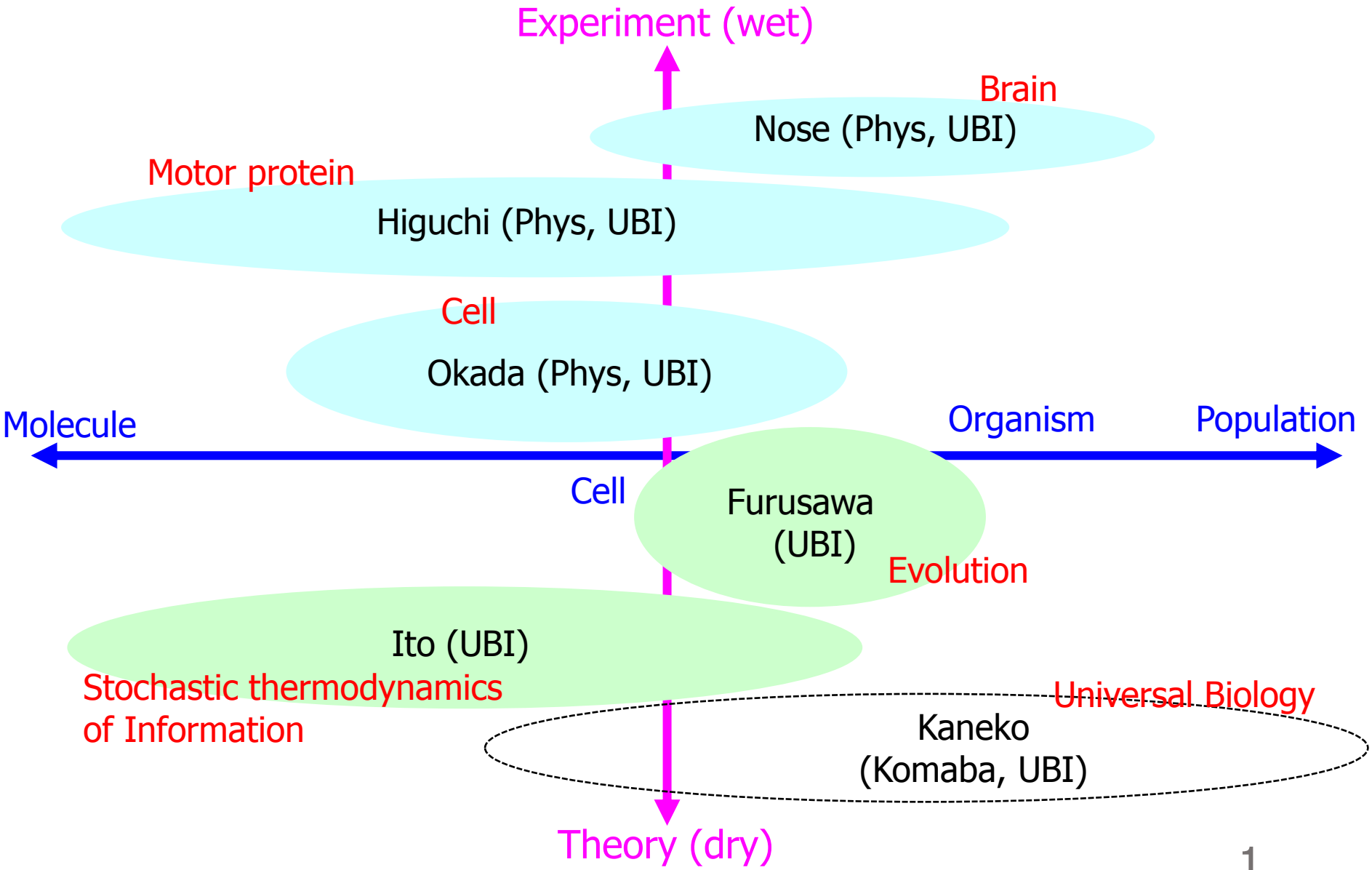
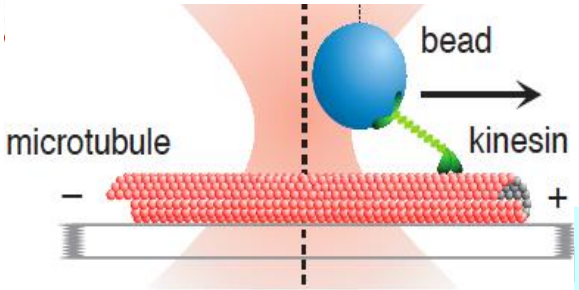
Sub course A7: Biophysics



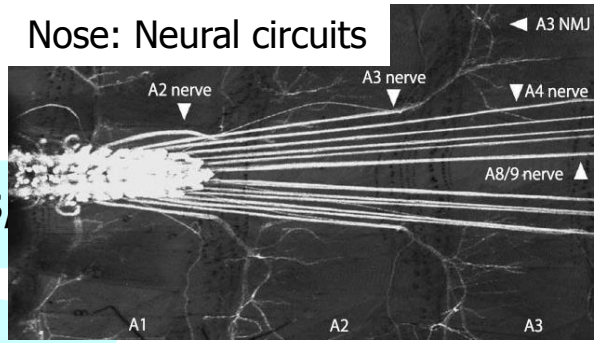
Sub course A7: Biophysics

Higuchi: Motor proteins

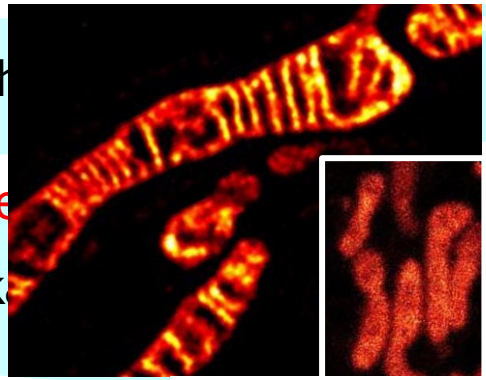


Experiment (wet)

Nose: Neural circuits

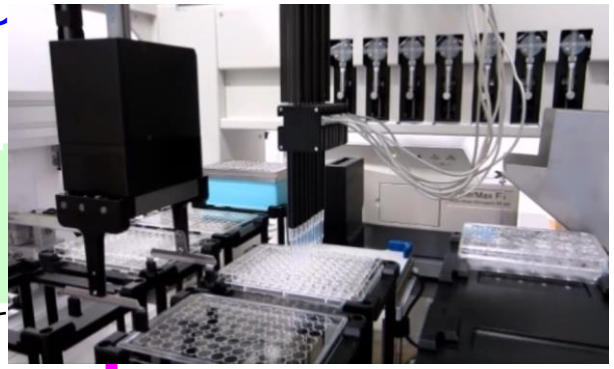


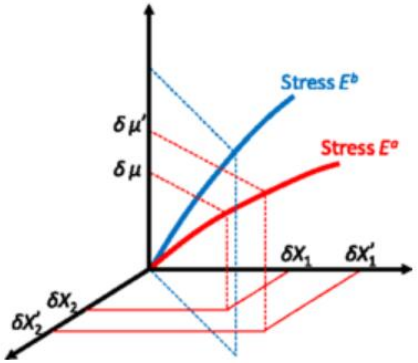
Okada: Live cell super-res imaging



Molecule ← → Organism → Population

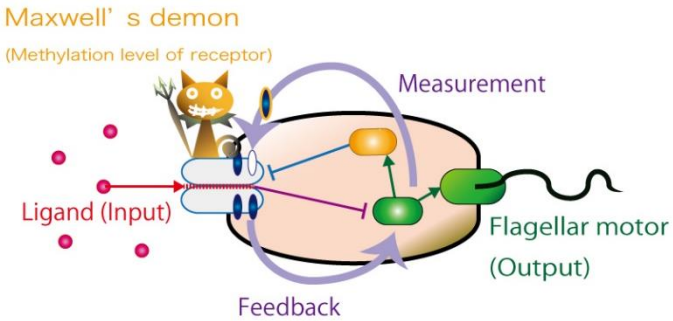
Furusawa: Experiment and theory of evolution





(Komaba, UBI)

Ito: Stochastic thermodynamics of information



Theory (dry)

Okada Lab

Yasushi Okada

Univ. Tokyo
- Department of Physics
- Universal Biology Institute
- International Research Center for Neurointelligence
- Department of Cell Biology and Anatomy

RIKEN
- Center for Biosystems Dynamics Research

14, Feb, 2020

Our research themes

Axonal transport
- Molecular function vs disease

Imaging technologies
- Super-res live imaging
- Single molecule imaging in live cells

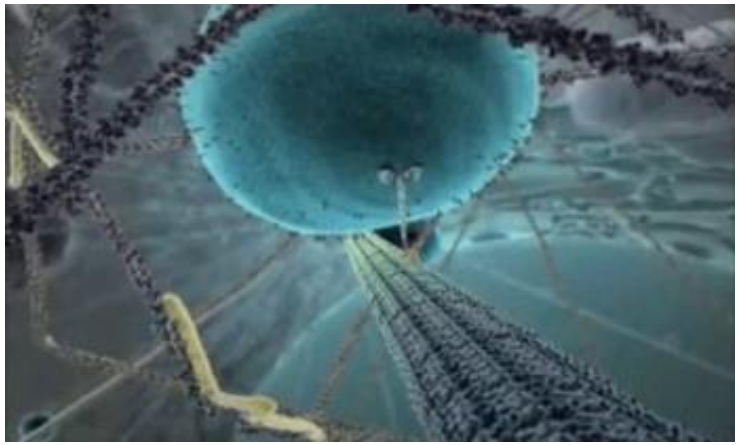
Crowded environment
- Live cytosol vs dead cytosol

More crowded environment

Nucleus
- Genome DNA packing
- Transcription

Probes for cell states
- Epigenome
- Green fluorescent RNA
- Metabolism

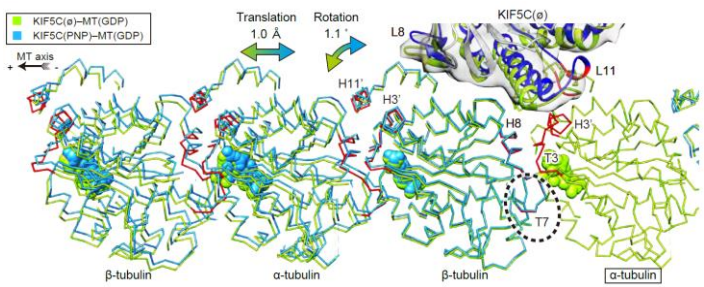
Studies on axonal transport



Axonal transport is

- an essential logistics in neurons.
- rate-limiting for learning and memory.
- associated with various diseases such as Alzheimer's disease or ALS.

What is the "road sign" to the proper destination?



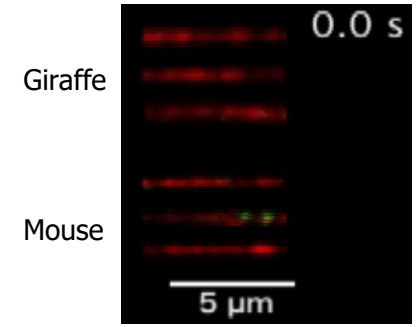
Conformational switching in the rail polymer, microtubule. (Shima et al., J Cell Biol. 2018)

How many motor proteins are working on each cargo? How much force is exerted?

$$\frac{F}{k_{\mathrm{B}}T_{\mathrm{eff}}} = \ln\left[P(\Delta X)/P(-\Delta X)\right]/\Delta X$$

Non-invasive measurement of force from imaging by using the fluctuation theorem. (Patent pending, Hayashi et al., MBoC 2018)

Does longer axon of giraffe require faster transport?

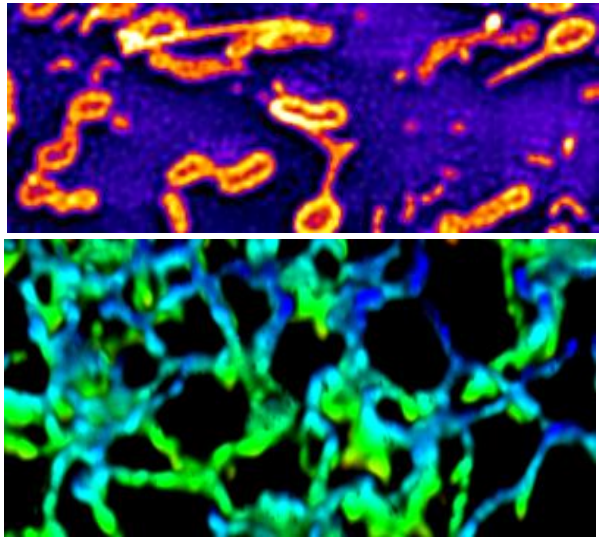


Giraffe kinesin moves faster by "long animal specific" mutations.

Super Resolution Live Cell Imaging

Spinning disk super-res microscope

SR live imaging
200 FPS,
100 nm res,
100 μm FoV.

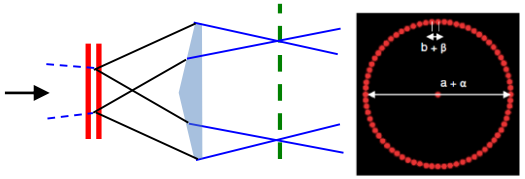


Commercialized
Award from MEXT

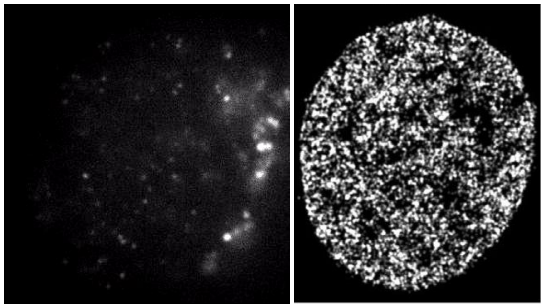
MBoC 2015

Ring-TIRF microscope for live cell STORM

3D SM imaging
at 1000 FPS

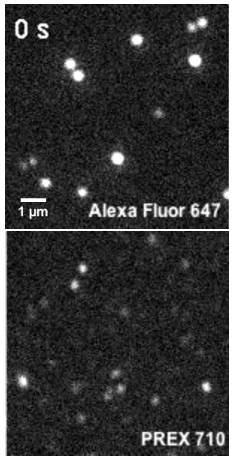


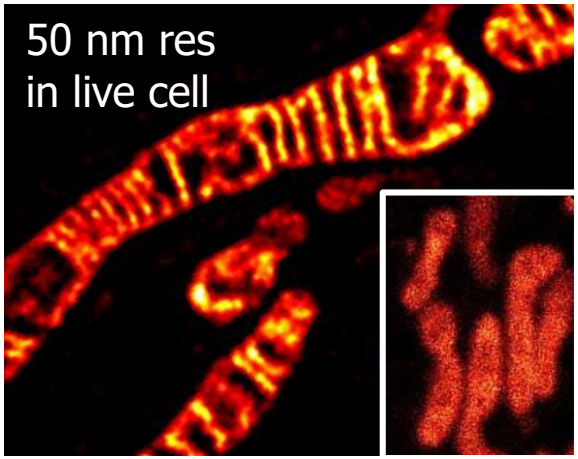
Live cell
STORM of H2B
at 20 nm acc.



Collaboration with
Maeshima-lab,
Mol Cell 2017

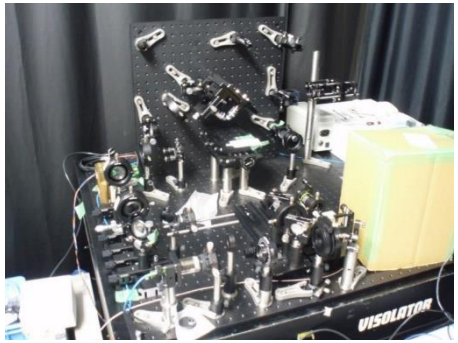
Super-stable dye for super-res imaging

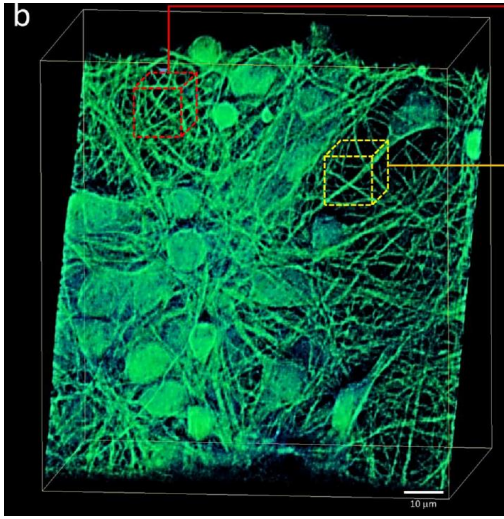




Collaboration with Drs Taki and Yamaguchi at Nagoya U
Angew Chem 2018; PNAS 2019

Bessel light sheet super-res live imaging



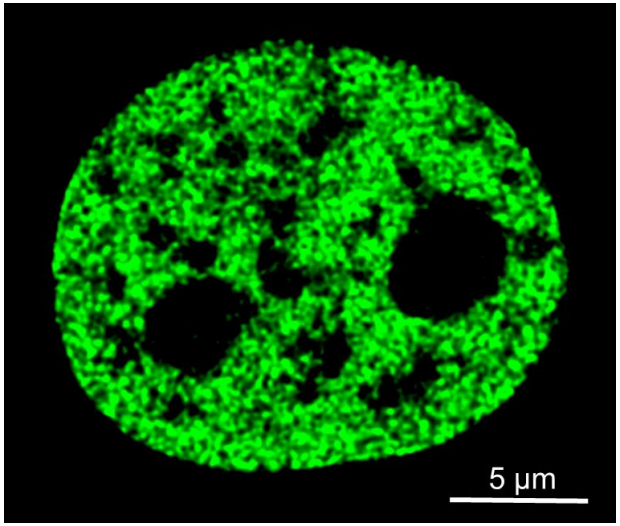


Collaboration with Peilin
Chen and Bi-Chang Chen
at Academia Sinica

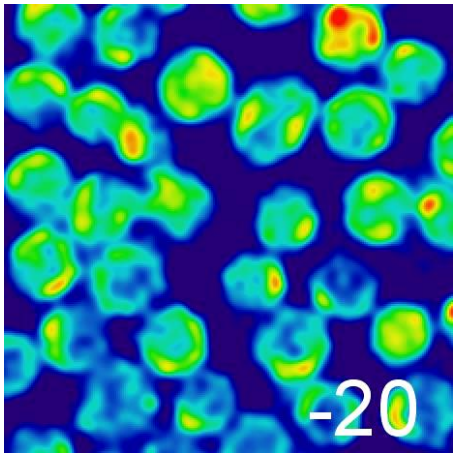
Commun Biol. 2019

Probes to read out the cell states by imaging

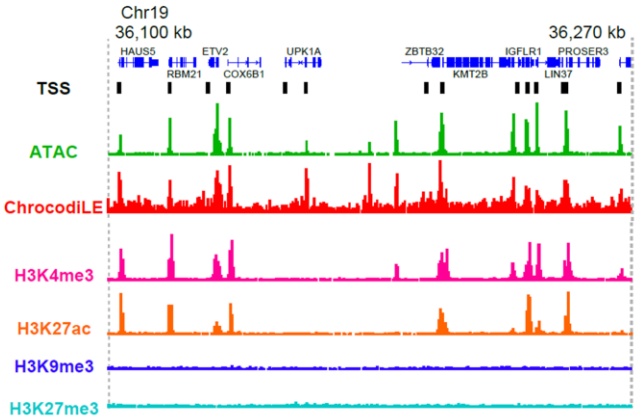
Epigenetic state by live imaging



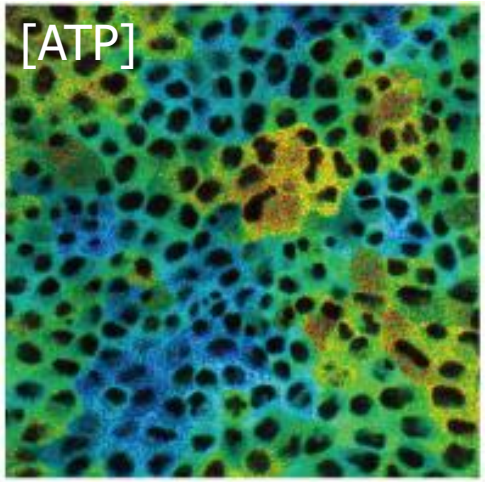
RNA expression dynamics

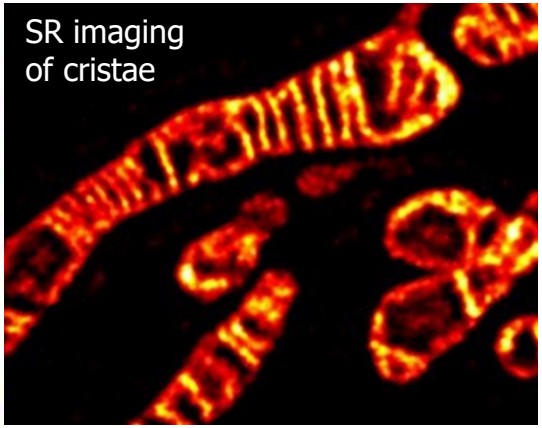


Single-cell sequencing



Metabolic states





PNAS 2019

New method for single molecule fluctuation analysis in cells.

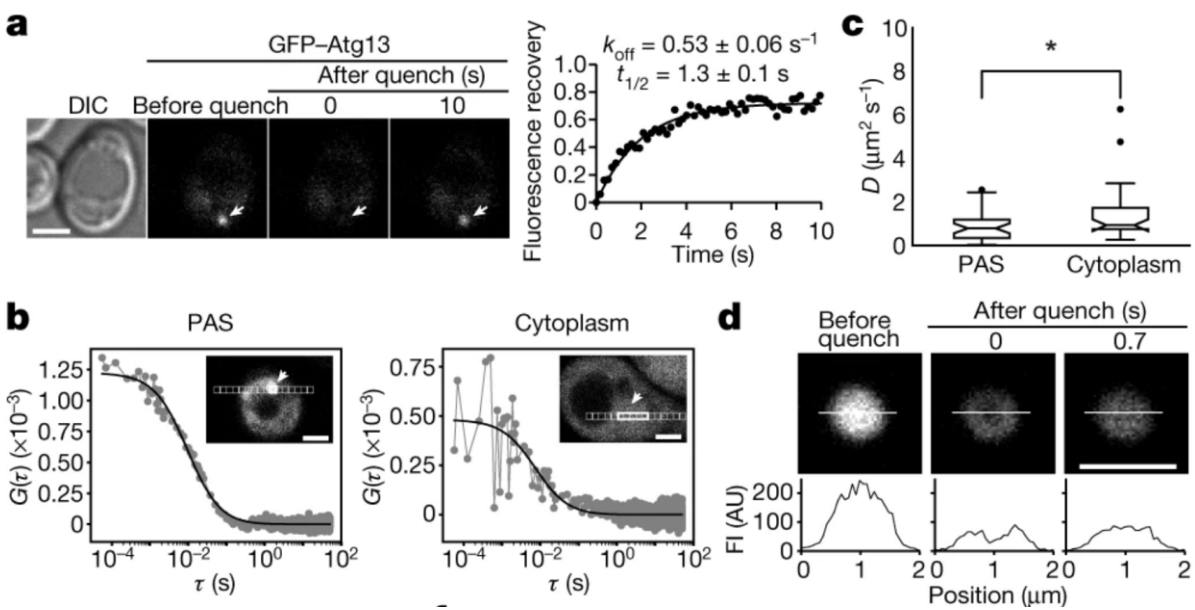
Article

Phase separation organizes the site of autophagosome formation

https://doi.org/10.1038/s41586-020-1977-6
Received: 17 December 2018
Accepted: 2 December 2019
Published online: 05 February 2020

Yuko Fujioka[1], Jahangir Md. Alam[1], Daisuke Noshiro[1,2], Kazunari Mouri[3], Toshio Ando[2], Yasushi Okada[3,4], Alexander I. May[5,6], Roland L. Knorr[7,8,9], Kuninori Suzuki[10,11], Yoshinori Ohsumi[5] & Nobuo N. Noda[1*]

Many biomolecules undergo liquid–liquid phase separation to form liquid-like



Line-scanning fluorescence correlation spectroscopy with denoising and trend removal by wavelet transformation.

$$G_\xi(\tau_\xi) = \frac{\langle \delta F_\xi(t_i)\delta F_\xi(t_i+\tau_\xi)\rangle}{\langle F_\xi(t_i)F_\xi(t_i+\tau_\xi)\rangle}$$

Enables to measure the dynamics of protein molecules in a small compartment of living cell.

Nature in press, 2019

External funding and collaborations

Grants-in-Aid for Scientific Research on Innovative Areas (FY2019-FY2023)
"Information Physics of Living Matters"

PI: Yasushi Okada

Ito (UBI), Takeuchi (A6)

+ 3 theoretical physicists

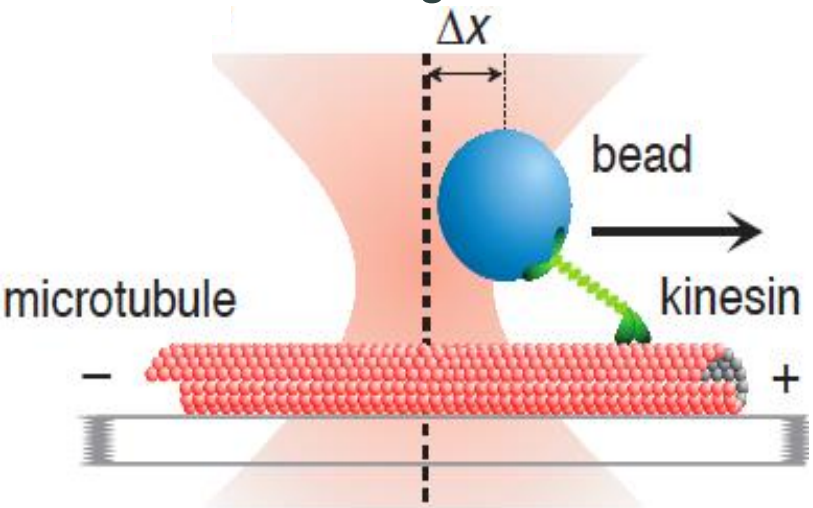
+ 6 experimental biophysicists

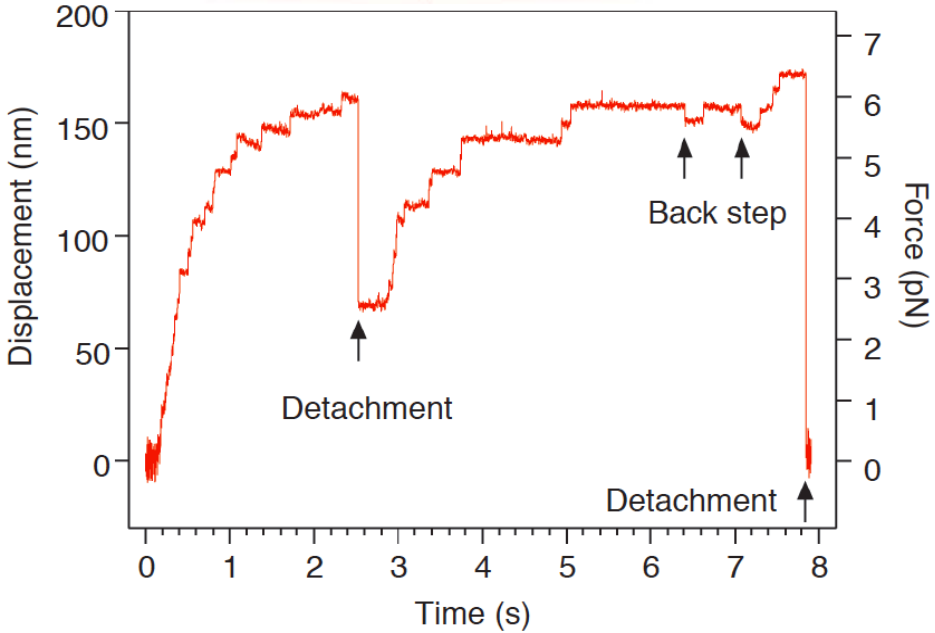
(~1,000 mil yen in total)

Motor proteins: From Molecule to Medicine application

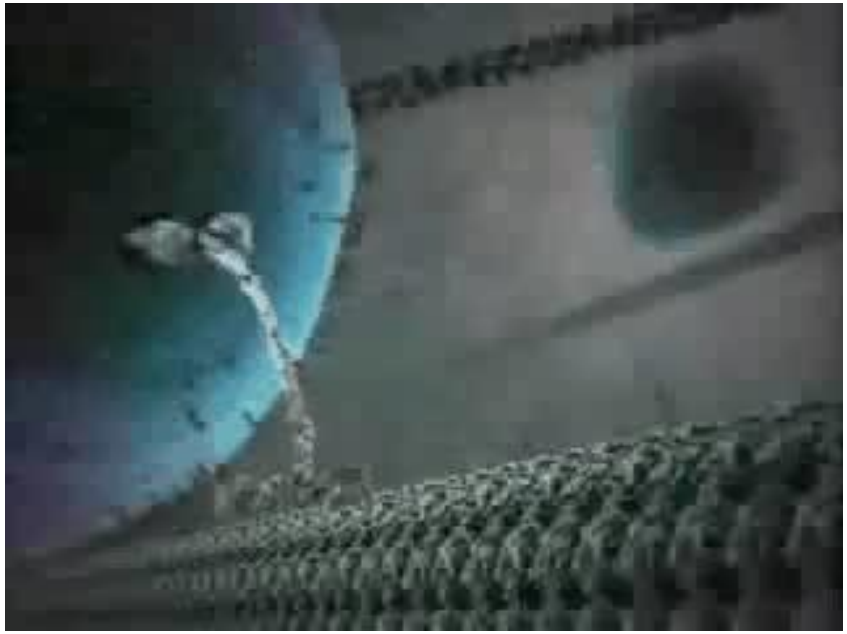
Hideo Higuchi (Dept. Phys. & UBI)

Movement of single kinesin molecules





Motor proteins, kinesin, dynein and myosin-V transport vesicles in cell.



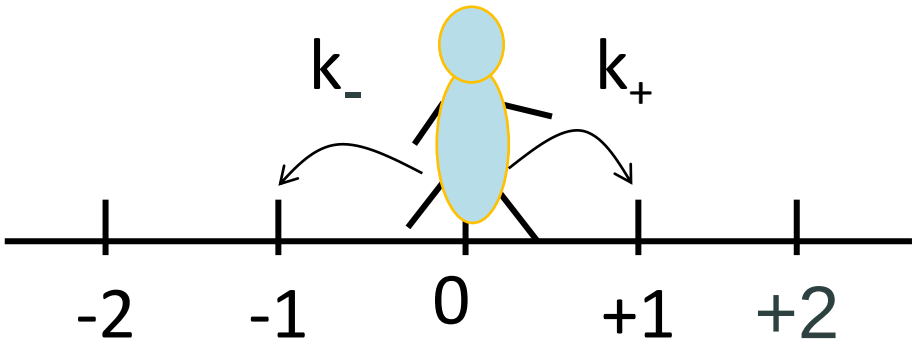
From BioVision

The motor steps explained by the model of biased Brownian walk

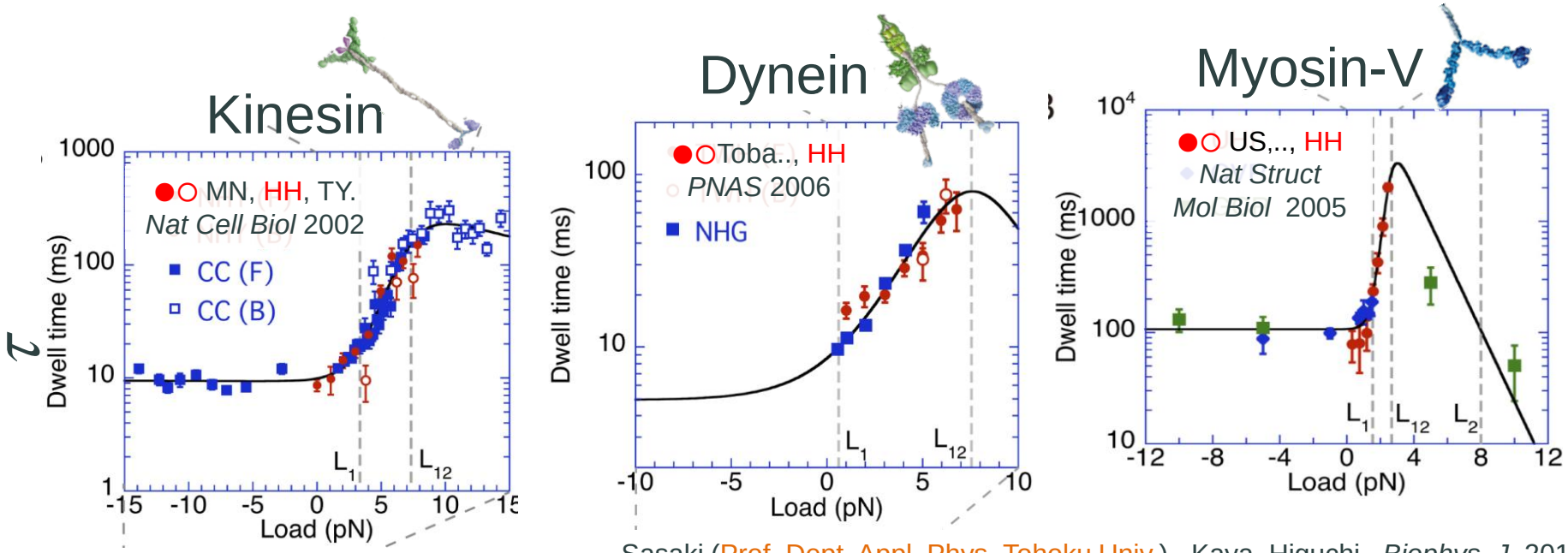
$$\frac{1}{k_+} = \frac{1}{\kappa_1} + \frac{1}{\lambda_1}\exp\left(\frac{d_1 L}{k_B T}\right)$$

$$\frac{1}{k_-} = \frac{1}{\lambda_2}\exp\left(-\frac{d_2 L}{k_B T}\right)$$

L: load

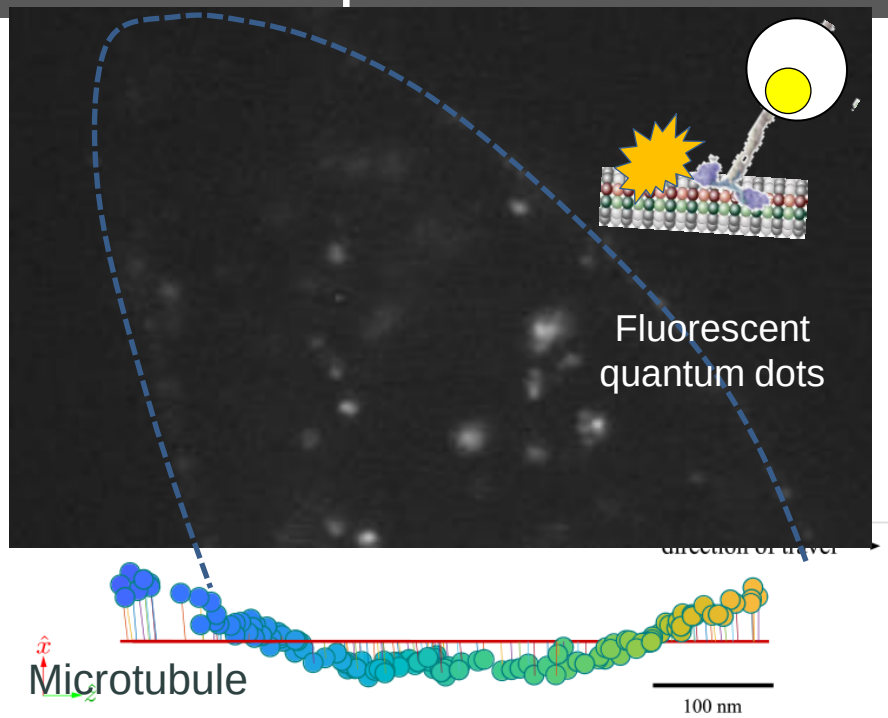


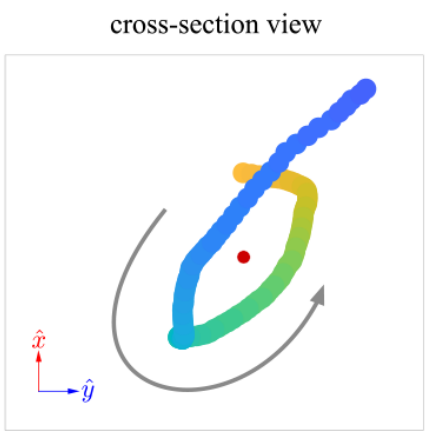
$$\tau = \frac{1}{k_+ + k_-} = \exp\left[\frac{d_2}{k_B T}(L - L_2)\right] + \exp\left[\frac{d_1 + d_2}{k_B T}(L - L_{12})\right]$$

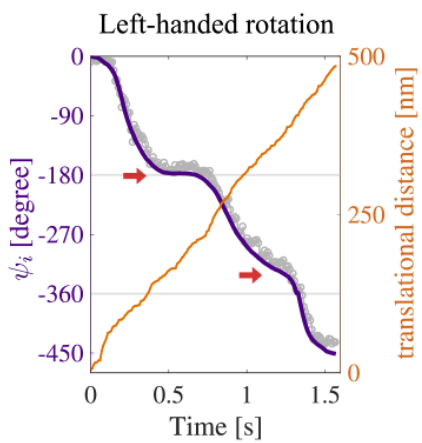


Sasaki (Prof. Dept. Appl. Phys. Tohoku Univ.) , Kaya, Higuchi. *Biophys. J.* 2018

Rotational vesicle-transport in cell

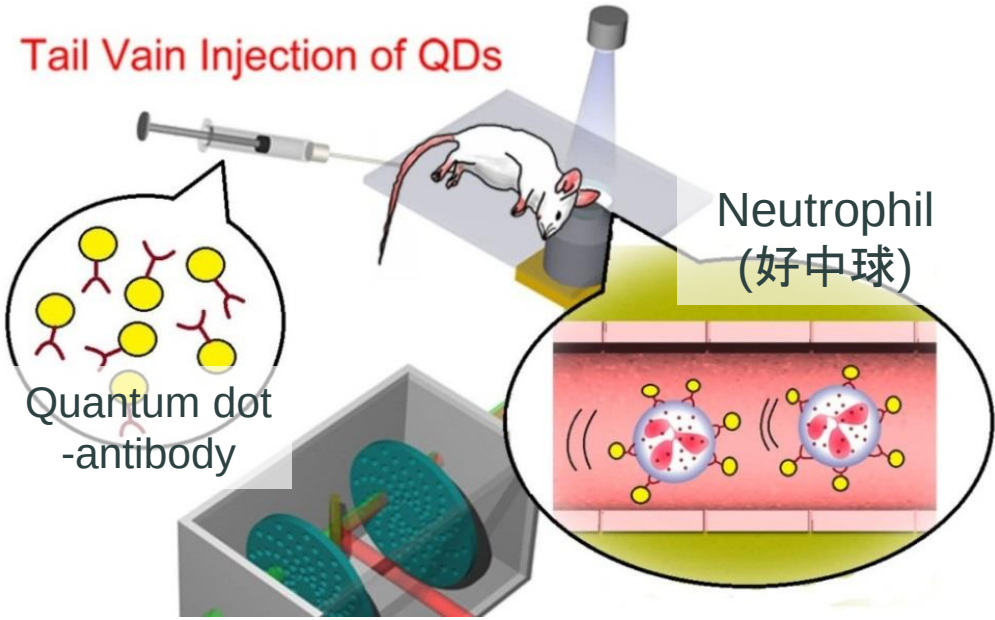


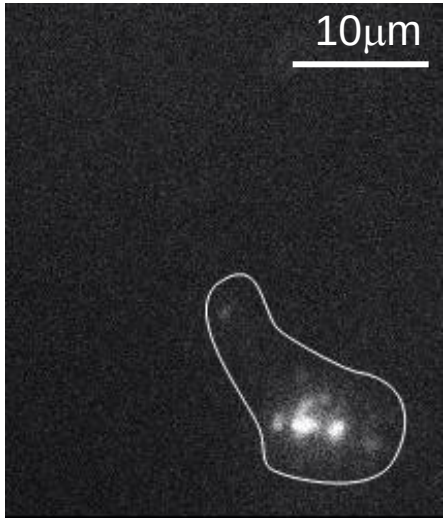


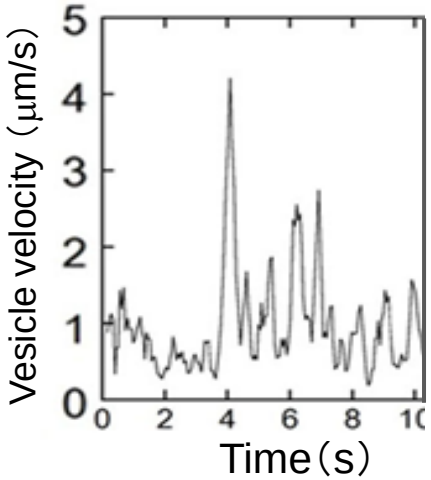


Lee (doctor student) & Higuchi. *Bio Opt Exp* 2019, *Opt Exp* 2018

Noninvasive imaging of vesicle transport in mouse

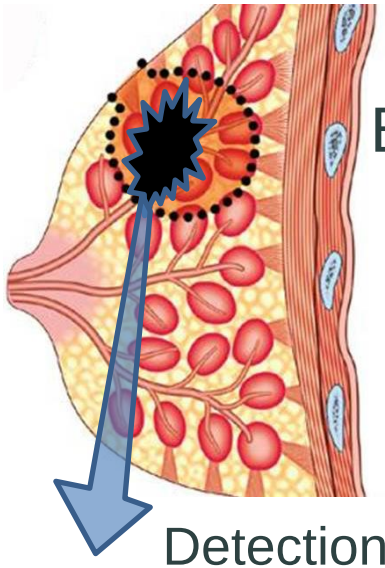






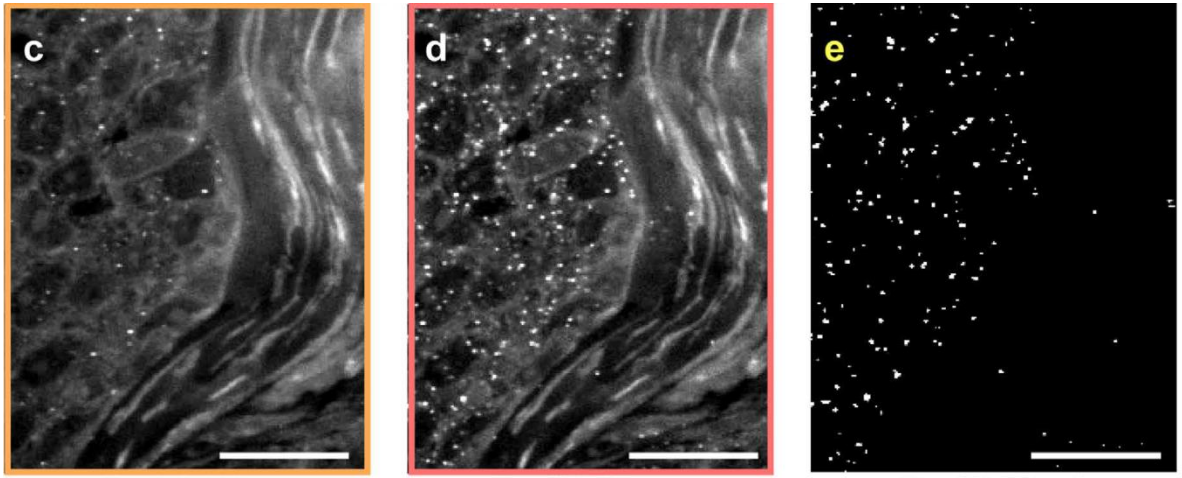
Kikushima (Postdoc), Kita, Higuchi. *Sci. Rep*. 2013

Predictive risk-diagnosis of human breast cancer by quantum dot imaging



Breast tissue dissected
by surgical oncologists (腫瘍外科医）

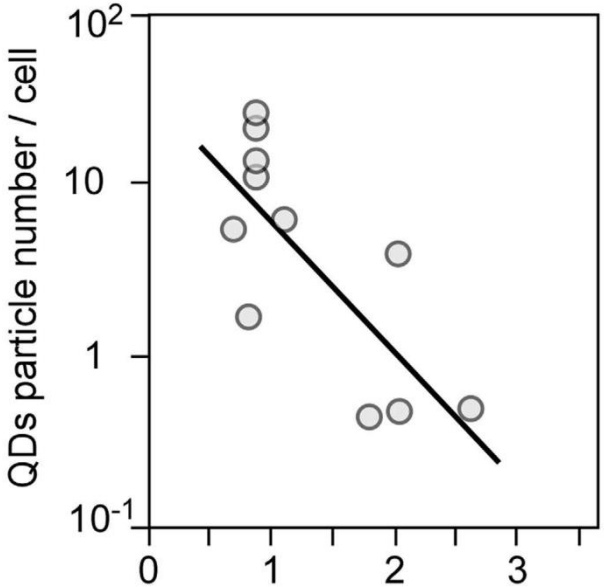
Detection of breast cancer cells in tissues



Em: 640-690nm
Background

Em: 695-740nm
AntiBody-Qdot

(Em: 695-740nm)
– (Em: 640-690nm)
Ab - background

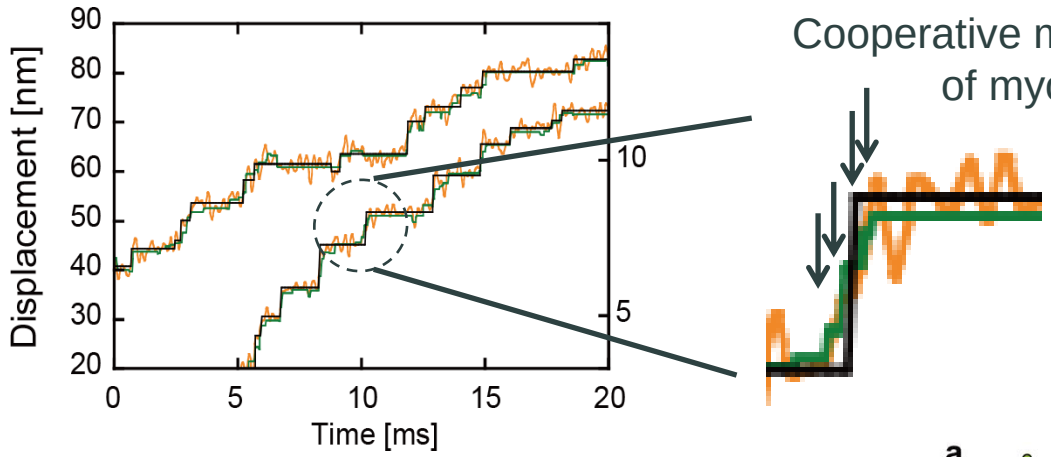


relapse-free survival
after surgery (year)
手術後再発期間(年）

Gonda (former assistant professor), MM, HH,…NO. 2019, *Sci Rep.* 2015

Other Research Highlights

1. M. Kaya (assistant professor and corresponding author), Y. Tani, T. Washio, T. Hisada & H. Higuchi. Coordinated force generation of skeletal myosins in myofilaments through motor coupling. *Nature Communications* (Aug. 2017)

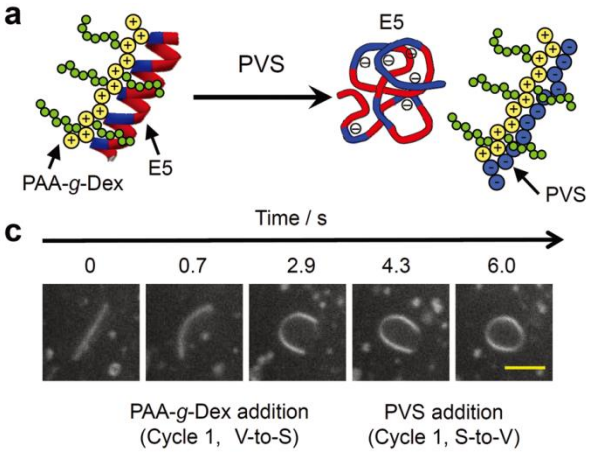


Cooperative multi-force generations of myosin assemble

2. N. Shimada (Tokyo Tech), H. Kinoshita, T.Umegae, S.Azumai, N.Kume, T.Ochiai, T.Takenaka, W. Sakamoto, T.Yamada, T. Furuta, T. Masuda, M.Sakurai, H.Higuchi, & A.Maruyama.

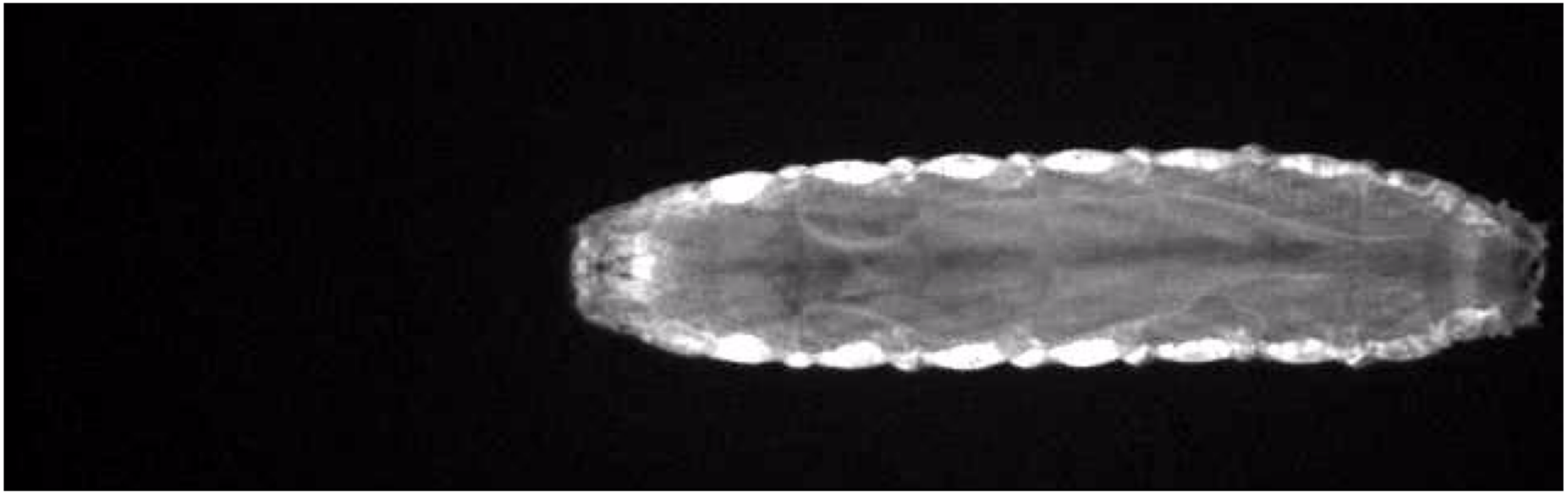
Cationic Copolymer-Chaperoned 2D–3D Reversible Conversion of Lipid Membranes.

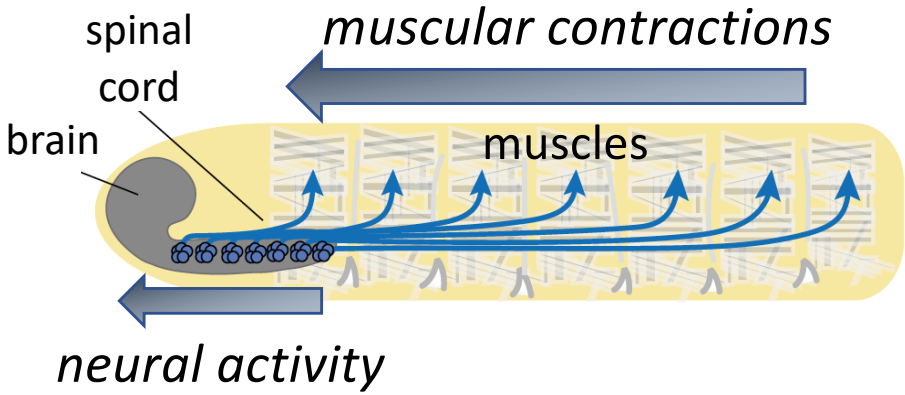
Advanced Materials (Sept. 2019) Impact factor, 25.8



Polymers (**amphiphilic peptides and cationic copolymers)** controlled the reversible conversion of lipid membranes

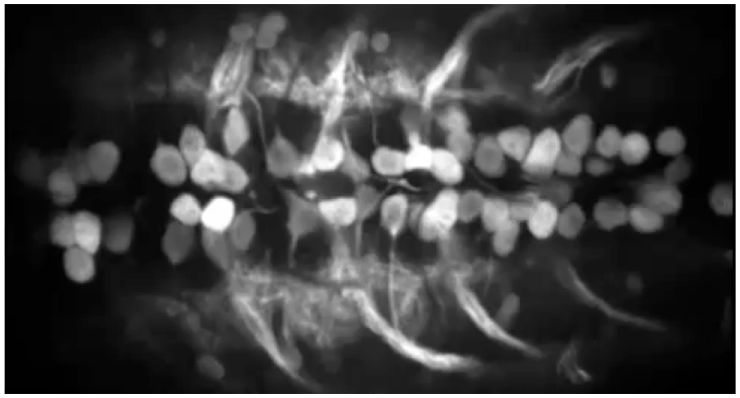
How neural circuits generate behavior?





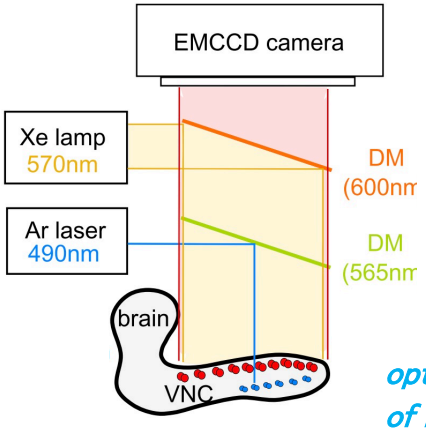
Experimental methods

Calcium imaging



visualization of neural activities

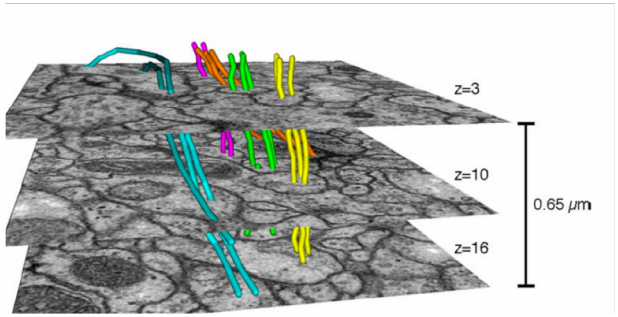
Optogenetics

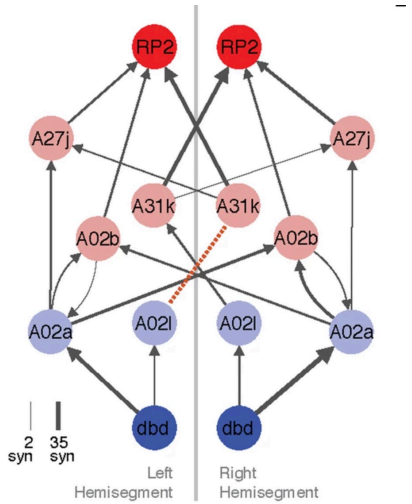


optical manipulation of neural activities

Connectomics

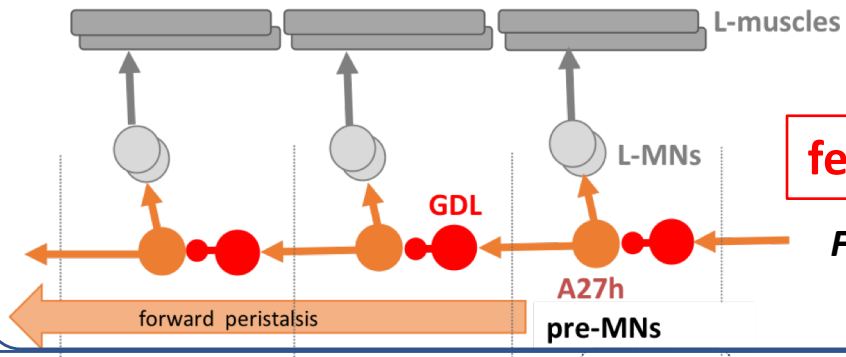
3D EM reconstruction of circuit diagram





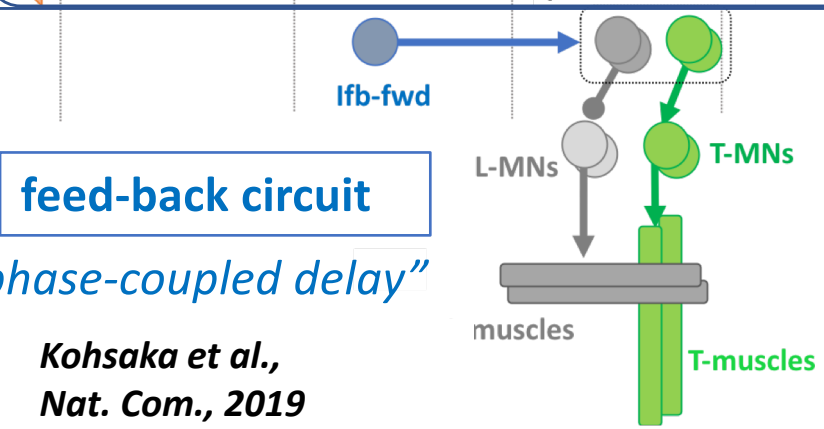
Cardona et al., eLife, 2016

Identified circuit motifs



feed-forward circuit

Fushiki et al., eLife, 2016



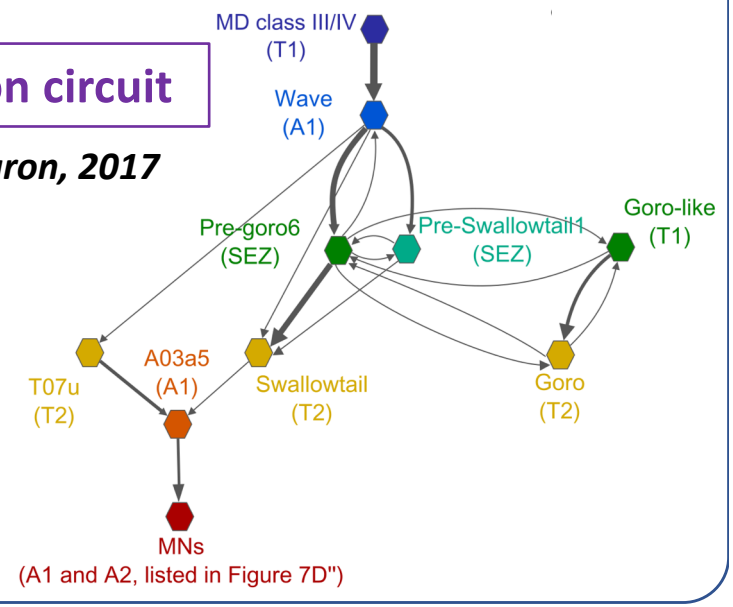
feed-back circuit

"phase-coupled delay"

Kohsaka et al., Nat. Com., 2019

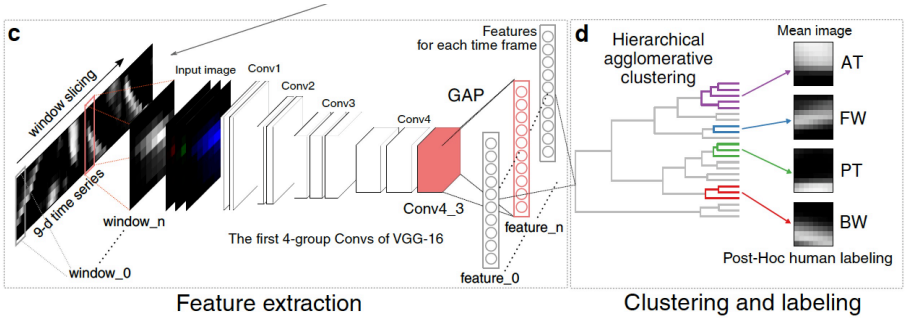
action-selection circuit

Takagi et al., Neuron, 2017



Data analyses and simulation

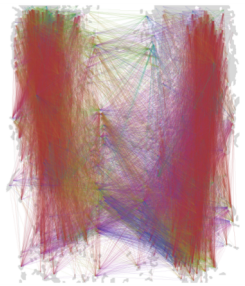
Clustering of neural activities by machine learning



Park et al., Sci. Report 2018

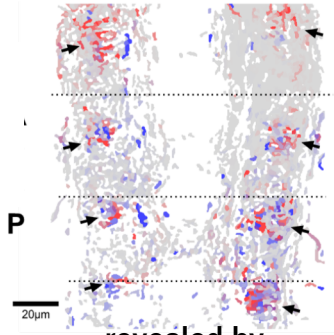
Network analysis of neural activities

network structure



constructed by transfer entropy

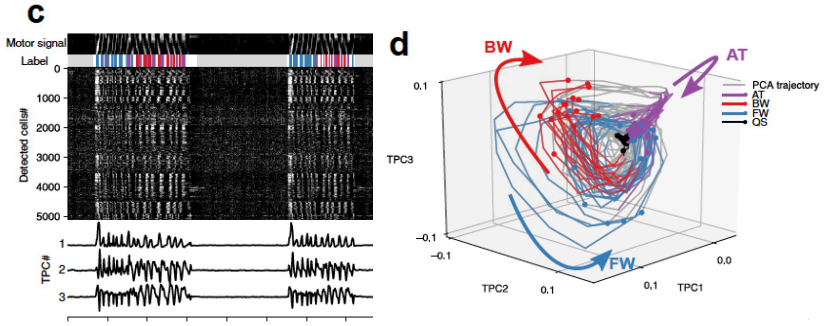
hub synapses



revealed by graph theory

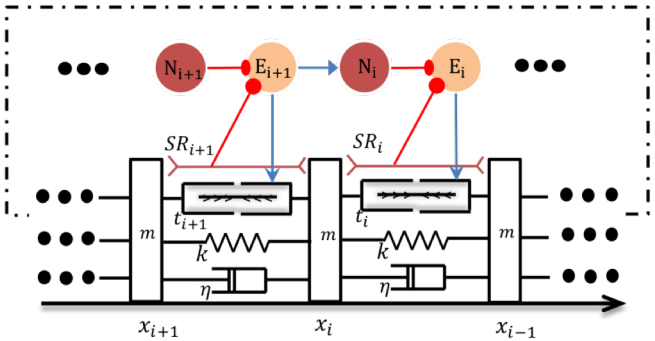
Fukumasu et al., in prep.

Dimensionality reduction of neural activities



Yoon et al., J. Neurogen. 2019

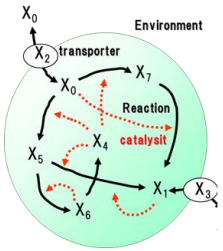
Neuromechanical model of larval locomotion

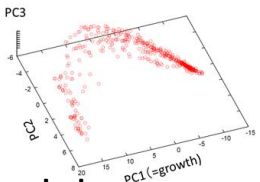


Sun et al., in prep.

Research direction (Furusawa group)

Extracting Universal features from complex biological systems (Universal Biology)

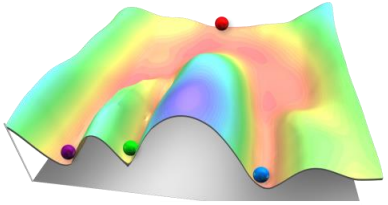


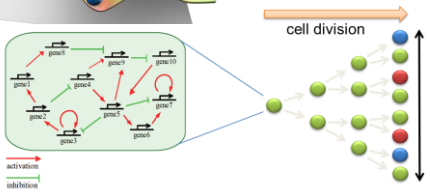


Theoretical model of adaptive evolution

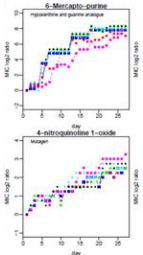
Theoretical analysis

"stemness" in development



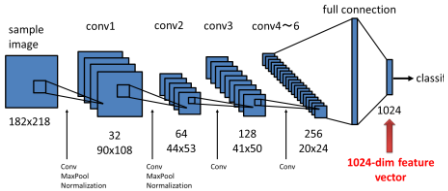


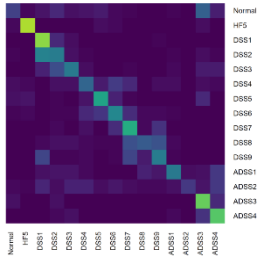
High-throughput experiments



Laboratory evolution of *E. coli*

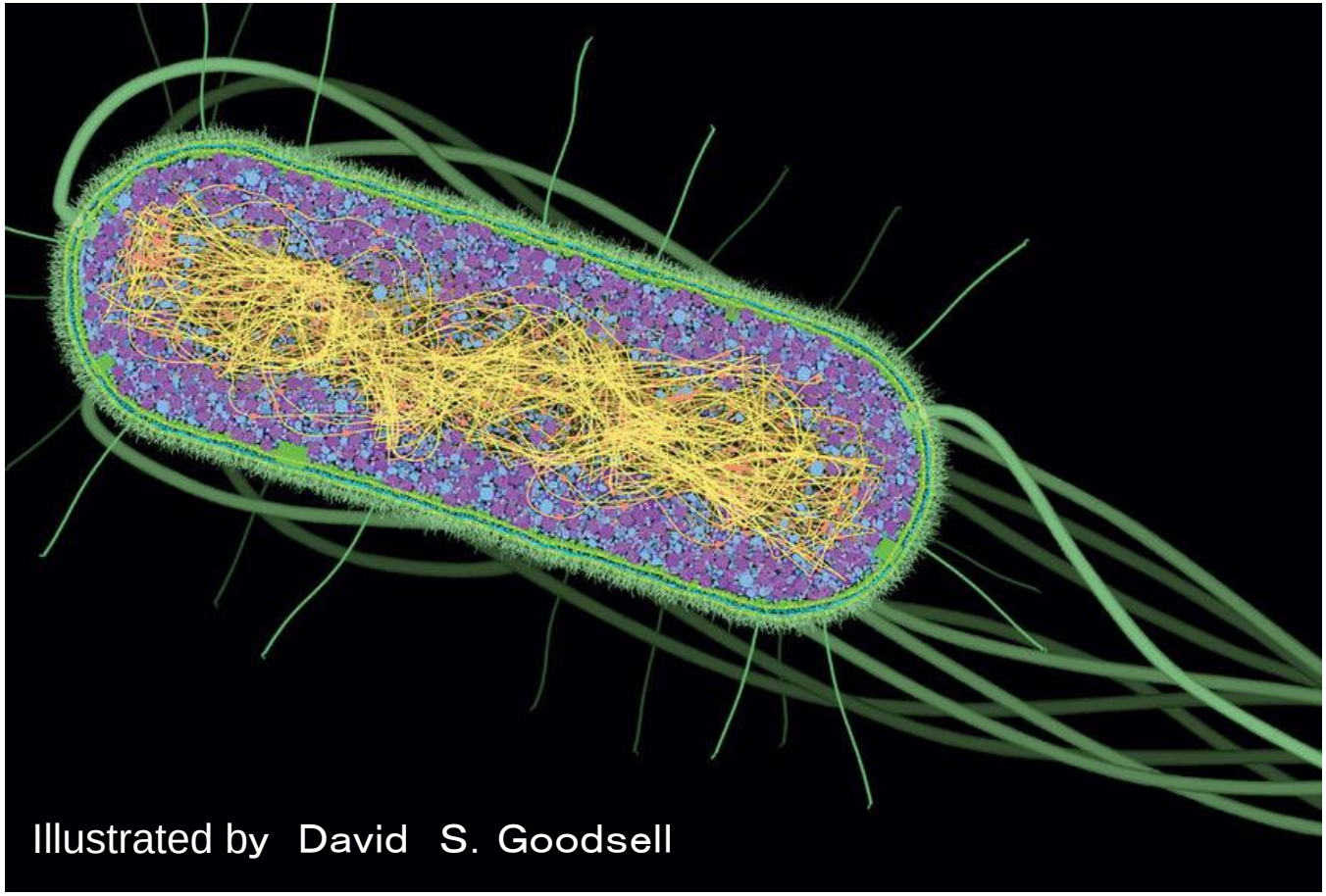
Data science





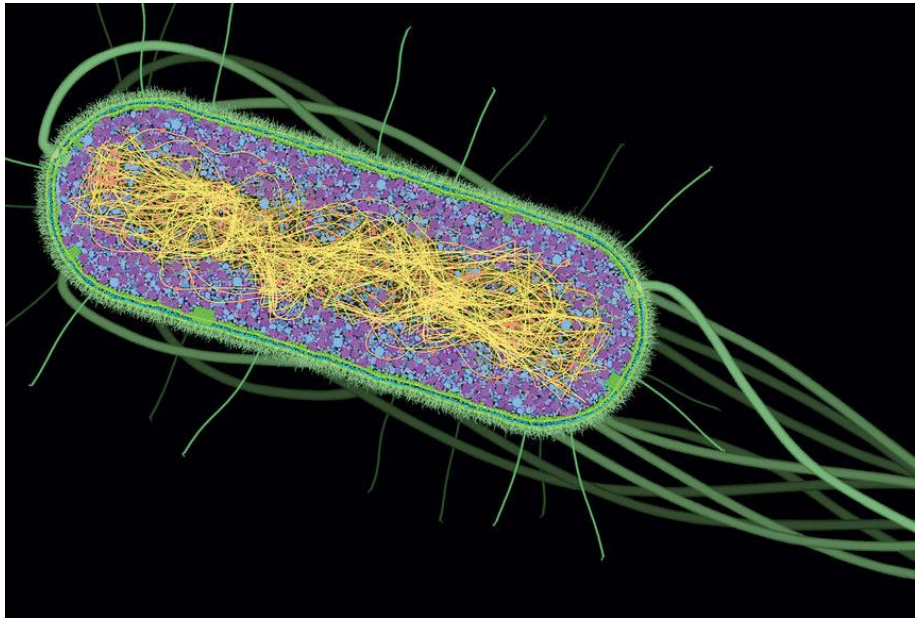
Decoding gut microbiota by neural networks

Adaptive Evolution of *E. coli*（大腸菌）



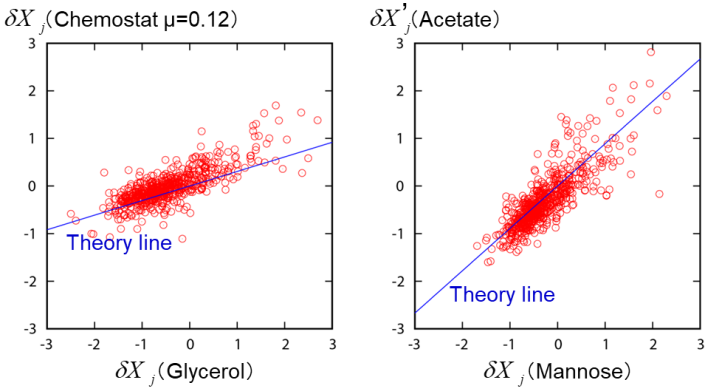
How many degrees of freedom are necessary to describe evolution of *E. coli*?

Constraints in Adaptive Evolution



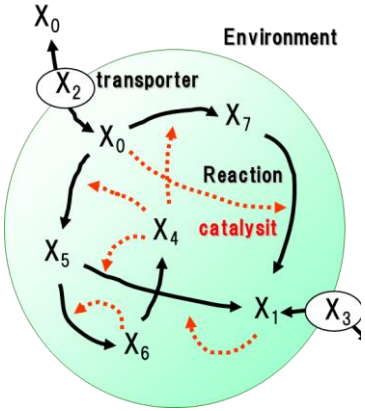
$$\delta X_j(E) = \delta\mu(E)\sum_i L_{ji}(1-\gamma_i/\alpha)$$

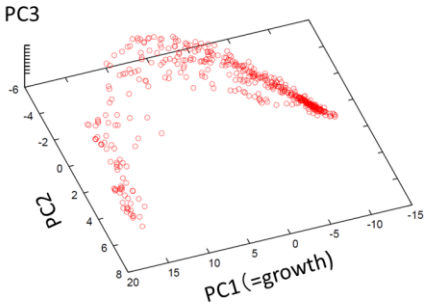
$$\boxed{\frac{\delta X'_j}{\delta X_j} = \frac{\delta\mu'}{\delta\mu}}$$



Theoretical studies suggested that:

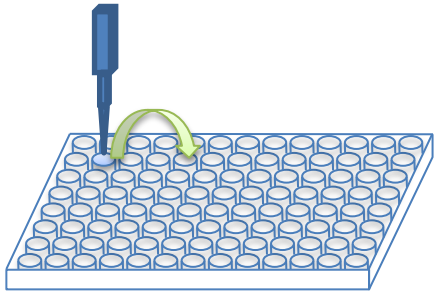
state of replicating cells are constrained on a low-dimensional dynamics.

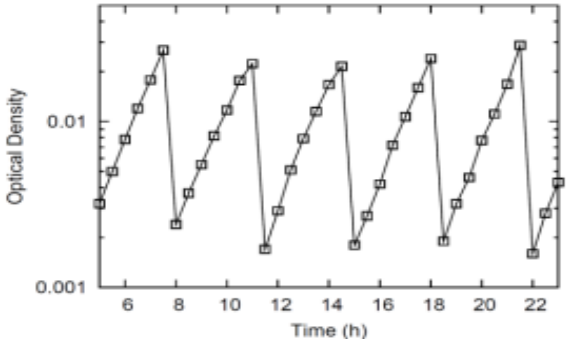




Kaneko, Furusawa, Yomo, **Phys. Rev. X** (2015)
Furusawa and Kaneko, **Jour. Roy Soc. Interface** (2015)
Furusawa and Kaneko, **Phys. Rev. E** (2018)

Automated System for Laboratory Evolution

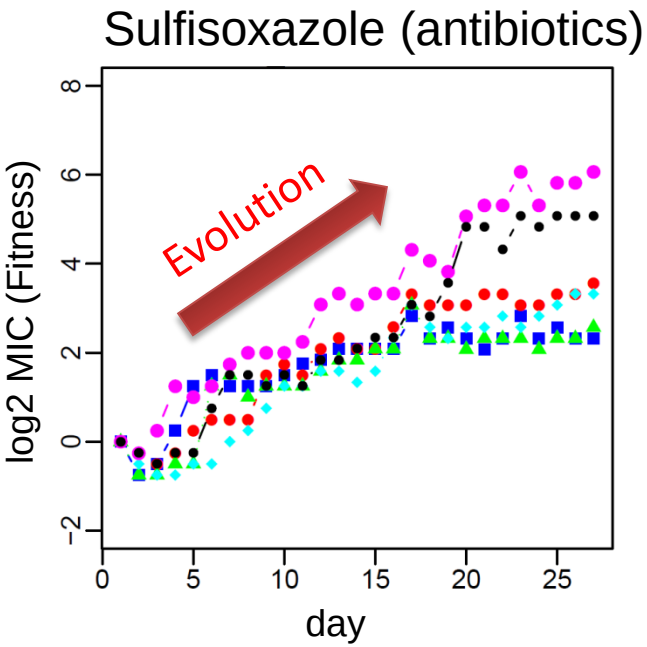


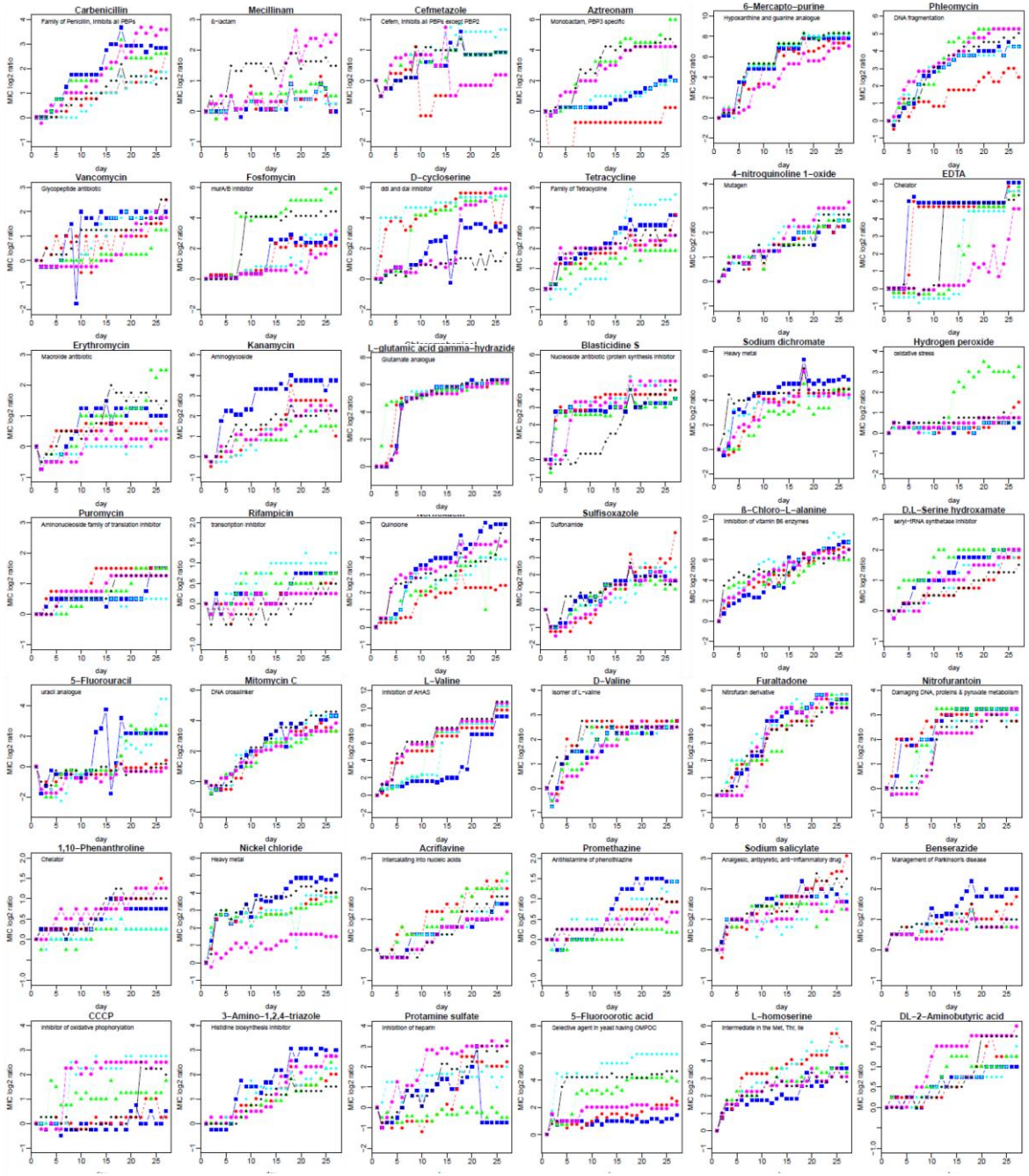


(x8 speed)

- ✓ About 16,000 independent cultures can be maintained automatically.

Horinouchi et al., **Jour. Lab. Automation** (2014)

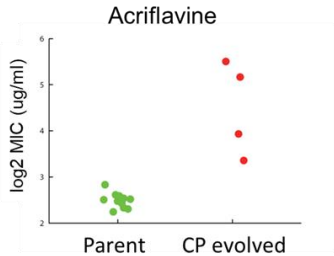




***E. coli* evolved to various stress conditions.**

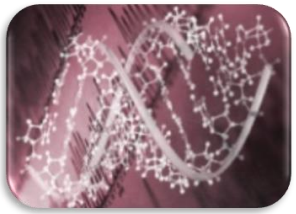
High-dimensional phenotype-genotype mapping

resistance/sensitivity

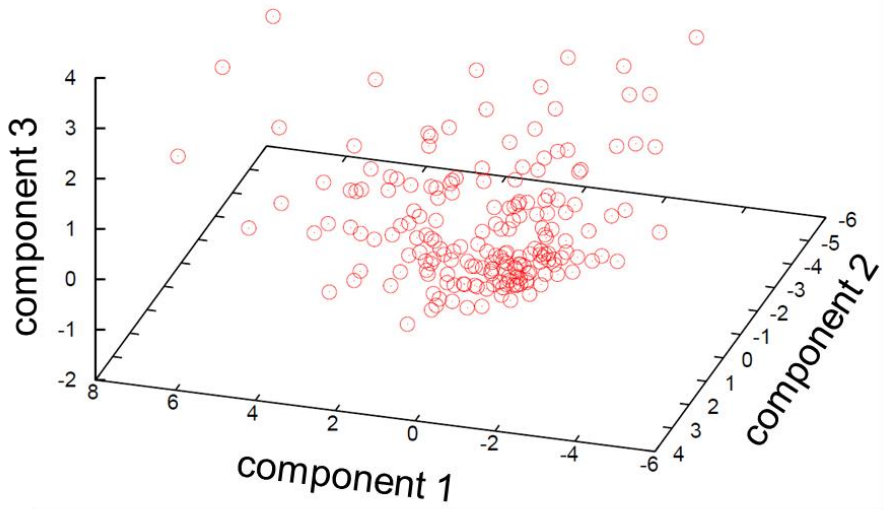


transcriptome data

genome sequence



Projection of transcriptome changes in a low-dimensional space



Extraction of "major axes" in phenotypic changes

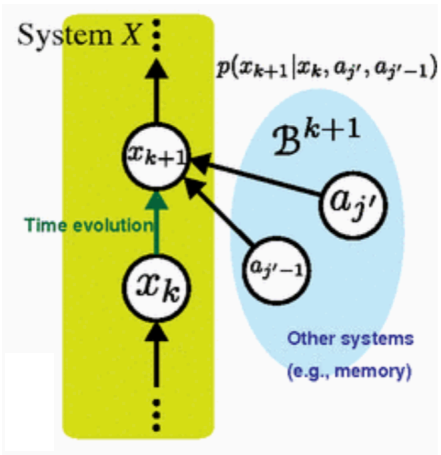
Prediction and control of evolutionary dynamics

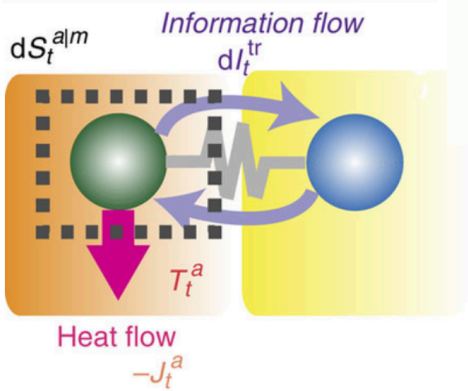
Suzuki et al., **Nature Comm.** (2014)
Horinouchi et al. , **BMC Evo. Biol.** (2015)
Furusawa, Horinouchi, Maeda, **Curr. Opin. Biotech.** (2018)

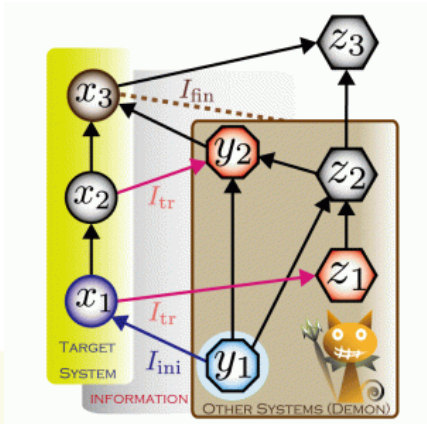
Research theme (Ito group)

To understand thermodynamic laws of information processing in biological systems (Universal Biology)

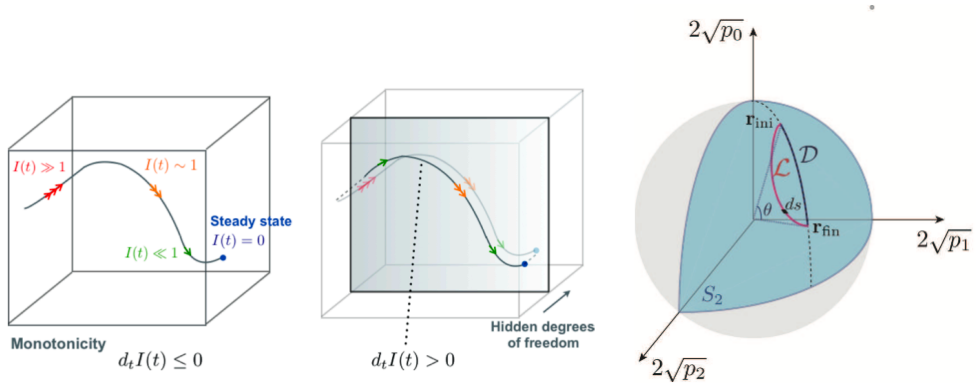
The second law of thermodynamics for subsystems with information flow



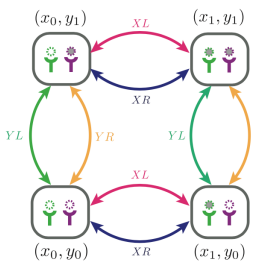


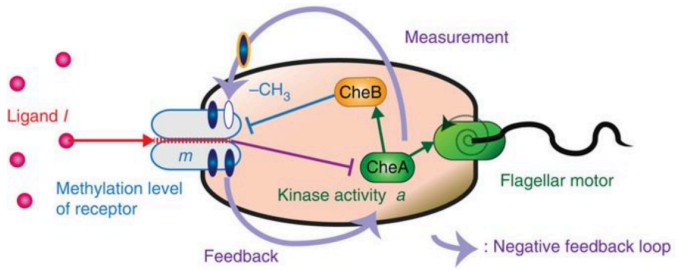


Thermodynamics of information geometry

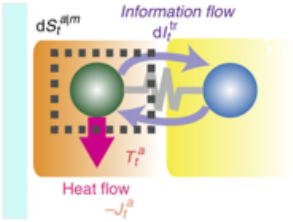


Thermodynamic cost of information processing in biological systems





Thermodynamic laws of information

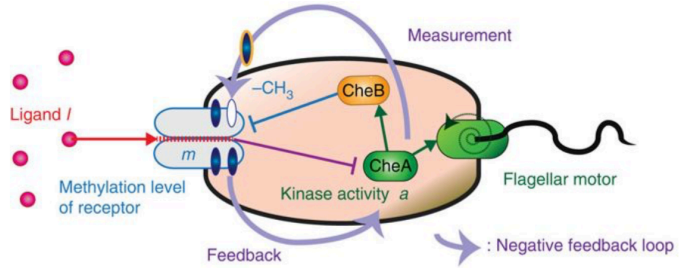


The second law of thermodynamics for subsystems with information flow

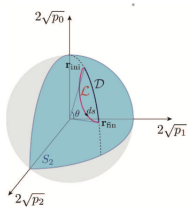
$$\sigma \geq \Theta_{\mathrm{info}}$$

Ito and Sagawa, Phys. Rev. Lett. (2013)
Shiraishi et. al, New J Phys. (2015)
Yamamoto et. al, Phys. Rev. E (2016)
Ito, Sci. Rep. (2016)

E. coli sensory adaptation



Ito and Sagawa, Nat. Commun. (2015)



Thermodynamics of information geometry

$$2\tau\mathcal{C} \geq \mathcal{D}^2$$

Ito, Phys. Rev Lett. (2018)
Ito, arXiv (2018)
Ito and Dechant, arXiv (2018)
Otsubo et al. arXiv (2020)

Uncertainty in biochemical reaction

Enzyme reaction

$$A + B \leftrightharpoons AB$$
$$A + X \leftrightharpoons AX$$
$$AX + B \leftrightharpoons AB + X$$

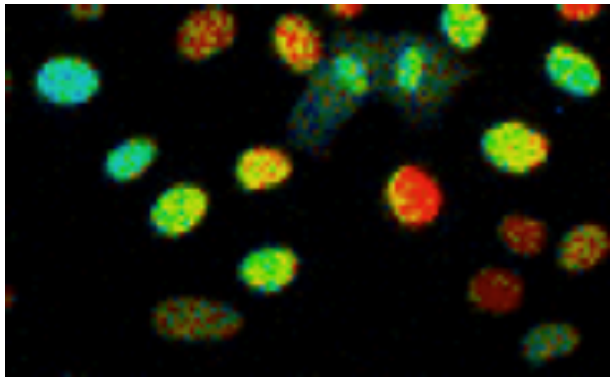
Autocatalytic reaction

$$X + Y \leftrightharpoons 2X$$

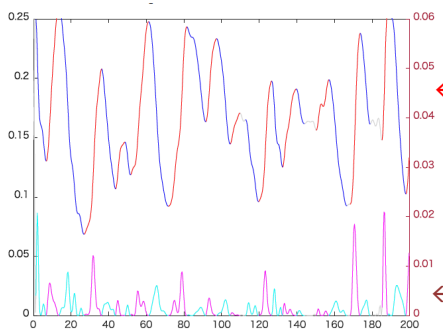
Ito, arXiv (2019)

Analysis of experimental data in biology

Information geometric analysis of ERK phosphorylation



ERK phosphorylation



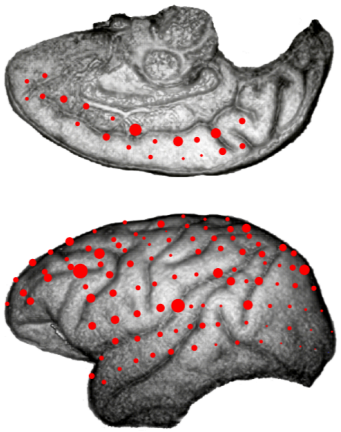
← ERK phosphorylation ratio

← Information geometric speed

Grant-in-Aid for Scientific Research on Innovative Areas

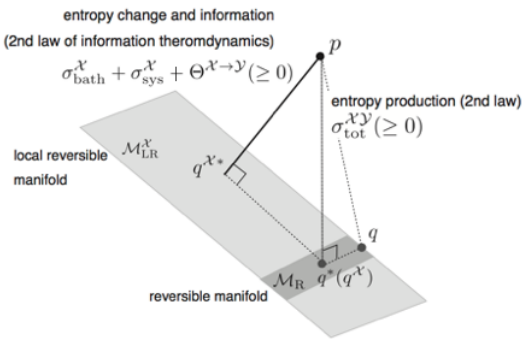
Information physics of living matters

Information thermodynamic analysis of neural data



The entropy production in monkey's brain

Information geometric method of estimating the entropy production



PRESTO sakigake

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