Dynamic polymerization of TDP-43 in health and disease

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TDP-43 is mislocalized and forms inclusions in ALS/FTD

Sporadic ALS – spinal cord
- affected cell
- healthy cell

FTD – frontal cortex
- Cytoplasmic TDP-43 aggregation and loss of nuclear localization

Igaz et al., Am J Path., 2008
Neumann et al., Science, 2006
Structural determinants of TDP-43 function and pathology

- NLS
- NES
- NTD
- RRM1
- RRM2
- Low complexity domain

- 80 106 176 191 262 414

nucleus
cytoplasm
Structural determinants of TDP-43 function and pathology

Lukavsky P., et al., NSMB, 2013

RNA metabolism
Structural determinants of TDP-43 function and pathology

Lukavsky P., et al., NSMB, 2013

Post-translational modifications and pathological signatures

Neumann et al., Int. J. Mol. Sci., 2009
What is the physiological state of functional TDP-43 and how does it form?

Afroz et al., Nature Commun., in press
What is the physiological state of functional TDP-43 and how does it form?

Afroz et al., Nature Commun., in press
What is the physiological state of functional TDP-43 and how does it form?

Protein dimerization
Nucleic acid binding
Inclusion formation

Lukavsky P., et al., NSMB, 2013
Romano V., et al., Prion, 2015
Zhang Y-J et. al., Human Molecular Genetics, 2013
Biophysical characterization of TDP-43 N-terminal domain (NTD)

2D-NMR spectrum of TDP-43 NTD

Protein is folded

30 °C, 500 MHz
Biophysical characterization of TDP-43 N-terminal domain (NTD)

2D-NMR spectrum of TDP-43 NTD

TDP-43 NTD crystals

Protein is folded

30 °C, 500 MHz
2.1-Å resolution crystal structure of TDP-43 NTD

<table>
<thead>
<tr>
<th>TDP-43 NTD</th>
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<tbody>
<tr>
<td><strong>Data collection</strong></td>
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<tr>
<td>Space group</td>
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<tr>
<td>Cell dimensions</td>
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<tr>
<td>$a$, $b$, $c$ (Å)</td>
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<tr>
<td>$\alpha$, $\beta$, $\gamma$ (°)</td>
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<td>Resolution (Å)</td>
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<td>$R_{\text{meas}}$</td>
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<tr>
<td>$R_{\text{merge}}$</td>
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<tr>
<td>$I/\sigma I$</td>
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<tr>
<td>$CC_{1/2}$</td>
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<tr>
<td>Completeness (%)</td>
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<tr>
<td>Redundancy</td>
</tr>
<tr>
<td>$\Delta a_{\text{env}} / \Delta a_{\text{ano}}$</td>
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| **Refinement** |
| Resolution (Å) | 49.15 – 2.10 |
| No. reflections | 12500 |
| $R_{\text{work}} / R_{\text{free}}$ | 0.166/0.206 |
| No. atoms | 1344 |
| Protein | 1192 |
| Ligand/ion |
| acetate | 1 |
| cacodylate | 4 |
| Water | 108 |
| B-factors |
| Protein | 48.46 |
| Ligand/ion | 54.16 |
| Water | 56.2 |
| R.m.s. deviations |
| Bond lengths (Å) | 0.031 |
| Bond angles (°) | 2.72 |
Head-to-tail arrangement of TDP-43 NTD monomers
Electrostatic interactions drive the head-to-tail polymerization of TDP-43 NTD.
Validation of inter-molecular interface in solution by nuclear magnetic resonance (NMR) spectroscopy.

Monomer → increasing concentration → Oligomer
Dynamic oligomerization of TDP-43 NTD monitored by NMR

\[ \text{Afroz et al., Nature Commun., in press} \]
Dynamic oligomerization of TDP-43 NTD monitored by NMR

Afroz et al., Nature Commun., in press
Dynamic oligomerization of TDP-43 NTD monitored by NMR

Afroz et al., Nature Commun., in press
Dynamic oligomerization of TDP-43 NTD monitored by NMR

Afroz et al., Nature Commun., in press
Dynamic oligomerization of TDP-43 NTD monitored by NMR

Dynamic self association is in low micro-molar range

Afroz et al., Nature Commun., in press
Chemical shift perturbations are consistent with the crystal structure interface.
Mutagenesis of residues at oligomerization interface based on crystal structure

Oligomerization interface is conserved across conserved various species.

Mutant proteins are folded

Afroz et al., Nature Commun., in press
Point mutations abrogate TDP-43 NTD oligomerization \textit{in vitro}

Afroz et al., \textit{Nature Commun.}, in press
Point mutations abrogate TDP-43 NTD oligomerization \textit{in vitro}

Afroz et al., Nature Commun., in press
Point mutations abrogate TDP-43 NTD oligomerization *in vitro*

Afroz et al., *Nature Commun.*, *in press*
Point mutations abrogate TDP-43 NTD oligomerization \textit{in vitro}

Afroz et al., Nature Commun., in press
Point mutations abrogate TDP-43 NTD oligomerization \textit{in vitro}

Afroz et al., Nature Commun., in press
Point mutations in the N-terminal domain do NOT affect TDP-43 subcellular localization.

Point mutations in the N-terminal domain attenuate TDP-43 oligomerization in cells.

In situ cross-linking with disuccinimidyl glutarate (DSG)
Functional role of TDP-43 oligomerization in RNA metabolism

endogenous TDP-43 depletion

motor neuron-like NSC-34 cells

Polymenidou et al., Nat Neurosc., 2011
Functional role of TDP-43 oligomerization in RNA metabolism

GFP + TDP-43 transfection

TDP-43 motor neuron-like NSC-34 cells

dependent endogenous TDP-43 depletion

splicing assay

Polimenidou et al., Nat Neurosc., 2011
Functional role of TDP-43 oligomerization in RNA metabolism

Sortilin 1

TDP-43 siRNA

Afroz et al., Nature Commun., in press
Functional role of TDP-43 oligomerization in RNA metabolism

Afroz et al., Nature Commun., in press
Visualization of TDP-43 physiological oligomers in cells
Tripartite GFP complementation (Split GFP)

GFP

11 stranded β-barrel
Tripartite GFP complementation (Split GFP)
Tripartite GFP complementation (Split GFP)

Non-fluorescent
Tripartite GFP complementation (Split GFP)

Non-fluorescent
Tripartite GFP complementation (Split GFP)

Non-fluorescent
Tripartite GFP complementation (Split GFP)
Tripartite GFP complementation (Split GFP)

Reconstituted Fluorescence

TDP-43
Tripartite GFP complementation

Afroz et al., Nature Commun., in press
TDP-43 oligomerization impedes inter-molecular low complexity domain interactions

Afroz et al., Nature Commun., in press
Does TDP-43 oligomerization antagonize its pathologic aggregation?

monitor aggregation in oligomerization mutants
Increased phosphorylation in TDP-43 oligomerization mutants

Afroz et al., Nature Commun., in press
Functional and dynamic TDP-43 oligomerization antagonizes pathologic aggregation
Functional and dynamic TDP-43 oligomerization antagonizes pathologic aggregation
Functional and dynamic TDP-43 oligomerization antagonizes pathologic aggregation

**Autoregulation?**
- splicing

Physiological functions

Dynamic TDP-43 oligomerization

Altered equilibrium

Narrative:
- NTD
- RRM1
- RRM2
- LCD

- NTD
- RRM1
- RRM2
- LCD

- NTD
- RRM1
- RRM2
- LCD
Increased proteolysis?

Autoregulation? – splicing

Functional and dynamic TDP-43 oligomerization antagonizes pathologic aggregation
Functional and dynamic TDP-43 oligomerization antagonizes pathologic aggregation.
1. Functional mechanisms

Functional and dynamic TDP-43 oligomerization antagonizes pathologic aggregation

2. Molecular basis of pathologic aggregation

Therapeutic strategy
Structure-based prevention of aggregation
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Thank you for your attention