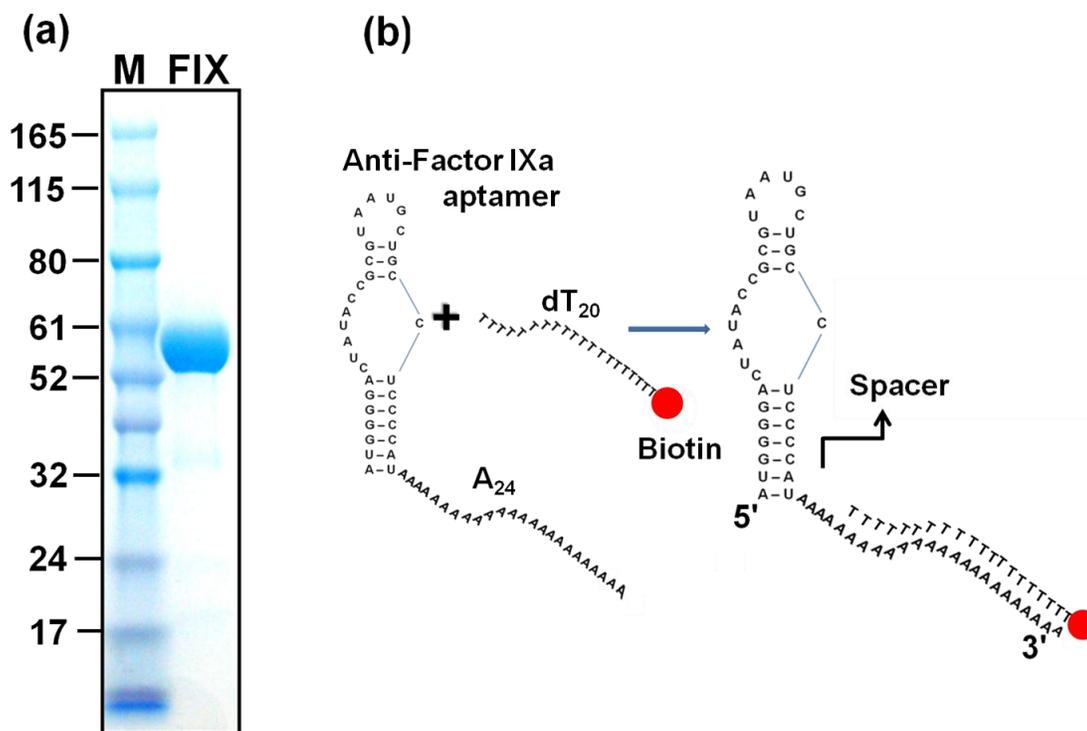


Supplementary information

**High-performance waveguide-mode biosensor for detection of Factor IX uses PEG-based blocking agents to suppress non-specific binding and improve sensitivity**

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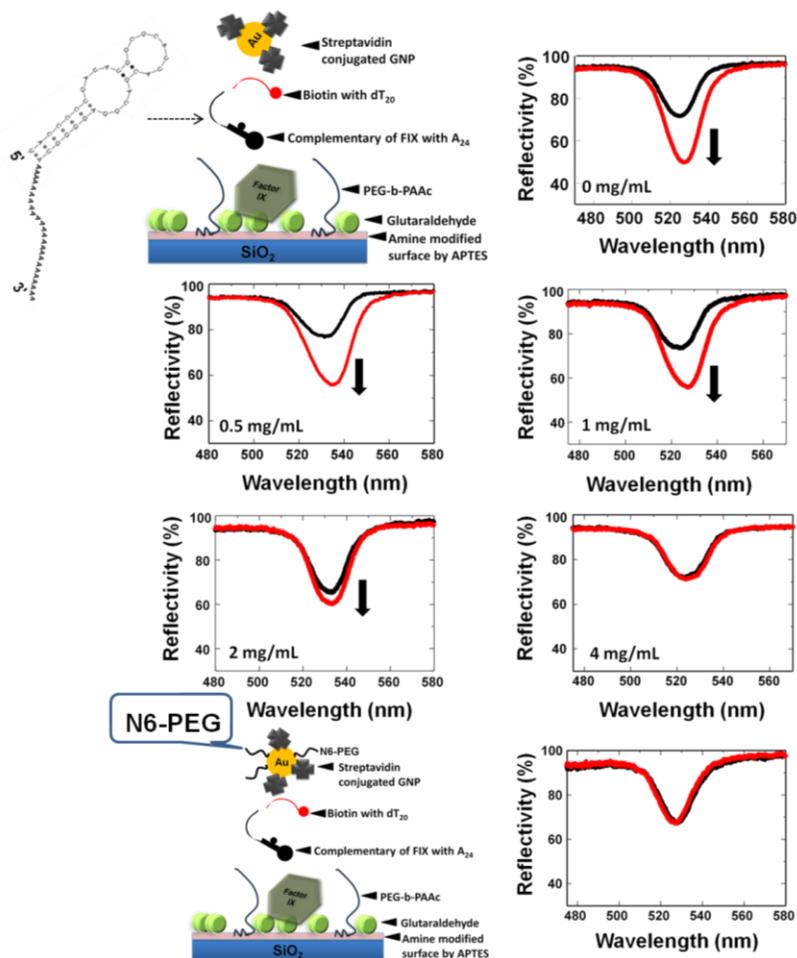
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**Supplementary Fig. S1**

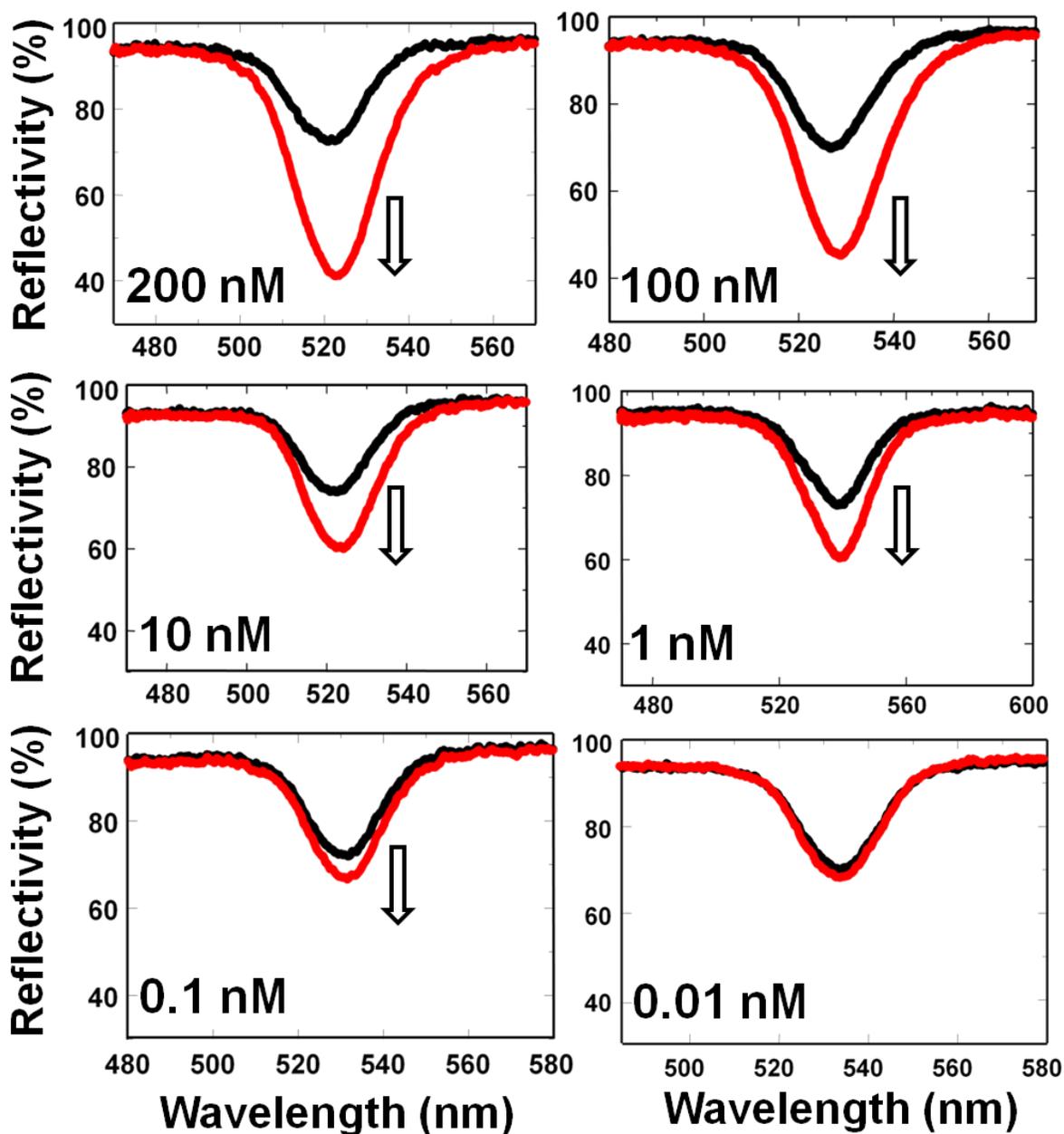
(a) Purity of FIX protein on SDS-PAGE. The FIX band was migrated at ~55,000 daltons. 3 µg of FIX was loaded. (b) Schematic diagram for the duplex formation of FIX aptamer with extended 3' end (A<sub>24</sub>) and biotin-dT<sub>20</sub>. Spacer region was created for flexibility.

**PEG-b-PAAc prevents non-specific binding on amine and Glu surfaces.** To check the origin of non-specificity, initially it was aimed to immobilize SA-GNPs directly on the amine surfaces. SA-GNPs have shown higher spectral changes as non-specific binding on amine surface. This non-specific attachment is due to the several factors such as electrostatic, hydrophobic and hydrogen bonding interactions occurred on sensor chip surfaces (Nagasaki, 2011). To suppress non-specific interaction of SA-GNP to sensor chip surface, PEG-b-PAAc was applied to surface. As can be seen in Supplementary Figure S2, significant signal was observed without PEG-b-PAAc. When 4 mg/mL of PEG-b-PAAc was applied to sensor chip surface prior to SA-GNPs, no signal change was observed at all. Negative character of PAAc segment in the block copolymer strongly interact with positive sensor chip surface and effectively avoid non-specific binding of SA-GNP, which is similar to our previous report on the surface modification of the positively charged rare earth ion-doped  $Y_2O_3$  nanoparticles surfaces by PEG-b-PAAc (Kamimura et al., 2008).



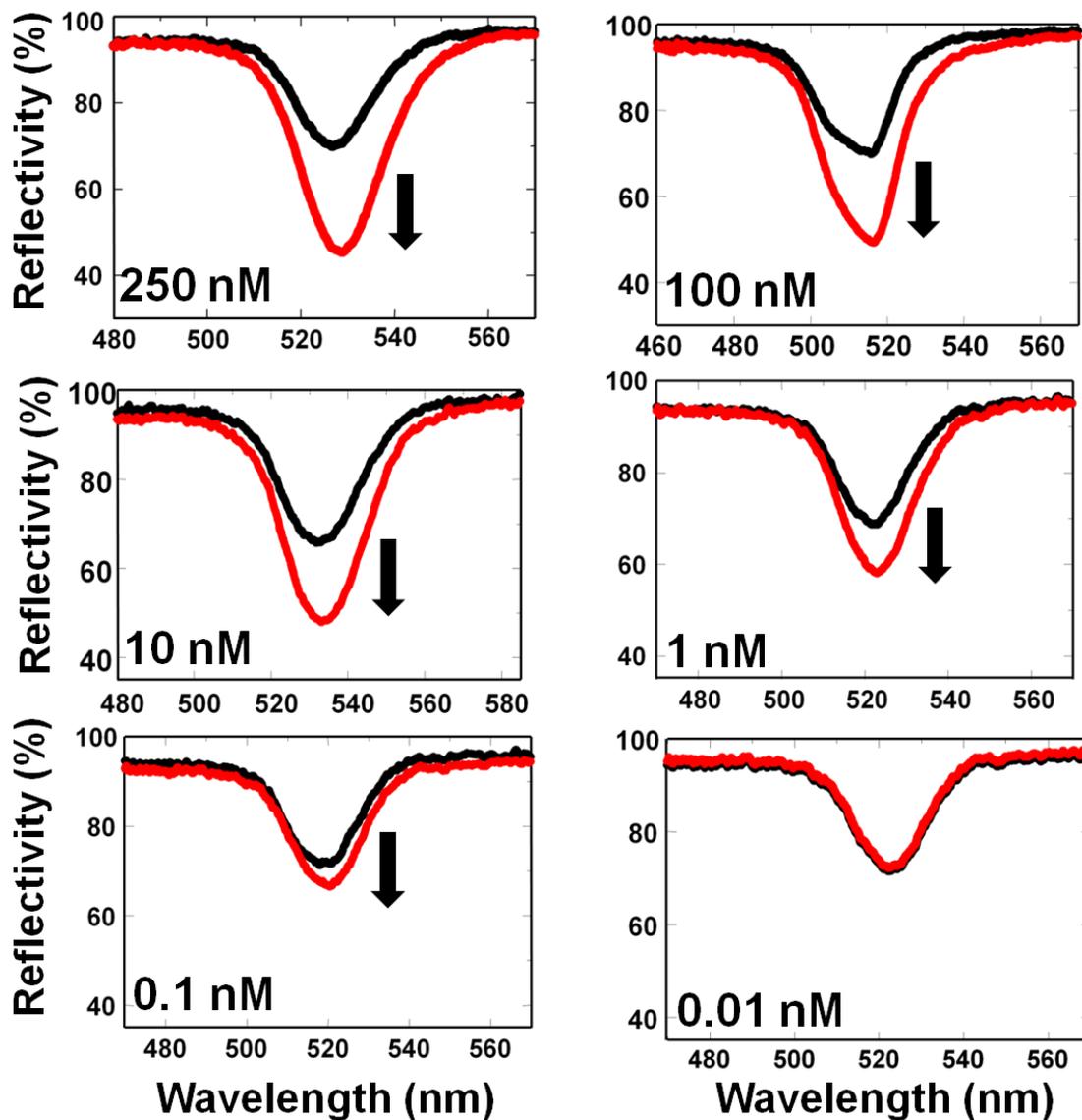
**Supplementary Fig. S2**

Control experiments with complementary sequences of FIX aptamer using different PEG-b-PAAc concentrations. Black and red spectrums indicate only buffer and attachment of streptavidin conjugated GNP, respectively. Predicted secondary structure with complementary sequences is shown. Arrows indicate the direction of changes in the spectrum. By the addition of N6-PEG (0.5 mg/mL) with SA-GNP, the spectrum remains same. 4 mg/mL of PEG-b-PAAc is sufficient for controlling non-specificity.



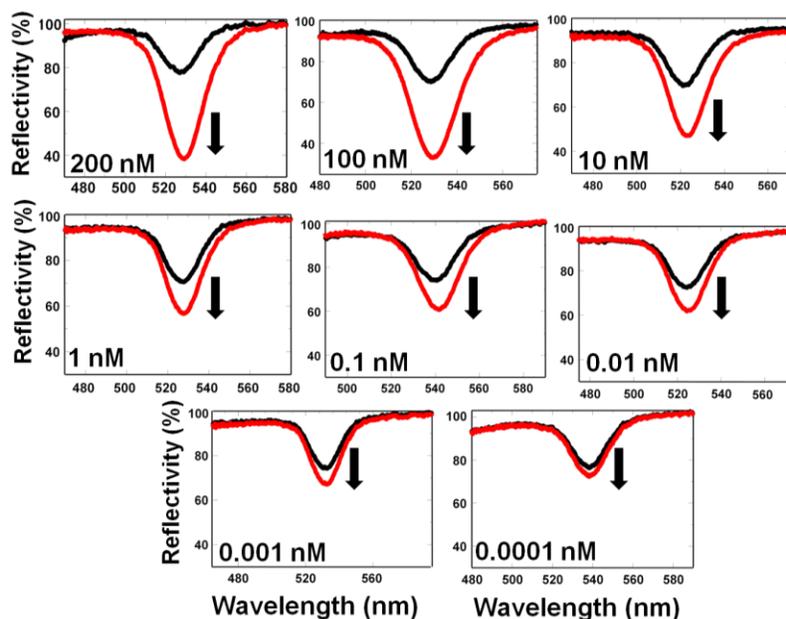
Supplementary Fig. S3

Aptamer titrations against constant FIX (250 nM). Aptamer titers were shown from higher concentration to lower concentration. Concentrations of other biomolecules were kept constant. Black and red spectra curves are the spectra before and after the attachment of SA-GNP, respectively. Arrows indicate the direction of changes in the spectrum



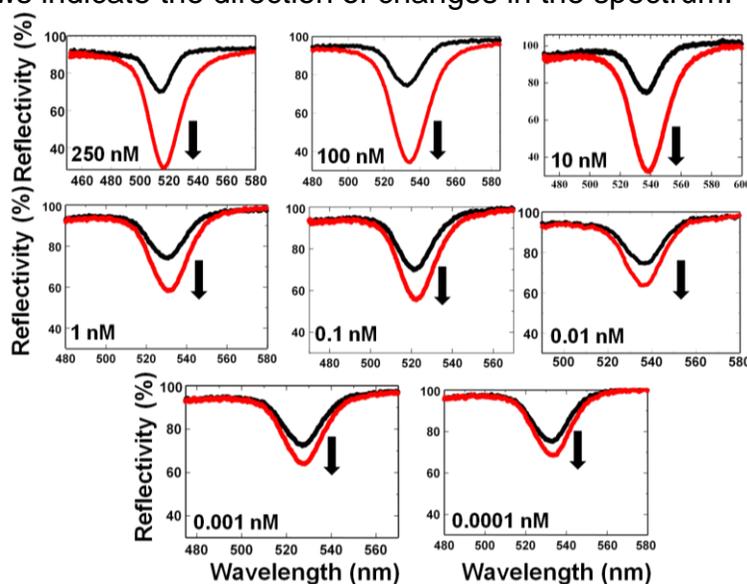
Supplementary Fig. S4

FIX titration with different against constant aptamer (100 nM) . FIX titer were shown with higher concentration to lower concentration. Concentrations of other biomolecules are constant. Black and red spectrums indicate only buffer and attachment of the streptavidin conjugated GNP, respectively. Arrows indicate the direction of changes in the spectrum.



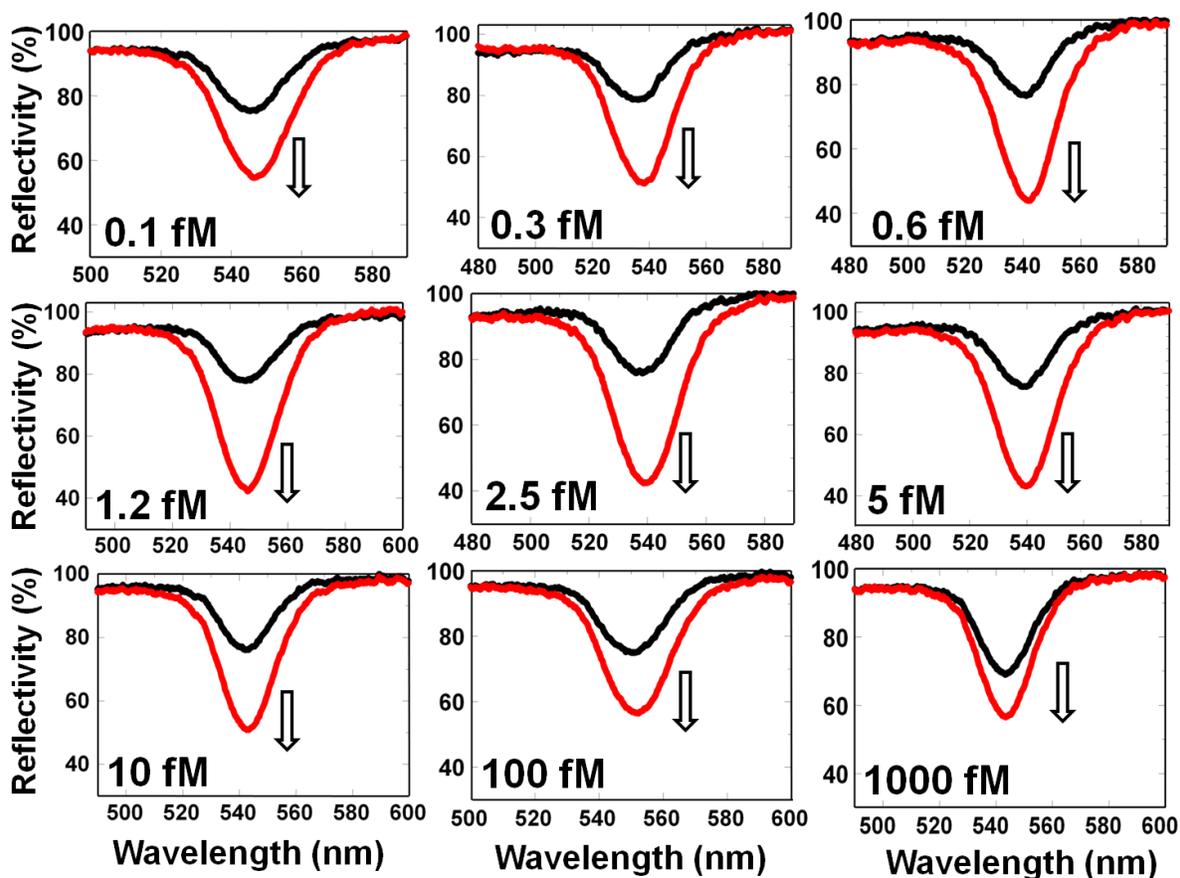
**Supplementary Fig. S5**

Aptamer titrations with different concentrations against constant FIX (250 nM). Aptamer titers were shown with higher concentration to lower concentration. Concentrations of other biomolecules are constant. Black and red spectrums indicate only buffer and attachment of streptavidin conjugated GNP, respectively. SA-GNP used with 0.5 mg/mL of N6-PEG. Arrows indicate the direction of changes in the spectrum.



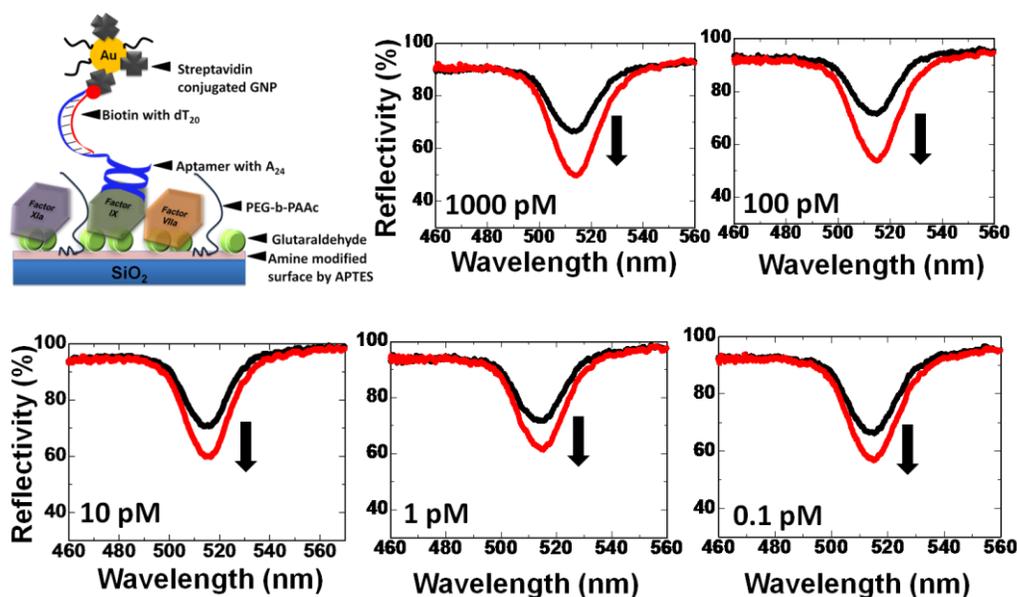
**Supplementary Fig. S6**

FIX titration with different against constant aptamer (100 nM). FIX titers were shown with higher concentration to lower concentration. Concentrations of other biomolecules are constant. Black and red spectrums indicate only buffer and attachment of streptavidin conjugated GNP, respectively. SA-GNP used with 0.5 mg/mL of N6-PEG. Arrows indicate the direction of changes in the spectrum.



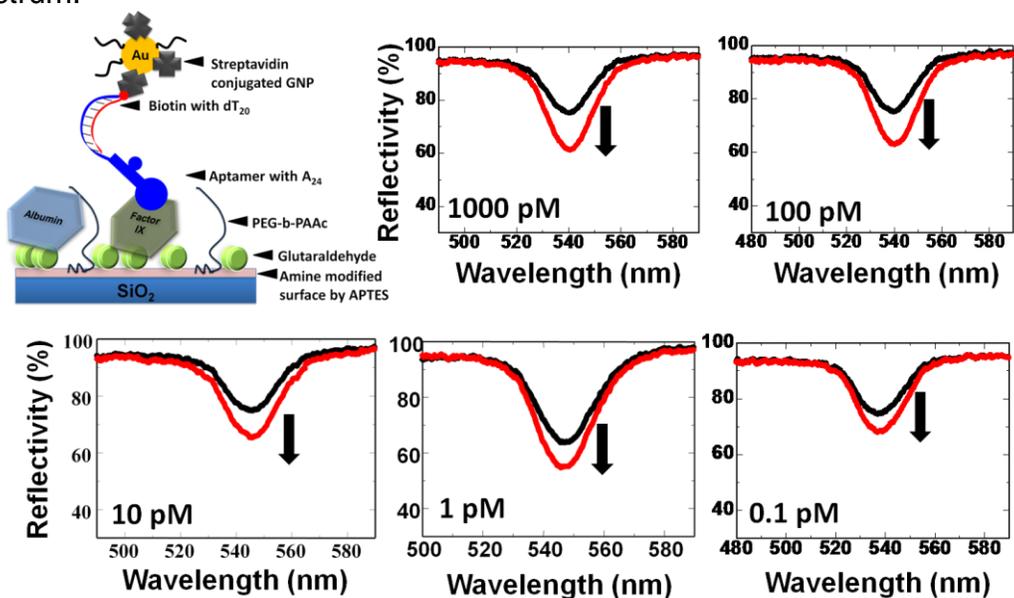
**Supplementary Fig. S7**

Competition assays using biotin. Different concentrations of biotin were added to SA-GNP before reacting with pre-immobilized biotinylated aptamers on the sensing surface. Spectral changes after applying the mixture to biotinylated aptamer-immobilized sensing surface. Black and red curves are the spectra before and after the attachment of SA-GNP, respectively. The concentrations indicated in the figures are those of the biotin. Arrows indicate the direction of changes in the spectra.



Supplementary Fig. S8

Selective binding of aptamer with FIX from the mixture of FIX, FXIa, FVIIa. Kept FXIa (6  $\mu\text{g/mL}$ ) and FVIIa (8.4  $\text{ng/mL}$ ) concentrations as constant and titrated with different concentrations of FIX. Black and red spectrums indicate only buffer and attachment of streptavidin conjugated GNP, respectively. Arrows indicate the direction of changes in the spectrum.



Supplementary Fig. S9

Selective binding of aptamer with FIX from the mixture of FIX and Albumin. Albumin concentration was kept as 84  $\text{nM}$  and titrated against different concentrations of FIX. Black and red spectrums indicate only buffer and attachment of streptavidin conjugated GNP, respectively. Arrows indicate the direction of changes in the spectrum.