Structural Understanding of Intrinsically Disordered Proteins

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While I Have Your Attention...

Take Home Points of Talk:

- I. Disorder is prevalent in the eukaryotic proteome.
- 2. Importance of the λN protein
 - E Forms a transient complex used to suppress Rho protein
- Macromolecules may impact (thermo)dynamic aspects of folding.

Overview

Background

- What are disordered proteins... Why do they matter?
- Where does the λN 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



Disorder Becomes Apparent

- Early discovery
 - Bovine serum albumin binding sites (Karush, 1950)
- Later...
 - Rapid rise of genomic data (~1990)
 - Predictors of natural disordered regions (PONDRs)
 - Early proton NMR experiments (Daniels et al, 1978)



Disorder, Disorder, (most)Everywhere!



Hosoda et al, 2011

300<

270<

human

atha

scer

ecoli

bsub

pfur

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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

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Nature Reviews | Molecular Cell Biology 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 nearphysiological 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





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Background

The λN protein

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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



More on Interaction with RNA



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The Case for $\lambda \, N$ 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

- Ist 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

•
$$\langle R_g \rangle \approx 30 \text{ Å}$$

Goals

Provide a set of atomistic properties

- Quantify correspondence with macroscopic ensembleaveraged experimental data
- Develop reference point for crowding studies

Context: Alzheimer's Disease

