細胞環境を考慮した生体分子シミュレーションの現状と今後

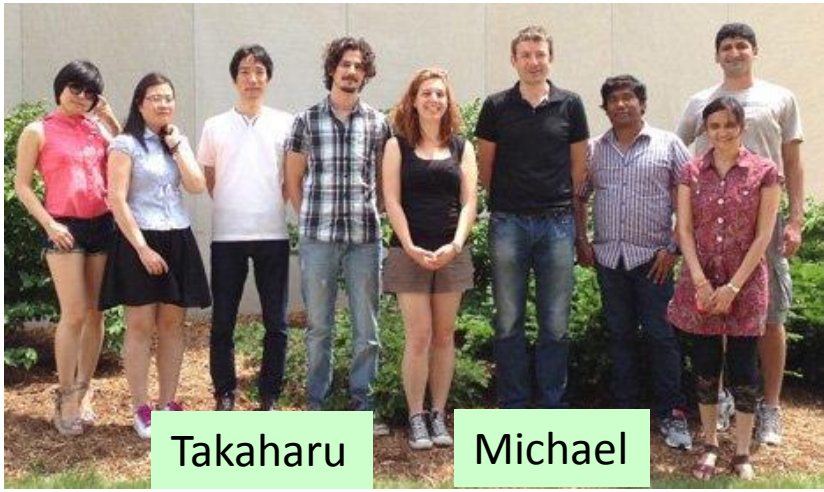
PCクラスタシンポジウム
2013/12/13

理化学研究所
杉田　有治

People who are working for the research

Sugita Group in RIKEN (Wako & Kobe)

Feig Group in Michigan State University

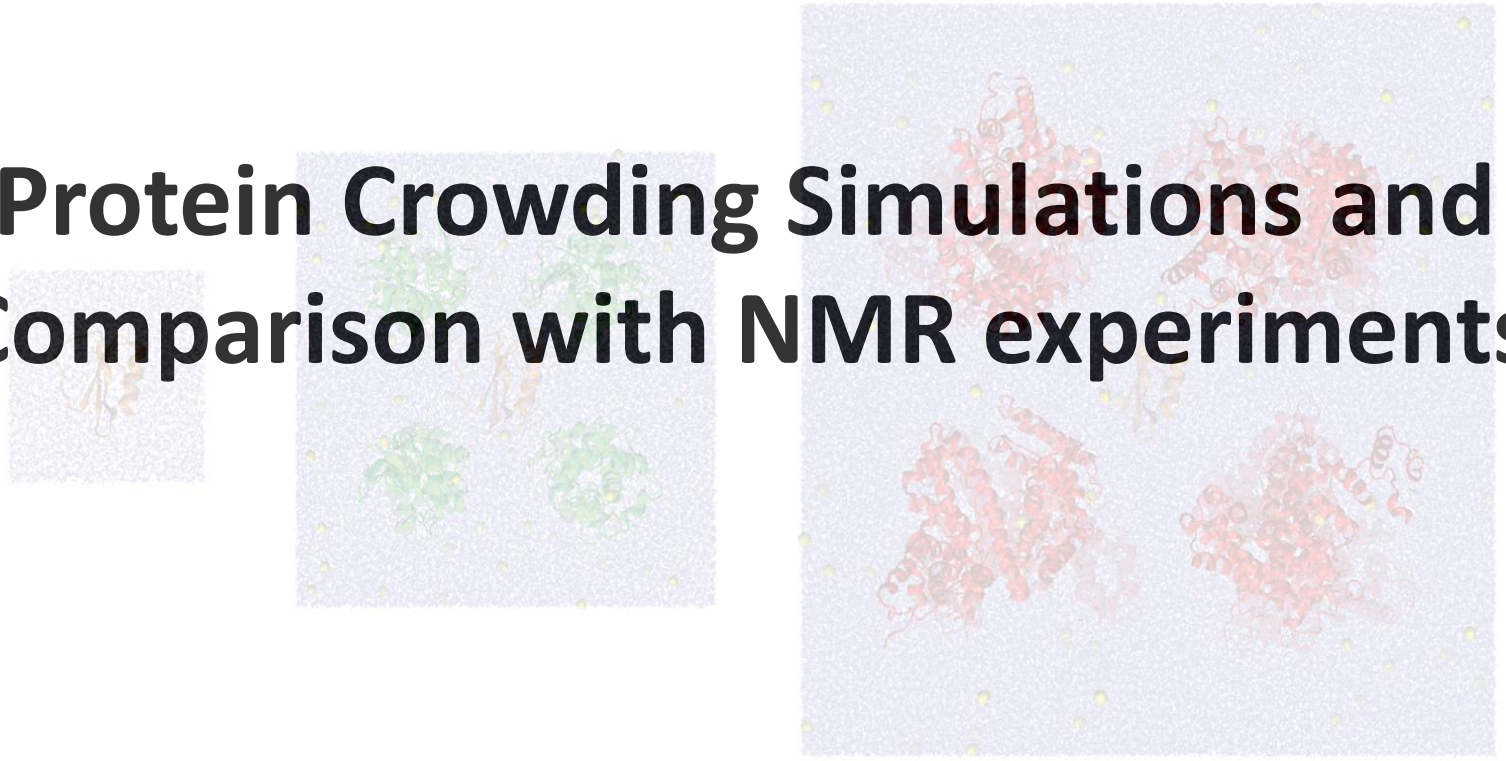


- **RIKEN Theoretical Molecular Science Laboratory**
  - **Dr. Isseki Yu**
  - **Dr. Takaharu Mori (Visiting Scientist in MSU)**
- **RIKEN AICS**
  - **Dr. Jaewoon Jung**
  - **Dr. Ryuhei Harada (→ Programming Environment Research Team)**

- **Michigan State University**
  - **Prof. Michael Feig**

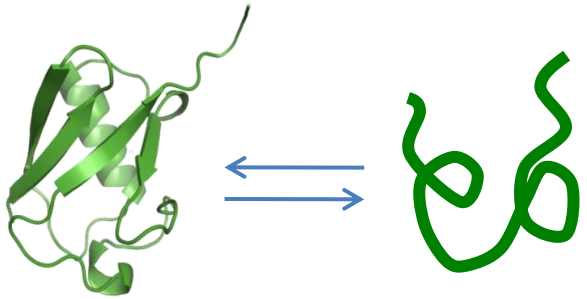
- **RIKEN QBiC (NMR experiment)**
  - **Dr. Takanori Kigawa**
  - **Dr. Naoya Tochio**

Protein Crowding Simulations and Comparison with NMR experiments



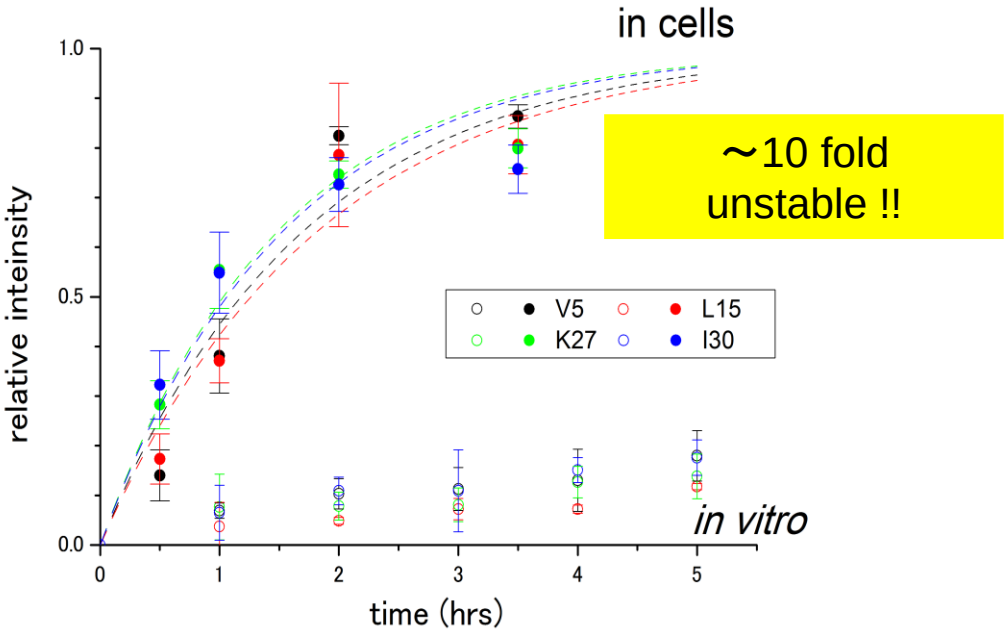
Why cellular environment is important ?

Inomata et al. Nature (2009)

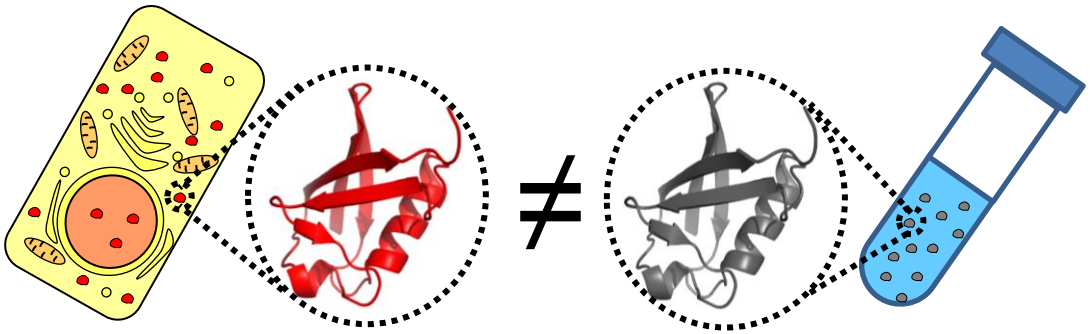


Folded state

Unfolded state



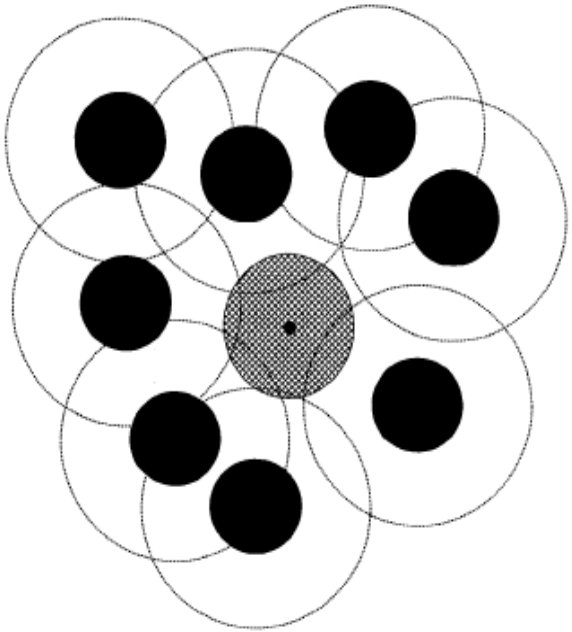
In-cell NMR suggests that conformational stability of ubiquitin in cells is lower than *in vitro*.



Protein dynamics
is different from *in vitro*.

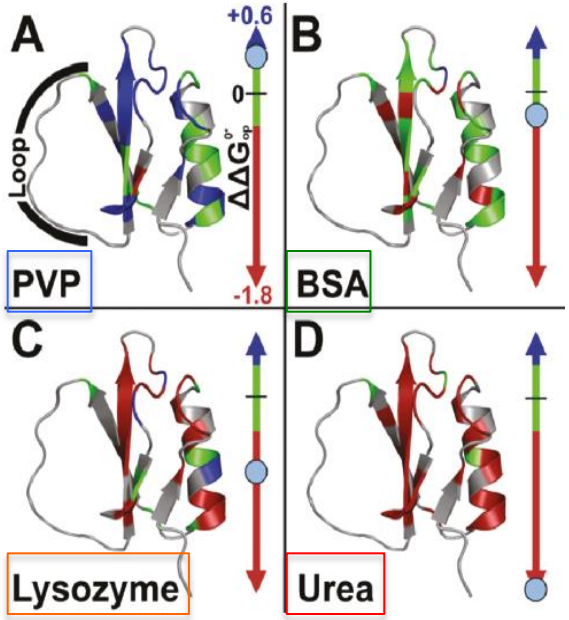
Macromolecular Crowding: Theory vs Experiment

★Hard sphere model (Minton,1993)
(***Entropy-centered*** model)



The *limitation* of the model

★NMR amide-exchange experiments of CI2 (Pielak, 2011)



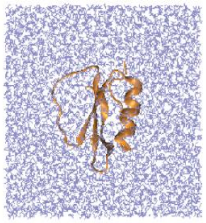
$\Delta G = \Delta H - T\Delta S$ **stabilization** **no-effect** **destabilization**

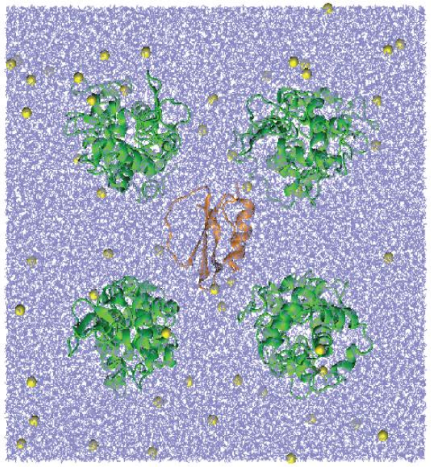
Compact ***native like*** structures are stabilized ⟺ Crowding ***stabilizes*** or ***destabilizes*** proteins

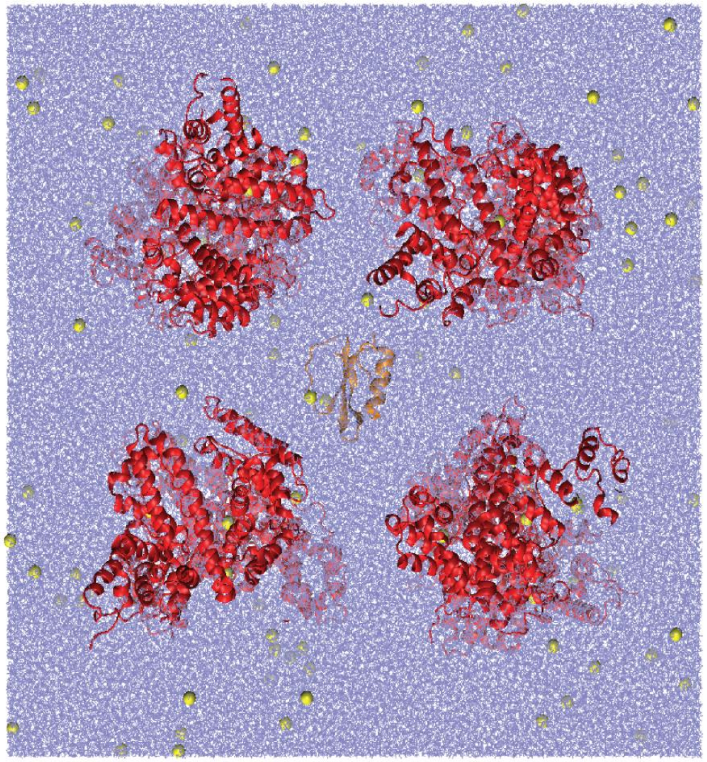
☆*Let's start crowding simulations using atomistic models with explicit solvents !*

Protein Crowding Systems for comparing with NMR experiments

Feig, Sugita: J. Phys. Chem B (2012) 116, 599-605







CI2
18K atoms
6K H_2O
infinite dilution

CI2 + 8 lysozymes
184K atoms
56K H_2O/64 Cl^-
108 g lysozyme/L
7% vol fraction

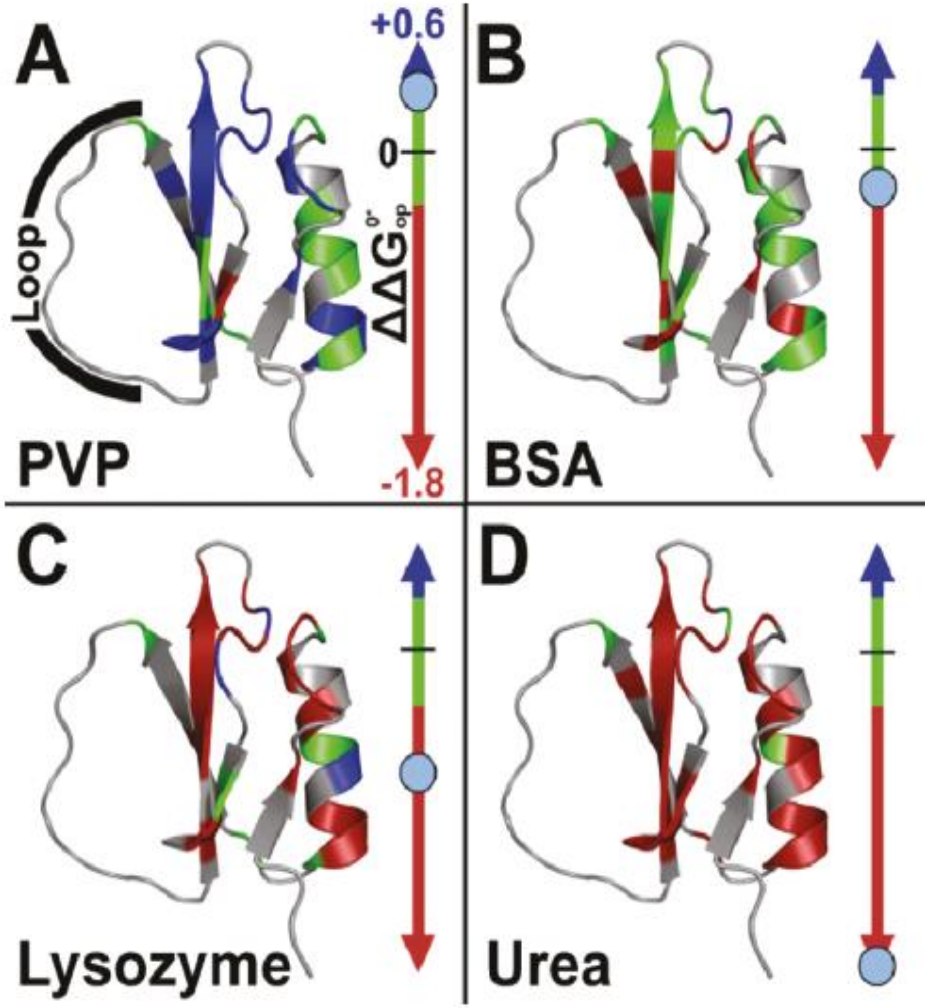
CI2 + 8 BSAs
835K atoms
253K H_2O/136 Na^+
104 g BSA/L solution
7% vol. fraction

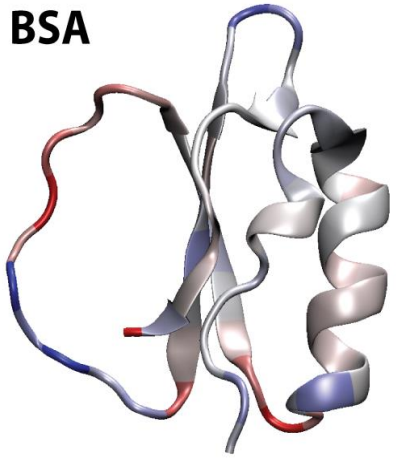
~160 ns MD

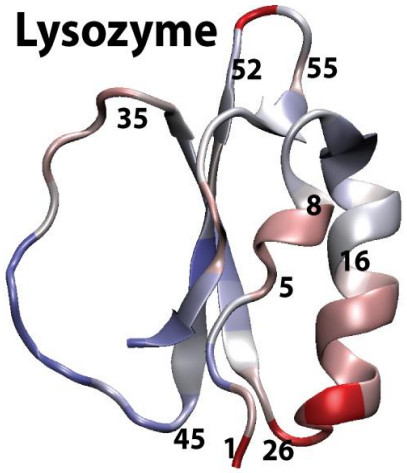
~250 ns MD

~120 ns MD

Protein Stability in NMR vs Protein Fluctuation in MD







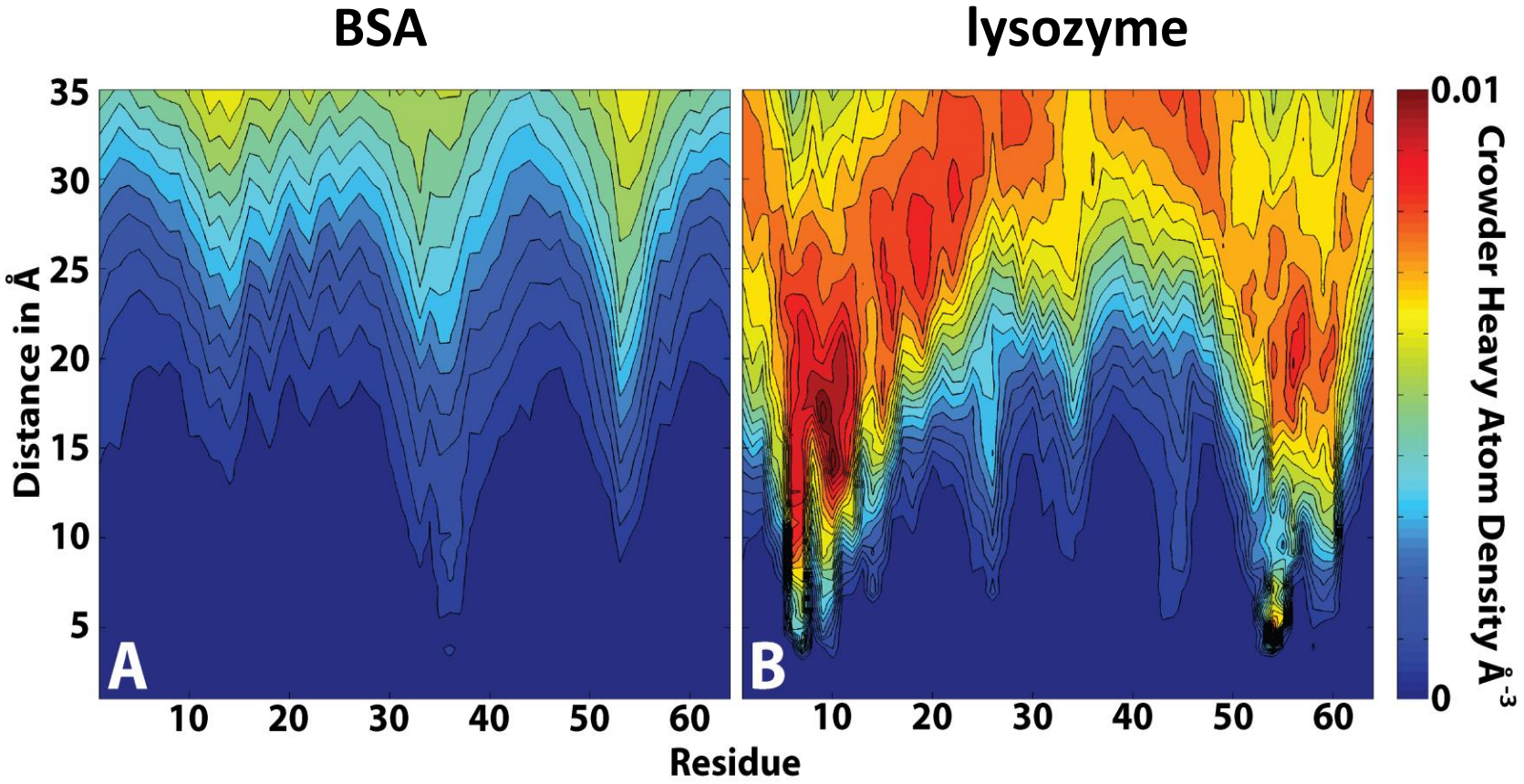
stabilization no-effect destabilization

increased/decreased fluctuations in MD

A. Miklos, M. Sarkar, Y. Wang, G. Pielak: JACS (2011) 133, 7116-7120

Feig, Sugita: J. Phys. Chem B (2012) 116, 599-605

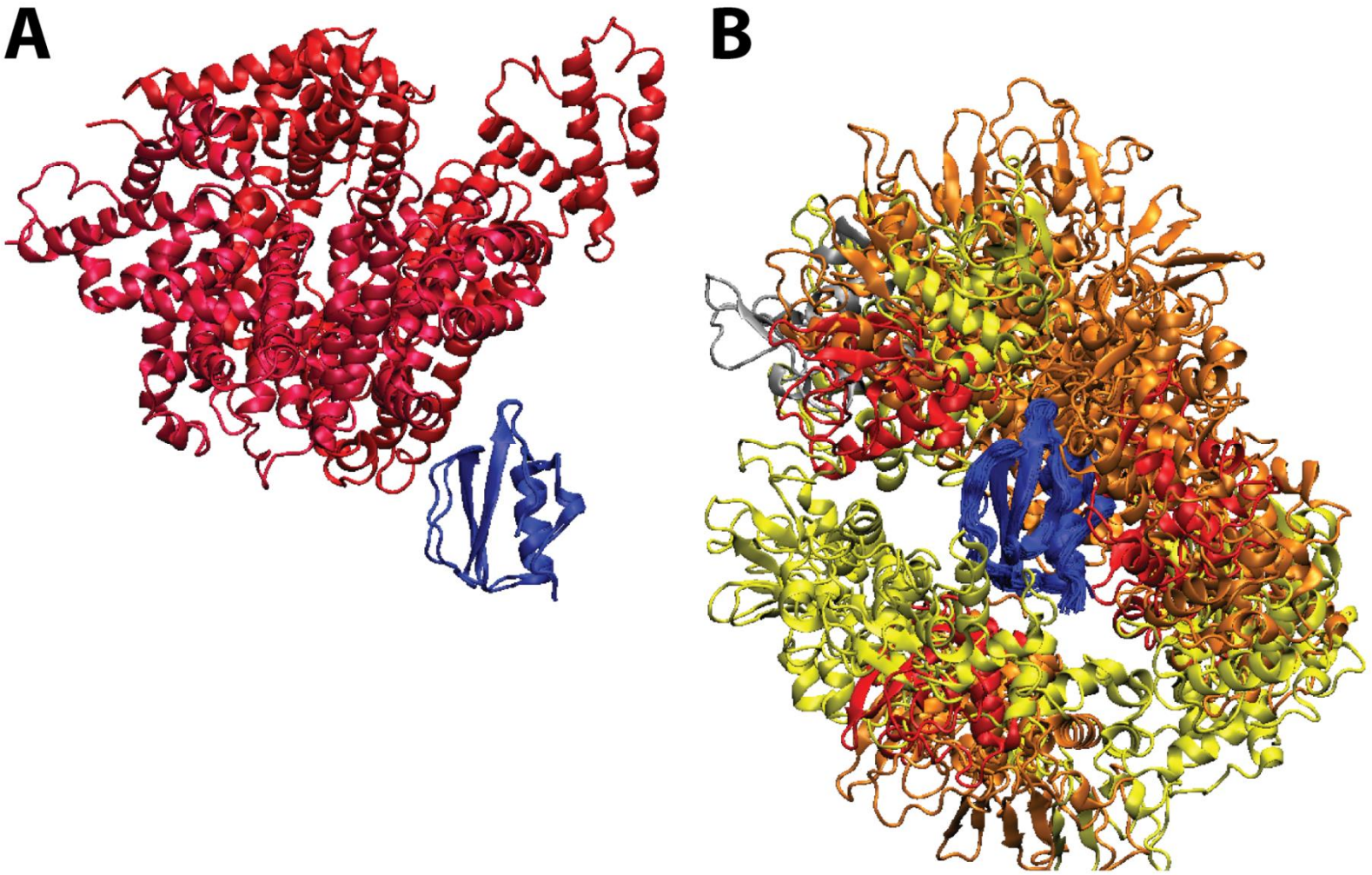
Interaction between CI2 and crowder proteins (BSA or Lysozyme)



Feig, Sugita: J. Phys. Chem B (2012) 116, 599-605

Interaction between CI2 and lysozyme is stronger than that between CI2 and BSA.

CI2 interaction with crowder proteins



on average

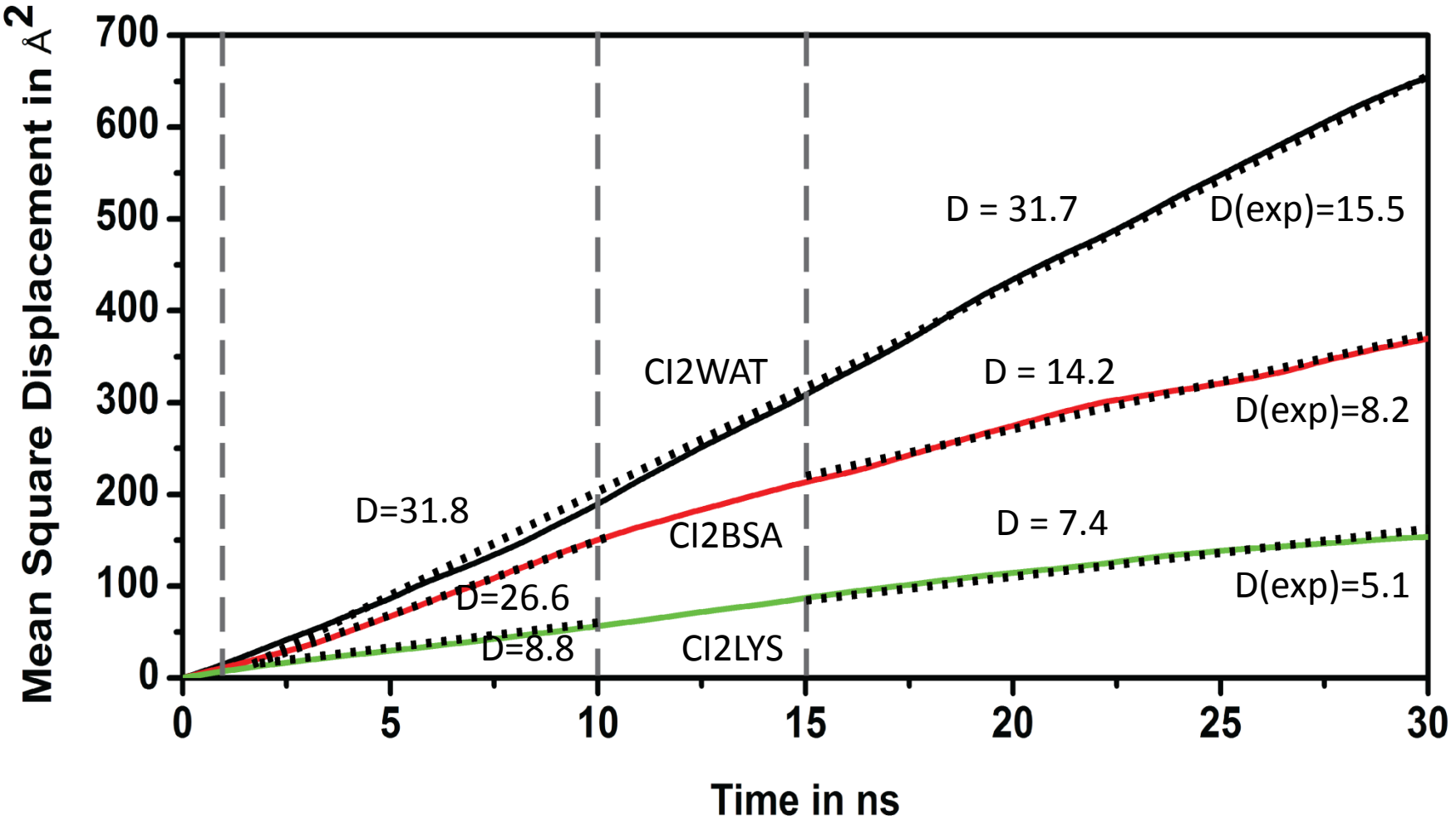
1.1 CI2-BSA contacts

2.6 CI2-lysozyme contacts

Feig, Sugita: J. Phys. Chem B (2012) 116, 599-605

Cellular Environment slow down diffusion of proteins

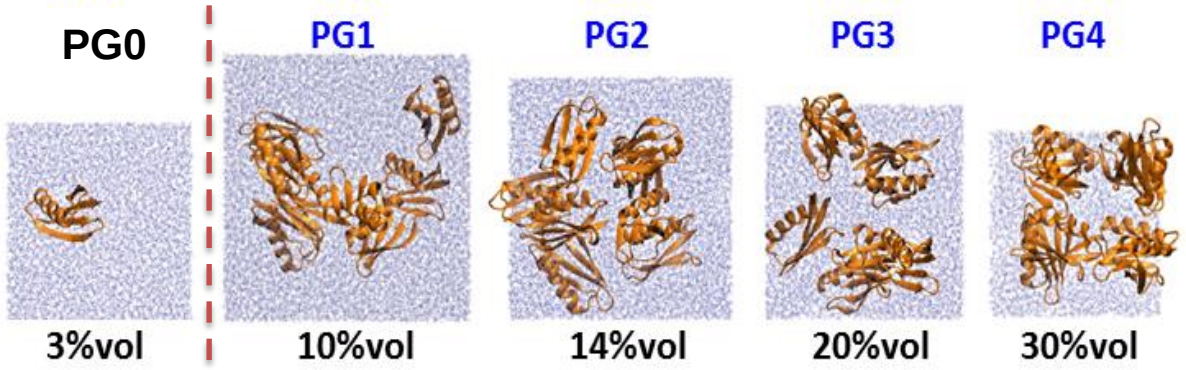
Feig, Sugita: J. Phys. Chem B (2012) 116, 599-605



[µm²/s] obtained according to Einstein relationship from slope of mean square displacement vs time.

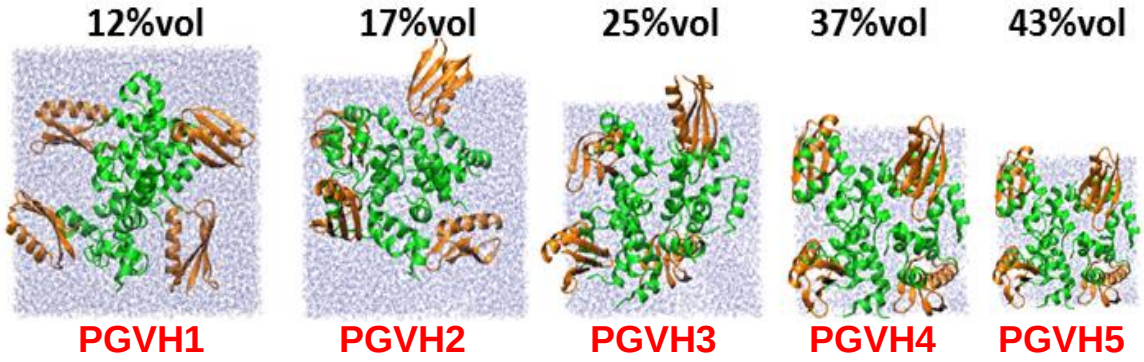
Crowding Systems with different concentration of proteins

PG0:
1 Protein G
Dilute



PG1-4:
8 protein G
Crowding

PGVH1-5:
4 protein G + **8 villin**
Crowding



Harada, Sugita, Feig, *J. Am. Chem. Soc.* 2012, 134, 4842-4849

NPT(1bar, 298K, 300ns)

Available volume for water within the first and second solvation shell vs bulk

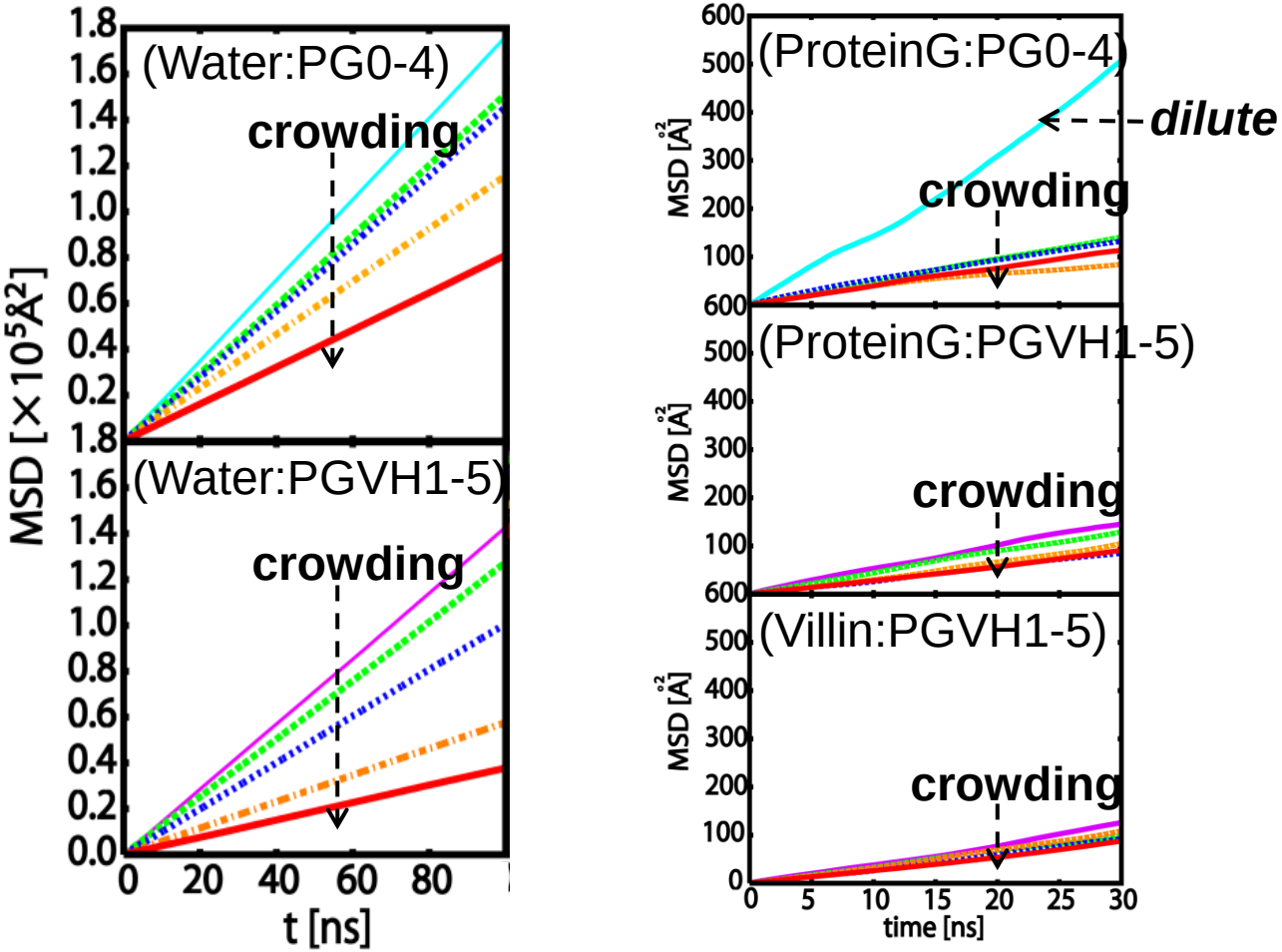
Harada, Sugita, Feig, *J. Am. Chem. Soc.* 2012, 134, 4842-4849

System	Protein volume fraction	1st solvation shell (r ≤ 4 Å) [%]	2nd solvation shell (4 Å < r ≤ 7 Å) [%]	Bulk (r >7 Å) [%]
PG1	0.10	22.4	14.4	63.3
PG2	0.14	31.7	20.4	47.9
PG3	0.20	44.0	24.6	31.5
PG4	0.30	65.6	26.7	7.7
PGVH1	0.12	28.1	16.3	55.6
PGVH2	0.17	37.7	19.1	43.3
PGVH3	0.25	53.4	22.2	24.5
PGVH4	0.37	76.2	19.6	4.2
PGVH5	0.43	86.4	13.0	0.6

★Almost no room for bulk water in highly crowded conditions (PG4, PGVH4, PGVH5).

Diffusion of Water and Protein Molecules in the Crowding Systems

$$MSD(t) = \left\langle | r(t'+t) - r(t') |^2 \right\rangle_{t'}$$

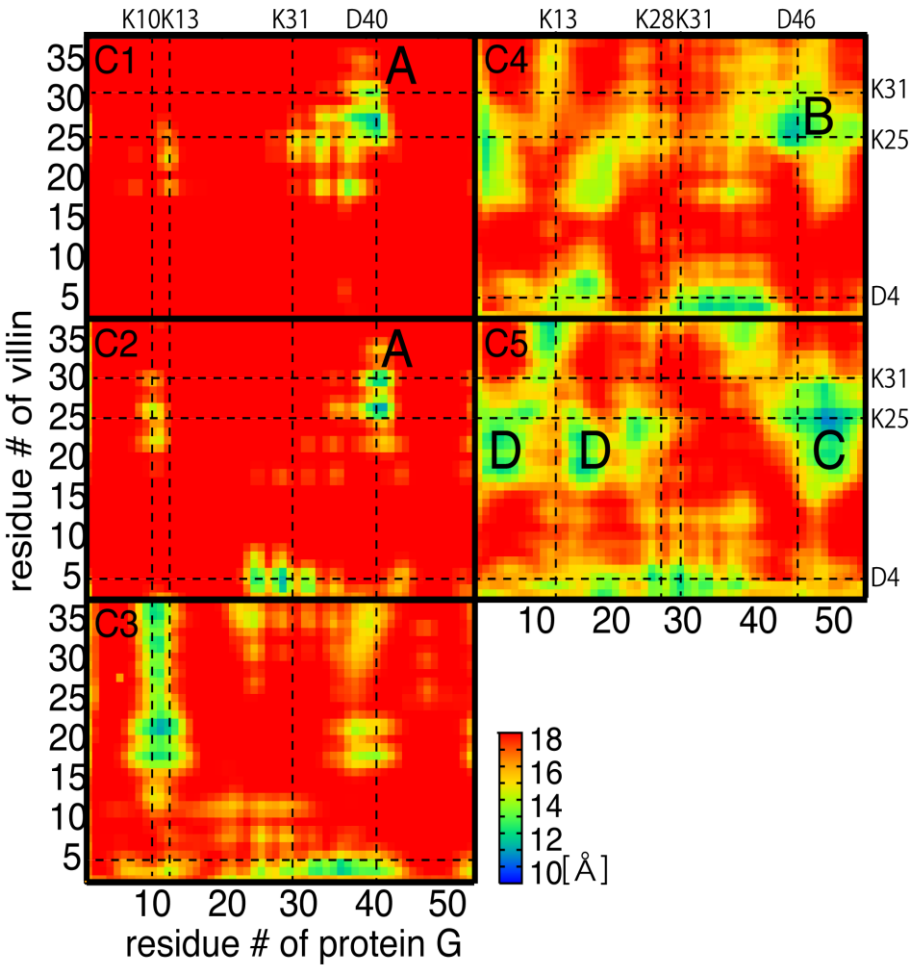


★ In crowded environment, diffusion of water and protein *significantly slow down*

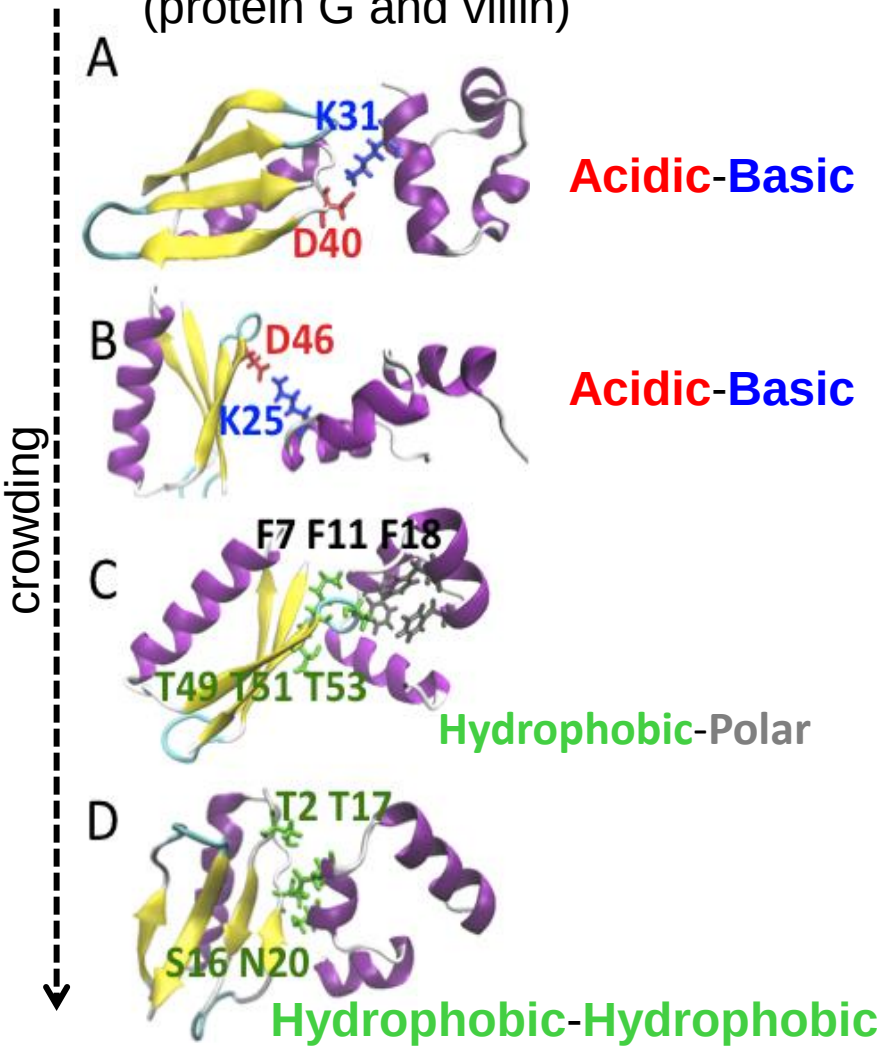
Harada, Sugita, Feig, *J. Am. Chem. Soc.* 2012, 134, 4842-4849

Protein-Protein Interactions in Crowding Systems

★***Contact maps*** between protein G and villin (***average minimum distances***)



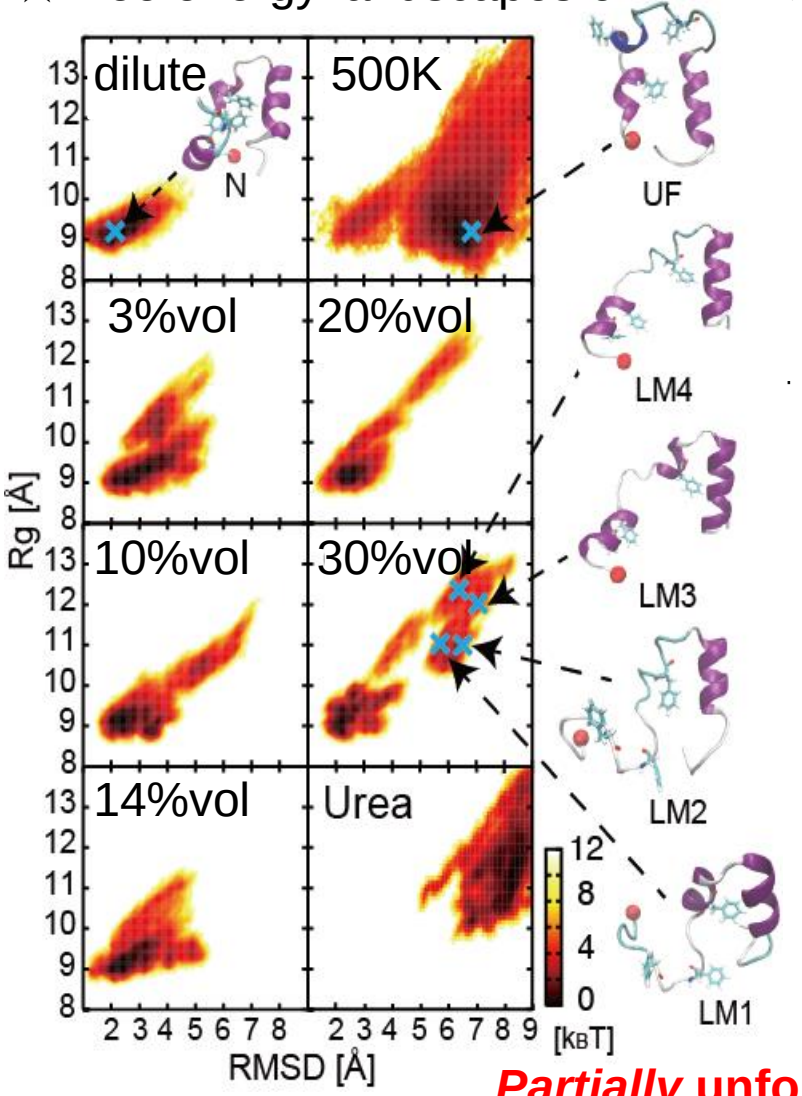
★Representative dimer structures (protein G and villin)



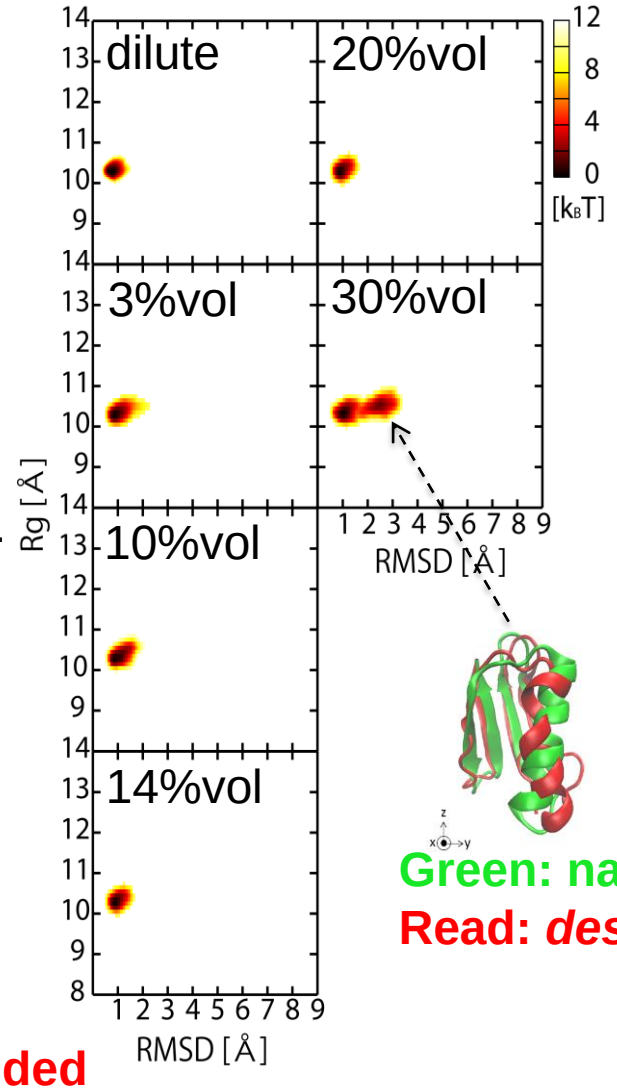
Harada, Tochio, Kigawa, Sugita, Feig, *J. Am. Chem. Soc.* 2013, 135, 3696-3701.

Conformational Stability of Villin and Protein-G in different crowding systems

★Free energy landscapes of ***villin***



★Free energy landscapes of ***protein G***



Green: native

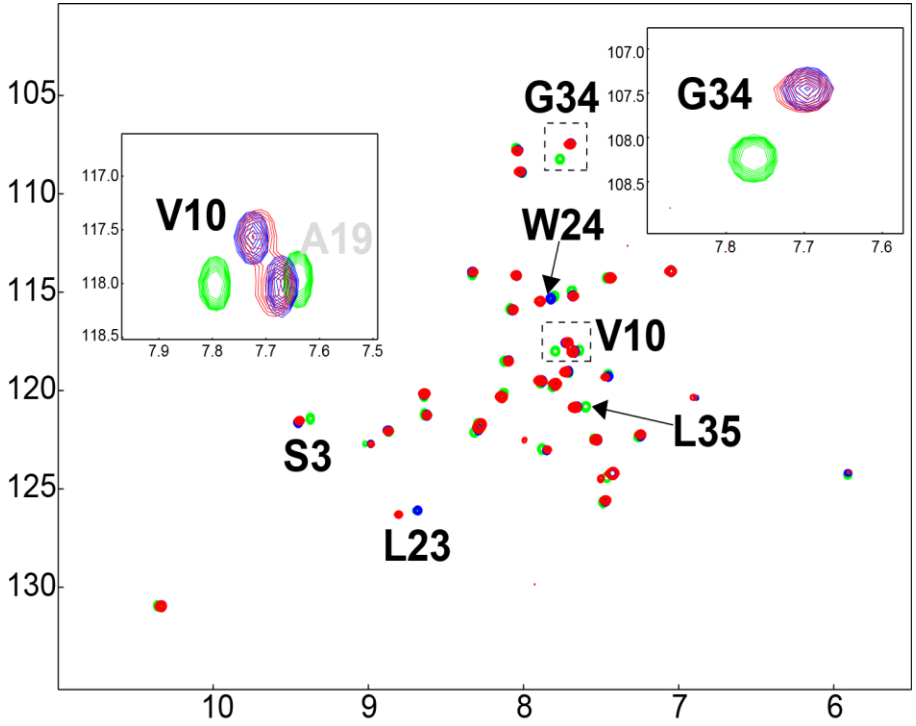
Read: *destabilized*

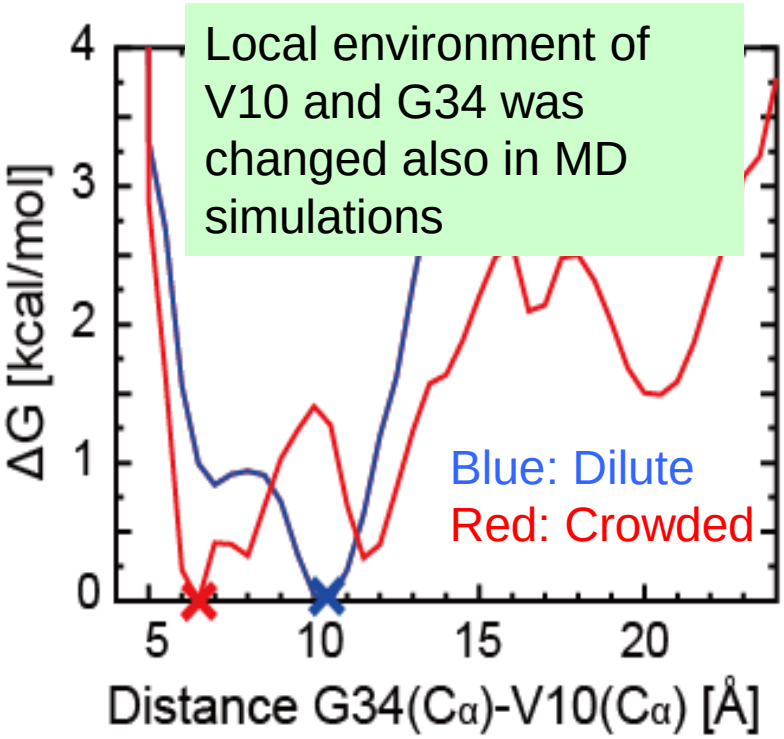
Partially **unfolded**

Harada, Tochio, Kigawa, Sugita, Feig, *J. Am. Chem. Soc.* 2013, 135, 3696-3701.

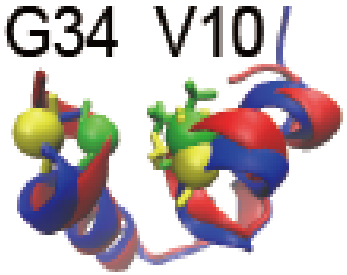
Comparison of protein crowding systems between MD and NMR

V10 and G34 changes their chemical shift due to crowding



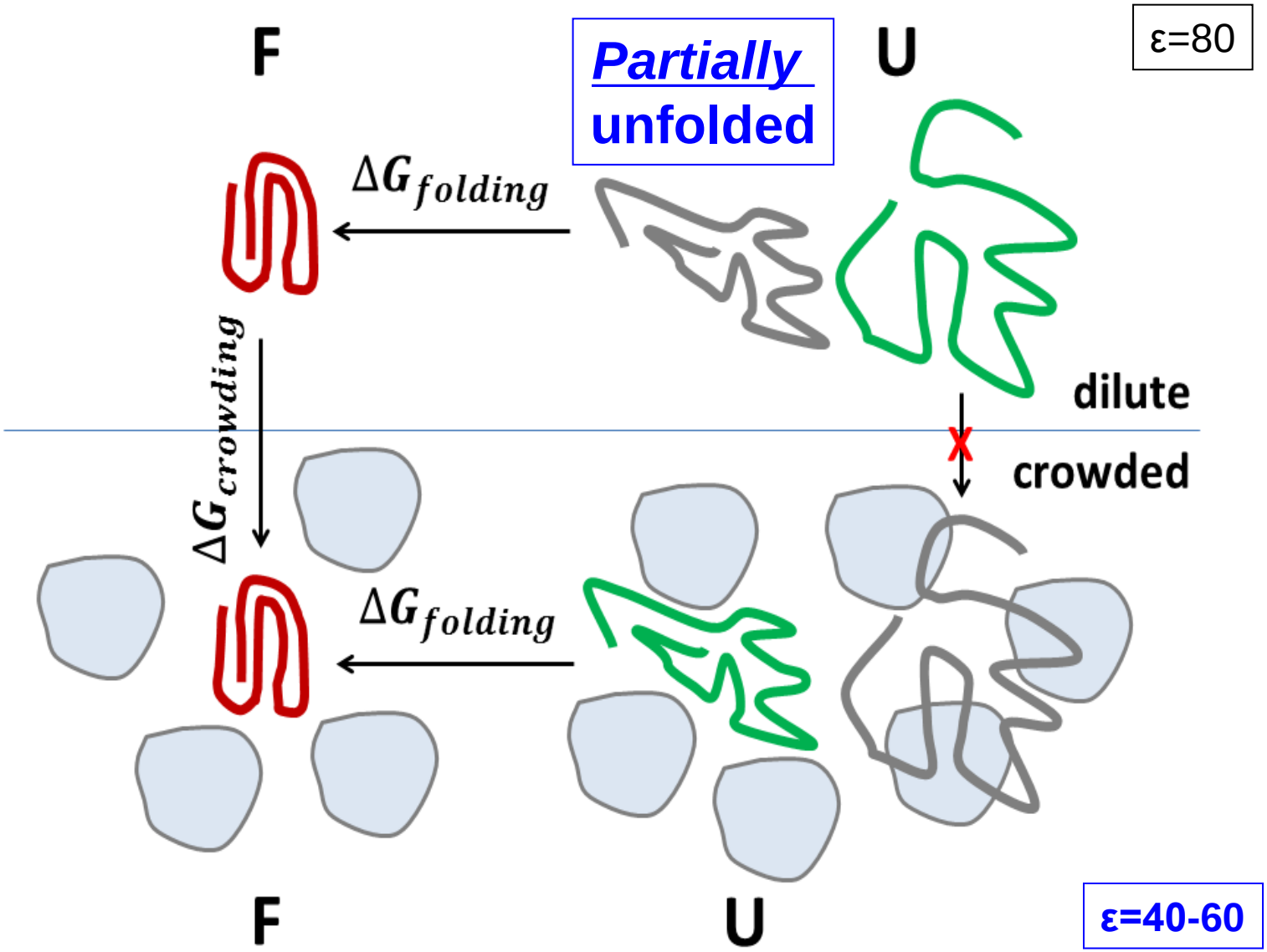


^{1}H, ^{15}N TROSY-HSQC spectra of ^{15}N-labeled villin
1mM villin (green),
32mM villin (blue),
32mM villin with protein G (red)
By Dr. Kigawa and Dr. Tochio (RIKEN SSBC)



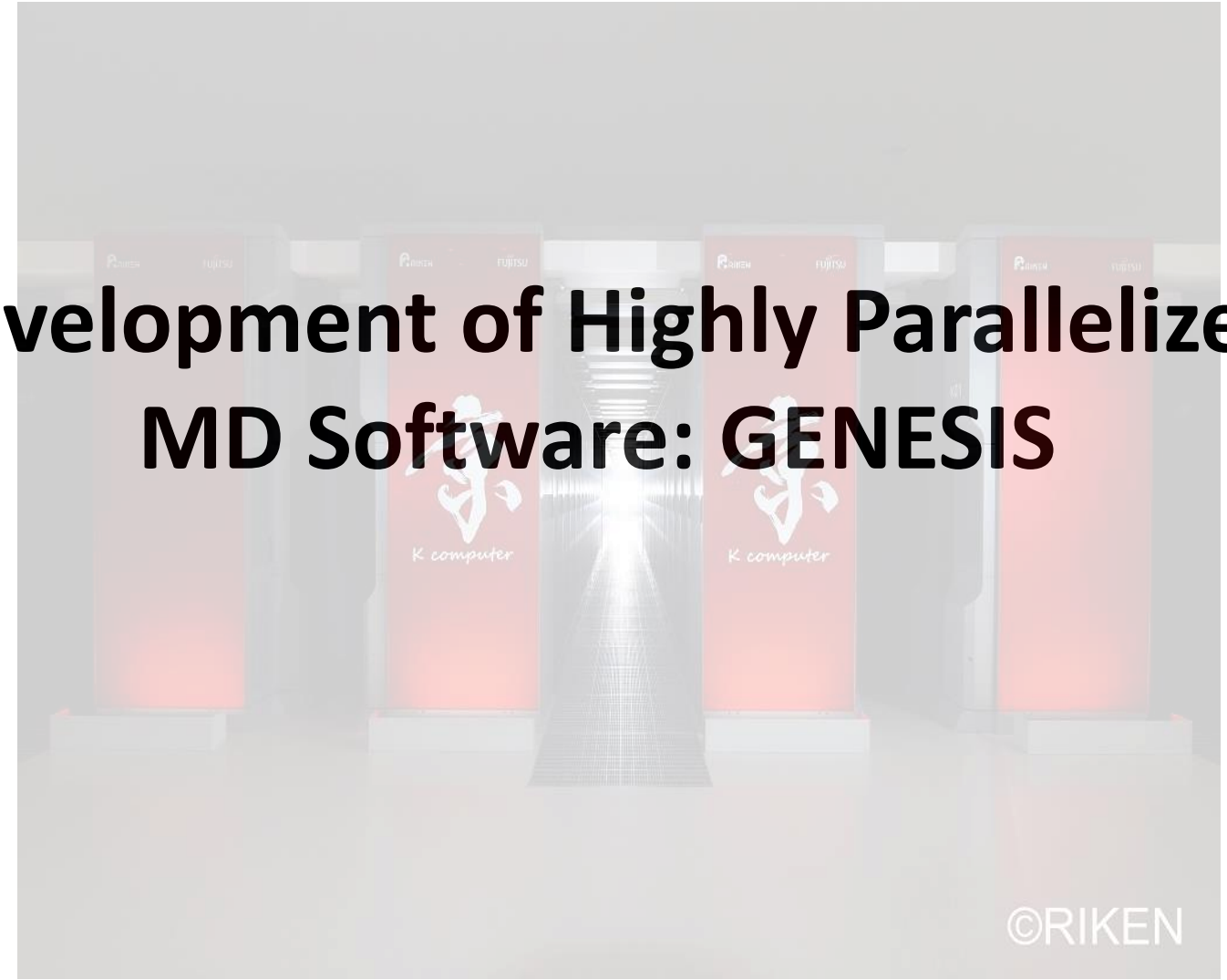
Harada, Tochio, Kigawa, Sugita, Feig, *J. Am. Chem. Soc.* 2013, 135, 3696-3701.

New views on macromolecular crowding



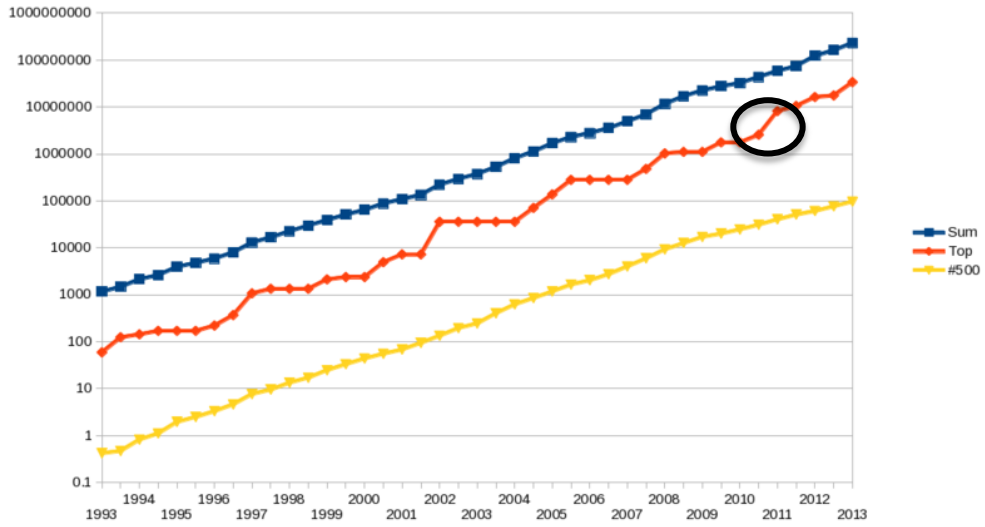
Harada, Tochio, Kigawa, Sugita, Feig, *J. Am. Chem. Soc.* 2013, 135, 3696-3701.

Development of Highly Parallelized MD Software: GENESIS



K computer at RIKEN Advanced Institute for Computational Science (AICS)

Performance of SuperComputer (from Top500)



Recently, the performance of supercomputers has been improved significantly. Top machine in 2013 is more than 100,000 times faster than that in 1993.

K computer (The 4th fastest computer)

Peak Performance: 10.51 PFLOPS

# of CPU Cores： 705,024

Total Memory：1.41PB（16GB per node）

Network: Tofu: 6D mesh / Torus

GENESIS (Generalized-Ensemble Simulation Systems)

1. Aims at developing efficient and accurate methodologies for free-energy calculations in biological systems

2. Efficient Parallelization - Suitable for massively parallel computers, in particular, K computer

3. Applicability for large scale simulation

4. Algorithms coupled with different molecular models such as coarse-grained, all-atom, and hybrid QM/MM

5. Generalized ensembles like Replica-Exchange Molecular Dynamics (T-REMD, REUS, MREM, Surface-tension REMD (New! T.Mori et al. JCTC in press.)

6. Open Source Code from this December

GENESIS (Generalized-Ensemble Simulation Systems)

GENESIS (V1) Development Team

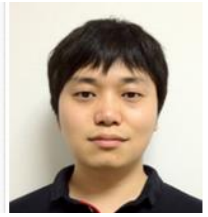
- Project Leader: Yuji Sugita
- Major Developers: **Jaewoon Jung, Takaharu Mori**
- Developers: **Chigusa Kobayashi, Yasuhiro Matsunaga, Takashi Imai, Takao Yoda (Nagahama Bio Institute), Norio Takase (Isogo Soft)**
- Other Contributors: Many members in Sugita Group


Y. Sugita


J. Jung

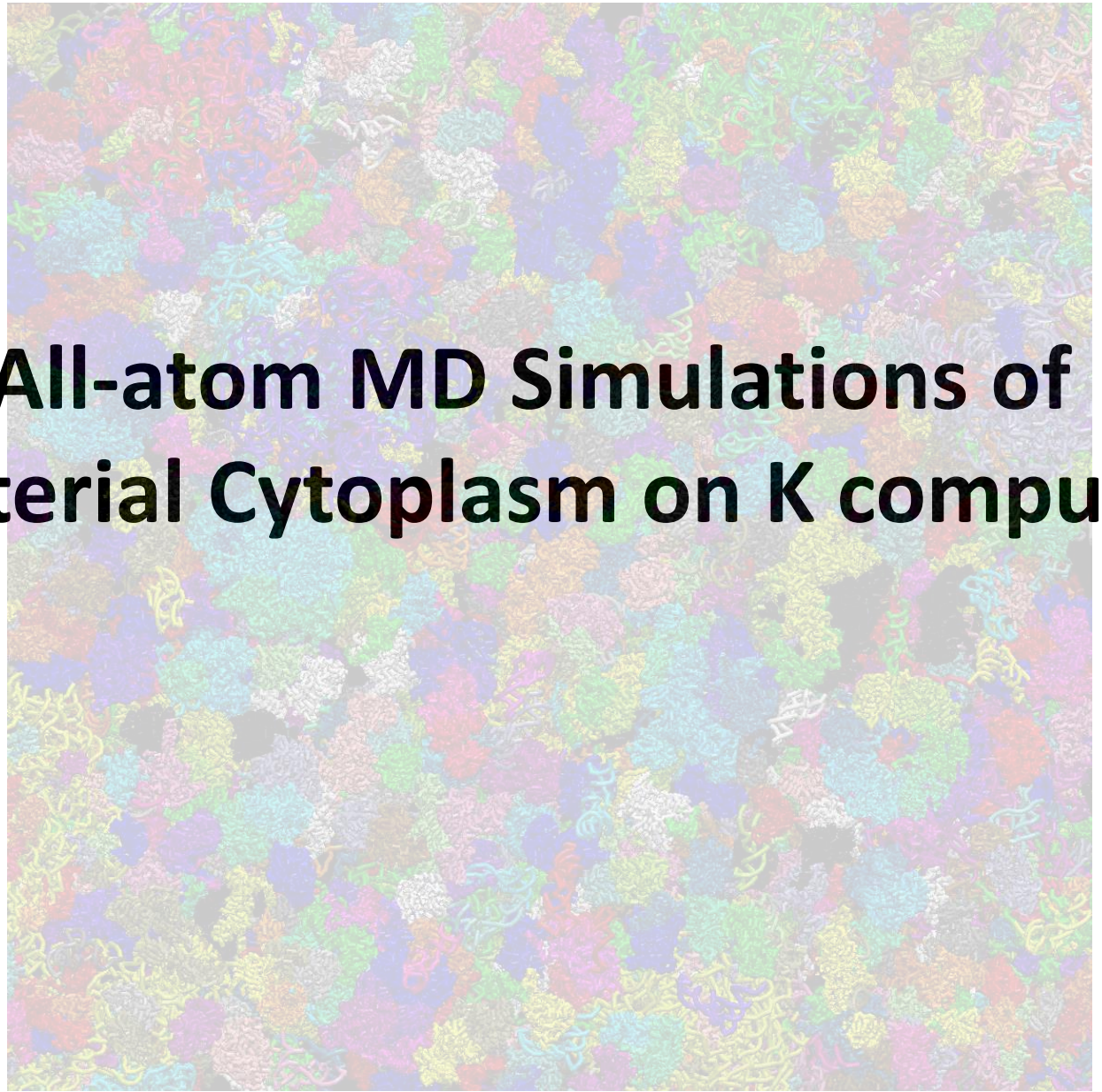

T. Mori


C. Kobayashi


Y. Matsunaga

New Features of GENESIS (V1)

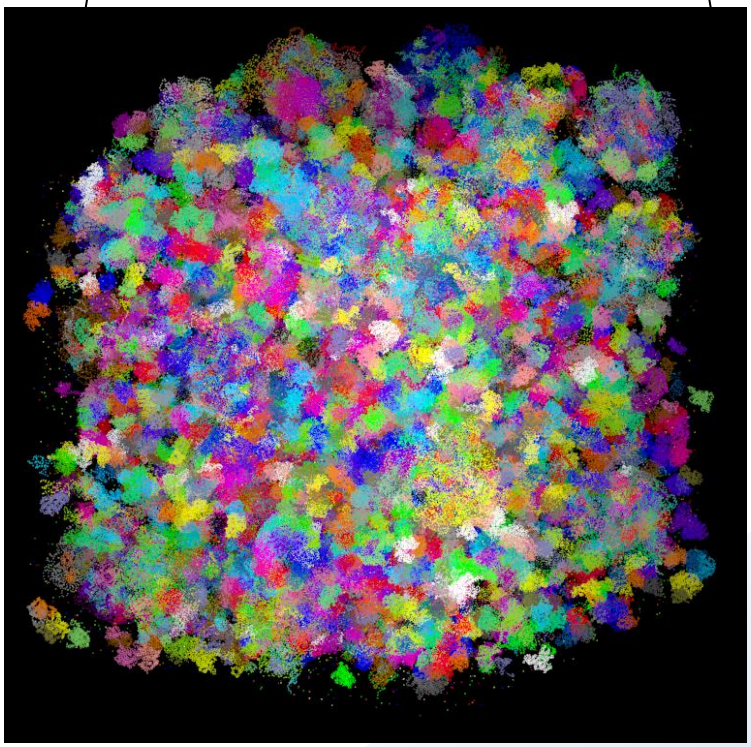
- Inverse Lookup Table Scheme for Nonbonded Interaction
  - J. Jung et al. J.Comp.Chem. 2013, 34, 2412-2420.
- Midpoint Cell Method for Hybrid Parallelization
- Fast 3D-FFT Calculations



All-atom MD Simulations of Bacterial Cytoplasm on K computer

Our Target System for All-atom MD Simulation

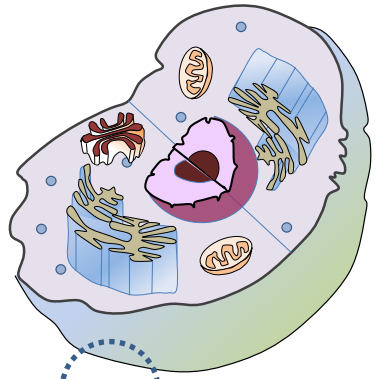
100nm = 1/10,000mm

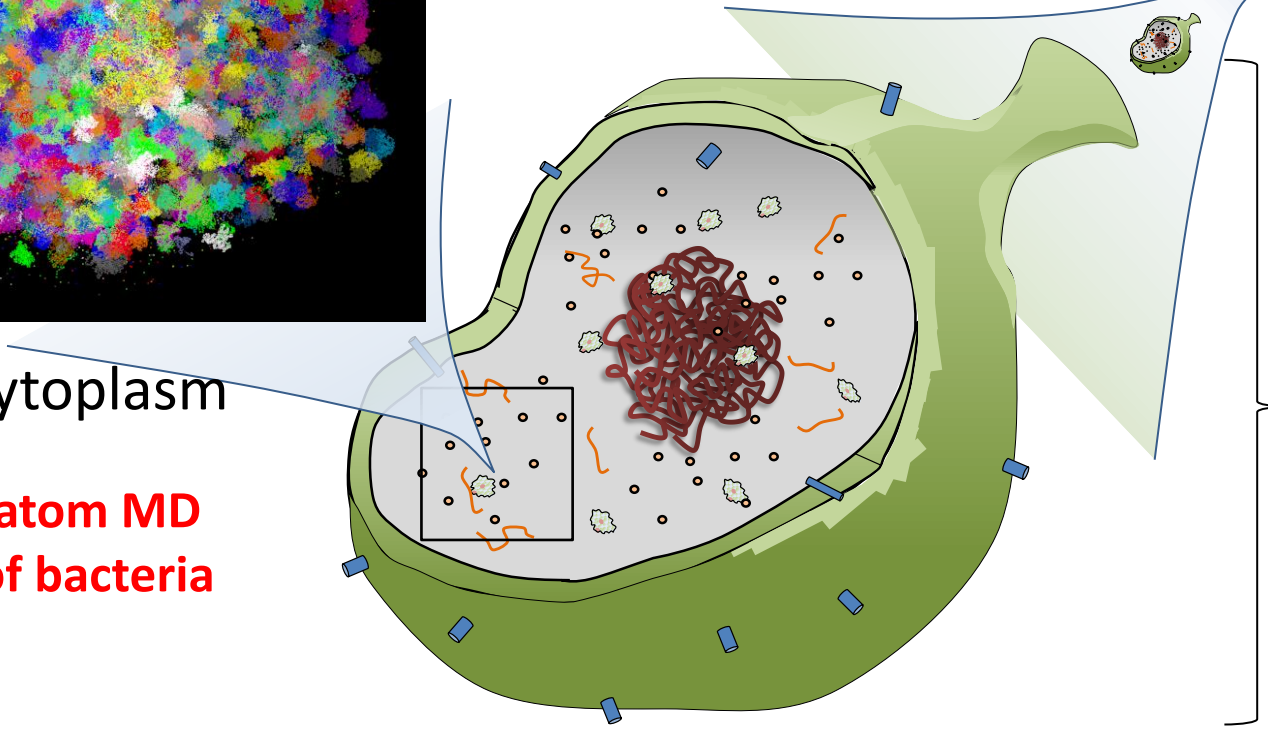


100x100x100 (nm^3)
100,000,000 atoms
（including water）
thousands of proteins !
vast amounts of metabolites !

animal cell

10μm
=1/100 mm





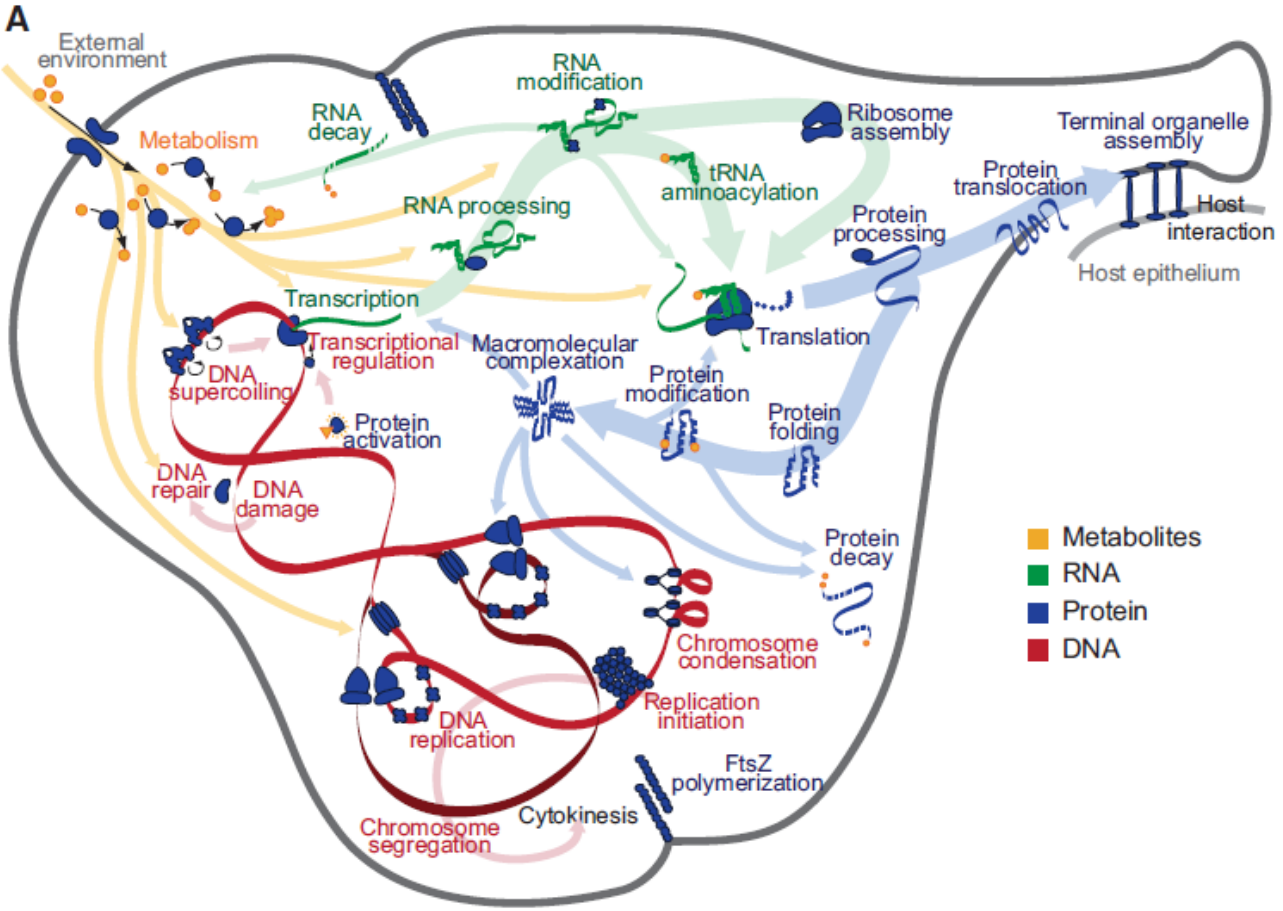
Mycoplasma Genitalium
（bacteria）

300nm
=3/10,000mm

Cytoplasm

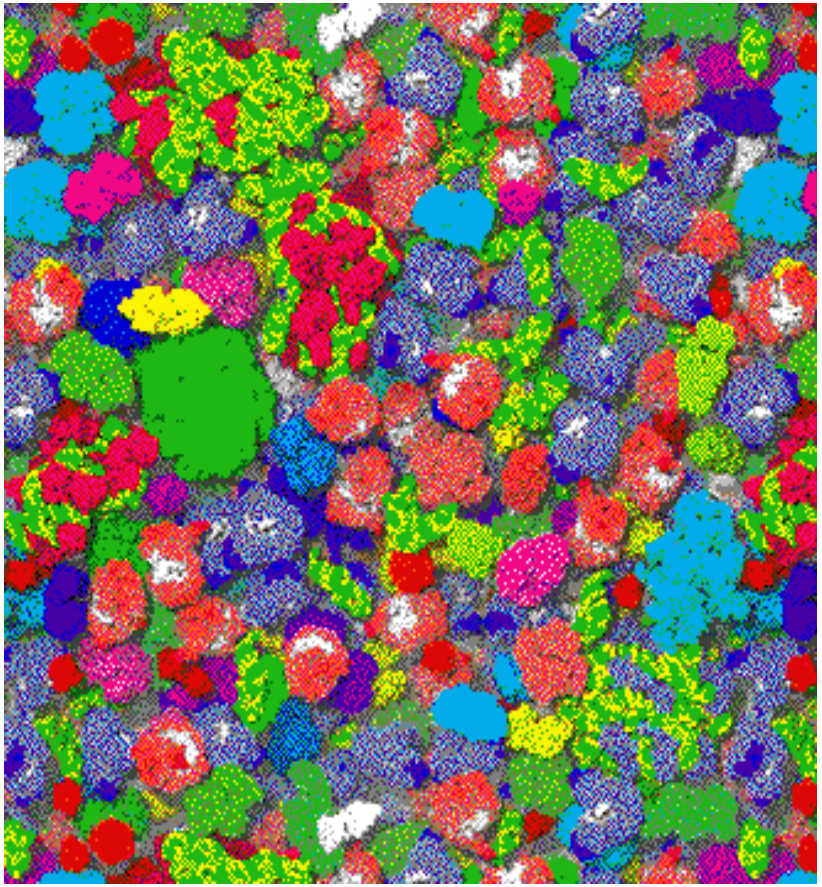
The first all-atom MD simulation of bacteria cytoplasm

Mycoplasma genitalium: a bacterium with the smallest known genome



Karr, J.R., Sanghvi, J.C., Macklin, D.N., Gutschow, M.V., Jacobs, J.M., Bolival, B., et al. A Whole-Cell Computational Model Predicts Phenotype from Genotype. Cell. 2012, 150, 389-401.

Brownian Dynamics Simulations by Elcock et al.

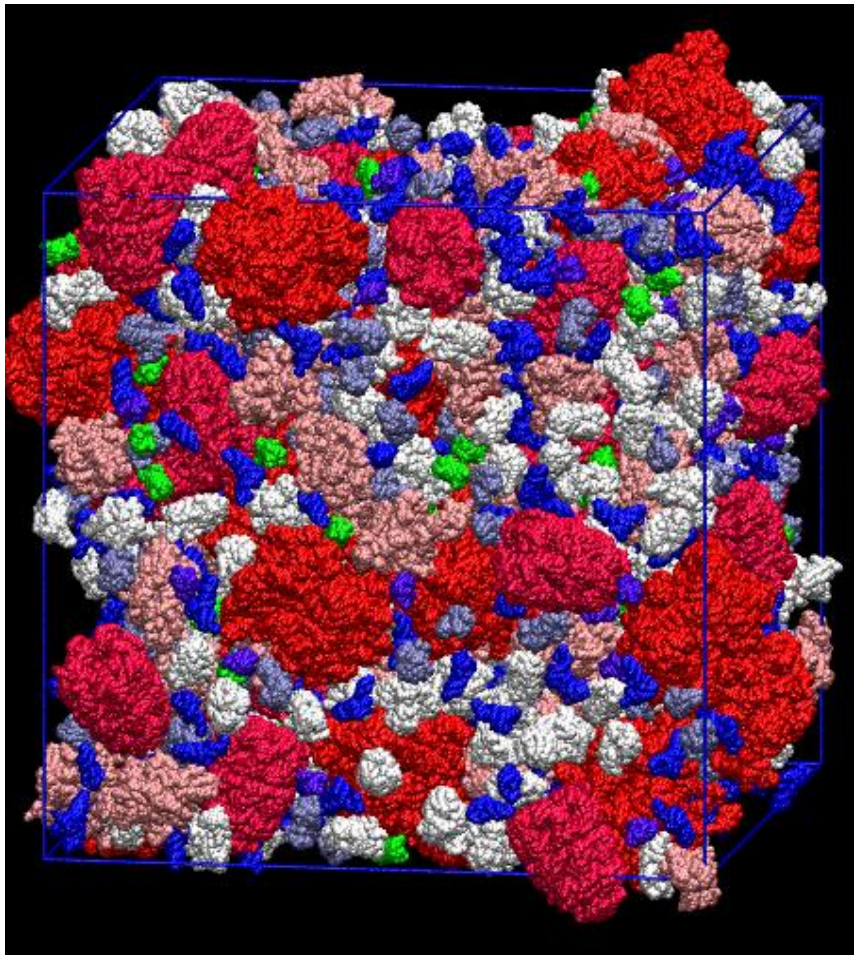


- PLoS Computational Biology 2010
- 80 X 80 X 80 (nm^3)
- 6 micro sec.
- 50 selected proteins from E. coli
- No metabolites/solvents
- Effective potential.
- Diffusion coefficients are provided as an input parameters.

- Focus on Thermodynamics (Protein Stability)
- Advanced treatment of molecules (full sampling and full scoring) is required to predict protein stability in crowded conditions.

Coarse-grained BD Simulations Without Hydrodynamic Effect

Brownian Dynamics Simulations by Ando and Skolnick



- PNAS 2010, 107: 18457-18462
- 100 X 100 X 100 (nm^3).
- A virtual cytoplasmic system of E. coli
- Over 1,000 macromolecules consisting of 15 different macromolecules in Brownian Dynamics (BD) simulations (50 micro sec)

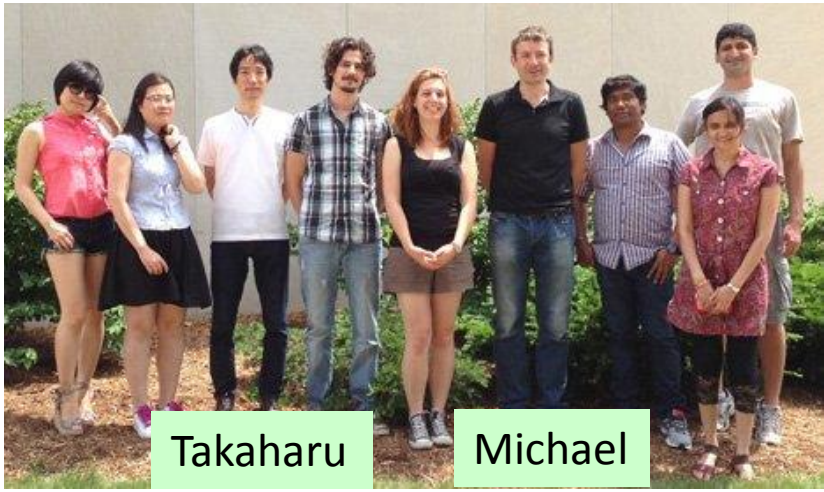
- Focus on the diffusion coefficient
- Hydrodynamic interactions greatly reduce the diffusion coefficient and create collective motions at cellular concentrations.

BD simulations with hydrodynamic effect on Spherical Model

Acknowledgement

Sugita Group in RIKEN (Wako & Kobe)

Feig Group in Michigan State University



- **Computational Resources**
  - RIKEN RICC
  - K computer
- **Research Fund**
  - SPIRE Field 1
  - RIKEN Internal funds (QBiC, AICS)