**Electronic Supplementary Information (ESI)**

# A Significant Enhancement of Color Transition from an On-off Type Achromatic Colorimetric Nanosensor for Highly Sensitive Multi-analyte Detection with Naked Eye

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## Additional experimental section

### Materials

Gold(III) chloride hydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), trisodium citrate dehydrate (TCEP), silver nitrate ($AgNO_3$), cetrimonium bromide (CTAB), sodium borohydride ($NaBH_4$), Immunoglobulin G (IgG), human serum albumin (HSA), bovine serum albumin (BSA), prostate specific antigen (PSA), lysozyme (LYZ), thrombin (THR), platelet-derived growth factor-AA (PDGF-AA), polyvinylpyrrolidone (average $M_w$: 40 kDa), poly(ethylene glycol) methyl ether thiol (average $M_n$: 6 kDa) and all other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium 3-methyl salicylate was purchased from Tokyo Chemical Industry (TCI). Deionized water (18.2 $M\Omega \cdot$cm) was prepared with a Sartorius Arium® pro Ultrapure water system. The oligonucleotides used in this work were prepared from either Integrated DNA Technologies Inc. (Coralville, IA, USA) or Bioneer Corp. (Daejeon, Korea) and were used after high performance liquid chromatography purification.

**Synthesis of AuNPs, AgNPs and AuNRs**

To synthesize AuNPs, we first prepared 100 mL of $HAuCl_4$ solution (1 mM) and mixed with 10 mL of sodium citrate dihydrate solution (38.8 mM) in a two-neck flask. The reaction was carried out through reflux. After 30 min of reaction, the synthesized AuNPs was cooled to room temperature (23-25°C) and stored in a dark room before use.[1] The AgNPs were synthesized by the citrate-stabilized method.[2, 3] First, 99 mL of sodium citrate dihydrate solution (0.3 mM) and $NaBH_4$ (1 mM) was prepared in one-neck flask. Subsequently, 1 mL of silver nitrate (0.01 M) was rapidly added to the mixed solution. The reaction was done with stirring in an ice-cold bath. After 20 min of reaction, the synthesized AgNP was stored in a dark room before use. The AuNRs were prepared by modifying seed-mediated growth method.[4] We first made a seed solution, which is composed of 5 mL of $HAuCl_4$ solution (0.5 mM), 5 mL of CTAB solution (0.2 M) and 0.6 mL of ice-cold $NaBH_4$ solution (0.01 M). The seed solution was aged at 30°C for 30 min. To make a growth solution, 62.5 mL of CTAB solution (0.2 M) was mixed with 62.5 mL of sodium 3-methyl salicylate (35.35 mM), and the mixed solution was sonicated in a water bath (50-70°C) for 1 h. After the mixed solution was cooled to room temperature (23-25°C), 3 mL of $AgNO_3$ solution (4 mM), 125 mL of $HAuCl_4$ solution (1 mM), and 0.5 mL of ascorbic acid (0.064 M) was added to the mixed solution with 15 min interval between each step. Finally, 0.4 mL of seed solution was injected into the growth solution. The reaction was proceeded for 12 h at 30°C for the growth of AuNRs. The synthesized AuNRs were isolated by centrifugation at 11,000 rcf for 45 min and then re-dispersed in 35 mL of deionized water (DI water).

**DNA functionalization on each individual nanoparticle**

First, 1 mL of each individual bare nanoparticle (AuNPs and AgNPs) and PVP (40kDa, 200 μM) capped AuNR (AuNR-$PVP_{40k}$) solution were placed in a vial, which was pretreated with 1 M sodium hydroxide solution and washed several times with DI water. Nine microliter of 1 mM DNA stock solution was mixed with 1 μL of 500 mM acetate buffer solution (pH 5.2) and 3 μL of 10 Mm trisodium citrate dehydrate (TCEP) and incubated for 1 h to activate thiol-tagged ssDNA. Subsequently, thiol-tagged ssDNA stock solution was transferred to 3 mL of bare AuNPs (3 nM), bare AgNPs (3 nM) and AuNR-$PVP_{40k}$ (2 nM) solution, respectively. After 18 h of incubation, we added 300 μL of 1 M NaCl solution to the samples to increase the chemical conjugation of the DNA on the nanoparticle surface. This solution was stored in a dark room for 18 h before use.

**Computational method of color analysis**

The computational analysis of the images was carried out by MATLAB *via* a house made algorithm. The analyzed images were obtained from digital camera of a smart phone (iPhoneS5). To separate samples images from the background, RGB (Red-Green-Blue) domain was converted to grayscale domain and both a low-pass filter and Gaussian smoothing was applied for pretreatment of the images. Canny edge detector was used to extract the edge images of the samples. We used dilate, fill, and erode morphological operations to soften the outlines of object images and to remove the protruding part of an object in images. Next, the analyzed images were transformed to saturation domain, which belongs to HSV (Hue-Saturation-Value) domain. Finally, we applied extracted images on analyzed saturation images to present the intensities of histogram. The flow chart of computational method for color analysis is shown in Fig. S15.

## Notes and references


1. J. H. Heo, K.-I. Kim, H. H. Cho, J. W. Lee, B. S. Lee, S. Yoon, K. J. Park, S. Lee, J. Kim, D. Whang and J. H. Lee, *Langmuir*, 2015, **31**, 13773-13782.
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3. N. G. Bastús, F. Merkoçi, J. Piella and V. Puntes, *Chem. Mater.*, 2014, **26**, 2836-2846.
4. X. Ye, L. Jin, H. Caglayan, J. Chen, G. Xing, C. Zheng, V. Doan-Nguyen, Y. Kang, N. Engheta, C. R. Kagan and C. B. Murray, *ACS Nano*, 2012, **6**, 2804-2817.

**Table S1.** The single stranded DNA (ssDNA) sequences used for each experiment.

| Use | Nomenclature | Sequence |
|---|---|---|
| For Achromatic nanoparticles experiment (Fig. 1) | Random DNA | 5'-SH-ATCGAACGTTCACAAGAA-3' |
| For target DNA1 detection (Fig. S7 and S8) | 5'-thiol-tagged ssDNA1$_A$ | 5'-SH-TAGCTAACAGCA-3' |
| | 3'-thiol-tagged ssDNA1$_B$ | 5'-CTCTAGATAAGT-SH-3' |
| | Target DNA1 | 5'-TATCTAGAGTGCTGTTAG-3' |
| For target DNA2 detection (Fig. S9 and S10) | 5'-thiol-tagged ssDNA2$_A$ | 5'-SH-GCGAGCTTCATT-3' |
| | 3'-thiol-tagged ssDNA2$_B$ | 5'-AGGACCCGTGAG-SH-3' |
| | Target DNA2 | 5'-ACGGGTCCTAATGAAGCT-3' |
| For target DNA3 detection (Fig. 3 and S6) | 5'-thiol-tagged ssDNA3$_A$ | 5'-SH-TAGTGGTATTGC-3' |
| | 3'-thiol-tagged ssDNA3$_B$ | 5'-GTTCGGGCGTCG-SH-3' |
| | Target DNA3 | 5'-CGCCCGAACGCAATACCA-3' |
| For non-target DNA4 detection (Fig. 3) | Non-target DNA4 | 5'-CTCGAACGGTCTCAGGAA-3' |
| For thrombin detection (Fig. S13 and S14) | Thrombin aptamer 1(Apt$_{Thrm1}$) | 5'-SH-TTTTTTTTTTTTTTTGGTTGGTGTGGTTGG-3' |
| | Thrombin aptamer 2 (Apt$_{Thrm2}$) | 5'-SH-TTTTAGTCCGTGGTAGGGCAGGTTGGGGTGACT-3' |
| For PDGF-AA detection (Fig. 5 and S11) | PDGF aptamer (Apt$_{PDGF}$) | 5'-SH-CAGGCTACGGCACGTAGAGCATCACCATGATCCTG-3' |

**Table S2.** Comparison of achromatic nanosensors for DNAs detection with other colorimetric sensors.

| Materials | Required process | Linear range | Spectrum Detection of limit | Naked eye detection of limit | Image processing detection of limit | References |
|---|---|---|---|---|---|---|
| Achromatic NPs & conjugated DNA | 1 step detection | 0.4 - 1 nM | 0.4 nM | 0.5 nM | Lower than 0.5 nM | This work |
| AuNPs & peptide nucleic acids (PNA) | 3 step detection | 50 - 1000 nM | 50 nM | 100 - 200 nM | Not available | 1 |
| AuNPs & salt | 3 step detection | 0 - 0.5 nM | 50 pM | 0.2 - 0.3 nM | Not available | 2 |
| Polydiacetylene (PDA) | 2 step detection | 20-100 nM | 20 nM | 100 nM | Not available | 3, 4 |
| AuNPs & conjugated DNA | 3 step detection | 15 - 60 nM | 5 nM | 500 nM | Not available | 5 |

**Table S3.** Comparison of achromatic nanosensor for proteins detection with other colorimetric sensors.

| Materials | Required process | Linear range | Spectrum Detection of limit | Naked eye detection of limit | Image processing detection of limit | References |
|---|---|---|---|---|---|---|
| Achromatic NPs & conjugated DNA aptamer | 1 step detection | 15 - 40 nM | 19 nM | 20 nM | Lower than 10 nM | This work |
| AuNPs & salt & DNA aptamer | 2 step detection | 0 - 167 nM | 0.83 nM | 83 nM | Not available | 6 |
| Aptamer & conjugated polydiacetylene (PDA) | 1 step detection | 0 - 10000 nM | 500 nM | 3000 nM | Not available | 7 |
| AuNPs & DNA aptamer | 2 step detection | 10 - 100 nM | 6 nM | 50 nM | Not available | 8 |
| AuNPs & strand displacement amplification (SDA) | 4 step detection | 4 - 40 nM | 1.1 nM | 10 nM | Not available | 9 |

**Notes and references (Table. S2 and S3)**


1. R. Kanjanawarut and X. Su, *Anal. Chem.*, 2009, **81**, 6122-6129.
2. P. Liu, X. Yang, S. Sun, Q. Wang, K. Wang, J. Huang, J. Liu and L. He, *Anal. Chem.*, 2013, **85**, 7689-7695.
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4. Y. K. Jung and H. G. Park, *Biosens. Bioelectron.*, 2015, **72**, 127-132.
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9. H. Zhang, F. Li, H. Chen, Y. Ma, S. Qi, X. Chen and L. Zhou, *Sens. Actuator B-Chem.*, 2015, **207**, 748-755.

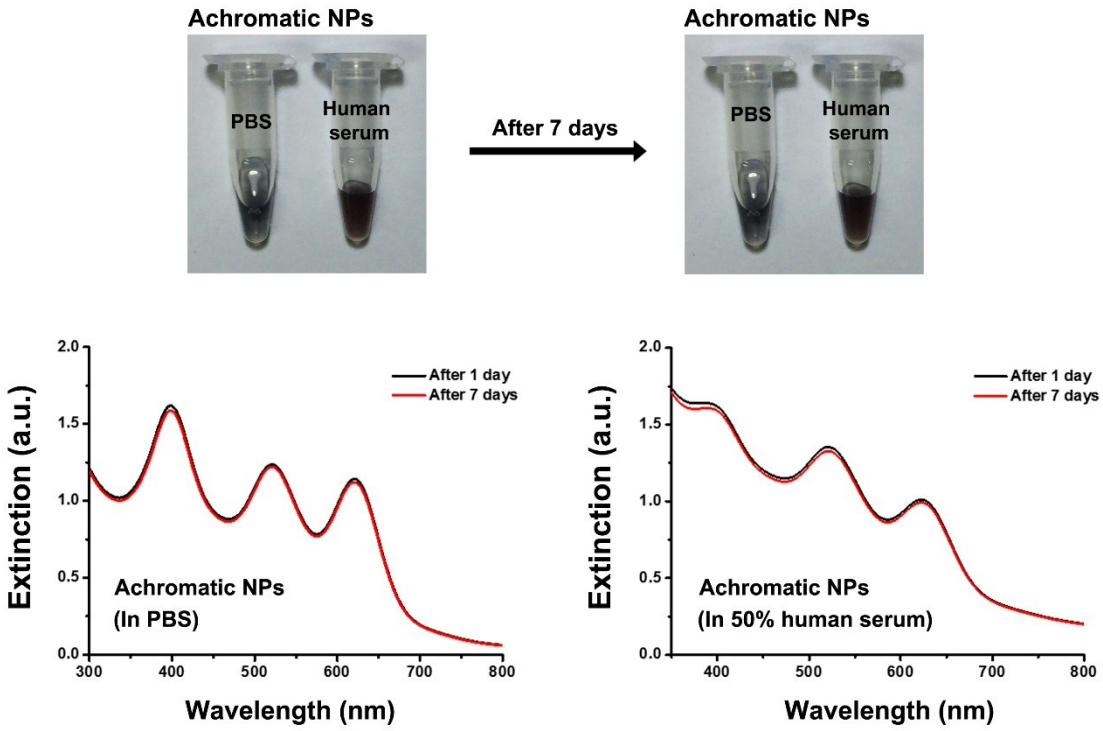


**Fig. S1** The variations of color change and extinction spectra of achromatic nanoparticles (achromatic NPs) in PBS and 50% human serum after 7 days. We incubated and monitored our sensor in 50% human serum and PBS to check their stability issue in biological media. When our sensor was placed in both media, we observed our sensor maintaining its black color even after 7 days (see Fig. S15). This negligible color change of our sensor was also verified by the insignificant shift and decrease of extinction spectra (see Fig. S15). This result demonstrates that our sensor can work in biological media without significant long term stability issue.

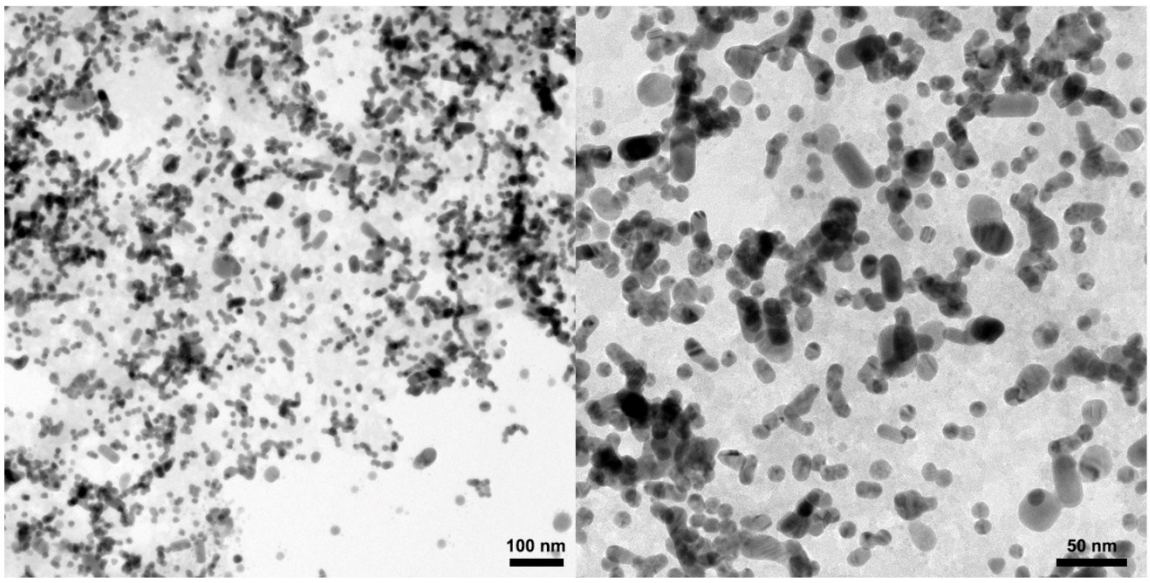


**Fig. S2** TEM images of achromatic (black) nanoparticles. DNA functionalized nanoparticles (AgNPs-DNA2$_{A\&B}$, AuNPs-DNA1$_{A\&B}$ and AuNRs-DNA3$_{A\&B}$) remained well dispersed.

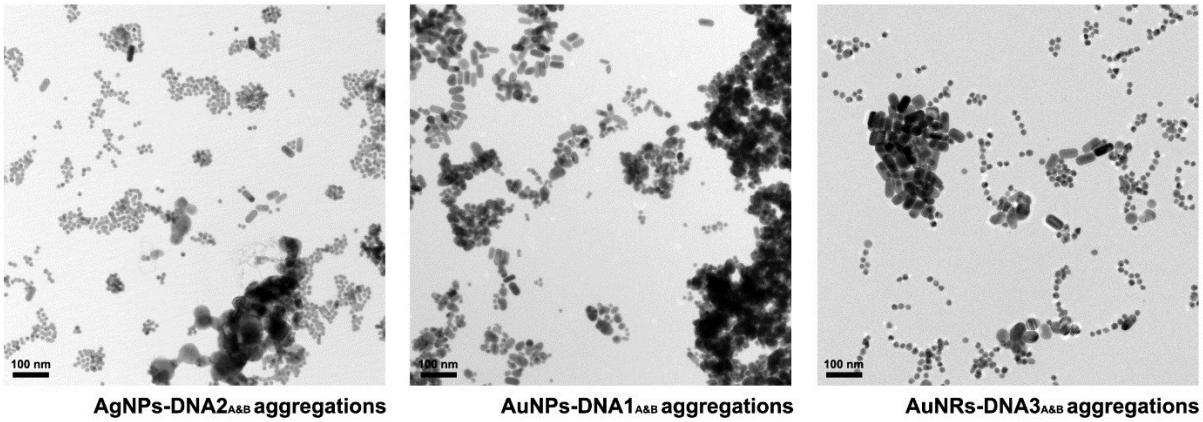


**Fig. S3** TEM images of achromatic nanoparticles (achromatic NPs) in the presence of target DNA. The AgNPs-DNA2$_{A\&B}$ were aggregated in the presence of target DNA2. The AuNPs-DNA1$_{A\&B}$ were aggregated in the presence of target DNA1. The AuNRs-DNA3$_{A\&B}$ were aggregated in the presence of target DNA3.

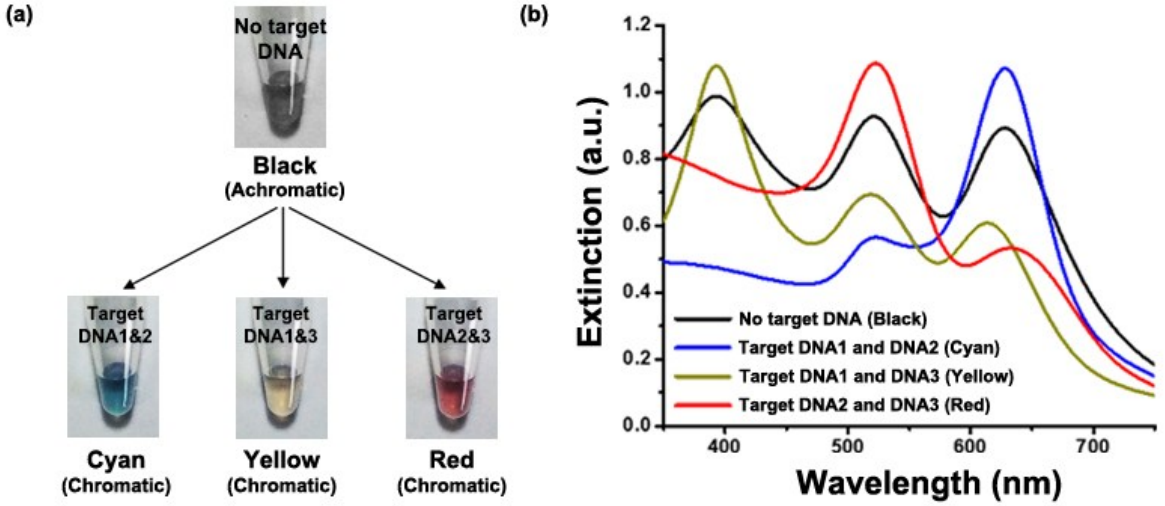


**Fig. S4** (a) Multi-color change of achromatic nanoparticles (achromatic NPs) depending on the two kinds of target DNAs. The concentration of each target DNA used is 50 μM. (b) The extinction spectra of achromatic NPs with two different target DNA strands.

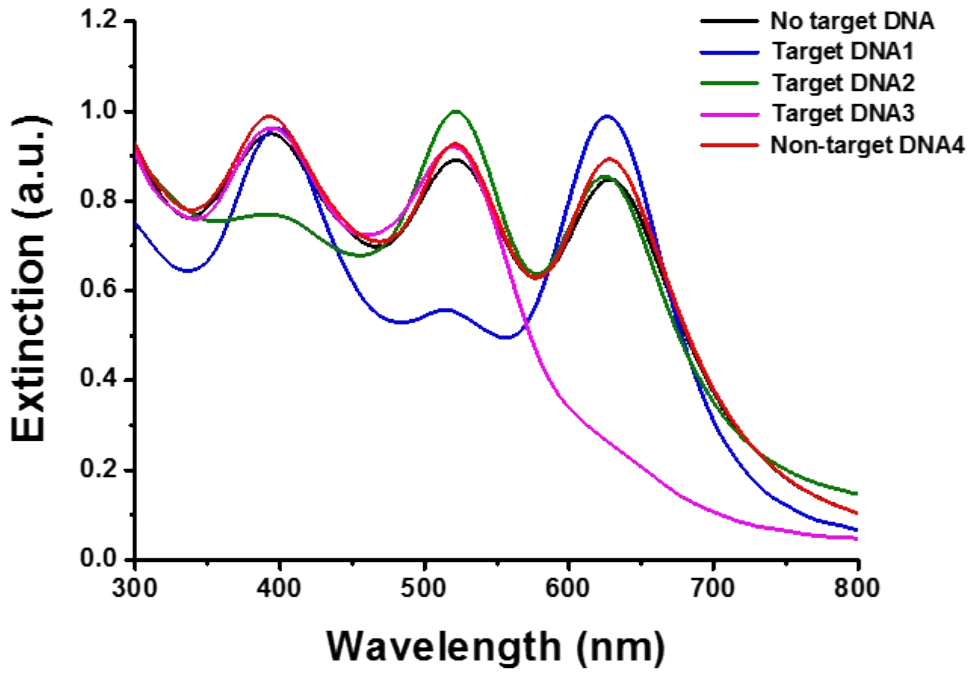


**Fig. S5** The extinction spectra of an achromatic nanosensor depending on the target DNA strands. The variations of an achromatic nanosensor in the extinction spectra with different targets (target DNA1, 2 and 3). The negligible change of extinction spectra in an achromatic nanosensor with a non-target DNA4.

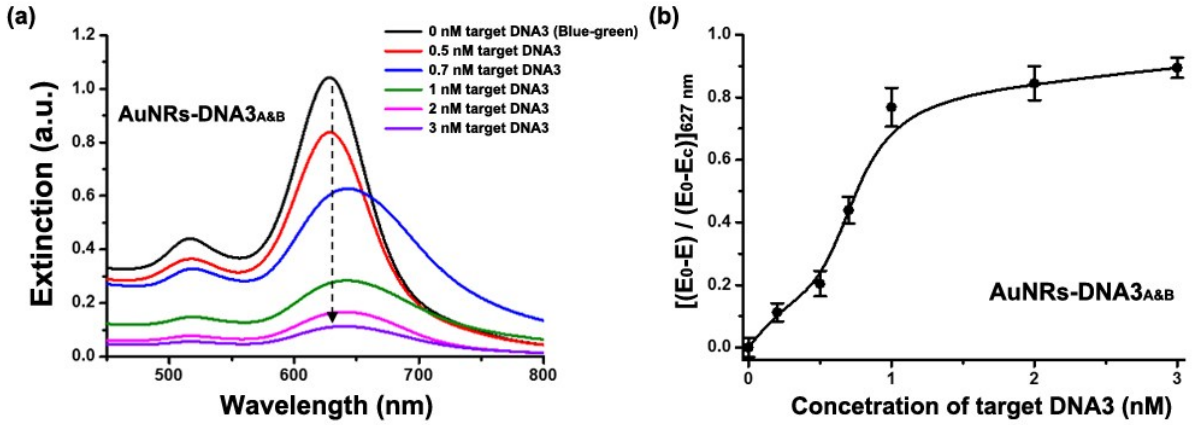


**Fig. S6** (a) The extinction spectra of AuNRs-DNA3$_{A\&B}$ with different concentrations of target DNA3. (b) The calibration curve of original AuNRs-DNA3$_{A\&B}$ obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 627 nm under the extinction spectra. $E_0$ designates the extinction of AuNRs-DNA3$_{A\&B}$ at 627 nm and $E_c$ specifies the extinction in the absence of AuNRs-DNA3$_{A\&B}$. The detection linear range of target DNA3 was approximately 0.4 nM - 1 nM. The limit of detection (LOD) calculated from calibration curve is 0.4 nM.

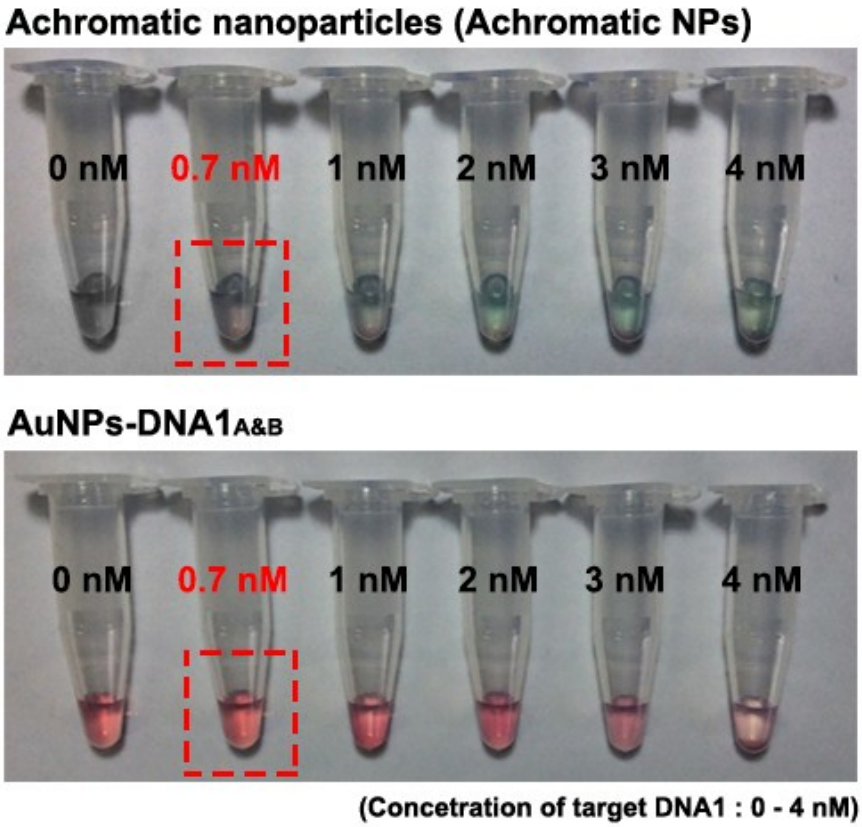


**Fig. S7** The color change of achromatic nanoparticles (achromatic NPs) and AuNPs-DNA1$_{A\&B}$ with different concentrations of target DNA1.

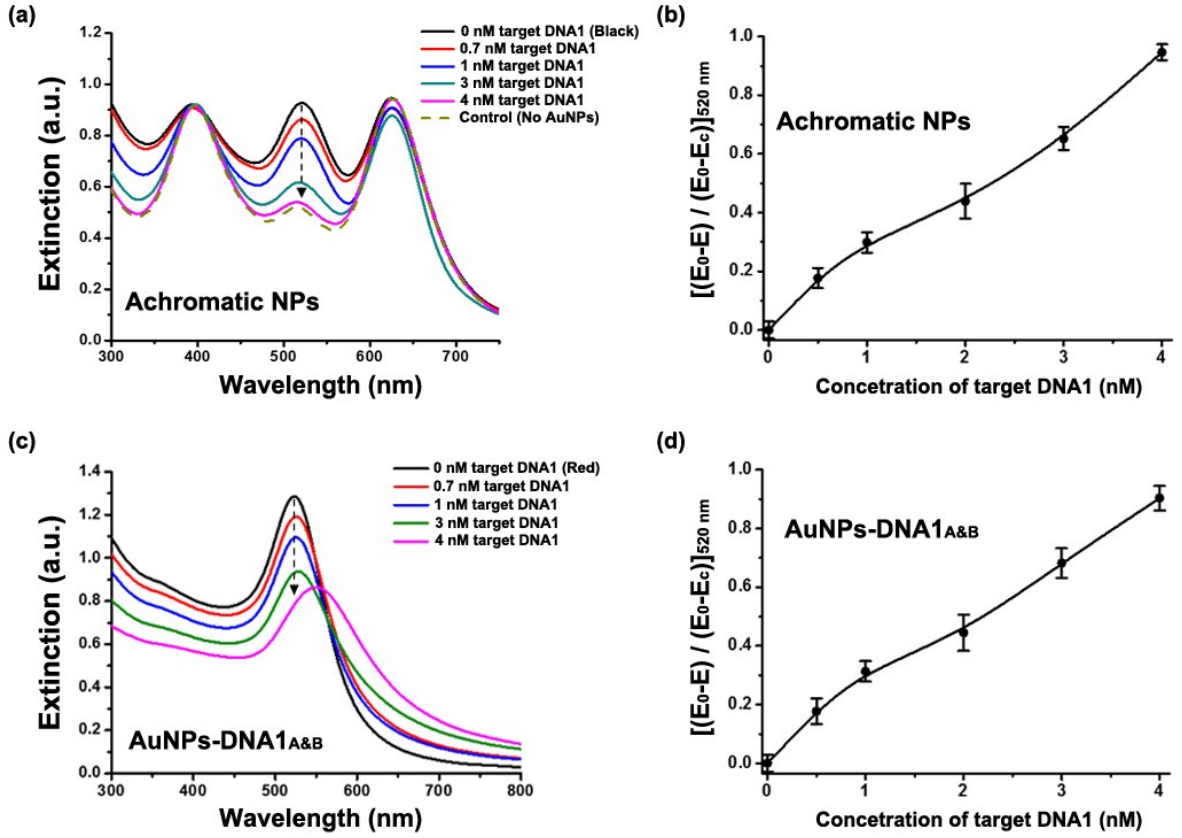


**Fig. S8** (a) The extinction spectra of achromatic nanoparticles (achromatic NPs) with different concentrations of target DNA1. (b) The calibration curve of achromatic NPs obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 520 nm under the extinction spectra. $E_0$ designates the extinction of achromatic nanosensor at 520 nm and $E_c$ specifies the extinction of achromatic nanosensor prepared without AuNPs-DNA1$_{A\&B}$ at the same wavelength. The detection linear range of a target DNA1 was approximately 0.5 nM - 3 nM. The limit of detection (LOD) calculated from calibration curve is 0.48 nM (c) The extinction spectra of original AuNPs-DNA1$_{A\&B}$ with different concentrations of target DNA1. (d) The calibration curve of original AuNPs-DNA1$_{A\&B}$ obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 520 nm under the extinction spectra. $E_0$ designates the extinction of AuNPs-DNA1$_{A\&B}$ at 520 nm and $E_c$ specifies the extinction in the absence of AuNPs-DNA1$_{A\&B}$. The detection linear range of target DNA1 was approximately 0.5 nM - 3 nM. The limit of detection (LOD) calculated from calibration curve is 0.5 nM.

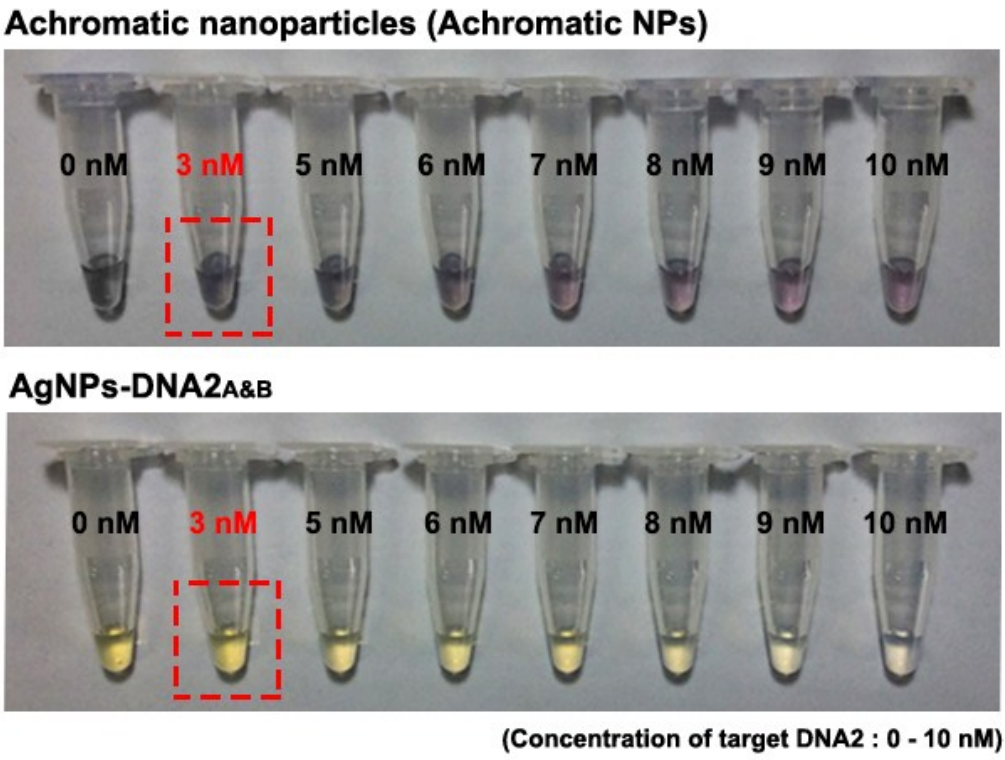


**Fig. S9** The color change of achromatic nanoparticles (achromatic NPs) and AgNPs-DNA2$_{A\&B}$ with different concentrations of target DNA2.

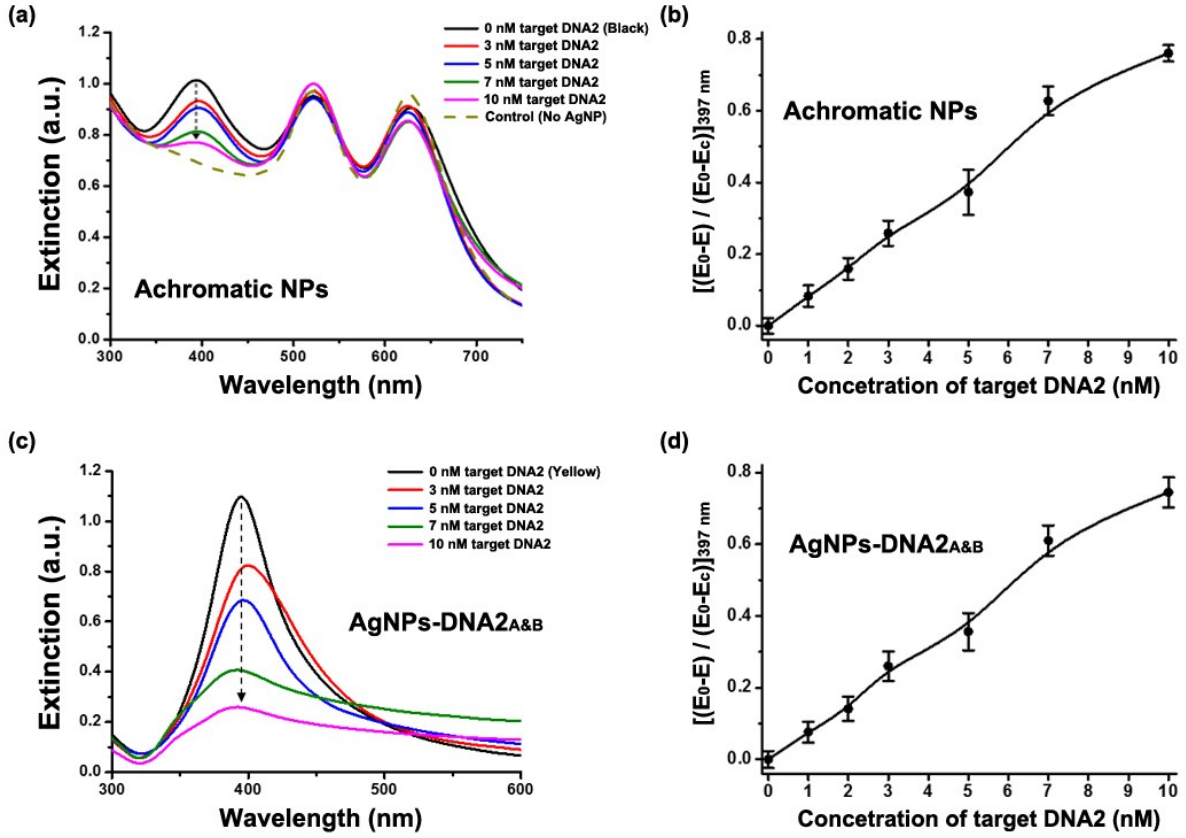


**Fig. S10** (a) The extinction spectra of achromatic nanoparticles (achromatic NPs) with different concentrations of target DNA2. (b) The calibration curve of achromatic NPs obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 397 nm under the extinction spectra. $E_0$ designates the extinction of achromatic nanosensor at 397 nm and $E_c$ specifies the extinction of achromatic nanosensor prepared without AgNPs-DNA2$_{A\&B}$ at the same wavelength. The detection linear range of target DNA2 was approximately 2 nM - 8 nM. The limit of detection (LOD) calculated from titration curve is 0.96 nM (c) The extinction spectra of AgNPs-DNA2$_{A\&B}$ with different concentrations of target DNA2. (d) The calibration curve of AgNPs-DNA2$_{A\&B}$ obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 397 nm under the extinction spectra. $E_0$ designates the extinction of AgNPs-DNA2$_{A\&B}$ at 397 nm and $E_c$ specifies the extinction in the absence of AgNPs-DNA2$_{A\&B}$. The detection linear range of target DNA2 was approximately 2 nM - 7.5 nM. The limit of detection (LOD) calculated from calibration curve is 0.98 nM

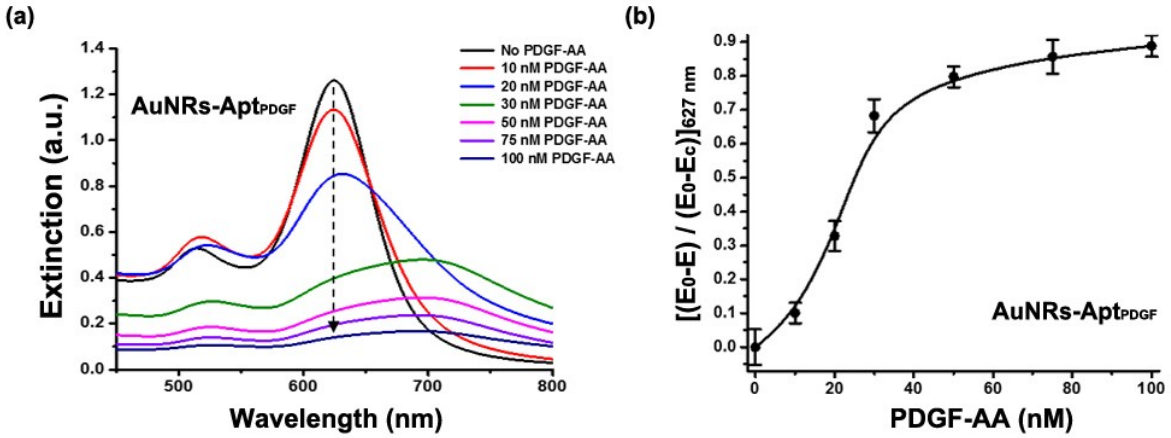


**Fig. S11** (a) The extinction spectra of PDGF aptamer functionalized AuNRs (AuNRs-Apt$_{PDGF}$) with different concentrations of PDGF-AA. (b) The calibration curve of PDGF aptamer functionalized AuNRs (AuNRs-Apt$_{PDGF}$) obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 627 nm under the extinction spectra. $E_0$ designates the extinction of AuNPs-AuNRs-Apt$_{PDGF}$ at 627 nm and $E_c$ specifies the extinction in the absence of AuNRs-Apt$_{PDGF}$. The detection linear range of PDGF-AA was approximately 15 nM - 40 nM. The limit of detection (LOD) calculated from calibration curve is 19 nM.

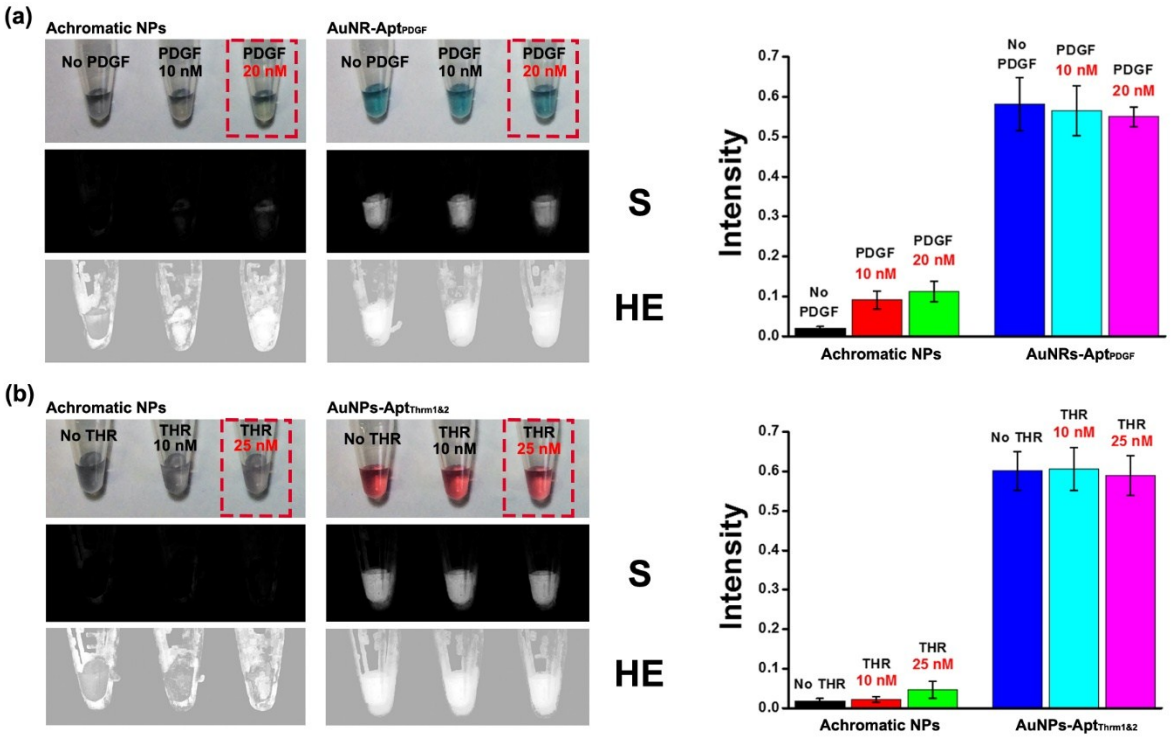


**Fig. S12** The analysis of color changes of achromatic nanoparticles (achromatic NPs) and aptamer functionalized individual nanoparticles in the presence of target proteins (thrombin and PDGF-AA) using the computational method. (a) Achromatic NPs and AuNRs-Apt$_{PDGF}$ in the presence of 0 to 20 nM PDGF-AA. (b) Achromatic NPs and AuNPs-Apt$_{Thrm1\&2}$ in the presence of 0 to 25 nM thrombin. It is possible to distinguish the presence of 25 nM thrombin by combining the achromatic nanosensor with the image processing method. This is close to the LOD (18 nM) obtained from UV-vis spectrophotometer.

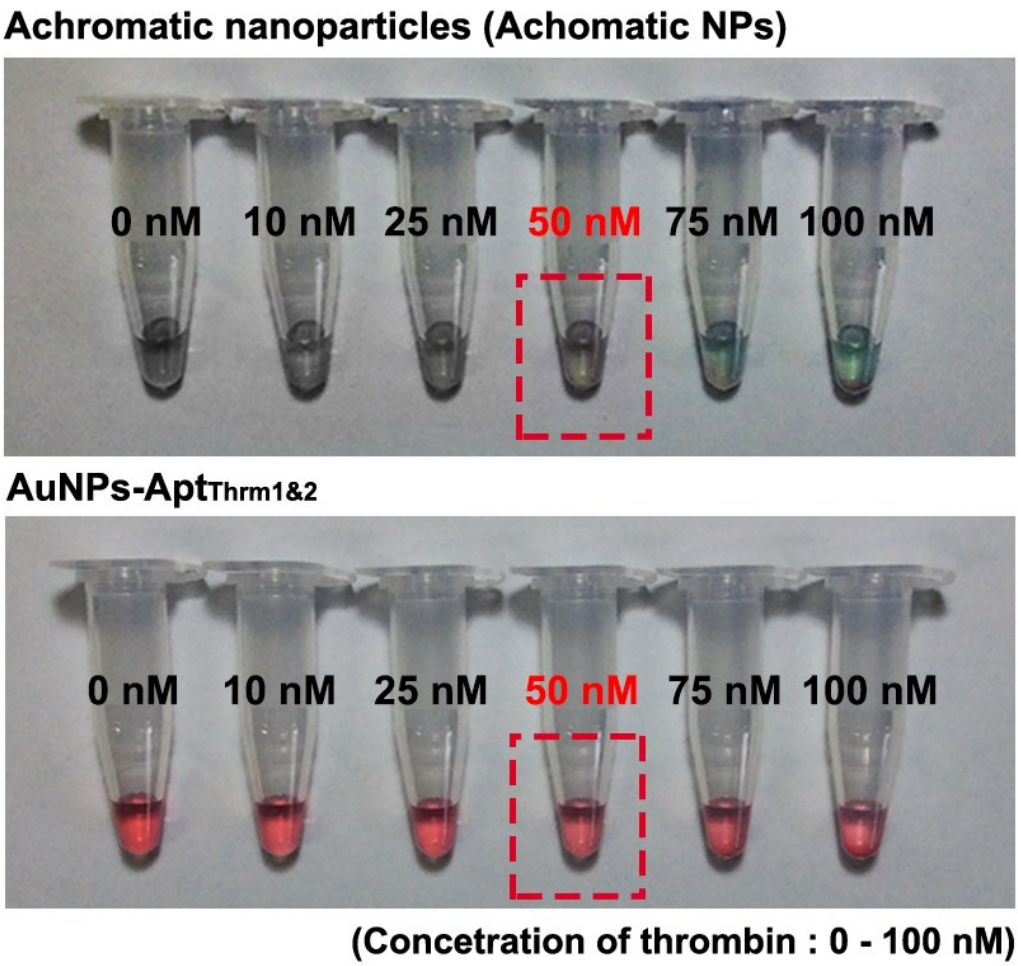


**Fig. S13** The color change of achromatic nanoparticles (achromatic NPs) and thrombin aptamer functionalized AuNPs (AuNPs-$Apt_{Thrm1\&2}$) with different concentrations of thrombin. A change was observed when the achromatic sensor was treated with 50 nM thrombin. However, it was very difficult to distinguish the color change occurring in the AuNPs-$Apt_{Thrm1\&2}$ sample treated with 50 nM thrombin.

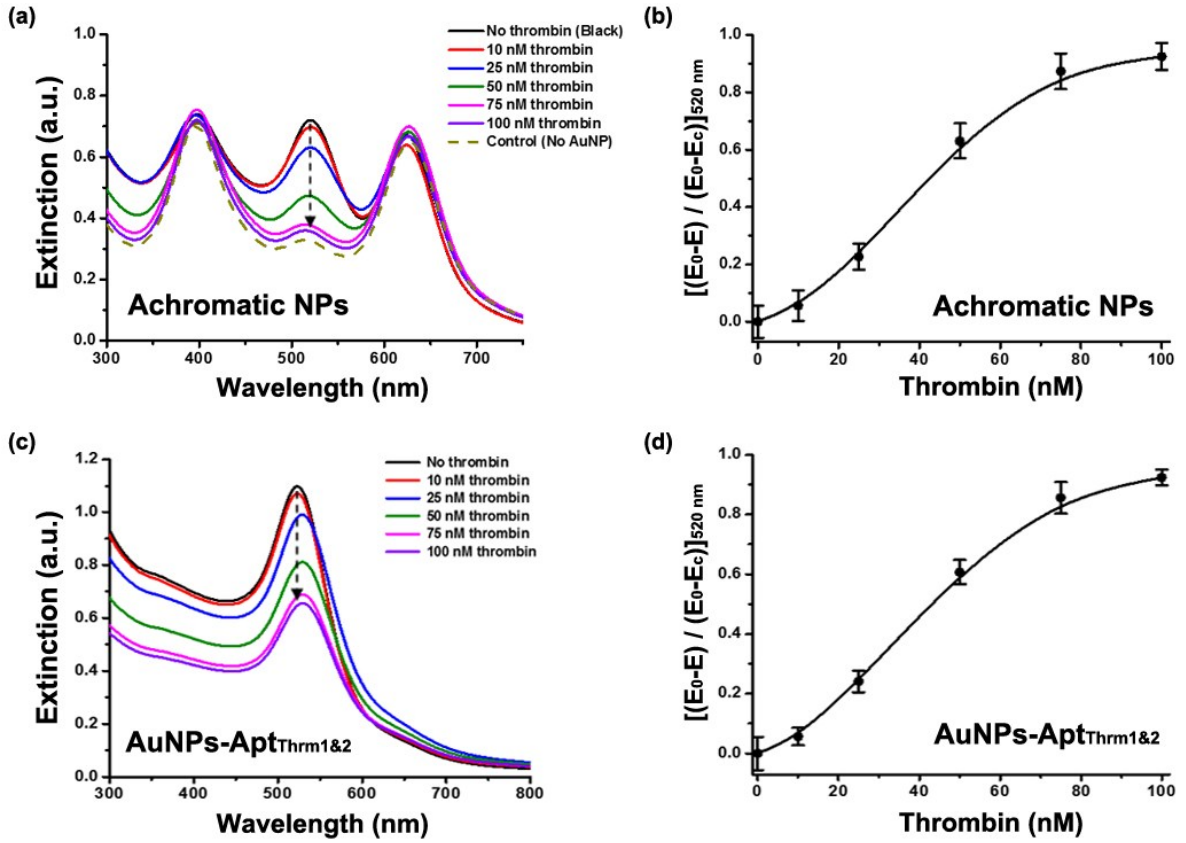


**Fig. S14** (a) The extinction spectra of achromatic nanoparticles (achromatic NPs) with different concentrations of thrombin. (b) The calibration curve of achromatic nanoparticle (achromatic NPs) obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 520 nm under the extinction spectra. $E_0$ designates the extinction of achromatic nanosensor at 520 nm and $E_c$ specifies the extinction of achromatic nanosensor prepared without AuNPs-$Apt_{Thrm1\&2}$ at the same wavelength. The detection linear range of thrombin was approximately 10 nM - 80 nM. The limit of detection (LOD) calculated from calibration curve is 20 nM. (c) The extinction spectra of thrombin aptamer functionalized AuNP mixture (AuNPs-$Apt_{Thrm1\&2}$) with different concentrations of thrombin. (d) The calibration curve of thrombin aptamer functionalized AuNP mixture (AuNPs-$Apt_{Thrm1\&2}$) obtained from the absorbance difference between $E_0$-E and $E_0$-$E_c$ at 520 nm under the extinction spectra. $E_0$ designates the extinction of AuNPs-$Apt_{Thrm1\&2}$ at 520 nm and $E_c$ specifies the extinction in the absence of AuNPs-$Apt_{Thrm1\&2}$. The detection linear range of thrombin was approximately 10 nM - 80 nM. The limit of detection (LOD) calculated from calibration curve is 20 nM.

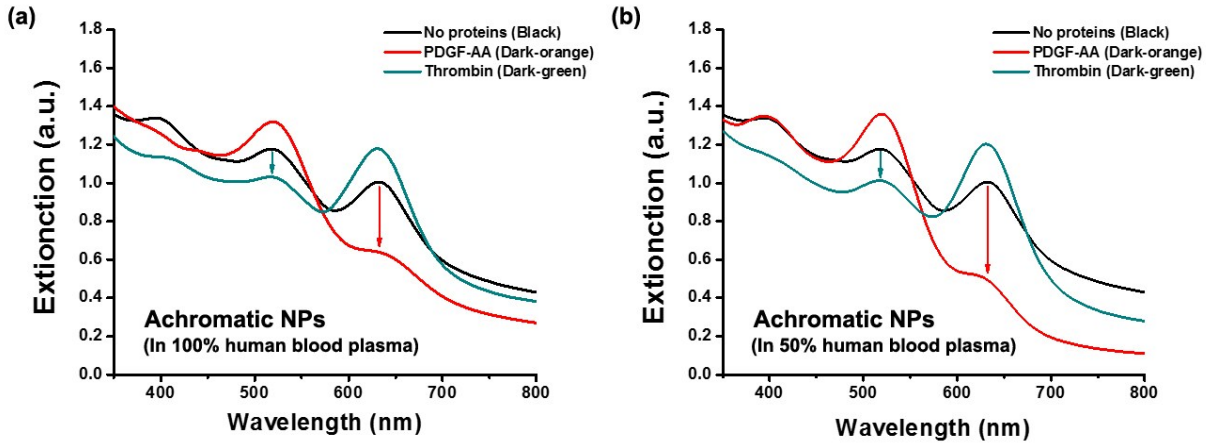


**Fig. S15** The extinction spectra of achromatic nanoparticles (achromatic NPs) in the presence of PDGF-AA or thrombin (a) in 100% human blood plasma and (b) in 50% human blood plasma.

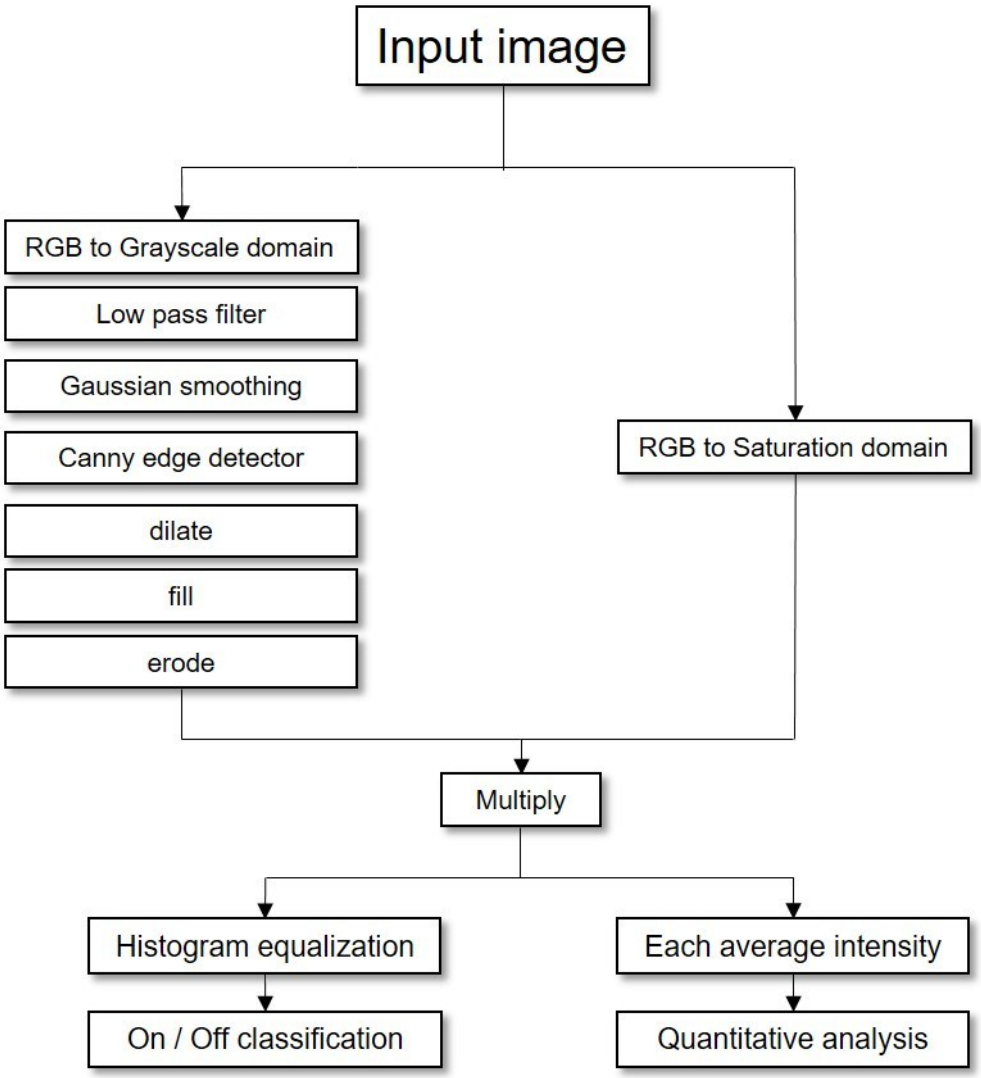


**Fig. S16** The flow chart of computational method for color analysis.