Nano Bio-functional Molecular Systems Research Team

Team Leader : Mizuo Maeda

Unpublished results are not included.

DNA sensing and SNP typing on a autonomous microchip with laminar flow-assisted dendritic amplification

K. Hosokawa, T. Sato, Y. Sato, M. Maeda,
***Proc. Micro Total Analysis Systems* (2009) 1061.**

We demonstrated DNA sensing and single-nucleotide polymorphism (SNP) typing with our two original technologies: (1) autonomous microchip, which is a microfluidic system requiring no external power sources for fluid transport; and (2) laminar flow-assisted dendritic amplification (LFDA), which is a signal amplification method suitable for our microchip. First, we optimized the experimental conditions using a biotinylated target (B-target) DNA (Fig. 1, Experiment 1), and obtained a limit of detection (LOD) of 1.7 pM with assay time of 17 min (Fig. 2). Next, we applied the optimized conditions to SNP typing of non-labeled target DNA (Fig. 1, Experiment 2), and succeeded in SNP typing of 10 pM target with assay time of 25 min (Fig. 3).

Since the proposed method is simple, rapid, and sensitive, we believe that it will be effective for point-of-care or field use, including medical diagnostics, identification of biowarfare agents, and forensic examinations.

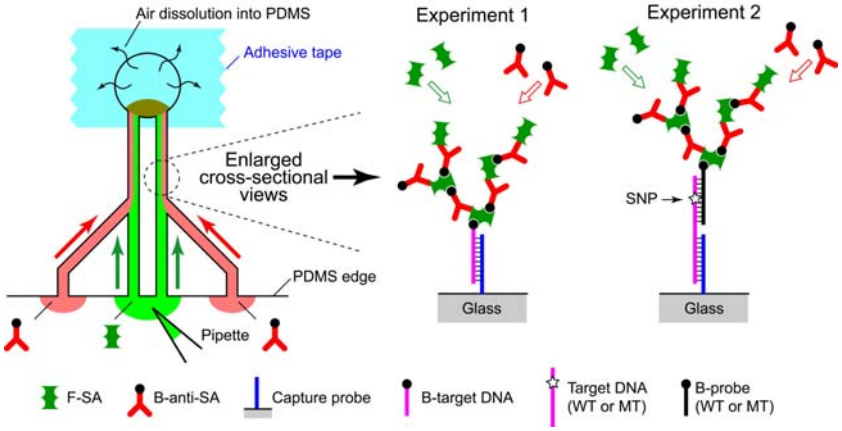

Fig. 1. Schematics of the LFDA step of the experiments: (Experiment 1) sensing of B-target DNA and (Experiment 2) SNP typing of non-labeled target DNA. F-SA = FITC-labeled streptavidin; B-anti-SA = biotinylated anti-streptavidin; B-target = biotinylated target; WT = wild type; MT = mutant type; B-probe = biotinylated probe.

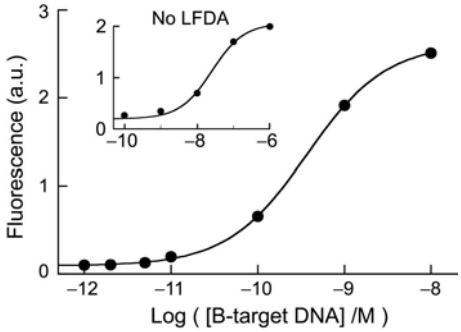

Fig. 2. Calibration plot of DNA sensing.

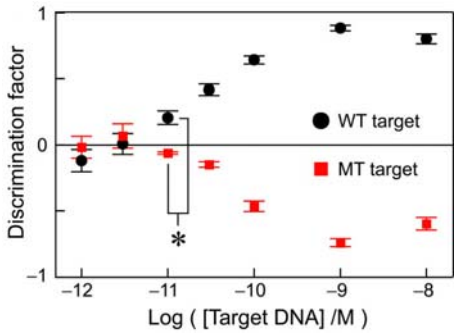

Fig. 3. Calibration plot of SNP typing.

Immunoassay on a power-free microchip with laminar flow-assisted dendritic amplification

K. Hosokawa, M. Omata, M. Maeda, *Anal. Chem*. 79 (2007) 6000.

We developed a rapid and ultrasensitive immunoassay on a microchip which needs no external power sources for fluid transport. Compared to our previous work, we improved the limit of detection (LOD) by 3 orders of magnitude by devising a new signal amplification method: laminar flow-assisted dendritic amplification (LFDA). Specifically, a sandwich immunocomplex with a biotinylated secondary antibody was constructed on the inner surface of the microchannel as described in the previous report. Onto the immunocomplex, solutions of FITC-labeled streptavidin (F-SA) and biotinylated anti-streptavidin (B-anti-SA) were supplied from laminar flow to grow a dendritic structure along the centerline of the microchannel (Figs. 1 and 2). As a result, human C-reactive protein (CRP) was detected in 23 min with an LOD of 0.15 pM (Fig. 3).

The combination of the power-free microchip and the LFDA will provide a new opportunity for ultrasensitive molecular diagnosis at the point of care.

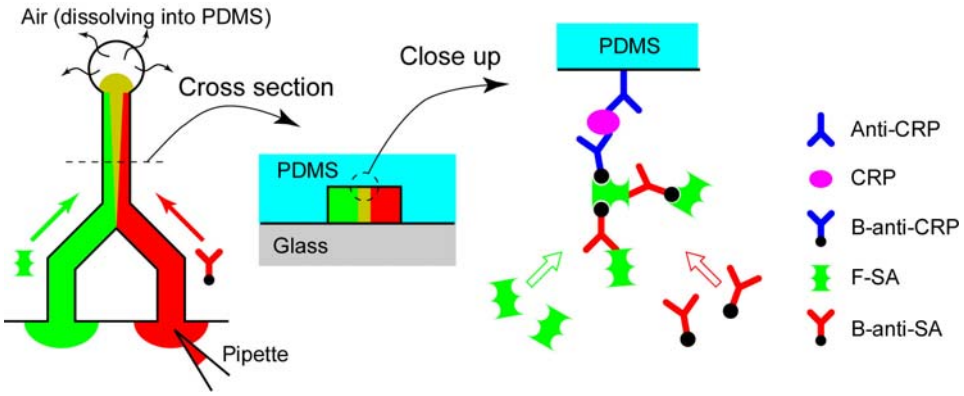

Fig. 1. Schematic description of LFDA on a power-free microchip.

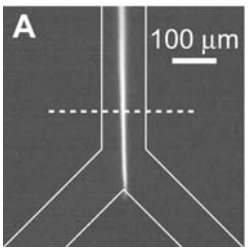

Fig. 2. Typical fluorescence image of LFDA.

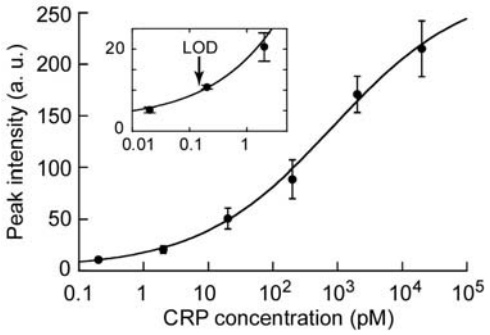

Fig. 3. Calibration plot of CRP detection.

Facile detection of toxic heavy metal ions using gold nanoparticles carrying double-stranded DNA

N. Kanayama, T. Takarada and M. Maeda *Polymer Preprints, Japan* 58 (2009) 4897

We prepared double-stranded DNA-functionalized gold nanoparticles containing a T-T mismatch pair (dsDNA-AuNP) as a detection probe for Hg^{2+} ions. The dsDNA-AuNP dispersed into an aqueous medium even at the high salt concentration. In the presence of Hg^{2+} ions, however, dsDNA-AuNP rapidly aggregated in a non-crosslinking manner, leading to a color change of the solution from red to light purple. The color change induced by surface plasmon resonance shift was easily detected by the naked eye within 5 min. The aggregation of dsDNA-AuNP with Hg^{2+} ions is attributed to formation of T-Hg^{2+}-T base pair, which should decrease the entropic repulsion between nanoparticles.

By optimizing the reaction conditions including the diameter of AuNP, the base number of dsDNA and the concentration of dsDNA-AuNP, an analytical device for simple, rapid and highly selective detection of toxic Hg^{2+} ions in aqueous solutions will be developed on the basis of the unique colloidal behavior of DNA-carrying nanoparticles.

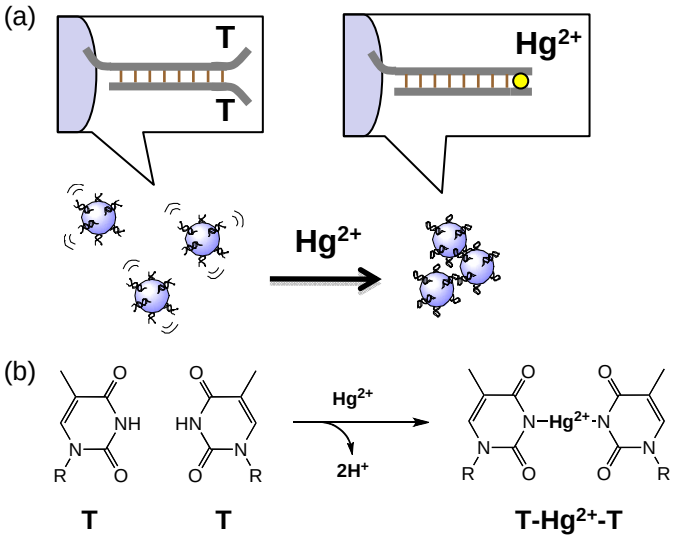

Fig 1. (a) Schematic illustration of non-crosslinking aggregation of dsDNA-AuNP induced by Hg^{2+} ions. (b) Formation of T-Hg^{2+}-T base pair.

Fig 2. Colorimetric detection of Hg^{2+} ions using dsDNA-AuNP (left: without Hg^{2+} ions, right: with Hg^{2+} ions).

A photoimmobilizable sulfobetaine-based polymer for a nonbiofouling surface

M. Sakuragi, S. Tsuzuki, S. Obuse, A. Wada, K. Matoba, I. Kubo, and Y. Ito,
***Mater. Sci. Eng.*, C30 (2010) 316**

A novel photoreactive polymer containing sulfobetaine polar groups was prepared by copolymerization of two kinds of methacrylic acids with sulfobetaine and azidoaniline. The polymer was photoimmobilized on polyester and polystyrene surfaces. Polymer immobilization altered both of the plain surfaces to becoming hydrophilic in a similar range of static contact angles. Micropattern immobilization was carried out on both polymers using a photomask. Measurements using time-of-flight secondary ion mass spectrometry detected an abundance of sulfur-containing ions in the patterned polymer, confirming that sulfobetaine had been immobilized. Protein adsorption and mammalian cell adhesiveness were reduced markedly on the immobilized regions. The reduction of cell adhesiveness was concentration-dependent for the immobilized polymer on polyester surfaces.

A novel sulfobetaine-containing polymer was immobilized photoreactively on conventional polymer surfaces and significantly reduced interactions with proteins and mammalian

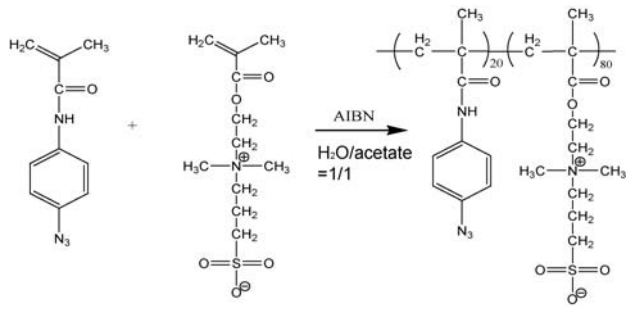

Fig. 1 Scheme for synthesizing the photoreactive sulfobetaine-containing polymer (Photo-SDA).

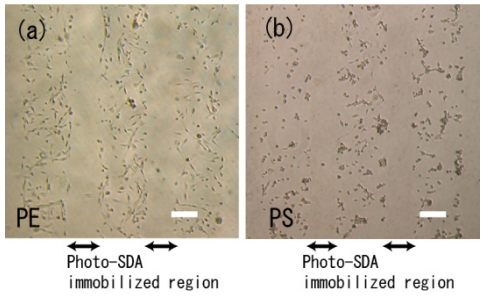

Fig. 3 Cell adhesion on a polymer immobilized region. Adhered STO cells on Photo-SDA micropatterned polyester (a) and polystyrene (b). PE: Polyester, PS: polystyrene.

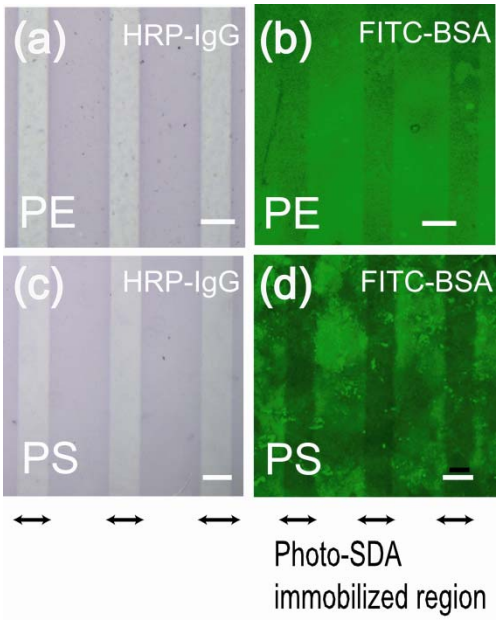

Fig 2 Phase contrast and fluorescent micrographs of HRP-IgG (a,c) and FITC-BSA (b,d) adsorbed onto a Photo-SDA micropatterned polyester (a,b) and polystyerene (c,d). The HRP conjugated protein was stained with a blue dye.

Formation of toxic protein nanoparticles by molecular chaperone prefoldin

M. Sakono, T. Zako, H. Ueda, M. Yohda and M. Maeda *FEBS J.* 275 (2009) 5982.

A number of proteins and peptides have been found to aggregate into nano-size fibrils or oligomer structures that cause various diseases. Amyloid β peptide (Aβ) forms typical fibrils and is known to cause Alzheimer's disease (AD). However, the pathological mechanism is still unknown. We found that molecular chaperone prefoldin induces a formation of toxic soluble Aβ oligomer of nano-size. In general, molecular chaperone proteins assist newly synthesized proteins for correct folding. Our results suggest that the interaction between prefoldin and Aβ oligomers prevents further aggregation and stabilizes the oligomer structure. Our result also suggests a novel function of molecular chaperone; i.e. formation of toxic nano-size protein assembly structure.

This finding suggests possible involvement of prefoldin in AD pathology. Our findings also suggest a new notion that the suppression of protein aggregation may cause the formation of toxic oligomeric species.

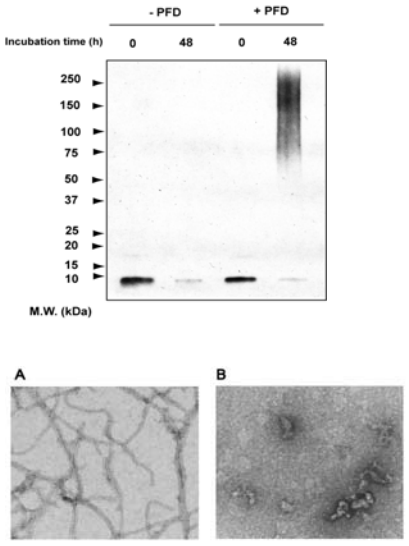

Fig. 1 Formation of soluble Abetaoligomer in the presence of molecular chaperone prefoldin. Upper panel: SDS-PAGE analysis using Abeta antibody. Lower panel: TEM images of (A) Abetafibrils formed in the absence of prefoldin and (B) Abeta oligomers formed in the presence of prefoldin

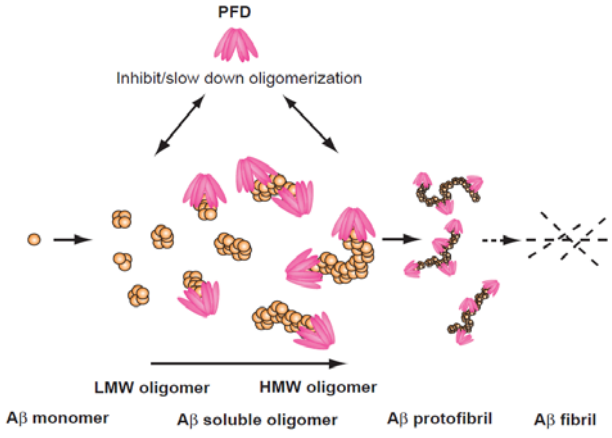

Fig 2. Schematic model of the formation of soluble Abeta oligomers in the presence of prefoldin.

Formation and toxicity of novel insulin ‘noodle’-like amyloid

T. Zako, M. Sakono, N. Hashimoto, M. Ihara and M. Maeda *Biophysical J.* 96 (2009) 3331.

Amyloid fibrils are associated with more than 20 diseases including Alzheimer’s disease and type II diabetes. Insulin is a 51-residue polypeptide hormone with its two polypeptide chains linked by one intra-chain and two inter-chain disulfide bonds, and has long been known to self-assemble *in vitro* into amyloid fibrils. We found that bovine insulin forms flexible filaments in the presence of a reducing agent, Tris (2-carboxyethyl) phosphine. Although both fibrils and filaments have beta-sheet structure, different binding properties of amyloid specific dyes such as thioflavin T suggest difference in their inner structure. Surprisingly, the cell toxicity of the insulin filaments was remarkably lower than that of the insulin fibrils.

This finding suggest that cell toxicity of amyloids correlates with their morphology. Detailed comparison between toxic fibril structure and the non-toxic filamentous structure should shed a new light on understanding of the mechanism of amyloid-associated diseases and possible pharmacological approaches to various diseases caused by amyloid fibrils.

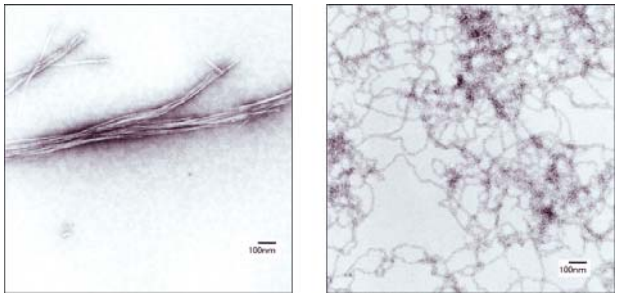

Fig. 1 Formation of ‘needle’-like insulin fibrils (left) and ‘noodle’-like insulin filaments (right)

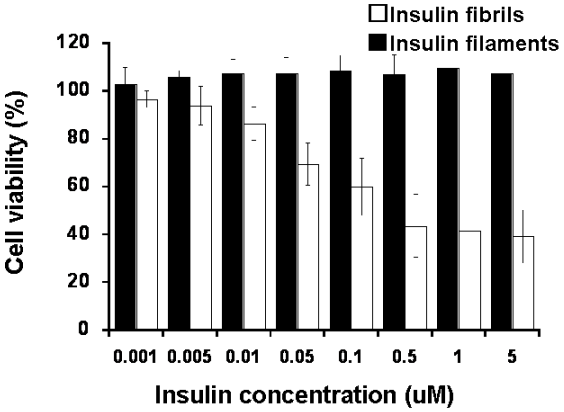

Fig 2. Toxicity of insulin amyloids. Insulin filaments are non-toxic.

Dumbbell-shaped nanocircular RNAs for RNA interference

N. Abe, H. Abe, and Y. Ito, *J. Am. Chem Soc.* 129 (2007) 15108.

We designed and synthesized dumbbell-shaped nanocircular RNAs for RNA interference applications, which consist of a stem and two loops1. RNA dumbbells are specifically recognized and cleaved by the human Dicer enzyme, and are thus transformed into double-stranded RNA in cells, although this RNA is resistant to degradation in serum. The structure was optimized to maximize its RNAi activity. The most potent activity was achieved when the stem length was 23 base pairs. The RNAi activity is prolonged by the shape of the molecule, an endless structure, compared with that of normal siRNA.

Dumbbell-shaped nanocircular RNA is potentially drug candidate for cancer or various diseases, which is based on RNA interference technology.

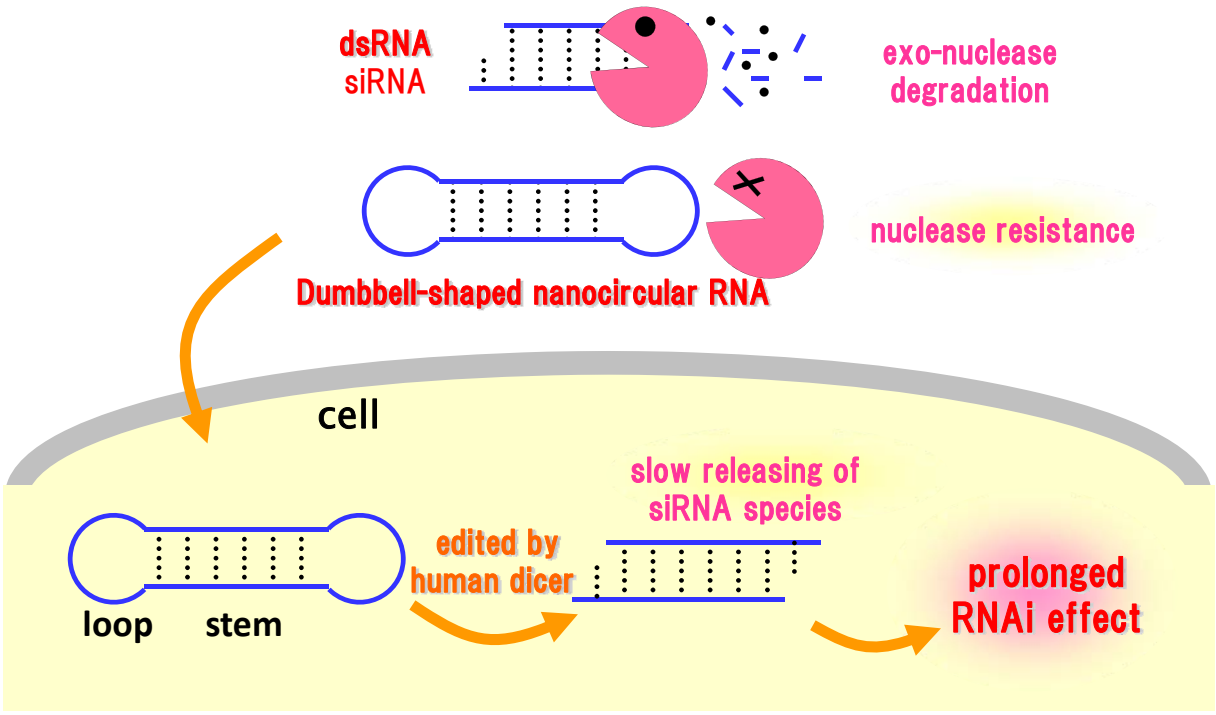

Fig 1 Dumbbell-shaped nanocircular RNA for prolonged RNA intereference

Matrix-binding growth factors for biomaterial surface modification

T. Kitajima. M. Sakuragi, H. Hasuda, T. Ozu, and Y. Ito, *Acta Biomaterialia 5* (2009) 2623.

We designed a novel chimeric protein of epidermal growth factor (EGF) with fibrin affinity for creating material surfaces with growth factor activity. The chimeric protein (FBD-EGF) was produced by the fusion of the fibronectin fibrin-binding domain (FBD) to EGF. It showed dose-dependent binding to fibrin and its binding was stable for at least 7 days, while native EGF showed little affinity. FBD-EGF promoted the growth of fibroblasts and keratinocytes in the fibrin-bound state as well as in the soluble state. Its potential was further studied on wound healing using a keratinocyte culture system in which fibrin was exposed upon injury of cell sheets. Fibrin-bound FBD-EGF promoted growth of the sheets over the injured area at a significantly faster rate than native EGF.

The present chimeric protein design will be extended to construct various growth factor fusion proteins with binding affinity. These protein will be applied to prepare material surfaces with biological activities, e.g. growth and differentiation and to tissue engineering (in vitro tissue construction etc).

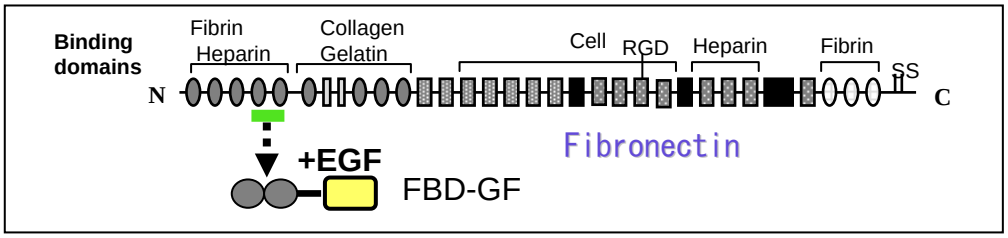

Fig. 1 Design of chimeric EGF protein.

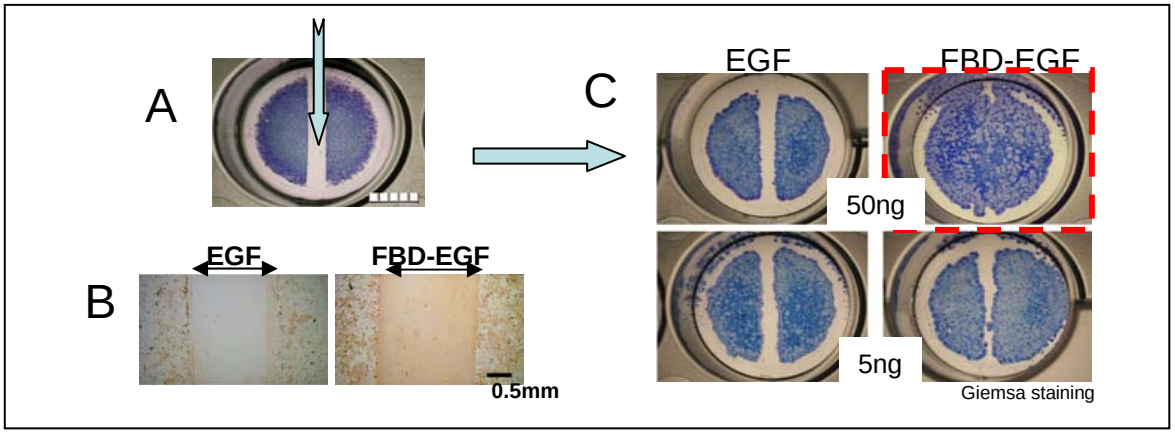

Fig. 2. Stable binding of chimeric EGF to fibrin is effective for wound healing.
A) Keratinocyte sheet grown on fibrin-coated surface was injured (2mm-wide) and EGF or FBD-EGF was applied. B) Confirmation of immobilized protein by anti-EGF staining.
C) Repair of injured sheet after 7 days. This repair process was inhibited by anti-EGF.

Localized strain effects on photoluminescence of quantum dots induced by nanoprobe indentation

K.Ozasa, M.Maeda, M.Hara, M.Ohashi, Y.H.Liang, H.Kakoi, Y.Arai,
***Physica E*, 40 (2008) 1920.**
K.Ozasa, M.Maeda, M.Hara, H.Kakoi, L.Xu, Y.H.Liang, Y.Arai,
***J.Vac.Sci.Technol. B*, 27 (2009) 934.**

We have examined localized strain effects on the photoluminescence (PL) of quantum dots (QDs) induced by nanoprobe indentation, and found that the fine PL peaks originating from single QDs show blue shifts and enhancement/decrease as indentation force increases [Fig 1]. By analyzing direct-to-indirect transition in the band line up of the QDs and surrounding GaAs matrix through numerical simulation, we concluded that the PL quench should be attributed to the crossover of Γ-band of InGaAs and X-band of InGaAs if the bowing parameter of InGaAs X-band of 1050±50 meV can be assumed, or to the electron repulsion from the nanoprobe due to conduction-band-energy increase.

The investigation shows a high possibility of localized strain to obtain improved device performances in quantum structures. The localized strain can also be applied to bio-molecules to modify their chemical properties.

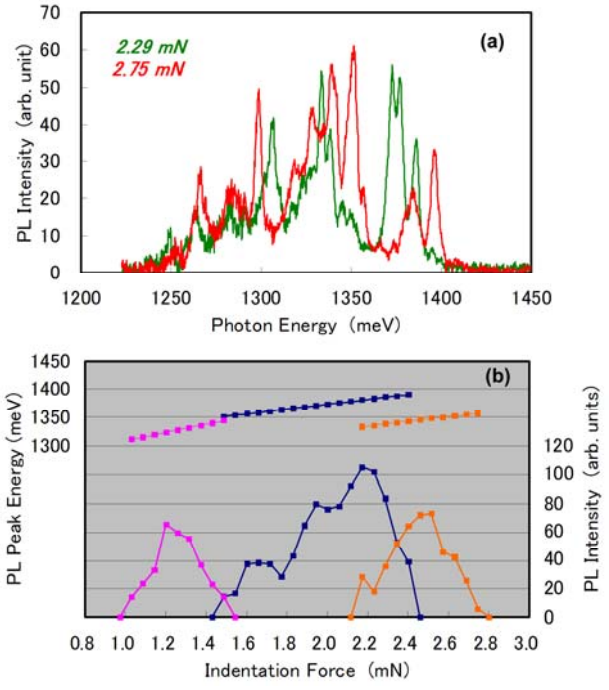

Fig. 1 (a) Full PL spectrum of the QDs with indentation force of 2.28 mN and 2.75 mN. (b) Three examples of dependence of peak energy and intensity on the indentation force.

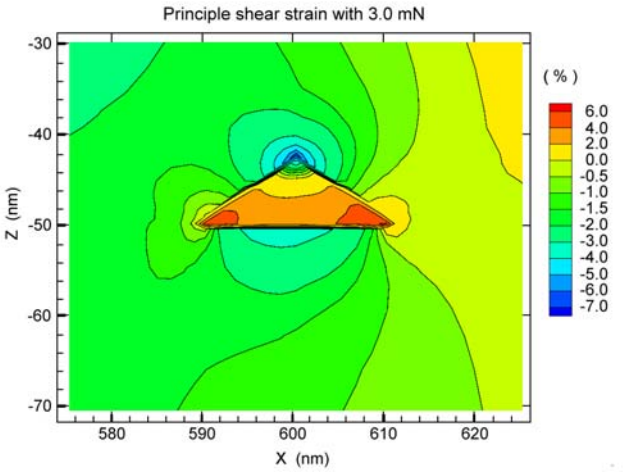

Fig. 2 Calculated distributions of principle shear strain, for the QD locating at the edge of the nanoprobe with 3.0 mN indentation.

Gene diagnosis in living cells

K. Furukawa, H. Abe, K. Hibino, Y. Sako, S.Tsuneda, and Y. Ito,
***Bioconjugate Chem* 20 (2009) 1026.**

Oligonucleotide-templated reactions are attracting attention as a method for RNA detection in living cells. We reported a novel probe for gene diagnosis in single cell, a reduction-triggered fluorescent amplification probe that is capable of amplifying a target signal. Azidomethyl fluorescein was newly synthesized and introduced into a probe. Azido-masked fluorescein on the probe showed a strong turn-on fluorescence signal upon oligonucleotide-templated Staudinger reduction. The catalytic reaction of the probe offered a turnover number of 50 as fluorescence readout within 4 h. Finally, probes were introduced into human leukemia HL-60 cells by use of streptolysin O pore-forming peptide. We successfully detected and quantitated the 28S rRNA and ┌-Actin mRNA signal above the background by flow cytometry. In addition, the same RNA targets were imaged by fluorescence microscopy. The data suggest that a reduction-triggered amplification probe may be a powerful tool in analyzing the localization, transcription, or processing of RNA species in living eukaryotic cells and moreover advanced gene diagnosis.

The new probe can be used to basic scientific tool for revealing role of RNA in living cells. In addition, it can be novel clinical method for gene diagnosis in living single cell that has never been reported.

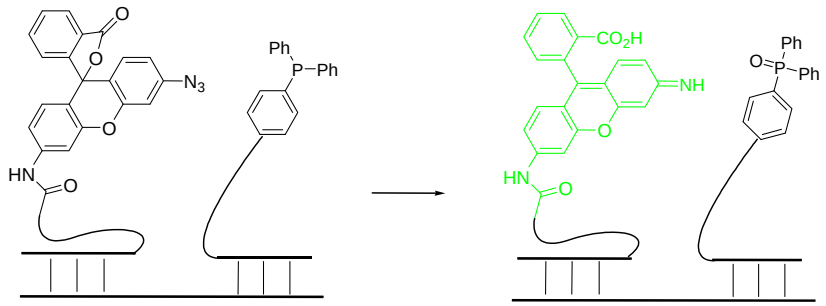

Fig 1 Chemical reaction-triggered fluorescent probe for genetic detection

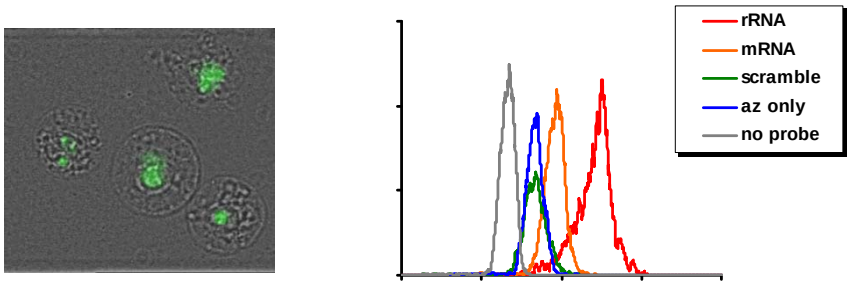

Fig 2 Imaging and quantitative analysis of gene expression in living cells.

Fluorescence probe for detection of protein function in living cells

T. Miyauchi, T. Yangida, Y. Sako, *Biophysics* 3 (2007) 63.

The Ras superfamily small GTPases are the major cell signaling protein regulating various cellular processes including proliferation, differentiation, movements, intracellualr transport. We developed a fluorescence probe which can be widely used to visulalize activation of small GTPases in submicrometer spatial resolution by detecting GDP/GTP exchange. In this probe, FRET from YFP-fused small GTPases to a fluorescent analogue of GTP, BODIPY(TR)-GTP is imaged. This approach allowed us to visualize confined localization of active (GTP-bound forms of) RhoA and Rac1, members of small GTPase that regulates cytoskeletons, in individual focal adhesions. Activated RhoA accumulated in immobile and long-lived focal adhesions but was not evident in unstable and temporary adhesions, while activated Rac1 was observed at every adhesion.

FRET-based indicators have already been developed to visualize RhoA and Rac1 activity in living cells. However, these indicators use one of the interactions between RhoA (Rac1) and the RhoA (Rac1)-binding domain of their effector proteins. Our probe that directly detecting activation (GDP-GTP exchange) of the small GTPases first achieved submicrometer resolution.

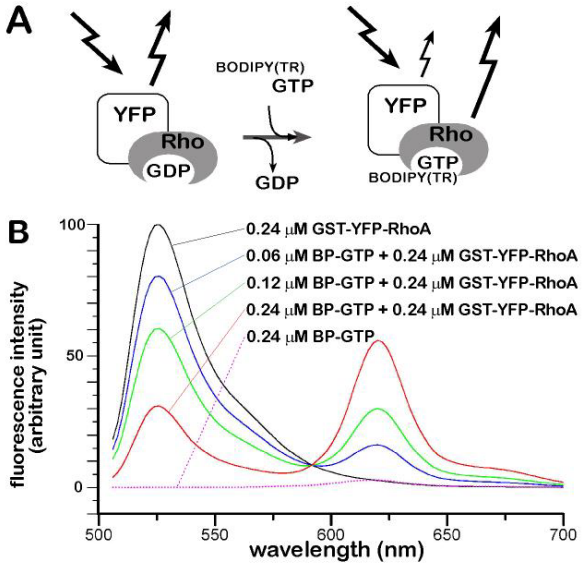

Fig 1. A FRET-based detection of GDP/GTP exchange on small GTPases. A. The principle for detecting GDP/GTP exchange. B. Fluorescence emission spectra of YFP-RhoA in the presence of various concentrations of BP-GTP.

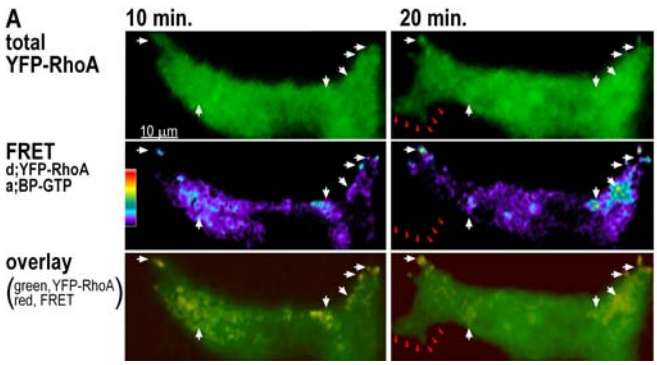

Fig 2. FRET signals of RhoA in a PC12D cell stimulated with NGF. Intracellular distribution of FRET signals were observed in a cell stimulated with NGF. BP-GTP was microinjected into the cell 10 min prior to the stimulation with NGF starting at time 0. Upper row: fluorescence from YFP-Rho. Middle row: FRET signals. Lower row: merged images of YFP Rho (green) and FRET signals (red). White arrows indicate positions with prominent FRET signals. Red arrowheads indicate locations of evident morphological changes of the cell observed after stimulation with NGF.

Two-color semiconductor Quantum dots wire utilizing the complementarity of DNA

S.-i. Tanaka, T. Miyata, T. Kato, K. Namba, T. Yangida, Y. Sako, S. Kawata, Y. Inouye,
"*DNA-Based Nanodevices*" (2008) 116

Arrangement of nano-particles at the molecular level is of crucial importance to realize multi-functional biosensors or molecular devices. We report a method for DNA-directed arrangement of Q-dot. A template DNA more than 1,000 bases in length with a repeat unit of 100 bases was synthesized by enzymatic reactions. Alternating Q-dots alignment was fabricated by using complementary binding between the template DNA and short fragments of DNA with two different sequences. Each of them was modified with two different colors of Q-dots by the avidin-biotin reaction and a reaction between an amino group and a sulfo-NHS group, respectively. Alignment of Q-dots on the template DNA was assessed by fluorescence microscopy, atomic force microscopy (AFM) and transmission electron microscopy (TEM). In AFM and TEM images, we have successfully observed Q-dots alignment on the template DNA.

Nano-particles possess size-tunable optical, electrical and magnetic properties. Especially, semiconductor nano-particle (Quantum dot (Q-dot)) can be used for multi-color biological imaging as well as for the construction of multi-functional biosensors and molecular devices. Our results provide a good starting point to the fabrication of two-color Q-dot wire on the template DNA.

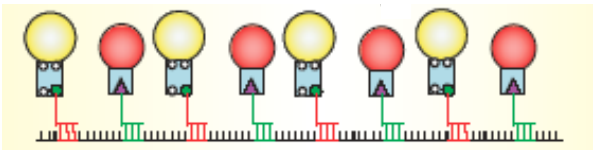

Fig 1. Arrignment of two-color semiconductor Quantam dots on a DNA template

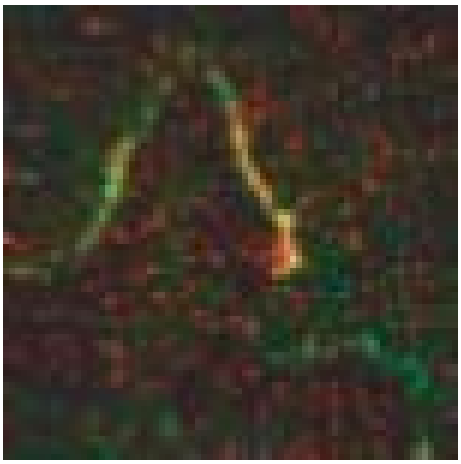

Fig 2. Observation of the two-color Quantam dots arringment using fluorescence microscopy

Controlling the number and positions of oligonucleotides on gold nanoparticles

K. Suzuki, K. Hosokawa, M. Maeda, *J. Am. Chem. Soc.* 131 (2009) 7518.

We developed a new method to immobilize a given number of oligonucleotides (ODNs) on gold nanoparticles (AuNPs) in a specific arrangement directed by a geometrical template made of DNA. The basic strategy is shown in Fig. 1. First, a set of thiolated ODNs for immobilization **1**, **2**, and a nonthiolated template **3** are hybridized to make a DNA nanostructure **4**. Next, the DNA nanostructure **4** is reacted with AuNPs **5** via the thiol groups to form a complex of the AuNP and the DNA nanostructure **6**. Finally, the intended AuNP/ODN conjugate **7** is obtained by removing the template **3** from the complex. The above strategy enables us to make various formats of AuNP/ODN conjugates simply by changing the design of the DNA nanostructure. We demonstrated proof-of-concept experiments using other AuNPs as labels for TEM imaging (Fig. 2).

The precisely controlled AuNP/ODN conjugates are promising new materials for biological sensing as well as for bottom-up nanotechnologies based on the Watson-Crick base pairing.

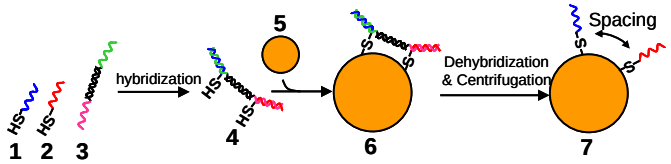

Fig. 1. Schematic illustration of the strategy for controlled immobilization of ODNs onto AuNPs.

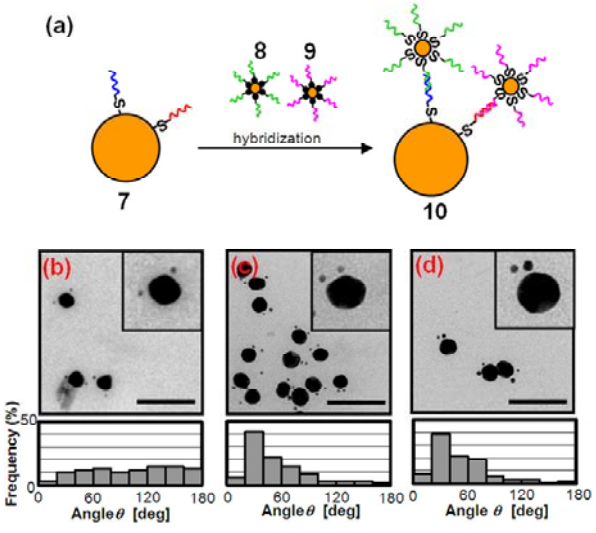

Fig 2. TEM observation of the AuNPs. (a) Labeling scheme. (b) AuNPs made without template. (c,d) AuNPs made with the template. scale bars, 100 nm.