ERLサイエンスワークショップ
2009.07.10

先端1分子計測と次世代放射光の役割

Advanced Single Molecular Observations and the task of Next X-ray Light Sources

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The University of Tokyo

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

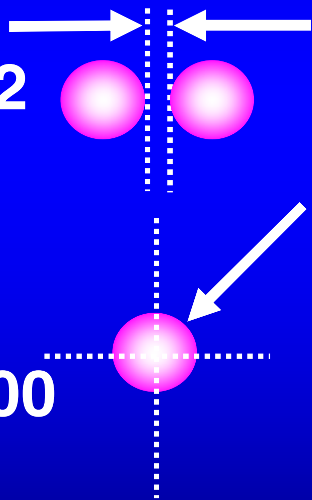
(1)Visible light

(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.

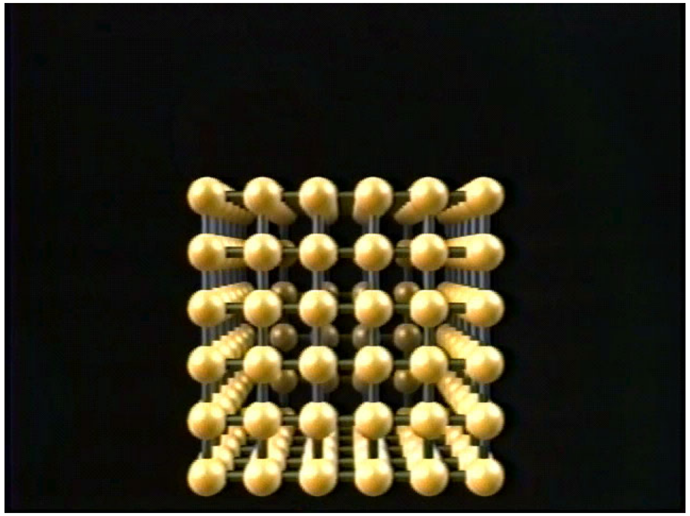
(2) X-ray (λ=1nm-0.01nm)

Tracking ~λ/100

Picometer-scale Dynamics

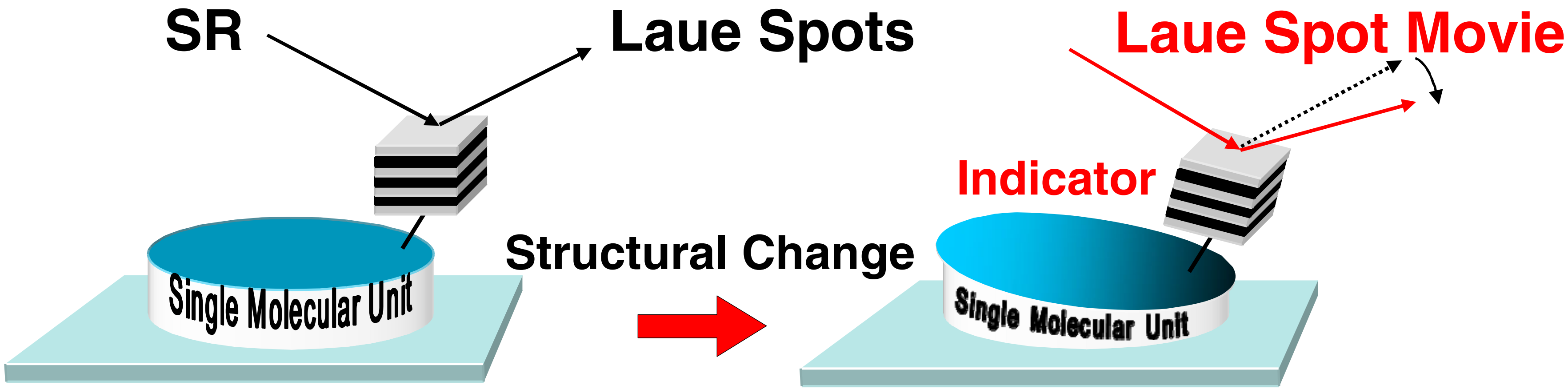
Principle: Diffracted X-ray Tracking (DXT)

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 from Chemical Conditions



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

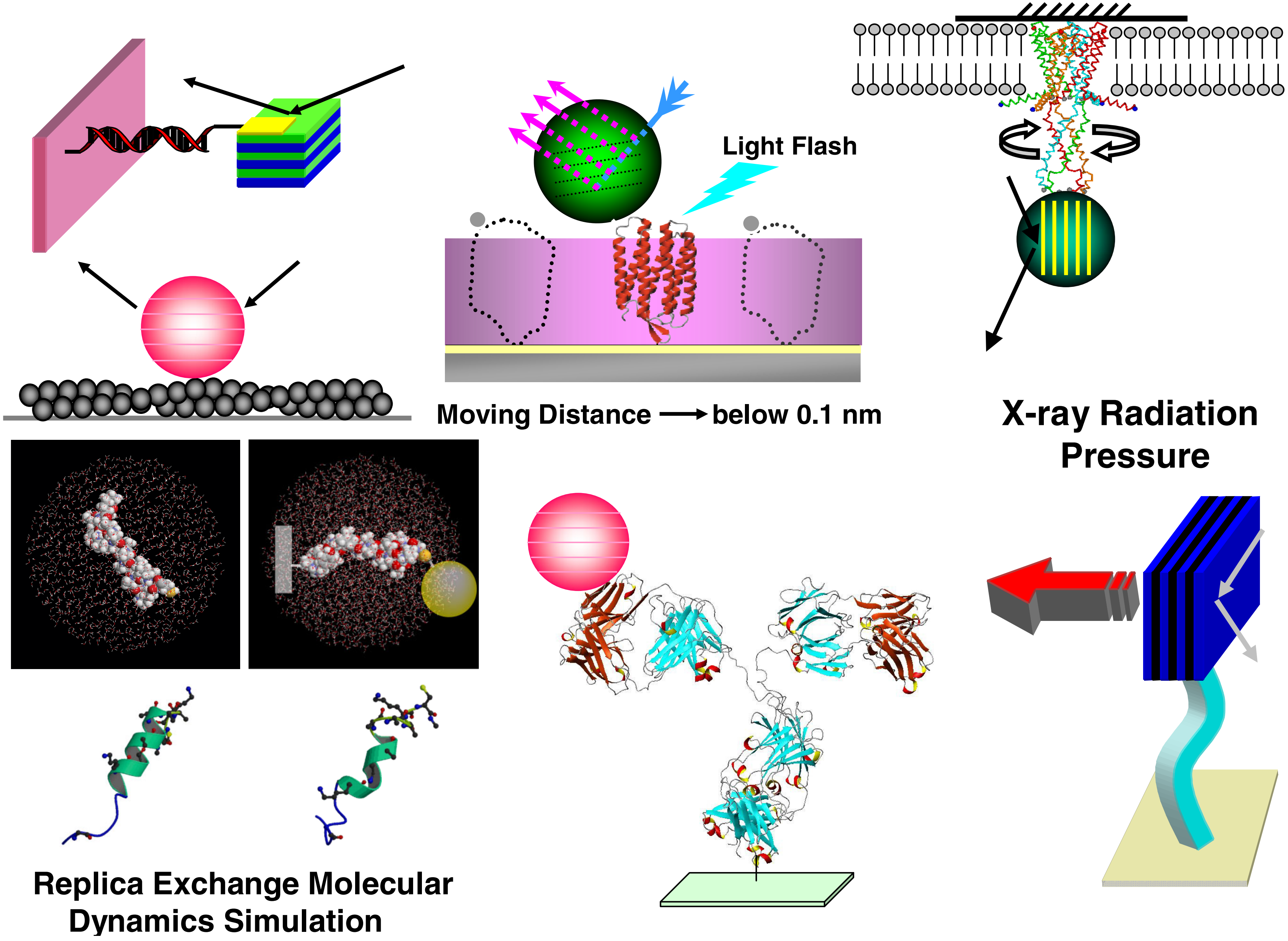
Single Molecular Measurements (comparison):

Fluorescent, FRET and X-rays (DXT)

FRET: Fluorescent Resonance Energy Transfer

	Fluorescent	FRET (Electron Transfer)	X-ray (DXT)
Labeled	Molecule Particle	Acceptor/Donor GFP, cy-3, FAD	Nanocrystal
Accuracy	-nm	-nm- 0.1nm	- 0.001nm
Chemical Infor.	Independence	Dependence	Independence
Intramolular Sturactural Infor.	△	○	◎
(Wavelength)	(300-800 nm)		(0.1-0.01nm)

Experimental Samples:



Experiment: Potassium Channel (KcsA)

H. Shimizu, M. Iwamoto, F. Inoue, T. Konno, Y.C. Sasaki, S. Oiki: ***Cell* 132**, 67 (2(

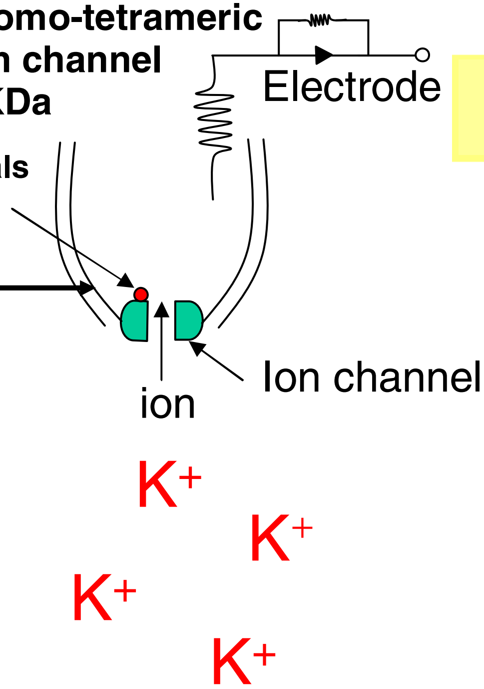
Final goal: Simultaneous Observations of Single Molecule

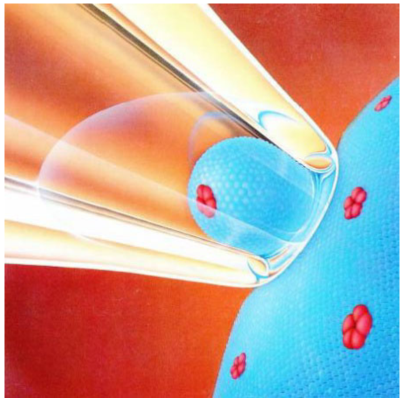
Expected Open and Close-state Structures of KcsA

a prokaryotic homo-tetrameric potassium channel 77KDa

Nanocrystals

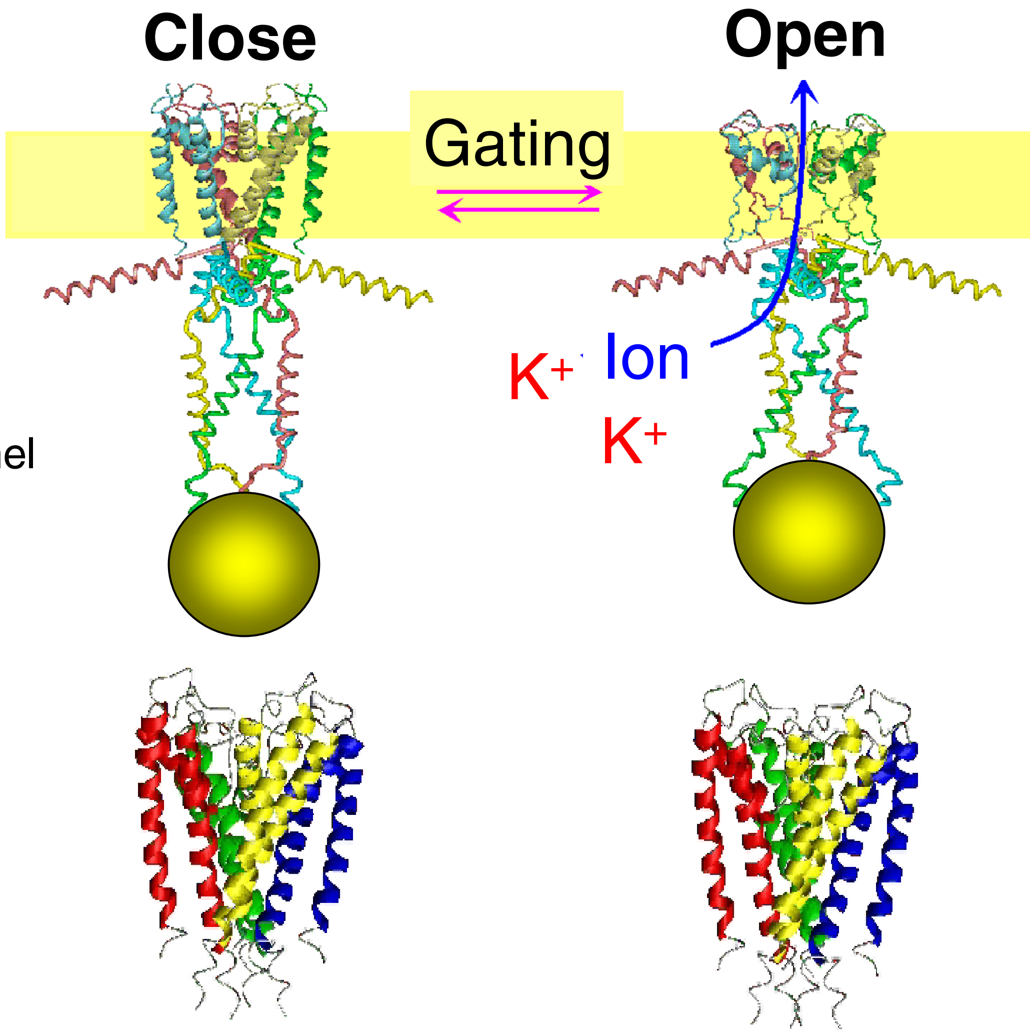
X-rays





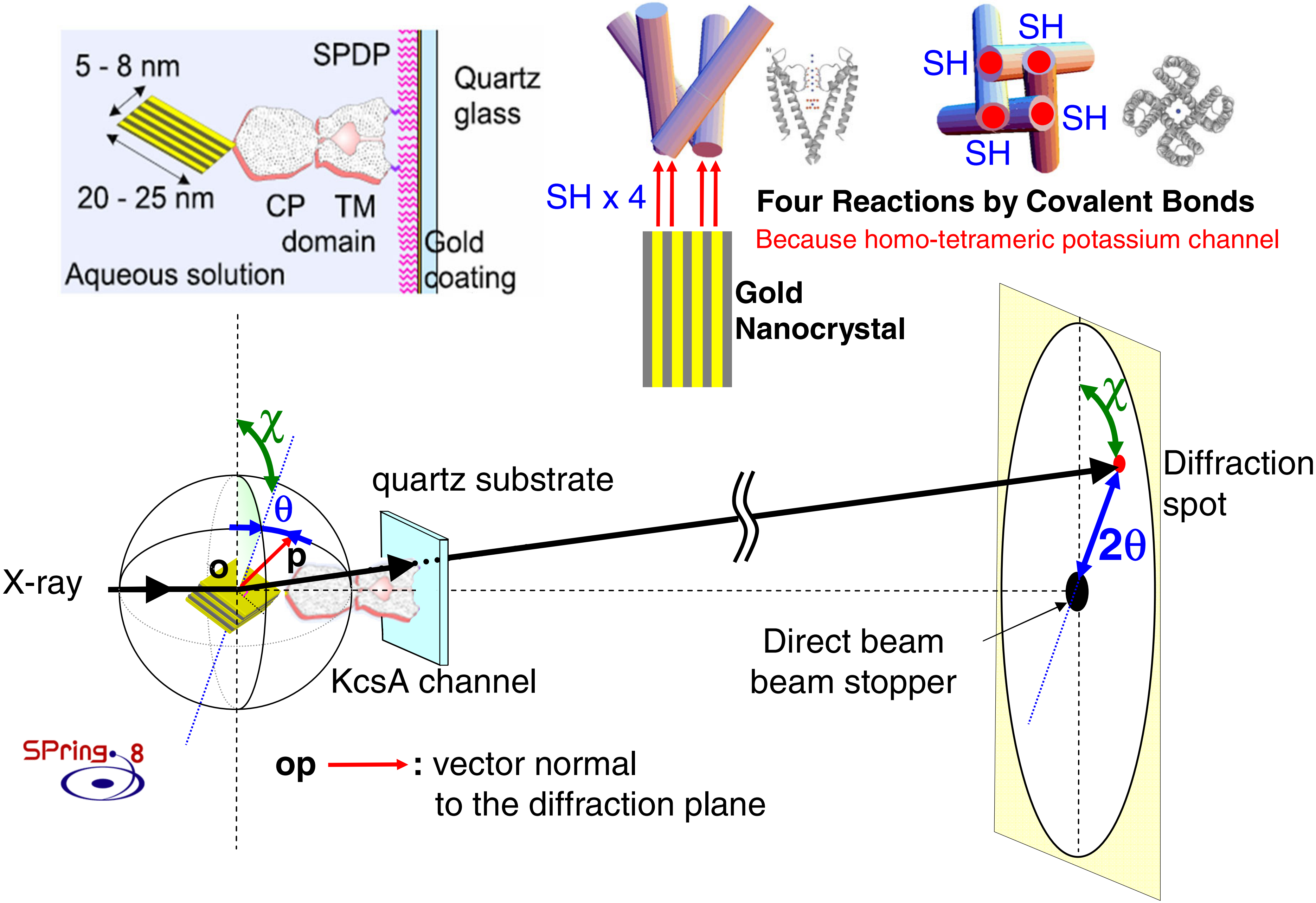
The patch clamp technique:

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



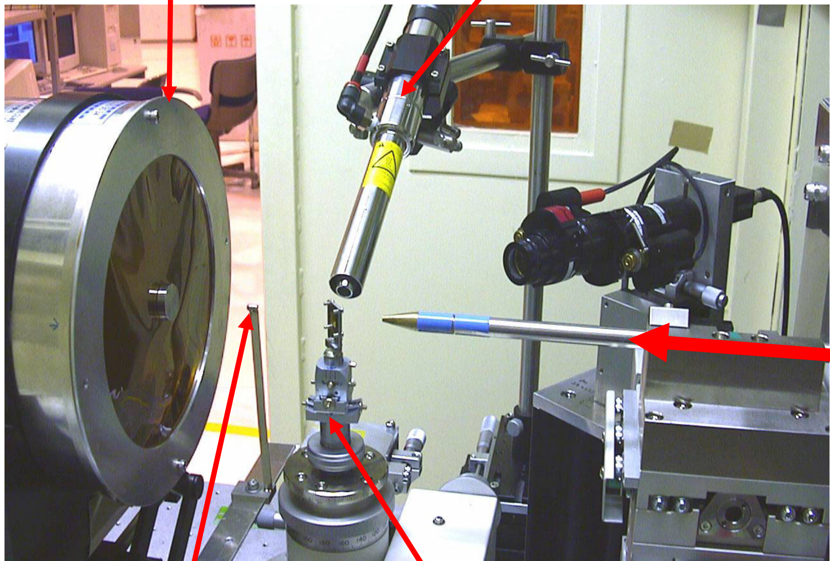
2003 Nobel Prize (Prof. R. Mackinnon)

Arrangements of Adsorbed KcsA and DXT



Instrumentation:

Image Intensifier
Cooling System



X-ray (SR)
(ϕ=200μm)

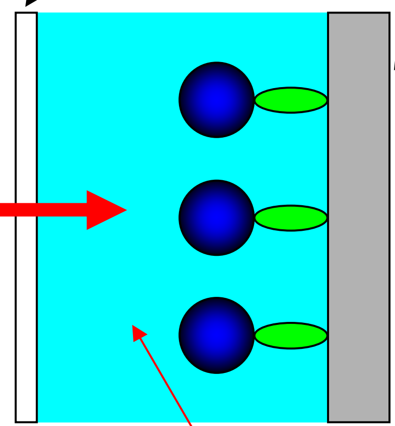
Direct Beam Stopper
Sample Holder

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:

Polyimide film(7.5μm)
quartz(70μm)



Aqueous Solution (7~10μm)

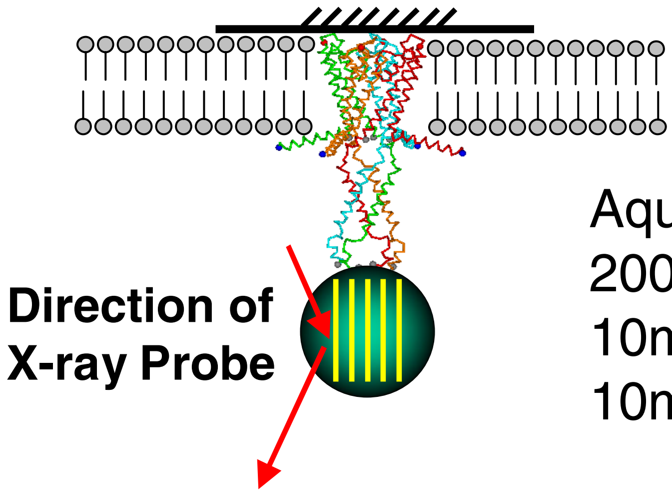
:gold nanocrystal
:molecule

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

DXT images:

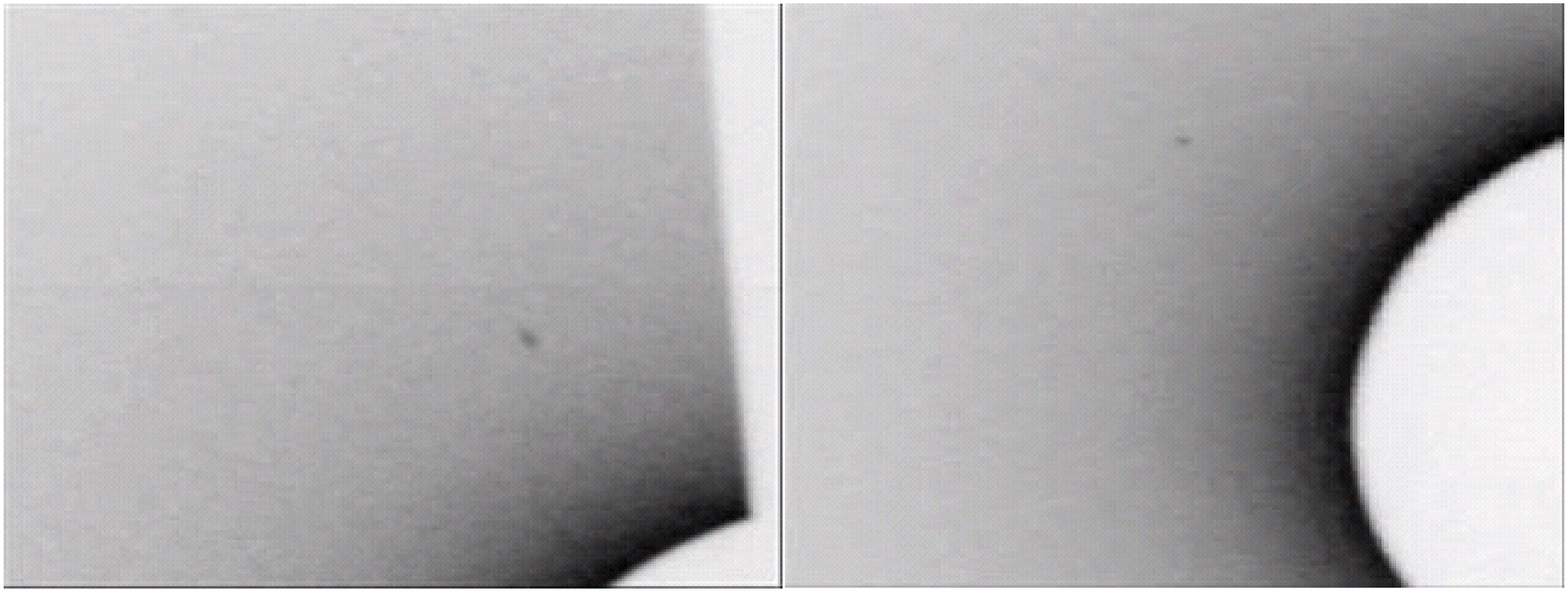
Diameter of Nanocrystal: 20-30nm

during 1s, Image Speed = 1/5

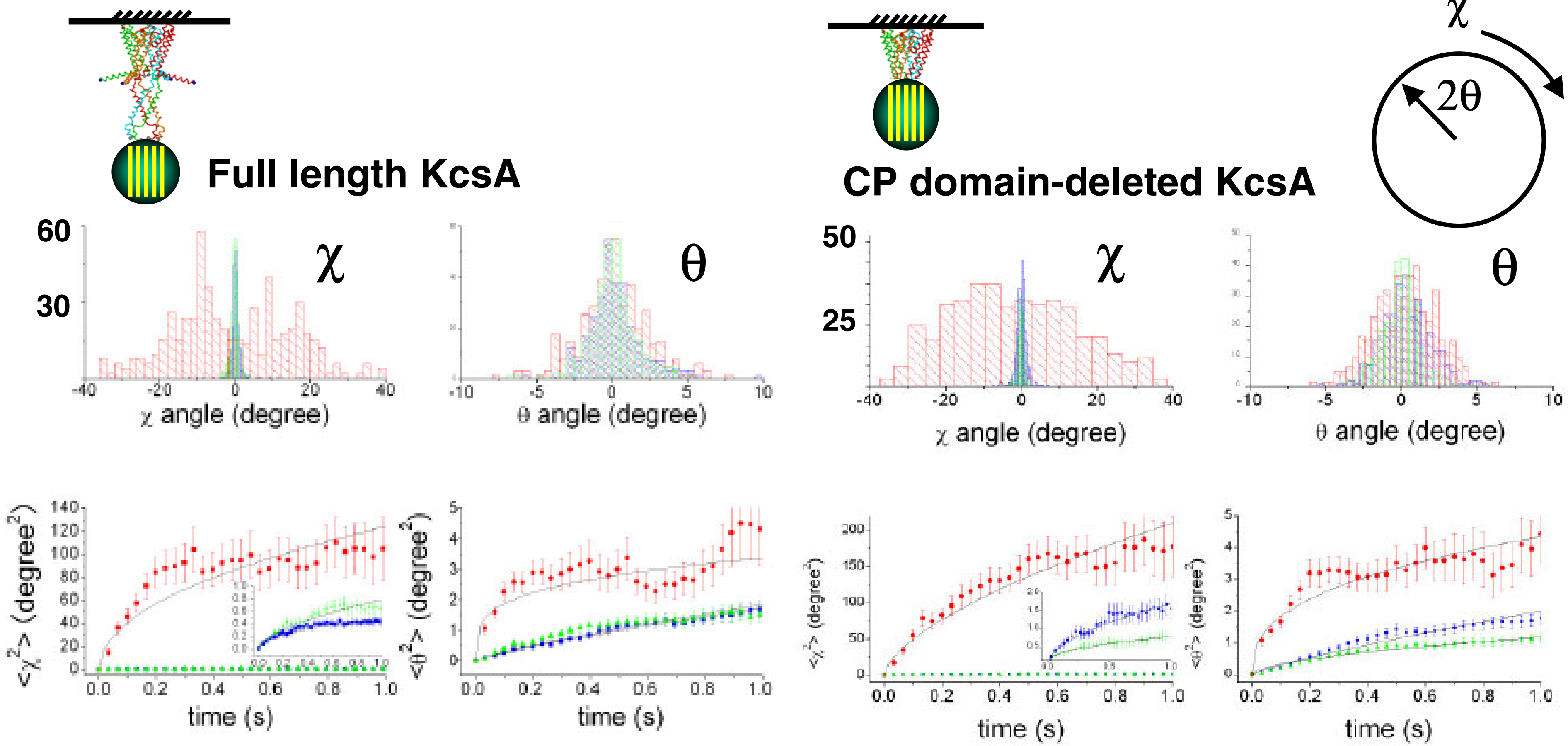


Aqueous Solution:
200mM KCl+
10mM HEPES for pH=7.5
10mM Succinic Acid for pH=4.0

pH=7.5 (closed state) **pH=4.0 (gating state)**



Histograms and MSD curves (from 400 spots)

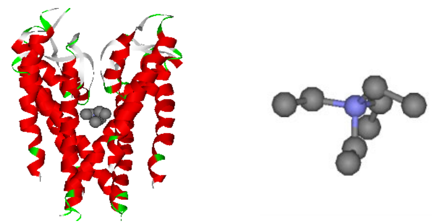


: pH=4.0 (gating state)

: pH=7.5 (closed state)

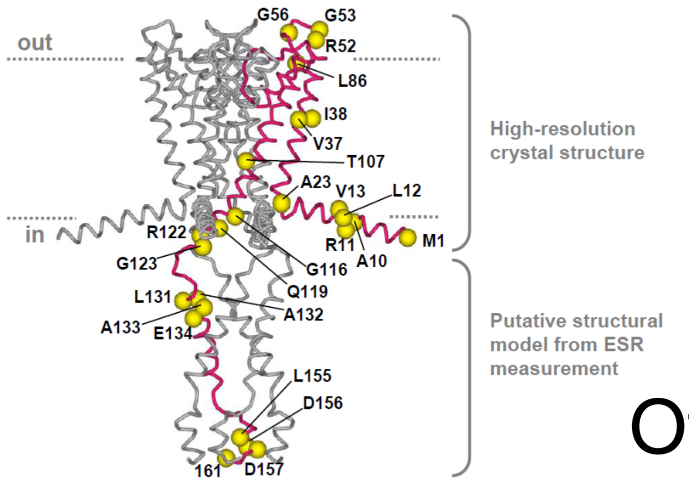
: pH=4.0 +TBA (open-locked state)

Position of Channel blocker



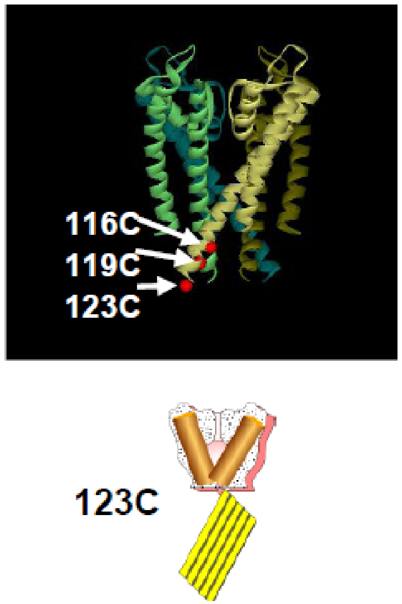
TBA（tetrabutylammonium）

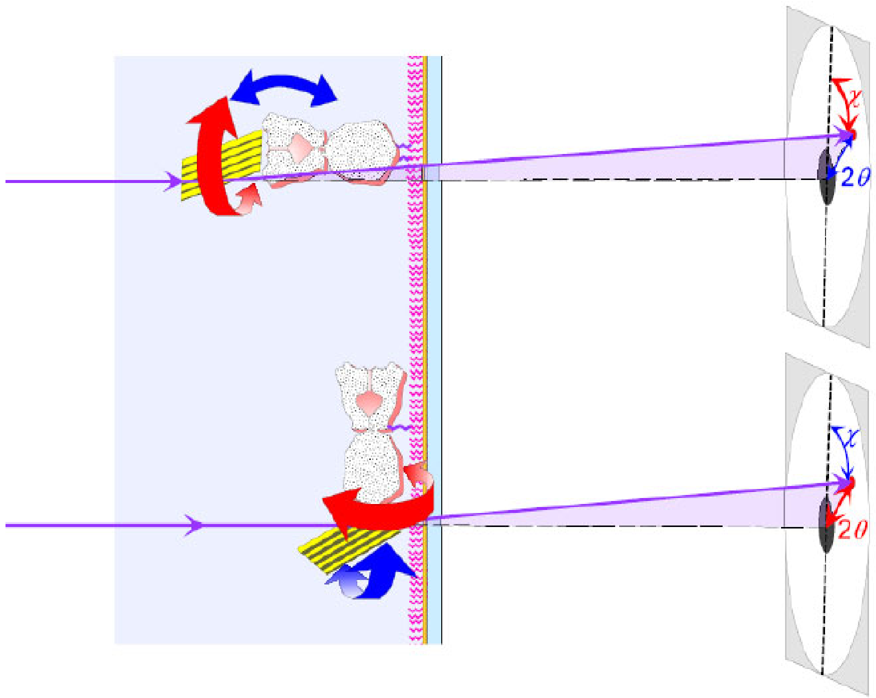
Other data (KcsA)



We utilized about twenty kinds of mutants of KcsA molecules in this experiment.

Other orientations of adsorbed KcsA

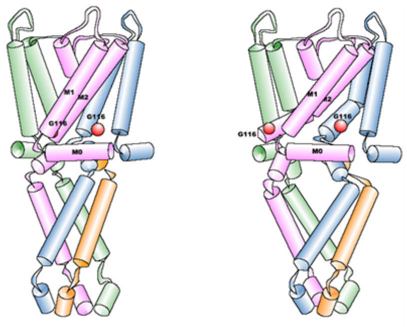


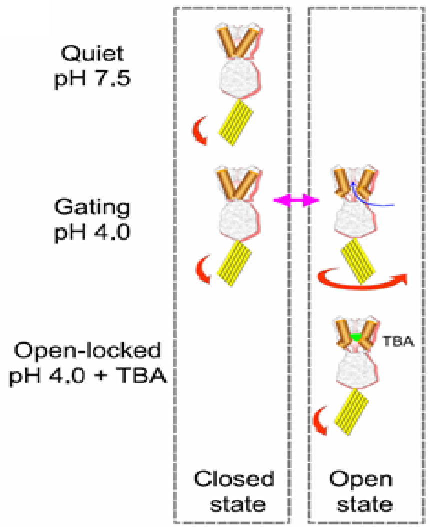


Other labeling sites

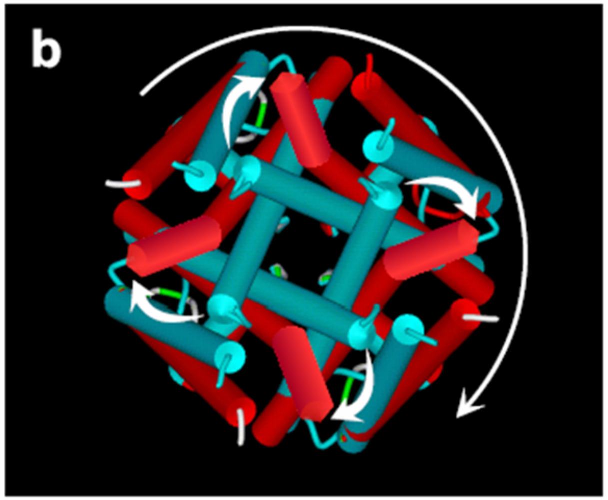
Twisting motions upon gating of KcsA

From structural data of KcsA crystals for opened and closed states, additionally, our dynamical observations, we can show the gating model.





Open direction upon gating

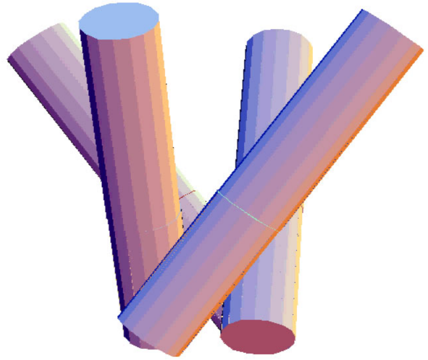


closed

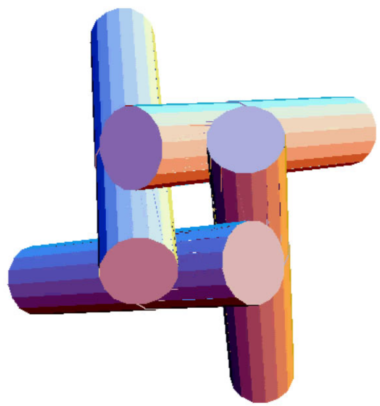
opened

View from CP domain (intracellular side)

Transmembrne domain of KcsA

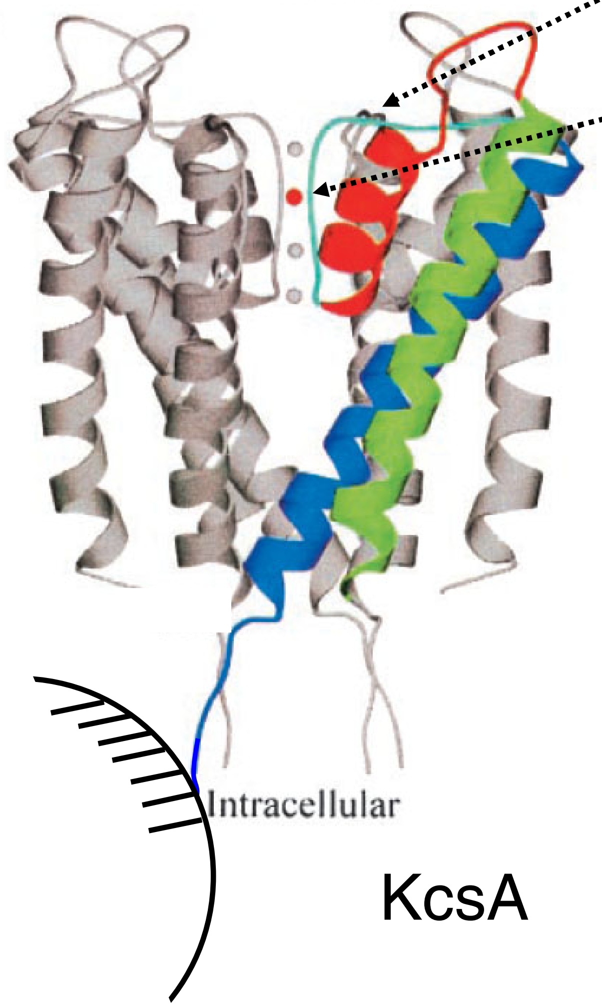


from side view



from CP domain side

Next observations (KcsA, KvAP, etc.,)



KcsA

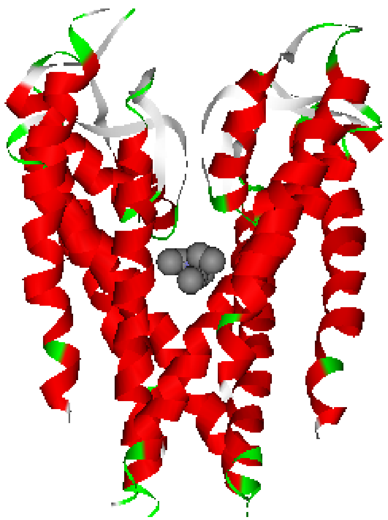
(a) Observations of extracellular domain.

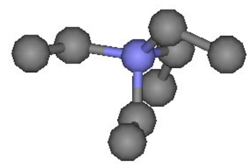
(b) Fast observations of filter part.

(c) Simultaneous observations.

(d) *In-vivo* observations.

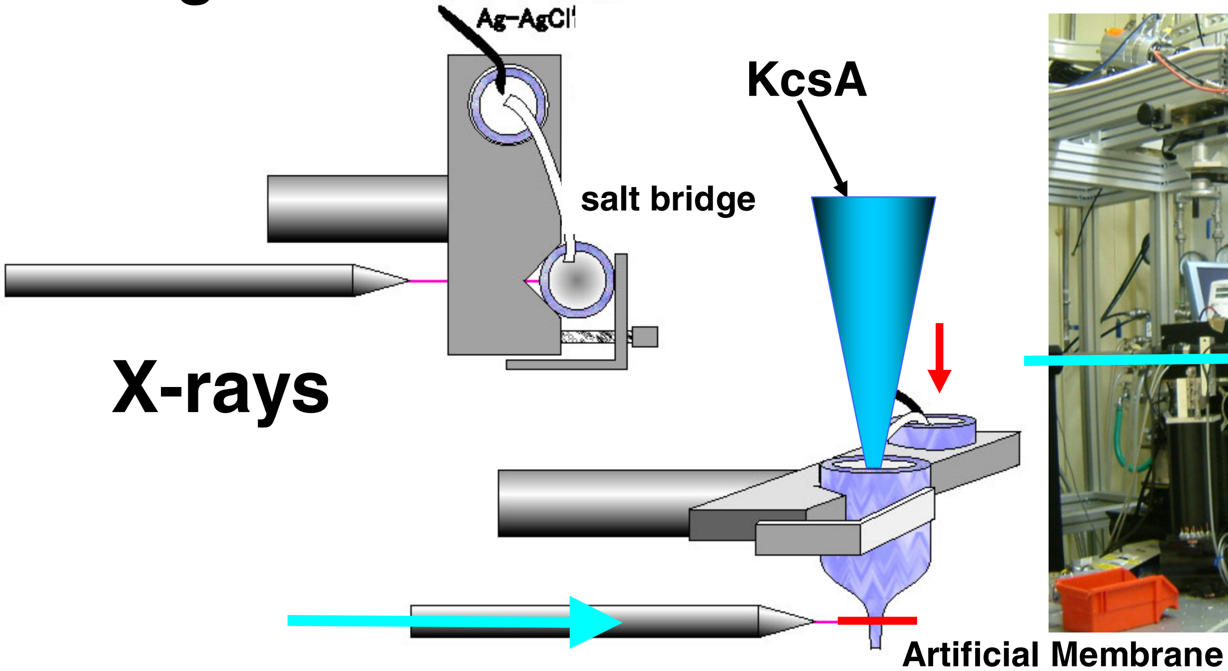
Position of Channel blocker

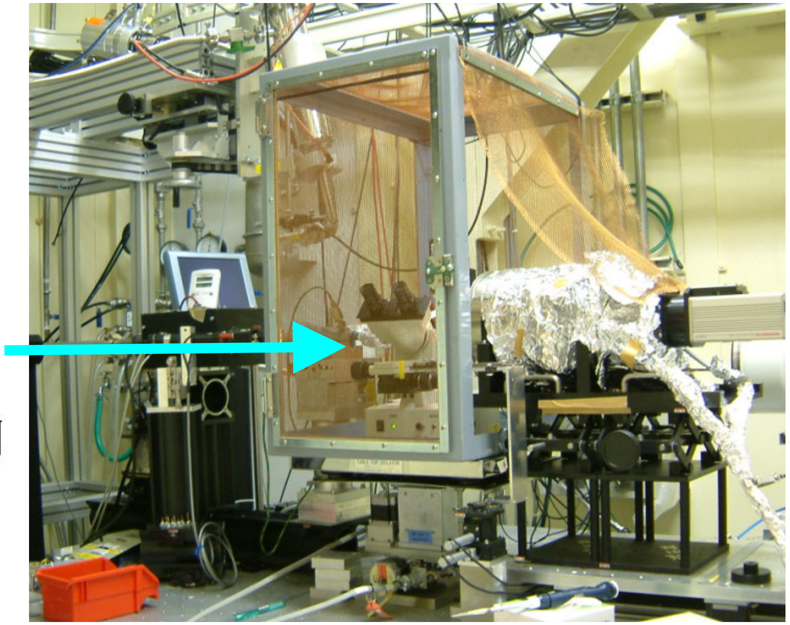


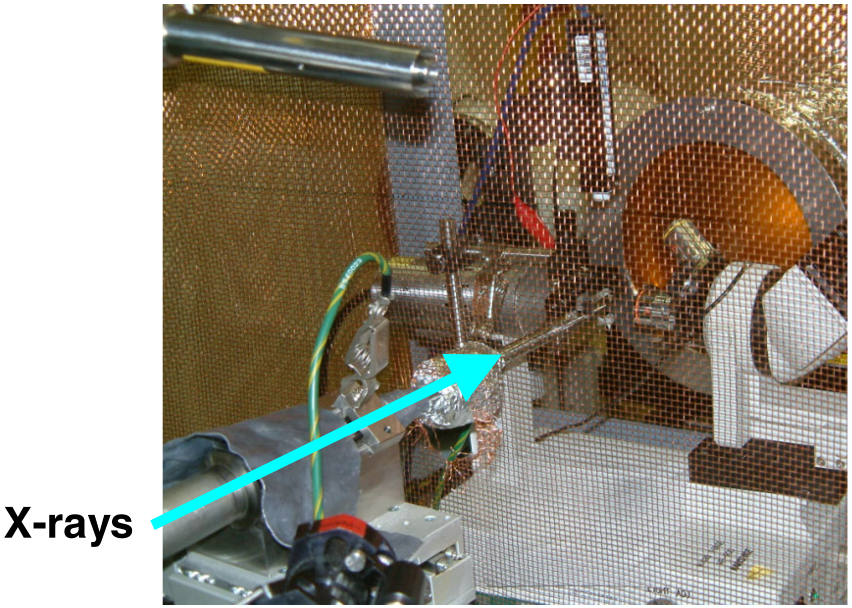


TBA（tetrabutylammonium）

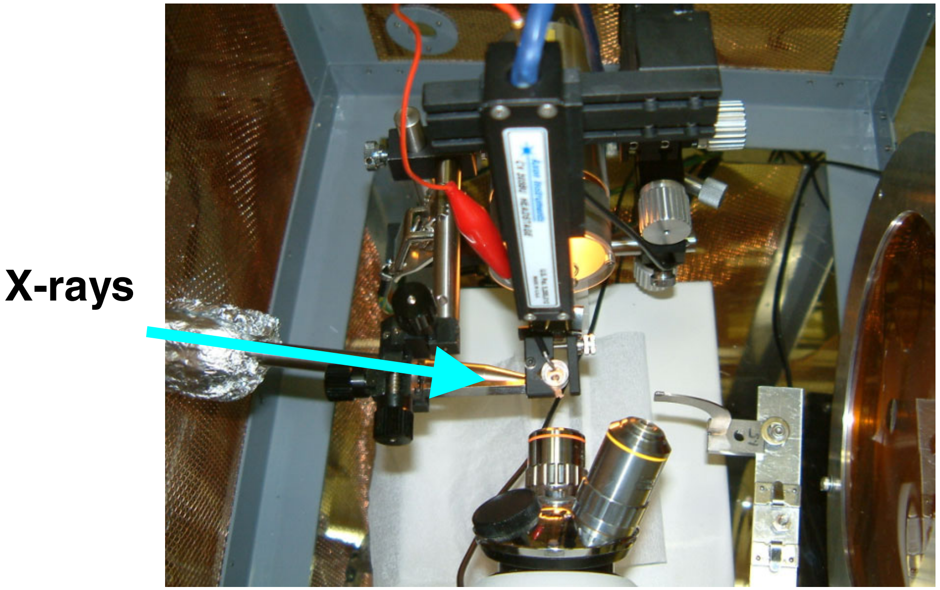
Arrangement of Simultaneous Observations





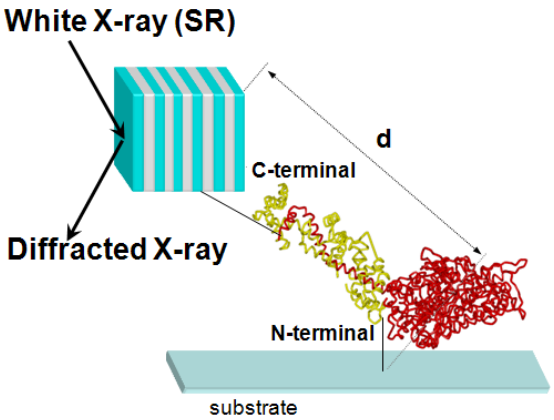


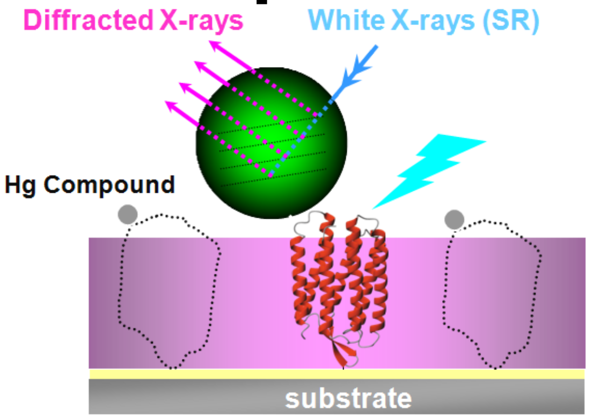
Front View

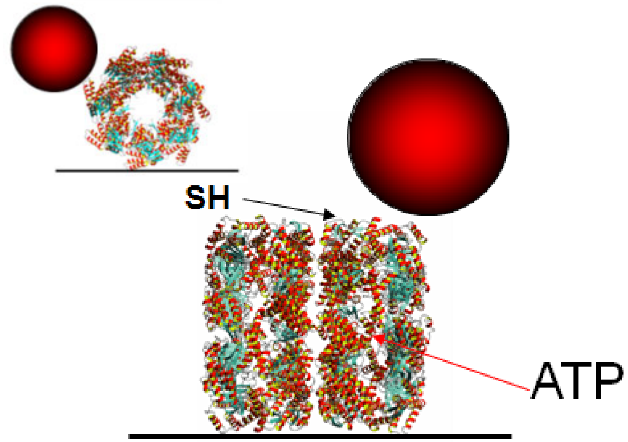


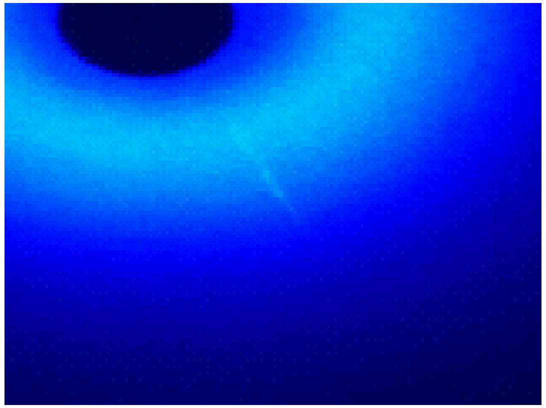
Upper View

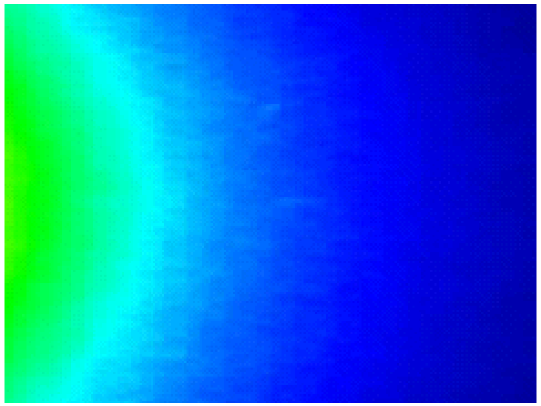
Other Experimental Samples:

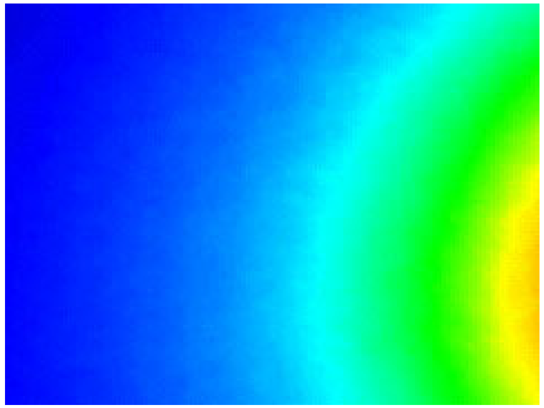


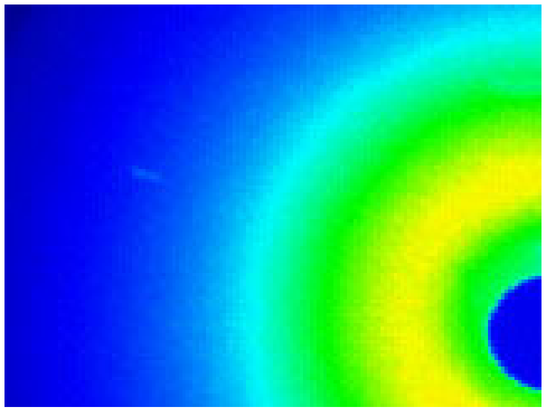


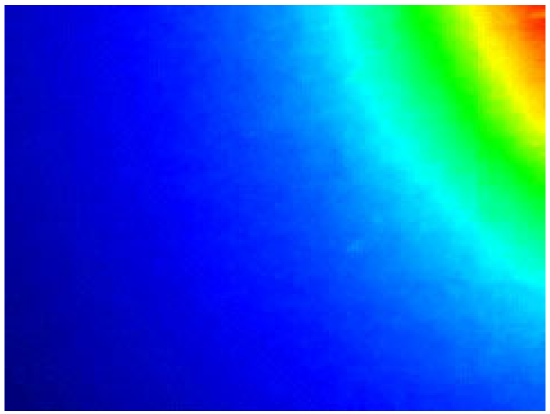


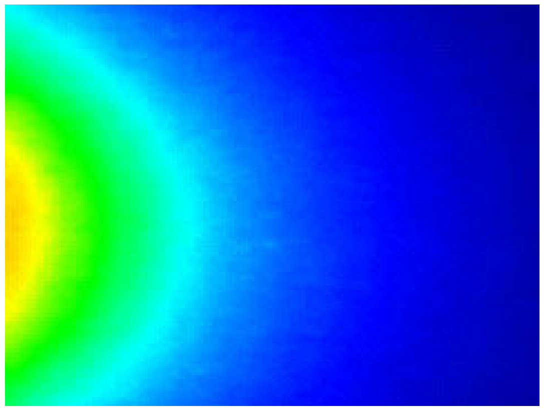

Mg-ATP(2mM)




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 , KvAP, AChR).

(2) *In vivo* observations.

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

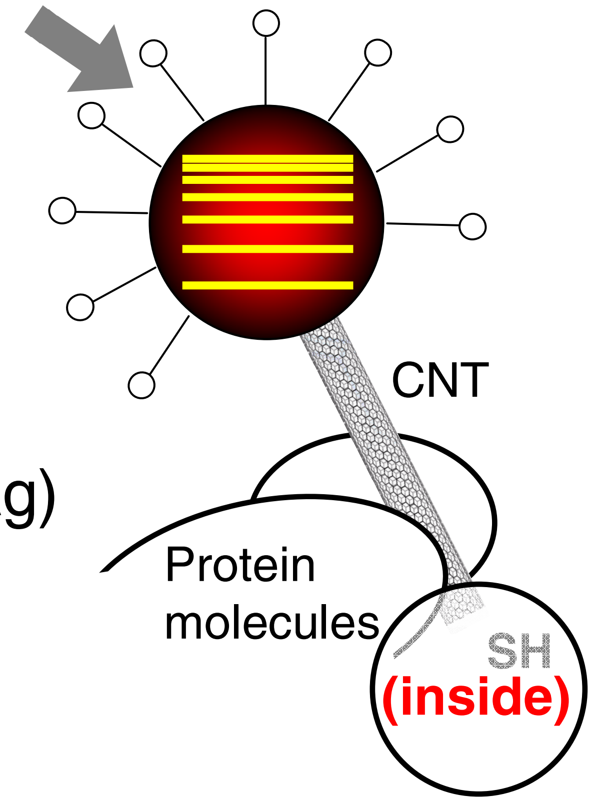
(4) DXT using monochromatic beam. We need multi-d-space nanoparticles.

(5) Fabrications of magnetic nanocrystal and other nanocrystal. (Fe_3O_4,Co, CdSe, Ag)

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

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

NanoProbe

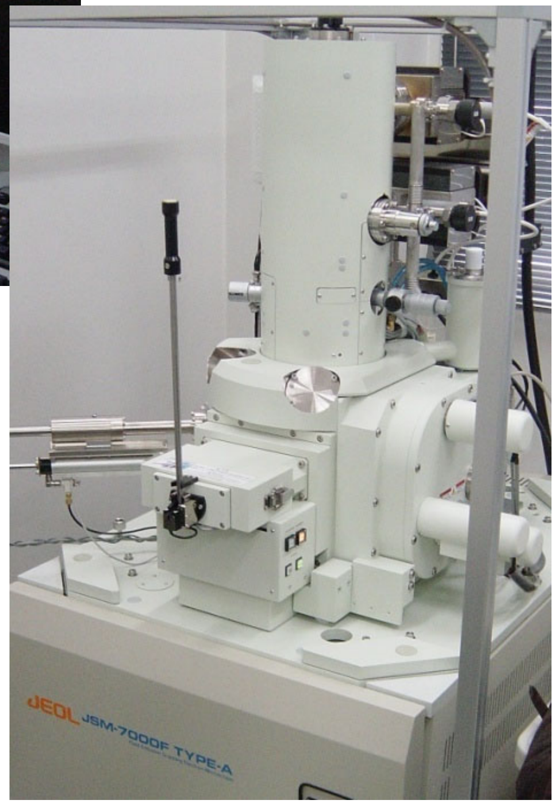


$$2d\sin\theta = n\lambda$$

Scanning Electron Microscope (JEOL JSM 7000F)

Laboratory Size

20cm



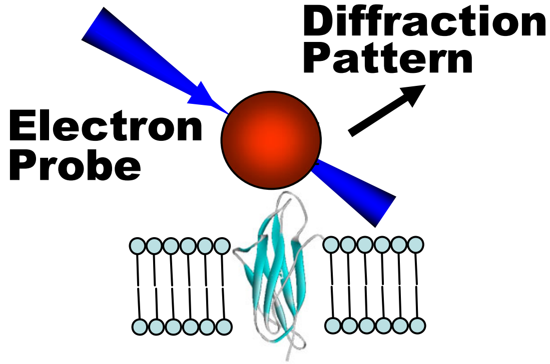
2km

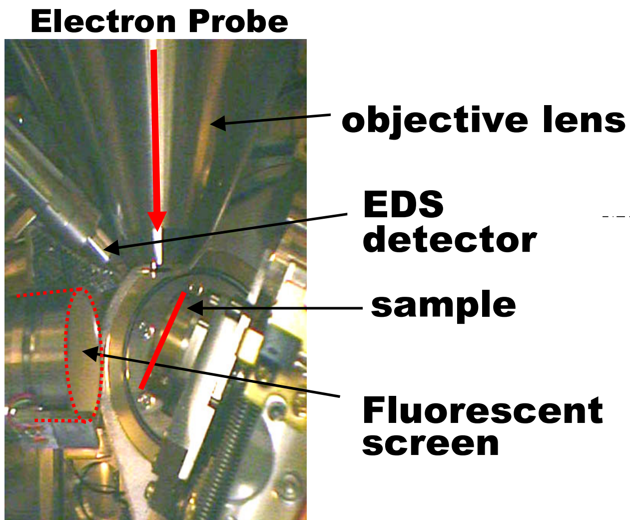
Synchrotron Orbital
Radiation Source
(SPring-8, Japan)

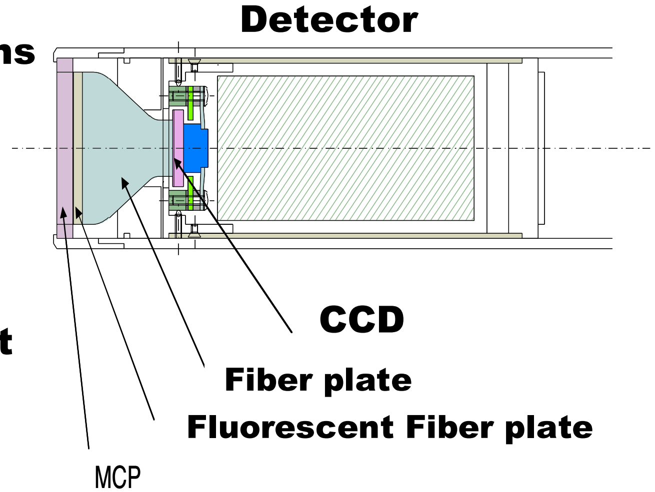
Comparison of X-ray and Electron Probe

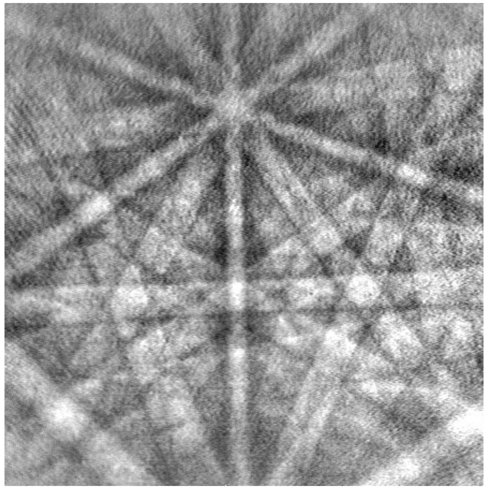
	X-ray (DXT)	Electron (DET)
Accuracy	0.01 deg	0.1deg
Dimension	2D to 3D	3D
Label Size	20-60 nm	10-40 nm
Damage	Small	Very Large
Instrument	Large Institute	Lab. level
Machine Time	1 day/month	Everyday

Concept of Single Molecular Detection using SEM:





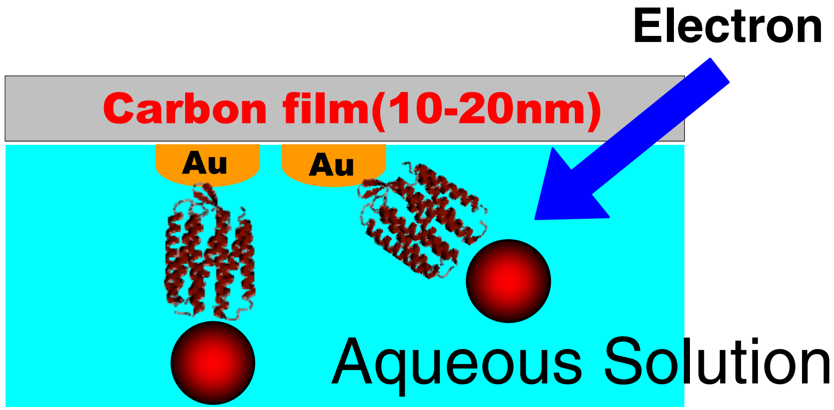




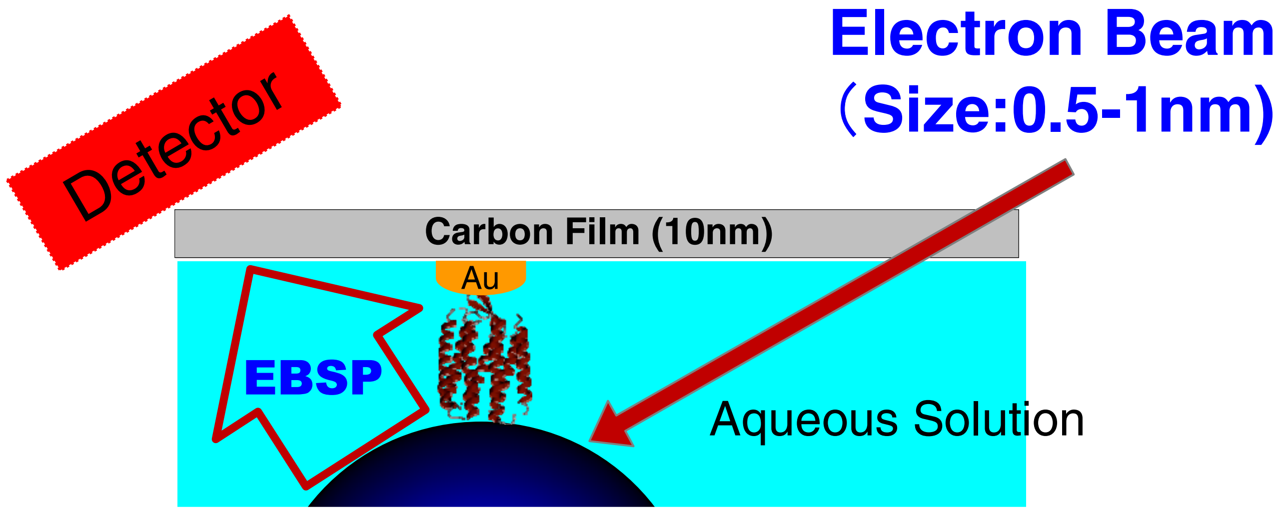
Electron Back Scattering Pattern (EBSP or EBSD)

Advantages:

Monochromatic beam, Nano-sized beam
Compact Instrumentation



Concept:
High speed detection before EBSP is attenuated

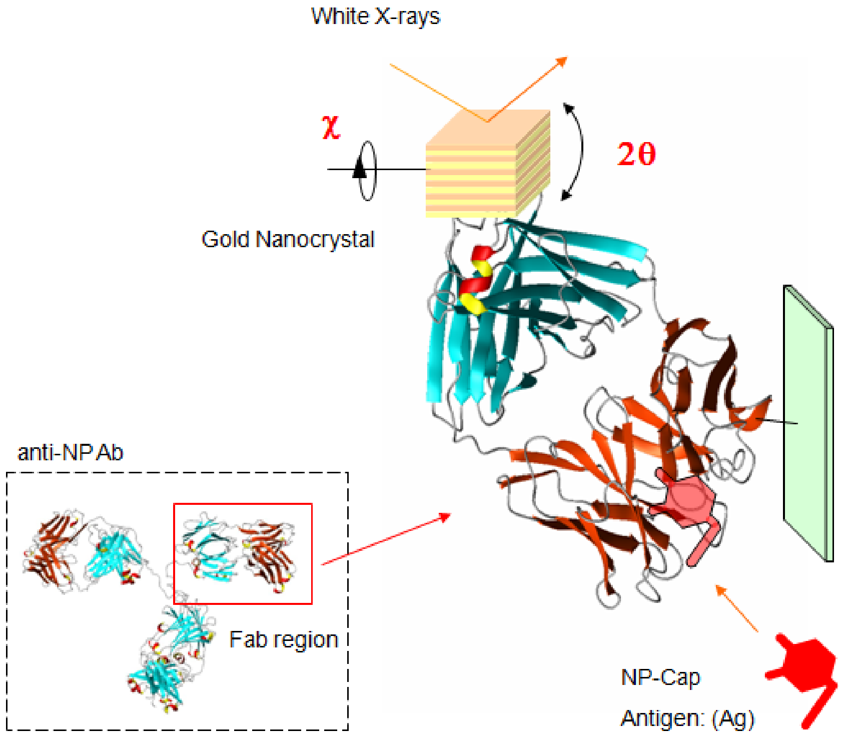


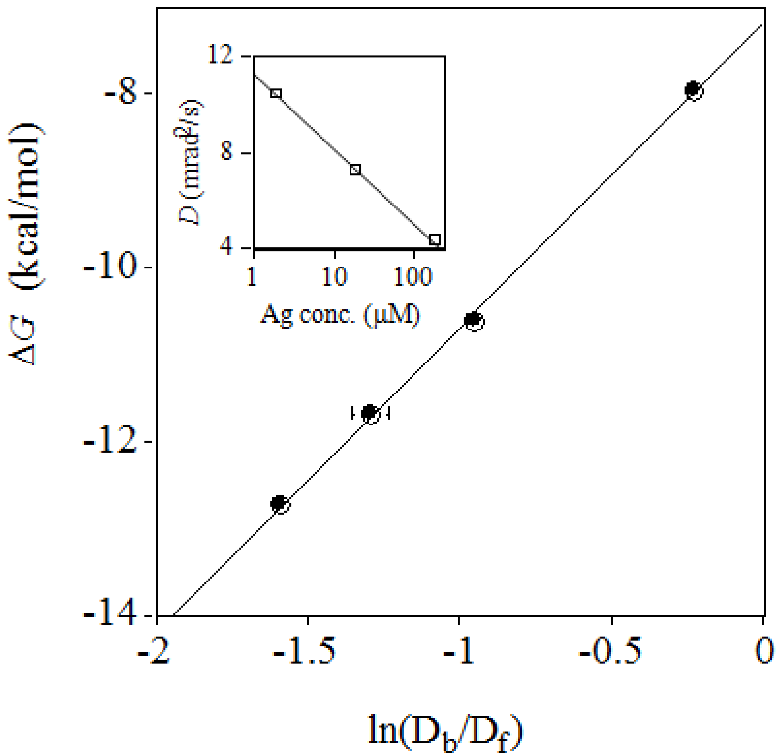
(1) Carbon Thin Film(10nm) and Gold(10nm).
(2) Pinpoint Electron Irradiation on Nanocrystal.

Mean free path (at 30KeV) :
Au (λ=3nm), **C**(λ=49nm), **H_2O**(λ=84nm)

Next Generation of Single Molecular World:

(1)Structural Determination from Single Molecules
(2) High Speed (ps-fs) and High Accuracy(pm)
(3)Single Molecular Calorimeter
(4) Control of Functional Dynamical Behaviors





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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

Diamond Light Source

K. Inoue

CREST/JST

T. Oshima, T. Igarashi

The University of Tokyo

K. Ichiyanagi

KEK/Institute of Materials Structure Science

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