March 19, 2008
Three Way Meeting,
Argonne National Laboratory

Skydeck (Sears Tower)
Adult:$12.95

Dynamical Single Molecular Observations of Functional Membrane Proteins

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New !?

Photo From The Hancock Observatory
Adult: $12.00 (16/March/2008) It's profitable!!

History of Single Molecular World:

T. Hirschfeld, Applied Optics (1975)
Optical microscopic observation of single small molecules.

A. Ashkin, Phys. Rev. Lett. (1978)
Trapping of Atoms by Resonance Radiation Pressure.

S. Asakura et al., J. Mol. Biol. (1980)
Dark-field Light Microscopic Study of the Flexibility of F-actin Complexes.

G. Binning et al., Phys. Rev. Lett. (1982)
Surface Studies by Scanning Tunneling Microscopy

J. A. Spudich et al., Nature (1983)
Movement of Myosin-coated Fluorescent beads on actin cables in vitro.

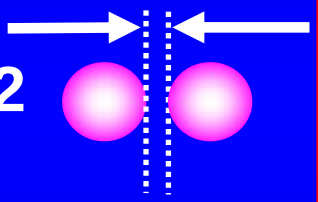
T. Yanagida et al., Nature (1984)
Direct observation of Motion of Single F-actin filaments in the Presence of Myosin.

Y. C. Sasaki et al., Phys. Rev. Lett. (2001)
Picometre-scale Dynamical X-ray Imaging of Single DNA molecules

Positioning Accuracy of Individual Molecules ?

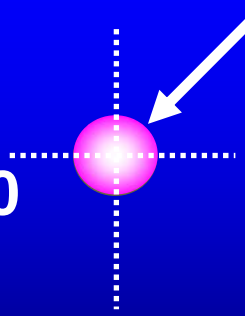
from visible light to x-rays

(1)Visible light
(λ=300nm-800nm)
Optical Diffraction limit
(imaging concept)



~λ/2

Tracking concept
~λ/100



Nanometer-scale Dynamics

We utilize the tracking concept
in the x-ray regions.

(2) X-ray (λ=1nm-0.01nm)

Tracking ~λ/100

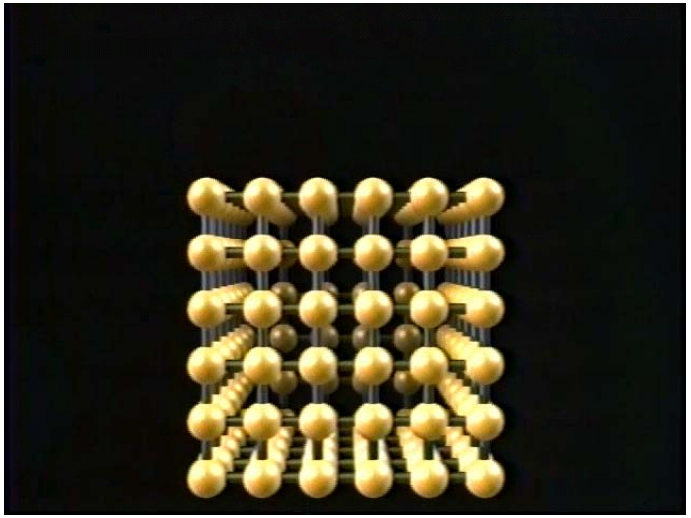
Picometer-scale Dynamics

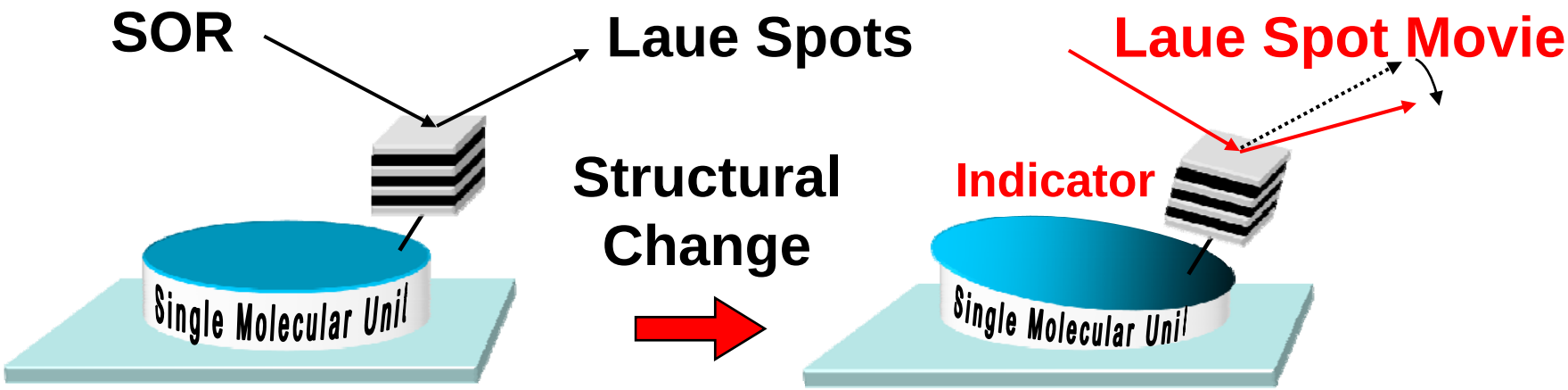
Principle: Diffracted X-ray Tracking (DXT)

DXT monitors x-ray diffraction from the individual nanocrystal, which is linked to the individual single protein molecule in bio-systems.

Features:

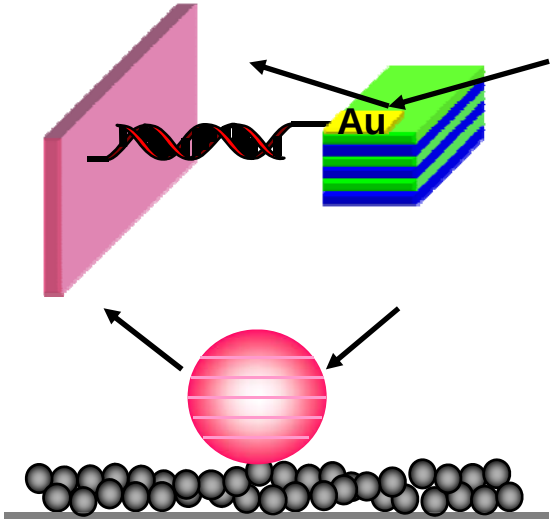
(1)High Accuracy (-pm = nm/1000)
(2)Time-resolved Information (from ms to μs)
(3) *In vivo* Measurement
(4) Independent Information from Chemical Conditions

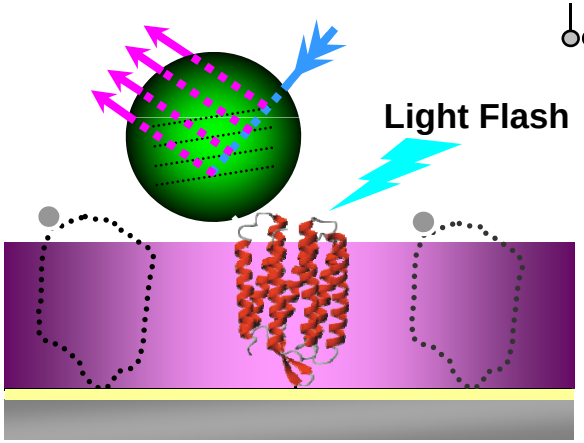




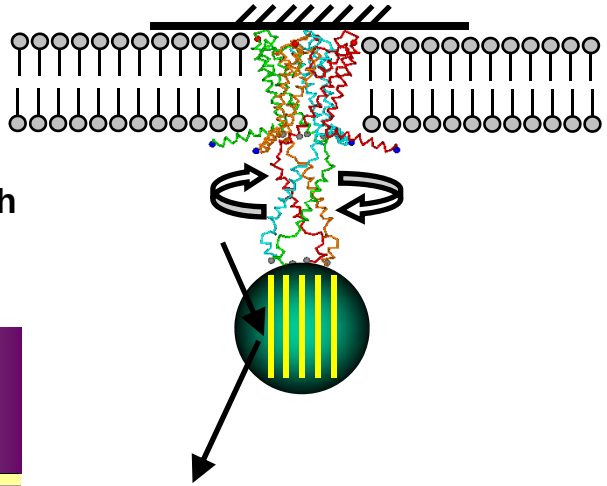
We assumed that motions of a specific site in individual proteins are equal to the observed orientations of nanocrystals.

Experimental Samples:

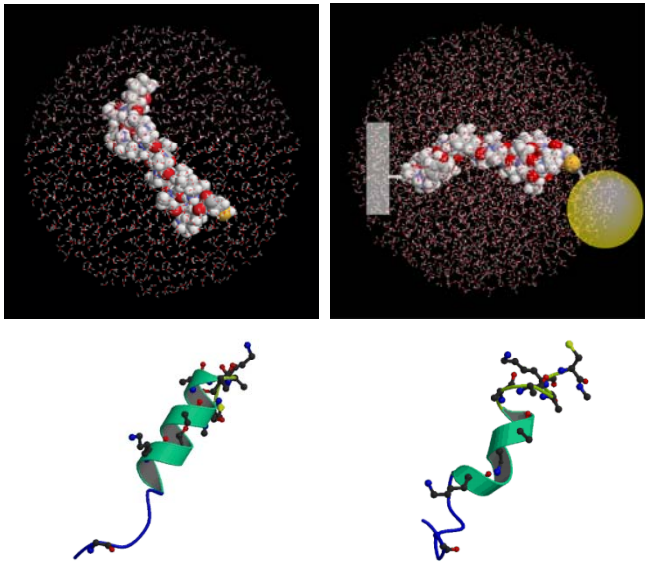




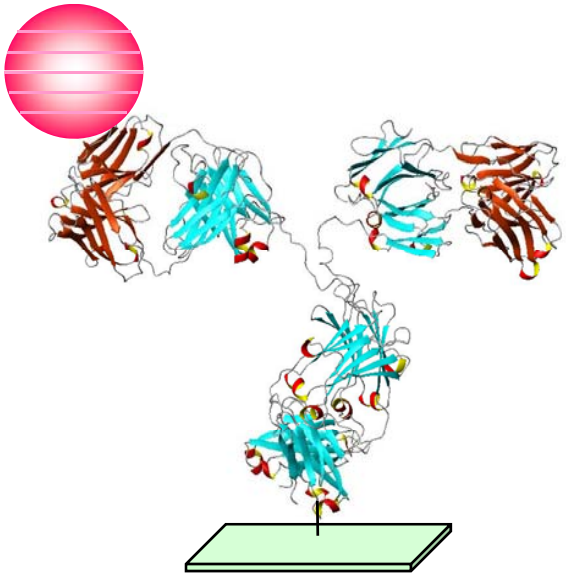
Moving Distance ⟶ below 0.1 nm

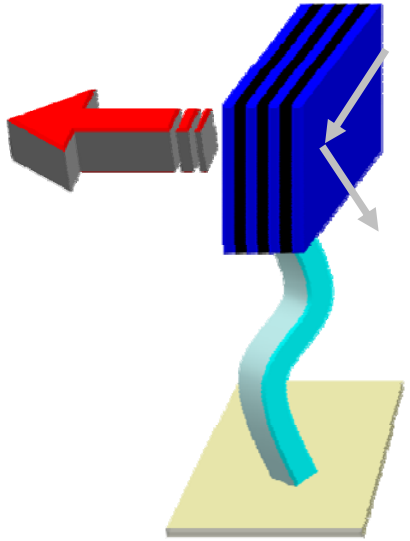


X-ray Radiation Pressure



Replica Exchange Molecular Dynamics Simulation



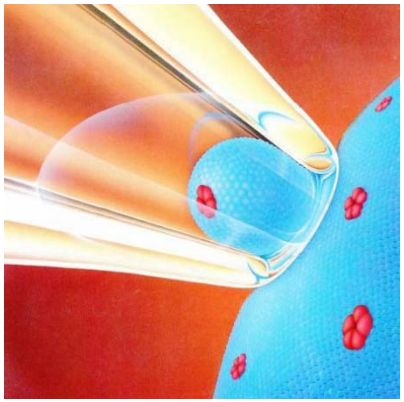


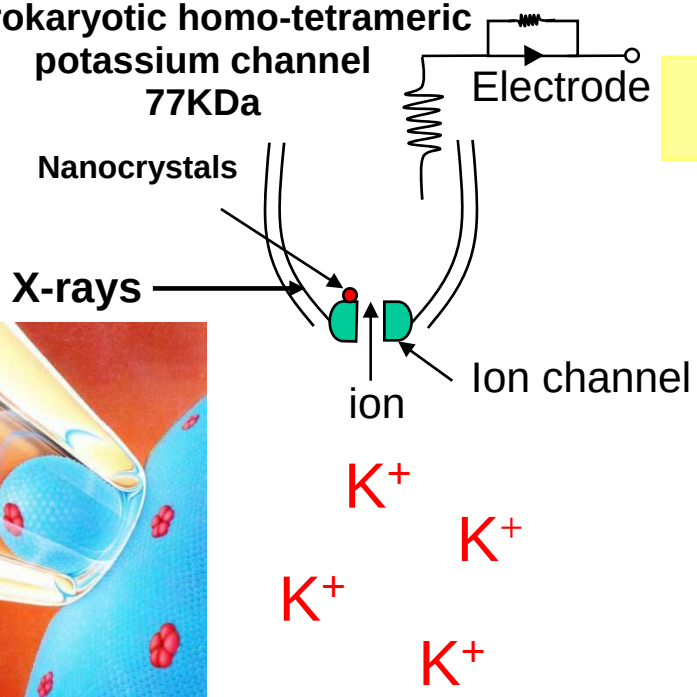
Experiment: Potassium Channel (KcsA)

H. Shimizu, M. Iwamoto, F. Inoue, T. Konno, Y.C. Sasaki, S. Oiki: ***Cell* 132**, 67 (2008)

Final goal: Simultaneous Observations of Single Molecule

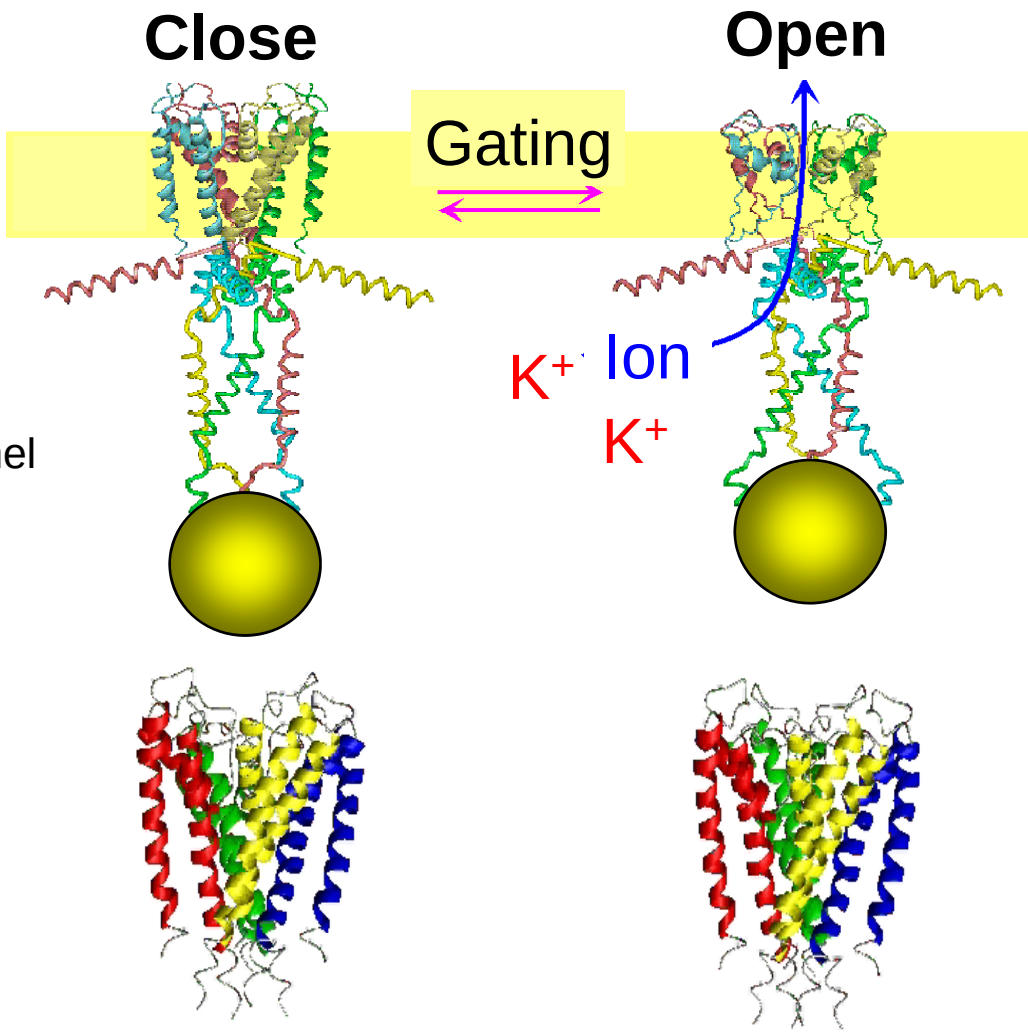
Expected Open and Close-state Structures of KcsA,
a prokaryotic homo-tetrameric potassium channel
77KDa





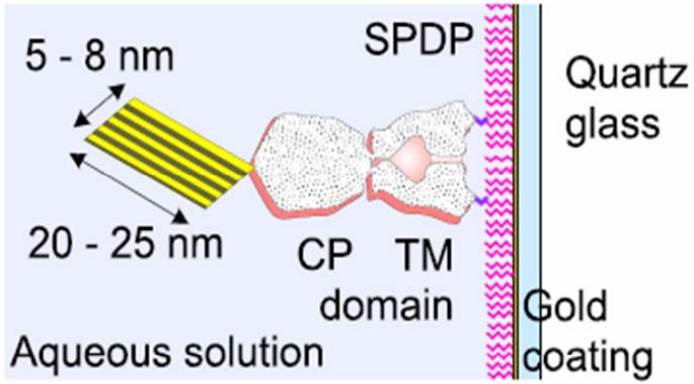
The patch clamp technique:

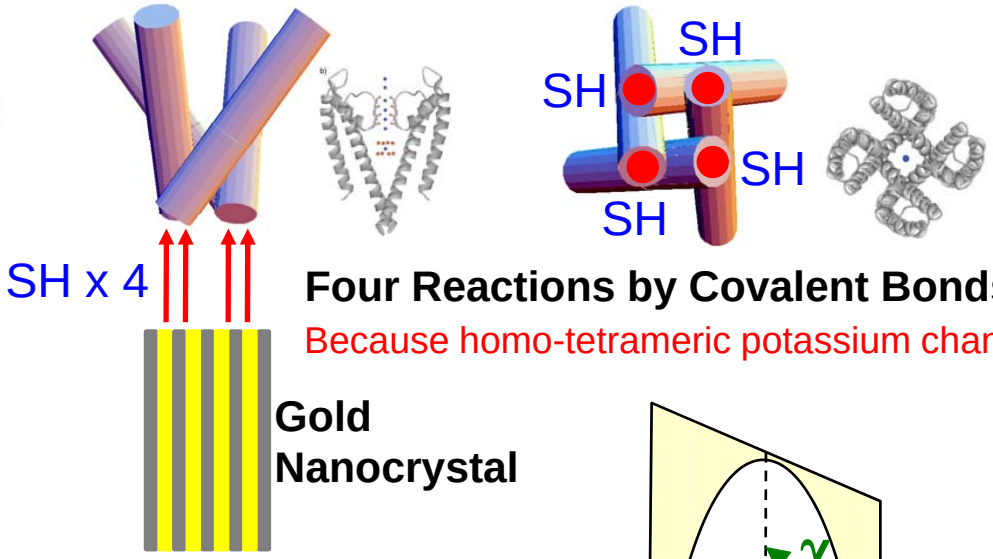
1991 Nobel Prize
(Prof. E.Neher and Prof.B.Sakmann)

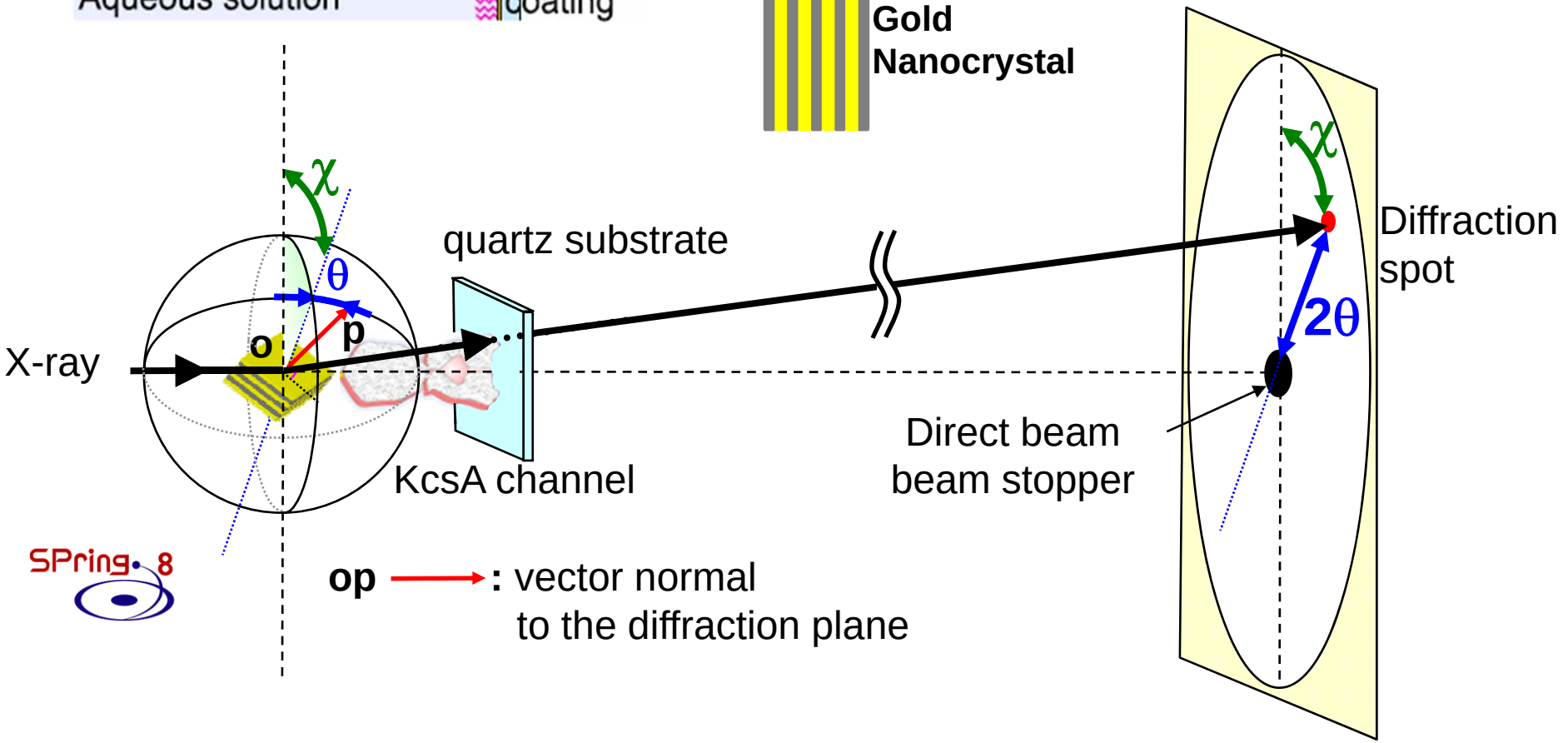


2003 Nobel Prize (Prof, R. Mackinnon)

Arrangements of Adsorbed KcsA and DXT







How to make artificial nanocrystal?

(many offered commercially nanocrystals are not perfectly crystallized.)

(1) (quasi) One-dimensional nanocrystal

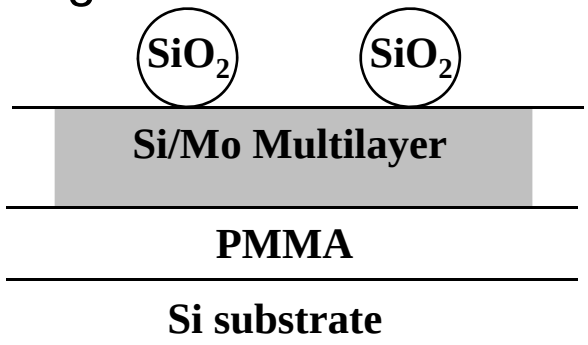
Gold nanocrystal (diameter:20-30nm)

The gold nanocrystals is fabricated by epitaxial-growth on NaCl(100) surface at 790 ^{o}C for 10 min.

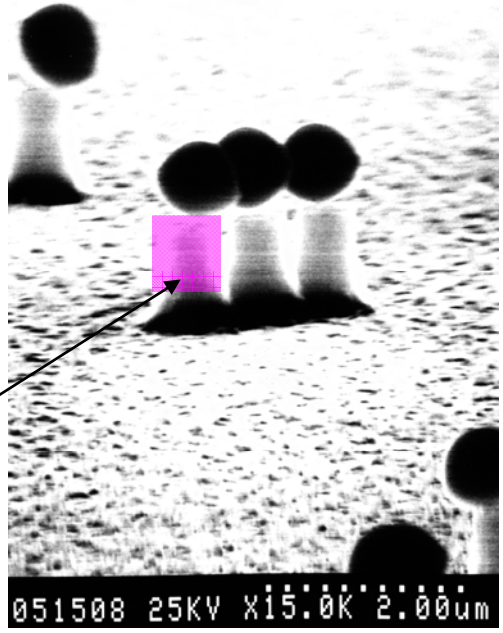
(2) One-dimensional nanocrystal

Nanoparticles of Si/Mo multi-layer (diameter: 40-80nm)

The artificial crystals is fabricated by a sequential process using silicon dioxide beads and the reactive ion etching processes.



Diameter of SiO_2beads
1.0 μm (600nm)
0.2 μm (50nm)
0.09 μm (30nm)



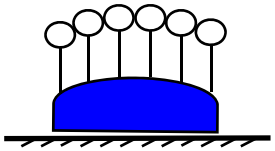
Protocol of Nanocrystal

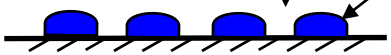
Evaporation

Growth of Au island

Hydrophobic surface

(are aggregated in aqueous solution)



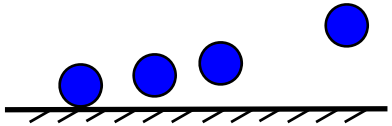


MgO, NaCl (100)

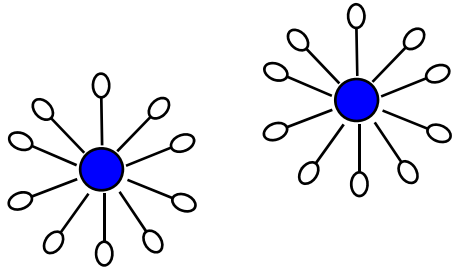
Dispersive nanocrystal

Annealing

Desorption using solutions

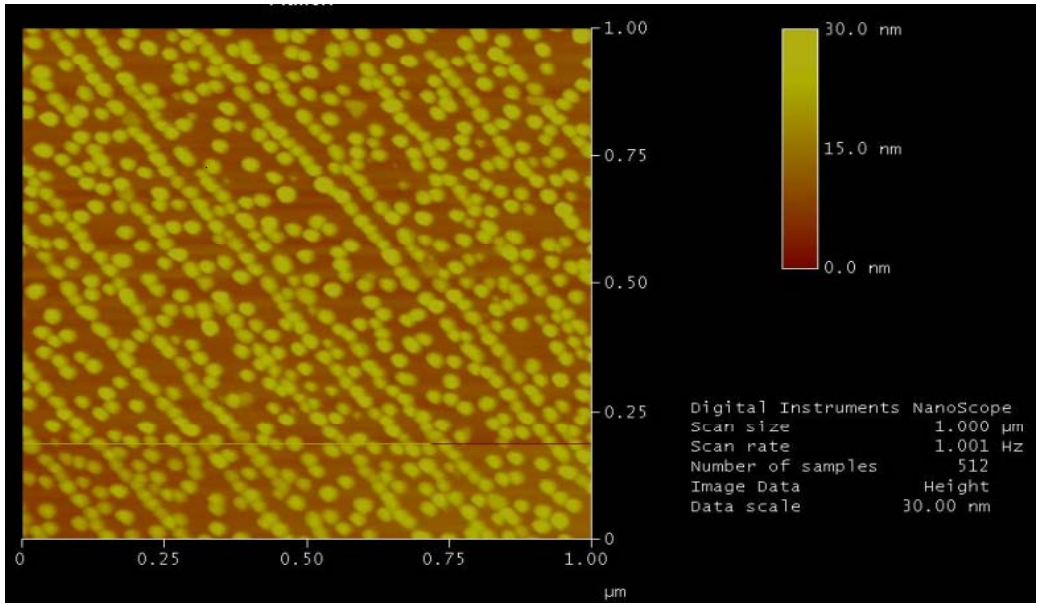


MgO, NaCl (100)



using some detergents

AFM image

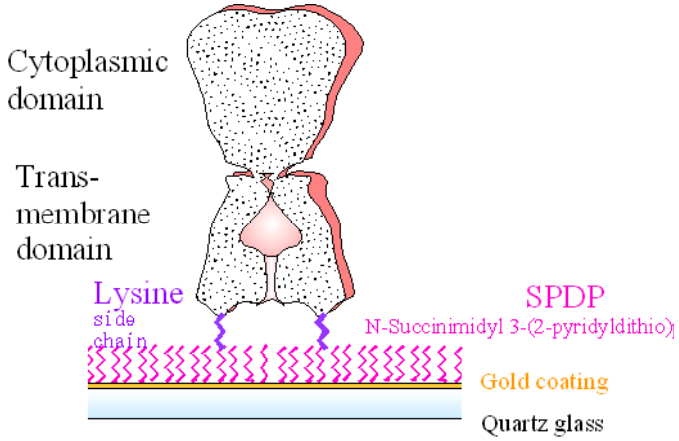


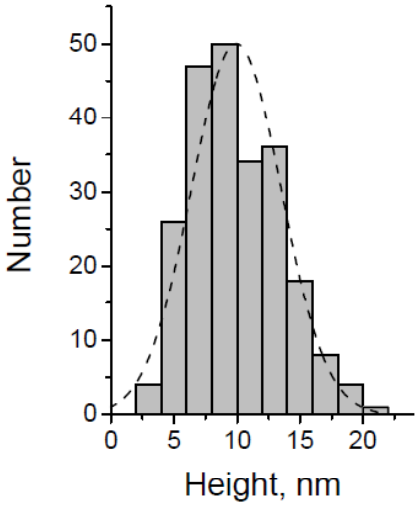
2nm(0.002nm/s)
NaCl(100)

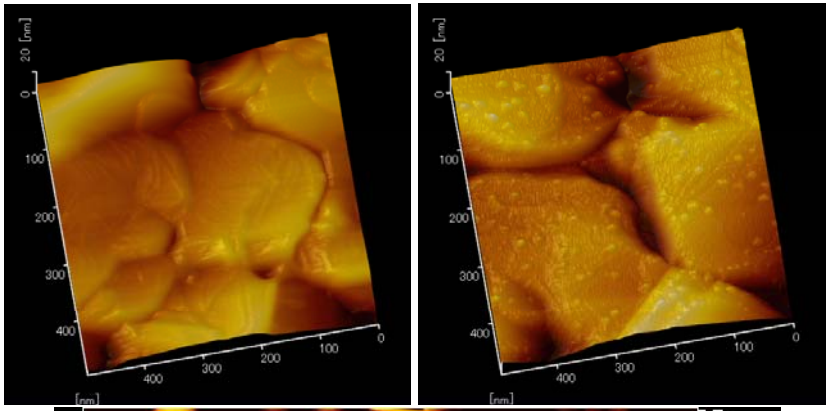
Temperature of substrate:
375^{o}C

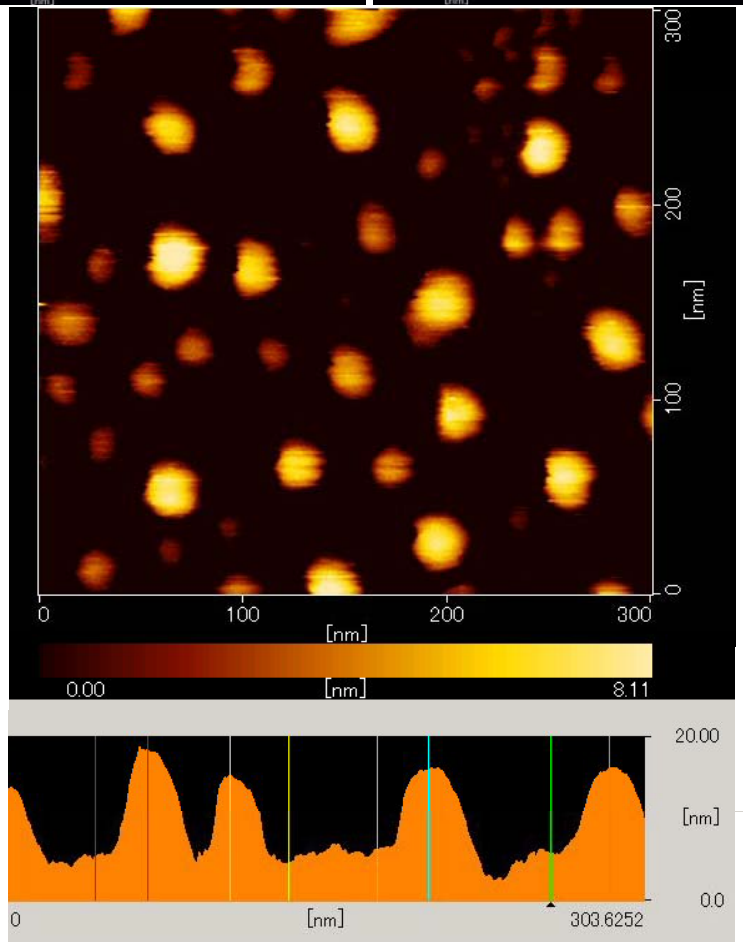
(1µm x 1µm)

AFM Images of Adsorbed KcsA



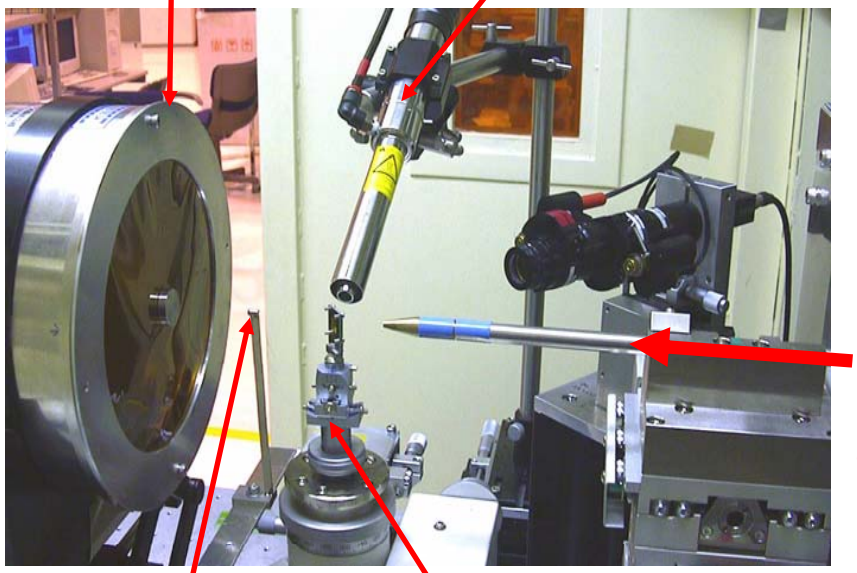






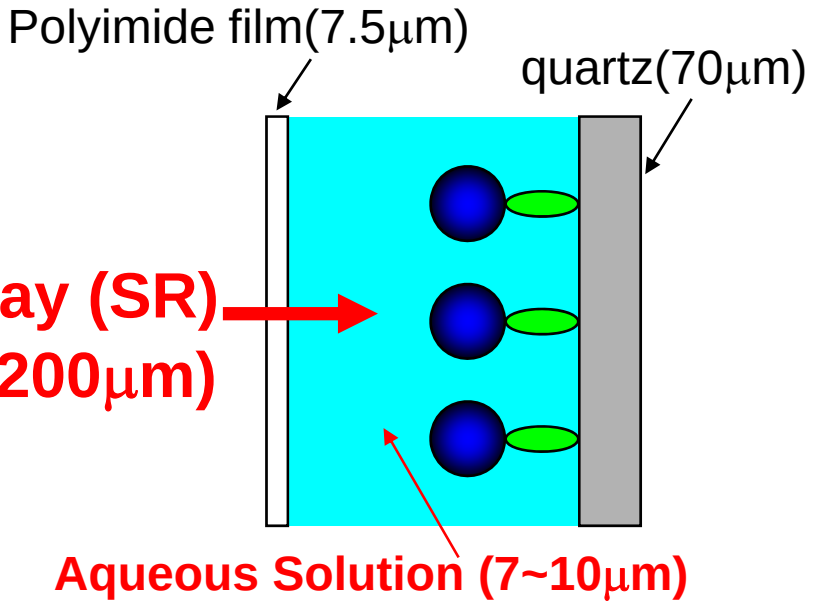
The histogram of the height of KcsA Channel on a flat gold surface.

Instrumentation:

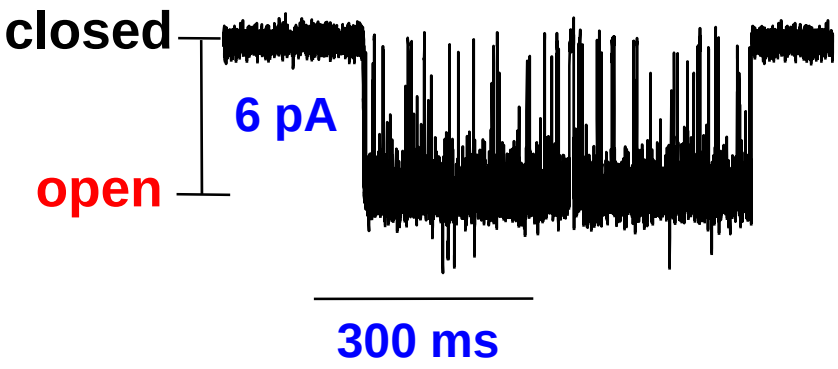


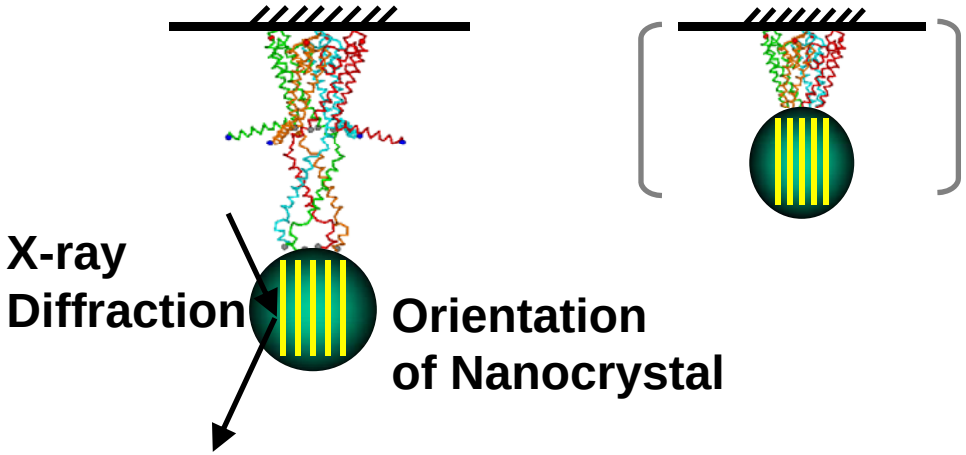
BL44B2 (White X-ray Mode)
at SPring-8(photon flux=
-10^{15} photon/sec/mm^2)
λ=1.7Å(7KeV)-0.4Å(30KeV)
Detector: X-ray Image Intensifier (Hamamastu, V5445P) with CCD
Specimen-Detector Distance: 10cm
Exposure time: within 1 second
(1-20 msec. x 33)

Cross-sectional view of the sample in DXT:

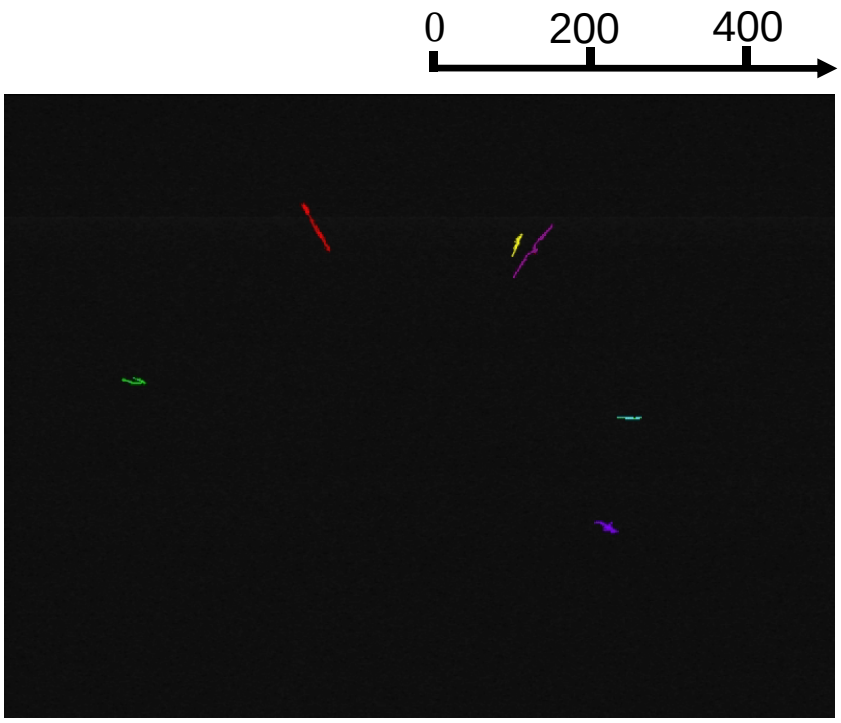


The important thing:
The diffraction spots must not overlap in one Laue image to distinguish the observed diffraction spots

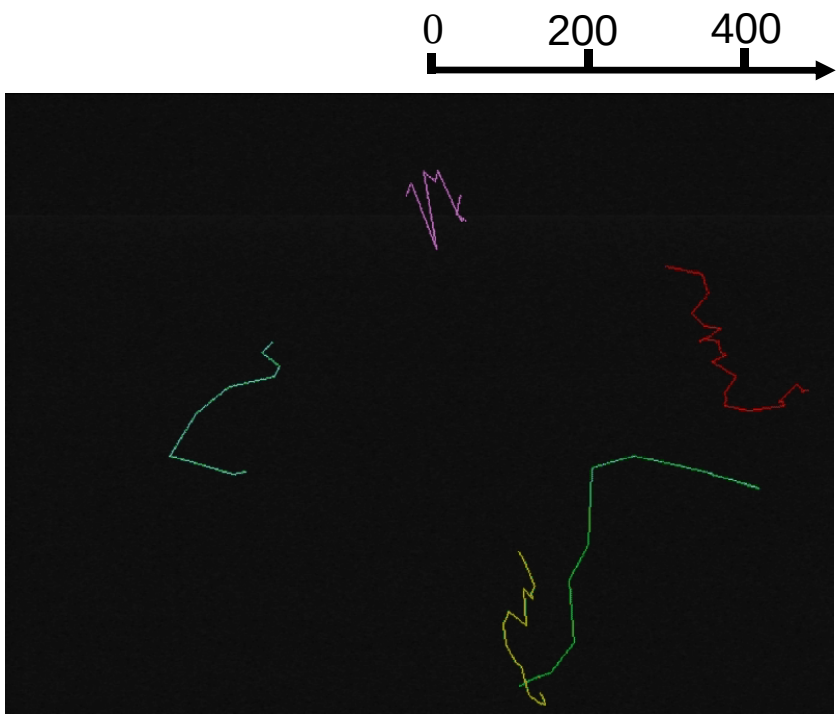




pH=7.5 (Closed State)

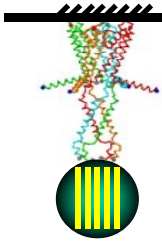


pH=4 (Open State)

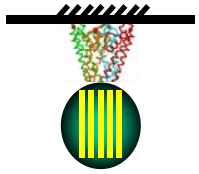


We conformed the movements using the inhibitor (TBA)

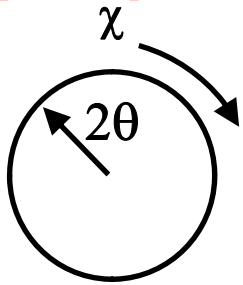
Histograms and MSD curves (from 400 spots)

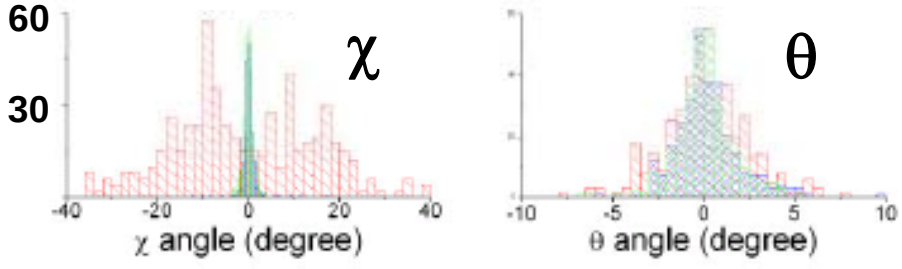


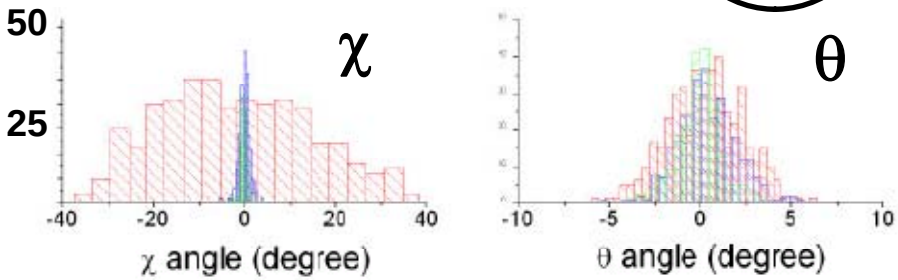
Full length KcsA

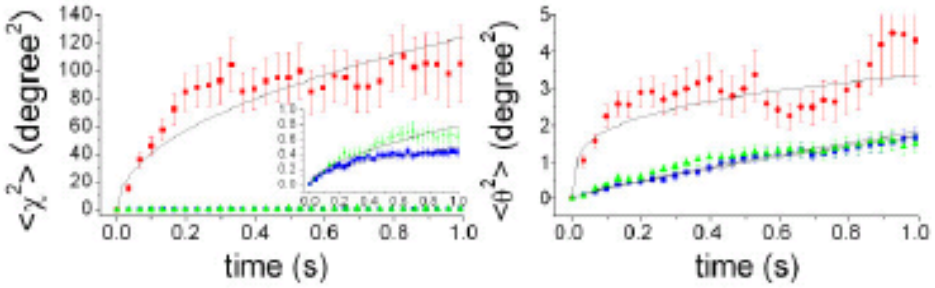


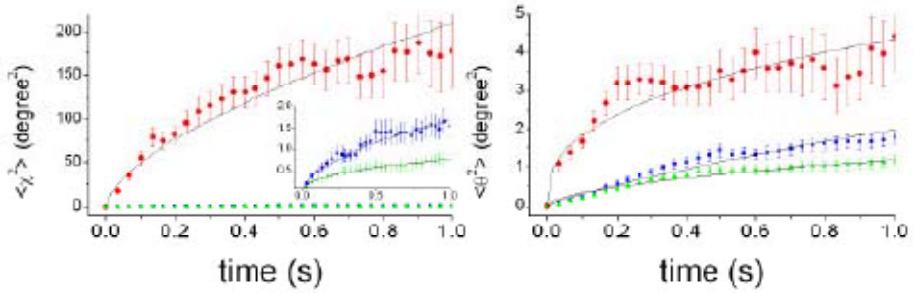
CP domain-deleted KcsA









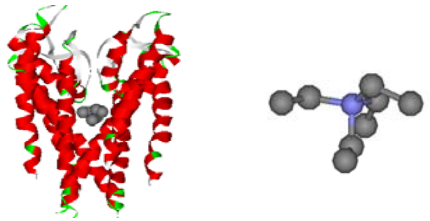


: pH=4.0 (gating state)

: pH=7.5 (closed state)

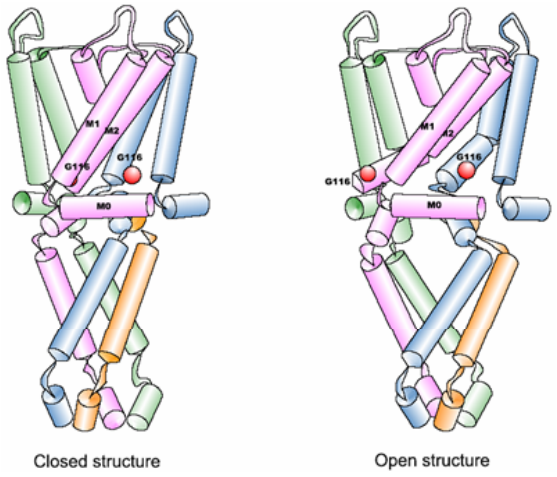
: pH=4.0 +TBA (open-locked state)

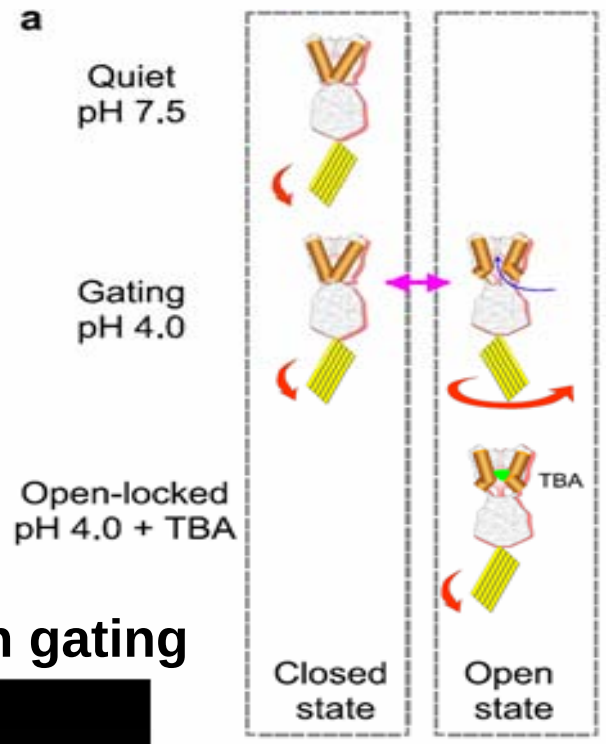
Position of Channel blocker



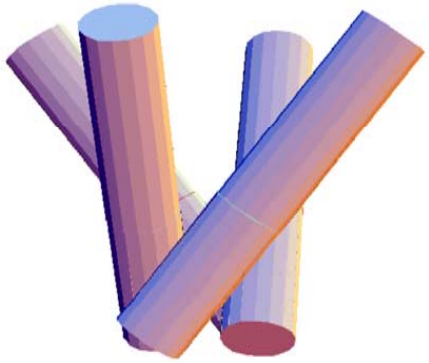
TBA（tetrabutylammonium）

Twisting motions upon gating of KcsA



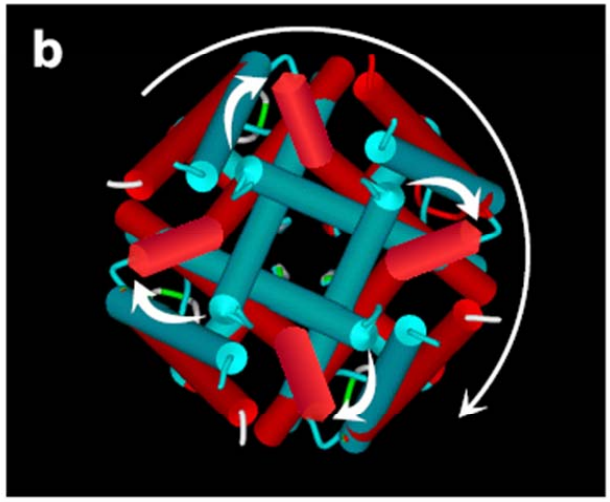


Transmembrne domain of KcsA



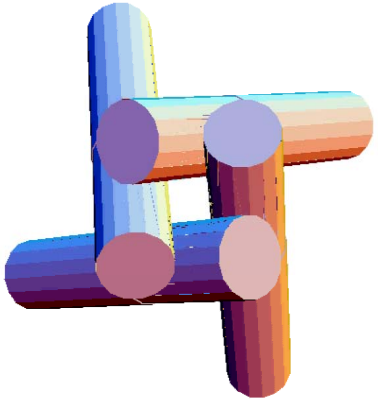
from side view

Open direction upon gating



closed

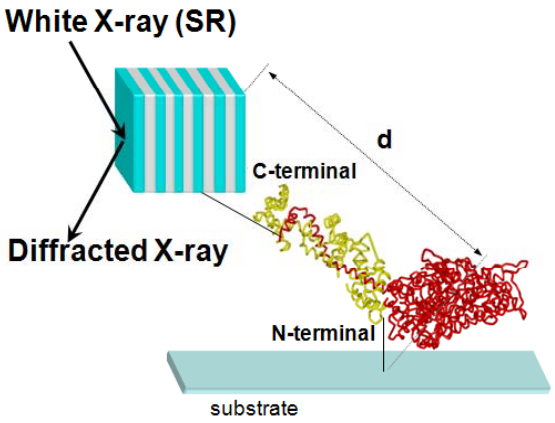
opened

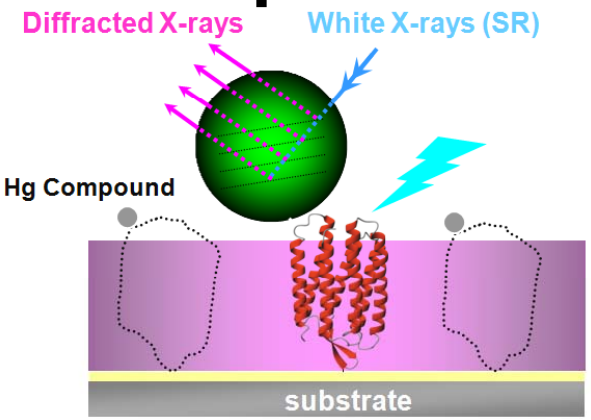


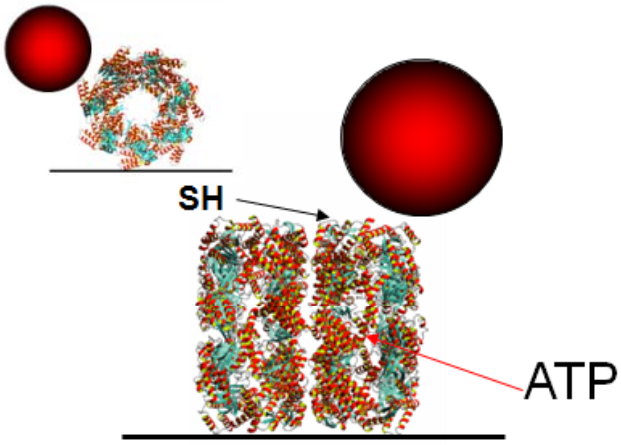
from CP domain side

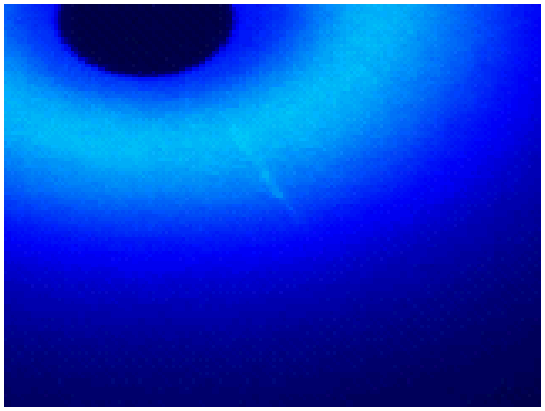
View from CP domain (intracellular side)

Other Experimental Samples:

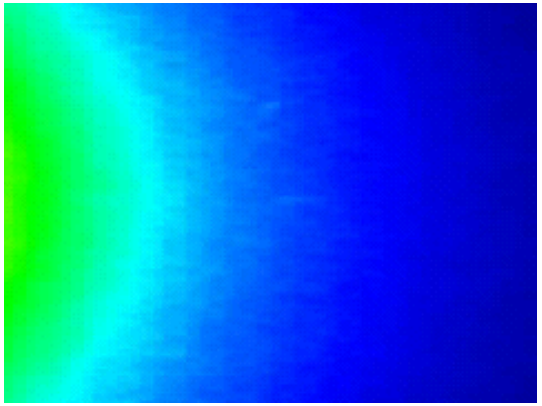


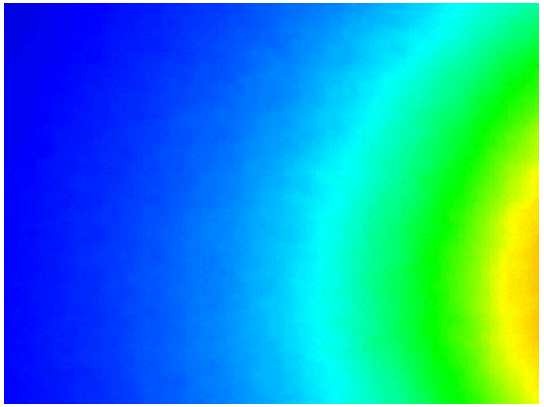




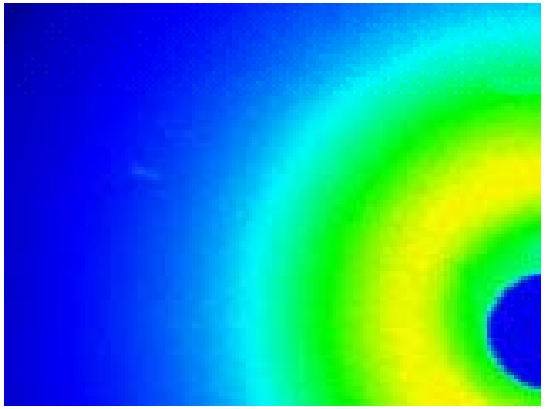


Mg-ATP(2mM)

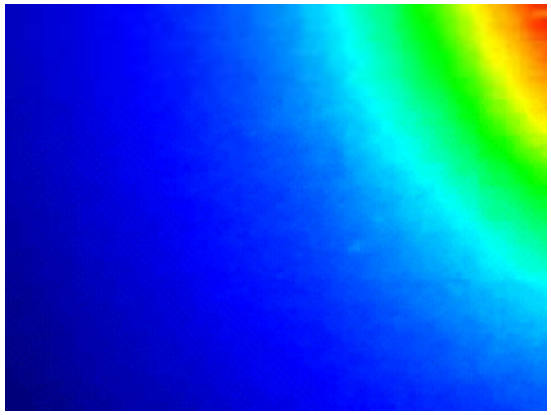


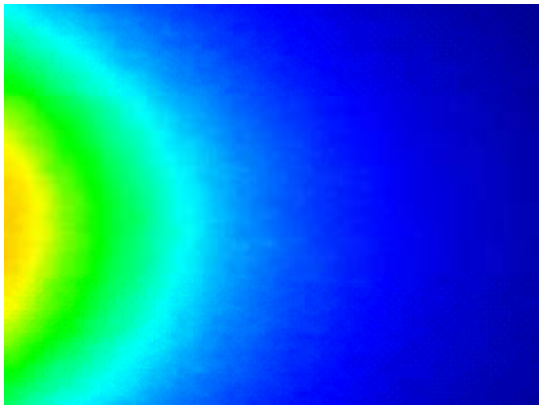


Without ATP(2mM)



Ca-ATP(2mM)





With ATP(2mM)

Next targets for DXT:

Still Photographs
(from crystal data)

⇩

Real-time Movie From Single Molecules

(1) Simultaneous observations of single molecule (KcsA or KvAP)

(2) *In vivo* observations

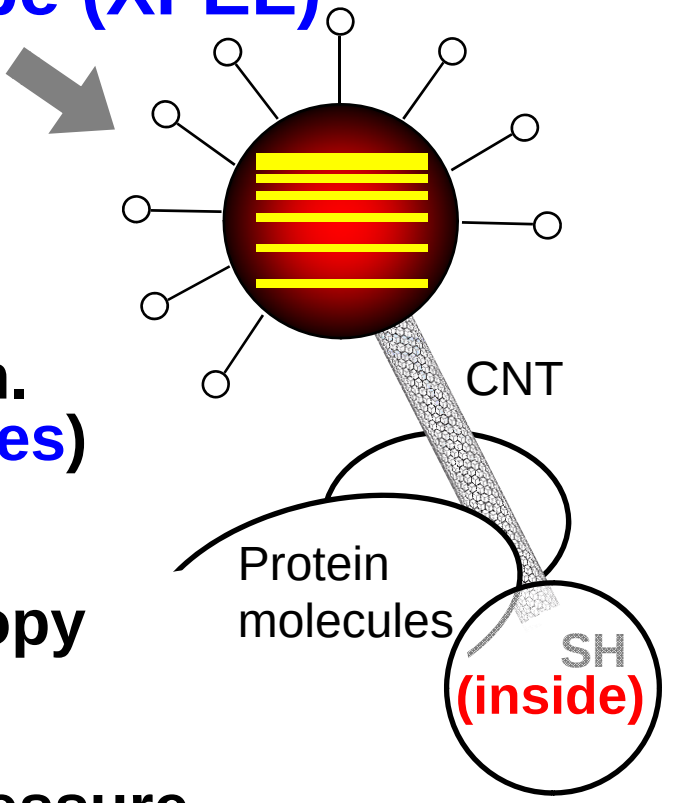
(3) High speed observations (-μs) using modified fast CCD.

(4) DXT using monochromatic beam.
(We need multi-d-space nanoparticles)

(5) Application to Electron microscopy (EBSP or EBSD).

(6) Application of X-ray radiation pressure force to new microscope.

NanoProbe (XFEL)

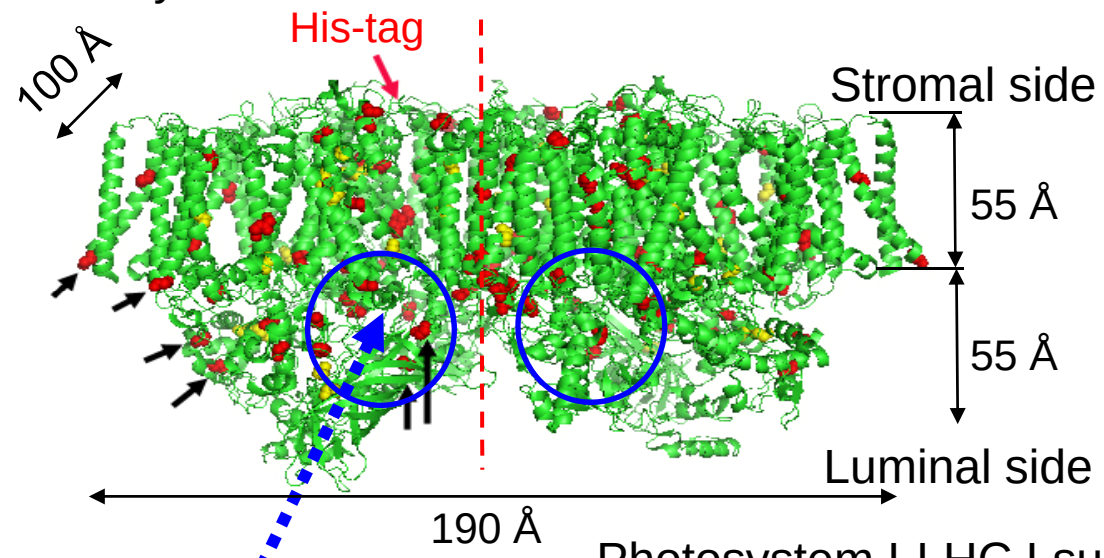


$2d\sin\theta = n\lambda$

Next Observation (1):

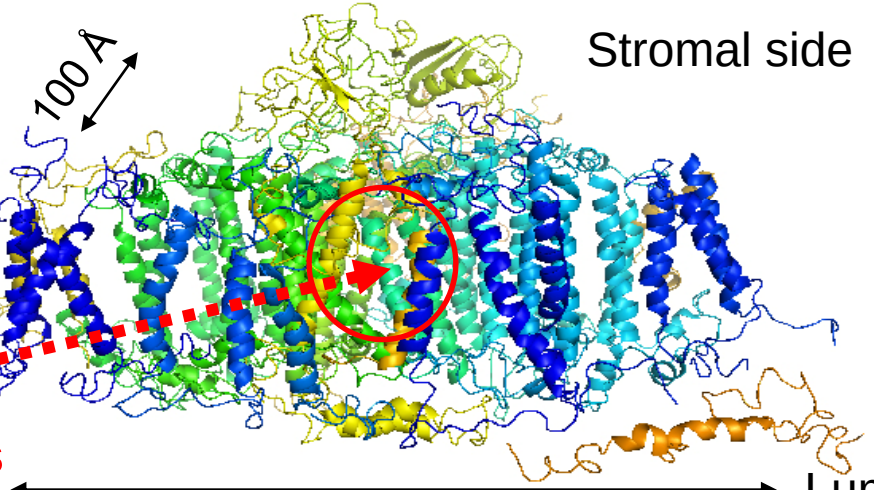
(Photosystem I , Photosystem II)

Photosystem II **dimmer** structure



Photosystem I-LHC I supercomplex structure (**monomer**)

Center Position
of Structural Changes



Center Position
of Structural Changes

A Special Thanks To:

SPring-8/JASRI Bio-soft Materials Group

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Fukui University (Department of Medicine)

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M. Iwamoto

Teikyo University (Bio-engineering Center)

N. Ohishi

Nara Institute of Science and Technology (NAIST)

M. Kataoka

University College London (London Centre for Nanotechnology)

I. Robinson

If you will try to measure DXT, I present you my gold nanocrystals !