Miniaturized multiplexed protein arrays

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Abstract: Miniaturization of microarrays is a crucial step towards development of portable biosensors for simultaneous detection of multiple (biological) targets. The main challenge in the process of miniaturization of the active site (spot) is the loss of signal (roughly proportional to the area), resulting in low Signal to Noise Ratio (SNR). Using our Nano-fountain pen (NFP) technique to print nanometer size structures of proteins[1-5], we have previously shown that the "first tier" of the sensor, i.e. the immobilization surface, plays a crucial role in maintaining high SNR and demonstrated the feasibility of spots with ~ 400 nm diameter and SNR of ~10 [6]. Here we concentrate on the "second tier", i.e. the layer of bioreceptors. In the case of IgG-based arrays, the binding site density (binding of target molecule to capture molecules) is severly reduced by randomly oriented immobilization of IgG. While this fact is well understood, reported methods for IgG orientation remain either complex, time consuming, expensive or unsubstantiated.

We present two new approaches to immobilize IgG molecules in a binding-favorable orientation:

a. An FC-receptor improved strategy (protein A as an example), where complexation with the IgG molecules is performed prior to immobilization on the sensor surface.

b. Electric-field assisted oriented immobilization, by addressing the electrical dipole of IgG molecules.

For both methods we demonstrate up to two-fold enhancement of signal and discuss the implications on further miniaturization of active site in multiplexed protein arrays. Moreover, we show that the protein A complexation approach is readily applicable for standard methods of micro-array manufacture, with the same signal amplification and significant reduction of costs.

References:

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