Conformational and Characteristic Modulation of Prothymosin Alpha following the Addition of Guanidinium Chloride investigated with X-ray/Neutron Scattering Techniques

JÜLICH Forschungszentrum

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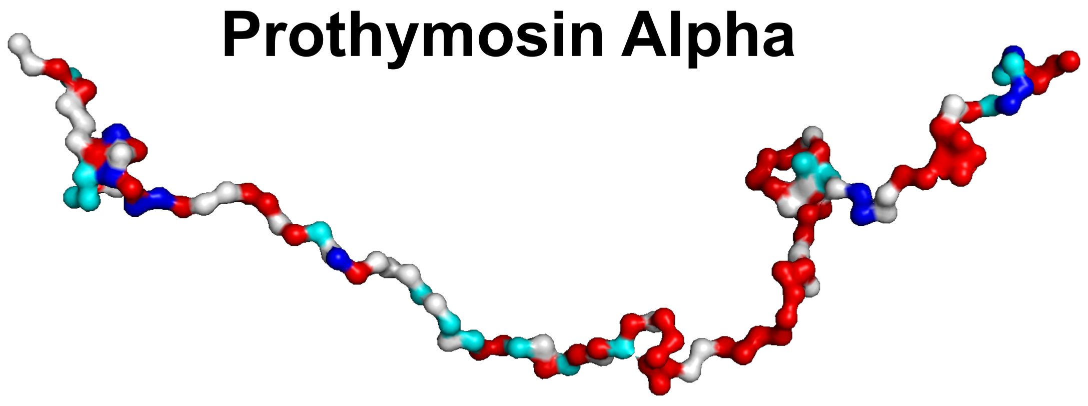
Intrinsically disordered protein

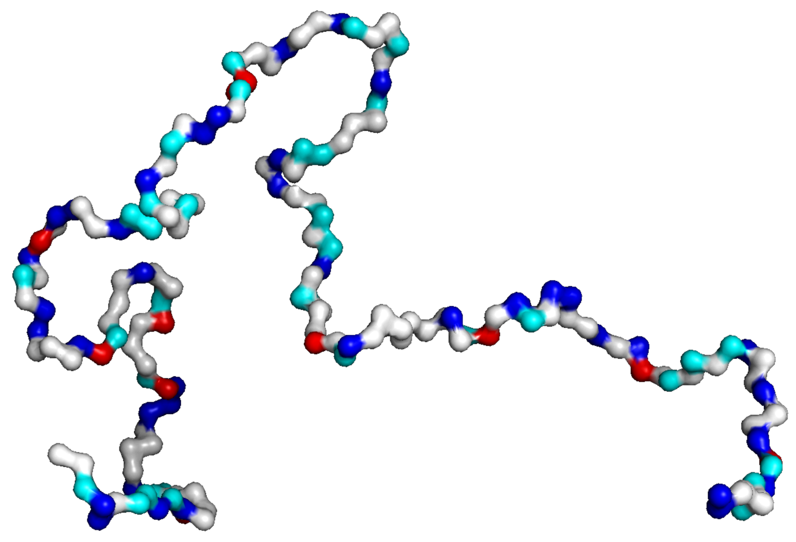
Prothymosin Alpha



- 112 amino acids: dominated by Aspartic acid and Glutamic acid
- No secondary structures found as a result of low number of hydrophobic residues

Negative | Polar | Positive | Hydrophobic

Myelin Basic Protein



- 169 amino acids: populated by positively charged residues and a number of hydrophobic ones
- Small portion of secondary structure exist in native condition

Introduction

Unlike protein in general, intrinsically disordered protein (IDP) derives its function from its low complexity domain. This domain confers sensitivity and adaptibility towards fluctuations in physico-chemical variables. Thus, they become indispensable in numerous cellular mechanisms. Examples include Prothymosin Alpha (ProTα) and Myelin Basic Protein (MBP). The former is known to form a complex with histone H1 in chromatin modification while the latter is able to undergo LLPS in myelin biogenesis.

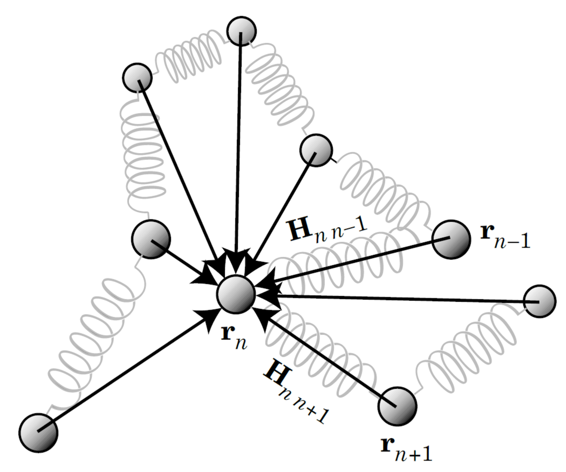
Aims

Quantitative description of the structure and dynamics of IDPs under the effect of GndCl. The influence of intrinsic properties of said IDP is also taken into account.

Methods

- Small angle X-ray scattering
  - Geometry
  - Compactness
- Neutron spin-echo spectroscopy
  - Nano second dynamics
- Theoretical model
  - Polymer with excluded volume
  - Flexible cylinder
  - Zimm / ZIF

Nanosecond dynamics and internal friction



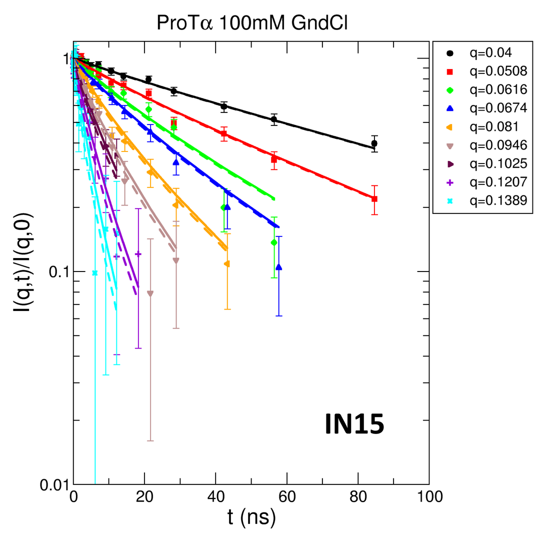
Teraoka, A. A. & Teraoka, I. Polymer Solutions: An Introduction to Physical Properties. (Wiley, 2002). p. 235

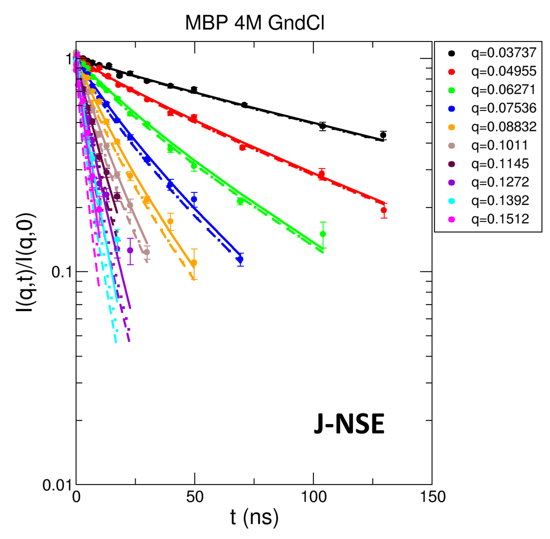
Zimm model

- Beads connected by entropic spring
- Rouse model with hydrodynamic interaction

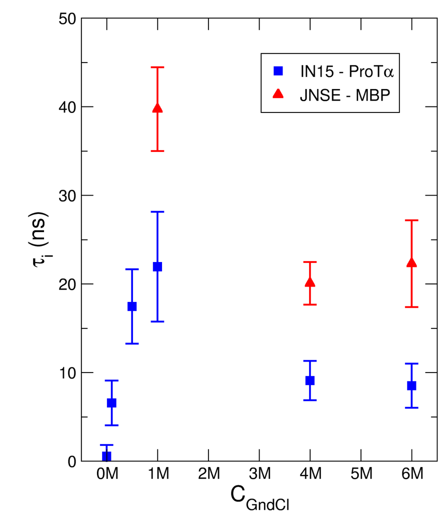
ZIF model

- Internal friction: microscopic interaction between beads





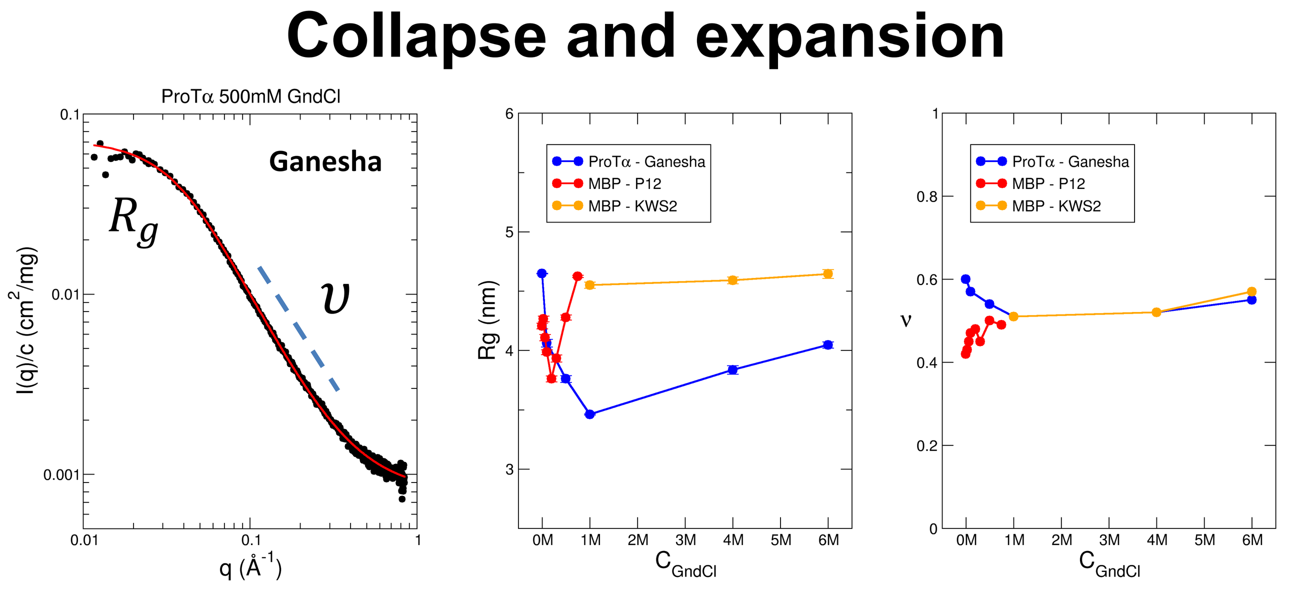
Internal friction



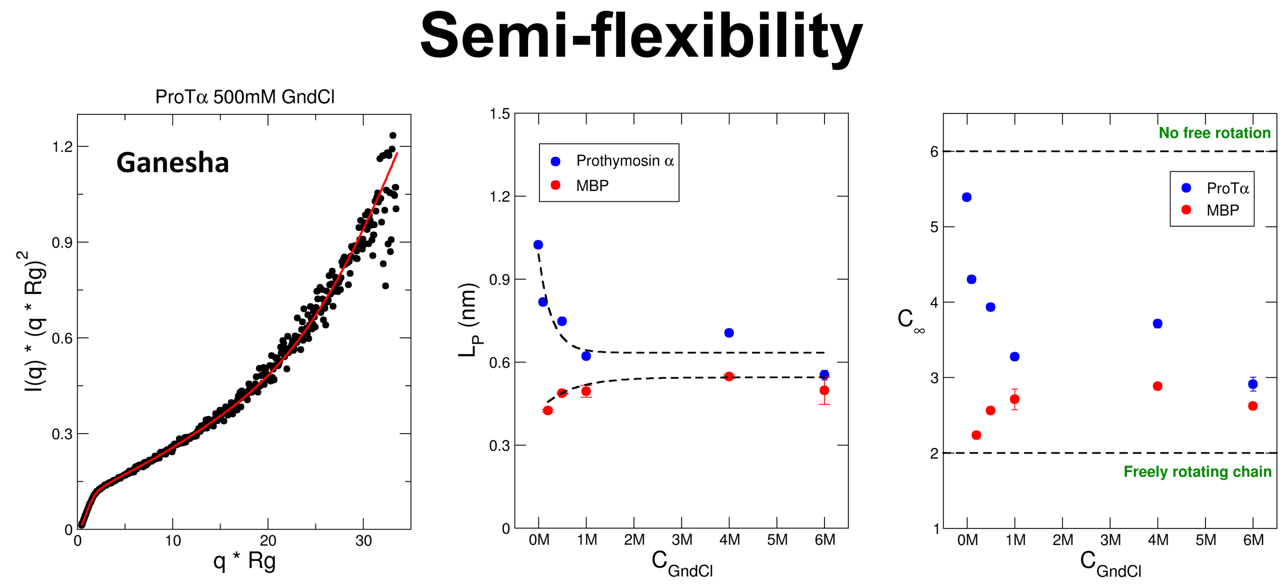
- ProTα (3 wt%) is measured at IN15 and MBP (5 wt%) at J-NSE.
- Dashed and solid line are fit of the data with Zimm and ZIF model respectively.
- τ_i is found to be 0 for ProTα at 0M GndCl. Then becomes larger as the protein collapse (1M) and finally converges at non zero value.
- At denatured state, MBP behaves similarly as Protα.

SAXS/SANS

Collapse and expansion



Semi-flexibility

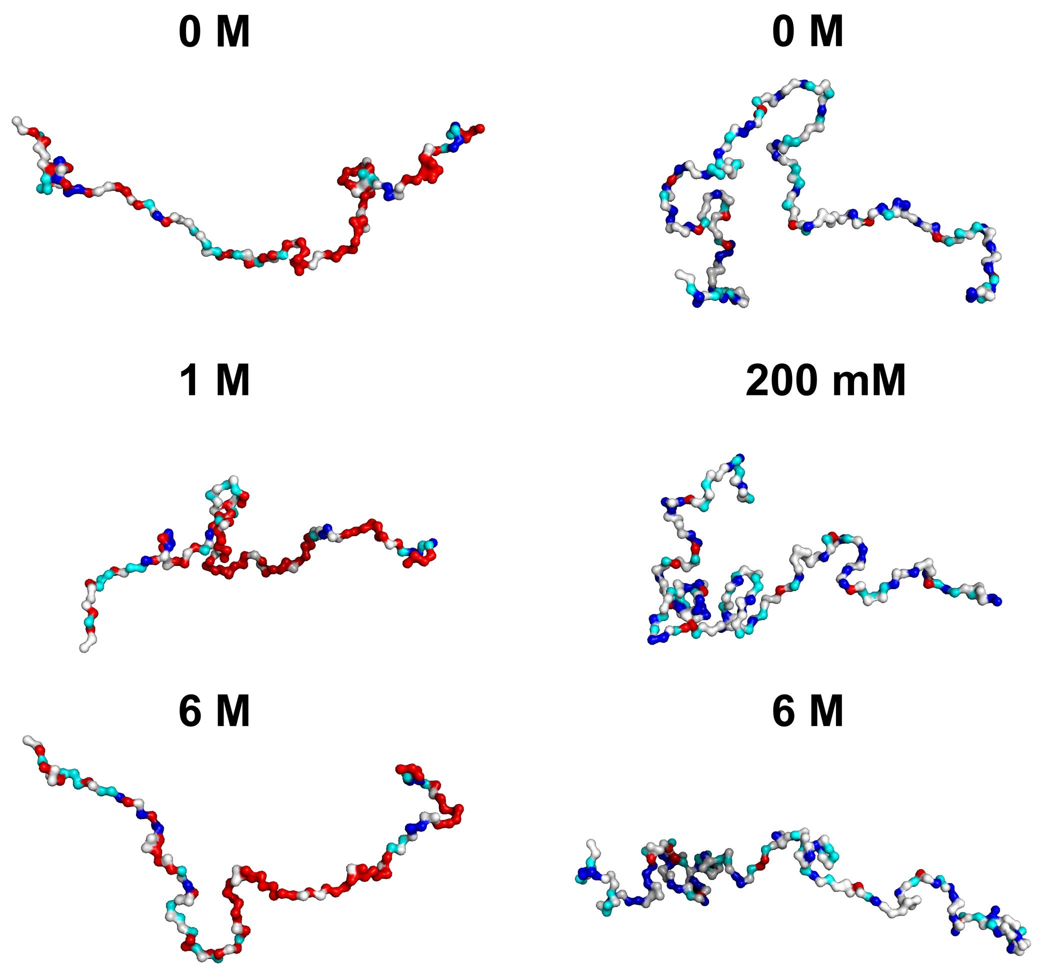


Generalized Gauss model

- GndCl has salting-in effect at lower concentration, followed by salting-out when further increased. Cut-off at 1 M for ProTα and 200 mM for MBP.
- The scaling exponent at 0M GndCl is 0.6 for ProTα and 0.42 for MBP. Indicating that ProTα is fully unfolded in native while MBP is relatively more compact.

Flexible Cylinder model

- Upon adding GndCl, ProTα becomes more flexible while MBP stiffer,
  - MBP at GndCl concentration lower than 200 mM is ignored due to the influence from secondary structure.
- Despite being different, both IDPs behave similarly at high GndCl concentration.



A model of ProTα (left) and MBP (right) generated by EOM. The chain is oriented such as it starts with the N terminus on the left and C terminus on the right.

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