

Supplementary Information

Development of a Multiplex Sandwich Aptamer Microarray for the Detection of VEGF₁₆₅ and Thrombin

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Figure S1. Images of a microarray slide after the incubation of Alexa555-labeled VEGF₁₆₅. Vap7(12T)NH₂ and Vap7-NH₂ were anchored on the glass slide as capture layer on the left and on the right, respectively. The green fluorescence (due to Alexa555 fluorophore) represents the bound labeled protein.

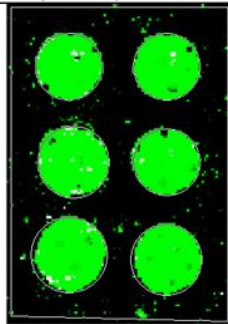
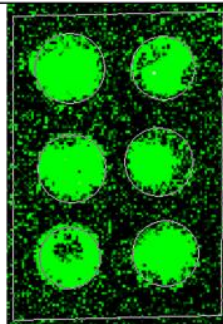
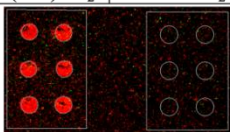
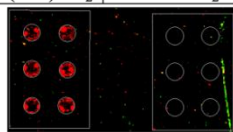
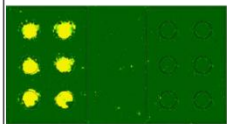
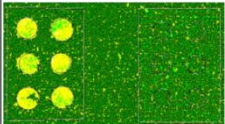
Capture layer	Vap7 (12T)NH ₂	Vap7 NH ₂
Chamber		
Protein	Alexa555-labeled VEGF ₁₆₅	

Table S1. Schematic representation of the SAM protocol for VEGF detection.

Sub-array	1		2		3		4	
Capture layer	Vap7 (12T)NH ₂	Random DNA-NH ₂	Vap7 (12T)NH ₂	Random DNA-NH ₂	Vap7 (12T)NH ₂	Random DNA-NH ₂	Vap7 (12T)NH ₂	Random DNA-NH ₂
Protein	VEGF ₁₆₅ (0,1μM)		Alexa555-VEGF ₁₆₅ (0,1μM)		VEGF ₁₆₅ (0,1μM)		Alexa555-VEGF ₁₆₅ (0,1μM)	
Detection layer	pre-complexed with VEa5-Cy5(0,5μM)		pre-complexed with VEa5-Cy5(0,5μM)		VEa5-Cy5(0,5μM)		VEa5-Cy5(0,5μM)	
Procedure	One-step				Two-steps			

Figure S2. Images of the microarray slide for the Sandwich Aptamer Microarray (SAM) for VEGF. In each subarray, Vap7(12T)NH₂ was printed on the left while the negative control (Random DNA-NH₂) was printed on the right. Red fluorescence represents binding by VEa5-Cy5. In chambers 2 and 4, in which Alexa 555-labeled VEGF₁₆₅ (500 nM) was incubated, the fluorescence of the yellow spots indicates the simultaneous co-localization of the protein (green) and of the detection aptamer (red).

One-step protocol				Two-steps protocol				
1		2		3		4		
Vap7 (12T)NH ₂	Random DNA-NH ₂	Vap7 (12T)NH ₂	Random DNA-NH ₂	Vap7 (12T)NH ₂	Random DNA-NH ₂	Vap7 (12T)NH ₂	Random DNA-NH ₂	
								Red fluorescence (Cy5)
								Green and red fluorescence merge (Alexa 555 + Cy5)