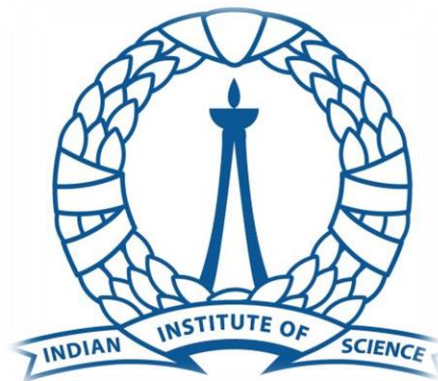


Nanoscale segregation of synaptic scaffolding protein SAP97 follows first order phase transitions

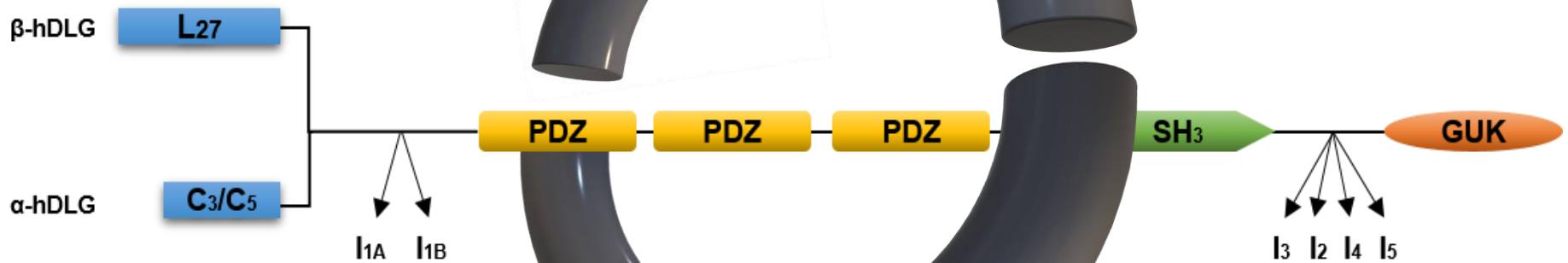


Premchand Rajeev
PhD Student
Centre for Neuroscience
Indian Institute of Science

DLG1/SAP-97/hDLG

Ubiquitous and evolutionarily conserved protein

Expression of multiple alternative splice variants

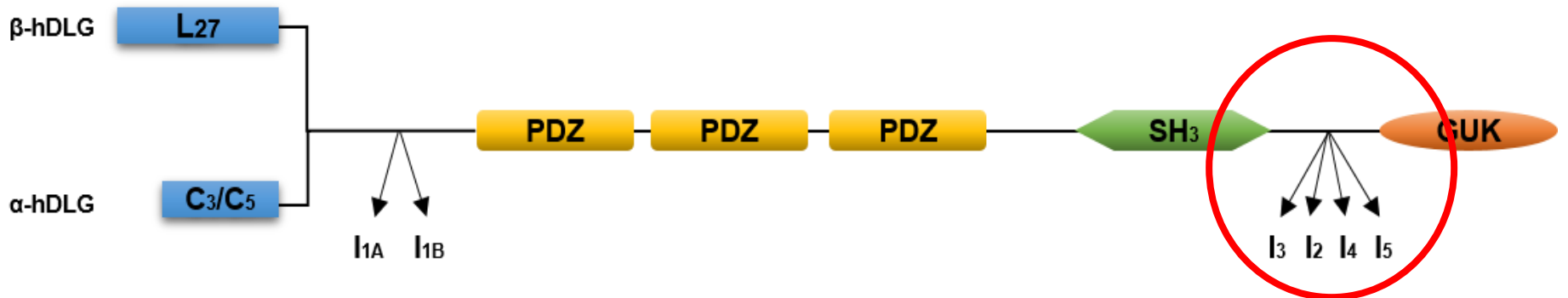


The only known MAGUK to directly interact with GluA1

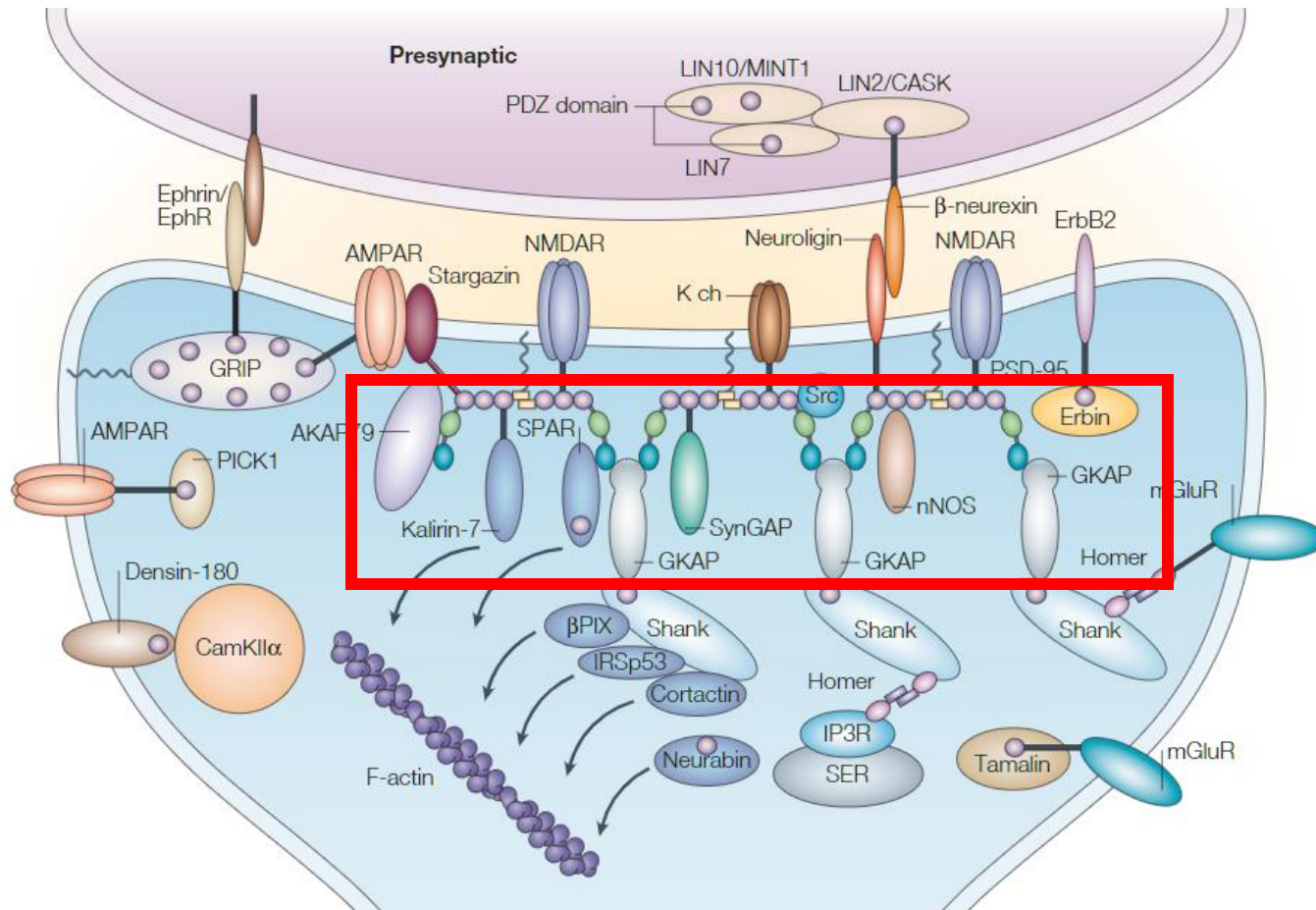
Ectopic expression and silencing of gene alters the spike frequency

Sap97/hDLG protein domains

L27 domain	<ul style="list-style-type: none"> • Myosin-6, CASK • (WU et al., 2002) (Roh et al., 2002)
I1A & B region	<ul style="list-style-type: none"> • P56,P38- kinase • (Hanada & Chisti, 1997)
PDZ domain	<ul style="list-style-type: none"> • APC, GluA1, Kir2.2, Kv1.5, KIF1B-α • (Folco et al., 2004) (Leonoudakis et al., 2004) (Leonard et al., 1998)
SH3 domain	<ul style="list-style-type: none"> • ADAM10 • (Marcelo et al., 2007,)
HOOK region	<ul style="list-style-type: none"> • Calmodulin, 4.1N protein, AKAP • (Hanada et al., 2003)(Nikandrova et al., 2010)(Rumbaugh et al., 2003)
GUK domain	<ul style="list-style-type: none"> • LGN proteins • (Zhu et al., 2011)



PSD as a phase separated compartment



Membrane less organelles undergoing LLPS

Liquid-Liquid Phase Separation in Physiology and Pathophysiology of the Nervous System

Yasunori Hayashi, Lenzie K. Ford, Luana Fioriti, Leeanne McGurk, and Mingjie Zhang


Journal of Neuroscience 3 February 2021, 41 (5) 834-844; DOI: <https://doi.org/10.1523/JNEUROSCI.1656-20.2020>

Liquid–liquid phase separation in human health and diseases

[Bin Wang](#), [Lei Zhang](#), [Tong Dai](#), [Ziran Qin](#), [Huasong Lu](#), [Long Zhang](#)  & [Fangfang Zhou](#) 

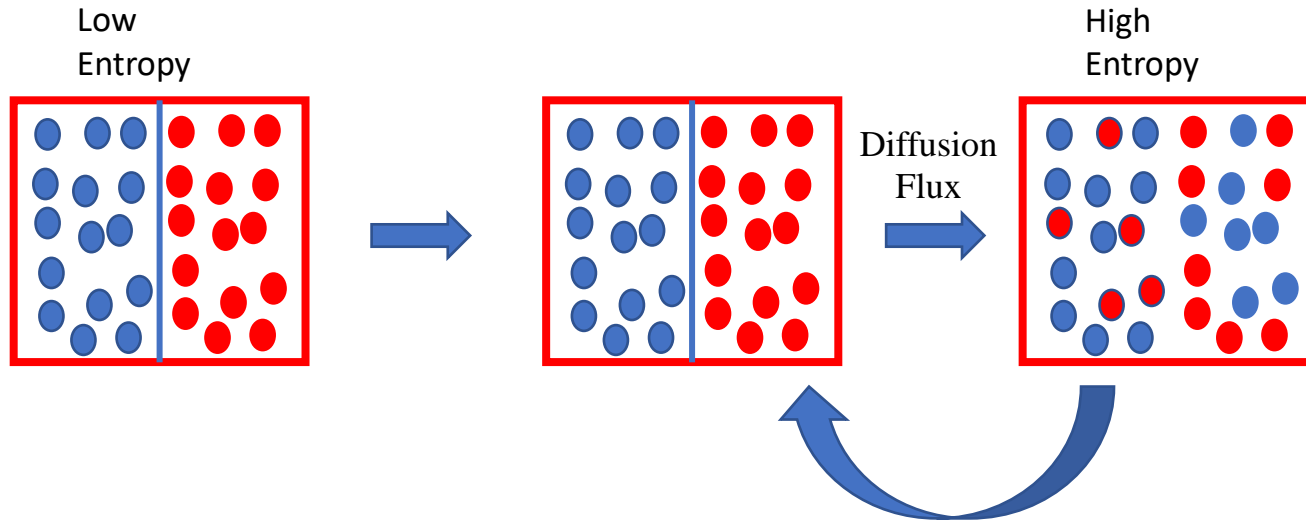
[Signal Transduction and Targeted Therapy](#) **6**, Article number: 290 (2021) | [Cite this article](#)

Protein phase separation hotspots at the presynapse

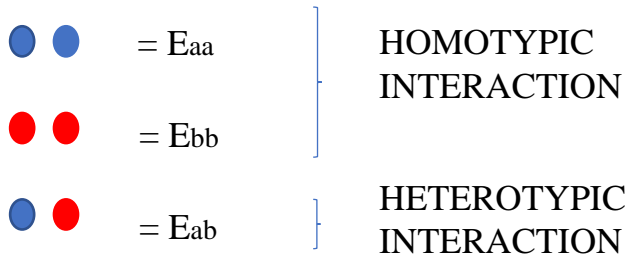
Janin Lautenschläger 

Published: 09 February 2022 | <https://doi.org/10.1098/rsob.210334>

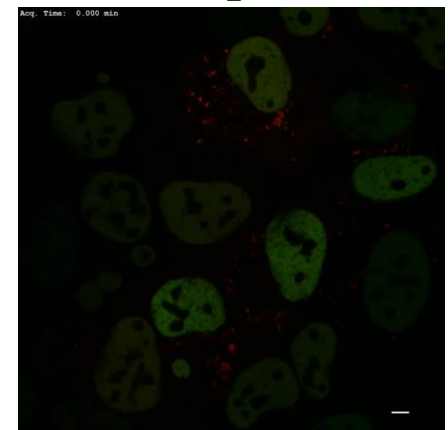
Phase transition, Entropy & Diffusion Flux



Is Phase separation possible???
What drives it?

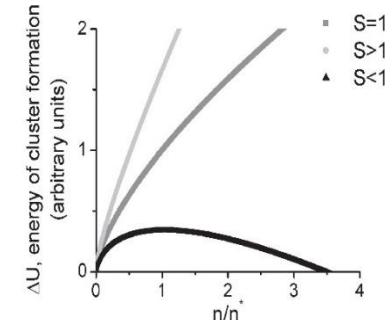
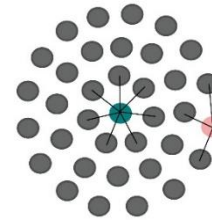
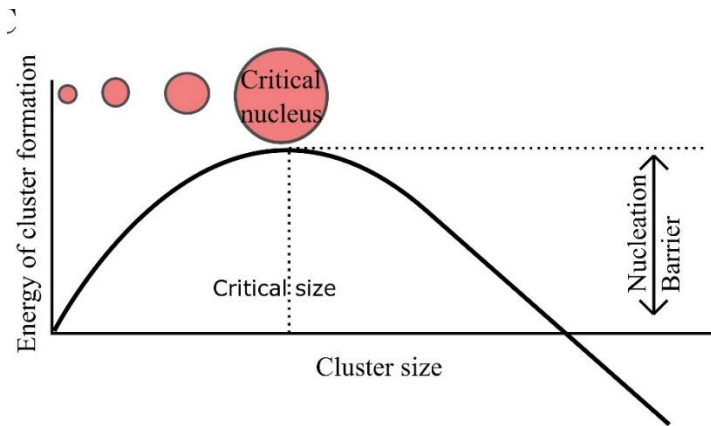


Phase Separation



AVERAGE HOMOTYPIC INTERACTION ENERGY < HETEROTYPIC INTERACTION ENERGY

Classical nucleation theory along with Super resolution imaging to explain the cluster formation



Sub critical clusters follows a Boltzmann distribution

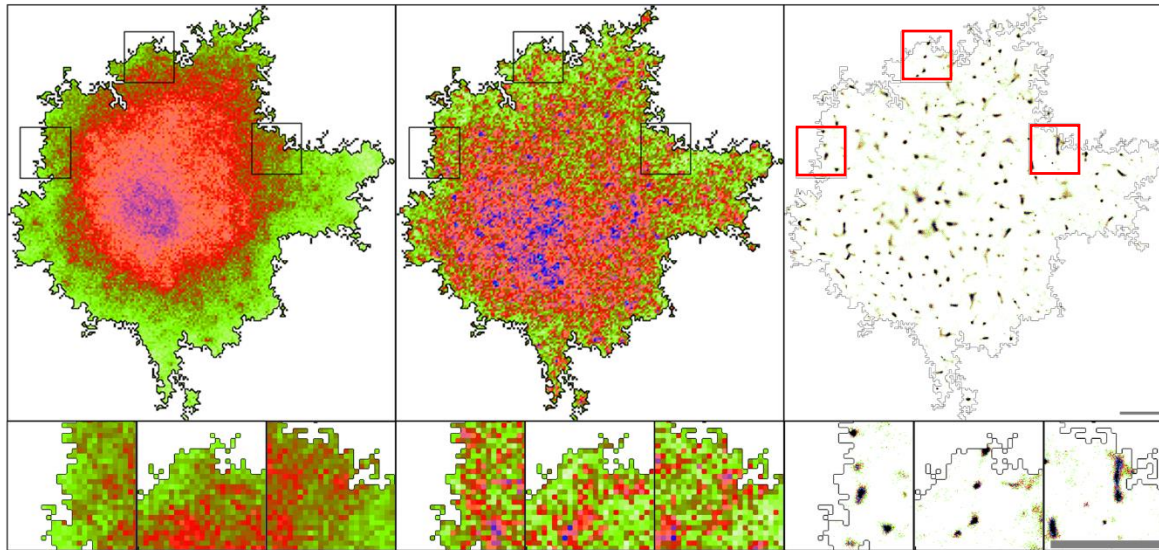
- $P(n) = Ae^{-\Delta G/k_B T}$
- $\Delta G(n) = -K_b T \text{Log} P(n)$

For a spherical Homogenous nucleation

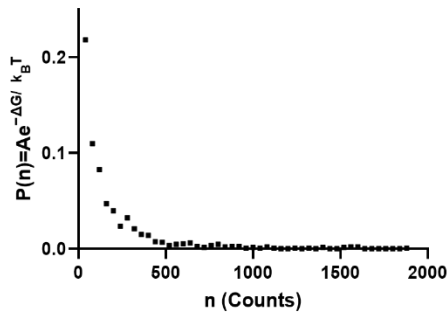
- $\frac{4}{3} \pi R_n^3 = v_n$
- $4\pi R_n^2 = A_n$
- $\Delta G_{surface} = \sigma A_n$
- $\Delta G_{bulk} = bn$ [$b = C_{amb}/C_{sat}$]
- $\Delta G = an^{2/3} \pm bn$
- $an^{2/3} = \text{Surface energy term}$
- $bn = \text{Bulk energy term}$

Phase transition of Sap97 clusters from Single Molecule Fluorescence Intensity

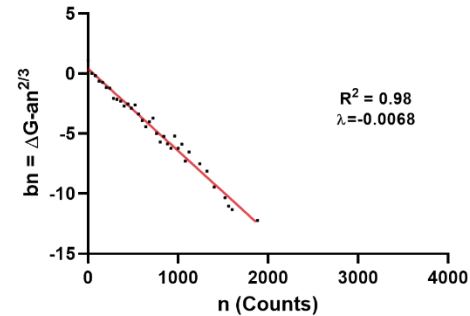
A.



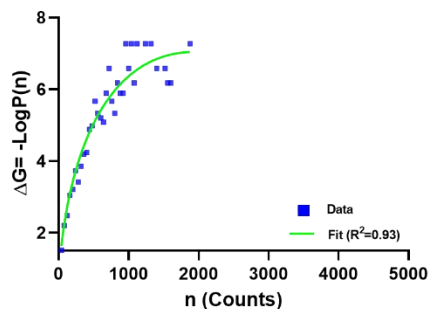
B.



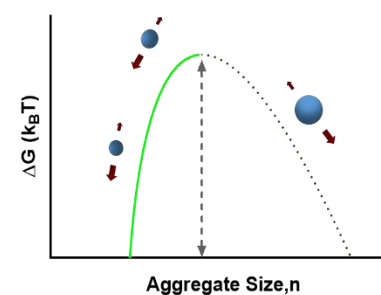
D.



C.



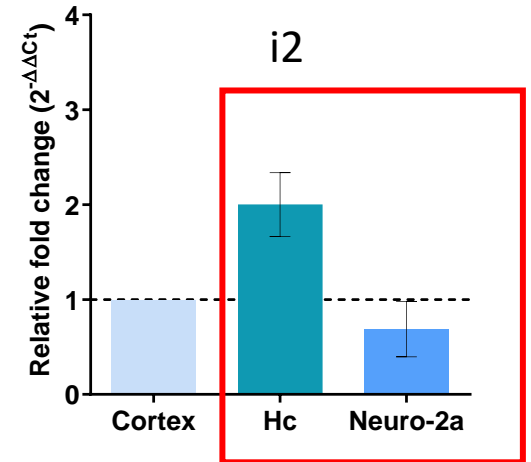
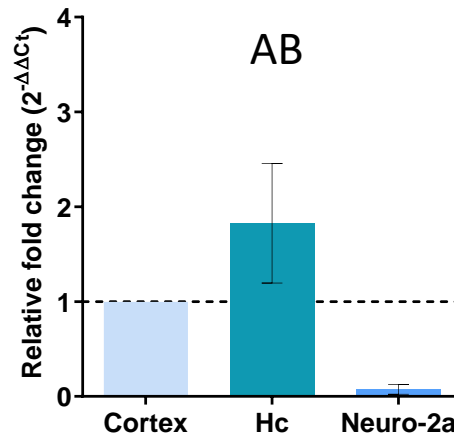
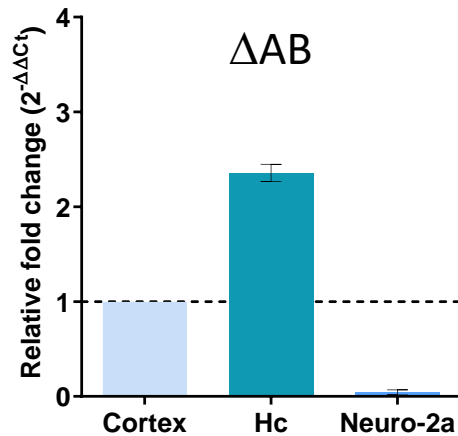
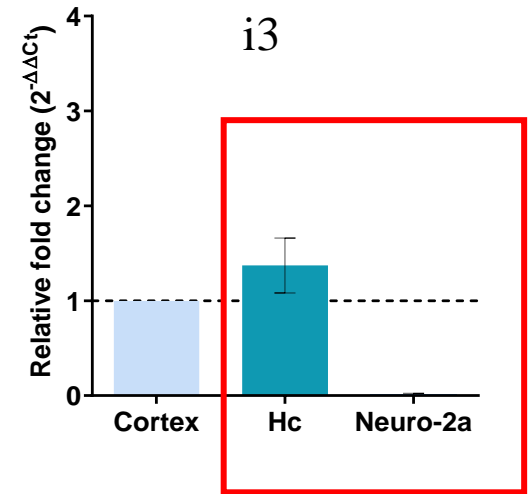
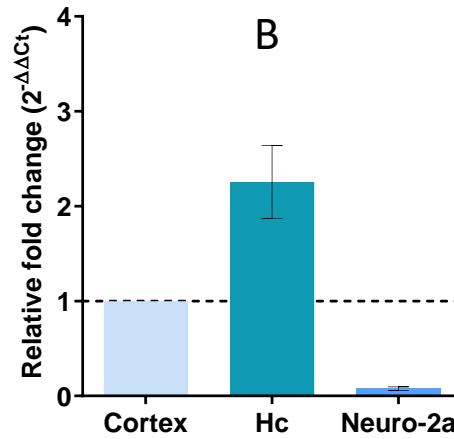
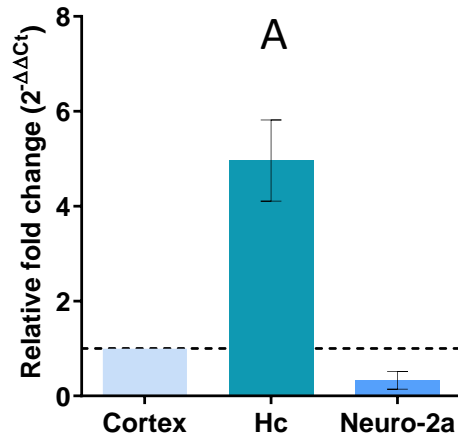
E.



More Questions???

- Which are these isoforms of SAP 97 we are looking at?
- Does SAP97 behave similarly in neurons?

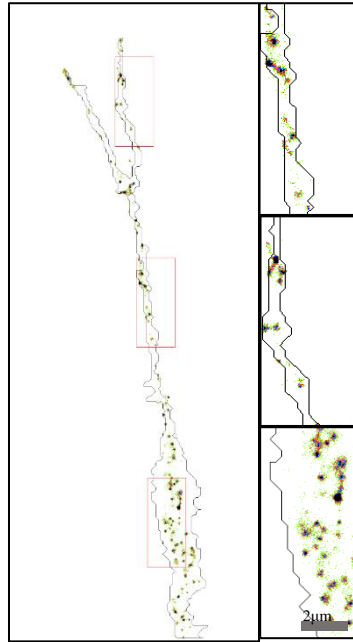
Relative quantification of mRNA levels of SAP97 in postnatal rodent brain and Neuroblastoma cells



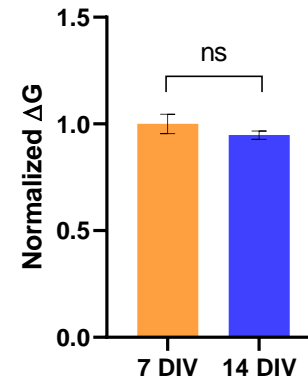
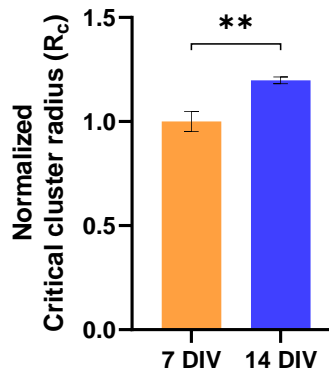
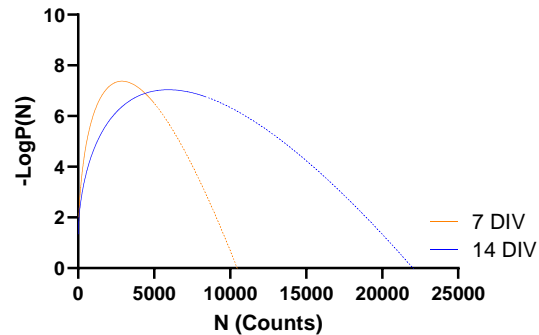
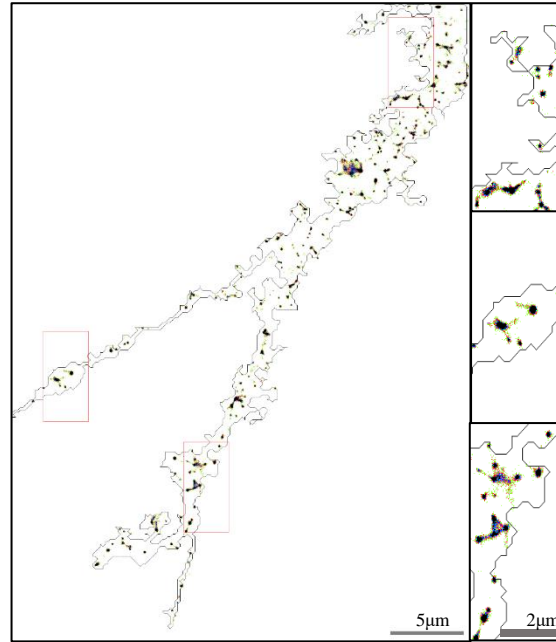
N = $\left. \begin{array}{l} 9 \text{ cortices} \\ 18 \text{ hippocampus} \\ 4 \text{ replicates of N2A} \end{array} \right\}$

Phase transition of Sap97 clusters in hippocampal pyramidal neurons

7-DIV



14-DIV



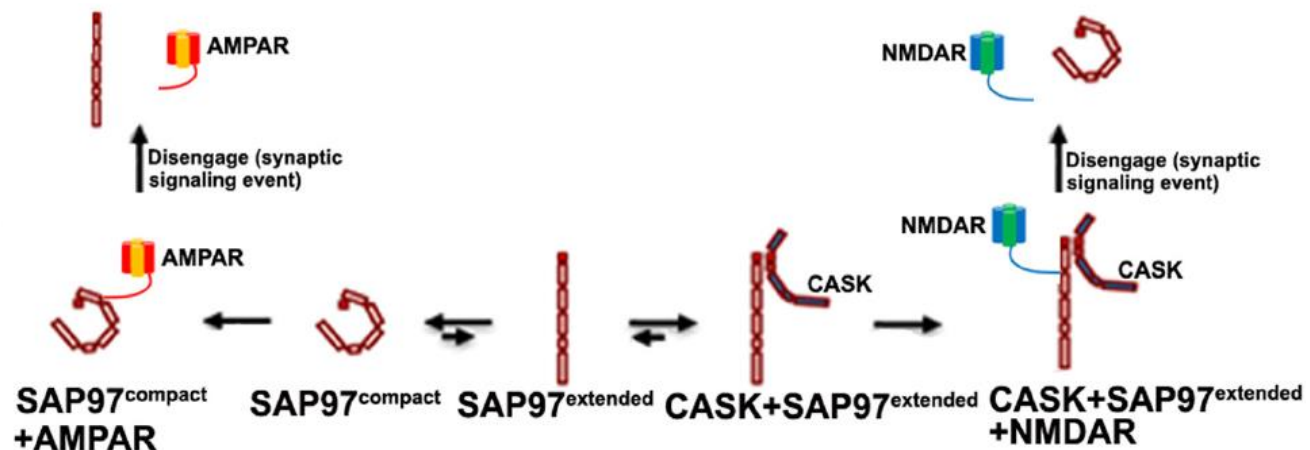
N=8 cells

Activity Dependent conformational change in SAP97

- Upon activation by Calcium entry the CaM gets activated and opens the C-Terminal (SH3-HOOK-GUK) conformation in SAP-97.

		net charge in 1-8-14 motif
SAP97	636-EVGVIPSKRRVEKKERARLKT ¹ VKKFN-660	4+
SAP102	575-QIGVIPSKRRVEKKERARLKT ¹ VKKFH-599	4+
PSD93/Chapsyn	592-EMGVIPSKRRVERKERARLKT ¹ VKKFN-616	4+
DLG	656-QIGVIPSKRRWERKMRARD ¹ RSVKKFQ-680	5+
PSD-95/SAP90	484-DIGFIPSKRRVERREWSRLK ¹ AKDWG-508	(3+)
smMLCK	1086-RRKWQKTGHAVRAIGRL ¹ SSM-1105	3+
α -Fodrin	1192-ASPWKSARLMVHTVAT ¹ ENSI-1211	2+
Caldesmon	454-AEGVRNIKSMWEKGNV ¹ ESSP-473	2+
Titin	4388-HTLIKKDLNMV ¹ SAARISCC-4407	2+
AdenylCyclase	315-ADAFKKIARELNTYIL ¹ RPV-334	2+
Mastoparan X	1-INWKGIAAMAKKL ¹ L-14	3+

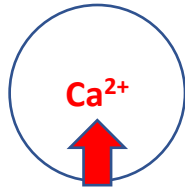
Paarmann et al., 2002



Lin, Jeyifous et al., 2013

Is it possible to alter the Phase separation of SAP97 by perturbing intra cellular Ca^{+2} levels?

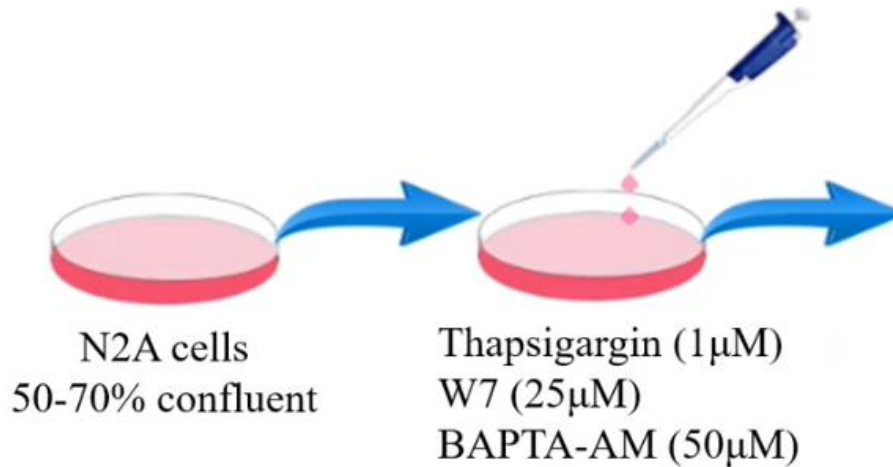
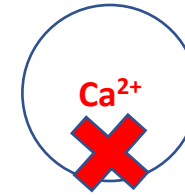
Thapsigargin (Tg)



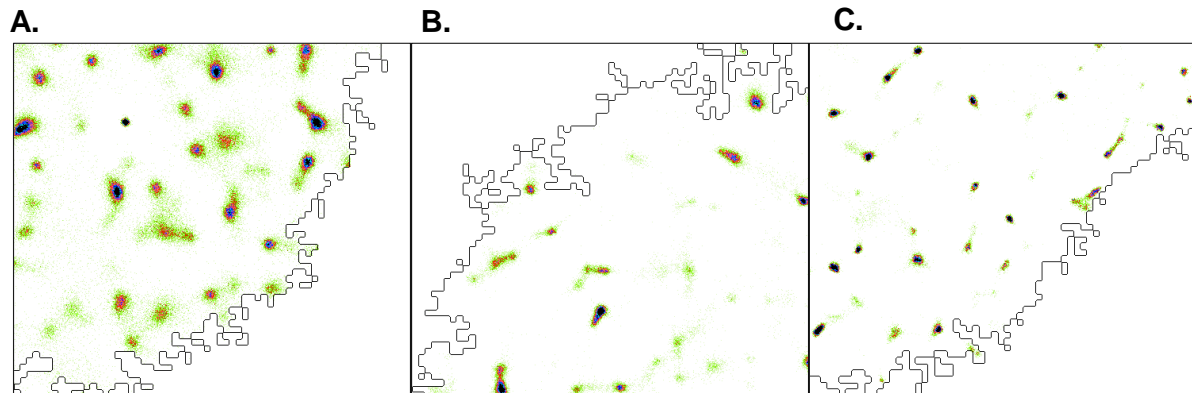
w7



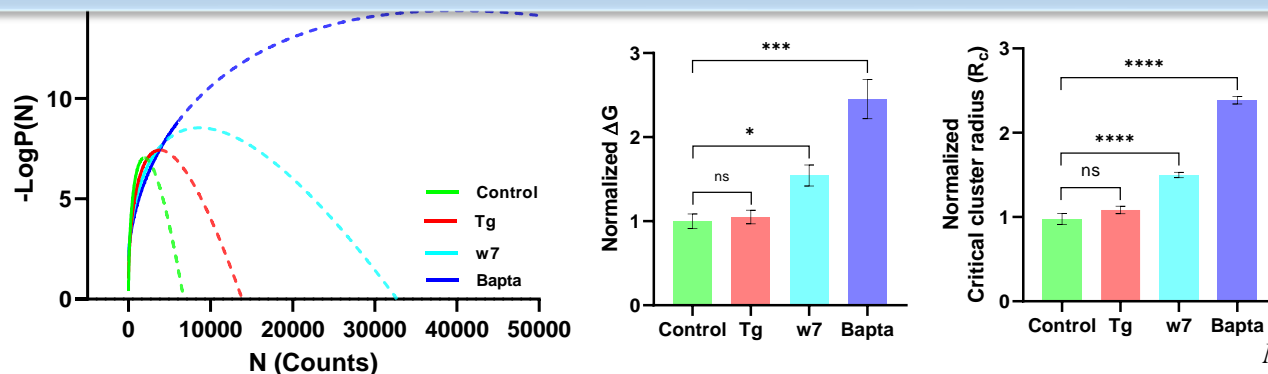
BAPTA-AM (Bapta)



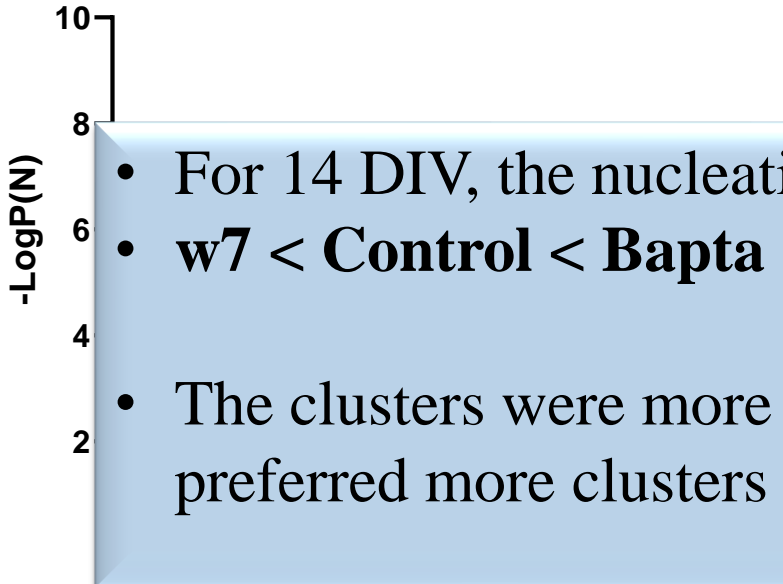
The effect of Ca^{2+} signalling in molecular phase separation of SAP97 clusters



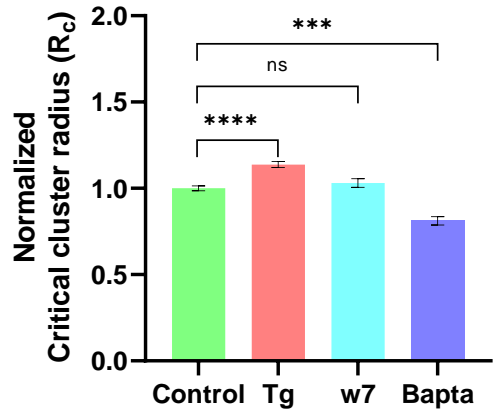
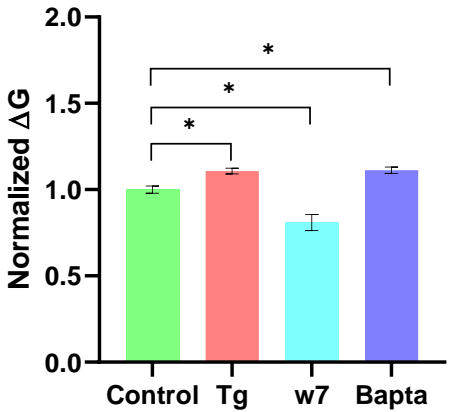
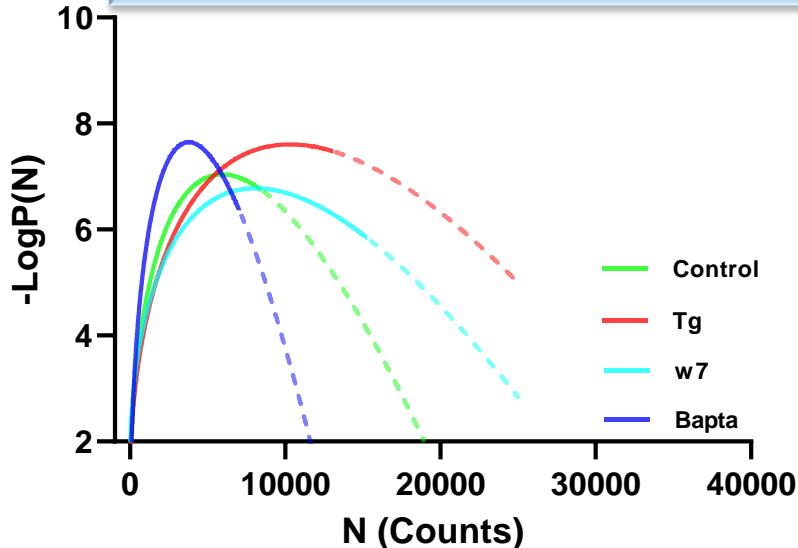
- The nucleation barrier was increasing
- **Control = Tg < w7 < Bapta**
- This indicates that I2 isoform, in the absence of Ca^{2+} and Calmodulin, forms large clusters.



SAP97 clusters in neurons are sensitive to intracellular Ca^{2+} fluctuation

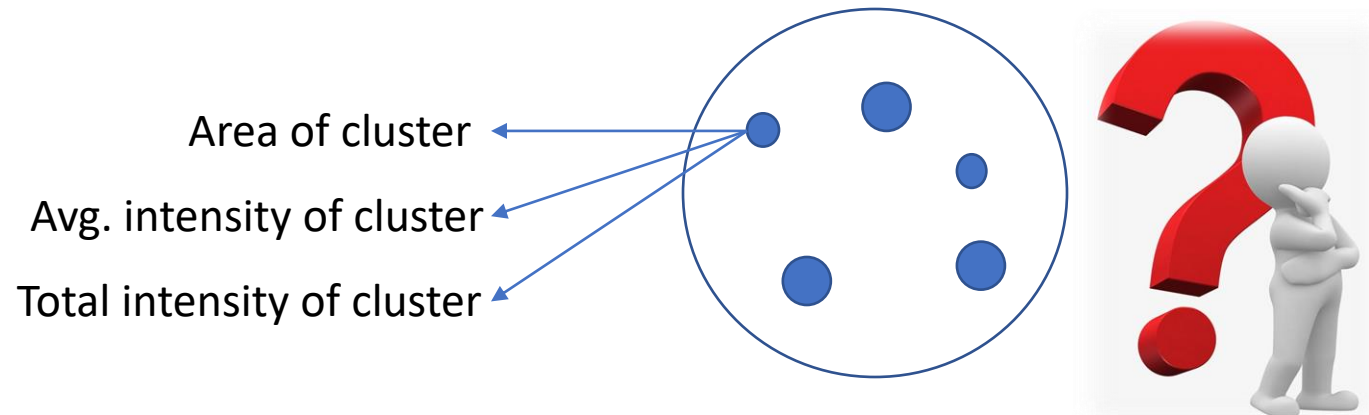


- For 14 DIV, the nucleation barrier was increasing
- **w7 < Control < Bapta = Tg**
- The clusters were more confined at low Ca^{2+} , while the system preferred more clusters in the absence of Calmodulin.



We have observed that SAP97 clusters are differentially regulated with altered Calcium levels and the isoform composition

To what extent are the cluster parameters changing??



Rank Order Analysis

Imaging

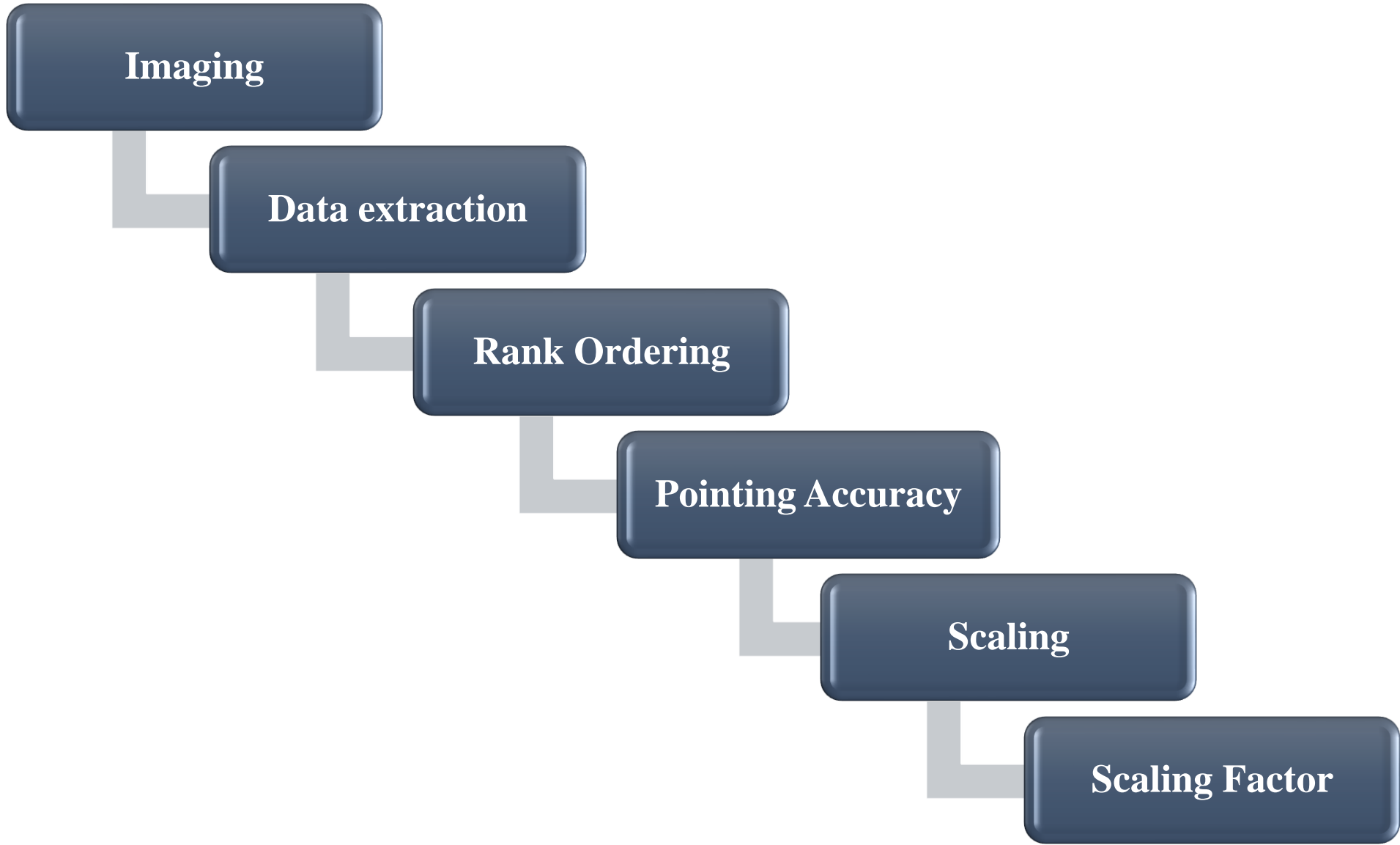
Data extraction

Rank Ordering

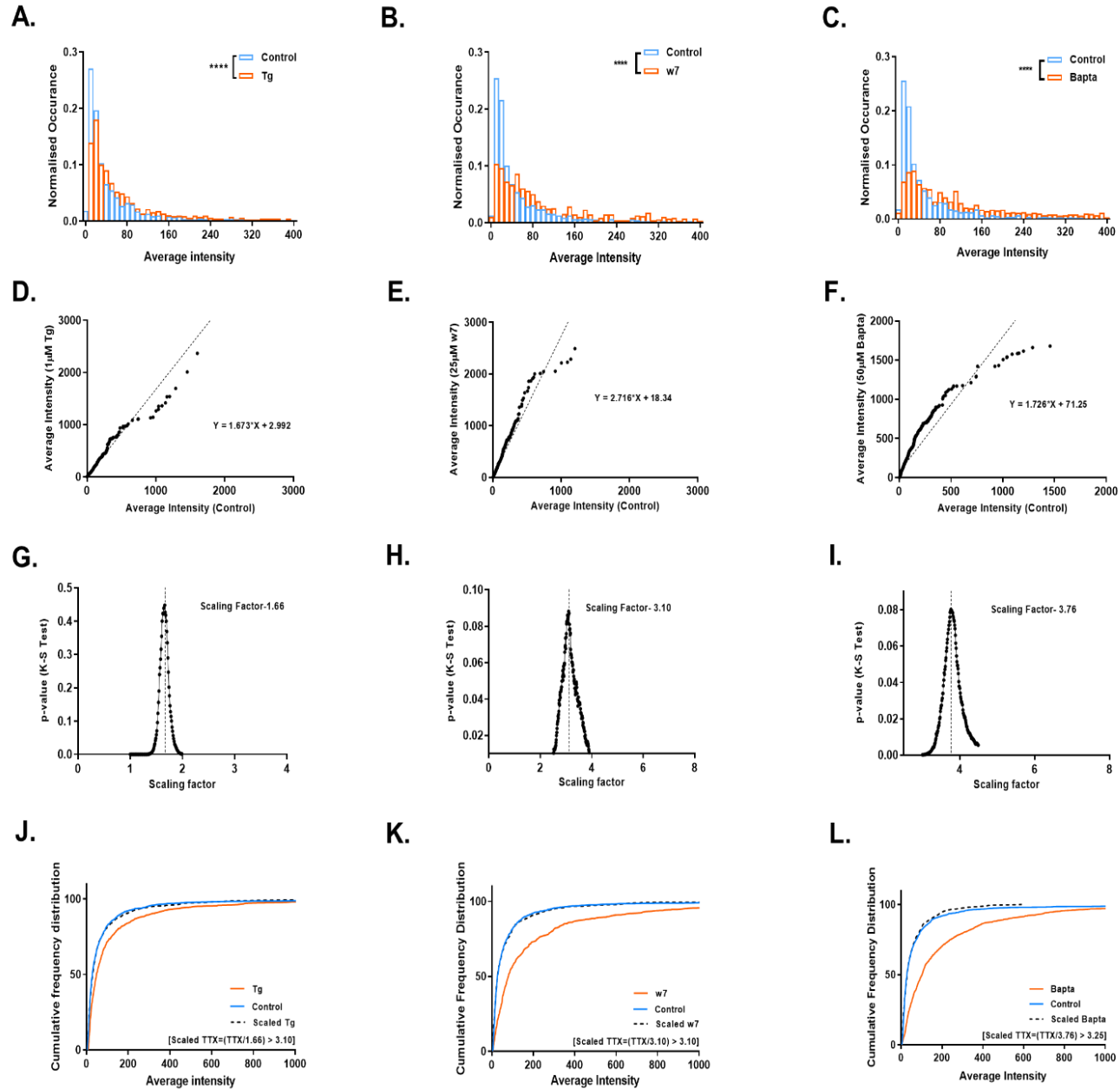
Pointing Accuracy

Scaling




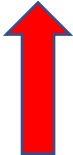


Scaling Factor



Endogenous Sap97 Cluster Analysis In Neuro-2a Cells Using Rank Order Method

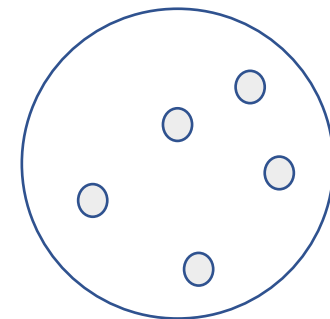
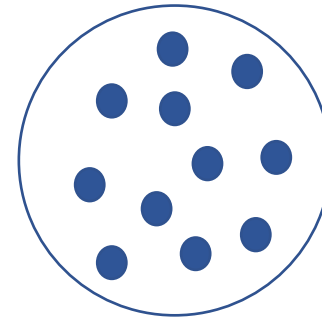
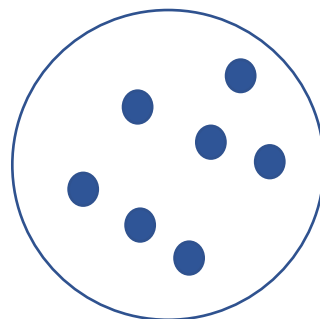
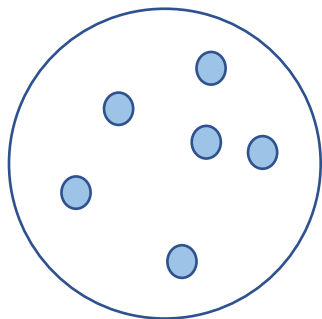


The cluster parameters were altered by Ca^{2+} perturbation in N2A cells

Conditions	Area	Average intensity
Control vs Tg	1.24 	1.66 
Control vs w7	1.22 	3.10 
Control vs BAPTA-AM	1.46 	3.76 

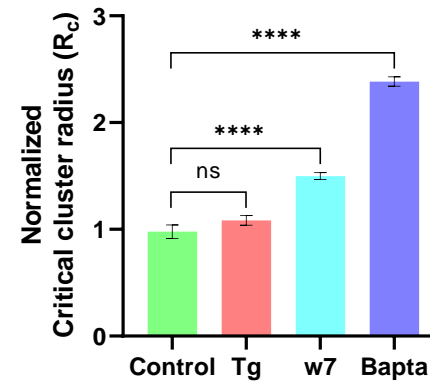
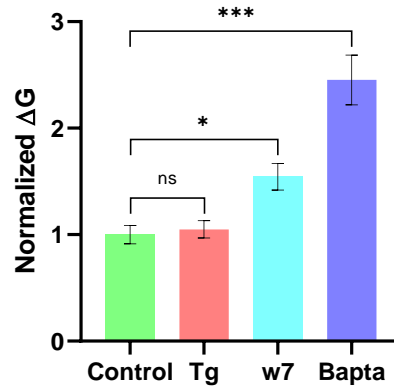
Sap97 protein in 14 DIV gets clustered more in the absence of Calmodulin and gets drastically reduced at low calcium levels

	Untreated	Tg	w7	Bapta
Area of cluster	(0.029±0.002)	(0.031±0.002) NS	(0.024±0.003) NS	(0.027±0.002) NS
Average intensity	(12.16 ±1.362)	(24.562±2.488) **	(44.000±6.910) ****	(4.89±0.305) ****
Total intensity	(1035 ±115)	(1587±192) **	(2435±464) ****	(284±33) ****

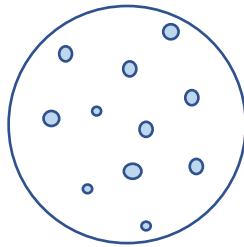


Conclusion

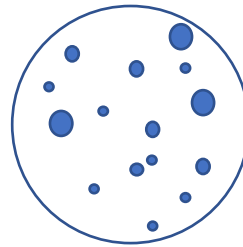
N₂A cells



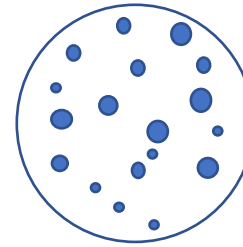
N₂A cells



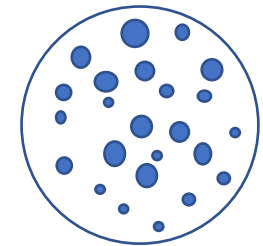
Untreated



Tg



w7

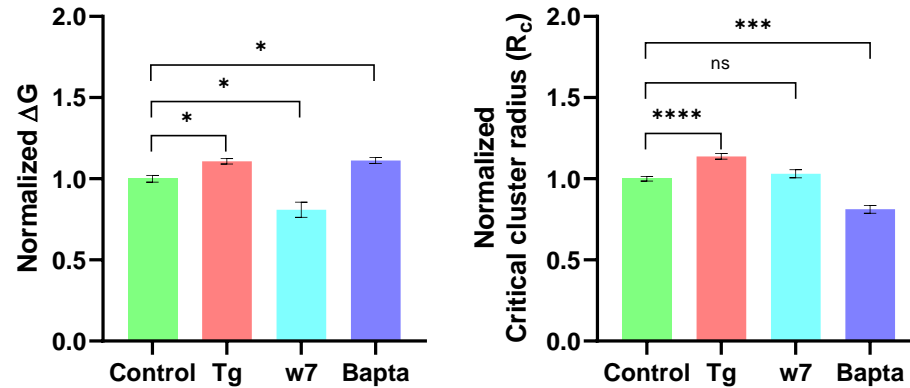


Bapta

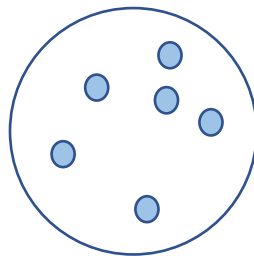
Sap97 isoform - I2 form large clusters and is recruited more into clusters when intracellular Ca²⁺ levels are perturbed.

Conclusion Contd...

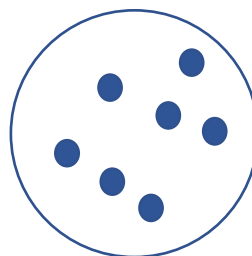
14 DIV Neurons



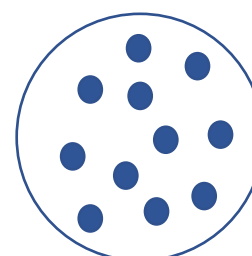
14 DIV Neurons



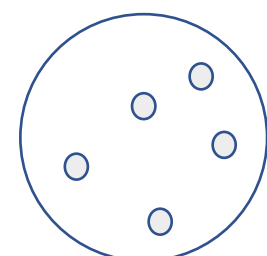
Untreated



Tg



w7



Bapta

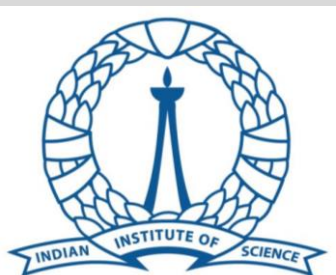
- Sap97 form large clusters in matured neurons
- More molecules get recruited into clusters in the absence of Calmodulin while it's reduced in the absence of Ca^{2+}

Data not shown

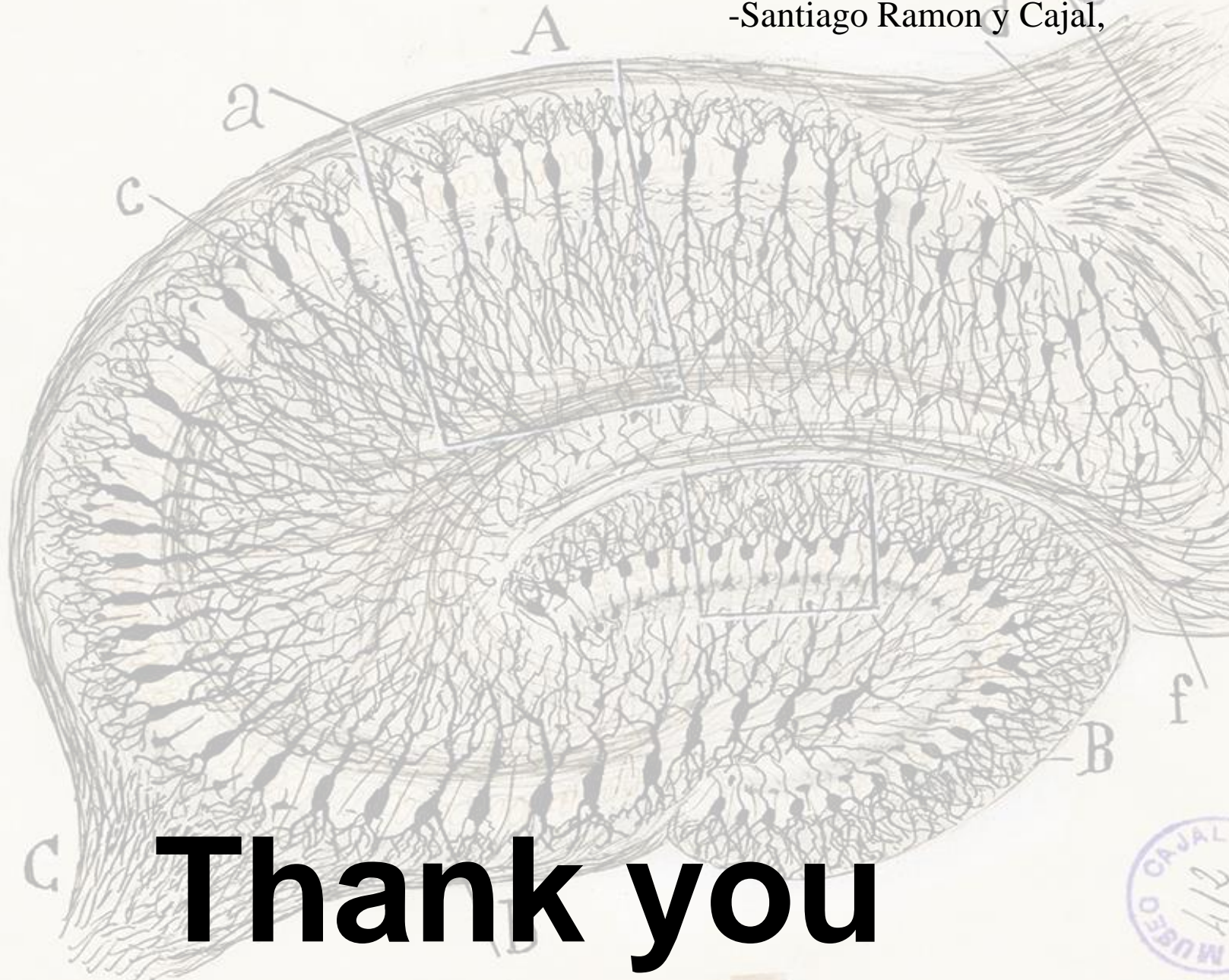
- FRAP studies have been performed to understand the mobility of SAP97 isoforms under variable Ca^{2+} levels.
- Further we will be looking at invitro imaging studies to look for Phase separation of SAP97

Wind beneath my wings...

- Dr. Deepak Nair
- Dr. Mini Jose Deepak and my colleagues
- CNS administration and all staffs
- NCBS imaging facility, IISc Animal facility
- IISc, MHRD and UGC for funding the research.



. "A look was enough. Dumbfounded, I could not take my eyes from the microscope"
-Santiago Ramon y Cajal,



Schuetese

*untere
1/3*

Thank you

