① RNAseq and RIPseq Bioinformatic analysis
→ DEG and CST-RNA binding targets

② Neuron-specific Functional modules characterization
Although DEG overlap is small, CST models share similar pathohistological hallmarks that suggest they regulate functionally related targets

Gene regulation Mechanism identification

Different regulation mechanisms

2.1 Neuron-PPI network
2.1.1 RNAseq profile of 15 fly tissues (FlyBase database)
            Neuron expressed proteins - above 1^{st}Q in neuron

2.1.2 Fly physical PPI data (APID repository)
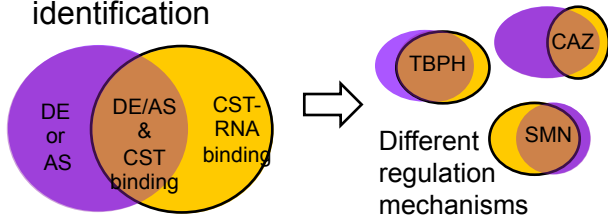
Common DEG target identification

Common DEGs

Small overlap

Global Network:

- **All tissues >1^{st}Q**
- **Neuron tissue >1^{st}Q**
- Other tissues

FEA result:
- **Function 1**
- **Function 2**
- Function 3
- Function 4

2.2 Neuron Net Functional Enrichment
Critical to identify housekeeping protein tissue-specific roles

2.3 Functional Module: Groups of PPI pairs annotated under the same enriched term

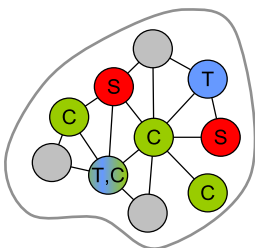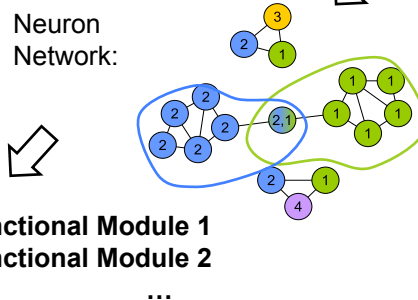Neuron Network:
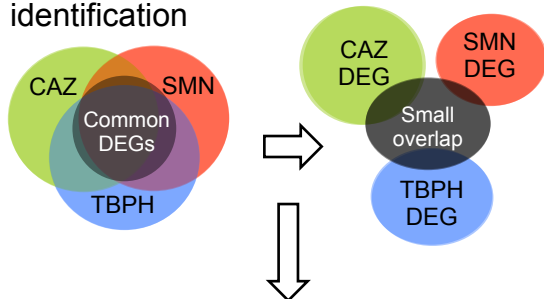
③ CST kd Functional impact evaluation
Functional module alteration is proportional to the number of elements deregulated by each kd

CAZ impact $\dfrac{\text{# CAZ DEG in Mod 1}}{\text{Total proteins in Mod 1}}$

SMN impact $\dfrac{\text{# SMN DEG in Mod 1}}{\text{Total proteins in Mod 1}}$

TBPH impact $\dfrac{\text{# TBPH DEG in Mod 1}}{\text{Total proteins in Mod 1}}$

Functional Module 1
Functional Module 2
…

Select Functional Modules with >20% minimal impact for all CAZ, SMN and TBPH kd

Cytoskeleton signaling regulation Is a hallmark along selected modules

④ Selected Functional Modules exploration:

Network includes all DEGs found on selected modules and highlights common DEGs in inner network

Combination of DEG and RNA binding info might help to decipher CST mechanisms of gene regulation on specific molecular pathways

Different network aesthetics help to evaluate molecular commonalities and relations between CAZ, SMN and TBPH targets

i)   Housekeeping protein functional roles in neuron context
ii)   DEGs molecular roles in cytoskeleton signaling according to FlyBase gene description