Surface plasmon resonance-based immunoassay for human C-reactive protein

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Direct Ab immobilization procedure (without Pr A/G)

A SPR Au chip was hydroxylated by treatment with 90 µL of 1% (w/v) KOH for 5 min followed by extensive washing with ultrapure water (UPW). The EDC-activated anti-human CRP Ab was prepared by incubating anti-human CRP Ab (990 µL, 0.100 mg mL^{-1}) with EDC (10 µL, 4 mg mL^{-1} in 0.1 M MES, pH 4.7) for 15 min at room temperature (RT). The EDC-activated anti-human CRP Ab was admixed in 1% (v/v) APTES (in the ratio of 1:1 v/v) and the resulting solution (90 µL) was injected over the KOH-treated Au SPR chip. After 30 min of incubation at RT in a fume hood, the Ab-functionalized SPR chip was washed extensively with HBS, docked into BIAcore 3000 and then primed. Thereafter, 20 µL of 1% (w/v) BSA was injected at 10 µL min^{-1} to block the non-specific protein binding sites on the anti-human CRP Ab-functionalized chip.

Previously developed multistep Ab immobilization procedure based on covalent binding of Pr A

Surface cleaning of SPR Au chip (SIA kit) and APTES functionalization

The Au chip was cleaned with Piranha etch [60 µL of H_2SO_4 (97.5%, v/v): 30 µL of H_2O_2 (30%, v/v)] for 2 min followed by extensive washing with UPW. The chip was then incubated with 100 µL of 2% (v/v) APTES for 1 h at RT in a fume hood followed by five washings with UPW.

EDC activation of Pr A

Pr A (990 µL of 100 µg/mL in HBS) was incubated at RT for 15 min with 10 µL of a cross-linking solution containing EDC (4 mg/mL) and sulfo-NHS (11 mg/mL) in 0.1 M MES buffer, pH 4.7. It leads to the activation of carboxyl groups on Pr A with EDC.

Antibody immobilization procedure

EDC-activated Pr A (50µL, 0.100 mg/mL) was injected over all four flow cells of an APTES-functionalized Au chip at 10 µL/min until the baseline was stabilized. This leads to the covalent binding of EDC-activated Pr A on the APTES-functionalized Au chip. Thereafter, 50 µL of anti-human CRP Ab (0.100 mg/mL) diluted in 10 mM HBS, pH 7.4 was injected over all flow cells at 10 µL/min. Non-specific binding sites on the Ab-bound chip were then blocked by injecting 20 µL of 1% (w/v) BSA at 10 µL/min.

Covalent Ab immobilization procedure on CM5-dextran chip[1]

A CM5 dextran-functionalized Au chip was docked into the BIAcore 3000 system and primed. The optimum pH of sodium acetate buffer for Ab immobilization, determined by pre-concentration studies, was 4.2. The CM5-dextran chip was activated by injecting a 50 µL cross-linking solution, containing 200 µg of EDC and 550 µg of sulfo-NHS in 0.1M MES buffer, pH 4.7, through all the flow cells at 10 µL/min. Thereafter, 50 µL of anti-human CRP Ab (0.100 mg/mL) was injected into the four flow cells of the APTES-functionalized Au chip at 10 µL/min and the baseline was allowed to stabilize. Non-specific binding sites on the chip were then blocked by consecutive injection of 20 µL of 1 M ethanolamine hydrochloride, pH 8.5 and 20 µL of 1% (w/v) BSA at 10 µL/min. Ethanolamine hydrochloride blocked unreacted ester groups of the CM5-dextran matrix, while BSA blocked non-specific binding sites on the chip surface.

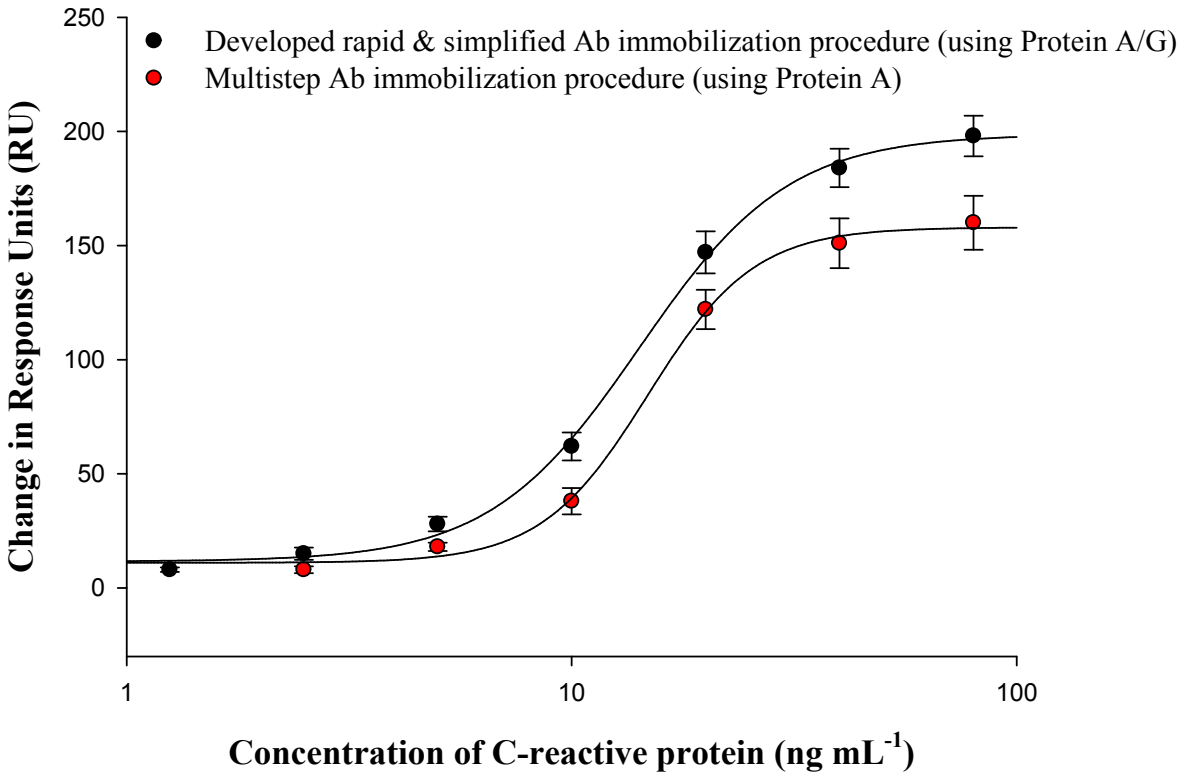

Fig. S1. Comparison of the SPR-based CRP IAs performed using the developed rapid and highly-simplified Ab immobilization procedure using Pr A/G and our previously reported multistep Ab immobilization procedure using Pr A. All experiments were performed in triplicate with the error bars representing the standard deviation.

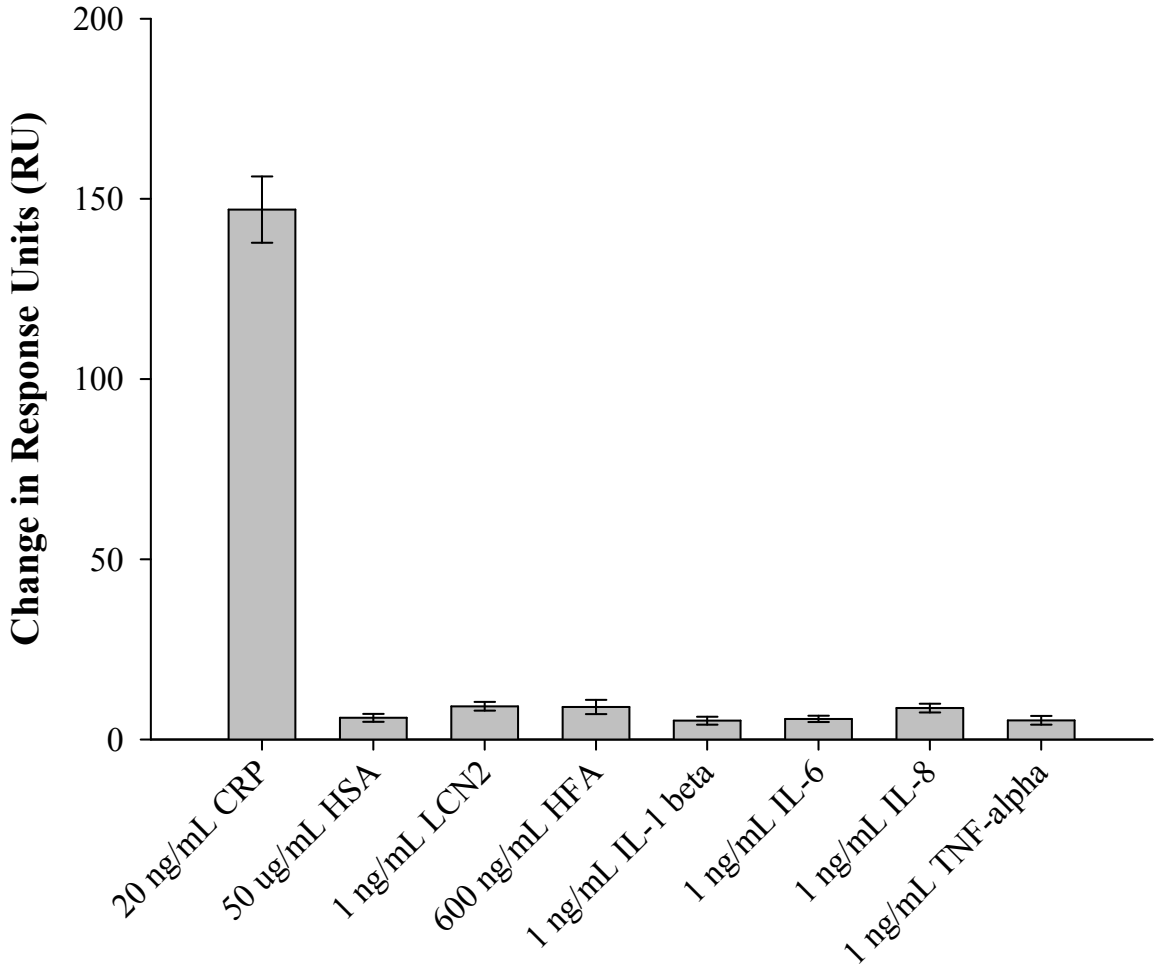

Fig. S2. Specific binding of human CRP by the developed SPR IA in comparison to the non-specific control proteins at relatively high concentrations compared to their physiological levels: human serum albumin (HSA), human lipocalin-2 (LCN2), human fetuin A (HFA), interleukin (IL)-1 beta, IL-6, IL-8 and tumor necrosis factor (TNF)-alpha. All experiments were done in triplicate with the error bars representing the standard deviation.

REFERENCES

(1) Vashist S. K.; Dixit, C. K.; MacCraith, B. D.; O'Kennedy, R. *Analyst* 2011, 136, 4431-4435.