Replica Exchange Simulations of Protein-Protein Binding and Multi-protein Complex Formation

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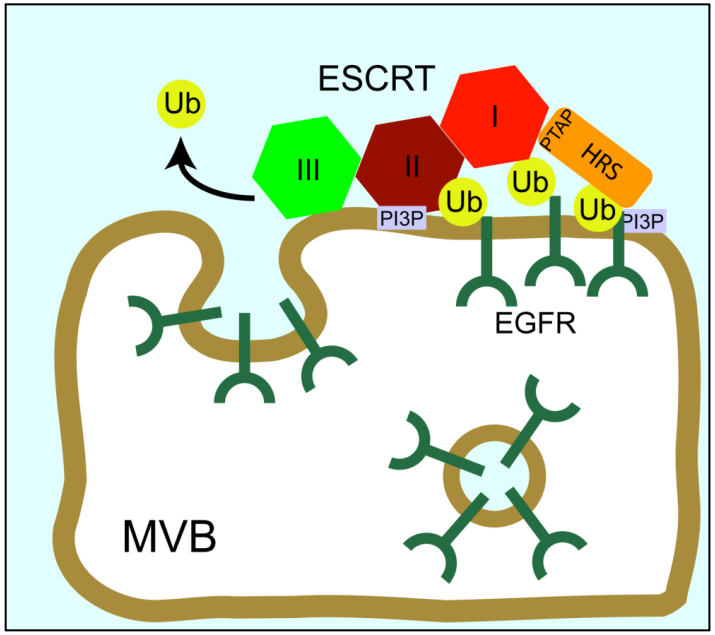
Background

- Many biological functions are carried out by large, multi-protein assemblies
  - DNA transcriptional regulation
  - Signal transduction
  - Nuclear pore complex
  - Membrane-protein trafficking
  - Viral entry and release



ESCRT machinery (Membrane trafficking)

Motivation

Understanding structure and dynamics of multi-protein assemblies

- Many multi-protein assemblies form only transiently
  - Held together by relatively weak pairwise interactions ($K_d > 1\mu M$)
- Multi-protein assemblies contain unstructured regions
  - Flexible polymeric linkers connecting structured domains

$\Rightarrow$ Challenges for traditional structural approaches

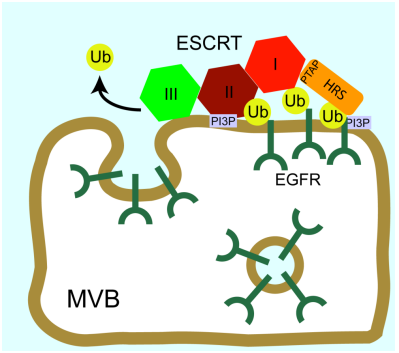
- X-ray crystallography: Difficult to crystallize weak complexes with unstructured regions
- NMR spectroscopy: Size limits
- Electron microscopy: Trapping of functional assemblies

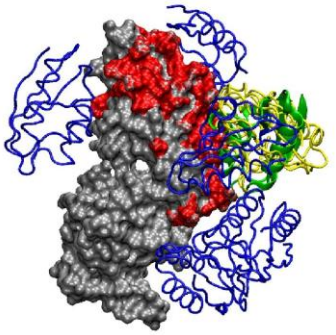
$\Rightarrow$ New opportunities for modeling, simulation, and theory!

- Complement experiments
- Provide predictions, insights, and new directions

Outline

1. Model and Method:
   - Validation: structures and binding affinities
2. Structure and dynamics of multi-protein assemblies
   - Vps27/Hse1: ESCRT protein sorting machinery
   - Collaboration with *James H. Hurley*, NIDDK
3. Transient encounter complexes in protein-protein complex formation
   - Paramagnetic relaxation enhancement NMR of protein-protein complexes
   - Collaboration with *G. Marius Clore*, NIDDK



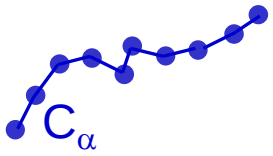


Coarse-grained model for multi-protein assemblies

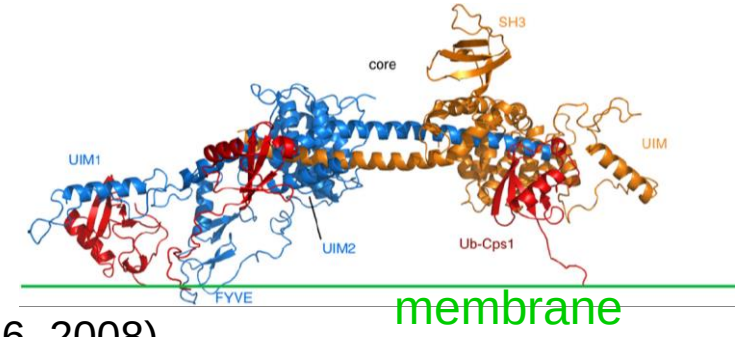
- Residue-level (C_α only) coarse-graining
  - Rigid body for structured domains

- Transferable energy function
  - Long-range Debye-Hückel electrostatic interactions
  - Residue-dependent short-range interactions (Miyazawa-Jernigan statistical contact potentials)
  - Experimental inputs: Lysozyme *osmotic protein second-virial coefficient* and Ub-CUE *protein binding affinity*
- Flexible linkers: polymer model
  - Harmonic stretching potential
  - Bending potential
  - Torsion angle potential

} Tested for protein folding



- Membrane interactions
  - Planar membrane
  - Short-range interactions between residues and membrane
  - Electrostatic interactions



(YCK, Hummer, *J. Mol. Biol*. **375**, 1416, 2008)

Simulation method

- Replica exchange Monte Carlo
  - Twenty replicas at different temperatures
  - Enhances equilibrium sampling
  - Implemented in the parallel architecture of Biowulf cluster

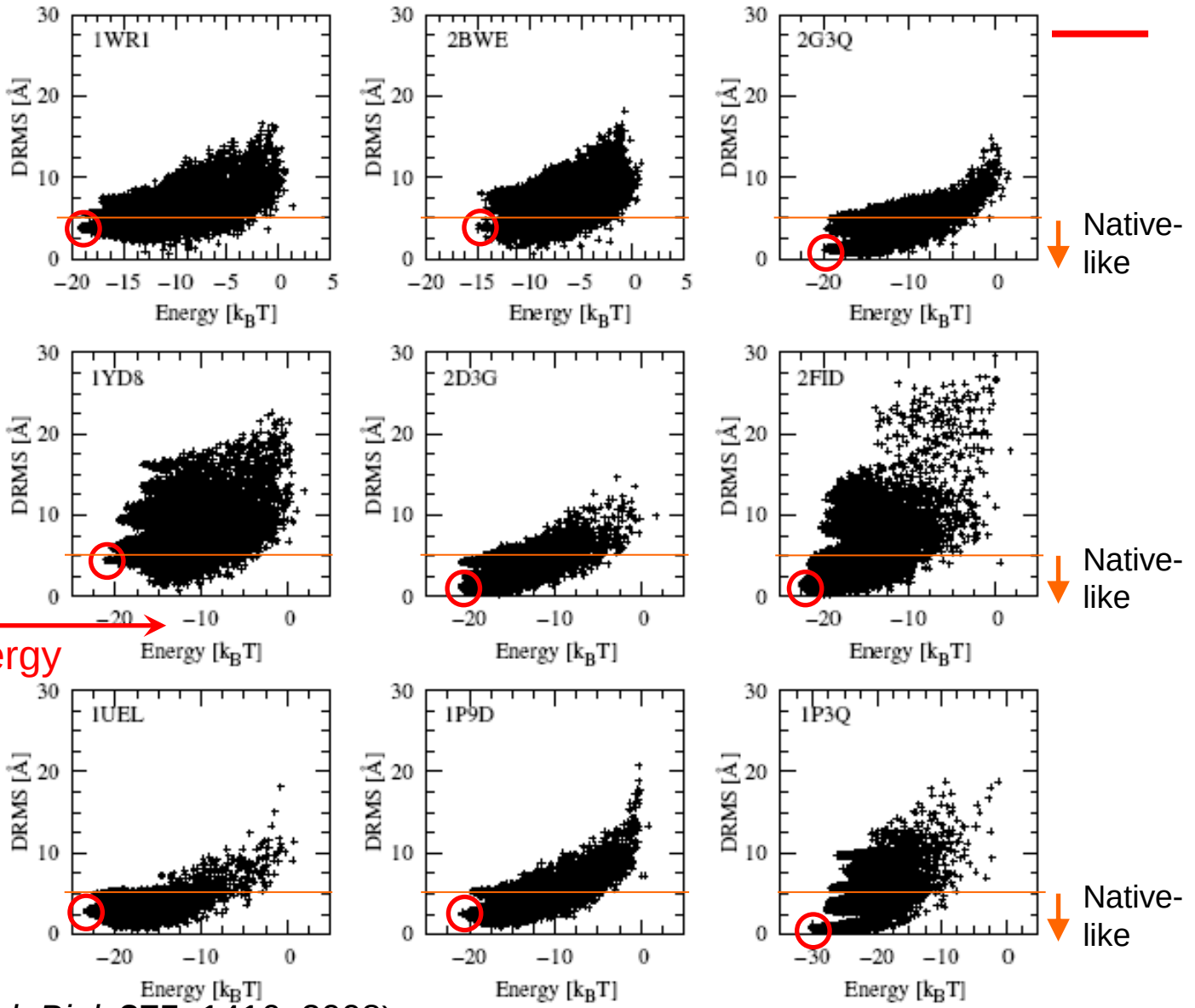
(YCK, Hummer, *J. Mol. Biol*. **375**, 1416, 2008)

Validation: complex structure

Ub/CUE
Ub/UIM1
Ub/UBA
Ub/GAT
Ub/DUIM
Ub/UIM2
UbL/UBA
UbL/UIM
UbL/UIM2

Distance to native structure

Energy

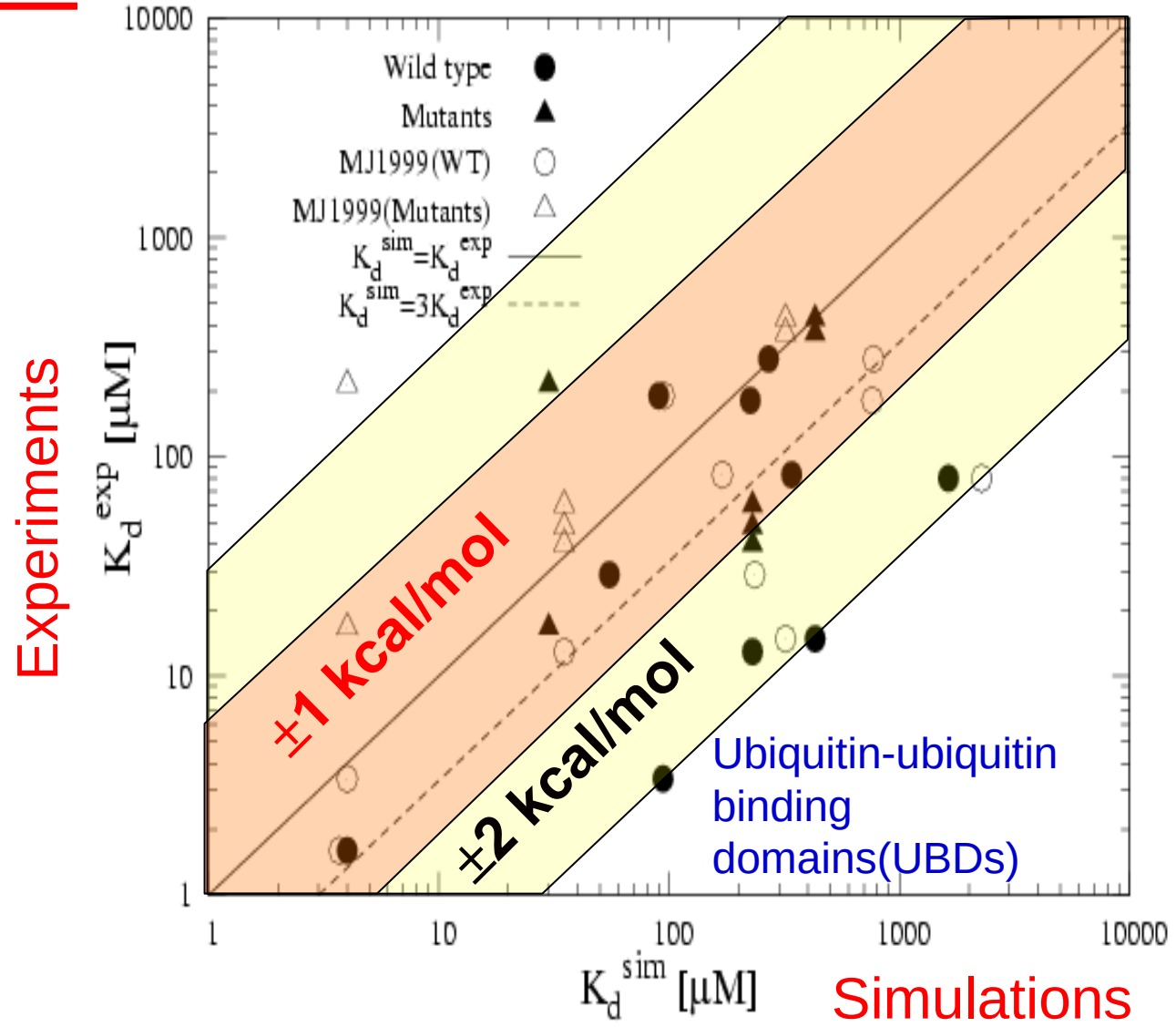


$$\mathrm{DRMS} = \frac{1}{N}\sum_{i,j}\left|d_{ij}^{\exp} - d_{ij}^{\mathrm{model}}\right|$$

(YCK, Hummer; *J. Mol. Biol*. **375**, 1416, 2008)

Validation: binding affinities

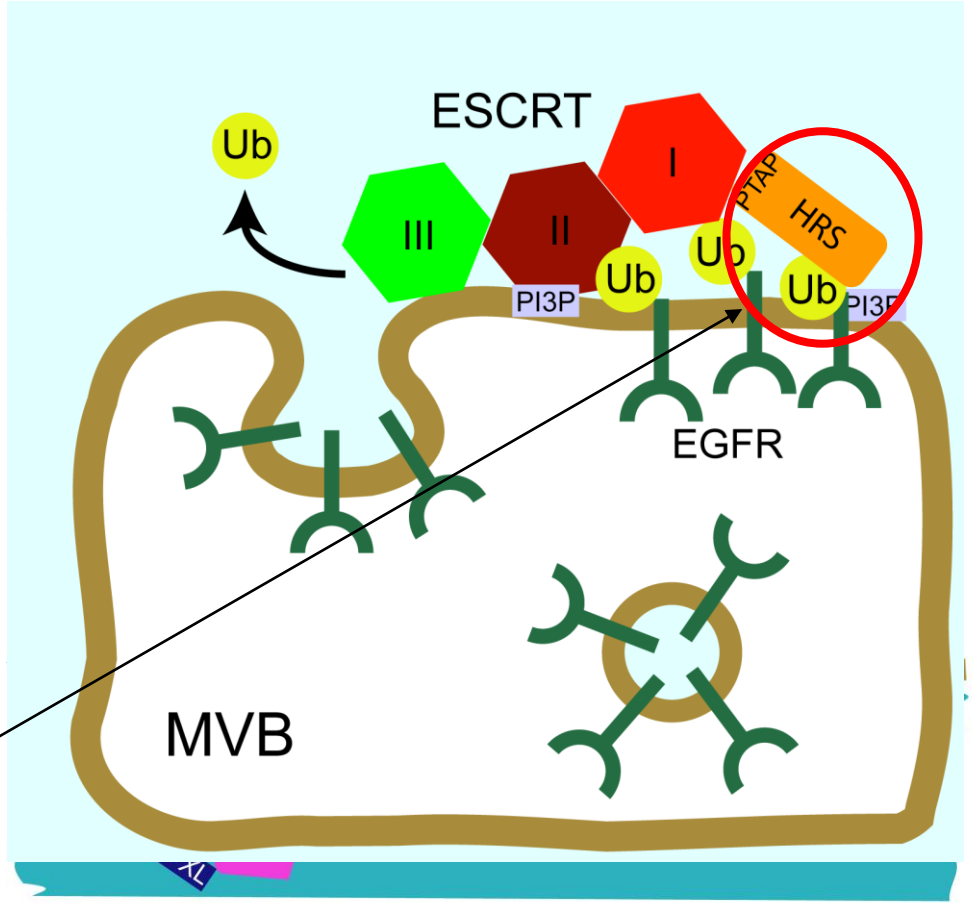
Ub/CUE
Ub/UIM1
Ub/UBA
Ub/GAT
Ub/DUIM
Ub/UIM2
UbL/UBA
UbL/UIM
UbL/UIM2



(YCK, Hummer; *J. Mol. Biol*. **375**, 1416, 2008)

Application I:multi-vesicular body (MVB) protein sorting machinery

- The ESCRT machinery targets ubiquitinated transmembrane proteins for degradation in the lysosome or yeast vacuole
- ESCRTs are required for HIV budding at the plasma membrane

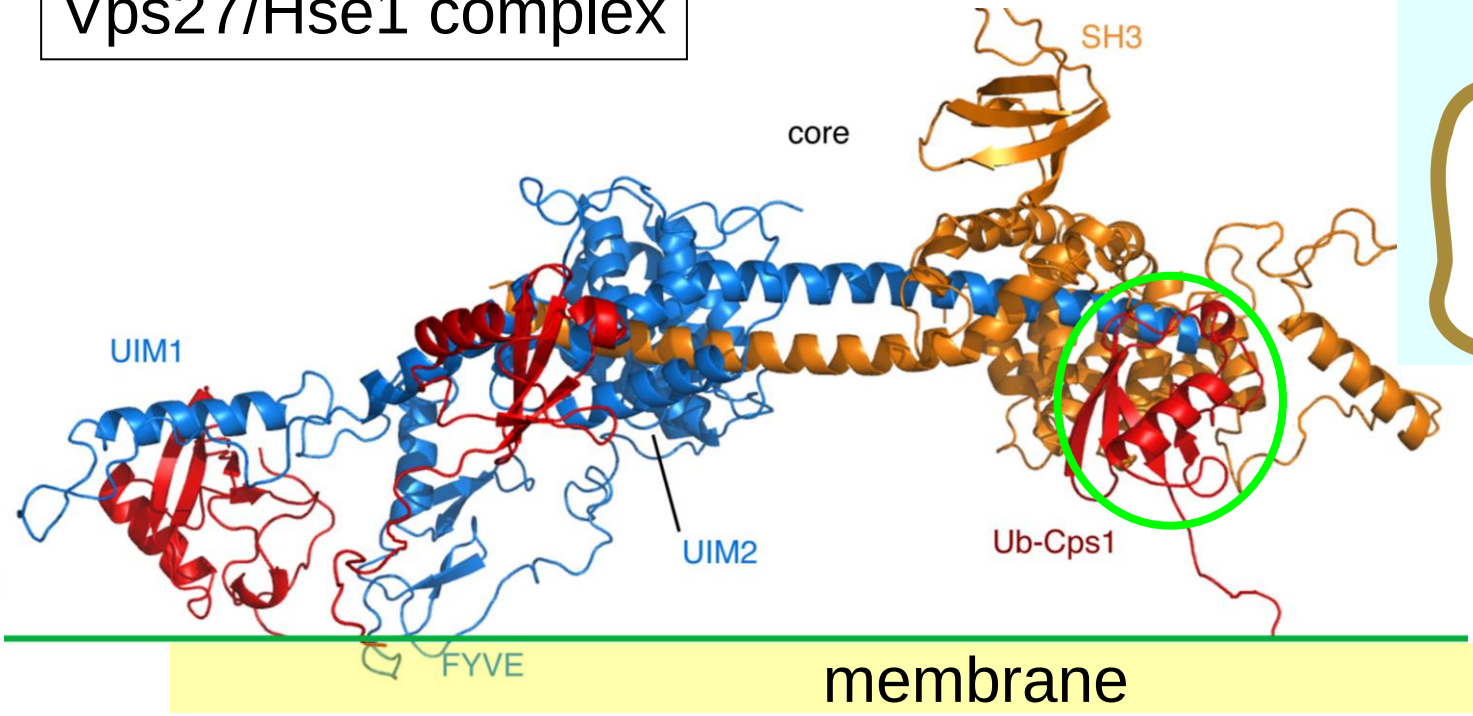


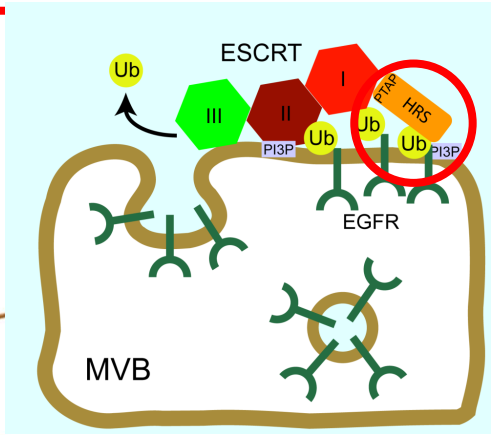
Vps27/Hse1(yeast)
Hrs/STAM(human)

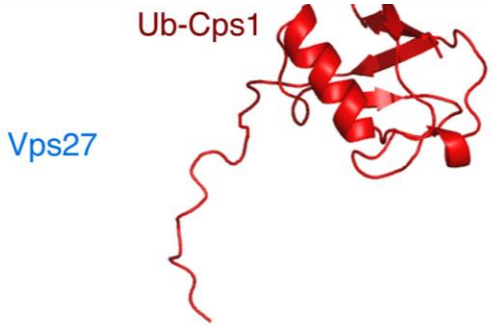
(Prag, Watson, YCK, Beach, Ghirlando, Hummer, Bonifacino, Hurley, *Dev. Cell* **12**, 973, 2007)

Structure of the assembled Vps27/Hse1 complex

Vps27/Hse1 complex

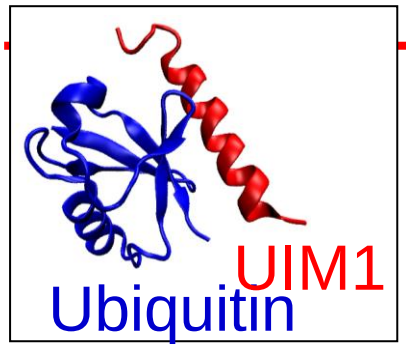


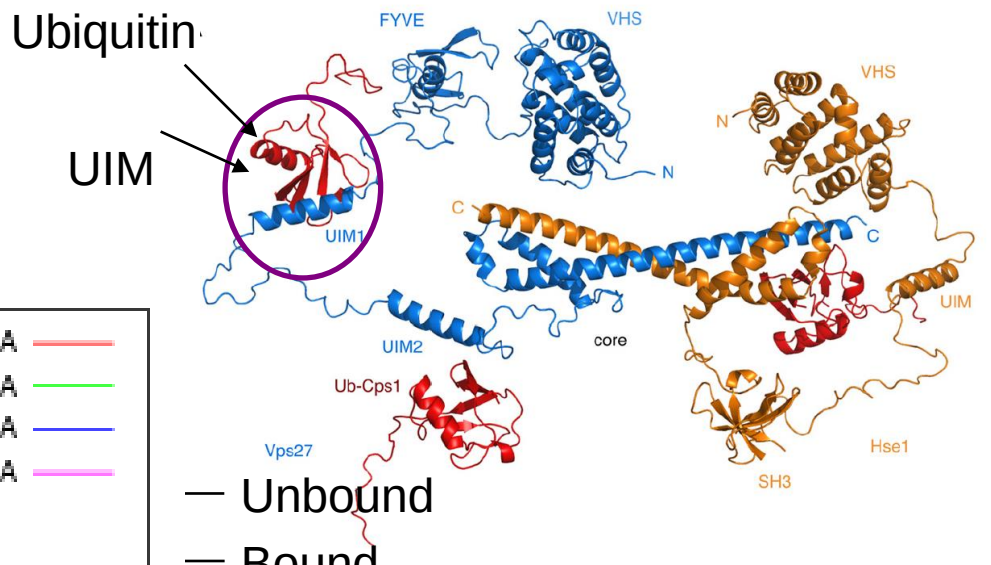


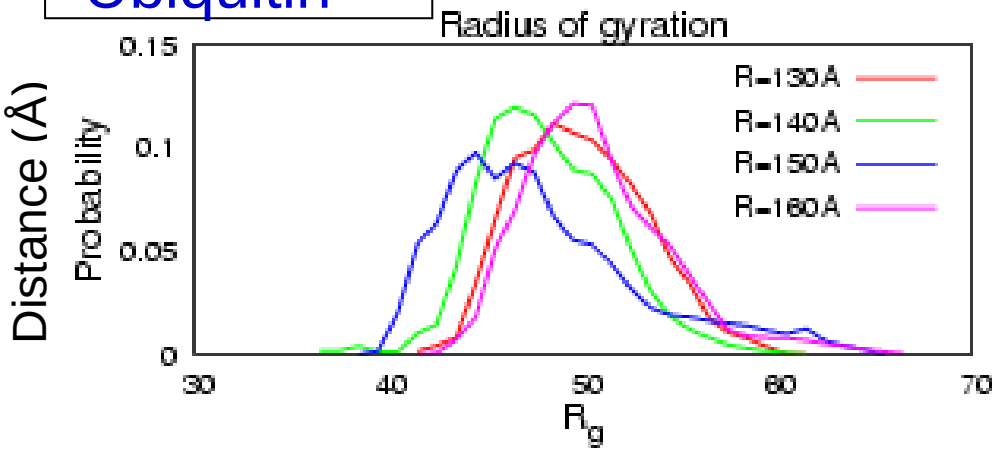


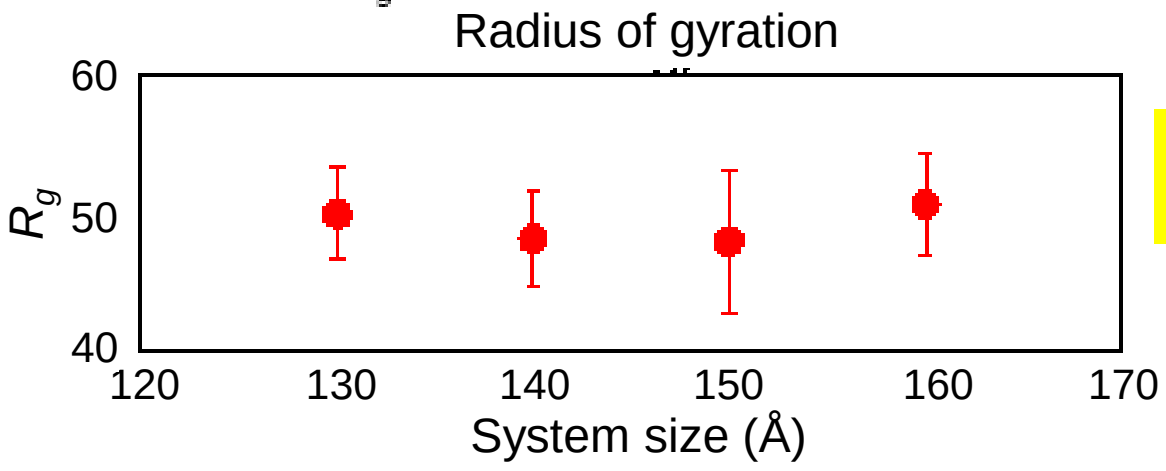
(Prag, Watson, YCK, Beach, Ghirlando, Hummer, Bonifacino, Hurley, *Dev. Cell* **12**, 973, 2007)

Vps27/Hse1 complex is dynamic and open



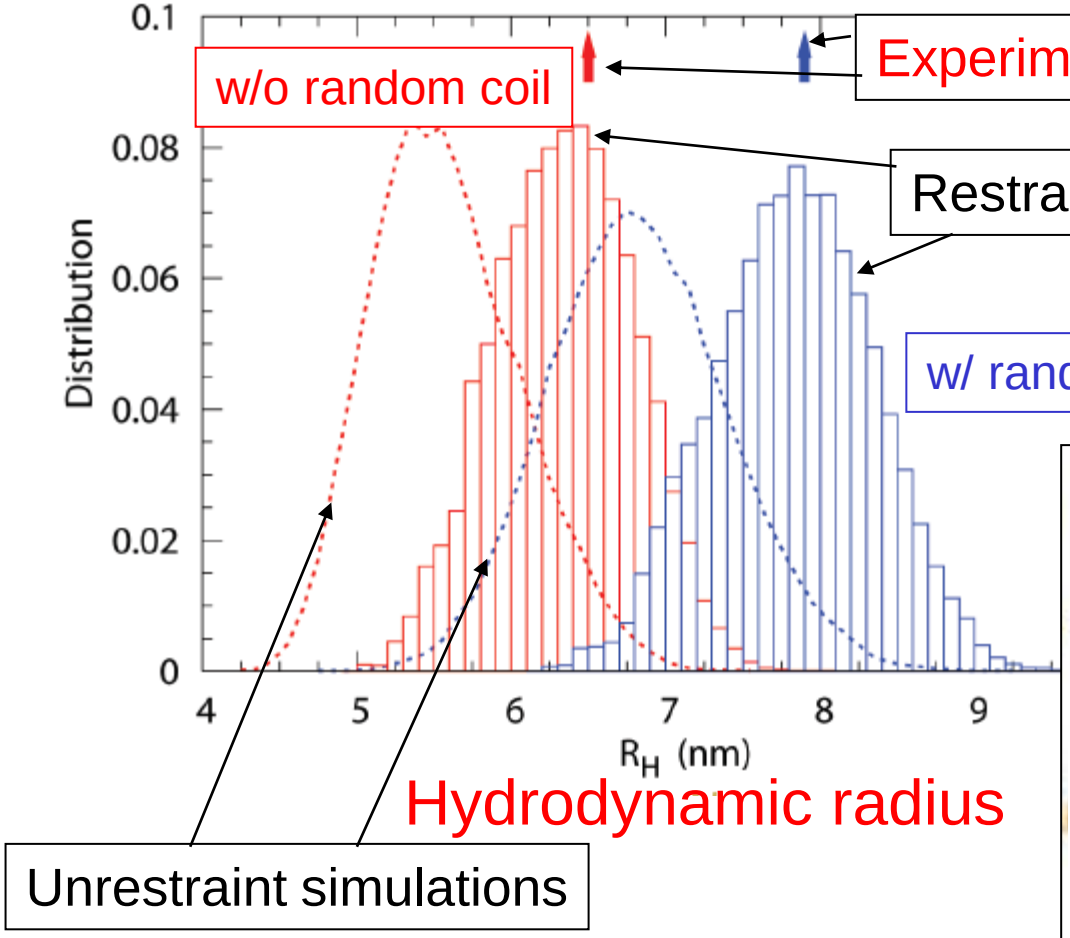


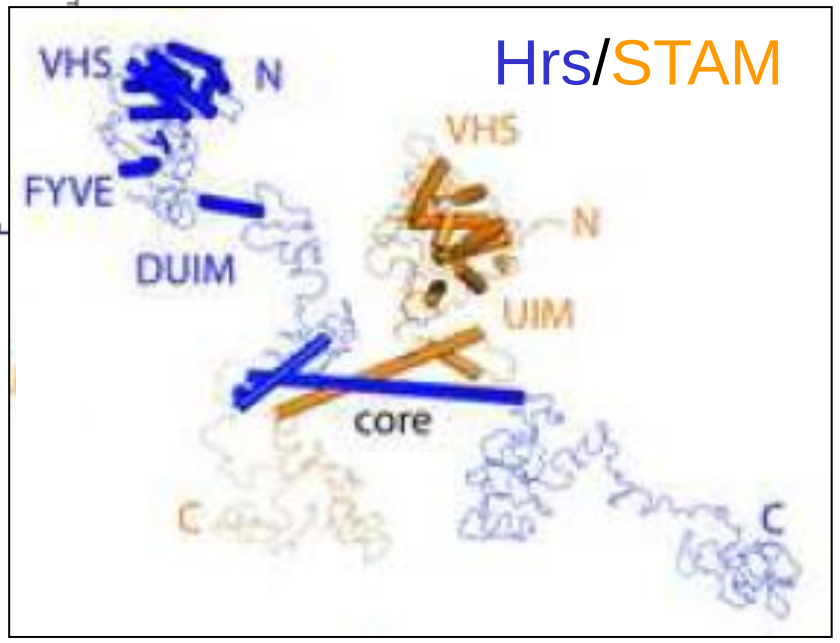




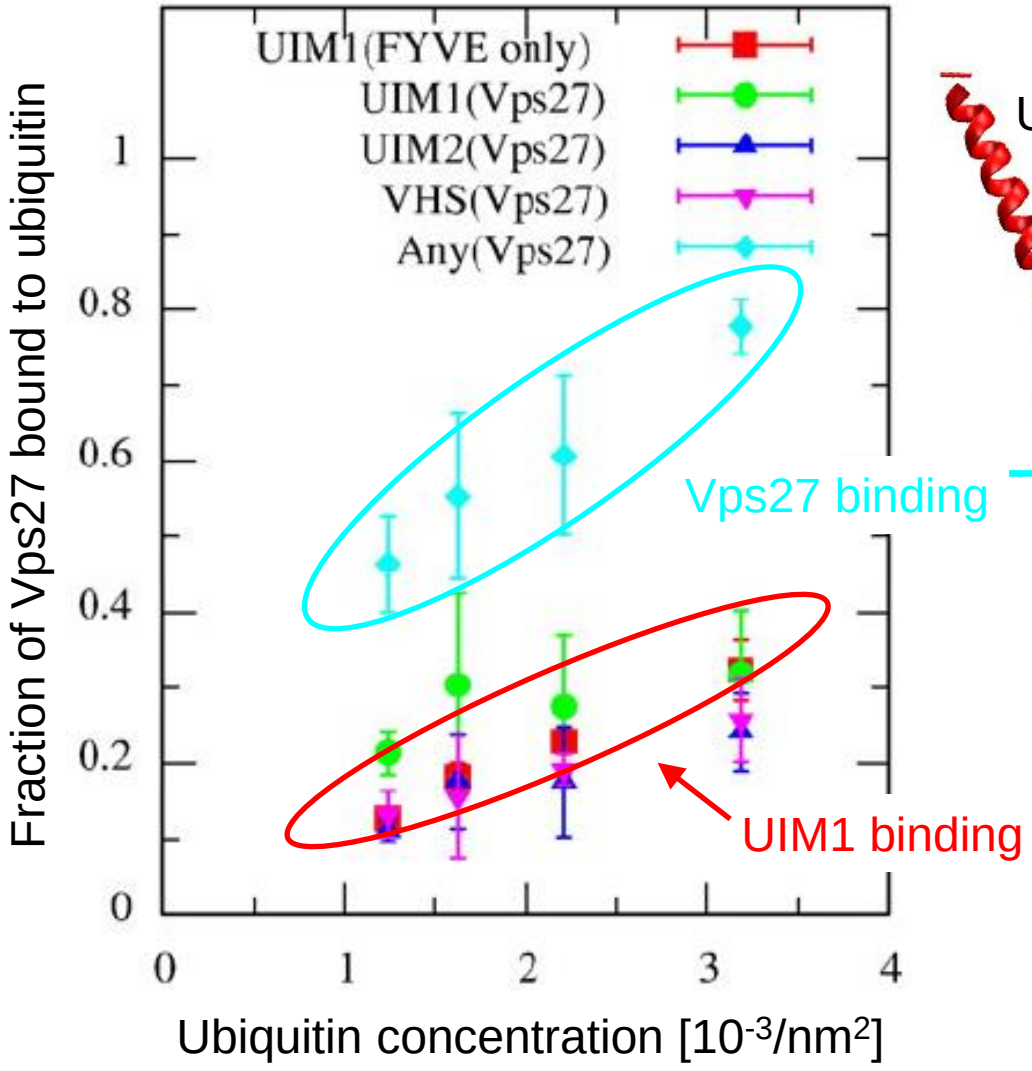
Globular protein:
R_g = 30Å

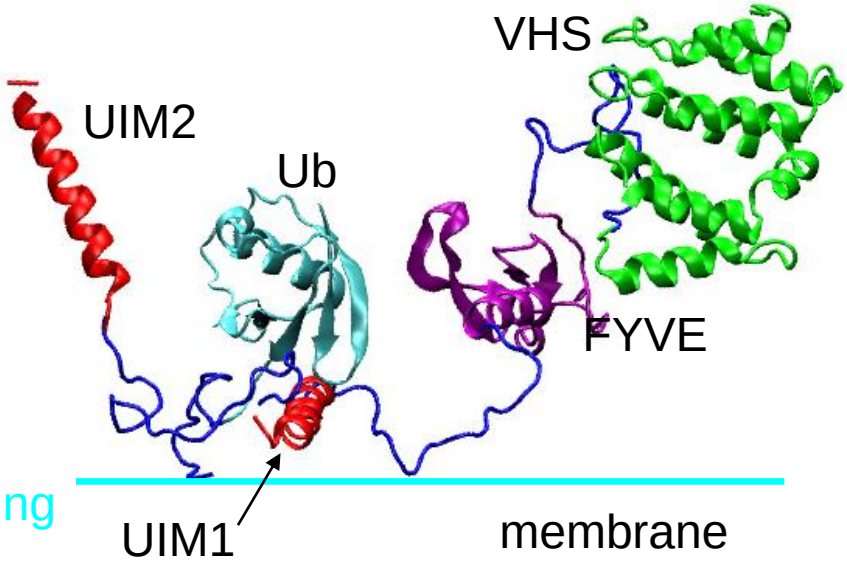
Hrs/STAM(human) complex also shows open structures





Positive cooperativity enhances Vps27 binding to ubiquitin





FYVE domain is tethered to the membrane

Proteins can move freely parallel to the membrane

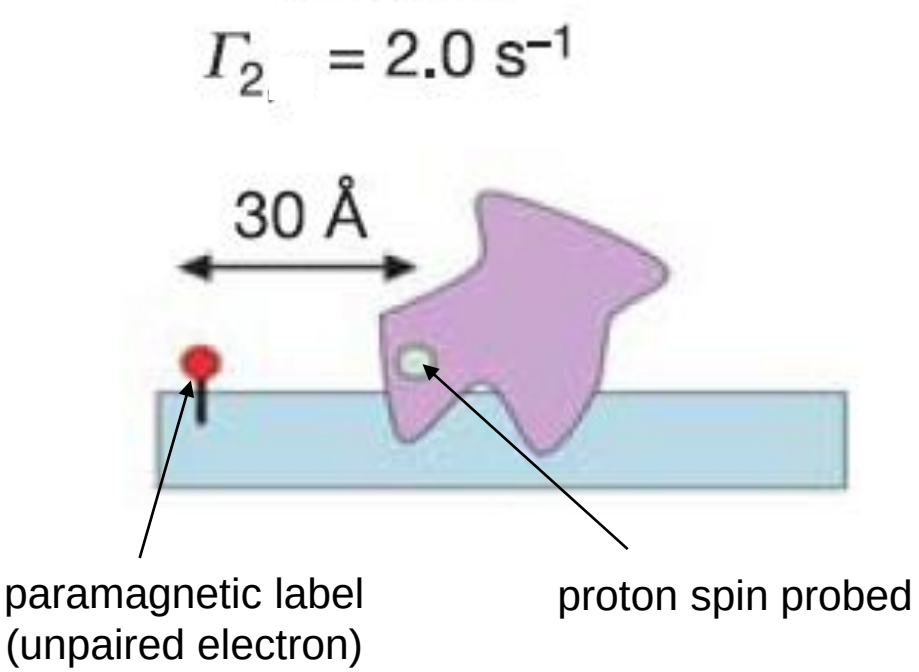
(YCK, Hummer; *J. Mol. Biol*. **375**, 1416, 2008)

Summary I: Simulations of Vps27/Hse1

- Dynamic and open structure
  - Important for targeting a variety of ubiquitinated cargos
- Cooperative binding of ubiquitin via nonspecific interactions
  - Essential for function at low biological concentrations
- Are nonspecific interactions detectable?

Application II: Transient encounter complexes probed by simulation and NMR

- Paramagnetic relaxation enhancement (PRE) probes the presence of low-population (<10%) transient encounter complexes

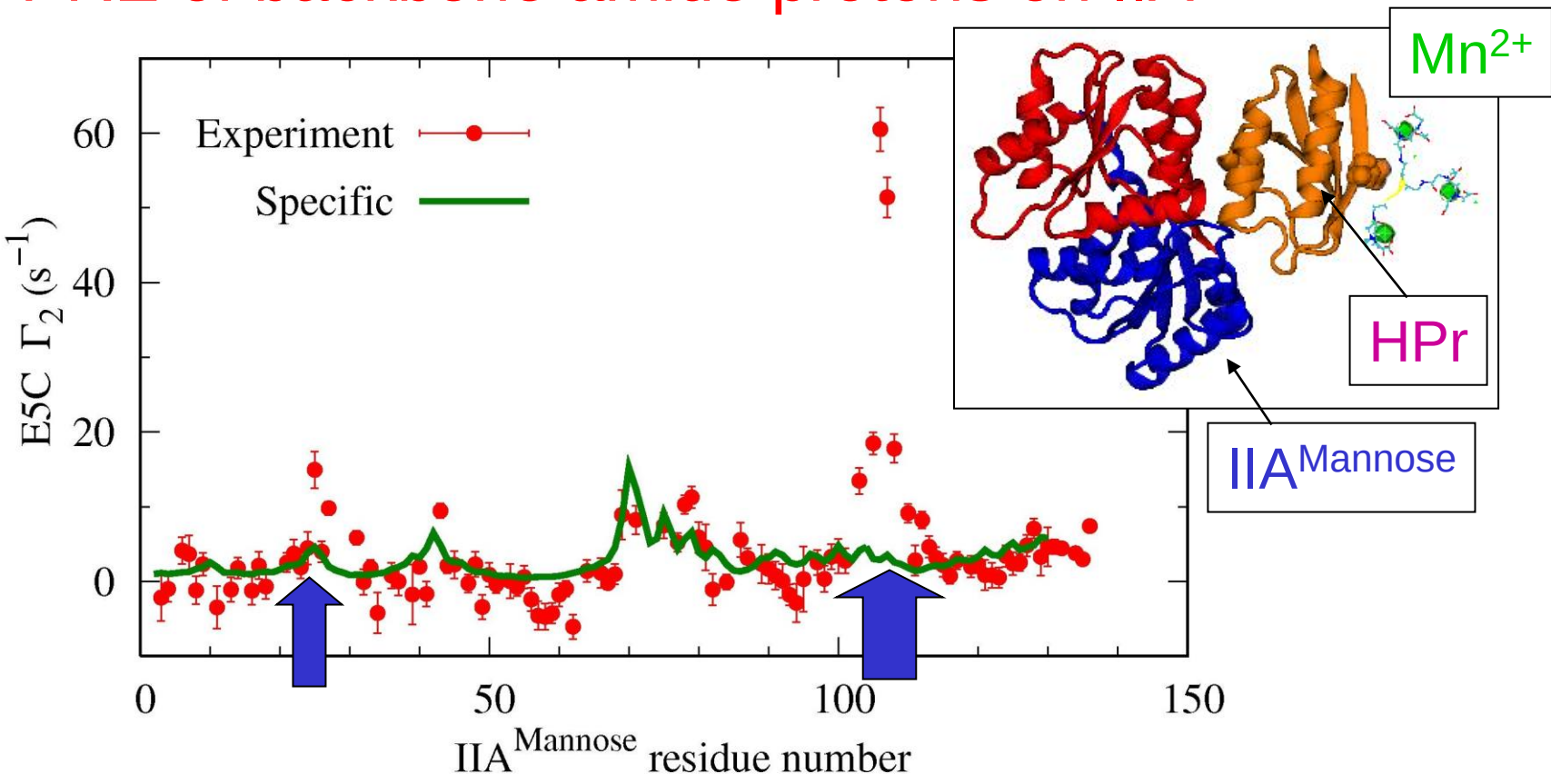


Iwahara, Clore, *Nature* **440**, 1227, 2006

PRE ~ $1/r^6$

NMR Paramagnetic Relaxation Enhancement (encounter complexes of HPr-IIA^{Mannose}?)

- PRE of backbone amide protons on IIA^{Mannose}



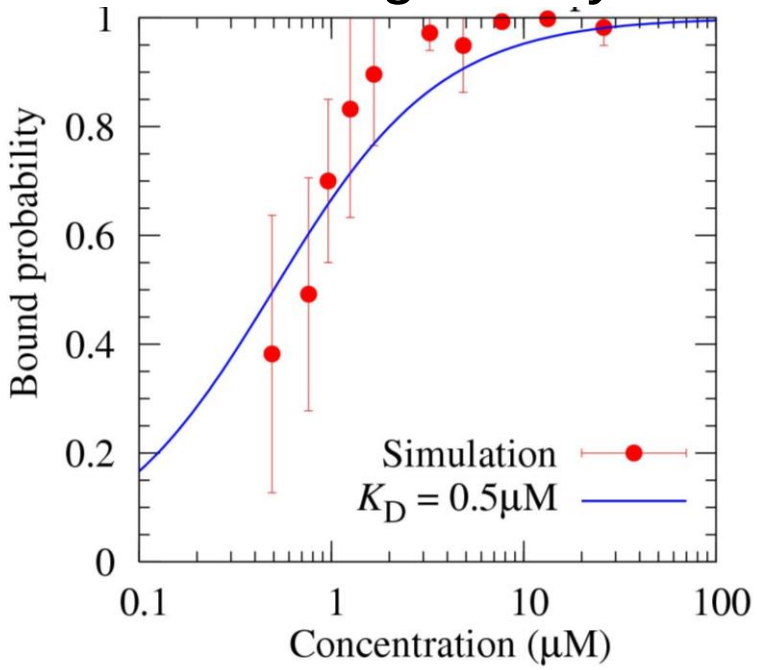
Can we simulate encounter complexes?

(Tang, Iwahara, Clore, *Nature* **444**, 383, 2006)

Replica-exchange simulations of HPr-IIA^{Man} complex

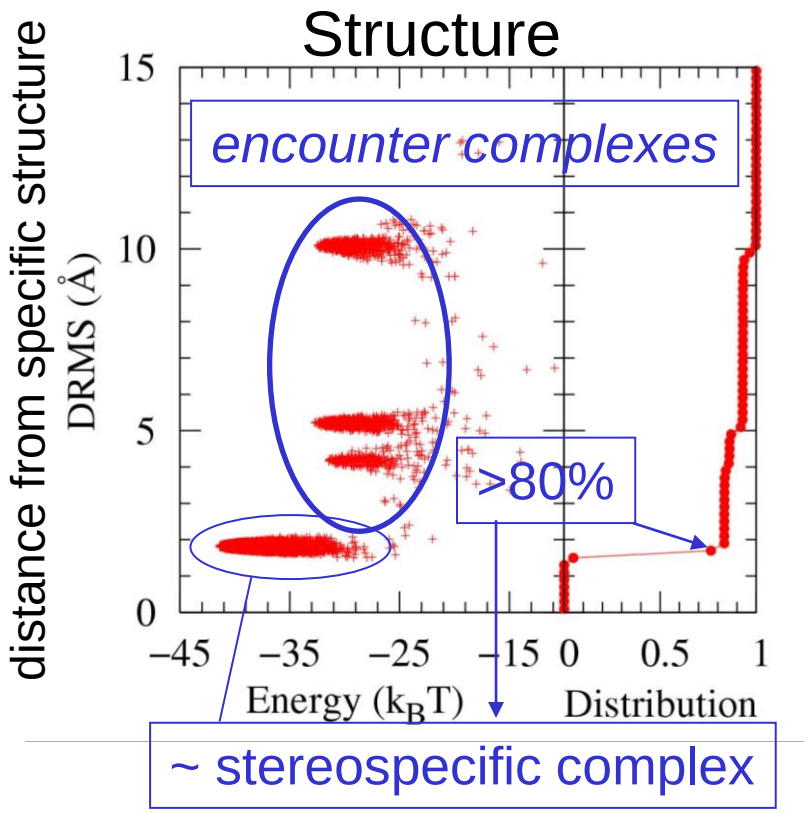
- Coarse-grained simulation model (YCK, Hummer; *J. Mol. Biol*. **375**, 1416, 2008)

Binding affinity



K_d(exp)~30 μM $\Rightarrow$ error ~ kT ln 60 ~ 2.5kcal/mol

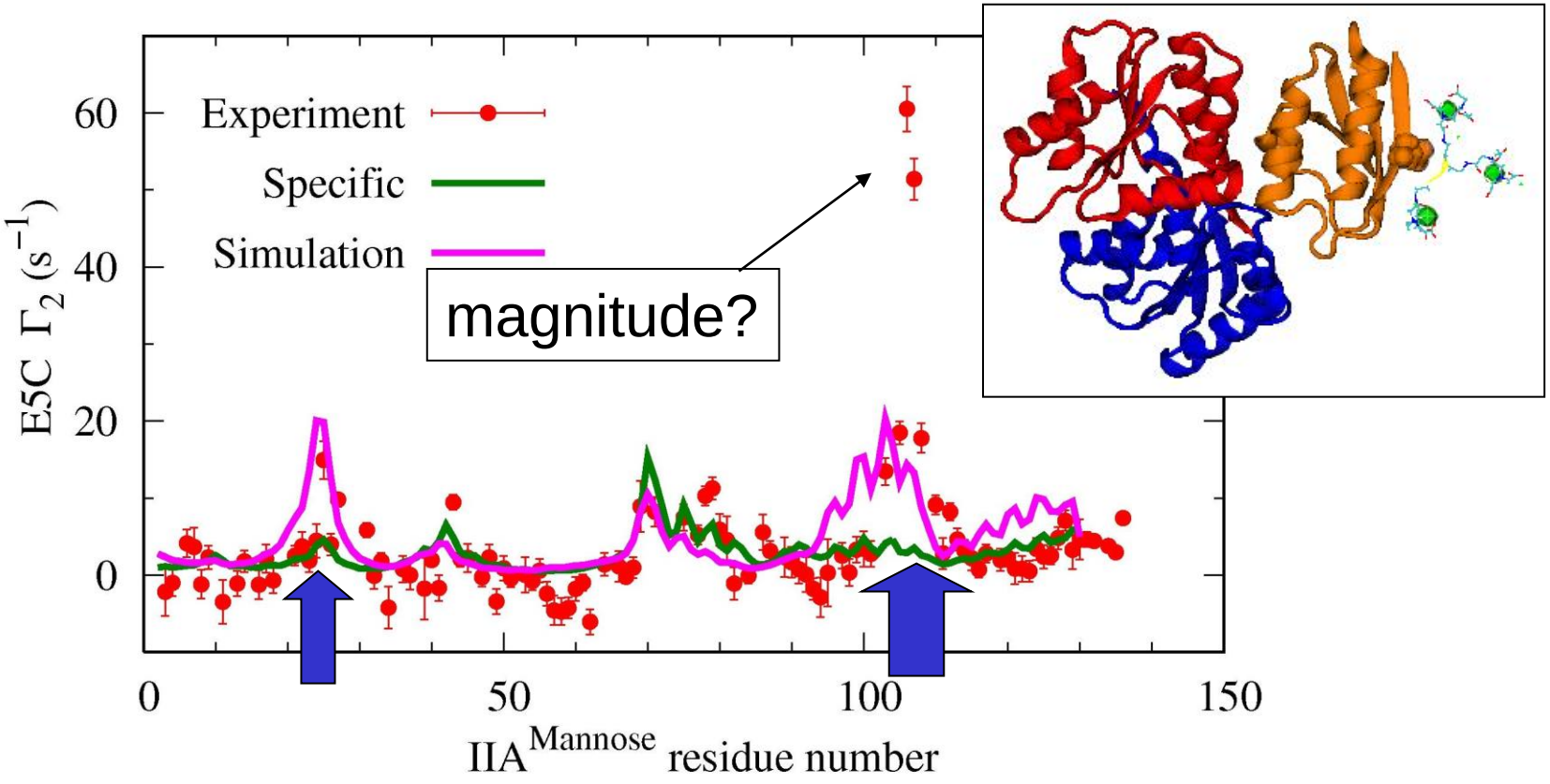
Structure



(YCK, Tang, Clore, Hummer, *PNAS USA* **105**, 12855, 2008)

PRE profiles of HPr-IIA^{Man} complex

- PRE of backbone amide protons on IIA^{Mannose}



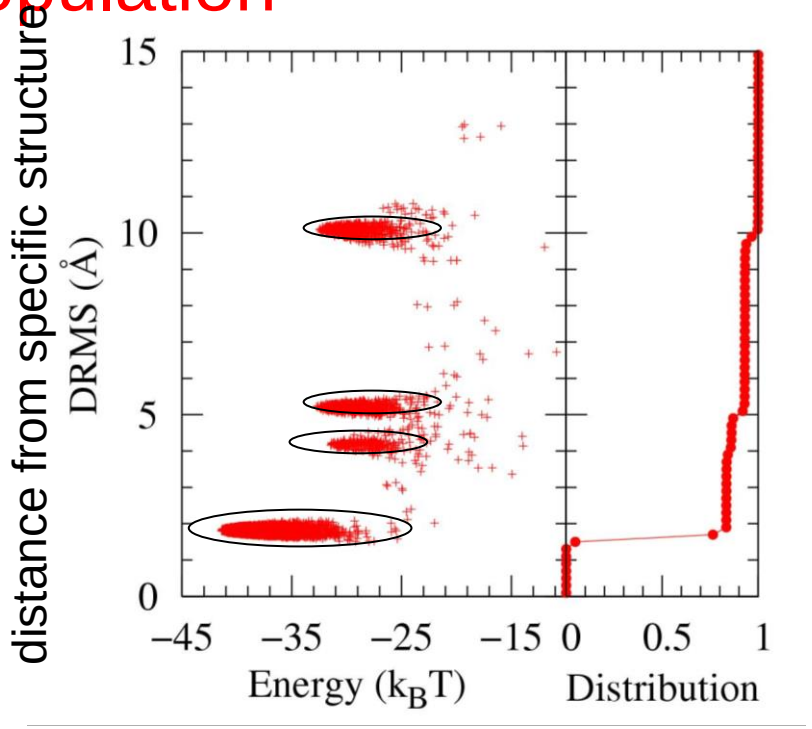
(YCK, Tang, Clore, Hummer, *Proc. Natl. Acad. Sci. USA* **105**, 12855, 2008)

Reweighting of simulation structures

- Simulation model should not be expected to produce accurate populations
- 2 kT binding free energy difference
  → 10-fold difference in population

⇒Cluster the structures of the specific and non-specific complexes

⇒Re-weight the populations of the clusters to match PRE profiles



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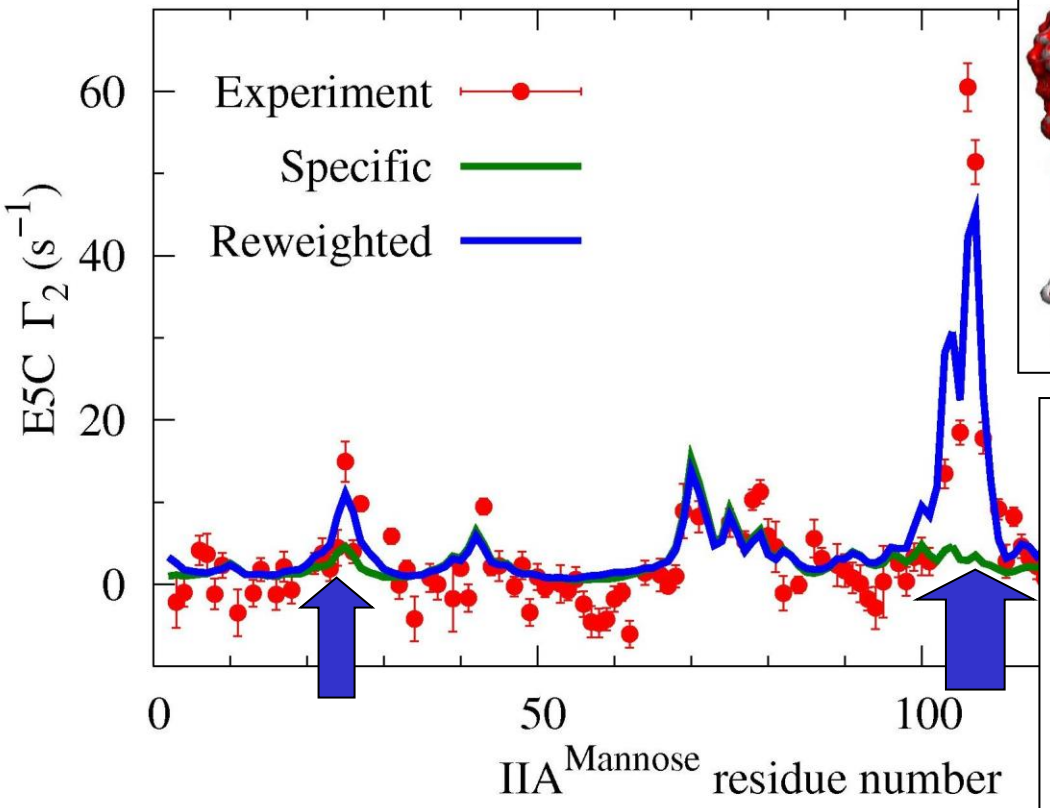
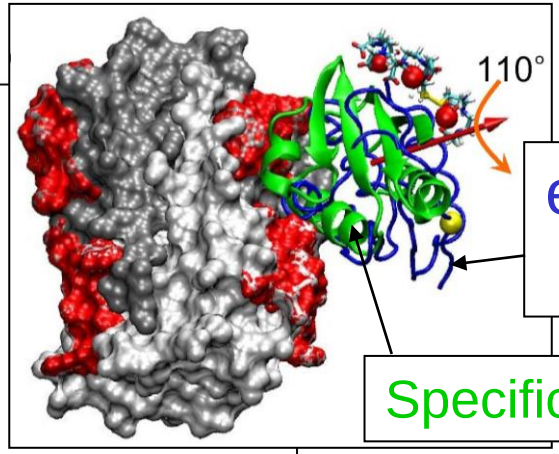


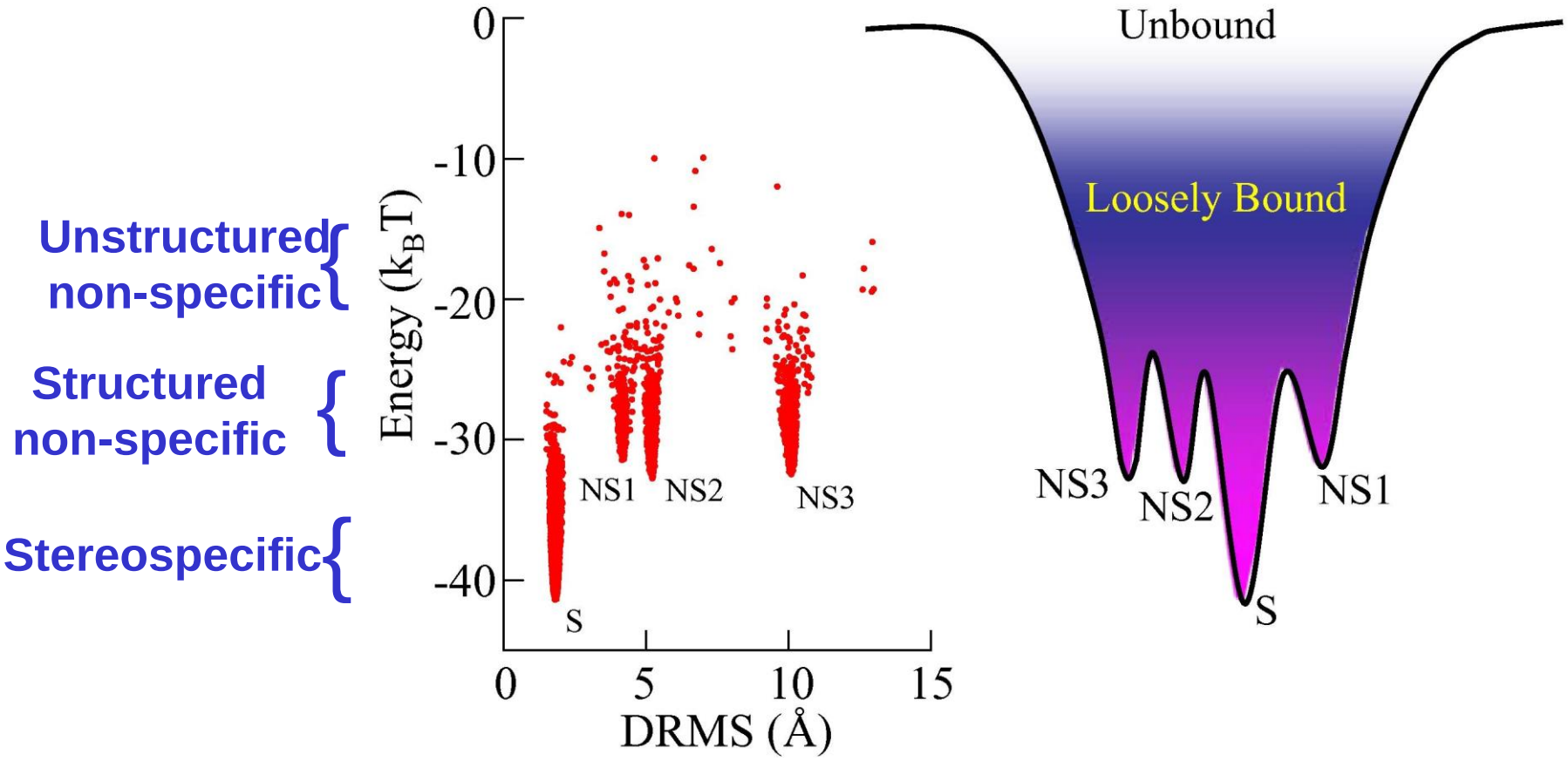


- Non-specific population <10%
- Single dominant non-specific cluster
  - 110^{o} rotation of stereospecific complex

(YCK, Tang, Clore, Hummer, *Proc. Natl. Acad. Sci. USA* **105**, 12855, 2008)

Energy landscape of protein complex formation

- Funnel-like energy landscape



(YCK, Tang, Clore, Hummer, *Proc. Natl. Acad. Sci. USA* **105**, 12855, 2008)

Summary II: Biology of transient encounter complexes

- Accelerated on-rate (barnase: Schreiber, Fersht, *Nat Struct Biol* **3**, 427, 1996)
- Strengthening of weak specific interactions in multi-protein assemblies (Vps27)
- Alternative binding modes (mannose transport: Hu *et al.* *J Biol Chem* **283**, 11024, 2008)
- Evolutionary remnants of earlier specific interactions?

Conclusion

- Coarse-grained model and transferable energy function provide valuable and complementary information regarding structures and dynamics of *multi-protein assemblies* and *transient encounter complexes*

Acknowledgments

- Gerhard Hummer (NIDDK, NIH)

ESCRT complex

- James Hurley (NIDDK, NIH)

PRE of encounter complexes

- G. Marius Clore (NIDDK, NIH)
- Chun Tang (U. Missouri)

Computational resources

- NIH Biowulf
- Helix Systems Staff