

binding to the labeling substance, the disulfide structure in the polypeptide is reduced to be used as a sulfhydryl group (SH). In the reduction of the disulfide bond, dithiothreitol (DTT),  $\beta$ -mercaptoethanol ( $\beta$ -ME), and mercaptoethylamine (MEA) can be used. Therefore, when the labeling substance has a functional group having high reactivity with an amino group or a thiol group (e.g. a succinimido group and a maleimide group), the labeling substance can be directly bound to the amino and thiol groups of polypeptide by mixing the polypeptide with the labeling substance. Examples of the labeling substance include Alexa Fluor750 modified with succinimide ester and Alexa Fluor790 (manufactured by Invitrogen).

**[0125]** When the labeling substance **93** has an amino group, a thiol group, an aldehyde group, and a carboxyl group, the labeling substance **93** can be easily bound to the polypeptide support **91** by binding the labeling substance **93** to the polypeptide support **91**, for example, via a chemical cross-linker; forming a dithiol bond between the labeling substance **93** and the polypeptide support **91**; and performing a general chemical reaction.

**[0126]** The cross-linker generally has a linear structure and is formed of a spacer having a succinimido group which reacts with amino and thiol groups as well as a maleimide group at the both ends. The use of the cross-linker as the first linker **94** allows the polypeptide support **91** to be linked to the labeling substance **93**. For example, when the labeling substance **93** has a thiol group, a cross-linker having a succinimido group at one end and having a maleimide group at the other end can be used in binding the labeling substance **93** to the amino group of polypeptide. In this case, the amino group in polypeptide is reacted with the succinimide group in the cross-linker, and the maleimide group of the cross-linker is exposed to the surface of polypeptide. The binding can be performed by reacting the maleimide group with the thiol group in the labeling substance. Here, the length of the spacer of the cross-linker is not particularly limited. Examples of the spacer include PEG chains and nucleic acids. Examples of the specific example of the cross-linker include N-[ $\alpha$ -maleimide acetoacetoxy]succinimide ester (AMAS), N-[ $\beta$ -maleimide propyloxy]succinimide ester (BMPSE), maleimide butyryloxy succinimide ester (GMBS), m-maleimide benzoyl-N-hydroxysuccinimide ester (MBS), succinimidyl trans-4-(N-maleimidyl methyl)-cyclohexane-1-carboxylate (SMCC), N-[ $\epsilon$ -maleimide caproyl oxy]succinimide ester (EMCS), succinimidyl-4-(p-maleimide phenyl)butyrate (SMPB), succinimidyl-6-[( $\beta$ -maleimide propionamide)hexanoate] (SMPH), succinimidyl-4-[N-maleimidemethyl]cyclohexane-1-carboxy-[6-amide caproate] (LC-SMCC), and NHS-PEGn-Maleimid. The cross-linker may be glutaraldehyde in which functional groups at both ends have reactivity with an amino group, a cross-linker which has two functional groups (an amine-reactive NHS ester group and a light-reactive diazirine group) at the end or the like.

**[0127]** When the labeling substance **93** has a thiol group, the binding is possible by reacting the thiol group of the labeling substance **93** with the thiol group of polypeptide to form a dithiol bond. When the labeling substance **93** has a carboxyl group, the labeling substance **93** can be bound to the amino group of polypeptide by activating using NHS. When the labeling substance **93** has an aldehyde group, a stable bond can be formed by forming a Schiff base with the amino group of polypeptide and reducing it.

**[0128]** As the method for binding the polypeptide support **91** to the labeling substance **93** by a non-covalent bond, a method for binding the labeling substance **93** to polypeptide by a non-covalent and a method for binding the labeling substance **93** via a substance bound to polypeptide by a covalent bond by a non-covalent bond are contemplated.

**[0129]** Examples of the method for binding the labeling substance **93** to polypeptide by a non-covalent bond include a method for utilizing binding of streptoavidin to a labeling substance labeled with biotin and the like. Examples of the method for binding the labeling substance via a substance bound to polypeptide by a covalent bond by a non-covalent bond include a method comprising covalently-binding DNA having an amino group at the end to polypeptide in the above manner and non-covalently binding complementary DNA to which the labeling substance is bound to the DNA by hybridization and the like.

**[0130]** As described above, when the polypeptide support **91** is used as a support of the labeling substance **93**, a labeling substance-binding substance in which the sum of labeling substances is accurately controlled can be easily produced as compared with the case where a support composed of an inorganic material is used.

**[0131]** The method for binding the first binding substance **92** to the polypeptide support **91** is performed by the same method as the method for binding the labeling substance **93** to the polypeptide support **91**.

**[0132]** Subsequently, the detection process is performed [see the detection process of FIG. 6D].

**[0133]** In the detection process, the user first injects an electrolytic solution through the sample inlet **30b** of the detection chip **20**. Thereafter, the user inserts the detection chip **20** into the chip insertion unit **11** of the detector **1** shown in FIG. 1. Then, the user gives an instruction to start measuring to the detector **1**. Here, the electrode leads **71**, **72**, and **73** of the detection chip **20** inserted into the detector **1** are connected to the ammeter **14** and the power source **15**. Then, an arbitrary potential based on the reference electrode **69** is applied to the working electrode **60** by the power source **15** of the detector **1**. As the potential to be applied to the electrode, a potential in which the current value (stationary current, dark current) is low when the analyte is not irradiated with excitation light and the photocurrent generated from the analyte becomes a maximum photocurrent is preferred. The potential may be applied to the counter electrode or the working electrode.

**[0134]** Thereafter, the light source **13** of the detector **1** emits excitation light to the labeling substance **93** on the working electrode **60**. Thus, the labeling substance **93** is excited to generate electrons. The generated electrons move to the working electrode **60**. As a result, current flows between the working electrode **60** and the counter electrode **66**. Then, the current flowing between the working electrode **60** and the counter electrode **66** is measured by the ammeter **14** of the detector **1**. The current value measured by the ammeter **14** correlates with the number of the labeling substance **93**. Therefore, the analyte S can be quantified based on the measured current value. The excitation light may be only light in a specified wavelength region, which is obtained using a spectrometer or a bandpass filter, if necessary.

**[0135]** Thereafter, a current value digitally converted by the A/D converting unit **16** is input into the control unit **17**. Then, the control unit **17** estimates the amount of the analyte in the sample from the digitally converted current value based on a