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Unique Contributions of PREs and DEER to PSI-Biology and Beyond

Michael A. Kennedy

Miami University, Oxford, Ohio

NESG NMR Workshop, Amherst, New York
August 27-28, 2012

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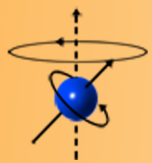

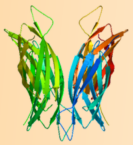

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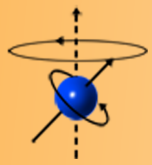
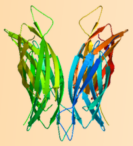
How do we harness PREs and DEER to push the limits of Magnetic Resonance in PSI Biology?

1. **PREs and DEER in Larger proteins (30-40 kDa) in combination with sparse constraints (ILV protonated, ^{2}H, ^{19}F)**

2. **Structural constraints for Larger homodimers and heterodimers, including protein/DNA complexes**

3. **Exploration of new biology**
   - ***Homodimer exchange kinetics***
   - ***Detection of transient, lowly populated states***





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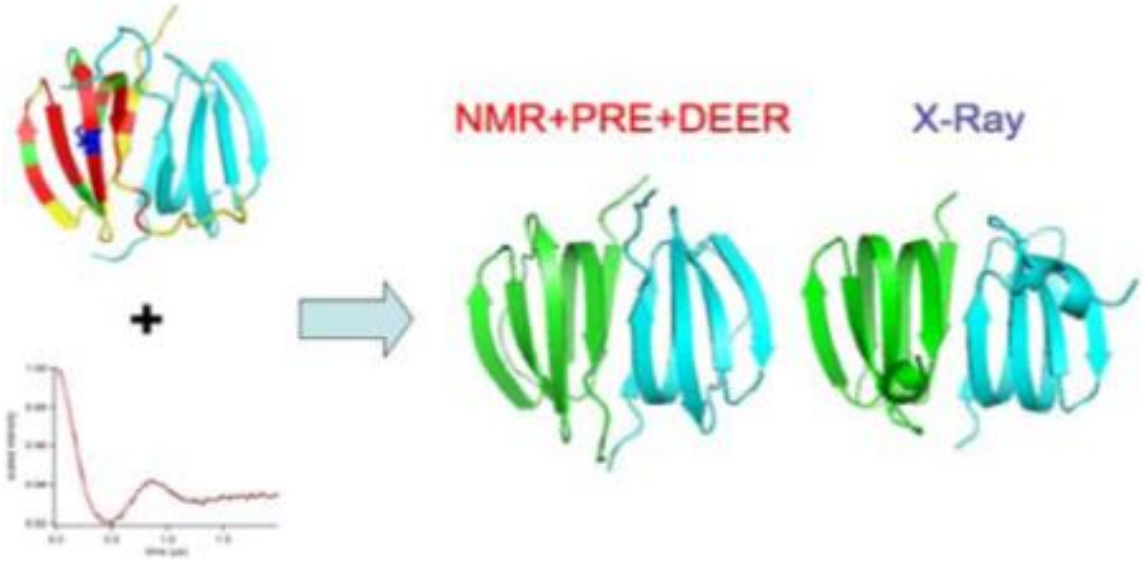
1. PREs and DEER in **Larger** proteins (30-40 kDa) in combination with sparse constraints (ILV protonated, ^{2}H, ^{19}F)

We have demonstrated with small systems (<100 aa).



What we need moving forward:

1. **access to targets in 30-40 kDa range**
2. **coordination within NESG to demonstrate in 30-40kDa systems**

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2. PREs and DEER for structural constraints for **Larger** homodimers and heterodimers, including protein/DNA complexes

Published in final edited form as:
J Am Chem Soc. 2010 September 1; 132(34): 11910–11913. doi:10.1021/ja105080h.

Combining NMR and EPR Methods For Homo-Dimer Protein Structure Determination

Yunhuang Yang[$,#], **Theresa A. Ramelot**[$,#], **Robert M. McCarrick**[$], **Shuisong Ni**[$], **Erik A. Feldmann**[$,#], **John R. Cort**[#,§], **Huang Wang**[#,+], **Colleen Ciccosanti**[#,+], **Mei Jiang**[#,+], **Haleema Janjua**[#,+], **Thomas B. Acton**[#,+], **Rong Xiao**[#,+], **John K. Everett**[#,+], **Gaetano T. Montelione**[#,+,%], and **Michael A. Kennedy**[$,#,*]

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OHIO ADVANCED EPR LABORATORY at MIAMI UNIVERSITY

DEER measurements were made using a Bruker ELEXSYS E580 EPR spectrometer equipped with a Super QFT-Q-band resonator

Measurements at Q-band give >10x greater sensitivity compared to X-band!

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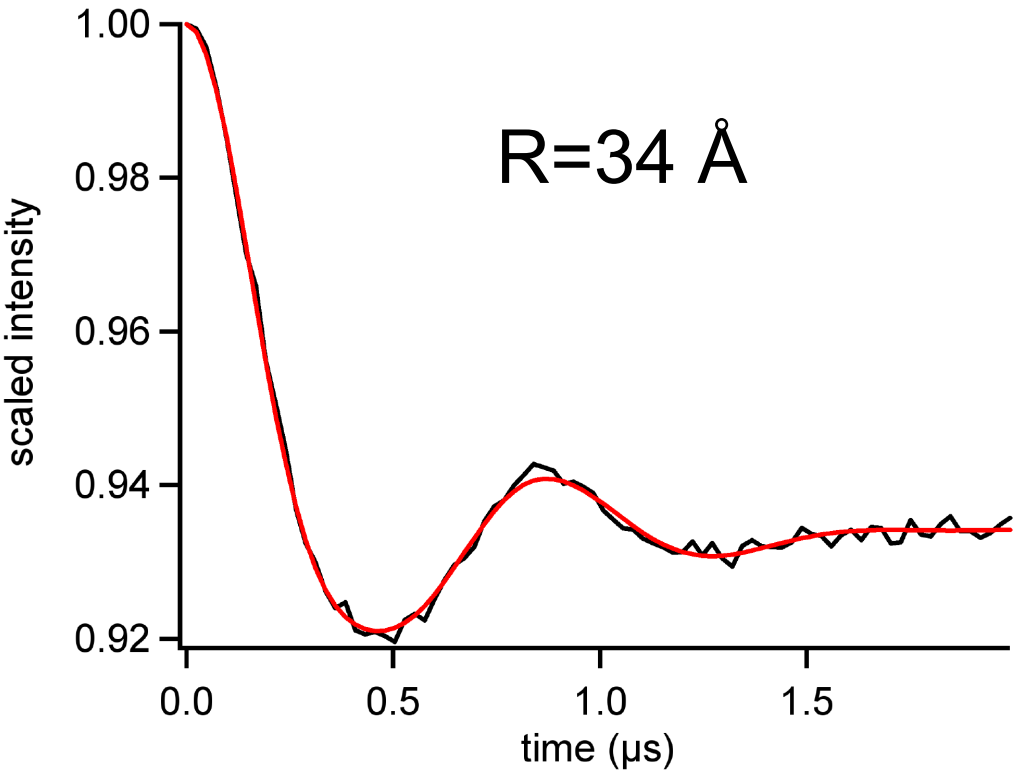
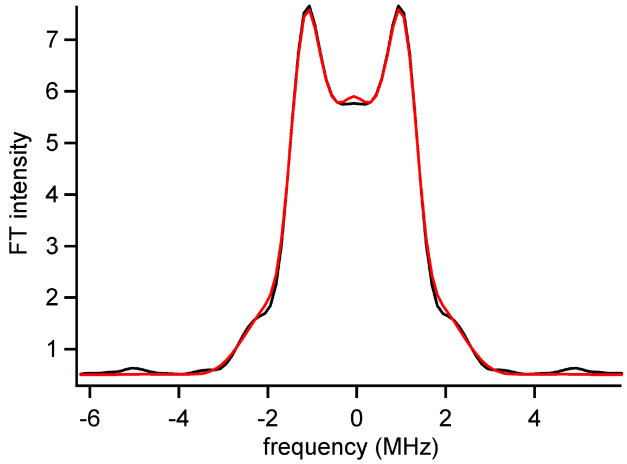
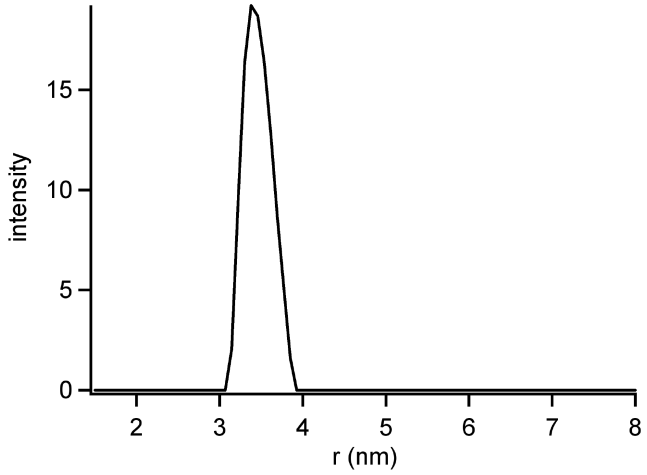
DEER Distance Measurements at Q-band







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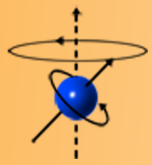

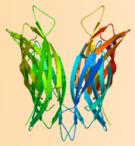

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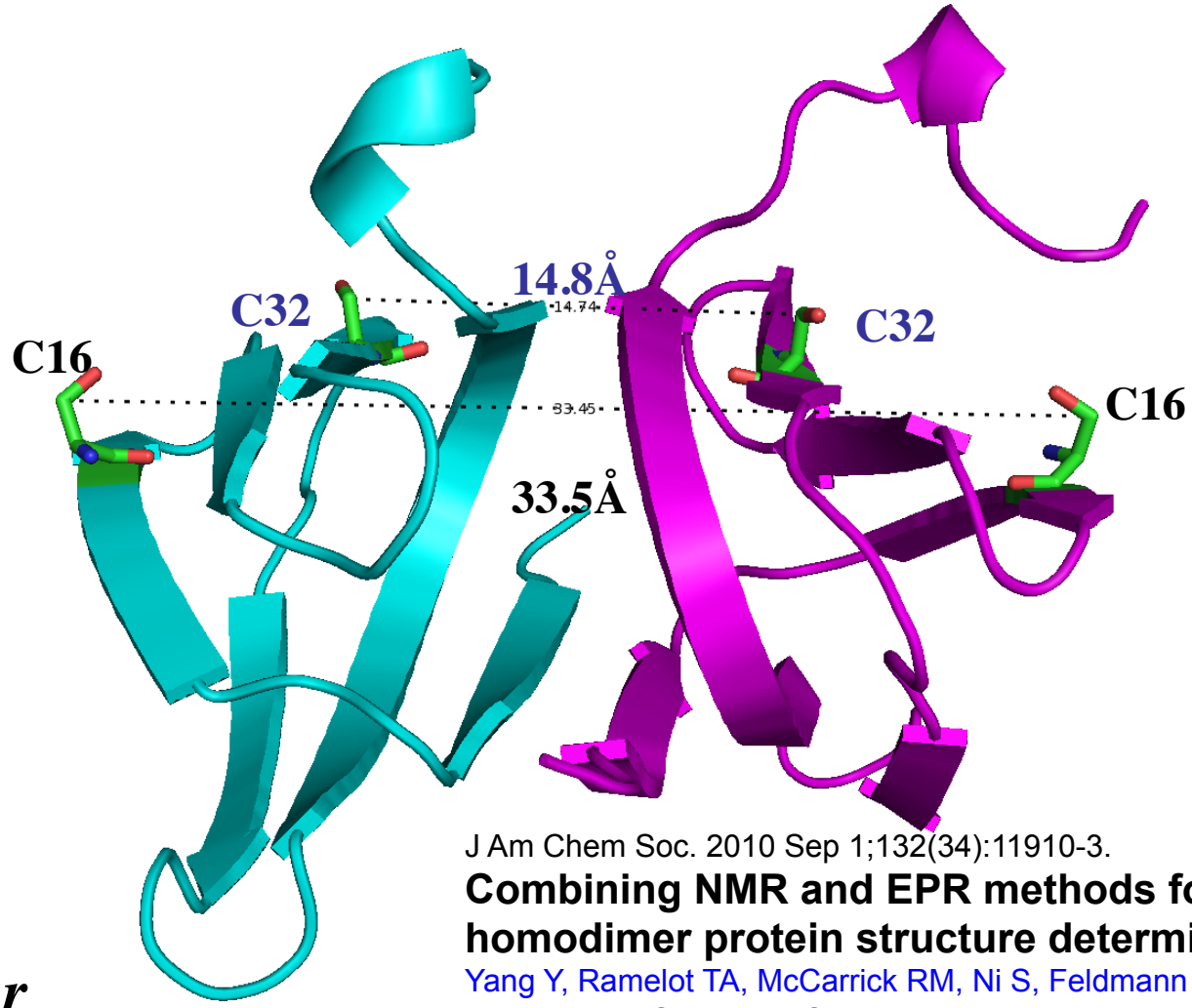
MTSL- Labeled DhR8C for DEER and PRE Measurements

DEER

C32-C32: ~20 Å

C16-C16: ~34 Å



62 aa / monomer

J Am Chem Soc. 2010 Sep 1;132(34):11910-3.

Combining NMR and EPR methods for homodimer protein structure determination.

Yang Y, Ramelot TA, McCarrick RM, Ni S, Feldmann EA, Cort JR, Wang H, Ciccosanti C, Jiang M, Janjua H, Acton TB, Xiao R, Everett JK, Montelione GT. Kennedy MA.

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What we need to move forward:

1. **Identification of 30-40 kDa model homodimer systems for methods development/demonstration**

2. **Identification of 30-40 kDa heterodimer systems for methods development/demonstration**

3. **Identification of Protein/DNA model systems for methods development/demonstration**

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3. Exploration of New Biology: Homodimer Exchange Kinetics

The Transient Nature of Protein-Protein Interactions is Important to their Biological, Biochemical, Cellular Function

"Protein-protein complexes that dissociate and associate readily, often depending on the physiological condition or environment, play an important role in many biological processes"

Nooren and Thornton, **Structural Characterization and Functional Significance of Transient Protein-Protein Interactions.** *J. Mol. Biol.* **325**, 991-1018 (2003)

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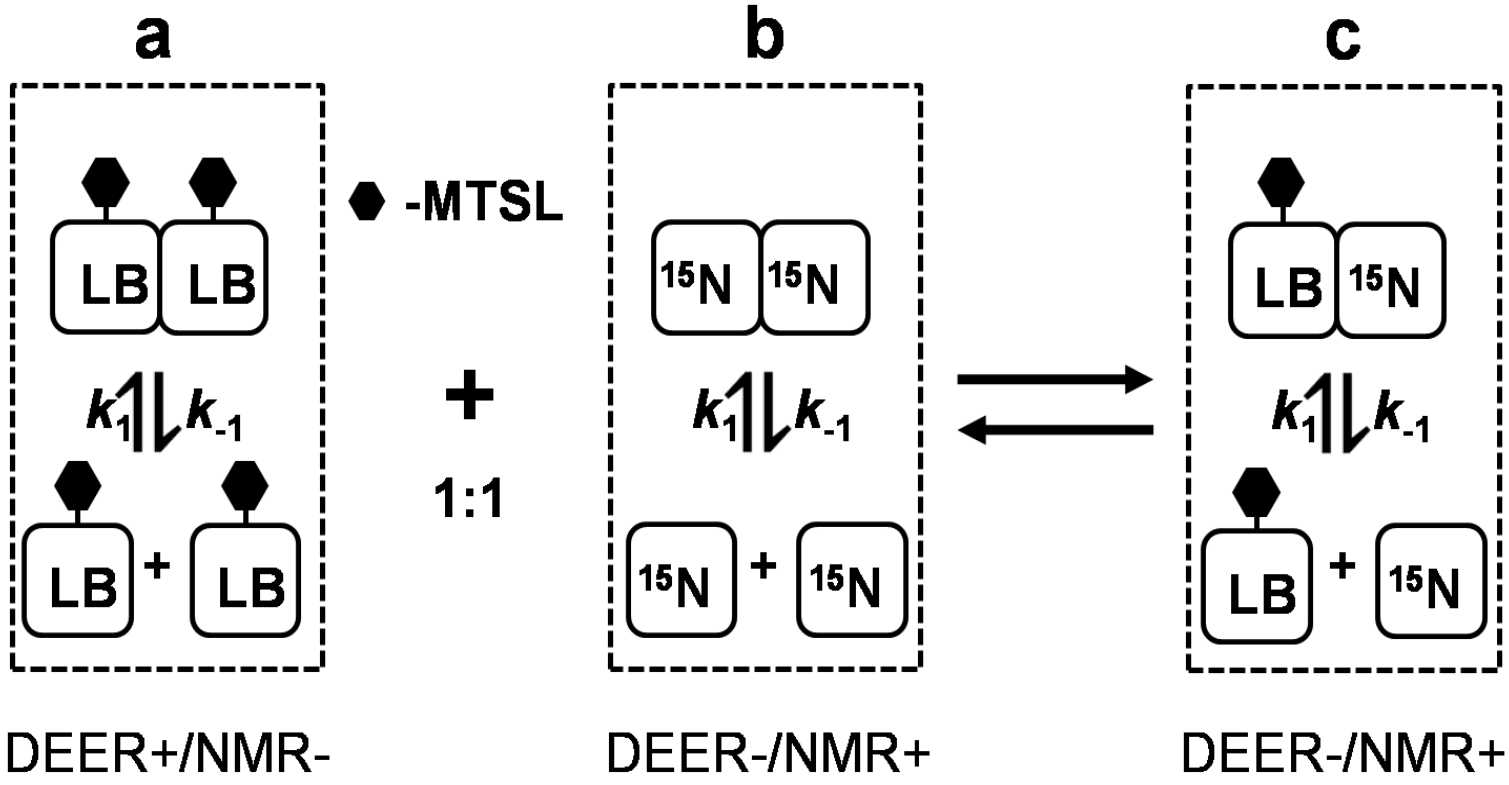
Spin- and Isotope-Labeling Strategy for Measurement of Rate Constants for Homodimer Subunit Exchange



Yang et al., J. Biomolecular NMR, under review, 2012

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Theoretical Simulations of DEER Response During Mixing

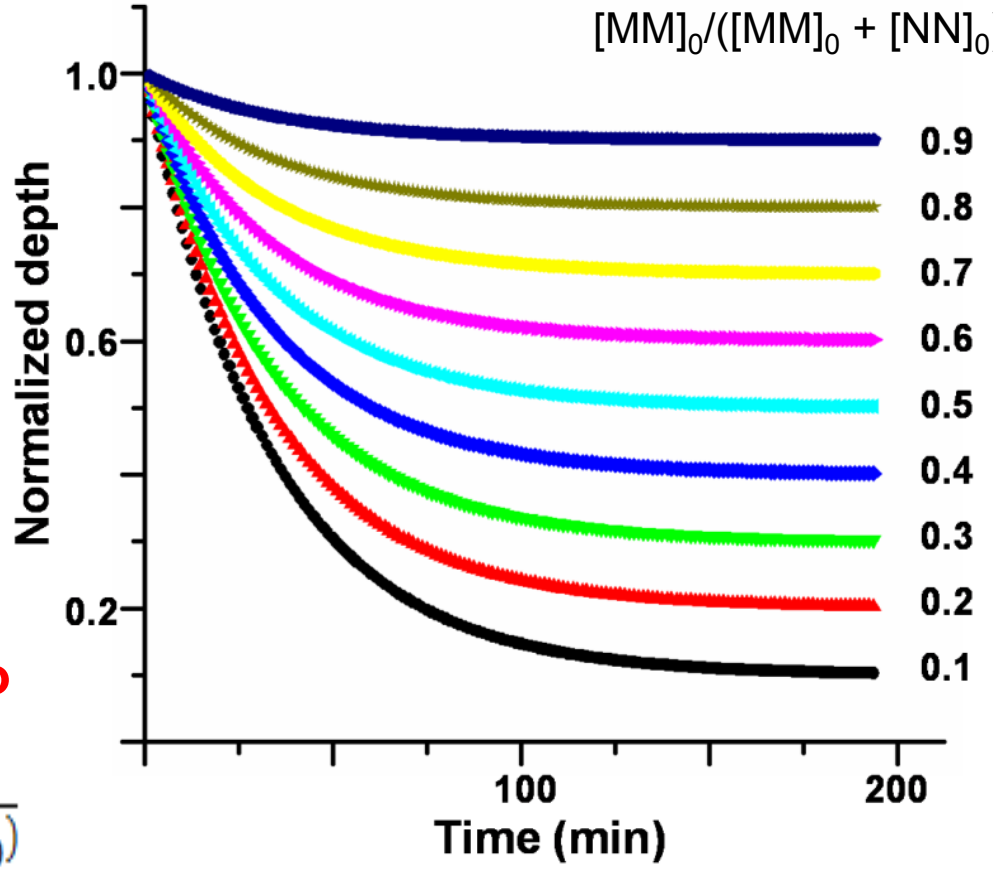
$$MM + NN \underset{k_{-1}}{\overset{k_{-1}}{\rightleftharpoons}} 2MN$$

For 1:1 mixing of MM:NN

$$\frac{I(t)}{I_0} = 0.5 \times \exp(-k_{-1}t) + 0.5$$

For mixing of MM:NN at any ratio

$$\frac{I(t)}{I_0} = \frac{[NN]_0}{([MM]_0 + [NN]_0)} \times \exp(-k_{-1}t) + \frac{[MM]_0}{([MM]_0 + [NN]_0)}$$



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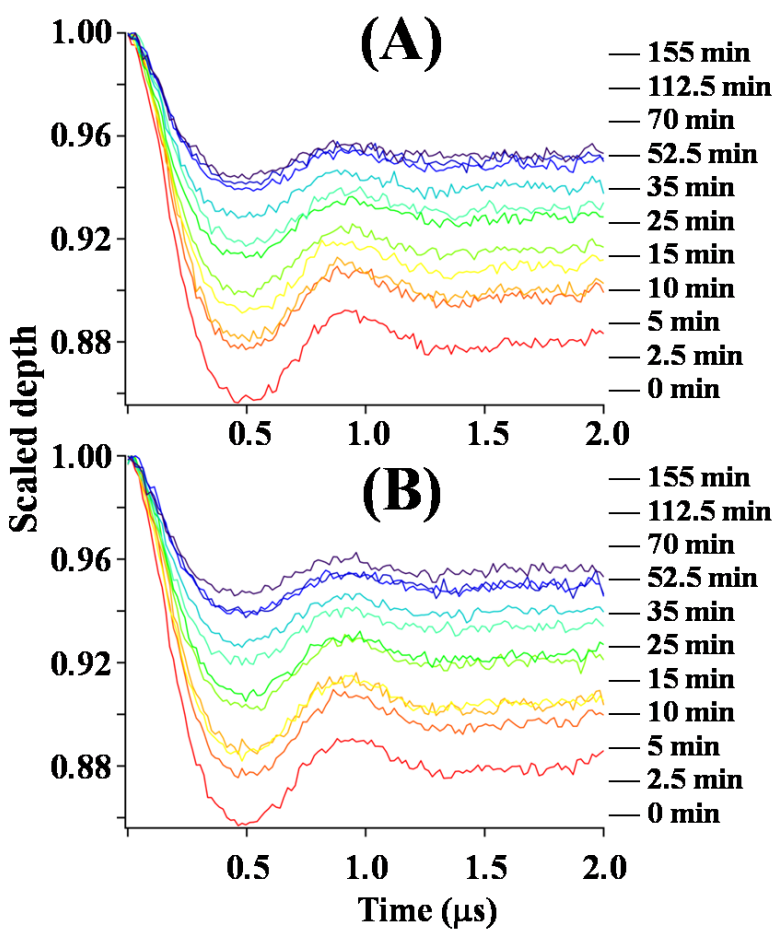
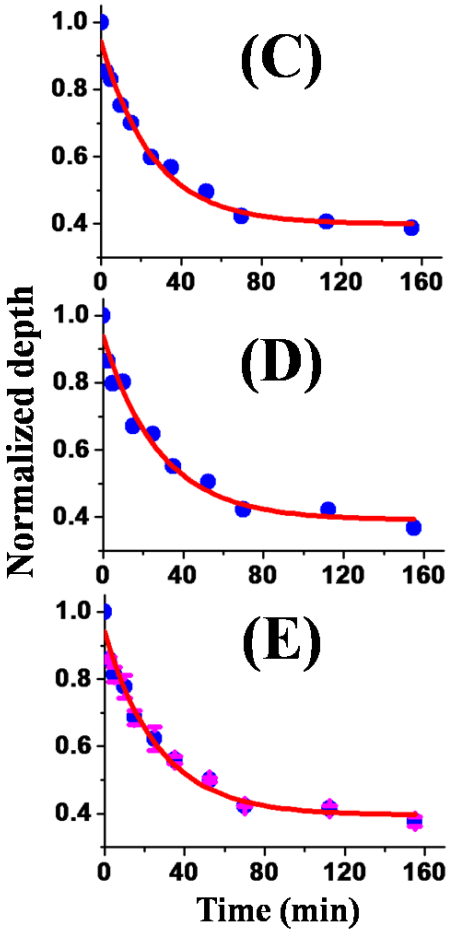
Repeated Measurement of DEER Decay





Set #1

k_{-1} = 0.039 ± 0.005 min^{-1}

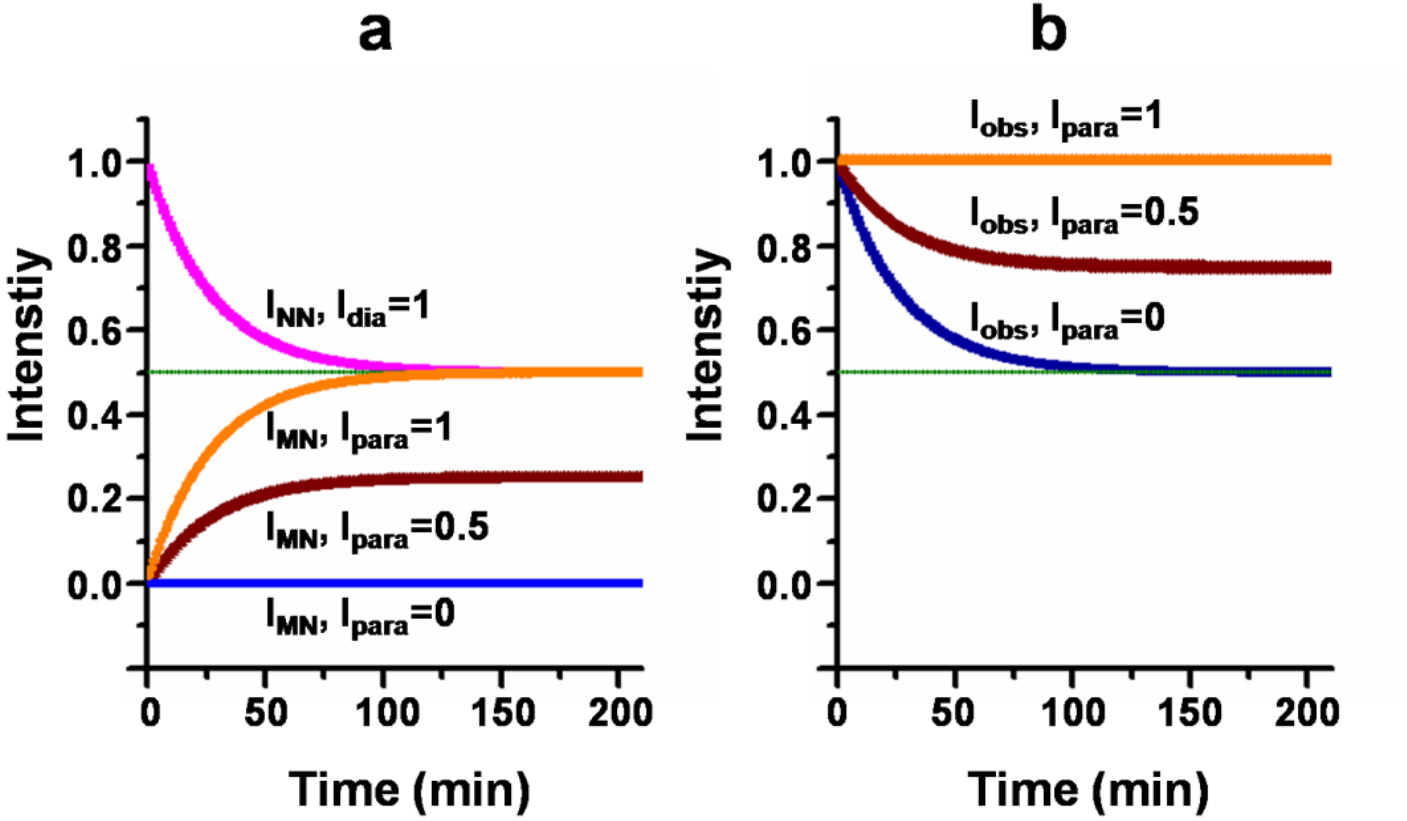
Set #2

k_{-1} = 0.035 ± 0.006 min^{-1}

Average

k_{-1} = 0.037 ± 0.005 min^{-1}

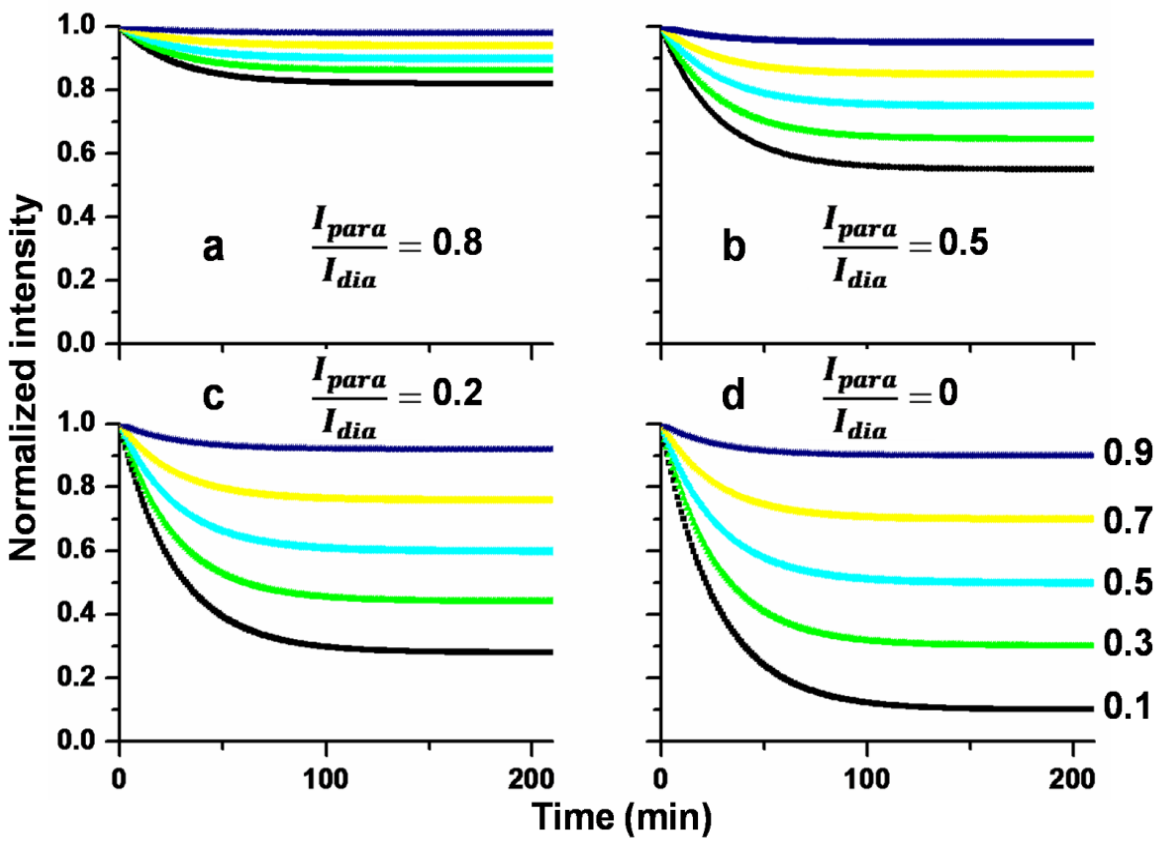
Theoretical Simulations of PRE Response During Mixing for special case of 1:1 Mixing of MM : NN



$$\frac{I(t)}{I_0} = \{0.5 \times \exp(-k_{-1}t) + 0.5\} + \frac{I_{para}}{I_{dia}}\{0.5 \times [1 - \exp(-k_{-1}t)]\} + \frac{I_N}{I_0}$$

Theoretical Simulations of PRE Response During Mixing for Mixing of MM : NN at General Ratio

$$\frac{I(t)}{I_0} = \left\{\frac{[MM]_0}{([MM]_0+[NN]_0)} \times \exp(-k_{-1}t) + \frac{[NN]_0}{([MM]_0+[NN]_0)}\right\} + \frac{I_{para}}{I_{dia}}\left\{\frac{[MM]_0}{([MM]_0+[NN]_0)} \times [1-\exp(-k_{-1}t)]\right\}$$



$[NN]_0/([MM]_0 + [NN]_0)$

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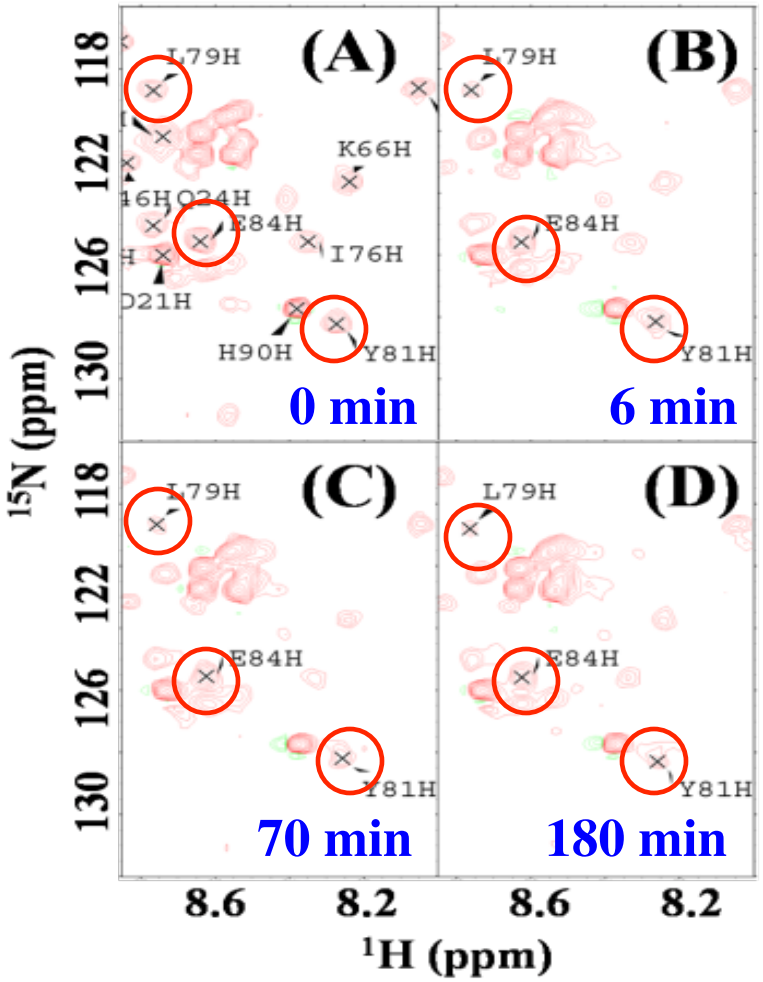
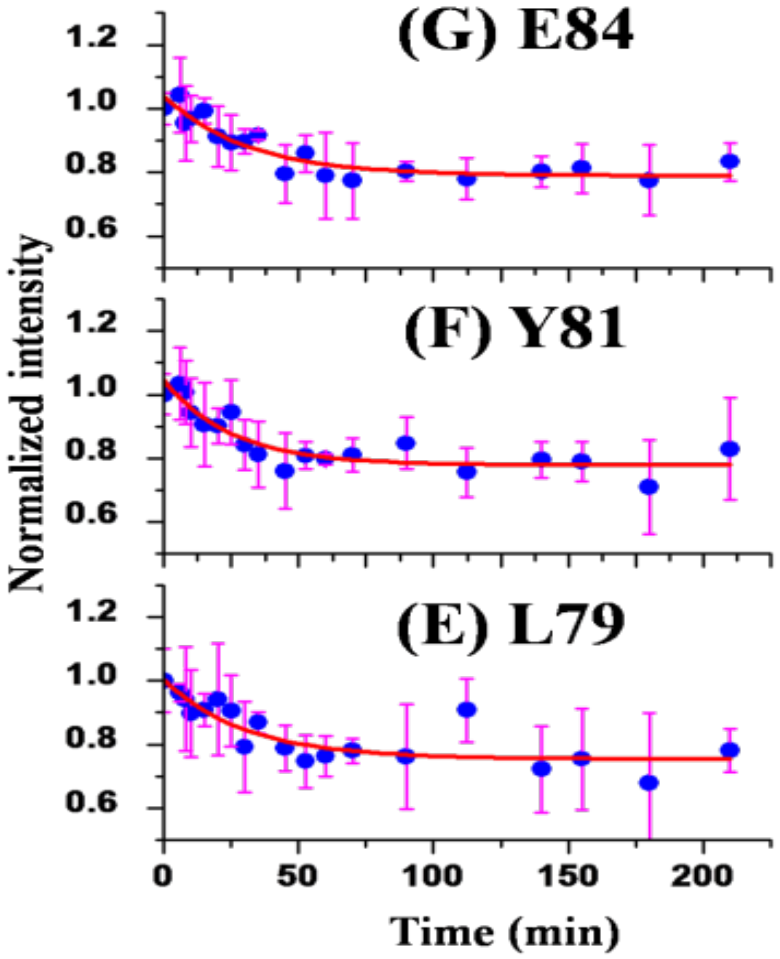
Experimental Determination of k_{-1} from Three Residues





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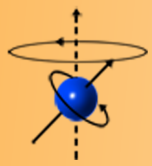
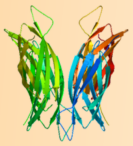
Pros and Cons of DEER and PREs

DEER	PRE
Requires single Cys	Requires single Cys
Requires single Para metal	Requires single Para metal
Effective over 20-70 Å	Effective up to ~20Å
Single measurement per sample	Multiple measurements per sample
Small sample requirement	Larger sample requirement
Superior Reproducibility	Inferior Reproducibility
Effectively no size limit	Normal size limit for Protein NMR
Easier data analysis	More difficult data analysis
Precise time determination	Less precise time determination
k_{off} minutes to months	k_{off} minutes to months
Requires Q-band pulsed EPR	Requires NMR instrumentation





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Where do we go from here?

1. Demonstrate DEER and PRE based measurements using ACTUN tag with paramagnetic metal binding

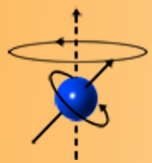
2. Investigate chain exchange kinetics in all 30 NESG homo-dimers

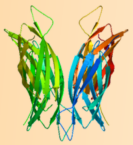
3. Extend DEER/PRE technologies to K_d determinations

4. Investigate effect of molecular crowding on chain exchange kinetics

5. Probe relationship between biological/biochemical activity and chain exchange kinetics

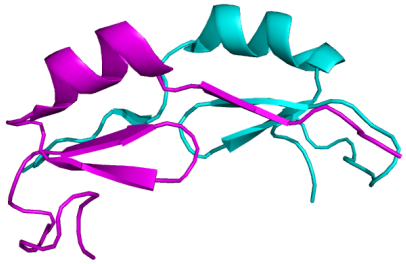
6. Support protein dimer design/engineering projects



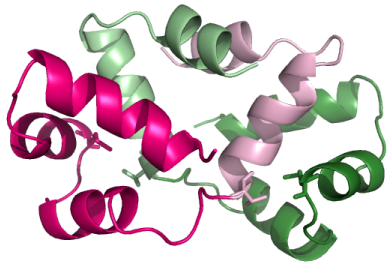


NESG NMR Homodimers (~ 30 NMR)

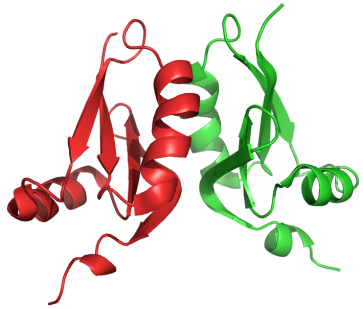
SR450 / YqaI



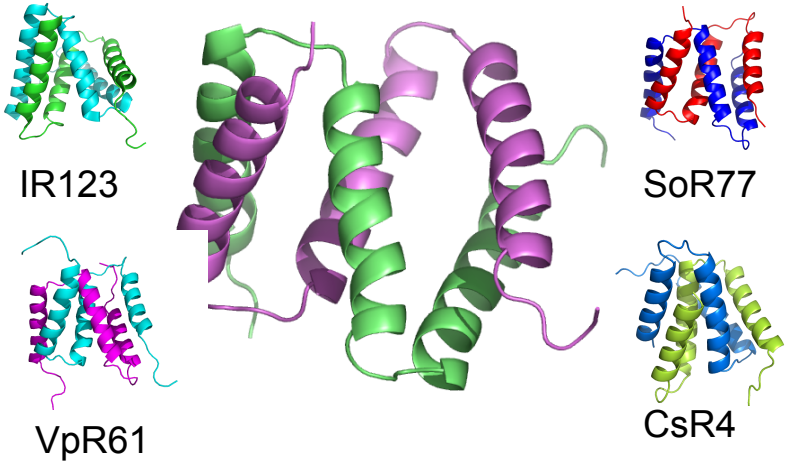
BtR244 / BT_p548217
HTH transcriptional regulator



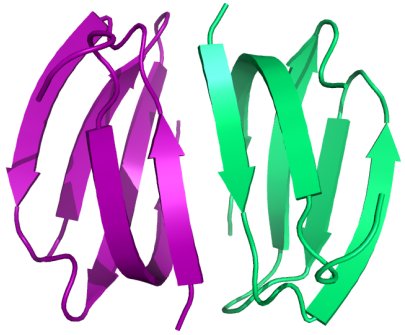
Net6 / NE0084
thioredoxin-like



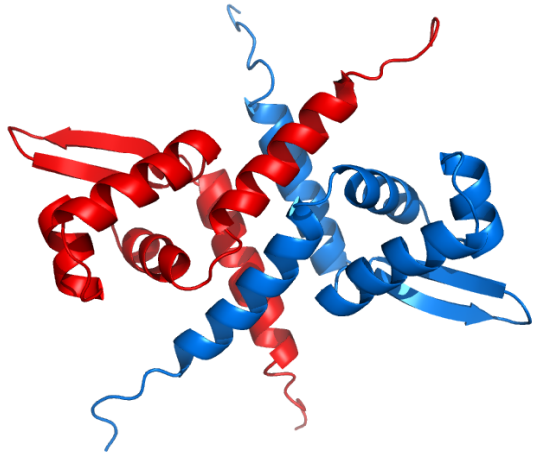
ER309 / YejL / UPF0352
And family: SoR77, CsR4, IR123, VpR61



DhR8C / DSY0195 (21-82)



MbR242E / Mb0332 (1-100)



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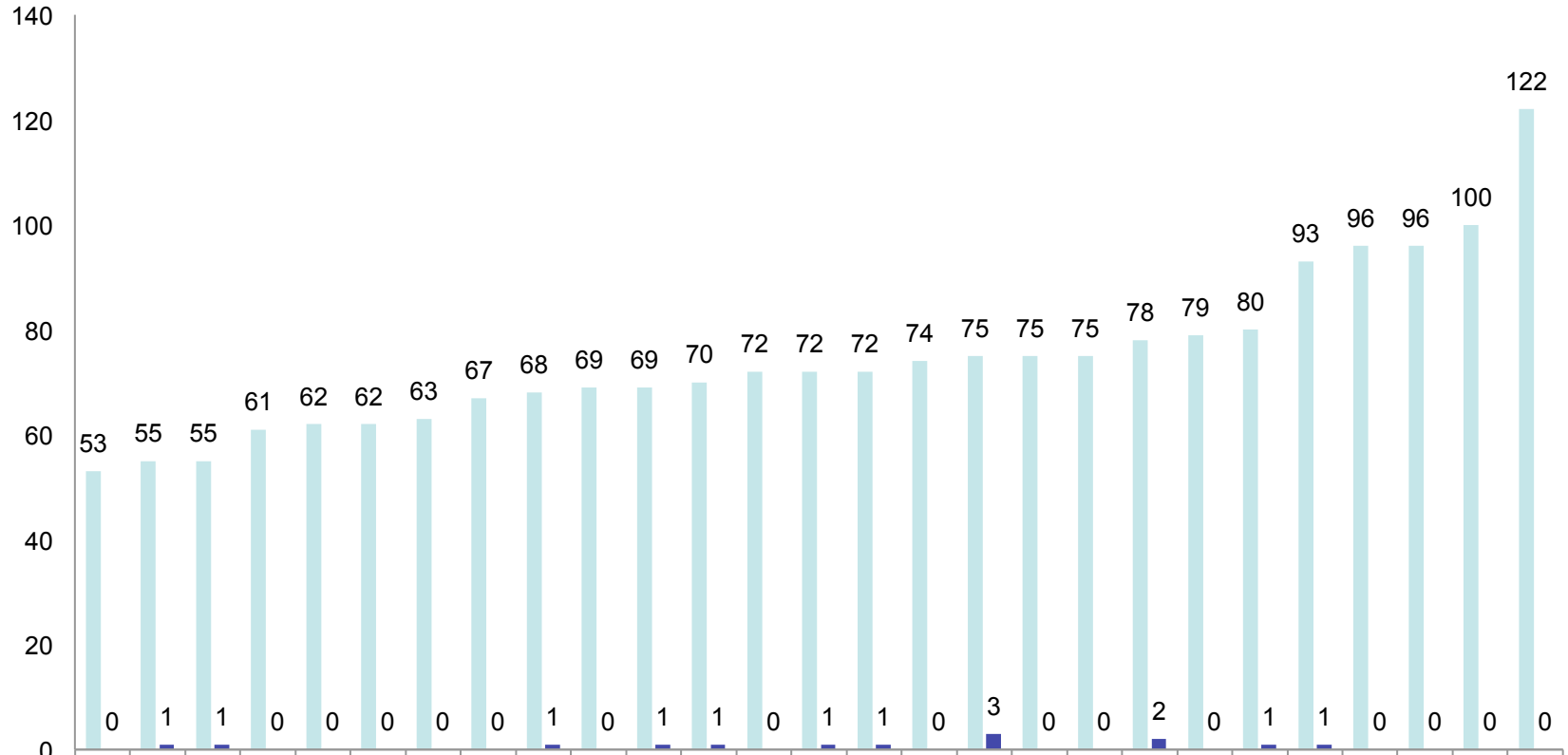
NESG Homo Dimers



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Series1	53	55	55	61	62	62	63	67	68	69	69	70	72	72	72	74	75	75	75	78	79	80	93	96	96	100	122
Series2	0	1	1	0	0	0	0	0	1	0	1	1	0	1	1	0	3	0	0	2	0	1	1	0	0	0	0

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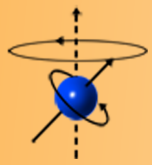
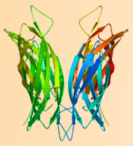
- **3. Exploration of New Biology:**
  Detection of transient, lowly populated states

The Transient Nature of Protein-Protein Interactions is Important to their Biological, Biochemical, Cellular Function

"Much less is known about lowly-populated, higher free energy states that are invisible to conventional structural and biophysical techniques "

Clore and Iwahara, **Theory, Practice and Applications of Paramagnetic Relaxation Enhancements for the Characterization of Transient Low-Population States of Biological Macromolecules and Their Complexes.** *Chem. Rev.*. **109**, 4108-4139 (2009)





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Clore, Iwahara, 2009

PRE amplification of lowly populated states

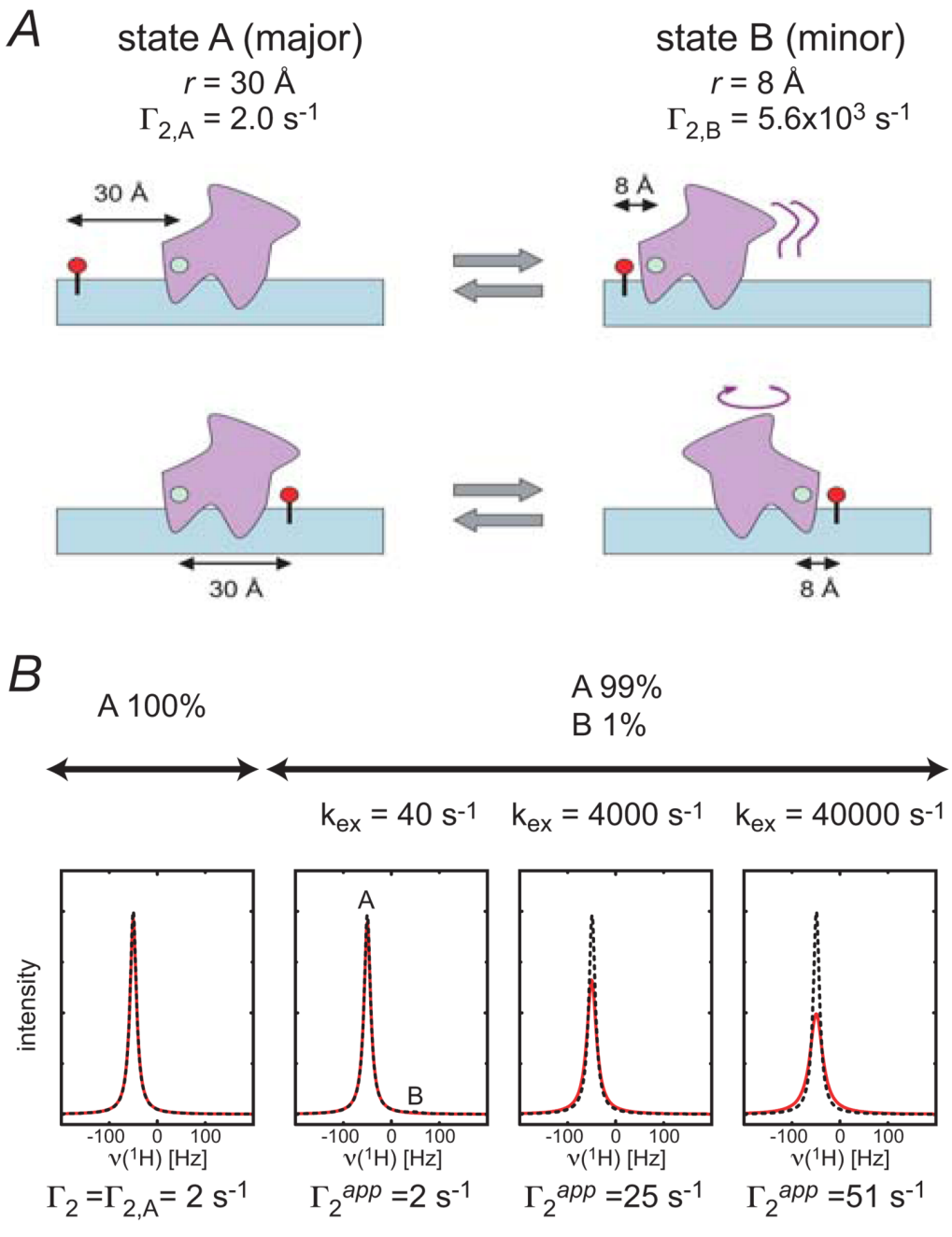

Figure 4.
PRE-based amplification of information on low-population states. (A) Two-site exchange

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Applications

1. **Investigation of target-search process in DNA-Protein Interactions**
2. **Encounter complexes in protein-protein association**
3. **Transient domain-domain interactions in proteins**

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SUMMARY:
How do we harness PREs and DEER to push the limits of Magnetic Resonance in PSI Biology?

1. **PREs and DEER in Larger proteins (30-40 kDa) in combination with sparse constraints (ILV protonated, ^{2}H, ^{19}F)**

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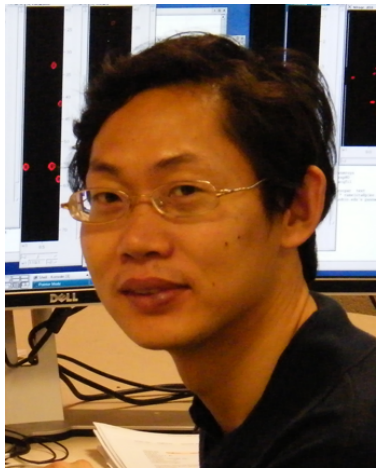

Yunhuang Yang

Theresa Ramelot

Shuisong Ni

Robert McCarrick

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Guy Montelione and Rutgers team
National Science Foundation
Miami University
Ohio Board of Regents

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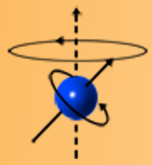

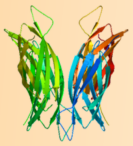

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