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Title:	Understanding Dynamics and Conformational Changes in Proteins
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Understanding Dynamics and Conformational Changes in Proteins

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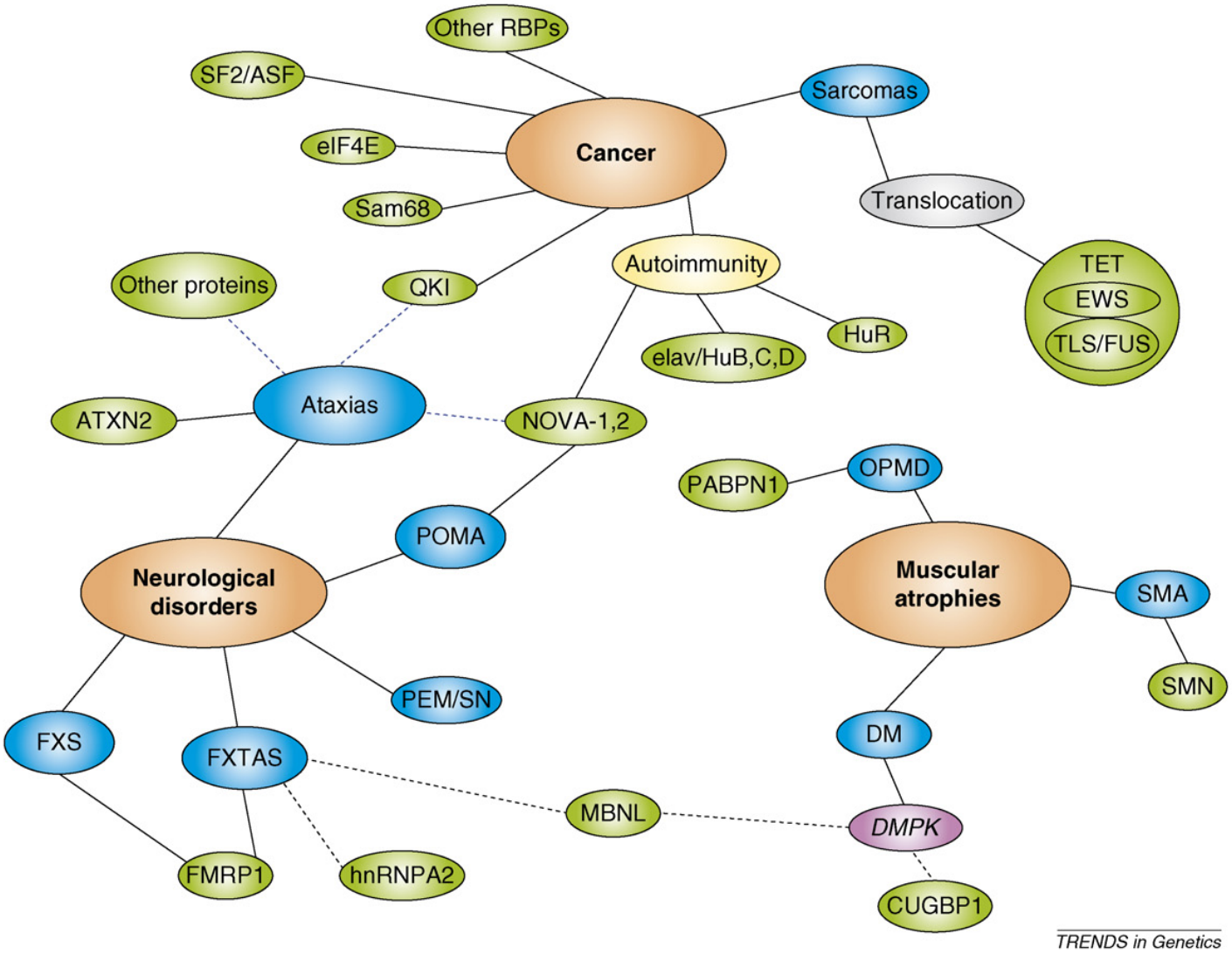
July 13, 2012

I. Protein dynamics in RNA-protein complex (U1A-SL2)

II. Deciphering the chaos in an intrinsically disordered protein (alpha-synuclein)

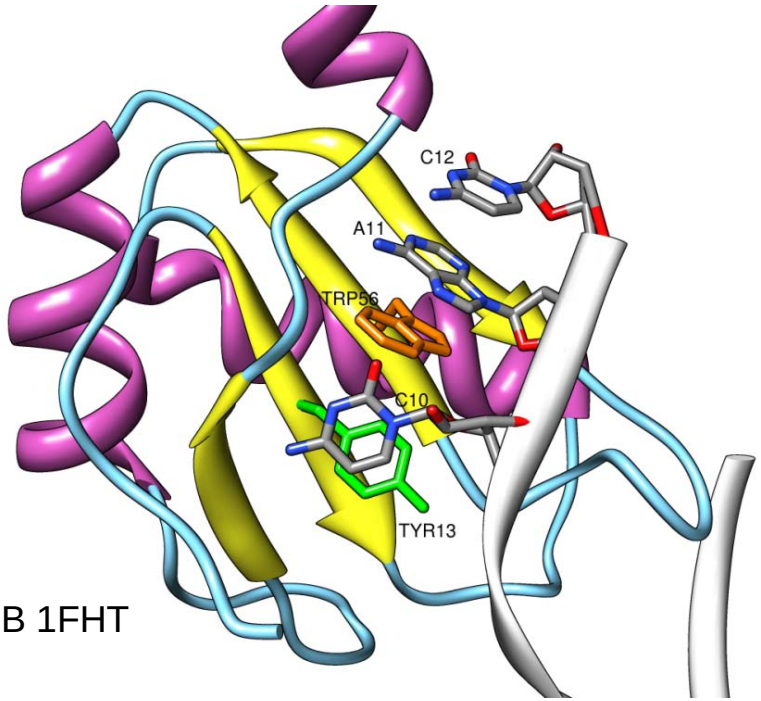


- **Gene expression – RNA editing and degradation**
- **Prediction and control**
- **Therapeutics – peptides, small molecules**

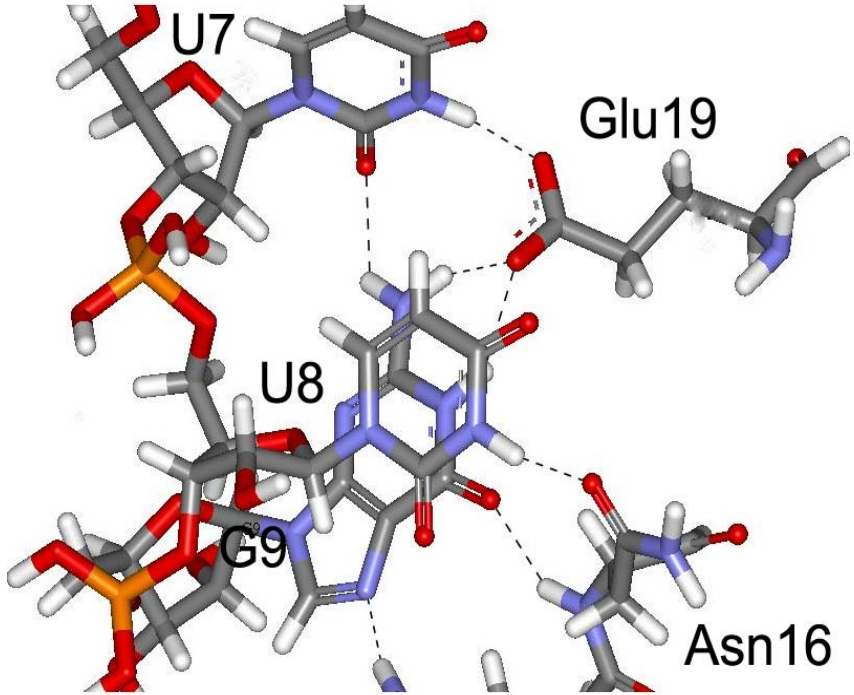
Lukong, et al. *Trends in Genetics,* **2008,** 24, 416

RNA-Protein recognition

- Individual interactions (H-bond, stacking, VDW)
- Dynamics (different time-scales) – essential in recognition
- Long-range cooperative interactions



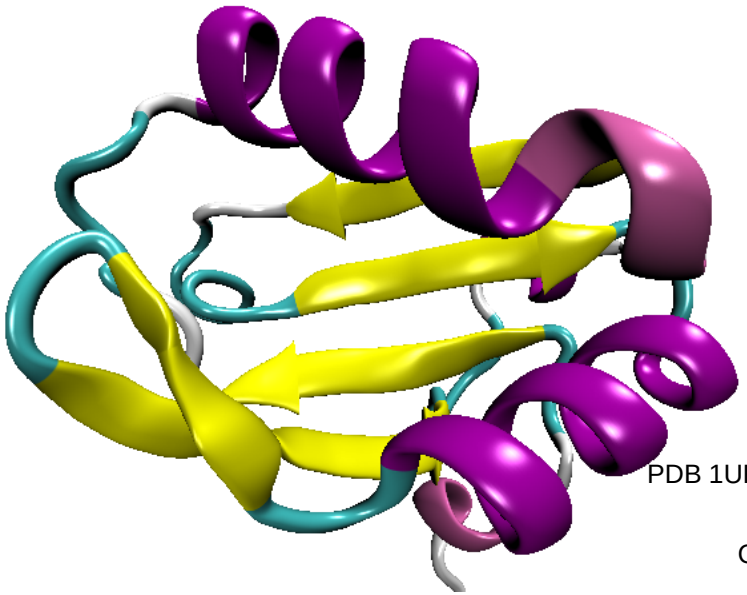
PDB 1FHT



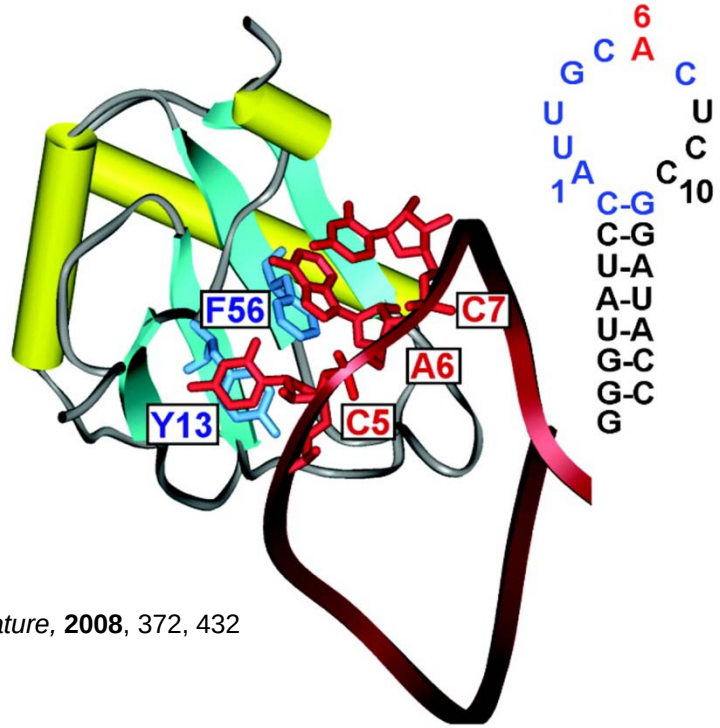
Baranger, et al. *BMC Biochemistry*, **2007**, 8, 22

What were we studying?

- RNA Recognition Motif (RRM) - common RNA binding domain
- Involved in gene expression and RNA metabolism
- 80 -100 amino acids in an RRM
- forms a scaffold for recognizing ssRNA; varied affinities
- U1A is a model system – picomolar affinity

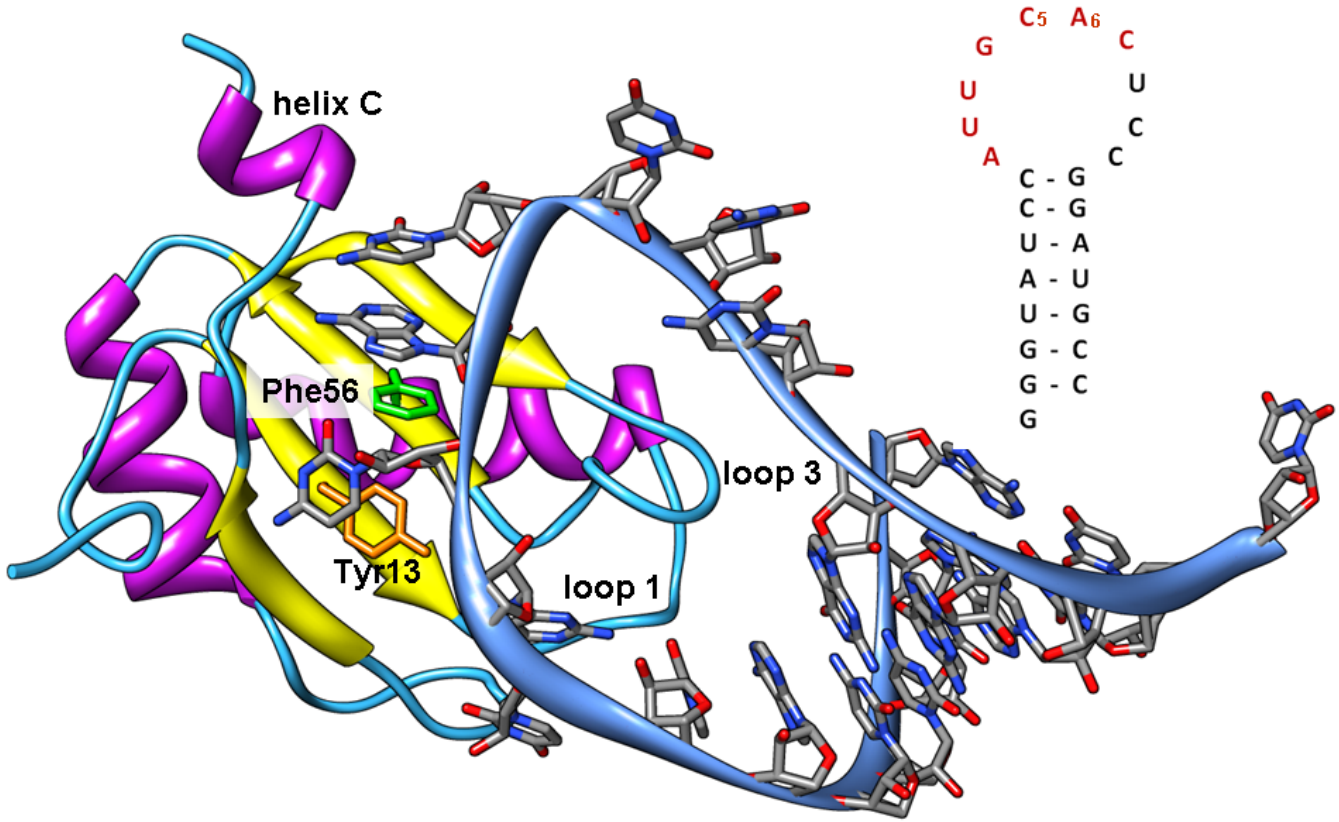


PDB 1URN



Oubridge, et al. *Nature,* **2008**, 372, 432

U1A-SL2 RNA complex



- **Picomolar binding affinity; single RRM**
- **Networks of H-bonds, electrostatics and stacking**
- **Conformational changes for U1A and RNA**

Oubridge, et al. *Nature,* **2008**, 372, 432

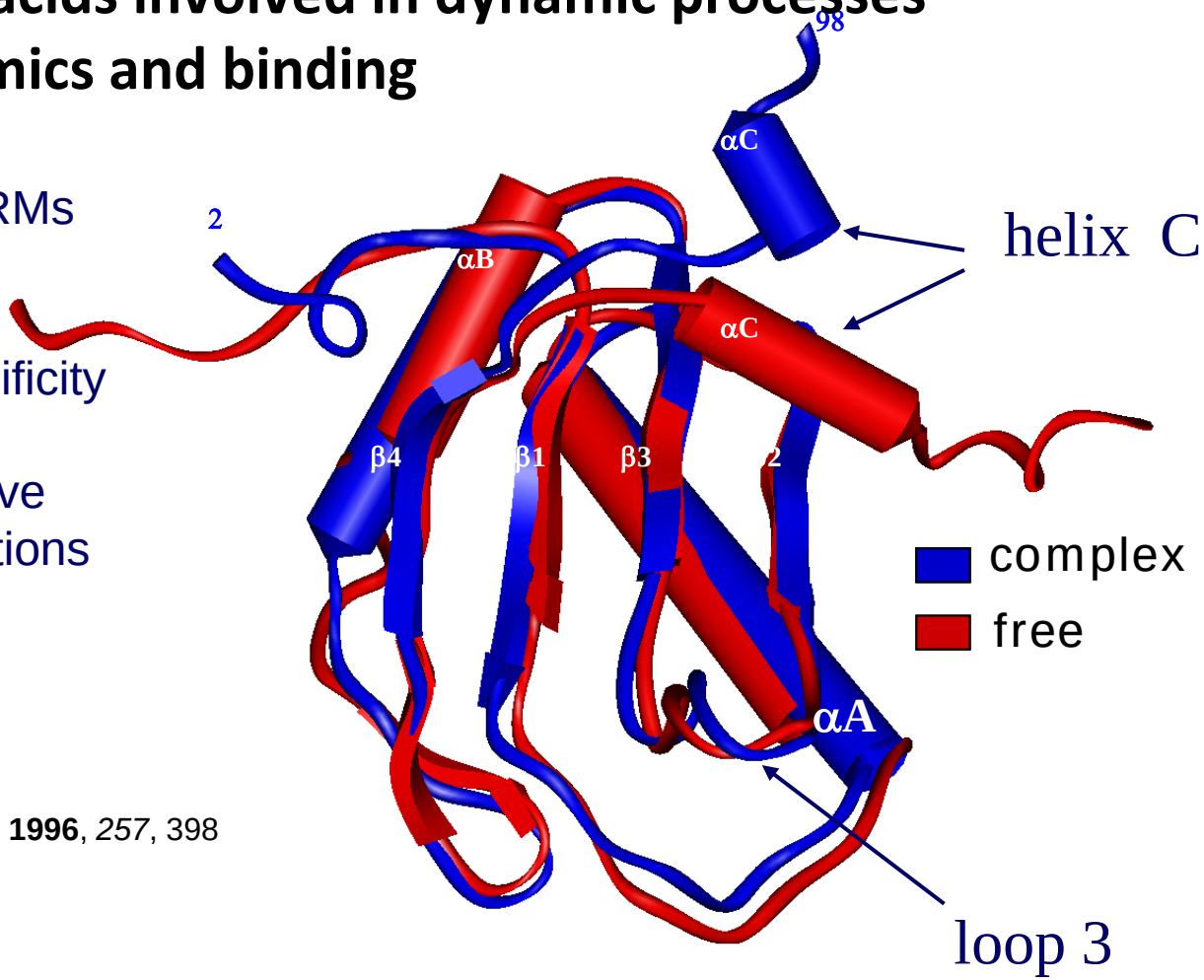
Research goals

- **Characterize dynamics and kinetics of U1A**
- **Identify amino acids involved in dynamic processes**
- **Correlate dynamics and binding**

- Often present in RRMs has different roles

- Contributes to specificity

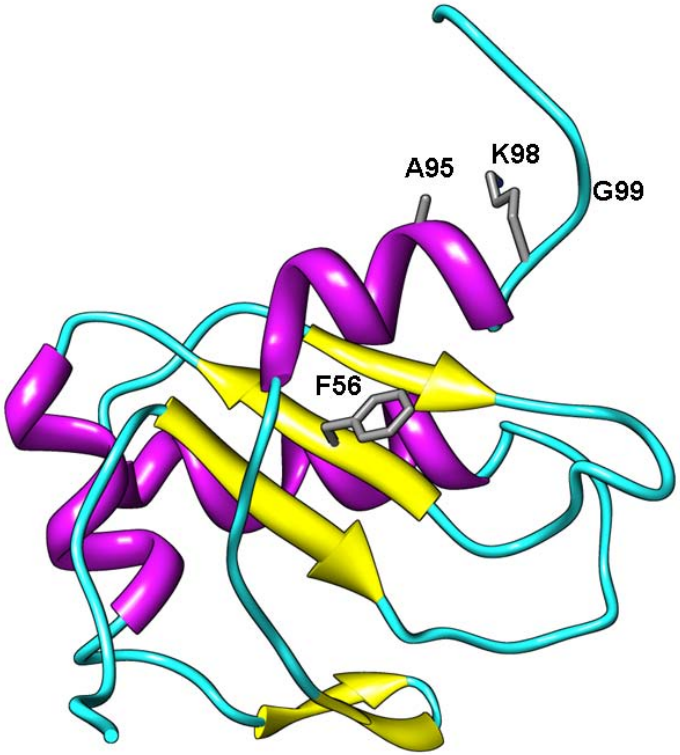
- Dynamics might have cooperative contributions to RNA recognition

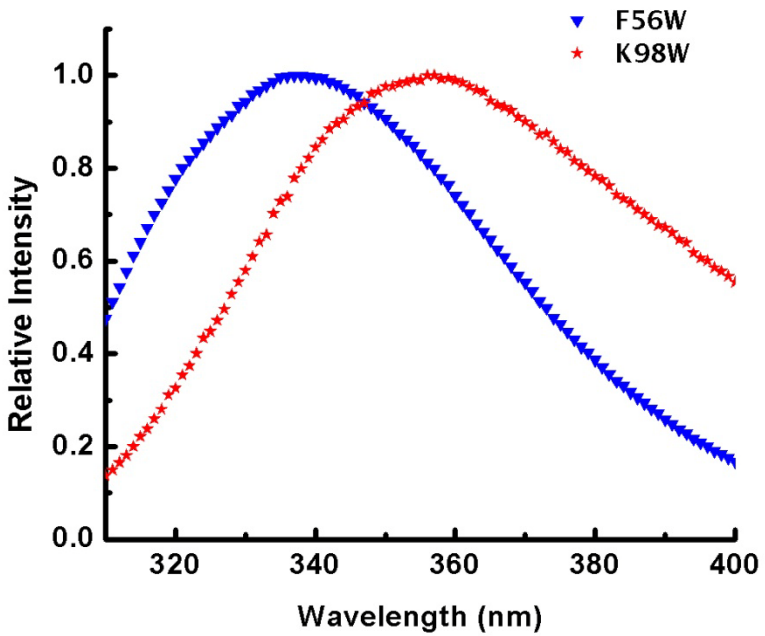


Kormos, Baranger, et al. *J. Mol. Biol.*, **1996**, *257*, 398

i.) Helix C dynamics

- **Fluorophore environment**



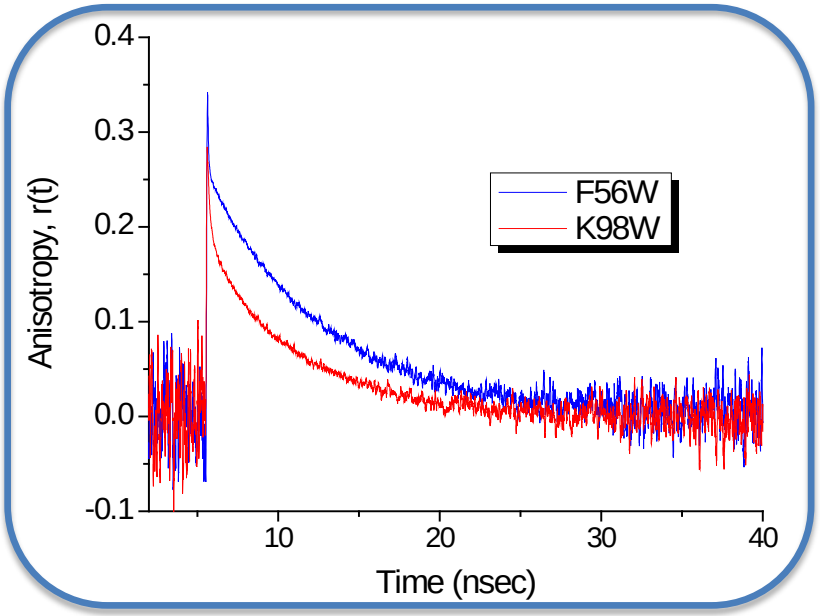


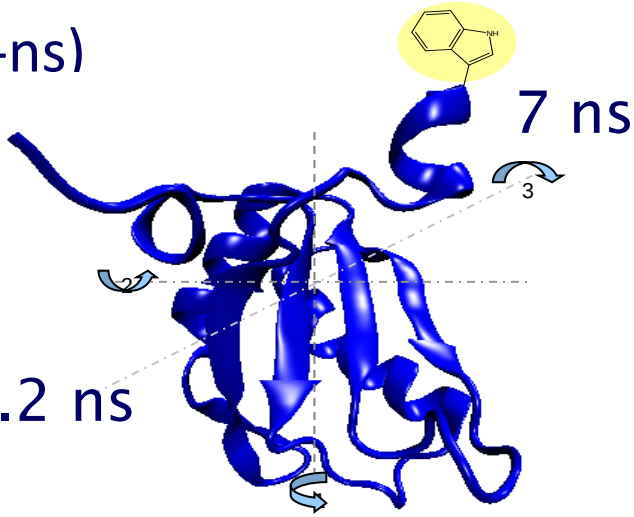
- **K98W and F56W bind similar as WT**
- **K98W is more solvent-accessible than F56W**

Anunciado, Baranger, et al. *J. Phys. Chem B*, **2008**, 112, 6122

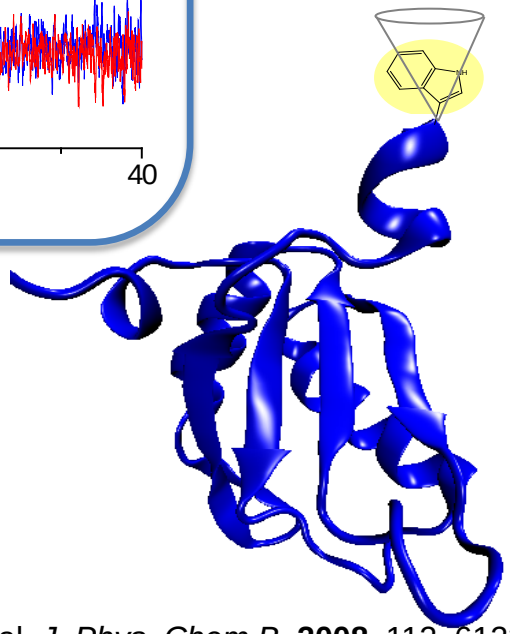
Fast protein dynamics

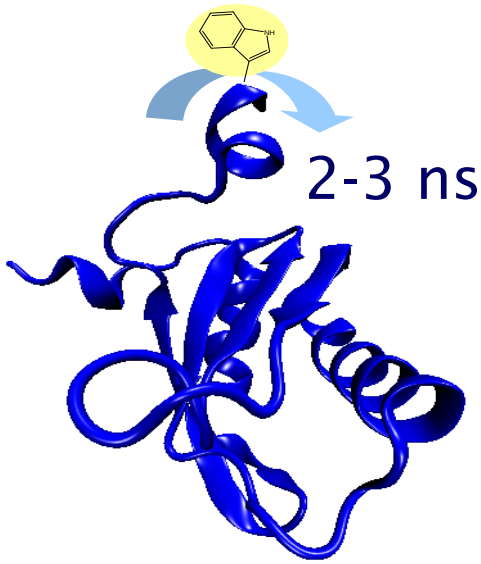
- time-resolved fluorescence anisotropy (ps-ns)





0.07 – 0.2 ns





Anunciado, Baranger, et al. *J. Phys. Chem B*, **2008**, 112, 6122

Fluorescence anisotropy

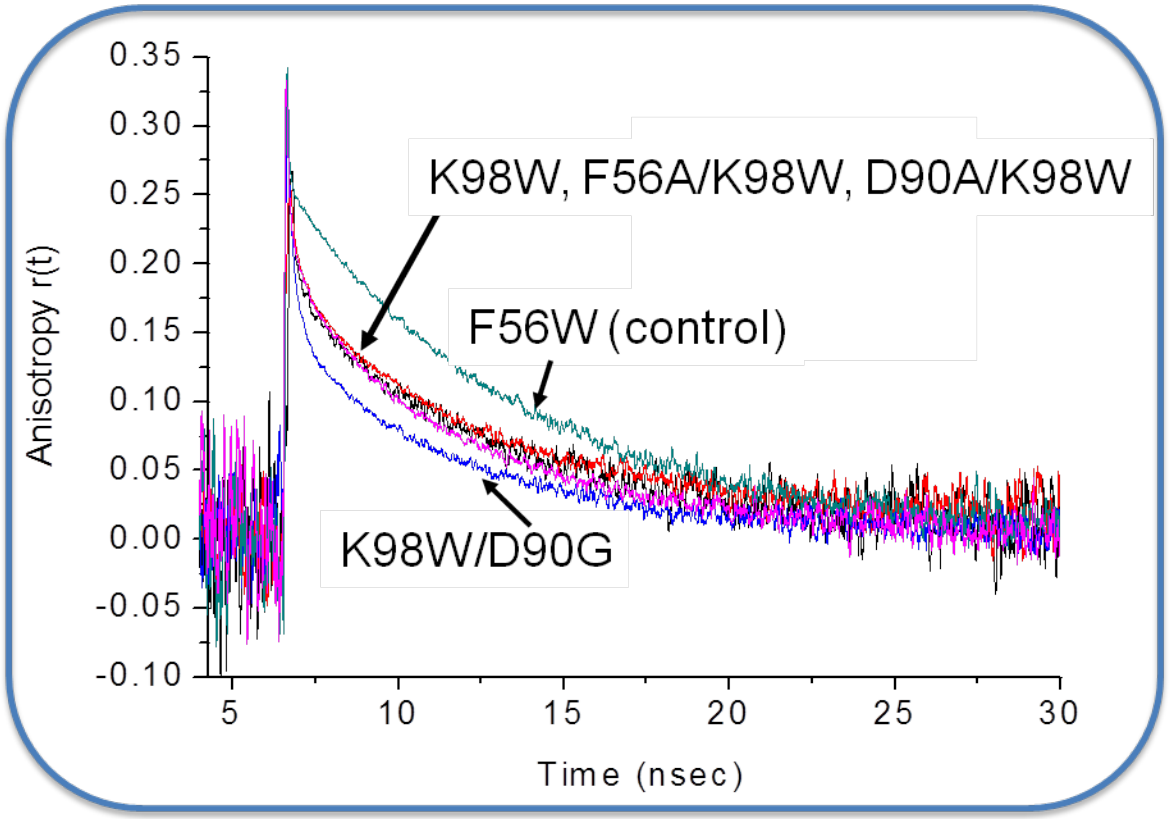
	K98W	G99W	F56W	F56AK98W	D90AK98W	D90GK98W
r_0	0.29 ± 0.03	0.27 ± 0.02	0.31 ± 0.06	0.29 ± 0.02	0.33 ± 0.05	0.29 ± 0.04
β_1	0.29 ± 0.03	0.25 ± 0.03	0.18 ± 0.12	0.27 ± 0.02	0.38 ± 0.11	0.43 ± 0.04
θ_L(ns)	0.09 ± 0.03	0.18 ± 0.03	0.12 ± 0.08	0.11 ± 0.03	0.08 ± 0.07	0.10 ± 0.05
β_2	0.22 ± 0.05	0.32 ± 0.04		0.24 ± 0.05	0.13 ± 0.03	0.20 ± 0.05
θ_S(ns)	2.58 ± 0.44	2.27 ± 0.30		2.57 ± 0.60	1.96 ± 0.88	2.22 ± 0.67
β_3	0.49 ± 0.04	0.43 ± 0.05	0.82 ± 0.16	0.49 ± 0.10	0.49 ± 0.05	0.37 ± 0.08
θ_R(ns)	7.56 ± 0.46	6.63 ± 0.76	7.06 ± 0.57	7.22 ± 0.31	6.77 ± 0.28	6.53 ± 0.55

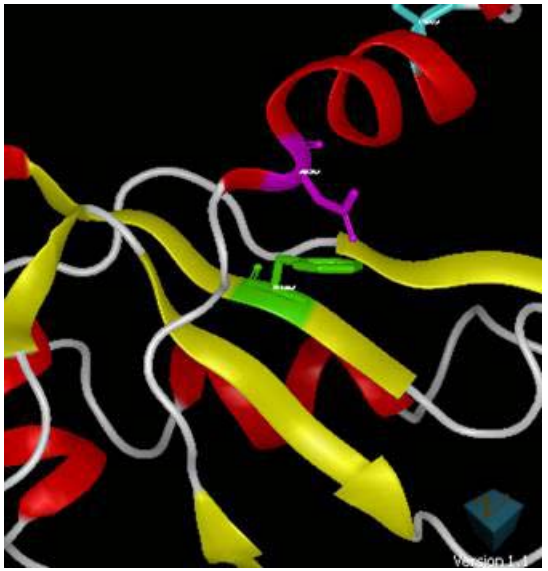
θ values are the correlation times; β values are the contribution of each component to the anisotropy decay

- Two correlational times for F56W and three for K98W
- Overall tumbling rotational constant agrees with calculation and in literature

Anunciado, Baranger, et al. *J. Phys. Chem B*, **2008**, 112, 6122

Mutations to increase dynamics?





- D90G mutation alters dynamics; destabilized the complex 100X
- F56AK98W destabilize of the complex; no effect on dynamics

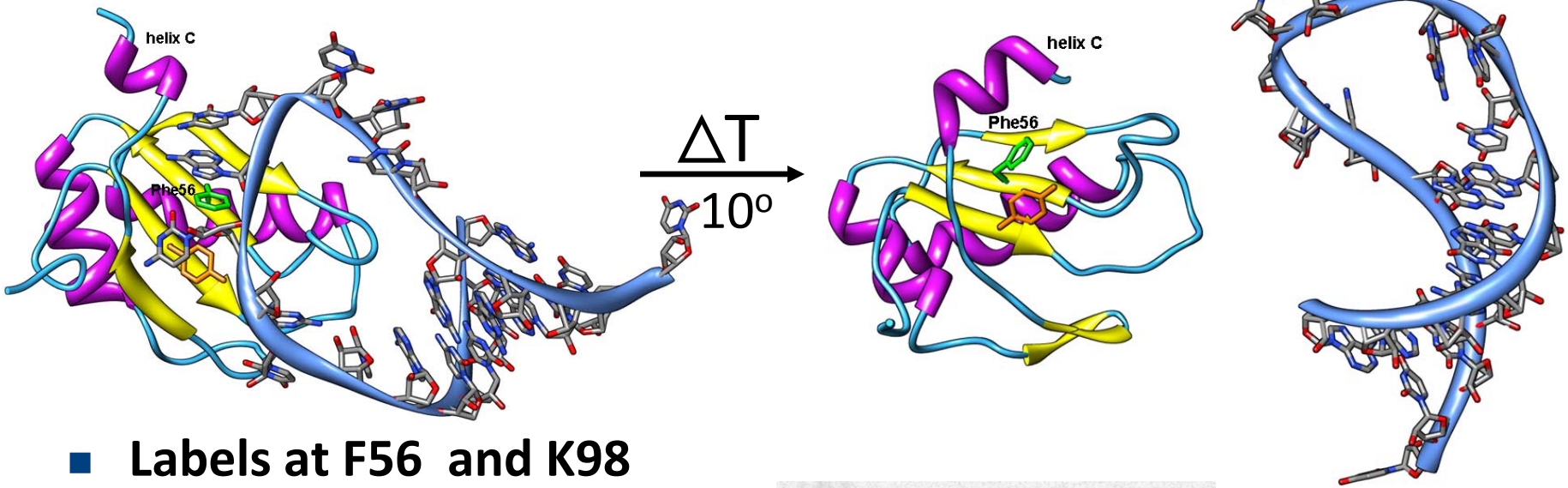
Anunciado, Baranger, et al. *J. Phys. Chem B*, **2008**, 112, 6122

i) Conclusions

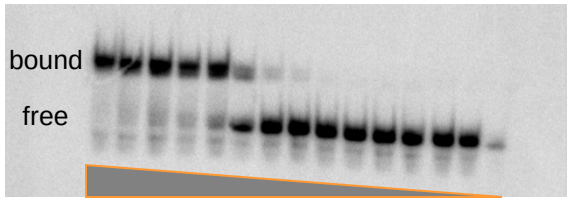
- Helix C is dynamic in 2-3 ns, 20^{o}
- Helix C motion is not a transition between open and closed protein conformations
- Data supports an induced fit mechanism of binding
- Mutations at the hinge of helix C (D90A and D90G) do not alter dynamics but cause complex destabilization
- MD simulation results agree with fluorescence anisotropy data

ii) Kinetic studies of the U1A-SL2 complex

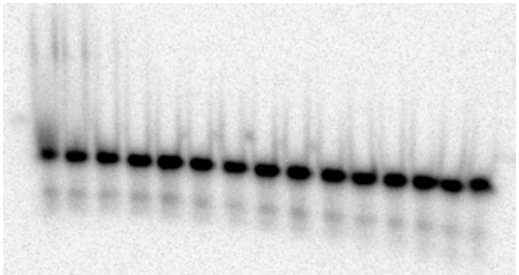
- **Study kinetics of complex dissociation (ns-s timescale)**
- **Laser-induced T-jump to perturb complex**



- **Labels at F56 and K98**



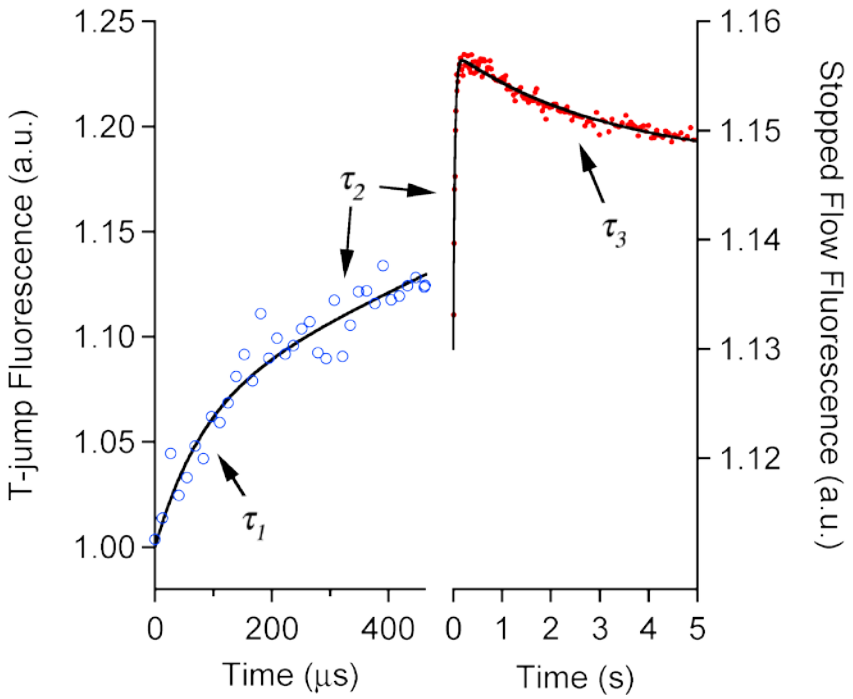
35C 300mM KCl-phoshate



45C 300mM KCl-phosphate

Anunciado, Baranger, et al. J. Mol. Biol. **(2011)** 408, 5, 896-908

Kinetics experiments

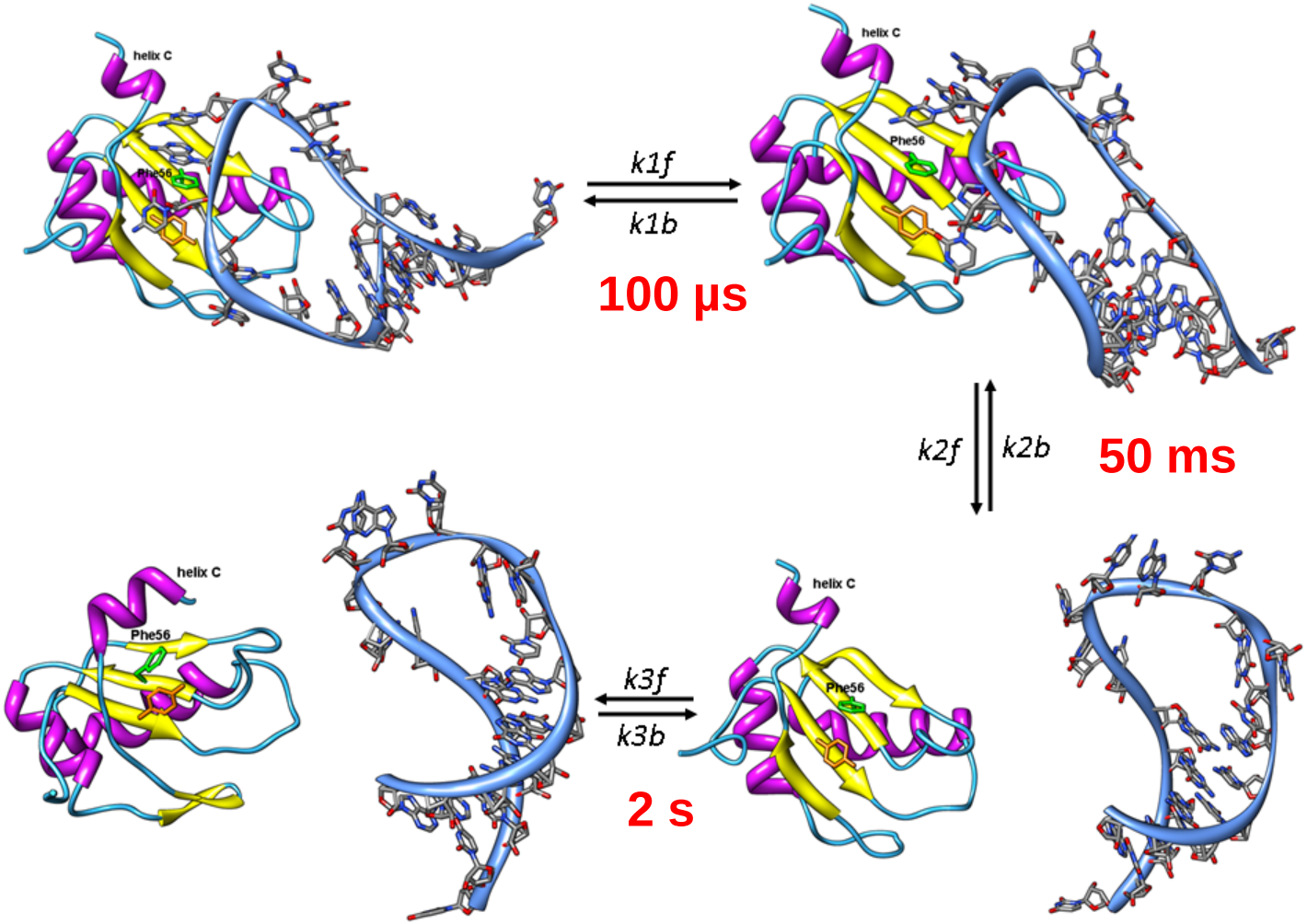


- Laser T-jump and stopped-flow experiments; 25-45 °C
- 1:1 U1A:RNA ratios, 5-20 μM conc.
- $\tau_1 \approx 100$ μs, $\tau_2 \approx 50$ ms and $\tau_3 \approx 2$ s
- τ_1 and τ_2 correspond to increase in fluorescence

Anunciado, Baranger, et al. J. Mol. Biol. **(2011)** 408, 5, 896-908

Kinetic scheme of dissociation

$$PR_{tight} \underset{k_{1b}}{\overset{k_{1f}}{\rightleftarrows}} PR_{loose} \underset{k_{2b}}{\overset{k_{2f}}{\rightleftarrows}} P + R \underset{k_{3b}}{\overset{k_{3f}}{\rightleftarrows}} P' + R$$



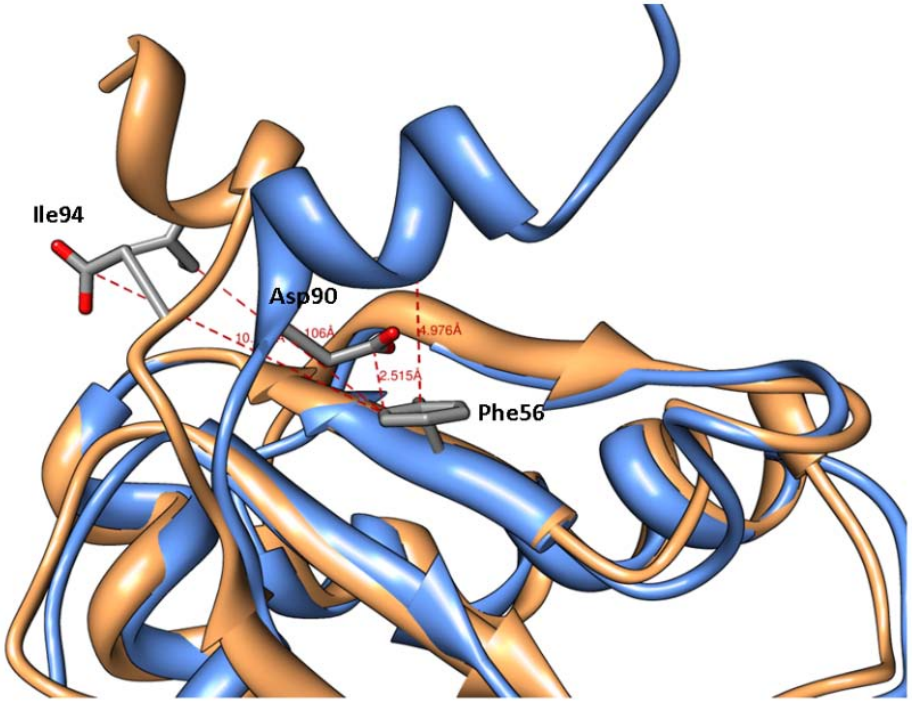
Anunciado, Baranger, et al. J. Mol. Biol. **(2011)** 408, 5, 896-908

ii) Conclusions

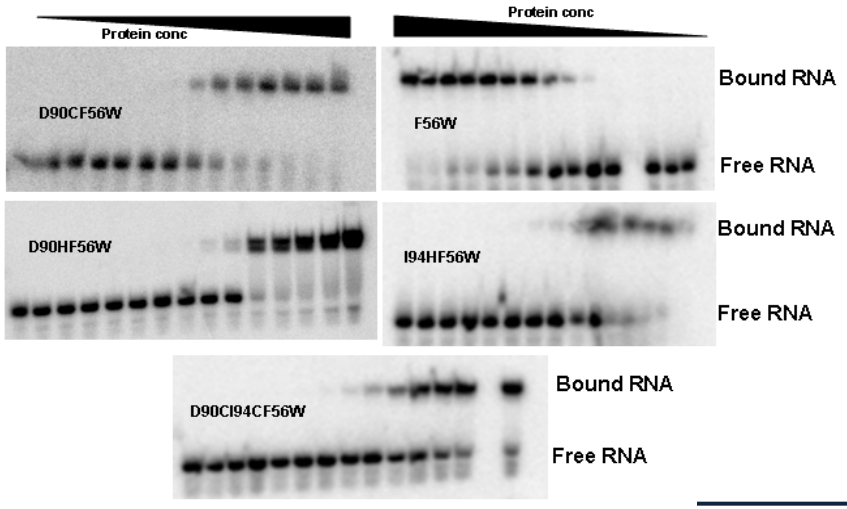
- **Global fit of T-jump, stopped-flow and equilibrium binding:**
  - Three-step dissociation, 2 intermediate states
  - Consistent with MD simulation studies (Nilsson)
  - Supported by SPR studies (Laird-Offringa)
- **First step – relaxation of close-range interactions**
  - Change in Trp56 orientation ; affected its interaction with A6
- **Second step – dissociation; release of electrostatic and non-specific interactions**
  - Removal of the RNA from the binding surface
  - Greater distance between Trp56 and A6 – fluorescence increase
- **Third step – slow decrease in fluorescence; protein conformational changes; helix C motion; reduction in Trp56 solvent accessibility**

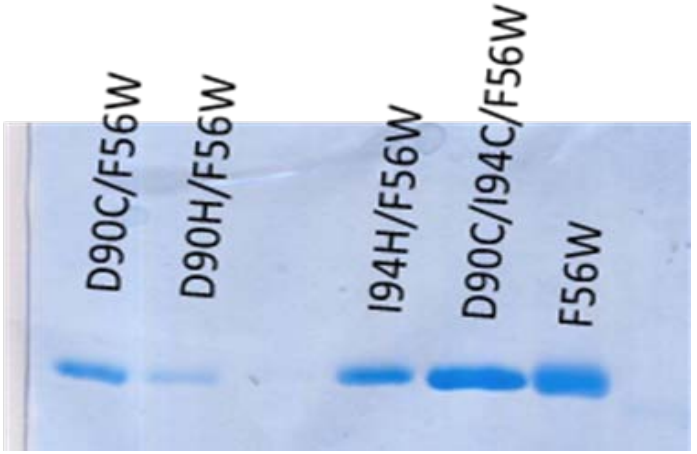
Probing helix C dynamics by Trp quenching

- **To study the effects of His, Cys introduced near F56W ~ 2-3Å in free U1A and 10-14Å in the complex**
  - D90H/F56W
  - D90C/F56W
  - I94H/F56W
  - I90C/F56W
  - D90C/I94C/F56W
  - D90H/I94H/F56W



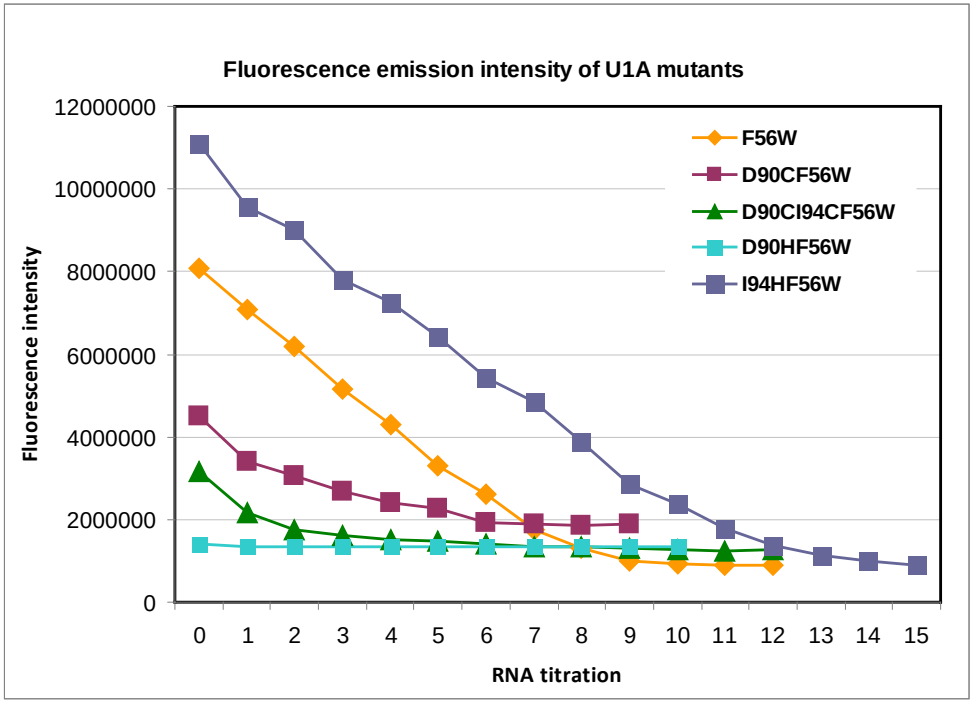
Probing helix C dynamics by Trp quenching





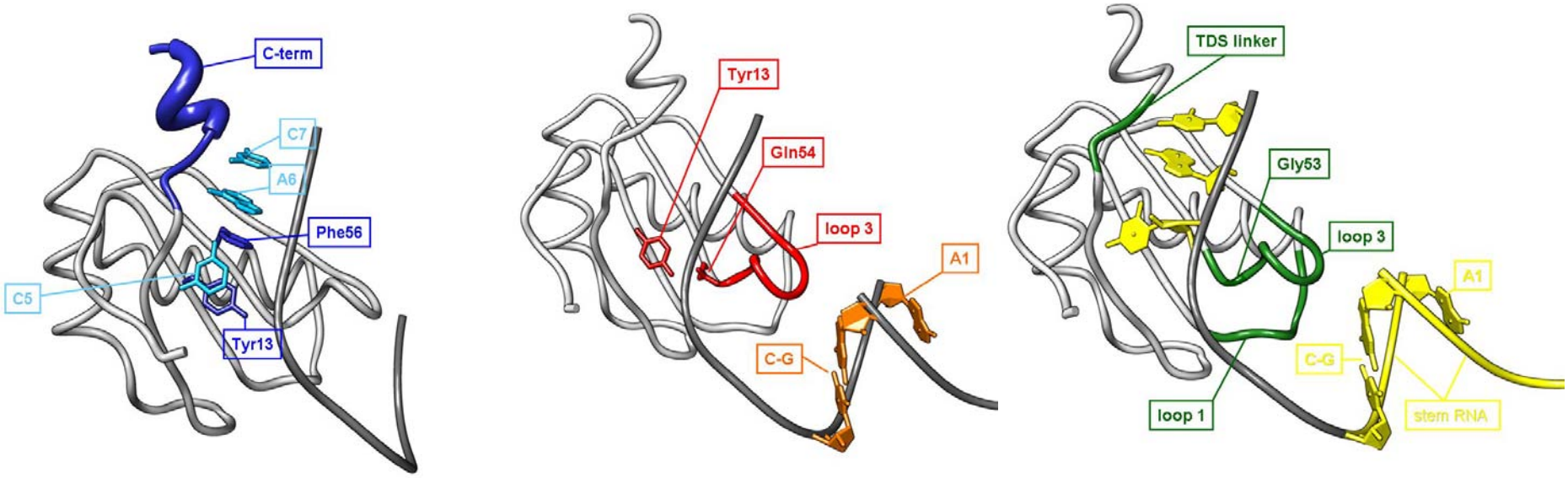
Protein	K_d (M)
WT	$1.0 (\pm 0.1) \times 10^{-10}$
F56W	$8.0 (\pm 1.0) \times 10^{-10}$
D90CF56W	$4.0 (\pm 3.0) \times 10^{-9}$
D90HF56W	$3.5 (\pm 0.9) \times 10^{-9}$
I94HF56W	$1.0 (\pm 0.8) \times 10^{-7}$
D90CI94CF56W	$2.0 (\pm 0.2) \times 10^{-7}$

Steady-state fluorescence



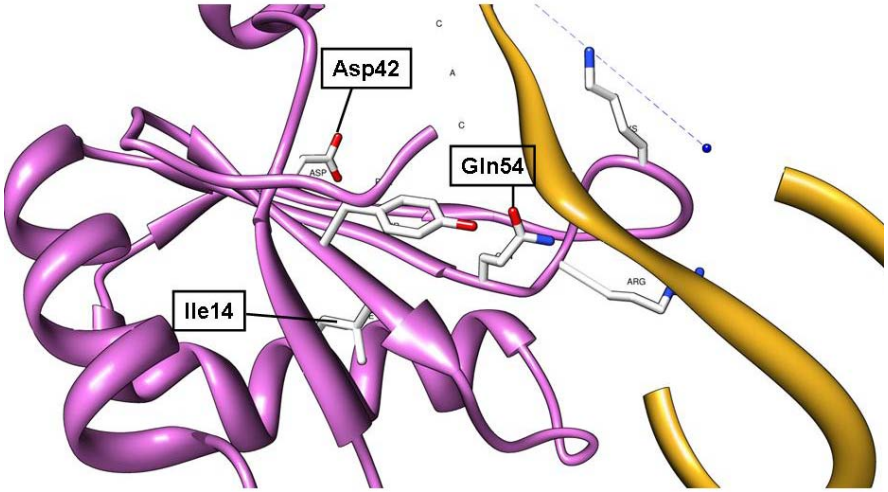
- preliminary data
- helix C dynamics data could support proposed kinetic model
- further kinetic experiments to be conducted – CD, stopped-flow and T-jump fluorescence

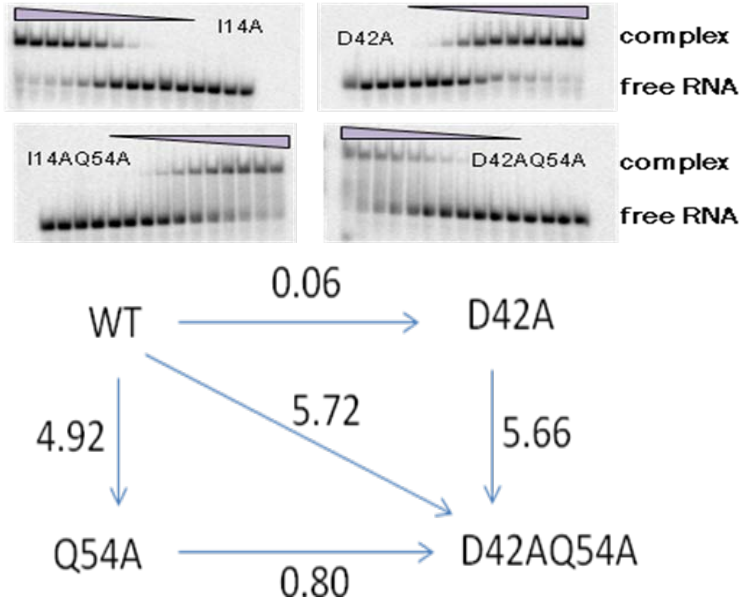
iii) Investigation of Cooperative Interactions



- **Network 1: Tyr13, Phe56 and C-term residues Lys96-Phe101**
- **Network 2: Tyr13, Gln54 and loop 3 residues**
- **Network 3: Gly53, loop1, loop3 and the TDS linker**
- **Investigate energetic coupling between: Ile14, Asp42 and Gln54 (MD-DCCM)**
- **Integrate computational results and experimental data – build a model of cooperative networks of interactions in the U1A system**

Ala mutations of Ile14, Asp42, and Gln54





- **I14AQ54A caused ~5.0 kcal/mol complex destabilization (5000x less binding)**
  - Coupling energy of ~0.08 kcal/mol
- **D42AQ54A caused ~5.72 kcal/mol destabilization (16,000x less binding)**
  - Coupling energy of ~0.74 kcal/mol
  - Negative local cooperativity

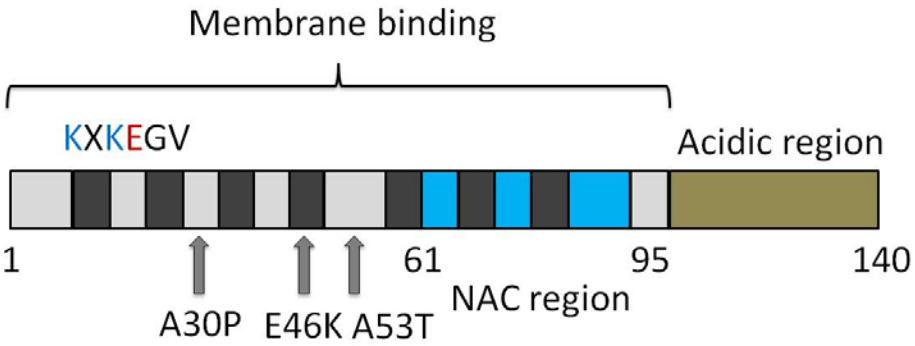
iii) Conclusions

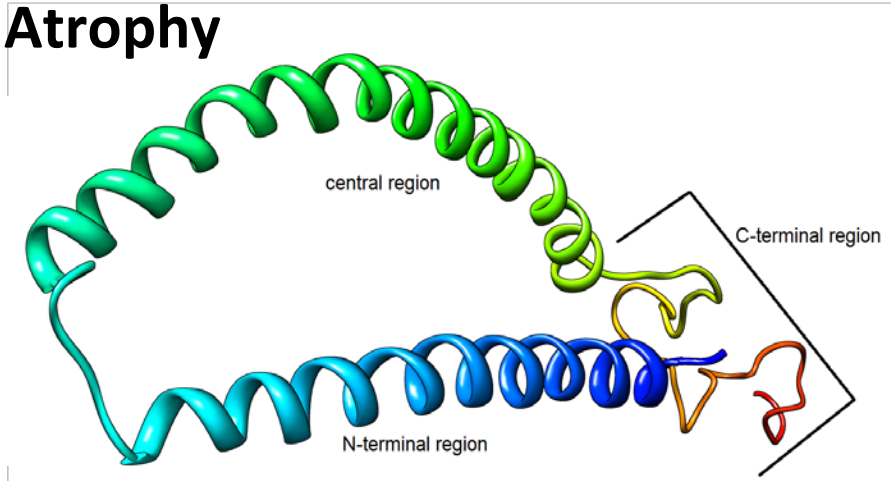
- **No cooperative interactions between Ile14 and Gln54 as opposed to findings in computational methods**
- **Asp42 and Gln54 shows negative local cooperativity**
  - any perturbation in the structure or dynamics in the β-sheet can be propagated through the entire binding surface
- **The mutations studied here are just a few of those pinpointed in the computational studies – further studies**

Deciphering the chaos in IDP

Alpha-synuclein

- Small (14kDa), heat-stable protein; Unfolded random coil in solution
- Unknown function; Located in presynaptic nerve terminals and interacts with phospholipid membranes
- Major component of Lewy bodies in Parkinson's disease and Dementia Lewy Bodies; neuronal and glial cytoplasmic inclusions in Multiple System Atrophy





PDB 1XQ8

J Biol Chem 2005, 280:9595-9603.
Science 2003, 302:819-822

Alpha-synuclein

- May regulate trafficking of lipid secretory vesicles
- Prevented aggregation of other proteins in chaperone-like manner
- Highly conserved KTKEGV (6x in N-terminal region)
- Mutations A53T, A30P and E46K in familial PD

Protein sequence

```
1          10         20         30         40         50
MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVH
51         60         70         80         90         100
GVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL
101        110        120        130        140
GKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA
```

Biochemistry 2007; 46(15):4499-509
J Biol Chem 2005, 280(10):9595-603

Research objectives

- To characterize structure and conformations of Alpha-synuclein in solution

- To elucidate the molecular mechanisms involved in the folding and binding of Alpha-synuclein with SDS to better understand its membrane binding and function

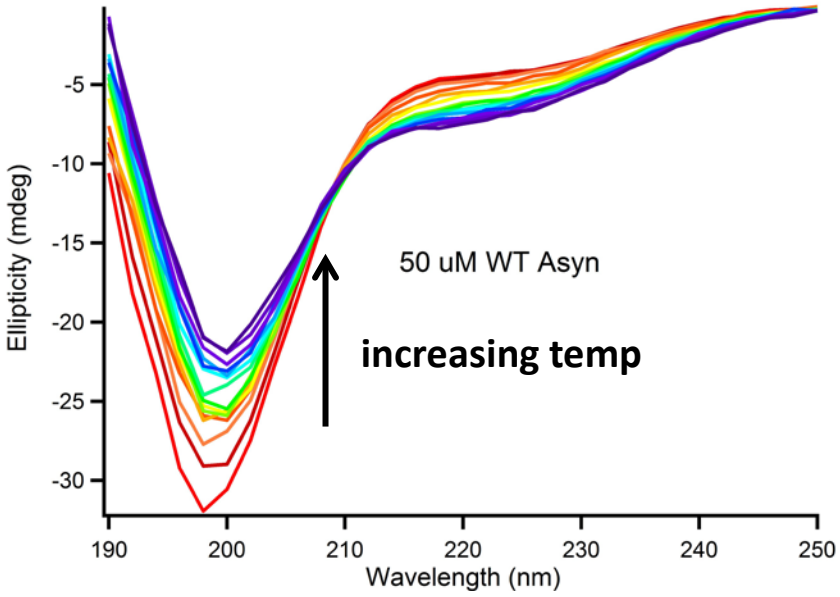
Methods

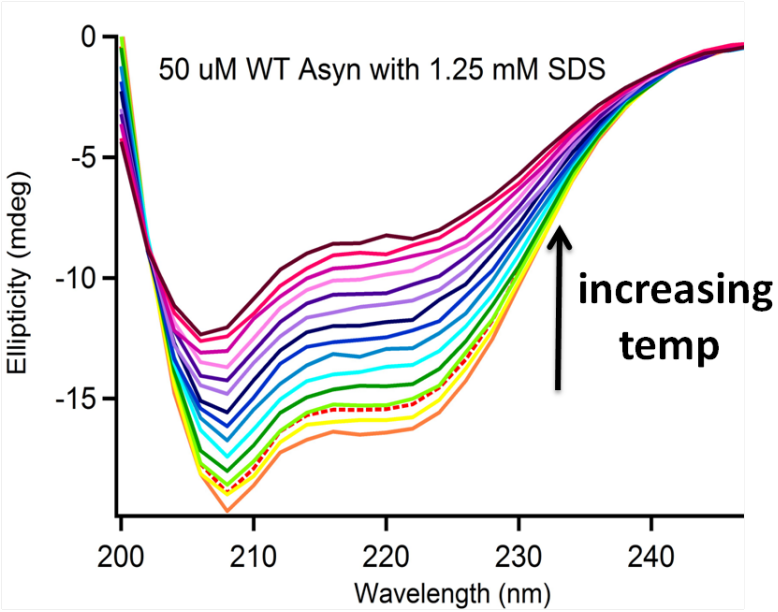
Fourier-transform Infrared spectroscopy

All atom MD simulation

Circular Dichroism

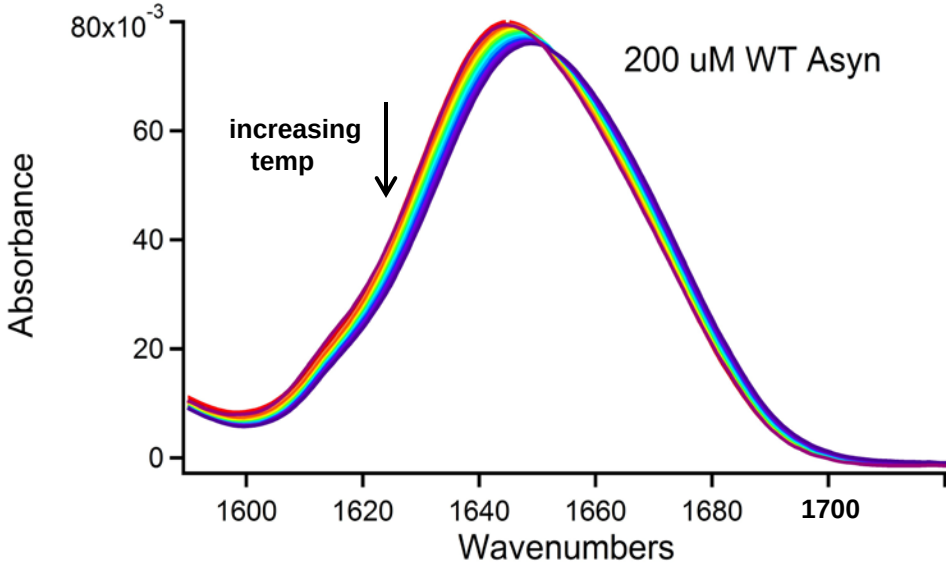
Small Angle Neutron Scattering

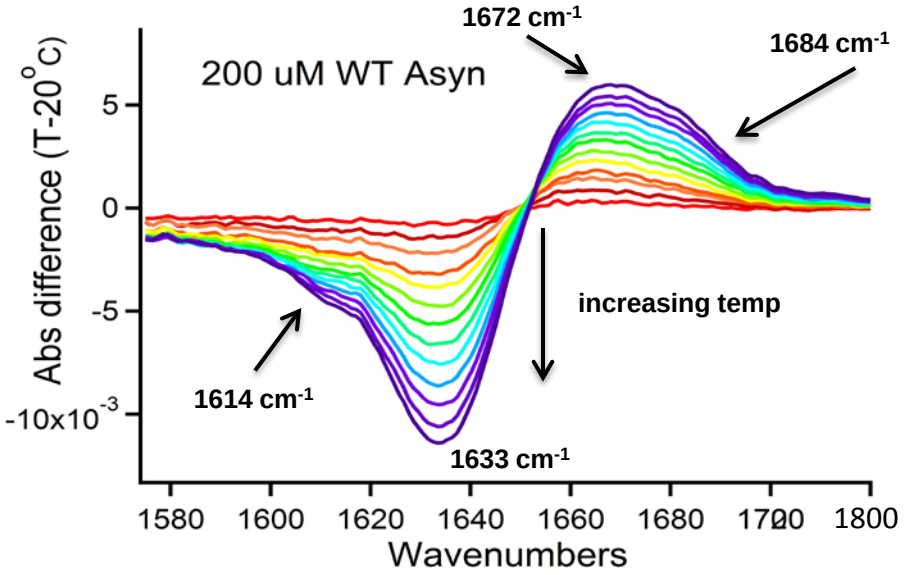


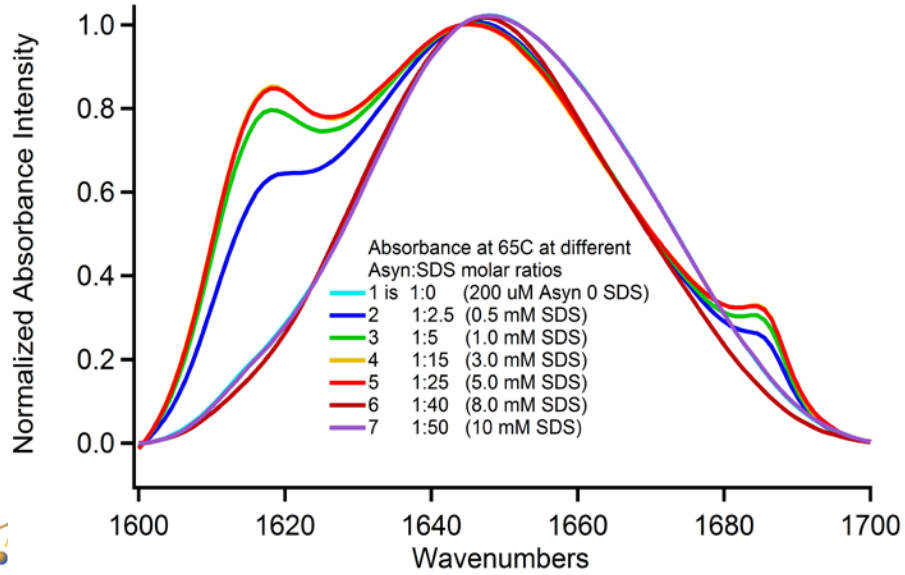


- WT Asyn showed alpha-helical structure with SDS interaction;
- Shift from random coil to helical structure seen even at 0.25 mM SDS;

Fourier Transform Infrared Spectroscopy

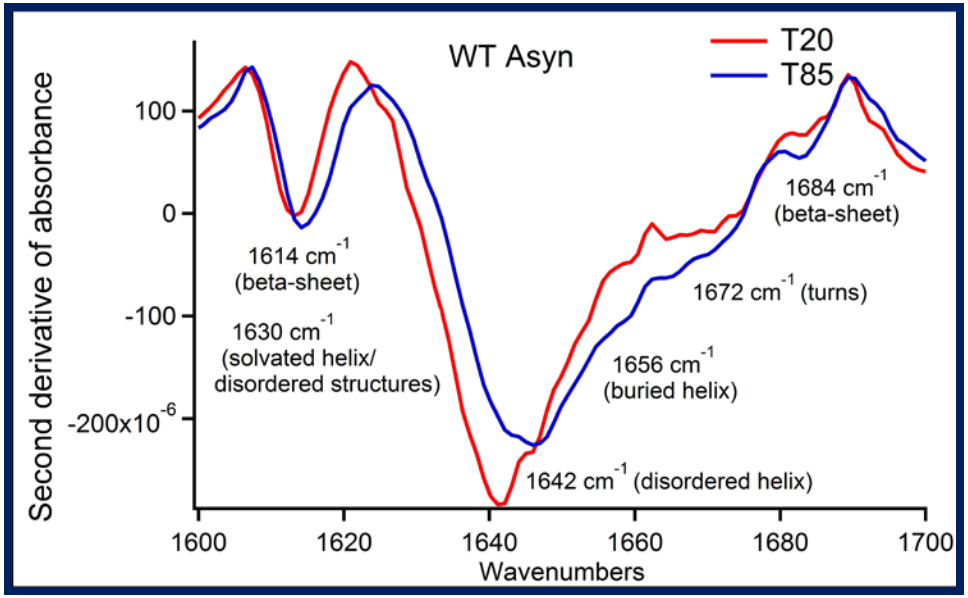


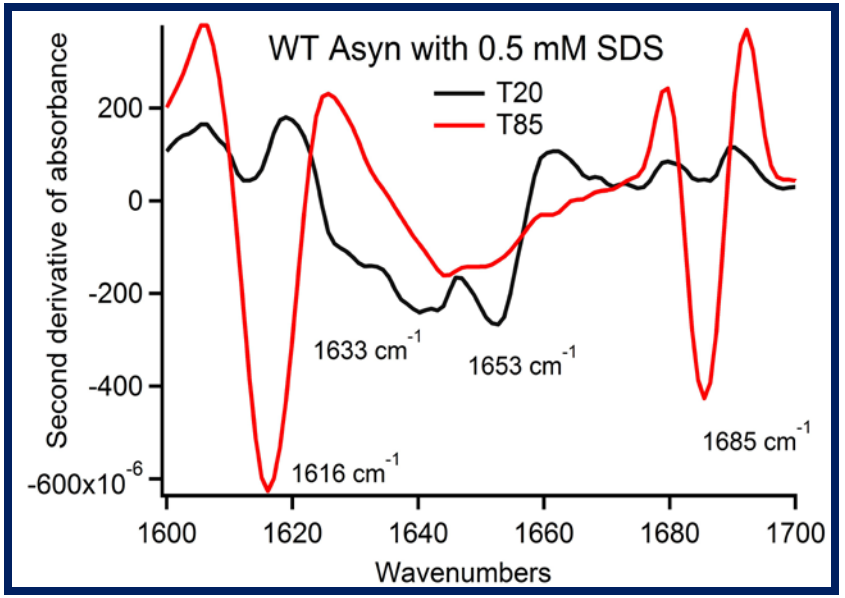




- Fibrillation occurs in Asyn:SDS molar ratios 1:2.5, 1:5, 1:15, 1:25 but not at 1:40, 1:50, 1:100 and 1:200
- Literature reports fibrillation with SDS at a 1:10-40, optimum at 1:25, our FTIR data showed fibrillation at 1:2.5-25 Asyn:SDS

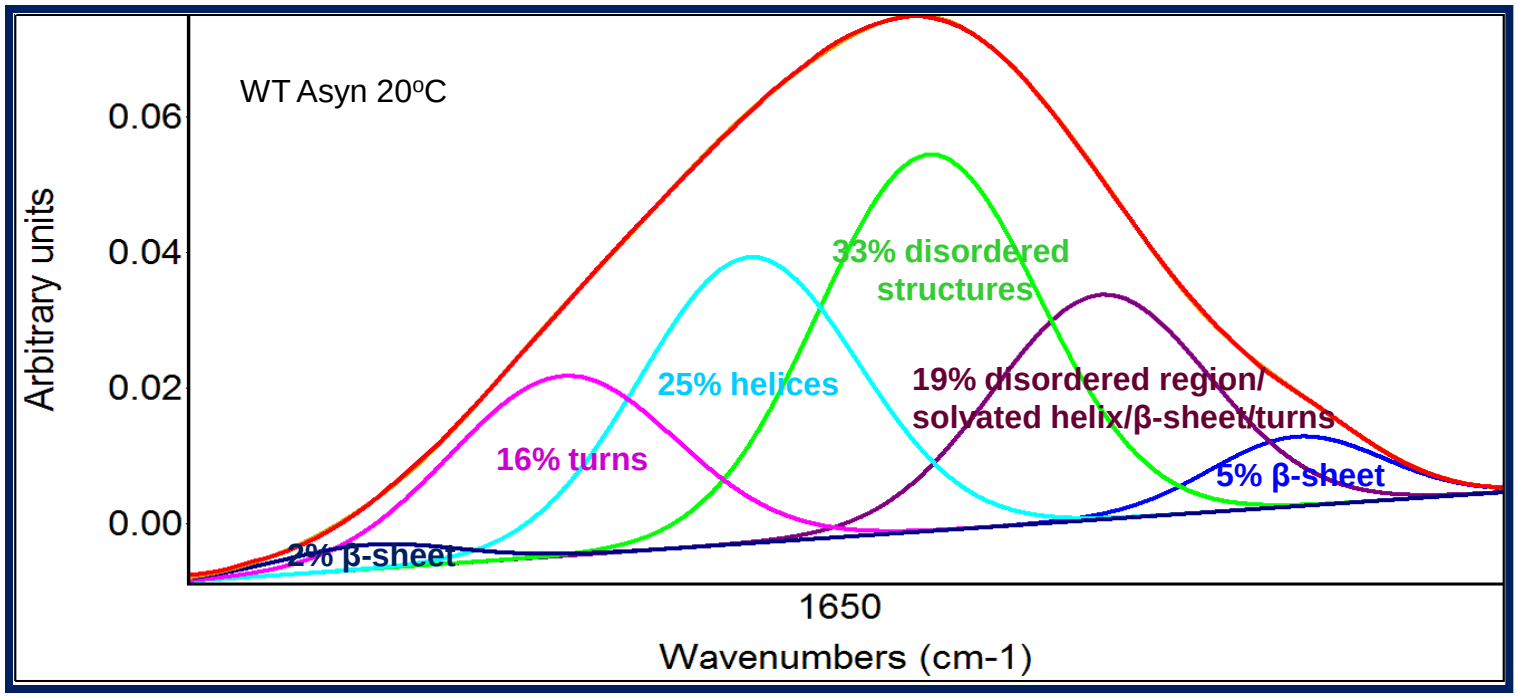
Effect of SDS on Alpha-synuclein



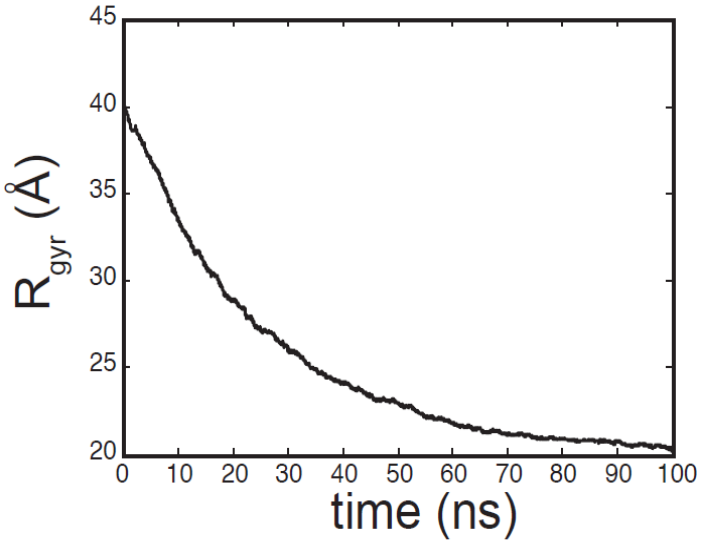


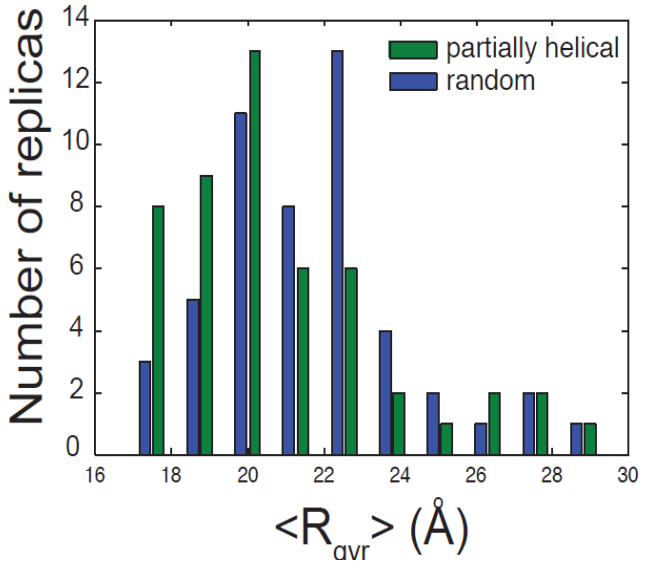
- **The change is subtle with SDS binding**
- **Overall secondary structure changes in Asyn that lead to loss in disorder and gain in folding**
- **Working on improving global fit analysis**

Amide I deconvolution and spectral assignment

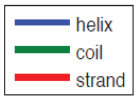


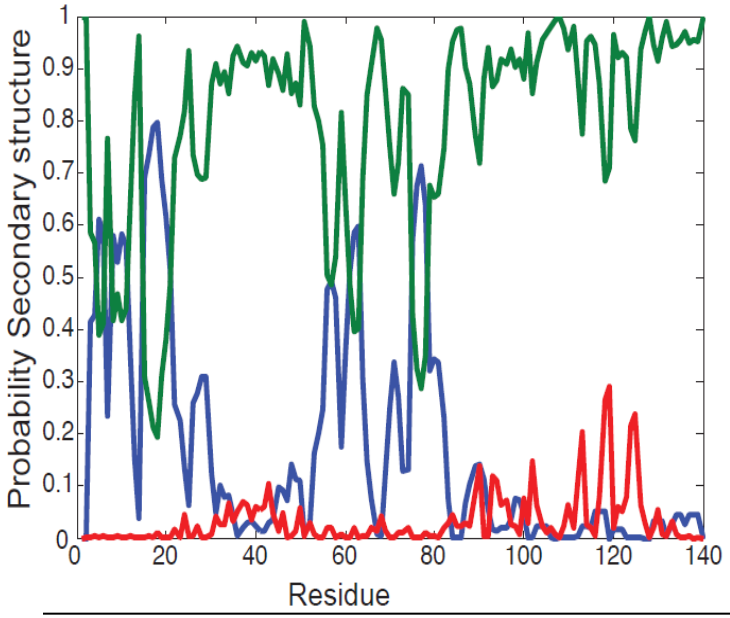
MD Simulation





Parameters: 200 and 350-ns all atom MD simulation using PDB 1XQ8, OPLS-AA force field, 100 simulations





S. Gnanakaran and Anurag Sethi

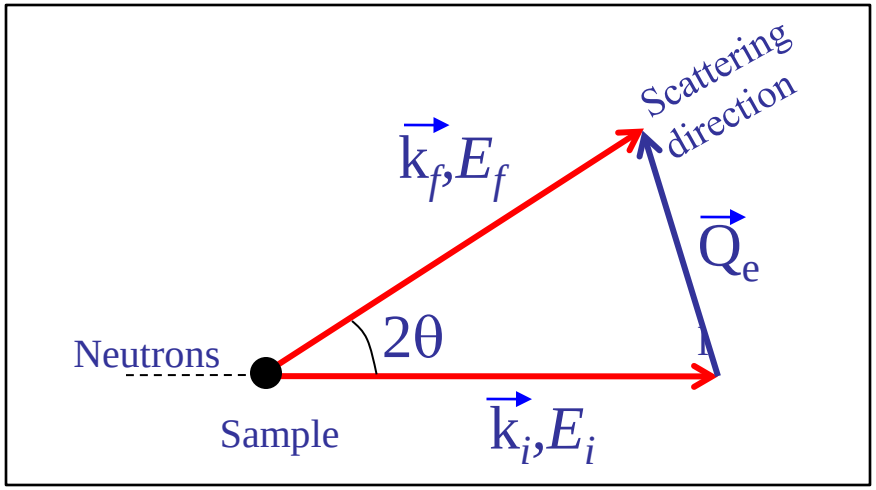
MD Simulation

- All simulations showed a heterogenous population of stable and compact Asyn
- Fast timescale decay in the Rg; Conformational rearrangements led to final collapsed structures with Rg = 16-30 Å
- Rg for a typical folded chain (140 aa) = 15 Å; Rg for totally unfolded chain (140 aa) = 52 Å
- The C-terminal tail of Asyn tends to form transient β-strand; residues 1-25 and 50-80 have the strongest propensity to form alpha-helical structures
- Asyn is more compact and has a non-random behavior than a typical fully extended polymer

Small angle neutron scattering

- **SANS:**

$$Q_{el} = 2k\sin\theta = \frac{4\pi\sin\theta}{\lambda} \xrightarrow{\text{small angles}} Q = \frac{4\pi\theta}{\lambda}$$



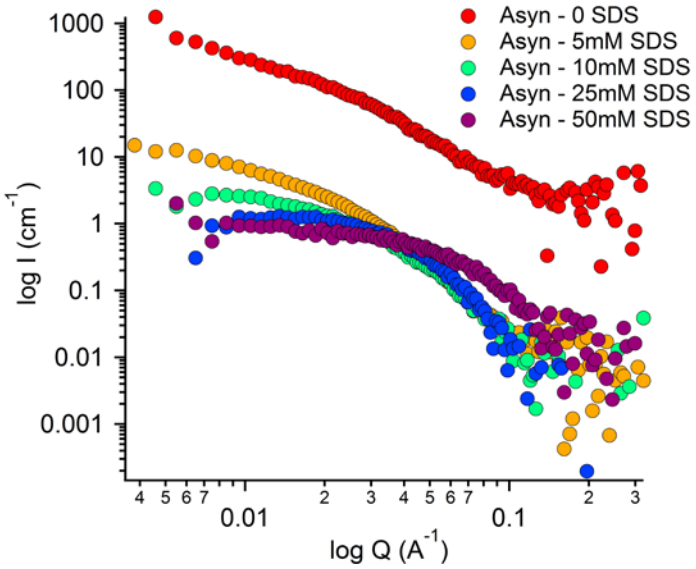
- **"Basic Equation"**

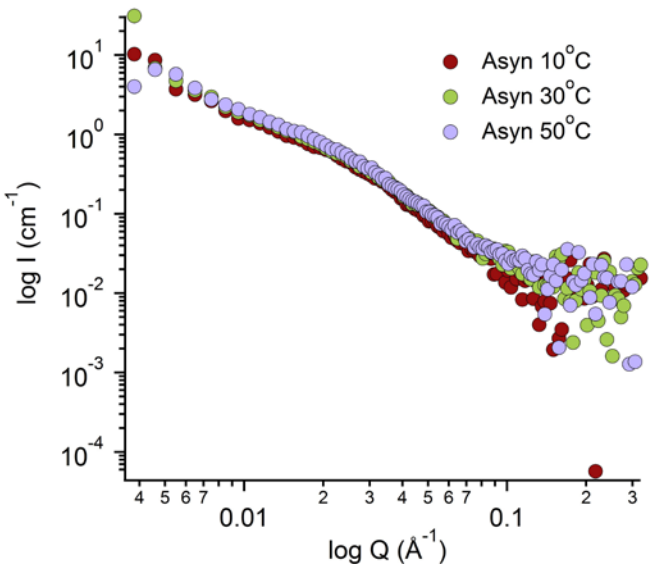
$$I(Q) = NV^2(\Delta\rho)^2 F(Q)S(Q) + B$$

N: number density
V: volume
$\Delta\rho$: contrast factor
F(Q): form factor (intraparticle structure factor)
S(Q): structure factor
B : background, constant term

Low-Q Diffractometer
Q-range: 0.003 - 0.5 Å^{-1}
Angular Range: 4-60 mrad (0.23 - 3.44°)
Wavelength Range (incoming): 1 - 16Å
Moderator: liquid H_2 at 20K (partially decoupled) – **Be reflector**
Detector: 2D area detector
Source/sample aperture: 2 - 8mm

Small angle neutron scattering



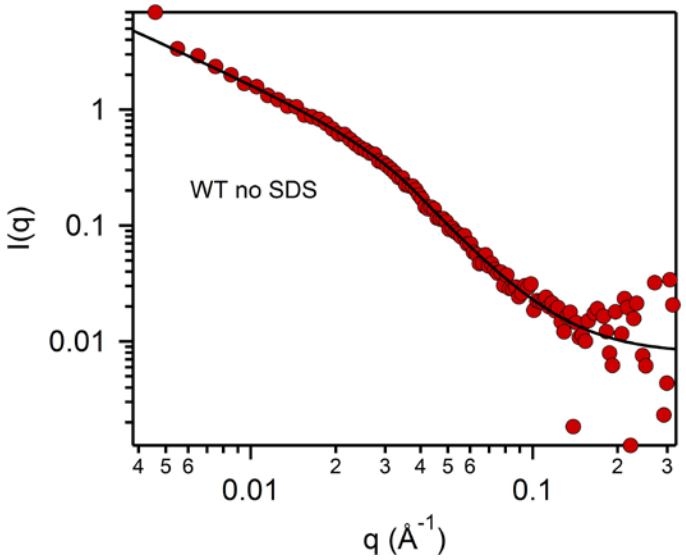


1mM WT Asyn

50 mM $NaHPO_4$-Na_2HPO_4

100 mM NaCl

Different SDS concentrations



Guinier-Porod analysis

	WT	WT 5mM SDS	WT 10mM SDS	WT 25mM SDS	WT 50mM SDS
Dimension Variable, s	1 ± 0 (rod)	1 ± 0 (rod)	1 ± 0 (rod)	0 ±0 (sphere)	0 ± 0 (sphere)
Rg, Å	**30.91 ± 0.0902**	**39.61 ± 0.0321**	**28.36 ± 0.0106**	**44.03 ± 0.0126**	**28.29 ± 0.0127**
Porod Exponent	3 ± 0	3 ± 0	4 ± 0	4 ± 0	3 ± 0
R, Å (based on s and Rg)	43.71	56.01	40.10	56.85	36.53
Correlation length (Zimm plot)	100.8	115.3	64.71	35.26	22.82

- Rg for WT Asyn is ~ 31Å; Rg from SAXS ~ 40Å
- Asyn adopts a rod-like structure that becomes spherical with SDS
- No significant changes observed with T-dependent (10-50^{o}C) results
- SDS micelles Rg ~ 18Å; does not change with concentration;
- SDS micelles are spherical, R_{sphere} ~ 23Å (consistent with literature)

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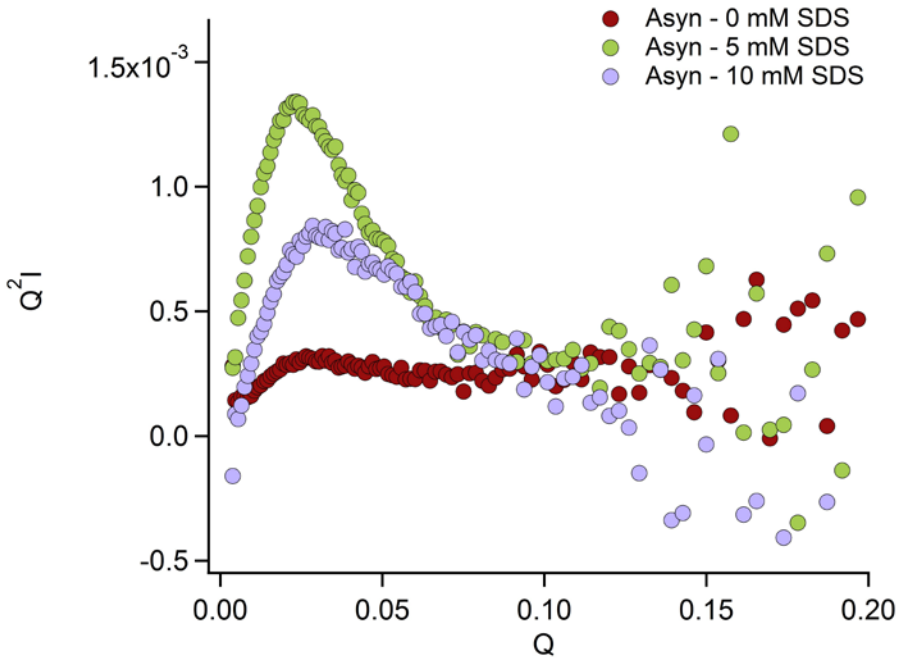
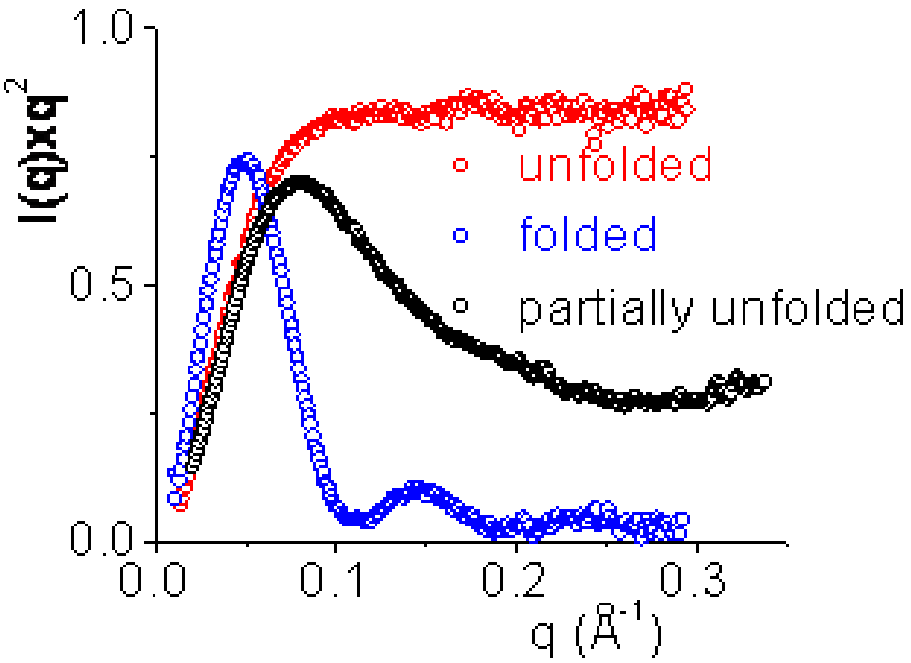
Kratky plot





- Kratky plot showed Asyn by itself is partially folded
- Addition of SDS makes Asyn more partially folded - peak maximum at lower Q values and sharper peaks
- All samples tend to be partially folded but with disordered regions (tail of plot)
- 10-50°C T-melt does not seem to affect the overall fold of Asyn except for the disordered region

Summary

- **Asyn forms a more compact state that is partially folded and may have transient α-helical and β-sheet structures associated with them.**
- **Asyn conformation in solution and its interaction with SDS and other ligands need careful analysis to gain better understanding of how its structure and conformation relate to its function and how they influence the pathogenesis of Parkinson's disease.**
- **In-progress:**
  - FTIR global fit for amide I analysis
  - SANS full model analysis

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