

第1回 先進的観測技術研究会
—局所構造解析, イメージングの最前線—

2012年12月26日
於 小林ホール、KEK

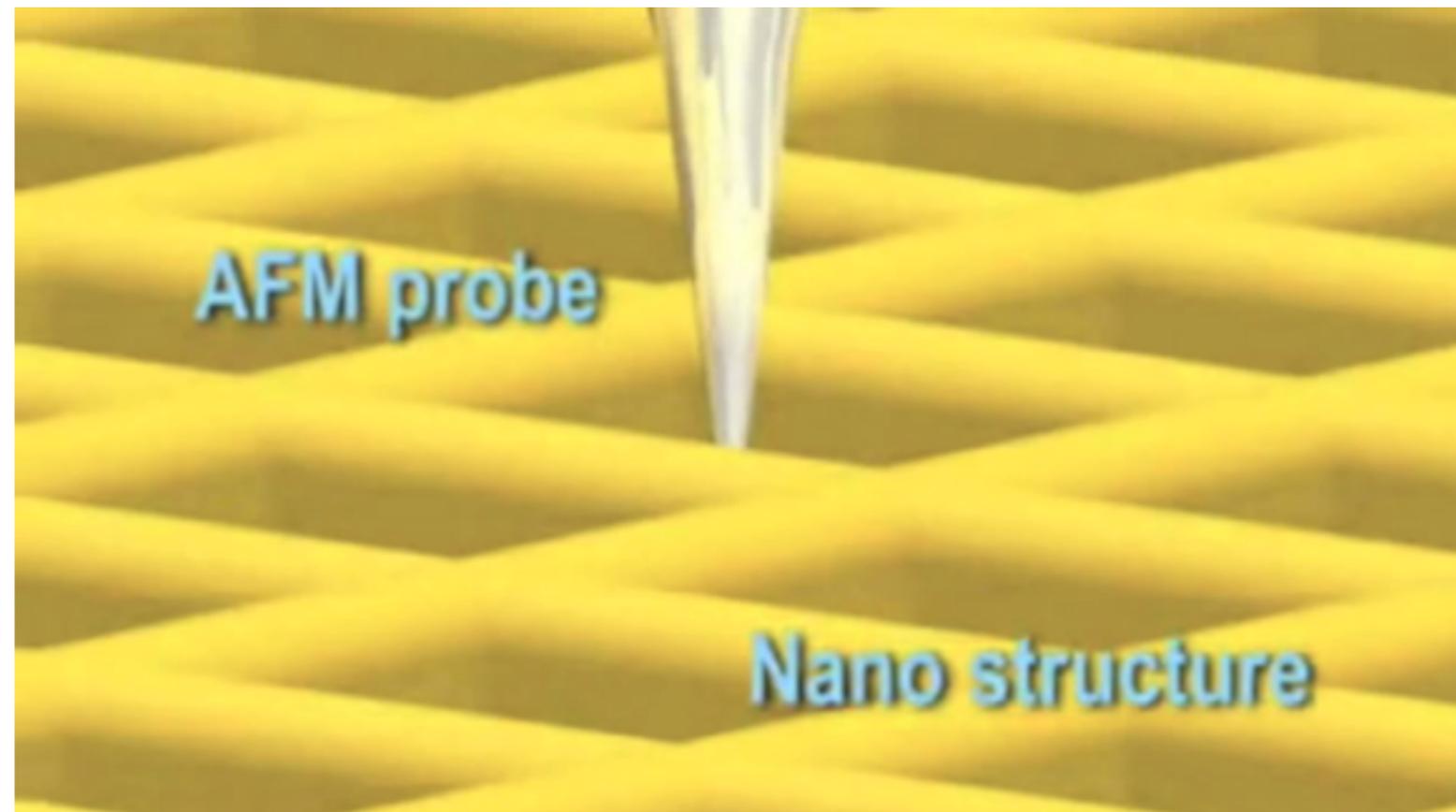
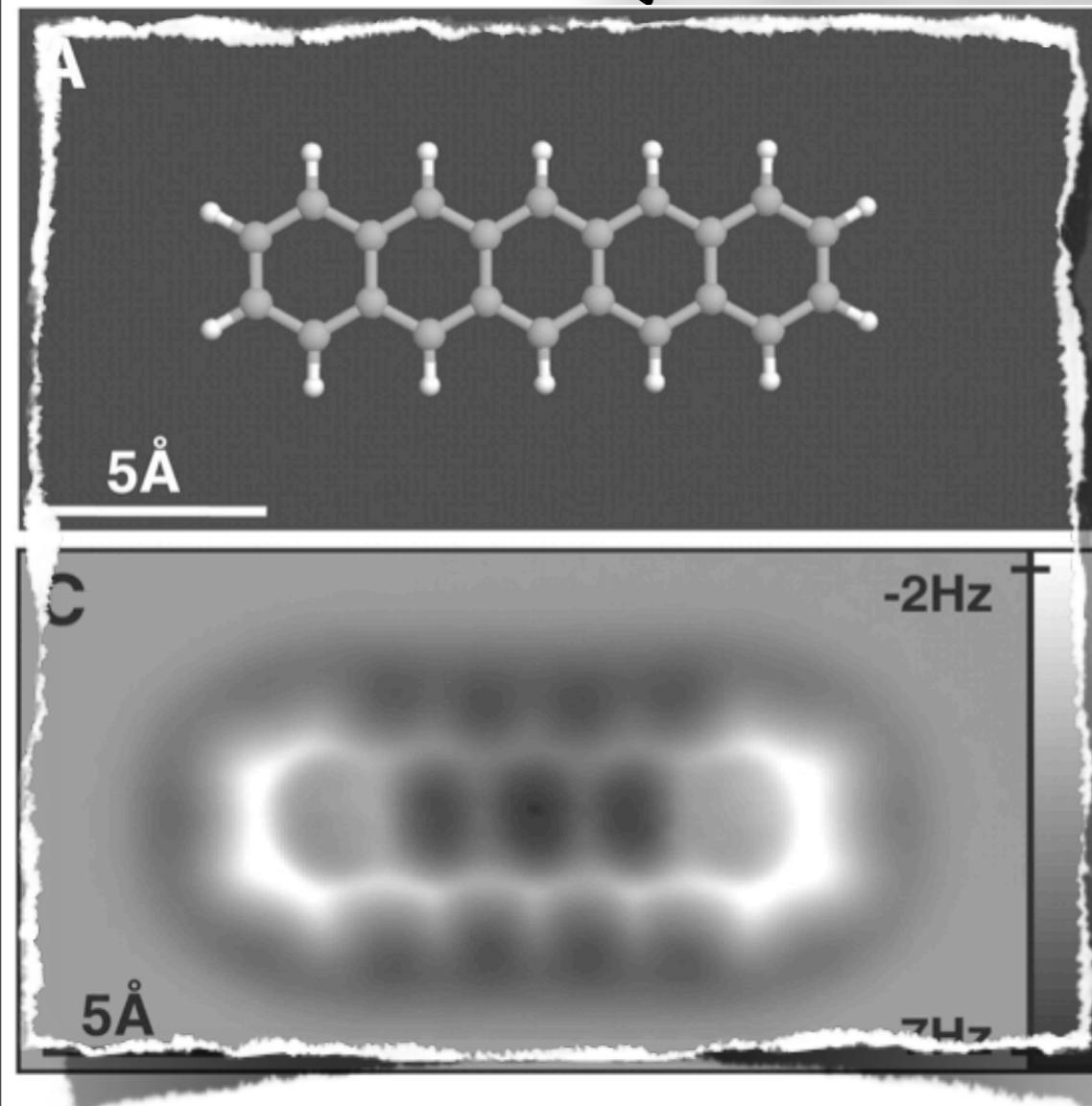
高速原子間力顕微鏡による生体 分子のダイナミクス解析

内橋 貴之, 安藤 敏夫

金沢大学 理工研究域・バイオAFM先端研究センター

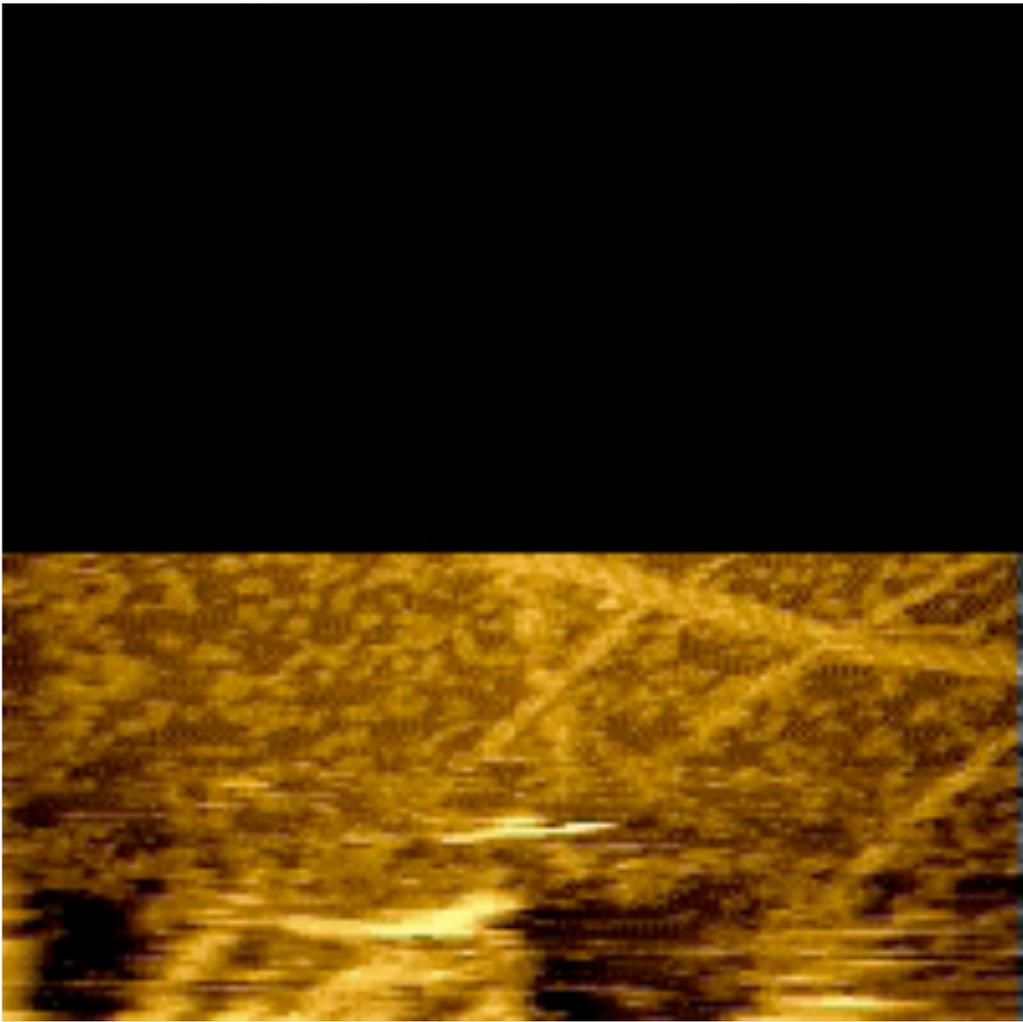
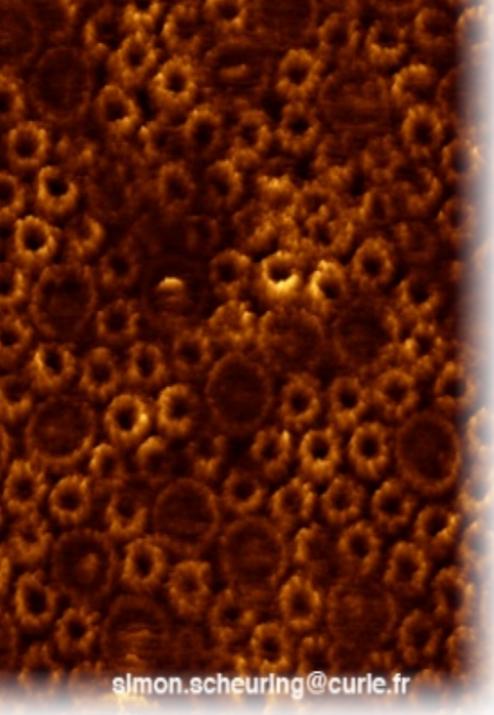
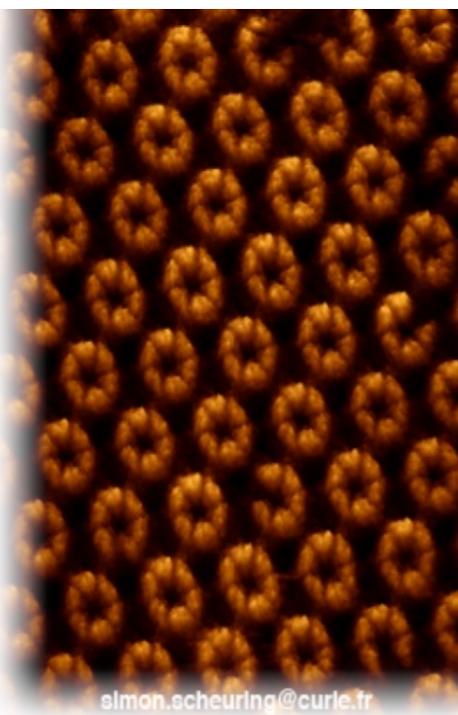
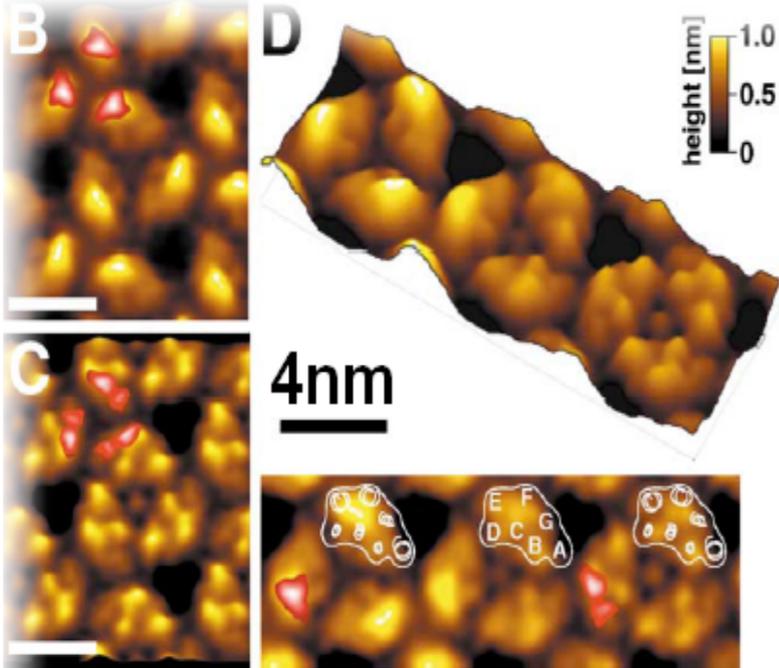


原子間力顕微鏡 (Atomic Force



- * 高い空間分解能 ($< 1 \text{ nm}$)
- * 試料 (絶縁性、導電性)
- * 測定環境 (大気、真空、溶液)

AFMによる生体試料イメージング



* 画像取得時間 (> 1 min)

➡ 静止画像

* 触針型プローブ

➡ 試料の破壊

初期のタイムラプスイメージング

1989年

Imaging Crystals, Polymers, and Processes in Water with the Atomic Force Microscope

B. DRAKE, C. B. PRATER, A. L. WEISENHORN, S. A. C. GOULD, T. R. ALBRECHT, C. F. QUATE, D. S. CANNELL, H. G. HANSMA, P. K. HANSMA

The atomic force microscope (AFM) can be used to image the surface of both conductors and nonconductors even if they are covered with water or aqueous solutions. An AFM was used that combines microfabricated cantilevers with a previously described optical lever system to monitor deflection. Images of mica demonstrate that atomic resolution is possible on rigid materials, thus opening the possibility of atomic-scale corrosion experiments on nonconductors. Images of poly-alanine, an amino acid polymer, show the potential of the AFM for revealing the structure of molecules important in biology and medicine. Finally, a series of ten images of the polymerization of fibrin, the basic component of blood clots, illustrate the potential of the AFM for revealing subtle details of biological processes as they occur in real time.

THE ATOMIC FORCE MICROSCOPE (AFM) (1) gives topographic images by scanning a sharp tip over a surface (2) and has been used to produce atomic-resolution images of both conductors (3) and nonconductors (4). Its published technological applications already include atomic-scale friction measurements (5), imaging of magnetic fields above thin-film recording heads (6), imaging of polymers (7), and imaging of photoresist on silicon (8).

The images we present in this report show that the AFM can be used on a large and important class of systems: nonconductors

covered with aqueous solutions. This class includes many important systems in biology, medicine, and technology, from mitochondria in cytoplasm to painted ships in seawater. The AFM obtains images fast enough (a few seconds per image) to observe many biological and chemical processes in real time.

A new, gentler and more reliable AFM

B. Drake, C. B. Prater, A. L. Weisenhorn, S. A. C. Gould, D. S. Cannell, H. G. Hansma, P. K. Hansma, Department of Physics, University of California, Santa Barbara, CA 93106.

T. R. Albrecht and C. F. Quate, Department of Applied Physics, Stanford University, Stanford, CA 94305.

SCIENCE, VOL. 243

fibrinの重合過程

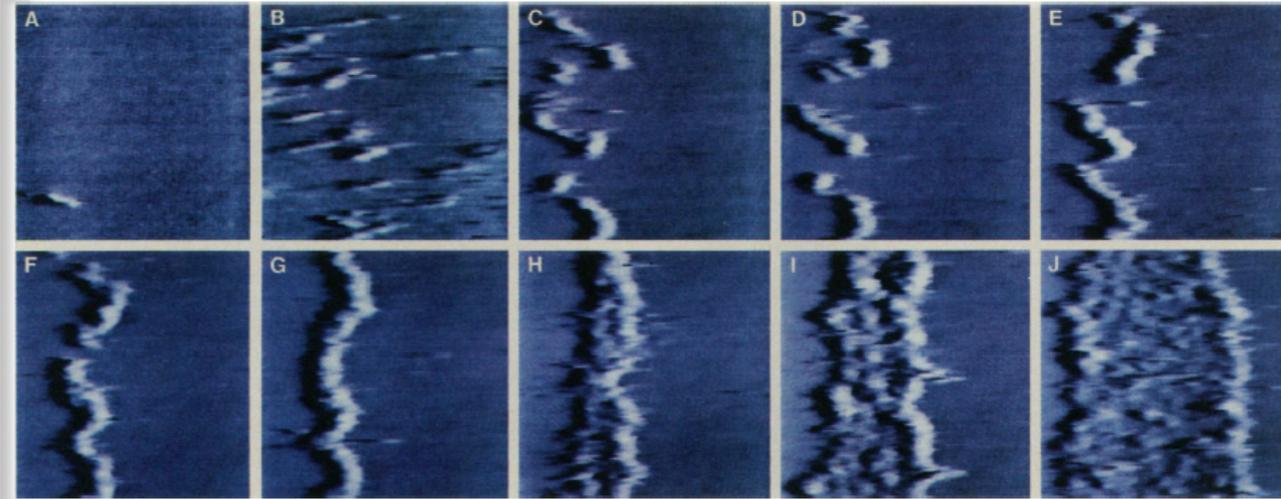


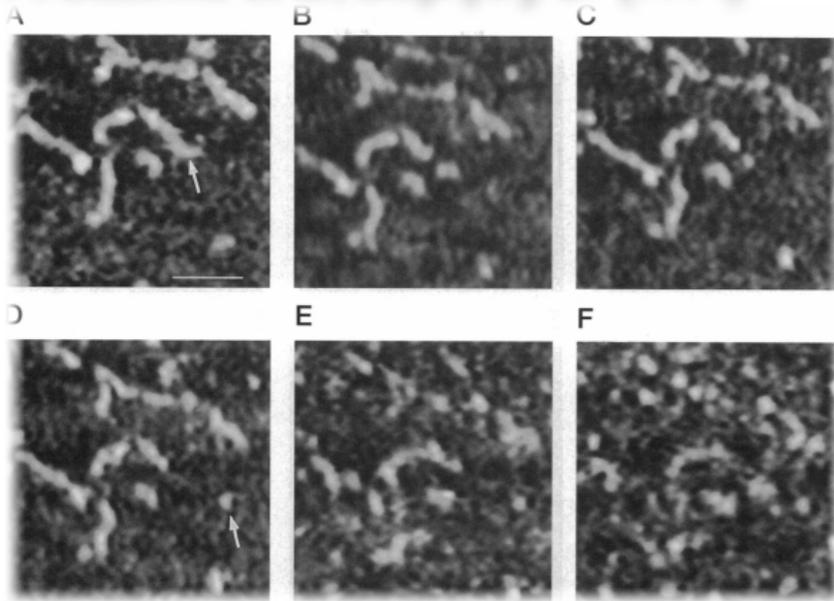
Fig. 3. Ten AFM images from a video cassette recorder tape that show clotting of the human blood protein fibrinogen in real time. The images were selected from before introduction of the clotting enzyme thrombin (A), and at various times after its introduction: 9 min (9'), 10 min 20 s (10'20"), 10'30", 11'20", 12'10", 12'40", 14'50", 17'10", 33' for (B) through (J). Each image area is 4500 Å by 4500 Å.

コンタクトモード

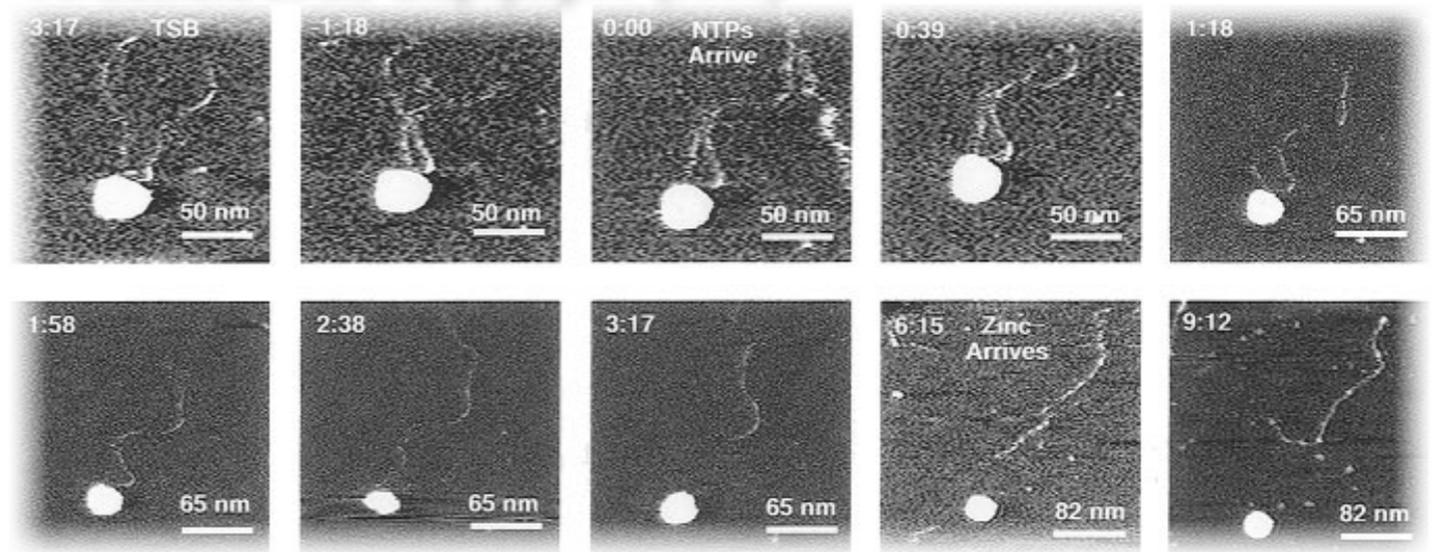
溶液中

時間分解能 ~1分

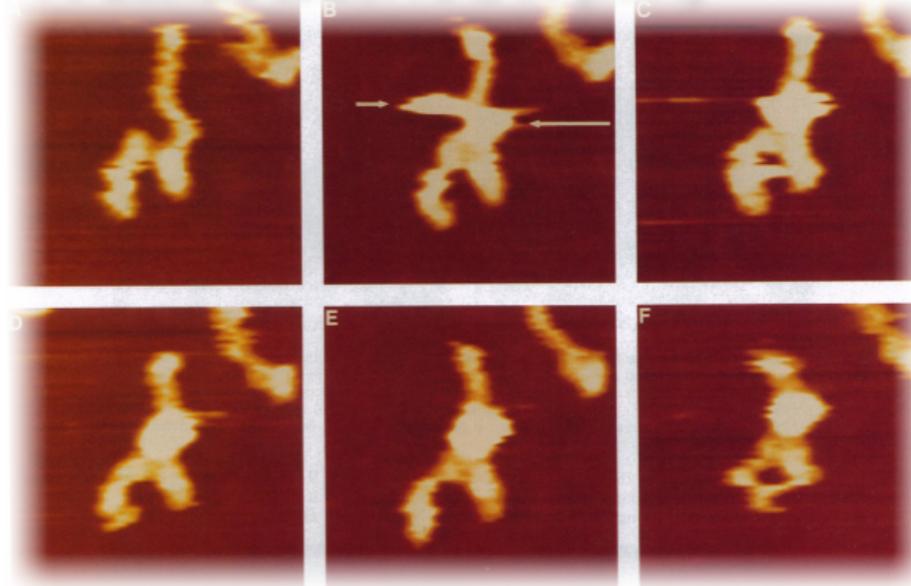
M. Bezanilla et al., Biophys. J. 67 (1994)



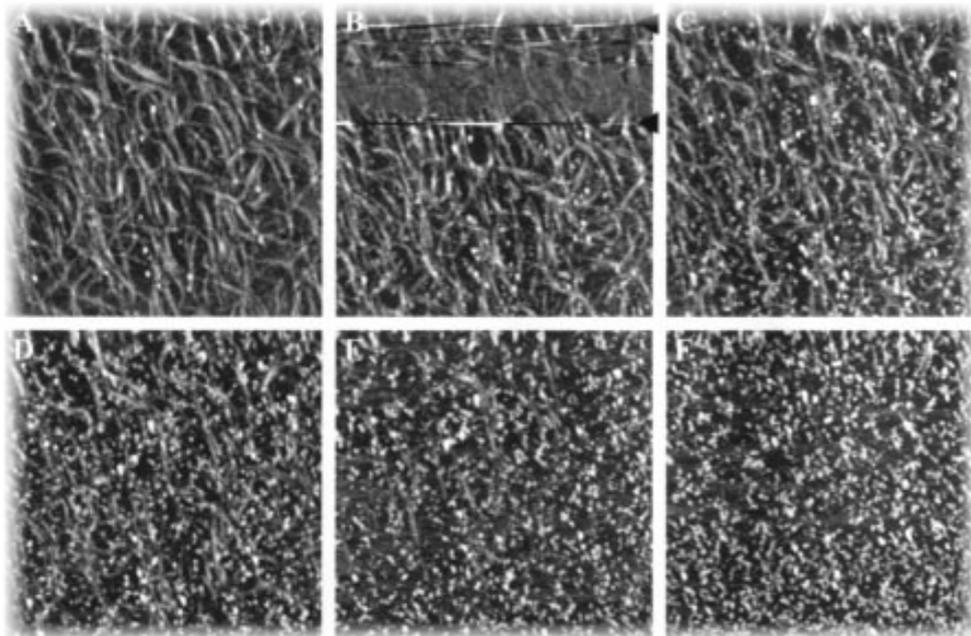
S. Kasas et al., Biochemistry 36 (1997)
M. Guthold et al., Biophys. J. 77 (1999)



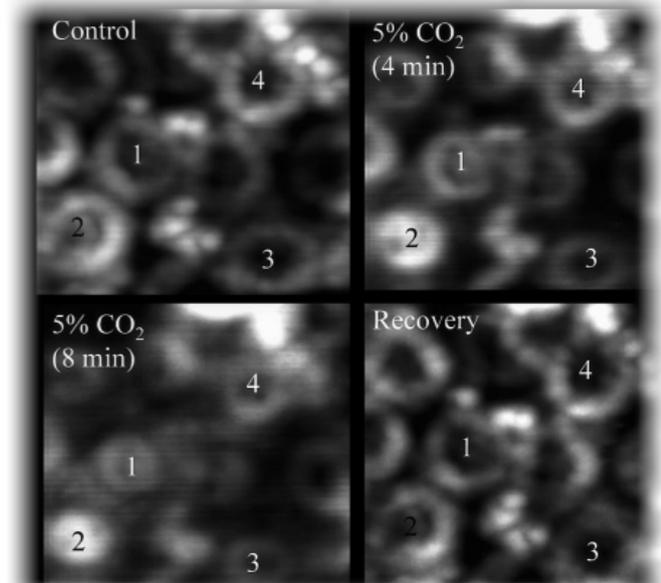
M. Guthold et al., PNAS 91 (1994)



H. Lin et al., Biochemistry 38 (1999)



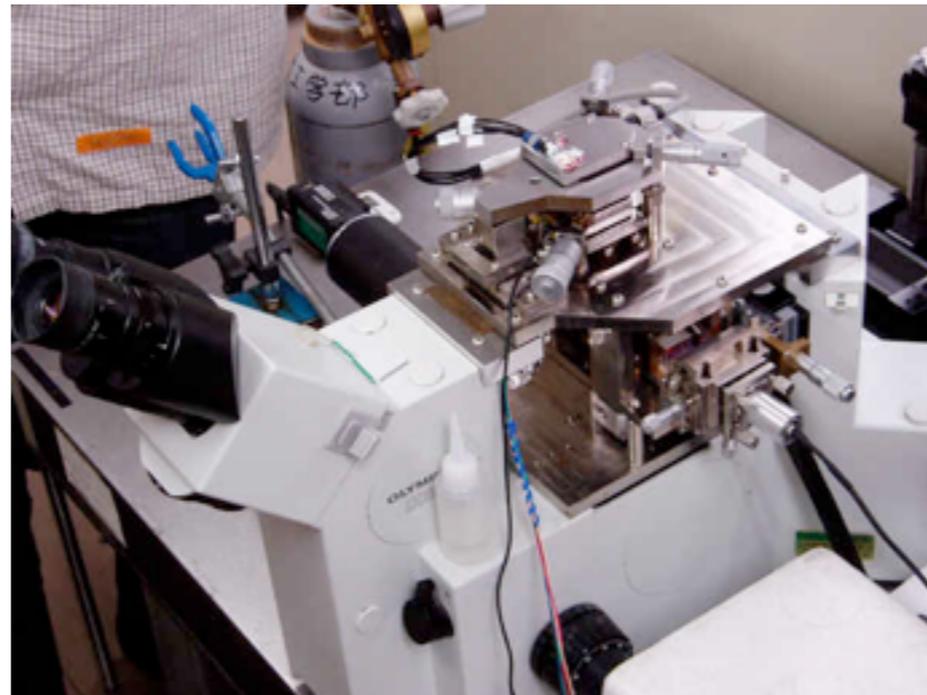
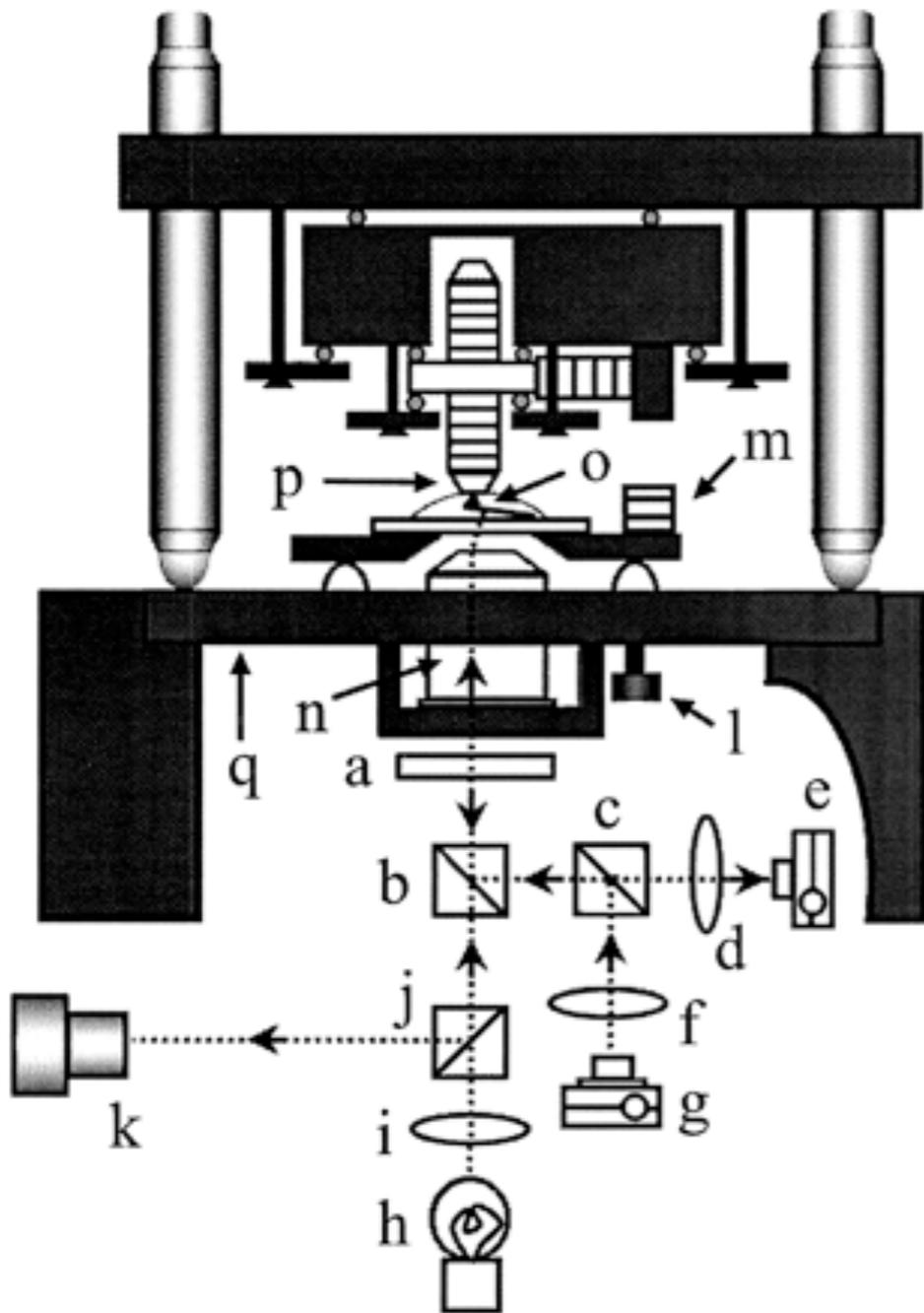
H. Oberleithner et al., Pflügers Arch – Eur J Physiol 439 (2000)



タッピングモード
~1分/frameで測定

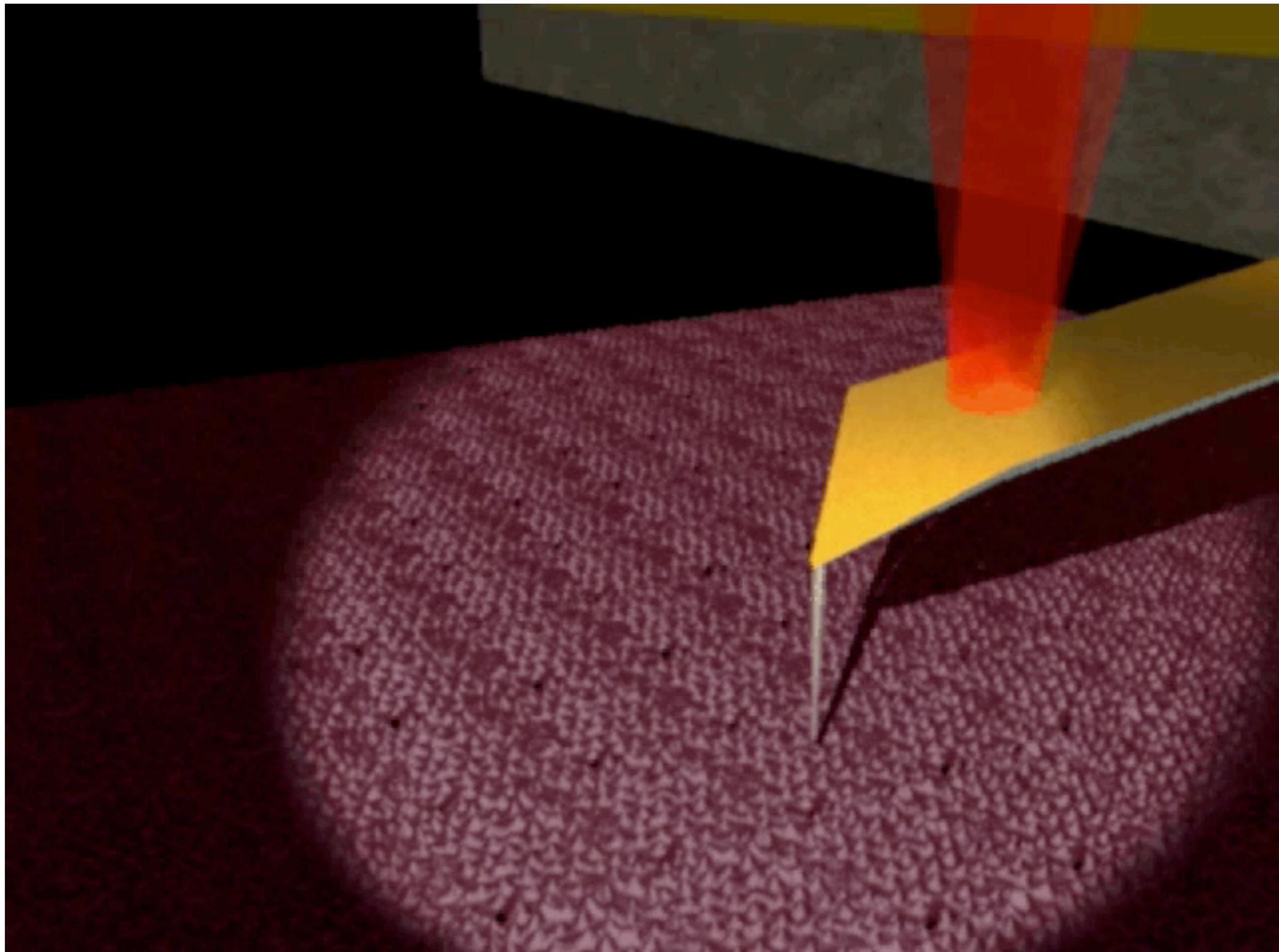
高速AFM (2001~)

T. Ando, N. Kodera, E. Takai, D. Maruyama, K. Saito, and A. Toda, A High-speed atomic force microscope for studying biological macromolecules, *Proc. Natl. Acad. Sci. USA* **98**:12468-12472 (2001).



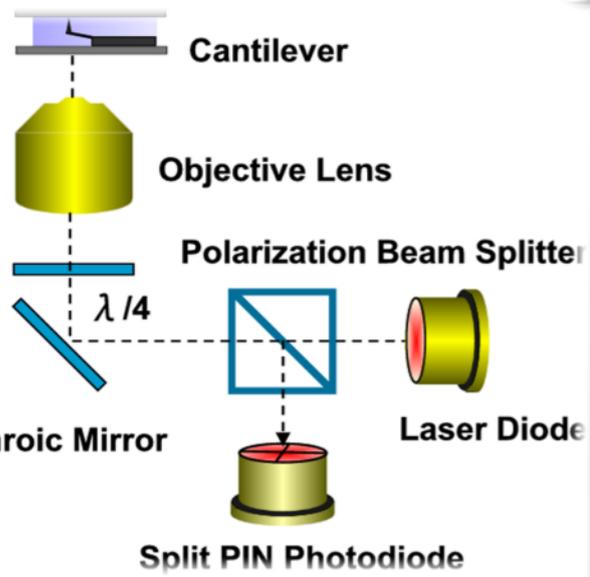
80ms/frame



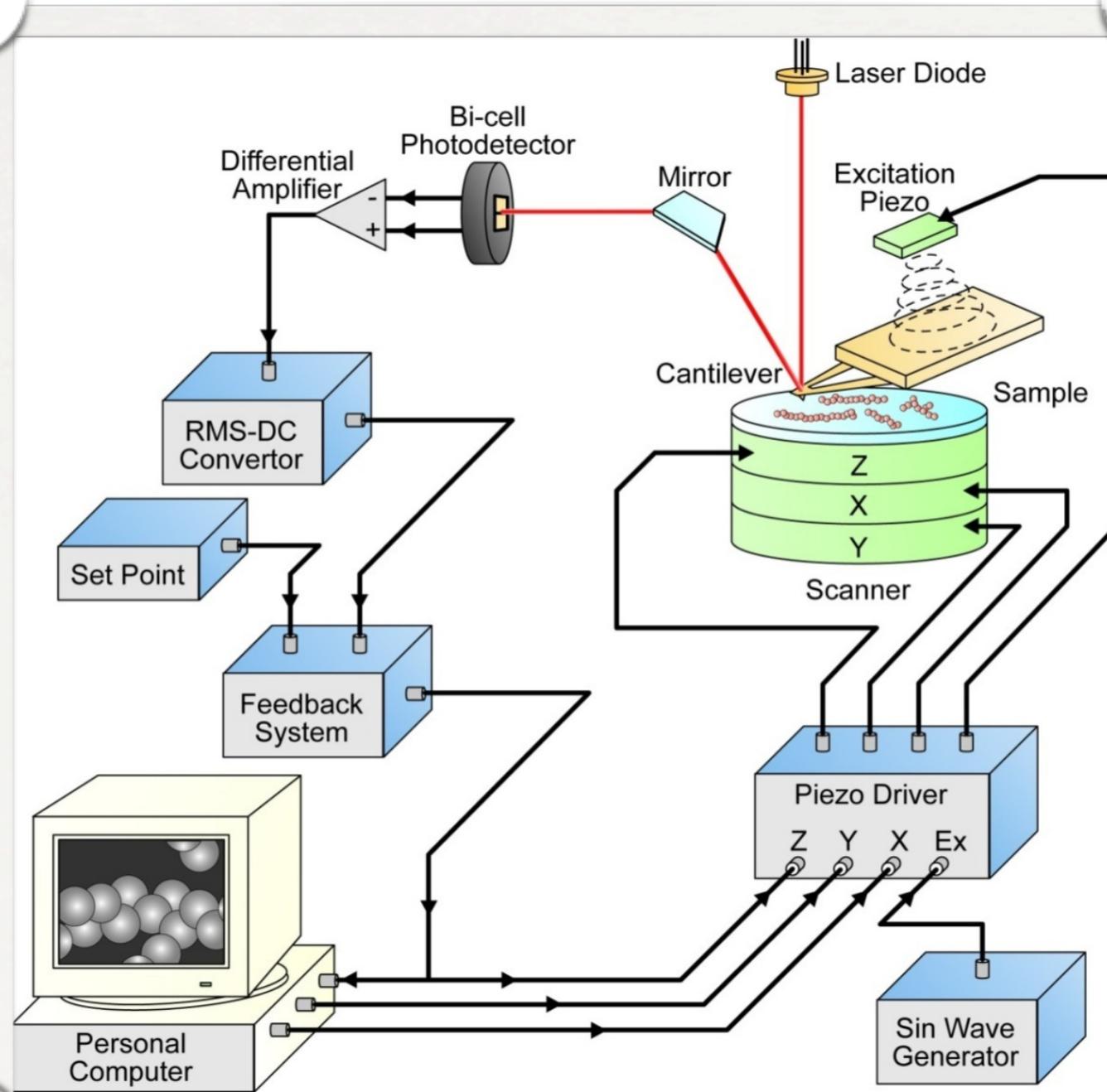
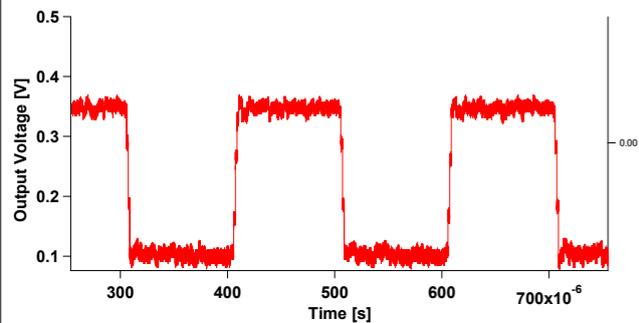


高速化のための要素技術

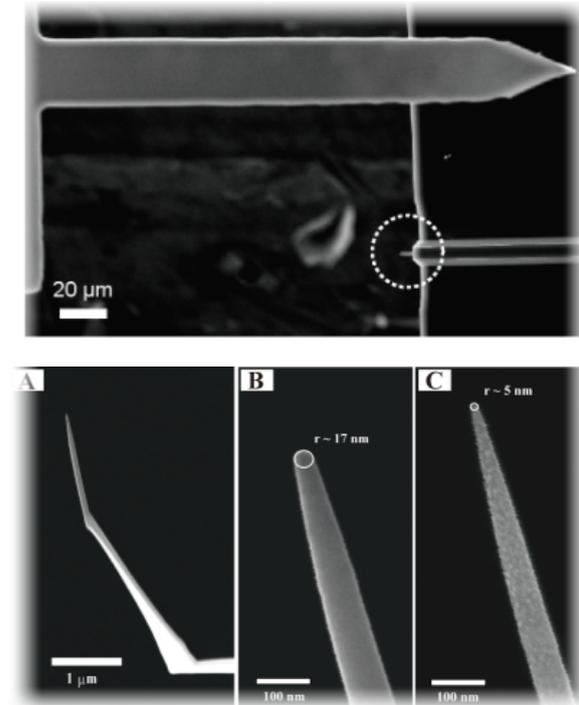
OBD detector



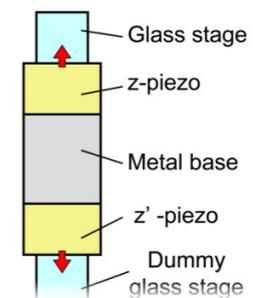
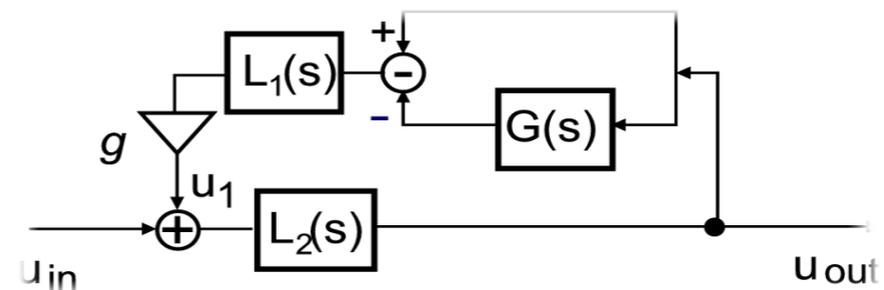
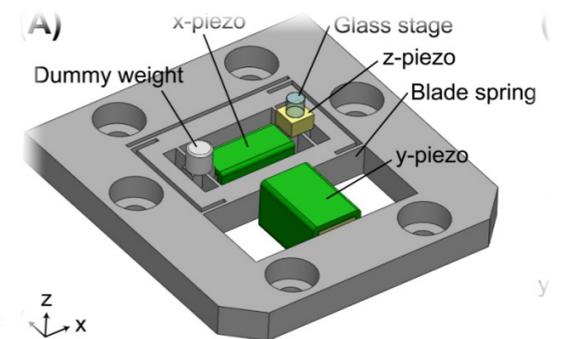
Fast amplitude detector



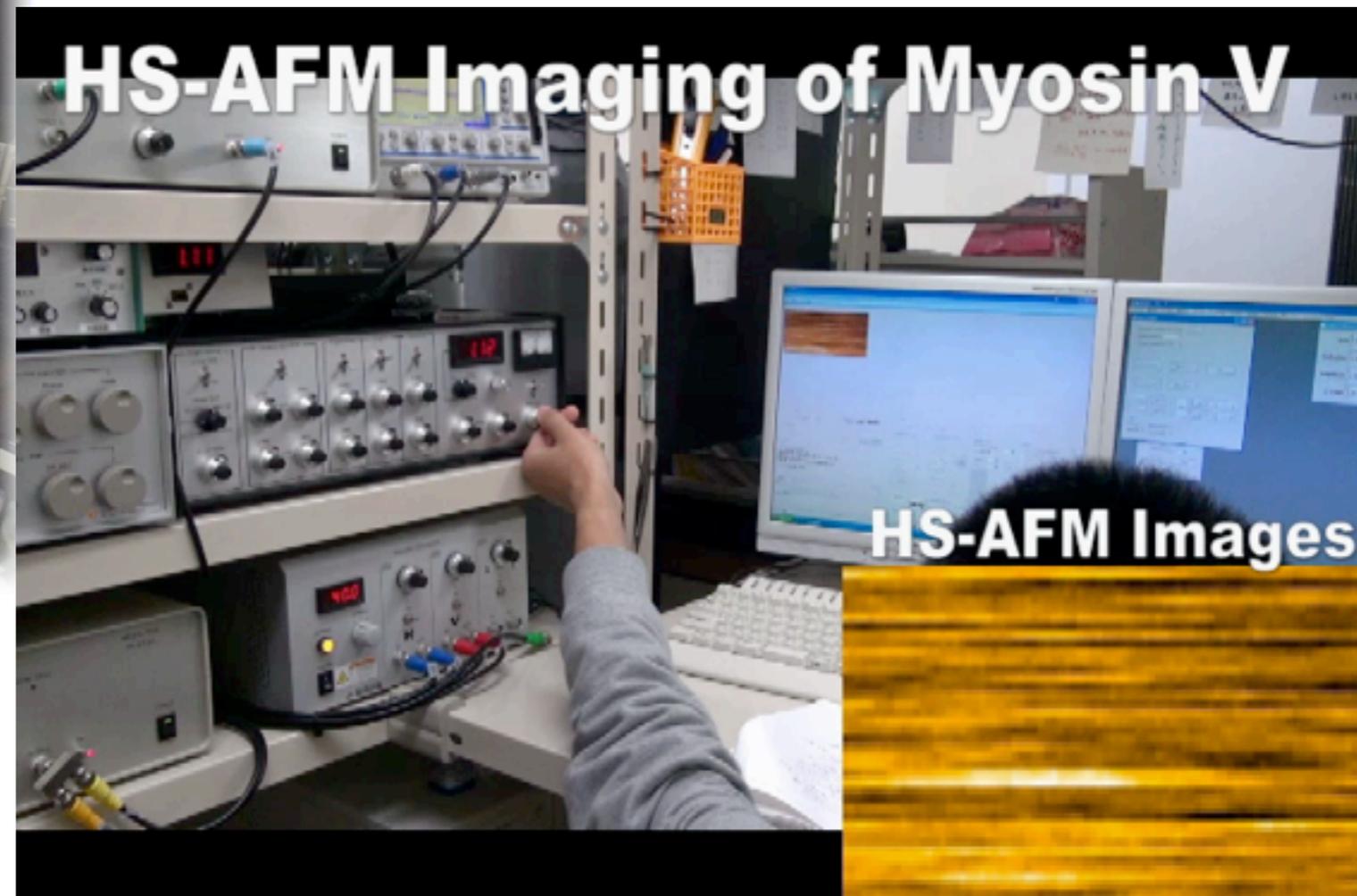
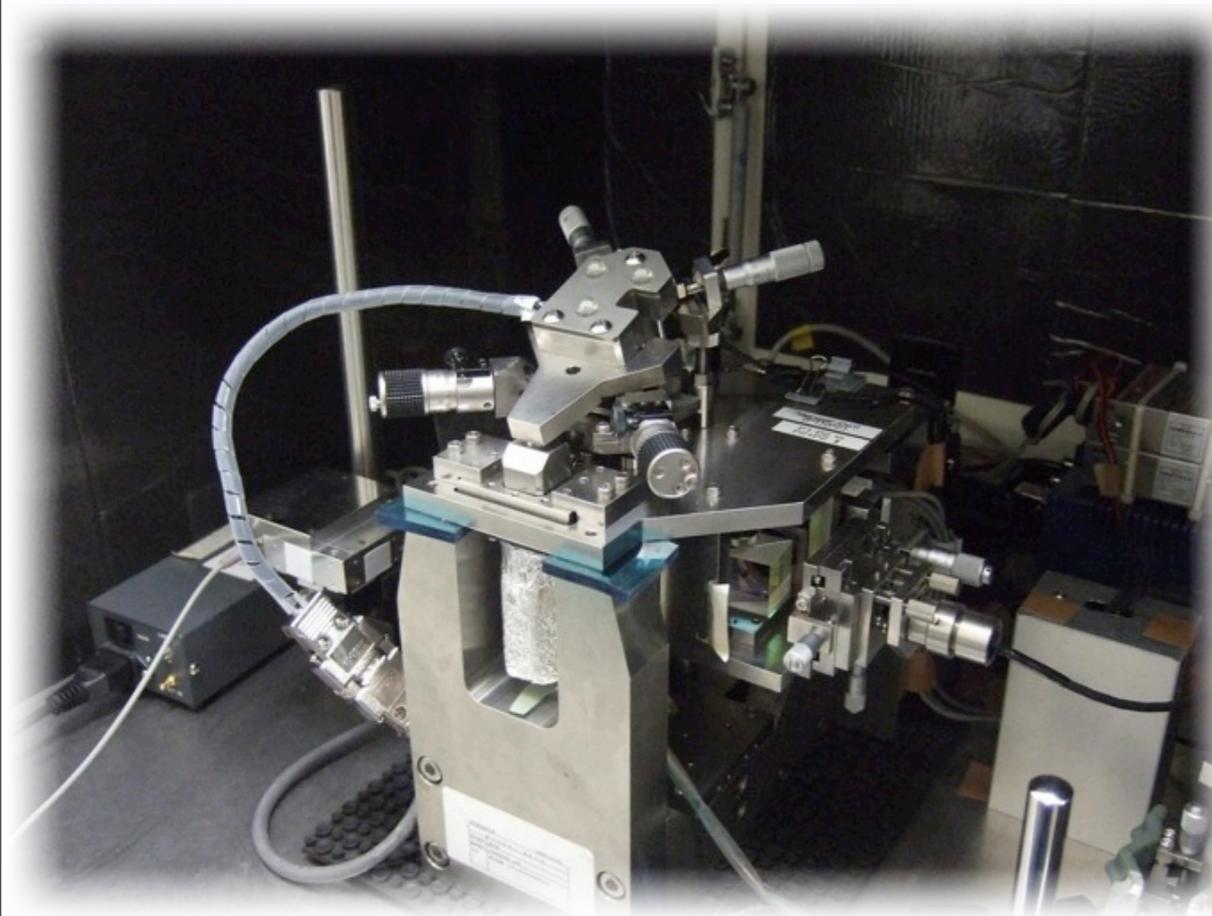
Small Cantilever



Scanner & Damping



高速AFMシステム



最速イメージングレート：40 ms/frame for $L=250$ nm, $N=100$

T. Uchihashi, N. Kodera and T. Ando, *Nature Protocols* **7**, 1193 (2012).

イメージング

1. タンパク質の機能動態

* モータータンパク質: ミオシンV, **F₁-ATPase**

セルラーゼ

* 膜タンパク質: バクテリオロドプシン

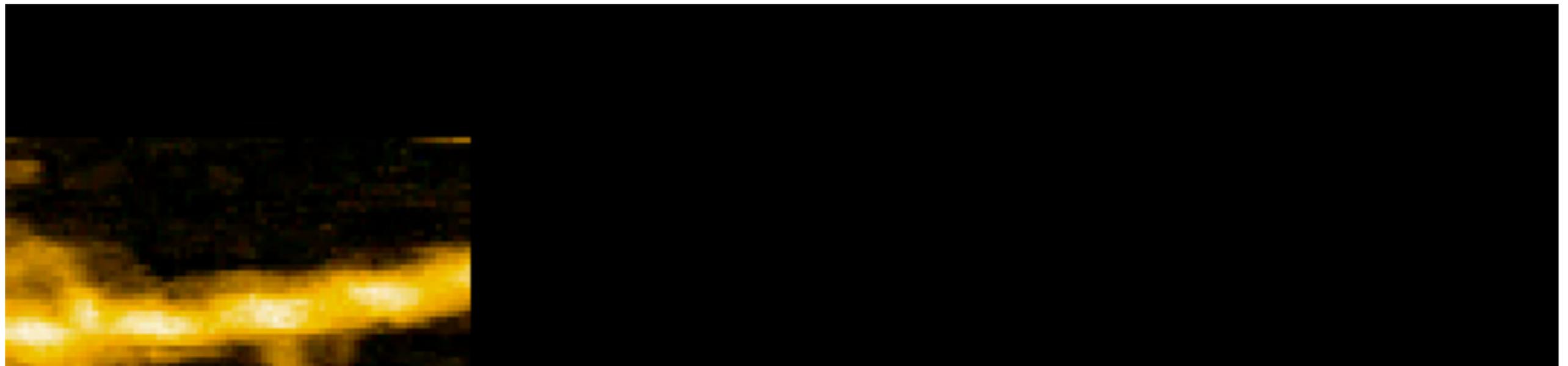
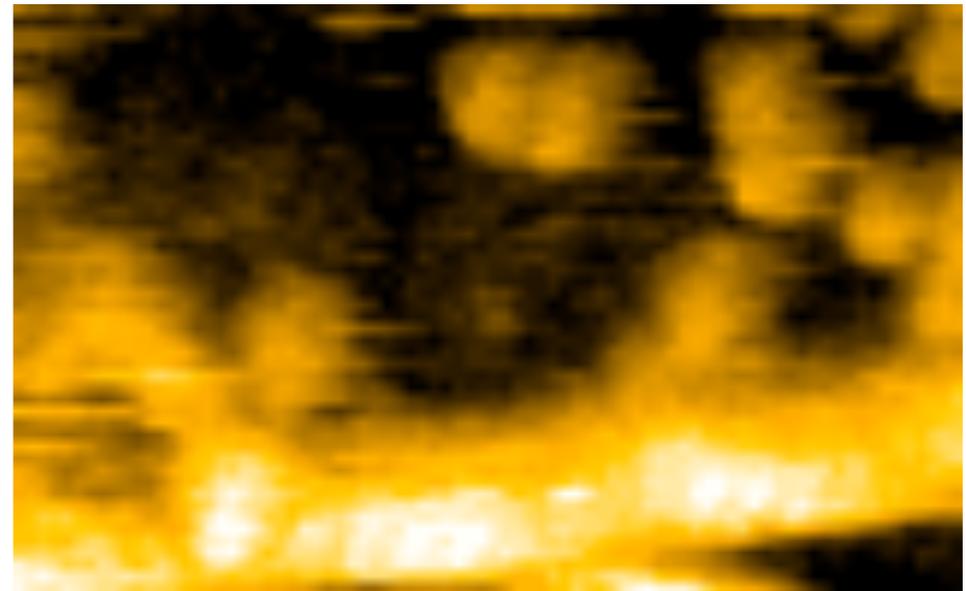
2. タンパク質結晶のダイナミクス

アネキシンV, ライセニン

ミオシンVの歩行観察



M. L. Walker et al., *Nature* (2000)

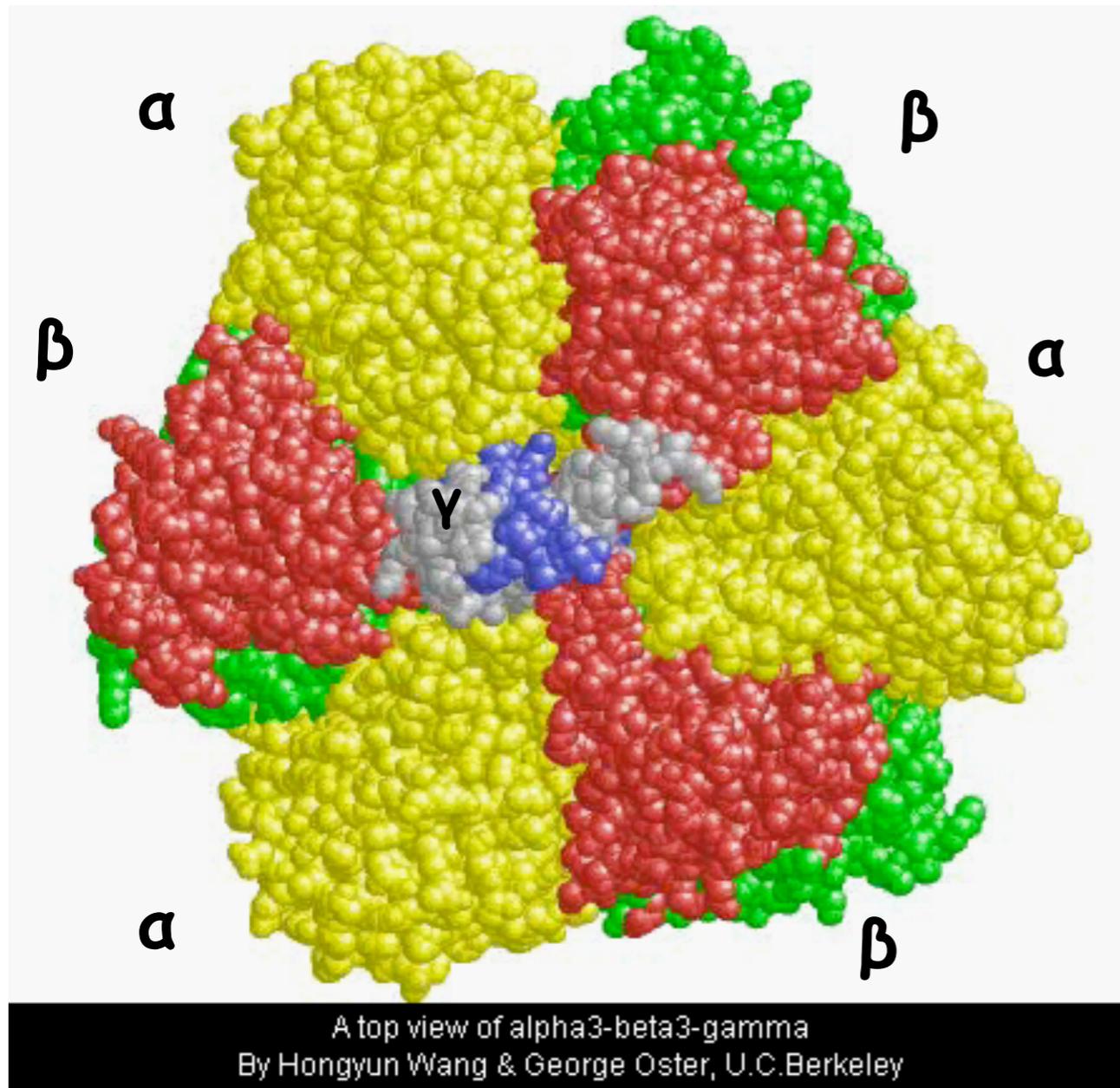


5 μ M-ATP, 150 nm x 75nm, 147ms/frame

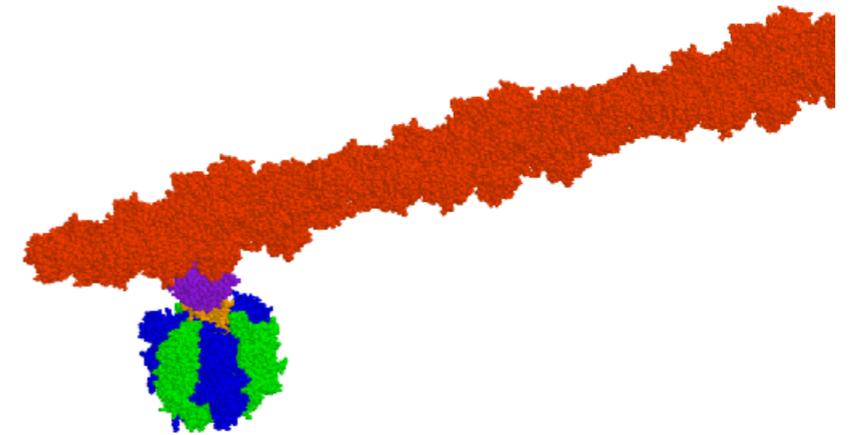
N. Kodera, D. Yamamoto, R. Ishikawa, and T. Ando, *Nature* **468**: 72-76 (2010).

F₁-ATPase

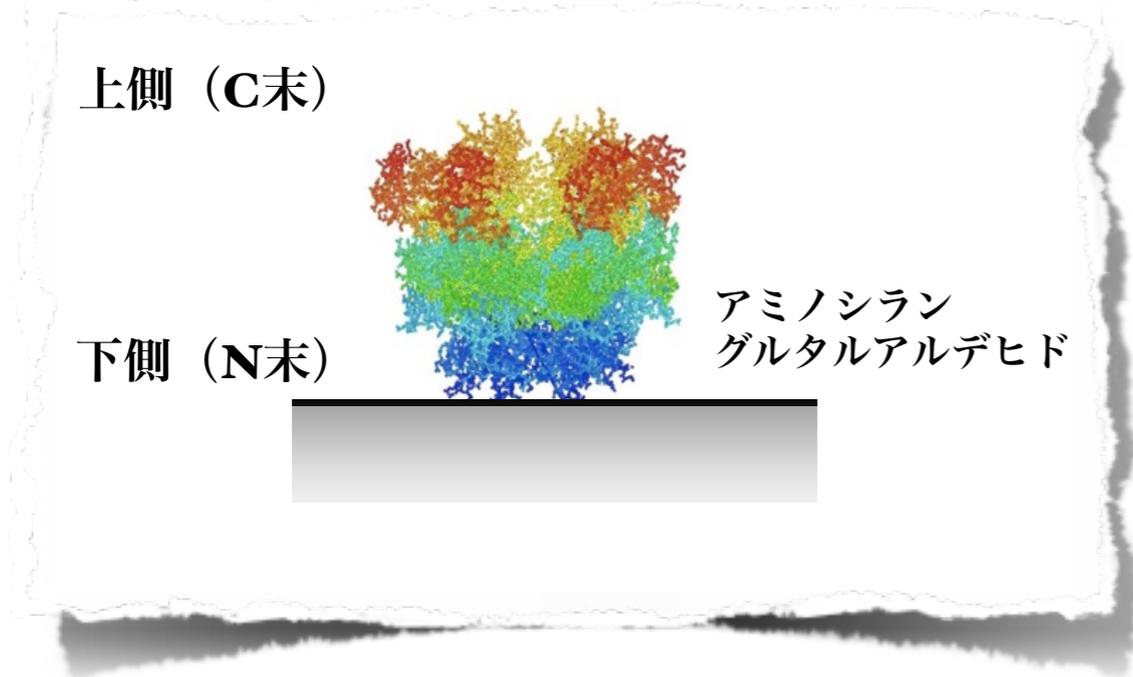
F₁-ATPase



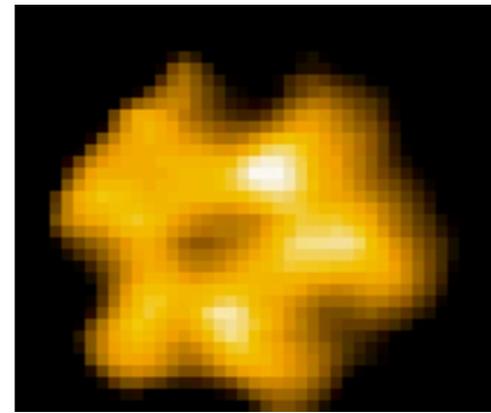
Wang and Oster, *Nature* **396** (1998) 279.



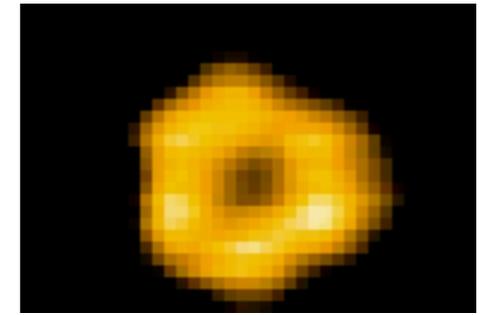
H. Noji et al., *Nature* **386** (1997) 299.



結晶構造



12.5 fps

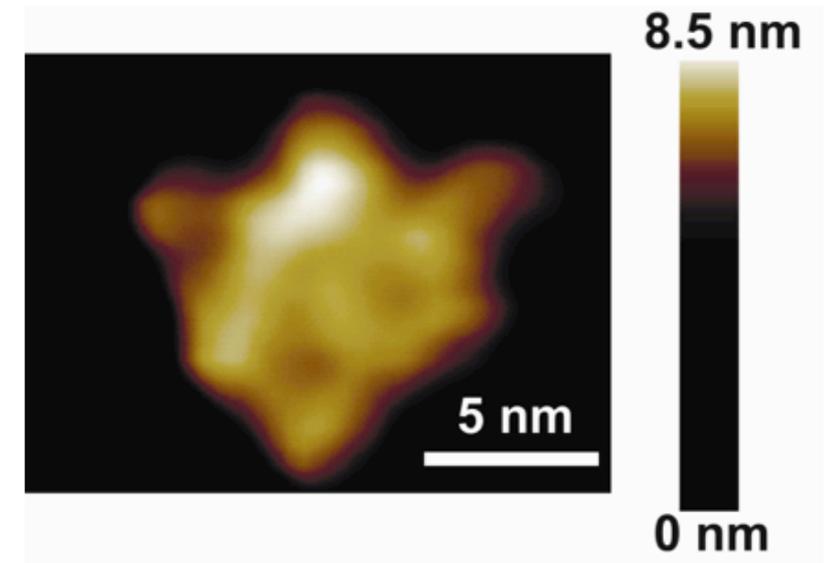
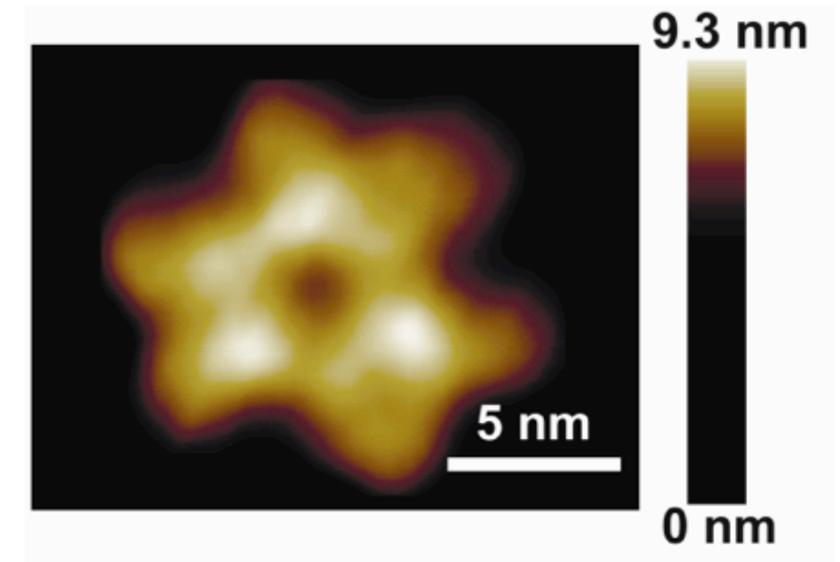
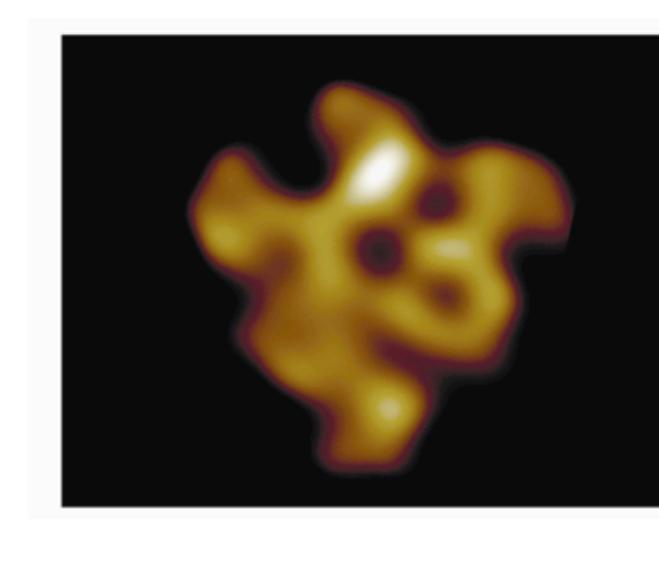
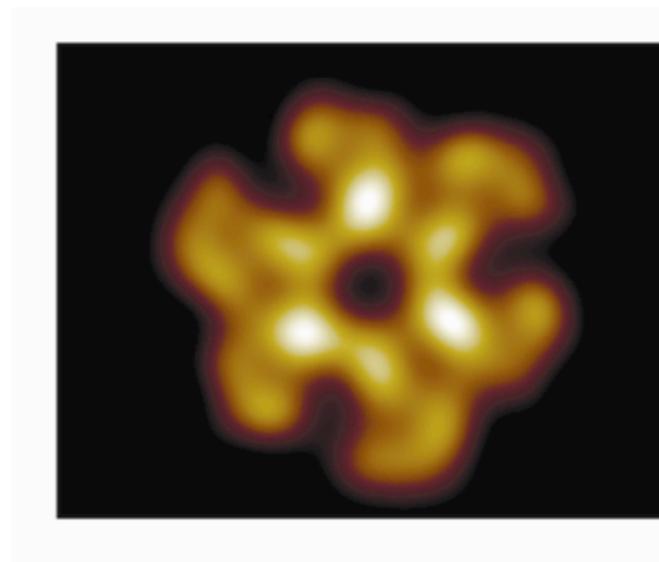
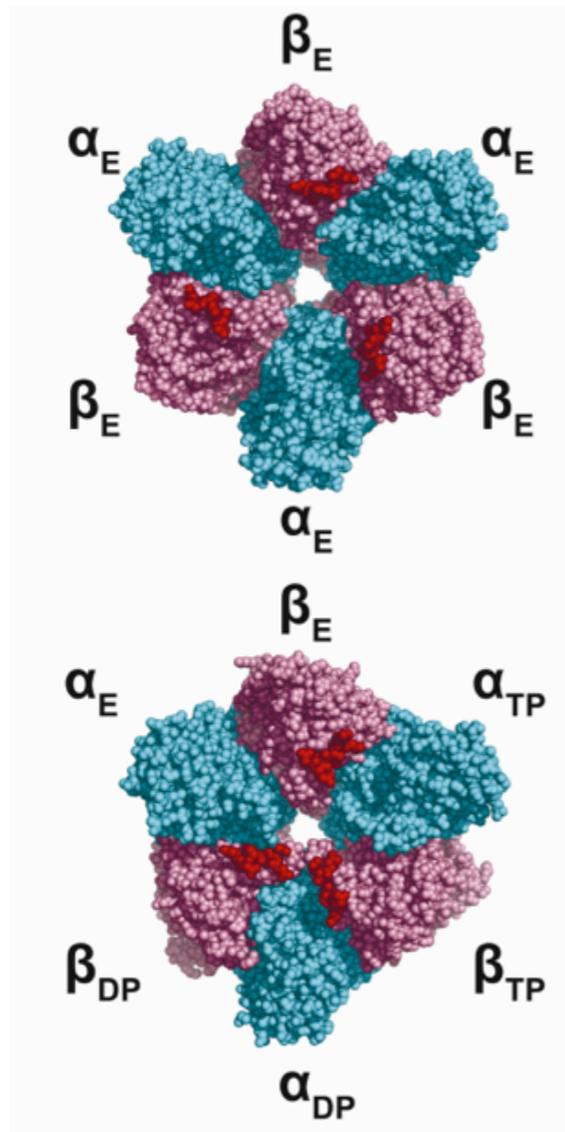


12.5 fps

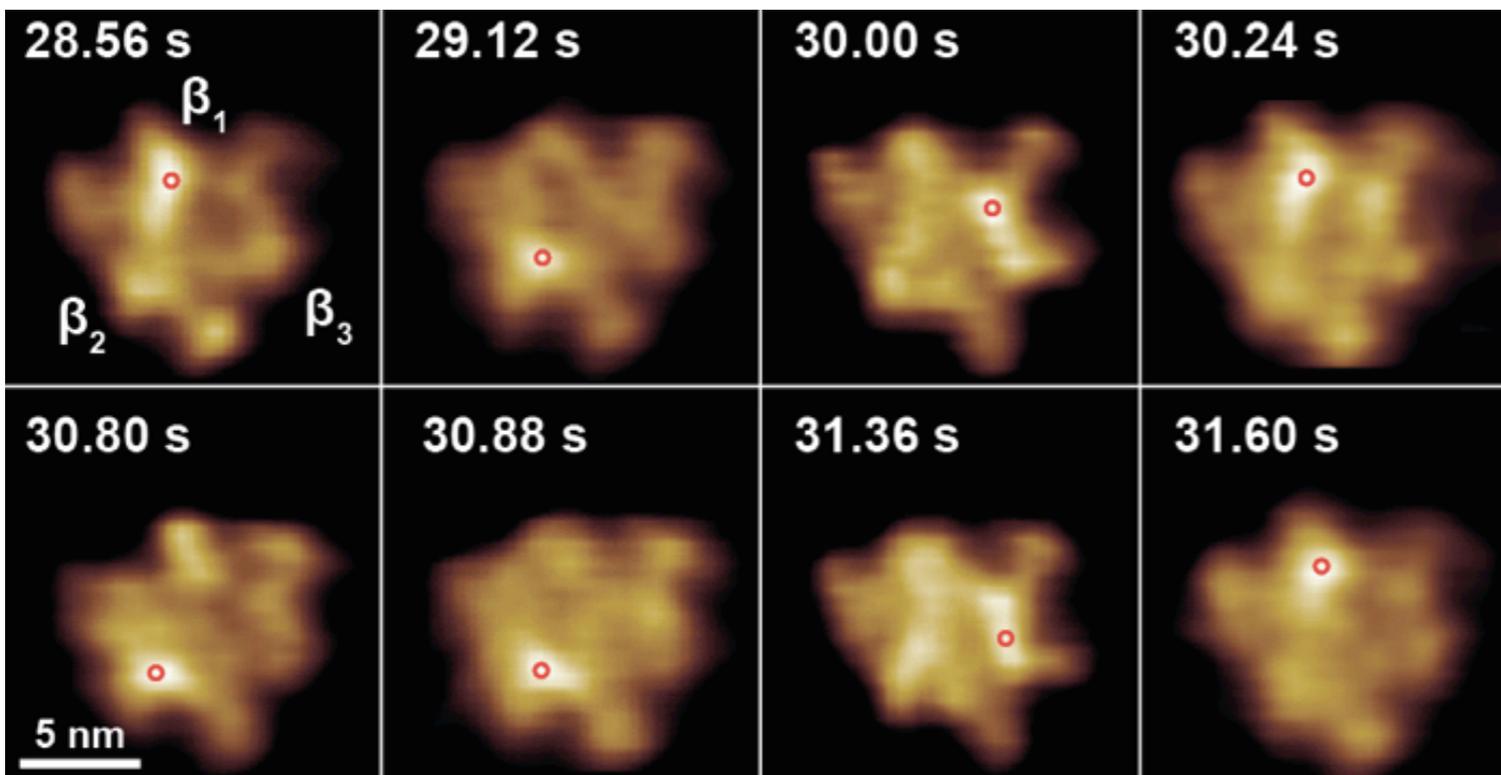
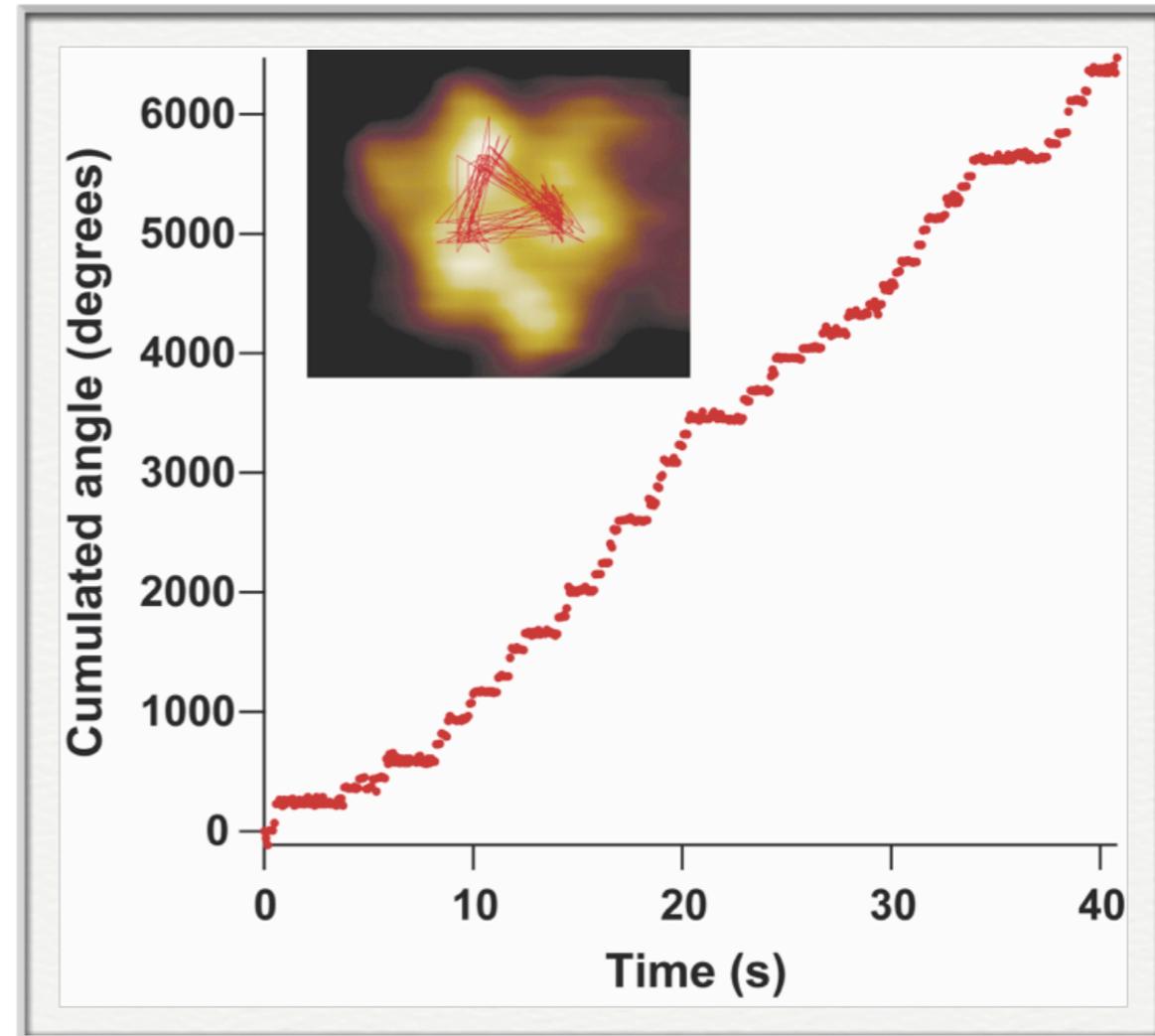
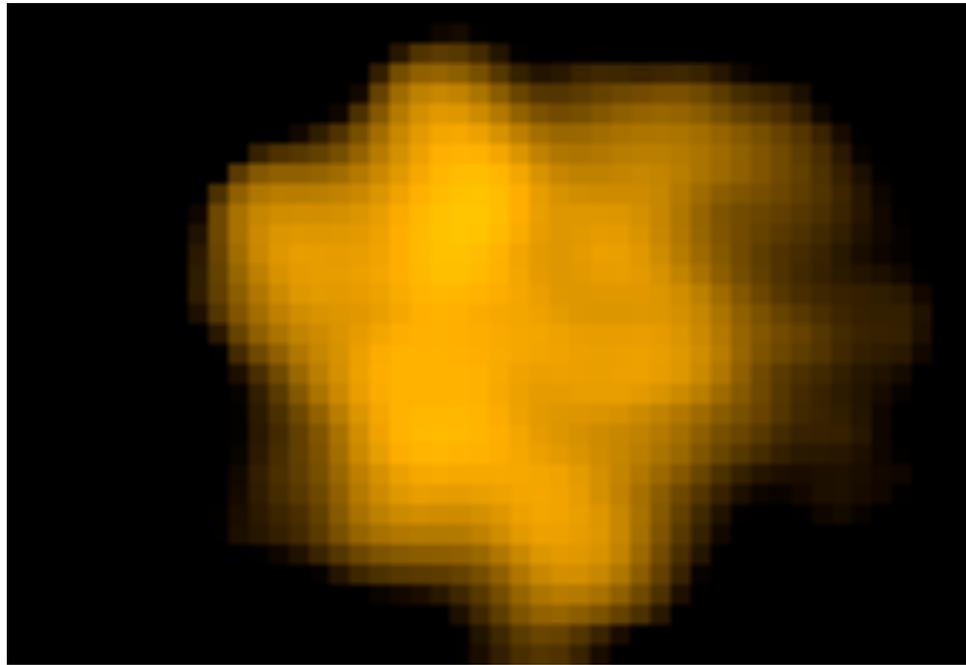
シミュレーション像

AFM像

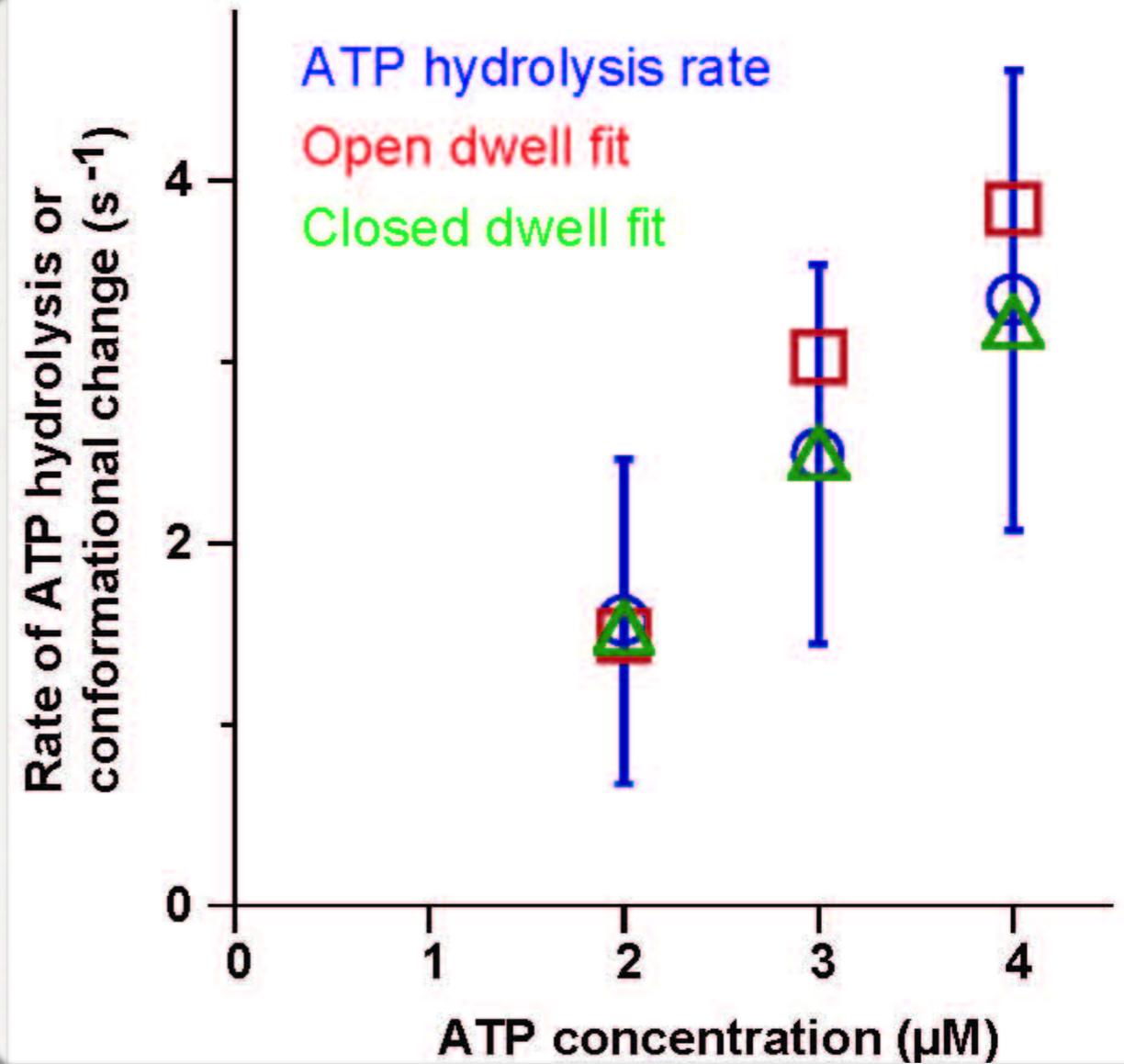
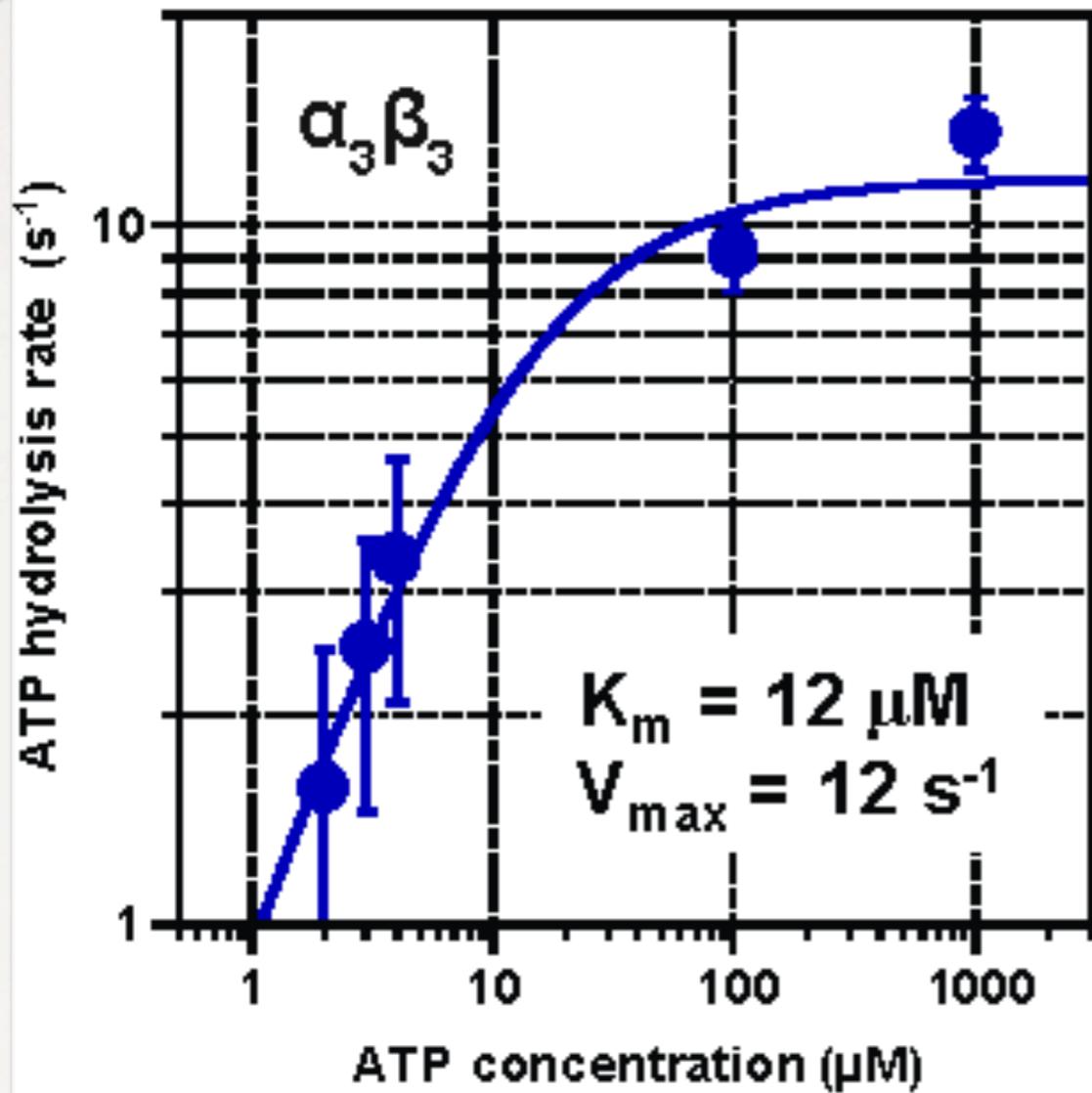
N.F.



構造変化の観察

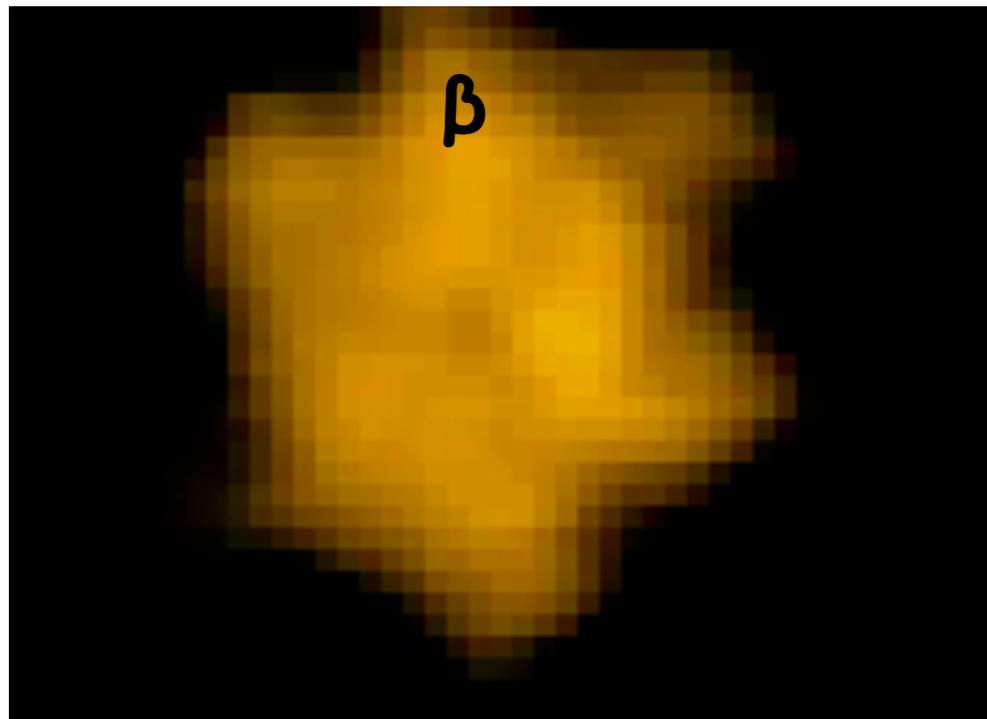


ATP濃度依存性

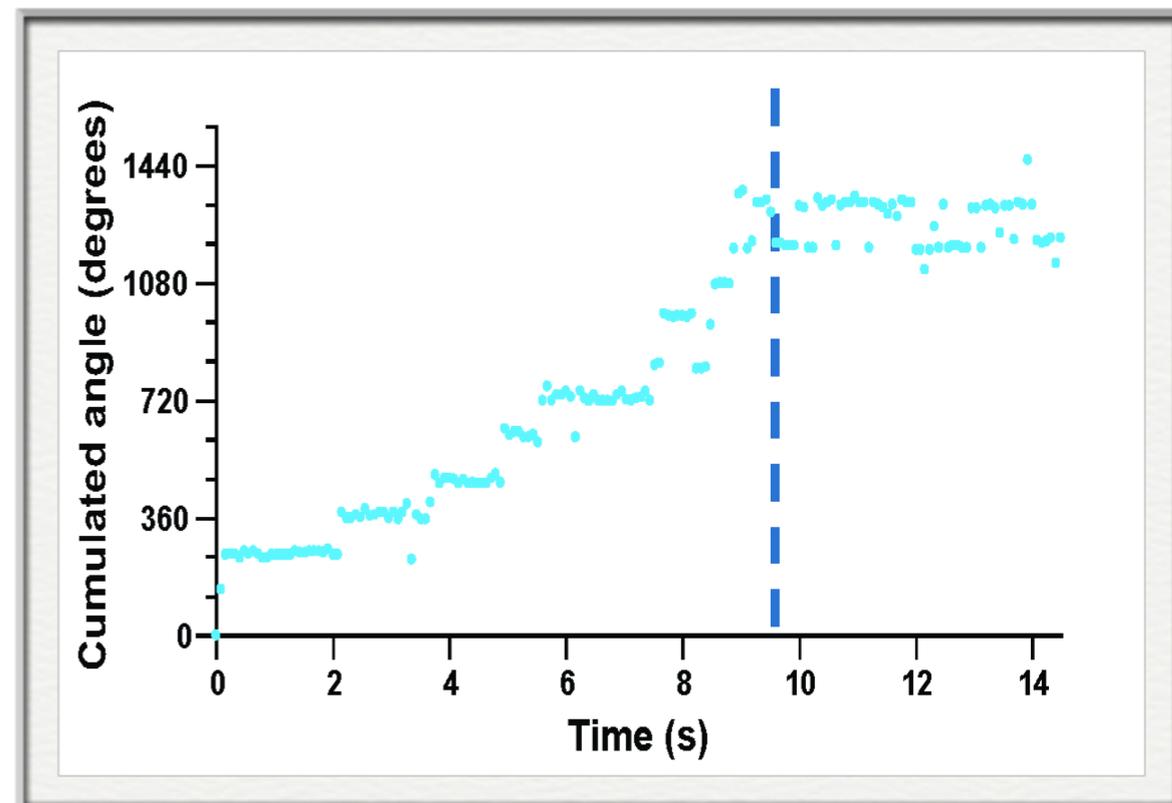
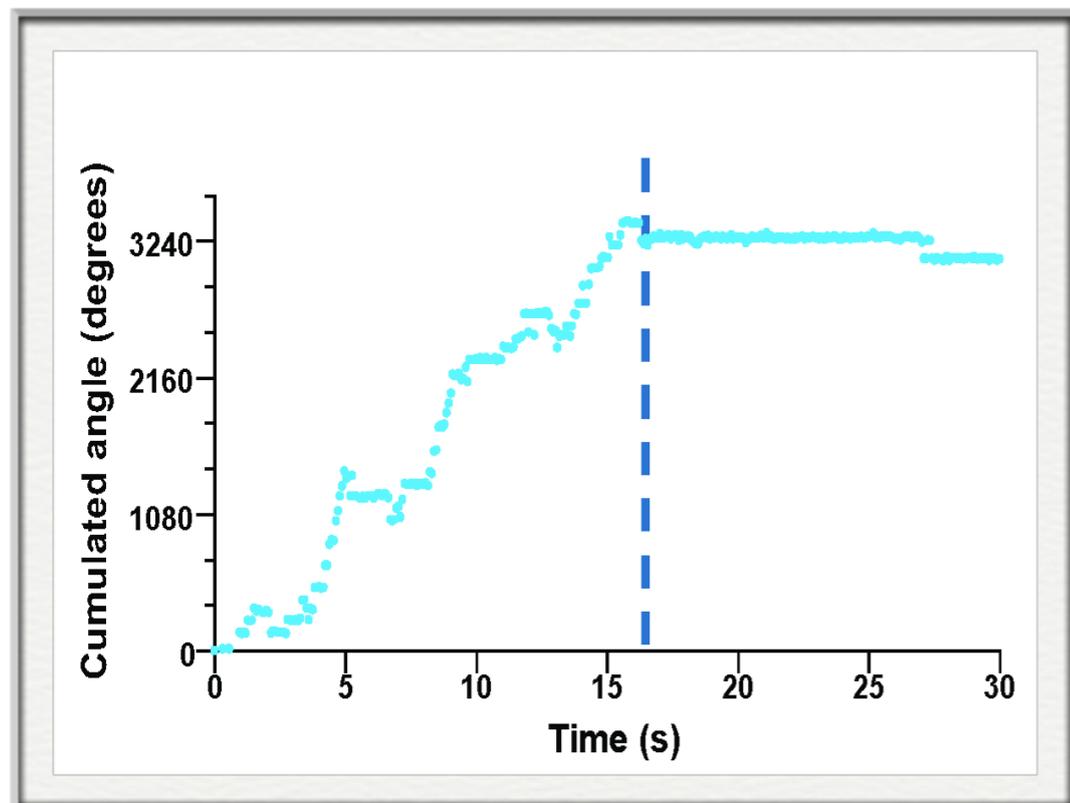
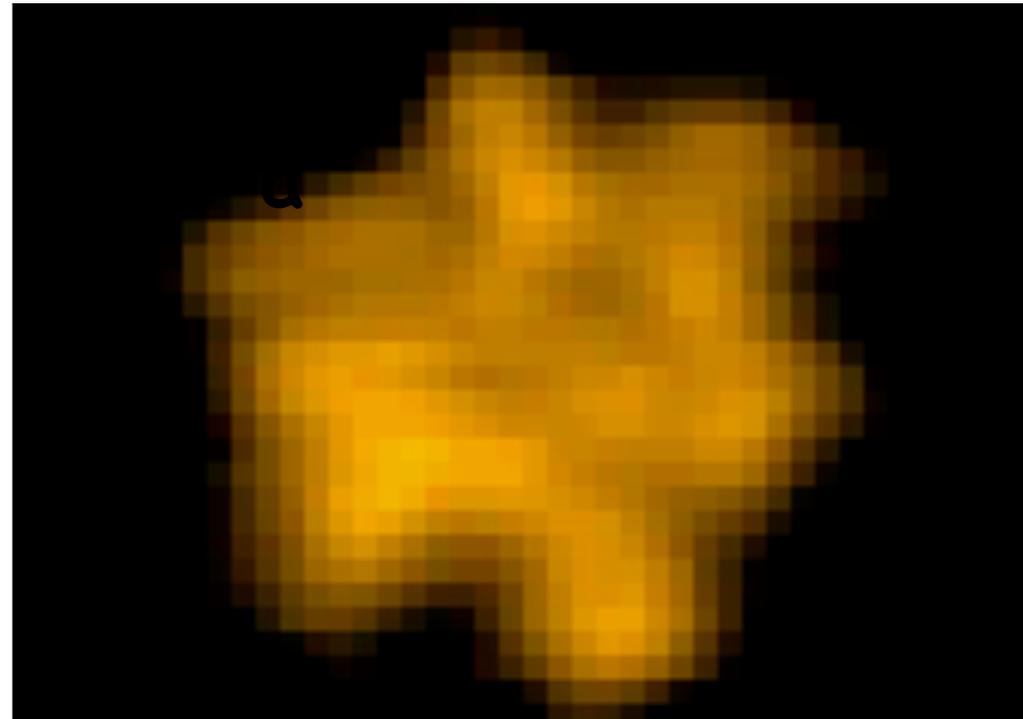


リング内の協同性

β の脱離

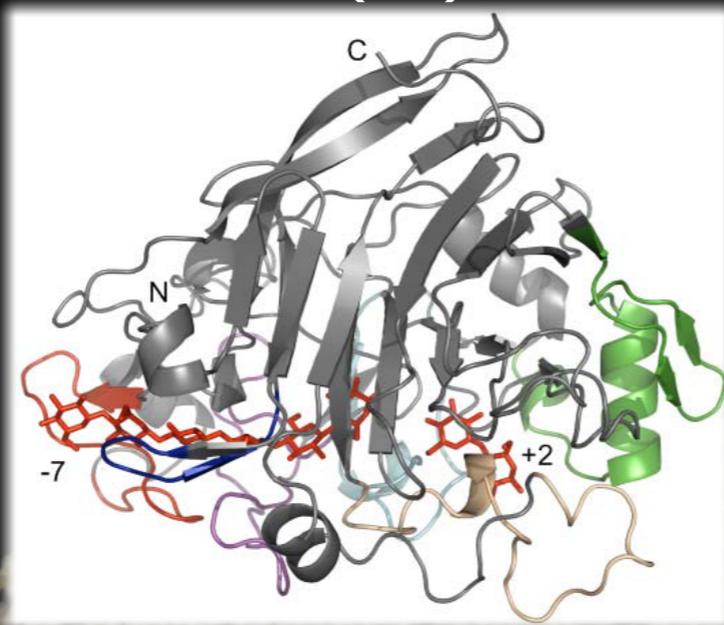


α の脱離

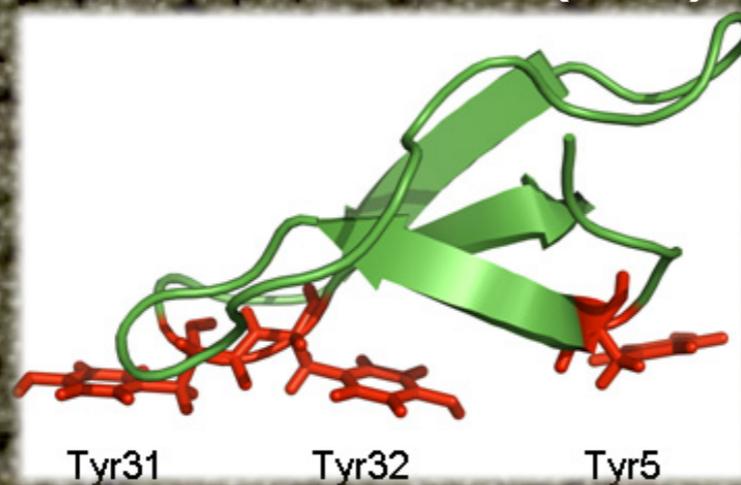


セルラーゼ

触媒ドメイン(CD): 50kDa



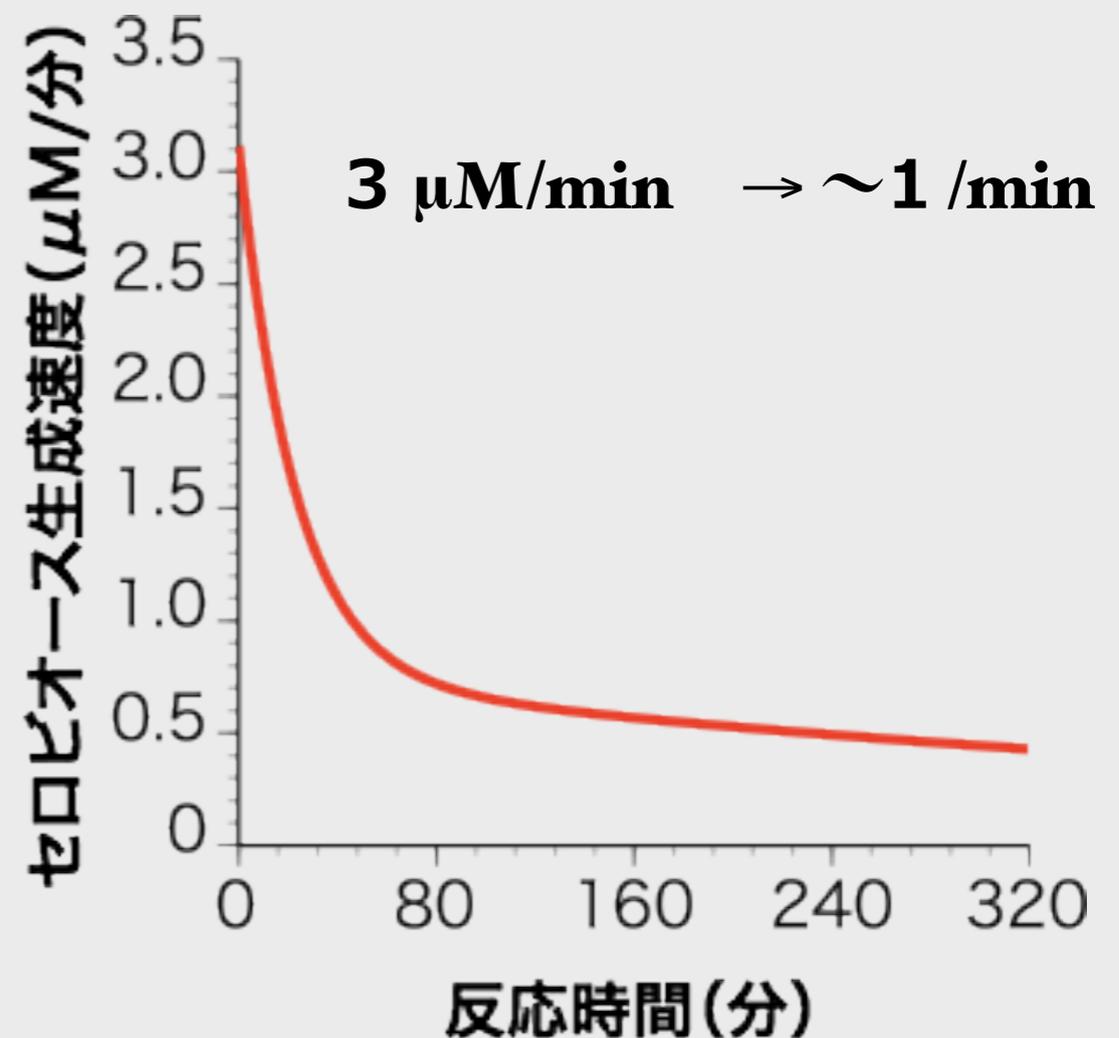
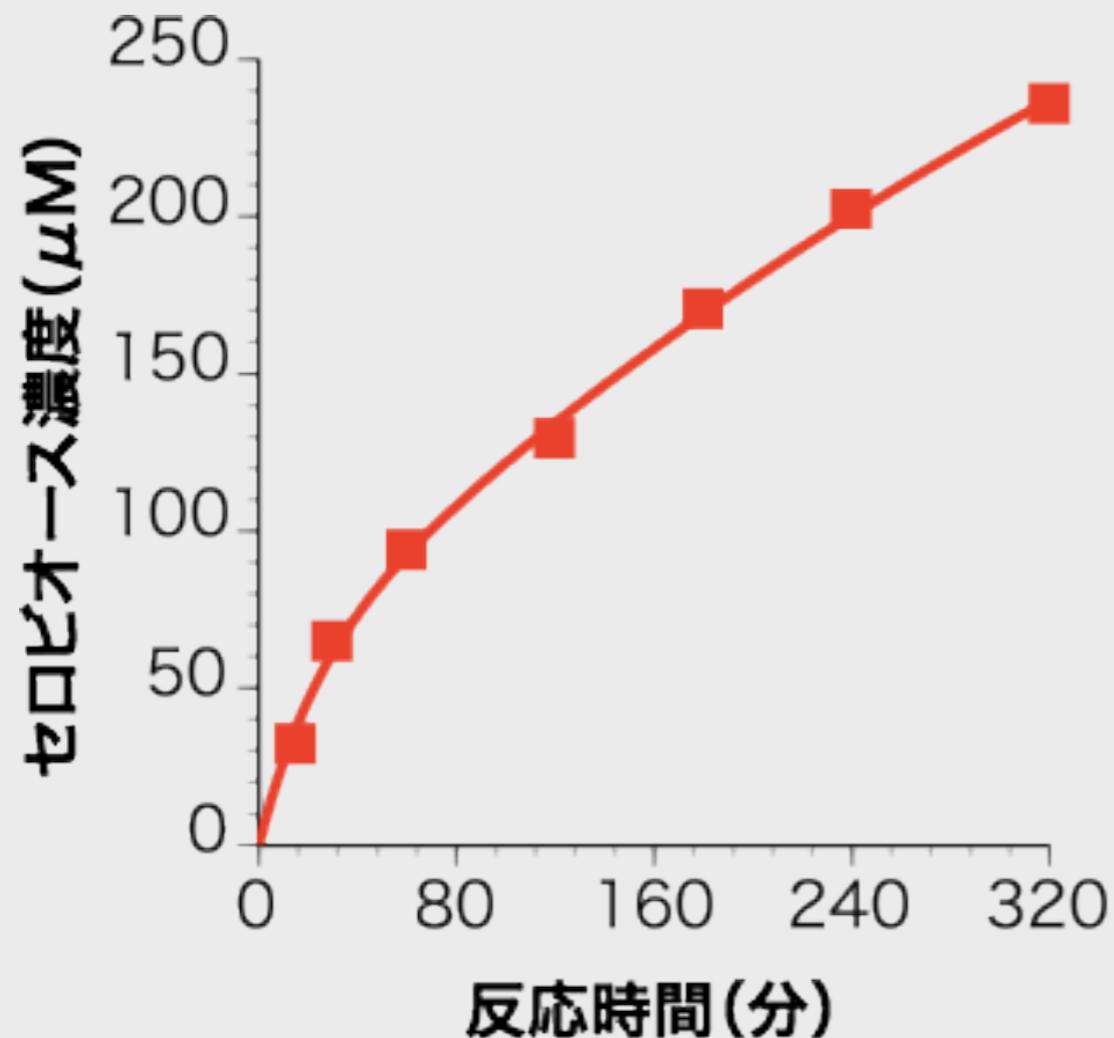
セルロース結合ドメイン(CBD): 3 kDa



結晶性セルロース

セルロースの加水分解

*Trichoderma*菌由来Cel7Aによる
天然セルロースの分解

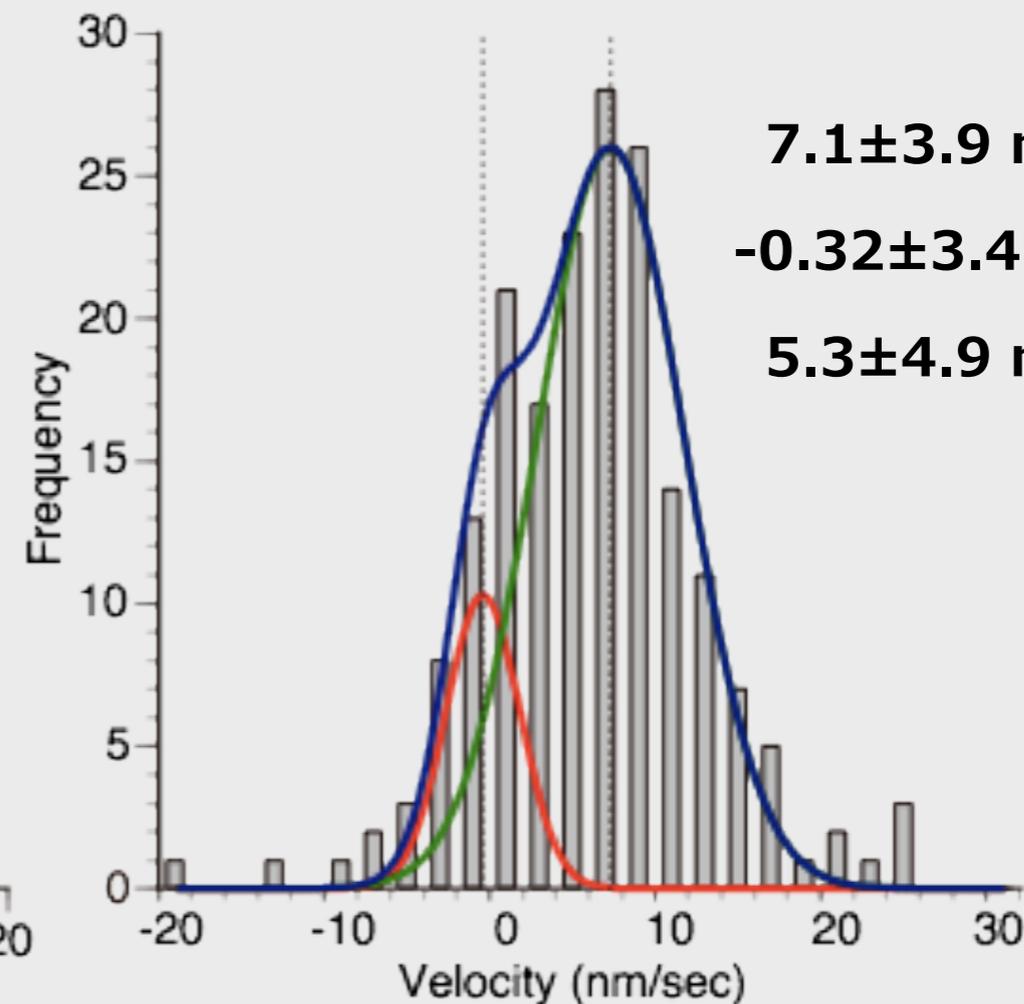
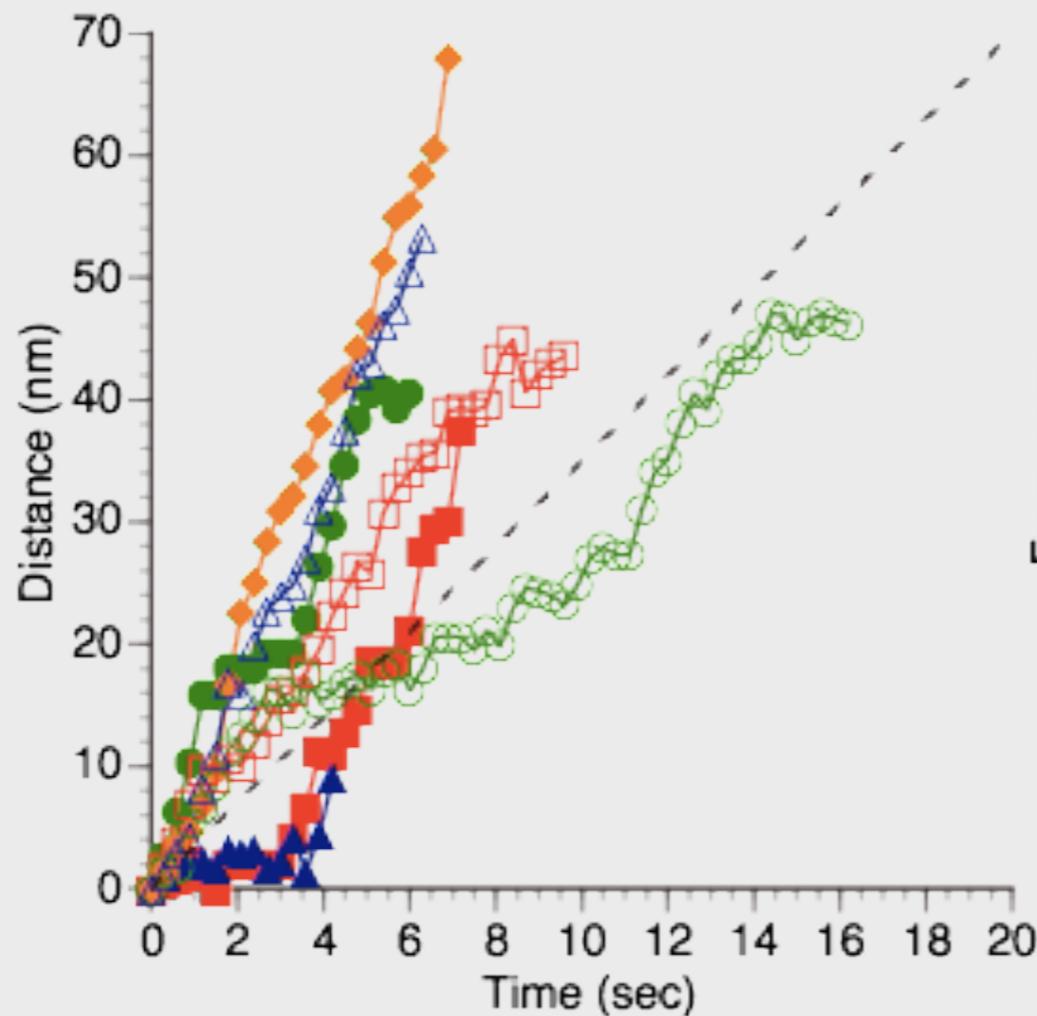


天然セルロース表面で
TrCel7Aはモノレールのよ
うに一列に並び、止まったり
進んだりする



0.3 s/frame
x10 sped-up

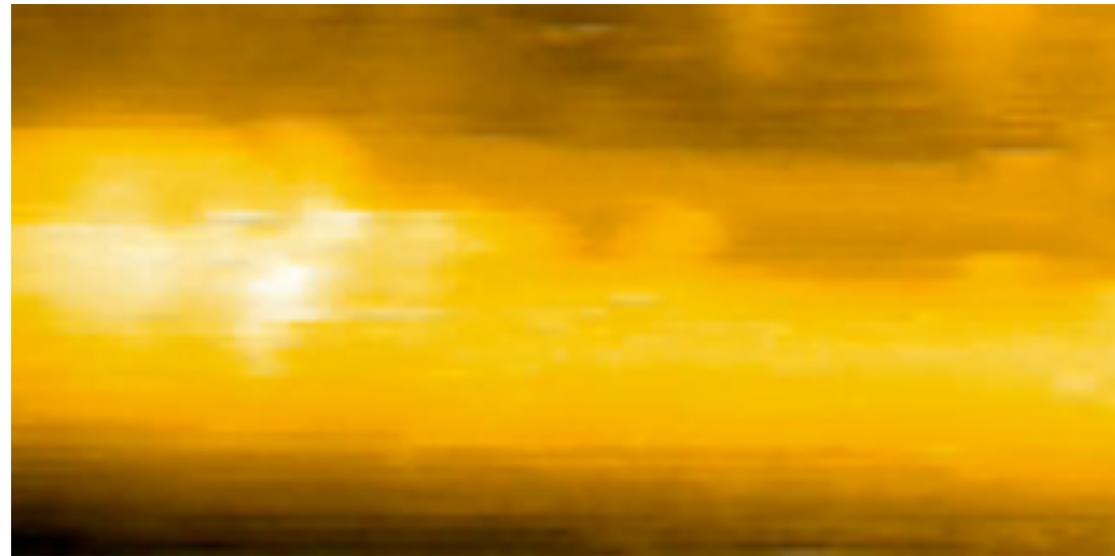
50 nm



7.1 nm/sec → 7 /sec : 400倍以上!

セルラーゼの交通渋滞

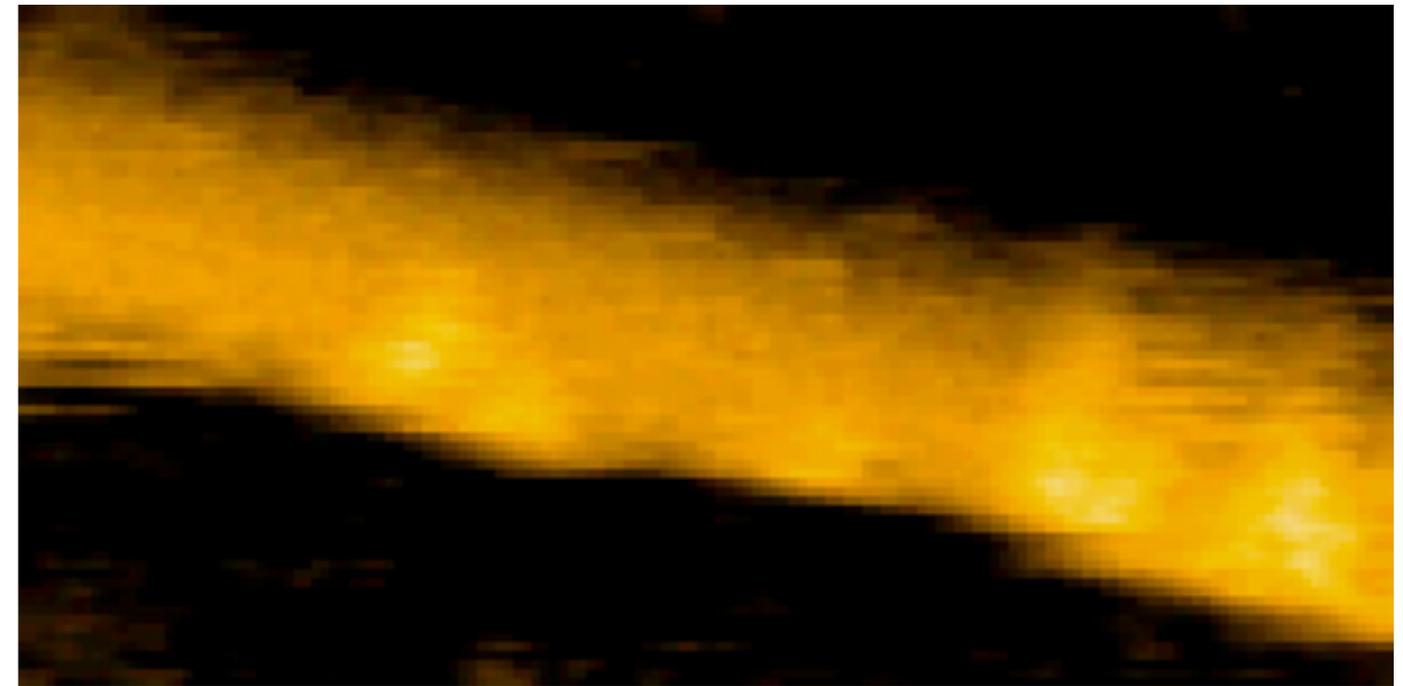
渋滞を起こすセルラーゼ分子



0.15s/frame
5倍速

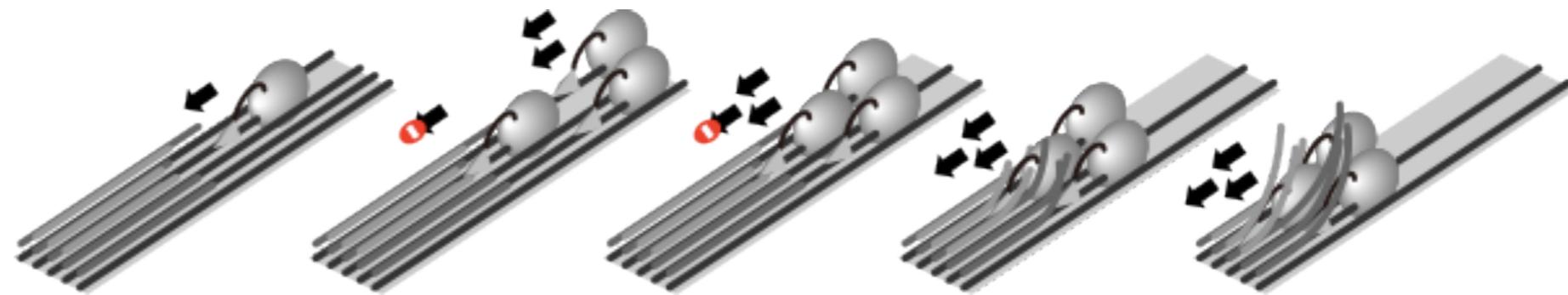
25 nm

セルロースを削りながら前に進むセルラーゼ分子



0.3s/frame
10倍速

50 nm

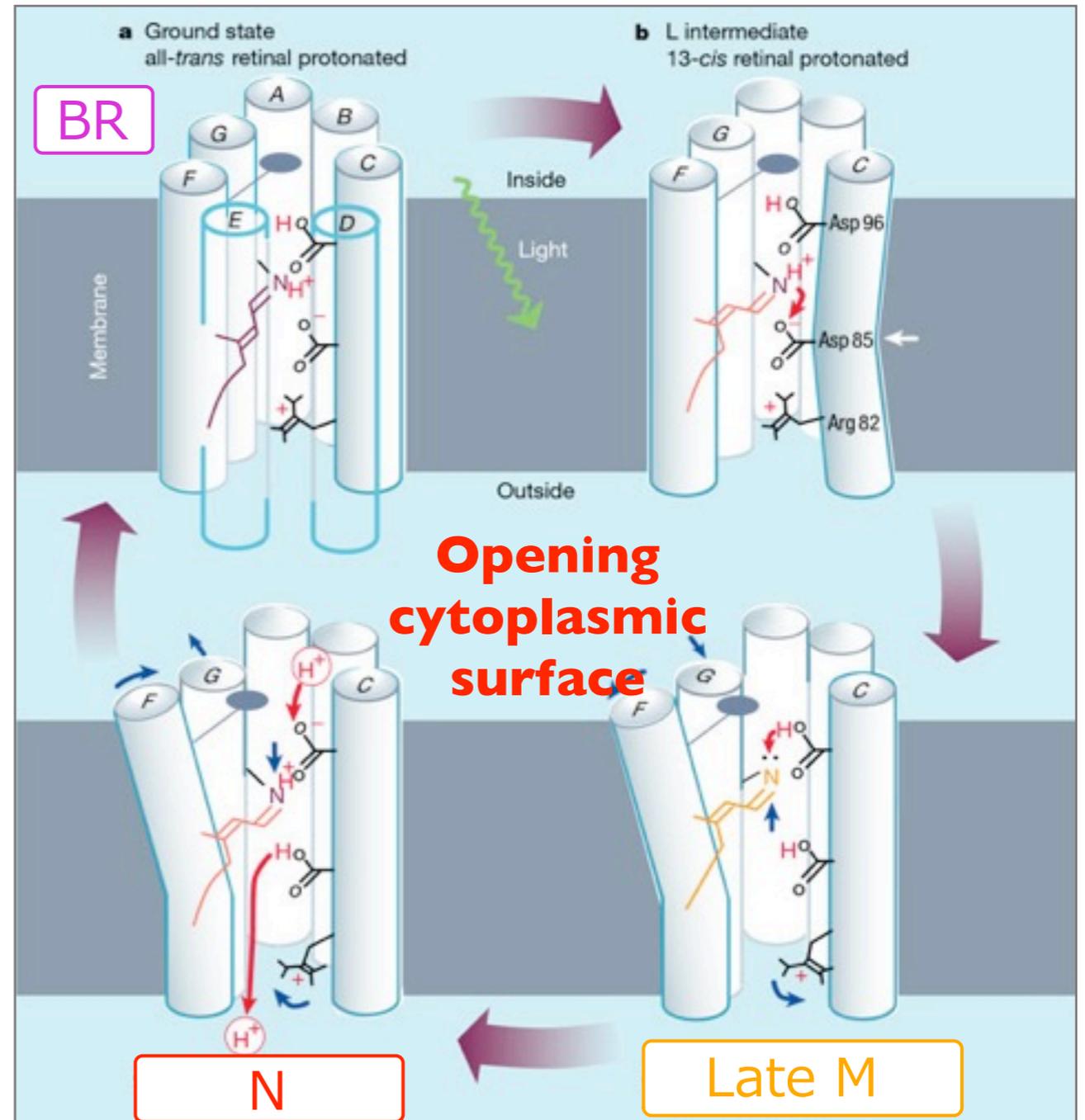


バテクテリオロドプシン

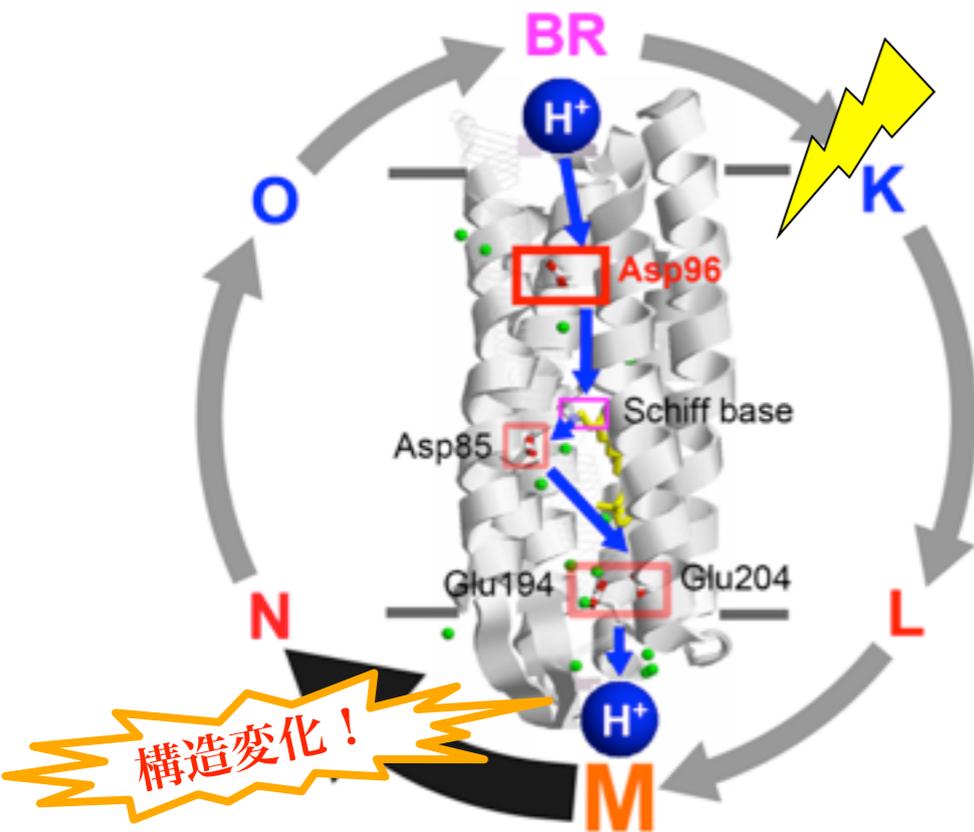


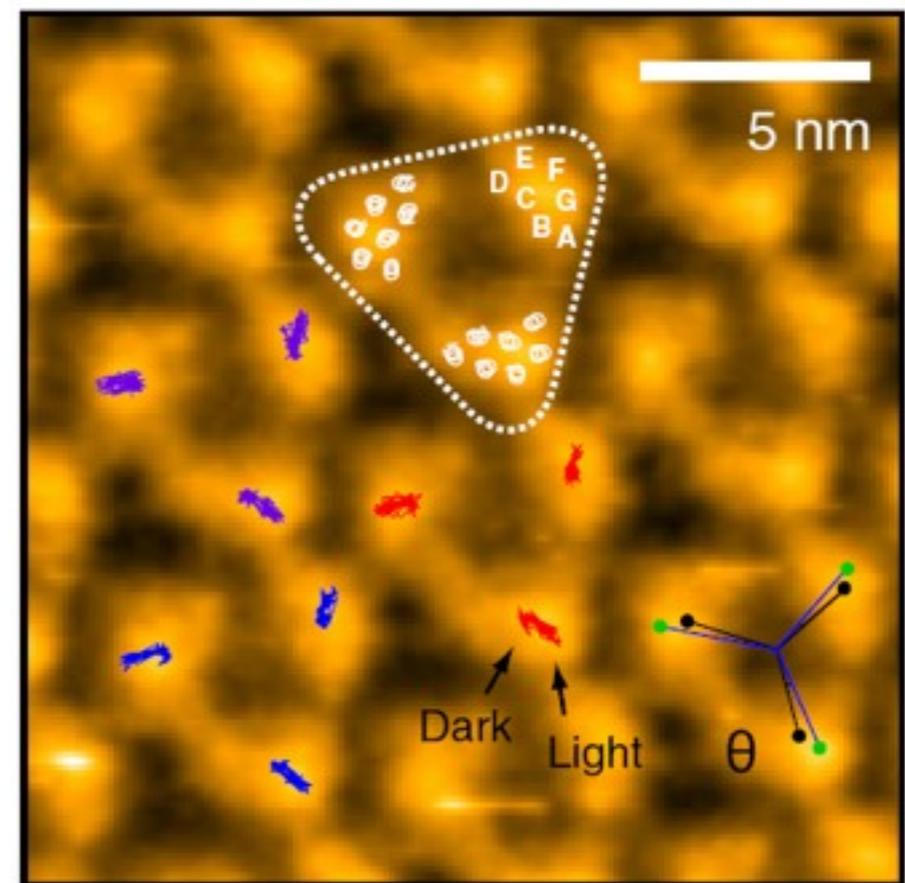
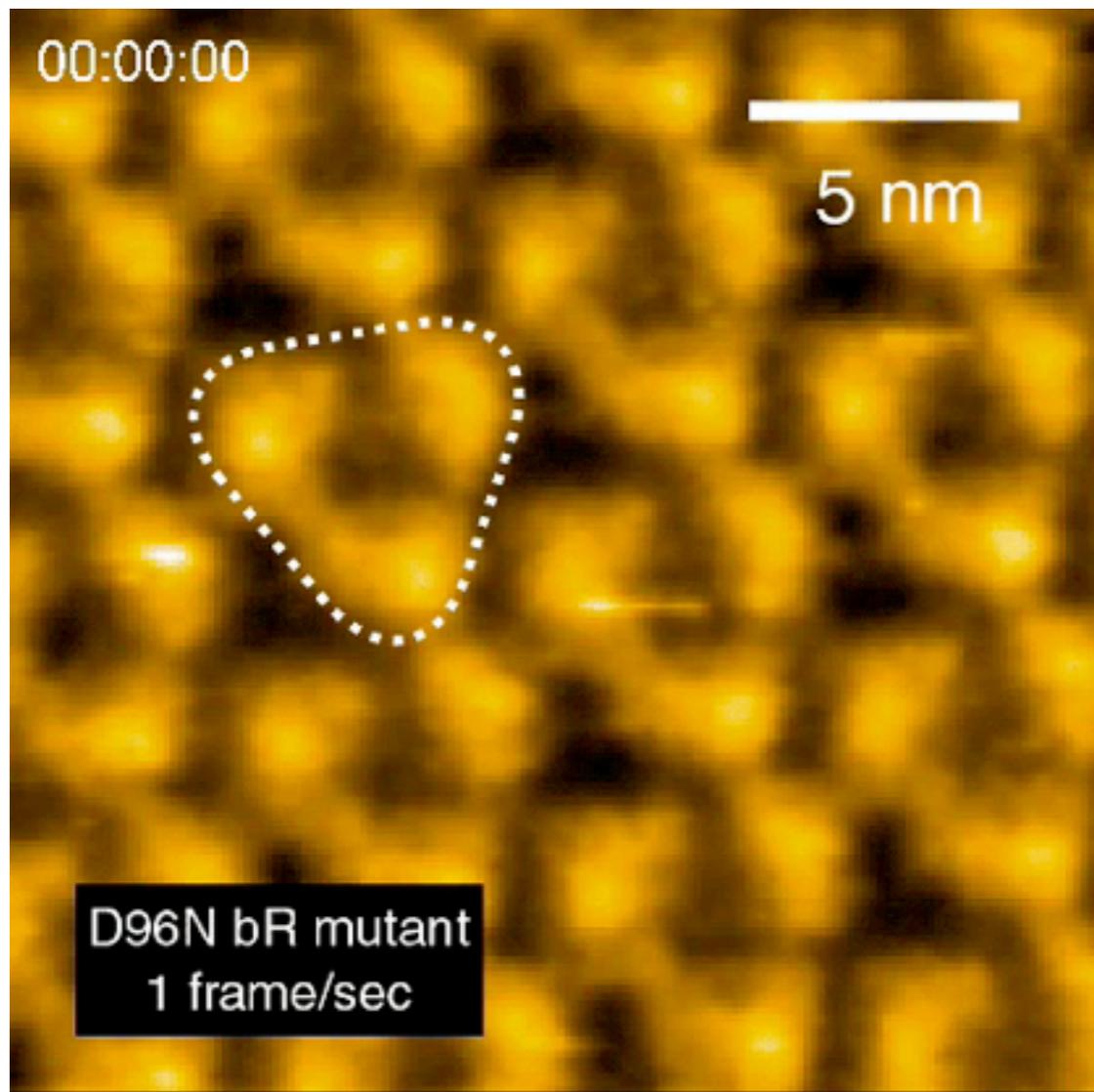
Diffraction technique:
electron, X-ray and neutron

Spectroscopy:
FTIR and EPR

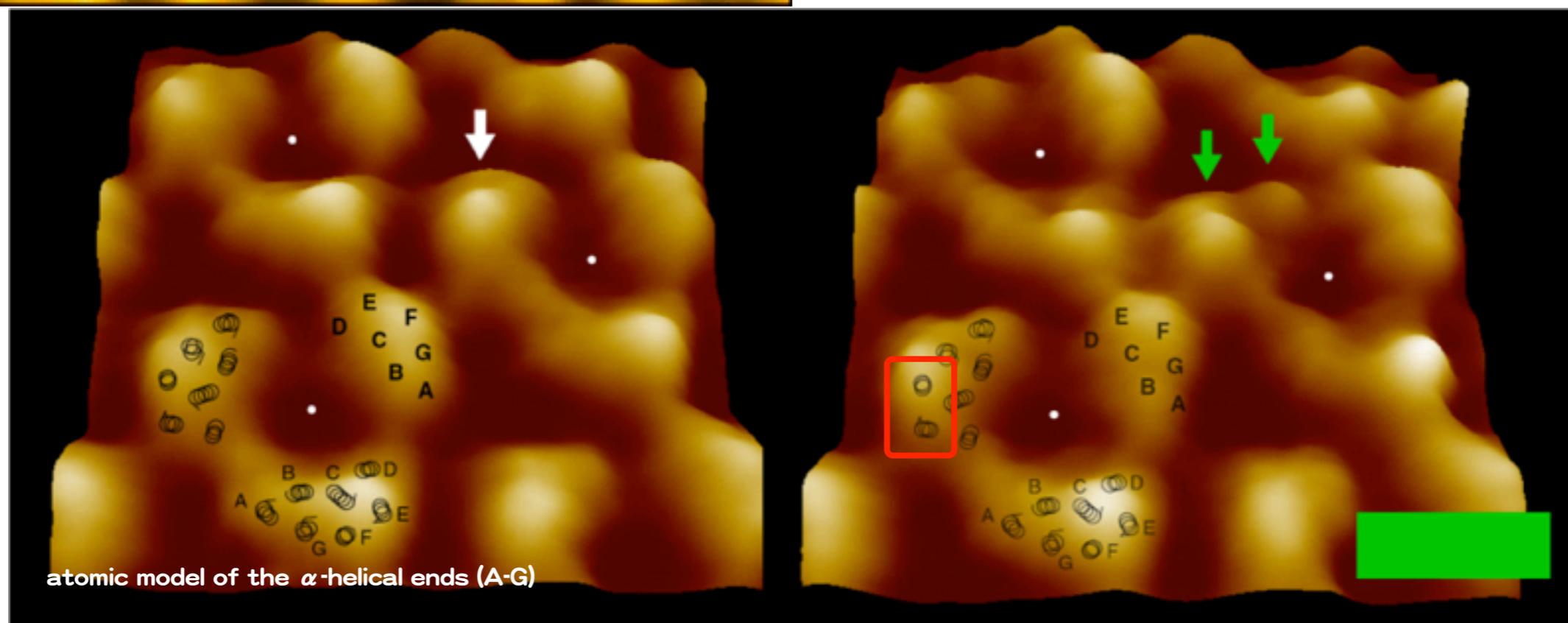


W. Kühlbrandt, *Nature* (2000)



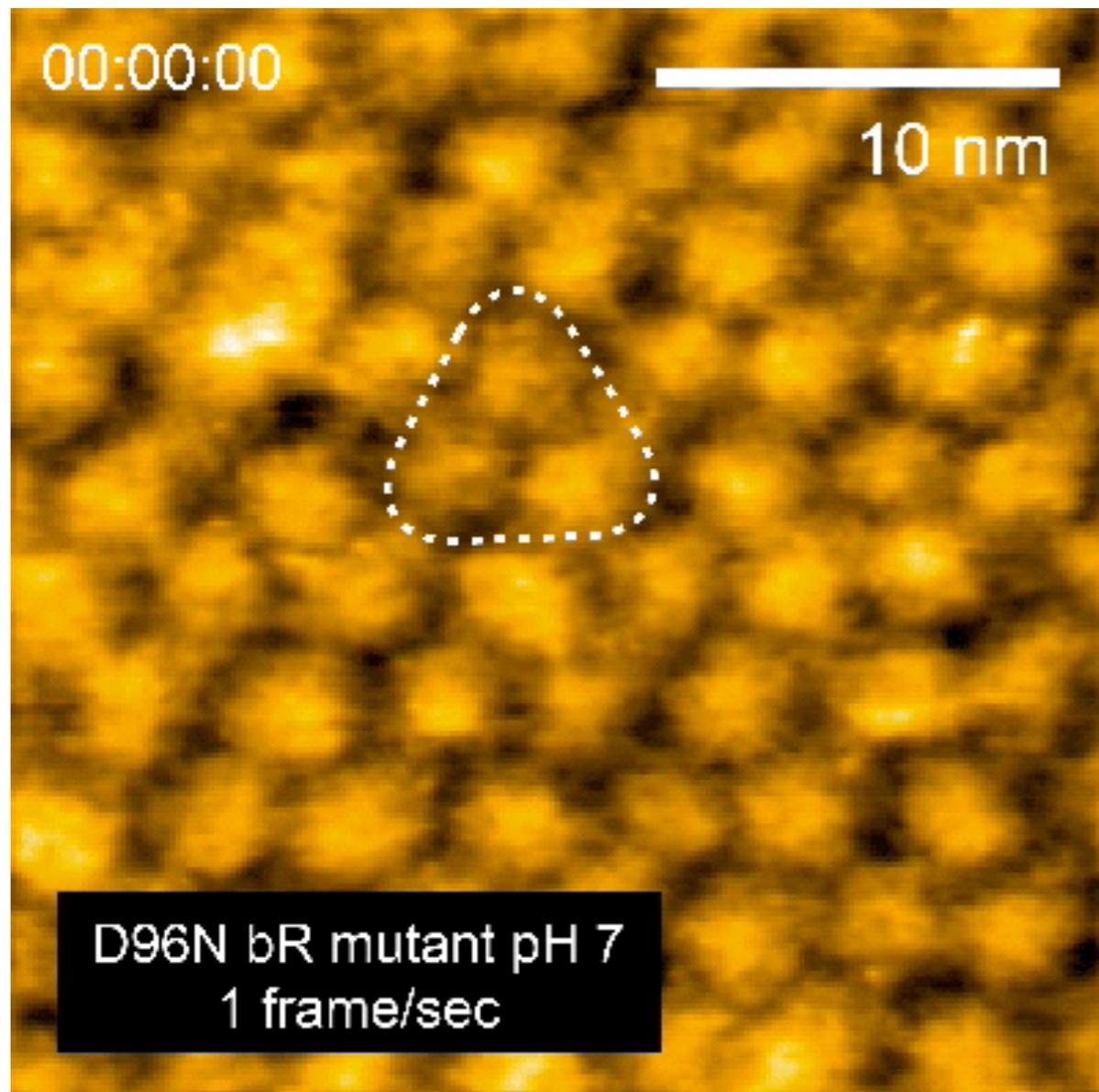


変位 : 0.69 ± 0.15 nm



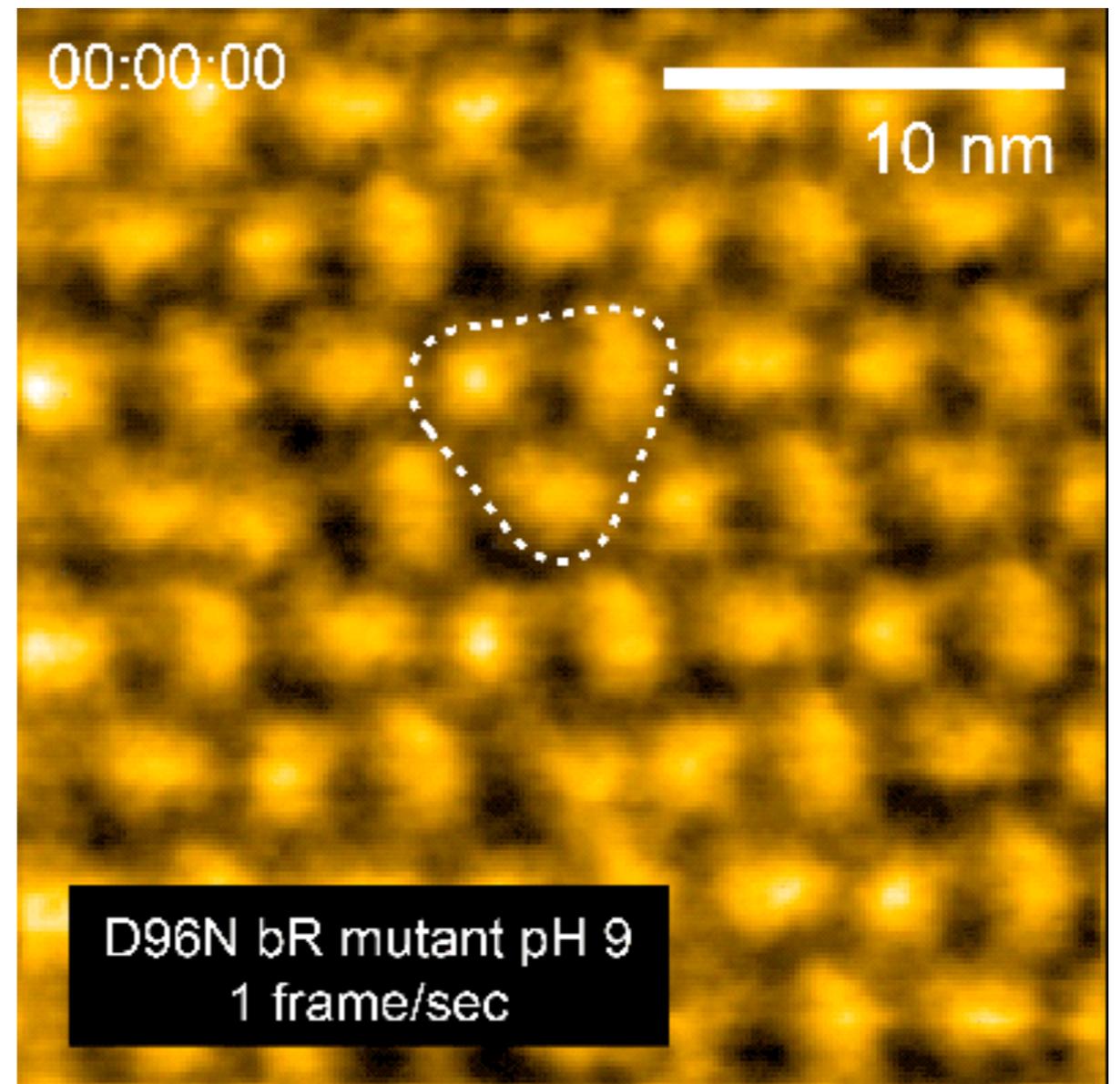
pH依存性

D96N at pH 7



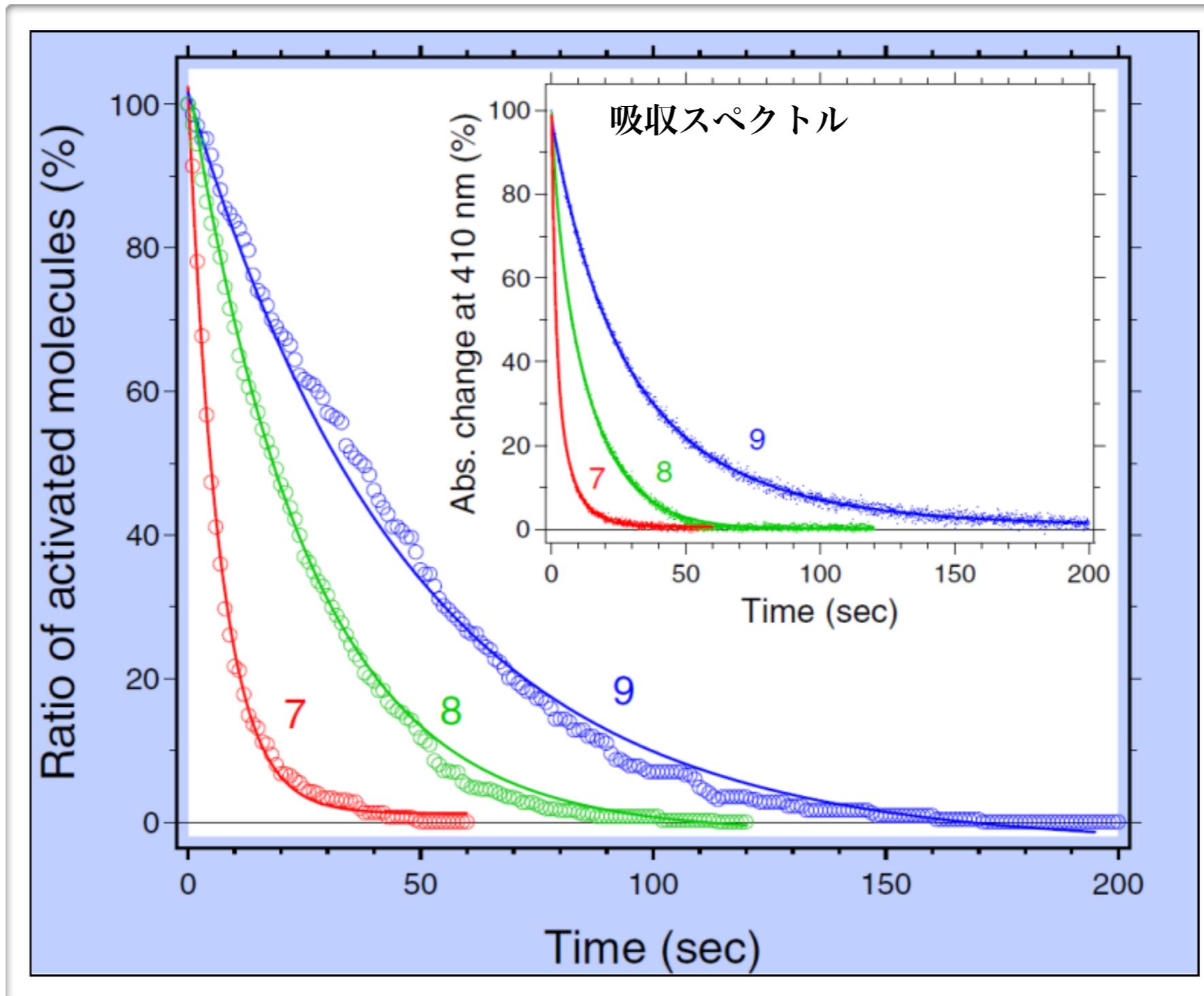
1 frame/sec, x10 play

D96N at pH9



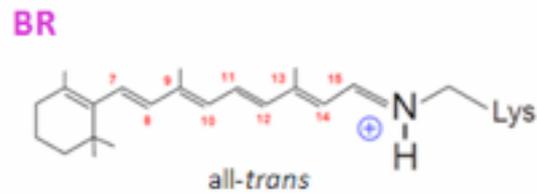
1 frame/sec, x10 play

pH依存性



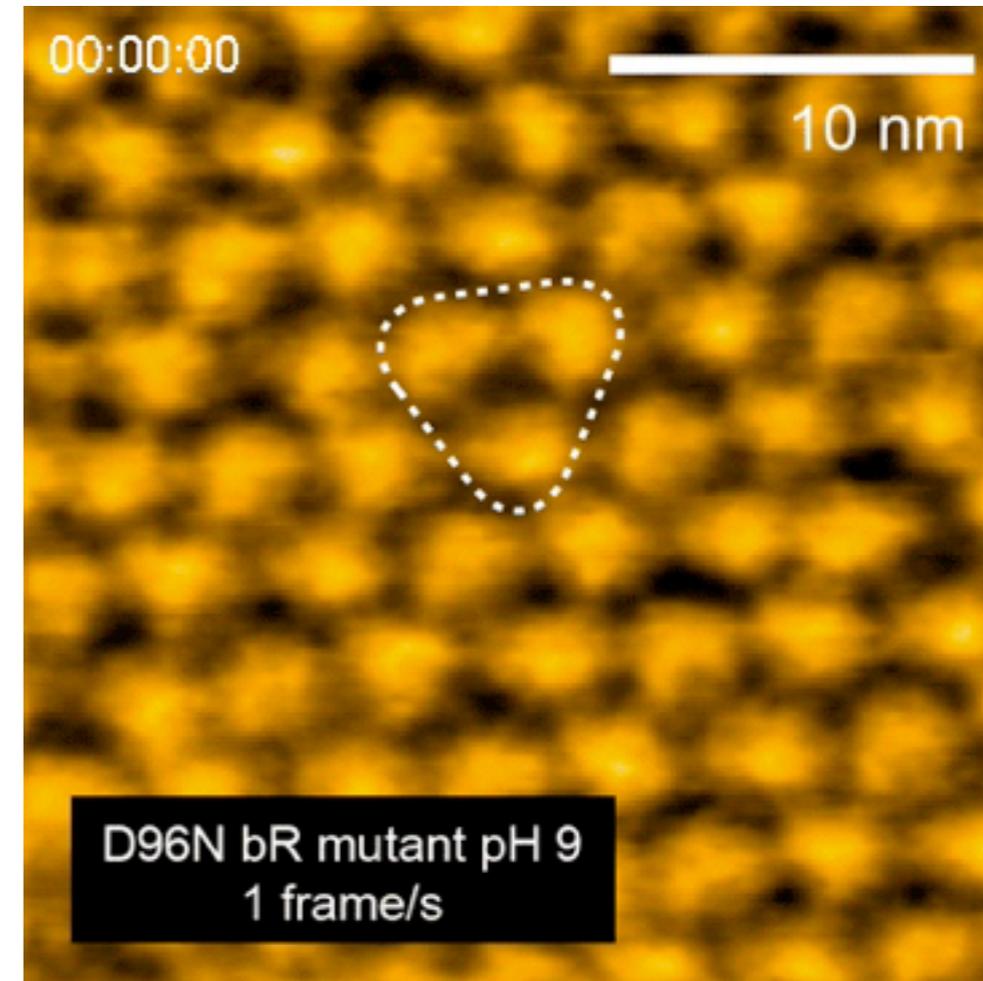
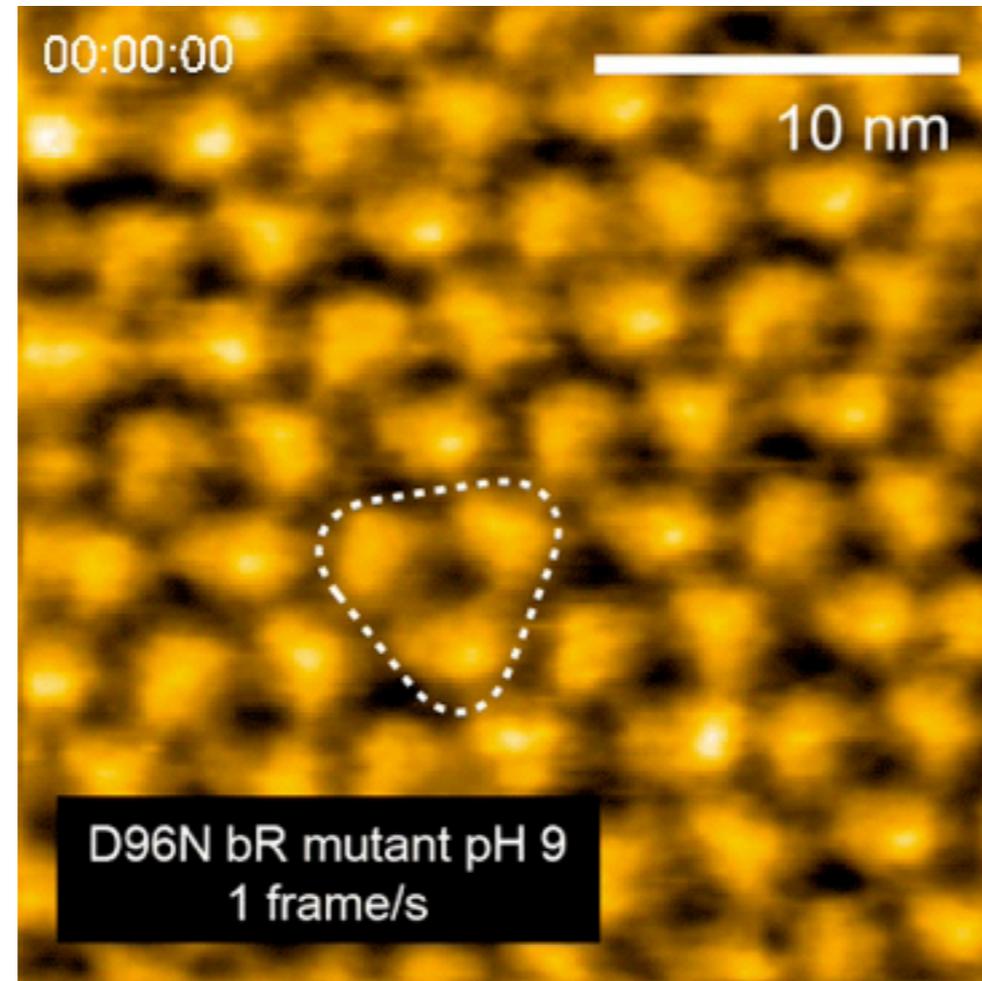
2色照射による逆反応

pH 8での緩和時間: $\tau_{1/2} = 40$ s



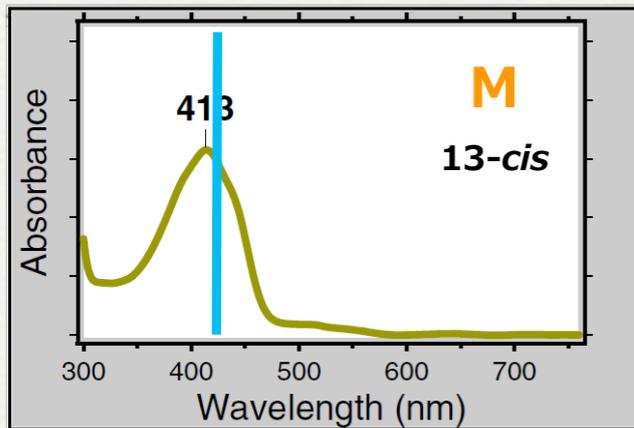
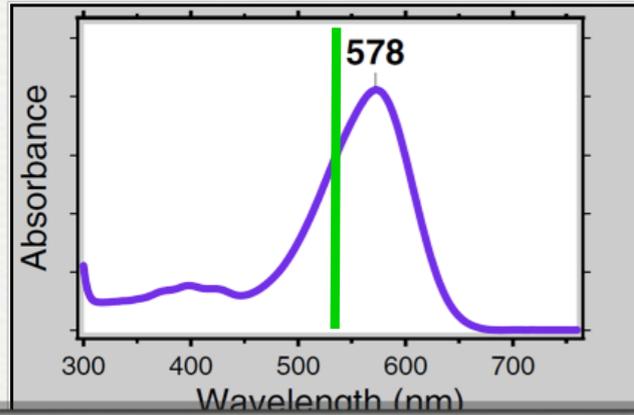
Green (10s) → Off (10s)

Green (10s) → Blue (10s)



10 mM tris-HCl pH 8, 300 mM KCl,
200x200 pixel², green laser: 532 nm

10 mM tris-HCl pH 8, 300 mM KCl,
200x200 pixel², green laser: 532 nm,
blue laser: 408 nm



イメージング

1. タンパク質の機能動態

* モータータンパク質: ミオシンV, **F₁-ATPase**

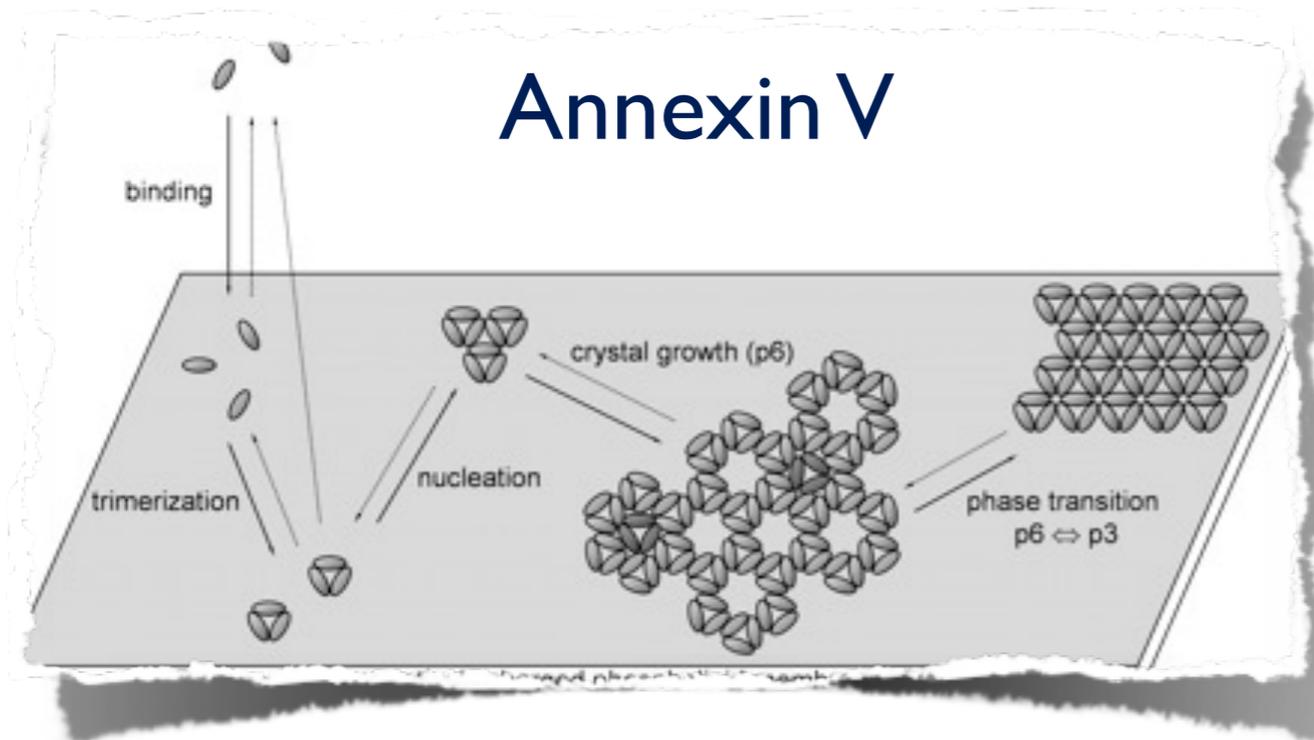
セルラーゼ

* 膜タンパク質: バクテリオロドプシン

2. タンパク質結晶のダイナミクス

アネキシンV, ライセニン

タンパク質結晶のダイナミクス



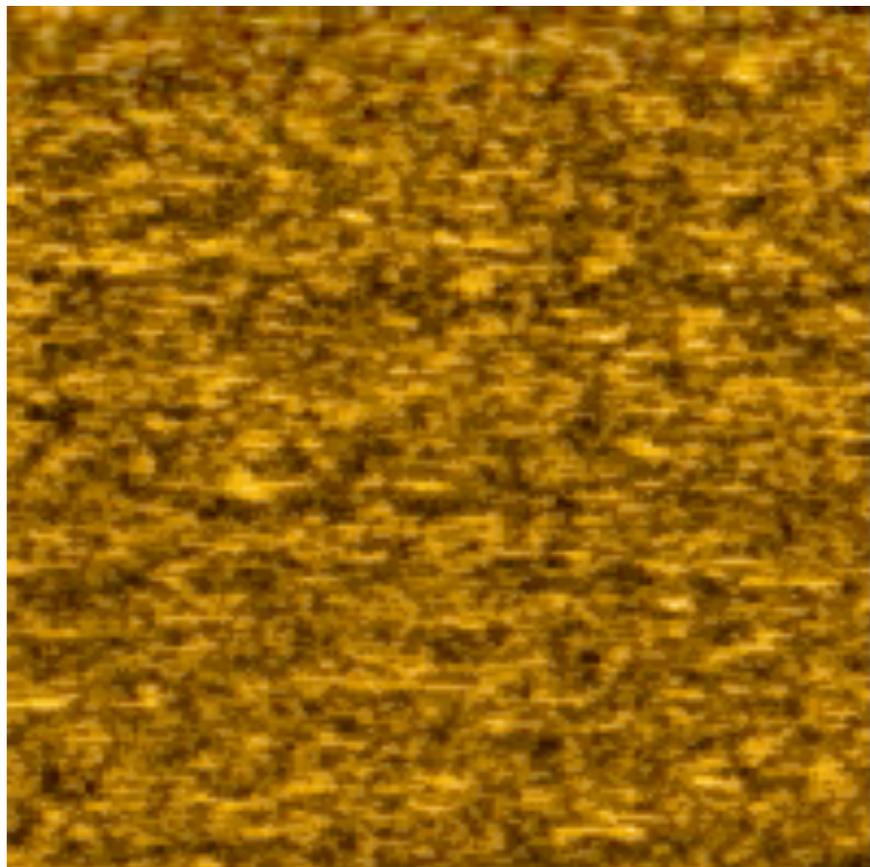
Annexin V

Lipid:

DOPC: DOPS:DOPE=5 : 2 : 3

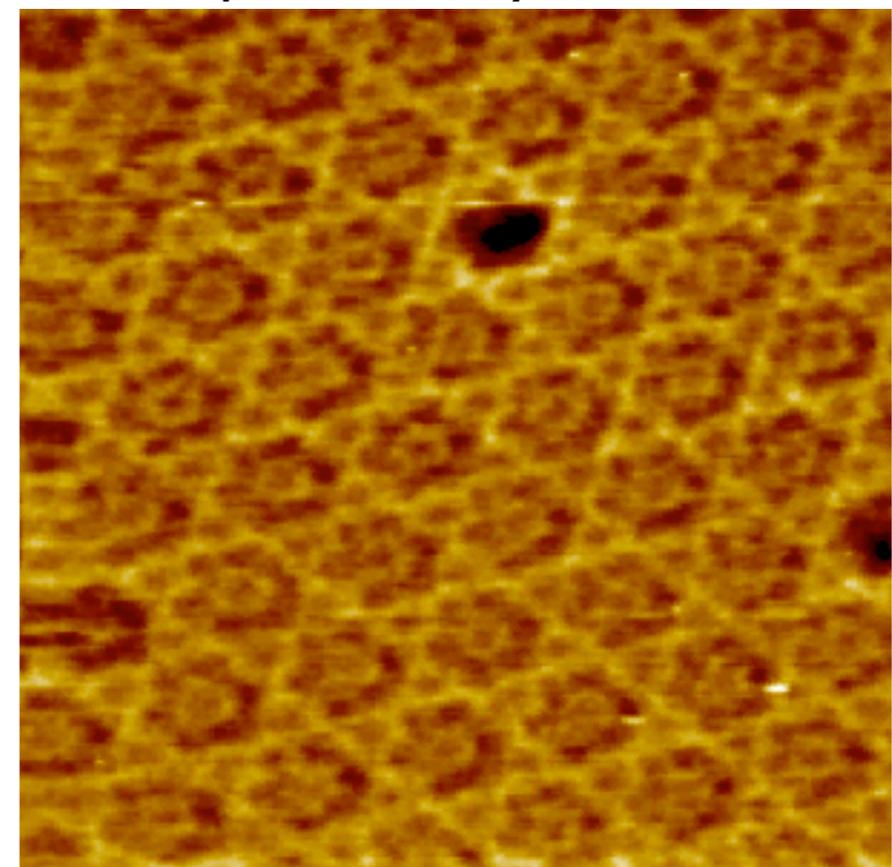
Buffer: 50 mM Tris-HCl pH 8.0,
5mM KCl, 2.5mM MgCl₂,

Assembly (3mM-CaCl₂)



400nm x 400nm 1 s/frame (x20 plays)

Equilibrium dynamics

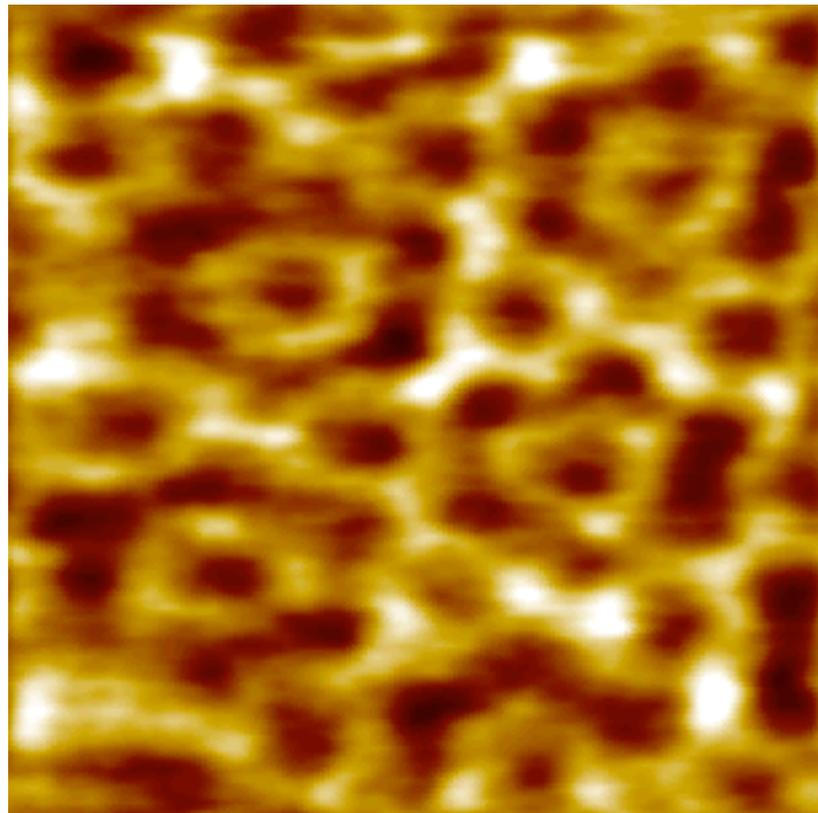


150nm x 150nm 500 ms/frame (x5 plays)

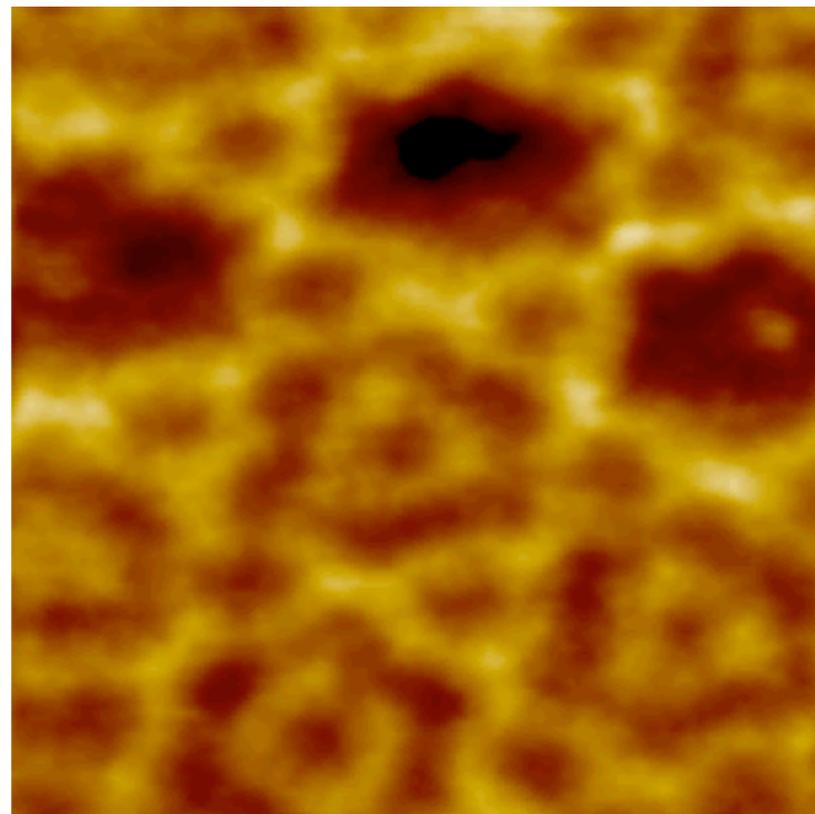
タンパク質結晶のダイナミクス

Annexin V

Rotational Diffusion

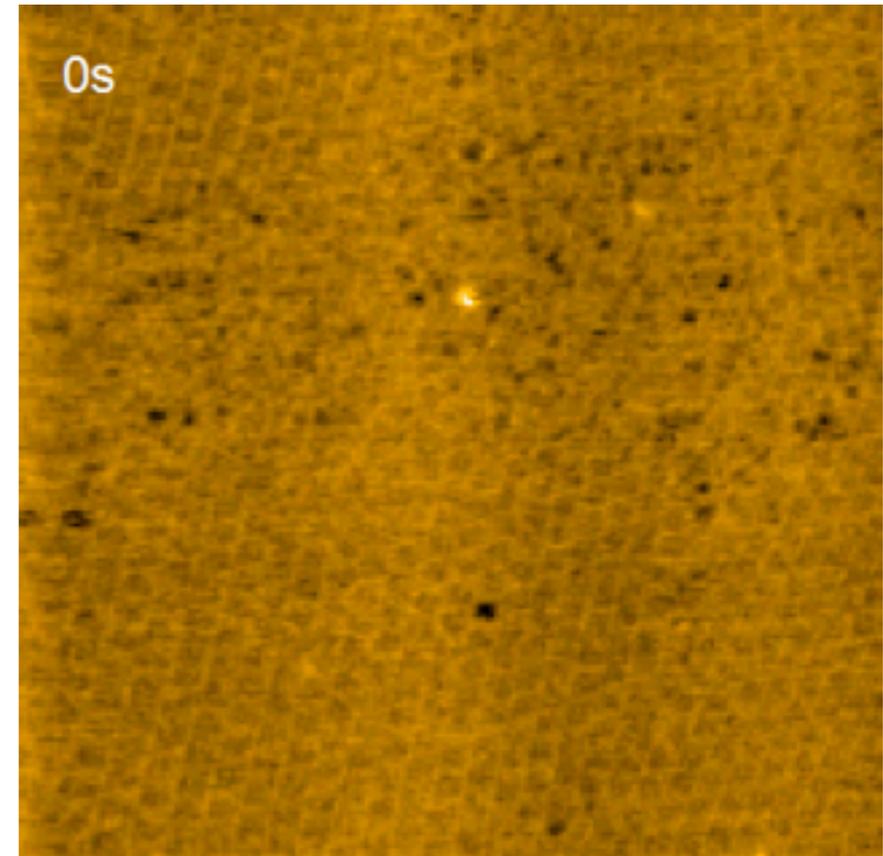


50nm x 50nm 500 ms/frame
(x5 plays)



50nm x 50nm 200 ms/frame
(x2 plays)

Disassembly (EGTA)

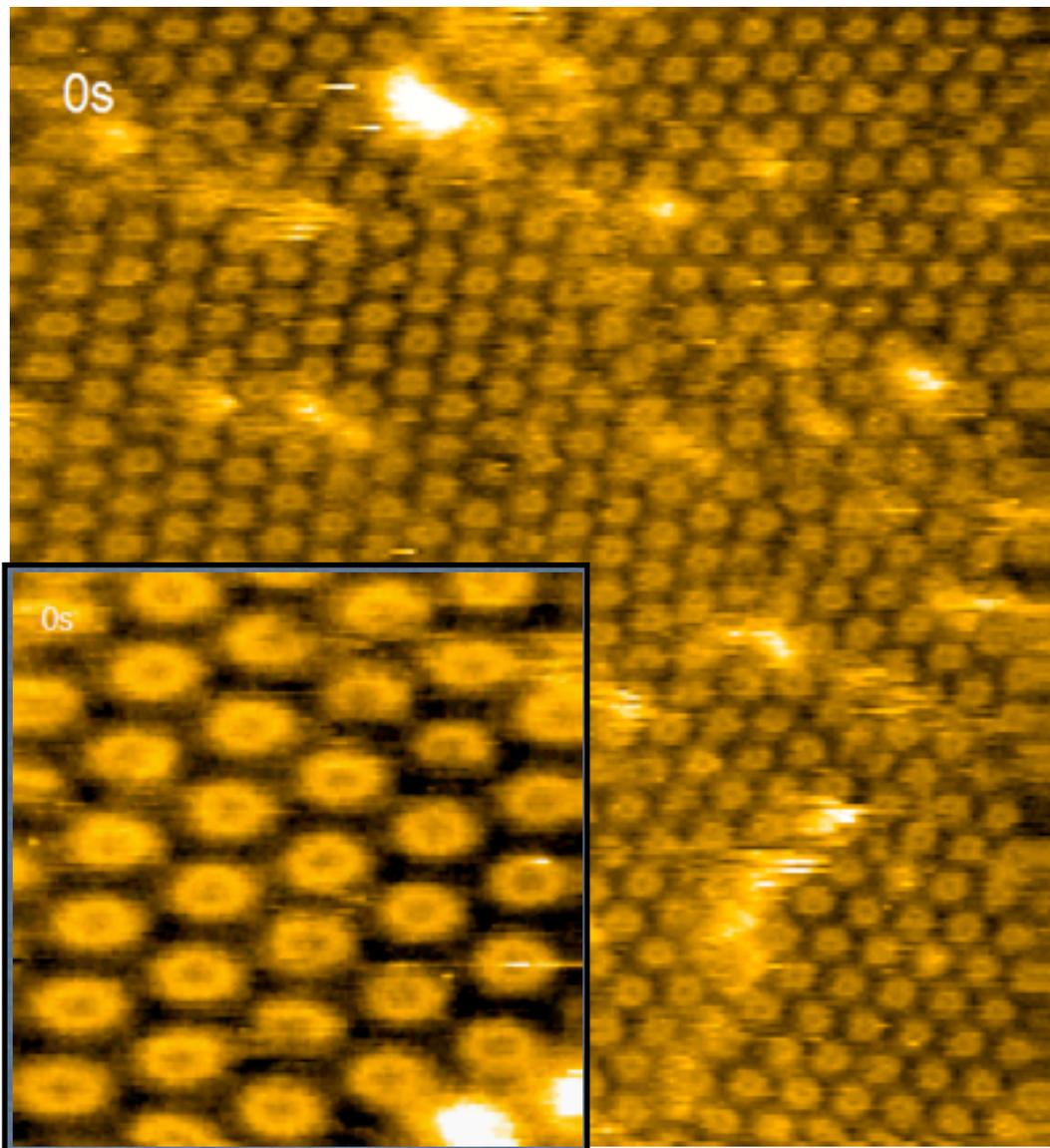


50nm x 50nm 1 s/frame
(x20 plays)

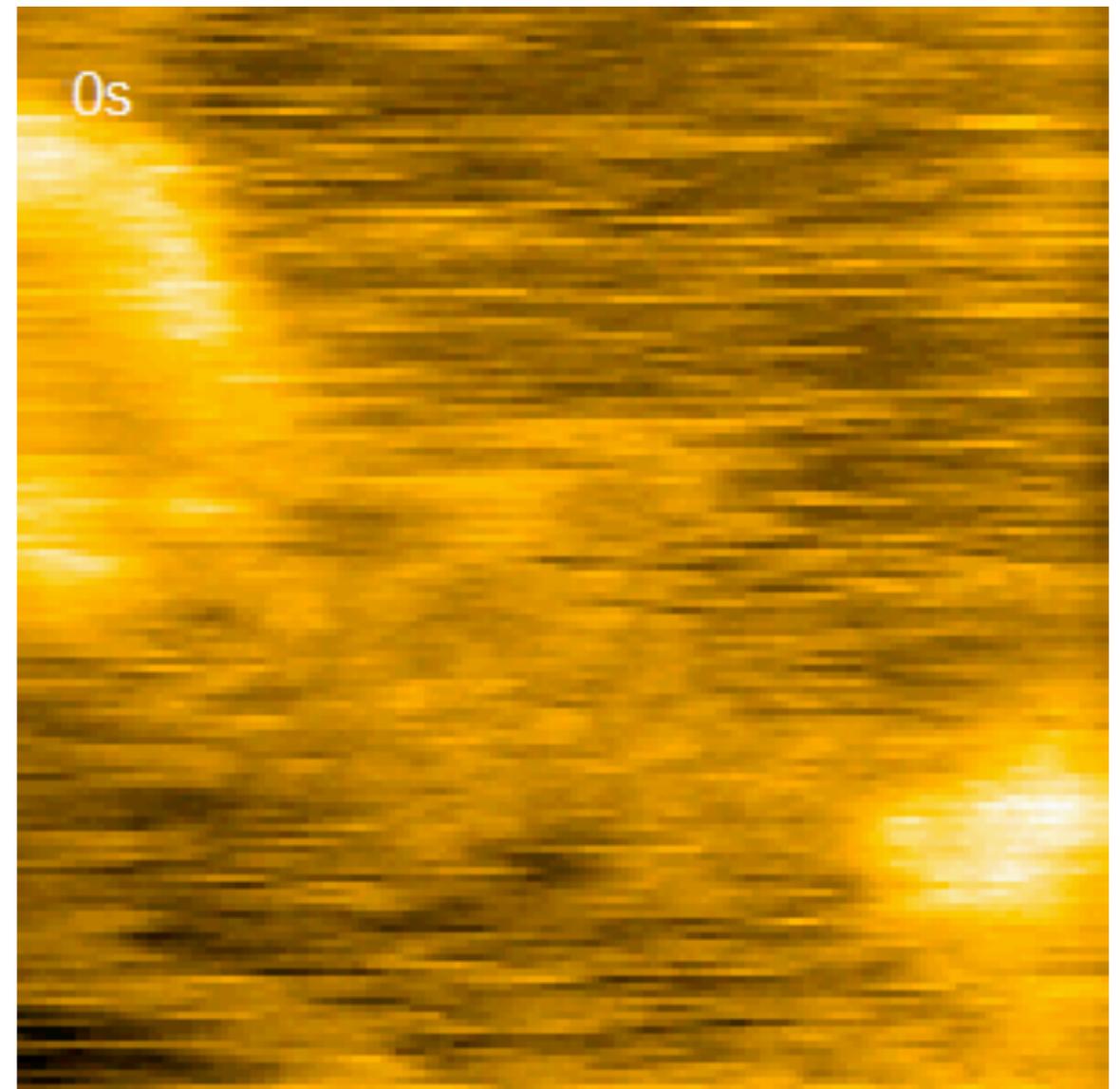
タンパク質結晶のダイナミクス

Lysenin/SM/cholesterol (1:1)

Assembly



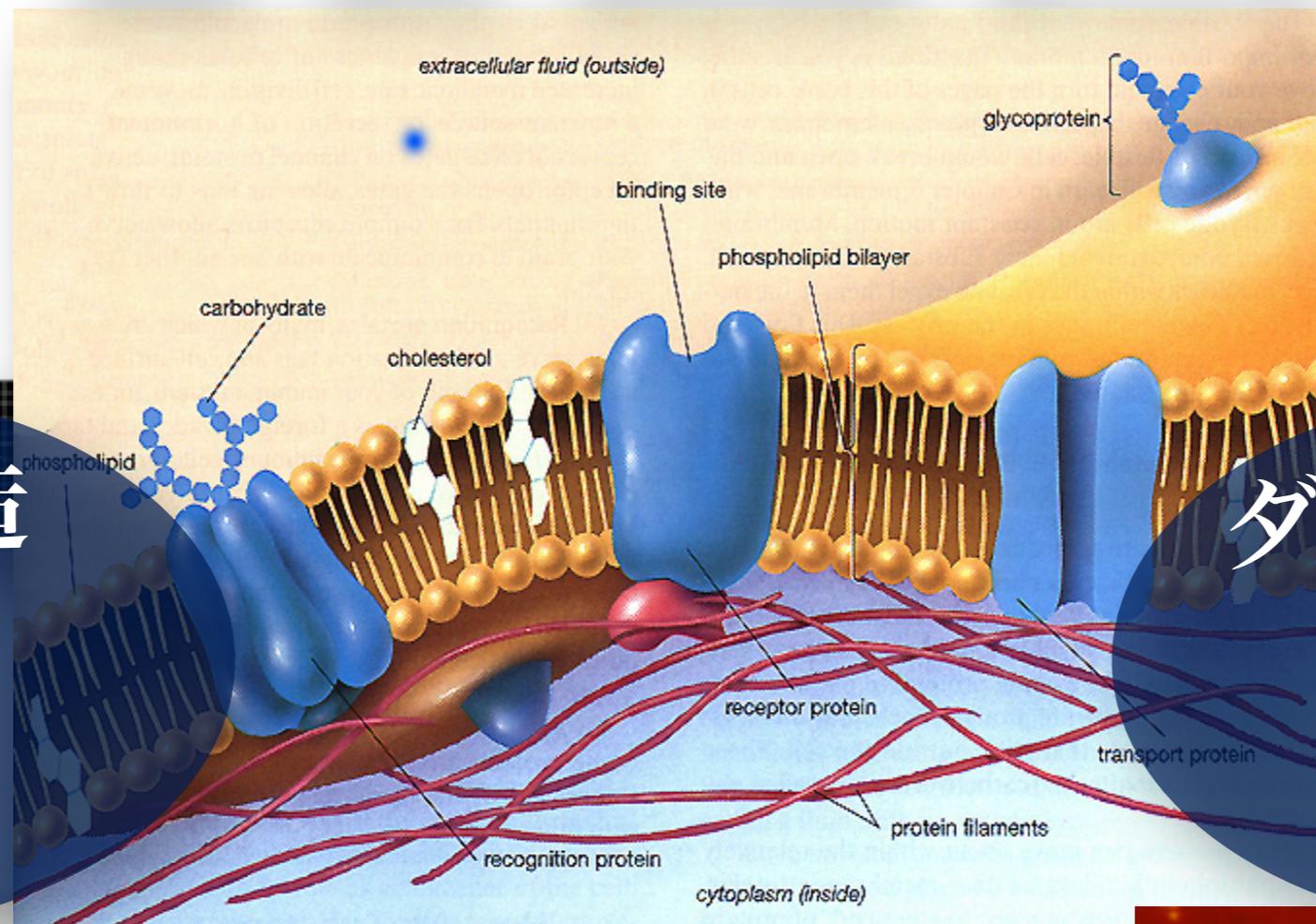
300nm x 300nm 1s/frame



250nm x 250nm 0.3s/frame
(x10 plays)

生体分子の解析技術

機能、構造、構造変換、相互作用



分子構造

回折

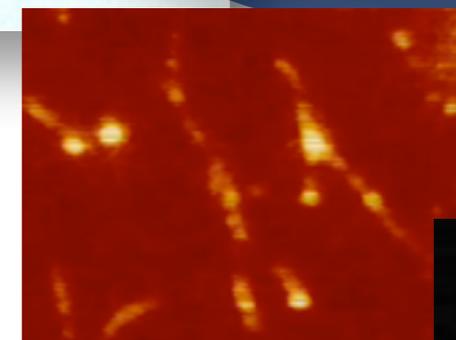
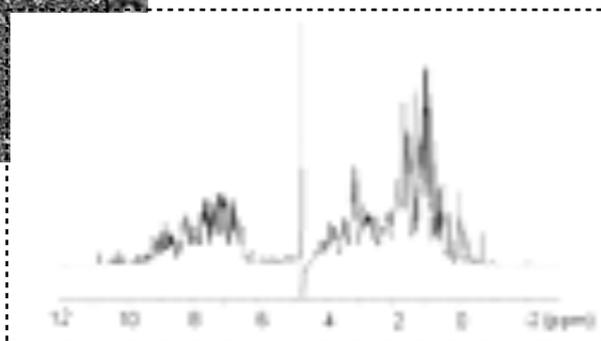
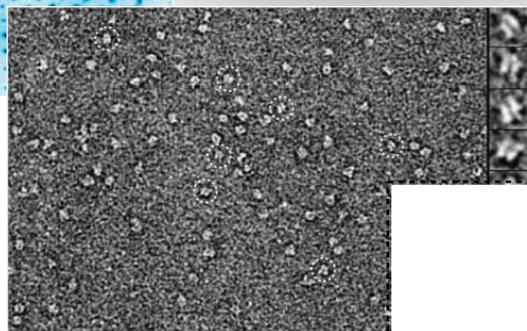
電子顕微鏡

NMR

ダイナミクス

光学顕微鏡

全反射・FRET・
光/磁気ピンセット

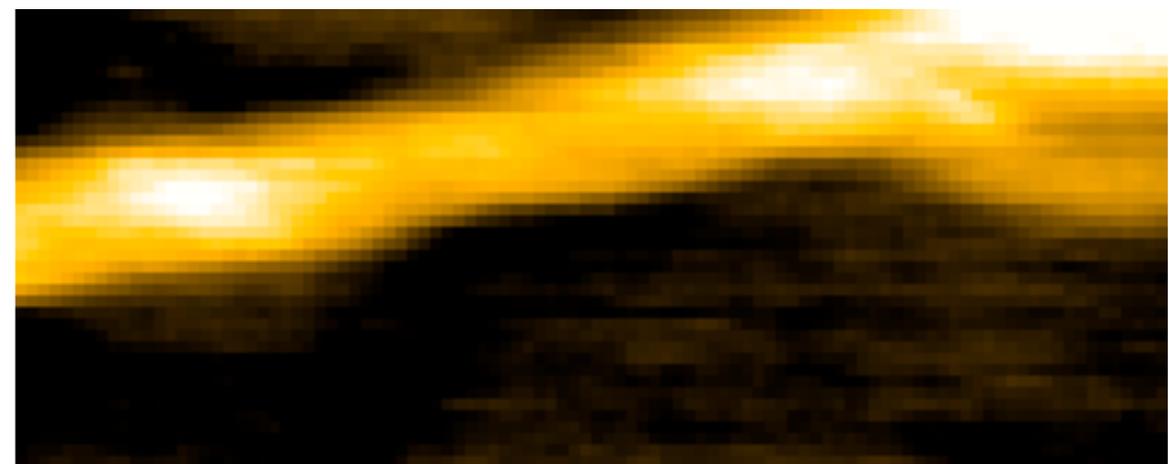
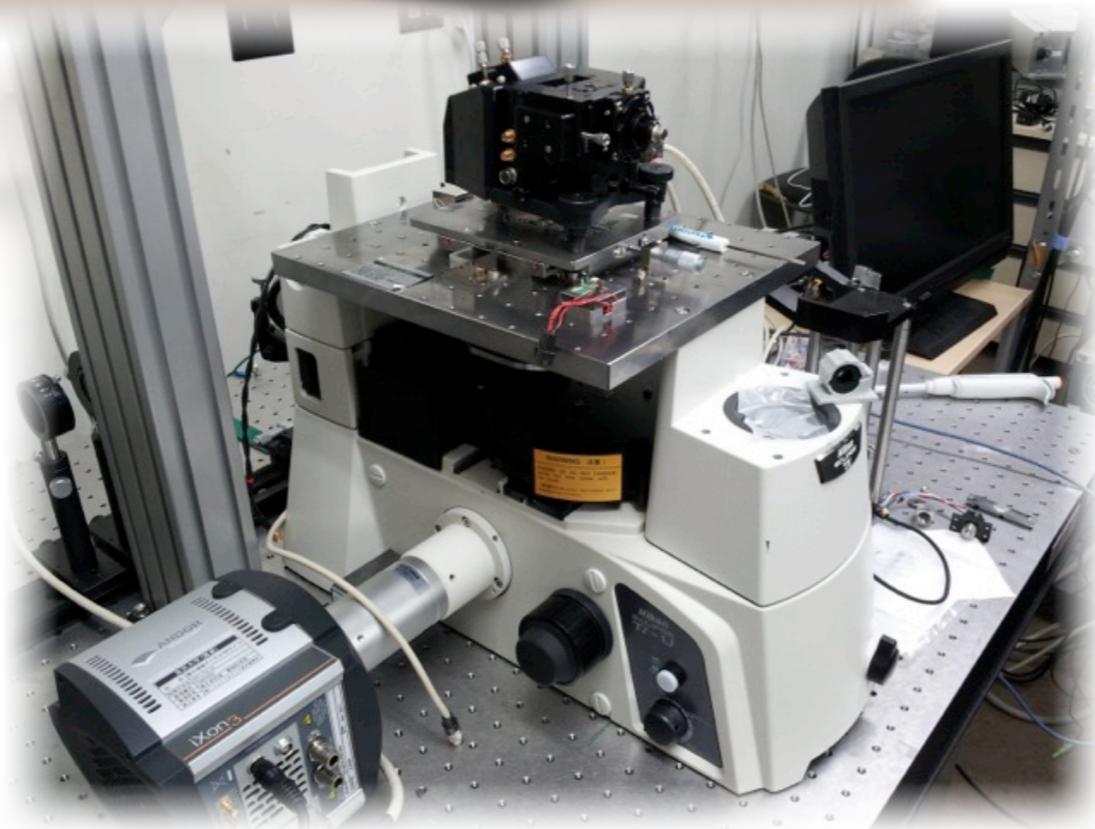
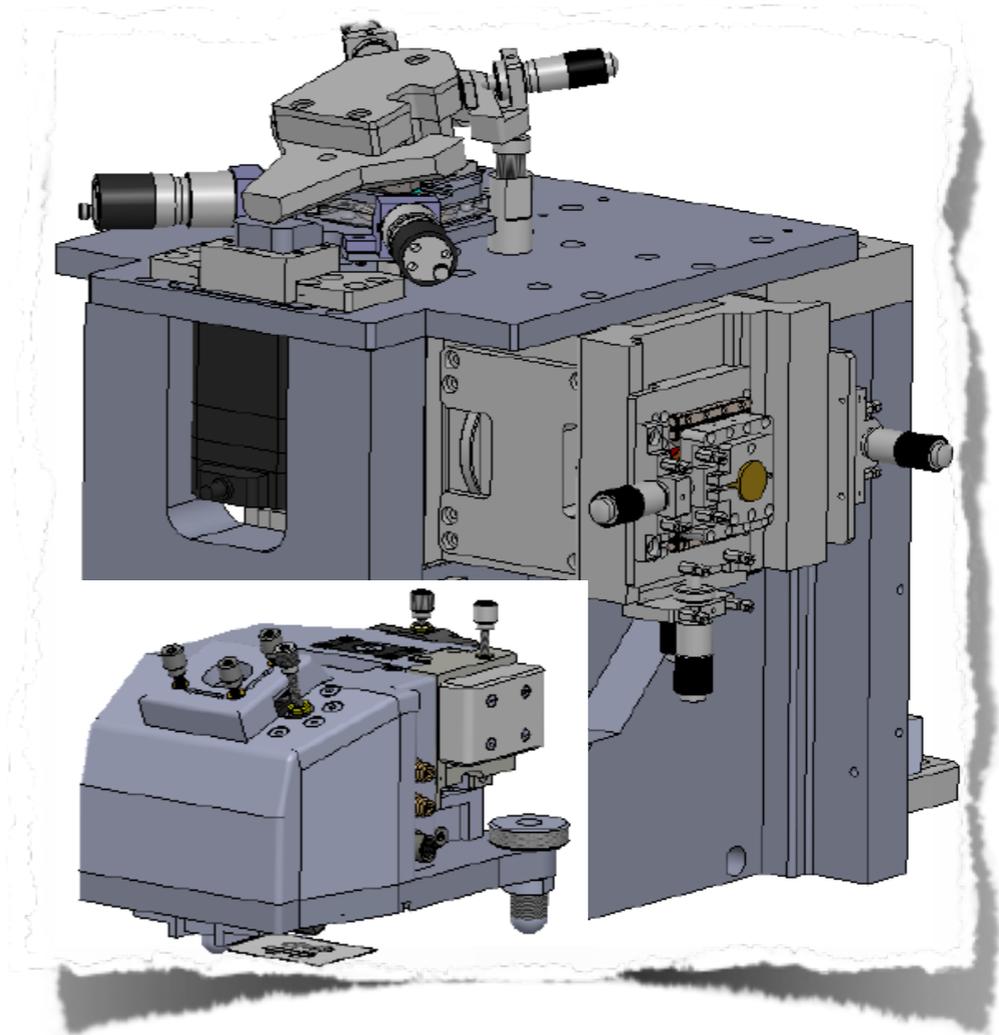
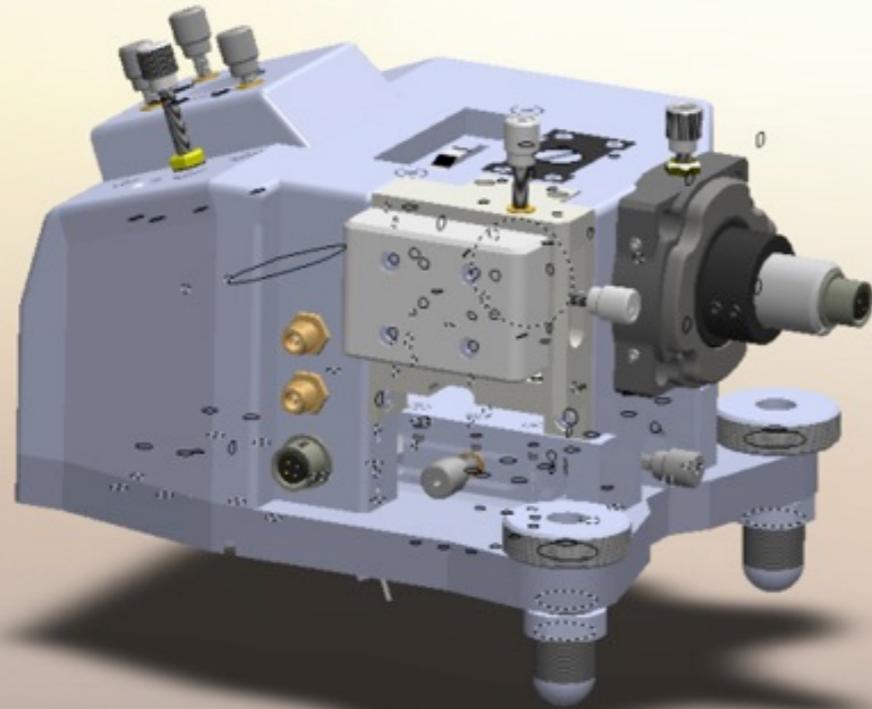


今後の展開 1

- 構造のみの観察：構造変換と基質結合
- 複数のタンパク質が関与する現象

1分子蛍光顕微鏡との複合化

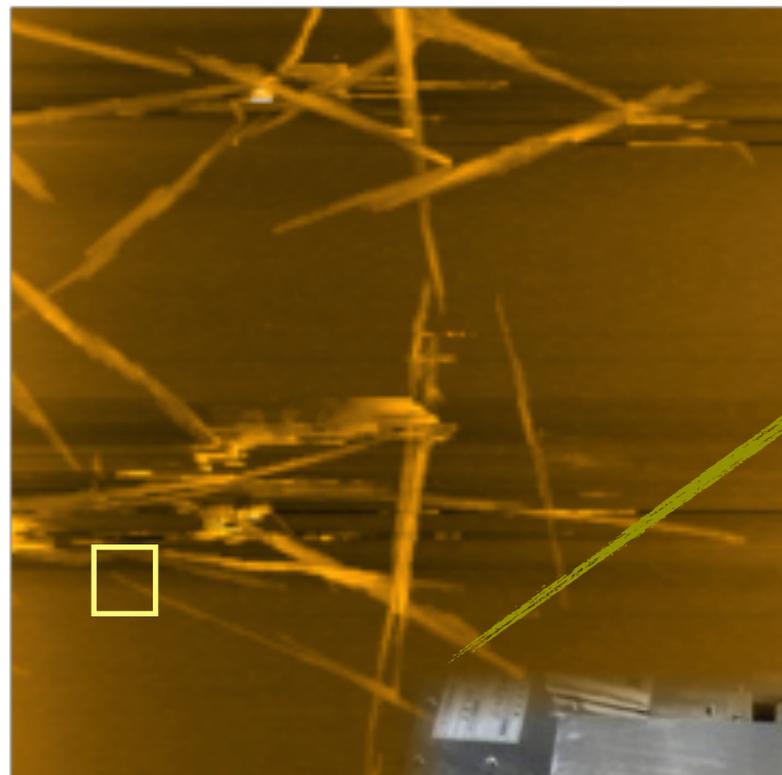
高速AFM/螢光顯微鏡



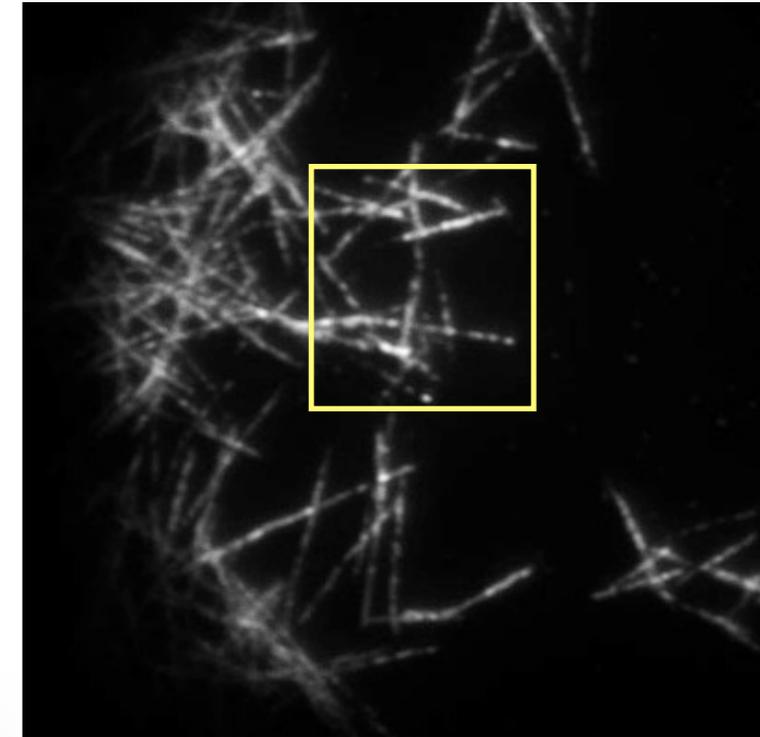
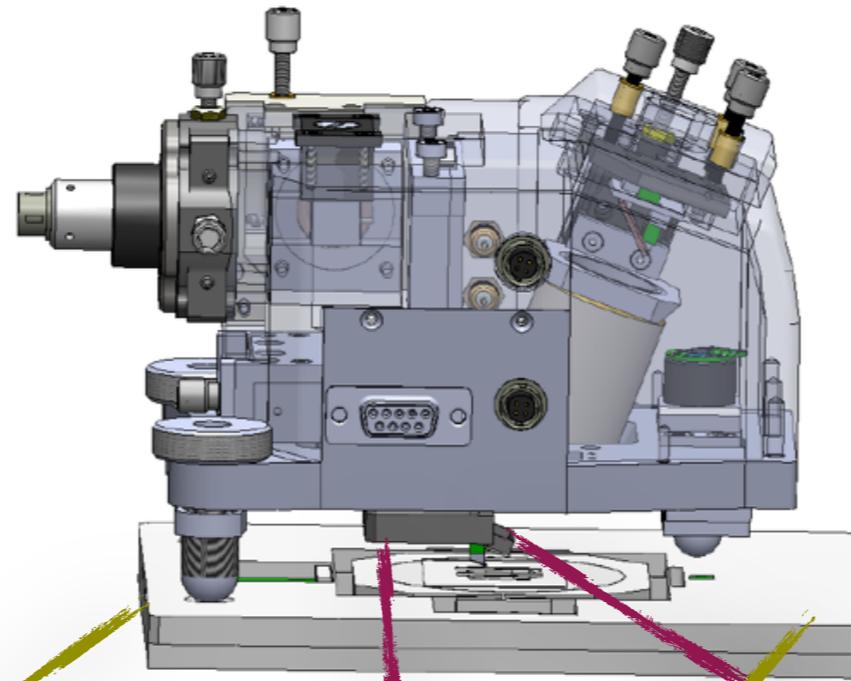
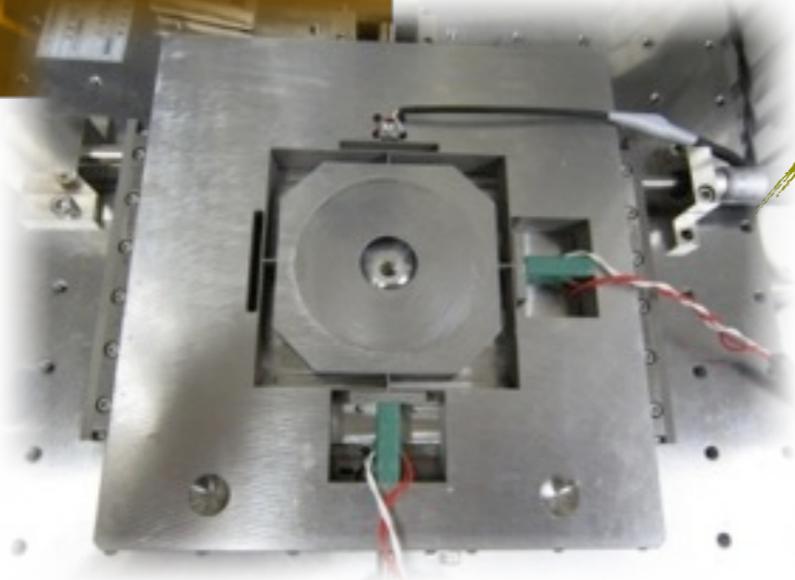
高速AFM/蛍光顕微鏡

低速・広範囲AFM

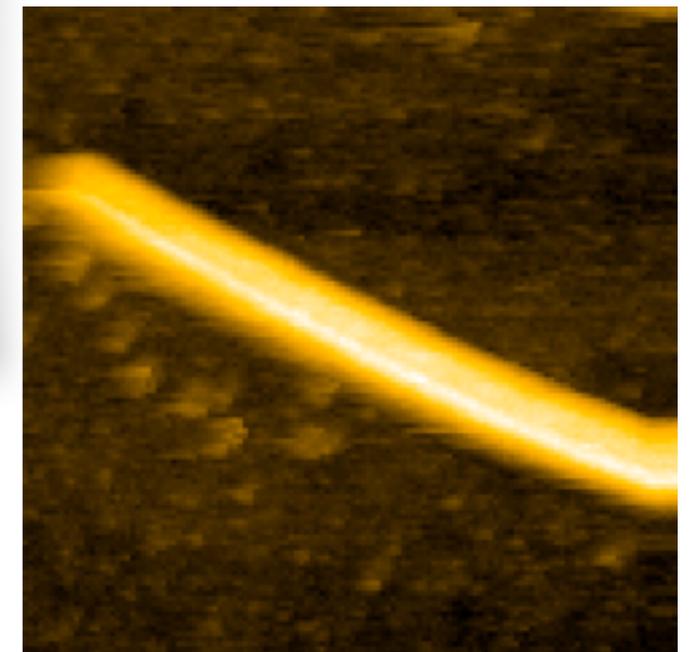
$8\ \mu\text{m} \times 8\ \mu\text{m}$



$6\ \mu\text{m} \times 6\ \mu\text{m}$

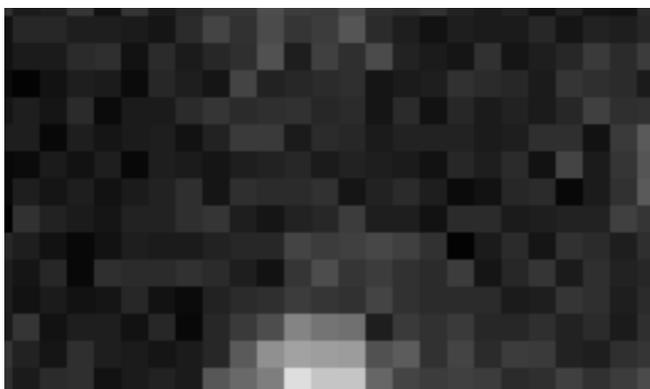
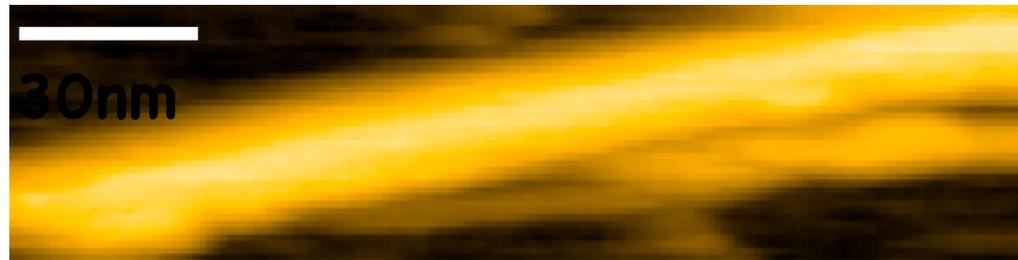


高速AFM

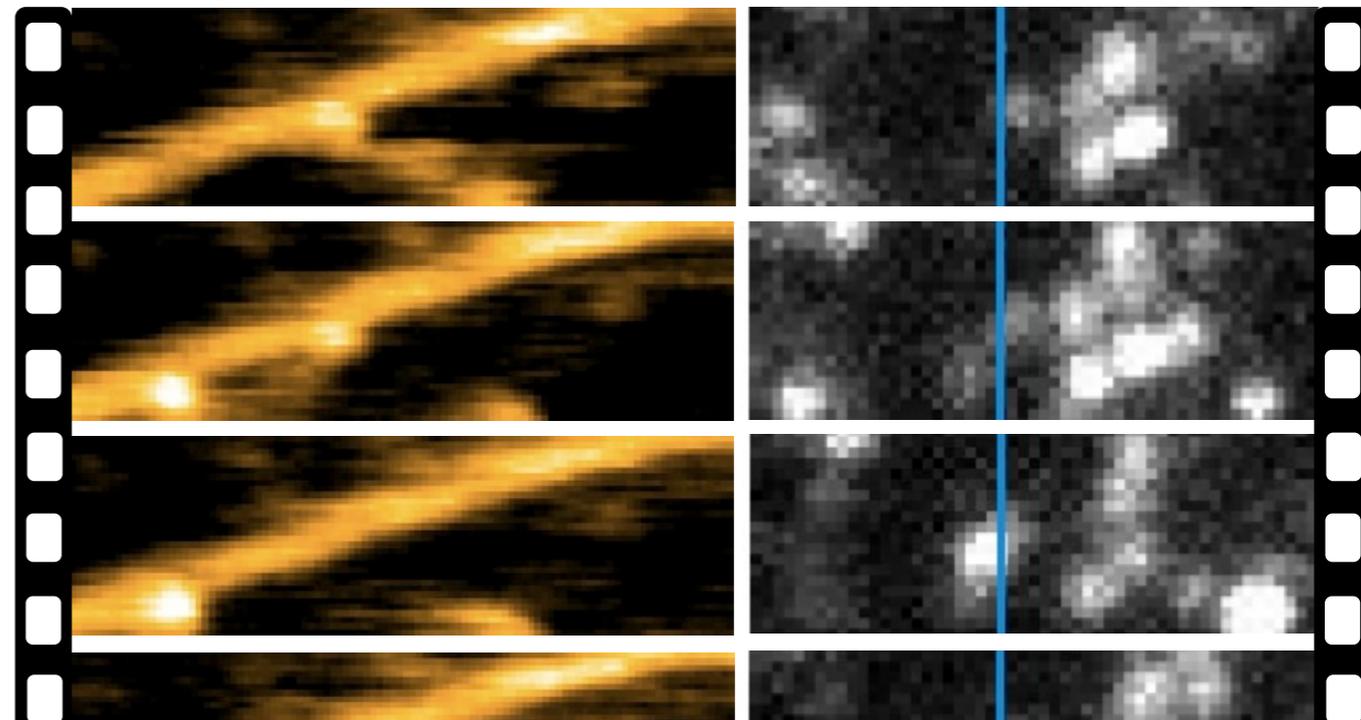
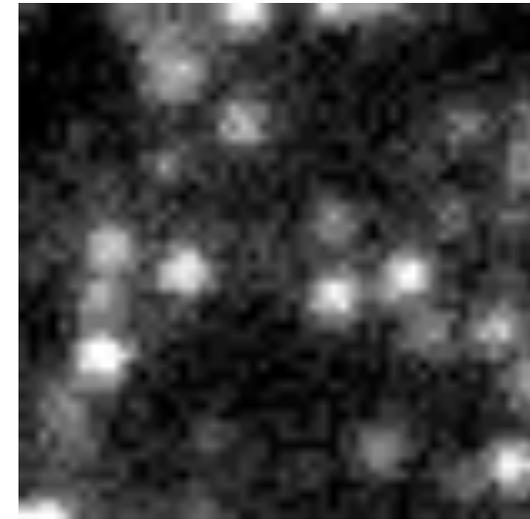
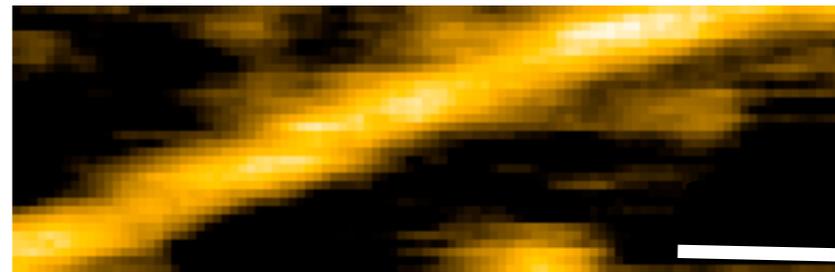


同時觀察例

cy3-chitinase/chitin
330 ms/frame



cy3-myosin V/actin
200 ms/frame



今後の展開 2

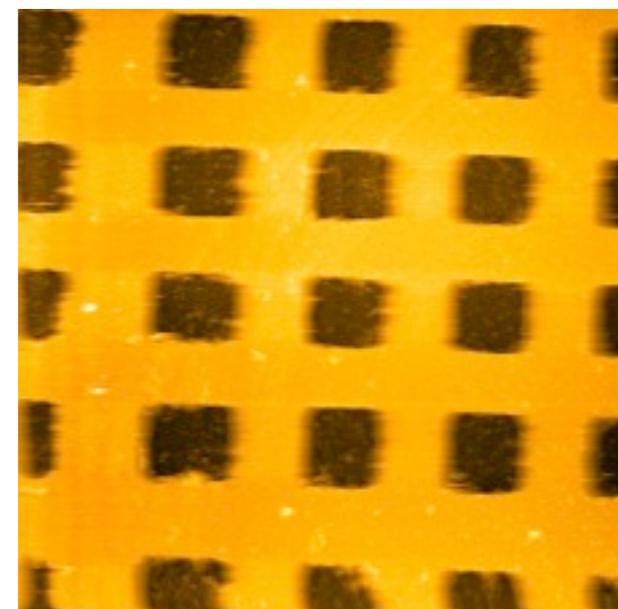
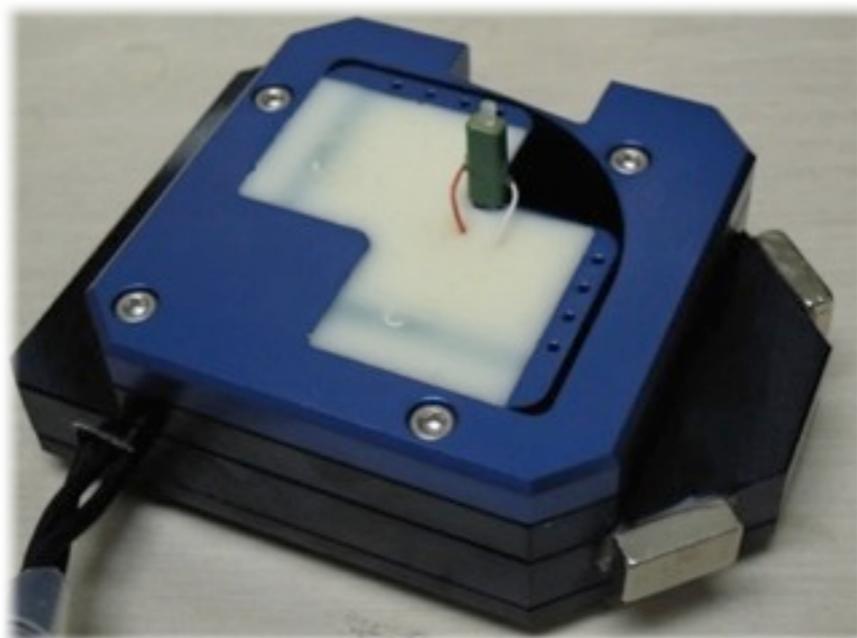
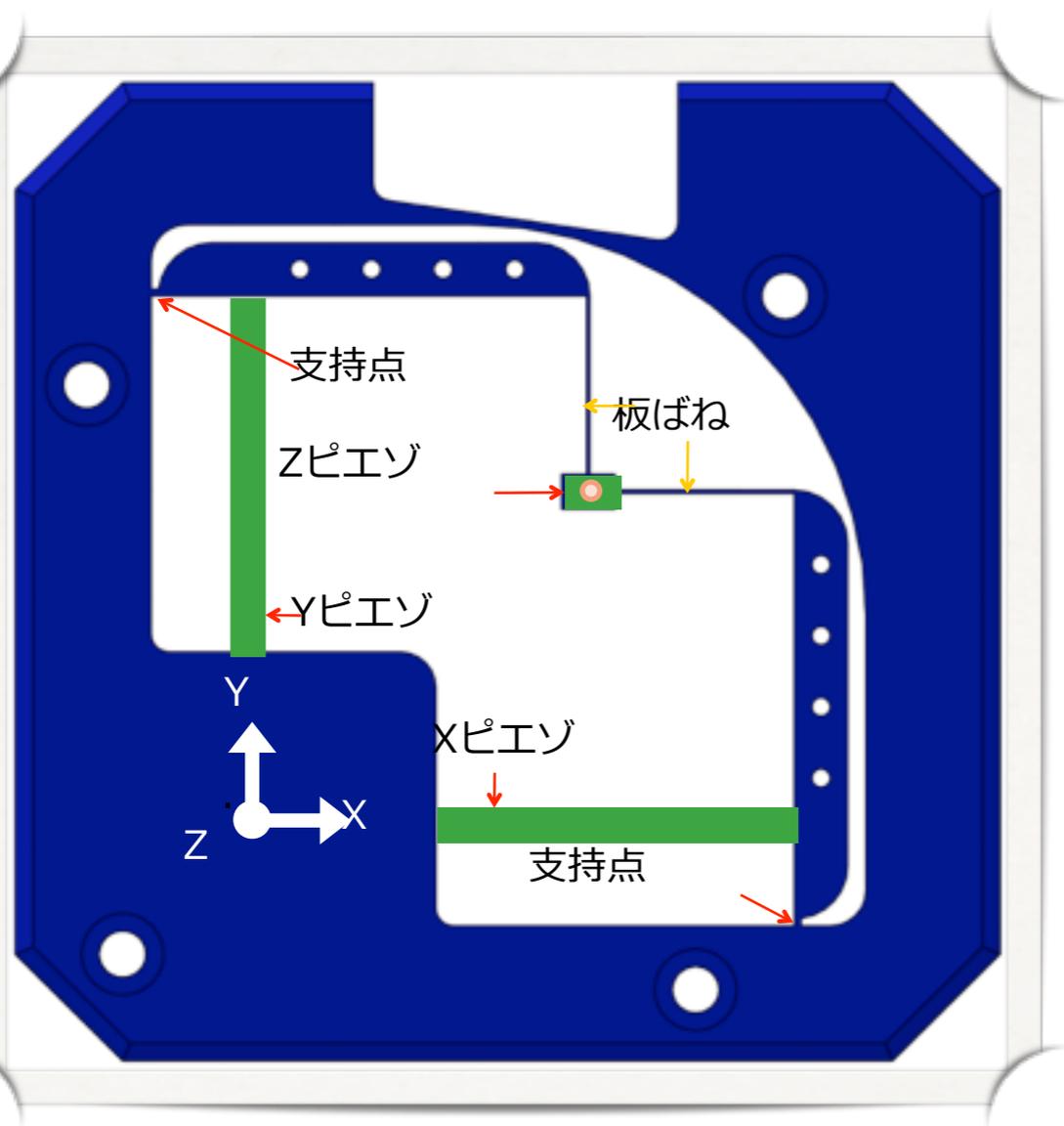
● 細胞表面の形態変化

高速 & 広範囲スキャナー

細胞のイメージング

広範囲/高速スキャナーの開発

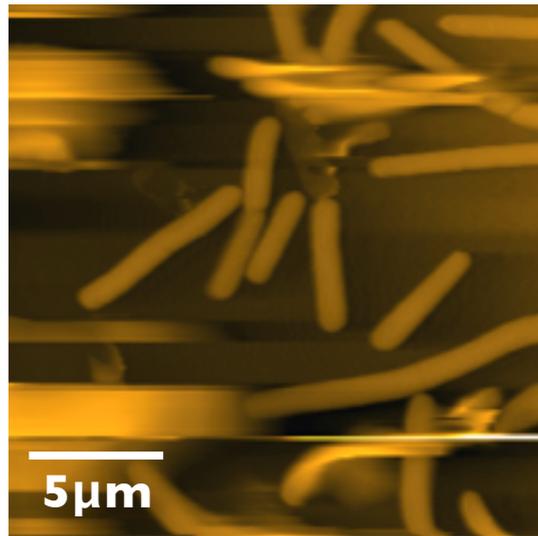
最大変位量 **X : 43.5 μ m、Y : 42.5 μ m**
Z : 4.3 μ m



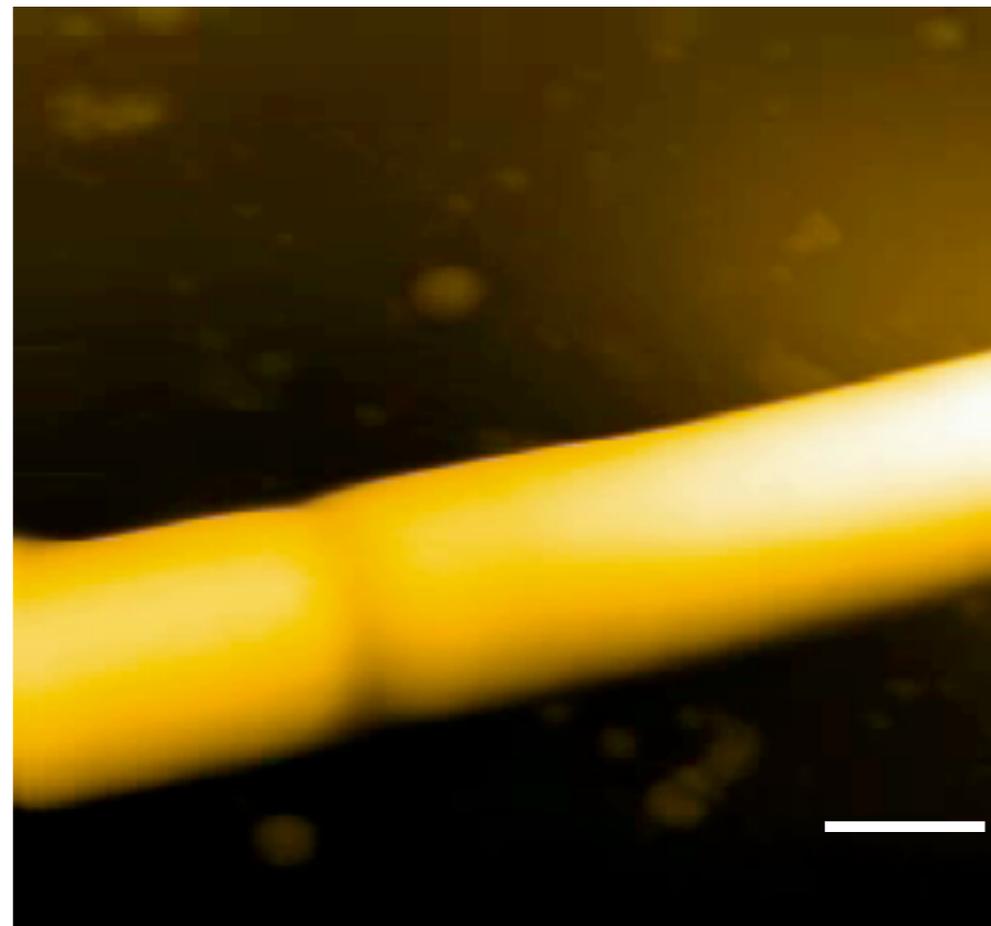
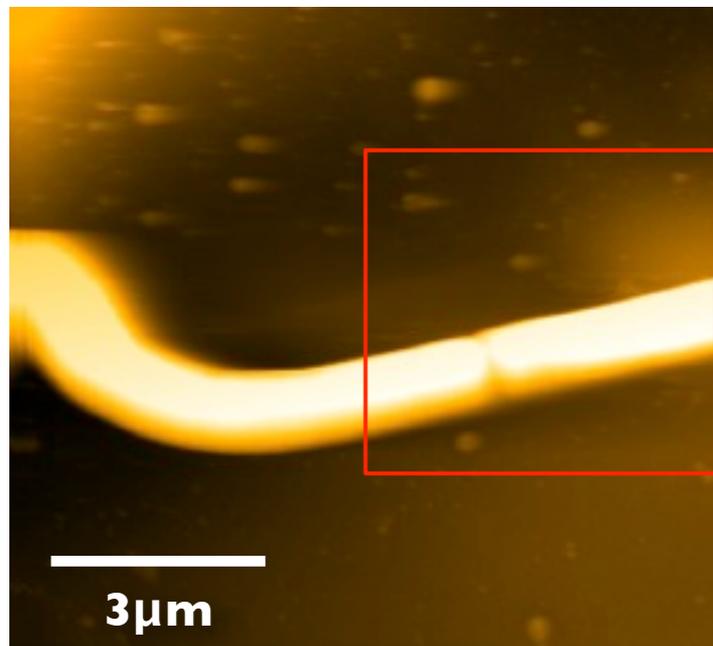
40 μ m \times 40 μ m
8s/frame

枯草菌の溶菌過程

ポリ-L-リジン / ガラス基板, リゾチウム最終濃度



8s/frame



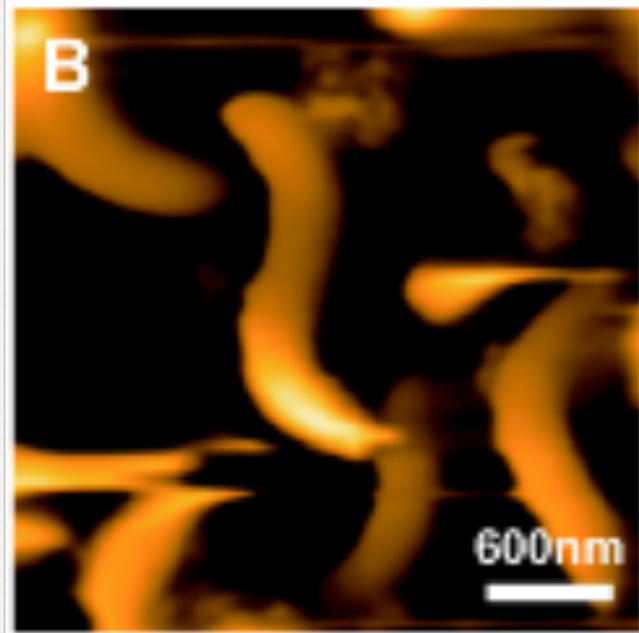
20 s/frame



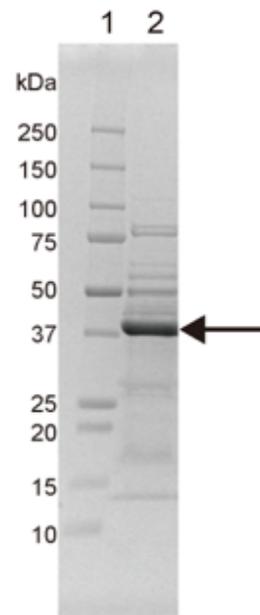
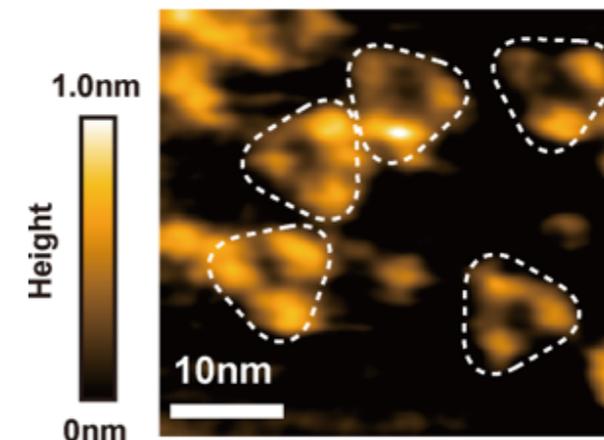
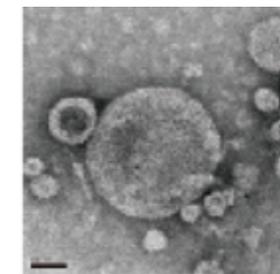
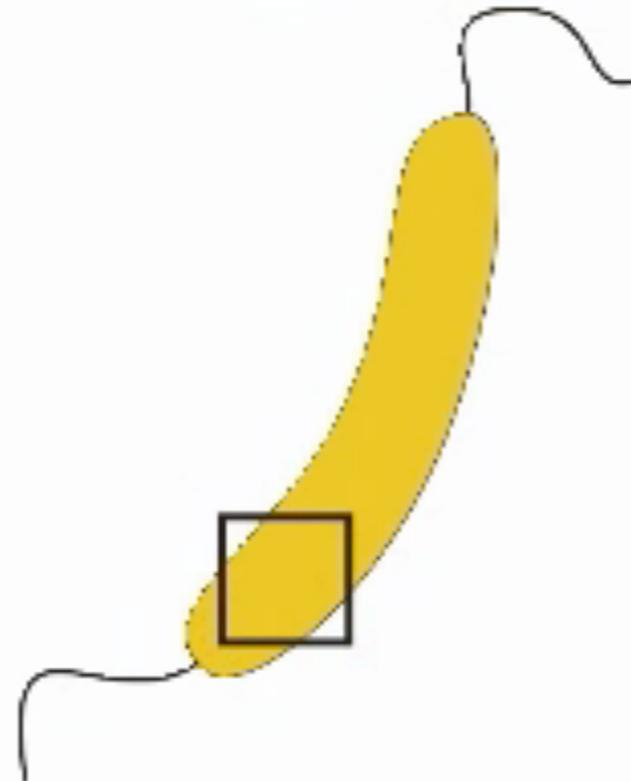
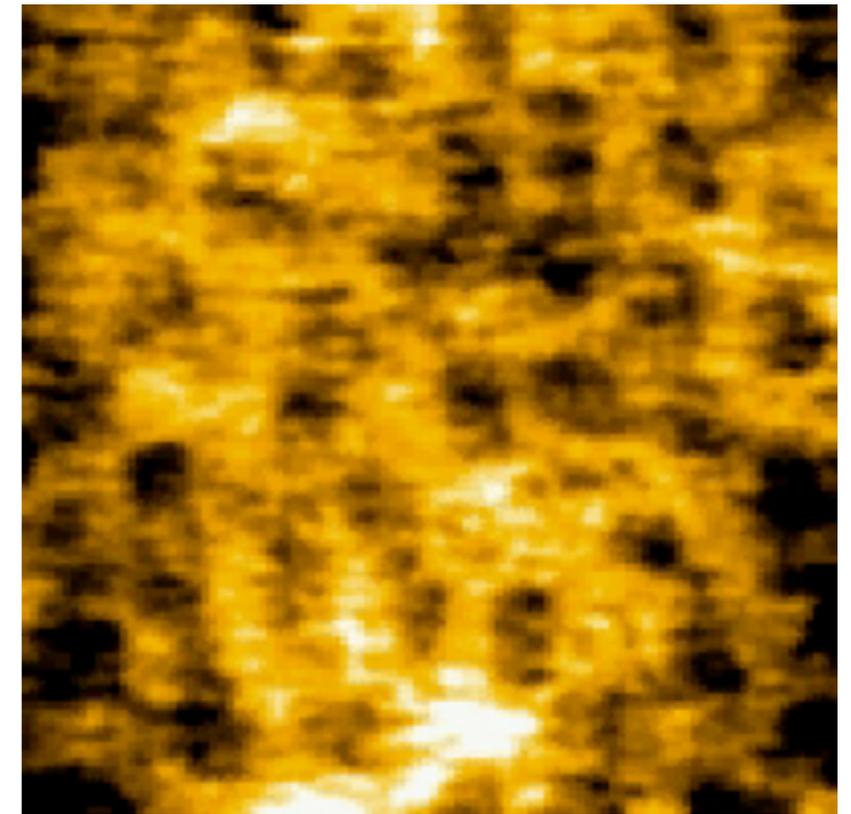
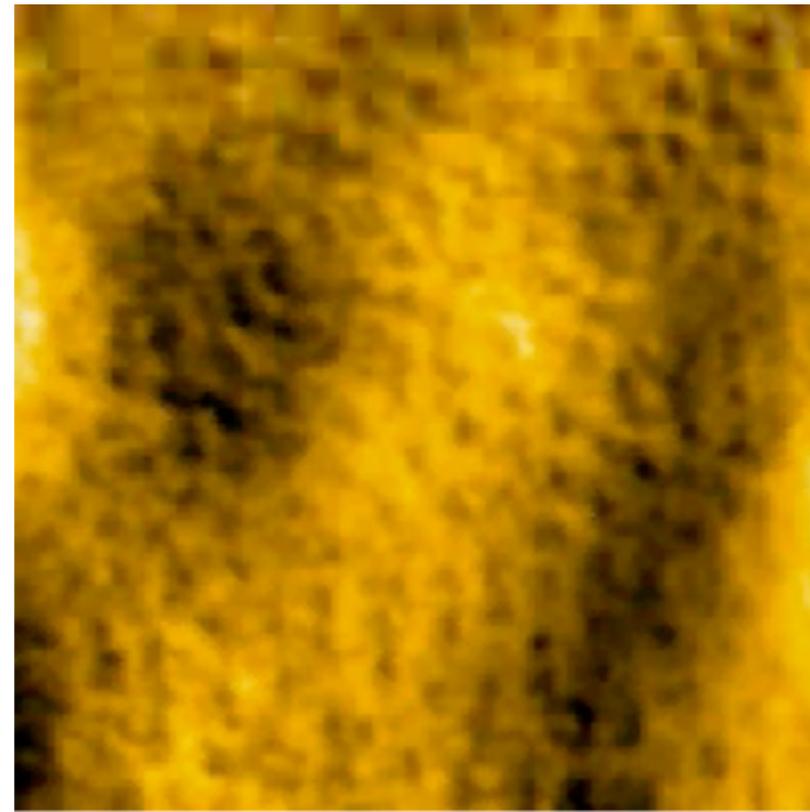
2 s/frame

バクテリア表面のタンパク質観察

磁性細菌



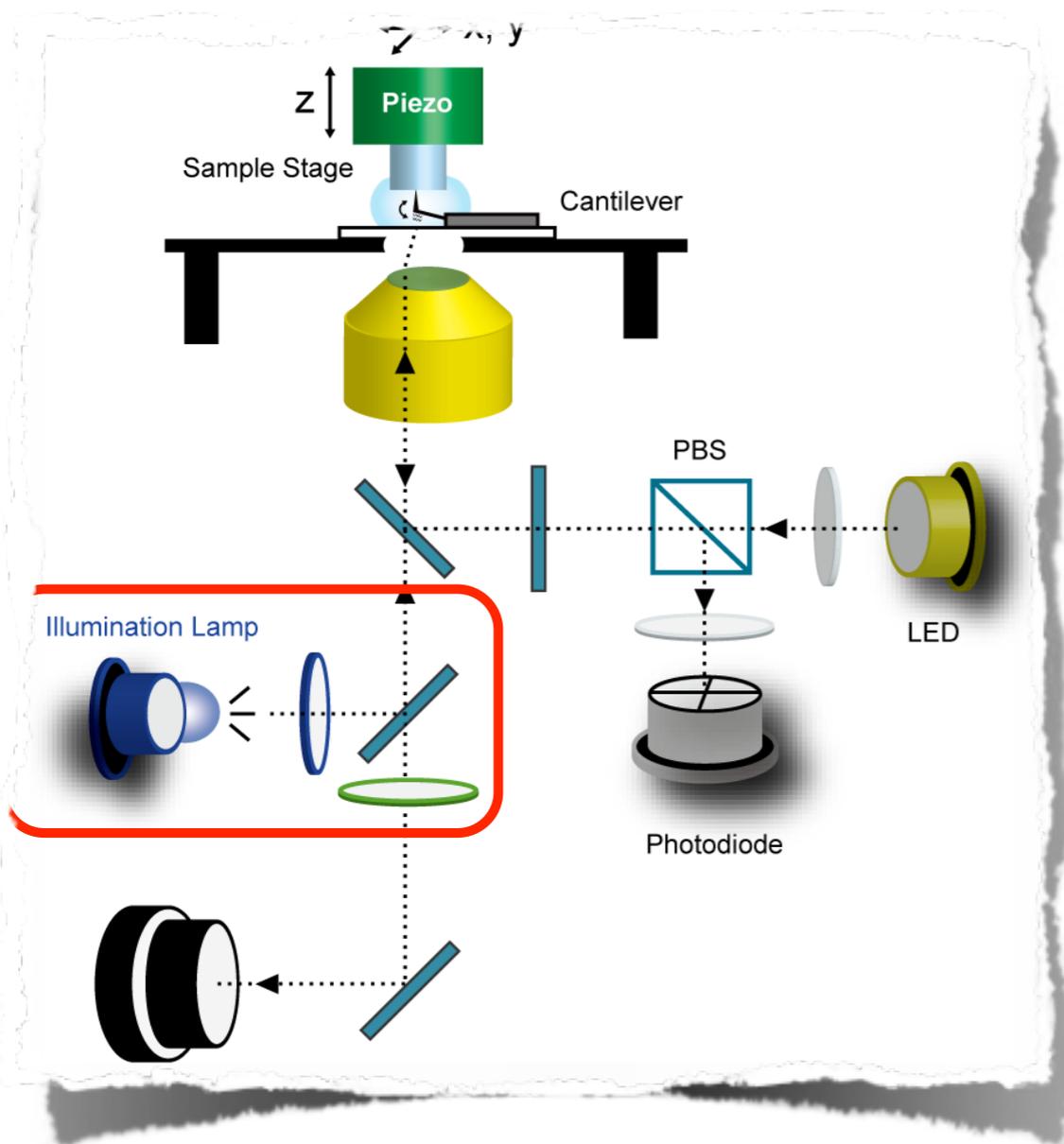
1100nm Height 0nm



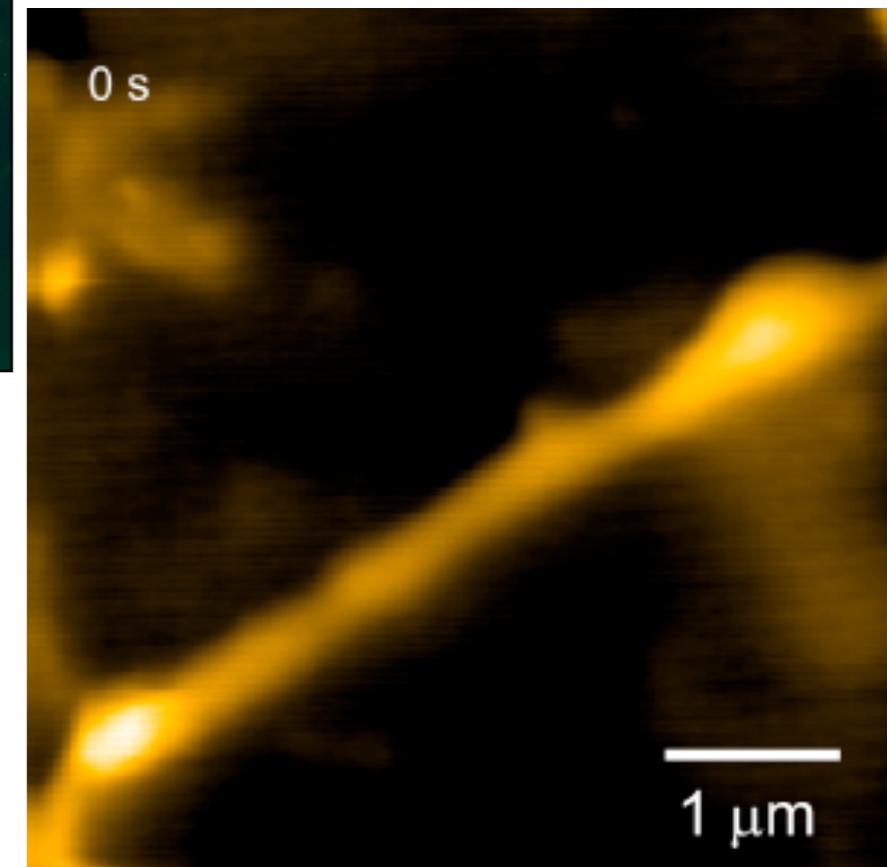
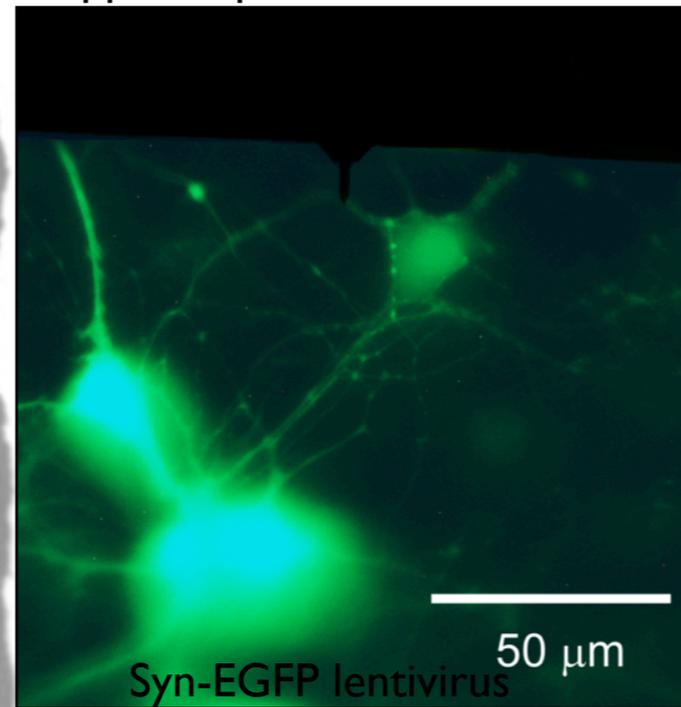
細胞表面のイメージング

細胞観察：広範囲スキャナーだけでは不十分

→ 光学顕微鏡による観察領域の設定

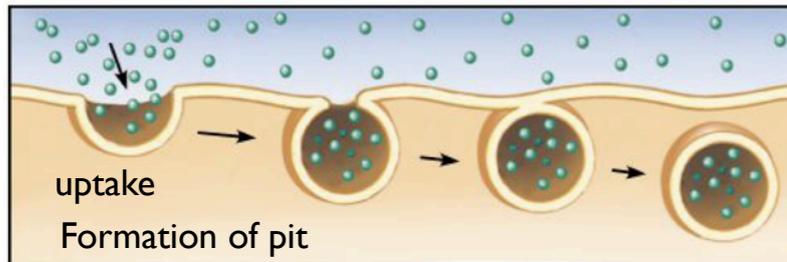


Hippocampal neurons 9 DIV

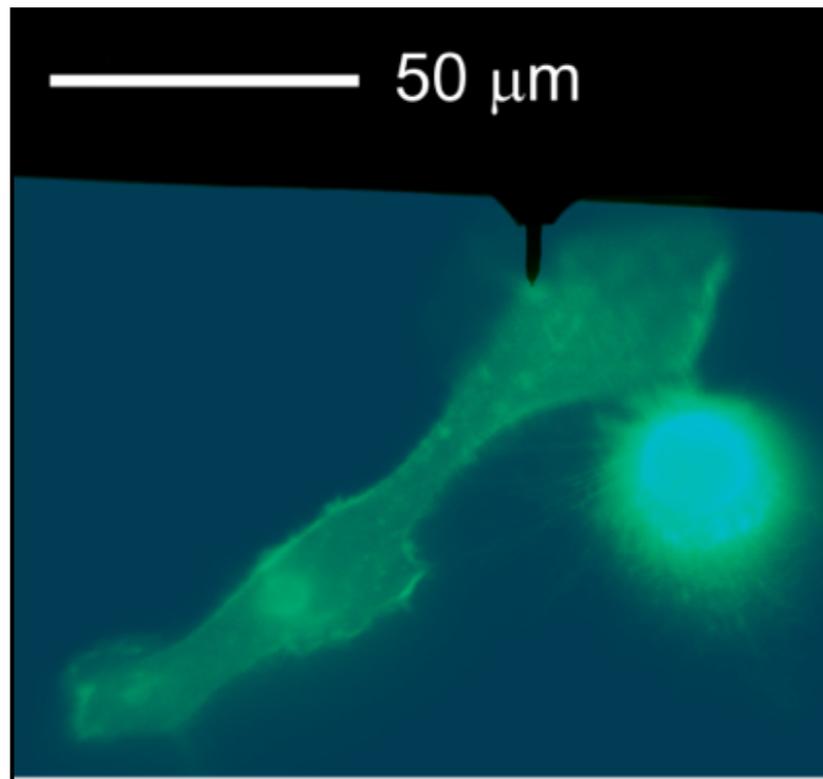


5 s/frame, x100 play

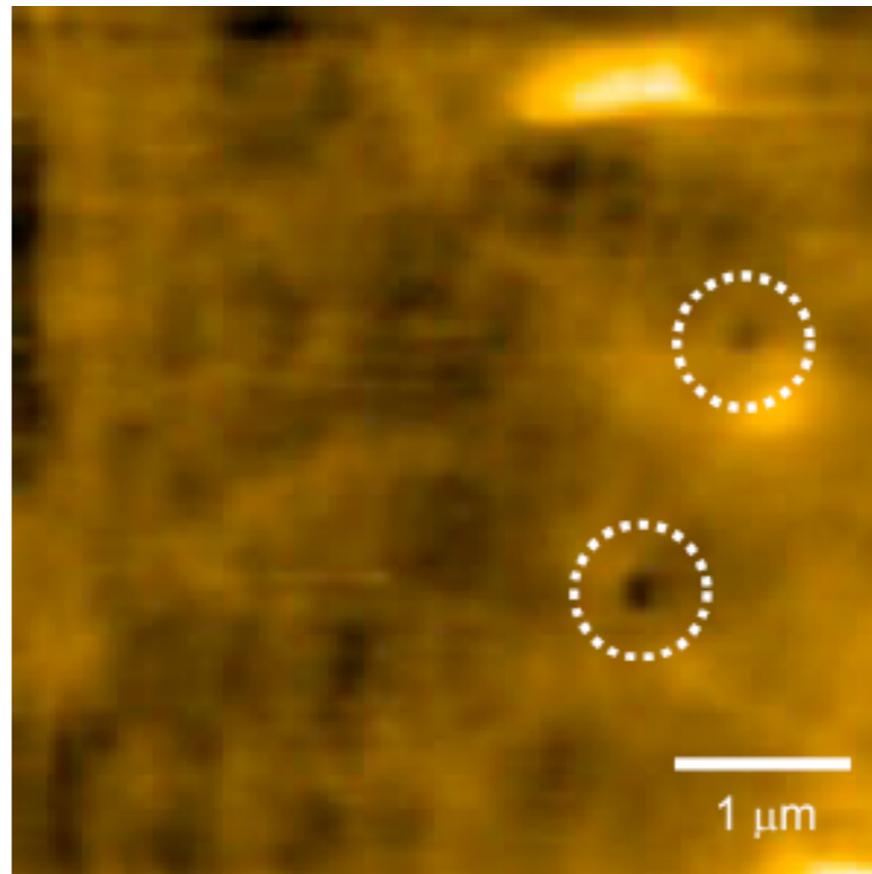
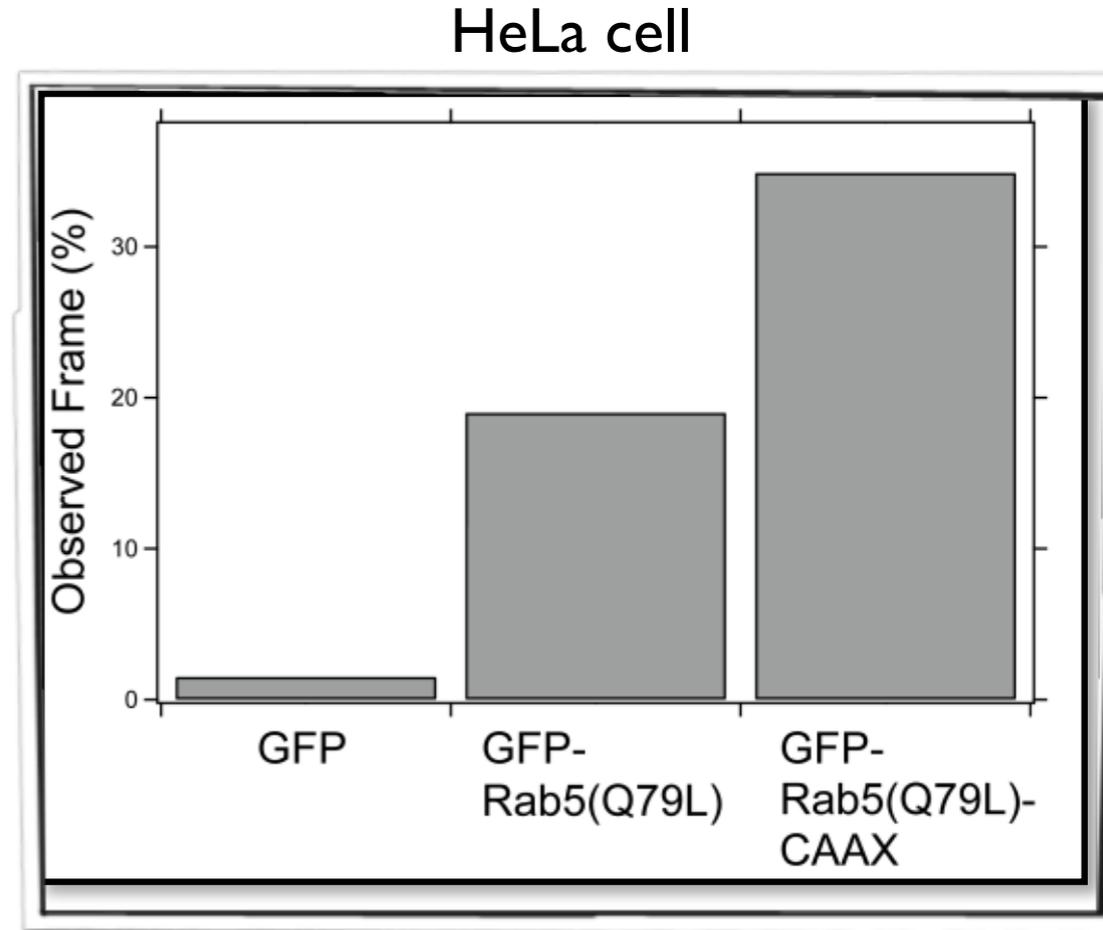
エンドサイトーシス



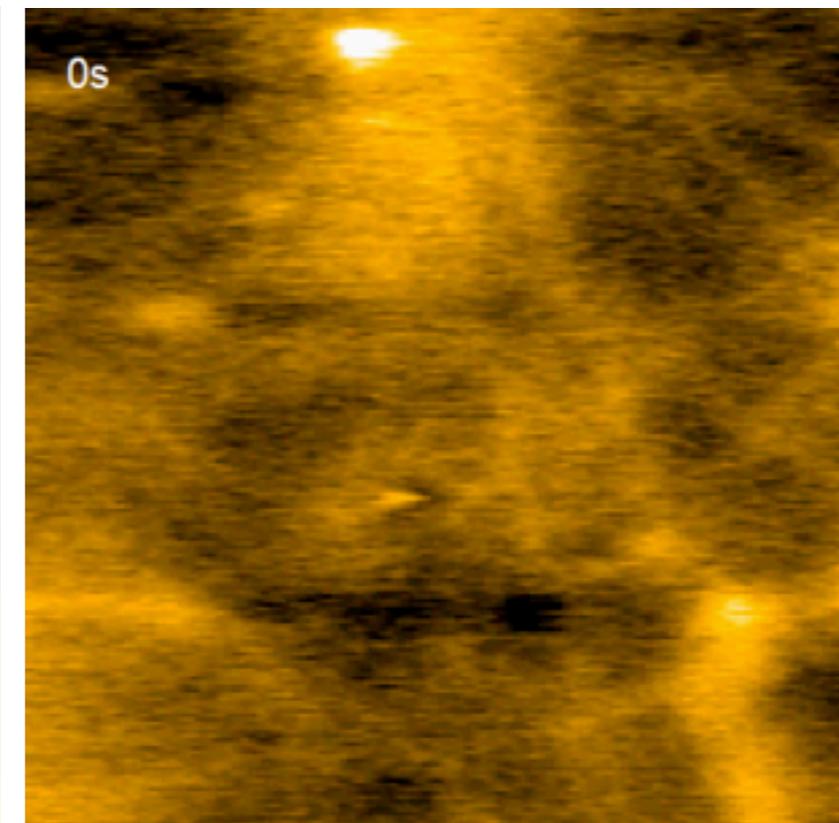
Rab5 regulates endocytosis
 Q79L, a constitutively active Rab5 mutant
 CAAX localizes to the cell membrane



HeLa cell
 mEGFP-Rab5(Q79L)-CAAX



5 s/frame, x50 play
 40



PtK2 Cell
 2 s/frame, x10 play