Isotachophoresis-Based Surface Immunoassay

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In the absence of amplification methods for proteins, the immune-detection of low-abundance proteins using antibodies is fundamentally limited by binding kinetic rates. In this talk I will present a new class of surface-based immunoassays in which protein-antibody reaction is accelerated by isotachophoresis (ITP). In recent work we demonstrated the use of ITP to pre-concentrate and deliver target proteins to a surface decorated with specific antibodies, where effective utilization of the focused sample is achieved by modulating the driving electric field (stop-and-diffuse ITP mode) or applying a counter flow that opposes the ITP motion (counterflow ITP mode). Using enhanced green fluorescent protein (EGFP) as a model protein, we carried out an experimental optimization of the ITP-based immunoassay and demonstrated a 10,000-fold improvement in limit of detection compared to a standard immunoassay, in a 6 min protein-antibody reaction. I will discuss the design considerations for such systems, and present analytical models for the two operation modes, elucidating the interplay between reaction, diffusion and accumulation time scales, and enabling the prediction and design of future immunoassays.

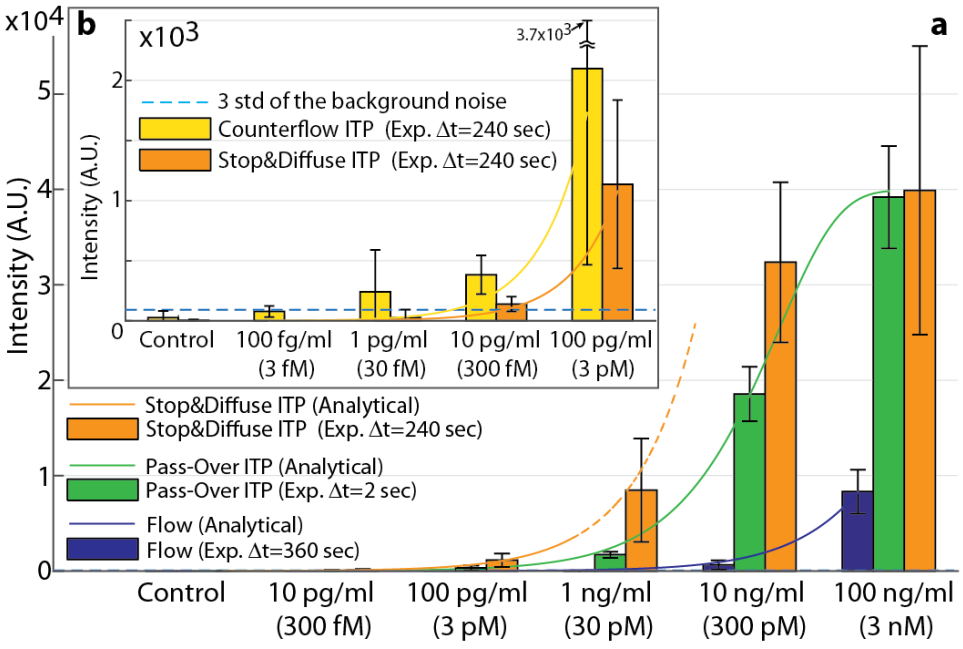

Figure 1. Analytical and experimental results comparing the signal of the ITP-based immunoassay with standard flow. ***a****, PO-ITP provides approx..100-fold improvement in LoD, whereas SD-ITP provides a total improvement between 100- and 1,000-fold. Solid lines are the fit to our theoretical model. The SD-ITP analytical model is valid only at low concentrations, and its extension to higher concentrations is indicated by a dashed line.* ***b****, At lower concentrations, an additional gain is obtained by the CF-ITP, enabling a further improvement by one order of magnitude compared with SD-ITP. The yellow line represents the CF-ITP model, plotted used the values of* k_{on} , α *and* b_m *, obtained by fitting the models to experimental data for the standard flow, PO-ITP and SD-ITP cases. Using CF-ITP and a standard microscopy imaging system, we demonstrate an immunoassay with a LoD of 30 fM, a 10,000-fold improvement compared with a standard immunoassay. The error bars represent 95% confidence of the mean (n = 3).*