Which process or biomolecule would you study with a chemical probe if you had one in hand?
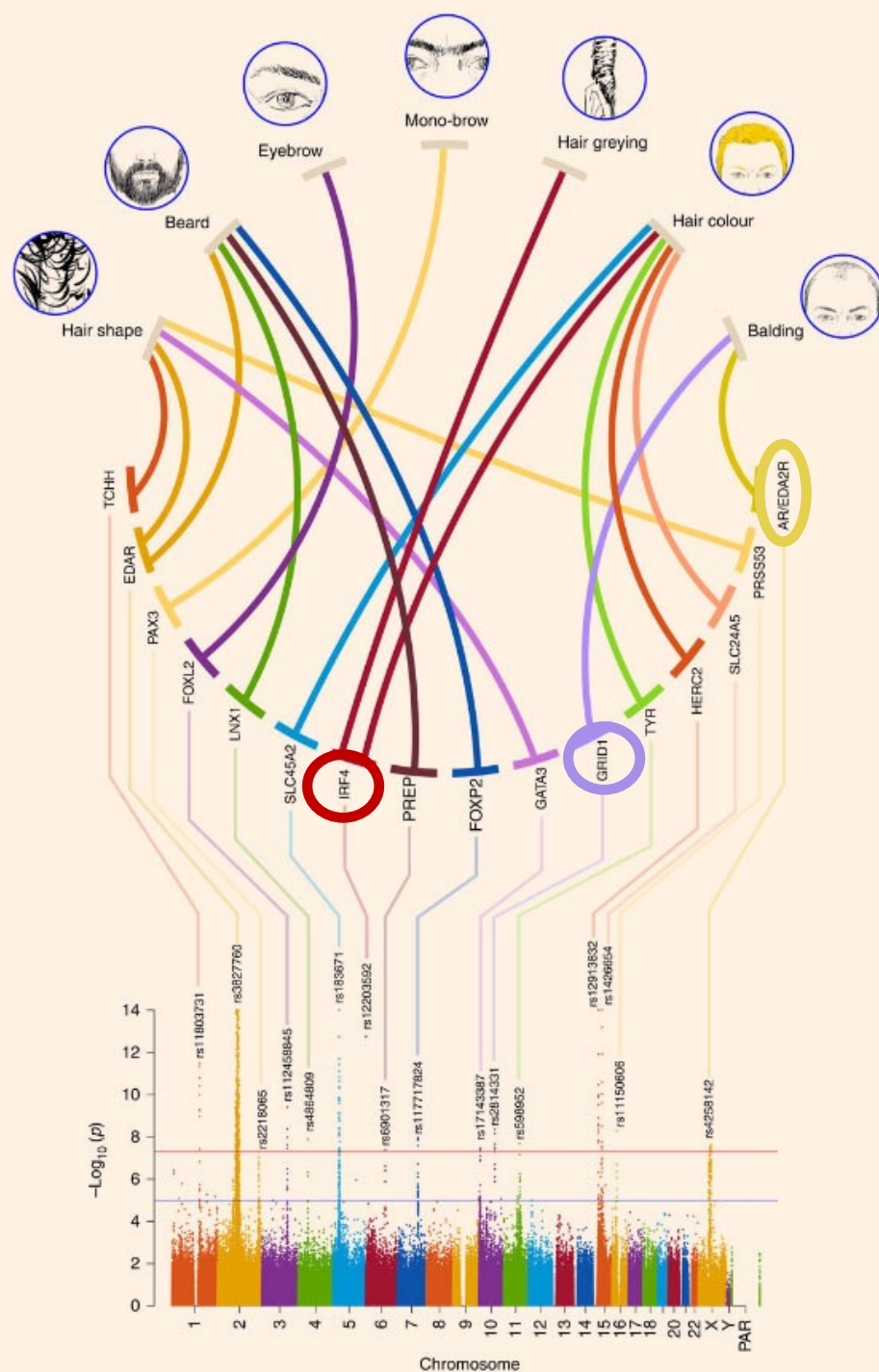
Genome Wide Association Study (GWAS):

hair-related phenotypes

GWAS: correlates single-nucleotide changes across the genome with specific traits

A genome-wide association scan in admixed Latin Americans identifies loci influencing facial and scalp hair features

The Cancer Dependency Map

interrogation of viability effects in cancer cell lines to map genetic dependencies

patient models (cell lines/organoids)
representing many tumors

molecular and
genetic information

genetic dependencies
and drug sensitivities

map of cancer
dependencies
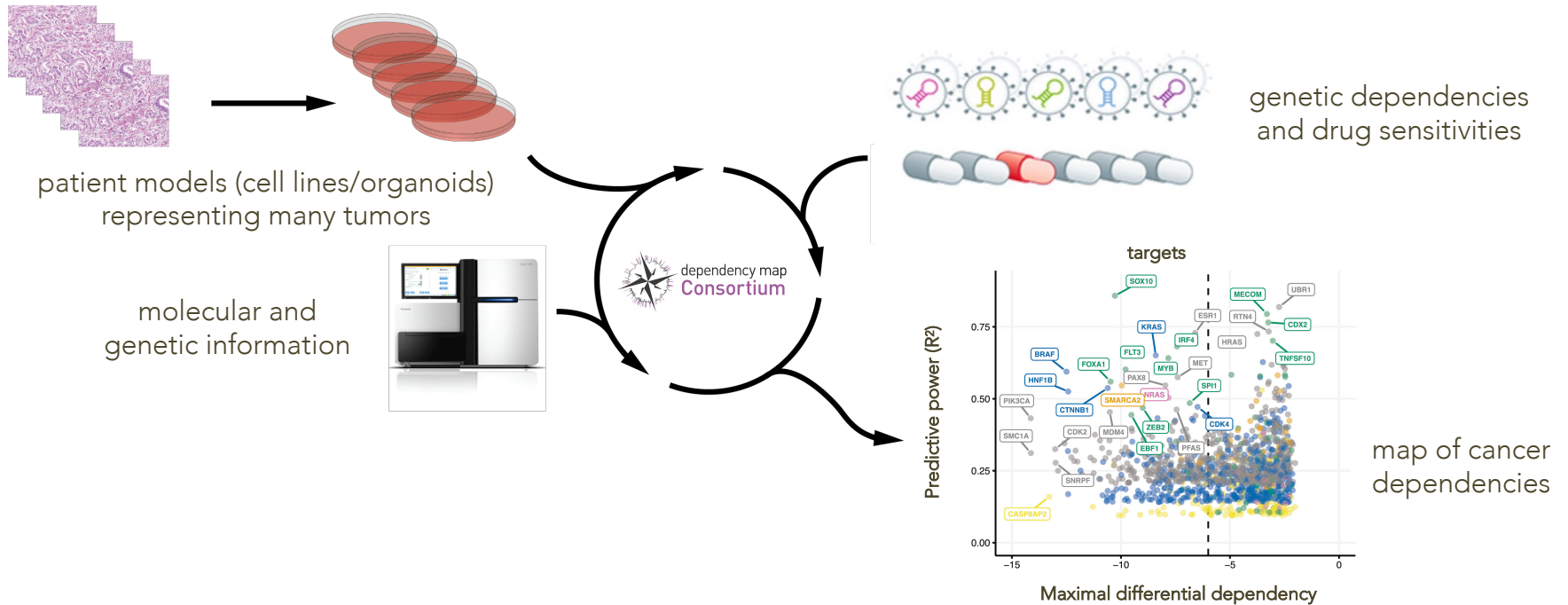
Discovery of potential TARGETS for therapeutic discovery

Discovery of PREDICTORS of patient response

Pre-publication DATA RELEASES to enable the scientific community at depmap.org

As of 11/21/21:
>2,000 cancer models
3913 genetic dependency screens
33 drug panels in sensitivity screens

Tsherniak et al., *Cell*, 170 (3): 564-576 (2017)
McDonald et al., *Cell*, 170 (3): 577-592 (2017)
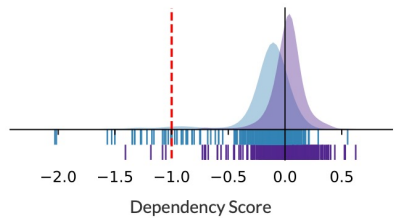Vazquez & Boehm, *Mol Sys Bio*, 16 (7): e9757 (2020)
Dharia et al., *Nat Genet*, 53 (4): 529-538 (2021)

IRF4 interferon regulatory factor 4

Overview | Dependency | Characterization | Description

Dependent Cell Lines

CRISPR: 42/558 STRONGLY SELECTIVE
RNAi: 11/711 STRONGLY SELECTIVE

Dependency Score

Dependency Score: Outcome from DEMETER2 or CERES. A lower score means that a gene is more likely to be dependent in a given cell line. A score of 0 is equivalent to a gene that is not essential whereas a score of -1 corresponds to the median of all common essential genes.

Strongly Selective: A gene whose dependency is at least 100 times more likely to have been sampled from a skewed distribution than a normal distribution (i.e. skewed-LRT value > 100).
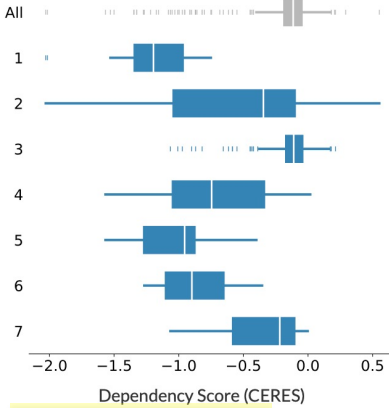
View more

The protein encoded by this gene belongs to the IRF (interferon regulatory factor) family of transcription factors, characterized by an unique tryptophan pentad repeat DNA-binding domain. The IRFs are
View more

Search external sites for IRF4
- PubMed (996 entries)
- GeneCards
- GTEx
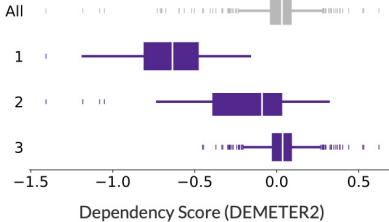- NCBI

View more

## Enriched Lineages

CRISPR

Dependency Score (CERES)

1. Multiple Myeloma (4.4e-79)
2. Haematopoietic And Lymphoid (3.4e-39)
3. Solid (4.5e-38)
4. Lymphoma (4.0e-15)
5. T-cell lymphoma Other (9.5e-12)
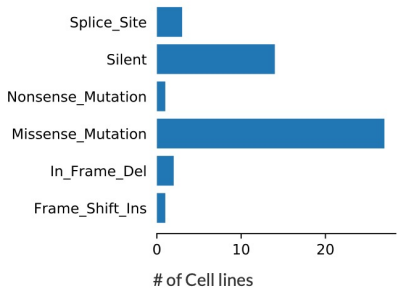6. ALCL (9.9e-07)
7. Melanoma (1.3e-04)

RNAi

Dependency Score (DEMETER2)

1. Multiple Myeloma (1.3e-72)
2. Haematopoietic And Lymphoid (3.3e-28)
3. Solid (6.5e-21)

Expression & CN

TPM (log2)

Copy Number (log2 relative to ploidy)
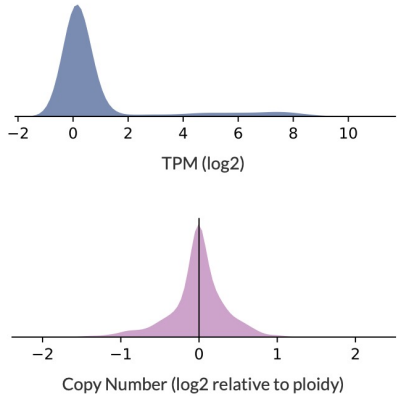
View more

Mutations

# of Cell lines

View more

Target Tractability

Bioactive Compounds	No
Druggable Structure	No
Druggable by Ligand Based Assessment	No
Enzyme	No

Original and additional data on *CanSAR*
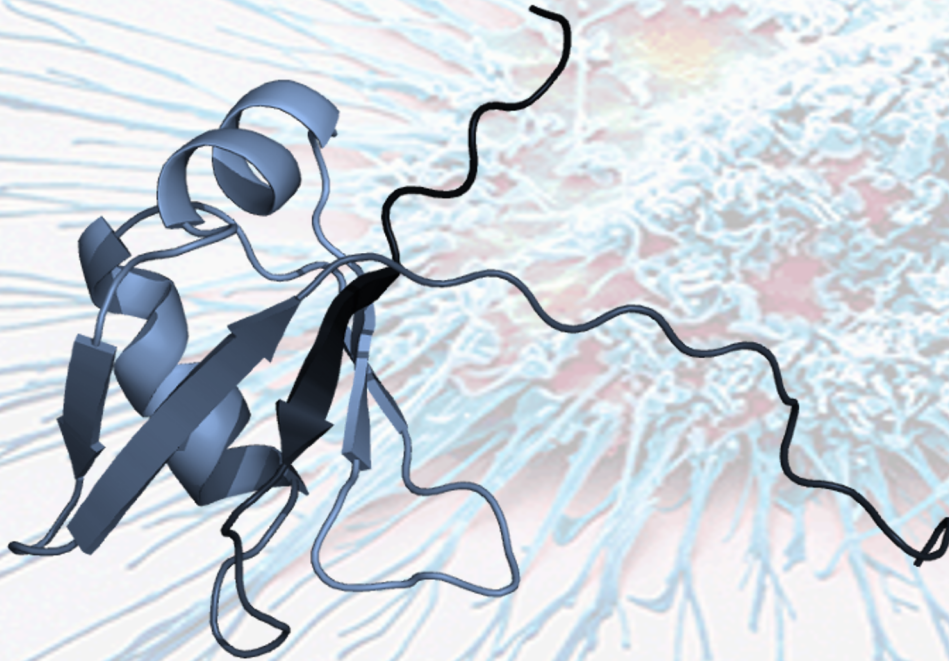
View mor

Top Co-dependencies

CRISPR (Avana) Public 19Q1

	Gene	Pearson correlation
Plot	PRDM1	0.58
Plot	POU2AF1	0.57
Plot	NFKB1	0.50
Plot	MEF2C	0.47
Plot	IKBKB	0.45

Combined RNAi (Broad, Novartis, Marcotte)

	Gene	Pearson correlation
Plot	NFKB1	0.35
Plot	ARMCX6	0.33
Plot	TLNRD1	0.32
Plot	SP2	-0.31
Plot	MYSM1	0.31

Our protein target - TDP-43

February 8, 2022

TDP-43
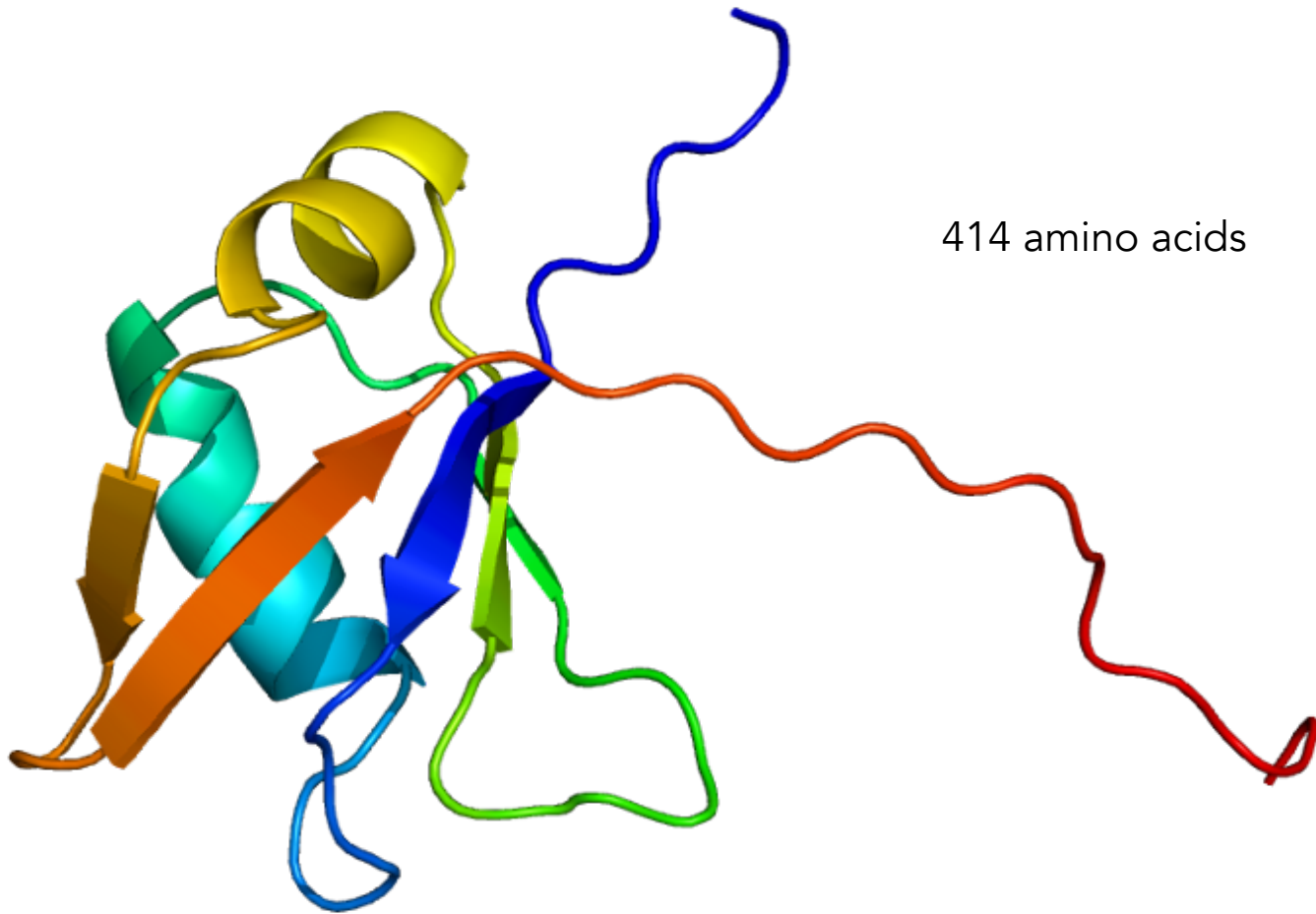
414 amino acids

TAR DNA-binding Protein that is 43 kilodaltons

How big is the typical protein?

E. coli [N=4,303]

median = 277

budding yeast [N=6,723]

median = 358

human HeLa [N=22,257]

median = 431

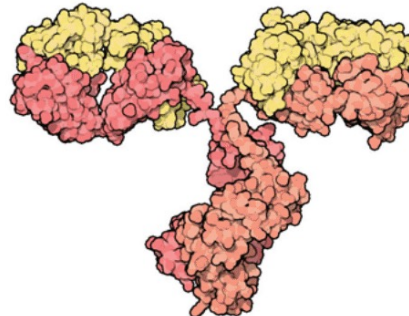frequency vs protein length (AA)
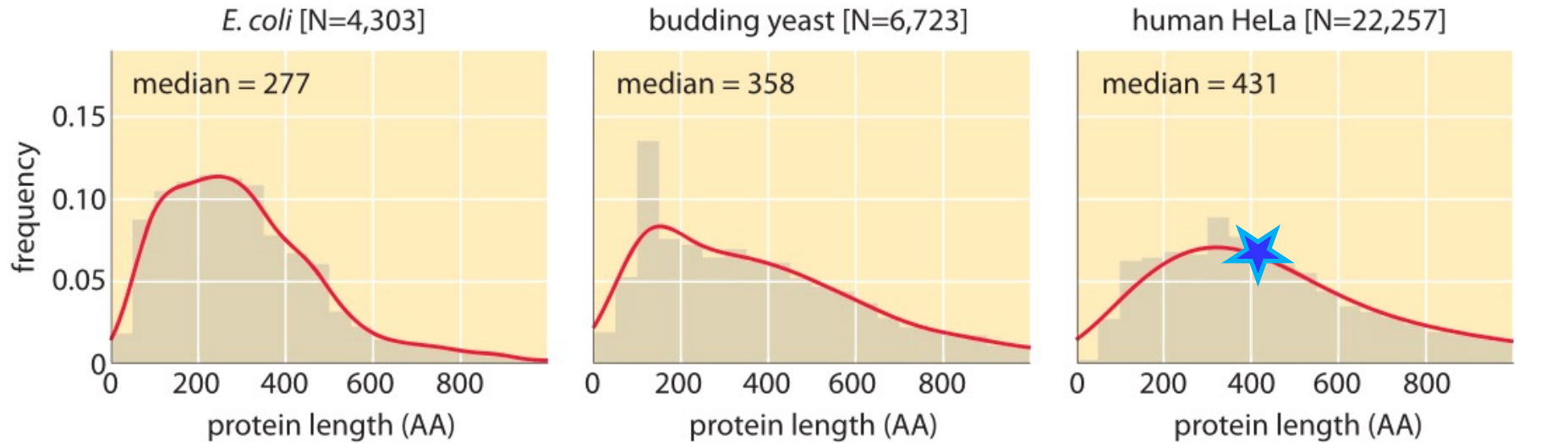
TDP-43

insulin
5.8 kDa

trypsin
23.3 kDa

hemoglobin
64.5 kDa

hexokinase
102 kDa

immunoglobulin G
150 kDa

ATP synthase complex
>500 kDa

Predicted Genomic Lengths, *Trends in Genetics* (2001)
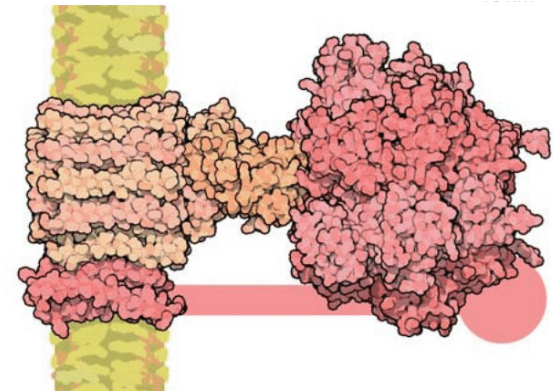
Domain structure of TDP-43
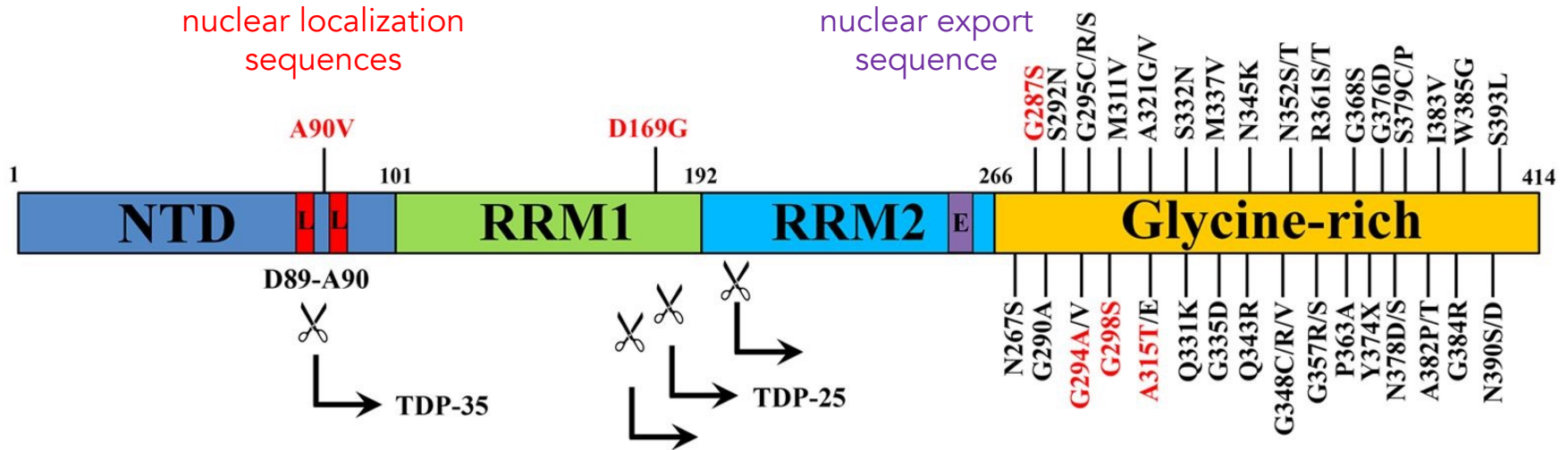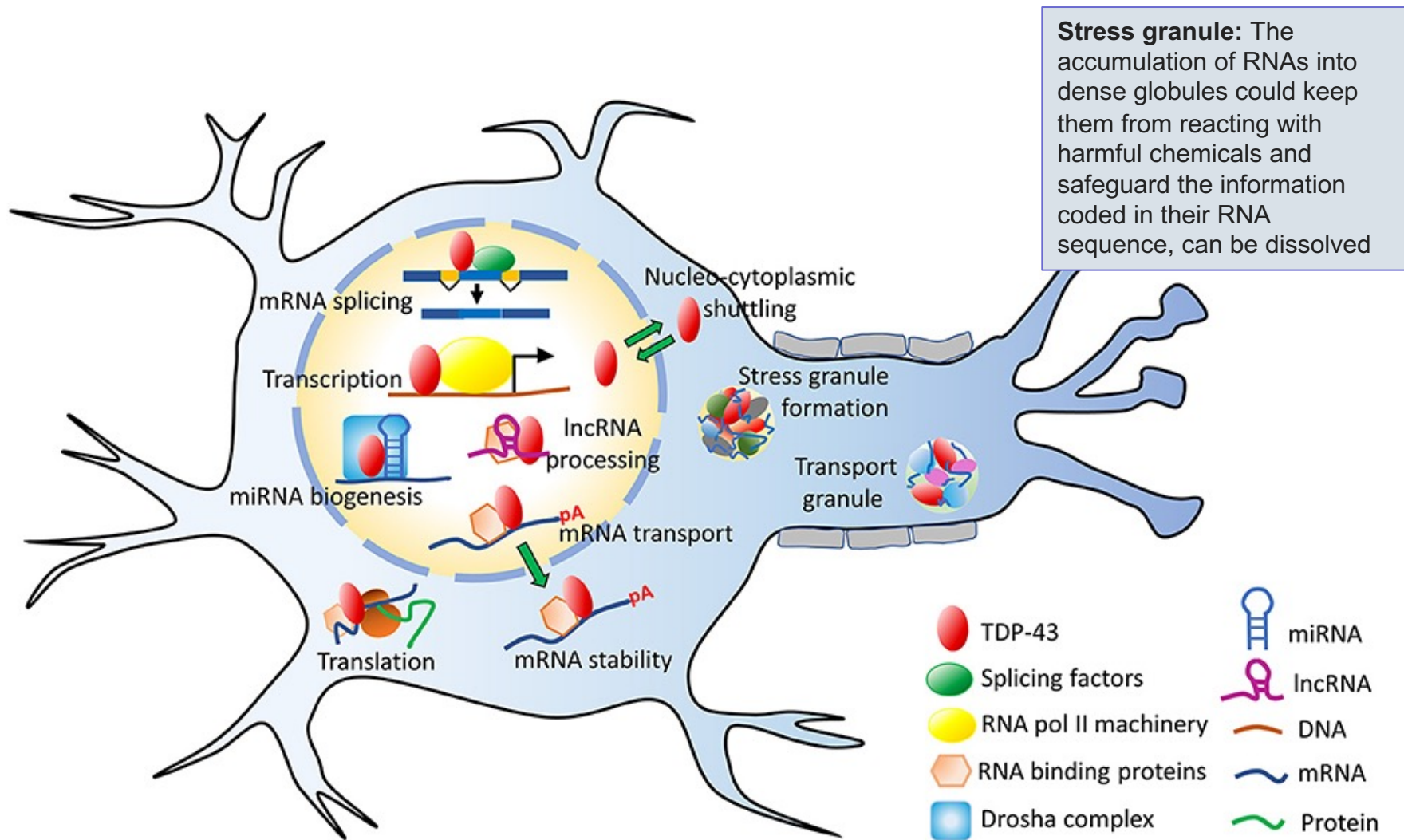

Four major domains

NTD   = N-terminal domain
RRM1   = RNA-recognition motif 1
RRM1   = RNA-recognition motif 2
Gly-rich = C-terminal glycine-rich domain

50 missense mutations
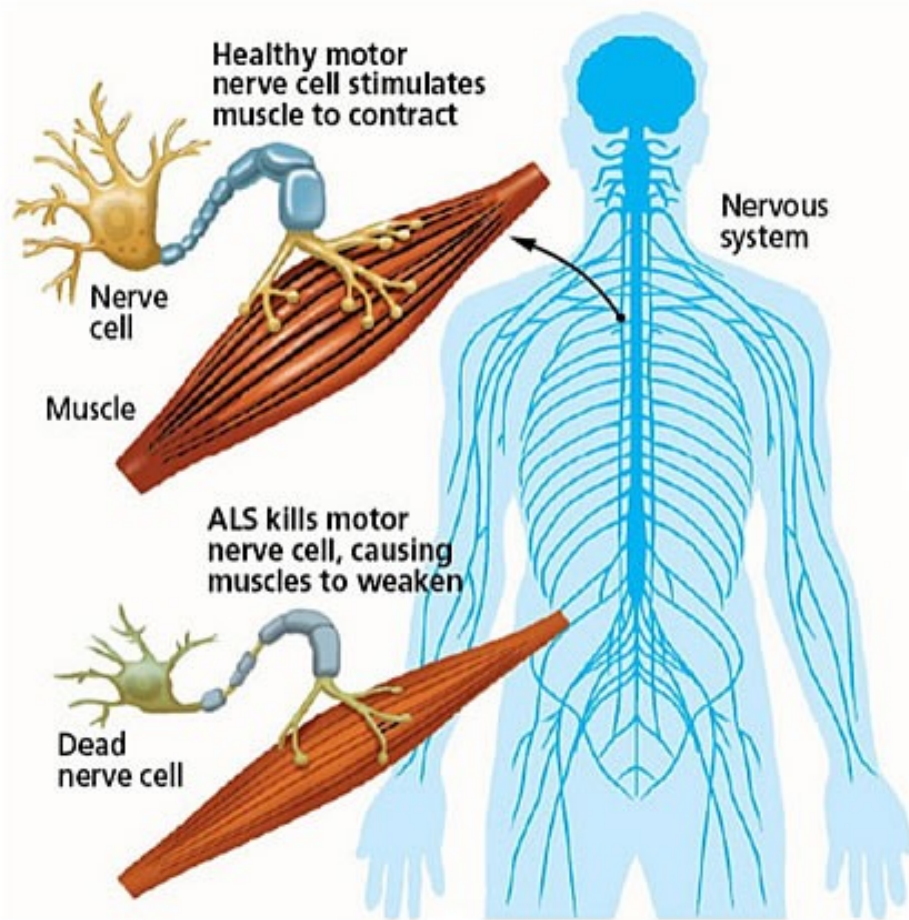identified in ALS patients
(prevalent mutations in red)

multiple cleavage products

Nuclear and cytoplasmic functions of TPD-43

Stress granule: The accumulation of RNAs into dense globules could keep them from reacting with harmful chemicals and safeguard the information coded in their RNA sequence, can be dissolved

mRNA splicing

Transcription

miRNA biogenesis

lncRNA processing

Nucleo-cytoplasmic shuttling

Stress granule formation

Transport granule

mRNA transport

Translation

mRNA stability

TDP-43
Splicing factors
RNA pol II machinery
RNA binding proteins
Drosha complex

miRNA
lncRNA
DNA
mRNA
Protein

Prasad et al, Frontiers in Molecular Neuroscience (2019)

Amyotrophic lateral sclerosis
ALS or Lou Gehrig's disease

Healthy motor nerve cell stimulates muscle to contract

Nerve cell

Muscle

ALS kills motor nerve cell, causing muscles to weaken

Dead nerve cell

Nervous system

- progressive neurodegenerative disease that destroys motor neurons

- when motor neurons cannot send impulses to muscle, the muscles begin to waste away

- nerve cell death makes it impossible for the brain to control muscles or signal for them to move

- eventually, all muscles are affected, including arms and hands, legs and feet, and those that control swallowing and breathing

ALS stats

- 90% of ALS cases are sporadic and strike any race or ethnic background, at any age

- ALS is responsible for 2 deaths per 100,000 people (annually), greater than Huntington's or multiple sclerosis

- ~5,000 patients diagnosed each year in the US, ~30,000 at any given time

- life expectancy from diagnosis is 2-5 years, 20% live longer

- 80% cases begin between at 40-70 yo

- Gulf War veterans develop ALS at ~2x the rate of the typical population

- care costs are high (avg. >$200k/yr)

Pete Frates, Who Promoted the Ice Bucket Challenge, Dies at 34

The former college baseball player's involvement in the viral trend helped raise more than $100 million toward fighting A.L.S.

Pete Frates and his wife, Julie, at a Boston Red Sox game in 2015. He helped raise more than $100 million toward fighting amyotrophic lateral sclerosis, a disease he learned he had in 2012. Elise Amendola/Associated Press

By Jonah Engel Bromwich

Dec. 9, 2019

PBS
NEWS
HOUR

‹

Go Deeper

als

amyotrophic lateral sclerosis

lou gehrig's disease

stephen hawking

By –
Katherine Harmon

Leave a comment

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f 🐦

How did Stephen Hawking live 55 years with ALS?

Science   Jan 9, 2012 10:59 AM EST

died age 76

Lou Gehrig
Baseball
died 3 yr pd

Ezzard Charles
Boxer
died 8 yr pd

Dwight Clark
Football
died 3 yr pd

Mao Zedong
died of heart attack

Hideyuki Ashihara
Karate Master
died 8 yr pd

Marián Čišovský
Soccer
died 6 yr pd

Joost van der Westhuizen
Rugby
died 6 yr pd
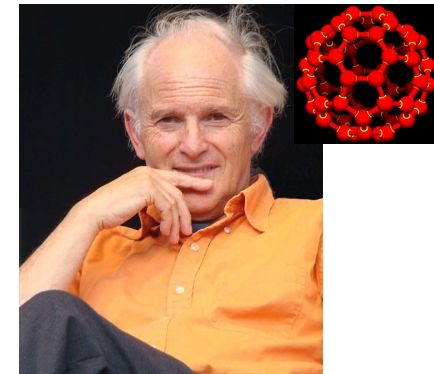
Nobel Laureate
Harry Kroto
died 2 yr pd

Women?

'sporadic' cases – 90%, usually in 50s
2x more frequent in men than women

- -

familial cases – 10%, younger onset
affects men and women equally

several gene mutations have been discovered in
familial disease (FALS):

hexanucleotide (GGGCCC) repeat expansion in non-coding
region of C9ORF72 gene on chromosome 9p21

SOD1 – Cu/Zn superoxide dismutase

TDP-43 – RNA processing protein that forms toxic
neuronal and glial inclusion bodies
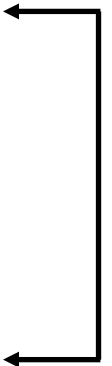
Massive unmet therapeutic need for ALS patients
only one drug available
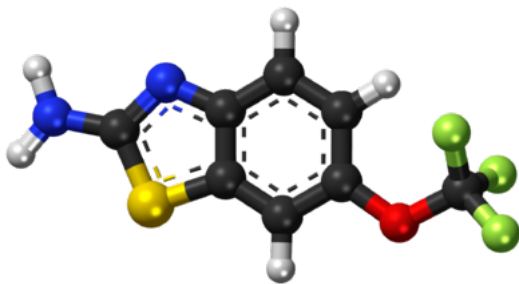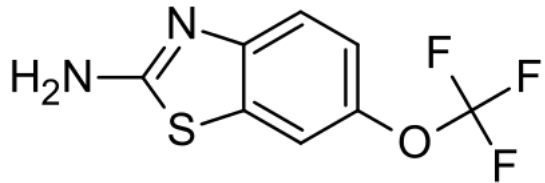
Riluzole

- delays the onset of ventilator-dependence

- may increase survival by 2-3 months

- 9% gain in probability of surviving 1 year

- many side effects

- interacts with sodium channels in damaged neurons

- may non-specifically interact with other receptors (kainite, NMDA, $GABA_A$)

- primary mechanism of action is stimulation of glutamate uptake

- glutamate lingers at synapses of damaged neurons and swift clearance is necessary

Domain structure of TDP-43
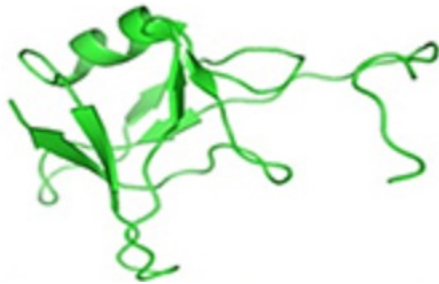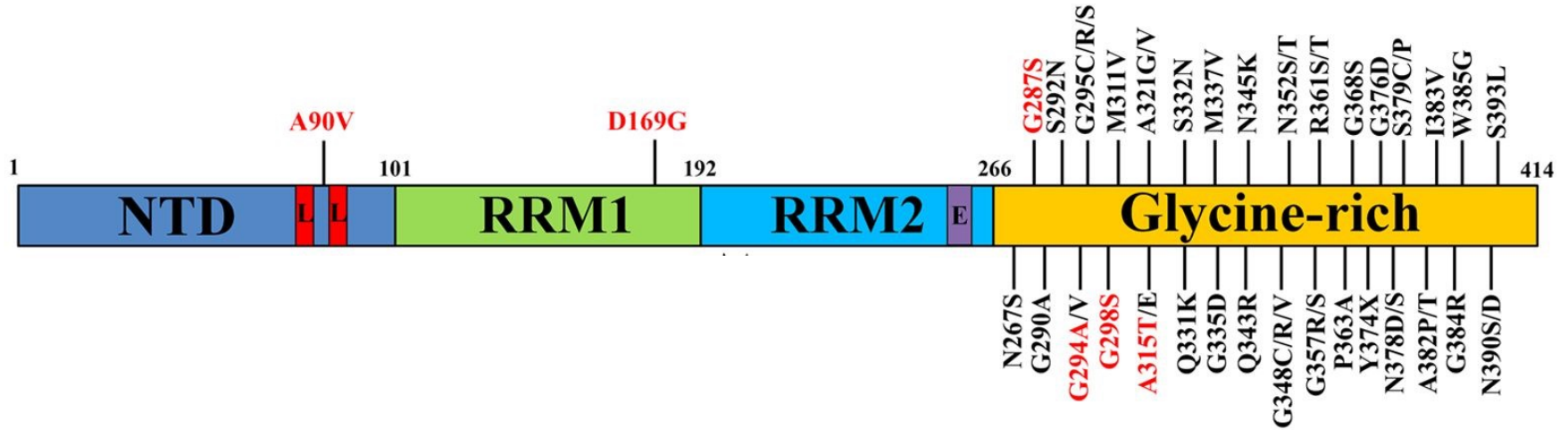
NTD region
aa 1-77
PDB ID = 2N4P

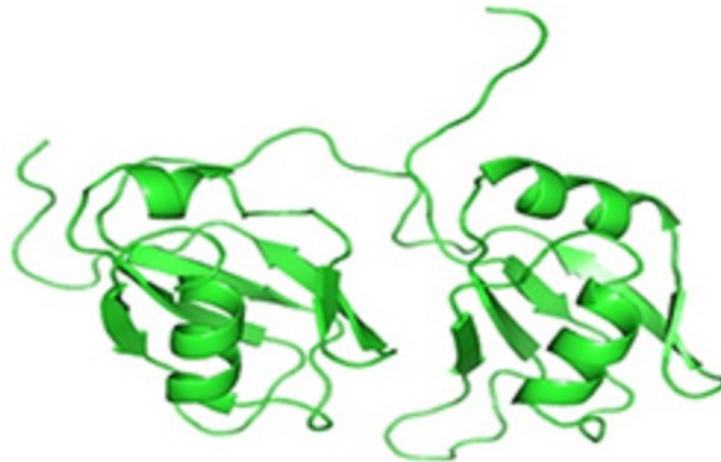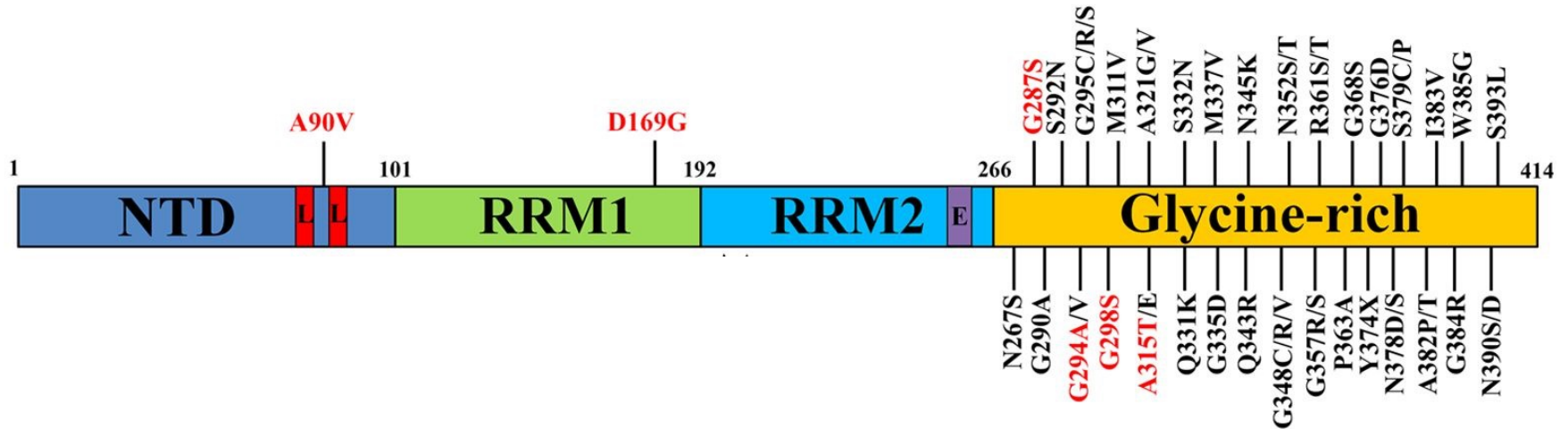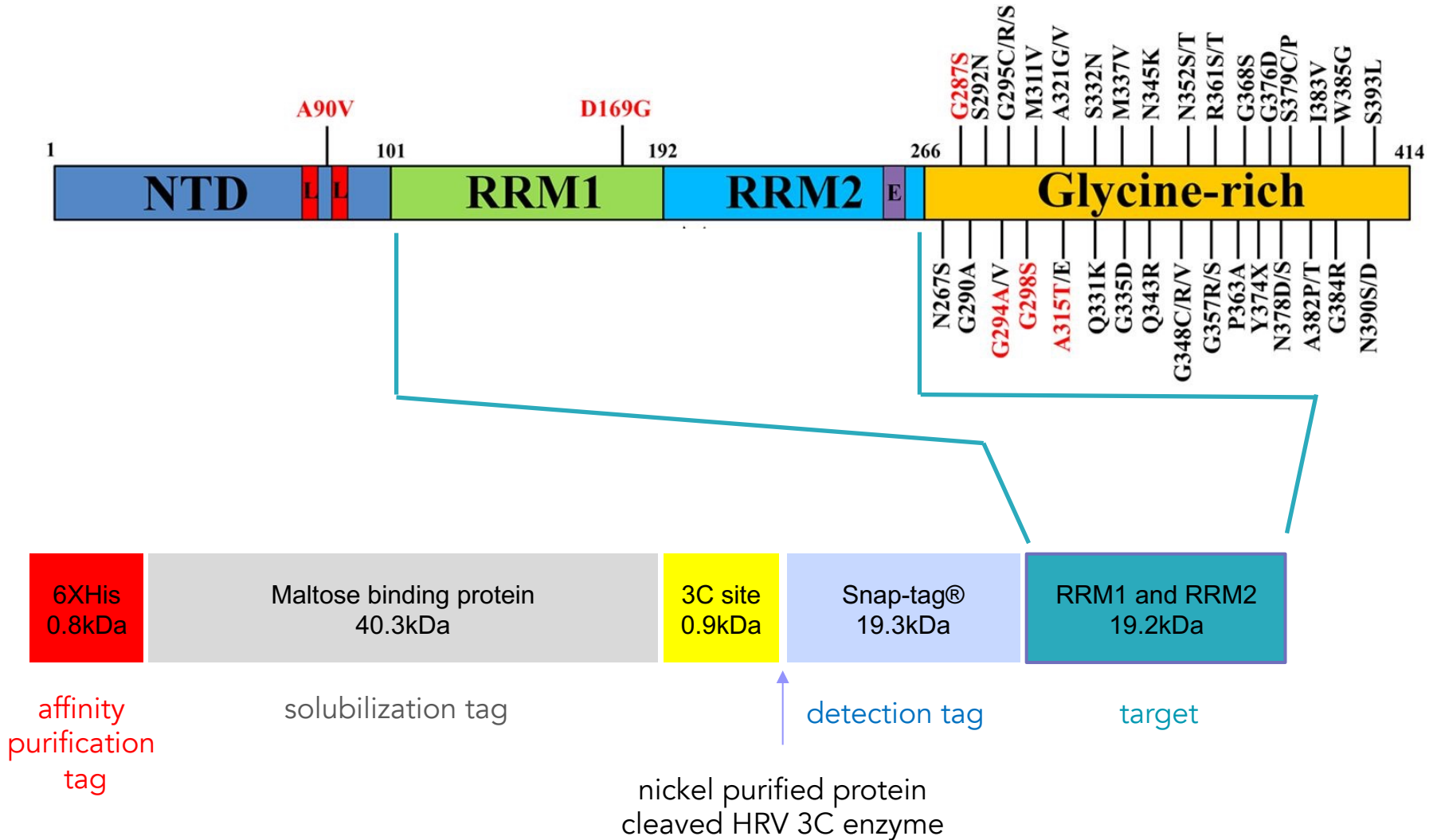# Domain structure of TDP-43
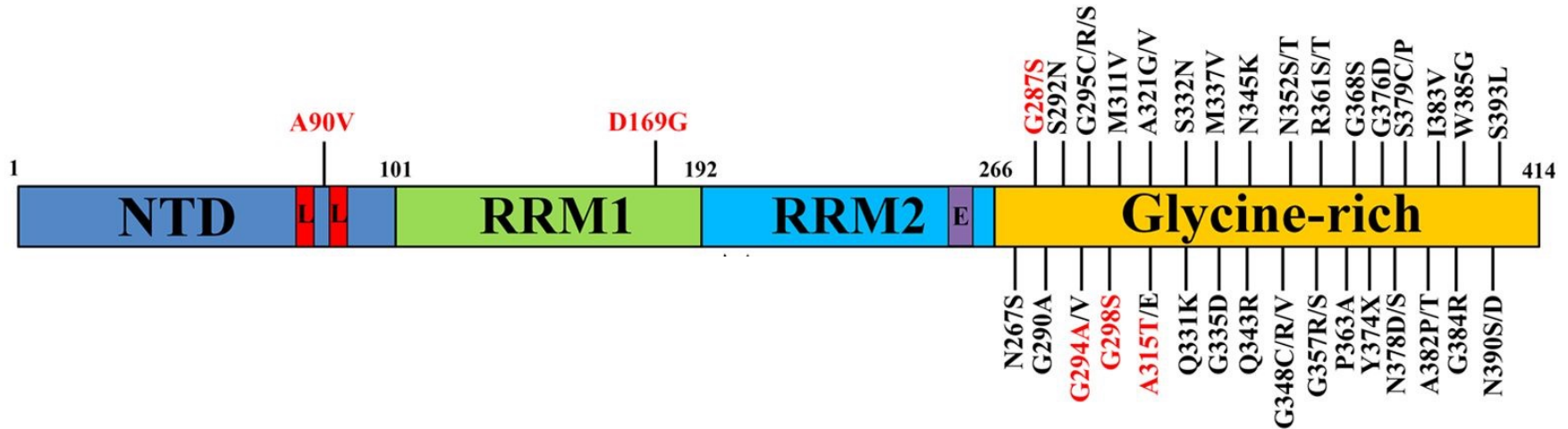
C-term
aa 311-360
PDB ID = 2N3X
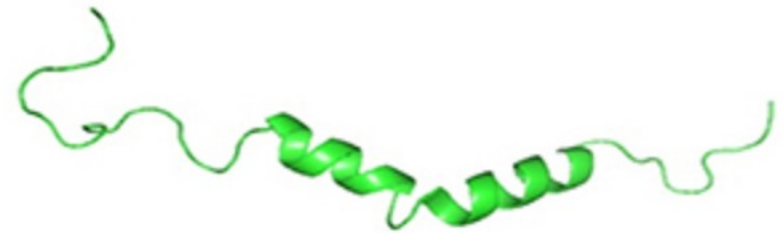
Low complexity domain (LCD)
tendency to aggregate

Domain structure of TDP-43

Tandem RNA-binding motifs
aa 102-269
PDB ID = 4BS2

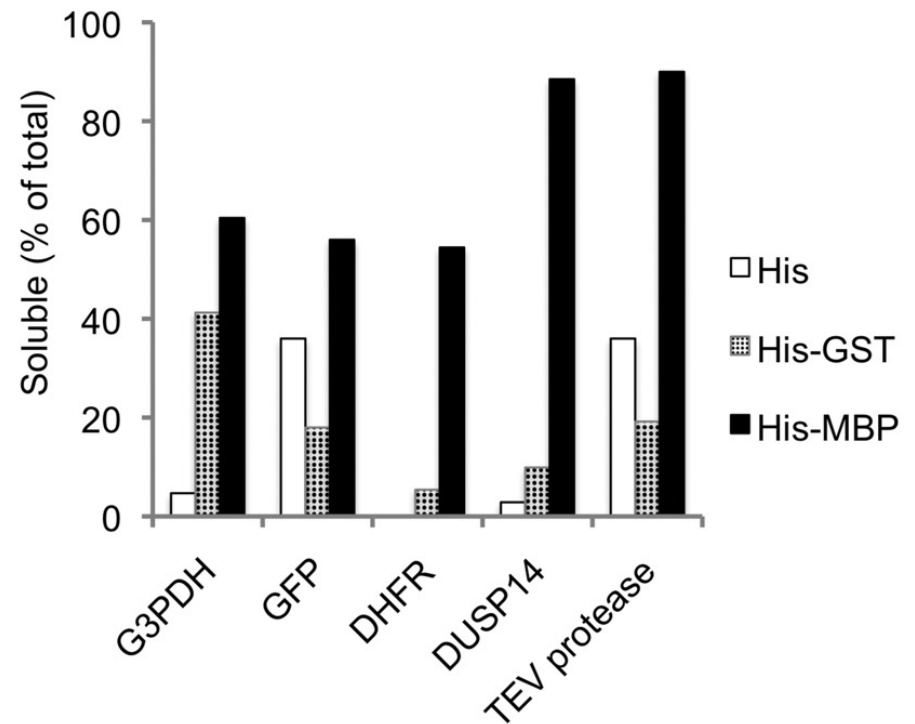# Domain structure of TDP-43

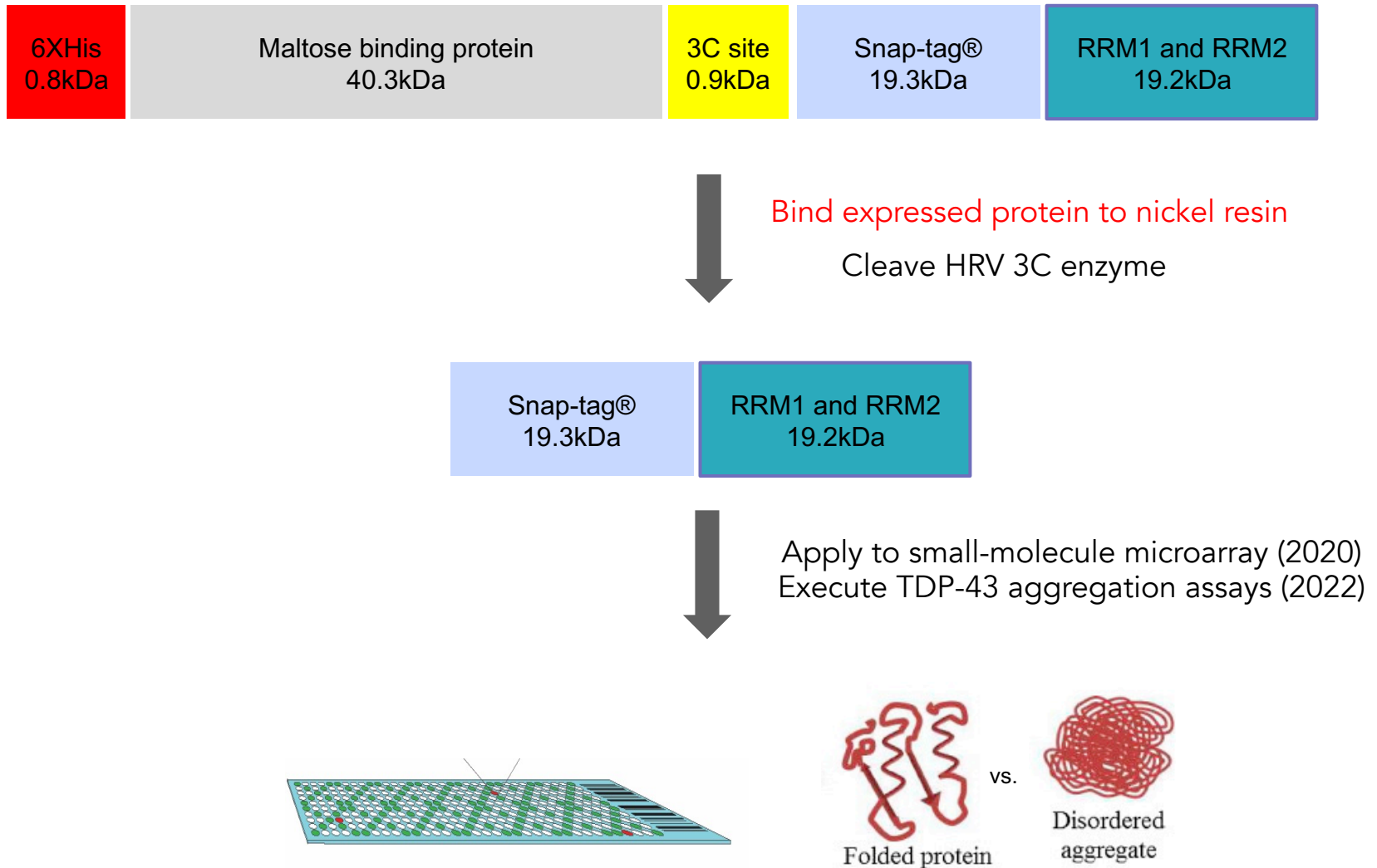# Maltose Binding Protein (MBP) is an *enhancer of protein solubility*

fusion protein architecture

comparison of solubility across multiple passengers

TDP-43 purification in 20.109 lab

6XHis 0.8kDa	Maltose binding protein 40.3kDa	3C site 0.9kDa	Snap-tag® 19.3kDa	RRM1 and RRM2 19.2kDa

Bind expressed protein to nickel resin

Cleave HRV 3C enzyme

Snap-tag® 19.3kDa	RRM1 and RRM2 19.2kDa

Apply to small-molecule microarray (2020)
Execute TDP-43 aggregation assays (2022)

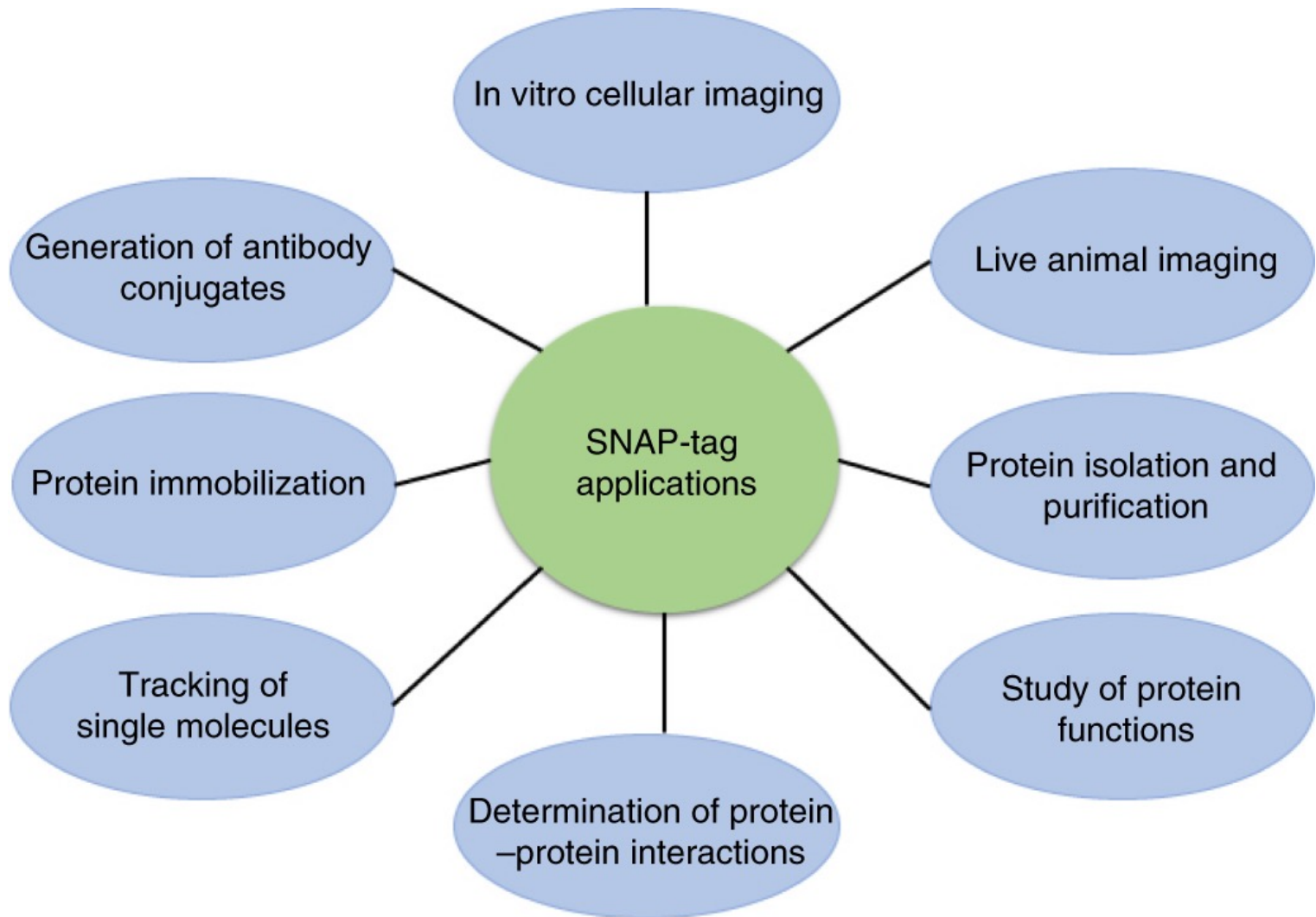Folded protein   vs.   Disordered aggregate

SNAP tags can be fused to proteins and further specifically and covalently tagged with a ligand

dye labeling reaction

engineered O-6-methylguanine-DNA methyltransferase (MGMT)

O^6-benzylguanine derivatives
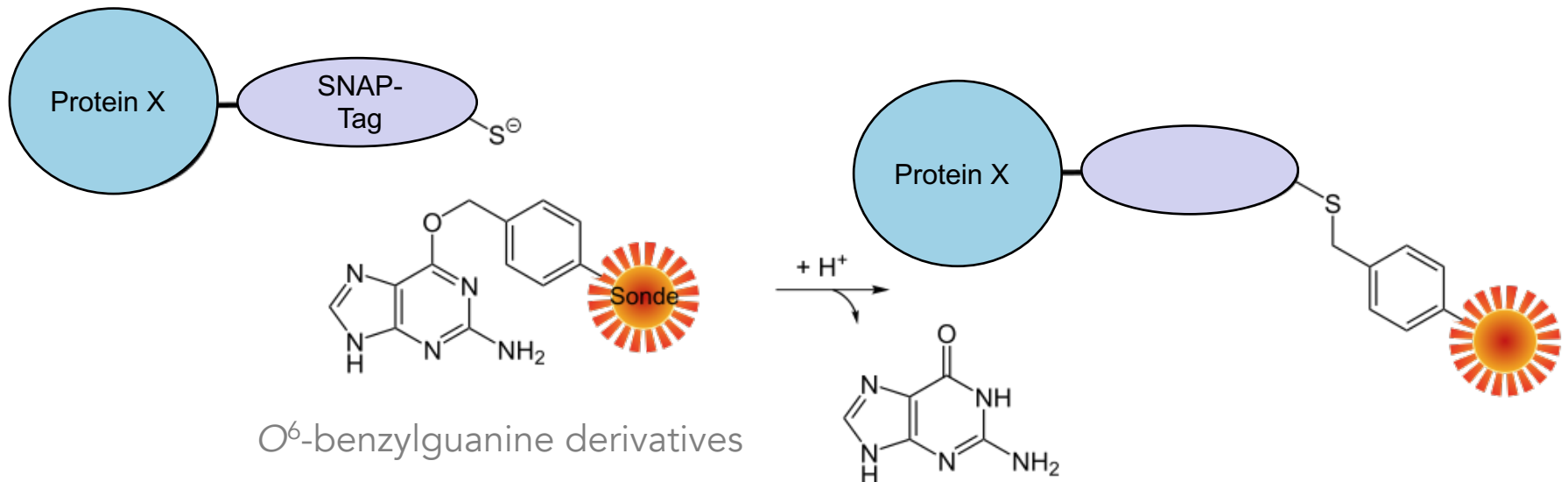
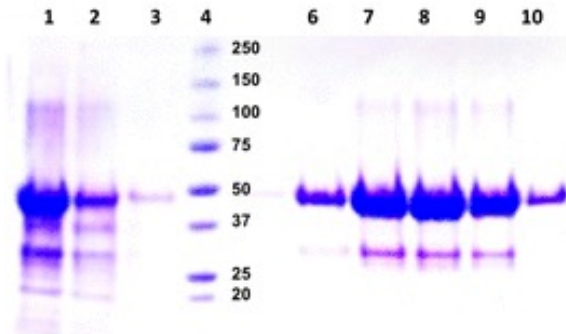# SNAP tags can be used in many applications

October 2019

Isolation and characterization of soluble human full-length TDP-43 associated with neurodegeneration

Mirella Vivoli Vega, Alessia Nigro, Simone Luti, Claudia Capitini, Giulia Fani, Leonardo Gonnelli, Francesca Boscaro, and Fabrizio Chiti ✉
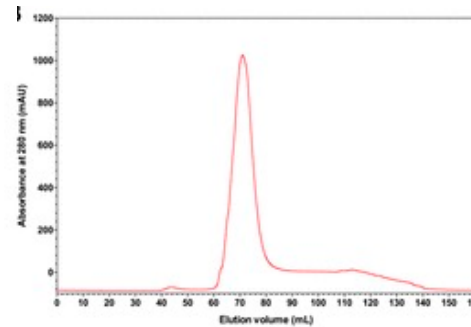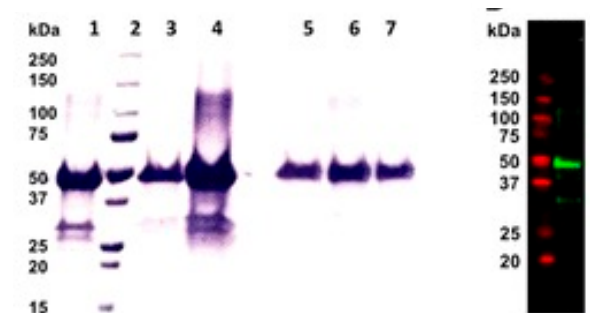
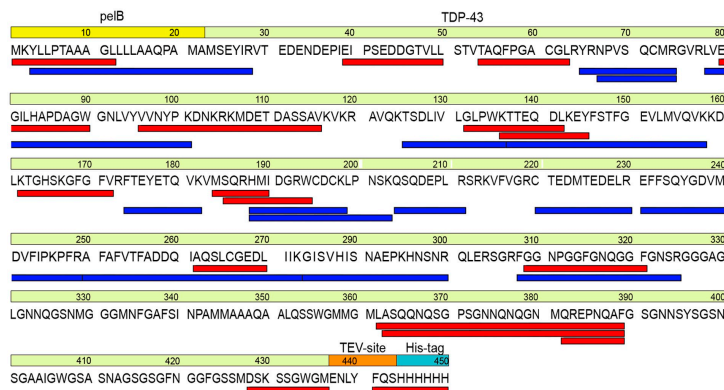nickel chromatography (denaturing conditions)

size exclusion column (denaturing conditions)

refolding & western blot

mass spectrometry - MW & sequence
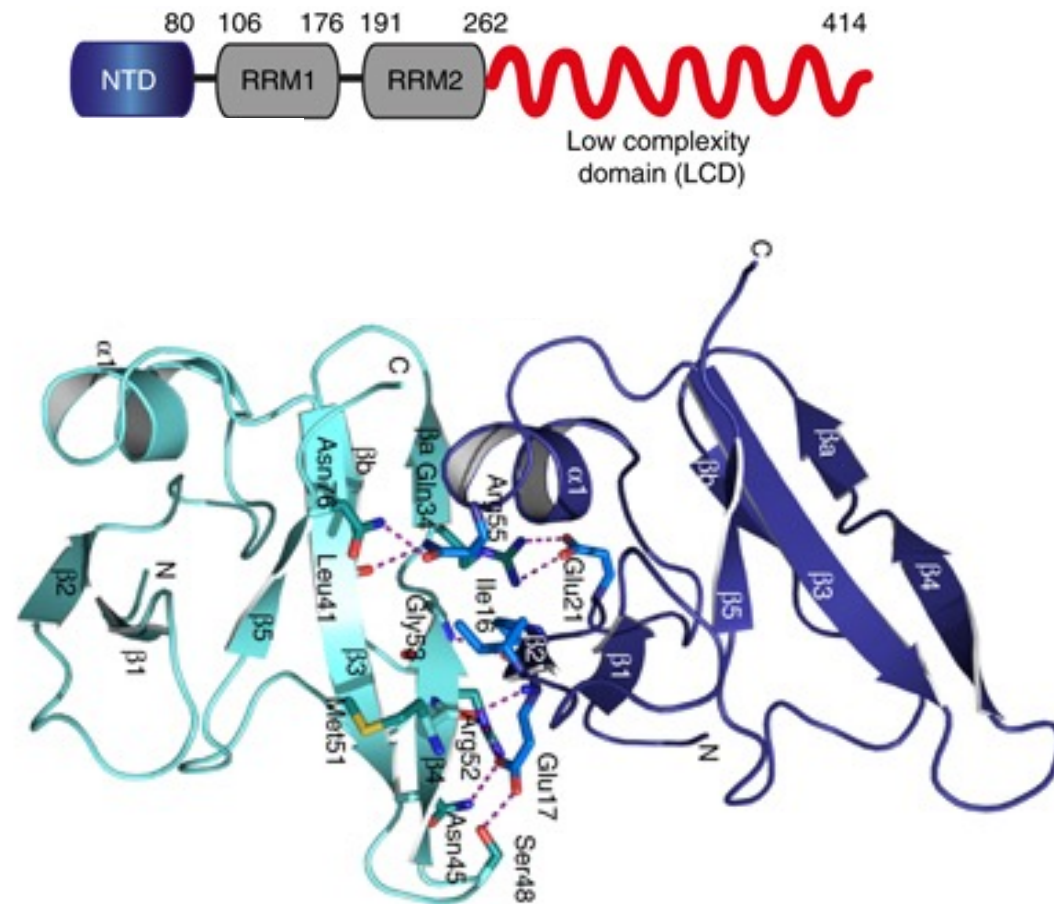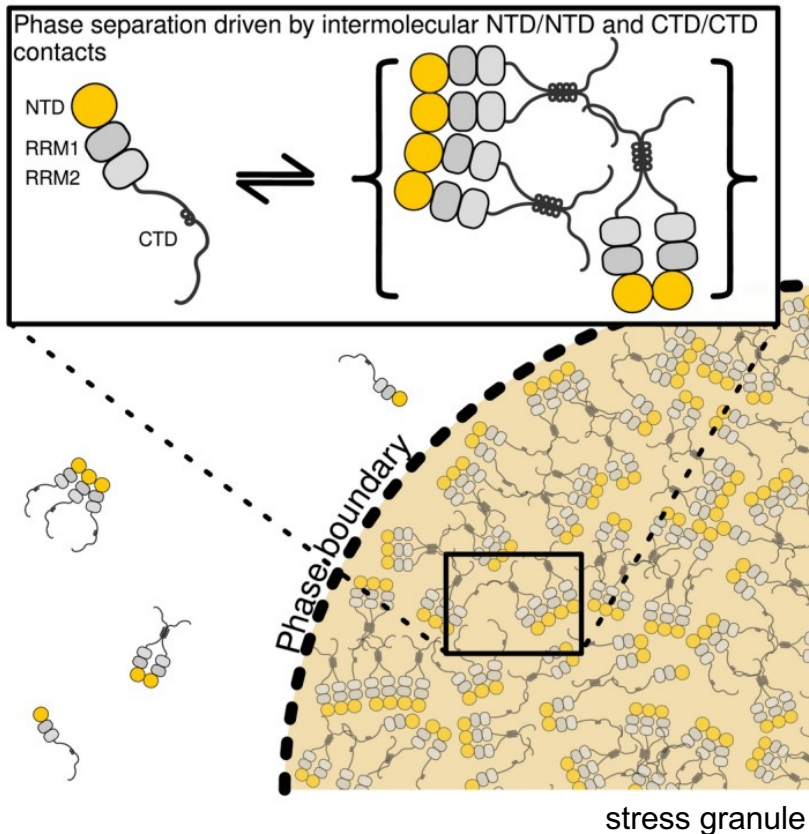
analytical SEC reveals a dimer!

$D_h = 130 \pm 3$ Å

$D_h = 196 \pm 3$ Å
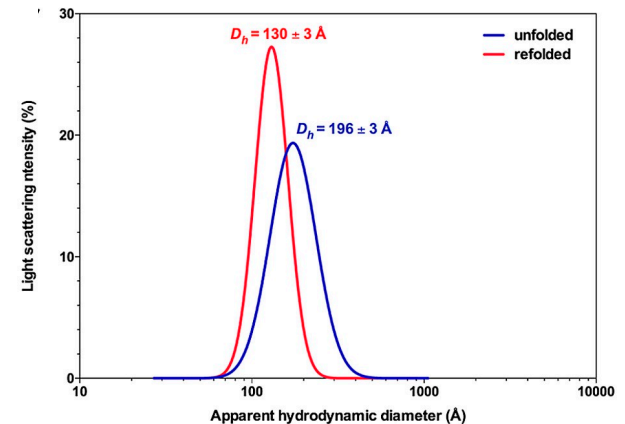
N-Terminal Domain (NTD) forms homodimers in crystal structures

Afroz et al, Nature Communications (2017)

NTD orientation plays a role in TDP-43 polymerization and phase separation
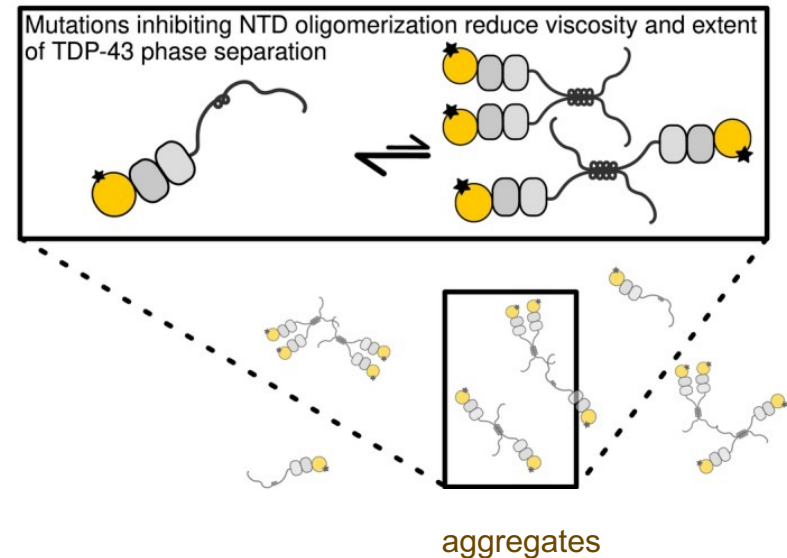

TDP-43 oligomers:
Functional high-order oligomers promote phase separation

NTD oligomerization-disrupting mutants:
Disruption of NTD/NTD contacts destabilizes phase separation

stress granule

aggregates

peptidomimetics or small molecules
as a therapeutic strategy
to sequester mutants from aggregation?

RNA binding assists liquid-liquid phase separation of TDP-43 in disease

Mann et al, Neuron 102 (2):321-338 (2019)
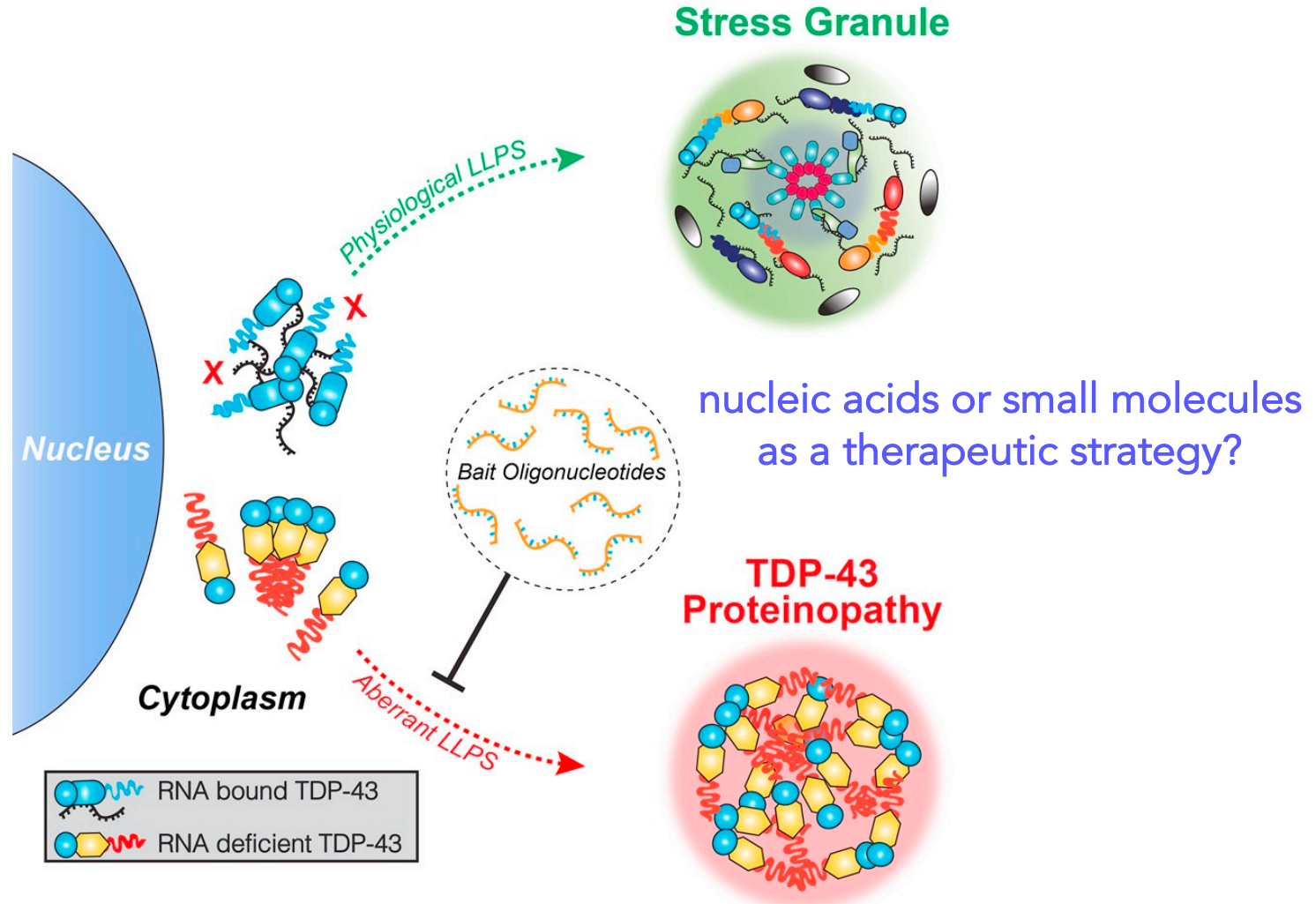
Companies are interested in agents to control phase separation in an effort to develop drugs for neurodegeneration and cancer

our knowledge is rapidly evolving

RESULTS BY YEAR   3,443 results

1995   2022

Disease-linked TDP-43 hyperphosphorylation suppresses TDP-43 condensation and aggregation

Lara A Gruijs da Silva [1] [2], Francesca Simonetti [1] [2] [3], Saskia Hutten [1], Henrick Riemenschneider [3], Erin L Sternburg [1], Lisa M Pietrek [4], Jakob Gebel [5], Volker Dötsch [5], Dieter Edbauer [2] [3] [6], Gerhard Hummer [4] [7], Lukas S Stelzl [1] [4] [8] [9], Dorothee Dormann [1] [6] [9]
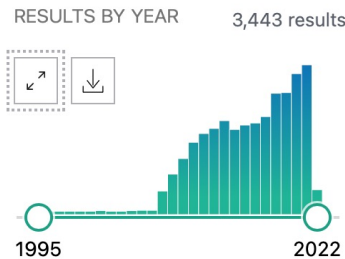
Affiliations + expand
PMID: 35112738   DOI: 10.15252/embj.2021108443

Low-level overexpression of wild type TDP-43 causes late-onset, progressive neurodegeneration and paralysis in mice

Chunxing Yang [1], Tao Qiao [1], Jia Yu [2], Hongyan Wang [1], Yansu Guo [1], Johnny Salameh [3], Jake Metterville [3], Sepideh Parsi [1], Issa Yusuf [1], Robert H Brown [3] [4] [5], Huaibin Cai [2], Zuoshang Xu [1] [4] [5]

Affiliations + expand
PMID: 35113871   PMCID: PMC8812852   DOI: 10.1371/journal.pone.0255710

TREM2 interacts with TDP-43 and mediates microglial neuroprotection against TDP-43-related neurodegeneration

Manling Xie [#] [1] [2], Yong U Liu [#] [3] [4], Shunyi Zhao [1], Lingxin Zhang [5], Dale B Bosco [1], Yuan-Ping Pang [6], Jun Zhong [7], Udit Sheth [2] [8], Yuka A Martens [8], Na Zhao [8], Chia-Chen Liu [8], Yongxian Zhuang [5], Liewei Wang [5], Dennis W Dickson [8], Mark P Mattson [9], Guojun Bu [8], Long-Jun Wu [10] [11] [12]

Affiliations + expand
PMID: 34916658   PMCID: PMC8741737 (available on 2022-06-16)
DOI: 10.1038/s41593-021-00975-6

Structure of pathological TDP-43 filaments from ALS with FTLD

Diana Arseni [1], Masato Hasegawa [2], Alexey G Murzin [1], Fuyuki Kametani [2], Makoto Arai [3], Mari Yoshida [4], Benjamin Ryskeldi-Falcon [5]

Affiliations + expand
PMID: 34880495   PMCID: PMC7612255 (available on 2022-07-01)
DOI: 10.1038/s41586-021-04199-3

Genome-wide identification of the genetic basis of amyotrophic lateral sclerosis

Sai Zhang [1], Johnathan Cooper-Knock [2], Annika K Weimer [1], Minyi Shi [1], Tobias Moll [2], Jack N G Marshall [2], Calum Harvey [2], Helia Ghahremani Nezhad [2], John Franklin [2], Cleide Dos Santos Souza [2], Ke Ning [2], Cheng Wang [3], Jingjing Li [3], Allison A Dilliott [4], Sali Farhan [4], Eran Elhaik [2], Iris Pasniceanu [2], Matthew R Livesey [2], Chen Eitan [6], Eran Hornstein [6], Kevin P Kenna [7], Project MinE ALS Sequencing Consortium; Jan H Veldink [7], Laura Ferraiuolo [2], Pamela J Shaw [2], Michael P Snyder [8]

Collaborators, Affiliations + expand
PMID: 35045337   DOI: 10.1016/j.neuron.2021.12.019

TDP-43 Modulation by Tau-Tubulin Kinase 1 Inhibitors: A New Avenue for Future Amyotrophic Lateral Sclerosis Therapy

Vanesa Nozal [1] [2], Loreto Martínez-González [1] [2], Marta Gomez-Almeria [1], Claudia Gonzalo-Consuegra [3], Paula Santana [4], Apirat Chaikuad [5] [6], Eva Pérez-Cuevas [1] [2], Stefan Knapp [5] [6], Daniel Lietha [7], David Ramírez [7], Sabrina Petralla [8], Barbara Monti [8], Carmen Gil [1], Angeles Martín-Requero [1] [2], Valle Palomo [1] [2], Eva de Lago [2] [3], Ana Martinez [1] [2]

Affiliations + expand
PMID: 34978799   DOI: 10.1021/acs.jmedchem.1c01942

Phosphorylated TDP-43 aggregates in peripheral motor nerves of patients with amyotrophic lateral sclerosis

Nilo Riva [1] [2], Francesco Gentile [1], Federica Cerri [1] [2], Francesca Gallia [3], Paola Podini [1], Giorgia Dina [1], Yuri Matteo Falzone [1] [2], Raffaella Fazio [2], Christian Lunetta [4], Andrea Calvo [5], Giancarlo Logroscino [6], Giuseppe Lauria [7] [8], Massimo Corbo [9], Sandro Iannaccone [10], Adriano Chiò [5], Alberto Lazzerini [11], Eduardo Nobile-Orazio [3], Massimo Filippi [2] [12] [13] [14], Angelo Quattrini [1]

Affiliations + expand
PMID: 35076694   DOI: 10.1093/brain/awab285

TDP-43 aggregation is observed in multiple diseases
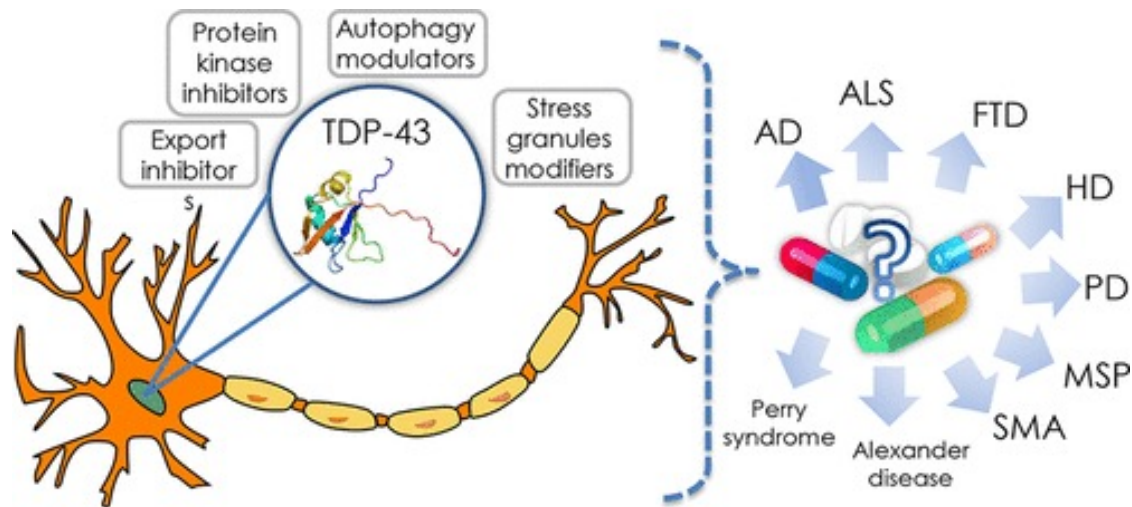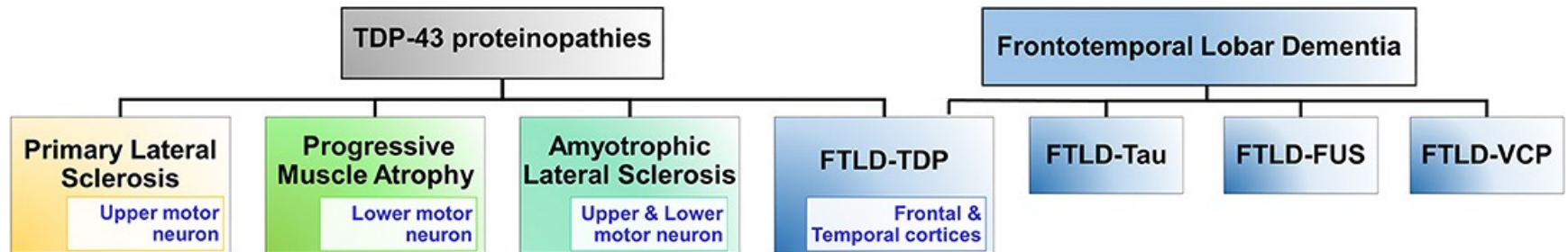
Upcoming lectures

2/3/22	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/8/22	Lecture 2	Our protein target: TDP-43
2/10/22	Lecture 3	Small molecule microarrays
2/15/22	Lecture 4	Quantitative evaluation of protein-ligand interactions
2/17/22	Lecture 5	A ligand discovery vignette: sonic hedgehog
2/22/22	No Class	
2/24/22	Lecture 6	Engineering transcriptional responses with a small molecule
3/1/22	Lecture 7	Wrap up discussion for Mod 1 experiments and report