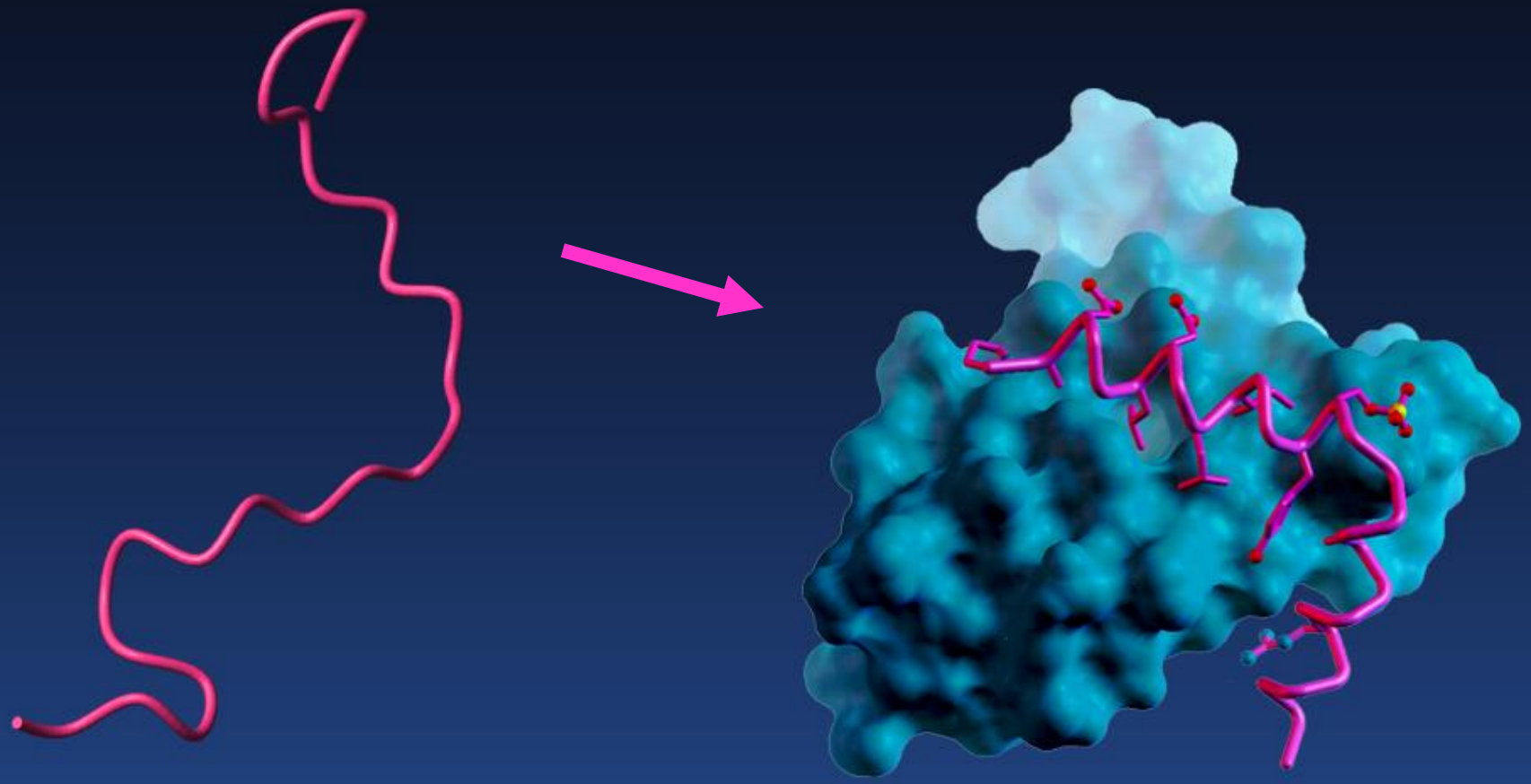
Solution NMR of intrinsically disordered proteins at ultrahigh fields

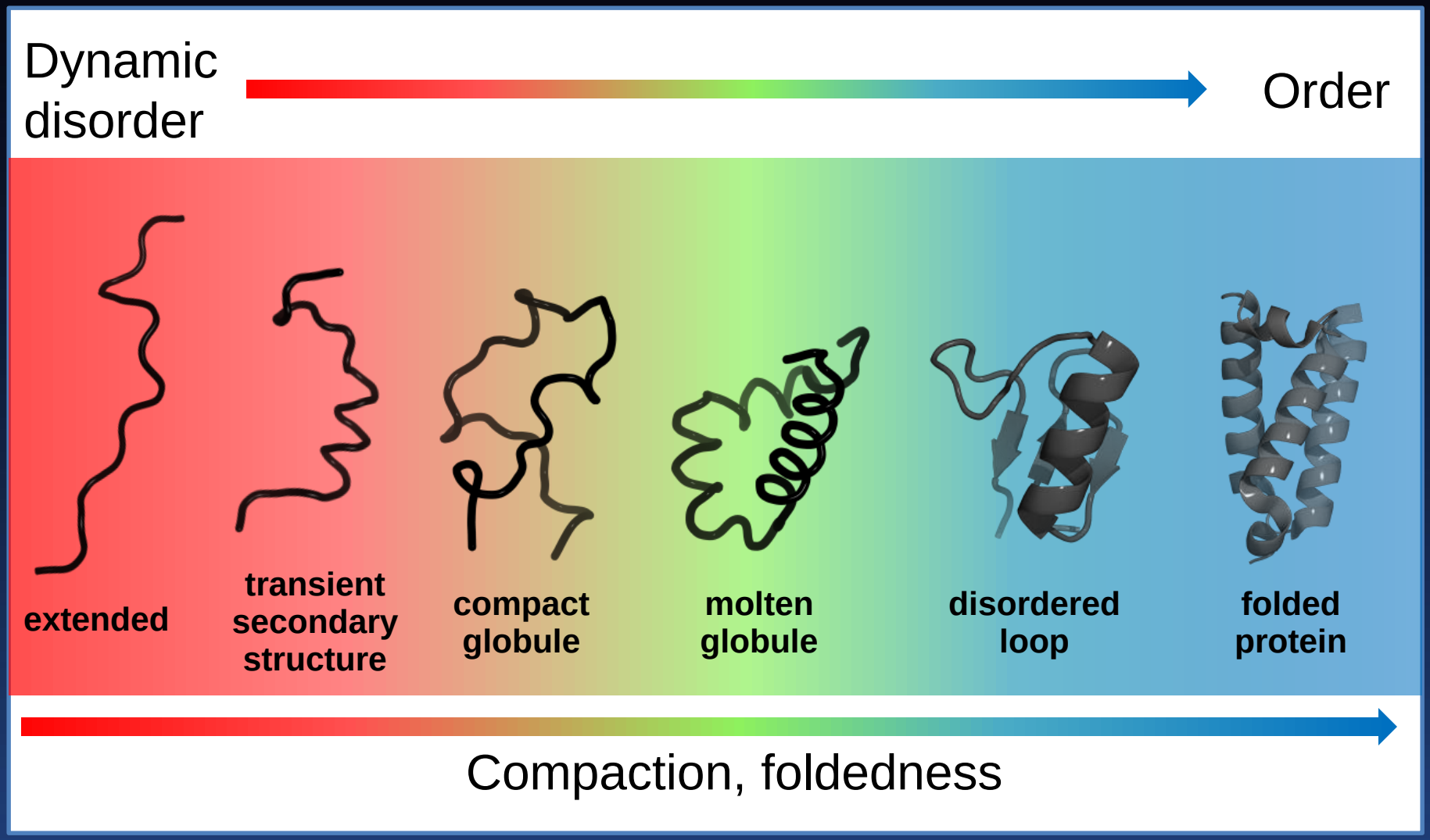


Intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs)

- unable to fold spontaneously into well-defined, stable globular 3D structures
- 30-50% of eukaryotic proteins are predicted to be partially or entirely disordered
- many eukaryotic proteins contain structured domains and long disordered regions

The abundance and importance of IDPs was not recognized until the mid-1990s

The protein conformational continuum



IDPs play a central role in dynamic regulatory and assembly processes in the cell

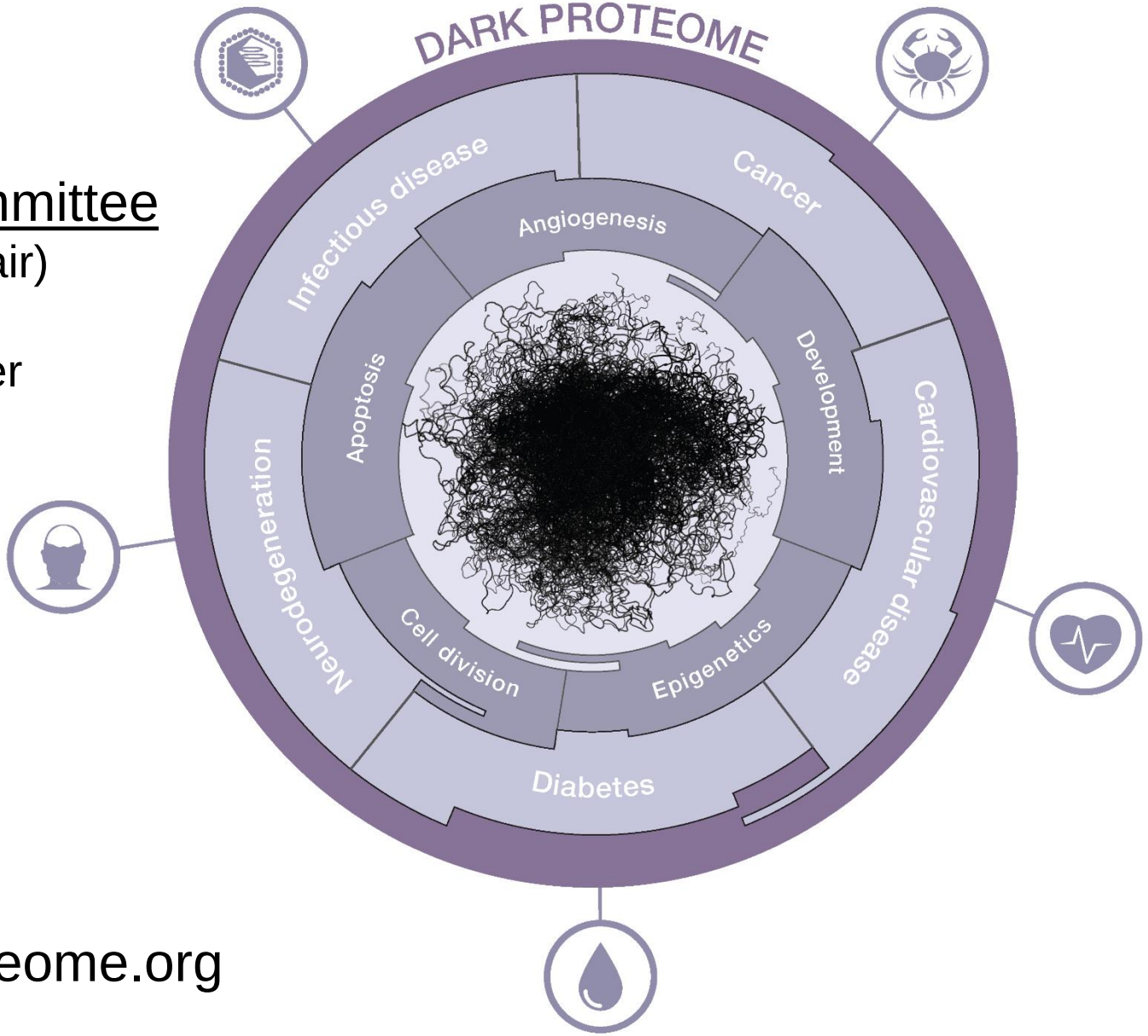
- central component of the control circuitry of the cell (transcription, translation, cell cycle control, signaling, etc.)
- associated with debilitating diseases (cancer, leukemia, cardiovascular disease, diabetes, infectious disease, neurodegenerative diseases, traumatic brain injury)
- disordered regions impart flexibility in assembly of macromolecular complexes
- cellular organization (form membrane-less compartments)
- ramifications for basic molecular and cell biology, bioenergy, and design of bio-inspired materials

IDPs play a central role in the dynamic regulation of key cellular processes

- unique characteristics of IDPs impart flexibility and complexity to dynamic cellular signaling networks
- IDP signaling modulated by posttranslational modifications (phosphorylation, acetylation, methylation, ubiquitination, SUMOylation, O-GlcNAc addition)
- PTMs modulate interactions with target proteins and determine signaling outcome (switches, rheostats, threshold responses)
- understanding of IDPs is central to understanding regulatory and signaling networks in the cell

Wright & Dyson, Nature Rev. Mol. Cell Biol. 16, 18, 2015

The Human Dark Proteome Initiative

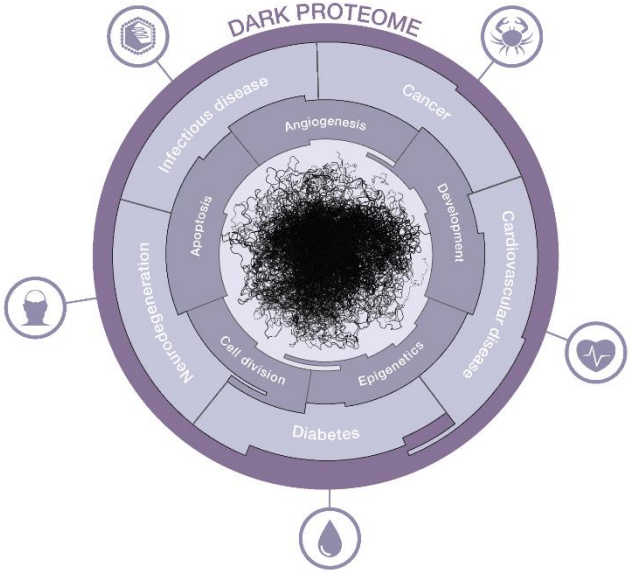


<u>Executive committee</u>

Peter Wright (chair)
Jean Baum
Steven Finkbeiner
Jeffrey Hoch
Richard Kriwacki
Arthur Palmer
Rohit Pappu

http://darkproteome.org

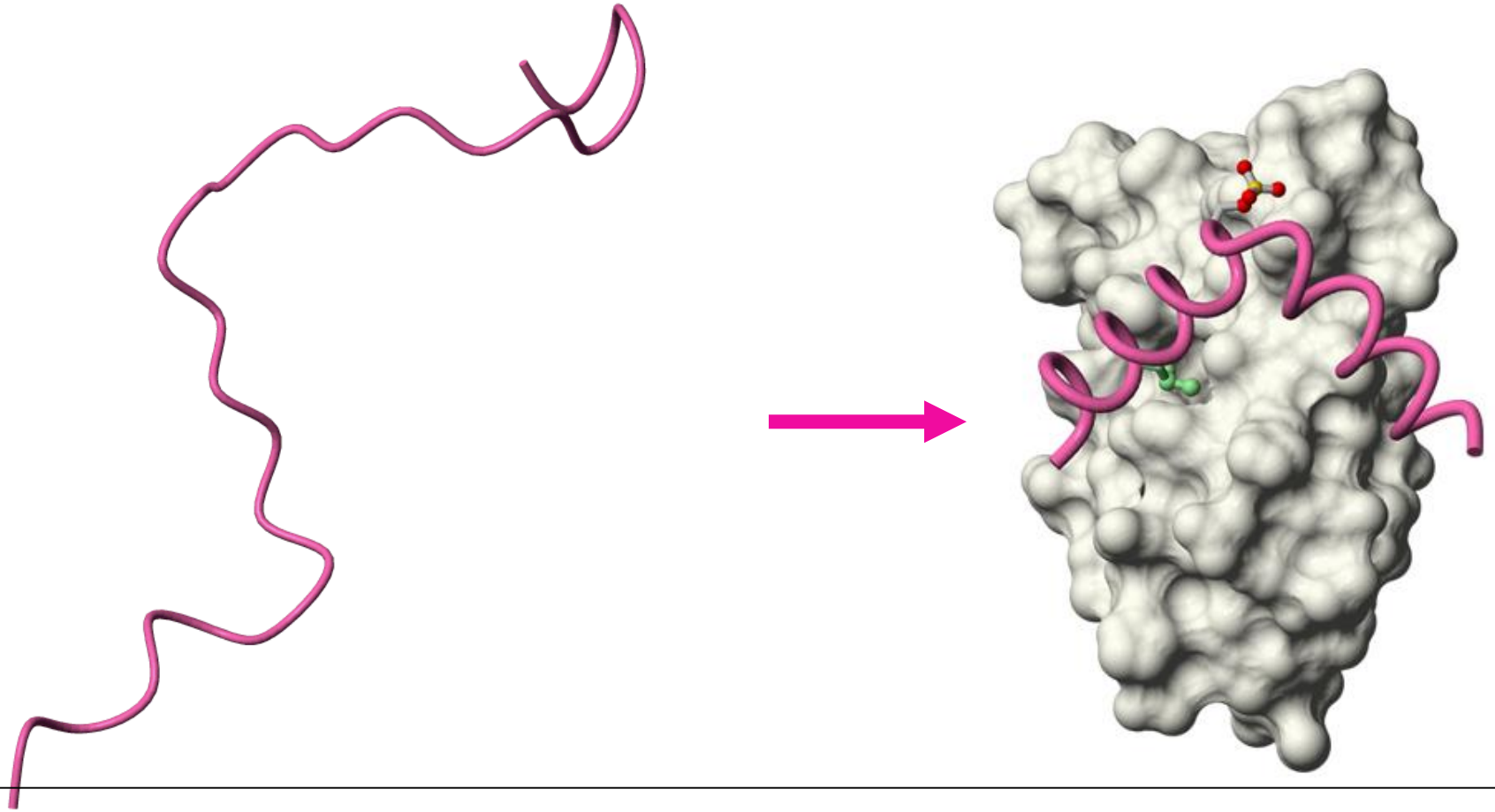
Goals of the dark proteome initiative



- Draw attention to the human dark proteome and its myriad roles in human biology and disease.
- Promote research on intrinsically disordered proteins and “invisible” excited states.
- Advocate for cutting edge infrastructure and creation of centers of excellence to research the human dark proteome and translate discoveries into cures for devastating diseases.

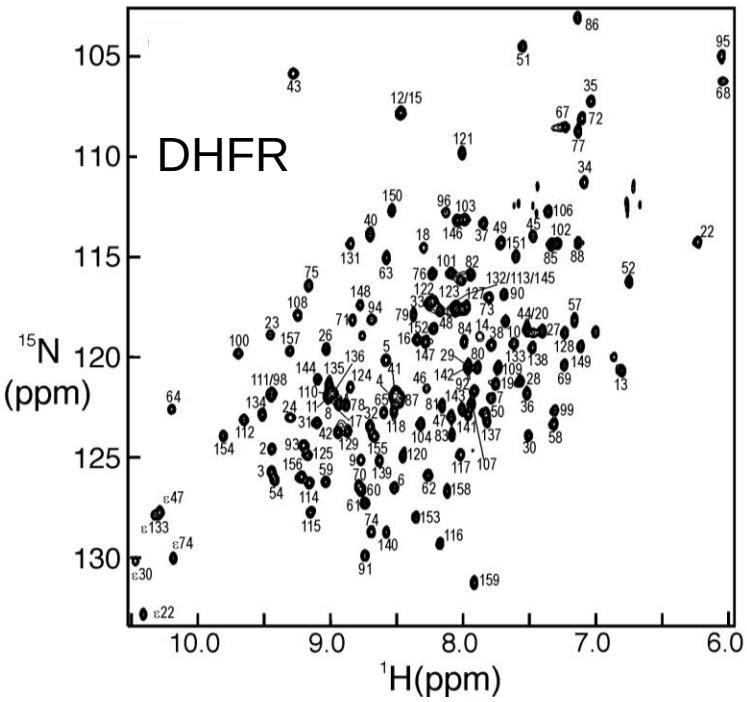
http://darkproteome.org

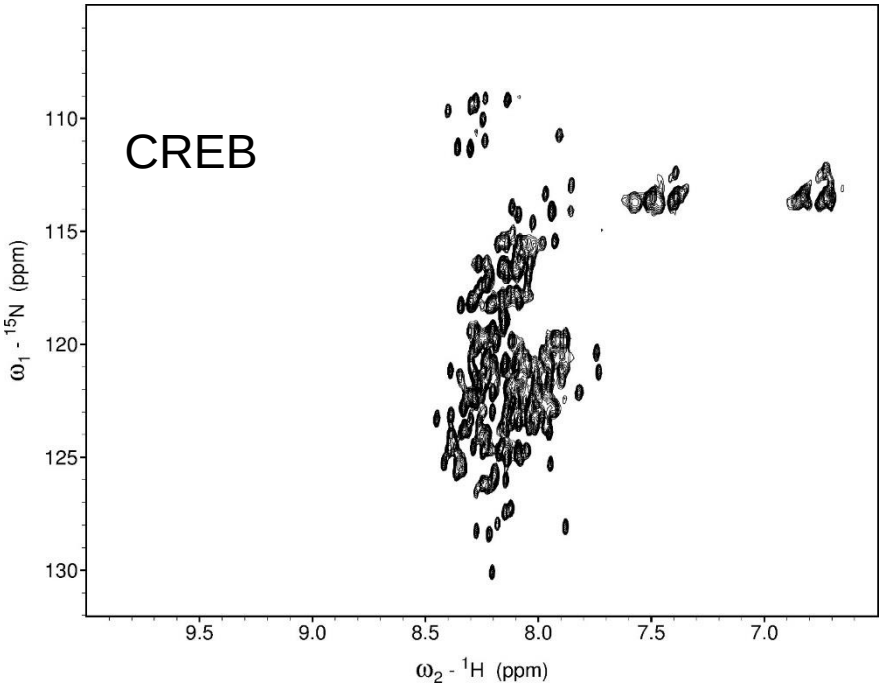
NMR is a unique tool for characterization of IDPs



Insights into structural ensemble, dynamics, interactions, and posttranslational modifications of IDPs - both free and bound to their targets

NMR spectra of IDPs





- limited dispersion and pathological signal overlap
- highly variable line widths
- frequently require < 10μM concentration
- exchange broadening in complexes

NMR spectra of IDPs

- limited dispersion and pathological signal overlap
- exchange broadening in complexes

Solutions:

- apply reductionist, divide-and-conquer strategy
- generate fusion constructs to minimize exchange broadening
- acquire high dimensional (5D, 6D) NMR spectra

Partial solutions only: need for UHF NMR

Divide-and-conquer approach

Pros:

- relatively straightforward, can provide valuable insights

Cons:

- protein domains do not function in isolation but act synergistically in the cell
- divide-and-conquer approach unable to reveal how complex proteins function
- results can be highly misleading

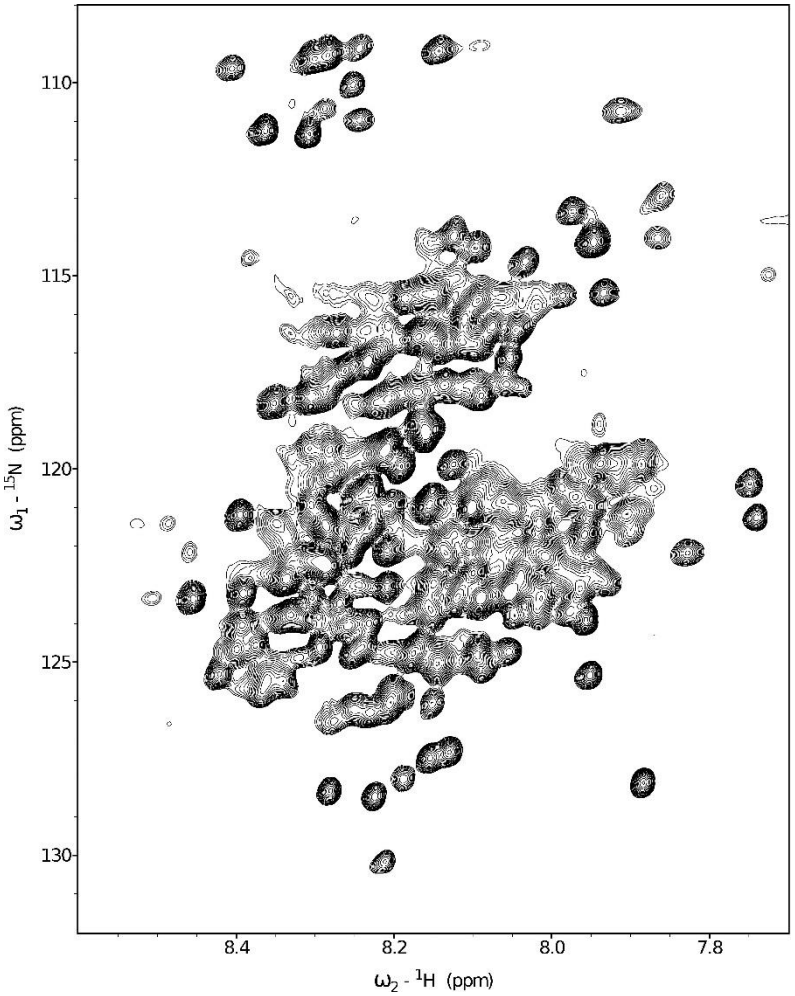
We need holistic approaches to characterize full-length IDPs and their interactions

TROSY spectrum of ^{15}N, ^{2}H CREB(1-341)

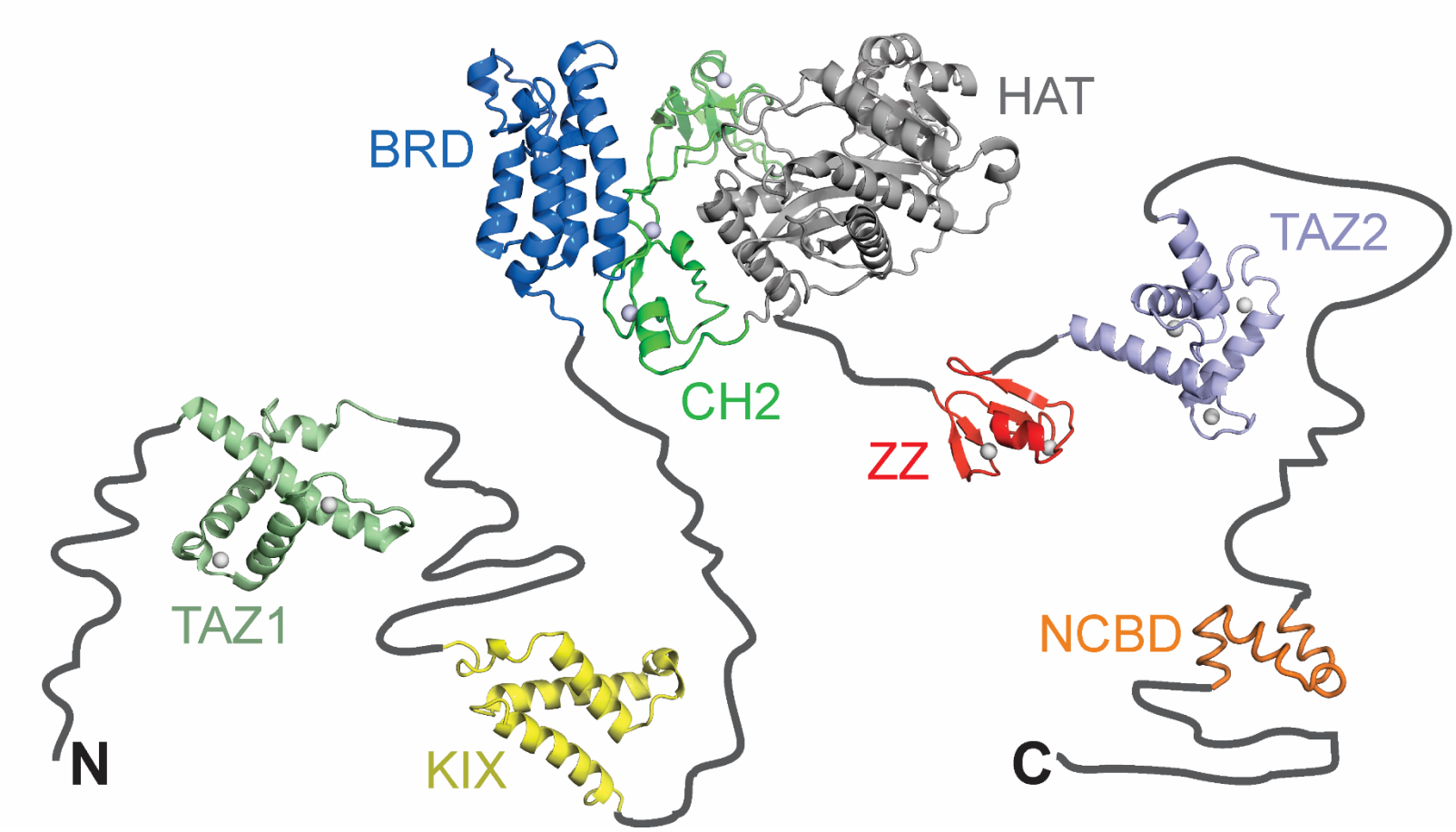
- pathologically overlapped, highly variable linewidth

- many cross peaks broadened
- fast relaxation makes higher dimensional (4D, 5D) experiments impossible
- such systems need UHF NMR

Full-length CREB dimer, 900 MHz



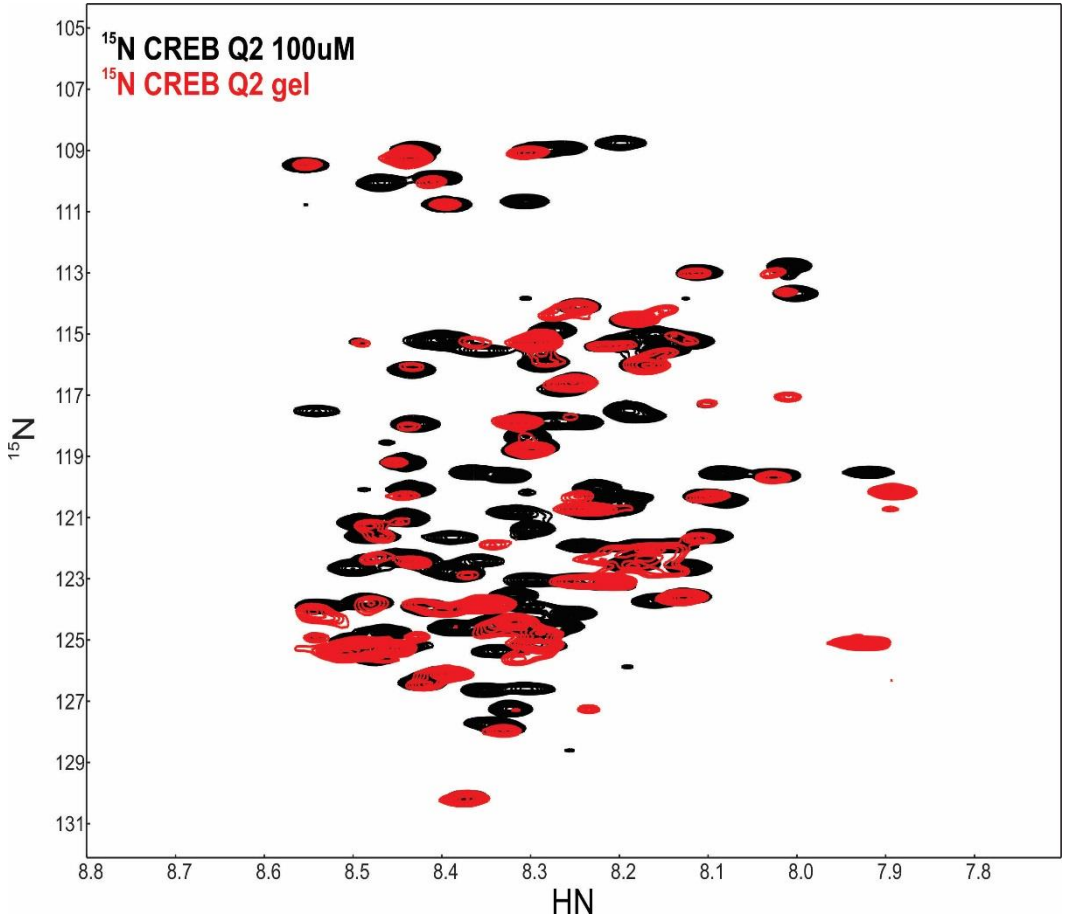
The challenge of “real” eukaryotic proteins with both structured and disordered regions



Transcriptional coactivator CBP/p300

IDP phase separation: membrane-less organelles

- hydrogel formation by Q2 domain of CREB
- insights into molecular interactions in phase-separated state



UHF NMR will be a central, enabling technology for characterization of IDPs and their interactions

- increased dispersion (increased resolution)
- increased sensitivity (low μM concentrations)
- enhanced capability to perform ^{13}C and ^{15}N direct detect experiments
- different exchange regime (may move from exchange broadening to slow exchange)
- extended frequency range for relaxation measurements, analysis of dynamics
- access faster timescales, lower excited state population in CPMG dispersion experiments