

Self-Assembled Monolayers Related Reagents ~Alkanethiol Derivative~

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1. Introduction

SAMs (Self-Assembled Monolayers) are organized layers of molecules which spontaneously forms on a solid surface. SAMs are easily modified at a single molecular level as well as at the assembled levels.

Due to the high applicability, SAMs has allowed various studies such as mechanism of electron transfer on molecular layers, and biosensor in recent years.

It is known that thiol and disulfide derivatives form high-density and unidirectional layers on a noble metal surface such as gold¹⁻⁵), silver^{4,6,7}), copper^{4,8,9}), palladium^{10,11}), and platinum¹²⁻¹⁴). SAMs formed with thiol and disulfide derivatives on gold-coated glass plate are used for biosensors (SPR¹⁵⁻¹⁷), QCM¹⁸⁻²⁰), functionalization of gold nanoparticle²¹⁻²⁴), and are applied as electronic materials (listography^{25,26}), photoelectric conversion^{27,28}), and molecular electronic device²⁹).

2. The Mechanism of SAM Formation

The molecules must have the following characteristics to form SAM. 1) they must have functional groups which reacts with metal atom on glass plate surfaces. 2) they must assemble by themselves and have intermolecular interaction to form uni-directional layers in high-density. When thiol derivative is used, functional grope will be have thiol or disulphide which forms strong bond with Au and interaction will be van der Waals force from alkane chain and π - π stacking between the aromatic hydrocarbon.

The formation scheme of alkane thiol is shown in fig. 1. When a gold-coated glass plate is immersed in a thiol derivative solution, alkane thiols begin to bind to the gold-coated glass plate as Au-S formation. When amount of Au-S bond increased, intermolecular interactions between each SAMs help to form more high-density and highly oriented SAMs.

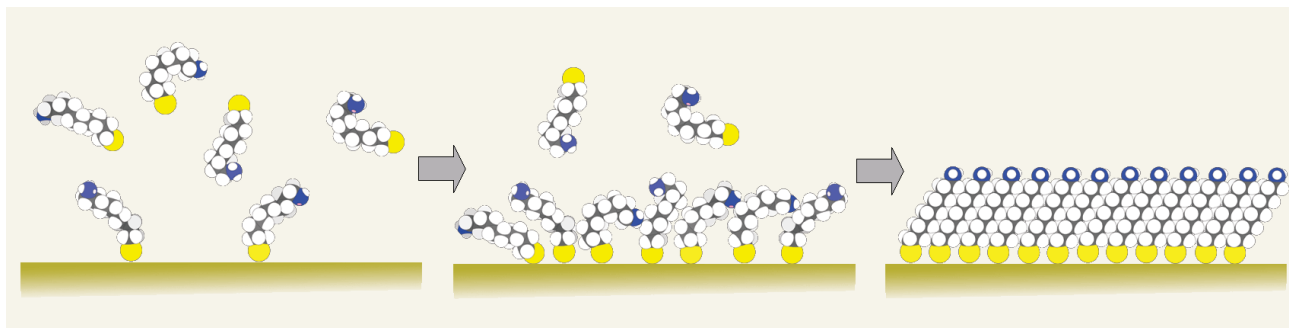


Fig. 1 Schematic of Alkanethiol SAM Formation

Fig. 2 shows *in situ* STM image when decanethiol is formed at closest packing on Au(III) surface. The distance between orange-colored spots is 0.5 nm, which is equal to $\sqrt{3}$ times the distance between each Au atoms (0.29 nm). Unit cell is $\sqrt{3}$ times in length as that of the Au. Since the direction of molecular arrangement slips off 30 degrees from atomic arrangement of the substance, it is known as ($\sqrt{3}\times\sqrt{3}$) R30° structure³⁰).

Alkanethiol derivatives also form SAMs on a gold crystal surface such as Au(100), Au(200), Au(220), and Au(311)^{3,31,32}. Therefore, a single-crystal Au(III) plate is not always required for applications.

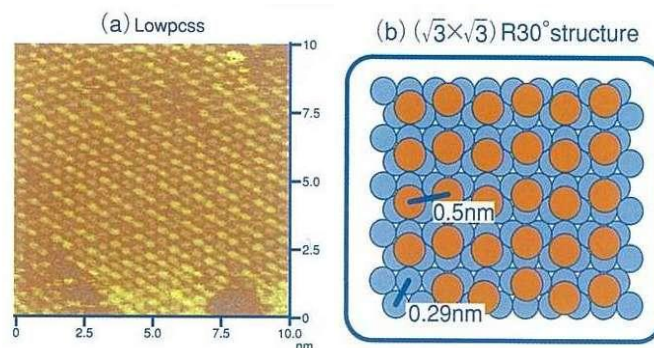


Fig. 2 (a) *in situ* STM picture of C10SH SAM on Au(111)
 (b) Schematic of adsorbed thiol molecule on Au(111)
 Dr. Uozaki, Hokkaido University

3. SAM Preparation

One of the features of SAM is a simple preparation. No special equipment is required to prepare SAM layers. The preparation is done by simple immersing of a gold-coated glass plate in a thiol/disulfide derivative solution in appropriate concentration. The overview and precaution of preparation for SAM on gold-coated glass plate is explained below.

<Procedure>

- (1) Prepare a gold-coated glass plate pre-washed with Piranha solution.*¹⁾
- (2) Immerse the gold-coated glass plate in 0.01~11mmol/l of thiol derivative solution for 30min ~ 24h.*²⁾
- (3) Wash the SAM-coated plate with ethanol and then distilled water.*³⁾
- (4) Dry the plate under nitrogen as necessary.*⁴⁾

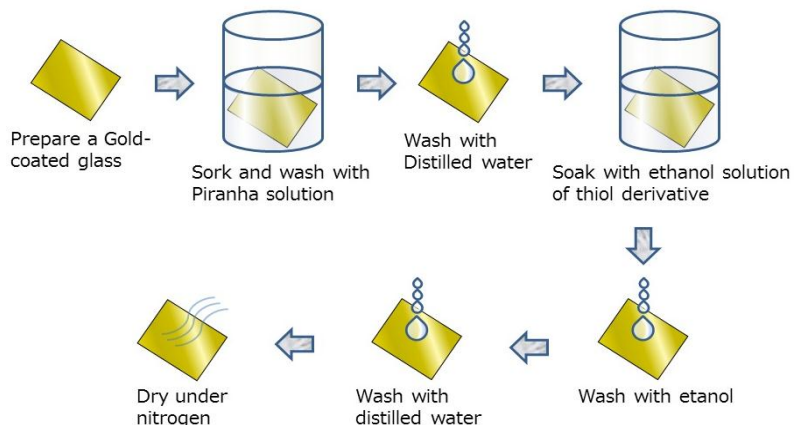


Fig. 3 Preparation method of SAM

*1) Bare metal surface tend to absorb organic substances, therefore it is necessary to remove the organic substances from metal surface before preparation of SAMs.

Piranha solution (sulfuric acid and 30% hydrogen peroxide, 3:1) is commonly used for washing of gold and platinum surfaces. After 10 to 15 minutes of immersing, wash it with

distilled water. It is important to understand that Piranha solution is an oxidizing agent. Piranha solution requires additional handling and disposal procedure, because it intensively reacts with organic substances. Although specialized equipment is required, UV Ozone is also used as effective washing method.

Among various metal plates, gold substrate is commonly used for applications of SAMs, because of 1) availability, 2) handling, 3) non-oxidizing ability, 4) adaptability to various analytical instruments such as SPR, QCM, RAIRS, and ellipsometry. Formation of SAM is still possible with other metal plates. However, minimization of oxide film is required.^{4, 6-14)}

- * 2) Formation of SAM depends on concentration of thiol derivative, immersing time, solvent, and immersing temperature.

<Density and Immersing Time>

Density and immersing time are related each other. A thiol solution with lower concentration requires longer immersing time for SAM formation^{33), 34)}.

A few mmol/l of thiol derivative solution starts to bind on a gold-coated glass plate within a few minutes. A plate is saturated with Au-S formation after re-oriented for a few hours. The higher concentration of thiol derivative solution, shorter immersing time may be required. However, it is proposed that higher oriented SAM is formed when a plate is immersed for longer time in lower concentrated thiol derivative solution.

<Solvent>

Various solvents can be used as long as each solvent dissolves thiol derivatives. However, ethanol is widely used as a solvent due to its capability for thiol derivatives containing various types of polar characteristics and molecular weight. High-grade ethanol is commercially available. Moreover, less toxicity of ethanol to human is also one of the reason. It has not been investigated as much, but there have been reported that the SAM formed by nonpolar solvent has poor orientation than that of SAM formed in ethanol^{33, 35)}.

Thiol derivative has a nucleophilic character. When using thiol derivative, make sure to choose a solvent which does not react with the nucleophile. There is no significant influence of dissolved oxygen when using gold plate. But when using oxygen-sensitive metallic plate, minimization of dissolved is require.

<Temperature>

It has been reported that if SAM layers are formed above 25°C, incomplete SAM layers will be formed.^{35,36)} Also, when temperature of a solution is higher than room temperature, desorption of impurities are increased and reorientation is accelerated.

- * 3) Dithiobis (succinimidyl undecanoate) is hydrolyzed by water. Therefore, it should not be washed with water. Wash it with appropriate solvent and dry.
- * 4) When storing the SAM modified plate, store it in dry condition rather than in the solution.³⁷⁾ In addition, it is recommended to store the plate under nitrogen gases and protect from light to prevent the oxidation of thiol site.

4. Effect of Molecular Structure of Alkanthiol Derivative

SAM can be formed having various function by differences such as length of alkyl chain, terminal functional group, presence of oligo ethylene glycol, and difference between thiol and disulfide. It is also possible to control character of SAM by using different derivatives simultaneously. This technique is called as "Mixed SAMs".

(1) Difference between Thiol and Disulfide

It is known that both thiol and disulfide derivatives form a same structural SAM.³⁸⁻⁴⁰⁾ It is considered that hydrogen will be produced when thiol forms Au-S bond. However, there is no academic report that says hydrogen had been detected. The solubility of thiol derivative is higher than disulfide derivatives. Thiol derivative is used more often than disulfide derivatives. However, if the terminal function group of a target reacts with thiol (e.g. activated ester, maleimido), it is necessary to use disulfide derivative for SAM layer preparation.

Alkanethiol



Symmetry Alkyldisulfide



Asymmetry Alkyldisulfide



Fig. 4 Schematic of Alkanethiol and alkyldisulfide derivative

(2) Effect of Alkyl Chain Length

Longer alkyl chained SAM enhances a stability of SAM layers. On Fig. 6, cyclic voltammetry (CV) shows reductive desorption of SAMs. According to this experiment, longer alkyl chain enables to create stronger Au-S. It is also known that a length of alkyl chain effects to electron transfer monitoring through SAMs.⁴¹⁻⁴³⁾

(3) Terminal Functional Group

There are various alkanethiol derivatives with different terminal function groups commercially available. There are many application using alkane thiol derivatives, For example, modification of metallic surface to be hydrophilic or water repellency, and immobilization of protein or antigen. Please see section 6 for additional information regarding application for biosensor and related products.

(4) Effect of Oligoethyleneglycol

Generally, a hydrophobic surface easily adsorbs a protein, whereas a hydrophilic surface suppresses a protein adsorption. Even polarity of oligoethyleneglycol (-EG₆OH) in between hydroxyl group(-OH) and amido group(-CONH₂).⁴⁴⁾ It has been reported that oligoethyleneglycol (-EG₆OH) suppresses nonspecific absorption of protein more its polar characteristic. It is considered because of steric repulsion effect of oligoethyleneglycol chain. Therefore, oligoethyleneglycol is suitable for producing a biosensor with less nonspecific adsorption of protein. Alkane thiol derivatives containing oligoethylene glycol therefore, has been widely used for biosensors to create desired SAM layers.

(5) Mixed SAMs

There are two ways to prepare Mixed SAM. One is to prepare by mixing two different solutions of alkane thiol derivatives, and the other is to use an asymmetric disulfide derivatives. The assembly process is a dynamic equilibrium that favors formation of the most energetically stable SAM. Even if either method is used, the composition ratio of the SAM deviates from the initial ratio of the components. Especially, by using thiol derivative of different alkyl chains length, ratio of longer alkyl chain is increased and phase-separated structure aggregated the same alkyl chain length is formed.⁴⁵⁻⁴⁸⁾

The one of the useful application of Mixed SAM is for biosensor using a alkane thiol derivative introduced oligoethyleneglycol moiety. In order to bind antigen and antibody on a glass plate through SAMs, thiol derivatives containing carboxyl group or amino group can be used. If either terminal function group is used alone, non-specific adsorption is caused by electrostatic interaction. However, if they are used with an oligoethyleneglycol (-EG₆OH) with hydroxylic acid terminal, occurrence of non-specific adsorption can be prevented significantly.⁴⁹⁾

5. Evaluation Method of SAMs

Formation process and orientation structures of the SAM layers have been studied in STM/AFM, XPS, SPR, QCM, contact angle, and CV. Fig. 5 shows the forming ability of SAM layers using 11-Amino-1-undecanethiol, hydrochloride [product code : A423] on a gold-coated glass plate in ethanol. After immediate formation of Au-S bonds, amount of formed-SAM layers are gradually increased and proceed towards steady state by reorientation.

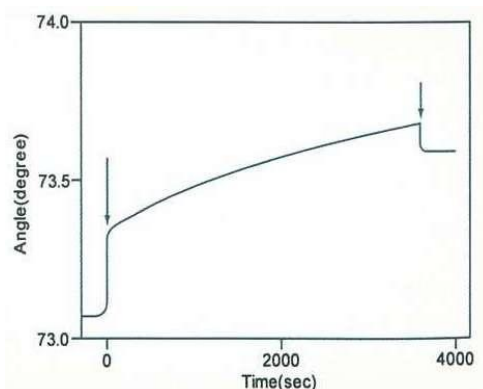


Fig. 5 SAM forming process(SPR)
*10 $\mu\text{mol/l}$ 11-AUT,HCl(22°C)
Dr. Tsutsui, Dr. Fujita, Dr. Yokoi,
Kyusyu University

<Evaluation of SAM Reductive Desorption by CV>

1) CV measurement conditions

- A) Working Electrode: SAMs
- B) Modified Electrode / Reference Electrode: Ag / AgCl electrode (KCl saturation)
- C) Return Electrode: Pt Plate
- D) Inception Voltage: -0.1V
- E) Maximum Sweep Voltage: -0.1V
- F) Minimum Sweep Voltage: -1.3V
- G) Sweep Rate: 50 mV/sec, 0.1 mmol/l KOH solution, under N₂(remove the dissolved oxygen by bubbling N₂ for 15 min. before measurement)

2) Information Obtained from Cyclic Voltammogram

Formed SAMs on a gold electrode can be confirmed by presence of irreversible cathode current from elimination of the thiolate anion on the gold surface.

The absorbed amount of thiol derivatives can be estimated as integral value of the area of the irreversible cathode current. In the cyclic voltammogram of Fig. 6, the adsorbed amount of each one of amino alkanethiol hydrochlorides with different alkyl chain is nearly 1.3 nmol/cm^2 with 5 minutes immersed time regardless of the chain length. From this result, it is considered that the forming rate of SAM will not be changed by chain length.

Each reductive desorption potential peak indicates a desorption potential of absorbed species from the gold surface. According to cyclic voltammogram shown on Fig. 6, the reductive desorption potential peak was shifted to negative potential when alkyl chain is longer. The longer alkyl chain of amino alkanethiol hydrochloride, are less likely to be eliminated from the gold electrode. Therefore, the stability of SAM is greater when the length of the alkane chain is longer.

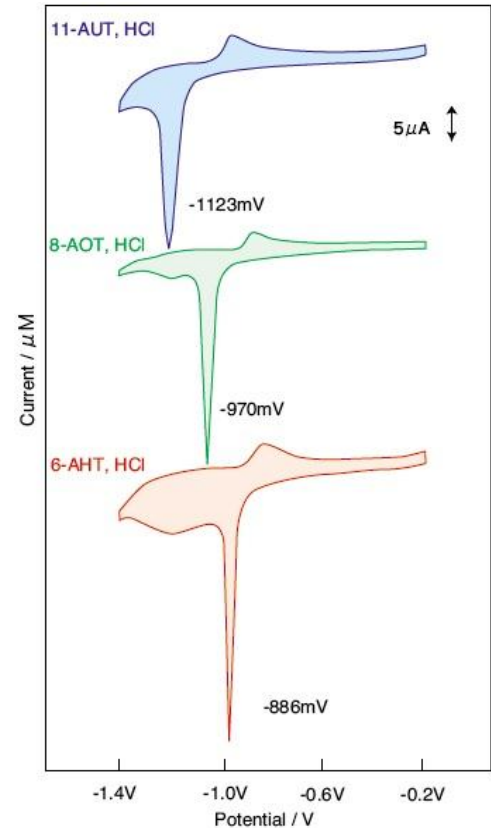


Fig. 6 Cyclic voltammogram of Aminoalkanethiol type

6. Biosensor Application of SAMs and Relative Products

SPR and QCM are widely used in biosensor applications because they can measure intermolecular interaction without labeling measuring objects by monitoring the changes of each refractive index and frequency of quartz (QCM).

SAM is widely used for immobilization of antibody, antigen, and DNA. The immobilization methods of SAM are listed in below.

- 1) SAM of terminal Carboxylic acid
Ester-activation by WSC(EDC)/NHS => Combine with amino group of objective substance
- 2) SAM of terminal amino group
Glutaraldehyde reaction => Combine with amino group of objective substance
- 3) SAM of terminal NTA
Ni complexation => Combine with His-Tag introduced objective substance
- 4) SAM of terminal biotin
biotin immobilization => Combine with streptavidine labeled objective substance

6-1. Application of Carboxylic Acid Terminal SAM

Protein such as antibody has reactive lysine residue. The method using activated ester is one of the most commonly used methods for protein immobilization. After immobilizing the SAM of carboxylic acid terminal, carboxylic acid is converted to NHS ester group using WSC (EDC) and *N*-Hydroxy succineimide (NHS). Amino group of protein reacts with NHS ester and forms a covalent bond (Fig. 7).

By using activated ester method, *Kyo et. al.* utilized SAM prepared with 10-Carboxy-1-decanethiol [Product Code: C385] and Carboxy-EG6-undecanethiol [Product Code: C445] to immobilize an antibody. The mixing ratio of 9 to 1 (Carboxy-EG₆-undecanethiol and Hydroxy-EG₃-undecanethiol) was used in their experiment to reduce nonspecific binding significantly.⁴⁹⁾

DOJINDO has developed Carboxylic acid-SAM Formation Reagent [Product Code: C488] which is optimized to suppress nonspecific adsorption and is available in convenient size.

In addition, Dojindo also offer Amine Coupling Kit [Product Code: A515] which contains a set of various buffer and reagents to activate carboxyl groups.

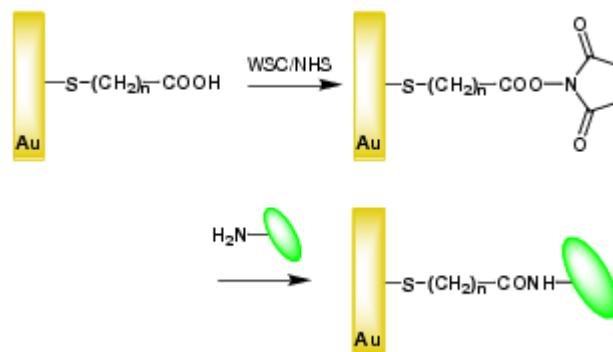
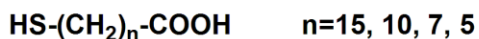


Fig. 7 Immobilization of protein by activation of carboxylic acid terminal SAM

<Related Products>

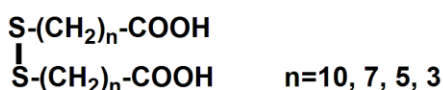
Carboxyl type

[C429] 15-Carboxy-1-pentadecanethiol
[C385] 10-Carboxy-1-decanethiol
[C386] 7-Carboxy-1-heptanethiol
[C387] 5-Carboxy-1-pentanethiol



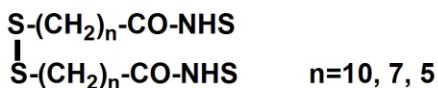
Carboxy disulfide type

[C404] 10-Carboxydecyl disulfide
[C405] 7-Carboxyheptyl disulfide
[C406] 5-Carboxypentyl disulfide
[D524] 4,4'-Dithiodibutyric acid



Carboxy disulfide NHS ester type

[D537] Dithiobis(succinimidyl undecanoate)
[D538] Dithiobis(succinimidyl octanoate)
[D539] Dithiobis(succinimidyl hexanoate)



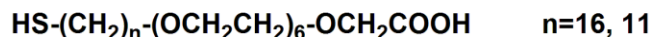
Hydroxyl type

[H394] 16-Hydroxy-1-hexadecanethiol
[H337] 11-Hydroxy-1-undecanethiol
[H338] 8-Hydroxy-1-octanethiol
[H339] 6-Hydroxy-1-hexanethiol



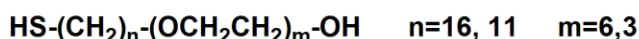
Carboxyl type introduced oligoethylene glycol

[C445] Carboxy-EG₆-undecanethiol
[C463] Carboxy-EG₆-hexadecanethiol



Hydroxyl type introduced oligoethylene glycol

[H354] Hydroxy-EG₃-undecanethiol
[H355] Hydroxy-EG₆-undecanethiol
[H395] Hydroxy-EG₃-hexadecanethiol
[H396] Hydroxy-EG₆-hexadecanethiol

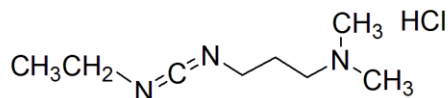


1 shot type

[C488-10] Carboxylic acid-SAM Formation Reagent
Unit : 1 μmol x 3

Activating reagent of Carboxyl group

[W001] WSC (EDC)



[A515] Amine Coupling Kit Unit : 2 ml x 4

6-2. Application of Amino Terminal SAM

For desired substance containing SH group, SAM of amino terminal with activated ester group and maleimido group cross-linking reagent is useful for immobilization of DNA introduced SH group and cysteine residue peptide. An Amino terminal on the surface of the SAM reacts with cross-linking reagent, and it is converted to a maleimido group. Then, Maleimido group forms covalent bond by reacting specifically with SH group. It results in immobilizing of the desired substance (Fig. 8). *Brockman et al.* has produced DNA array by immobilizing of SH-introduced DNA.⁵⁰⁾ In addition, *Williams et al.* has prepared peptide array using peptide containing terminal cysteine.⁵¹⁾

Amino terminal SAM is also utilized for immobilization of an object with amino group by simultaneous using of glutaraldehyde which has multiple aldehyde groups. Glutaraldehyde acts as a cross-linker. The amino group reacts with the aldehyde group and forms a Schiff base as shown in the Fig. 9.

Solanki et al. has produced about cholesterol sensor (SPR) by immobilizing a cholesterol oxidase through glutaraldehyde.⁵²⁾

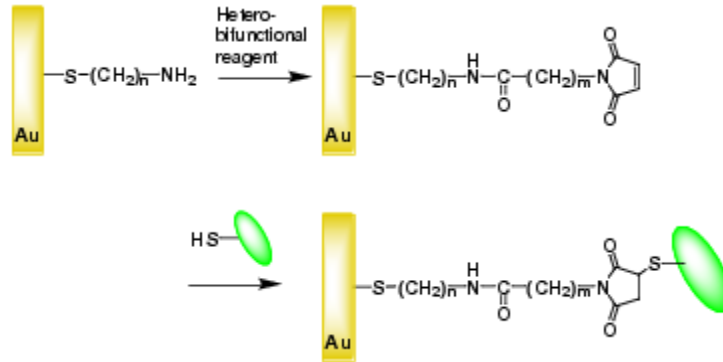


Fig. 8 Immobilization of DNA using Amino terminal SAM and cross-linking reagent

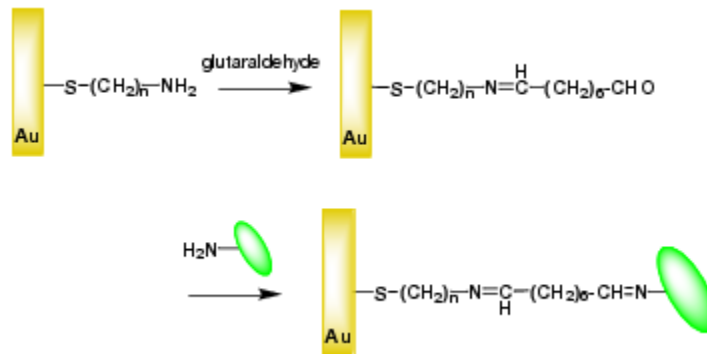
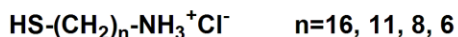


Fig. 9 Immobilization of protein using amino terminal SAM and glutaraldehyde

<Related Products>

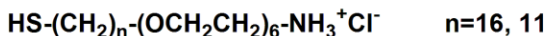
Amino type

[A458] 16-Amino-1-hexadecanethiol, hydrochloride
[A423] 11-Amino-1-undecanethiol, hydrochloride
[A424] 8-Amino-1-octanethiol, hydrochloride
[A425] 6-Amino-1-hexanethiol, hydrochloride



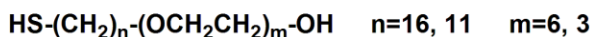
Amino type introduced oligoethylene glycol

[A483] Amino-EG₆-undecanethiol, hydrochloride
[A505] Amino-EG₆-hexadecanethiol, hydrochloride



Hydroxy type introduced oligoethylene glycol

[H354] Hydroxy-EG₃-undecanethiol
[H355] Hydroxy-EG₆-undecanethiol
[H395] Hydroxy-EG₃-hexadecanethiol
[H396] Hydroxy-EG₆-hexadecanethiol



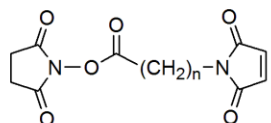
Hydroxy type

[H394] 16-Hydroxy-1-hexadecanethiol
[H337] 11-Hydroxy-1-undecanethiol
[H338] 8-Hydroxy-1-octanethiol
[H339] 6-Hydroxy-1-hexanethiol



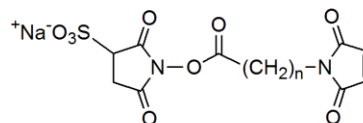
Cross-linking reagent

[K214] KMUS
[H257] HMCS
[E018] EMCS
[G005] GMBS



n=10,7,5,3

[S250] Sulfo-KMUS
[S026] Sulfo-HMCS
[S024] Sulfo-EMCS
[S025] Sulfo-GMBS



n=10,7,5,3

6-3. Application of NTA Terminal SAM

NTA forms a stable complex with heavy metals such as Ni, which allows introduction of a metal ion to compound bond NTA, biological molecule, and solid surface to metal ion. These metal chelate compounds have been used for detection and isolation of specific substance interacting with metal ion such as His-Tag protein. As for immobilization of NTA onto SAMs, there are various derivatives of NTA available. Therefore, SAM can be selected based on the type of NTA derivative.

Signal et al. has examined that immobilize NTA on the SAM containing oligoethyleglycol and combine His-Tag receptor through Ni detects of ligand. They have reported that the immobilization of His-Tag receptor using NTA-SAM results in accurate data and higher reproductively than amine coupling method (dextran gel film).⁵³⁾

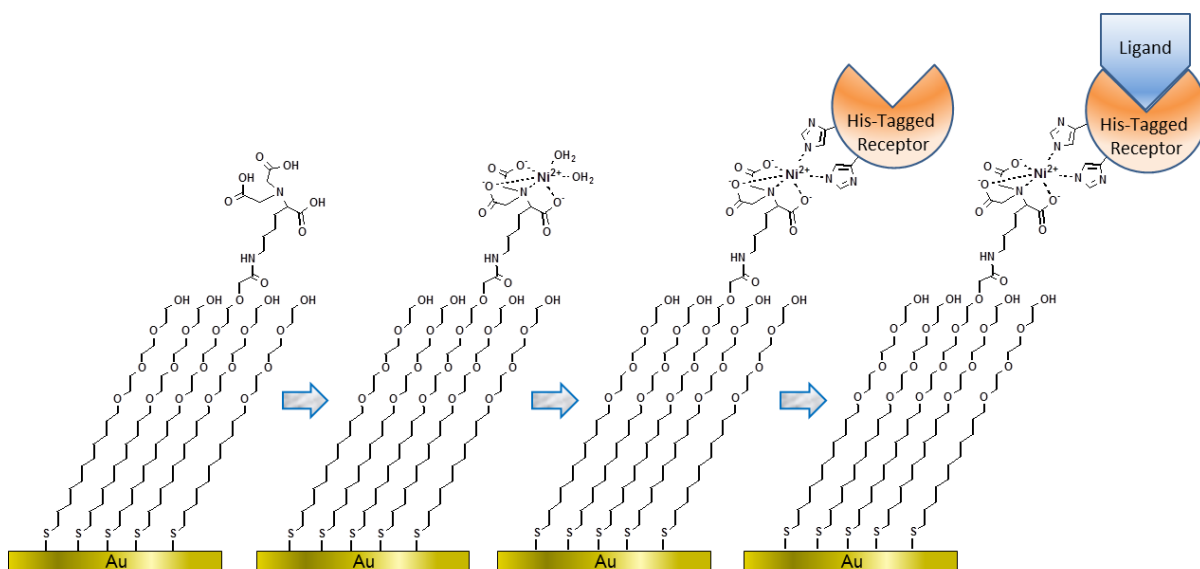
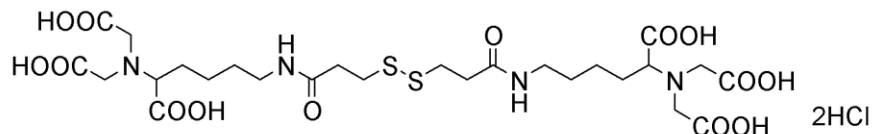


Fig. 10 Immobilization of His-Tag protein to plate immobilized NTA.

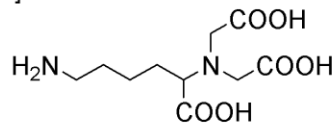
<Related Products>

[D550] Dithiobis(C₂-NTA)

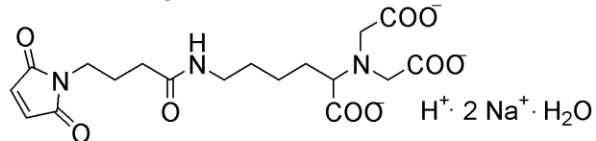


SAM reagents can be conjugated to NTA derivative below are also available.

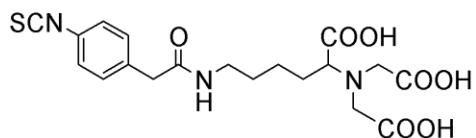
[A459] AB-NTA free acid



[M035] Maleimido-C₃-NTA



[I279] Isothiocyanobenzyl-NTA



6-4. Application of Biotin Terminal SAM

Avidin-Biotin method has been widely used in enzyme immunoassay (EIA). Avidin-Biotin method allows rapid and strong immobilization of protein. This applies in an immobilization of the substance on the SAM layers. Avidin-Biotin method also allows immobilization of biotinylated antibody, peptide, and DNA.

Biotin can be introduced onto amino terminal SAM using bitin NHS. However, optimization of biotinylation conditions is necessary to be optimized. DOJINDO offers pre-optimized Biotin-SAM Formation Reagent [Product Code:B564] for convenient use. This section represents examples of use of B564 for SPR.

<Formation of SAM using Biotin-SAM Formation Reagent>

- 1) Add 1-2 ml of ethanol to Biotin-SAM Formation Reagent (Final concentration should be 1 mmol/l when 1 ml of ethanol is added).
- 2) Prepare Piranha solution(mixture concentration: Sulfuric acid 700 ul: 30% hydrogen peroxide solution 300 ul). Drop 20 ul of Piranha solution on the gold film of sensor chip.
- 3) Rinse the sensor chip surface by ultrapure water for a couple of ten seconds, and dry it by air within 30 minutes after rinsing. Immerse the sensor chip in the solution of Biotin-SAM formation prepared during step 1.
- 4) Incubate over 12 hours in a dark room and at ambient temperature.

<Preparation of sensor immobilized antibody and AFP detection study in contaminants>

- 1) After immobilization of Biotin, set a sensor chip to the device.
- 2) Using buffer prime, equilibrate all flow channels by PBST.
- 3) ConFig a flow rate with 20 ul/min, and start SPR sensor gram.
- 4) Flow 80 ul streptabivin solution(concentration: 0.1 mg/ml) for 4 minutes.
- 5) Switch to PBST and wash the surface of the sensor.
- 6) Flow 80 ul of 0.3 mg/ml Biotinylated anti-rabbit IgG antibody solution for 4 minutes.
*Capture method using secondary antibody.
- 7) Switch to PBST and wash the surface of the sensor.
- 8) Flow 80 ul of 0.03 mg/ml anti-AFP antibody solution for 4 minutes.
- 9) Switch to PBST and wash the surface of the sensor.
- 10) Flow 80 ul of 0.01 mg/ml AFP solution(PBST) for 4 minutes.
- 11) Switch to PBST and wash the surface of the sensor.
- 12) Flow 80 ul of 0.01 mg/ml AFP solution(PBST containing 10 mg/ml BSA) for 4 minutes.
*Detection study of AFP(alpha-fetoprotein) in contaminants.
- 13) Switch to PBST and wash the surface of the sensor.

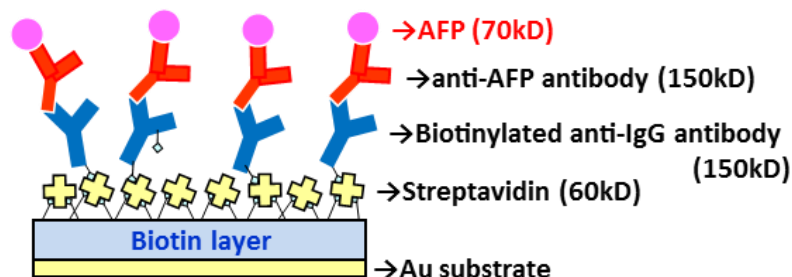


Fig. 11 AFP detection sensor

<Comparison Data between DOJINDO and Competitor>

Biotin terminal layers prepared with DOJINDO's Biotin SAM Formation reagent gives only 1.7 mdeg of nonspecific adsorption when 10 mg/ml of BSA added(Fig.12).

On the other hand, a high signal is observed at 47.4 mdeg, even only 1/1000 equivalent ratio of AFP(0.01 mg/ml) to BSA is added to sample solution. The result shows that the immobilized layers prepared with Biotin-SAM formation reagent is appropriate for a biosensing or an antibody screening when sample contains many contaminating proteins, such as serum and medium. On the other hand, immobilized layers prepared with commercially available Biotin-SAM Reagent of Company A results in concern about accuracy and reproducibility due to the increased noise (S/N=4.3).

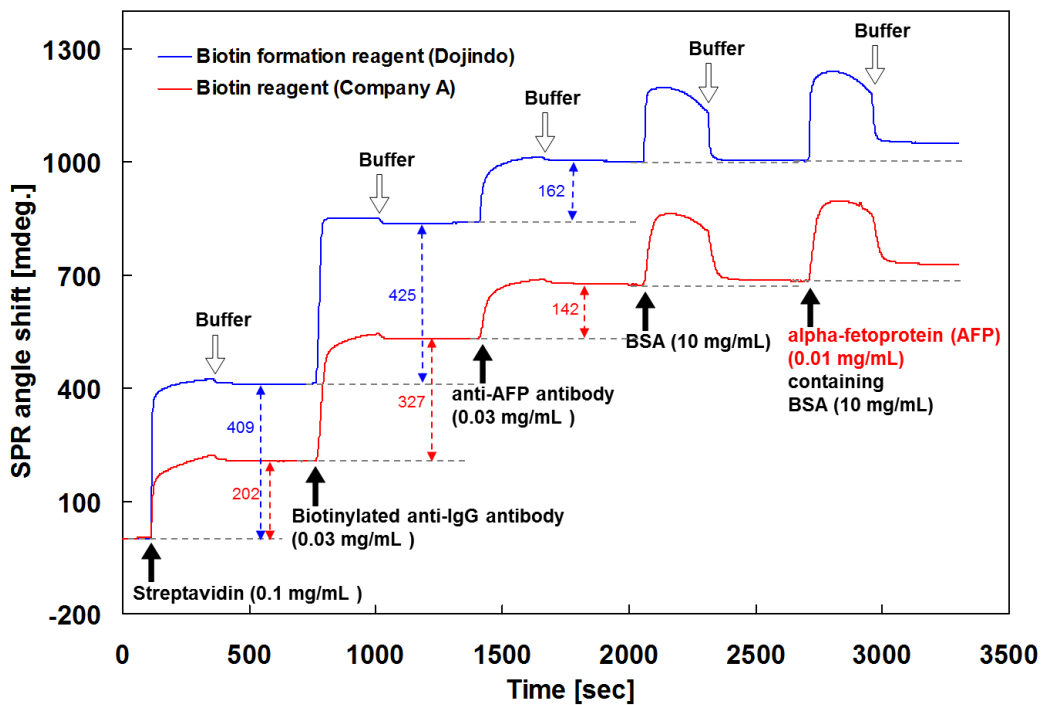


Fig. 12 SPR sensor gram of protein detection in contaminants by sensor formed by Biotin-SAM Formation Reagent and competitor

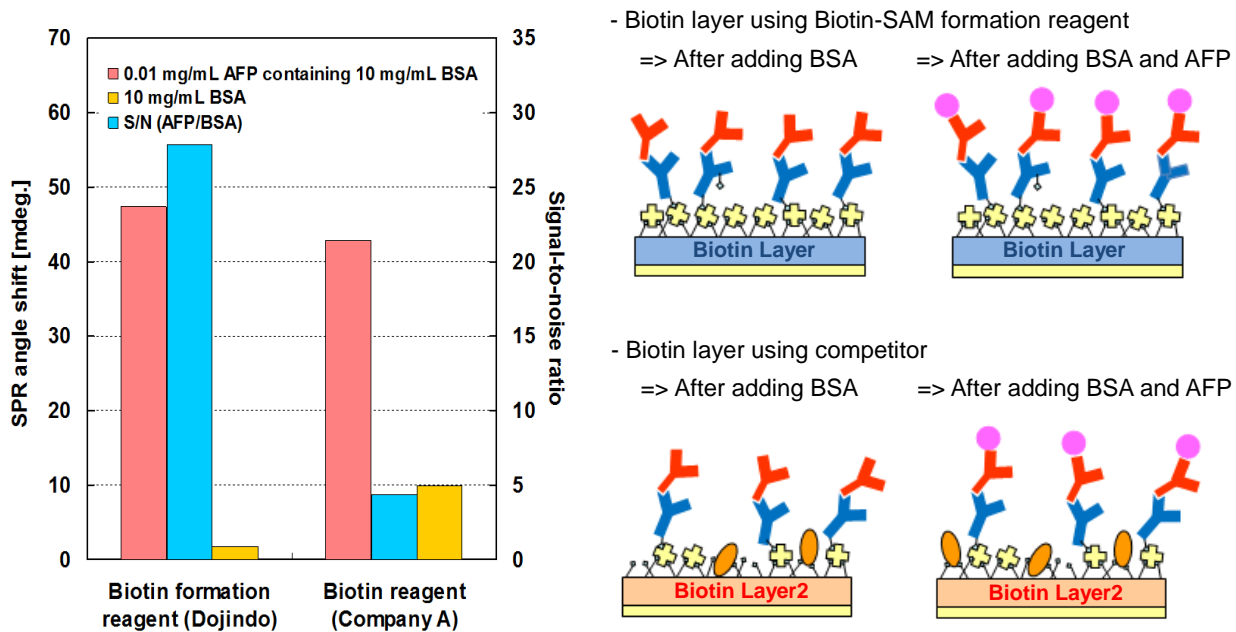


Fig. 13 S/N Comparing of protein detection in contaminants by sensor formed by Biotin-SAM Formation Reagent and competitor

<Related Products>

[B564-10] Biotin-SAM Formation Reagent, unit : 1 μ mol x 3

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7. Relative Reagent of Other SAMs

7-1. Ferrocenyl type

Ferrocenyl moiety allows simple one-electron redox reactions. Therefore, it is possible to create electrochemically active SAMs a gold surface and this technique has been used for a molecule film modified sensor. For example, *Uozaki et al.* has reported that Ferrocenyl moiety works as a mediator of electron transfer and has also reported about expression of rectification of oxidation due to the presence of the monolayer. They have prepared monomolecule films on a gold electrode surface using 11-Ferrocenyl-1-undecanethiol [product code: F246], and have measured the responses of reversible redox reactions of chemicals in solution by cyclic voltammetry(CV). Regarding adsorption state of ferrocenyl alkanethiol, there is also interesting report which suggests that orientation of ferrocenyl SAM oxidation of ferrocenyl SAM.

In addition, *S. Rubin et al.* has reported sensor device using ferrocenyl SAM. They have prepared mixed monomolecule film on a gold electrode surface using ferrocenyl alkanethiol and amino alkanethiol, and then immobilized glucoseoxidase via amino terminal of amino alkanethiol. They have prepared a sensor device using gluoxidase as a redox active site and ferrocenyl terminal as a mediator, and have studied about correlation between alkyl chain lengths and sensing abilities for glucose.

Generally, melting point of these series of Ferrocenyl SAM, especially for 6-Ferrocenyl-1-hexanethiol [product code: F269], is around 30°C. Therefore, please be aware of melting might be happened during your handling.

[F246] 11-Ferrocenyl-1-undecanethiol (CH₂)_n SH

[F247] 8-Ferrocenyl-1-octanethiol

[F269] 6-Ferrocenyl-1-hexanethiol

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7-2. Fmoc-Amino Type

Alkanethiol with Amino terminal is suitable when introduce various peptides, proteins, and others that will be molecular recognition sites. After preparing of SAM layers using amino alkane thiol which Fmoc-protected on a gold coated glass plate, amino terminal can be exposed by deprotection of the Fmoc. *J. M. Brockman et. al* have carried out an imaging of protein-DNA interactions by chemical modification of the SAM immobilized DNA after light irradiation patterning. Interaction of amino terminal and gold surface can be avoided by using Fmoc-protected amino alkane thiol. For

example, Fmoc-protected amino alkane thiol was used when SAM layers were patterning by deprotection by ultraviolet irradiation and chemical modification. The deprotection can be achieved easily by immersing the substrate in a basic solution.

[F287] *N*-Fmoc-Aminoundecanethiol

[F288] *N*-Fmoc-Aminooctanethiol

[F289] *N*-Fmoc-Aminohexanethiol

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7-3. Other Alkanethiol Derivative

It has been reported that the sulfobetaine terminal SAMs have particularly higher effects of nonspecific adsorption under 200 mmol/l or more ionic strength or weakly-basic conditions. It is expected that a high sensitive biosensor can be prepared by simultaneous using of thiol or disulfide derivative. In addition, *Ostuni et al.* have used DOJINDO's Sulfobetaine3-undecanethiol [product code: S350] for patterning research of bacteria and mammalian cell. Sulfobetaine3-undecanethiol is also expected to have further application for biomaterials patterning.

Thermal stability of Amido terminal SAMs has been reported due to its hydrogen bonding effects compared to other SAMs with different terminal. *Mosley et al* have studied about polymer sheet which can reversibly detach because of hydrogen bonding using amido terminal SAMs. Moreover SAMs with amido terminal have been used for relation study between hydrophilicity and protein adsorption, and its reducing effect of non specific protein adsorption has been confirmed.

[S350] Sulfobetaine3-undecanethiol

[A510] 10-Amido-1-decanethiol

[A509] 7-Amido-1-heptanethiol

[A508] 5-Amido-1-pentanethiol

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