Microbead agglutination based assays

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INTRODUCTION

We report a simple and rapid room temperature assay for point-of-care (POC) testing that is based on specific agglutination. Agglutination tests are based on aggregation of microbeads in the presence of a specific analyte thus enabling the macroscopic observation. Such tests are most often used to explore antibody-antigen reactions. Agglutination has been used for protein assays using a biotin/streptavidin system as well as a hybridization based assay (Figure 1a). The agglutination systems are prone to self-termination of the linking analyte (Figure 1b and 1d), prone to active site saturation and loss of agglomeration at high analyte concentrations (Figure 1c and 1e).

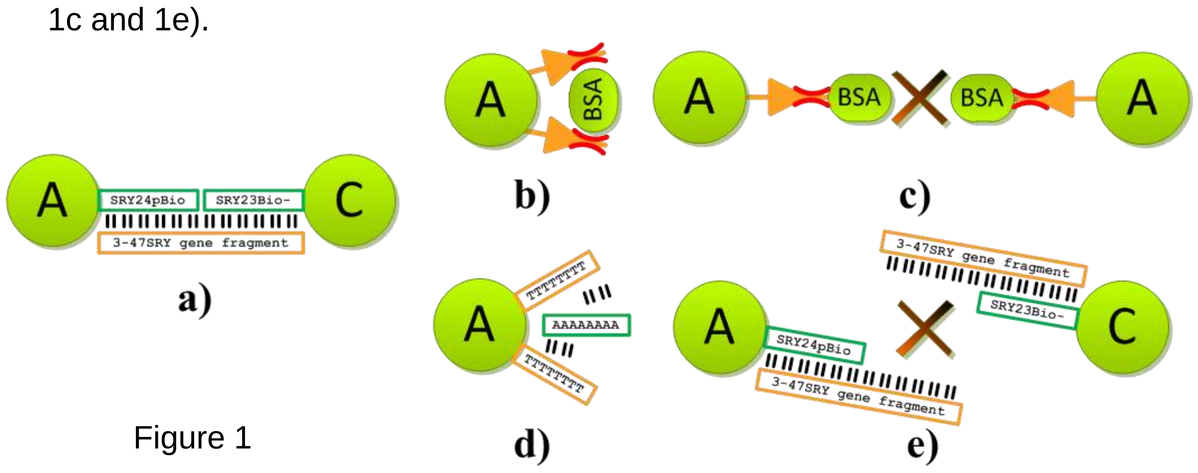

Figure 1

OPTICAL IMAGE PROCESSING

Two different methods for determining the agglutination strength were developed (Figures 2, 3).

- The first method (the grayscale method) is based on the distribution of the image's grayscale values (ranging from 0 for black to 1 for white).
- The second method (the binary method) is based on processing of a binary image via thresholding, with black values assigned to the background, and white values assigned to the areas occupied by agglutinated particles.

THREE-COMPONENT ASSAY USING DNA

SRY gene hybridization based agglutination example. Target DNA (3-47SRY) concentration titration by mixing with oligo SRY24pBio, SRY23Bio-functionalized microbeads in a solution (A + B + C). Sequence of photographs, each labeled with its µM final concentration of (3-47SRY) oligonucleotide (Figure 5).

THREE-COMPONENT STEPPED ASSAY

Same SRY gene hybridization based agglutination example. Beads functionalized with oligo SRY24pBio were mixed with various concentrations of target DNA (3-47SRY). After hybridization and washing away non-hybridized DNA, SRY23Bio-functionalized microbeads were introduced ((A + B) + C) with following agglutination (Figure 6).

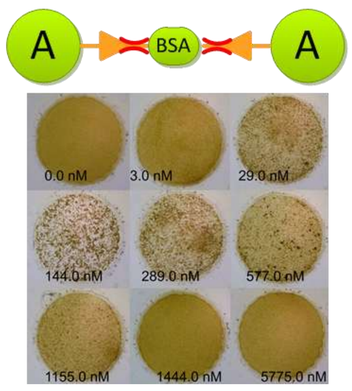

Figure 4

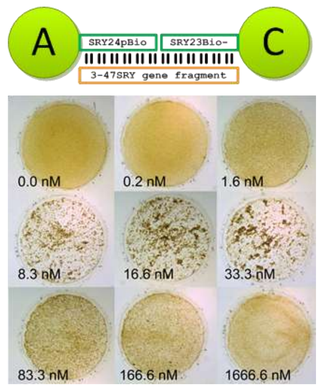

Figure 5

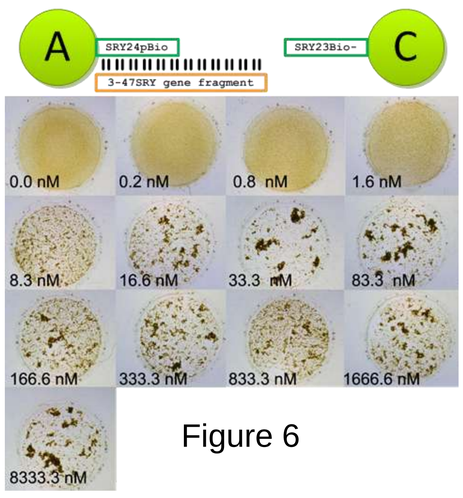

Figure 6

RESULTS AND DISCUSSION

For three-component agglutination assays (A + B + A), concentrations as low as 2.9 nM of biotinylated BSA could be detected by agglutination, whereas, for DNA based assays (A + B + C), ssDNA with concentration as low as 1.6 nM could be detected (Figure 7). The stepped assay ((A + B) + C) avoids the loss of signal at high analyte concentration; therefore, the detection range is extended (in the range of tens of µM).

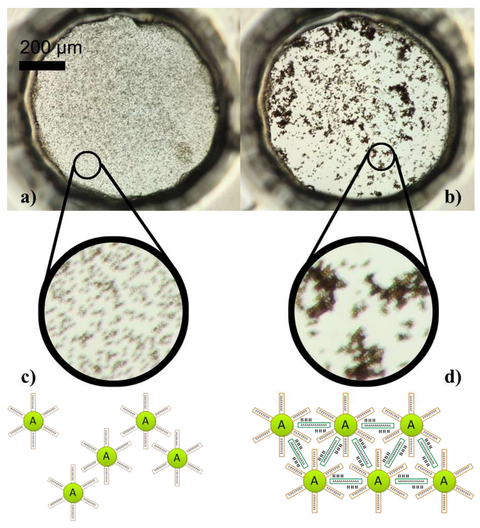

Figure 2

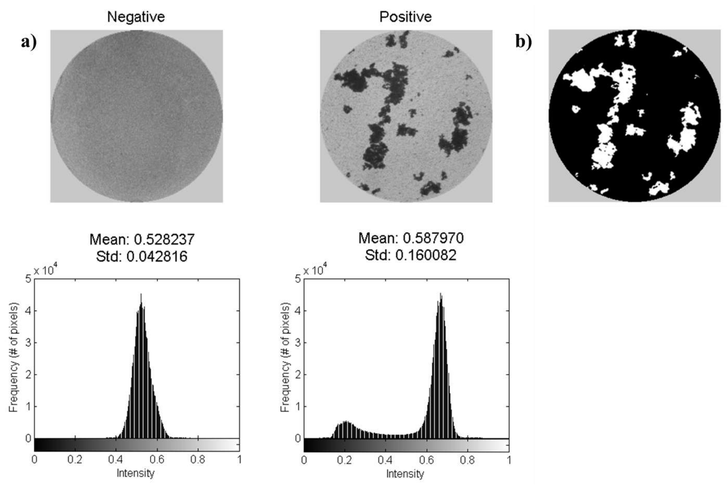

Figure 3

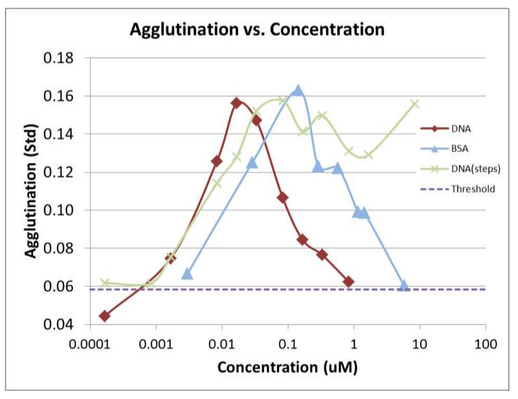

Figure 7

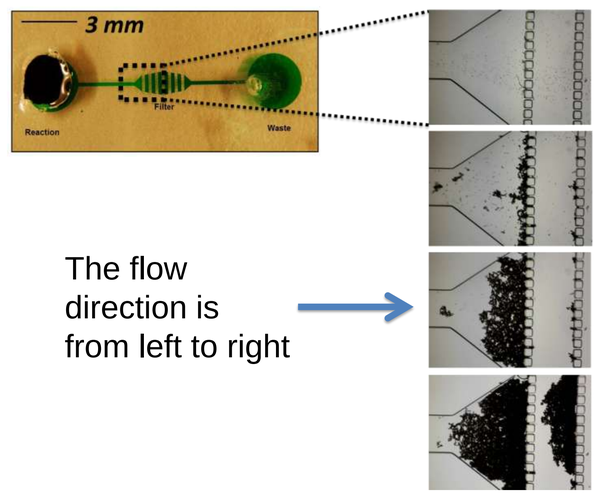

Figure 8

METHODS

We investigated the molecular target/ligand interaction, explaining the common agglutination problems related to analyte self-termination, linkage of the analyte to the same bead instead of different microbeads. We classified the agglutination process into three kinds of assays: a two-component assay, a three-component assay and a stepped three-component assay.

THREE-COMPONENT ASSAY USING PROTEIN

Titration of biotinylated BSA protein concentration for the agglutination assay with streptavidin coupled beads in a solution (A + B + A). Sequence of photographs, each labeled with its nM final concentration of biotinylated BSA protein (Figure 4).

DETECTION OF AGGREGATES IN MICROFLUIDIC DEVICE

Non-bound microbeads are flowing freely in PDMS microfluidic device. Agglutinated beads get trapped at the rows of columns and are visible by naked eye. The input well where beads were pipetted, the agglutinated beads held by the trapping columns and the output channel where negative pressure was applied, can be seen (Figure 8).

CONCLUSIONS

Although we compared these three kinds of assays for recognizing DNA and protein molecules, the assay can be used for virtually any molecule, including ions and metabolites. In total, the optimized assay permits detecting analytes with high sensitivity in a short time, 5 min, at room temperature. Such a system is appropriate for POC testing.

References for further reading:
1. Vaidya, H.C., et al., *Extremely high values of prostate-specific antigen in patients with adenocarcinoma of the prostate; demonstration of the "hook effect".* Clin Chem, 1988. **34**(10): p. 2175-7.
2. Rogers, P.H., et al., *Selective, controllable, and reversible aggregation of polystyrene latex microspheres via DNA hybridization.* Langmuir : the ACS journal of surfaces and colloids, 2005. **21**(12): p. 5562-9.
3. Leslie, D.C., et al., *New detection modality for label-free quantification of DNA in biological samples via superparamagnetic bead aggregation.* J Am Chem Soc, 2012. **134**(12): p. 5689-96.