Structural Studies of Hetero-Amyloid Signaling Complexes Using SSNMR

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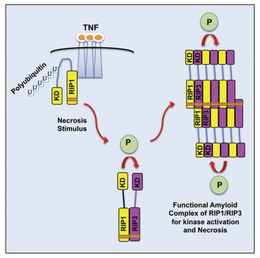
Amyloids are protein aggerates that have a fibrillar morphology with beta-sheet secondary structures. Many amyloids are pathogenic and associated with multiple human diseases, while some of them play indispensable roles in the biological system, termed as functional amyloids. Our lab are specifically interested in the functional amyloids that are responsible for signaling.

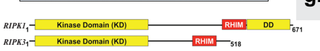
Core structure of RIPK1/RIPK3 revealed by SSNMR

--------the first solved structure of a hetero-amyloid

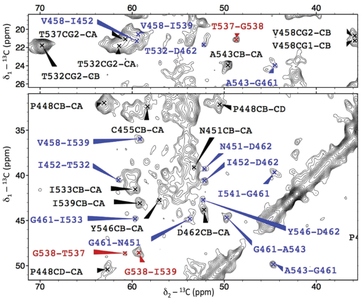
Biological Background



- **Receptor-interacting protein kinase 1 and 3** (RIPK1 and RIPK3) are key determinants in tumor necrosis factor (TNF)-induced cell-fate regulation.
- They share a unique segment of homologous sequences, **RIP homotypic interaction motifs (RHIMs)**.
- They can form a **necrosome** mediated by RHIMs to initiate a unique cell death pathway known as **programmed necrosis or necroptosis**.

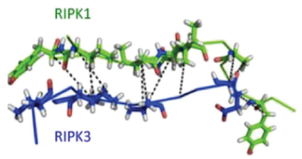


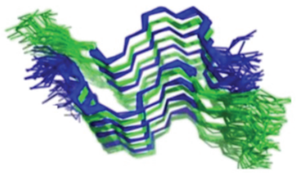
Results



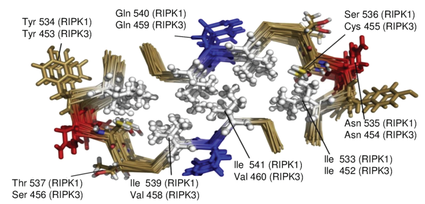
- RIPK1/RIPK3 complex has a **robust core flanked by flexible regions**. The spectrum illustrates contacts within the RIPK1- RIPK3 core complex.(intermolecular labeled blue, intra-residue labeled black, and sequential labeled red)

Long-range intermolecular contacts are selected to to define parallel beta sheet strands.





The 20 lowest-energy conformers obtained with inclusion of low-ambiguity, resolved restraints.



Structual Highlights
- Hydrophobic interface and core
- AsN and Gln H-bonds
- Try, Ser and Thr stack
- Cys-Ser ladder

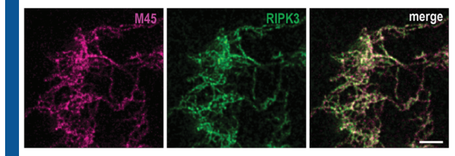
Structual Studies of Hetero-Amyloid M45/RIPK3

Biological Background

- The **murine cytomegalovirus protein M45** protects cells from necroptosis induced by TNFR activation.
- The N-terminal 90 residues of the M45 protein,containing a **RHIM** domain, sufficiently protects against TNFR-induced necroptosis.
- This region drives rapid self-assembly into homo-oligomeric amyloid fibrils and interacts with the RHIMs of RIPK1 and RIPK3 to form **hetero-amyloid** fibrils in vitro.

Questions to be answered

How does M45 protect cells from necroptosis?
What's the underlying structual mechanism?

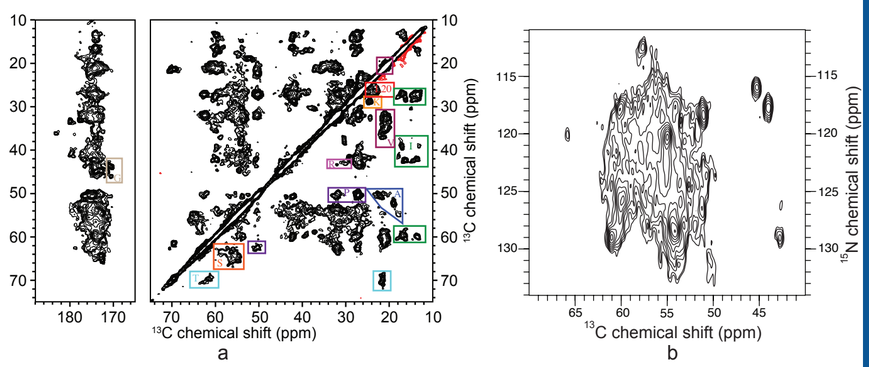


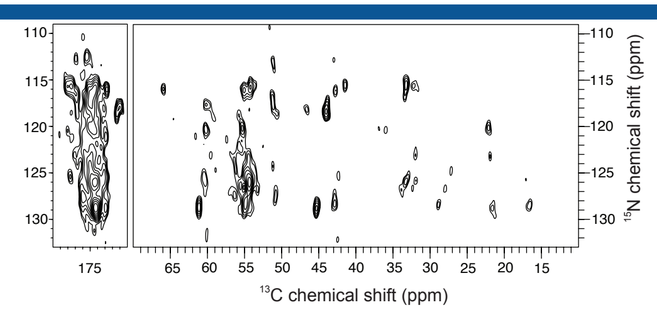
Super-resolution STED image of YPet-RIPK3$_{387-518}$ (green) and mCHERRY-M45$_{1-90}$ (red) in amyloid fibrils, indicating co-localization of host and viral proteins in the hybrid amyloid network.

Results

1D spectra of M45 in M45/RIPK3 complex.
(a) ^{13}C INEPT, (b) ^{13}C Cross Polarization, (c) ^{15}N Cross Polarization

- No protein signal seen in the INEPT spectrum, indicating it's mostly **rigid.**
- Types assignments in the DARR spectrum indicating most of them are **beta-sheet.**
- A few strong and well resolved peaks shown in NCA, while the rest of them less resolved, likely indicating a **rigid core flanked by mobile regions**.
- In the N(CO)CX spectrum, magnetization on some COs can be transferred through the complete spin system, indicating **some residues are rigid and well-structured.**





2D spectra of M45 in M45/RIPK3 complex.
(a)^{13}C-^{13}C DARR with 75ms mixing time, (b) NCA selective CP,
(c) N(CO)CX reduced 3D

Scaled recoupling of chemical shift anisotropies at high magnetic field

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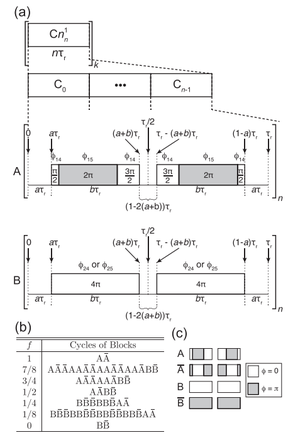
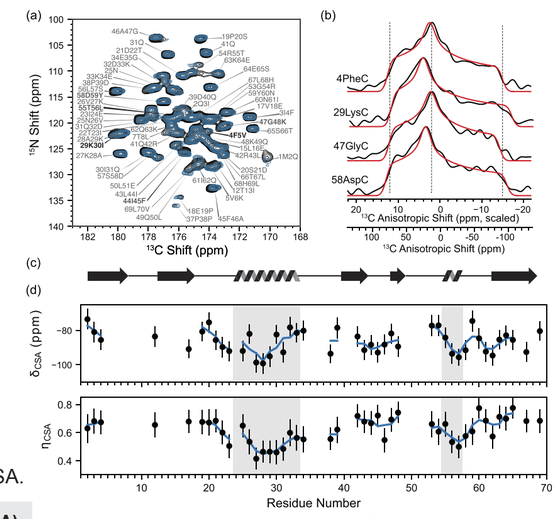


f	Cycles of Blocks
1	$A\bar{A}$
7/8	$A\bar{A}\bar{A}\bar{A}A\bar{A}\bar{A}\bar{A}A\bar{A}\bar{A}\bar{A}A\bar{A}B\bar{B}$
3/4	$A\bar{A}\bar{A}A\bar{A}B\bar{B}$
1/2	$A\bar{A}B\bar{B}$
1/4	$B\bar{B}B\bar{B}B\bar{A}A$
1/8	$B\bar{B}B\bar{B}B\bar{B}B\bar{B}B\bar{B}B\bar{B}A\bar{A}$
0	$B\bar{B}$

Pulse sequence diagram for fROCSA.



Three-dimensional NCO-1/2-ROCSA(0.0329, 0.467) experiment applied to microcrystalline Ubiquitin.

- **Chemical shift anisotropy(CSA)** provides valuable information on the **structure** and **dynamics** of proteins.
- **fROCSA** recovers the static CSA lineshapes in an indirect dimension with a **customized scaling factor**.
- It makes it possible to study CSA with **large spans** at reasonable experimental conditions.

- **Site-specific CSA** tensor parameters for the **backbone carbonyl** were measured.
- Clear trends were seen in the protein sequence, in correspondence with **secondary structures**.

Acknowledgement

The RIPK1/RIPK3 project was in collaboration with **Wu's lab at Harvard Medical School**. **Miguel Mompeán, Wenbo Li, Jixi Li, Ségolène Laage, Ansgar B Siemer, and Gunes Bozkurt** contributed to the project.

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References

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