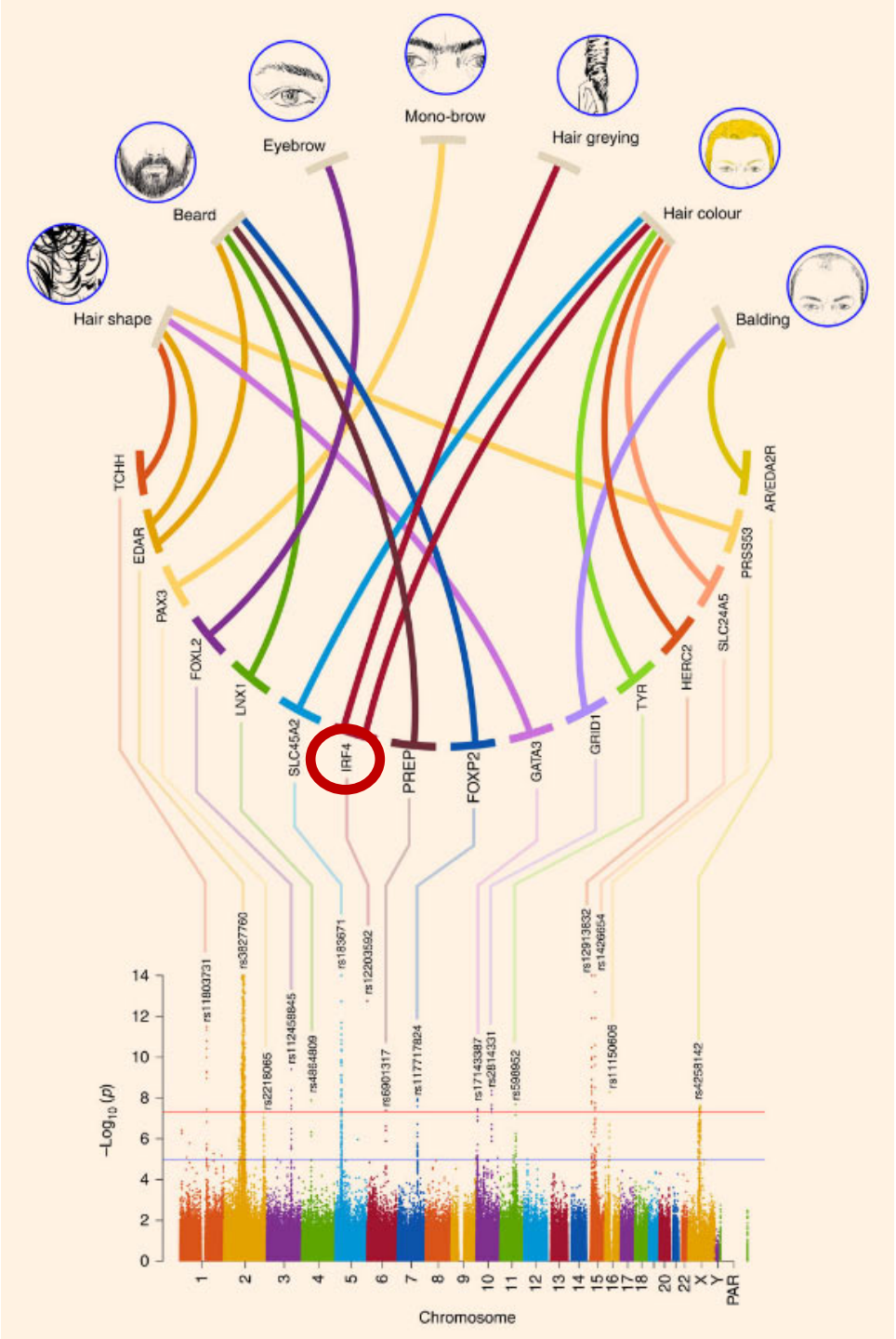
Which process or biomolecule would you study with a chemical probe if you had one in hand?

# Genome Wide Association Study:

Hair-related phenotypes




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


Kaustubh Adhikari et al. Nature Communications, **2016**

**doi: 10.1038/ncomms10815**.


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

depmap

# depmap.org

## Look up dependencies

Enter a gene, cell line, lineage or compound

**Use this portal to:**

UNDERSTAND Dependency profiles at genome-scale across more than 500 human cell lines

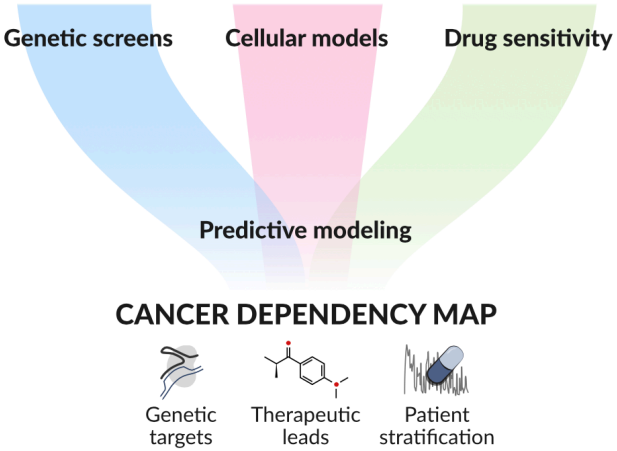
FIND Detailed genetic and pharmacologic characterization of over 1000 cell lines

IDENTIFY Genetic drivers that have functional importance as potential drug targets

SEARCH For cell line models that best represent your research interests

EXPORT Presentation-quality figures

**The goal of the Cancer Dependency Map is to create a comprehensive preclinical reference map connecting tumor features with tumor dependencies to accelerate the development of precision treatments.** Our strategy is to systematically characterize cellular models of cancers and to test those models for sensitivity to genetic and small-molecule perturbations. By integrating data beyond those collected at the Broad, DepMap hopes to develop a complete understanding of the vulnerabilities of cancer, identify targets for therapeutic development, and design strategies to optimize patient responses to those therapies.

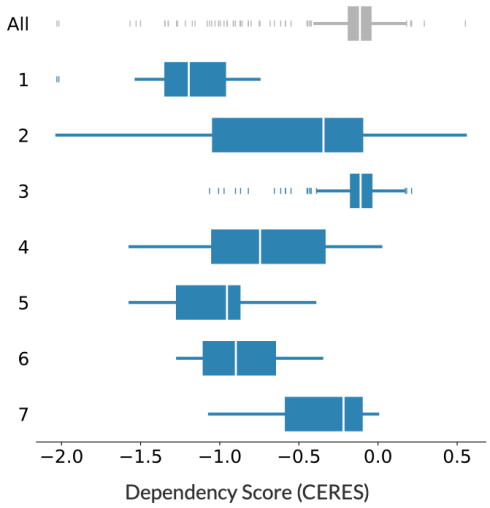



To date DepMap has profiled more than 500 cell lines. Over the next several years we will greatly expand the diversity of cell lines profiled for genetic vulnerabilities with quarterly data release. Additionally, limited drug sensitivity data are available.

# IRF4

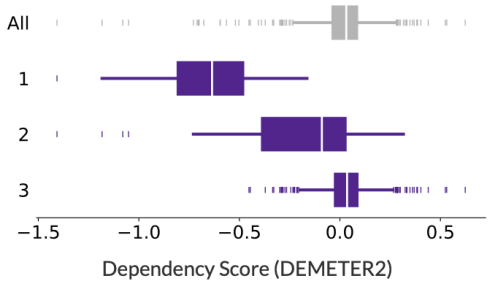
## Enriched Lineages

### CRISPR




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)

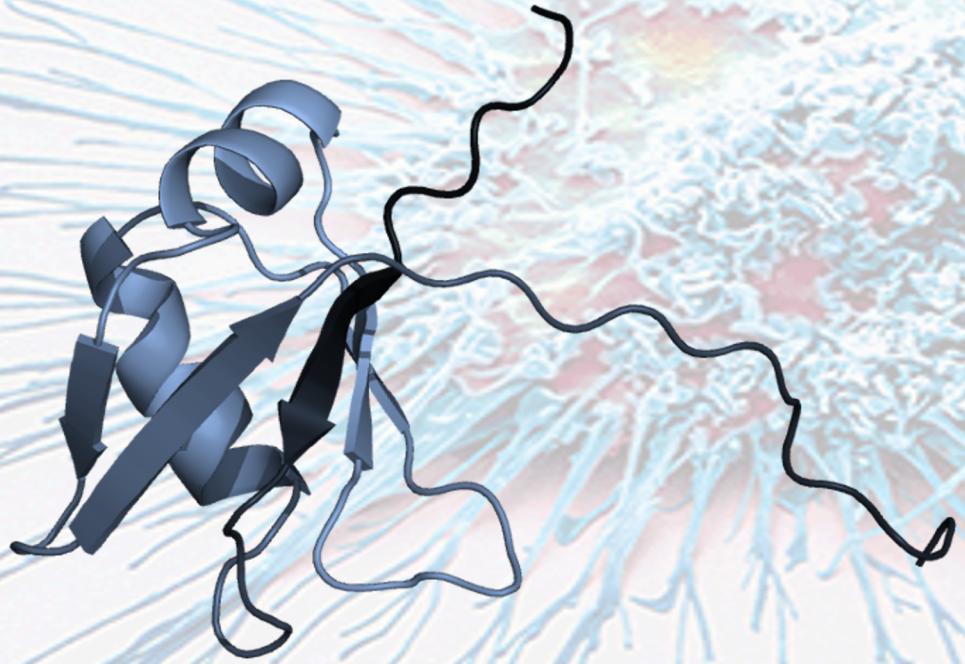
### RNAi




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

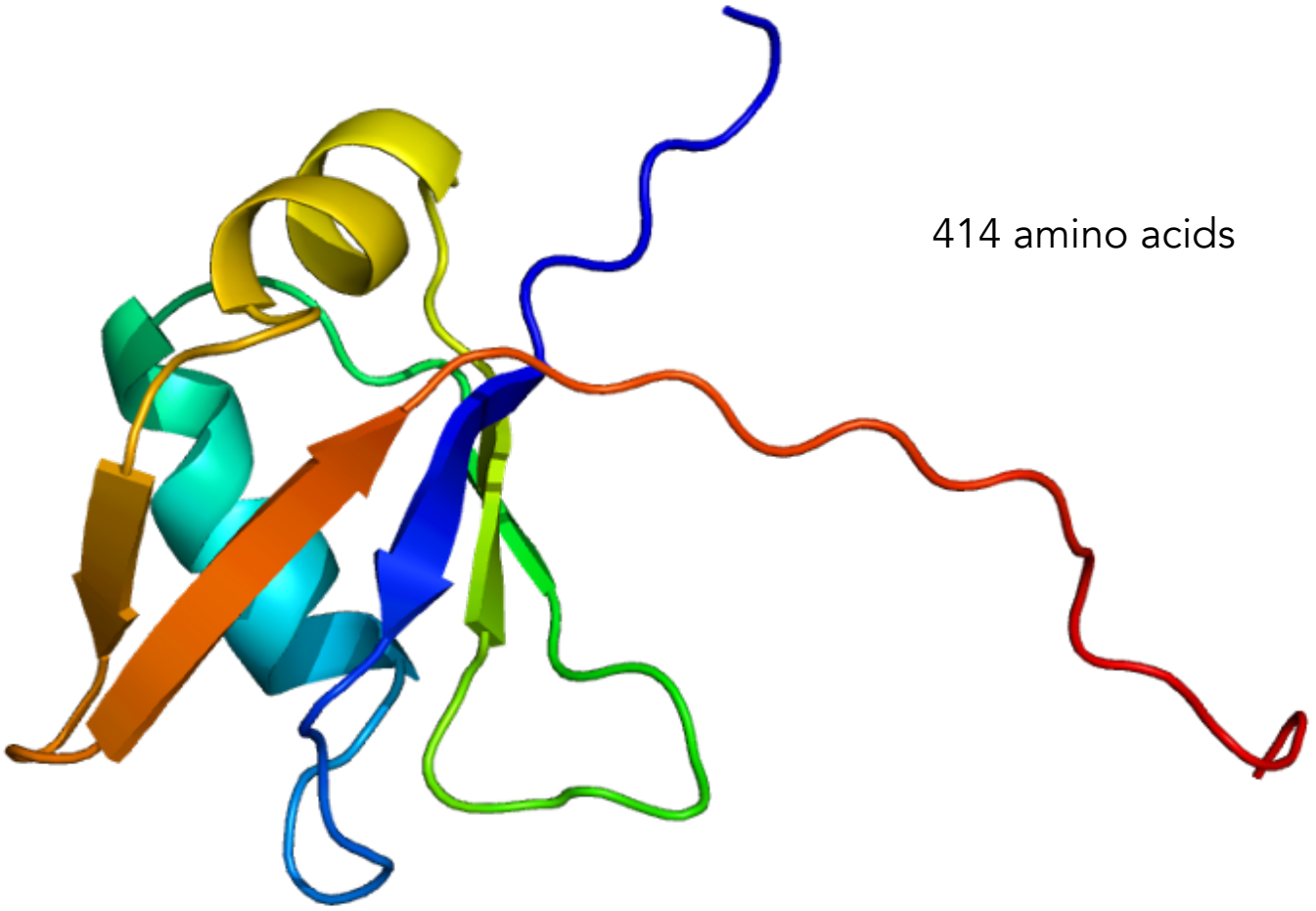
# Our screening target

## TDP-43



February 11, 2020

# TDP-43



414 amino acids

TAR DNA-binding Protein that is 43 kilodaltons

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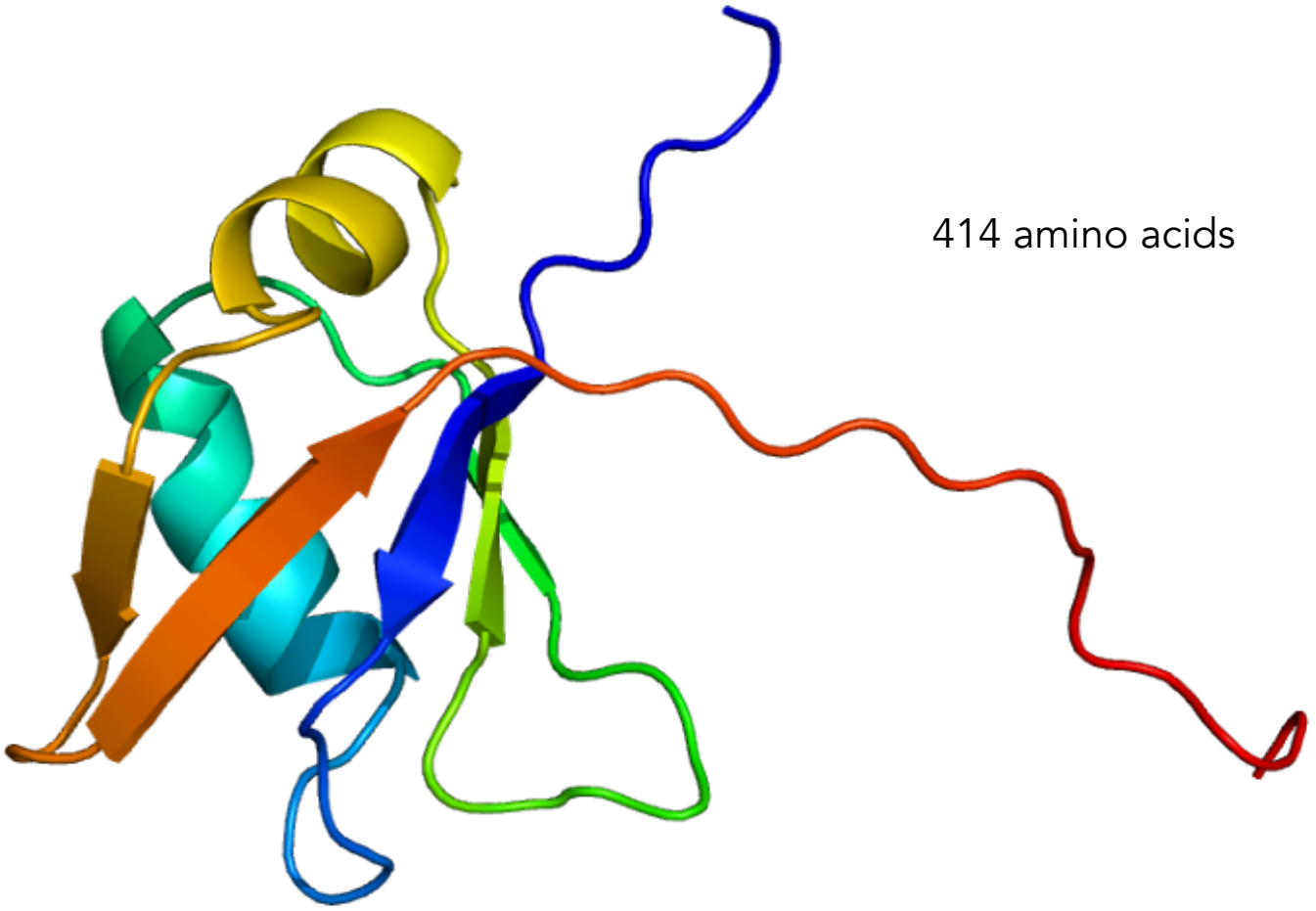

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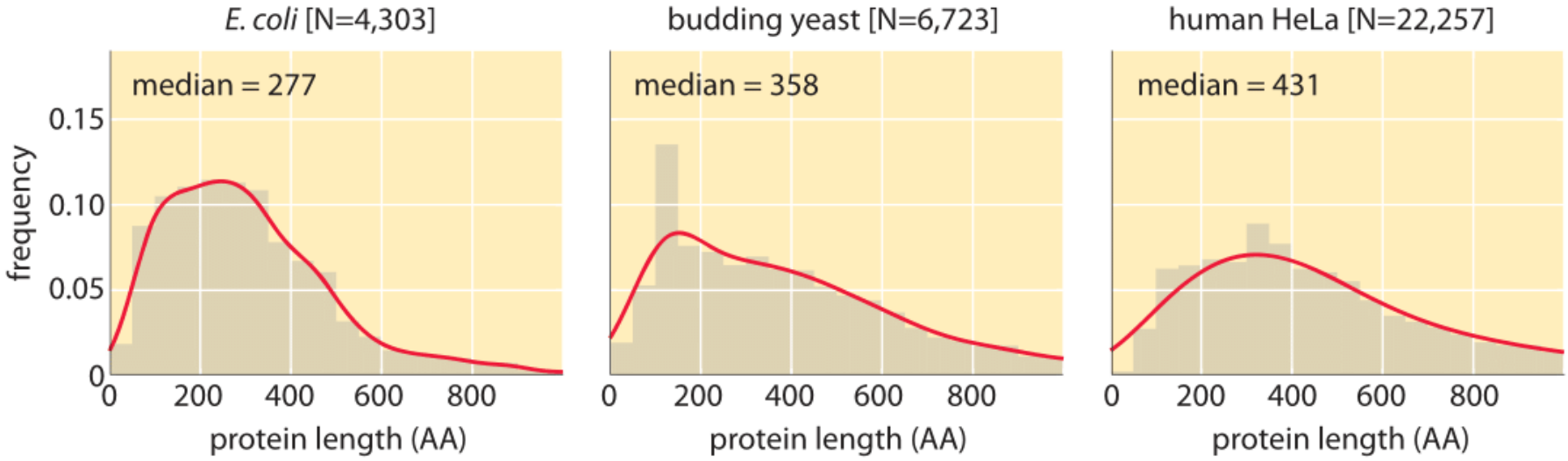


Predicted Genomic Lengths, *Trends in Genetics* (2001)

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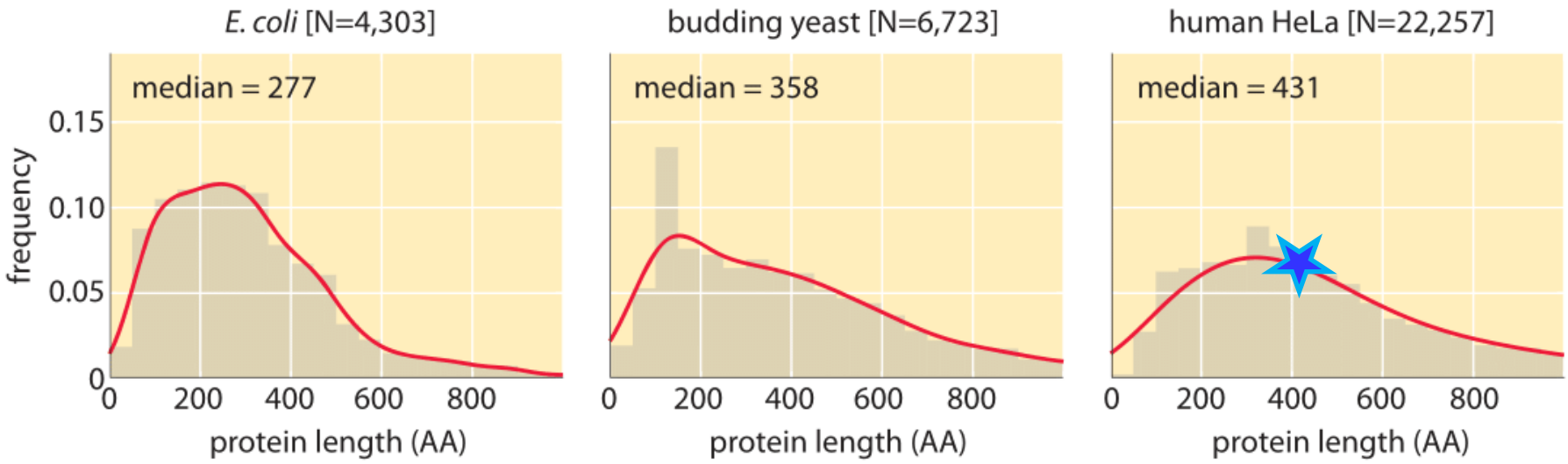


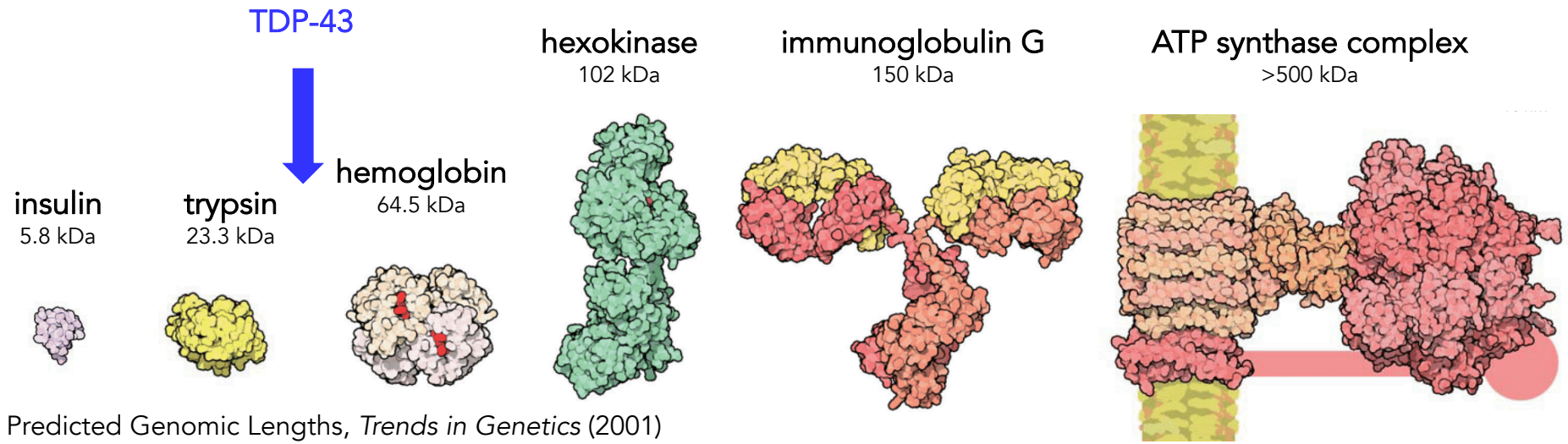


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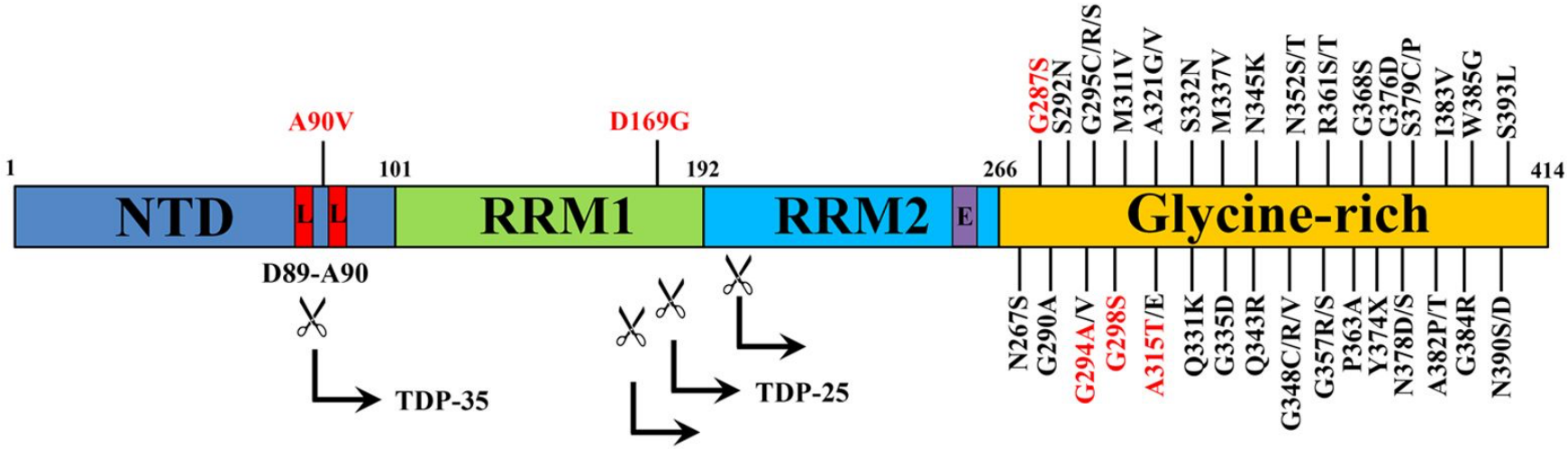


Four major domains

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

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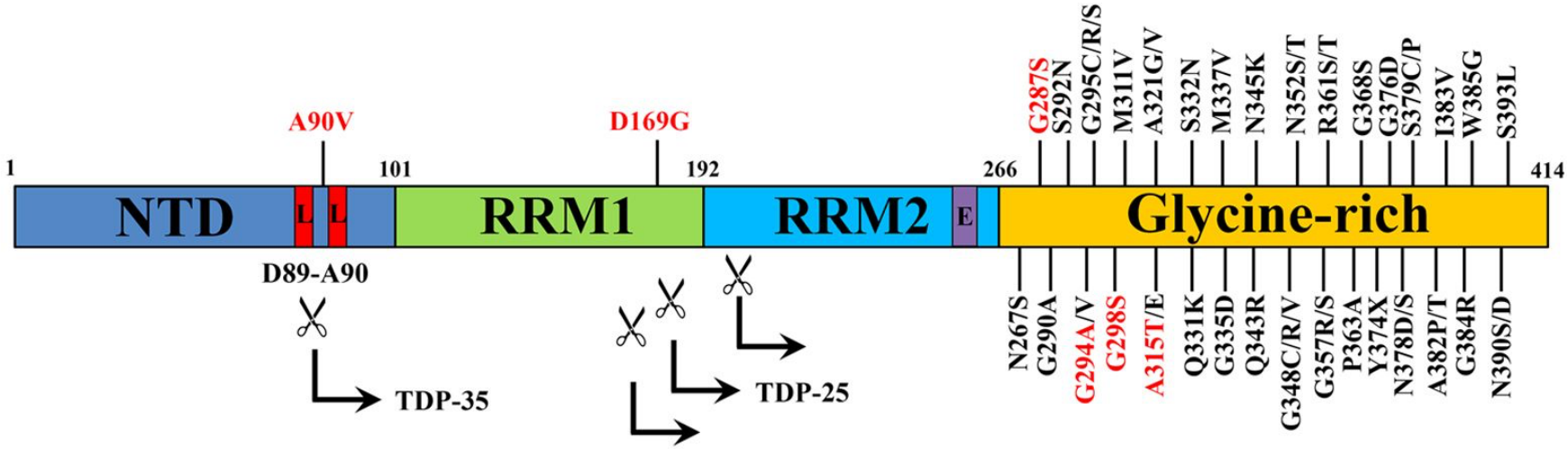


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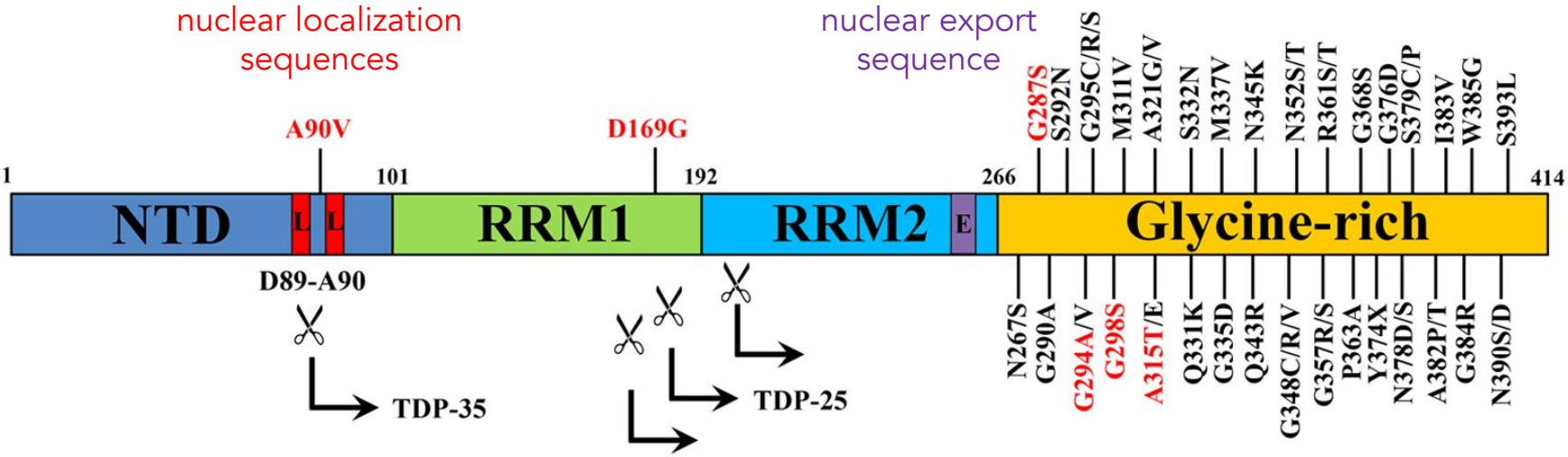


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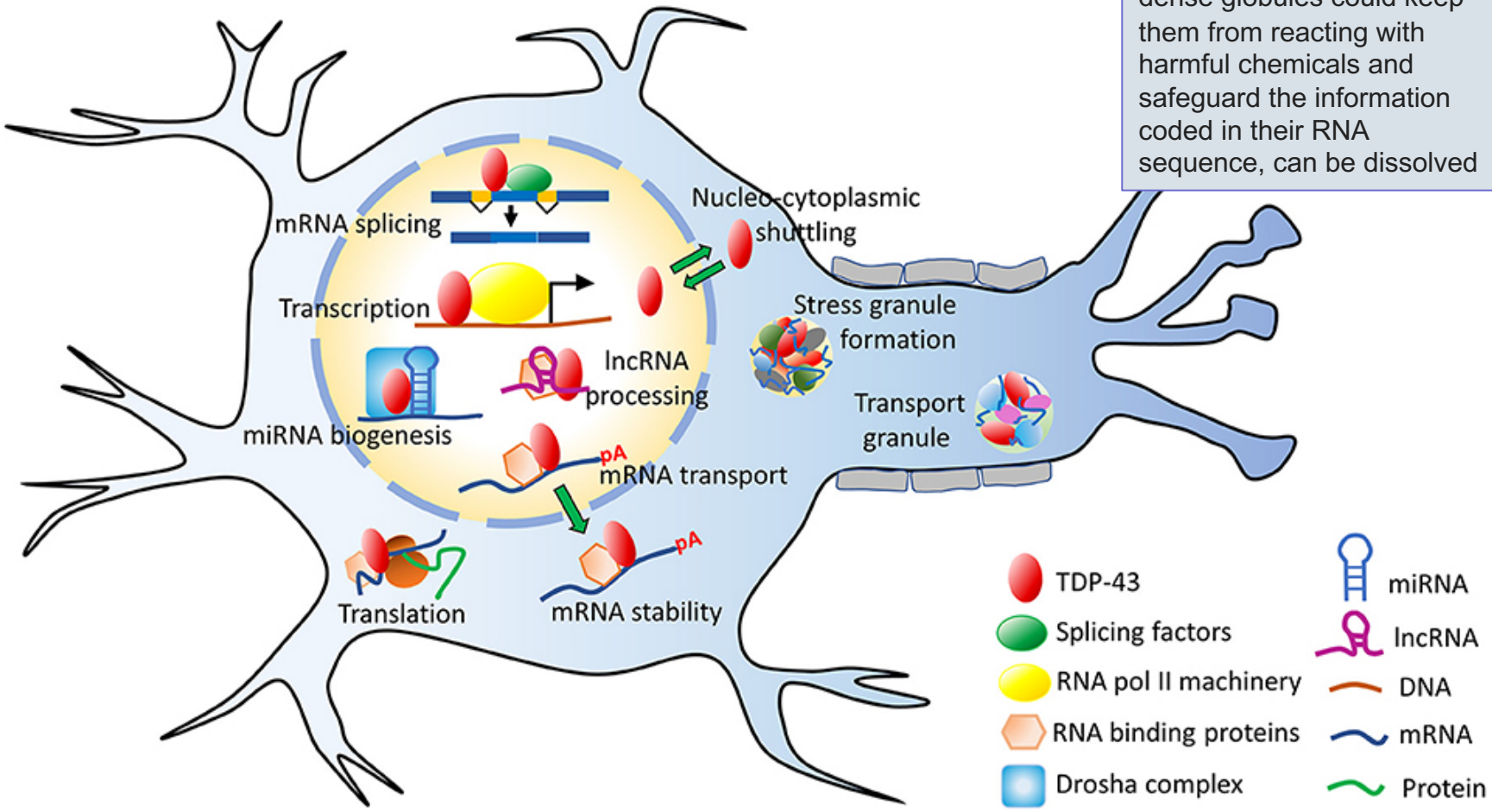
# Nuclear and cytoplasmic functions of TPD-43

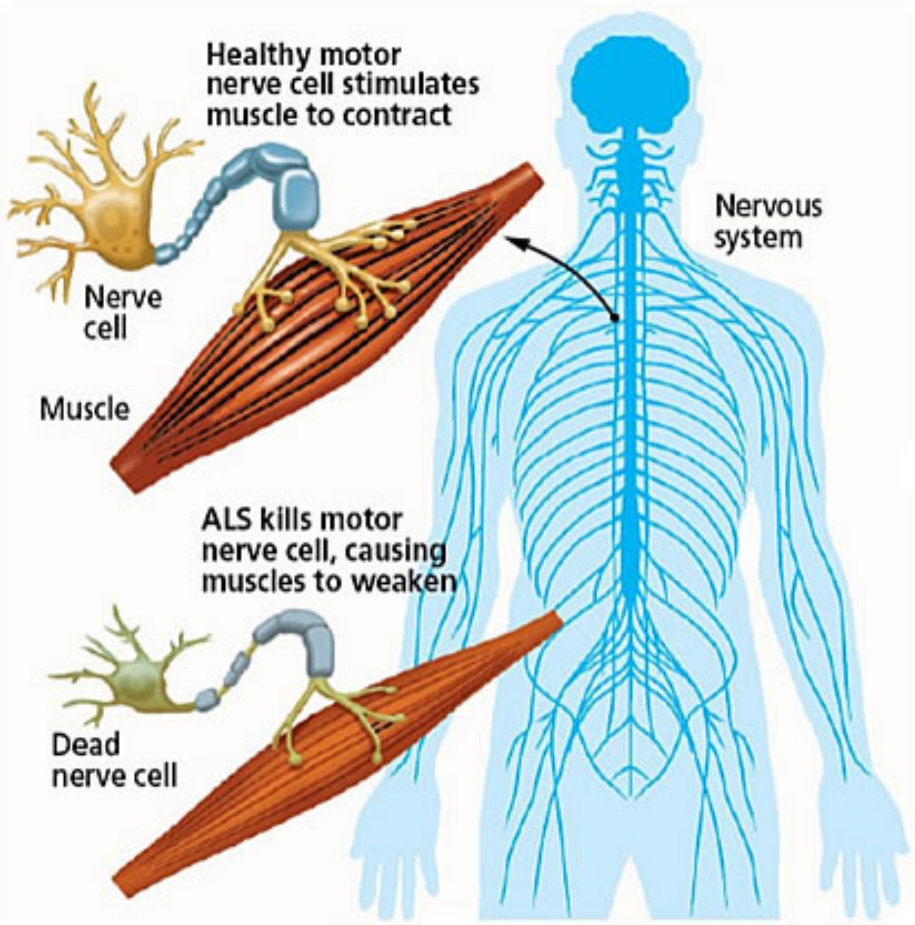
**Stress granule:** The accumulation of RNAs into dense globules could keep them from reacting with harmful chemicals and safeguard the information coded in their RNA sequence, can be dissolved




Prasad et al, Frontiers in Molecular Neuroscience (2019)

# Amyotrophic lateral sclerosis

## Lou Gehrig's disease

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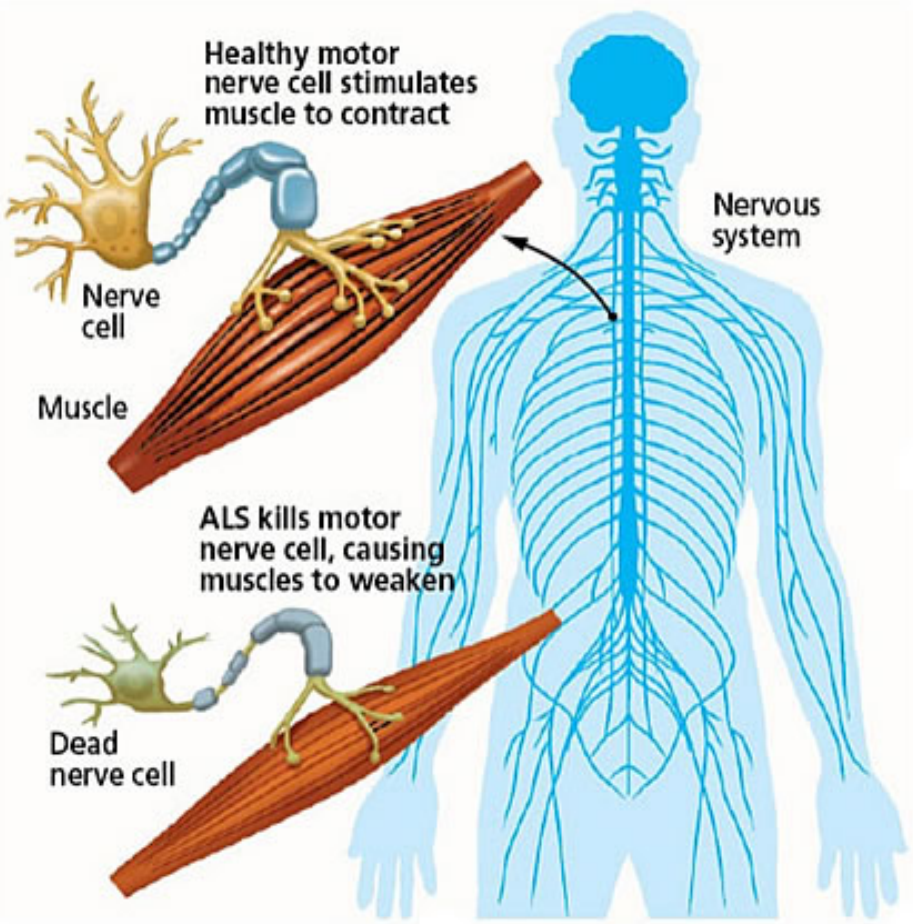


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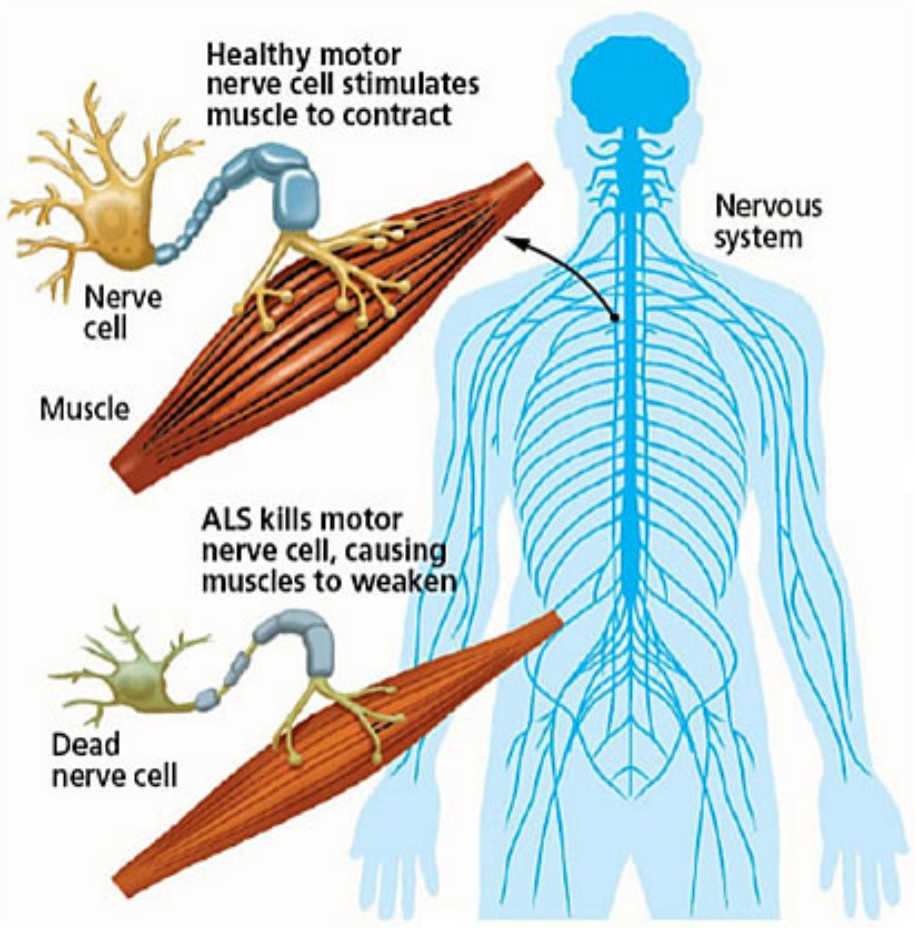


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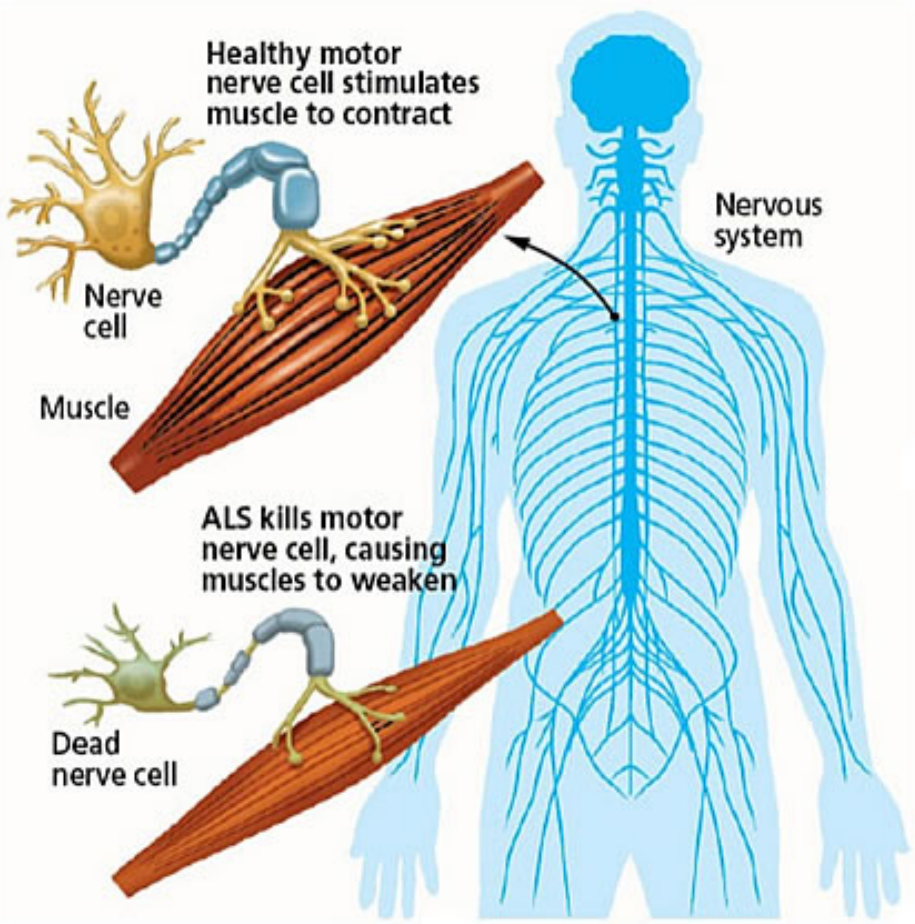


- progressive neurodegenerative disease that destroys motor neurons
- when motor neurons cannot send impulses to muscle, the muscles begin to waste away
- nerve cell death makes it impossible for the brain to control muscles or signal for them to move
- eventually, all muscles are affected, including arms and hands, legs and feet, and those that control swallowing and breathing

# ALS stats

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- 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

The New York Times

By Jonah Engel Bromwich

Dec. 9, 2019

*Left:* Cosmologist Stephen Hawking on October 10, 1979 in Princeton, New Jersey. Photo by Santi Visalli/Getty Images

*By –*
Katherine Harmon

# How did Stephen Hawking live 55 years with ALS?

Science Jan 9, 2012 10:59 AM EST

Leave a comment

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## Go Deeper

als

amyotrophic lateral sclerosis

lou gehrig's disease

stephen hawking

died age 76

Lou Gehrig
died 3 yr pd

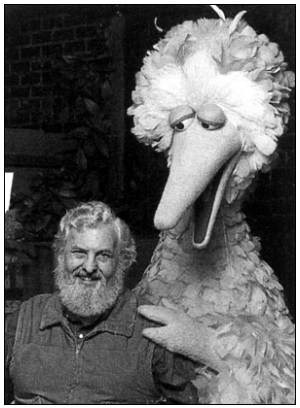
Ezzard Charles
died 8 yr pd

Dwight Clark
died 3 yr pd

Mao Zedong
died of heart attack

Tim Green
alive 2 yr pd

Steve Gleason
alive 9 yr pd
Congressional Medal of Honor,
Jan 2020



Jon Stone
died 4 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):

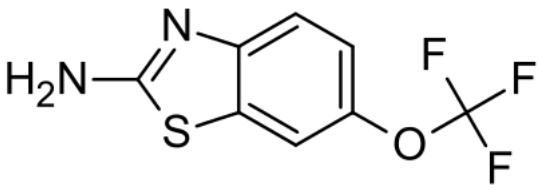
**hexanucleotide (GGGCCC) repeat expansion** in non-coding region of C9ORF72 gene on chromosome 9p21

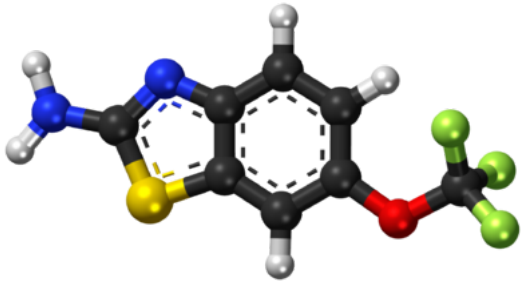
**SOD1** – Cu/Zn superoxide dismutase

**TDP-43** – RNA processing protein that forms toxic neuronal and glial inclusion bodies

# Massive unmet therapeutic need for ALS patients

only one drug available

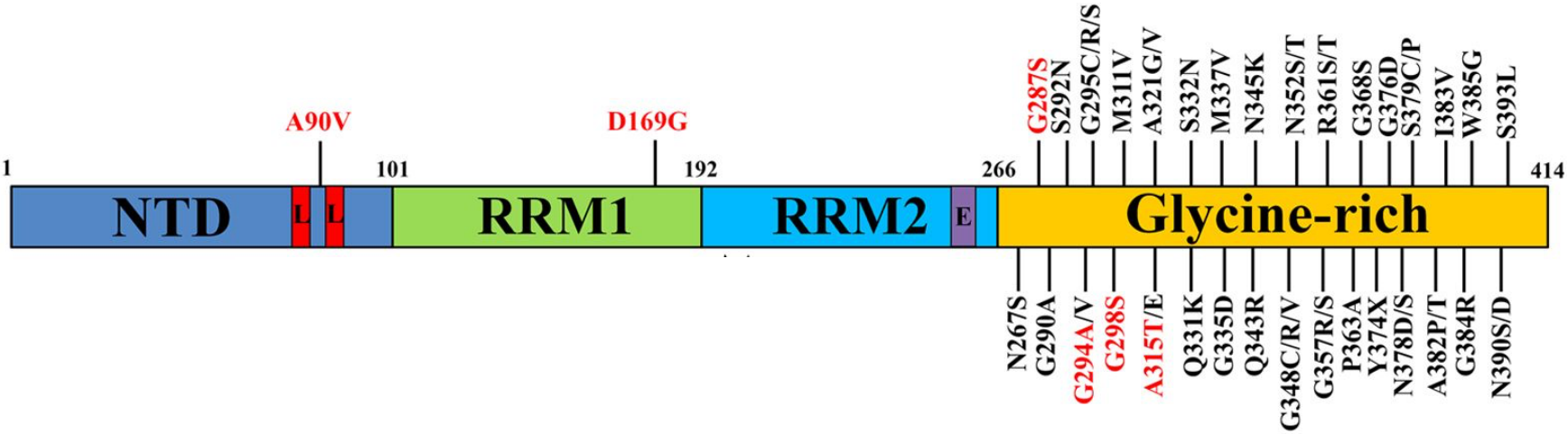


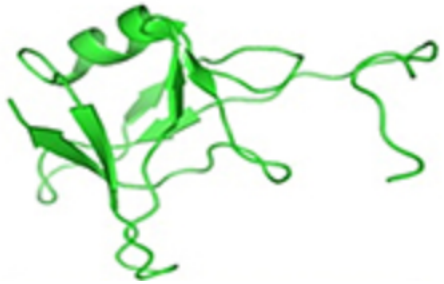



Riluzole

- 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

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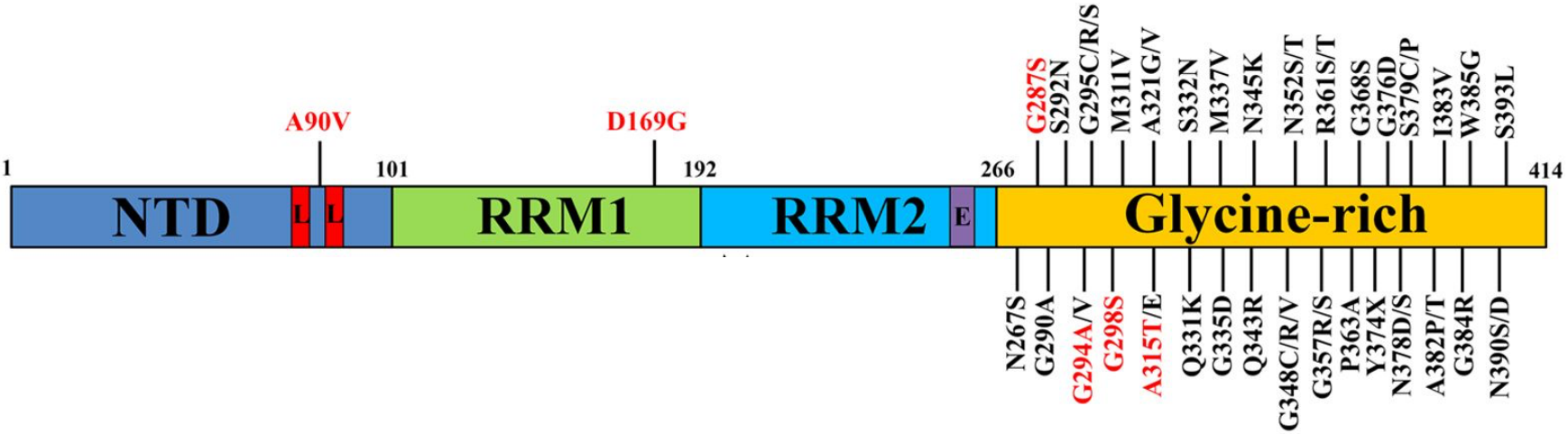
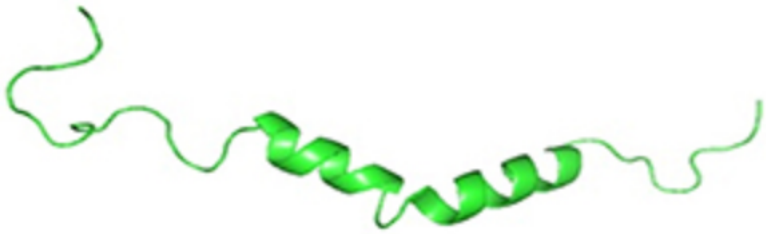



C-term
aa 311-360
PDB ID = 2N3X



Low complexity domain (LCD)
tendency to aggregate

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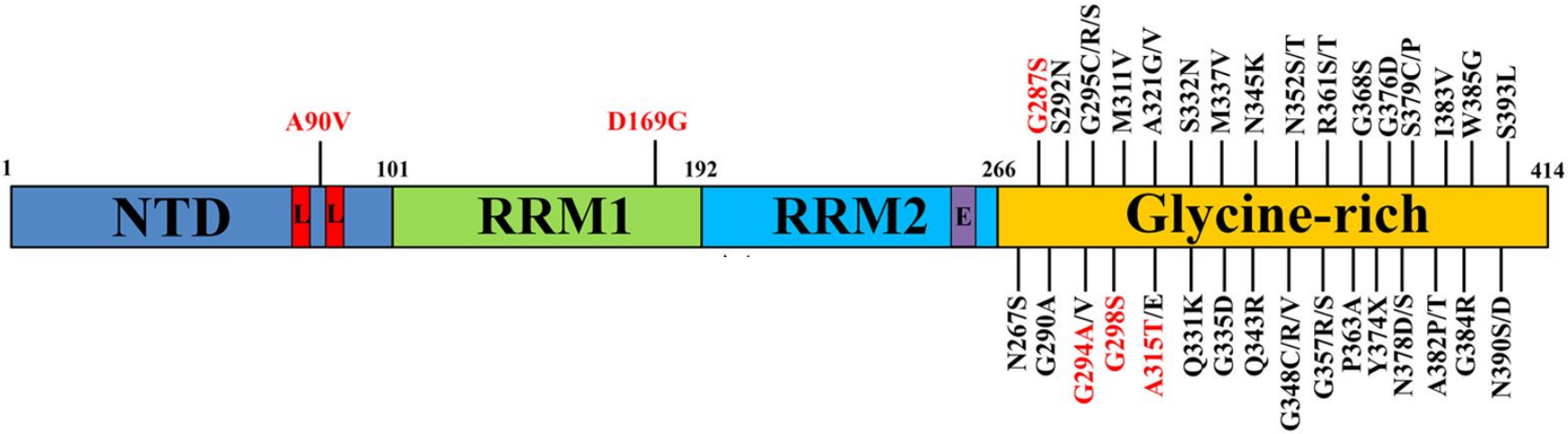


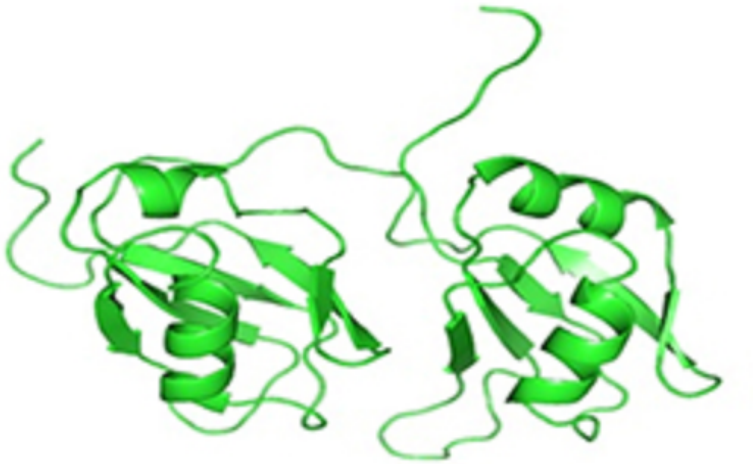

Tandem RRMs
aa 102-269
PDB ID = 4BS2

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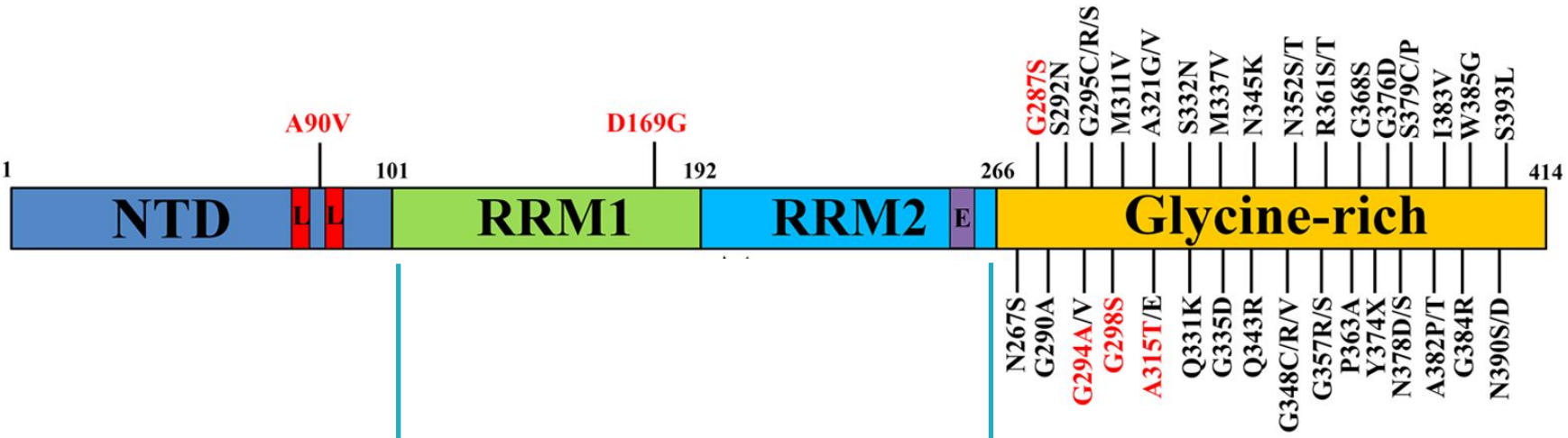
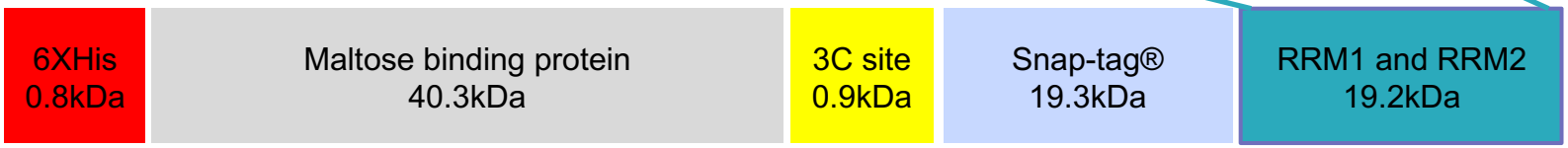


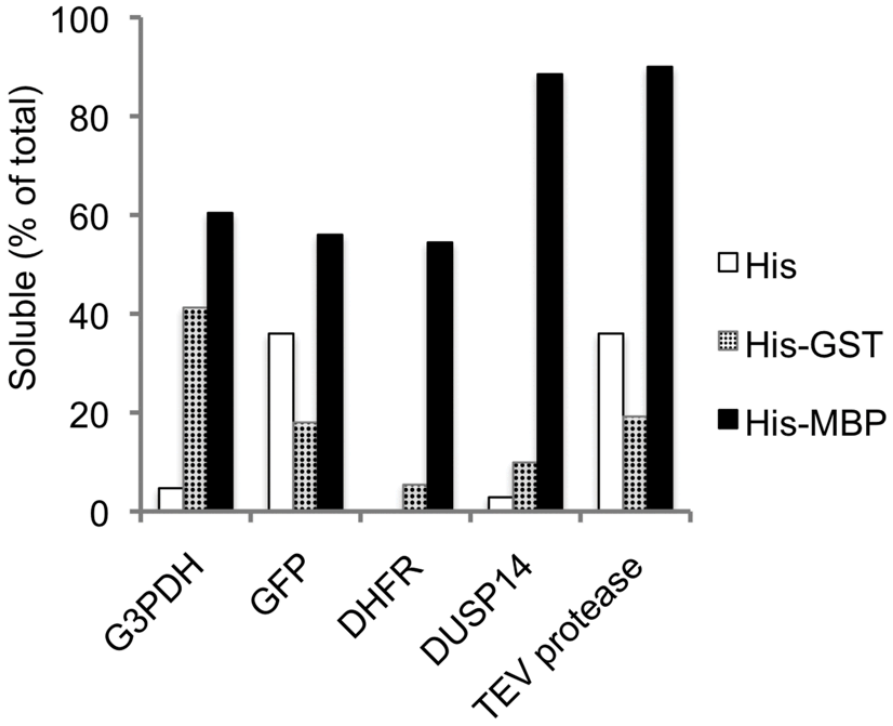

affinity purification tag

nickel purified protein will be cleaved HRV 3C enzyme

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

fusion protein architecture


comparison of solubility across multiple passengers

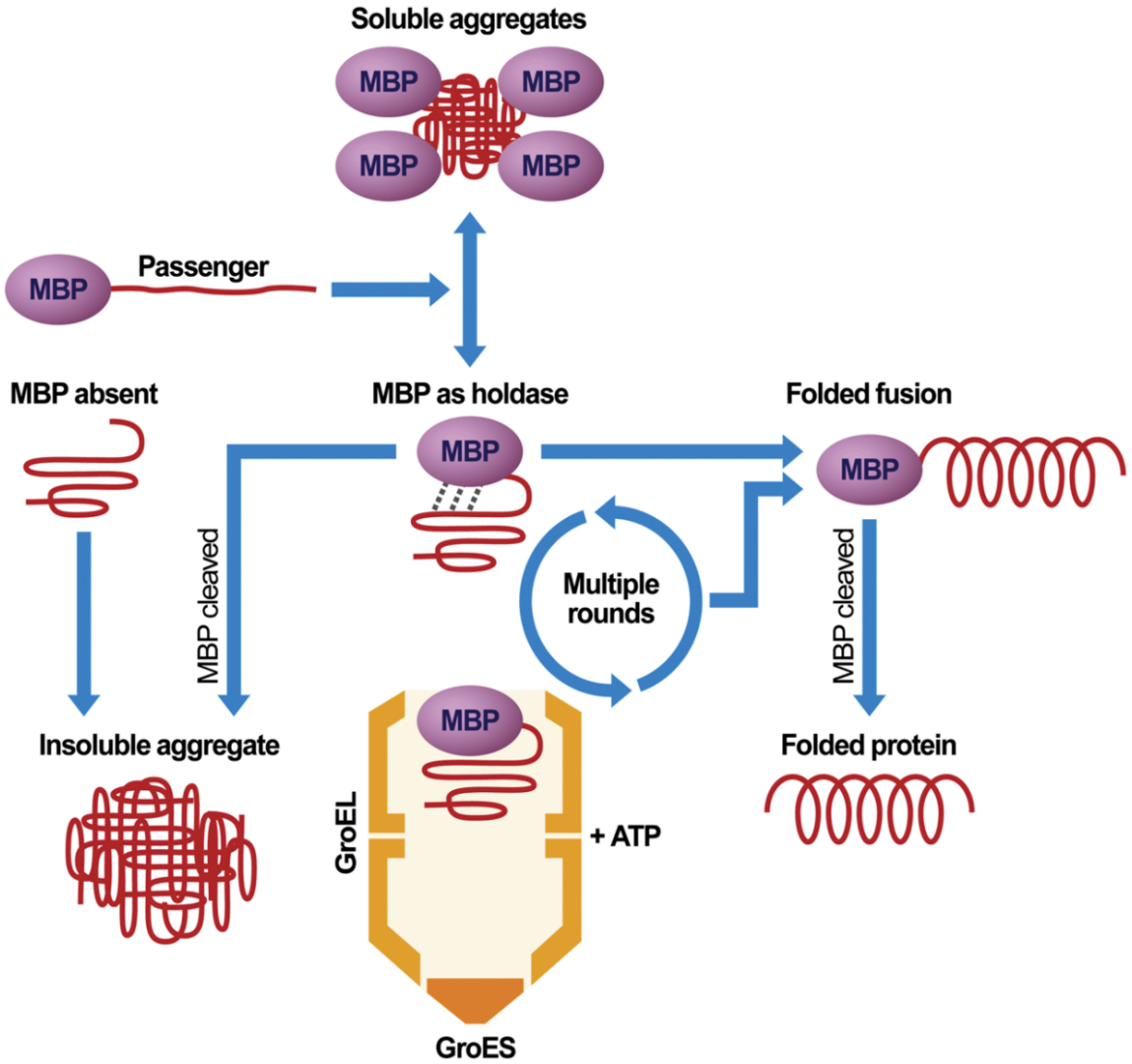


OPEN ACCESS PEER-REVIEWED
RESEARCH ARTICLE

# The Ability to Enhance the Solubility of Its Fusion Partners Is an Intrinsic Property of Maltose-Binding Protein but Their Folding Is Either Spontaneous or Chaperone-Mediated

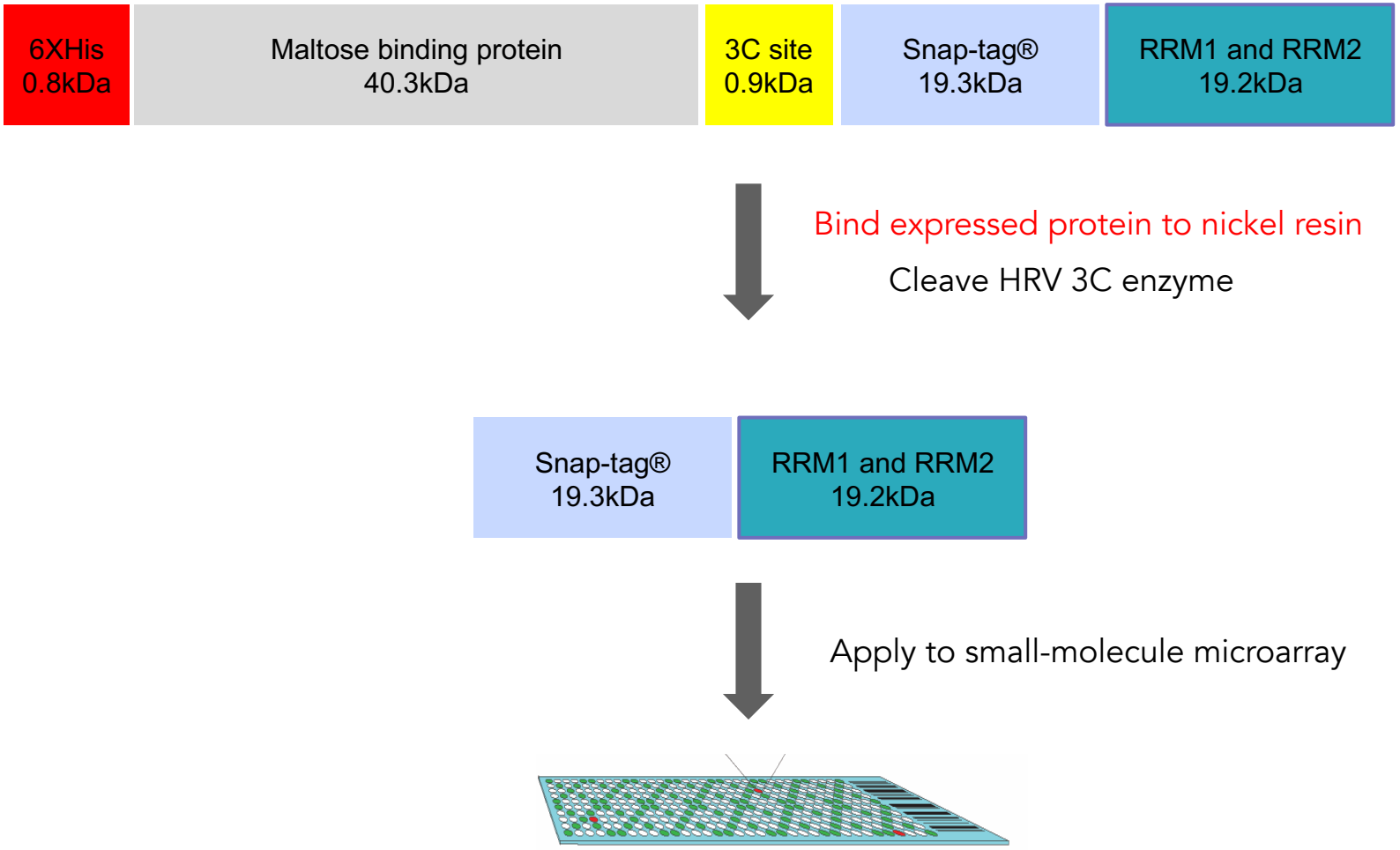
Sreejith Raran-Kurussi, David S. Waugh

Published: November 16, 2012 • https://doi.org/10.1371/journal.pone.0049589



# TDP-43 purification in 20.109 lab

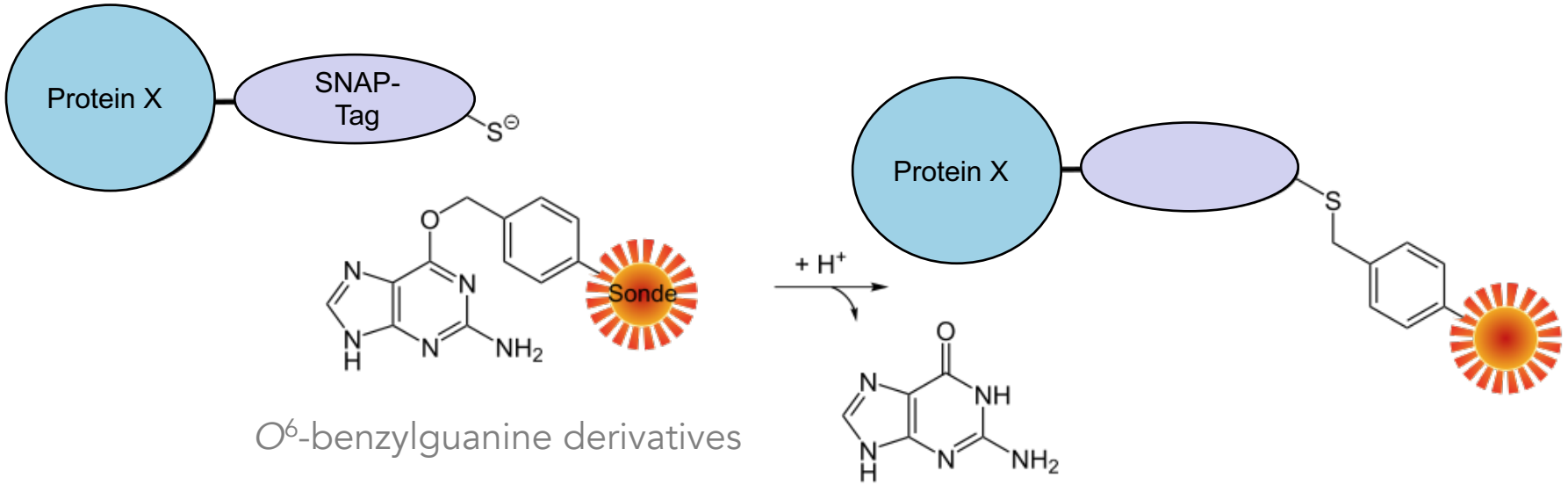
*more details in lab lecture*



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

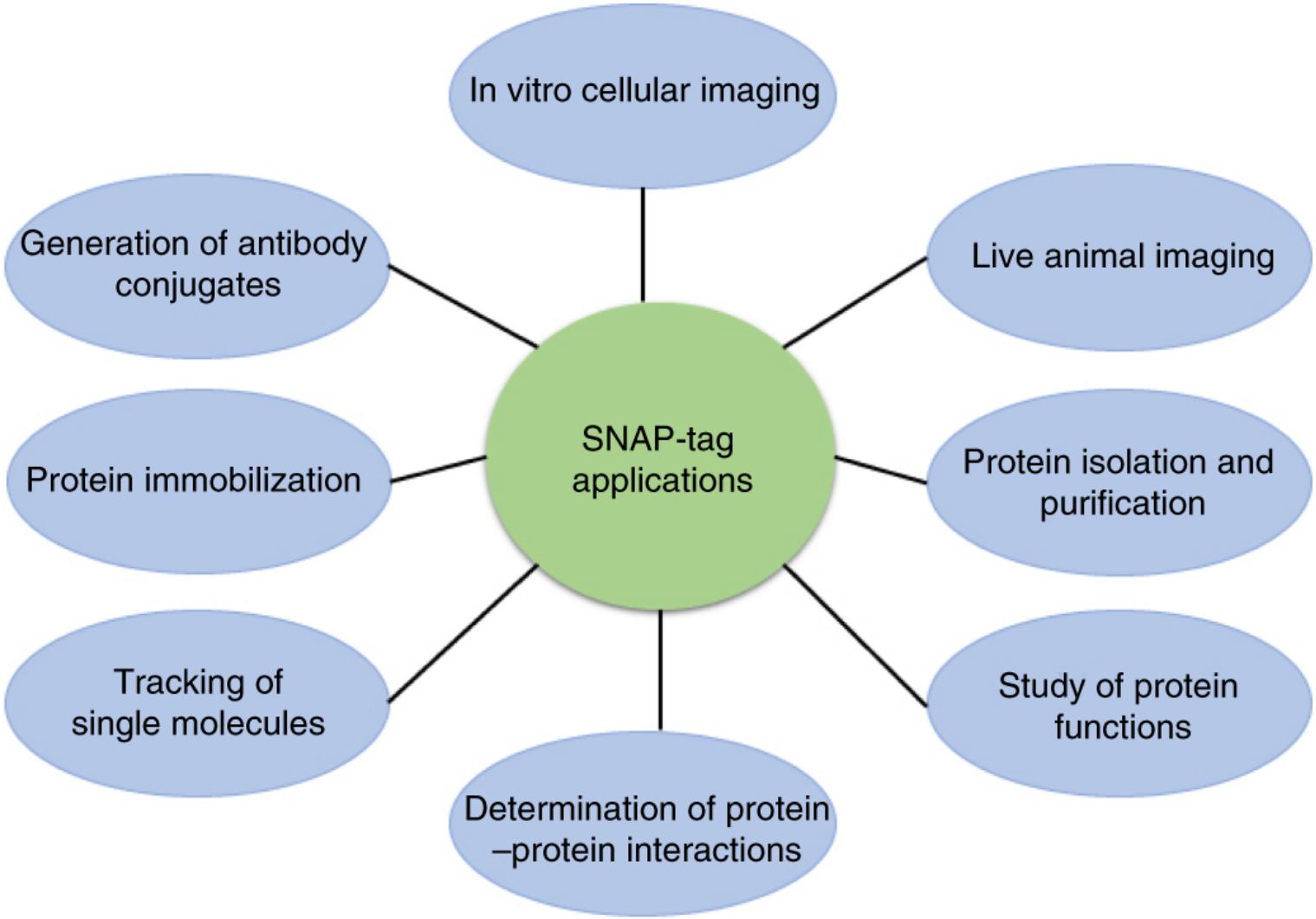
dye labeling reaction

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




$O^6$-benzylguanine derivatives

# SNAP tags can be used in many applications



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

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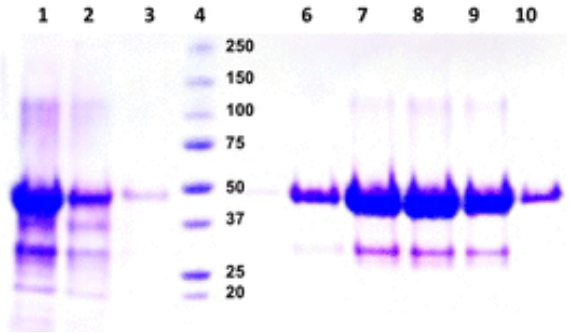
**Published Online:** 1 Oct 2019 | https://doi.org/10.1096/fj.201900474R

THE FASEB JOURNAL

nickel chromatography
(denaturing conditions)



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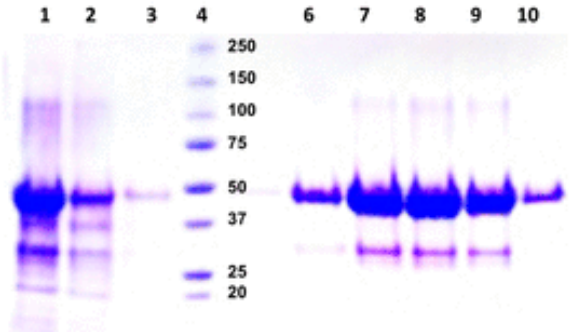


size exclusion column
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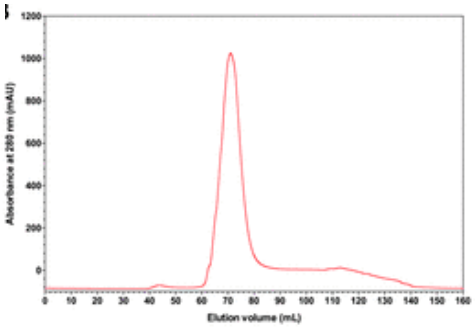

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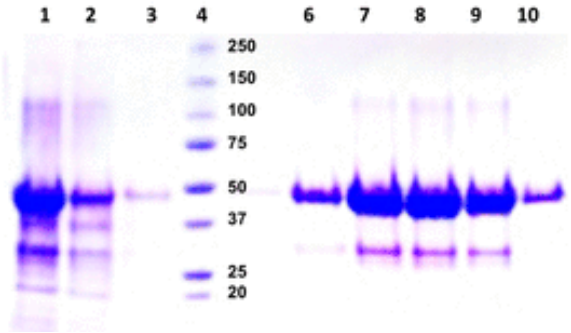
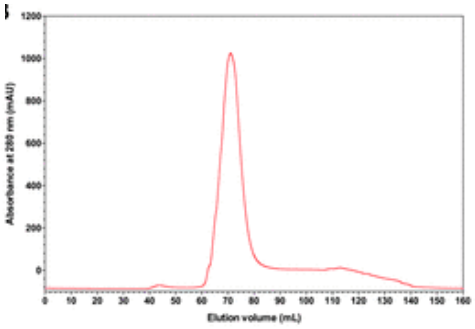
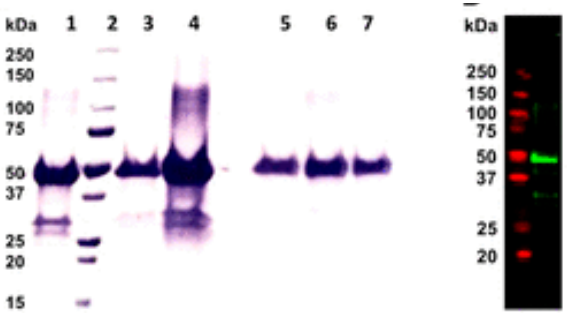



size exclusion column
(denaturing conditions)




refolding & western blot



October
2019

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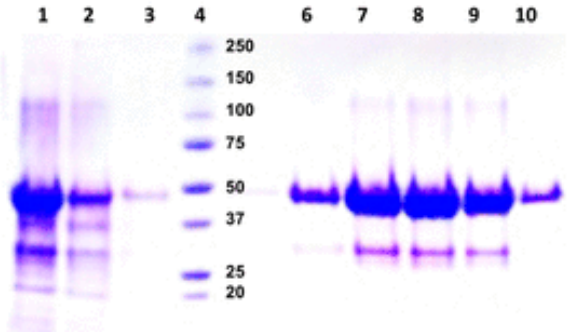
# Isolation and characterization of soluble human full-length TDP-43 associated with neurodegeneration

Mirella Vivoli Vega, Alessia Nigro, Simone Luti, Claudia Capitini, Giulia Fani, Leonardo Gonnelli, Francesca Boscaro, and Fabrizio Chiti

**Published Online:** 1 Oct 2019 | https://doi.org/10.1096/fj.201900474R

THE FASEB JOURNAL

nickel chromatography
(denaturing conditions)




size exclusion column
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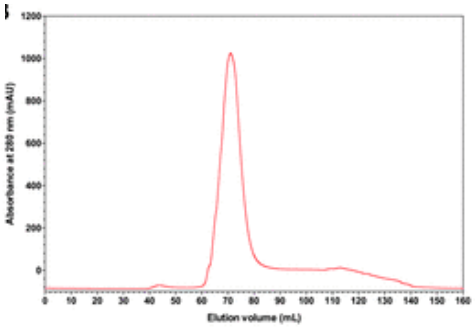


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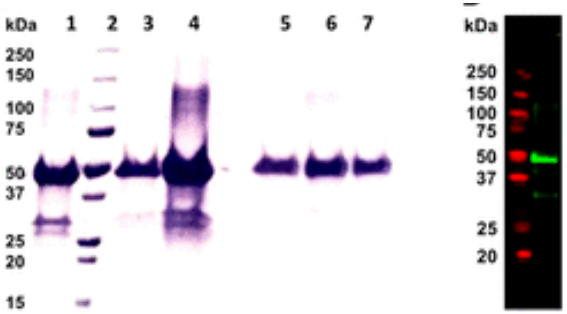
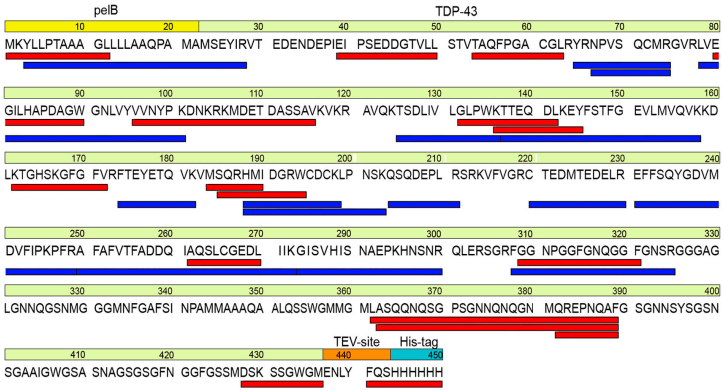



mass spectrometry - MW & sequence



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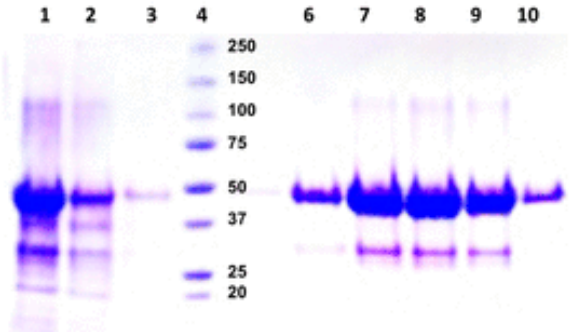


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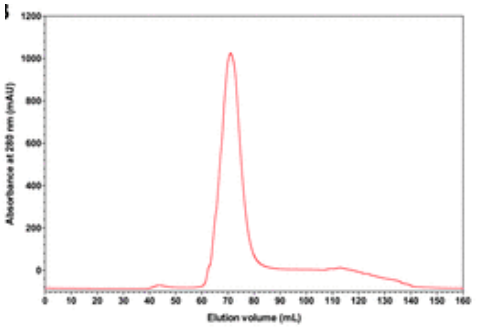


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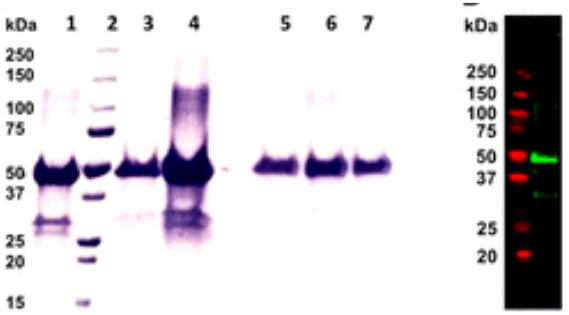
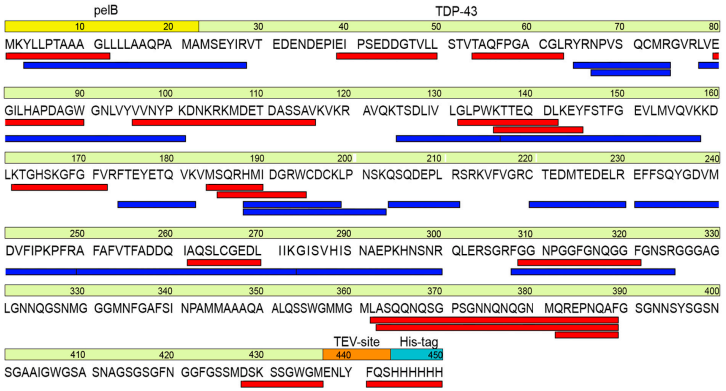
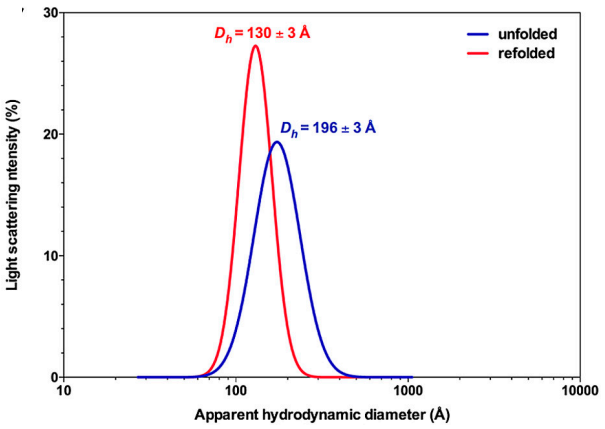


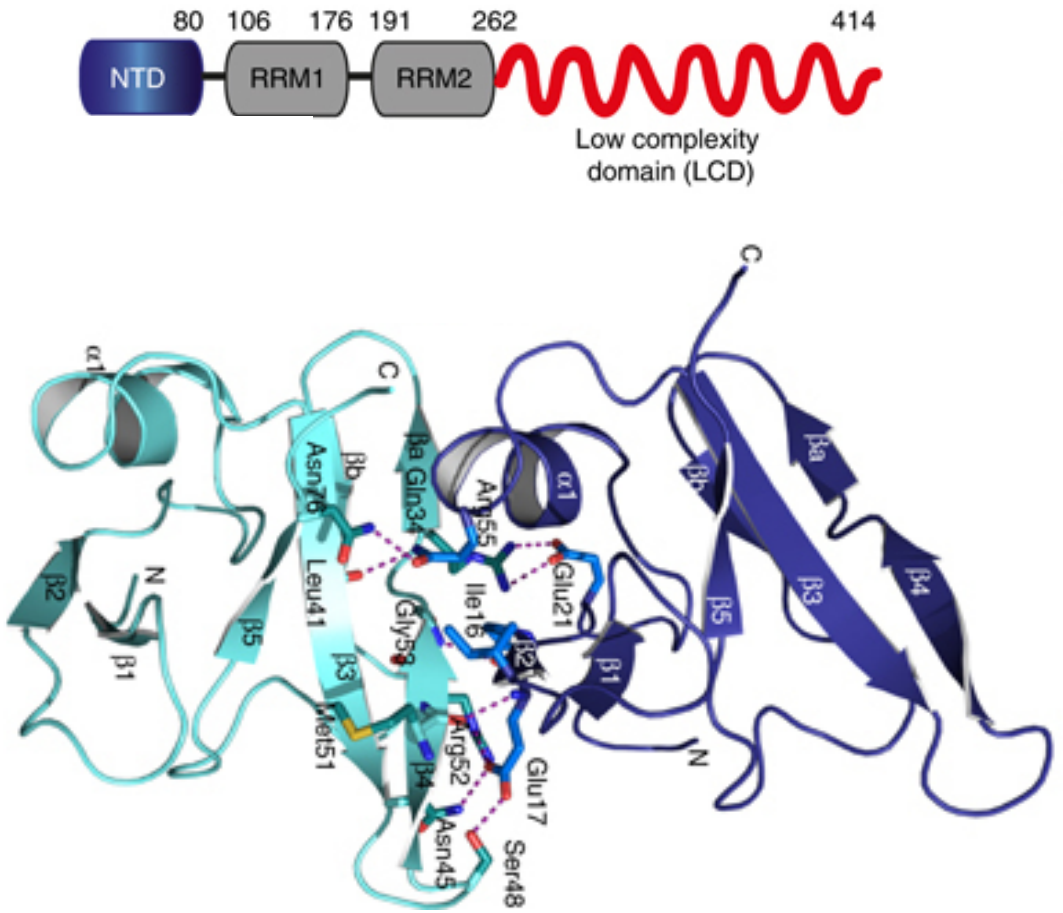

mass spectrometry - MW & sequence




analytical SEC reveals a dimer!

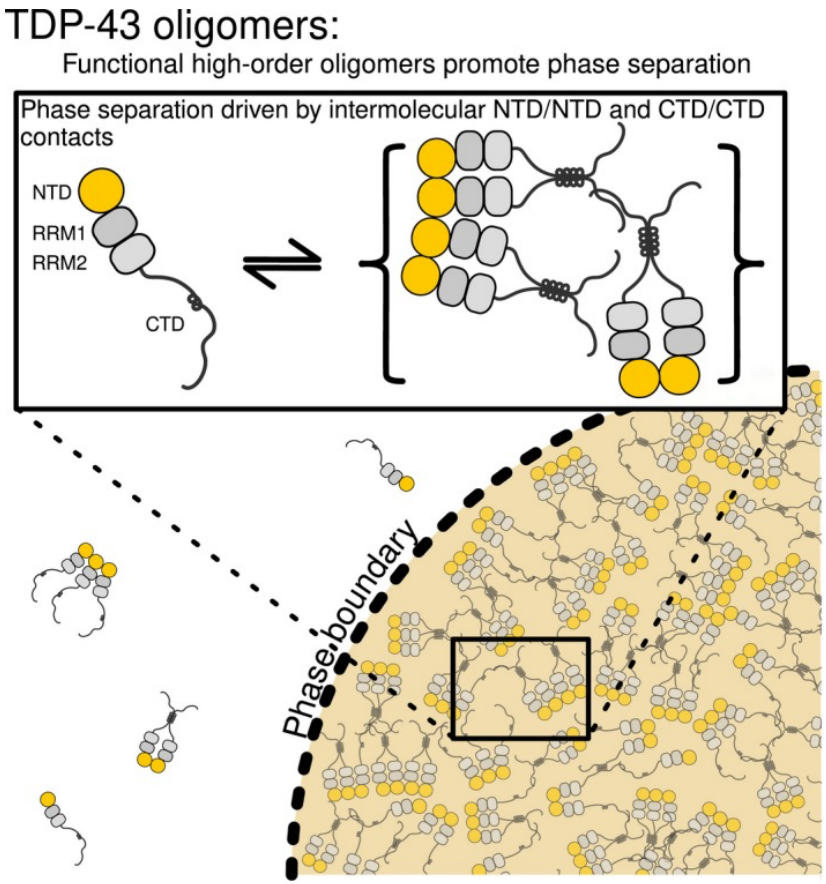


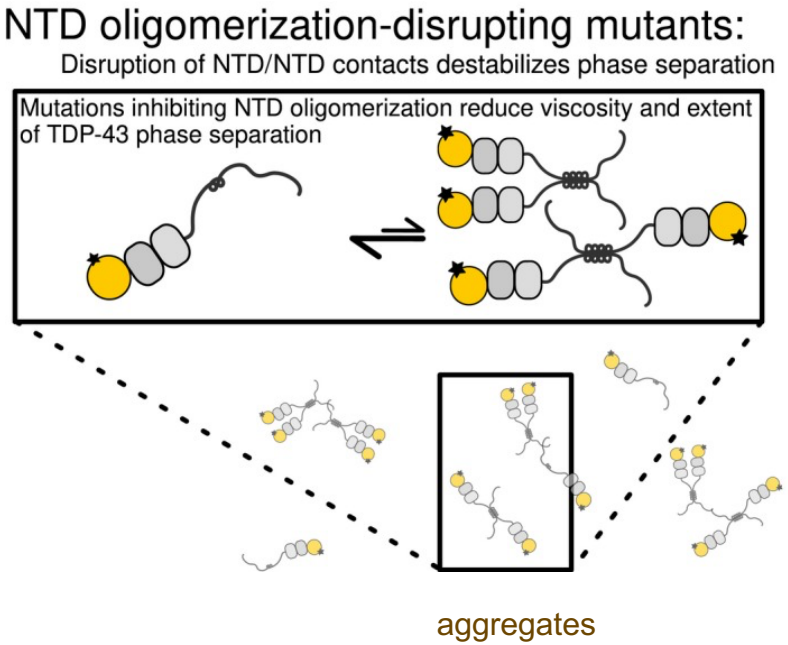
# NTD forms homodimers in crystal structures

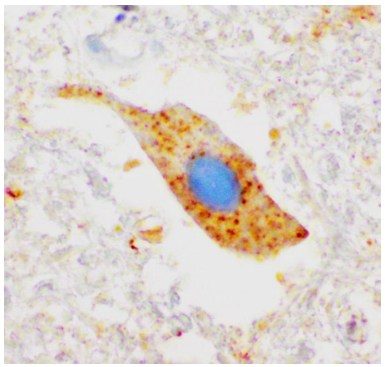



Afroz et al, Nature Communications (2017)

# NTD orientation regulates TDP-43 polymerization and phase separation




Wang et al, EMBO J, 37(5): e97452 (2018)

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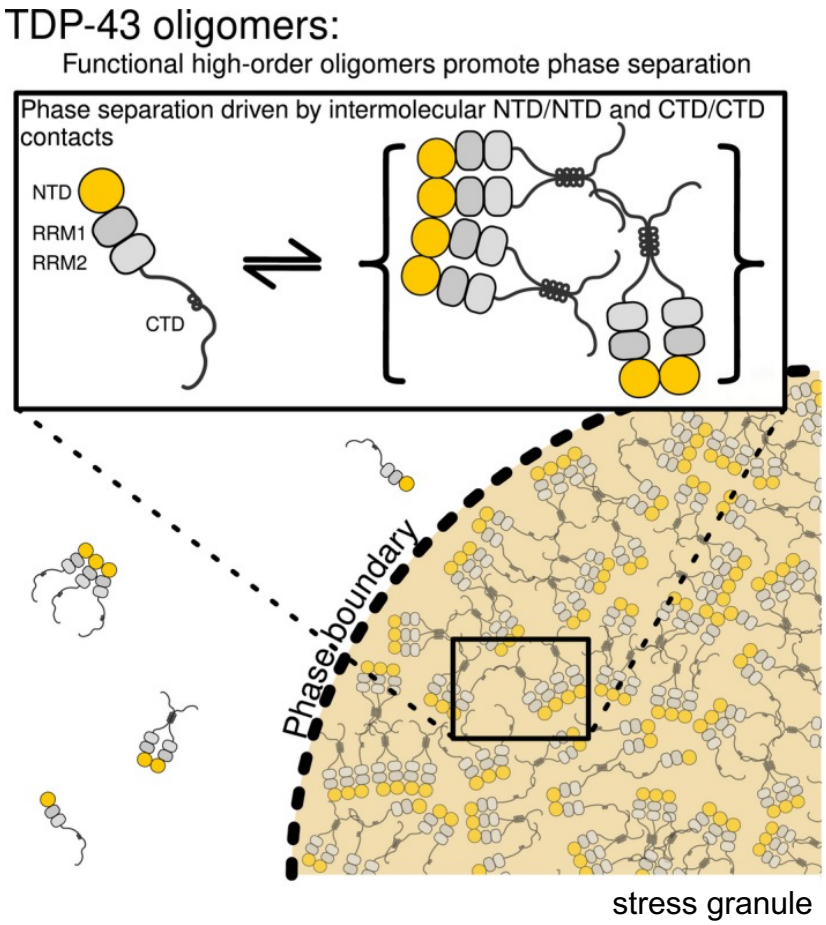
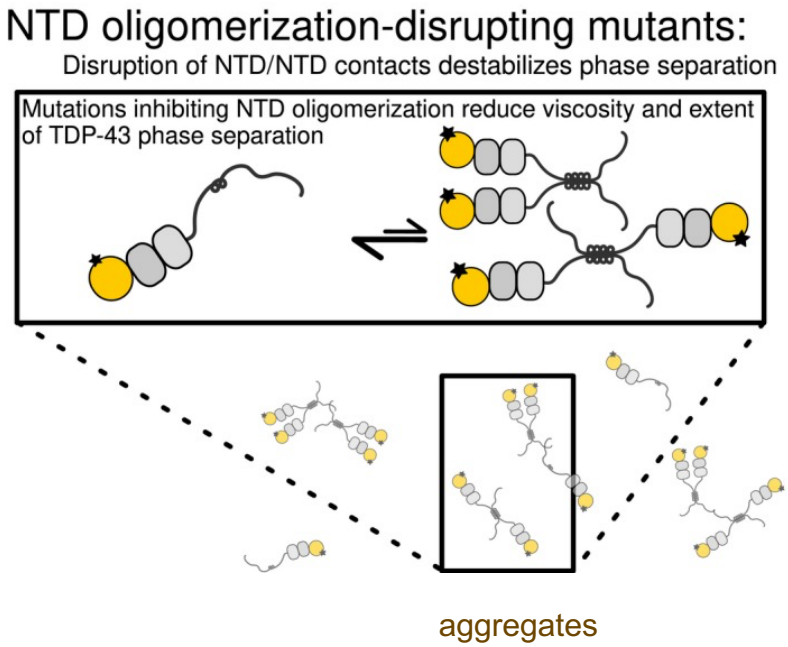
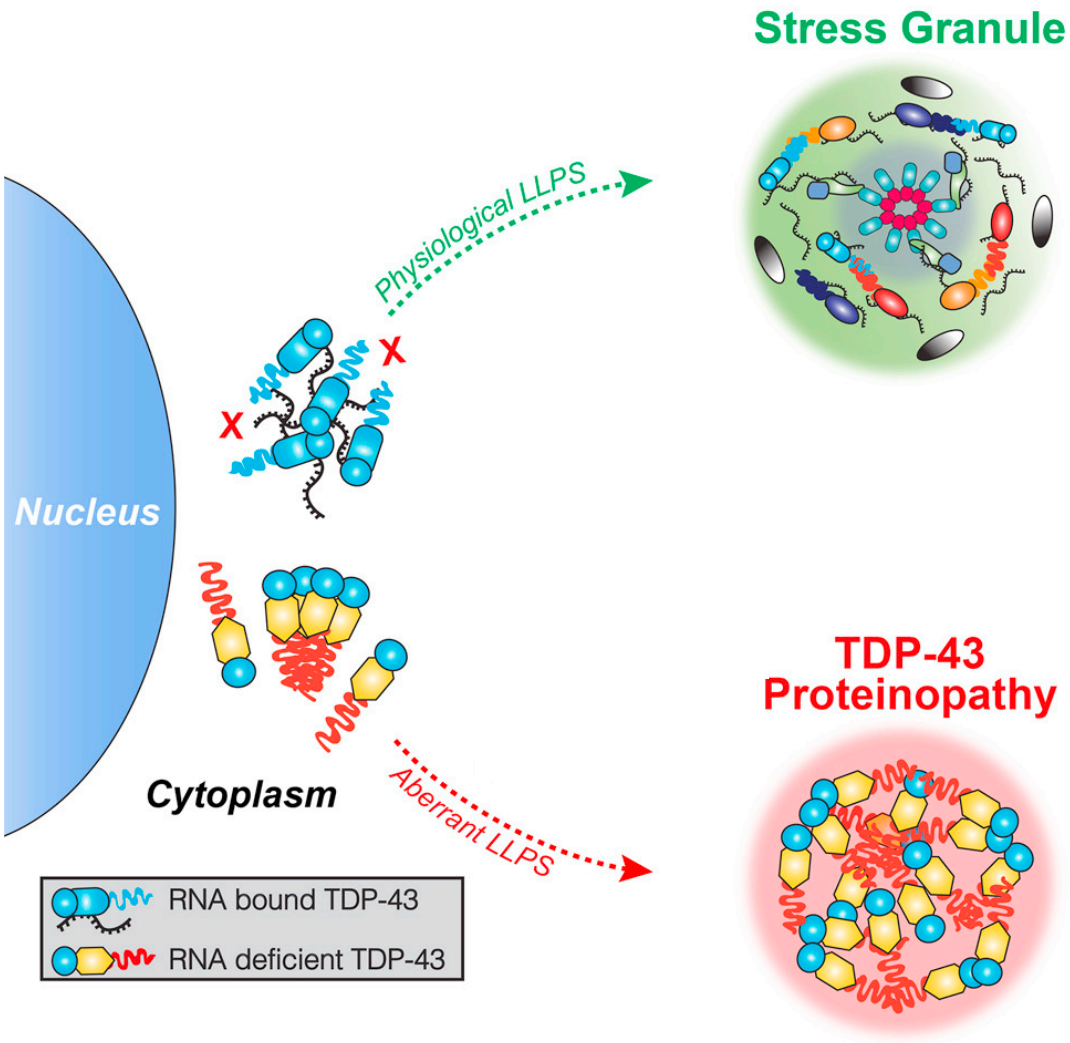



peptidomimetics or small molecules
as a therapeutic strategy
to sequester mutants from aggregation?

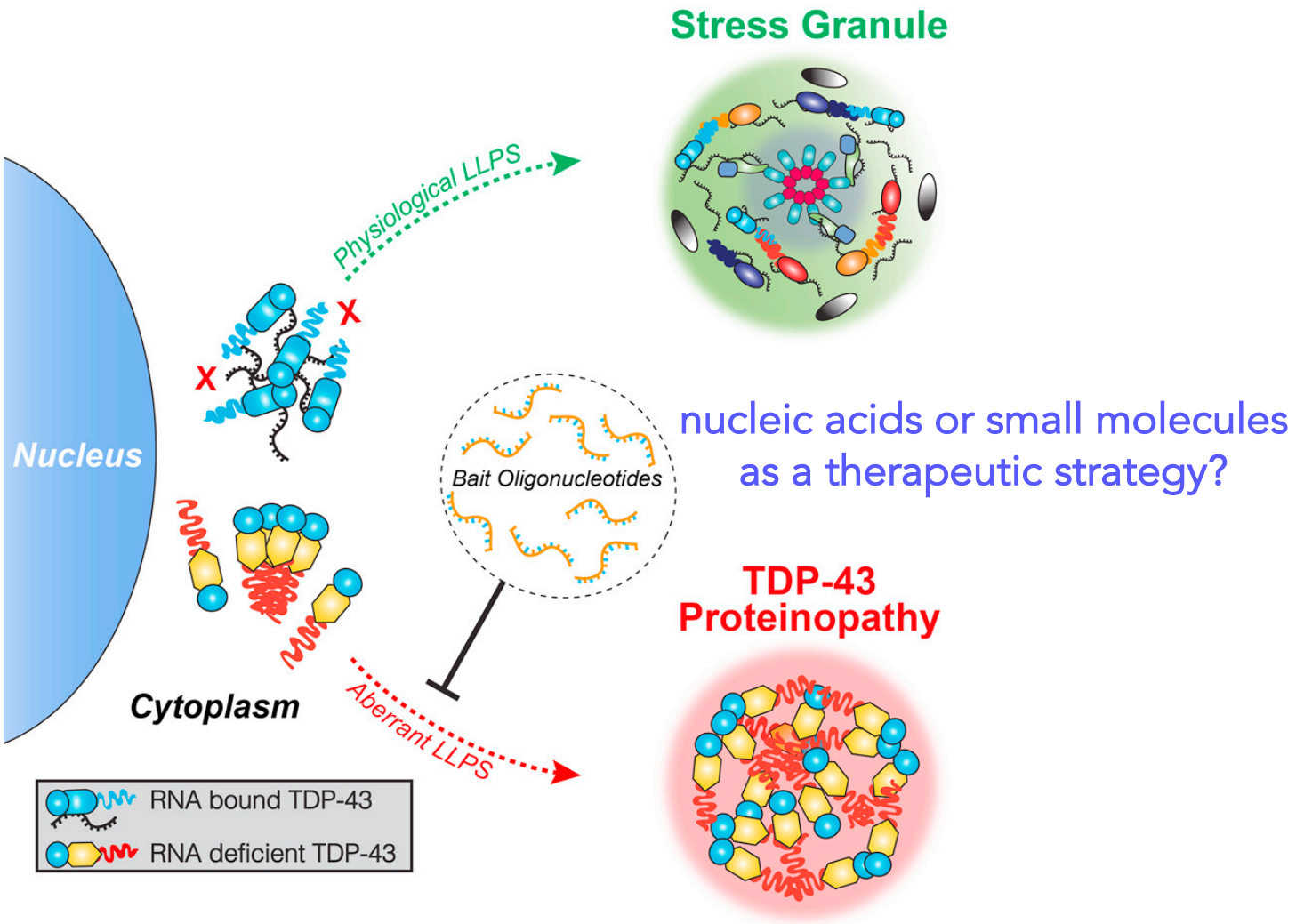
Wang et al, EMBO J, 37(5): e97452 (2018)

# Liquid-liquid phase separation of TDP-43 in disease




Mann et al, Neuron 102 (2):321-338 (2019)

# 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

dewpointx

Cambridge, MA








Companies are interested in agents to control phase separation in an effort to develop drugs for neurodegeneration and cancer

dewpoint$_x$

Cambridge, MA

nature biotechnology

News in Brief | Published: 09 January 2020

## Bayer makes first move into condensates

*Nature Biotechnology* **38**, 5(2020) | Cite this article

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German pharma Bayer has signed a $100 million deal with Dewpoint Therapeutics to jointly pursue biomolecular condensates for drug discovery. The companies will combine Dewpoint's condensates expertise with Bayer's small-molecules library to discover drugs for heart diseases and gynecological indications.

Companies are interested in agents
to control phase separation in an effort
to develop drugs for neurodegeneration and cancer

dewpointx

Cambridge, MA

BAYER

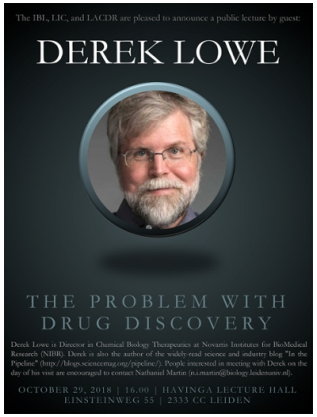
nature reviews
drug discovery

NEWS · 10 APRIL 2019

## Biomolecular condensates pique drug discovery curiosity

Transient liquid-like droplets made up of proteins and RNA are scattered throughout the cell — with potentially broad drug discovery implications.

NOVARTIS



SCIENTIFIC REPORTS

Article | Open Access | Published: 26 September 2016

# TDP-43 aggregation mirrors TDP-43 knockdown, affecting the expression levels of a common set of proteins

S. Prpar Mihevc, Marco Baralle, Emanuele Buratti & Boris Rogelj

*Scientific Reports* **6**, Article number: 33996 (2016) | Cite this article

**631** Accesses | **21** Citations | **10** Altmetric | Metrics

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## ALS-causing D169G mutation disrupts the ATP-binding capacity of TDP-43 RRM1 domain

Mei Dang, Jianxing Song*

*Department of Biological Sciences, Faculty of Science, National University of Singapore, 10 Kent Ridge Crescent, 119260, Singapore*

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## Early Metastable Assembly during the Stress-Induced Formation of Worm-like Amyloid Fibrils of Nucleic Acid Binding Domains of TDP-43

Meenakshi Pillai and Santosh Kumar Jha*

**Cite this:** *Biochemistry* 2020, 59, 3, 315-328
Publication Date: January 3, 2020
https://doi.org/10.1021/acs.biochem.9b00780


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## Using Tetracysteine-Tagged TDP-43 with a Biarsenical Dye To Monitor Real-Time Trafficking in a Cell Model of Amyotrophic Lateral Sclerosis

Janice S. W. Ng, Maya A. Hanspal, Naunehal S. Matharu, Teresa P. Barros, Elin K. Esbjörner, Mark R. Wilson, Justin J. Yerbury, Christopher M. Dobson and Janet R. Kumita*

**Cite this:** *Biochemistry* 2019, 58, 39, 4086-4095
Publication Date: September 6, 2019
https://doi.org/10.1021/acs.biochem.9b00592


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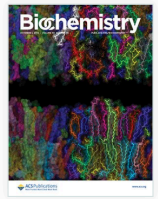


Biochemistry

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Molecular Brain

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Micro report | Open Access | Published: 20 January 2020

## ALS-linked TDP-43$^{M337V}$ knock-in mice exhibit splicing deregulation without neurodegeneration

Seiji Watanabe, Kotaro Oiwa, Yuri Murata, Okiru Komine, Akira Sobue, Fumito Endo, Eiki Takahashi & Koji Yamanaka

*Molecular Brain* **13**, Article number: 8 (2020) | Cite this article

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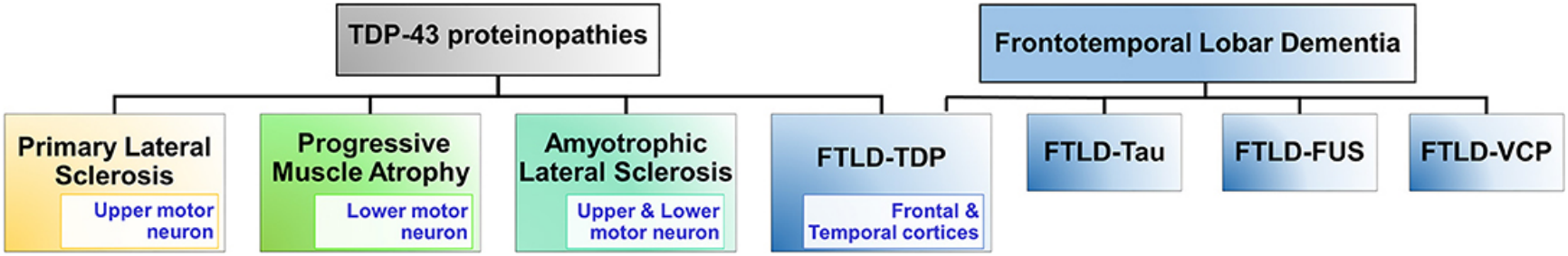
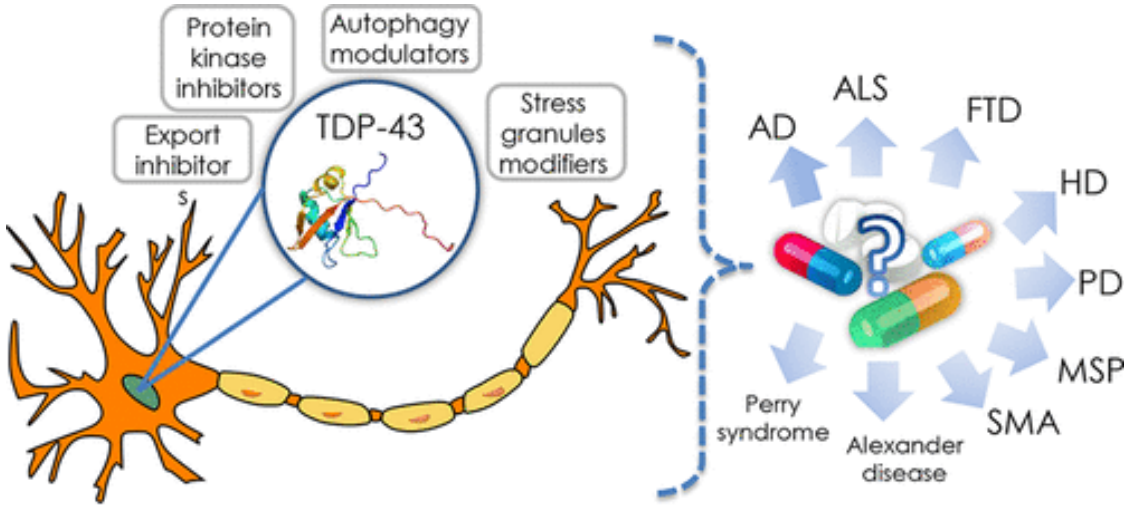
our knowledge is rapidly evolving

# TDP-43 aggregation is observed in multiple diseases



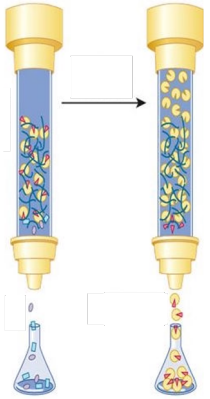



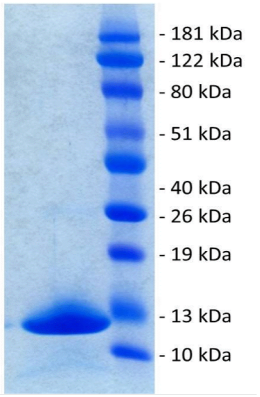
# Spring 2020 Mod 1 path to probe discovery




*in silico* cloning; overexpress TDP-43

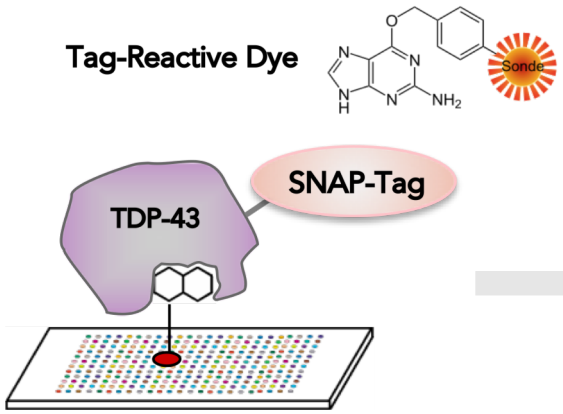
lab day 1





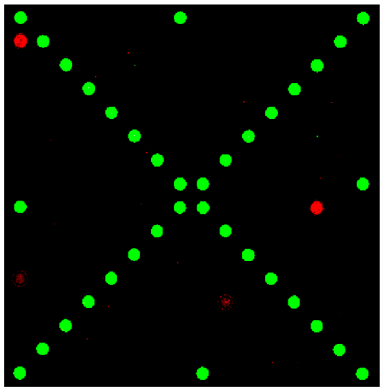

purify and analyze TDP-43 concentration

lab days 2 and 3



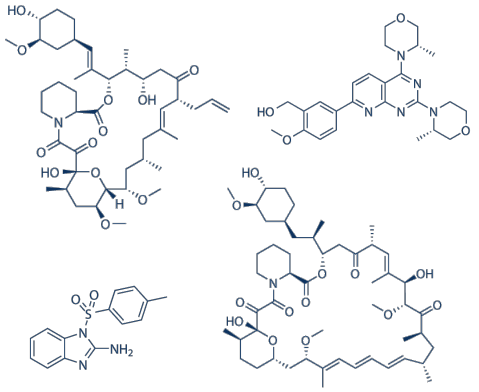

ligand discovery screen

lab day 5



scan images and analyze data

lab days 5 and 6



compare hit lists for teams

lab day 7

# Our path to finding ligands - lectures

| | | |
|---|---|---|
| 2/5/20 | Lecture 1 | Intro to chemical biology: small molecules, probes, and screens |
| 2/11/20 | Lecture 2 | Our protein target: TDP-43 |
| 2/13/20 | Lecture 3 | Small molecule microarrays |
| 2/18/20 | No Lecture | |
| 2/20/20 | Lecture 4 | Quantitative evaluation of protein-ligand interactions |
| 2/25/20 | Lecture 5 | A ligand discovery vignette: sonic hedgehog |
| 2/27/20 | Lecture 6 | Engineering transcriptional responses with a small molecule |
| 3/3/20 | Lecture 7 | Wrap up discussion: suggestions for how to report your findings |