Expanding a portfolio of (FO-) SPR surface chemistries with the Co(III)-NTA oriented immobilization of His_6-tagged bioreceptors for applications in complex matrices

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EXPERIMENTAL SECTION

FO-SPR platform (FOx Biosystems)

The compact FO-SPR platform (White FOx 1.0) commercialized by FOx Biosystems (Diepenbeek, Belgium) is illustrated in Figure S1. The optical assembly of the device is protected and covered by a black container. Four fiber-optic (FO) probes are integrated into the platform as multiple sensing channels. The white light is delivered to each FO probe by a bifurcated fiber and the reflected SPR signal is collected by the same bifurcated fiber to a spectrometer. User-friendly software is used to control the automated robot system, enabling a flexible movement of the FO probes and recording the obtained data.

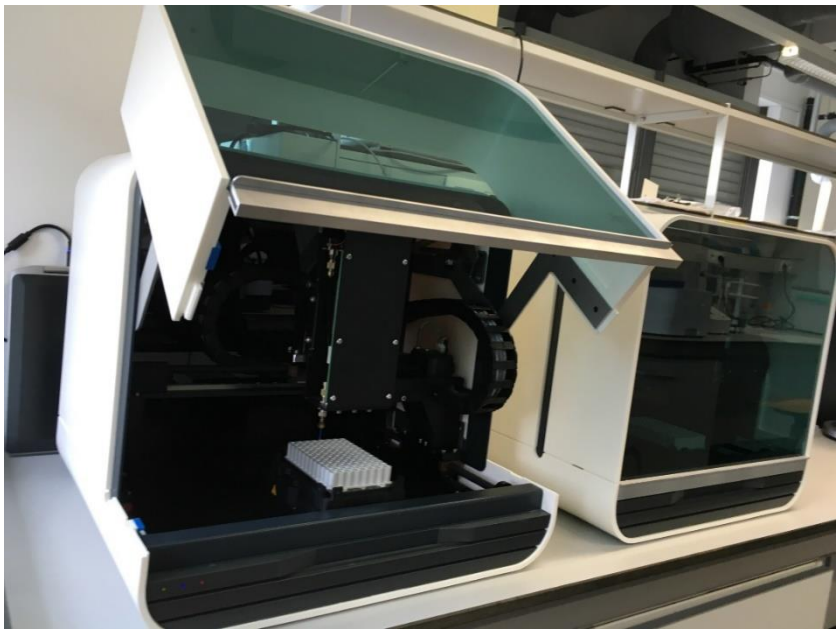

Figure S1. The FO-SPR platform (FOx Biosystems) used in this study

RESULTS AND DISCUSSION

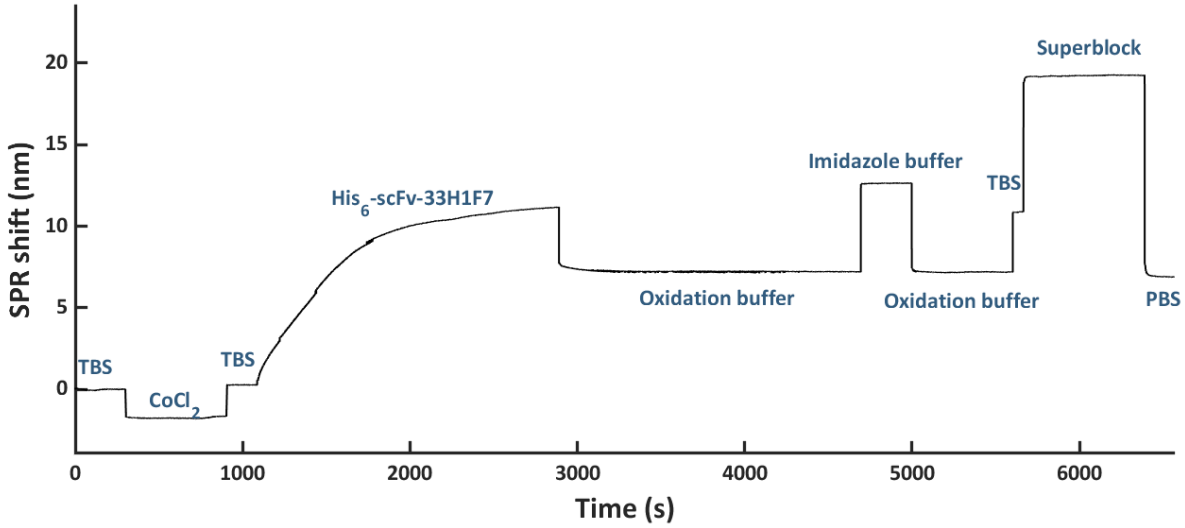

Figure S2. The sensorgram depicting functionalization of the FO probe, with each step labelled. Almost no signal decrease was observed during the step with imidazole, which proved the successful and robust oxidation of Co^{2+} to Co^{3+} without elution of His_6-tagged scFv-33H1F from the sensor surface.

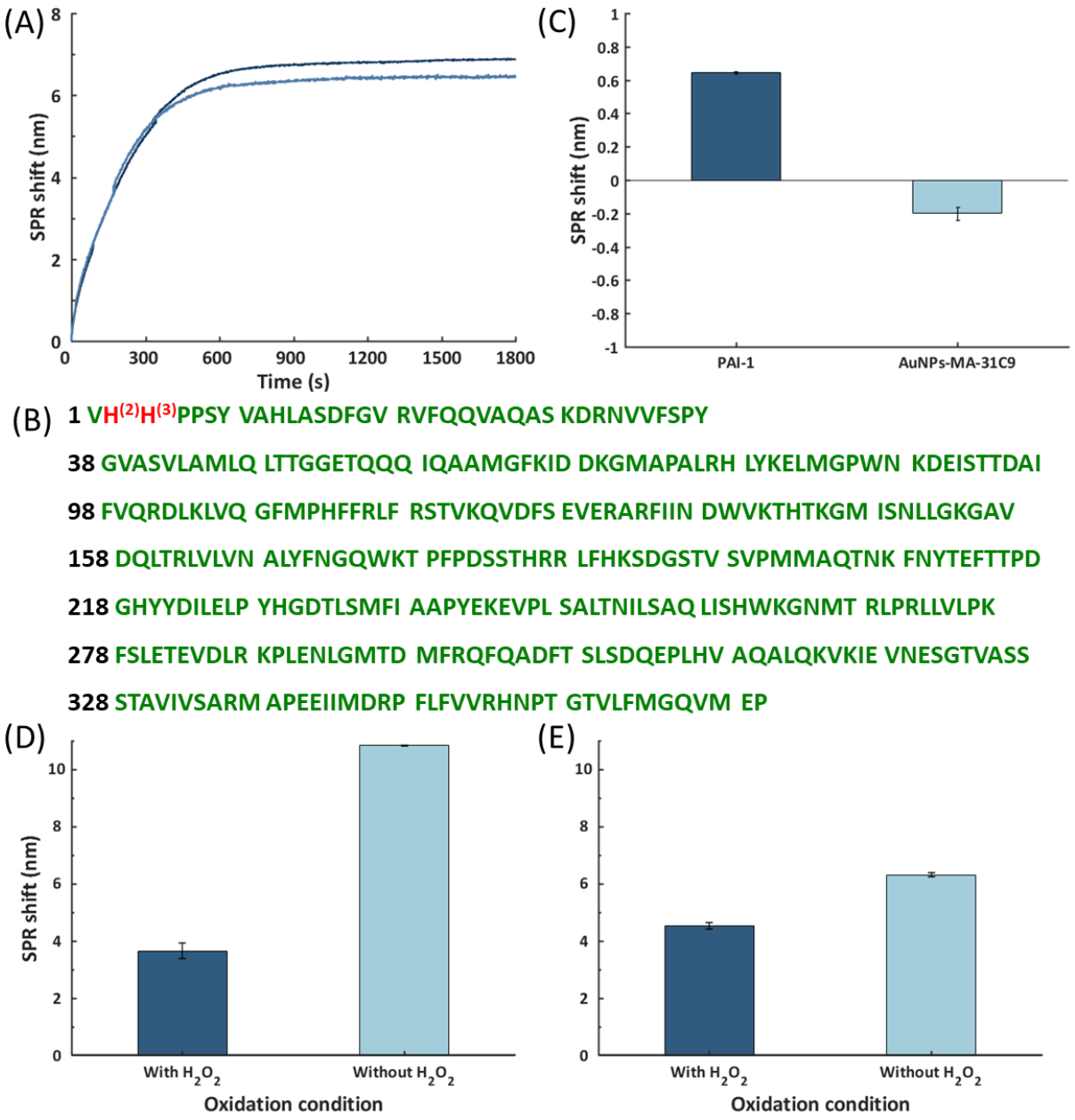

Figure S3. Analysis of PAI-1 sequence and non-specific binding to the FO-SPR surface. (A) Kinetic curves. 10 µg/mL of PAI-1 in TBS/Tween20 buffer was immobilized on the Co(II)-NTA surface of the FO probe (n_s = 2). (B) The amino acid sequence of PAI-1 (Homo sapiens), with two consecutive histidines highlighted in red. H^3 has been previously shown to be part of the epitope for binding to MA-31C9.[1] (C) SPR shifts obtained from the negative control experiment, where no bioreceptor was immobilized on the FO probes to perform sandwich bioassay for detecting PAI-1 (400 ng/mL spiked in buffer) using AuNPs functionalized with MA-31C9 for signal amplification (n_s = 2). SPR shifts obtained from the immobilization of (D) scFv-33H1F7 and (E) PAI-1 at 10 µg/mL in TBS buffer, after the FO probes coated with the chelate (Co(II)-NTA-H_2O) were immersed in PBS buffer pH 6.5 with or without H_2O_2 for 30 min. Error bars represent standard deviations (n_s = 2).

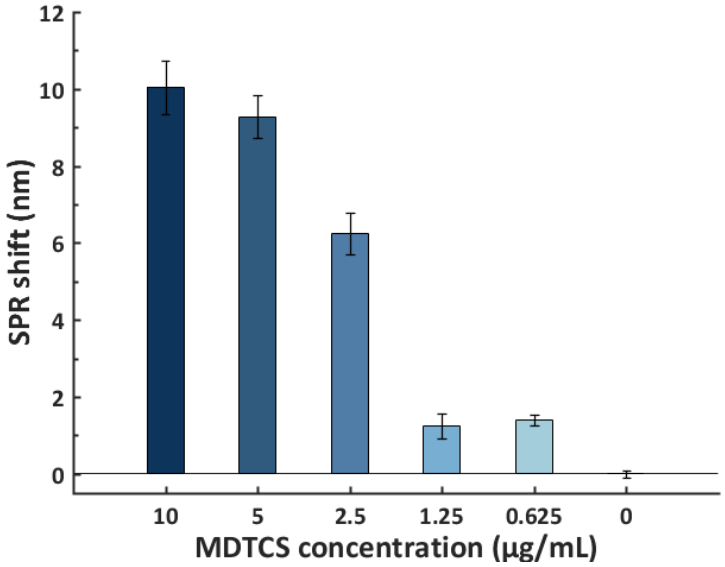

Figure S4. SPR shifts obtained for immobilization of different concentrations of MDTCS bioreceptors on the FO probe. Error bars represent standard deviations (n_s = 2).

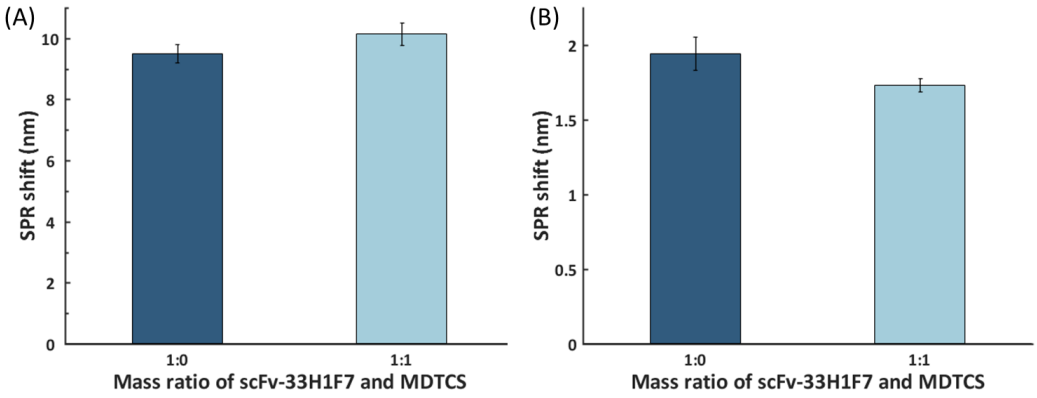

Figure S5. Interaction between PAI-1 and the mixture of scFv-33H1F7/MDTCS. (A) SPR shifts of immobilizing the mixture of scFv-33H1F7/MDTCS at the mass ratios of 1:0 and 1:1 separately (at a total amount of 10 μg/mL). (B) SPR shifts of detecting 1 μg/mL of PAI-1 using the FO probe immobilized with the corresponding mass ratio of scFv-33H1F7/MDTCS. Error bars represent standard deviations (n_s = 2).

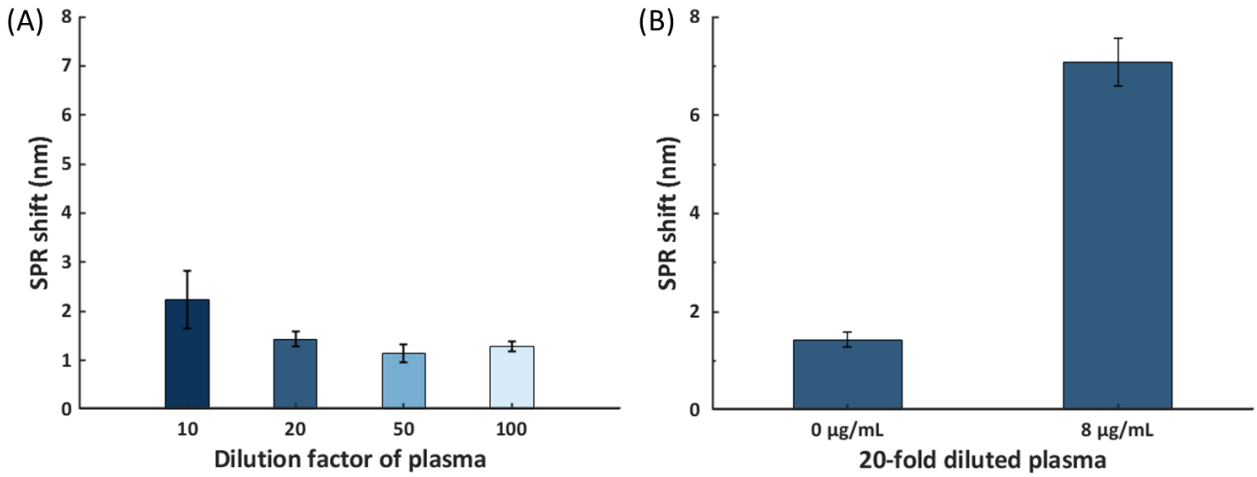

Figure S6. (A) Summary of the non-specific signal obtained for different dilutions (10, 20, 50 and 100-fold) of plasma samples. (B) SPR shifts obtained from 20-fold diluted plasma samples spiked with 0 or 8 µg/mL of PAI-1. The FO probes were functionalized with 10 µg/mL of scFv-33H1F7 for 30 min of the label-free bioassay. Error bars represent standard deviations (n_s = 2).

Table S1. The calculated p-values from a t-test to compare LOD estimates in different bioassays at various conditions. Based on the calculated p-values from the table with none being less than 0.05, there is no significant difference in calculated LOD estimates between every two bioassays.

Bioassay type			**Label-free bioassay**				**Sandwich bioassay**			
Sample matrix			**Buffer**			**20x plasma**	**Buffer**			**10x plasma**
scFv-33H1F7 concentrations (µg/mL)			**20**	**10**	**5**	**10**	**20**	**10**	**5**	**10**
Label-free bioassay	**Buffer**	20		0.29	0.81	0.46				
		10			0.19	0.99				
		5				0.37				
	20x plasma	10								
Sandwich bioassay	**Buffer**	20						0.24	0.20	0.26
		10							0.67	0.99
		5								0.70
	10x plasma	10								

References

(1) Gils, A.; Lester, ; Meissenheimer, M.; Compernolle, G.; Declerck, P. J.; Gils, A. Species-Dependent Molecular Drug Targets in Plasminogen Activator Inhibitor-1 (PAI-1). *J. Thromb. Haemost.* **2009**, No. 02, 609–610.