Structural Understanding of Intrinsically Disordered Proteins

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While I Have Your Attention…

- Take Home Points of Talk:
  1. Disorder is prevalent in the eukaryotic proteome.
  2. Importance of the λ N protein
     1. Forms a transient complex used to suppress Rho protein
  3. Macromolecules may impact (thermo)dynamic aspects of folding.

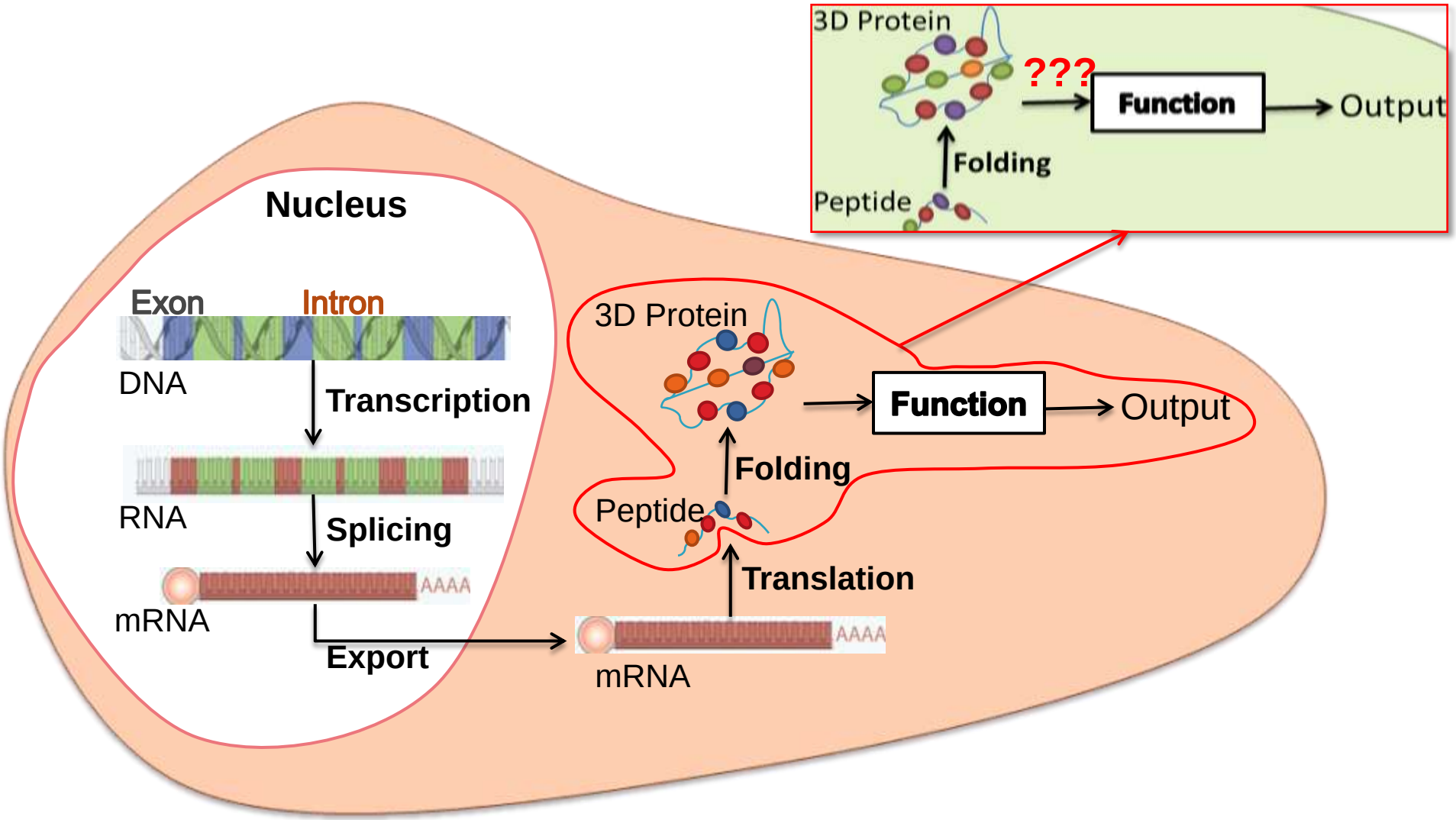
Overview

- Background
  - What are disordered proteins… Why do they matter?
  - Where does the λ N fit in?
- Experimental motivation
  - Interesting questions, goals, hypothesis
- Current Work
  - Setup, techniques, preliminary results
- Future Work

Background

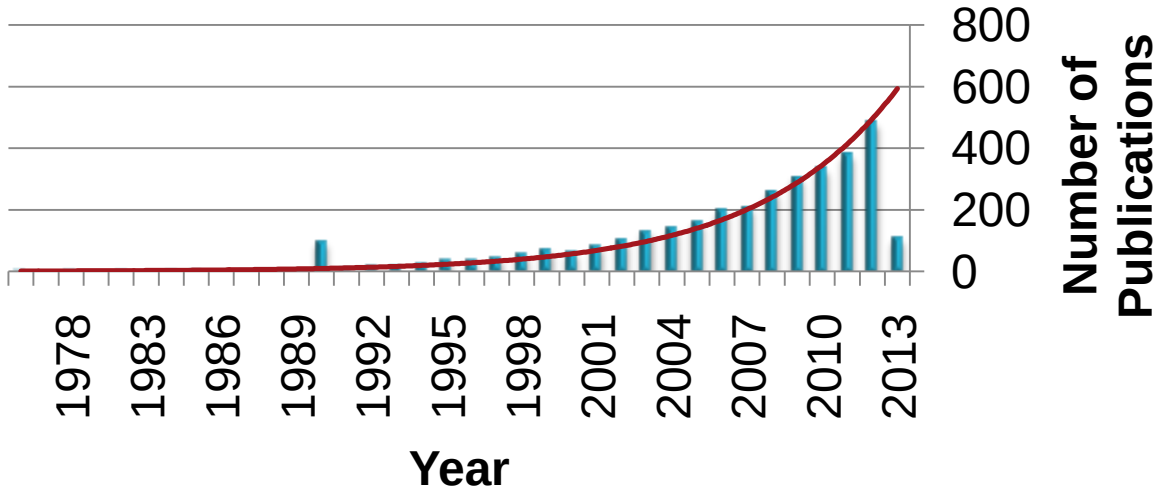
Rise, Prevalence, and Possible Roles of Disorder in Proteins

Structure / Function Paradigm



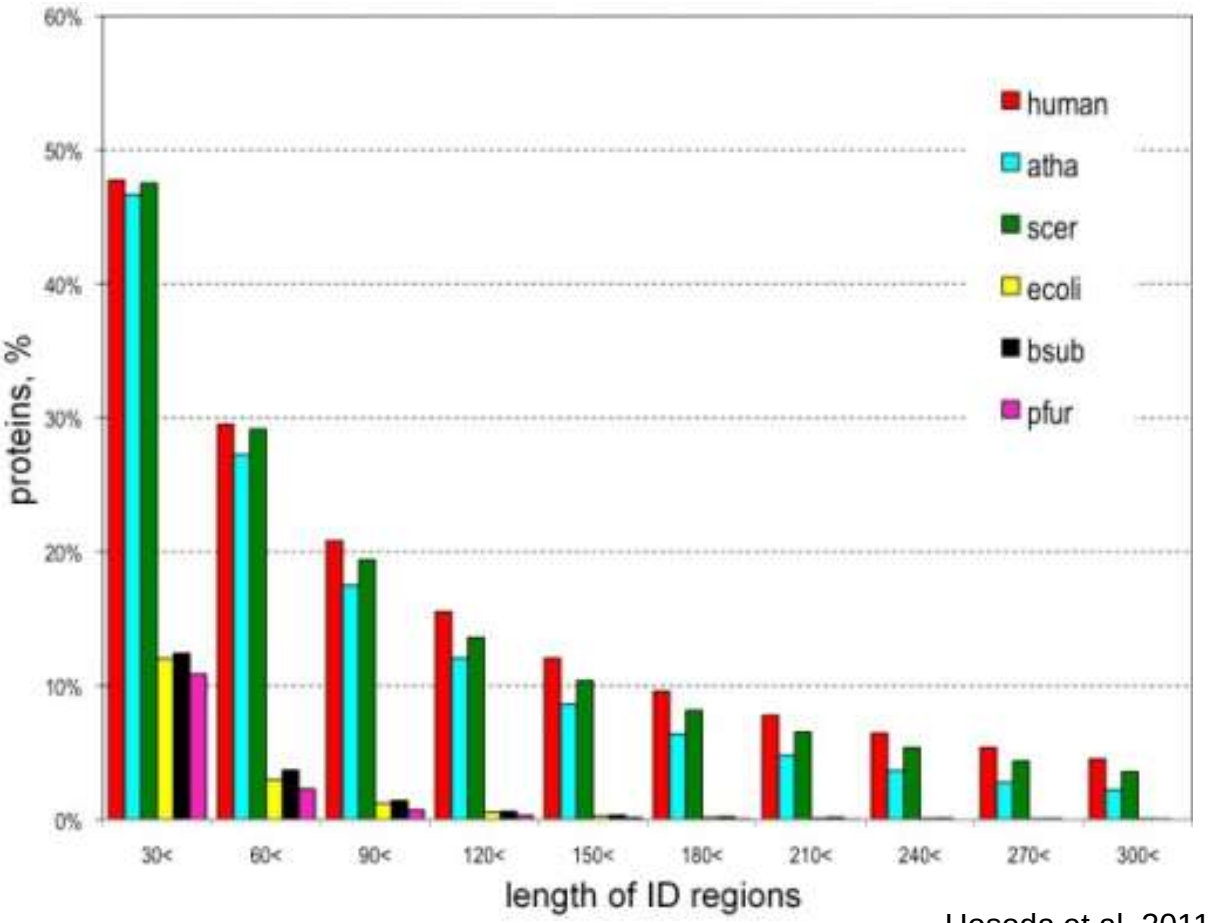
Disorder Becomes Apparent

- Early discovery
  - Bovine serum albumin binding sites (Karush,1950)
- Later…
  - Rapid rise of genomic data (~1990)
    - ***P***redictors ***o***f ***n***atural ***d***isordered **r**egions (PONDRs)
  - Early proton NMR experiments (Daniels et al, 1978)



Disorder, Disorder, (most)Everywhere!

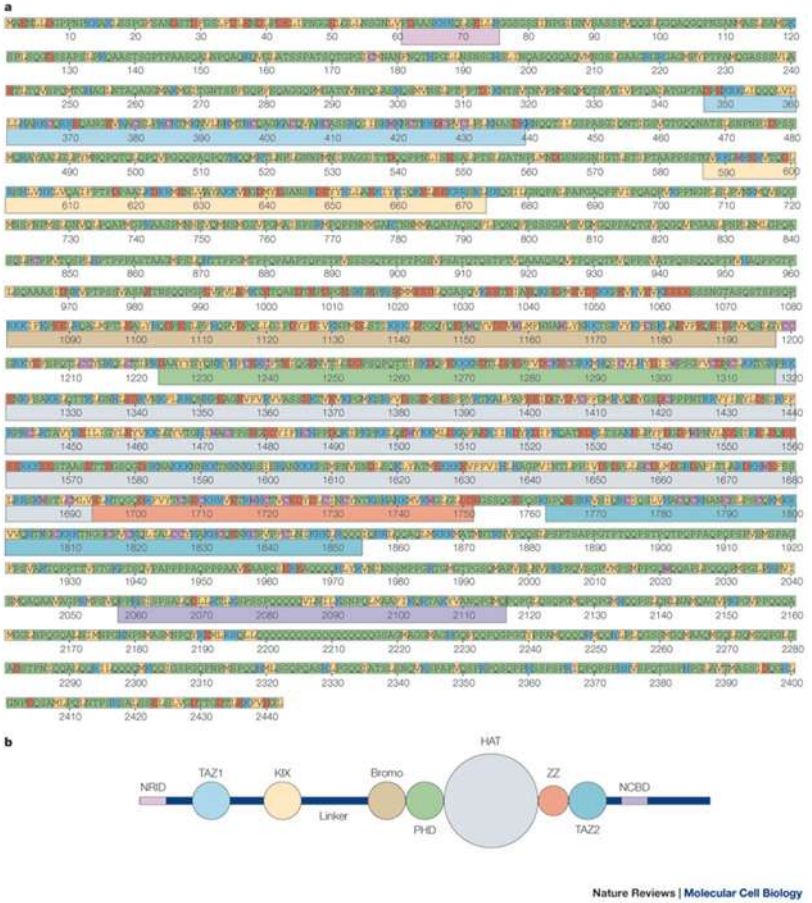
- Generally:
  - ↑complexity of organism → ↑disorder
  - Some exceptions
- **35–51%** of eukaryotic proteome (Dunker et al, 2000)



Hosoda et al, 2011

Why Did We Miss It?

- Unobserved
  - Bias of experiment
  - Access to genomic data limited before ~1990
  - Crystal structure relatively uninformative
- Ignored
  - Crystal structure artifacts dismissed
  - Disorder thought to be an artifact



Dyson & Wright, 2005

What is Disorder in Proteins?

- Definition:
  - A protein that does not adopt a well-defined native structure when isolated in solution under near-physiological conditions (Eliezer, 2009)
- 2 types
  - Denatured state ensembles (DSEs)
  - Intrinsically disordered proteins (IDPs)
- Vast and malleable configurational ensembles (CEs)
- Charged
- What can impact disorder?

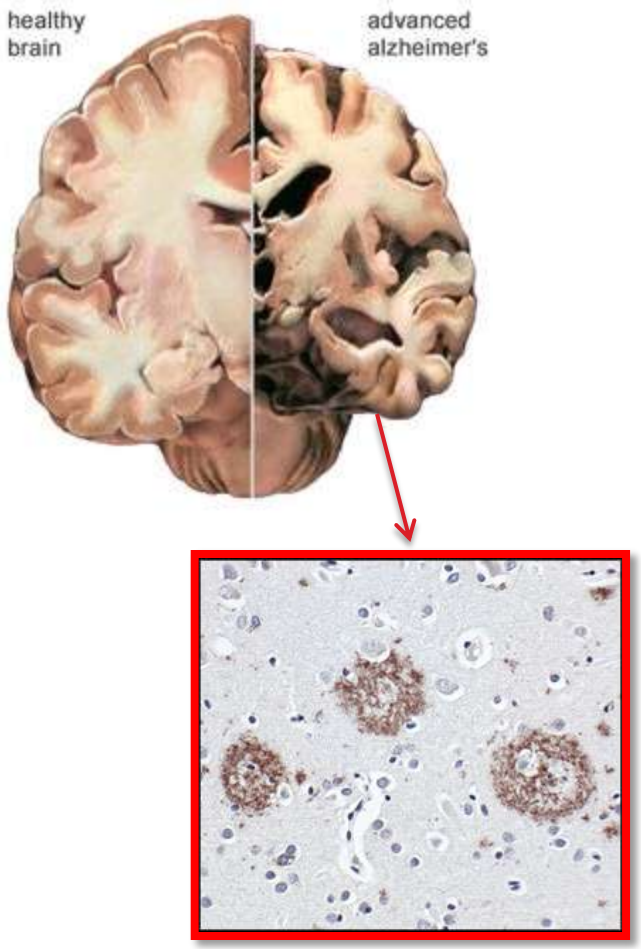
Why are IDPs Interesting?

Diverse Roles!

- Regulatory
  - Homeostasis of signaling pathways
  - Translation/Transcription
- Structural
  - Flexible Linkers

AND….. They can kill you.

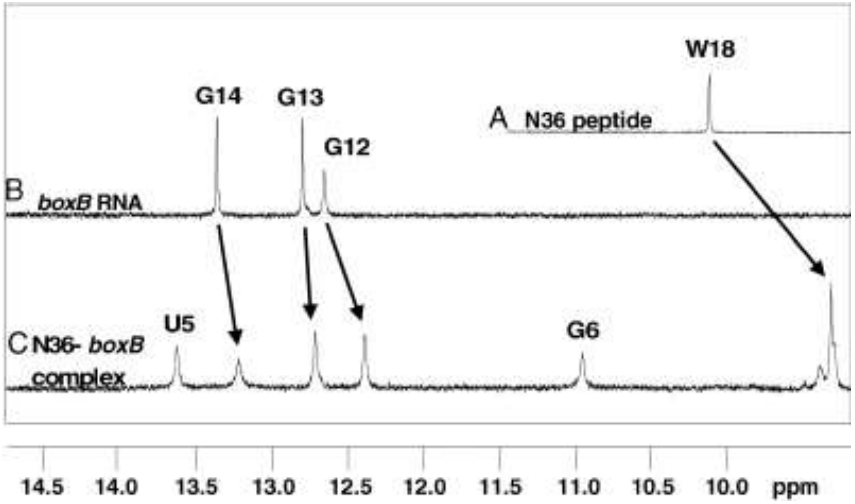
- Disease states
  - Cancer (lack of cell cycle regulation)
  - Brain (amyloid plaque formation)



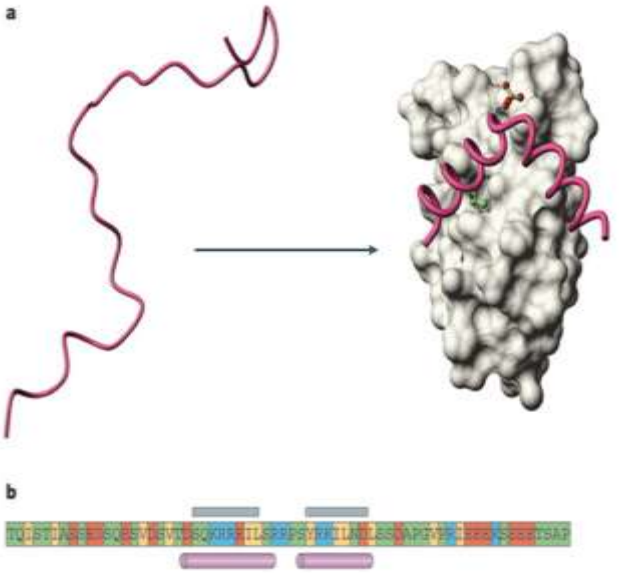
Lee et al, 2003

Proposed Mechanisms

- Regulation
  - Folding upon binding
  - Highly specific / low affinity binding
- Multiple interaction sites
- Aggregation



Schärpf et al, 2001



Nature Reviews | Molecular Cell Biology

Dyson & Wright, 2005

Background

The λ N protein

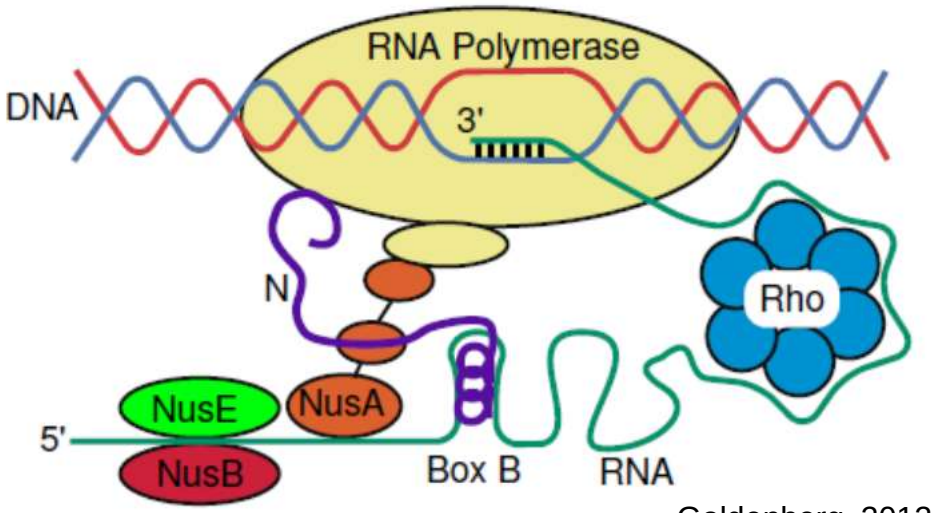
Structure

- 107 residues (1799 atoms)
- Positively charged side chains
  - Proportion of arginine to lysine: 22%

Structure	Position	Length (residues)	Visual Alpha Helix Beta Sheet
Helix	4-10	7	
Helix	12-20	9	
Helix	23-25	3	
Beta Strand	26-29	4	

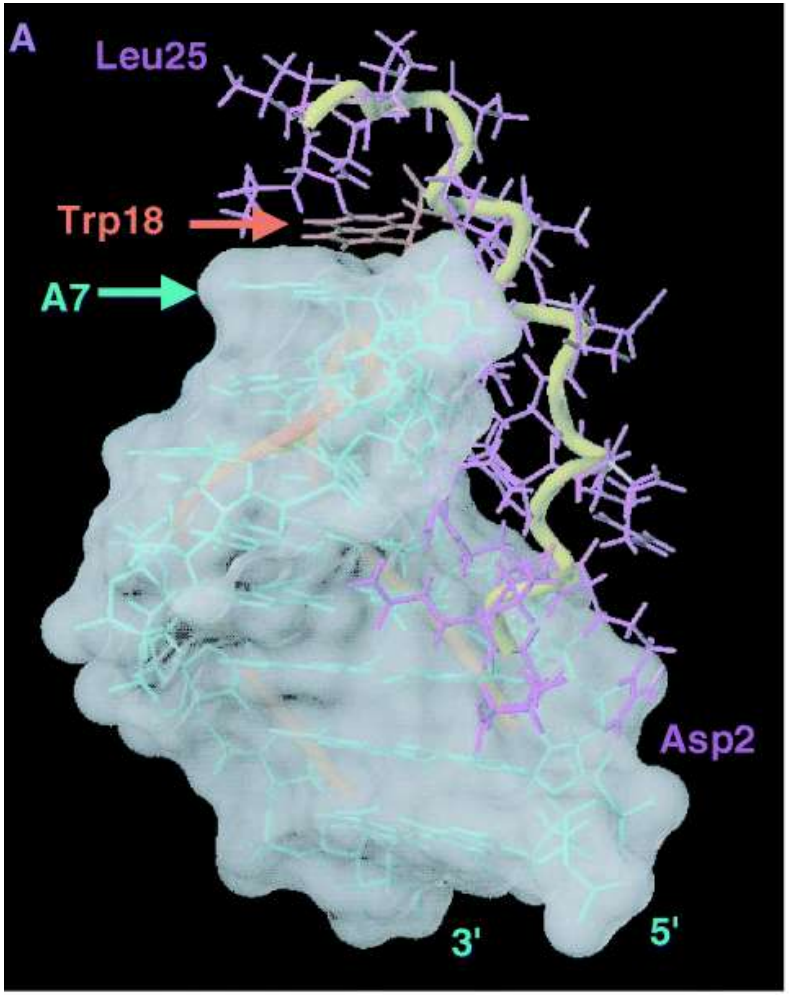
Function

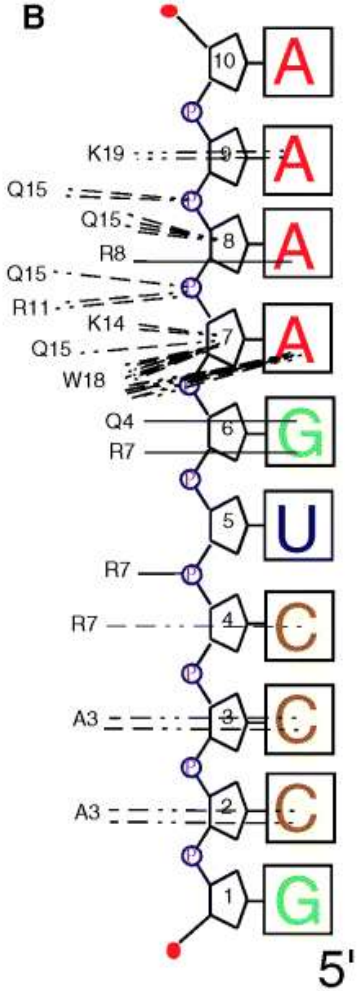
- Transient complex
- Interacts with
  - RNA
  - RNA polymerase
- End Result
  - Prevent termination of transcription



Goldenberg, 2012

More on Interaction with RNA





Schärpf et al, 2001

The Case for λ N as a Model System

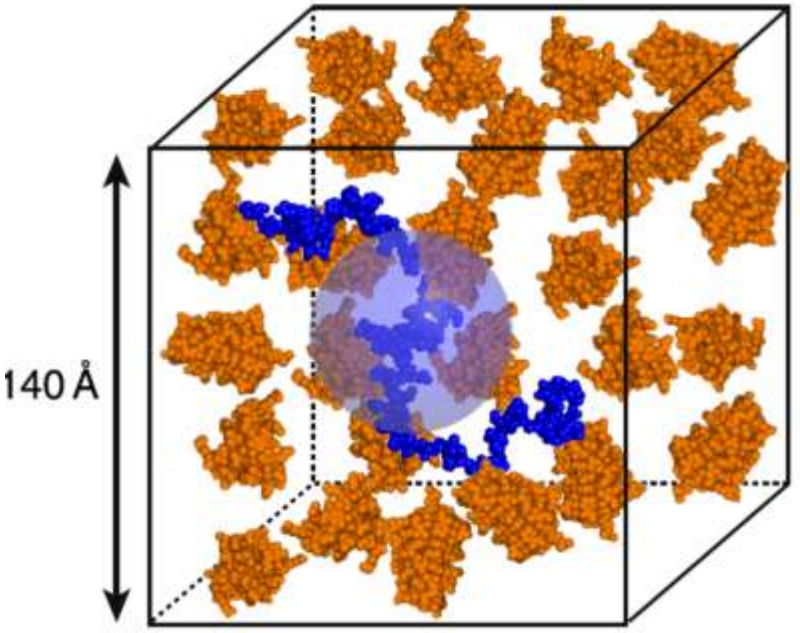
- Regulatory function
- Multiple interaction partners
- Extensively unfolded in isolation
- Flexible structure

Background

Questions, hypothesis, and goals

What Are We Trying to Address?

- Question
  - Which structural characteristics lead to the (thermo)dynamic propensity of IDPs to remain denatured?
- Importance
  - 1st step in addressing how the CEs of λ N are modified in response to molecular crowding stress
    - Establishes a baseline for comparison



Goldenberg, 2011

Hypothesis/Expected Outcomes

- CEs of the prototype λ N
  - Sensitive to changes in solution conditions
  - Local structure may be modulated more readily than global structure
  - Can be modulated through different levels of molecular crowding stress
- Will remain extensively unfolded
  - $\langle R_g \rangle \approx 30$ Å

Goals

- Provide a set of atomistic properties
  - Quantify correspondence with macroscopic ensemble-averaged experimental data
- Develop reference point for crowding studies

Context: Alzheimer's Disease

NIH / National Institute on Aging