Efficient Bioconjugation of Protein Capture Agents to Biosensor Surfaces Using Aniline-Catalyzed Hydrazone Ligation

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SUPPORTING INFORMATION

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Table S1. Summary of peak shifts before and after glycine rinse at all pH conditions and the estimated immobilized antibody density

	pH (# of rings)	Peak shift before glycine rinse (pm)	Peak shift after glycine rinse (pm)	Estimated immobilized antibody density (ng/mm^2)	Estimated area/antibody (nm^2/antibody)
Without aniline	4.5 (n=12)	72.4 ± 8.7	60.6 ± 8.8	0.9 ± 0.13	280 ± 40
	6.0 (n=12)	139.6 ± 37.0	111.3 ± 37.4	1.6 ± 0.5	150 ± 50
	7.4 (n=11)	102.8 ± 27.8	20.4 ± 20.4	0.3 ± 0.3	800 ± 800
With aniline	4.5 (n=10)	443.4 ± 21.8	437.5 ± 21.7	6.4 ± 0.3	39 ± 2
	6.0 (n=12)	371.3 ± 8.2	358.6 ± 8.3	5.3 ± 0.12	47 ± 01
	7.4 (n=11)	329.1 ± 12.4	302.6 ± 10.2	4.4 ± 0.15	56 ± 2

Aniline-catalyzed immobilization of anti-thrombin antibody at different flow rates and pHs

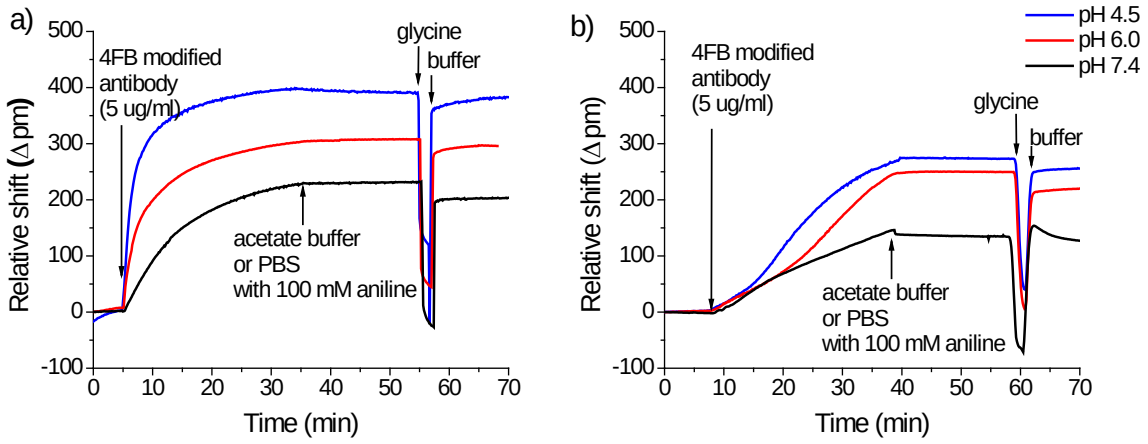

Figure S1. Real-time shifts in resonance wavelength from representative microrings upon covalent immobilization of anti-(human thrombin) antibody onto the sensor surfaces at three different pHs in the presence of 100 mM aniline at 150 μL/min (a) and 6 μL/min (b). In each trace the sensors were initially incubated in buffer (with or without aniline, respectively) and a 5 μg/mL solution of 4FB-modified antibody with 100 mM aniline was flowed for 30 min. After switching back to the original buffer for 20 minutes, non-covalently attached antibody was removed with a low pH (pH 2.2) glycine buffer rinse. The sensors were then returned to the respective buffer at t = 57 min to determine the residual net shift corresponding to the amount of covalently immobilized antibody

Comparison of succinimidyl ester/amine conjugation and aniline-catalyzed hydrazone ligation at pH 7.4

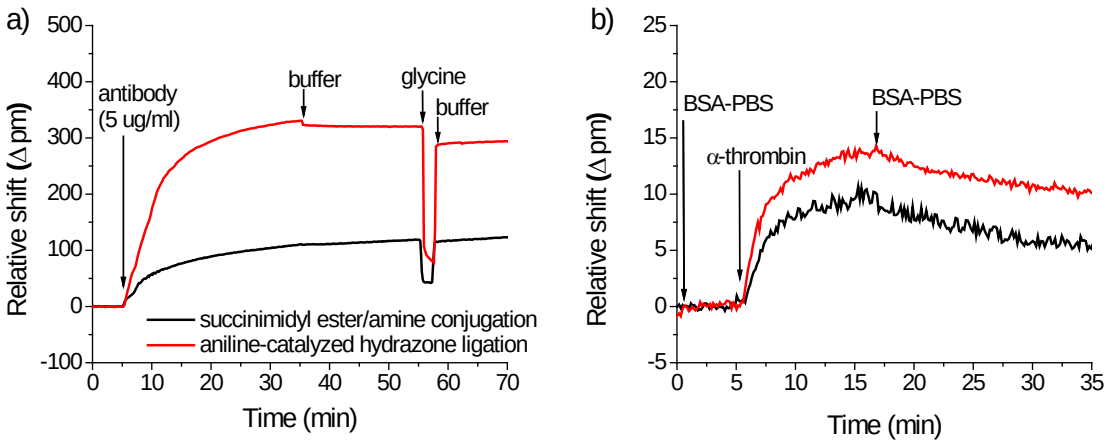

Figure S2. (a) Thrombin antibody immobilization on the succinimidyl ester modified surface (black) and 4FB-modified antibody immobilization on the HyNic presented surface in the presence of aniline (red). In each trace the sensors were initially incubated in buffer (PBS at pH 7.4 or 100 mM aniline in PBS at pH 7.4, respectively) and a 5 μg/ml solution of unmodified or 4FB-modified antibody was incubated for 30 min. After buffer rinse (20 min), any non-covalently attached antibody was removed by a rinse with low pH (pH=2.2) glycine buffer; (b) Detection of human α-thrombin using microrings modified with antibody at pH 7.4 by succinimidyl ester/amine conjugation (black) and aniline-catalyzed hydrazone ligation (red). The microrings are initially in BSA-PBS buffer and thrombin is introduced at t = 5 min. Following 10 min incubation with thrombin, the flow cell is rinsed with BSA-PBS to observe antigen dissociation.

Comparison of responses from human α-thrombin binding to microrings functionalized with or without aniline at pH 6.0

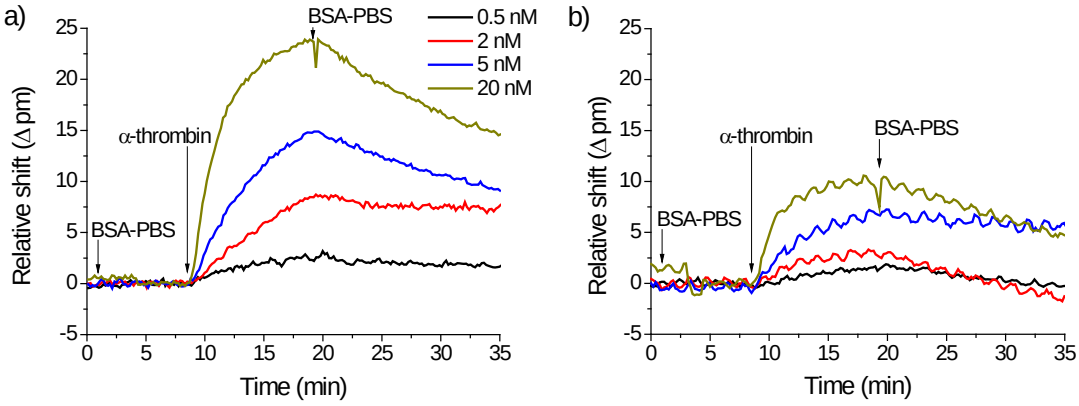

Figure S3. Time-resolved detection of human α-thrombin using microrings modified with anti-(human thrombin) antibody at pH 6.0 in the presence (a) and absence (b) of aniline. Following 10 minutes exposure to thrombin, the antibody surface was rinsed with BSA-PBS for 20 minutes and regenerated by exposure to glycine buffer for two minutes before returning to BSA-PBS.

Comparison of responses from human α-thrombin binding to microrings functionalized with or without aniline at pH 4.5

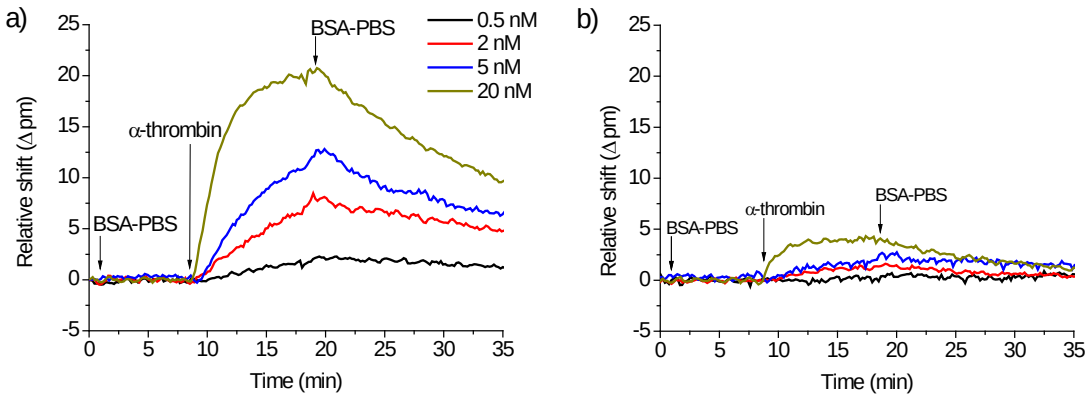

Figure S4. Time-resolved detection of human α-thrombin using microrings modified with anti-(human thrombin) antibody at pH 4.5 in the presence (a) and absence (b) of aniline. Following 10 minutes exposure to thrombin, the antibody surface was rinsed with BSA-PBS for 20 minutes and regenerated by exposure to glycine buffer for two minutes before returning to BSA-PBS.