Structural Heterogeneity in Intrinsically Disordered Proteins

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Introduction

Intrinsically disordered proteins (IDPs) lack a rigid 3D structure as opposed to folded proteins.

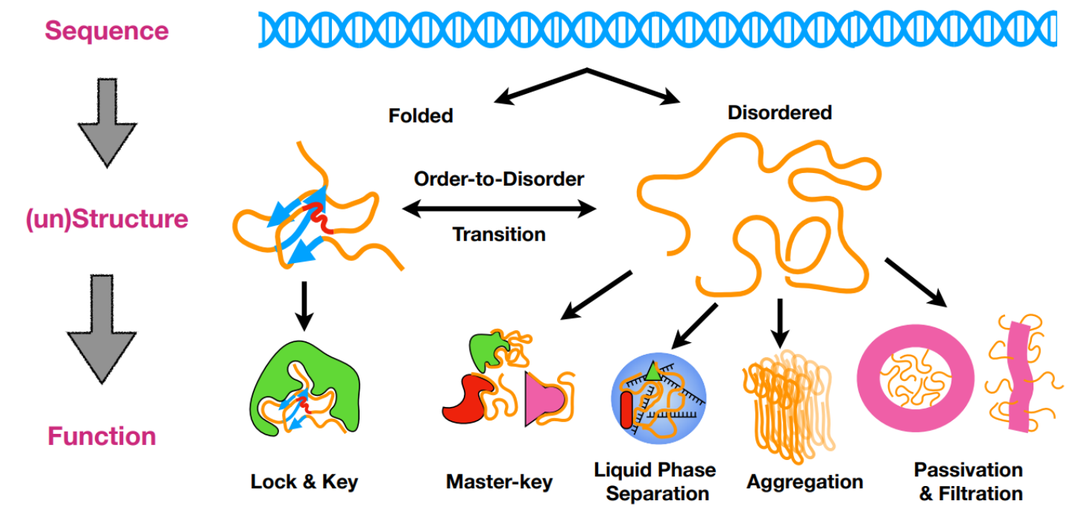

Figure 1. Folded and disordered conformations, and the transition between the two, lead to different biological function. Adapted from Ref [1]

The unique structural plasticity of IDPs enables one to harness the power of statistical mechanics and polymer physics. For example, Flory's mean-field theory relates a polymer's ensemble structure by $R_g \sim N^{\nu}$.

Here, R_g is the polymer's radius of gyration, N is the number of its monomers and ν is the Flory exponent (Fig. 4).

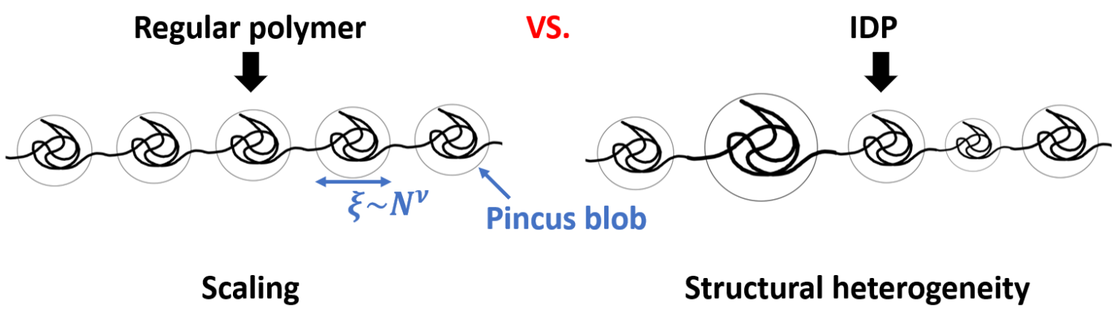

Figure 2. Traditional regular polymer versus IDP.

Recent studies suggest that such mean-field approximation fails to describe heteropolymers such as intrinsically disordered proteins where a complex interaction network may lead to structural heterogeneity (Fig. 2, Ref. 2).

Methods and Materials

- We purify the intrinsically disordered neurofilament-low carboxyl tail (NFLt) domain as a model system to IDP [3].
- Seven truncated segments from the NFLt sequence were synthesized, each of 20 amino acids long, with some overlaps (segments 1 to 7).
- We quantify the ensemble-averaged radius of gyration (R_g), Flory exponent (ν), asphericity (δ), and the ensemble representation conformations using small-angle X-ray scattering (SAXS) measurements.

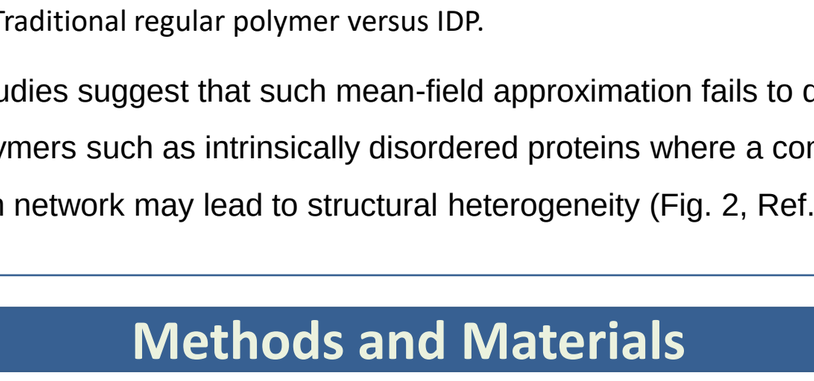

Figure 3. SAXS measurement setup.

- We labeled the peptides with Naphthyl (donor) and Dansyl (acceptor) Förster resonance energy transfer (FRET) pairs. Using a time-correlated single-photon counting method, the end-to-end probability distribution (R_{ee}) was measured and compared with SAXS data.
- The FRET measurements were done on free peptides in solution and as part of the NFLt.

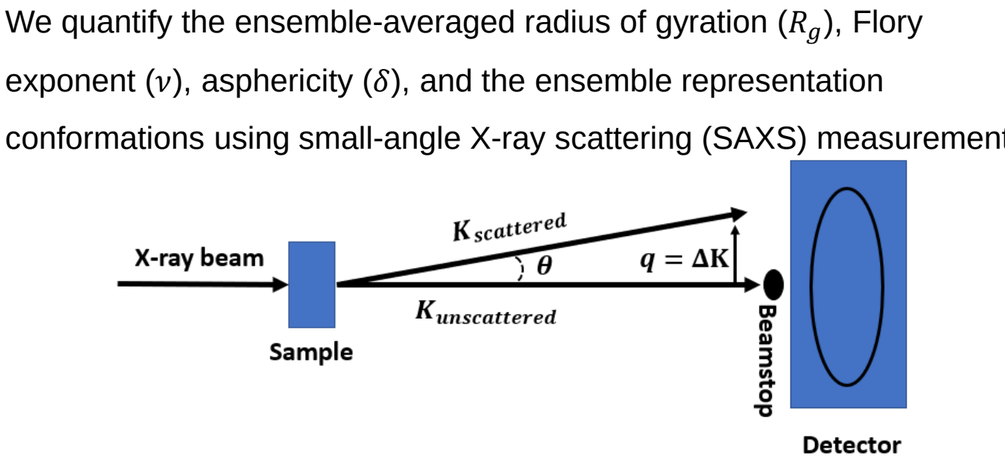

Figure 4. Schematic representation of R_{ee} and R_g as measured by FRET and SAXS, respectively.

Results

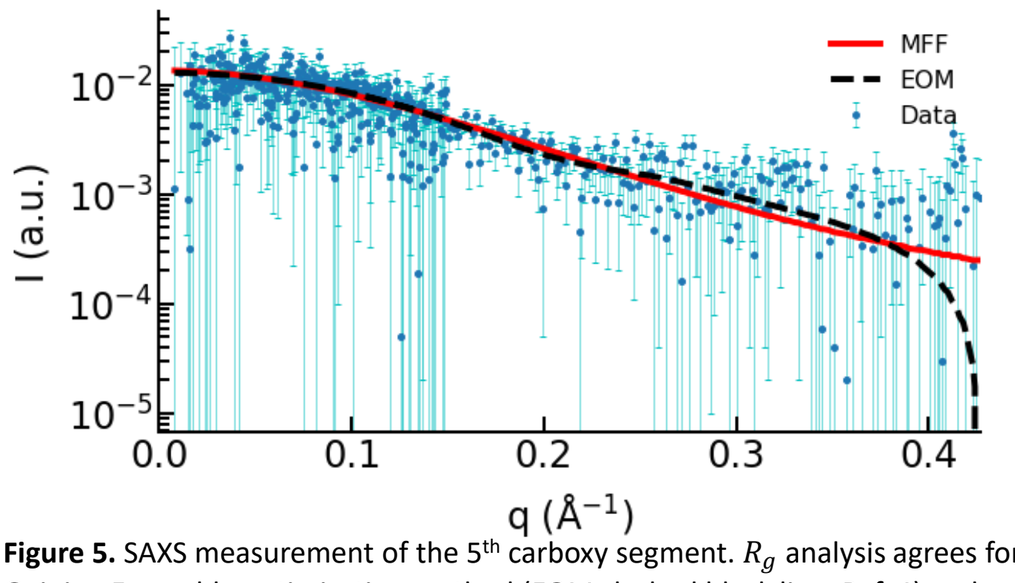

Figure 5. SAXS measurement of the 5th carboxy segment. R_g analysis agrees for Guinier, Ensemble optimization method (EOM-dashed black line, Ref. 4) and molecular form factor (MFF-red solid line, Ref. 5).

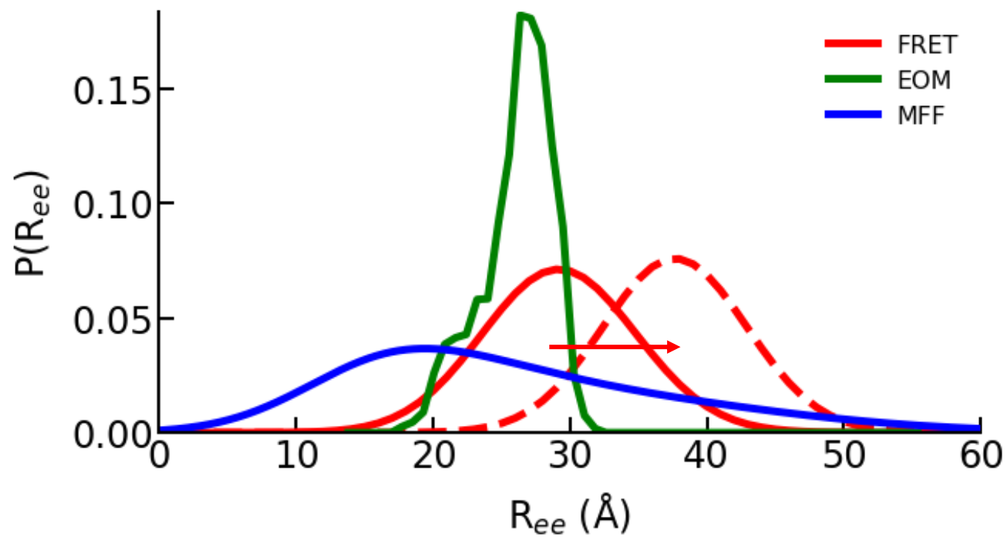

Figure 6. End-to-end distance distributions (R_{ee}) of the 5th segment as was extracted from the EOM and MFF fits to SAXS data (Fig. 5), and as was extracted from the FRET measurement. A free segment in solution and a segment as part of the NFLt are marked in solid and dashed lines, respectively. We find the segment expands when measured in the context of the entire NFLt (red arrow).

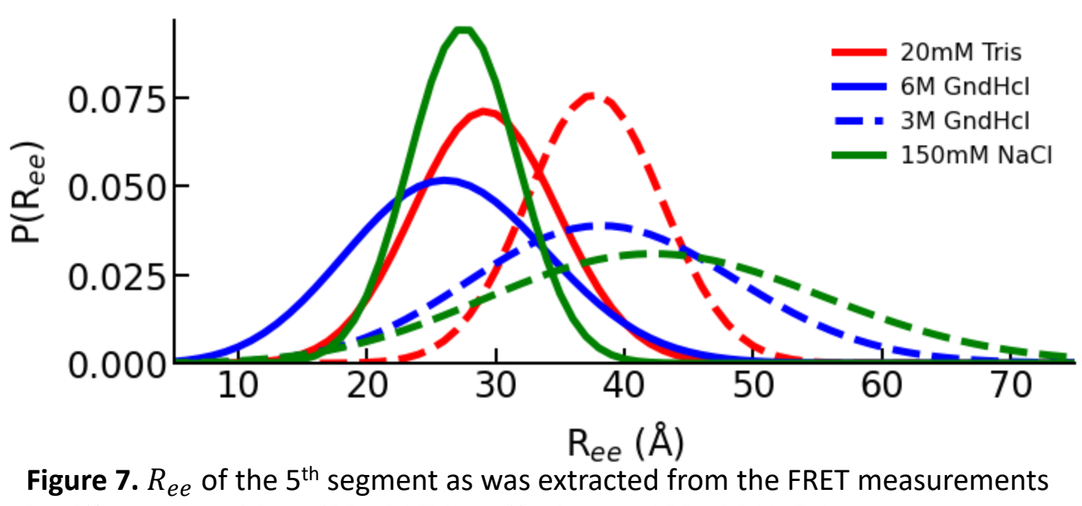

Figure 7. R_{ee} of the 5th segment as was extracted from the FRET measurements in different conditions (20mM Tris buffer in red, 150mM NaCl in green, and GndHcl in blue). A free segment in solution and the same segment within the NFLt are marked in solid and dashed line, respectively. Here, again, we find an expansion of the peptide when it is measured as part of the entire protein.

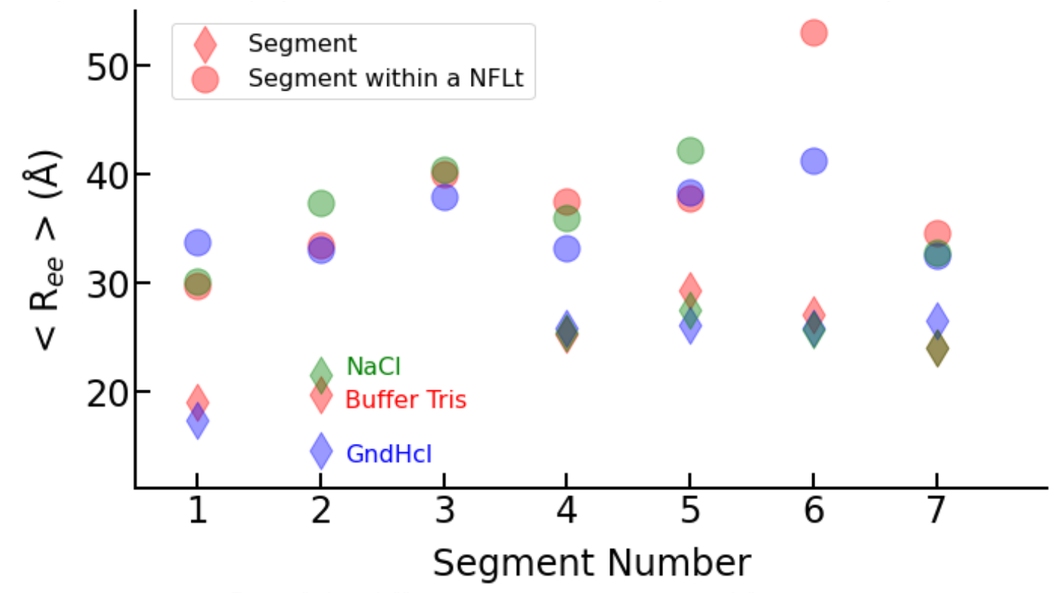

Figure 8. Mean R_{ee} of the different segments extracted from FRET measurements in different conditions (same colors as in fig 7). Measurement of the free segments in solution and segments within the NFLt are labeled as diamonds and circles, respectively. Free segments in solution are expended when measured as part of the protein.

Discussion & Conclusions

- We directly demonstrated structural heterogeneity with alternative polymer statistics for the segments.
- We found that the amino-acid context plays a critical role in determining the structure of NFLt.
- Our findings demonstrate that long- and short-ranged interactions within IDPs are required to explain the ensemble structural statistics in physiological conditions.
- This study further highlights IDPs' unique characteristics in comparison to flexible homopolymers.

Acknowledgements

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References

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