The Effects of Zn2+ on the Binding of the BDNF Prodomain with SorCS2 on Neuronal Function and Structure: Implications for Learning and Memory

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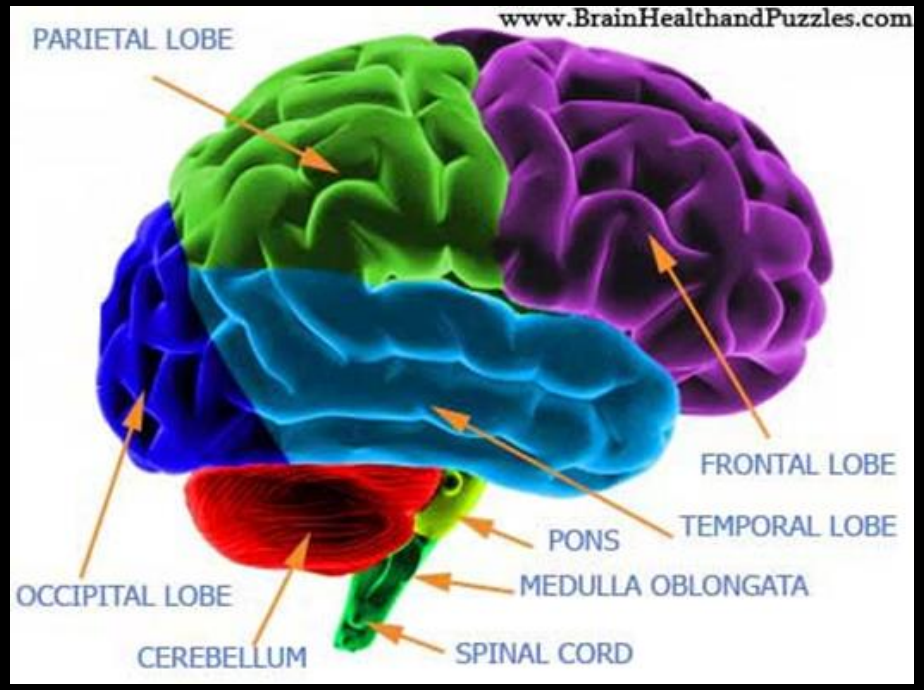
Introduction

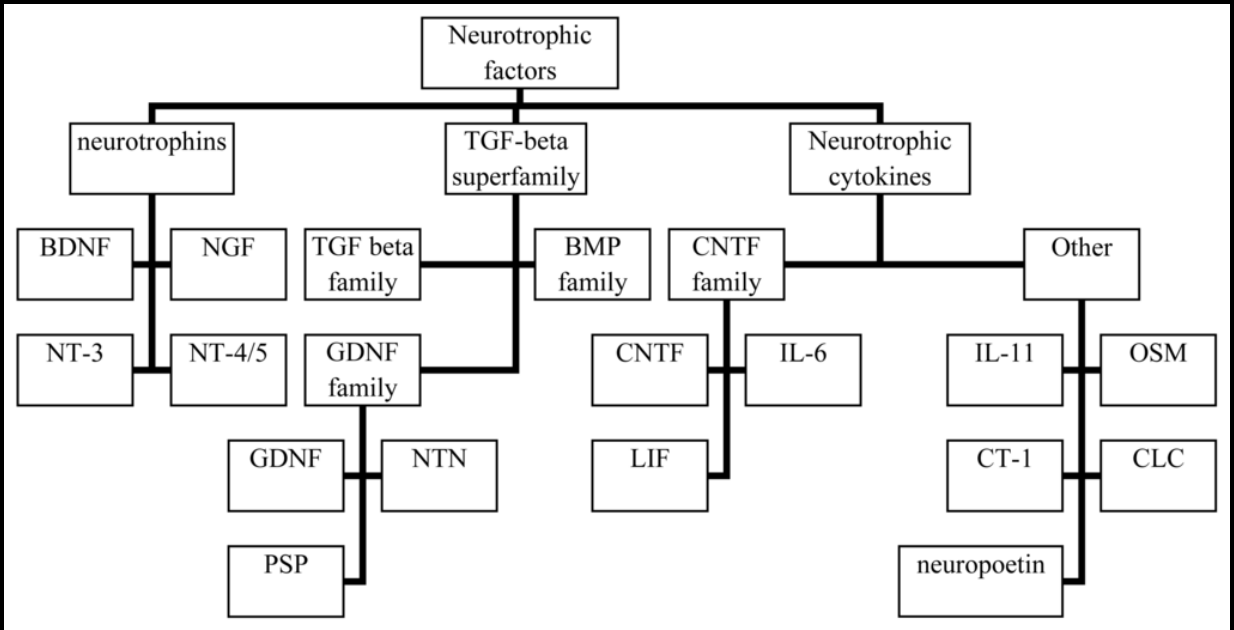
- Neurons -> Brain
- Hippocampus = Control Center
- Neural connections -> Brain-Derived Neurotrophic Factor (BDNF)



Ligands

- Regulate cell proliferation, differentiation
- Axon and dendrite growth, synaptogenesis, and synaptic function and plasticity

(Reichardt, 2006, Lu, B, Pang, PT, Woo. N.H., 2005, Minichiello L, 2009)



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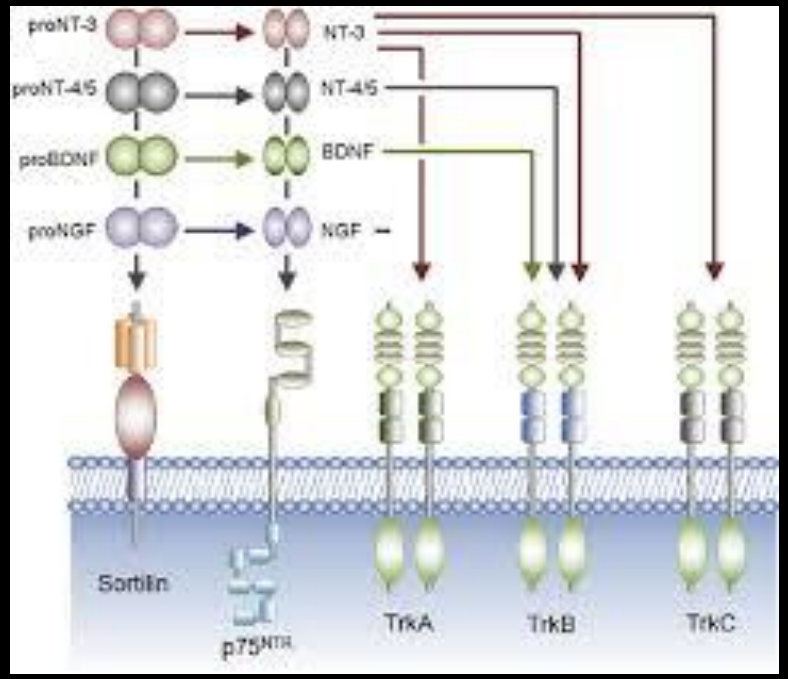
Pro-Neurotrophins

- Proteolytically cleaved in trans-Golgi by furin or in secretory granules by pro-protein contervases]
- Extracellular cleavages created in mature domain formation show how to control synaptic functions of neurotrophins (Lu, 2003)
- proBDNF regulates hippocampal structure, synaptic transmission, and plasticity (Yang et al., 2014)
- Induce apoptotic signaling (Nykjaer et al. 2004; Teng et al. 2005; Jansen et al. 2007; Willnow et al. 2008; Yano et al. 2009)



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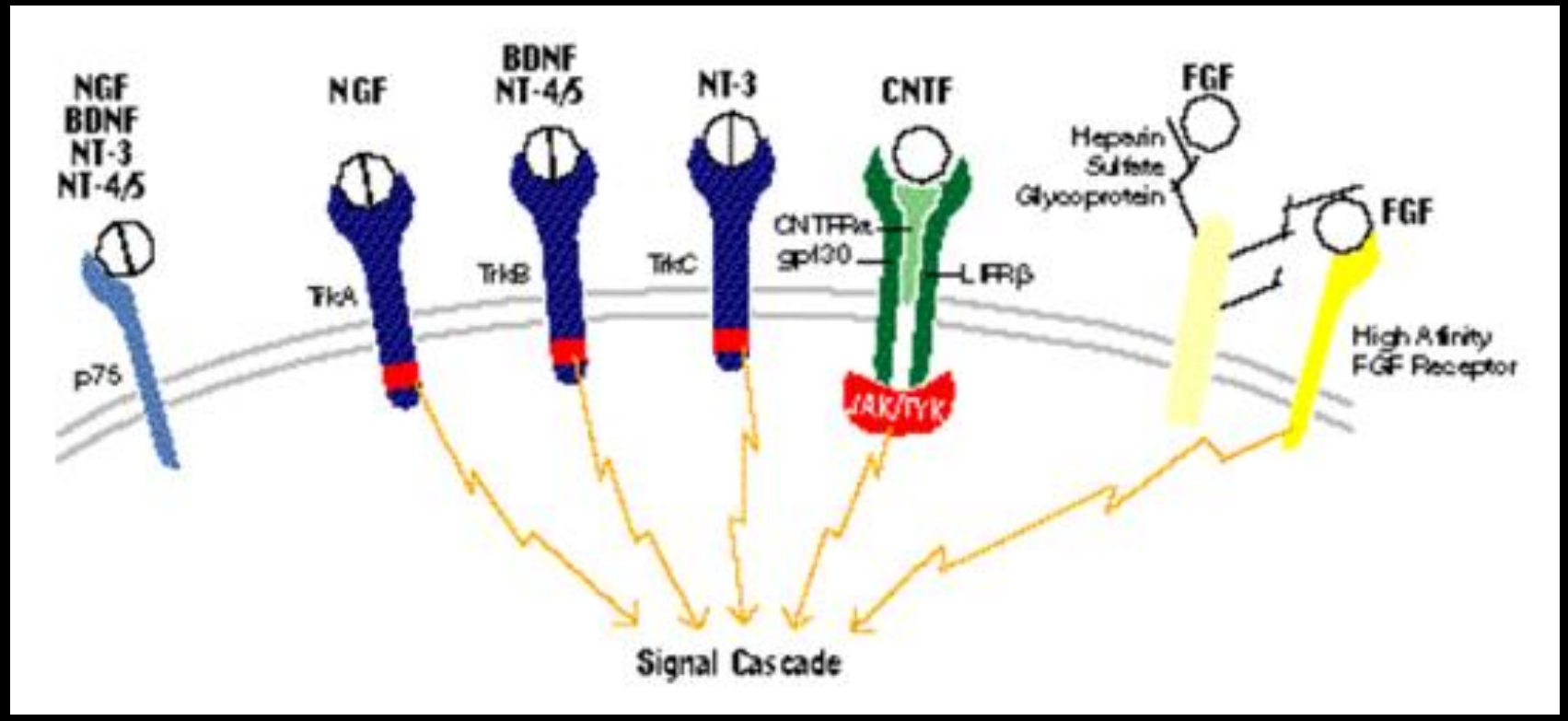
Mature-Neurotrophins

- Formation of mature neurotrophins as a result of the removal of the “pro” region (Seidah el al., 1996)



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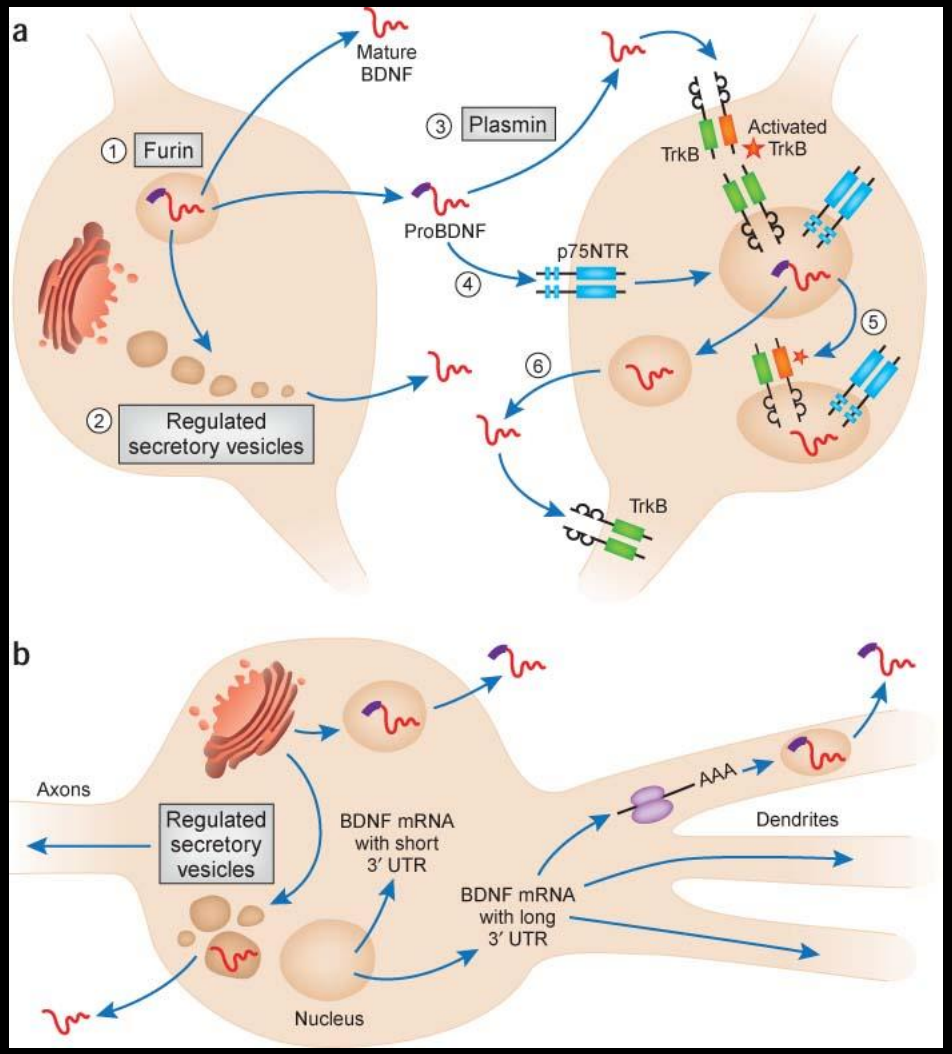
BDNF

- BDNF and NT-4/5 “functionally redundant” (Chao 2003)
- Blood and plasma BDNF levels reflect brain-tissue BDNF levels (Klein et al, 2010)
- Secreted in an activity and calcium ion dependent matter
- Controlling synaptic transmission and long-term synaptic plasticity (Lu, 2003)



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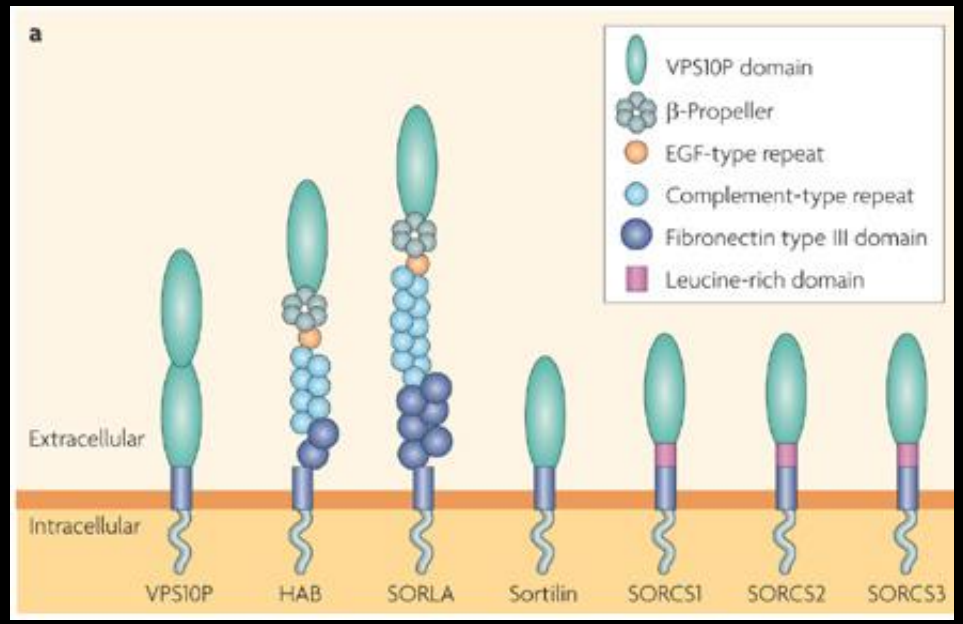
Alterations in Genes

- Genes promote protein production
- Single Nucleotide Polymorphism (SNP) of BDNF -> Val66Met substitution in pro domain region
- Linked with depression, memory alterations, and anxiety disorders
- Val66Met substitution induces structural changes (Anastasia et al., 2013)

SorCS2

- Receptor and gene found within the nervous system
- Member of the sortilin family, this receptor binds with a variety of ligands to mediate neuronal activities.
- Neurotrophin receptor targeting the pro-domain of proNGF with high affinity (Nykjaer, et al, 2004)
- Deficiency does not affect developmentally regulated apoptosis of sympathetic neurons, or prevent age-dependent degeneration (Jansen, et al., 2007)



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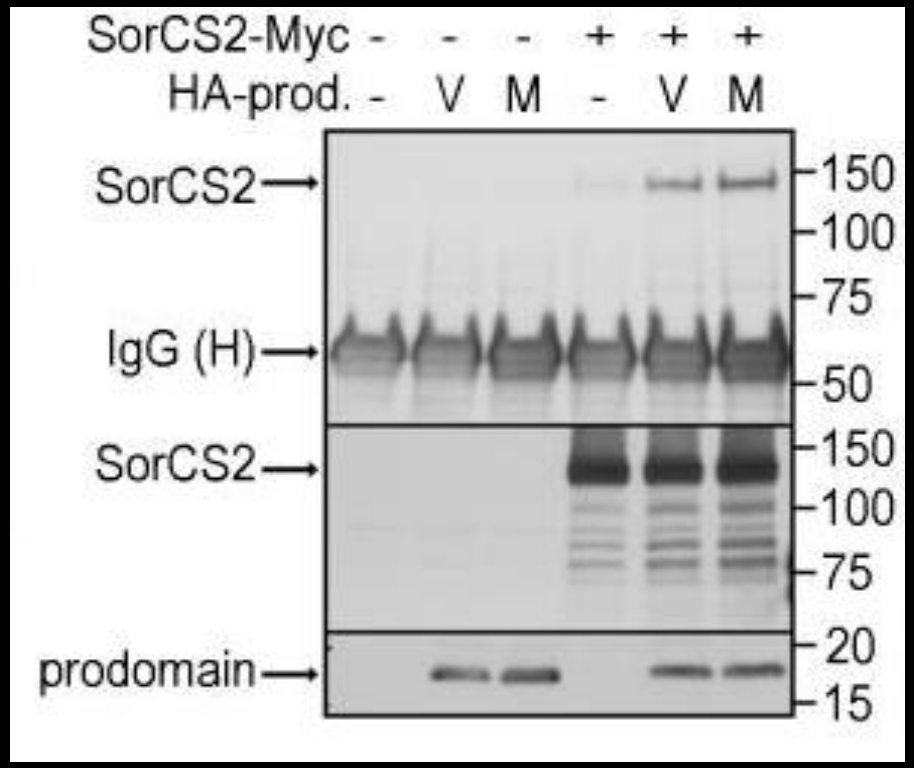
Purpose

- To determine
  - The consequences of growth contraction and synapse elimination
  - If prodomain interactions with SorCS2 and Zn2+ are causing the formation of plaques that may lead to memory loss or obstruction.
- Previously, a 28% binding differential of the Val and Met isoforms was seen (Anastasia, 2013)
- Biocone vs. Avidin Beads

Research Questions and Hypotheses

- Q1: How does SorCS2 interact with the prodomain?
- Q2: How does the met influence prodomain effects?
  - H0 Null Hypothesis: They interact differently
  - H1 Refined Hypothesis: Met prodomain -> more pronounced
  - H2 Protein Purification Hypothesis: knock out SorCS2 -> Met prodomain will not have any effect

Binding of Prodomain with SorCS2



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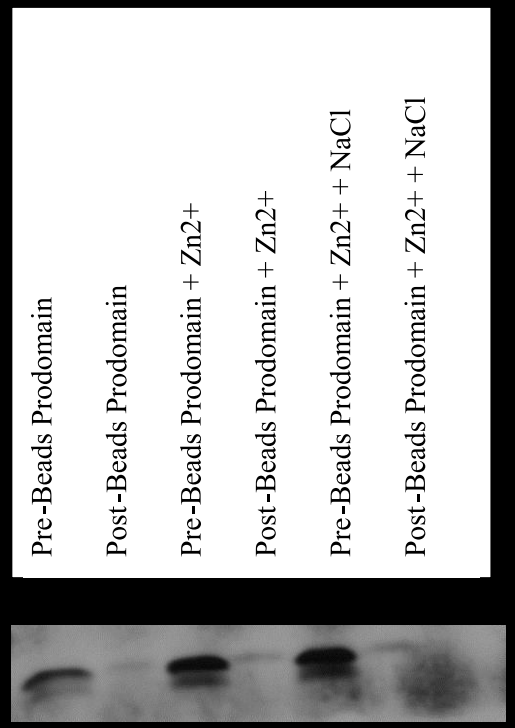
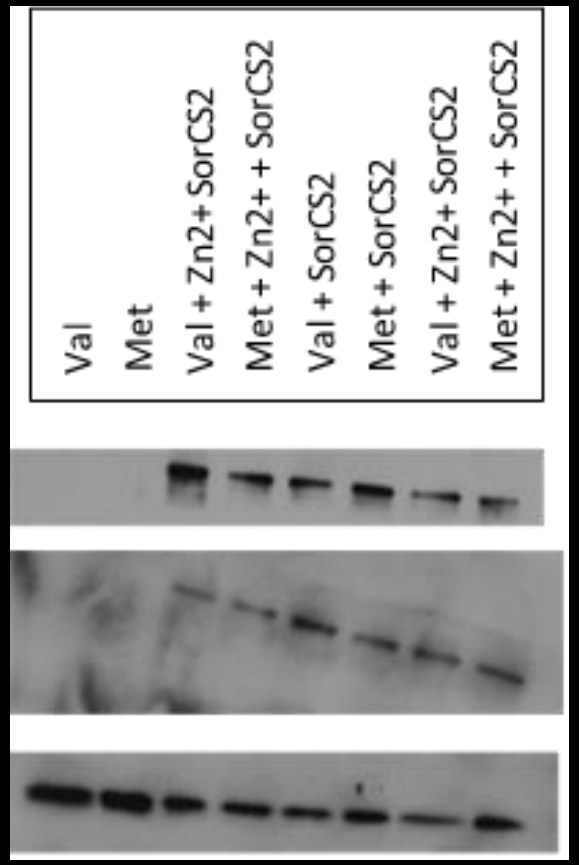
- Met66 binding with SorCS2 compared to Val66 (Anastasia, 2013)
- Differences in function and structure of prodomain can result from binding differences
- Impact of Zn2+ -> examine

Testing Prodomain Binding to Avidin Agarose beads Test Trial Using Co-IP



- Incubated with proBDNF Gene Copeoia antibody (1:10,000 dilution) followed by HRP
- *Results:*
  - To determine nonspecific binding
- Are there prodomain alterations dependent on SorCS2 binding with Zn2+?
- Demonstrates prodomain complex

Prodomain/Zn2+ Complex Binding to SorCS2 using Co-IP/ Striping and Probing For Biotin and SorCS2



- SDS- Page -> incubation with Ab SorCS2 (at a 1:1000 dilution) -> Ab Shp HRP (at a 1:5000 dilution)
- Vectastain ABC Kit to detect protein
- *Results:*

SorCS2

Biotin

Prodomain

- Biotinylation successful
- Lack of interfere with Prodomain presence
- 10:1 ratio and 1/10 binding - SorCS2 binds
- Val and Met present

Prodomain/Zn2+/SorCS2 Complex Binding with SorCS2 and Prodomain Using Co-IP

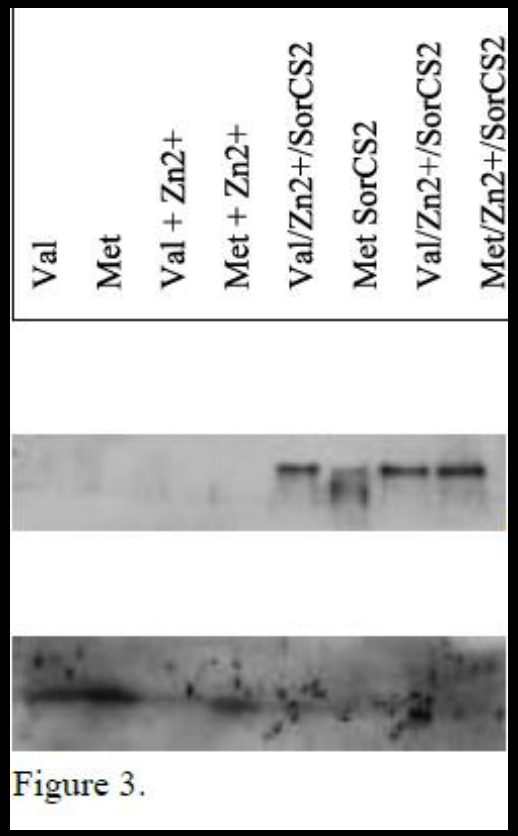
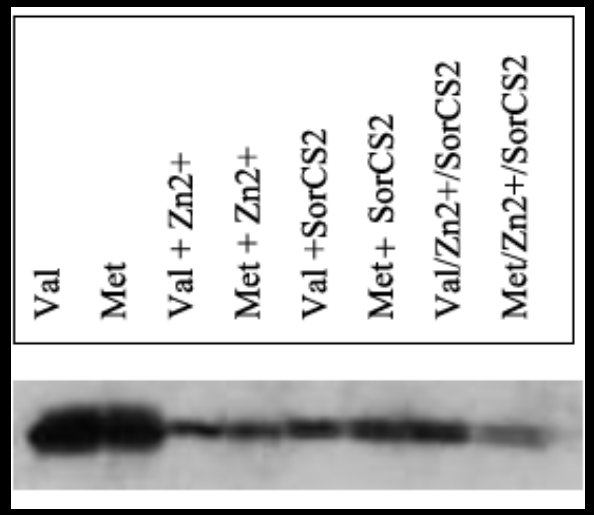


Figure 3.

- Co-IP followed by SDS-Page with Zn2
- SorCS2 antibody (1:10 dilution) and proBDNF Gene Copeoia antibody (1:10,000 dilution) followed by HRP.
- Results:
  - Nonspecific binding with avidin beads
  - Aggregation greater in Met than in Val isoform

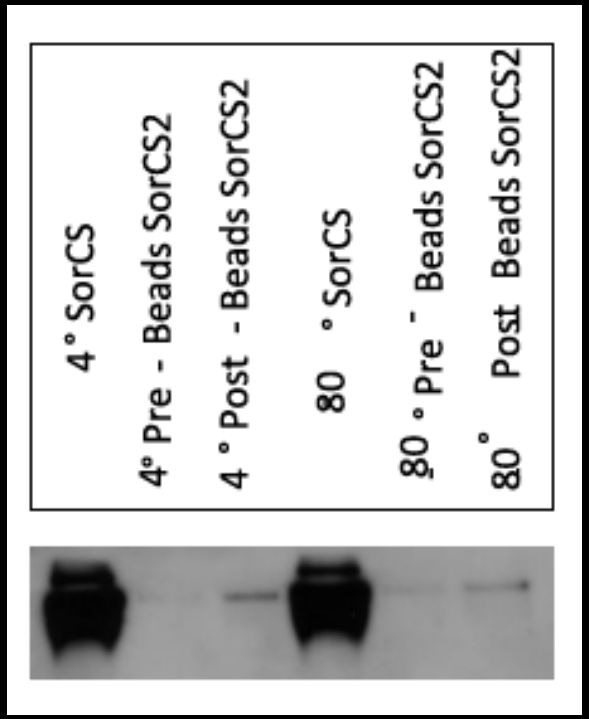
Prodomain/Zn2+/SorCS2 Complex Binding with SorCS2 Using Co-IP



- Co-IP followed by SDS-Page with Zn2+
- Probed for SorCS2 antibody (1:10 dilution) followed by HRP

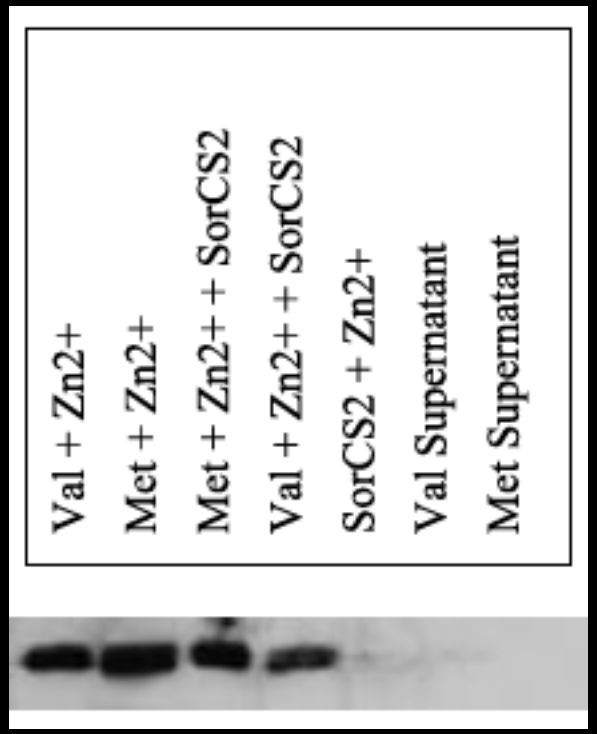
- Results:
  - Lack of difference in amount of Val and Met
  - Binding in presence of SorCS2

SorCS2 Binding to Agarose Avidin Beads using Co-IP



- Co-IP performed followed by SDS-Page
- Blot probed using SorCS2 antibody (1:10 dilution) followed by HRP
- Results:
  - Demonstrate ability to bind with Agarose beads
  - 4° binds slightly better

Prodomain/Zn2+ Complex Binding to SorCS2



- Incubation -> Co-IP performed followed by SDS-Page
- Probed using proBDNF antibody and SorCS2
- Results:
  - Nonspecific binding of SorCS2
  - Conditions are not right

Discussion/Conclusion

- Fast dissociation -> long washes
- Isoforms of prodomain -> induction structures -> different functions in the molecule
- Met and Val prodomains interact with SorCS2 differently
- Met prodomain should have demonstrated more binding (neither proven nor disproved)
- Possible problems in biotinylation of SorCS2 or best possible results at amount of SorCS2
- Nonspecific binding coming down in the Met more than the Val
- Binding of the Met and Val isoforms appeared the same
- Conditions of the experiment were not right

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