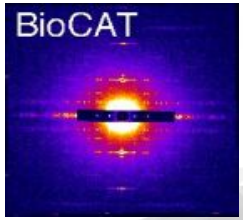


MICROGRADIENT FLUIDICS

University of Massachusetts Medical School

The unfolded state of proteins viewed with time-resolved FRET, unnatural amino acids and microfluidic mixing

Osman Bilsel

Microgradient Fluidics LLC

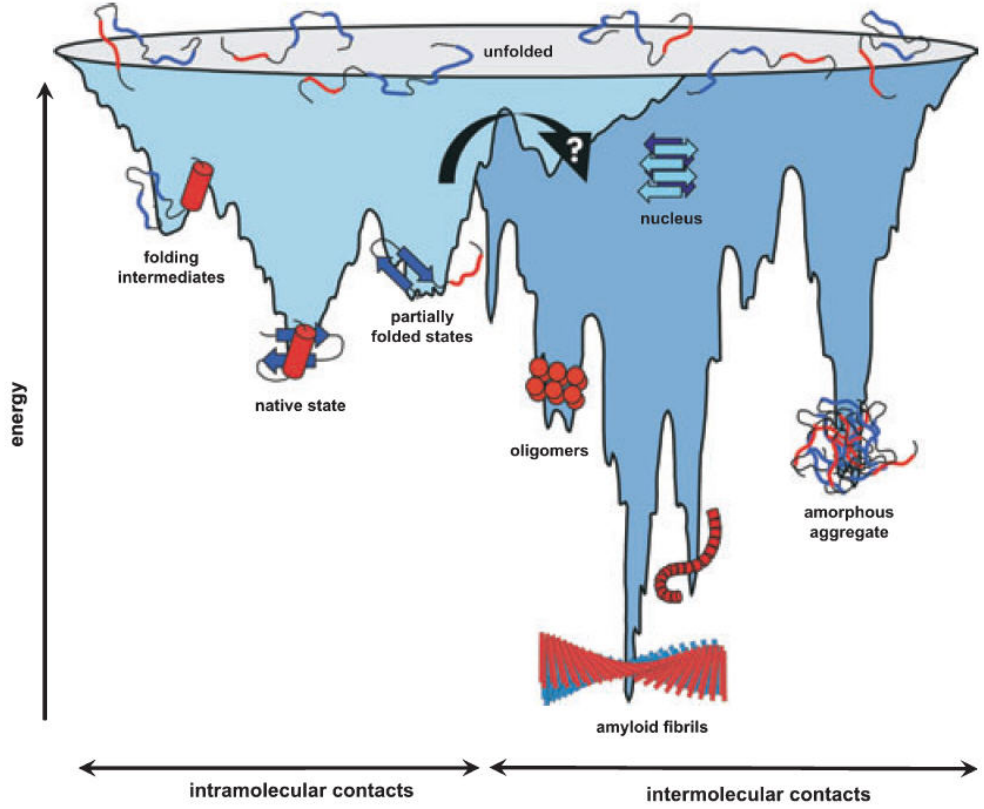
University of Massachusetts Medical School

HOS 2021

Bethesda, MD

April 14, 2021

Mapping the folding landscape of a protein is important for understanding biological function and human disease

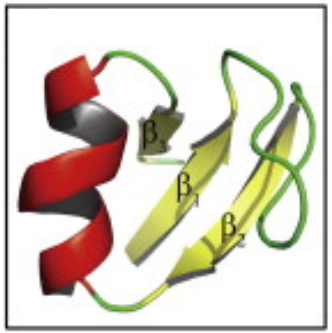


Jahn and Radford, *FEBS Journal* **272** (2005) 5962

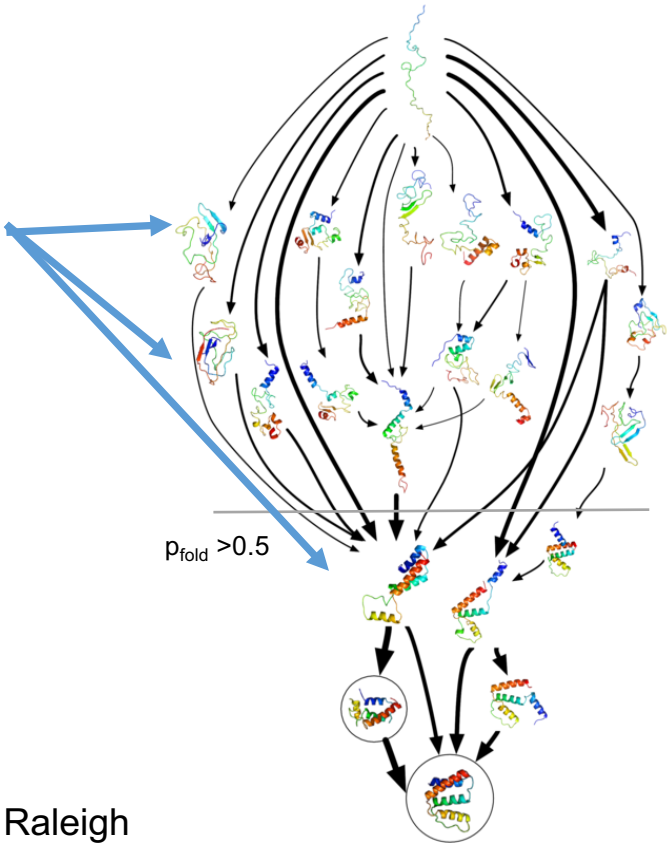
Overall goal is atomistic understanding of molecular interactions

Challenges:

- Low equilibrium populations
- Heterogeneous populations
- (statistical descriptions)
- Short-lived and dynamic



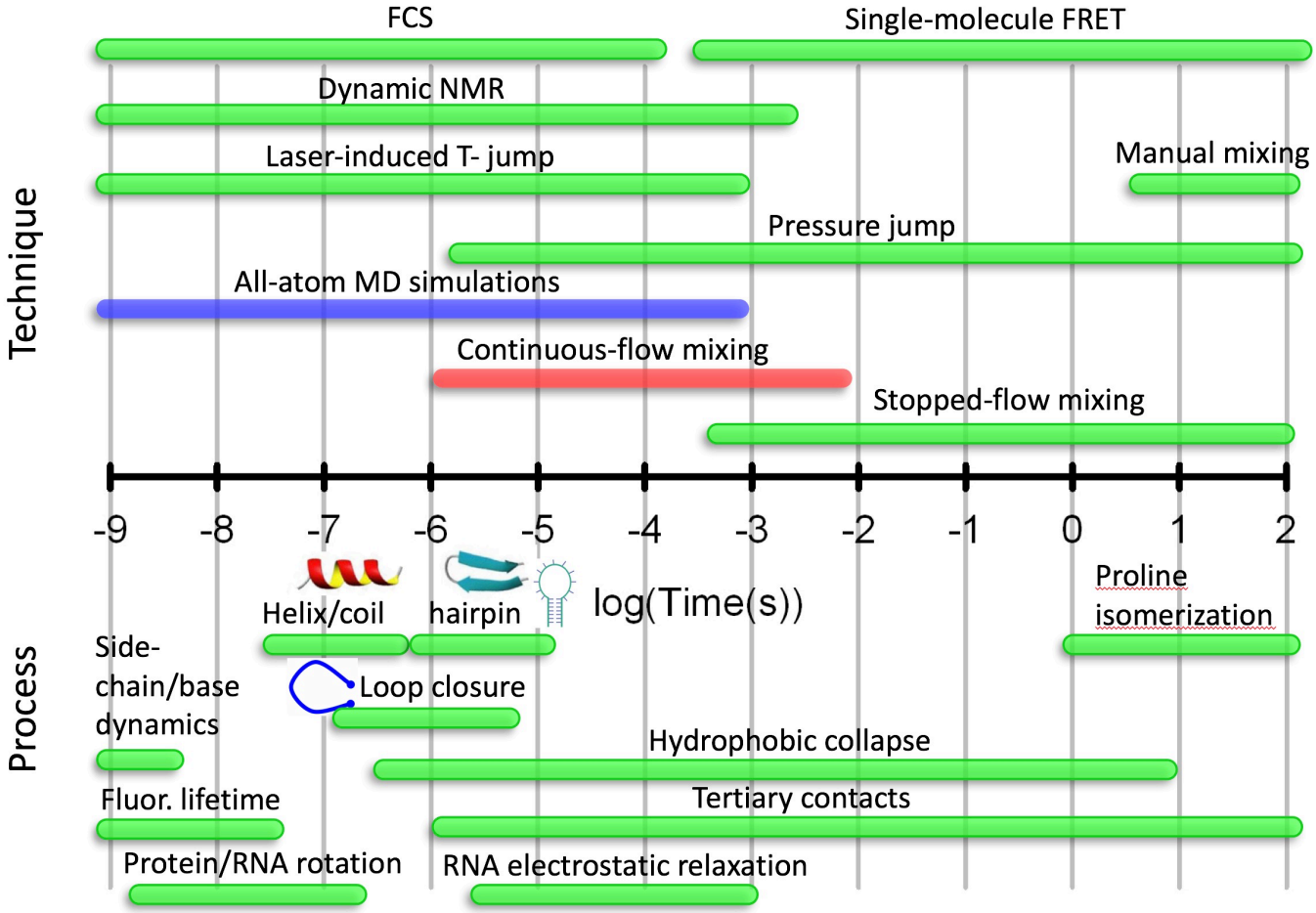
Ntl9



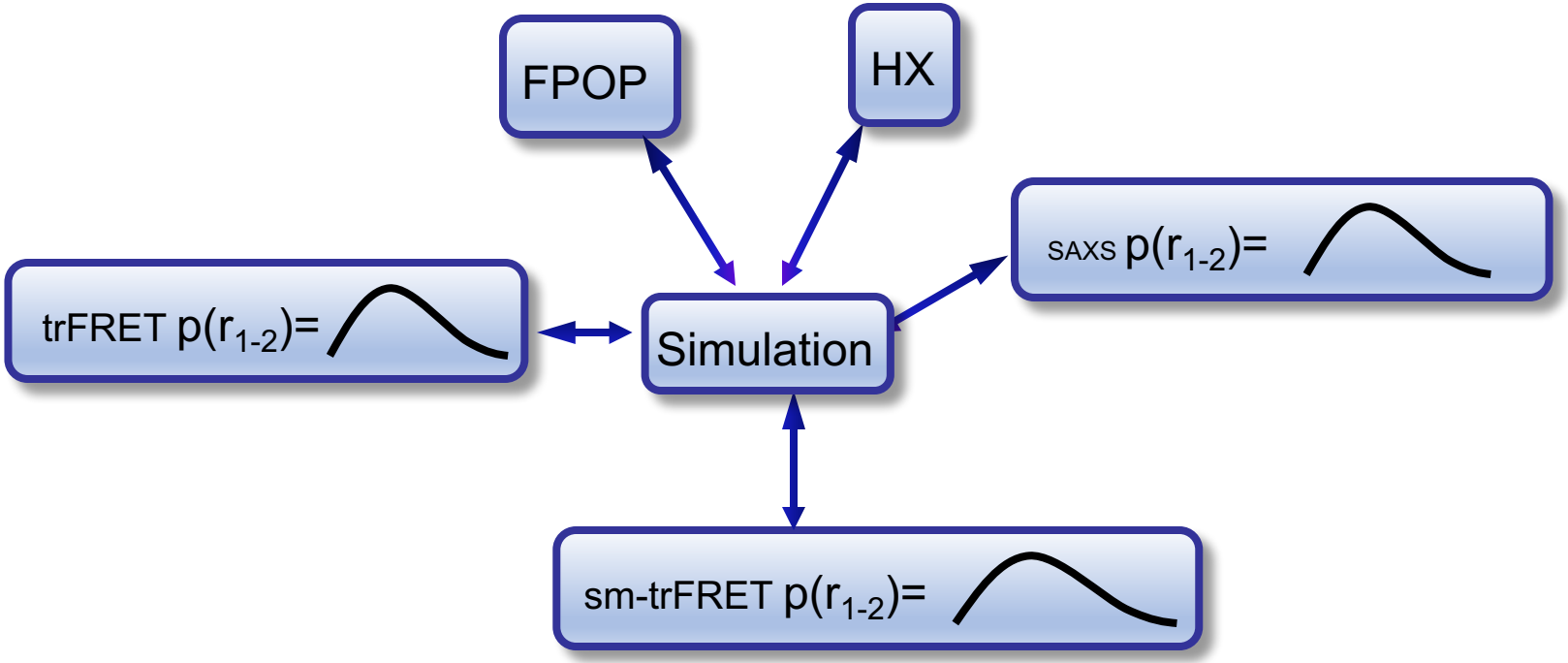
Collaboration with Ivan Peran, Isaac Carico and Dan Raleigh (Stony Brook), Alex Holehouse and Rohit Pappu (WashU)

Voelz *et al* J Am Chem Soc. **2012** 134(30):12565

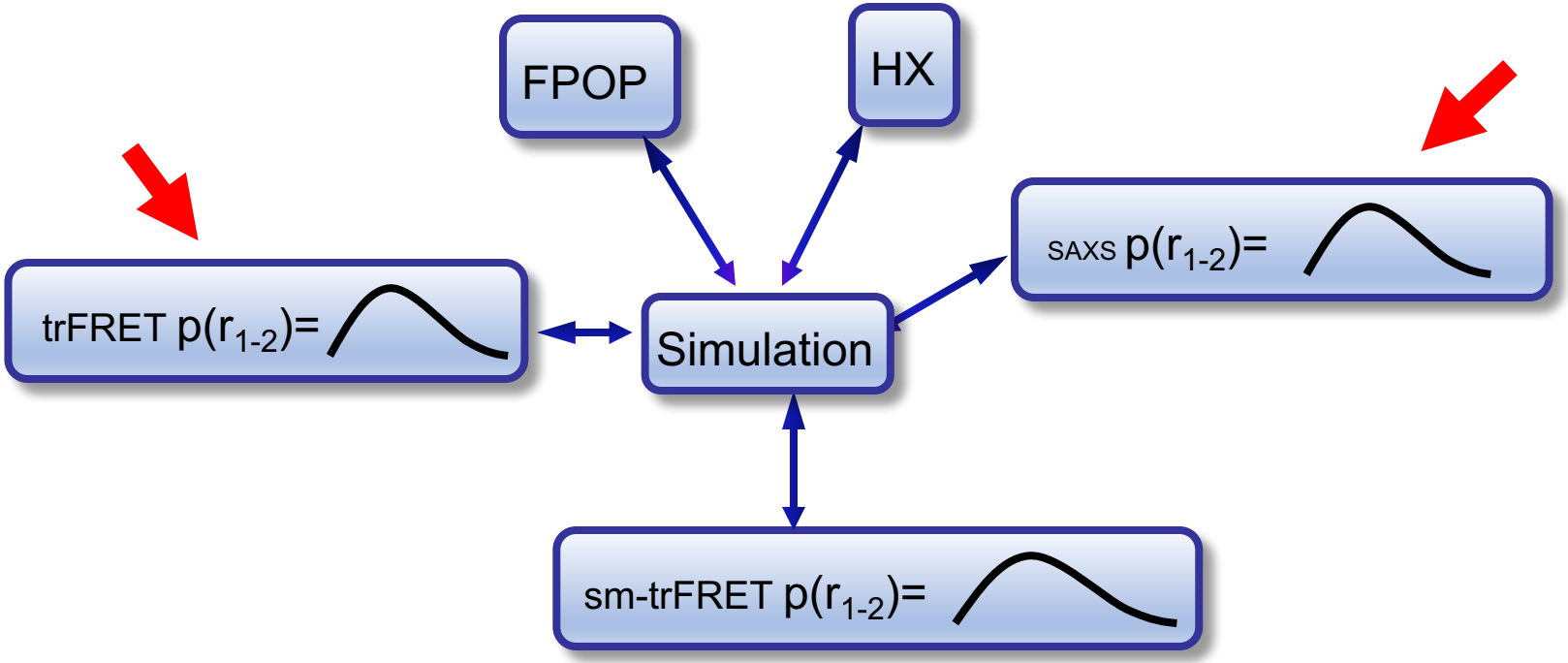
Timescales for protein and RNA dynamics span large dynamic range



Integrating complementary experimental structural probes with simulation



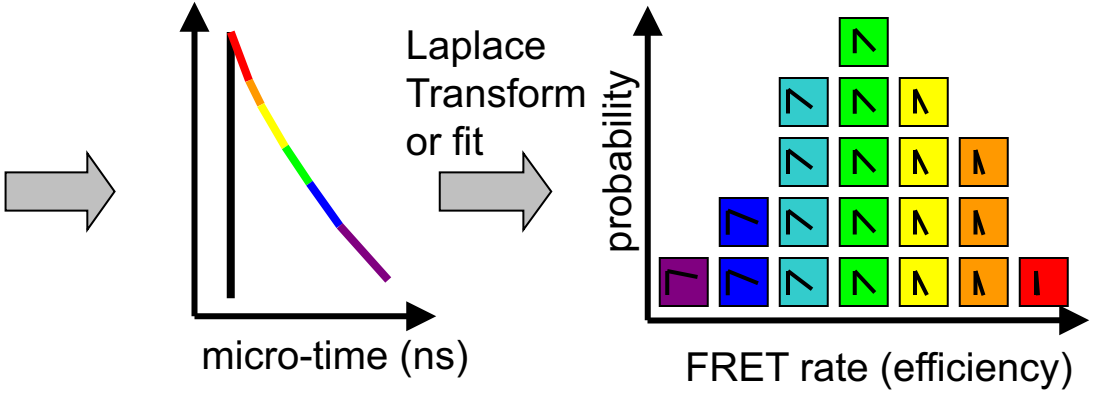
Integrating complementary experimental structural probes with simulation



Ensemble time-resolved FRET can yield distance distributions

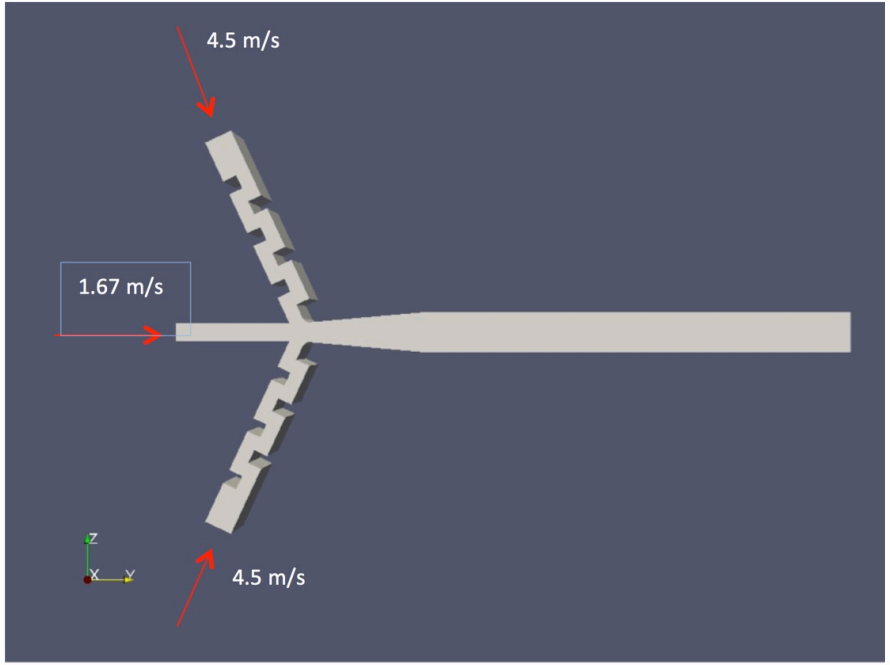
Add up all of the blocks (individual molecule) at a single experiment time (nanoseconds).

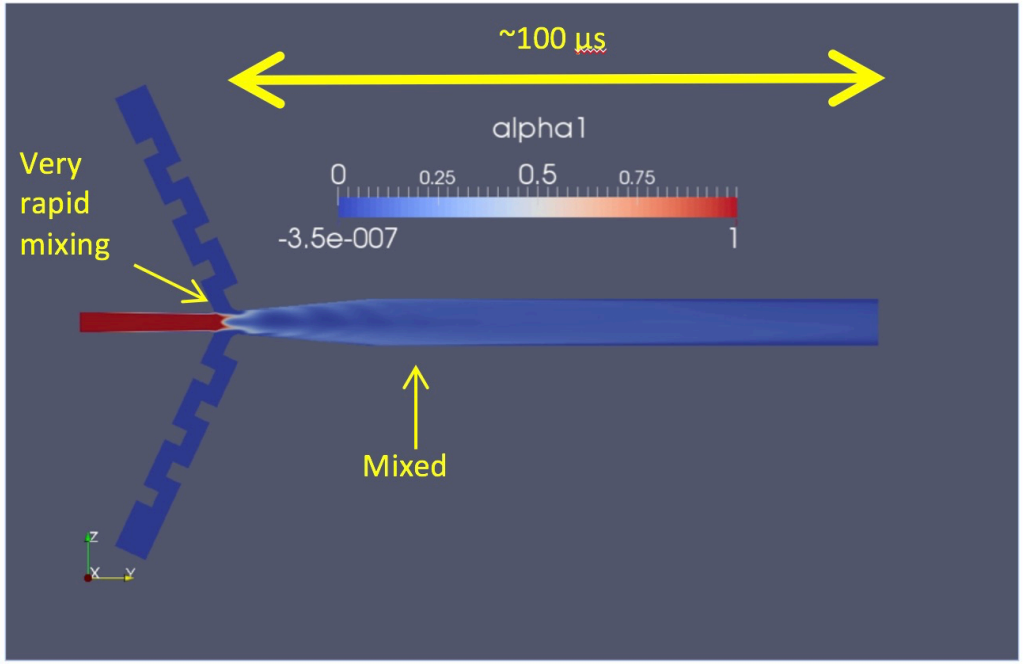
$$I(t) = \int_{k=0}^{\infty} p(k_d, k_{ET}) e^{-(k_d + k_{ET})t} dk$$



- ❑ trFRET contains distance distribution information
- ❑ Averaging time is ns (vs. ms in most smFRET studies)
- ❑ Not shot noise limited

Computational fluid dynamics facilitate mixer design





Design parameters:

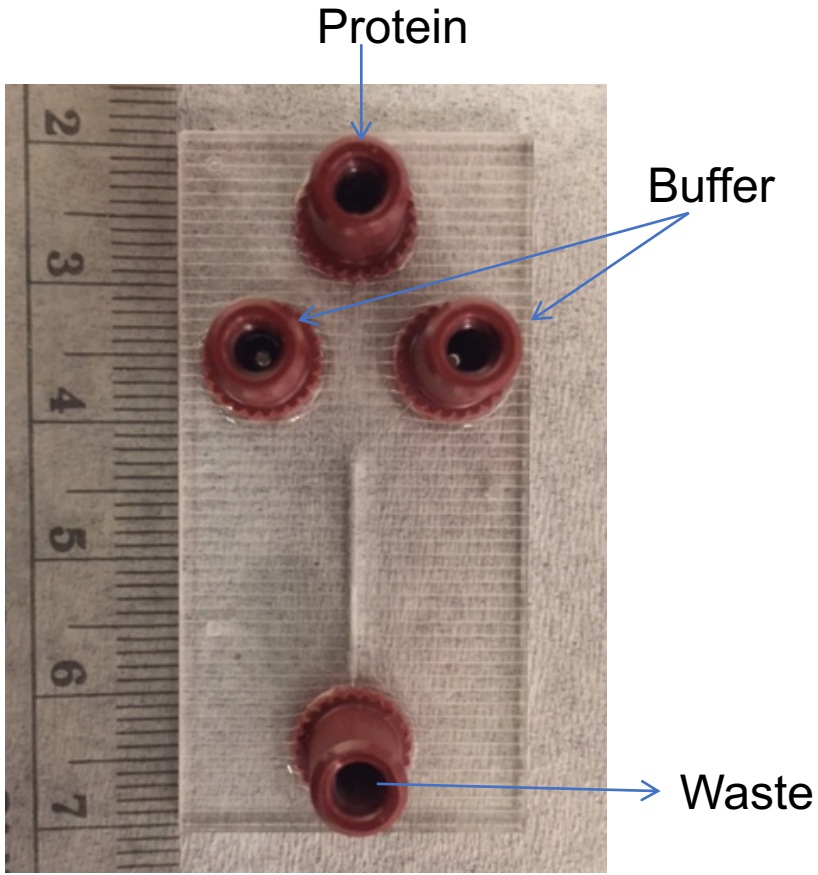
- Fast and efficient mixing to minimize sample consumption
  Uniform flow velocity profile
- Avoid cavitation
- Minimize potential shearing

(~2 µs transition time)

Venkatesh Inguva and J. Blair Perot

All-quartz microfluidic chip

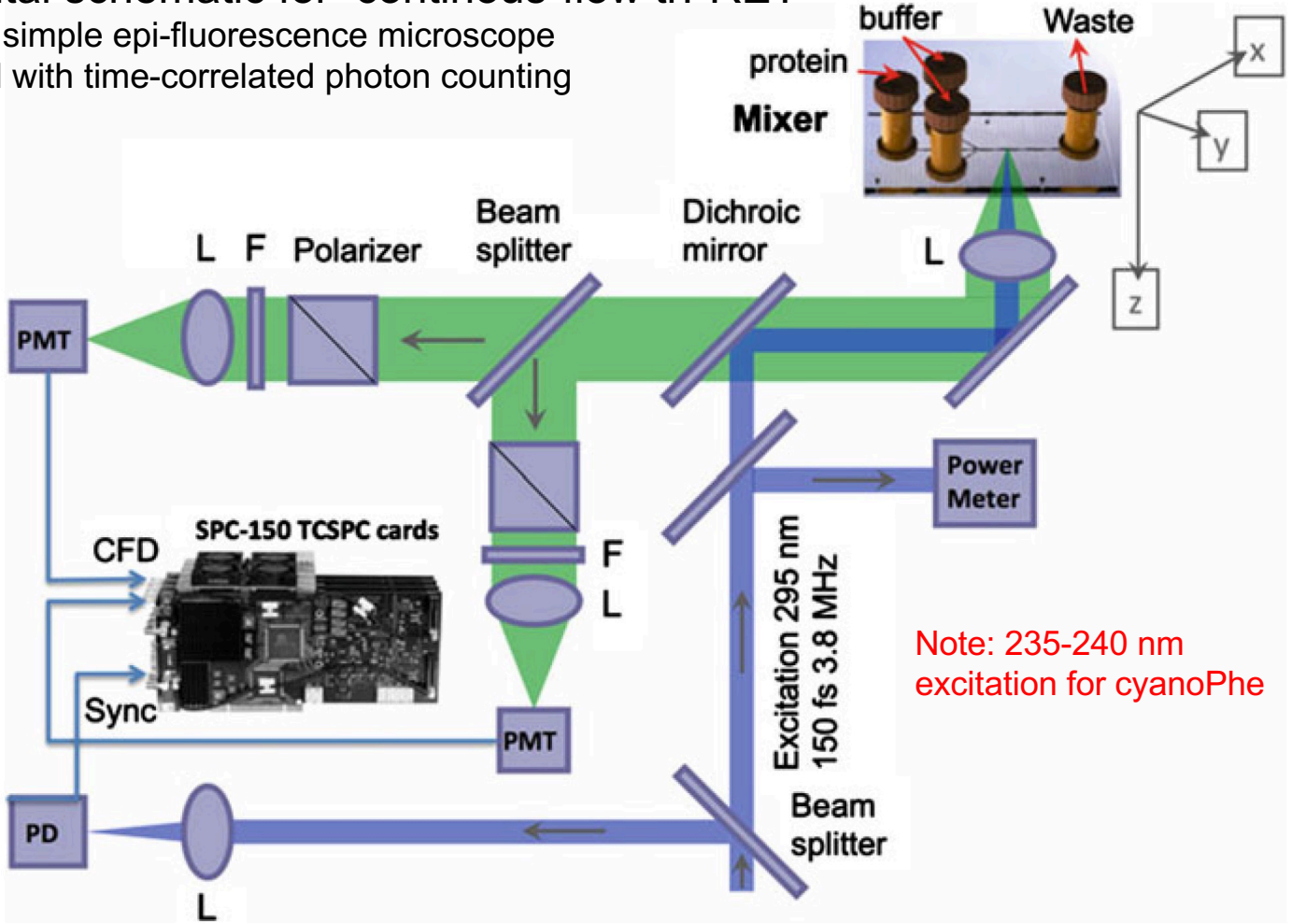
- Suitable for fluorescence and SAXS
- Robust to >1000 psi



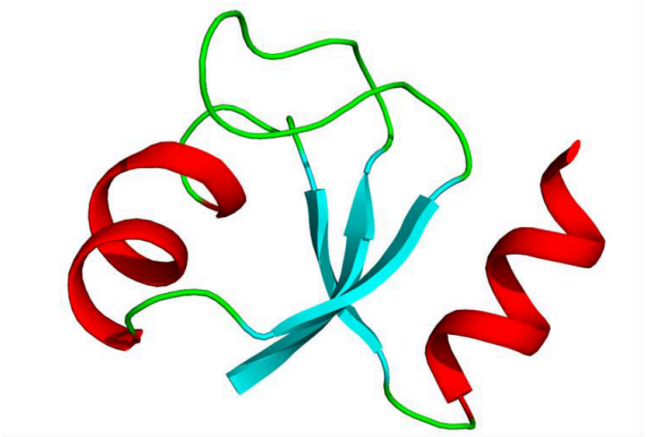
Ali Said (Translume)

Experimental schematic for continous-flow trFRET

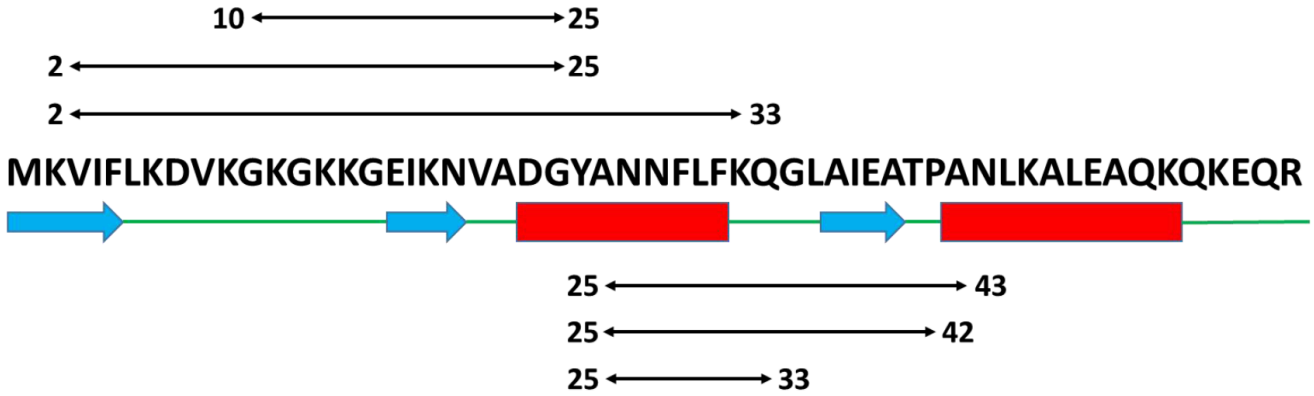
- Based on simple epi-fluorescence microscope
- Integrated with time-correlated photon counting



N-terminal Domain of L9 (NTL9)

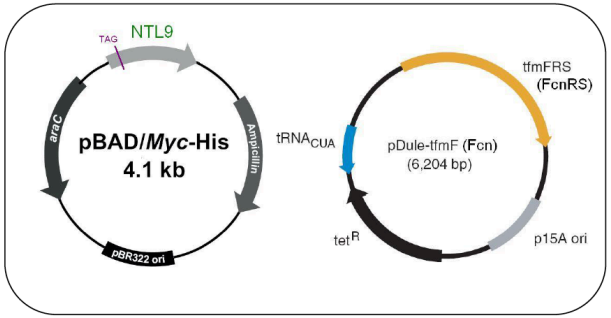


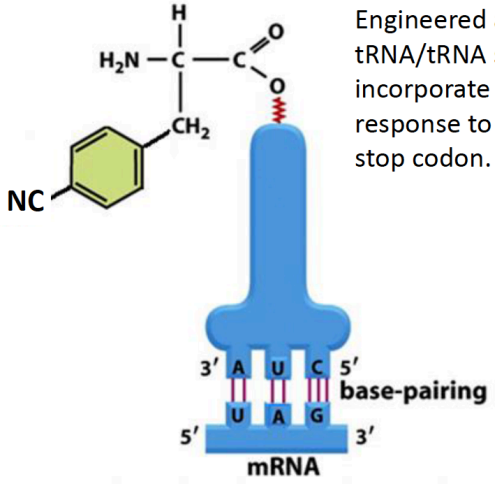
- 56 residue protein that folds cooperatively in a two-state fashion over a wide range of experimental conditions. The folding time constant is about 2 ms.
- The figure below the structure shows residue pairs that were probed via FRET.



p-Cyanophenylalanine (F_{CN})

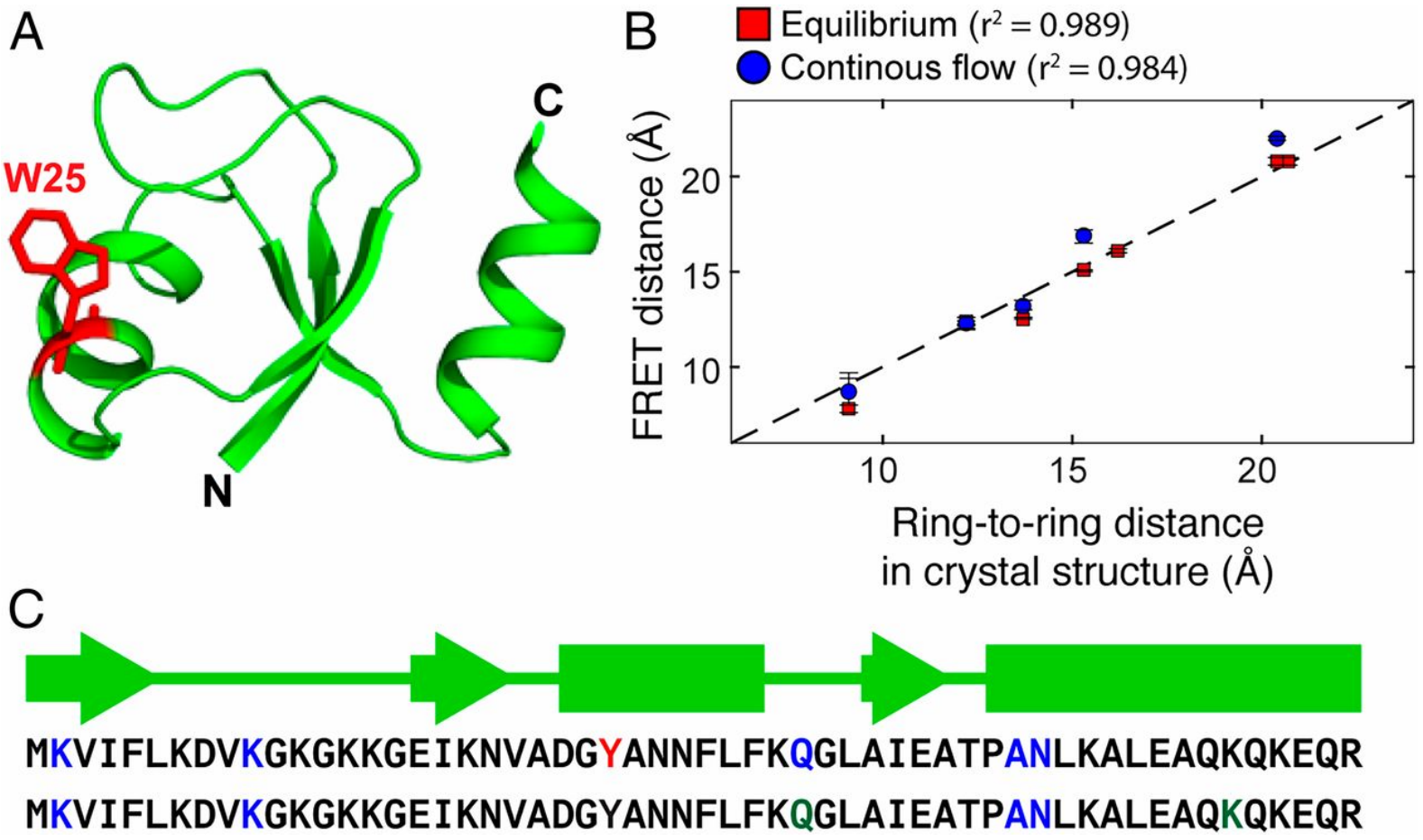
- Analog of Tyr that minimally perturbs the protein structure.
- Selectively excited at 240 nm with an emission maximum at 290 nm.
- Excited-state decay is single exponential with a lifetime of 7.0 ns in water.
- Is the donor in a FRET pair with Trp with R_0=16Å.
- Incorporated recombinantly using 21st pair technology developed by the Schultz and Mehl labs.





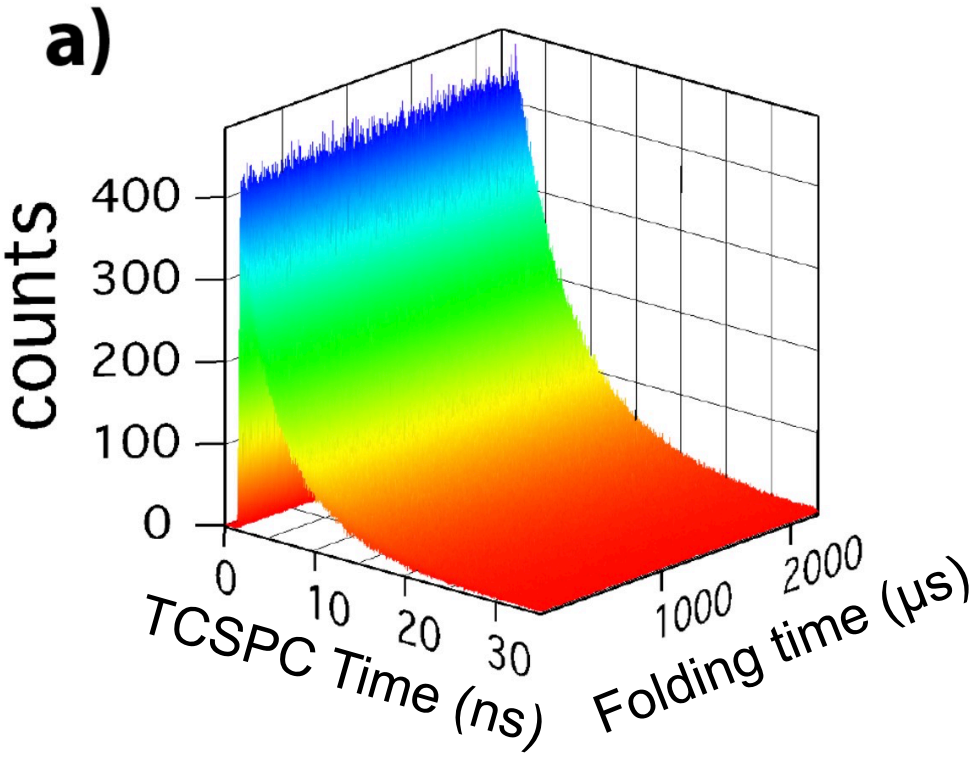
Engineered amino-acyl tRNA/tRNA synthetase incorporate F_{CN} in response to an amber stop codon.

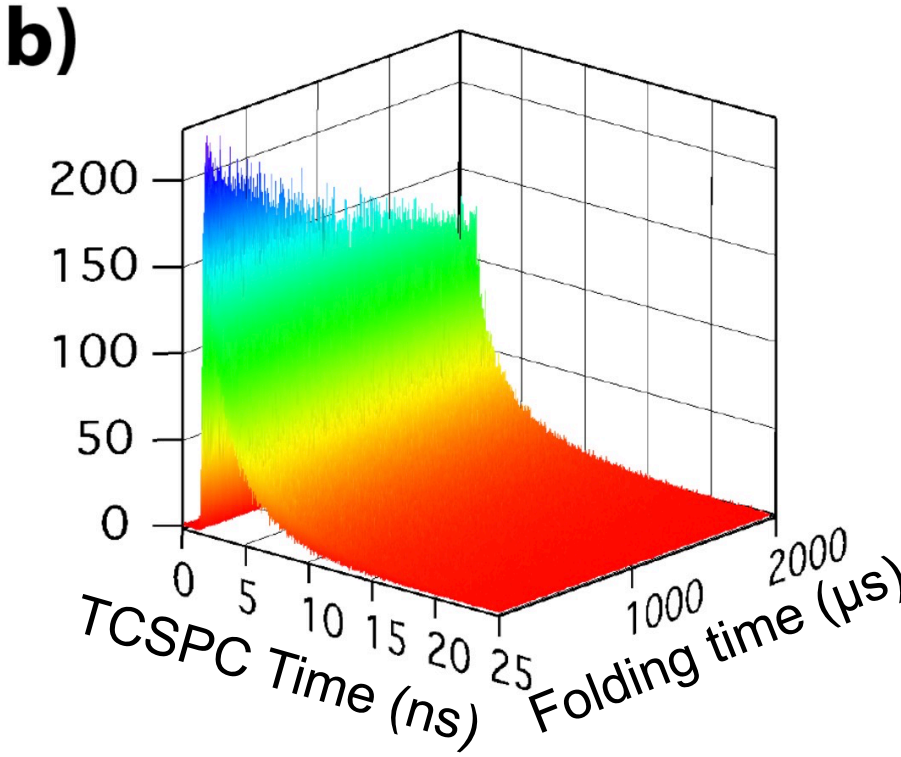
trFRET derived distances for the native state agree with crystal structure



Donor only

Donor + Acceptor





Global analysis of donor-only and donor-acceptor data sets

$$I_d(t_{TCSPC}) = I_d(0) \cdot \sum_{}^{N} \alpha_i e^{-k_{d_i} \cdot t_{TCSPC}} + const$$

Donor-only

$$I_{da}(t_{TCSPC}) = \int_0^{\infty} \sum_{i}^{N} \alpha_i e^{-k_{d_i} \cdot t_{TCSPC}} \cdot p(k_{ET}) \cdot e_{ET}^{-k_{ET} \cdot t_{TCSPC}} + const.$$

Donor-acceptor

$$p(r) = \sum_i \frac{a_i}{\sigma\sqrt{2\pi}} e^{-(r-\omega_i)^2/2\sigma_i^2}$$

Gaussian

$$p(r, t_{kin}) = \sum_i c_i(t_{kin}) \cdot p_i(r)$$

$$r^6 = R_0^6 \cdot \left(\frac{k_{Dave}}{k_{ET}}\right)$$

$$p(r) = \frac{4\pi a N r^2}{l_c^2\left(1 - \left(\frac{r}{l_c}\right)^2\right)^{9/2}} \exp\left(\frac{-3l_c}{4l_p\left(1 - \left(\frac{r}{l_c}\right)^2\right)}\right)$$

Worm-like chain

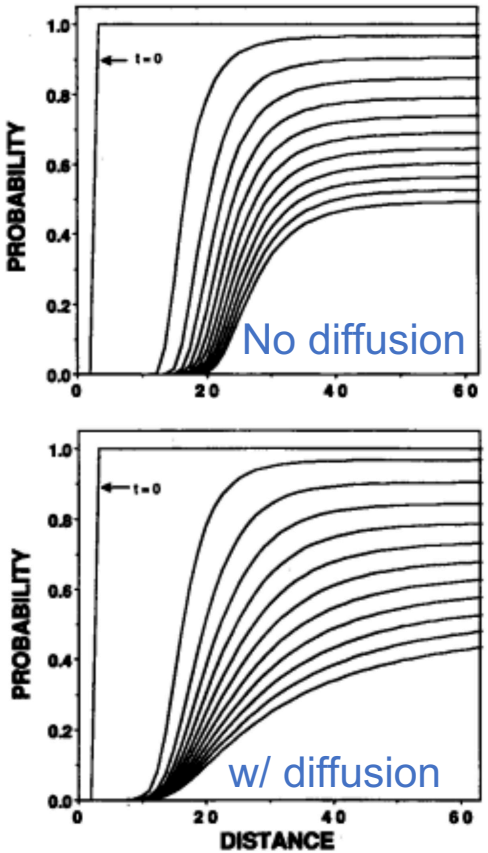
Diffusion during excited state needs to be considered

$$\frac{\partial \bar{N}(r, t_{TCSPC})}{\partial t} = \left\{ \sum_i \frac{\alpha_i}{\tau_i} \left[1 + \left(\frac{R_0}{r} \right)^6 \right] \right\} \cdot \bar{N}(r, t_{TCSPC})$$

$$+ \frac{1}{N_0(r)} \frac{\partial}{\partial r} \left[N_0(r) D(r) \frac{\partial \bar{N}(r, t_{TCSPC})}{\partial r} \right]$$

$$\bar{N}(r, t_{TCSPC}) = \frac{N^*(r, t_{TCSPC})}{N_0(r)}$$

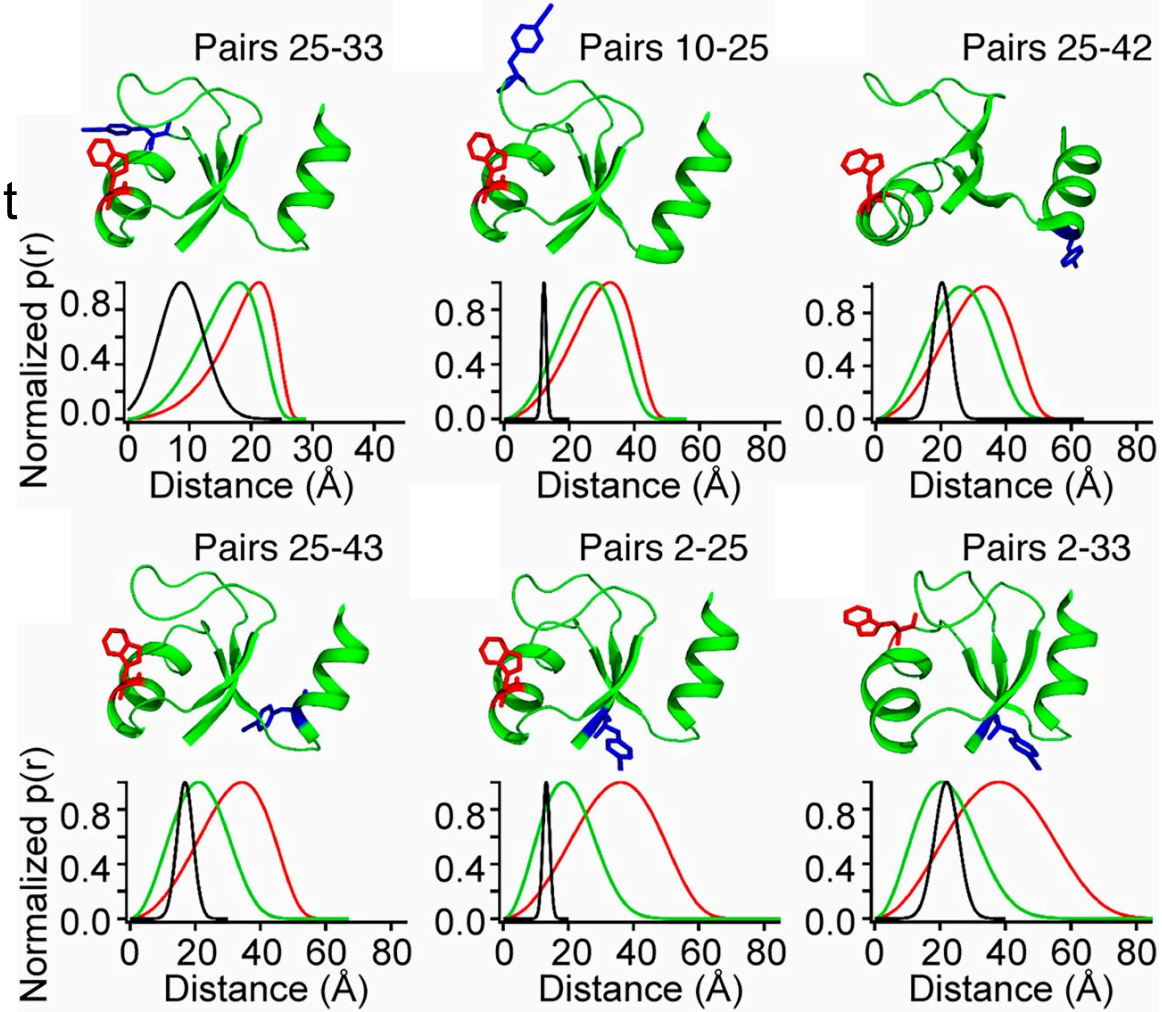
- Faster decrease of longer distance fractions
- Enhancement of shorter distances



Beechem and Haas, Biophys J. 1989

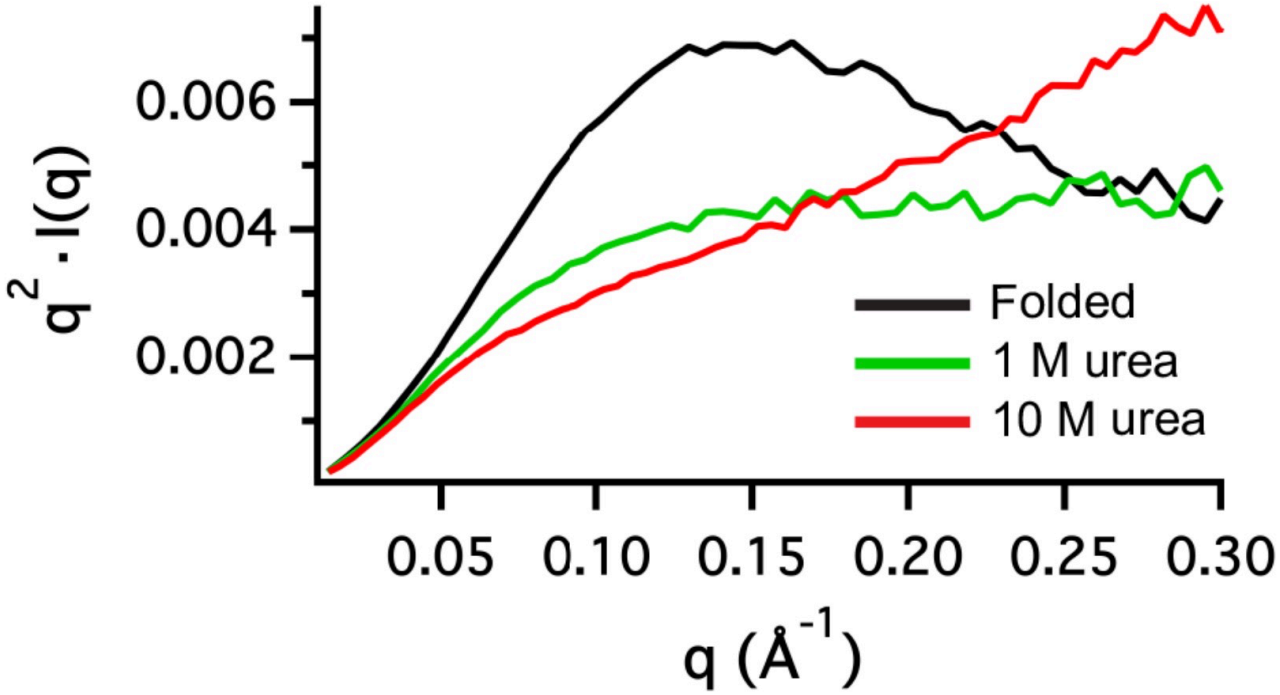
Distance distributions from global fit

10 M Urea
1 M Urea
Native



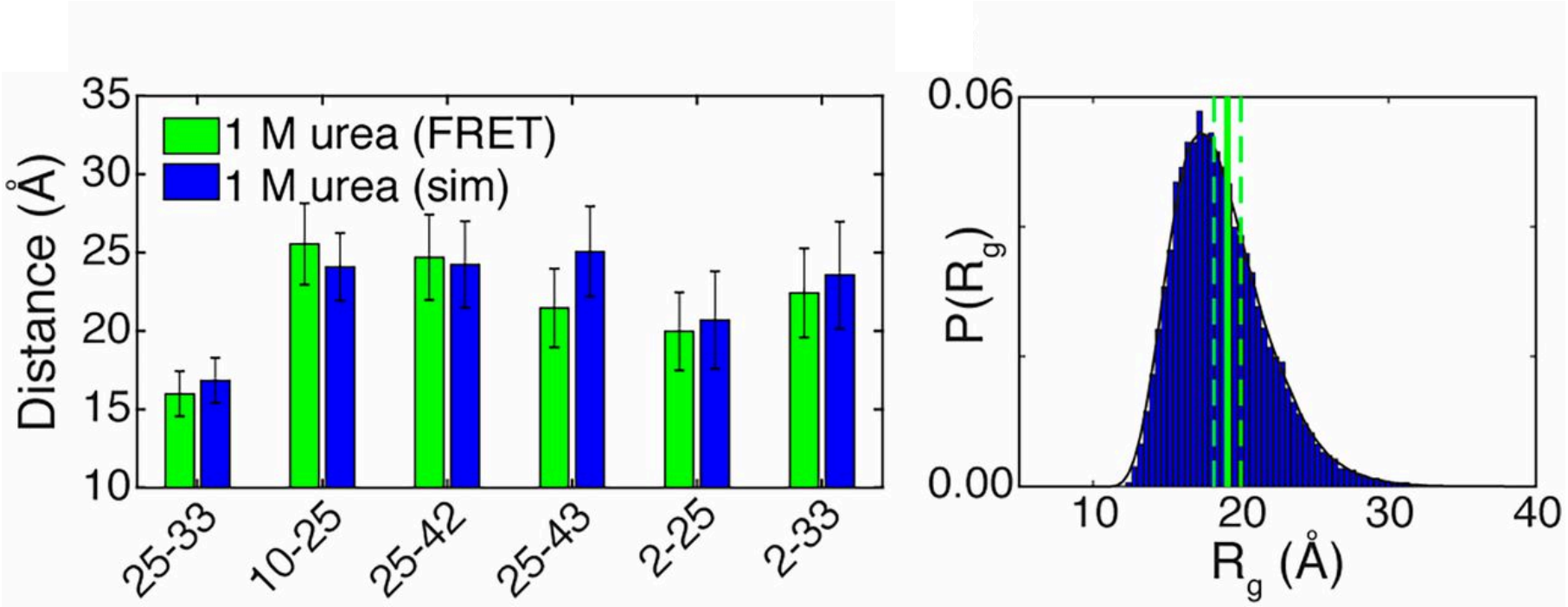
Ivan Peran, Alex Holehouse et al. PNAS doi:10.1073/pnas.1818206116

Small-angle x-ray scattering consistent with compaction



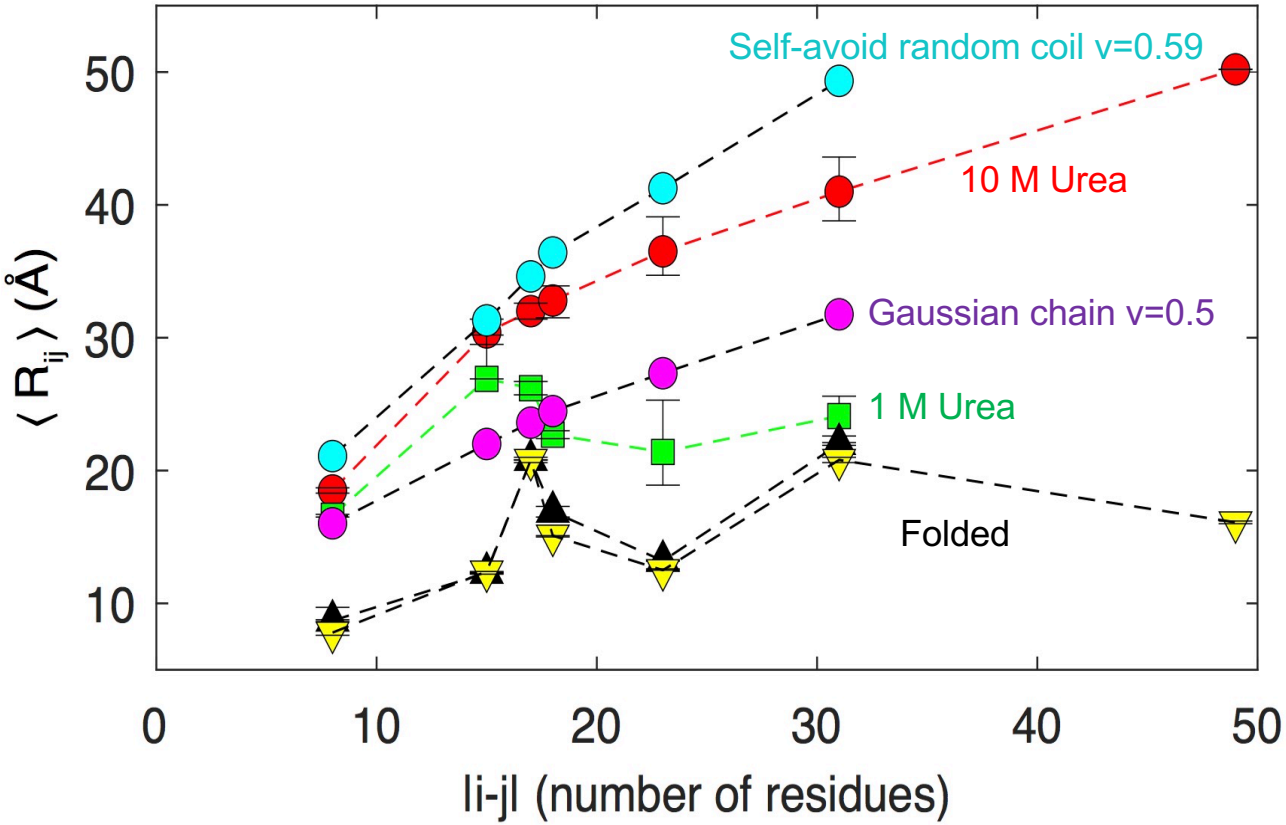
Ivan Peran, Srinivas Chakravarthy, Sagar Kathuria

All-atom Monte Carlo simulations provide ensemble consistent with *both* trFRET and SAXS data



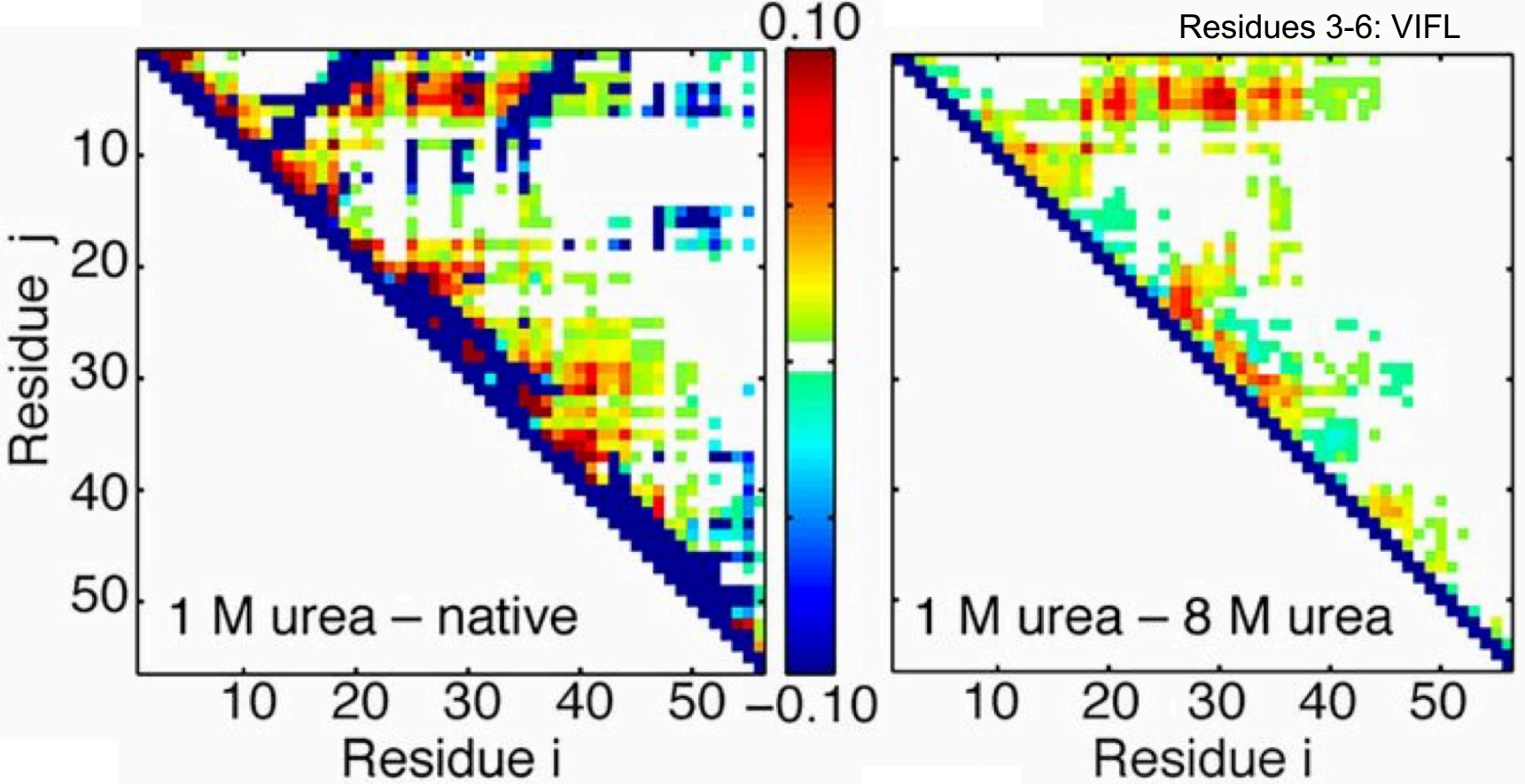
Ivan Peran, Alex Holehouse et al. PNAS doi:10.1073/pnas.1818206116

Distance vs sequence separation comparison



Ivan Peran, Alex Holehouse et al. PNAS doi:10.1073/pnas.1818206116

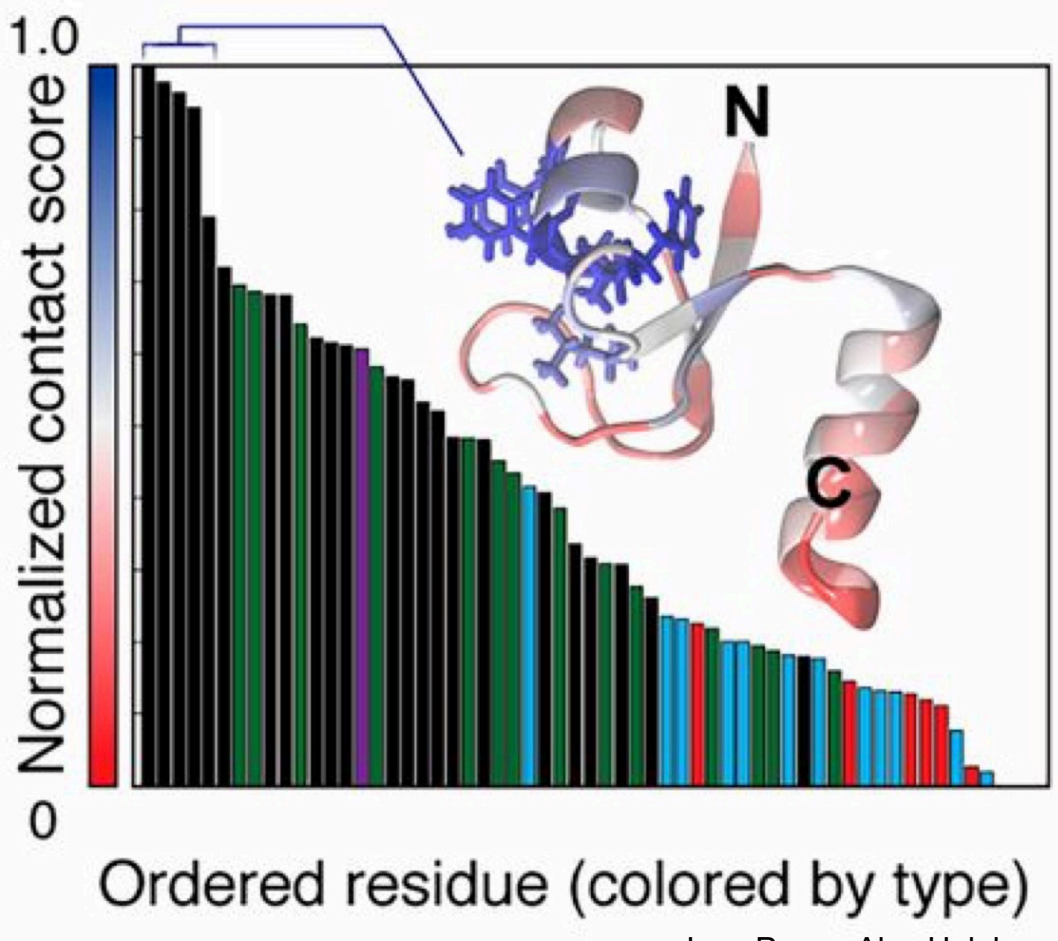
Simulations reveal additional contacts in unfolded state at 1M urea



Alex Holehouse

Ivan Peran, Alex Holehouse et al. PNAS doi:10.1073/pnas.1818206116

Hydrophobic contacts (ILVF) significant in unfolded state



Color code:

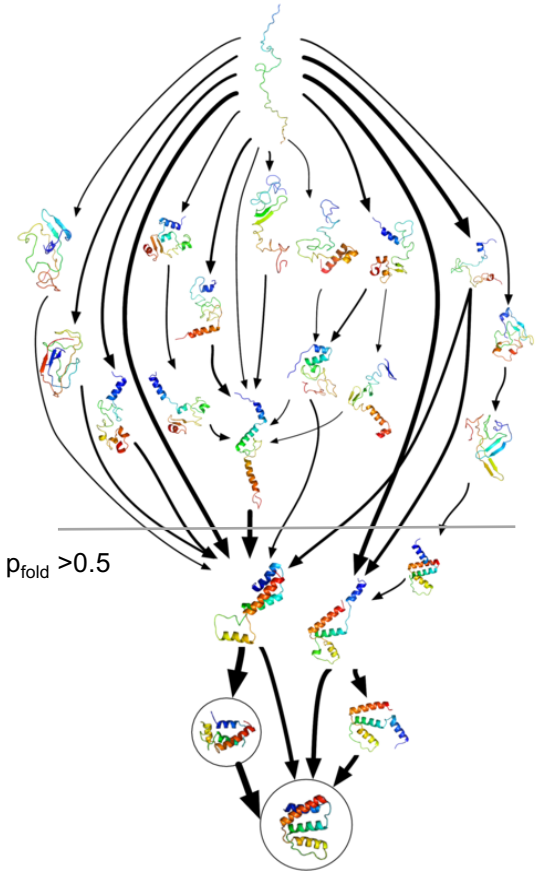
Hydrophobic/Aromatic
Polar
Proline
Positive
Negative

Alex Holehouse

Ivan Peran, Alex Holehouse et al. PNAS doi:10.1073/pnas.1818206116

Summary

- Microfluidic mixing coupled with time-resolved FRET yields structural insights into higher energy states (unfolded).
- Alternative detection techniques, e.g. CD, FPOP.
- Minimal perturbation with unnatural a.a.
- Unfolded state is more compact under refolding conditions.
- Transient interactions present.
- Hydrophobic/aromatic residues dominate.



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Acknowledgements

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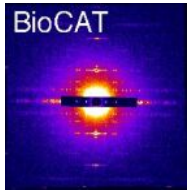
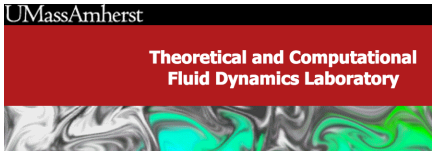
UMASS

Sagar Kathuria
Bob Matthews



Srinivas Chakravarthy
Jesse Hopkins
Tom Irving



Venkatesh Inguva
J. Blair Perot

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NSF

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United States of America