epilepsy
  - Peripheral neuropathies
  - Motoneuron diseases
  - Neuropathic pain
  - Chronic inflammatory pain
  - …

**Oncology:**

- **TrkA inhibitors**
  - Papillary thyroid carcinomas
  - AML
  - Neuroblastoma
  - Breast
  - Pancreas
  - Prostate
  - Skin
  - Lung
  - Ovary
  - …

- **TrkB inhibitors**
  - Neuroblastoma
  - Liver
  - Pancreas
  - Prostate
  - Ovary
  - …

- **TrkC inhibitors**
  - Breast
  - Kidney
  - …
Trks are activated by **neurotrophins** (secreted ~26 kDa homodimer proteins)

- **NGF**  
  Nerve Growth Factor

- **BDNF**  
  Brain-Derived Neurotrophic Factor

- **NT-3**  
  Neurotrophin-3

- **NT-4/5**  
  Neurotrophin-4/5

NGF is the preferred ligand for **TrkA**  
BDNF & NT-4/5 are preferred for **TrkB**  
NT-3 is the ligand of **TrkC**

“difficult” druggability  
High selectivity

“easy” druggability  
Low selectivity

Very different neurotrophins

Very different effects
Trk extracellular domains

Wiesmann et al. Nature 1999

Ultsch et al. JMB 1999
Trk intracellular (kinase) domain

TrkA, TrkB, and TrkC

Close relationship with MuSK, InsR and IGF-1R (structures known)

<table>
<thead>
<tr>
<th></th>
<th>InsR</th>
<th>IGF1R</th>
<th>MuSK</th>
<th>TrkA</th>
<th>TrkB</th>
</tr>
</thead>
<tbody>
<tr>
<td>InsR</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF1R</td>
<td>55.4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MuSK</td>
<td>39.2</td>
<td>41.7</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TrkA</td>
<td>39.1</td>
<td>36.8</td>
<td>45.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>TrkB</td>
<td>37.6</td>
<td>38.1</td>
<td>41.6</td>
<td>75.3</td>
<td>100</td>
</tr>
</tbody>
</table>

(% identity – kinase domain)
TrkA and TrkB apo crystal structures

- **His-TEV-TrkA-497-795**
  - PEG3350
  - Na Citrate 0.1M pH 5.6 at 4°C
  - P4_{1,2,2} – 2.3Å – 2 mol/ASU (4F0I)

- **His-TEV-TrkB-543-838**
  - Na Formate pH 8.0 at 4°C
  - P2_{1,2,2} – 1.7Å – 1 mol/ASU (4ASZ)

Apo-TrkA and TrkB are in an inactive DFG-out conformation.

Apo-TrkA Activation segment is different between the 2 mol/ASU but is restrained by crystal packing.
Comparing TrkA and TrkB apo crystal structures

- Apo-TrkA and apo-TrkB are very similar but the packing of TrkA involves at least:
  - The KID region
  - The activation loop with a blocked DFG motif in the ATP binding site
- The packing of apo-TrkA was unfavorable to soaking experiments
- Several unsuccessful co-crystallization attempts were tried

→ TrkB was used as a platform for structural studies
TrkB apo crystal structure – typical kinase fold

- Partly unstructured P-loop
- DFG-out blocking ATP site
- Fully visible Activation loop with dephosphorylated Tyrosine residues

(a)

N-lobe

- Hinge Region
- Partly unstructured KID

C-lobe
TrkB in complex with GW2580

- GW2580 was reported to be TrkB selective versus TrkA (17.5 fold)
- In-house data suggest less selectivity (4 fold)

<table>
<thead>
<tr>
<th></th>
<th>GW2580</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kd (nM)</td>
<td></td>
</tr>
<tr>
<td>TrkA</td>
<td>630</td>
</tr>
<tr>
<td>TrkB</td>
<td>36</td>
</tr>
<tr>
<td>TrkC</td>
<td>120</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IC₅₀ (µM)</th>
<th>GW2580</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrkA</td>
<td>0.162</td>
</tr>
<tr>
<td>TrkB</td>
<td>0.038</td>
</tr>
<tr>
<td>TrkC</td>
<td>0.041</td>
</tr>
</tbody>
</table>

P2₁,₂,₂ – 1.7Å – 1 mol/ASU (4AT5)

DFG-out inhibitor

Interacting residues are conserved between TrkA and TrkB
Where to find selectivity handles within Trks?

- Compared to apo-TrkB, TrkB-GW2580 has the hinge tip pushed back to accommodate the diaminopyrimidine moiety of the ligand
  - might not be possible in TrkA due to the presence of the KID loop restricting hinge conformations

2 possible handles to selectivity:

1. Hinge constraints in TrkA
2. KID direct interactions in TrkA

Yellow/Bronze = Apo TrkA
Green/Purple = TrkB-GW2580
(1) Hinge constraints in TrkA?


- **4GT5 (H32 – 1 mol/ASU)**
  - DFG-out
  - Hinge Cα trace has identical path to 4F0I
  - KID partially visible (involved in packing) folding back towards the hinge region similarly to 4F0I

- **4AOJ (I222 – 3 mol/ASU)**
  - DFG-in inhibitor
  - Hinge Cα trace has different path from 4F0I
  - KID not visible (not involved in packing)
    - Hinge free of any KID constrains
  - TrkA can adopt an unrestrained hinge structure
(2) Interacting with the Kinase Insert Domain?

Already proposed in IRAK-4 kinase

“The generally low sequence conservation in this front pocket offers an appealing opportunity for the design of selective inhibitors for IRAK-4.”

Conclusions

- Structures of the kinase domain of TrkA and TrkB confirms that selectivity towards Trk isoforms is not straightforward
  - Different hinge Cα trace of TrkB apo or liganded structures while apo TrkA has its hinge restrained by KID suggested one handle to selectivity

...BUT...

- New crystal structures of TrkA suggest hinge could also be flexible as in TrkB

...HOWEVER...

- The observed hinge plasticity could (still) be of interest for selectivity
- The KID might (still) be used for achieving selectivity as already proposed for IRAK-4
**Acknowledgements**

<table>
<thead>
<tr>
<th>LGCR Sanofi</th>
<th>Oncology Sanofi</th>
<th>CNS Sanofi</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. Bertrand</td>
<td>H. Bonnevaux</td>
<td>H. Bester (now</td>
</tr>
<tr>
<td>A. Dupuy</td>
<td>C. Delaisi</td>
<td>Pharmacovigilance)</td>
</tr>
<tr>
<td>A. Lebrun</td>
<td>C. Delorme</td>
<td>S. Boularand</td>
</tr>
<tr>
<td>M. Mathieu</td>
<td>E. Parmentier</td>
<td>M-C. Burgevin</td>
</tr>
<tr>
<td>A. Rak</td>
<td>B. Ronan</td>
<td>G. Dargazanli</td>
</tr>
<tr>
<td>C. Souaille</td>
<td>L. Schio</td>
<td>N. Mahmudi</td>
</tr>
<tr>
<td>G. Touyer</td>
<td>F. Viviani</td>
<td>M-A. N’Zoutani</td>
</tr>
</tbody>
</table>

**Biologics Sanofi**

| P.F. Berne           | H-P. Biemann                |
| J-Y. Crenne          | S. Davis                    |
| C. Faure             | T. Gladysheva               |
| N. Lounis            | M. Kothe                    |
| C. Valtre            | J. Lillie                   |

**Genzyme**

| J. Liu               |

...and all those I might have forgotten...