Supporting information

Co-immobilized poly(ethylene glycol)-block-polyamines promote sensitivity and restrict biofouling on gold sensor surface for detecting Factor IX in human plasma

Thangavel Lakshmipriya¹, Yukichi Horiguchi¹ and Yukio Nagasaki^{1,2,3*}

¹Department of Material Science, Graduate School of Pure and Applied Sciences, ²Master's School of Medicinal sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8573, ³International Center for Materials for Nanoarchitectonics Satellite (WPI-MANA) National Institute of Material Science (NIMS) and University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8573, Japan.

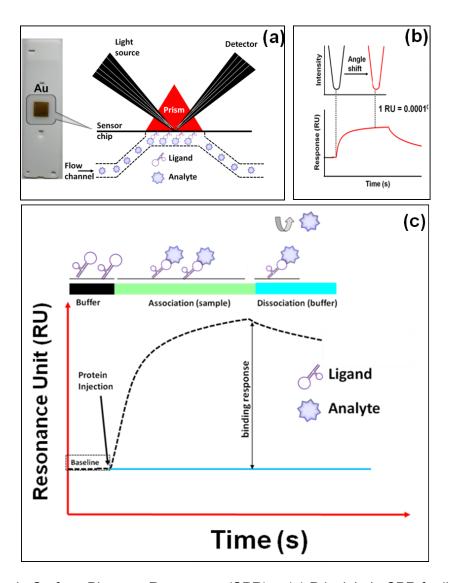


Figure S1. Setup in Surface Plasmon Resonance (SPR). (a) Principle in SPR for ligand and analyte interaction. The sensing surface (Au) used in SPR-Biacore is shown. (b) typical angular changes in SPR, upon the complex formation of ligand and analyte. (c) Association and dissociation process in

SPR for ligand and analyte interactions.

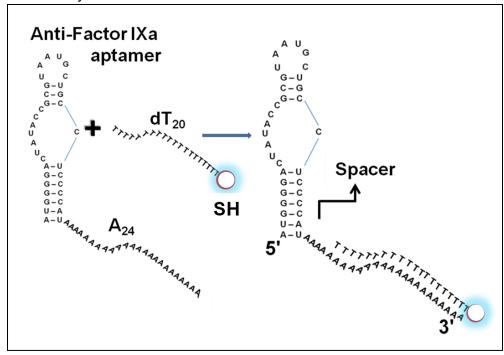


Figure S2. Evaluation of immobilization of SH- dT_{20} and aptamer- A_{24} duplex formation on Au surface.

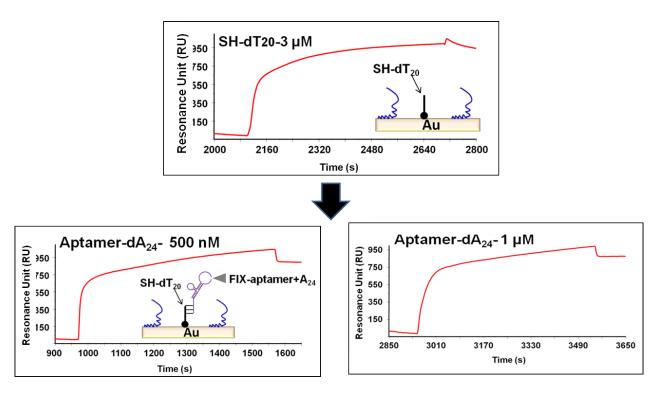


Figure S3. Evaluation of immobilization of SH-dT $_{20}$ and aptamer-A $_{24}$ duplex formation by SPR. SH-dT $_{20}$ was saturated with 3 μ M, and aptamer-A $_{24}$ duplex formation was saturated with 500 nM. Figure inset shown with cartoon.

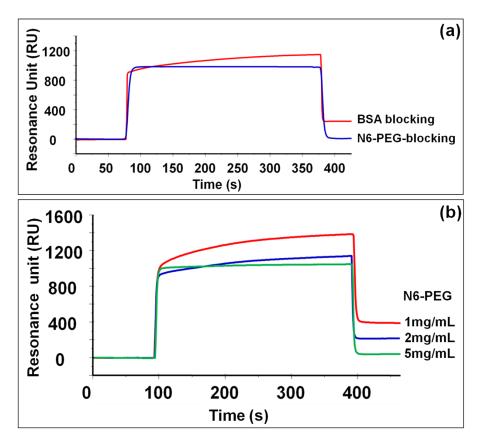


Figure S4: Determination of non-specific attachment of mouse-IgG-GNP in the absence of FIX. (a) BSA blocked surface (Red) and N6-PEG blocked surfaces (blue). The attachments are as follows; PEG-b-PAMA, SH-d T_{20} , aptamer- A_{24} , BSA/N6 PEG, FIX antibody, mouse-IgG-GNP. (b) Concentration dependent effect of N6-PEG blocking.

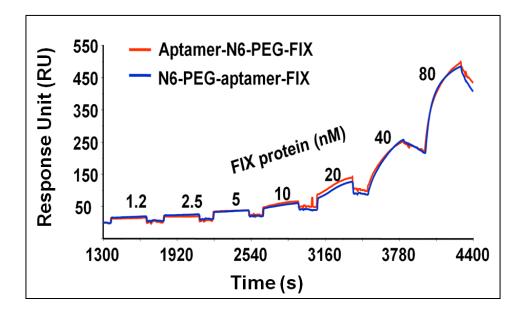


Figure S5: Determination of stability of aptamer upon N6-PEG attachment. Immobilization of FIX on Aptamer-N6-PEG modified surface (Red) and N6-PEG-aptamer modified surfaces (blue).

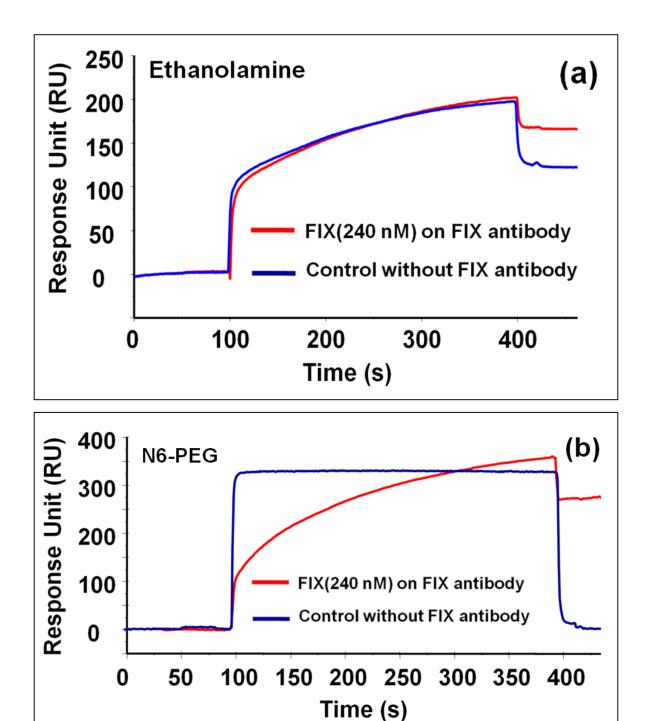


Figure S6. Comparative analyses between ethanolamine and N6-PEG for FIX-antibody and FIX interaction. SPR analyses for the specific and non-specific interaction in the presence of (a) ethanolamine and (b) N6-PEG.

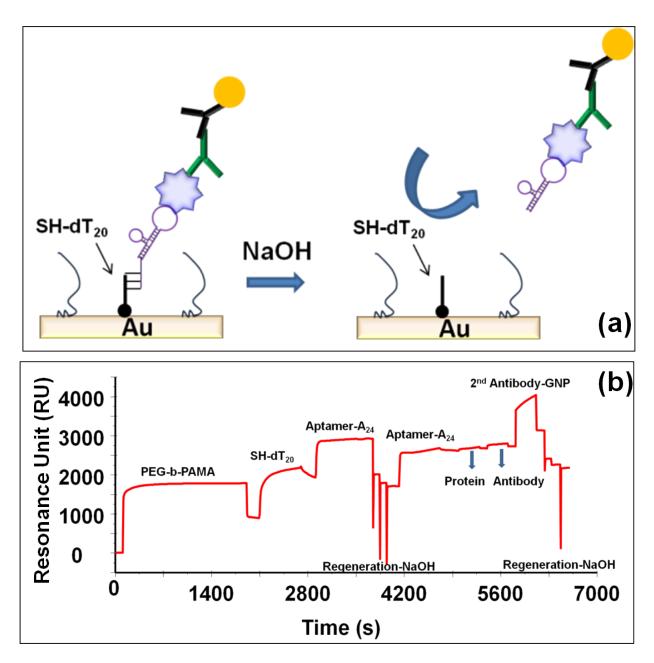


Figure S7. Regeneration on the sensing surface (a) cartoon illustrates the regeneration process. (b) Regeneration by SPR. Regeneration was performed by 10 mM NaOH, injected at the flow rate of 60 μ l/min, for 2 x 5 sec. Performed with 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl.

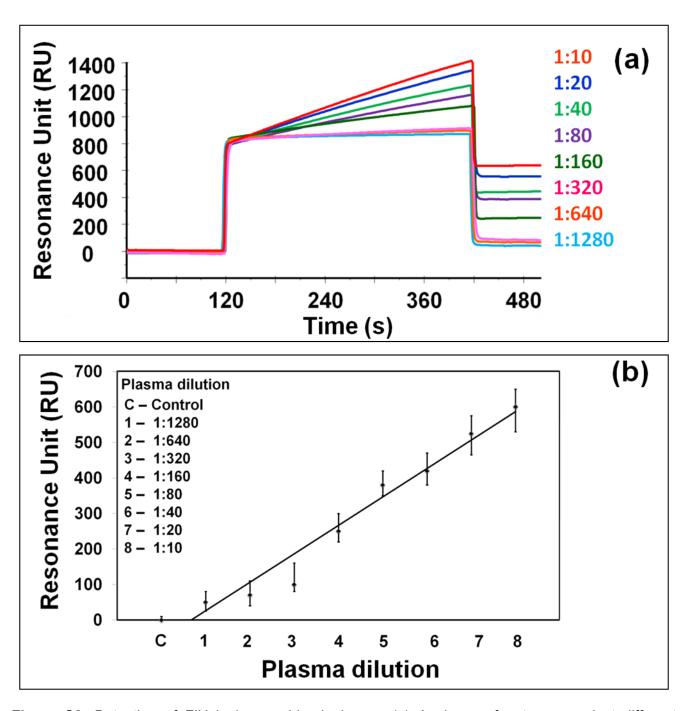


Figure S8. Detection of FIX in human blood plasma. (a) Analyses of aptamer against different dilutions of human blood plasma containing FIX by SPR. (b) Graphical representation. The real concentrations are indicated. Samples were injected at the flow rate of 10 μ L/min. All experiments were performed with 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl and 2 mM CaCl₂. Samples were injected at the flow rate of 10 μ L/min.

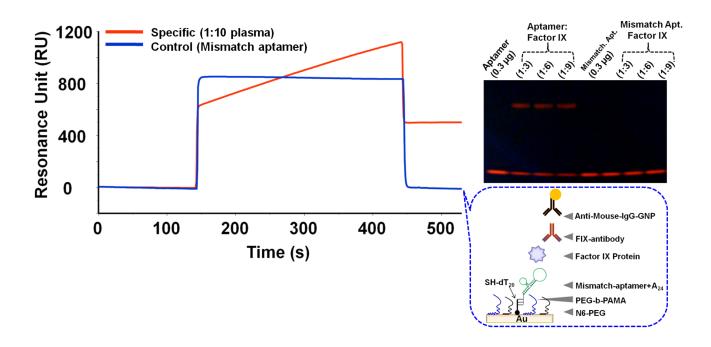


Figure S9. Analyses of FIX in plasma. Human plasma with dilution 1:10 was injected on the FIX aptamer immobilized surfaces (Instead of FIX protein) followed by FIX antibody and detected by mouse –IgG GNP. Control experiment was done with mismatch of FIX aptamer. Figure inset is cartoon for control experiment. Samples were injected at the flow rate of 10 μL/min. Native-PAGE for the specific aptamer-FIX and complementary aptamer-FIX complexes is shown by gel-shift assay. Shift and super-shifts were shown with aptamer-FIX. All experiments were performed with 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl and 2 mM CaCl₂.

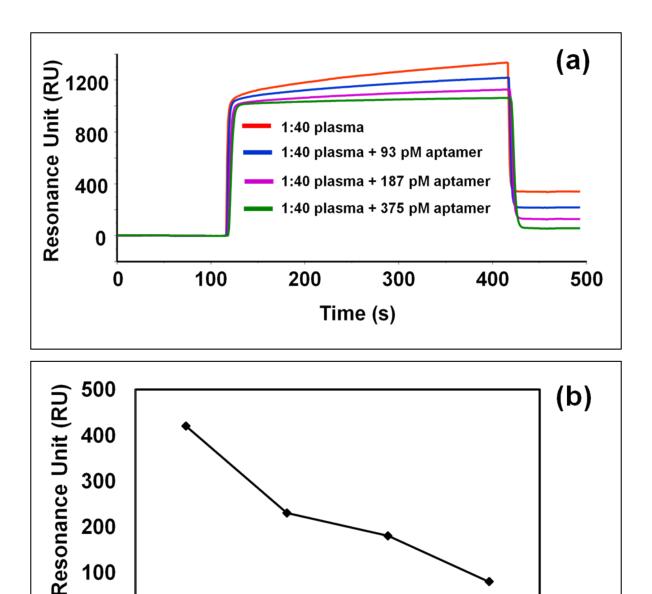


Figure S10. (a) Analyses of FIX in plasma with pre-mixed aptamer. After immobilization of aptamer and blocking with N6-PEG on Au surface, plasma containing FIX (1: 40 dilution) was premixed with different concentrations of specific aptamer (0 to 375pM) without poly-A tail. Samples were injected at the flow rate of 10 μ L/min. All experiments were performed with 10 mM HEPES buffer (pH 7.4) containing 150 mM NaCl and 2 mM CaCl₂. (b). Graphical representation of analyses of aptamer against constant (1:40 dilution) human plasma mixed with different concentration of aptamer without poly-A tail.

1: 40 plasma + Aptamer (pM)