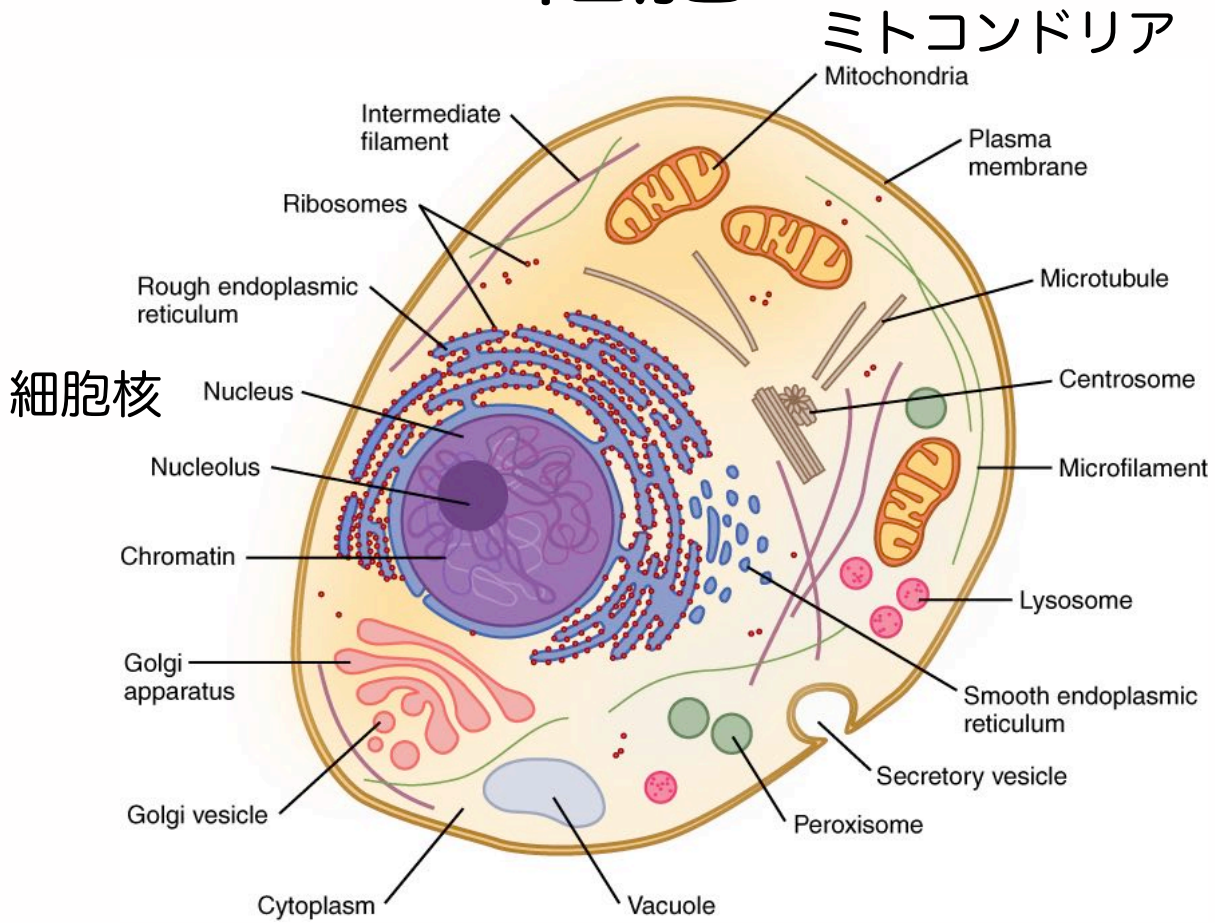
スパコンで迫る生体分子の働き

宮下治

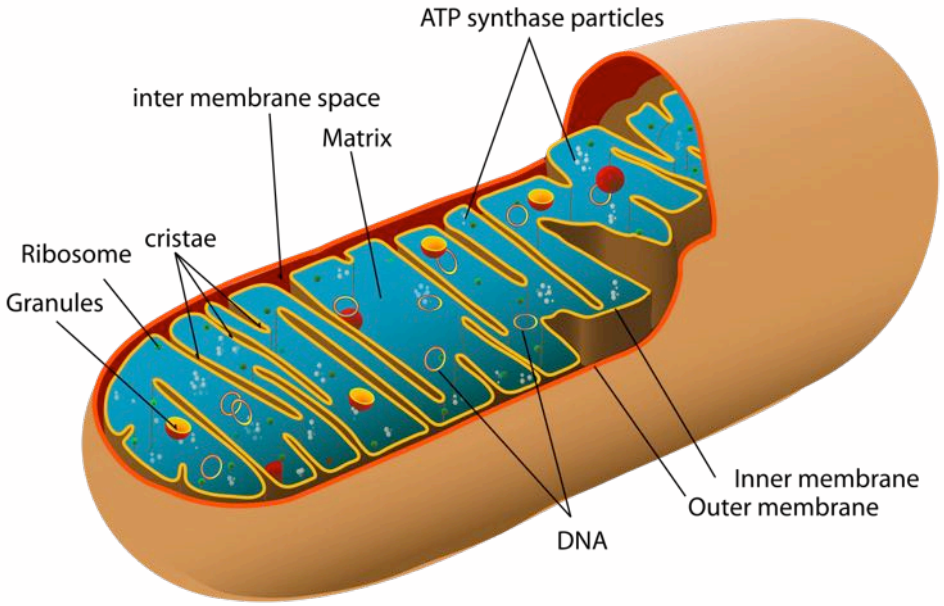
理化学研究所
計算科学研究機構
RIKEN Advanced Institute for Computational Science

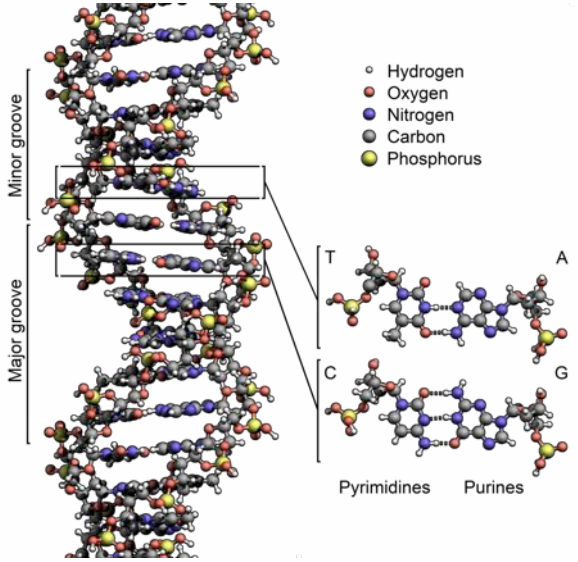
細胞

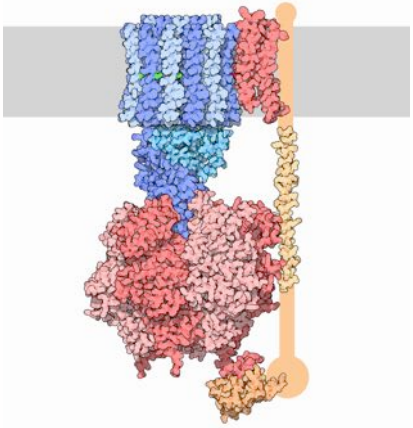


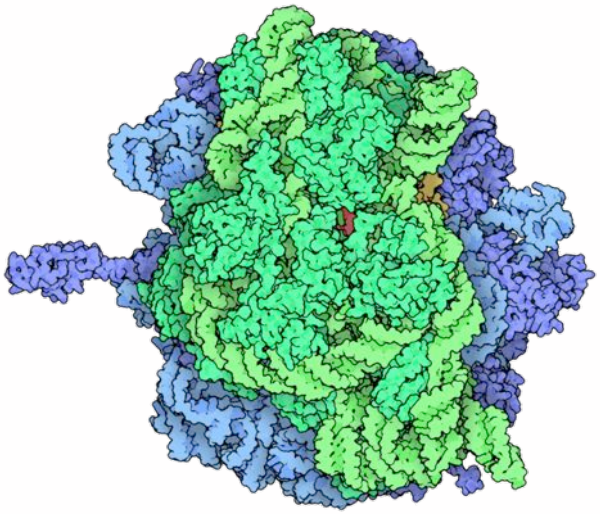
OpenStax College - Anatomy & Physiology, Connexions Web site.
http://cnx.org/content/col11496/1.6/

細胞から分子へ







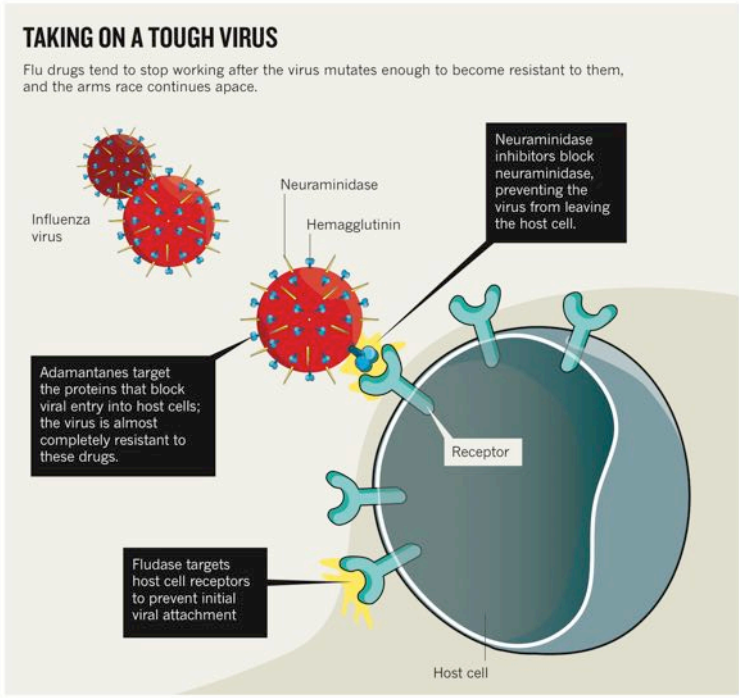


Credit: Cell: Mariana Ruiz Villarreal, ribosome & ATP synthase David Goodsell - RCSB PDB Molecule of the Month, DNA Zephyris

目的：生物分子を理解し医学へつなげる

インフルエンザウイルスが細胞に取り込まれる過程

R. Palmer, Nature 2011



DRUGS

Lines of defence

Antiviral treatments are a critical component of an effective healthcare response to influenza, but drug resistance to the treatment-of-choice has public health officials searching for other options.

Zachary Taylor, an infectious disease fellow at the Kaiser Permanente Fontana Medical Center in Sacramento, California. In part to safeguard against the possibility of such game-changing developments, drug developers are slowly filling the pipeline with alternative therapies (see 'Drugs to treat influenza infection'). Each drug come with side effects, which make them only worthwhile for those whom the flu could be potentially lethal — the elderly and the immunocompromised.

Given the wily history of the influenza virus, any sudden appearance of drug resistance is certain to concern public health officials. The first antiviral drugs to combat the disease — the adamantanes, which target the M2 channel protein to block virus entry into host cells — are now essentially useless. The US Centers for Disease Control and Prevention (CDC) found that 100 % of seasonal H3N2 flu in the 2009–2010 season and 99.8% of 2009 pandemic H1N1 flu were resistant to adamantanes.

Oseltamivir belongs to a class of drugs called neuraminidase inhibitors. These agents block the active site of a viral protein called neuraminidase (N), thereby arresting the influenza virus' ability to leave the host cell after it proliferates. The most common way for the influenza virus to evade oseltamivir is via the H275Y mutation (also known as H274Y) of neuraminidase, which replaces a single histidine amino acid with a tyrosine. This alteration interferes with the drug's ability to bind to the protein — a problem acknowledged by the maker of oseltamivir. "There remains a medical need and room for additional treatment options, especially for the management of severe infections and for improved pandemic preparedness," says Klaus Klumpp, Roche's top virologist. Klumpp says the Roche is supporting research into new therapies targeting viral replication as well as other mechanisms, but notes that these efforts are preclinical.

Fortunately, viruses with the H275Y mutation are still susceptible to a different neuraminidase

Mechanism-Based Covalent Neuraminidase Inhibitors with Broad-Spectrum Influenza Antiviral Activity

Jin-Hyo Kim,[1*†] Ricardo Resende,[1*] Tom Wennekes,[1*‡] Hong-Ming Chen,[1*] Nicole Bance,[2] Sabrina Buchini,[1§] Andrew G. Watts,[3] Pat Pilling,[4] Victor A. Streltsov,[4] Martin Petric,[5] Richard Liggins,[6] Susan Barrett,[4] Jennifer L. McKimm-Breschkin,[4] Masahiro Niikura,[2] Stephen G. Withers[1||]

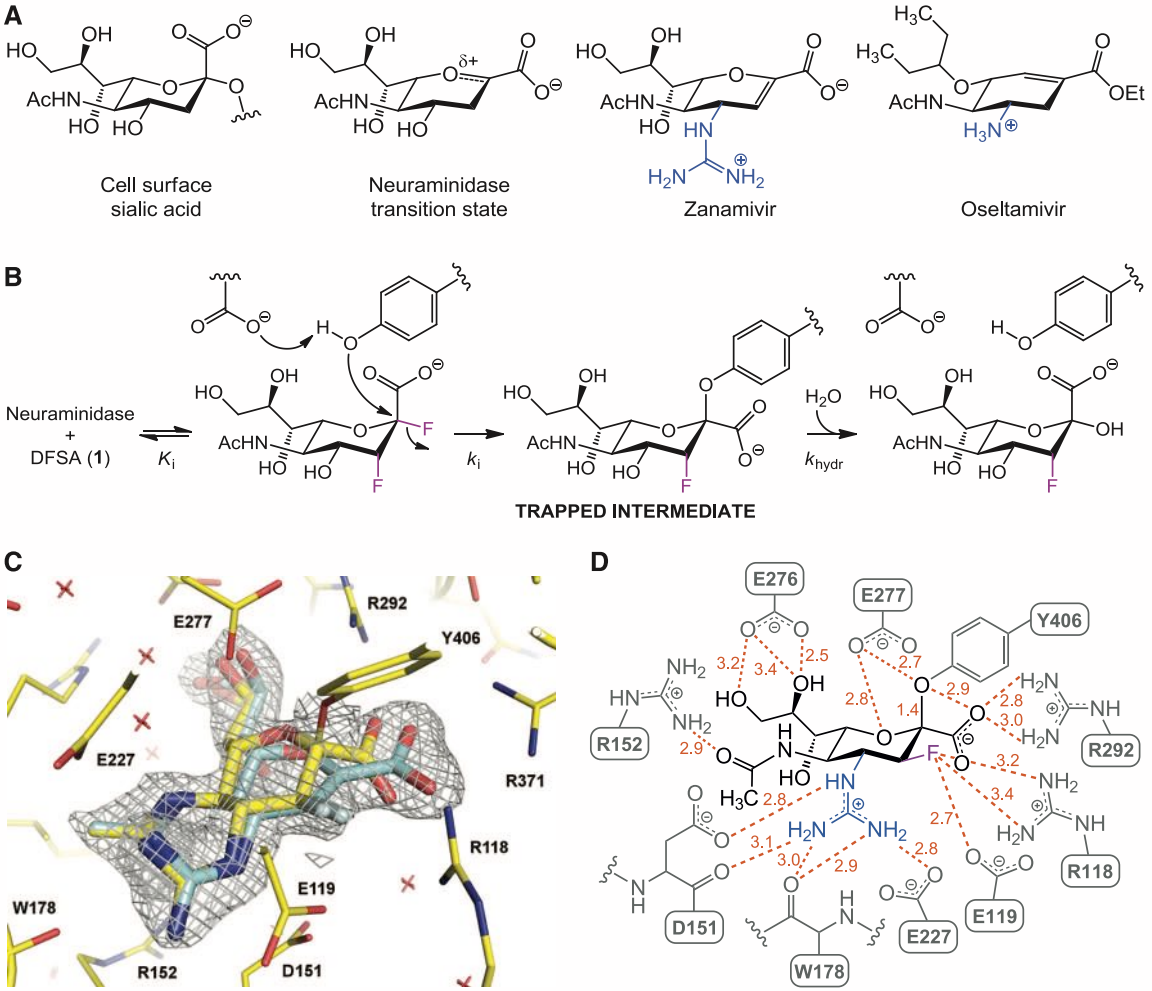


Fig. 1. Structures of key influenza therapeutics, mechanism of action of DFSAs, and x-ray structure of inhibited enzyme. (**A**) Chemical structures of cell surface sialic acids, the neuraminidase transition state, zanamivir (Relenza), and oseltamivir (Tamiflu). (**B**) Mechanism of action of the DFSAs. (**C**) X-ray crystallographic structure of the active site of the enzyme trapped as its 3-fluoro(eq)-4-guanidino-sialyl-enzyme intermediate (elimination product is in pale cyan) overlaid with omit (*22*) electron density map shown as a gray mesh contoured at 1σ within 1.6 Å of ligands. The electron density extends from the ligand molecule to Y406, suggesting a covalent link between the inhibitor's C-2 atom and the OH of Y406. (**D**) Diagram of interactions (orange dashed lines; distances in Å) with the sialic acid in the covalently inhibited enzyme. The corresponding diagram of interactions for the elimination product is shown in fig. S4.

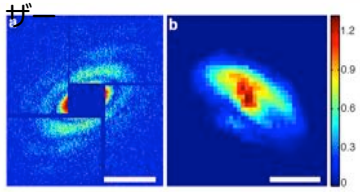
医学生物学に役立つ コンピュータによるデータ解析とシミュレーション

- 薬の開発には分子の原子構造と動きを理解する必要がある
- タンパク質は小さすぎて見えない
- X線や電子線を使った観測とデータ解析
- シミュレーションで分子の動きを再現し，理解する

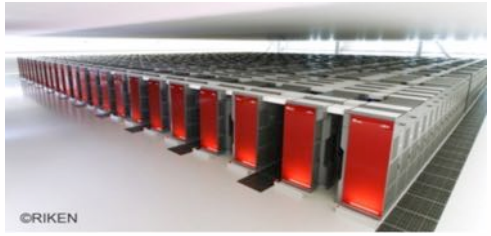
生物分子を見る実験方法

SPring-8 SACLA

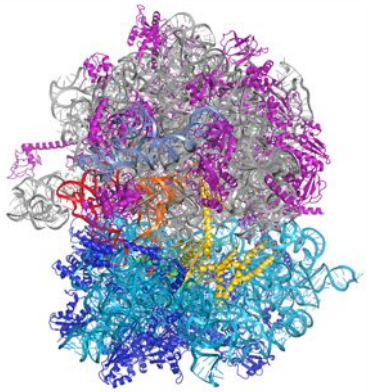
X線結晶解析，自由電子レーザー

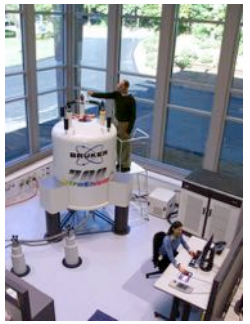


RNA sponge, Song, Gallagher-Jones, et al

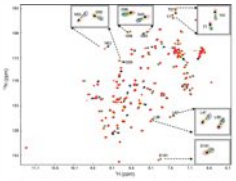


データ解析



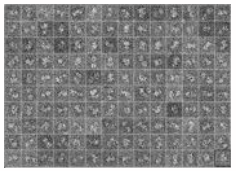


NMR



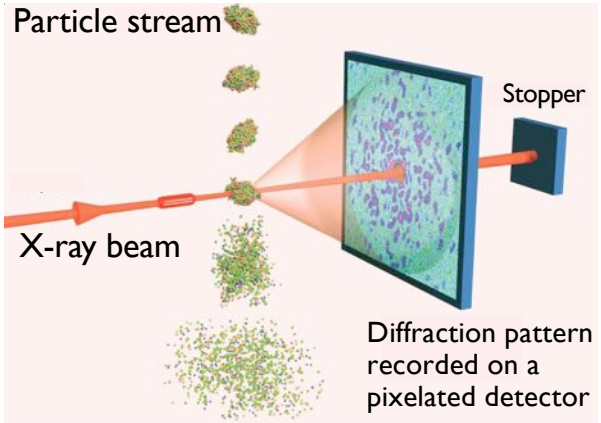
Cyanovirin-N, Sandstom, et al

電子顕微鏡

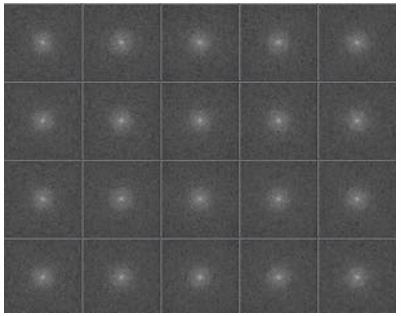


David J Morgan from Cambridge, UK - Tecnai 12 Electron Microscope

多数のデータを使った立体像の構築

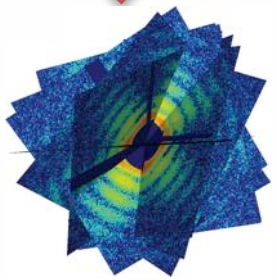


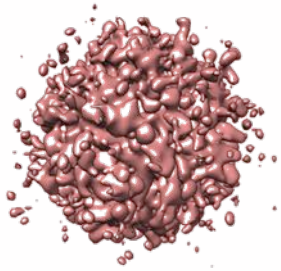
K. J. Gaffney and H. N. Chapman, Science (2007) 316 1444-1448



実験データ

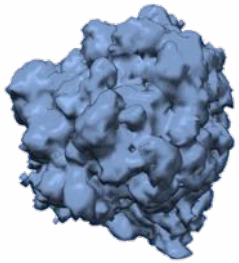
角度の推定





Structure Factor (3D structure in Fourier space)

phase retrieval



立体構造モデル

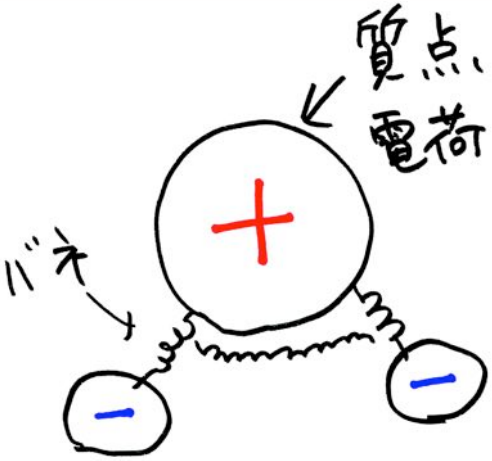
<u>スーパーコンピューターにより百万画像の解析を可能にする</u>

- 実験データをもとに決定した生体分子の構造は静止画像
- 実際は常に動いているが，観測は難しい
- 分子動力学シミュレーションにより，運動の様子を知ることができる．

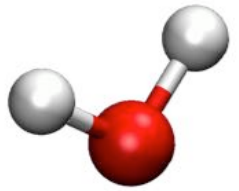
分子をプログラムで表現

水分子 H_2O

量子力学

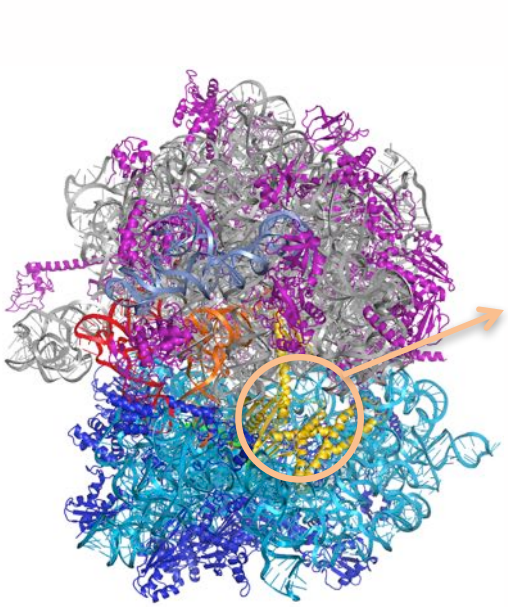


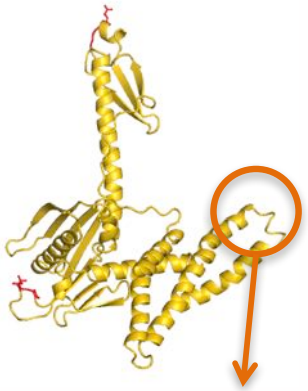
古典力学

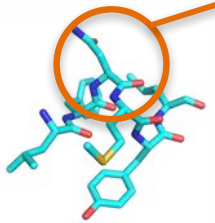


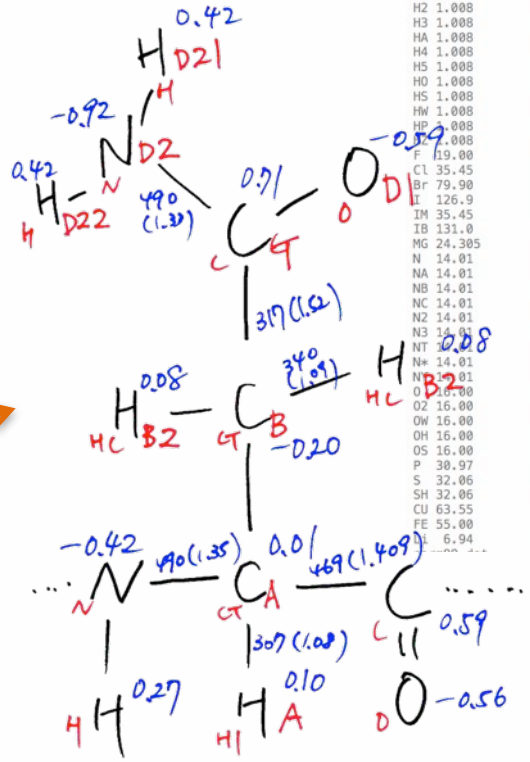
2013ノーベル化学賞

分子をプログラムで表現







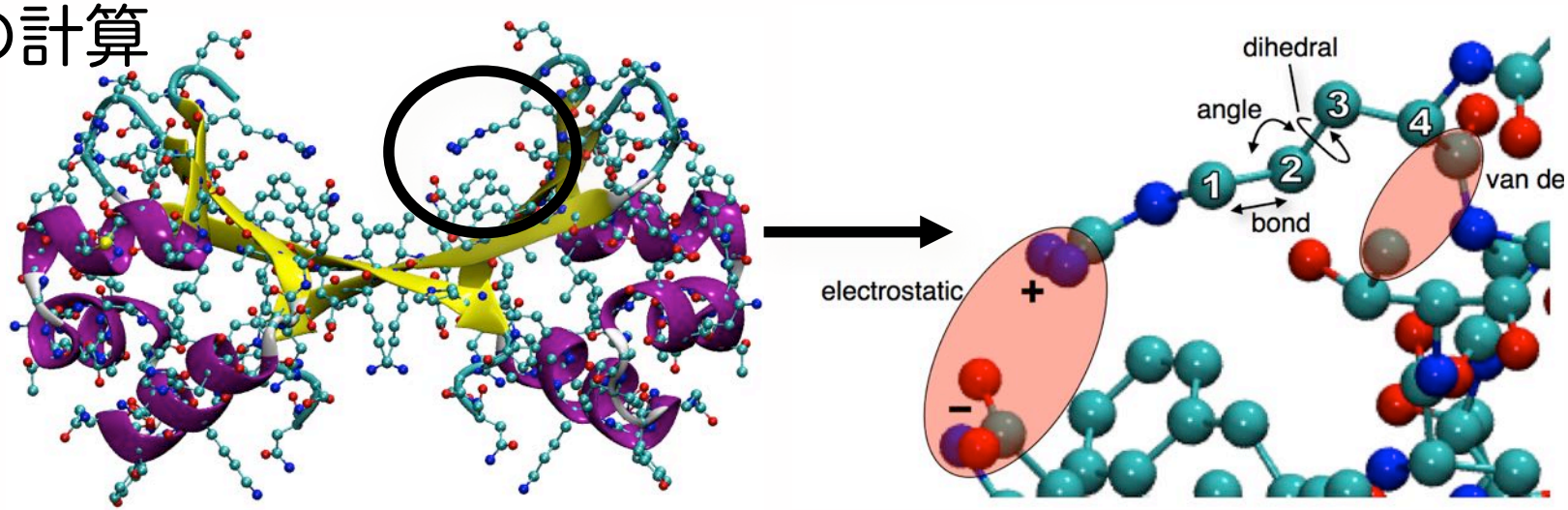


```
PARM99 for DNA,RNA,AA, organic molecules, TIP3P wat. Polariz.& LP incl.02/04/99
C  12.01          0.616  !            sp2 C carbonyl group
CA 12.01          0.360               sp2 C pure aromatic (benzene)
CB 12.01          0.360               sp2 aromatic C, 5&6 membered ring junction
CC 12.01          0.360               sp2 aromatic C, 5 memb. ring HIS
CD 12.01          0.360               sp2 C atom in the middle of: C=CD-CD=C
CK 12.01          0.360               sp2 C 5 memb.ring in purines
CM 12.01          0.360               sp2 C  pyrimidines in pos. 5 & 6
CN 12.01          0.360               sp2 C aromatic 5&6 memb.ring junct.(TRP)
CQ 12.01          0.360               sp2 C in 5 mem.ring of purines between 2 N
CR 12.01          0.360               sp2 arom as CQ but in HIS
CT 12.01          0.878               sp3 aliphatic C
CV 12.01          0.360               sp2 arom. 5 memb.ring w/1 N and 1 H (HIS)
CW 12.01          0.360               sp2 arom. 5 memb.ring w/1 N-H and 1 H (HIS)
C* 12.01          0.360               sp2 arom. 5 memb.ring w/1 subst. (TRP)
CY 12.01          0.360               nitrile C (Howard et al.JCC,16,243,1995)
CZ 12.01          0.360               sp C (Howard et al.JCC,16,243,1995)
C0 40.08                              calcium
H  1.008          0.161               H bonded to nitrogen atoms
HC 1.008          0.135               H aliph. bond. to C without electrwd.group
H1 1.008          0.135               H aliph. bond. to C with 1 electrwd. group
H2 1.008          0.135               H aliph. bond. to C with 2 electrwd.groups
H3 1.008          0.135               H aliph. bond. to C with 3 eletrwd.groups
HA 1.008          0.167               H arom. bond. to C without elctrwd. groups
H4 1.008          0.167               H arom. bond. to C with 1 electrwd. group
H5 1.008          0.167               H arom.at C with 2 elctrwd. gr,+HCOO group
HO 1.008          0.135               hydroxyl group
HS 1.008          0.135               hydrogen bonded to sulphur (pol?)
HW 1.008          0.000               H in TIP3P water
HP 1.008          0.135               H bonded to C next to positively charged gr
H  1.008          0.161               H bond sp C (Howard et al.JCC,16,243,1995)
F  19.00          0.320               fluorine
Cl 35.45          1.910               chlorine  (Applequist)
Br 79.90          2.880               bromine  (Applequist)
I  126.9          4.690               iodine  (Applequist)
IM 35.45          3.235               assumed to be Cl- (ion minus)
IB 131.0                              'big ion w/ waters' for vacuum (Na+, 6H2O)
MG 24.305         0.120               magnesium
N  14.01          0.530               sp2 nitrogen in amide groups
NA 14.01          0.530               sp2 N in 5 memb.ring w/H atom (HIS)
NB 14.01          0.530               sp2 N in 5 memb.ring w/LP (HIS,ADE,GUA)
NC 14.01          0.530               sp2 N in 6 memb.ring w/LP (ADE,GUA)
N2 14.01          0.530               sp2 N in amino groups
N3 14.01          0.530               sp3 N for charged amino groups (Lys, etc)
NT 14.01          0.530               sp3 N for amino groups amino groups
N* 14.01          0.530               sp2 N
NY 14.01          0.530               nitrile N (Howard et al.JCC,16,243,1995)
O  16.00          0.434               carbonyl group oxygen
O2 16.00          0.434               carboxyl and phosphate group oxygen
OW 16.00          0.000               oxygen in TIP3P water
OH 16.00          0.465               oxygen in hydroxyl group
OS 16.00          0.465               ether and ester oxygen
P  30.97          1.538               phosphate,pol:JACS,112,8543,90,K.J.Miller
S  32.06          2.900               S in disulfide linkage,pol:JPC,102,2399,98
SH 32.06          2.900               S in cystine
CU 63.55                              copper
FE 55.00                              iron
LI  6.94          0.029               lithium, ions pol:J.PhysC,11,1541,(1978)
```

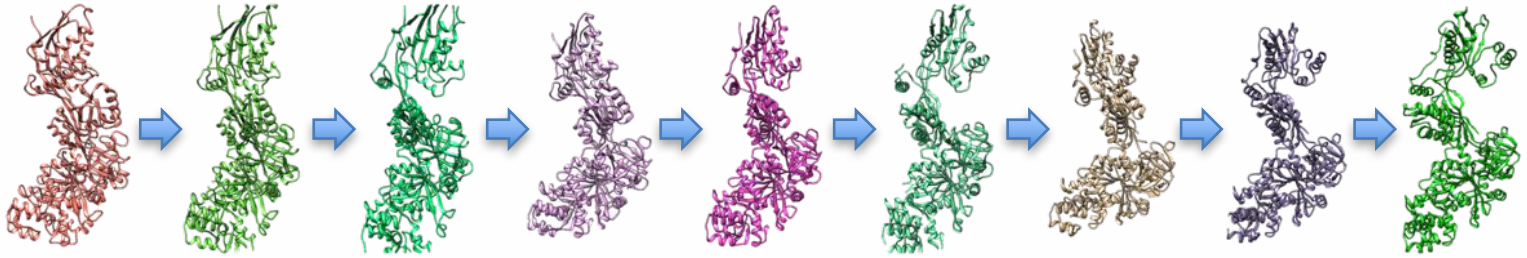
パラメーター

分子の動きをコンピューターで再現

1．力の計算



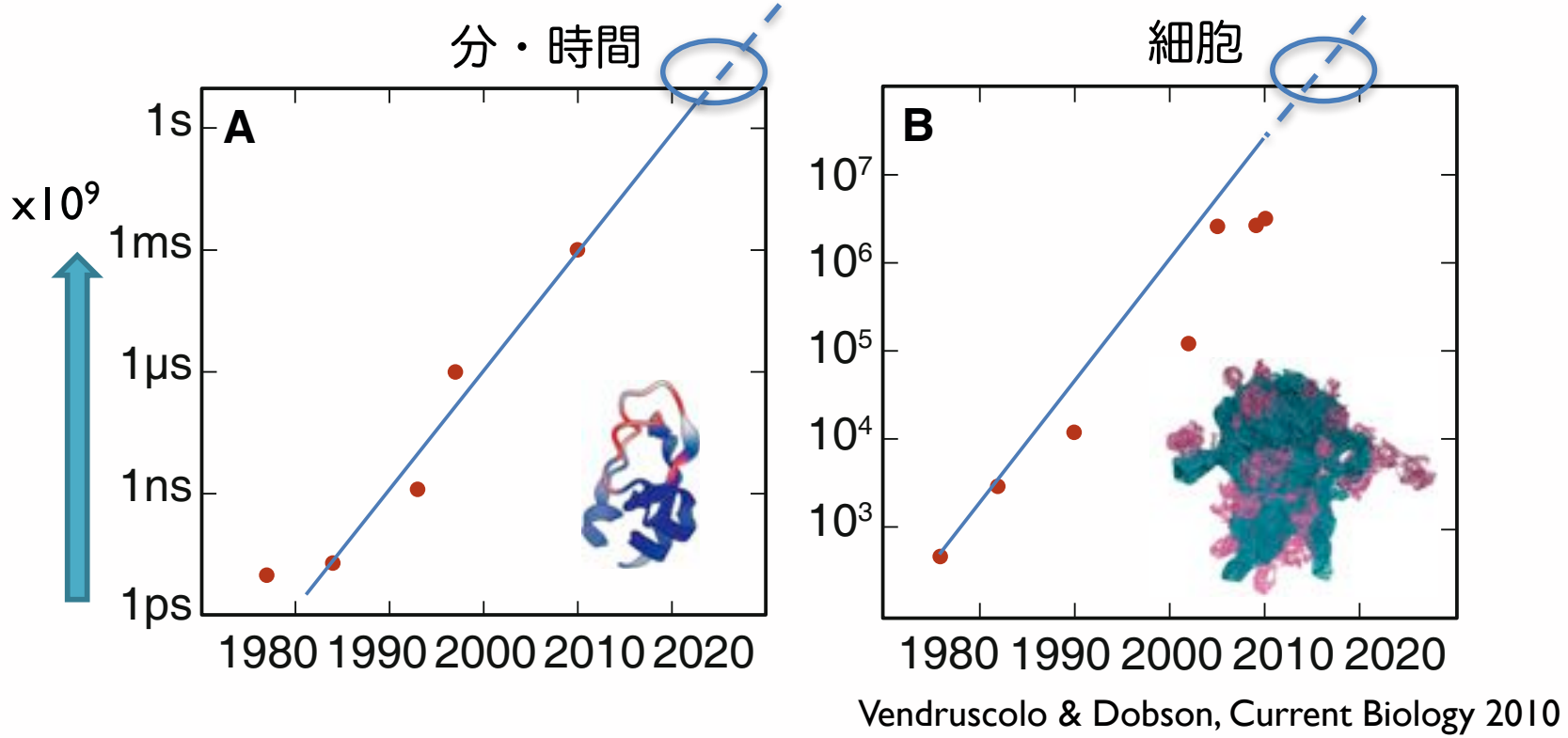
2．ニュートンの運動方程式を解く（少しずつ近似的に）



1回のサイクル
=フェムト秒
=10^{-15}秒

膨大な計算量

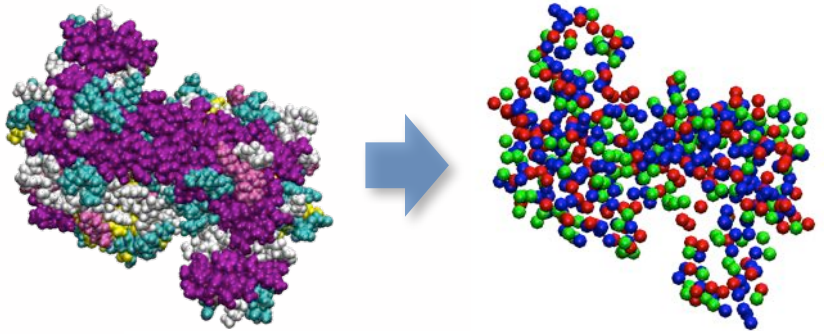
- 生物は複雑
- 現在のシミュレーションは，単純なごく一部のほんの一瞬



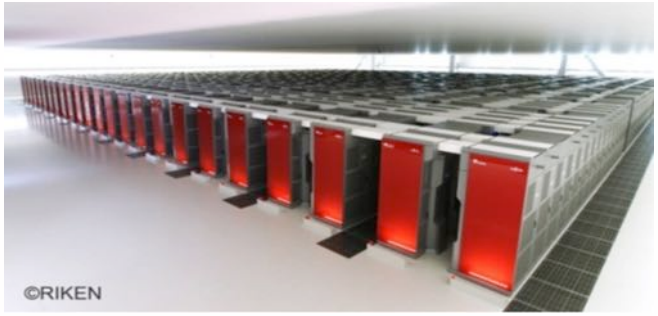
Vendruscolo & Dobson, Current Biology 2010

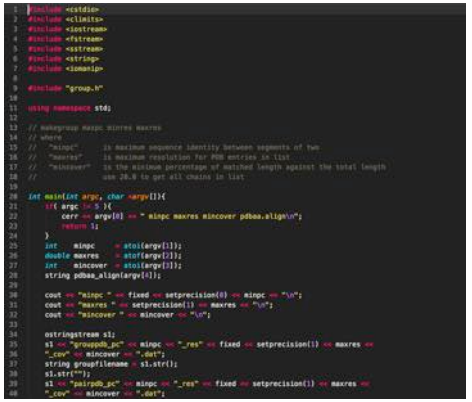
長いシミュレーションをする工夫

- 粗視化モデル
- 拡張シミュレーション
- スーパーコンピューター
- 並列化プログラム



$$P(k_i \leftrightarrow k_j) = \begin{cases} 1 & \text{for } \Delta \le 0 \\ \exp(-\beta\Delta) & \text{for } \Delta > 0 \end{cases}$$





Atomistic bacterial cytoplasm simulation

eLIFE Research article Biophysics and Structural Biology | Computational and Systems Biology

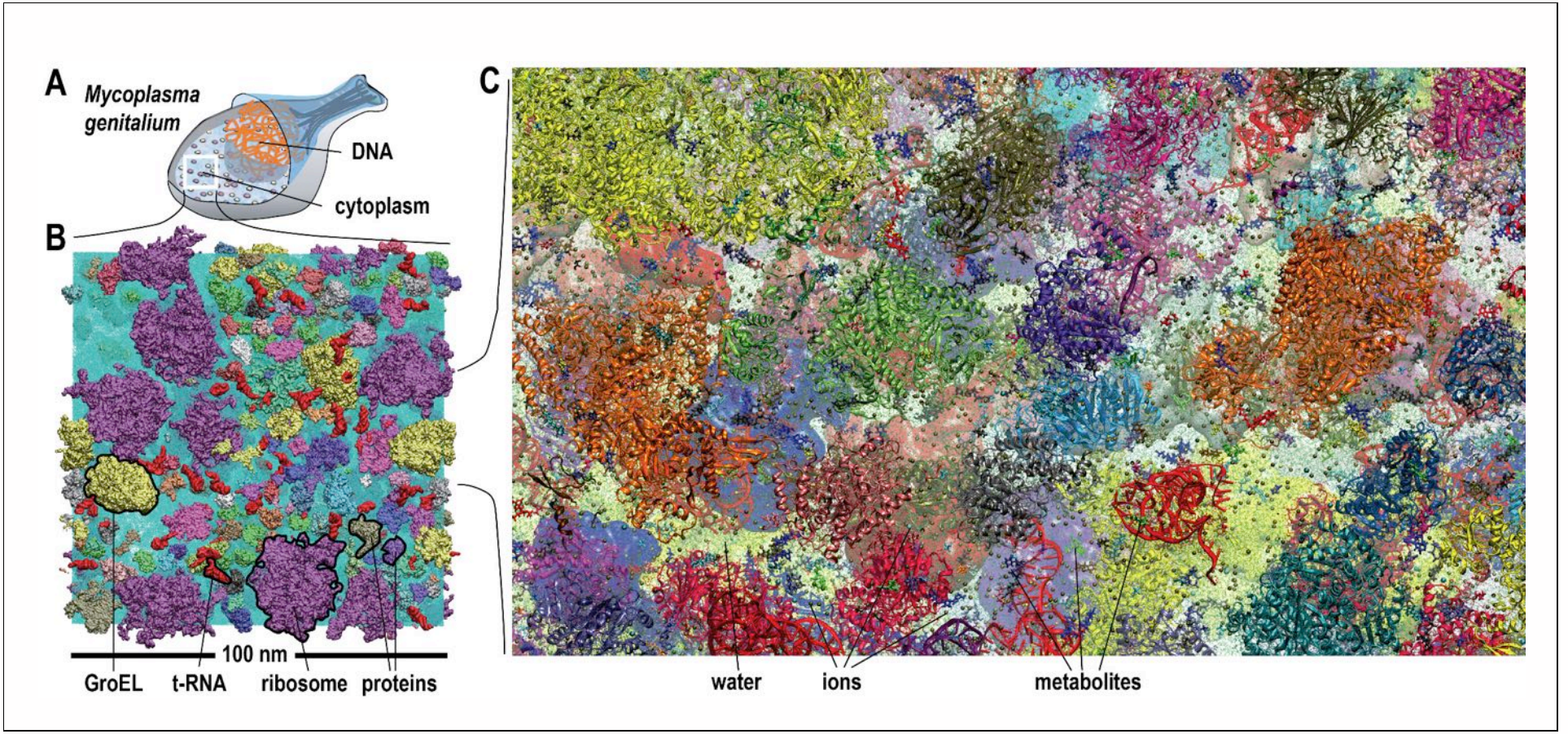


Figure 1. Molecular model of a bacterial cytoplasm. (**A**) Schematic illustration of *Mycoplasma genitalium* (*MG*). (**B**) Equilibrated MG_h system highlighted with proteins, tRNA, GroEL, and ribosomes. (**C**) MG_h cl ose-up showing atomistic level of detail. See also supplementary ***Figures 1*** and ***2*** for structures of individual macromolecules and metabolites as well as supplementary ***Figure 3*** for initial configurations of the simulated systems.

DOI: 10.7554/eLife.19274.003

スパコンで迫る生体分子の働き

- 生体分子の働く仕組みを知り，医療につなげる.
- 実験による観測とコンピューターによるデータ解析
- 動きを理解するための運動シミュレーション

謝辞

- 理研AICS
  松永康佑，杉田有治，Florence Tama，土井陽子
- 公益財団法人計算科学振興財団
  研究拠点（COE)形成推進事業