Analytical Chemistry

Annual Research Review

(1) Molecular Sensors Based on Biological Signal Transduction

Fluorescent Indicators for Imaging Protein Phosphorylation in Single Living Cells

To visualize signal transduction based on protein phosphorylation in living cells, we have developed genetically encoded novel fluorescent indicators, named phocuses. As phocuses, two different color mutants of green fluorescent protein (GFP) were joined by a tandem fusion domain composed of a substrate domain for protein kinase of interest, flexible linker sequence and phosphorylation recognition domain that binds with the phosphorylated substrate domain. Intramolecular interaction of the substrate domain and the adjacent phosphorylation recognition domain within phocus was dependent upon phosphorylation of the substrate domain by protein kinase, which changed efficiency of fluorescent resonance energy transfer (FRET) between the GFPs within phocus. In the present study, phocuses were exemplified using insulin signaling proteins, with which protein phosphorylation by insulin receptor was visualized.

A-1) *Nature Biotechnol*., **20**, 287-294 (2002)

Noninvasive Imaging of Protein-Protein Interactions in Living Subjects

We have developed bioluminescence-imaging strategies to noninvasively and quantitatively image protein-protein interactions in living mice by using a cooled charge-coupled device camera and split reporter technology. We validated intein-mediated reconstitution of split firefly luciferase proteins driven by the interaction of two strongly interacting proteins, MyoD and Id. The mouse implanted with cells transfected with both probes showed significant signals upon MyoD-Id interactions.

A-6)*Proc. Natl. Acad. Sci. USA*, **99**, 15608-15613 (2002)

(2) Ion-Channel Sensors Based on Self-Assembled Monolayers

Detection of oligonucleotides using PNA modified gold electrodes

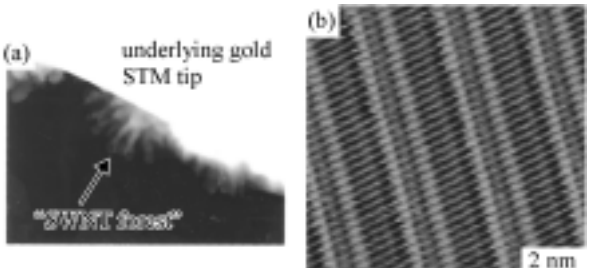
Gold electrodes modified with self-assembled monolayers composed of the peptide nucleic acid (PNA) probe and 8-amino-1-octanethiol were used for the detection of a complementary oligonucleotide with a detection limit of 5.1×10^{-10} M and a relative standard deviation of 1.5 % in a pH 7.0 phosphate buffer solution. In contrast, no responses to a non-complementary oligonucleotide were observed. This allows the indirect detection of the complementary oligonucleotide.

A-5) *Electroanalysis*, **14,** 1405-1410 (2002)

(3) Chemical Sensing Based on Chemically Modified STM Tips

Carbon Nanotube Tips

Carboxy-terminated single-walled carbon nanotubes (SWNTs) were successfully immobilized from solution phases onto the apex of gold tips for STM from solution phases. Gold STM tips were first modified with self-assembled monolayers of 4-mercaptobenzoic acid, and its carboxy groups were used to anchor carboxylated SWNTs through Zn^{2+} ion bridged coordination in-between. These SWNT tips gave high-resolution STM images of a diether monolayer formed on graphite surface. In addition and more importantly, the ether oxygens of the sample molecules were selectively observed as bright spots with the SWNT tips with significantly high reproducibility, which is due to the facilitation of electron tunneling through hydrogen bond interactions between the ether oxygens and carboxy groups at the end of the SWNT tips.



Figures. (a) Electron micrograph of SWNT tips prepared from solution phases. (b) STM image of diether molecules observed with an SWNT tip.

A-4) *Anal. Chem*., **74**, 4275-4278 (2002)

分析化学研究室

研究レビュー

(1) 細胞内情報伝達過程を検出するセンサー

生きた単一細胞内での蛋白質リン酸化を可視化する蛍光プローブ

蛋白質リン酸化に基づく細胞内情報伝達を可視化検出するために，新しい蛍光プローブを遺伝子工学的手法を用いて開発し，phocus（フォーカス）と名付けた．この phocus は緑色蛍光蛋白質（GFP）の2つの異色変異体と，検出目的のキナーゼによりリン酸化される基質配列，リンカーおよびリン酸化認識ドメインからなる．基質ドメインのリン酸化依存的に phocus の構造変化が誘起され，phocus 分子内に連結した蛍光蛋白質間の蛍光共鳴エネルギー移動（FRET）が変化することを示した．本研究では，インスリン受容体による蛋白質リン酸化の可視化検出を例示した．

A-1) *Nature Biotechnol.*, **20**, 287-294 (2002)

生きたマウス個体内での蛋白質間相互作用を検出する発光プローブ

自己触媒的蛋白質組み継ぎ反応（プロテインスプライシング）を利用した蛋白質間相互作用検出プローブを用いて，生きたマウス個体内でのタンパク質間相互作用を非侵襲的に検出する方法を開発した．スプリットした生物発光蛋白質（firefly luciferase）に，スプライシング蛋白質（DnaE）のN末及びC末を連結しプローブ分子とした．相互作用するタンパク質には MyoD-Id を用いた．マウス個体内での MyoD-Id 間相互作用によりプロテインスプライシング反応が起こり，個体内での luciferase 形成に伴う発光を，冷却 CCD カメラで検出することに成功した．

A-6) *Proc. Natl. Acad. Sci. USA*, **99**, 15608-15613 (2002)

(2) 自己集合単分子膜に基づくイオンチャンネルセンサー

ペプチド核酸（PNA）プローブ混合自己集合膜修飾金電極による DNA 検出

ペプチド核酸（PNA）と 8-amino-1-octanethiol（AOT）との混合自己集合膜（SAM）修飾電極を用いて，相補的配列を持つ DNA を 5.1×10^{-10} M（S/N = 3.0）の検出下限で選択的に検出することに成功した．相補的配列を持つ DNA をサンプルとして含む溶液中で$[Ru(NH_3)_6]^{3+}$をマーカーとしてボルタモグラムを測定した．DNA 濃度が上昇するに従って観測される電流が上昇し，還元ピークが正方向にシフトした．本研究で作製されたセンサーは，相補的な DNA に対してのみ選択的に検出できるセンサーであることが分かった．

A-5) *Electroanalysis*, **14**, 1405-1410 (2002)

(3) 化学修飾探針に基づく走査型トンネル顕微鏡を用いた化学センシング

カーボンナノチューブ探針

末端にカルボキシ基を持つ単層カーボンナノチューブ(SWNTs)を溶液中から STM の探針上に固定できることを初めて示した．金の STM 探針を 4-メルカプト安息香酸(4MBA)の自己組織化単分子膜(SAMs)で修飾し，その 4MBA および SWNT の両者のカルボキシ基を Zn^{2+}イオンを介して架橋することにより SWNTs を固定した．この SWNT 探針により，非常に高分解能での STM 観察が可能であることが明らかとなった．さらに，未修飾金探針を用いた場合とは異なり，試料分子中のエーテル酸素が選択的に明るく観察された．これは，SWNT 探針末端のカルボキシ基がエーテル酸素と水素結合を形成することにより，トンネル電流が促進されたためであると考えられる．

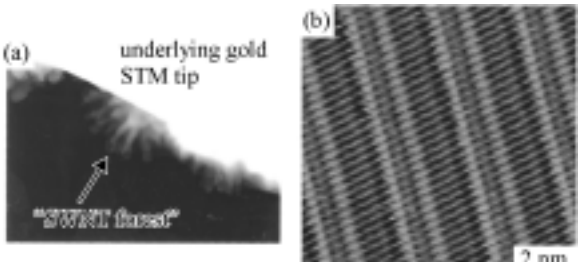


図1．(a)SWNT 探針の電子顕微鏡写真．(b) SWNT 探針を用いて観察した diether 分子の STM 像．

A-4) *Anal. Chem.*, **74**, 4275-4278 (2002)

A. Original Papers

1) M. Sato, T. Ozawa, (K. Inukai), (T. Asano), and Y. Umezawa: “Fluorescent Indicators for Imaging Protein Phosphorylation in Single Living Cells” *Nature Biotechnol*., **20**, 287-294 (2002).

2) Y. Umezawa, (K. Umezawa), P. Bühlmann, N. Hamada, J. Nakanishi, H. Aoki, M. Sato, Y. Nishimura and K. P. Xiao; “Potentiometric Selectivity Coefficients of on-Selective Electrodes. Part II. Inorganic Anions” *Pure Appl. Chem*., **74**, 923-994 (2002).

3) Y. Umezawa, P. Bühlmann, (K. Umezawa) and N. Hamada: “Potentiometric Selectivity Coefficients of Ion-Selective Electrodes. Part III. Organic Ions” *Pure Appl. Chem*., **74** 995-1099 (2002).

4) T. Nishino, T. Ito and Y. Umezawa: “Carbon Nanotube Scanning Tunneling Microscopy Tips for Chemically Selective Imaging” *Anal. Chem*., **74**, 4275-4278 (2002).

5) H. Aoki and Y. Umezawa: “High Sensitive Ion-Channel Sensors for Detection of Oligonucleotides Using PNA Modified Gold Electrodes” *Electroanalysis*, **14**, 1405-1410 (2002).

6) (R. Paulmurugan), Y. Umezawa and (S. S. Gambhir): “Noninvasive Imaging of Protein-Protein Interactions in Living Subjects by Reporter Protein Complementation and Reconstitution Strategies” *Proc. Natl. Acad. Sci. USA*, **99**, 15608-15613 (2002).*

B. Reviews

1) Y. Umezawa: “Detection of Phosphate Ion and Protein Phosphorylation — Crystal Surfaces, Ionophore Monolayers, and Protein Interactions—” *J. Supramol. Chem.*, **2**, 233-245 (2002).

2) T. Ozawa and Y. Umezawa: “Peptide Assemblies in Living Cells. Methods for Detecting Protein-Protein Interactions” *Supramol. Chem.*, **14**, 271-280 (2002).

3) Y. Umezawa, T. Ozawa and M. Sato: “Probing Chemical Processes in Living Cells: Application for Assay and Screening of Chemicals that Disrupt Cellular Signaling Pathways” *Bull. Chem. Soc. Jpn. (Accounts)*, **75**, 1423-1433 (2002).

4) Y. Umezawa, T. Ozawa and M. Sato: “Methods of Analysis for Chemicals that Promote/Disrupt Cellular Signaling” *Anal. Sci.*, **18**, 503-516 (2002).

5) Y. Umezawa, T. Ozawa and M. Sato: “Assay and Screening Methods for Chemicals that Disrupt Cellular Signaling Pathways. Risk Assessment for Potential Endocrine Disruptors” *Environmental Sci.*, **9**, 23-35 (2002).

6) Y. Umezawa: “Assay and Screening Methods for Bioactive Substances Based on Cellular Signaling Pathways” *Rev. Mol. Biotechnol.*, **82**, 357-370 (2002).

7) M. Sugawara, A. Hirano, P. Bühlmann and Y. Umezawa: “Design and Application of Ion-Channel Sensors Based on Biological and Artificial Receptors” *Bull. Chem. Soc. Jpn (Accounts)*, **75**, 187-201 (2002).

8) 梅澤喜夫：“物理･化学･生物的分析法と信頼性”「ケミカルエンジニアリング」**47**, (2002).

9) 佐藤守俊, 梅澤喜夫：“細胞内のタンパク質リン酸化を生きたまま観察する”「現代化学」No. 377, 48-54 (2002).

D. Books

1) 梅澤喜夫:「理科年表」(化学部) 監修 梅澤喜夫, 文部省国立天文台編, 丸善 (2002).

2) 梅澤喜夫:「機器分析実験」梅澤喜夫, 本水昌二, 渡會 仁, 寺前紀夫 編著, 東京化学同人 (2002).