A RANDOM-WALK BASED MODEL TO EXPLAIN ULTRASENSITIVE MAGNETIC BEAD-BASED IMMUNOASSAYS

M. Cornaglia*, R. Trouillon, H.C. Tekin[+], T. Lehnert, and M.A.M. Gijs
Ecole Polytechnique Fédérale de Lausanne, SWITZERLAND

ABSTRACT

We introduce a new modeling approach for understanding the fundamental principle of bead-based surface coverage immunoassays that rely on the specific interaction between surface-bound "small" magnetic beads (1 μm in diameter) and a flow of "large" magnetic beads (3 μm in diameter). The latter ones carry target antigens and are simultaneously used as detection labels [1]. This immunoassay technique allows attaining extremely low (attomolar) limits of detection, with strongly sub-linear dose-response curves, but the origin of these features remained unclear up to now. Our comprehensive theoretical model allows complete understanding of these unsolved issues.

KEYWORDS: Surface-based immunoassays, protein detection, superparamagnetic particles, magnetic particle-scanning, random-walk model

INTRODUCTION

Capturing antigens (Ags) from clinical sample solutions is often performed by using magnetic beads functionalized with specific antibodies (Abs) [2]. Ag-carrying beads can be transported to a detection surface covered with Abs where quantification is often performed by counting the beads captured on the surface via Ab-Ag-Ab immunocomplex bonds [3]. When molecules are tethered to surfaces, their immunoreaction kinetics is substantially reduced compared to freely diffusing species [4] and the probability of immunocomplex formation becomes an unlikely event. Nevertheless, we experimentally observed extremely low (attomolar) limits of detection. Moreover, dose-response curves obtained with this assay, as well as with other similar bead-based methods, always show a puzzling non-linear dependence on Ag concentration, which was so far unexplained. Here, we introduce a new "random-walk based" model, in order to explain the features of this new class of ultrasensitive magnetic bead-based immunoassays.

THEORY AND EXPERIMENTAL

The principle of our magnetic bead-based assay is shown in Fig. 1a. An array of Ab-functionalized small (∅ 1 μm) superparamagnetic beads is patterned at the bottom of a microfluidic channel. Large (∅ 3 μm) superparamagnetic beads are used to isolate specific Ags from a sample under test. Large beads are then injected into the microchannel and transported by the flow, while being magnetophoretically attracted onto the detection area. Large beads slide over the substrate and "scan" the functionalized surface of the small beads. When carrying an Ag, they can eventually bind to the small beads by forming an Ag-Ab-Ag immunocomplex (Fig. 1b). If no Ag is present instead, large beads cannot be retained on the array and they are removed by the flow. Simple counting of the number of captured large beads provides a measure of the amount of Ag molecules carried by the beads (Fig. 1c). Our modeling approach for this kind of assay is based on the concept of "magnetic bead scanning": each moving large bead, while transported by hydrodynamic forces, is actually subjected to successive stochastic reorientations induced by magnetic dipolar interactions with the substrate-bound small magnetic beads. This phenomenon can be theoretically described as "random walk" of the contact point between an Ag-carrying large bead and the small bead pattern. It can be modeled by considering stochastic moves over the surface of the mobile bead, until this point coincides with the position of an Ag, hence resulting in the bead capture (Fig. 1d). Moreover, the total number of specifically captured beads clearly depends on the amount of large beads which are actually scanning the small bead array. This number can be predicted by tridimensional (3D) particle tracking simulations, obtained by combining microfluidic and magnetic Finite Element Method (FEM) simulations with analytical calculations (Fig. 1e).

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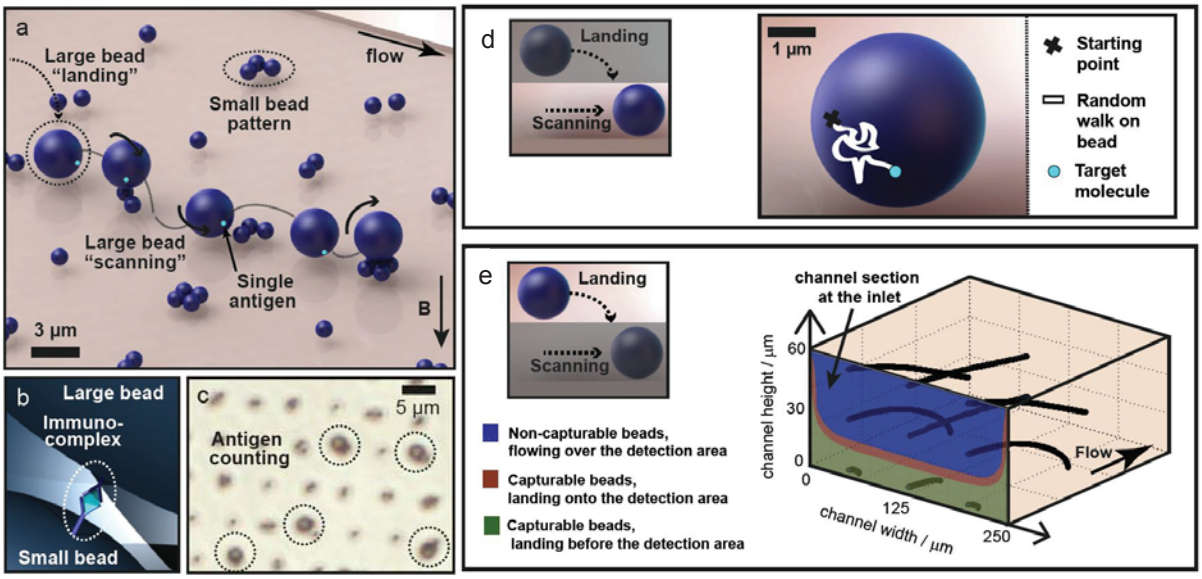

Figure 1: (a) Schematic representation of the principle of our magnetic bead-based assay. (b) Specific immunocomplex formation at the interface between a large and a small bead is responsible for the capture of large beads on the array. (c) Counting of the number of captured large particles provides the measure of the Ag concentration in the sample under test. (d) Schematic representation of the modeling approach used to describe the bead "scanning" phase of the assay: the displacement of the large bead over the small bead array is modeled by taking the large bead as reference system and considering a "random walk" of the point of contact between the large bead and a pattern of small beads. (e) Schematic representation of the modeling tool developed to describe the bead "landing" phase of the assay. The trajectory of a large bead entering the channel at any position can be predicted via 3D particle tracking simulations, obtained by combining FEM simulations with analytical calculations.

RESULTS AND DISCUSSION

The extremely low limit of detection attained with this kind of assay can be fully understood by considering the probability of single immunocomplex formation, which is dramatically enhanced by the bead-scanning mechanism (Fig. 2a-b). When large magnetic beads move in a magnetic field in absence of immobilized small beads, the field "pins" their magnetic moment, forcing them to slide over the substrate and probe it at one point [5], hence providing a single chance for ligand-receptor binding (modeled as a single throw of dices in Fig. 2a, case i). When instead large beads move in presence of both the external magnetic field and the small bead array, the contact point describes a different line on the surface of the large bead at each bead reorientation. This fact dramatically enhances the chance for successful binding (modeled as multiple throws of dices, Fig. 2a, case ii). By calculating the probability of large bead capture for both the aforementioned situations and for different bead sizes, we estimated the capture probability enhancement introduced by the bead-scanning mechanism (Fig. 2b). By using the random walk-based modeling approach, we can then explain and formally predict the particular behavior of the assay dose-response curves, over a wide range of Ag concentrations and for two distinct ligand-receptor systems: biotin – streptavidin (Fig. 2c) and TNF-α – anti TNF-α (Fig. 2d).

CONCLUSION

Because of the pioneering nature of our model in explaining the principle of ultra-low sensitivity detection, we believe that the proposed model will represent a powerful tool for the design of novel magnetic bead-based immunoassays with potentially unmatched performance and limit of detection.

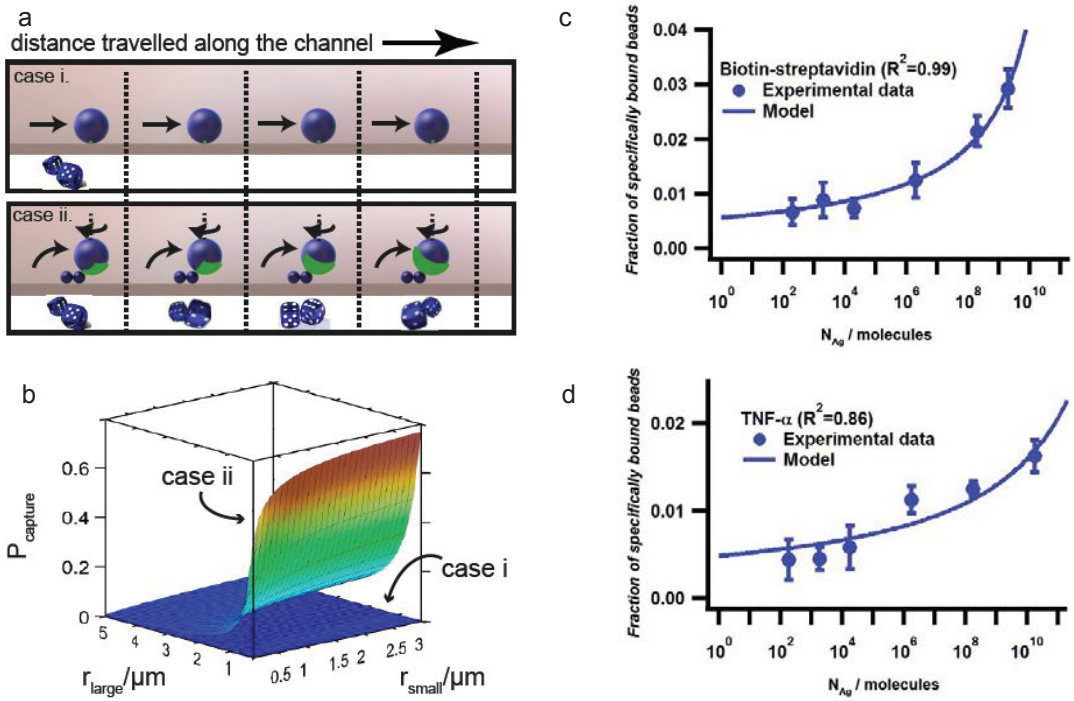

Figure 2: (a) Schematic representation of the bead scanning mechanism, (case i) in absence and (case ii) in presence of immobilized small beads. A different section of the large bead surface is explored at each interaction with a small particle group, providing higher chance of successful binding, probabilistically modeled as multiple throws of dices. (b) Probability of specific capture of single large beads ($P_{capture}$), calculated for the two cases reported in Fig. 2a, as function of the radii of large and small beads. (c,d) Experimental results of detection of (c) biotinylated anti-streptavidin and (d) TNF-α, spiked as Ag in 5 μL fetal bovine serum (FBS), as obtained from [1] and fitted according to our model predictions.

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CONTACT

*M. Cornaglia, tel: +41 21 693 48 34; matteo.cornaglia@epfl.ch; [+]present address: Stanford University School of Medicine, Canary Center Early Cancer Detection, Stanford, CA 94305-5101, USA.