

3 4

9 10

	212	26	12

17 10 21 3

7  
1

3

23

11

2

6

3

6  
1

20	9	6
20	12	
21	2	
21	3	
21	3	
21	4	

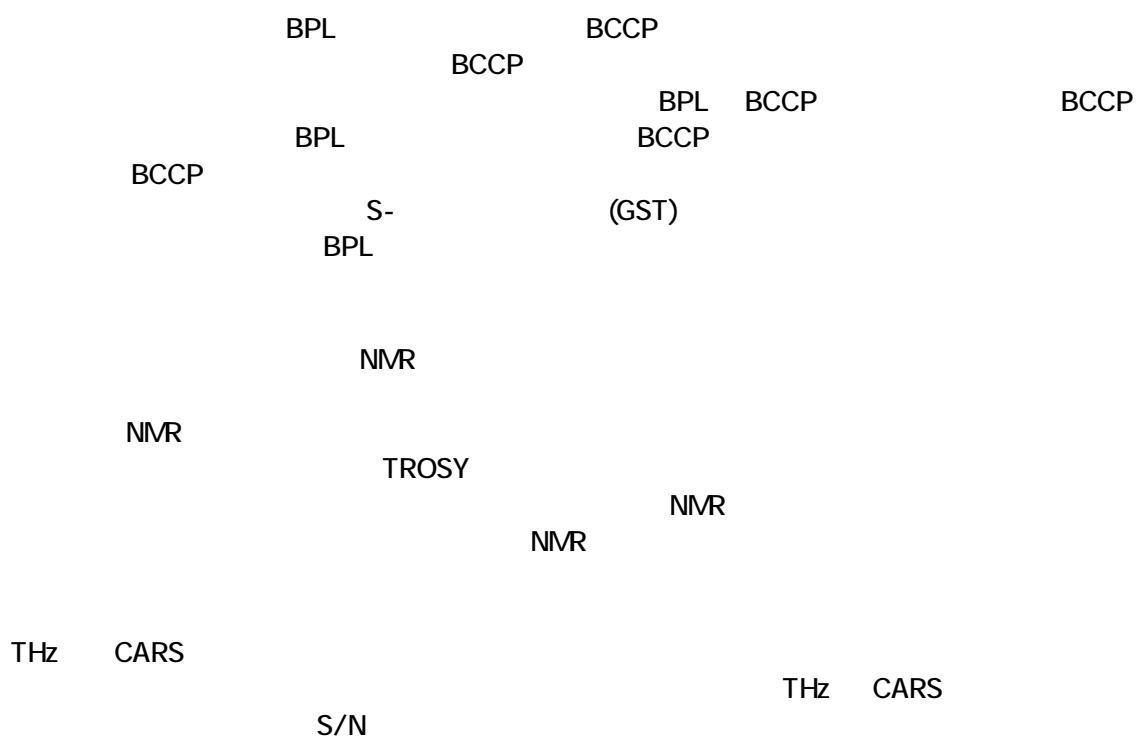
(KaiA, KaiB, KaiC) ATP  
X

CREB

in silico

SEM

in vivo



\*1

1 19 3

	0	50	50
	130	71	201
	23	3	26
	153	124	277

21 3

14	1	15

21 3

18 7 III Internatinal Conference on Small- angle Scattering  
SAS Young Scientist Prize  
19 12 19  
20 4 20

18 4 18 21

20 11 20

20 3 19

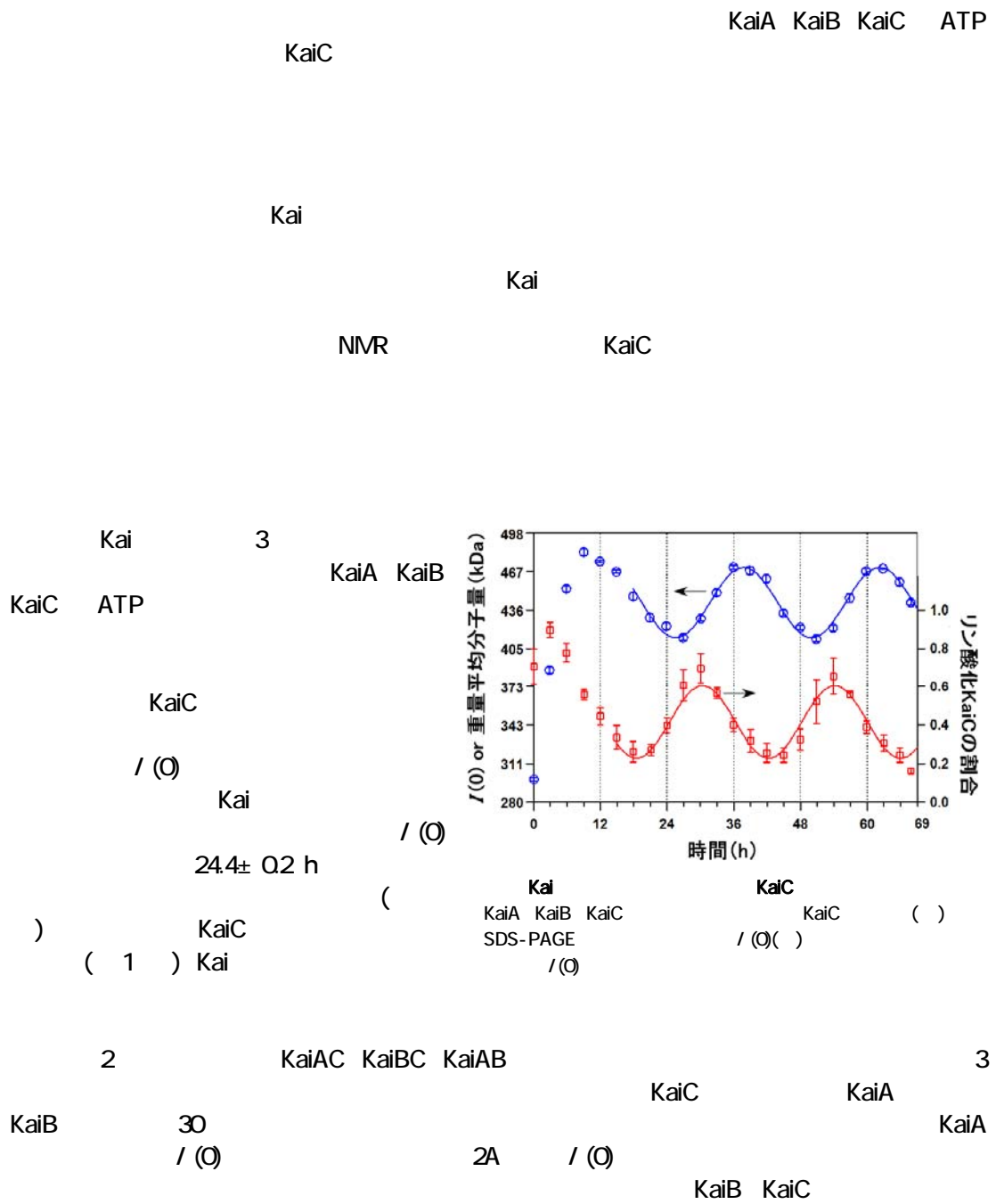
18 9 55

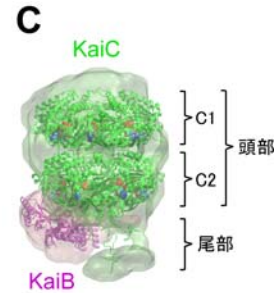
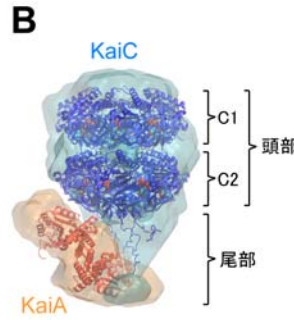
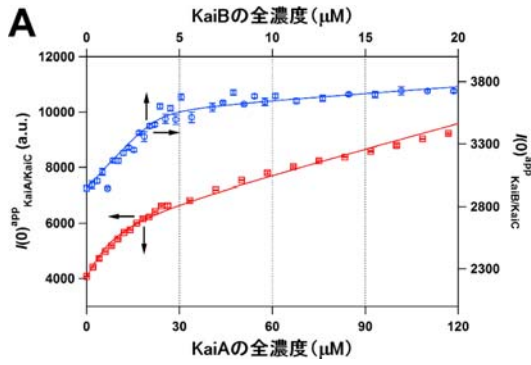
31

55

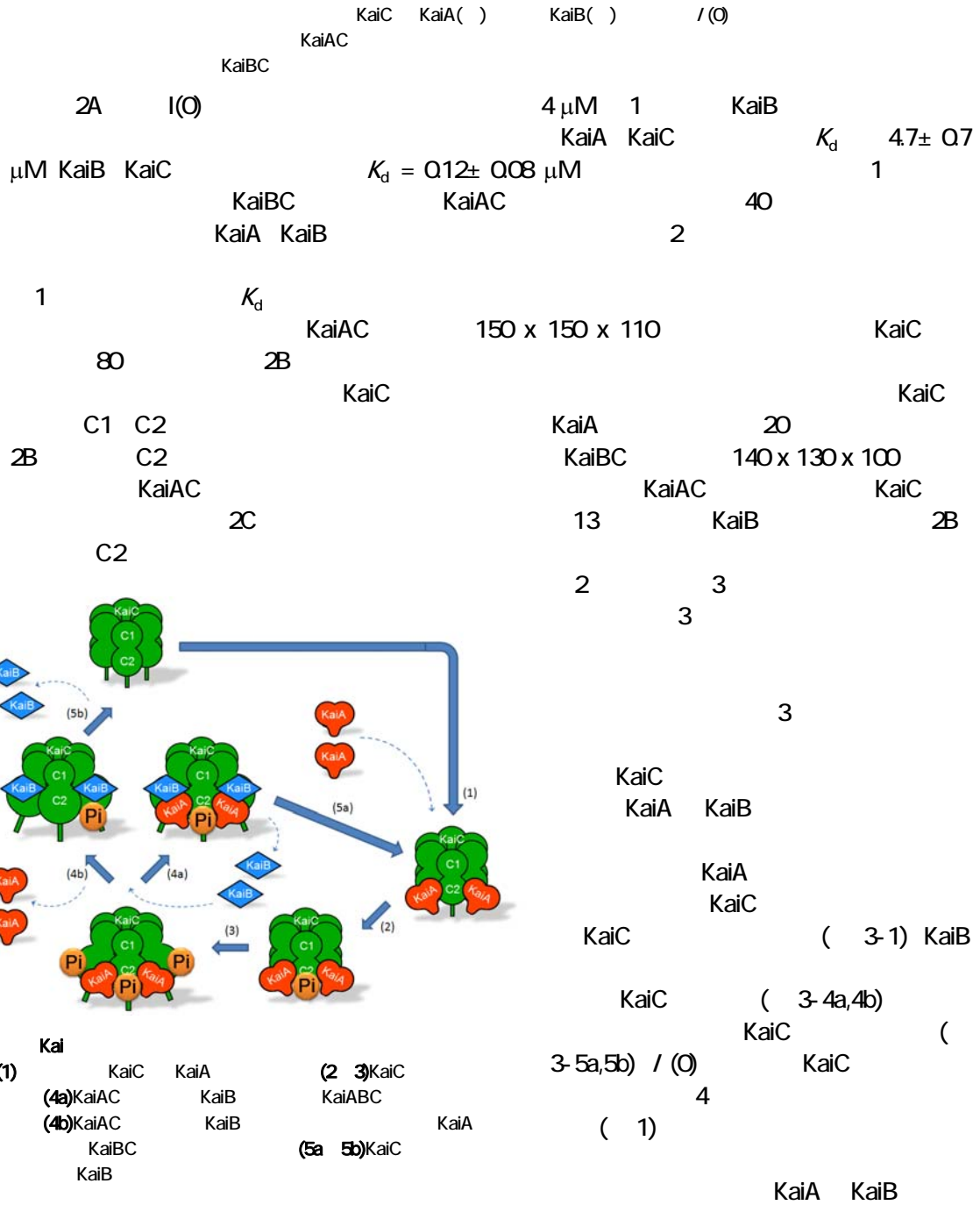
21 3

			( )
	in silico		
	in vivo	University College London Senior Research Fellow	
	NMR		
	THz CARS		



Kai



KaiB KaiAC ( 3-1) KaiC KaiB KaiA  
 3-4a KaiABC KaiA  
 3-4b KaiB KaiA KaiA KaiB 2B C KaiBC  
 KaiC KaiA KaiB 3-4b KaiA  
 KaiAC KaiABC KaiB C KaiA  
 KaiB ( 3-5a,5b) KaiBC KaiABC  
 / (O) Kai  
 / (O)  
 C KaiA KaiC KaiB KaiC 1 1 2B  
 2 1  
 KaiC X  
 mg/ml 1 mg/ml Kai  
 6  
 Spring-8 BL  
 XU Kai 4 X  
 3  
 (KaiA, KaiB, KaiC) ATP X

S. Yamada, S. Akiyama, H. Sugimoto, H. Kumita, K. Ito, T. Fujisawa, H. Nakamura and Y. Shiro, "The Signaling Pathway in Histidine Kinase and the Response Regulator Complex Revealed by X-ray Crystallography and Solution Scattering", *J. Mol. Biol.* 362, 123-139 (2006).

S. Akiyama, A. Nohara, K. Ito and Y. Maéda, "Assembly and Disassembly Dynamics of the Cyanobacterial Periodosome", *Molecular Cell*, 29, 703-714 (2008).

S. Akiyama, XIII International Conference on Small-angle Scattering, 2006 SAS Young Scientist Prize 2006 7 .

, 2007 12 .

, 2008 4 .

, " , " , 47, 133-138 (2007)

S. Akiyama, A. Nohara, K. Ito and Y. Maéda, "Real-time Small-angle X-ray Scattering Observation of Assembly and Disassembly Dynamics of Cyanobacterial Periodosome", *SPRING-8 Research Frontiers 2007*, 24-25, (2008).

" , " , 21, 305-312 (2008).

" , " , in press (2009)

S. Akiyama, K. Ito, Y. Maéda and T. Kondo, "Small-angle X-ray Scattering Studies on Assembling-Disassembling Complexes of Cyanobacterial Circadian Clock Proteins", Fifth East Asian Biophysics Symposium & Forty-Fourth Annual Meeting of the Biophysical Society of Japan (2006/11/12-16).

A. Nohara, K. Ito, Y. Maéda, T. Kondo and S. Akiyama, "Real-Time SAXS Observation of Assembling-Disassembling Cycles of Cyanobacterial Circadian Clock Proteins", Fifth East

Asian Biophysics Symposium & Forty-Fourth Annual Meeting of the Biophysical Society of Japan (2006/11/12-16).

S. Akiyama, A. Nohara, K. Ito and Y. Maéda, "Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Circadian Clock Proteins", The 67th Okazaki Conference (2007/11/10-12).

S. Akiyama, A. Nohara, K. Ito and Y. Maéda, "Assembly and Disassembly Dynamics of the Cyanobacterial Periodosome", IUCr2008 Satellite Meeting (2008/8/23-24).

S. Akiyama, "Assembly and Disassembly Dynamics of Cyanobacterial Clock Proteins", JGFoS (2008/10/31-11/2).

S. Akiyama, "Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Circadian Clock Proteins", (2007/6/23).

S. Akiyama, "Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Circadian Clock Proteins", (2007/12/23).

S. Akiyama, "Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Circadian Clock Proteins", (2008/6/27).

S. Akiyama, "Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Circadian Clock Proteins", (2008/11/8-9).

S. Akiyama, A. Nohara, K. Ito, Y. Maéda, T. Nishiwaki and T. Kondo, "Small-angle X-ray Scattering Study on Cyanobacterial Clock Proteins", (2008/12/3).

S. Akiyama, S. Yamada, H. Sugimoto, H. Kumita, K. Ito, T. Fujisawa, H. Nakamura and Y. Shiro, "Signal Transduction Pathway in Histidine Kinase and Response Regulator Complex Revealed by Joint Usage of Crystallography and Small-Angle X-ray Scattering", XIII International Conference on Small-angle Scattering (2006/7/11)

S. Akiyama, "Real-time SAXS Observation of Assembling-Disassembling Complexes of Cyanobacterial Circadian Clock Proteins", 9th International Conference on Biology and Synchrotron Radiation 2007/8/12-16.

S. Akiyama, "Real-time Small-Angle X-ray Scattering Study of Assembly and Disassembly Cycles of Cyanobacterial Circadian Clock Proteins", American Crystallographic Association 2008 Annual Meeting 2008/6/2.

S. Akiyama, A. Nohara, K. Ito and Y. Maéda, "Real-time SAXS Observation of Assembly

and Disassembly Dynamics of Cyanobacterial Clock Proteins", IUCr2008 (2008/8/27).

S. Akiyama, A. Nohara, K. Ito, Y. Maeda and T. Kondo, "Real-time SAXS Observation of Assembly and Disassembly Dynamics of Cyanobacterial Clock Proteins", NSRRC Workshop X-ray Crystallography / Spectroscopy (2008/10/9).

, " 45  
(2007/12/21).

, " X  
, ERL 2008/3/17 .

T. Uzawa C. Nishimura, S. Akiyama, K. Ishimori, S. Takahashi, H. J. Dyson and P. E. Wright, "Hierarchical folding mechanism of apomyoglobin revealed by ultra-fast H/D exchange coupled with 2D NMR", PNAS, 105, 13859-13864 (2008).

K. Inaba, M Suzuki, K. Maegawa, S. Akiyama, K. Ito and Y. Akiyama, "A pair of circularly permuted PDZ domains control RseP, the S2P family intramembrane protease of E. coli", J. Biol. Chem, 283, 35042-35052 (2008).

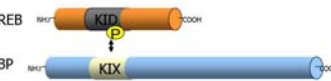
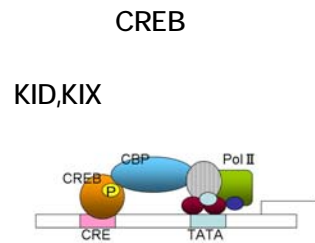
, " (2008/3/3).

21 , " (2008/8/2). ,

CREB  
 CREB cAMP response element binding protein

CBP CREB binding protein  
 CREB CBP  
 CREB

CREB  
 CREB  
 CRE

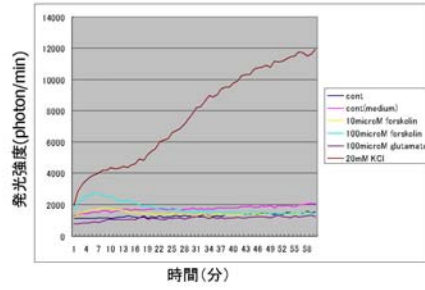


KIDがリン酸化され、KIXに結合し、転写を促進することが知られている。  
 シナプス可塑性、記憶形成に重要なことが報告されている。

C N  
 A, B  
 A, B  
 C N  
 KID KIX  
 N C KID KIX  
 KID KIX  
 KC

KID KIX  
 KID KIX  
 cAMP

KID KIX



KC

CREB

CRE



培養神経細胞に対してKCl刺激によって、発光強度が上昇した。

1

KID KIX

1

HEK293

N

HEK293

C

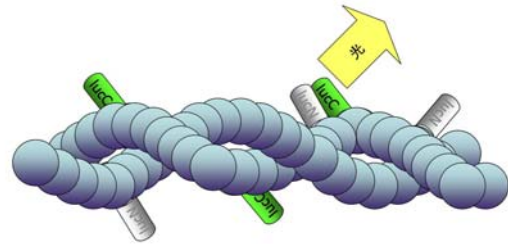
N

C

N

C

N

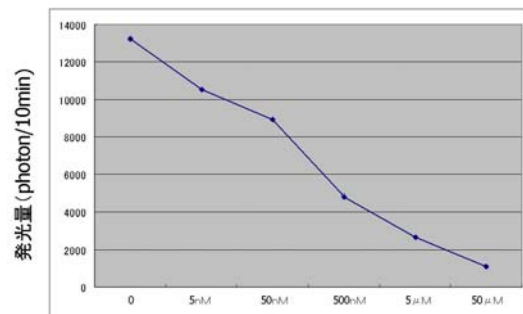


アクチン重合を計測するプローブ設計。

A

A

2



ラトランクリンA (アクチン重合阻害剤) 濃度

ラトランクリンA投与量依存的に発光強度が減少する。

IRES

HEK293

CREB

DNA

CREB

50

CREB

20 8 12

"

"  
2009/3/16-18

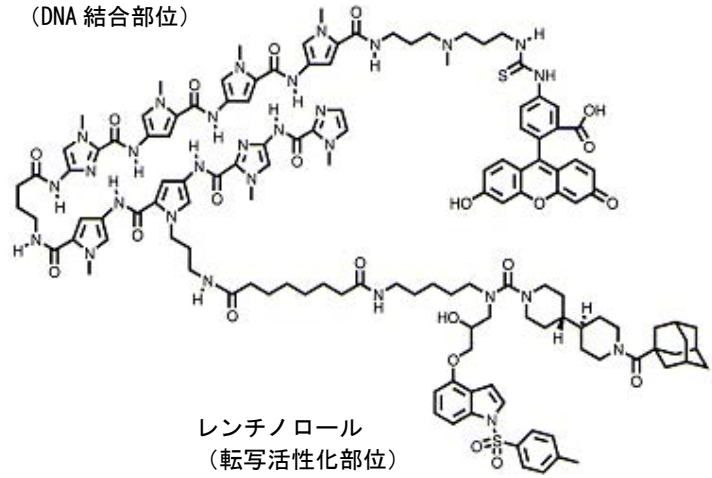
DNA

ヘアピンポリアミド分子  
(DNA 結合部位)

(JACS,  
DNA

126, 3461)

DNA



(JACS, 126 図1 小分子転写因子の化学構造。

15940)  
DNA  
Dervan  
5-TGACCAT

M  
5-TGACCAT

DNA

Sur-2 RNA

II

---

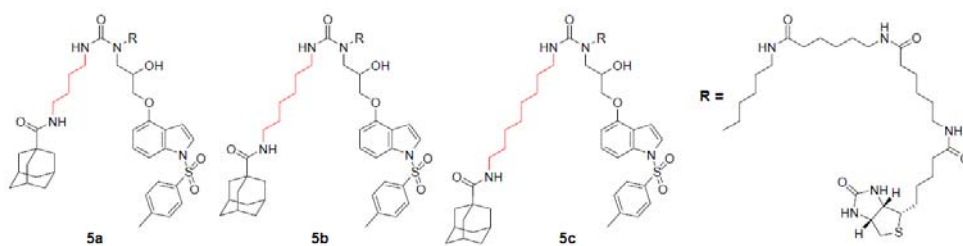
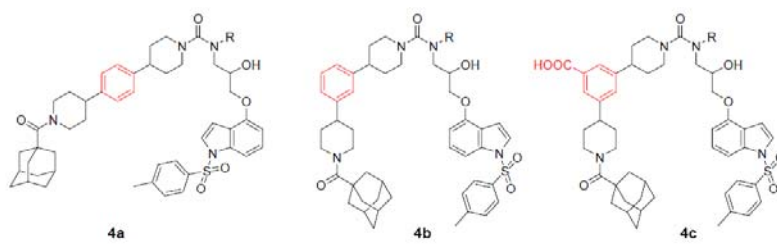
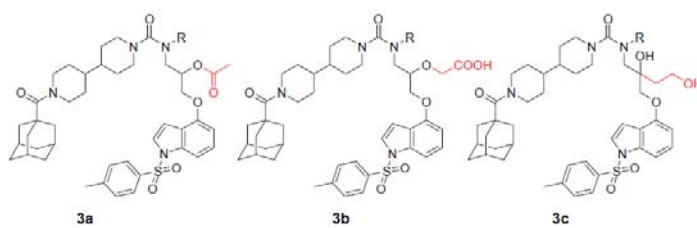
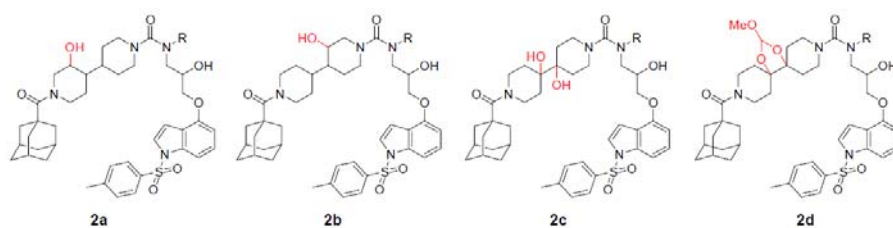
ESX

Sur2

2a 3c  
(4a)

(4b, 4c)

(5a 5c)



---

*in vitro*

Gal 4 SA plasmid  
reporter plasmid

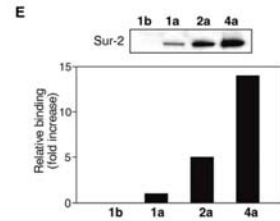
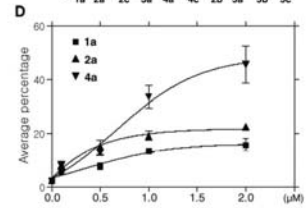
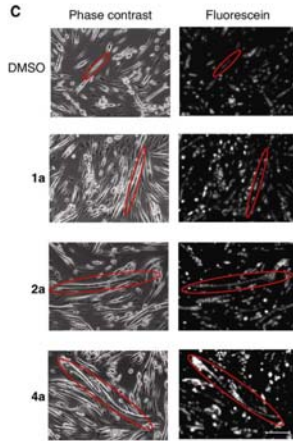
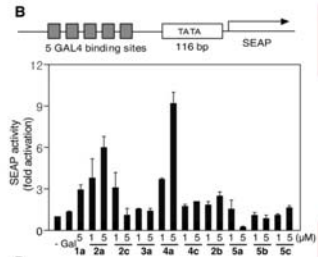
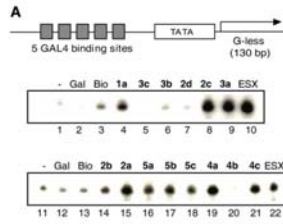
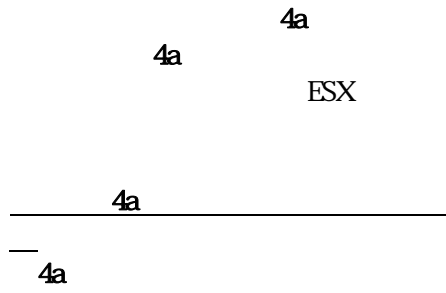
SEAP

*in vitro*

RI

mRNA

9



4a  
ESX  
MyoD  
4a

MyoD DNA  
(MyoD-SA)

C2C12  
2% horse serum

4a 1μ M

MyoD-SA

Hoechst 33342  
( )  
4a  
4a Sur2 ( ) 4a  
Sur2 4a Sur2  
4a 0.32 μM 4a 0.86 μM 2.6  
Sur2

Choi, Y., Shimogawa, H., Murakami, K., Ramdas, L., Zhang, W, Qin, J., Uesugi, M Chemical genetic identification of the IGF-linked pathway that is mediated by STAT6 and MFP2 *Chem Biol.* 13, 241- 249 (2006).

Sato, S., Kwon, Y., Kamisuki, S., Srivastava, N., Mao, Q., Kawazoe, Y., Uesugi, M Polyproline-rod approach to isolating protein targets of bioactive small molecules: Isolation of a new target of indomethacin. *J. Am. Chem. Soc.* 129(4), 873- 880 (2007).

Jung, D., Shimogawa, H., Kwon, Y., Mao, Q., Sato, S., Kamisuki, S., Kigoshi, H., Uesugi, M. Wrenchnolol Derivative Optimized for Gene Activation in Cells. *J. Am. Chem. Soc.* 131, 4774-4782 (2009).

2008 6 18

18 4

21

Jung, D., Choi Y., Uesugi, M Small organic molecules that modulate gene transcription. *Drug Discovery Today* 11, 452- 457 (2006).

(2007).

Vol.45, No.4

(2007).

58(5) 58- 66

5- 6 (2008).

Medical Science Digest 34(1)

Uesugi, M "Chemical biology of gene expression and cell differentiation." 6th Australian Peptide Conference. 2005, 10.

Uesugi, M "Chemical biology of gene expression and cell differentiation." ICOB-5 & ISCNP-25 IUPAC, International Conference on Biodiversity and Natural Products. 2006, 7.

Uesugi, M "Chemical Biology of Gene Expression." 2006 Japanese-American Kavli Frontiers of Science Symposium 2006, 12.

Uesugi, M "Chemical biology of gene expression." 19<sup>th</sup> FAOBMB Seoul Conference. 2007, 5.

Uesugi, M "Isolating and identifying the targets of bioactive small molecules." The 22<sup>nd</sup> Naito Conference on Chemical Biology. 2008, 9.

Henderson, Y. C., Frederick, M J., Jayakumar, A., Choi, Y., Kang, Y., Spring, P. M, Uesugi, M, and Clayman, G. L. Human LBP- 32/MGR is a repressor of the P450sc in human choriocarcinoma cell line JEG- 3. Placenta. 28, 152- 160 (2006).

in silico

3

S/N

1

Simulated-Annealing Neural-Network

1

Simulated-Annealing

Simulated-Annealing

Back-projection

( )

(

) Back-projection

P( L)

-secretase

( A)

TRPC3

15

3

B

14

TRIC

C

TRP-M2

D

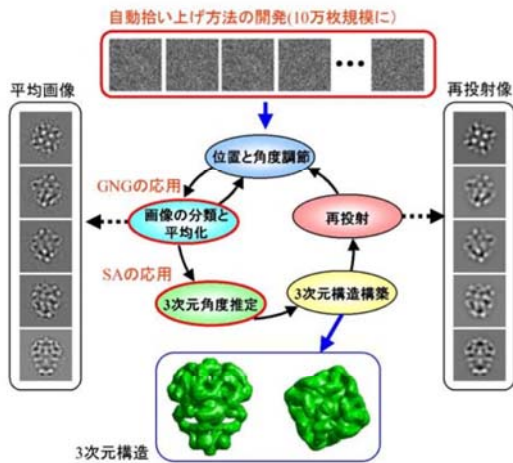


図1 単粒子構造解析の概要、数千から数万枚のタンパク質電顕画像から100クラス程度の平均画像を求め、これから3次元構造を解析する。

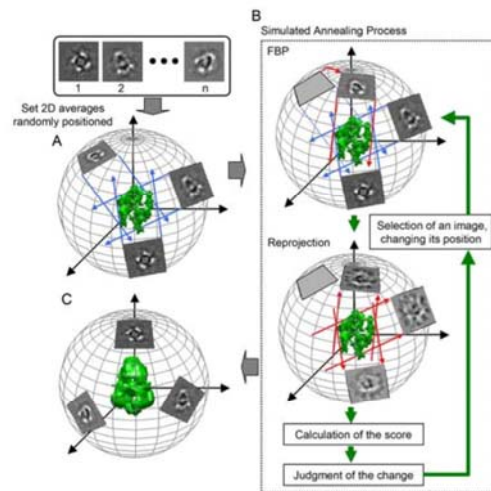


図2 Simulated-Annealingを応用した3次元角度推定方法の概要、数十枚の平均画像のオイラー角を自動的に決定する。

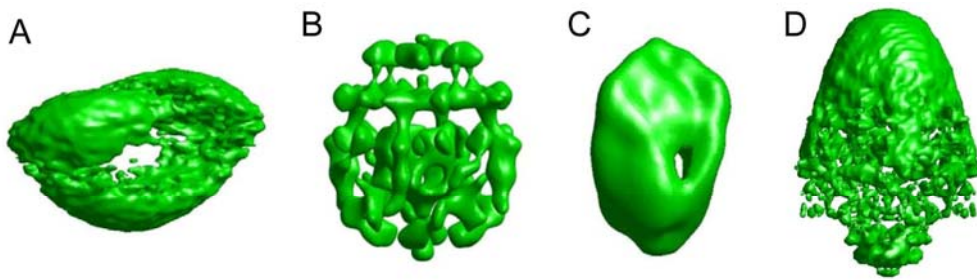


図3 本手法を用いた様々な膜タンパク質の単粒子構造解析の結果、A:アルツハイマー病関連膜タンパク質γセクレターゼの構造、B: TRPC3チャンネルの構造、C:筋細胞の細胞内小胞に存在するTRICチャンネルの構造、D:TRP-M2チャンネルの3次元構造。

S/N

SEM

SEM

eV

(ISEC) ISEC SEM

1nm

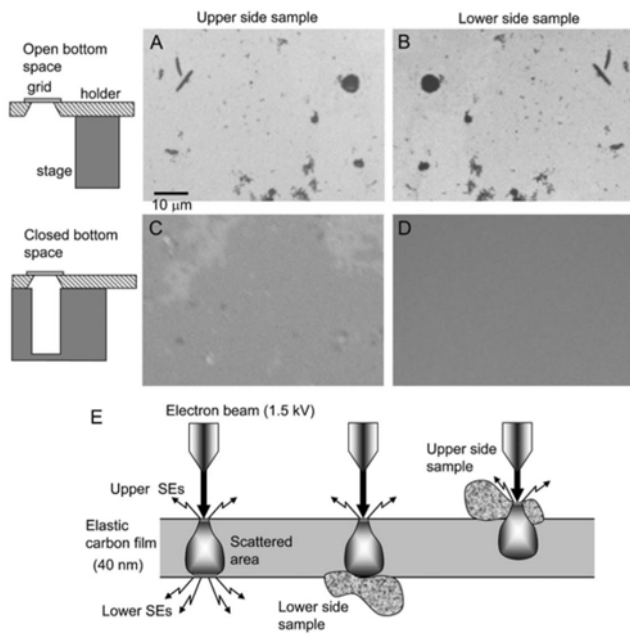


図4 SEMによるカーボン薄膜の非染色バクテリアサンプルの観察画像とISEC条件の概要。A:片持ち状態のカーボン薄膜上の画像。B:カーボン薄膜下の画像。C:アルミチューブ上にカーボン薄膜を設置し、薄膜上にサンプルを固定した時の画像、サンプルのコントラストが消失。D:アルミチューブ上でカーボン膜下のサンプル画像、完全にコントラストが消失。これらの画像は、40nmカーボン膜を用いて1.5kV加速条件で観察した。E:ISEC条件の概念図、40nmカーボン膜に1.5kVの電子線を照射すると、膜内を電子が散乱しカーボン膜内に吸収される。しかし、下部表層に到達した電子により下面より二次電子が放出される。カーボン膜下のサンプルは、この2次電子を吸収することで高いコントラストが生じる。

3 SEM

3

8

80

SEM SEM

2

ISEC

1nm

3

SEM

Toshihiko Ogura and Chikara Sato, "A fully automatic 3D reconstruction method using simulated annealing enables accurate posterioric angular assignment of protein projections", *J. Struct. Biol.*, Vol.156, p371- 386 (2006)

Yusuke Maruyama, Toshihiko Ogura, Kazuhiro Mo, Shigeki Kiyonaka, Kenta Kato, Yasuo Mouri and Chikara Sato, "Three-dimensional reconstruction using transmission electron microscopy reveals a swollen, Bell-shaped structure of transient receptor potential melastatin type2 cation channel", *J. Biol. Chem.*, Vol.282, p36961- 36970 (2007)

Kazuhiro Mo, Toshihiko Ogura, Muneyo Mo, Hiroyasu Shimizu, Tzyh-Chang Hwang, Chikara Sato and Yoshiro Sohma, "Three-dimensional reconstruction of human cystic fibrosis transmembrane regulator chloride channel revealed an ellipsoidal structure with orifices beneath the putative transmembrane domain", *J. Biol. Chem.*, Vol.283, p30300- 30310 (2008)

Toshihiko Ogura, "A high contrast method of unstained biological samples under a thin carbon film by scanning electron microscopy", *Biochem Biophys. Res. Commun.*, Vol.377, p79- 84 (2008)

Toshihiko Ogura, "Analyzing indirect secondary electron contrast of unstained bacteriophage T4 based on SEM images and Monte Carlo simulations", *Biochem Biophys. Res. Commun.*, 308, p 254- 259 (2009)

2008 4 25

2008 10 17

2008 12 22

2009 2 16

1

"

"

Vol.25, No.3 p236-241 2006

"

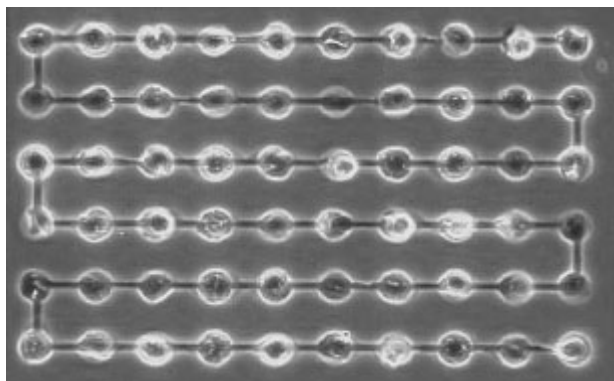
3

"

2007/6/16-17

( )

( 1)

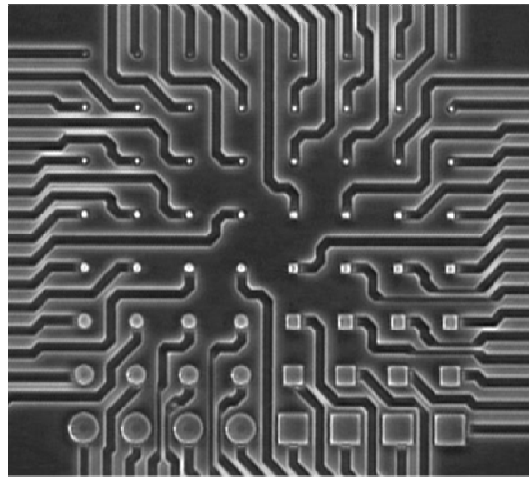


1 60

1

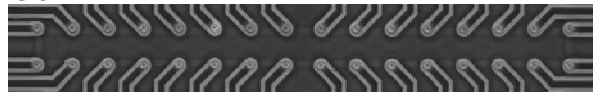
電極サイズ

( $\mu\text{m}$ )

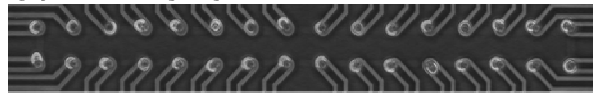


2

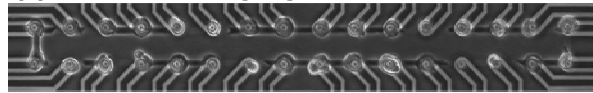
(a) MEA with AMC



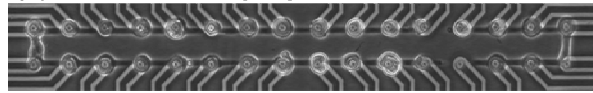
(b) Cardiomyocytes on MEA



(c) 1 DIV cardiomyocytes



(d) 2 DIV cardiomyocyte network



100  $\mu\text{m}$

20  $\mu\text{m}$

2 4 6 8 10

15 20 30  $\mu\text{m}$

( 2 )

(a)

(b)

(c)

(d)

10  $\mu\text{m}$

10  $\mu\text{m}$

( 3a )

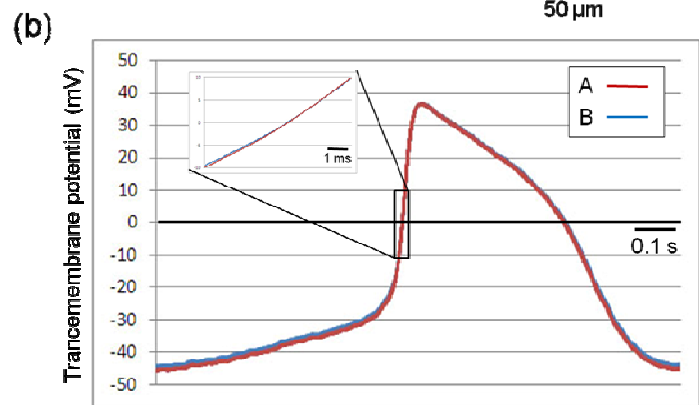
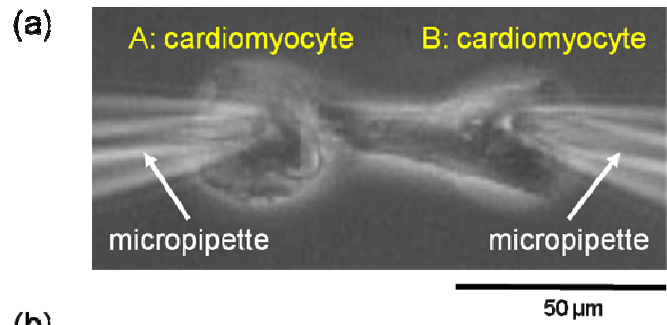
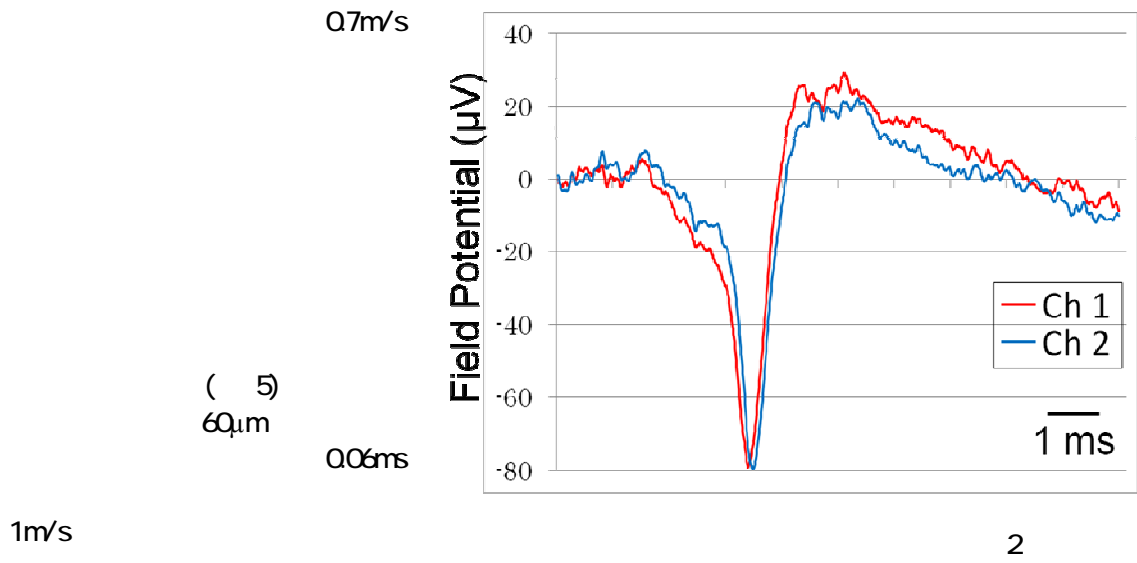
( 3b )

( 3d )

( 4 )

50  $\mu\text{m}$

0.07ms



(a) 2

(b) 2

( )

( )

K. Kojima, T. Kaneko and K. Yasuda, "Role of the community effect of cardiomyocyte in the entrainment and reestablishment of stable beating rhythms," *Biochem Biophys. Res. Commun.*, Vol. 351, pp. 209-215 (2006)

T. Kaneko, K. Kojima and K. Yasuda, "Dependence of the community effect of cultured cardiomyocytes on the cell network pattern," *Biochem Biophys. Res. Commun.*, Vol. 356, pp. 494- 498 (2007)

T. Kaneko, K. Kojima and K. Yasuda, "An on-chip cardiomyocyte cell network assay for stable drug screening regarding community effect of cell network size," *Analyst*, Vol. 132, pp. 892- 898 (2007)

T. Kaneko, K. Kojima and K. Yasuda, "Study of community effects of the cardiomyocytes with an agarose microchamber system," In "Frontiers in Life Sciences," Edited by Makoto Fujiwara, Shoichi Ishiura, and Naoki Sato, *Research Signpost*, pp.27- 38 (2006)

( )

Tomoyuki Kaneko, Kensuke Kojima, Ikuro Suzuki, Yoshihiro Sugio, and Kenji Yasuda, "Individual-cell-based measurement of the field potential in a cardiomyocyte by multi-electrode array with agarose microchambers," *Fifth East Asian Biophysics Symposium & Forty-Fourth Annual Meeting of the Biophysical Society of Japan*, 2006

Tomoyuki Kaneko, Kensuke Kojima, Ikuro Suzuki, Yoshihiro Sugio, and Kenji Yasuda, "Measurement of the field potential in individual cardiomyocytes by multi-electrode array with agarose microchambers," *46th Annual Meeting of the American Society for Cell Biology*, 2006

Tomoyuki Kaneko, Ikuro Suzuki, Kentaro Ando, Fumimasa Nomura, Tetsuo Kitamura, Jyunko Hayashi and Kenji Yasuda, "Delay Time of the Action Potential in Two Cardiomyocytes Connected by Fibroblasts," *47th Annual Meeting of the American Society for Cell Biology*, 2007

Tomoyuki Kaneko, Fumimasa Nomura, Yuki Tomoe, Ikuro Suzuki, Jyunko Hayashi and Kenji Yasuda, "Single-Cell Level Measurement of Conduction Velocity in Cardiomyocytes Network," *48th Annual Meeting of the American Society for Cell Biology*, 2008

( )

" 46  
2008

" 43  
2005 11 24

2007.4.20  
2007-111322

2007.6.8  
2007-152692

2007.6.8  
2007-152696

2007.6.8  
2007-152711

*in vivo*

*in vivo*

2

2

*in vivo*

2

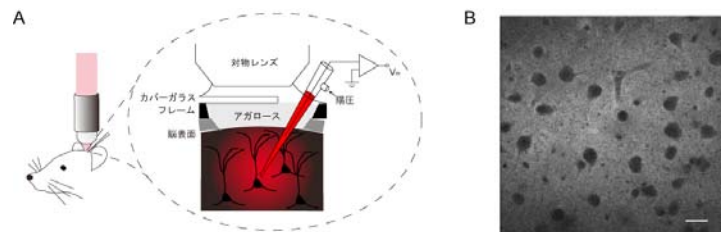
蛍光標識を必要としない単一ニューロンの可視化とシャドウパッチング法

2

2

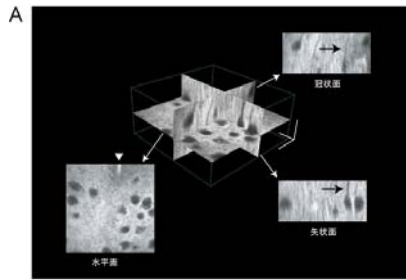
Alexa dye

2

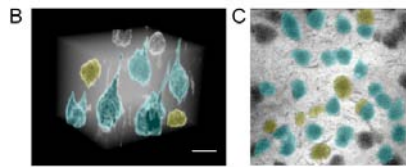


標識されていないニューロンを動物個体脳内で可視化する

2  
 150-200  $\mu\text{m}$       2 3      200-300  $\mu\text{m}$   
 10  $\mu\text{m}$       20  $\mu\text{m}$   
 87%      56%      100%



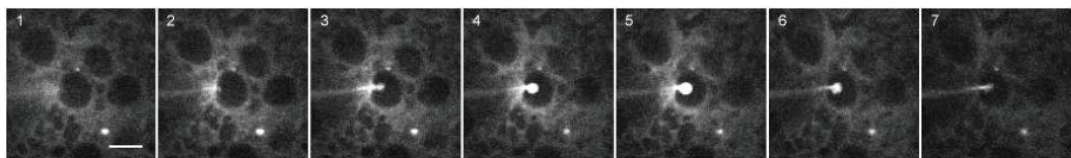
個体脳内におけるニューロンの可視化と同定 (A)



(B)

(C)  
 20  $\mu\text{m}$ .

3



シャドウパッチング法  
 $\mu\text{m}$  Alexa 594  
 2, 3      2.5      1      20  
 6      4, 5      7

4B

2 3

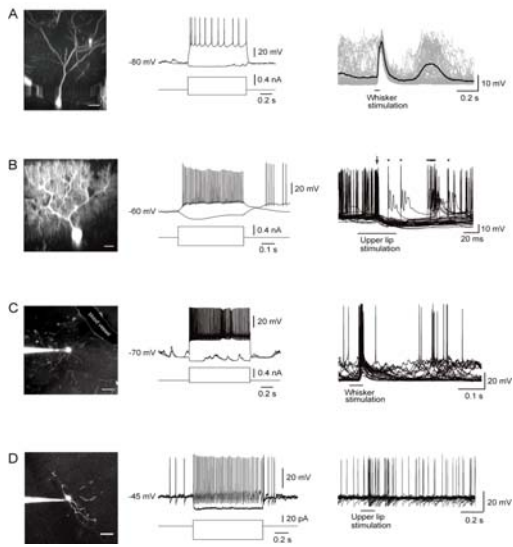
4A  
 4C, D

2

2  
 70%

20 M $\Omega$   
 30

2

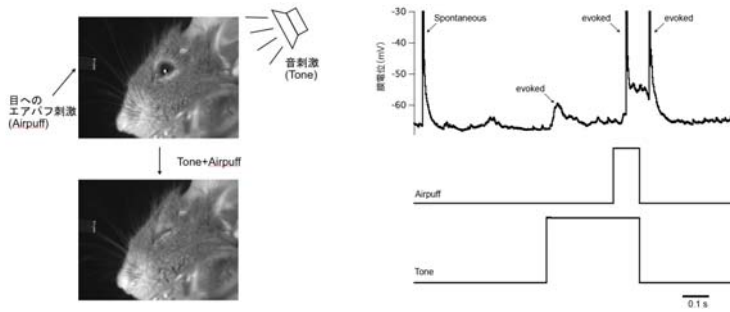


マウス個体脳における大脳皮質、小脳皮質ニューロンのシャドウパッチング  
2  
20 μm

A  
2 3 B  
C 2 3  
D

覚醒個体脳内における単一ニューロンからの選択的ホールセル記録

2



2

Kitamura, K., Judkewitz, B., Kano, M, Denk, W & Häusser, M: Targeted patch-clamp recordings and single-cell electroporation of unlabeled neurons in vivo. *Nature Methods*, 5, 61- 67 (2008).

2008 11

\_\_\_\_\_ In vivo 2 . Vol.26, No.12  
157-164 (2008).

\_\_\_\_\_ 2  
2009 2 .

Kitamura, K., Denk, W. & Häusser, M Targeted patch-clamp recordings from unlabeled neurons in the mammalian brain, *Neuroscience* 2006

\_\_\_\_\_ Targeted patch-clamp recordings from unlabelled neurons in vivo.  
2006

Kitamura, K.: Targeted whole-cell recordings from unlabeled neurons in vivo. NIPS-JST

2008.4.18

\_\_\_\_\_ in vivo 2  
2007.8.22

\_\_\_\_\_ 2  
31 2008.7.11

\_\_\_\_\_ in vivo  
2008.10.2

\_\_\_\_\_ 2009.2.13

Judkewitz, B., Rizzi, M, Kitamura, K. & Häusser, M: Targeted in vivo single- cell electroporation. Nature Protocols, in press.

Schultz, S. R., Kitamura, K., Post-Uiterweer, Krupic, J. & Häusser, M: Spatial pattern coding of sensory information by climbing- fiber evoked calcium signals in cerebellar Purkinje cell dendrites. Journal of Neuroscience, in press.

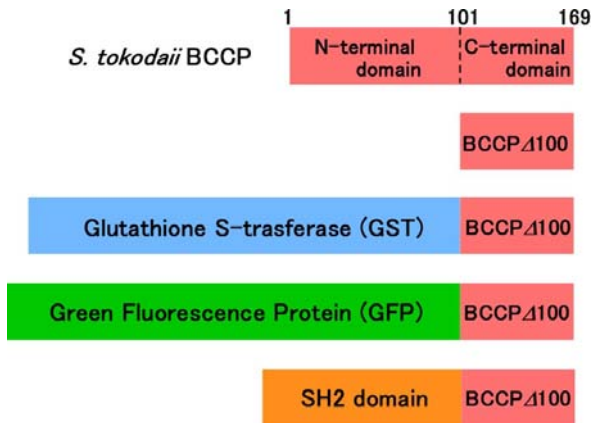
Y. Kawamura, H. Nakayama, K. Kitamura, M Kano Developmental changes in climbing fiber responses of cerebellar Purkinje cells revealed by whole- cell patch-clamp recordings in vivo. Neuroscience 2008.

\_\_\_\_\_ in vivo  
2008.7.9.

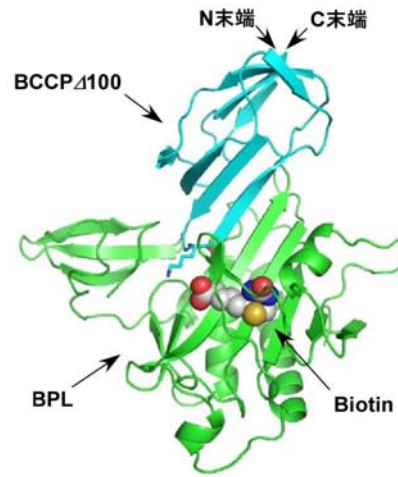
BPL BCCP  
*Sulfolobus tokodaii*  
 BPL BCCP Holo BCCP  
*S. tokodaii* BPL Holo BCCP  
BCCP  
BPL

---

*S. tokodaii*  
*S. tokodaii* BPL BCCP  
 BCCP N C C  
*S. tokodaii* BCCP 169  
100 N N  
 100 BCCP BCCP 100 BPL  
N 100 BPL  
 BPL BCCP SPR  
BPL Holo BCCP 100 K<sub>d</sub> 1.2 (nM)

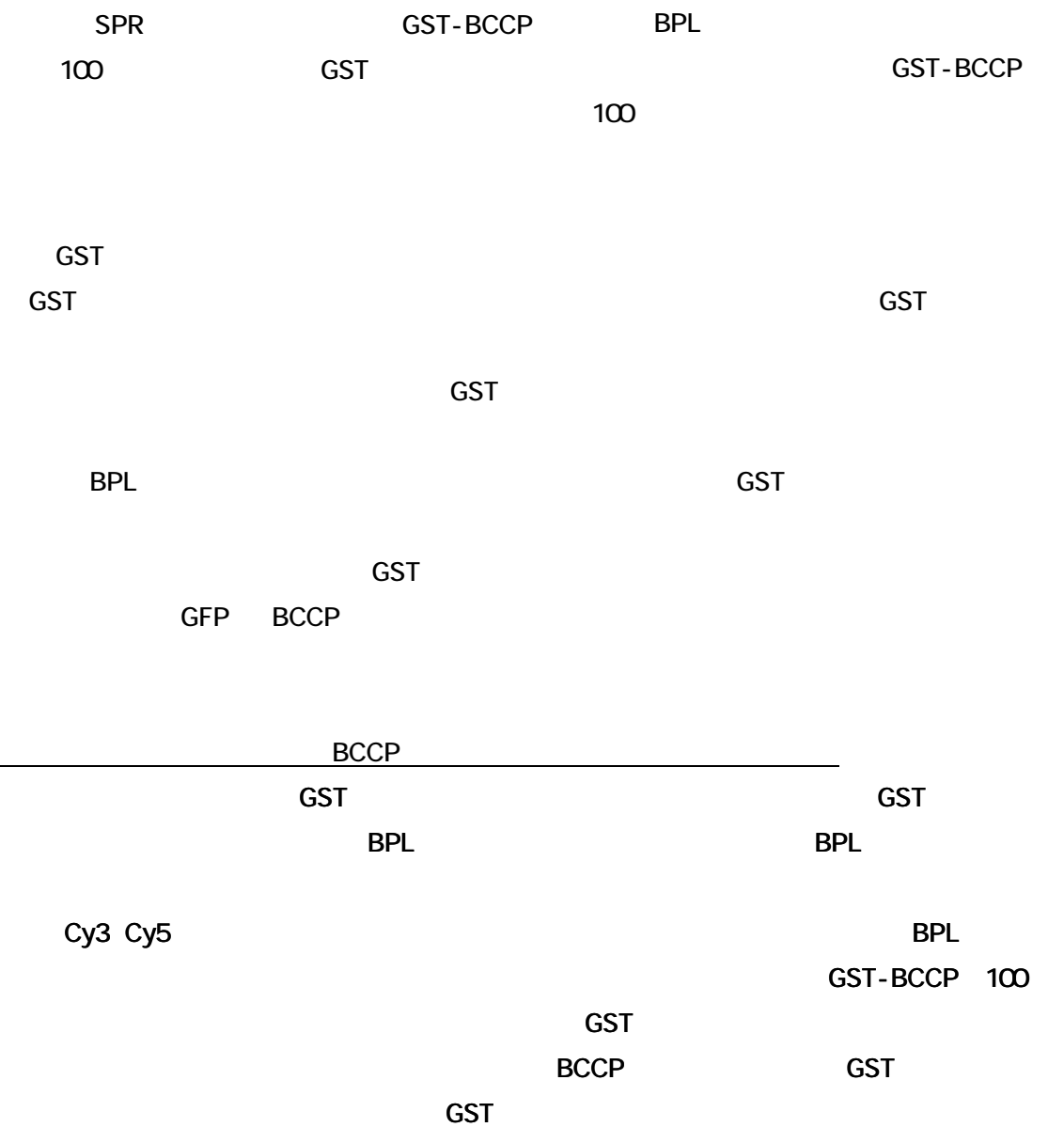
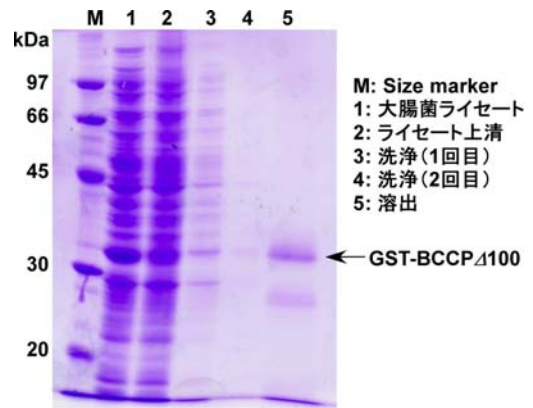
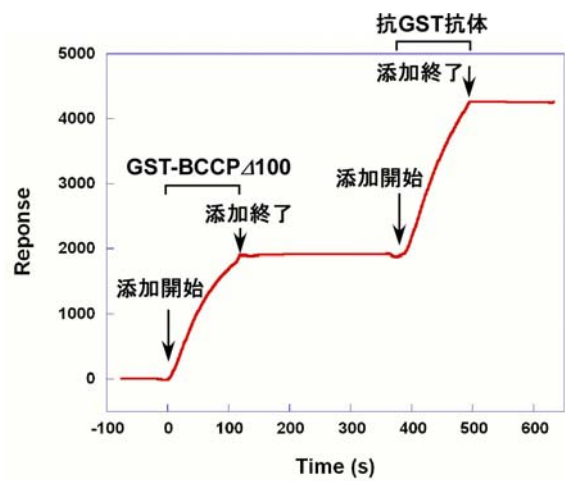


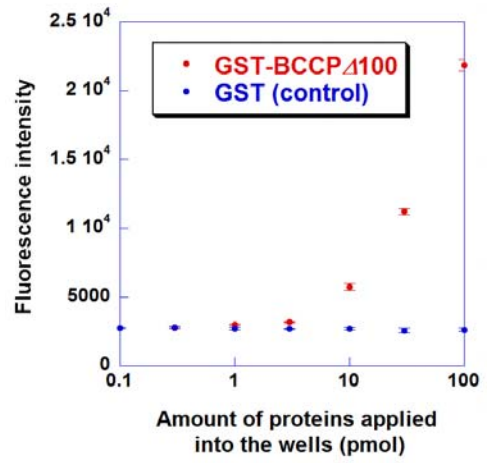
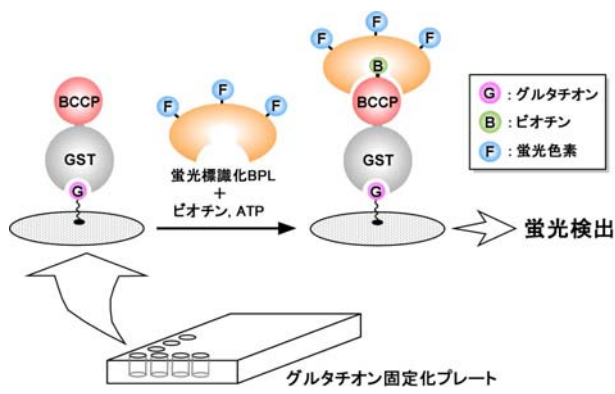
*S. tokodaii* BCCP



*S. tokodaii* BPL BCCP C

100	$K_d$	33 ( $\mu$ M)	Apo	BCCP	Apo	BCCP
Holo BCCP	BPL				$K_d$	BCCP
BPL						
BPL	BCCP					
					<i>S. tokodaii</i>	BPL BCCP
					BCCP	C N
C	BPL					
	BCCP					
BPL					BCCP	
BCCP						
	Glutathione S-transferase	GST		C	BCCP	100
	GST-BCCP	100			SPR	
	BPL					





BPL

GST

Src homology-2 (SH2) domain BCCP

1

BPL

*S. tokodaii*

BPL

BCCP

BCCP

BPL

SPR

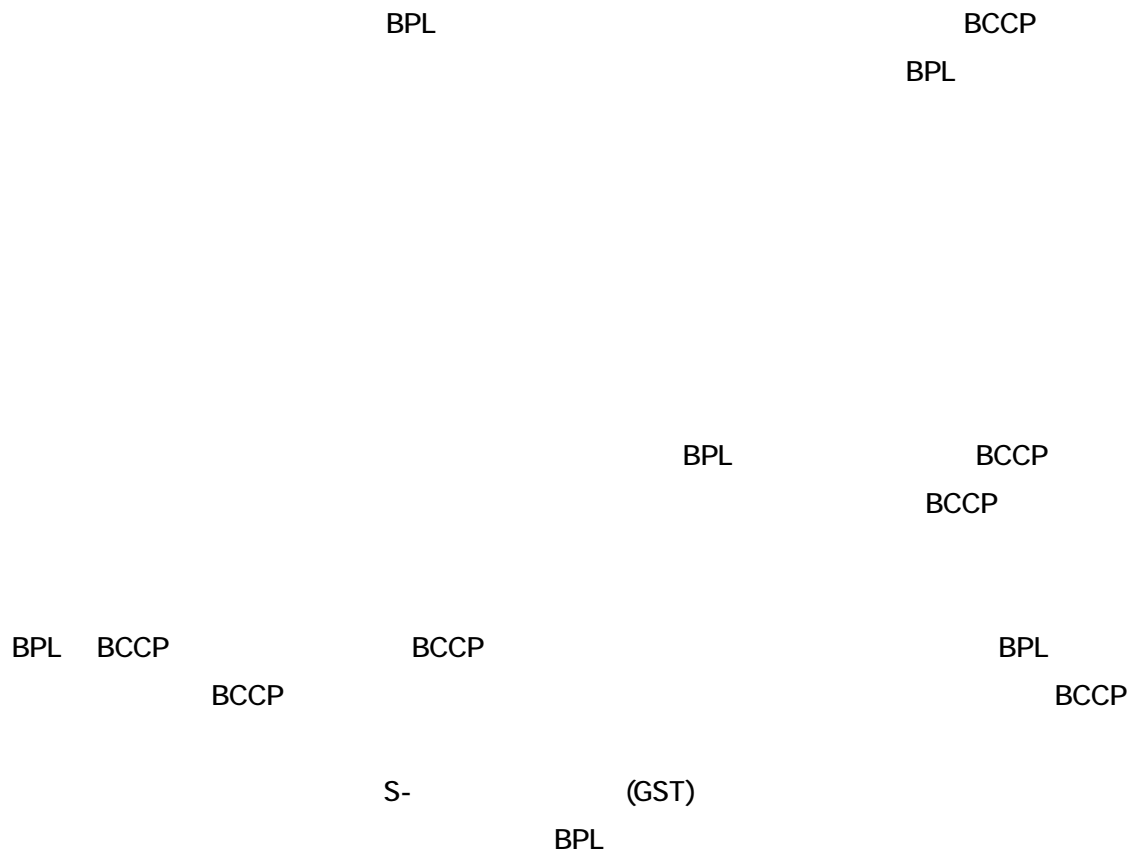
GST

GST

BPL-BCCP

BPL

BCCP



Yan-Qiu Li, Shinji Sueda, Hiroki Kondo, Yutaka Kawarabayasi, "A unique biotin carboxyl carrier protein in archaeon *Sulfolobus tokodaii*", *FEBS Letters*, 580, 1536- 1540 (2006)

Shinji Sueda, Yan-Qiu Li, Hiroki Kondo, Yutaka Kawarabayasi, "Substrate specificity of archaeon *Sulfolobus tokodaii* biotin protein ligase", *Biochemical and Biophysical Research Communications*, 344, 155- 159 (2006)

19 3 28

2007-085384

Shinji Sueda, Yan-Qiu Li, and Hiroki Kondo, "A unique biotinylating system in archaeon *Sulfolobus tokodaii*", 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress, 2006

Hiroki Kondo, Yan-Qiu Li, and Shinji Sueda, "Unusually high stability of an enzyme-product complex in the *Sulfolobus tokodaii* biotinylation system", 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress, 2006

Shinji Sueda, Hitoshi Tanaka, Masanori Yamagishi, Hiroki Kondo, "Development of a new tagging system for immobilization and detection of proteins", The first Japan-Korea joint symposium on bio-microsensing technology, 2008

Shinji SUEDA, "Development of a novel protein tagging system using a unique biotinylation reaction", KETI-RCBT 1st Workshop on Biosensing technology: Integration between dry and wet technology aiming for future biosensor, 2009

, "

", 32

, 2008

NMR

30kDa

30kDa

NMR

NMR

\_\_\_\_\_

dipolar coupling

20kDa

RDC Residual

RDC

$^{15}\text{N}$

$^1\text{H}$ - $^{15}\text{N}$

1A

RDC

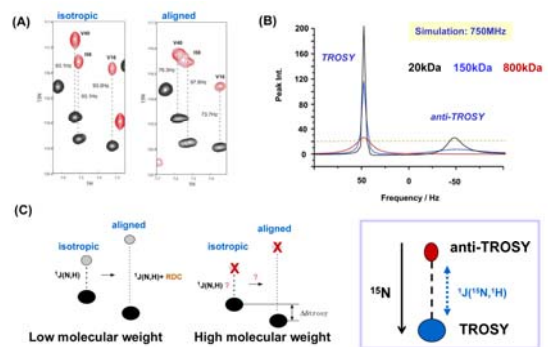
Trosy

1A

RDC

(Tjandra and Bax, Science, 278, 1111 (1997)).

NH



RDC

RDC

NMR

RDC

20kDa

RDC  
NMR

1B

anti-Trosy

100kDa

Trosy

100kDa  
RDC

Trosy

Trosy

DIORITE Determination of the Induced ORientation by  
Trosy experiments

DIORITE -

---

DIORITE

Trosy

Trosy

NMR

Trosy

RDC

30kDa

30kDa

1B

DIORITE

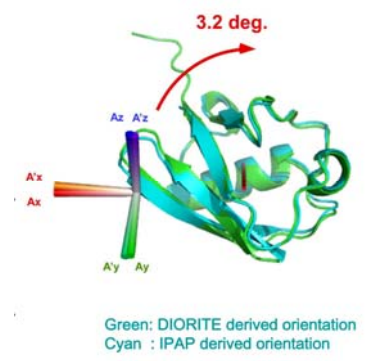
Trosy

DIORITE -

---

DIORITE

TROSY



10kDa- 50kDa

2 RDC  
DIORITE

900MHz  
600MHz

1.2

50kDa

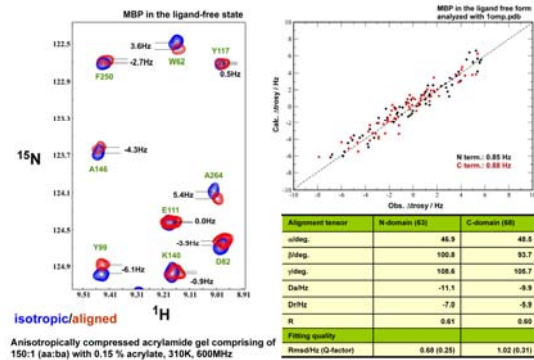
DIORITE

DIORITE

RDC

(8kDa)

RDC  
DIORITE  
DIORITE  
600MHz  
1.2  
15N  
DIORITE



MBP DIORITE

Trosy

900MHz  
3.2  
2

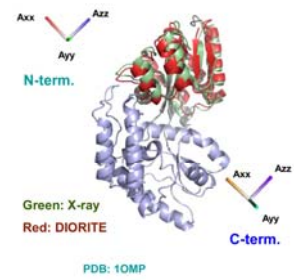
DIORITE

DIORITE

(MBP; 42kDa)  
MBP

DIORITE

3



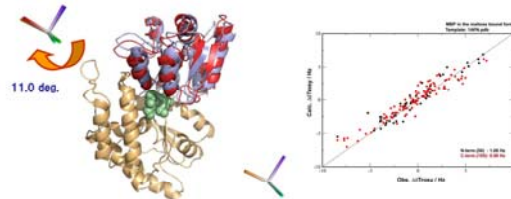
Hinge rotation angle difference between X-ray and NMR: 6.0deg.

Trosy

4: DIORITE  
MBP

MBP

80



Alignment tensor	N-domain (83)	C-domain (100)
ω/deg	51.7	59.3
β/deg	90.4	84.6
γ/deg	123.4	114.8
Da/Hz	13.6	-12.3
Dr/Hz	6.3	-4.4
R	0.48	0.26
Fitting quality		

Structural analysis using the X-ray coordinate for the MBP in the complex with maltose.

DIORITE  
MBP

5  
11 N  
MBP

DIORITE

N

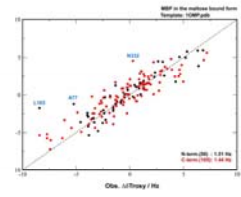
C

329

325 N  
DIORITE

( 6 )

DIORITE



Structural analysis using the X-ray coordinate for the MBP in the **ligand free form**.

PDB: 1OMP

Hinge rotation angle estimated from the x-ray coordinates 1OMP vs 1ANF was 32.5 deg.

Alignment measure	N-domain (20)	C-domain (20)
collog	82.8	82.4
fitlog	106.3	85.0
rllog	91.6	116.1
Darke	13.2	-12.4
Dette	6.5	-4.1
R	0.46	0.42
Fitting quality		
Rmsd(Å) (Q-factor)	1.61(0.46)	1.46(0.42)

DIORITE

mRNA

(CE)

CE 38kDa

20° C 3%

α

MBP

3%

50kDa

GTP

DIORITE

open  
GTP

CE

CE

7 DIORITE

CE

CE

( 7 )

NMR

Trosy

pH

50kDa

NMR

NMR

NMR

TROSY

NMR

NMR

Tate,S. Structure and mode of ligand recognition of the oxidized LDL receptor, LOX-1 (review article) in “Functional and structural biology on the lipo-network” Research Signpost, Kerala, India, 179-198 (2006).

Ishigaki,T., Ohki, I, Naoko Utsunomiya-Tate and \*Tate,S. Chimeric structural stabilities in the coiled-coil structure of the NECK domain in human lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), J.Biochem (Tokyo),141: 855-866 (2007).

\*Tate,S. Oxidized low-density lipoprotein receptor, LOX-1, on the endothelial cell - The receptor structure and functions of LOX-1 in atherogenesis. J.Biomol.Macromol. (review article), J.Biomol.Macromol. 7:11-22 (2007).

\*S.Tate. Anisotropic Nuclear Spin Interactions for the Morphology Analysis of Proteins in Solution by NMR Spectroscopy. Anal. Sci. 24: 39-49 (2008).

\_\_\_\_\_ 8 NMR ESR 1.NMR 1.5  
p40-69 (2006).

\_\_\_\_\_ 8 NMR ESR 3 NMR 3.2  
p162-192 (2006).

6, 234-235 (2008) NMR

Shin-ichi Tate  
Transient folding of the mediator binding domain of TFIIEb and its functional significance  
NMR association for structural biology in Yokohma “Intrinsically disordered proteins”

(2006.11, Yokohama)

Shin-ichi Tate

Structure and ligand recognition mode of oxidized LDL receptor LOX-1  
INPEC2006 (2006.6, Elsinore, Denmark)

Shin-ichi Tate

Molecular alignment determination only using orientation dependent TROSY shift changes  
The international workshop on "Perspectives on stable isotope aided NMR methods for  
protein structural analysis" (2007.03, Osaka, Japan).

Okuwaki,M, Kato,K., Shimahara,H., Tate,S., and Nagata,K. (2005): Assembly and  
disassembly of nucleosome core particles containing histone variants by human nucleosome  
assembly protein-1, *Mol. Cell Biol.*, 25:10639- 10651.

Takeshima,H., Suetake,I., Shimahara,H., Ura,K., Tate,S. and Tajima,S. "Distinct DNA  
methylation activity of Dnmt3a and Dnmt3b towards naked and nucleosomal DNA",  
*J.Biochem (Tokyo)*, 173:503- 515 (2006).

Shimahara,H., Yoshida,T., Shibata,Y., Shimizu,M, Kyogoku,Y., Sakiyama,F., Nakazawa,T.,  
Tate,S., Ohki,S., Kato,T., Mbriyama,H., Kishida,K.i., Tano,Y., Ohkubo,T., and Kobayashi,Y.  
Tautomerism of Histidine 64 Associated with Proton Transfer in Catalysis of Carbonic  
Anhydrase. *J. Biol. Chem* 282 9646- 9656 (2007).

2 (38- 44) 2008

THz CARS

CARS(Terahertz

Coherent Anti-Stokes Raman Scattering, THz-CARS)  
CARS

(THz)

(THz-TDS)

THz

( THz )  
THz  
THz-TDS

THz

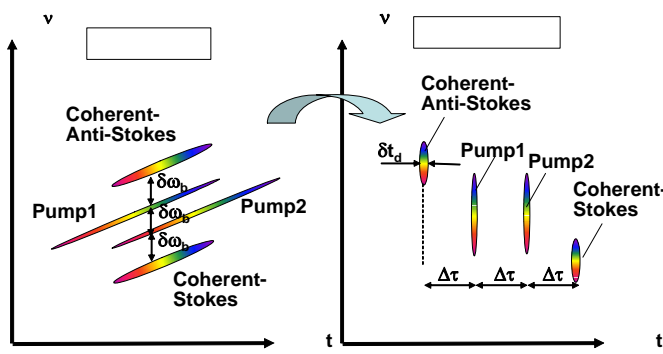
1 $\mu$ m

Coherent

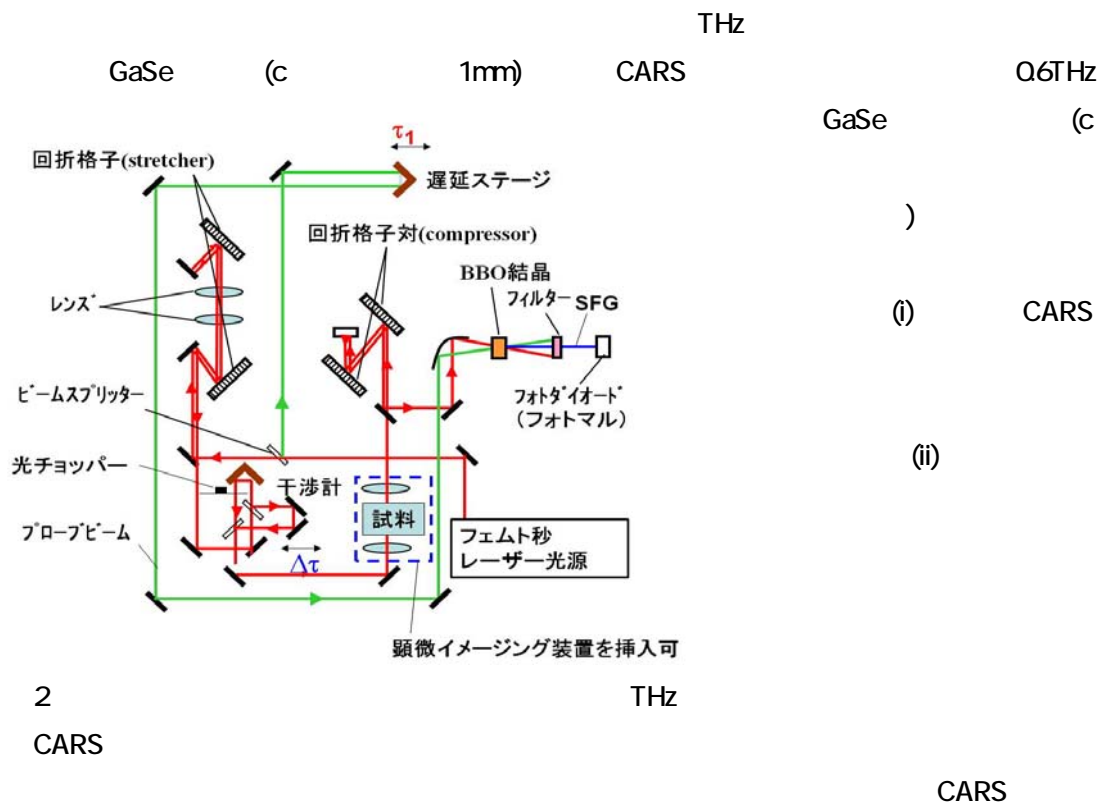
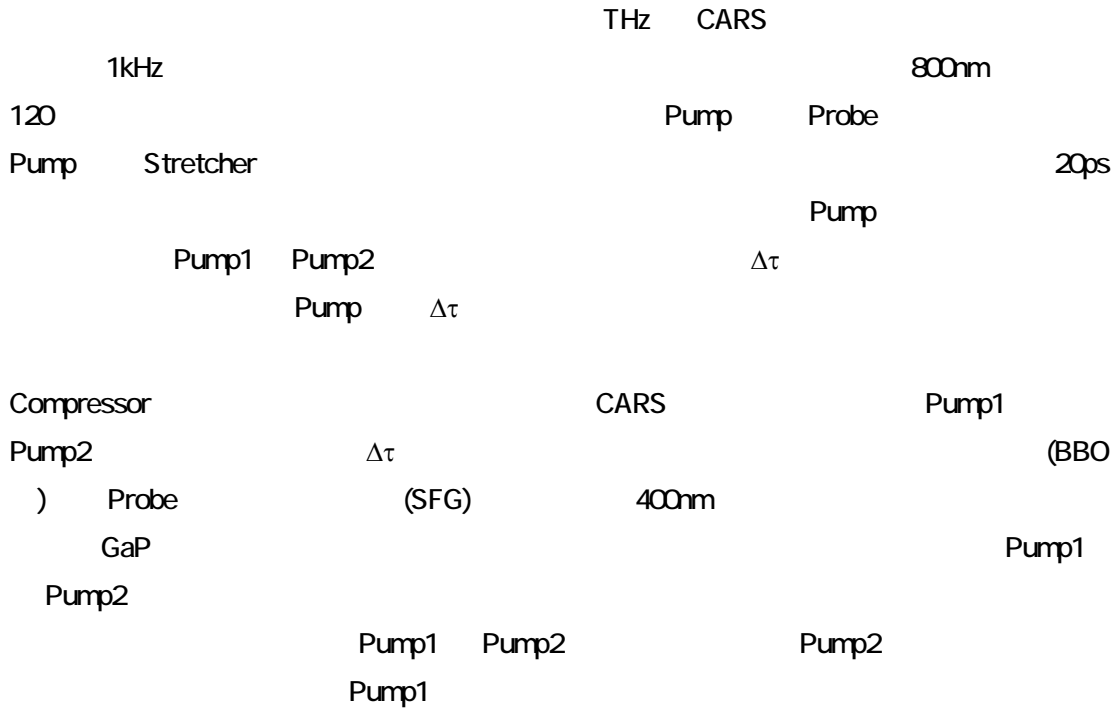
Anti-Stokes Raman Spectroscopy (CARS) THz  
THz (<6THz=200cm<sup>-1</sup>)

THz

CARS Pump Stokes  
 ( ) (i)  $10^4$   $10^5$   
 ( ) (ii) Anti-Stokes Pump Stokes  
 CARS Pump Stokes  
 2  
 THz ( $<30\text{cm}^{-1}$ )  
 ( $<1\text{cm}^{-1}$ )  
 SN  
 CARS THz 10THz  
 (Pump1 Pump2 )  $(\Delta\tau)$   $\Delta\tau$   
 ( $\delta\omega$  THz)  
 $\delta\omega$  Up-shift Coherent-Anti-Stokes  
 ( 1 ) Coherent-Anti-Stokes  $\delta\omega$   
 Pump1 Pump2  
 ( 1 )  
 Coherent-Anti-Stokes Up-conversion



1  
 Anti-Stokes ( )  
 -  
 Pump Stokes  
 (Inverse Raman Spectroscopy, IRS)  
 (Stimulated Raman Gain Spectroscopy, SRGS)  
 Pump2  
 Coherent Stokes  
 Coherent Stokes Raman Spectroscopy (CSRS)  
 Pump1  
 Pump2



GaSe

( ) CARS

CARS SN

3

CARS

CARS p-CARS

Pump2

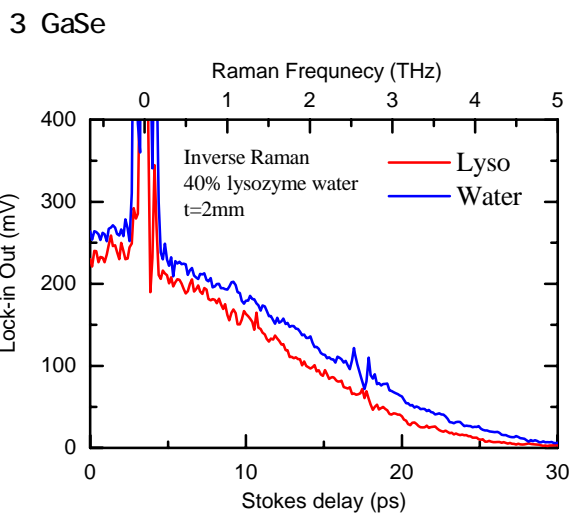
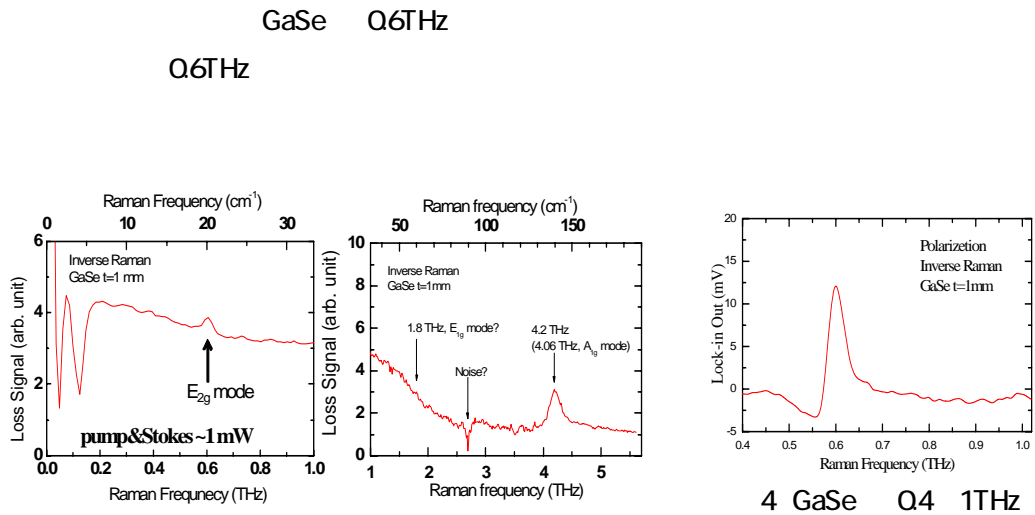
3      0.6THz      4THz

CARS

Probe       $\tau_1$   $\Delta\tau$

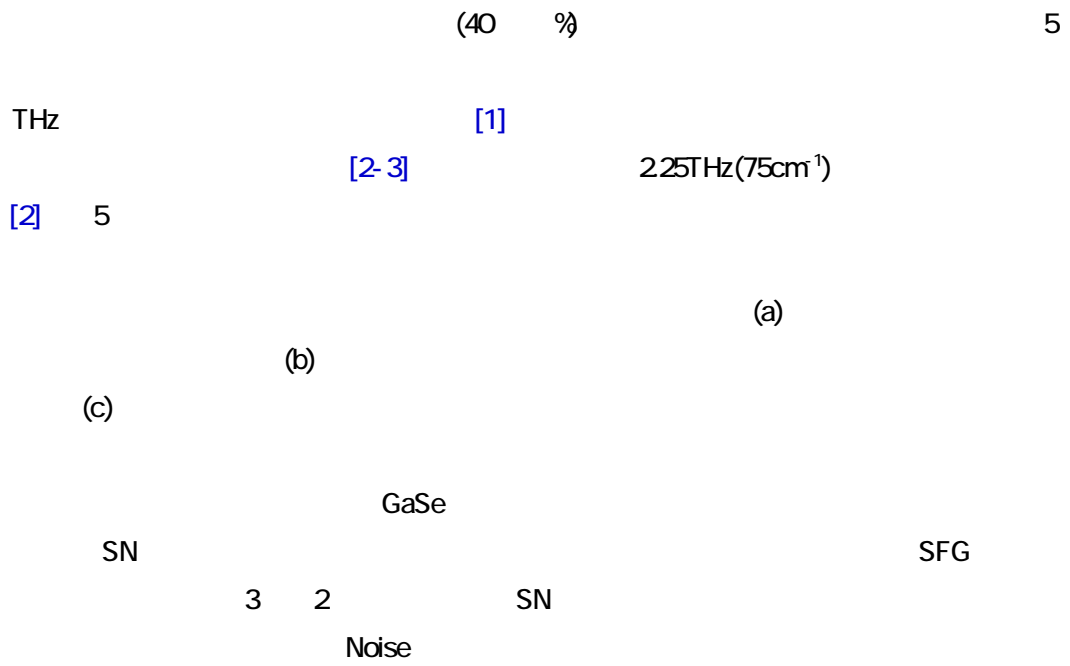
CARS

Pump1



5 (40

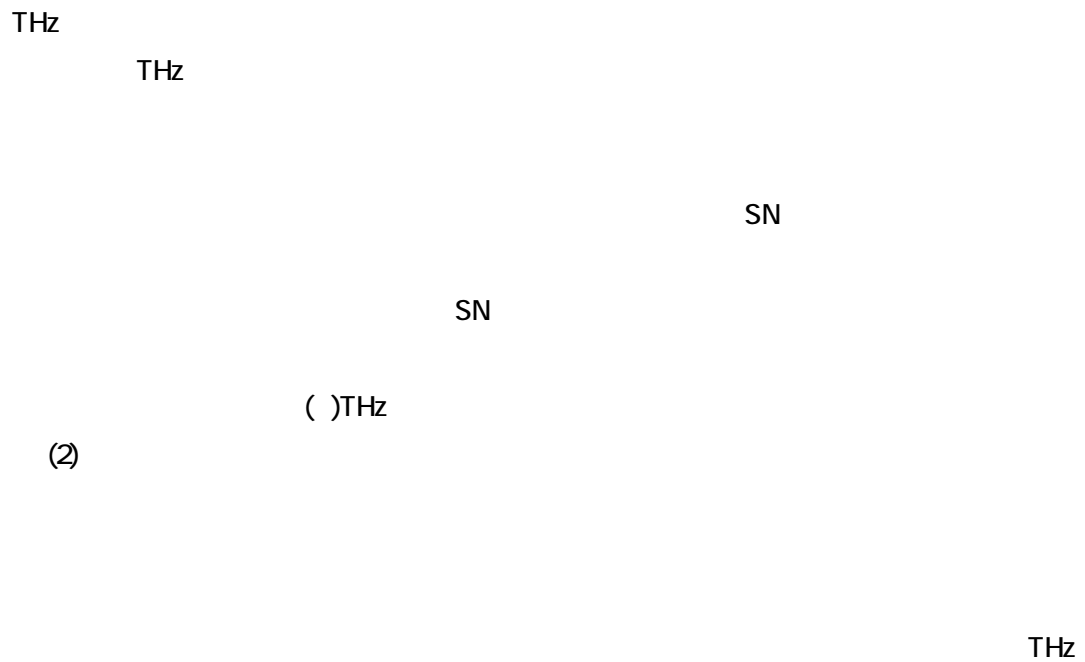
%)



[1] Brooks, *et al.*, Proc. Natl. Acad. Sci. USA, **82**, 4995 (1985).

[2] Genzel, *et al.*, Biopolymers, **15**, 219 (1976).

[3] Urabe, *et al.*, Biophysical Journal, **74**, 1533 (1998).



Masahiko Tani, T. Koizumi, H. Sumikura, M Yamaguchi, and M Hangyo: "Time-domain CARS signal detection for THz vibrational spectroscopy using chirped fs-pulses, " pp.275-276, Proceedings of the XXI International Conference on Raman Spectroscopy ICORS 2008 (21st August 2008, Uxbridge, West London, UK)

Masahiko Tani, Toshiyuki Koizumi, Hisashi Sumikura, Mariko Yamaguchi, and Masanori Hangyo: "Coherent Anti-Stokes Raman Scattering Spectroscopy in Terahertz Region Using Chirped Optical Pulses," The 33rd International Conference on Infrared, Millimeter, and Terahertz Waves (IRMMW-THz 2008), MB5.1197 (September 15-19, 2008, California Institute of Technology, Pasadena, CA, USA)

Christopher Que \_\_\_\_\_  
 CARS 2007 3  
 2 3 3

\_\_\_\_\_  
 CARS  
 (P-1, IPC) 2007 11 21 II 22

\_\_\_\_\_  
 CARS  
 OPJ 2007 , 2007 11 26

THz CARS 2008 \_\_\_\_\_ 55

30p-ZH-3 (

2008 3 30 )

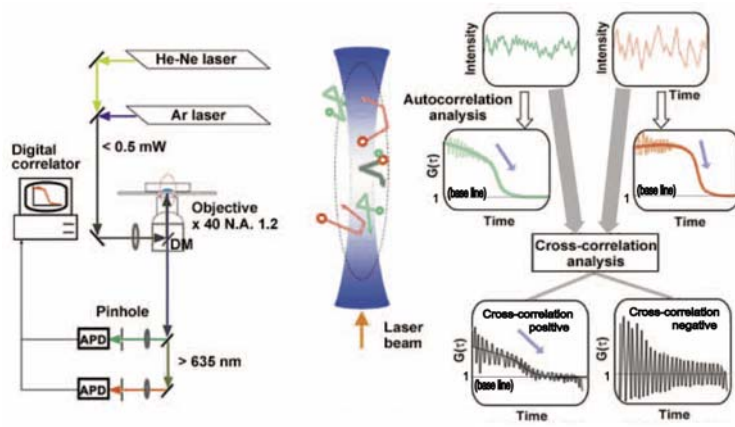
M. Tani, H. Sumikura, T. Nagashima, and M. Hangyo: "Coherent Raman Scattering Spectroscopy in Terahertz Frequency Region Using Chirped Optical Pulses," 20 P30 (Poster) ( 2008 11

19 21 )

\_\_\_\_\_ in THz CARS  
2008/ MMF  
2008 11 7

" "
   
 " " " "
   
 "
   
 " "

1-

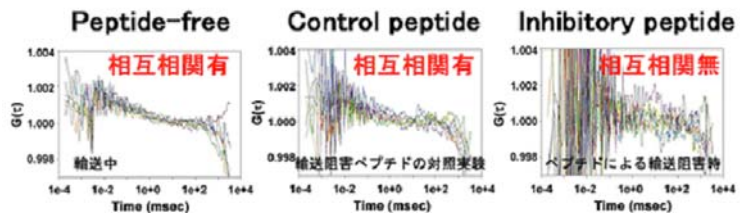


2



2

### Cross-correlation



3

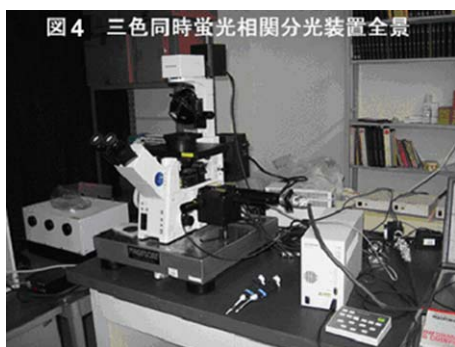
“ ”

3

“

”

4



$t_0$   
 $t$

$t_0 + t$   
 $n$

$n \times n$

“

”

19

2008 3

Neuroscience 27 : 4-5, 2009

Clinical

Neuroscience 27(2): 124-125, 2009

Clinical

Clinical Neuroscience 27(3), 2009

Hoshino M| Yamazoe S, Uesugi M| Terada S. Adhesamine, a newly synthesized chemical compound, is a very useful substrate for culturing mouse primary-cultured hippocampal neurons. The 48th Annual Meeting of the American Society for Cell Biology, San Francisco, December 13-17, 2008.

adhesamine

31

81

2008 12 9

12

adhesamine

114

2009 3 28 30

Communication in biosystems as observed by fluorescence correlation spectroscopy (FCS) Part 6 2009 3 27

2008 3

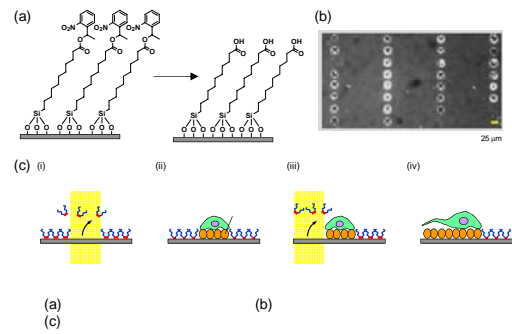
Hori H., Ozeki Y., Terada S., Kunugi H. Functional near-infrared spectroscopy reveals altered hemispheric laterality in relation to schizotypy during verbal fluency task. Prog

Neuropsychopharmacol. Biol. Psychiatry 32:1944-1951, 2008

Neuro2008      2008 7 9      11      : NIRS      31

2-

1a

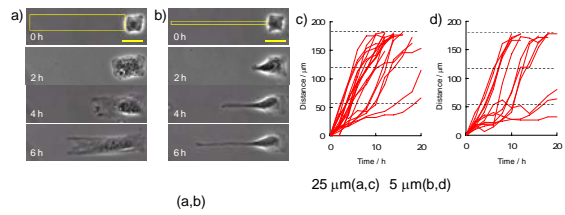


25 x 25  $\mu\text{m}^2$   
NIH3T3

25

$\mu\text{m}$

5  $\mu\text{m}$



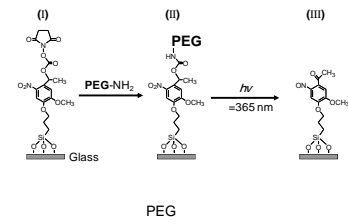
a,b

b

c d

5  $\mu\text{m}$

25  $\mu\text{m}$



PEG

AFM

PEG

NIH3T3

a,b

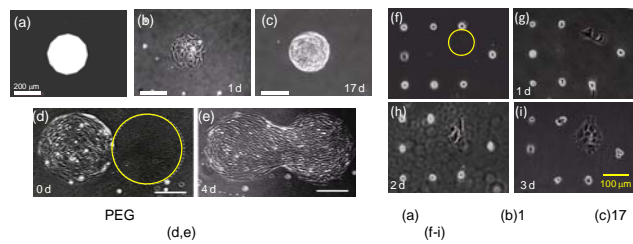
17

c

d,e

2

f-i



a

5 nm

PEG

*o*-

2-

b

HeLa

Ca<sup>2+</sup>

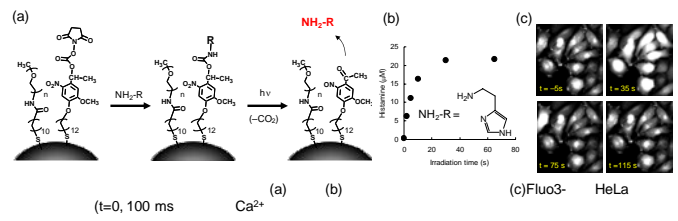
100 ms

Fluo 3

Ca<sup>2+</sup>

Ca<sup>2+</sup>

Ca<sup>2+</sup>



J. Nakanishi, Y. Kikuchi, S. Inoue, K. Yamaguchi, T. Takarada, M. Maeda, "Spatiotemporal Control of Migration of Single Cells on a Photoactivatable Cell-Microarray", *Journal of the American Chemical Society*, 129, 6694-6695 (2007)

J. Nakanishi, T. Takarada, K. Yamaguchi, M. Maeda, "Recent advances in cell micropatterning techniques for bioanalytical and biomedical sciences", *Analytical Sciences*, 24, 67-72 (2008)

Y. Kikuchi, J. Nakanishi, H. Nakayama, T. Shimizu, Y. Yoshino, K. Yamaguchi, Y. Yoshida, and Y. Horiike, "Grafting Poly(ethylene glycol) to a Glass Surface via a Photocleavable Linker for Light-induced Cell Micropatterning and Cell Proliferation Control", *Chemistry Letters*, 37, 1062-3 (2008)

Y. Kikuchi, J. Nakanishi, H. Nakayama, S. Inoue, K. Yamaguchi, H. Iwai, Y. Yoshida, Y. Horiike, T. Takarada, M. Maeda, "Arraying Heterotypic Single Cells on Photoactivatable Cell-Culturing Substrates", *Langmuir*, 24, 13084-95 (2008)

J. Nakanishi, H. Nakayama, T. Shimizu, H. Ishida, Y. Kikuchi, K. Yamaguchi, Y. Horiike, "Light-Regulated Activation of Cellular Signaling by Gold Nanoparticles That Capture and Release Amines", *Journal of the American Chemical Society*, 131, 3822-3823, 2009.

2007.9.15  
2007-240292

2008.7.15  
2008-184326

2006 9

55

23, 6-11, 2006

218, 125-128 (2006)

43-47 (2007).

52

1613-1618 (2007).

7, 18-26 (2008).

J. Nakanishi, Y. Kikuchi, T. Takarada, S. Inoue, K. Yamaguchi, M. Maeda, "Controlling cell adhesion, migration and protrusion on a functional cell-culturing substrate", , 2006

J. Nakanishi, Y. Kikuchi, S. Inoue, K. Yamaguchi, T. Takarada, M. Maeda, "Spatiotemporal control of cell protrusions and cell migration on a photoactivatable cell-culturing substrate", Gordon Research Conference –Signal Transduction by Engineered Extracellular Matrices–, 2006

J. Nakanishi, Y. Kikuchi, H. Nakayama, T. Shimizu, K. Yamaguchi, T. Takarada, M. Maeda, Y. Horiike, "Dynamic Cell Patterning on a Glass Functionalized with an Alkylsiloxane Having a Photocleavable Group", 1st Asian Biomaterial Congress, 2007

J. Nakanishi, Y. Kikuchi, H. Nakayama, T. Shimizu, H. Ishida, K. Yamaguchi, Y. Horiike, "Dynamic Micropatterning of Biomolecules and Cells Based on Substrates Presenting a Succinimidyl Ester via a Photocleavable Group", 8th World Biomaterials Congress, 2008

J. Nakanishi, "Photoresponsive Biointerfaces for Cell Analysis", MANA International Symposium, 2009

55 2006

J. Nakanishi, Y. Kikuchi, Y. Horiike, S. Inoue, K. Yamaguchi, T. Takarada, M. Maeda, "Spatiotemporal control of migration of single cells on a photoactivatable cell-microarray", 49, 59, 2007

69 2008

18 2008

57 2008

J. Nakanishi, "Dynamic Control of Cellular Microenvironment Based on Caged Compounds", First International Symposium on Interdisciplinary Materials Science, 2008

J. Nakanishi, "Photo-induced cell patterning technology", 2nd Int. Symposium on Atomic Technology for Biomaterials Science, 2008

2006

127 2007

22

2007

2008

2008

126



図1 広狭域2重2光子励起顕微鏡

高空間解像度2光子蛍光イメージング(①)、単一シナプス活性化用の2光子マイクロ刺激(①)、単一神経細胞活性化用の2光子マクロ刺激(②)、神経細胞集団活性化用の1光子刺激(③)が可能である。

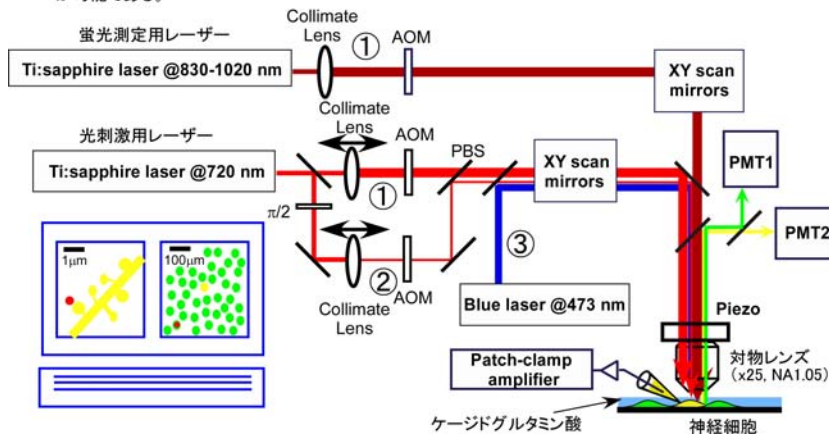
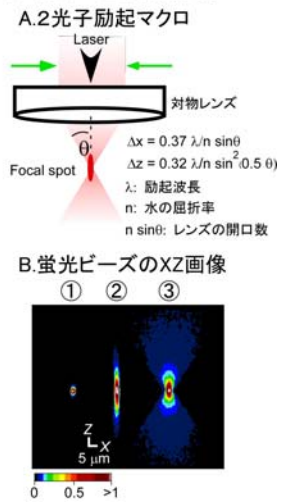


図2 光刺激の空間解像度



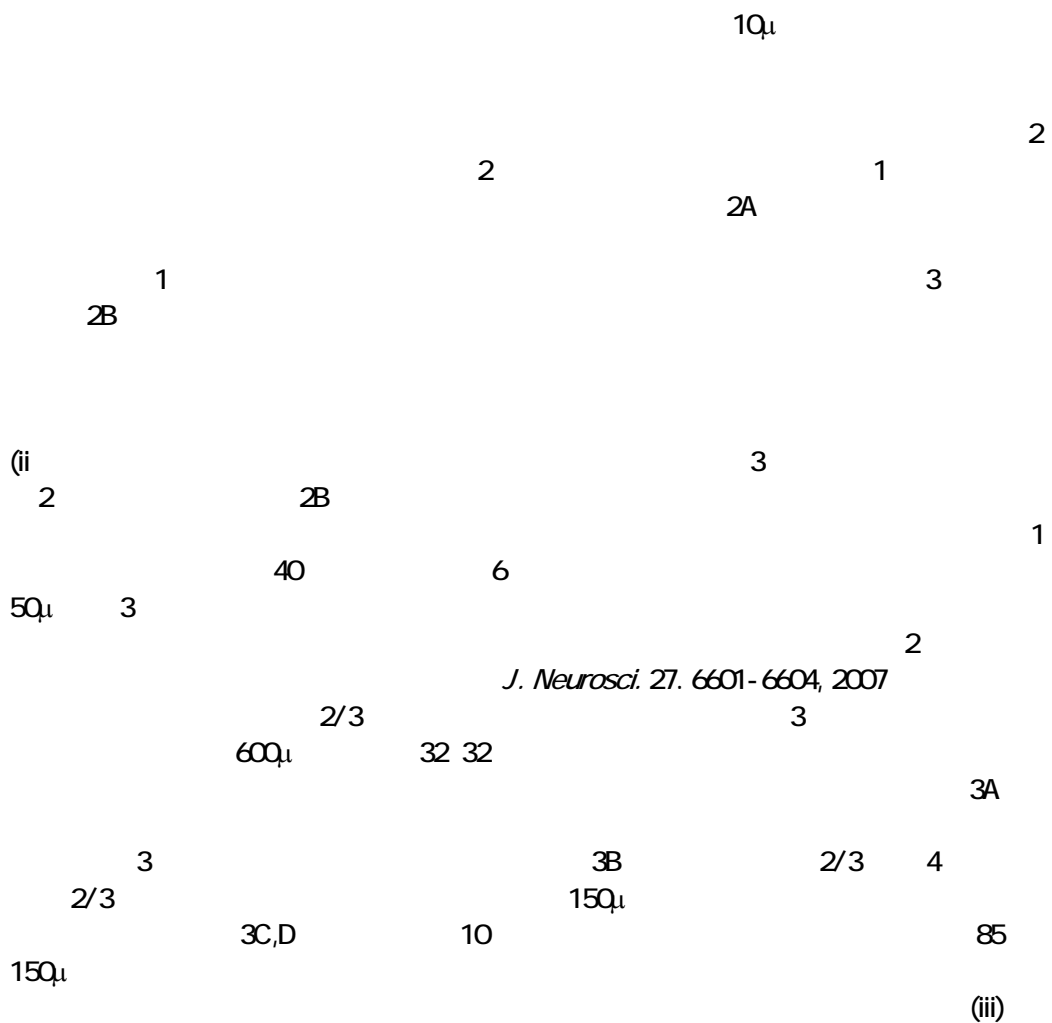


図3 第2/3層錐体細胞に入力する興奮性回路のマッピング

- (A)記録細胞の2光子蛍光イメージング。橙色の領域で光刺激が行われた。
- (B)シナプス電流を誘発する部位の3次元マッピング。シナプス電流の大きさが色コードされている。Zはマップ平面と記録細胞の細胞体との距離を表す。
- (C)BのマップをZ軸方向にスタックしたもの。
- (D)6つの記録細胞のスタック画像を平均化したもの。

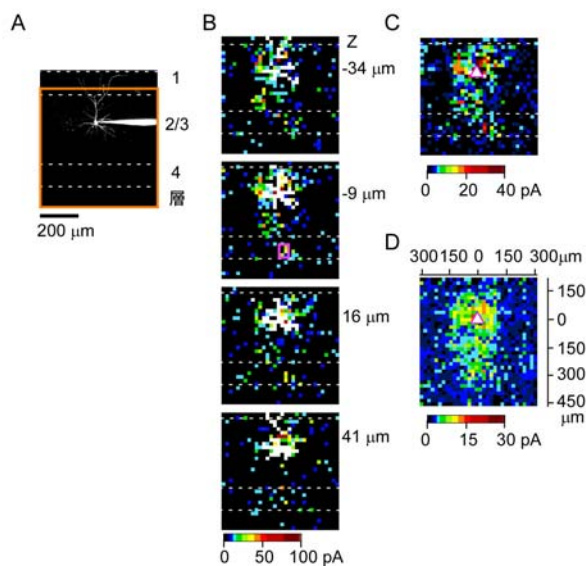
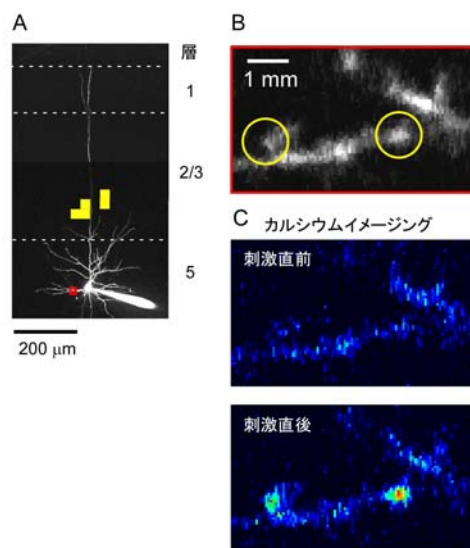
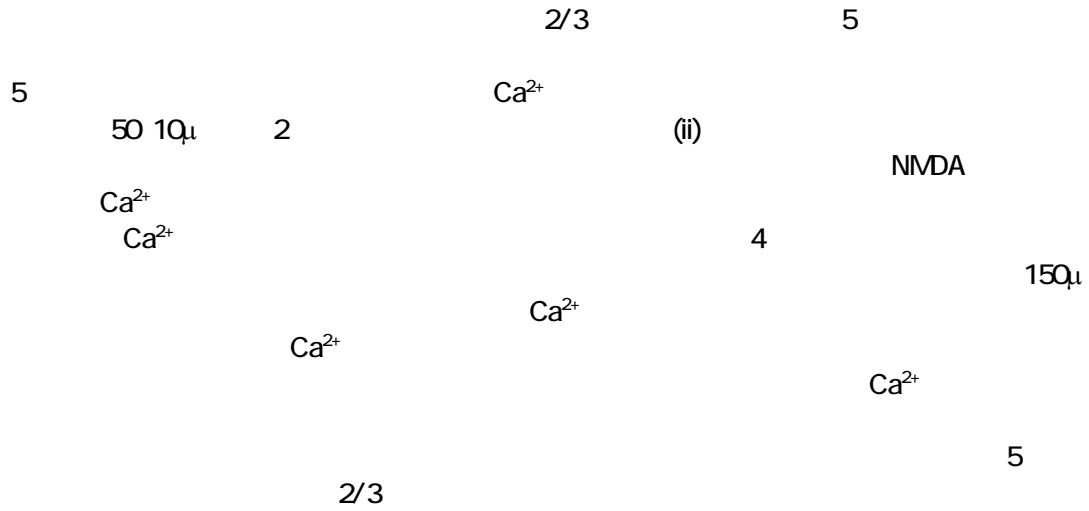


図4 シナプス入力細胞の位置と結合スパインの同定

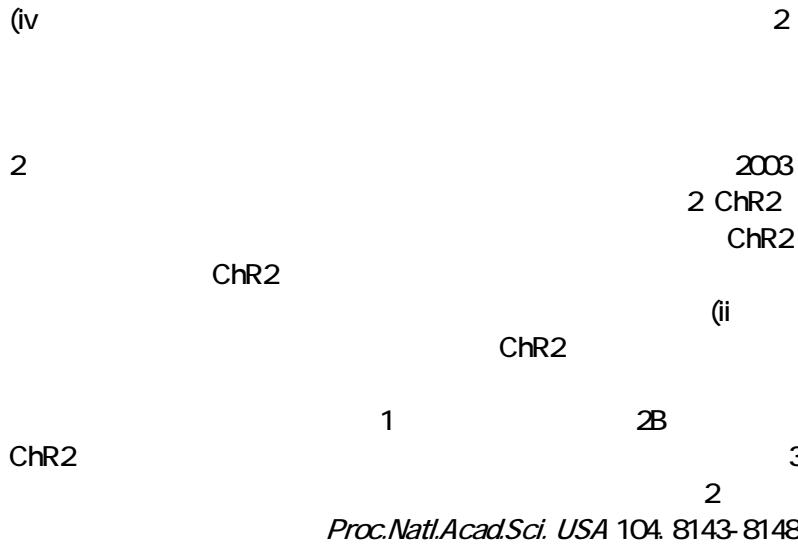
- (A)第5層の記録細胞の2光子蛍光イメージング。黄色の部位を光刺激するとCの反応が得られた。赤色の領域でイメージングが行われた。
- (B)イメージング領域の樹状突起とスパイン形態。黄色丸内のスパインがAの黄色部位刺激で反応した。
- (C)黄色部位の刺激前後でのカルシウムイメージングの一例。



(iii)



(iv)



(v)

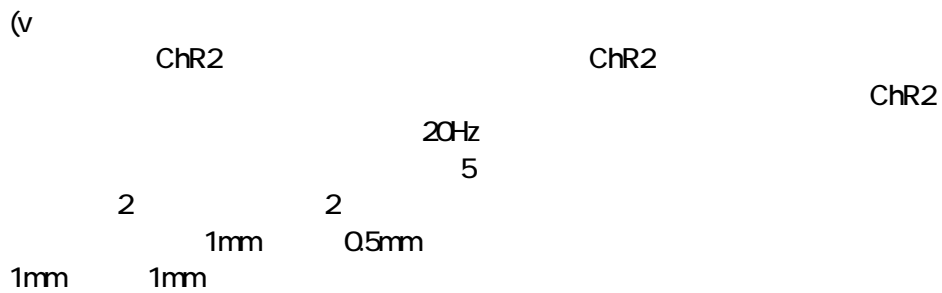
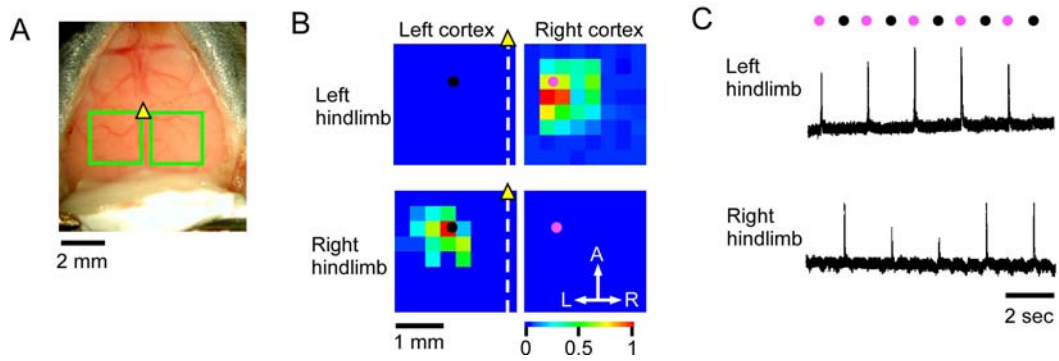


図5 個体マウス大脳皮質運動野の光機能マッピング

- (A)ChR2マウスの頭皮を剥いで、頭蓋骨越しにみた大脳皮質の画像。緑色の領域でマッピングが行われた。黄色三角印はブレグマを表す。
- (B)上は左後肢の支配領域のマッピング、下は右後肢の支配マッピングを表す。それぞれのマッピング領域は8x8に分割され、それぞれのピクセルで光刺激を行った。
- (C)Bの紫色の部位と黒の部位を交互に1Hzで光照射した時の左後肢と右後肢の動き。



2 2

ChR2

\*Wang H., \*Peca J., \*Matsuzaki M, Matsuzaki K., Noguchi J., Qiu L., Wang D., Zhang F., Boyden E., Deisseroth K., Kasai H., Hall WC., Feng G., Augustine G.J. High-speed mapping of

synaptic connectivity using photostimulation in Channelrhodopsin-2 transgenic mice. Proceedings of the National Academy of Sciences of the United States of America 104. 8143-8148, 2007. (\* equal contribution)

Ellis-Davies G.C.R., Matsuzaki M, Paukert M, Kasai H. and Bergles D.E. 4-carboxymethoxy-5,7-dinitroindoliny-glu: an improved caged glutamate for expeditious ultraviolet and 2-photon photolysis in brain slices. Journal of Neuroscience 27. 6601-6604, 2007.

Matsuzaki M, Ellis-Davies G.C.R. and Kasai H. High-resolution mapping of synaptic connections by two-photon macro photolysis of caged glutamate. Journal of Neurophysiology 99. 1535-1544, 2008

\*Tanaka J., \*Horiike Y., Matsuzaki M, Myazaki T., Ellis-Davies G.C.R. and Kasai H. Protein-synthesis and neurotrophin dependent structural plasticity of single dendritic spines. Science 319. 1683-1687, 2008 (\* equal contribution)

Hira R., Honkura N., Noguchi J., Maruyama Y., Augustine G.J., Kasai H. and Matsuzaki M Transcranial optogenetic stimulation for functional mapping of the motor cortex. Journal of Neuroscience Methods 179,258-263,2009.

2007 11 2

vol. 26, 298-302 2007

37th Annual Meeting of Society for Neuroscience

Matsuzaki M, Ellis-Davies G.C.R. and Kasai H. High-resolution mapping of synaptic connections in rat visual cortex by defocused two-photon uncaging of glutamate. ( 2007 11 )

29

Matsuzaki M, Tanaka , Myazaki T., Ellis-Davies G.C.R.. Kasai H. Plasticity and stability of single dendritic spines. ( 2006 7

30

Ellis-Davies Graham C.R.

2

2007 9

SSF/JST-PRESTO Joint Symposium  
Matsuzaki M Optical stimulation of synapses, neurons, and the cortex  
2008 5