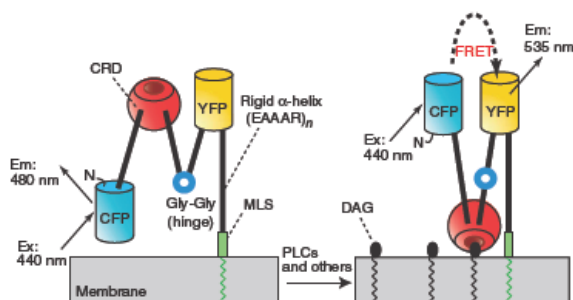


**Annual Research Highlights****(1) Imaging Diacylglycerol Dynamics at Organelle Membranes**

Fluorescence imaging is a powerful technique to visualize spatiotemporal dynamics of biomolecules in living cells. We describe fluorescent indicators for a lipid second messenger, diacylglycerol (DAG), which allow the localized analysis of DAG dynamics at subcellular membranes. We have thus pinpointed that DAG concentrations increase and/or decrease at not only the plasma membrane but also organelle membranes such as endomembranes and mitochondrial outer membranes.



**Fig. 1** Principle of the fluorescent indicator that detects DAG at cellular membranes. Upon binding of DAG to the CRD within the indicator, a flip-flop-type conformational change occurs, which changes the efficiency of FRET.

1.(1)-6) *Nature Methods*, **3**, 797–799 (2006).

**(2) Imaging the nanomolar range of nitric oxide with an amplifier-coupled fluorescent indicator in living cells**

Nitric oxide (NO) is a small uncharged free radical that is involved in diverse physiological and pathophysiological mechanisms. NO is generated by three isoforms of NO synthase, endothelial, neuronal, and inducible ones. When generated in vascular endothelial cells, NO plays a key role in vascular tone regulation, in particular. Here, we describe an

amplifier-coupled fluorescent indicator for NO to visualize physiological nanomolar dynamics of NO in living cells (detection limit of 0.1 nM). This genetically encoded high-sensitive indicator revealed that  $\approx 1$  nM of NO, which is enough to relax blood vessels, is generated in vascular endothelial cells even in the absence of shear stress. The nanomolar range of basal endothelial NO thus revealed appears to be fundamental to vascular homeostasis.

1.(1)-15) *Proc. Natl. Acad. Sci., USA*, **102**, 14515–14520 (2005).

**(3) Complementary base-pair-facilitated electron tunneling for electrically pinpointing complementary nucleobases**

Molecular tips in scanning tunneling microscopy can directly detect intermolecular electron tunneling between sample and tip molecules and reveal the tunneling facilitation through chemical interactions that provide overlap of respective electronic wave functions, that is, hydrogen-bond, metal-coordination-bond, and charge-transfer interactions. Nucleobase molecular tips were prepared by chemical modification of underlying metal tips with thiol derivatives of adenine, guanine, cytosine, and uracil and the outmost single nucleobase adsorbate probes intermolecular electron tunneling to or from a sample nucleobase molecule. We found that the electron tunneling between a sample nucleobase and its complementary nucleobase molecular tip was much facilitated compared with its noncomplementary counterpart. The complementary nucleobase tip was thereby capable of electrically pinpointing each nucleobase. Chemically selective imaging using molecular tips may be coined “intermolecular tunneling microscopy” as its principle goes and is of general significance for novel molecular imaging of chemical identities at the membrane and solid surfaces.

1.(1)-9) *Proc. Natl. Acad. Sci., USA*, **103**, 10–14 (2006).

## 1. 原著論文

### (1) Refereed Journals

- 1) M. Sato, T. Nakajima, M. Goto and Y. Umezawa, "A cell-based indicator visualizes picomolar dynamics of nitric oxide release from living cells", *Anal. Chem.*, in press.
- 2) A. Kanno, T. Ozawa and Y. Umezawa, "A Genetically Encoded Optical Probe for Detecting Release of Proteins from Mitochondria toward Cytosol in Living Cells and Mammals", *Anal. Chem.*, in press.
- 3) S. B. Kim, Y. Natori, T. Ozawa, Y. Umezawa and H. Tao, "A method for determining the activities of cytokines based on the nuclear transport of nuclear factor-kappa B", *Analytical Biochemistry*, in press.
- 4) M. Awais, M. Sato and Y. Umezawa, "A Fluorescent indicator to visualize ligand-induced receptor/coactivator interactions for screening of peroxisome proliferator-activated receptor ligands in living cells", *Biosensors & Bioelectronics*, in press.
- 5) Tetsushi Furukawa, Chang-Xi Bai, Asami Kaihara, Eri Ozaki, Takashi Kawano, Yutaka Nakaya, Muhammad, Awais, Moritoshi Sato, Yoshio Umezawa, Junko Kurokawa, "Ginsenoside Re, a Main Phytosterol of Panax Ginseng, Activates Cardiac Potassium Channels via a Non-Genomic Pathway of Sex Hormones", *Molecular Pharmacology*, 106.028134v1(2006), in press.
- 6) M. Sato, Y. Ueda and Y. Umezawa, "Imaging diacylglycerol dynamics at organelle membranes", *Nature Methods*, **3**, 797-799 (2006).
- 7) H. Shirakawa, M. Ito, M. Sato, Y. Umezawa and S. Miyazaki, "Measurement of intracellular IP<sub>3</sub> during Ca<sup>2+</sup> oscillations in mouse eggs with GFP-based FRET probe", *Biochemical and Biophysical Research Communications*, **345**, 781-788 (2006).
- 8) M. Awais, M. Sato, X. Lee and Y. Umezawa, "A Fluorescent Indicator to Visualize Activities of the Androgen Receptor Ligands in Single Living Cells", *Angew. Chem. Int. Ed.*, **45**, 2707-2712 (2006).
- 9) T. Ohshiro and Y. Umezawa, "Complementary base-pair-facilitated electron tunneling for electrically pinpointing complementary nucleobases", *Proc. Natl. Acad. Sci., USA*, **103**, 10-14 (2006).
- 10) A. Kanno, T. Ozawa and Y. Umezawa, "Intein-Mediated Reporter Gene Assay for Detecting Protein-Protein Interactions in Living Mammalian Cells", *Anal. Chem.*, **78**, 556-560 (2006).
- 11) Y. Itoh, Y. Ueda, M. Sugawara, M. Kataoka, H. Sato, and Y. Umezawa, "Nitrogenous Synergists Induced Potentiometric Response to Metal Ions with Polymeric Liquid Membranes Containing Thenoyltrifluoroacetone as an Ionophore", *Anal. Sci.*, **22**, 219-223 (2006).
- 12) S. B. Kim, T. Ozawa and Y. Umezawa, "A genetically encoded indicator for assaying bioactive chemicals that induce nuclear transport of glucocorticoid receptor", *Analytical Biochemistry*, **347**, 213-220 (2005).
- 13) S. B. Kim, R. Takao, T. Ozawa and Y. Umezawa, "Quantitative Determination of Protein Nuclear Transport Induced by Phosphorylation or by Proteolysis", *Anal. Chem.*, **77**, 6928-6934 (2005).
- 14) S. B. Kim, T. Ozawa and Y. Umezawa, "Genetically Encoded Stress Indicator for Noninvasively Imaging Endogenous Corticosterone in Living Mice", *Anal. Chem.*, **77**, 6588-6593 (2005).
- 15) M. Sato, N. Hida and Y. Umezawa, "Imaging the nanomolar range of nitric oxide with an amplifier-coupled fluorescent indicator in living cells", *Proc. Natl. Acad. Sci., USA*, **102**, 14515-14520 (2005).
- 16) H. Radecka, I. Szymanska, M. Pietraszkiewicz, O. Pietraszkeiewicz, H. Aoki and Y. Umezawa, "Intramolecular Ion-Channel Sensors Using Gold Electrodes Immobilized with Macrocyclic

Polyamines for Voltammetric Detection of Adenine Nucleotides”, *Chem. Anal. (Warsaw)*, **50**, 85-102 (2005).

- 17) T. Nishino, T. Ito and Y. Umezawa, “A Fullerene Molecular Tip Can Detect Localized and Rectified Electron Tunneling within a Single Fullerene-Porphyrin Pair”, *Proc. Natl. Acad. Sci., USA*, **102**, 5659-5662 (2005).
- 18) T. Ozawa, K. Nishitani, Y. Sako and Y. Umezawa, “A high-throughput screening of genes that encode proteins transported into the endoplasmic reticulum in mammalian cells”, *Nucleic Acids Res*, **33**, e34 (2005).
- 19) M. Sato, Y. Ueda, M. Shibuya and Y. Umezawa, “Locating Inositol 1,4,5-trisphosphate in the Nucleus and Neuronal Dendrites with Genetically Encoded Fluorescent Indicators”, *Anal. Chem.*, **77**, 4751-4758 (2005).

## 2. 総説・解説

- 1) 小澤岳昌, 梅澤喜夫: 「新しい蛍光タンパク質とその応用」, *ゲノム医学 特集 “再生医療とゲノム”*, **6**, No. 3, 53-56 (277-280), (2006-10).
- 2) Y. Umezawa, “Methods of Analysis for Imaging and Detecting Ions and Molecules”, *Award Accounts*, in press (Aug. 7, 2006).
- 3) Y. Umezawa, “Illuminating molecular processes in living cells”, *Chem. Asian J. (Focus Review)*, **1**, 303-312 (2006).
- 4) 大城敬人, 梅澤喜夫: 「STM 分子探針を用いて核酸塩基配列を決定する 分子間トンネル効果による相補的核酸塩基ピンポイント可視化検出」, *BIO INDUSTRY*, **23**, 68-73 (2006).
- 5) 大城敬人, 梅澤喜夫: 「STM 分子探針で核酸の塩基配列を読む 電子トンネル効果による相補的核酸塩基の単分子レベルピンポイント検出」, *BIONICS*, **3**, 72-73 (2006).
- 6) 西野智昭, 梅澤喜夫: 「分子探針による化学選択性 STM」, *表面科学*, **27**, 27-33 (2006).
- 7) T. Nishino and Y. Umezawa, “Chemically Modified STM Tips.”, *Sensor Letters*, **3**, 231-236 (2005).
- 8) Y. Tani and Y. Umezawa, “Ion-Selective Electrodes”, *Sensor Letters*, **3**, 99-107 (2005).
- 9) 西野智昭, 梅澤喜夫: 「分子探針を用いる走査型トンネル顕微鏡」, *分析化学 総合論文, 特集号 “ナノ空間と分析化学”*, **54**, 417-426 (2005).
- 10) Y. Umezawa, “Genetically Encoded Optical Probes for Imaging Cellular Signaling Pathways”, *Biosens. Bioelectron.*, **20**, 2504-2511 (2005).
- 11) Y. Umezawa et al., “Methods of Analysis for Chemicals that Disrupt Cellular Signaling Pathways: Risk Assessment for Potential Endocrine disruptors”, *Environmental Sciences*, **12**, 049-064 (2005).
- 12) Y. Umezawa, “Genetically encoded optical probes for molecular processes in living cells”, *TrAC Trends Anal. Chem.*, **24**, 138-146 (2005).

## 3. 著書

- 1) M. Sato and Y. Umezawa, “FRET-based reporters for intracellular enzyme activity”, *Encyclopedia of Proteomics*, Michael Dunn, Jeremy Tavaré (Eds.), John Wiley & Sons, in press.

- 2) Y. Umezawa, "Split Luciferase Systems for Detecting Protein-Protein Interactions in Mammalian Cells based on Protein Splicing and Protein Complementation", *Photoproteins in Bioanalysis*, Sylvia Daunert and Sapna K. Deo (Eds.), Wiley-VCH, in press.
- 3) 梅澤喜夫：「分析化学」東京化学同人 (2006. 3. 16).
- 4) T. Ozawa and Y. Umezawa, "Inteins for split-protein reconstitutions and their applications", *Inteins and Homing Endonucleases*, Marlene Belfort, Barry L. Stoddard, David W. Wood, Victoria Derbyshire (Eds.), 307-323, Springer-Verlag (2006).
- 5) M. Sato and Y. Umezawa, "Fluorescent Indicators for Imaging Protein Phosphorylation in Single Living Cells", *Cell Biology: A Laboratory Handbook*, 3<sup>rd</sup> Edition, Julio E. Celis (Ed.), Chap. 42, 325-328, Elsevier (2005).
- 6) 梅澤喜夫 編著, 「化学測定の事典 — 確度・精度・感度 — 」, 朝倉書店 (2005).

#### 4. その他（新聞掲載・特許など）

##### (1) 特許

- 1) 「キナーゼ阻害性融合タンパク質と医薬組成物」
  - ・出願人：梅澤喜夫
  - ・出願日：平成 18 年 8 月 2 日（特願 2006-211072）
- 2) 「タンパク質リン酸化インディケーター」
  - ・出願人：東京大学
  - ・出願日：平成 18 年 3 月 6 日（特願 2006-059615）
- 3) 「真核細胞におけるタンパク質 — タンパク質相互作用を検出するためのキットと方法」
  - ・出願人：東京大学
  - ・出願日：平成 17 年 9 月 30 日（特願 2005-288971）
  - ・PCT 出願：2006 年 9 月 29 日（PCT/JP2006/319716）
- 4) 「核酸塩基の検出方法」
  - ・出願人：科学技術振興機構
  - ・出願日：平成 16 年 12 月 28 日（特願 2004-381406）
  - ・PCT 出願中
- 5) 「一酸化窒素検出用センサー細胞とそれを用いた一酸化窒素の検出・定量方法」
  - ・出願人：科学技術振興機構
  - ・出願日：平成 16 年 7 月 5 日（特願 2004-198240）
  - ・PCT 出願：2005 年 7 月 5 日（PCT/JP2005/012721）
- 6) 「一酸化窒素検出・定量用プローブとそれを用いた一酸化窒素の検出・定量方法」
  - ・出願人：科学技術振興機構
  - ・出願日：平成 16 年 7 月 5 日（特願 2004-198239）
  - ・PCT 出願：2005 年 7 月 5 日（PCT/JP2005/012722）
- 7) 「イノシトール 1,4,5-三リン酸検出・定量用プローブおよびそれを用いたイノシトール 1,4,5-三リン酸検出・定量方法」

- ・ 出願人：科学技術振興機構
- ・ 出願日：平成 16 年 5 月 21 日（特願 2004-152484）
- ・ PCT 出願：2005 年 5 月 17 日（PCT/JP2005/009299）

8) 「蛋白質核内移行検出用プローブとそれを用いた蛋白質核内移行の検出・定量方法」

- ・ 出願人：科学技術振興機構
- ・ 出願日：平成 16 年 3 月 9 日（特願 2004-066424）
- ・ PCT 出願：2005 年 3 月 9 日（PCT/JP2005/04591）

9) 「核内レセプターのアゴニスト・アンタゴニスト検出用プローブとそれを用いた核内レセプターに対するアゴニストおよびアンタゴニストのスクリーニング方法」

- ・ 出願人：科学技術振興機構
- ・ 出願日：平成 16 年 2 月 12 日（特願 2004-035678）
- ・ PCT 出願：平成 17 年 2 月 14 日（PCT/JP2005/002660）