Electronic Supplementary Material (ESI) for Analyst

SUPPLEMENTARY CONTENT

Fluorescence Signal Transduction Mechanism for Immunoassay Based on Zinc Ion Release from ZnS Nanocrystals

Chad L. Cowles, Xiaoshan Zhu, and Nelson G. Publicover

Department of Electrical and Biomedical Engineering

University of Nevada Reno, Reno, Nevada, 89557-0260, USA

Section 1: Specificity of Fluozin-3 to Zinc Ions

The experiment was performed as follows. First, a microplate was washed using 1 × TAE buffer to minimize potential divalent ion contaminants in wells, and the wells were further washed using HEPES pH5.5 to remove residual TAE. Second, 10 nM $ZnCl_2$, 100 nM NaH_2PO_4, 100 nM $PbCl_2$, 100 nM $CaCl_2$, 100 nM $MgCl_2$, 100 nM KNO_3, 100 nM KCl, and 100 nM EDTA were prepared in HEPES pH5.5. Third, 50 μL of each chemical solution, including a blank (just HEPES pH5.5), were added to the wells, and sequentially 50 μL of 2.5 μM Fluozin-3 (prepared in HEPES pH5.5) were added to each well to react with chemical ions. After several minutes vortexing, the microplate was put into the microplate reader for fluorescence measurement. The above experiment was performed in triplicate.

Figure S1 shows the fluorescence signals generated from the reaction between Fluozin-3 and all tested chemicals.

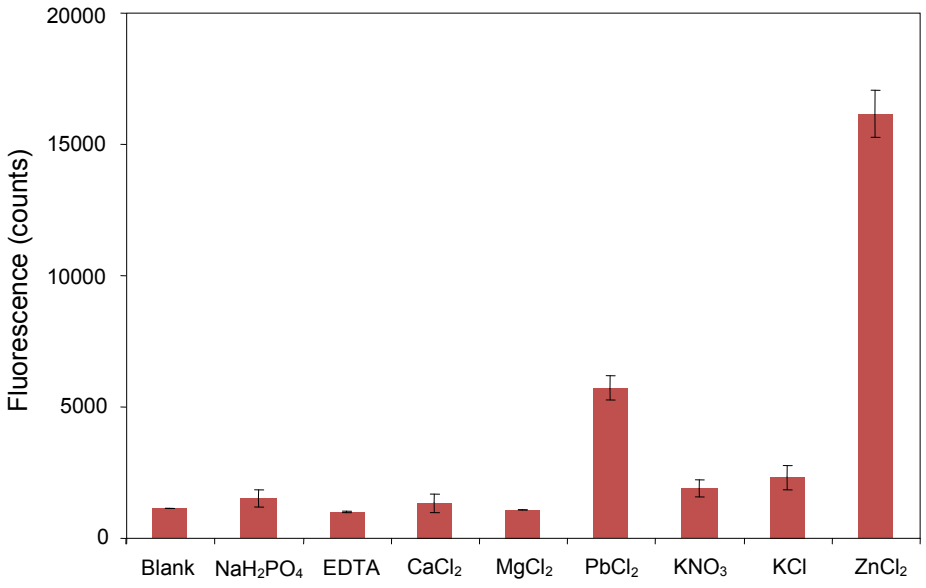

Figure S1. The potential interferences of other ions or chemicals on the reaction of zinc ion and Fluozin-3

Section 2: Optimization of Detection Antibody Concentration in Immunoassay

The experiment was performed as follows. First, 10 μL of Magnabind beads conjugated with goat anti-mouse IgG were added to the wells of a microplate and washed three times using PBS-R. Second, 50 μL of 1 μM mouse IgG were added to each well for 30 minute antigenic capture by the beads. After the incubation, the beads were washed using PBS-R. Because the antigen (mouse IgG) is of high concentration, and the bead surface in each well will be saturated with mouse IgG. Third, 50 μL of detection antibody (biotinylated goat anti mouse IgG) prepared in a wide concentration range (from 0 to 1 μM) were added to the wells and incubated with the beads for 30 min, and then the beads were washed 3 times using PBS-R. Fourth, 50 μL of 0.03 mg/mL streptavidin beta-galactosidase conjugate were added to each well to bind with detection antibody for 5 minutes. The beads were washed three times with PBS-R followed by two washes using PBS-D. Fifth, the beads were re-suspended in 20 μL of PBS-D, and 100 μL of 0.2 mM FDG were added to each well for 30 second incubation. The enzyme reaction was quenched using 100 μL of 5 × TAE. Finally, the beads were pulled down and 100 μL of the supernatant were transferred to each well for fluorescence measurement. The above experiment was performed in triplicate.

Figure S2 shows the fluorescence signal vs. the concentration of detection antibody. In this Figure, the turnover point for signal saturation, which is estimated at around 30 nM, is considered as the maximum loading concentration of detection antibody on the bead surface.

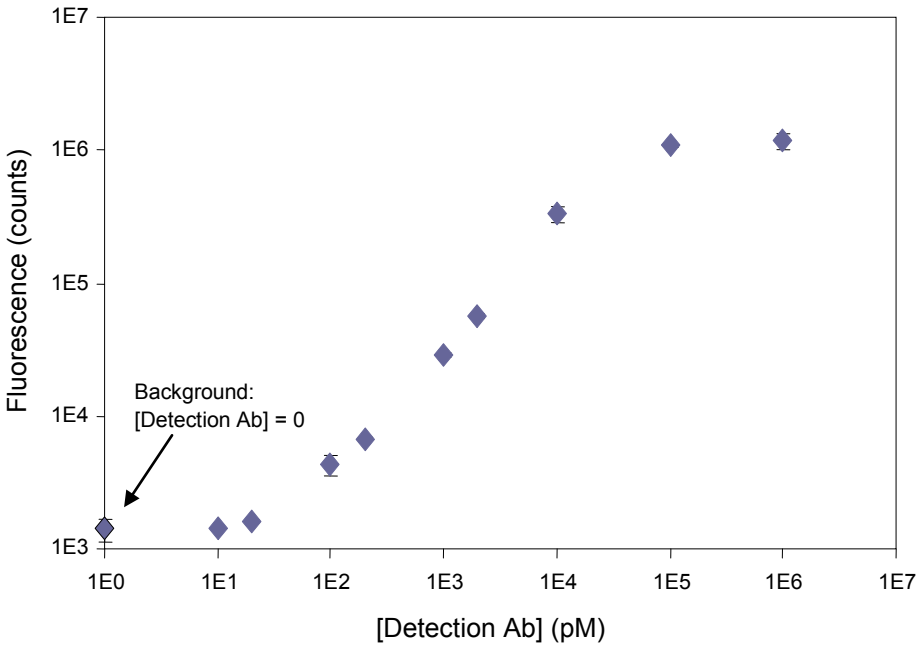

Figure S2. The plot to study the maximum loading of detection antibody (biotinylated goat anti mouse IgG) when beads are saturated with antigen mouse IgG

Section 3: Maximum Enzyme Concentration in Immunoassay

The experiment was performed as follows. First, 10 μL of Magnabind beads conjugated with goat anti-mouse IgG were added to the wells of a microplate and washed three times with PBS-R. Second, 50 μL of 1 μM mouse IgG were added to each well to incubate with the beads for 30 min, and then the beads were washed three times using PBS-R. Third, 50 μL of 100 nM biotinylated detection antibody, was added to each well and incubated with the beads for 30 minutes. Fourth, after the beads were washed, 50 μL of serial dilutions of streptavidin beta-galactosidase conjugate in the concentration range of 0 to 0.1 mg/mL were added to each well and incubated with the beads for 5 min. The beads were further washed using PBS-R and PBS-D. Fifth, the beads were re-suspended in 20 μL of PBS-D, and 100 μL of 0.2 mM FDG were added to each well for 30 second incubation. Sequentially, the enzyme reaction was quenched using 100 μL of 5 × TAE and 100 μL of the supernatant were transferred to new wells for fluorescence measurement. The above experiment was performed in triplicate.

Figure S3 demonstrates the fluorescence signal vs. the concentration of enzyme. In this Figure, the turnover point for signal saturation, which is estimated at around 0.03 mg/mL, is considered as the maximum loading concentration of enzyme on the bead surface when saturating concentrations of capture antibody, antigen, and detection antibody are used during immunoassay.

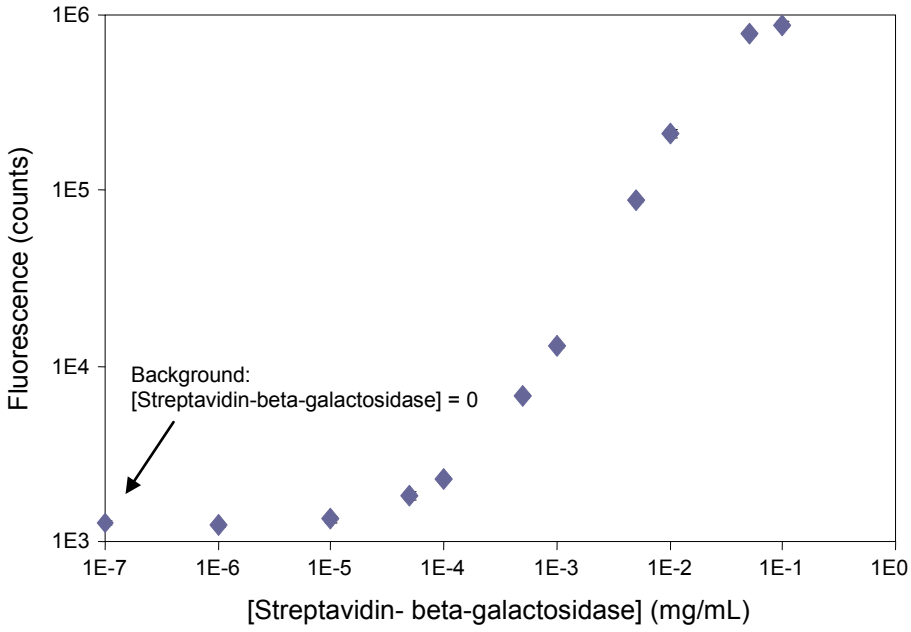

Figure S3. The plot to study the maximum loading of streptavidin conjugated beta-galactosidase when beads are saturated with antigen mouse IgG and biotinylated anti mouse IgG detection antibody

Section 4: Effects of TAE on Immunoassay

To investigate the possible negative effects of TAE on immunocomplexes (e.g. causing the dissociation of immunocomplexes), the following experimental steps were conducted on the basis of enzyme-based immunoassay. Two identical bead sets were prepared during steps one through four. First, 10 μL of Magnabind beads conjugated with goat anti-mouse IgG were added to the wells of the plate and washed. Second, 50 μL of serially diluted mouse IgG in the concentration range of 0 to 1000 nM were added to each well and incubated with the beads for 30 minutes. After incubation, the beads were further washed. Third, 50 μL of 100 nM biotinylated detection antibody were added to each well and incubated with the beads for 30 min, and afterwards the beads were washed. Fourth, 50 μL of 0.03 mg/mL streptavidin beta-galactosidase conjugate were added to each well and incubated with the beads for 5 min, and the beads were further washed. Fifth, one set of the beads (or the immunocomplexes) prepared through the above four steps was washed with 1 × TAE containing 0.05% Tween 20, and concurrently the other set of the beads was washed with PBS-R. Sixth, two sets of beads were further washed with PBS-R and then PBS-D. Each set of the beads were re-suspended in 20 μL of PBS-D, and 100 μL of 0.2 mM FDG was added to each well for 30 second incubation. The enzyme reaction was quenched with 100 μL of 5 × TAE. Finally, 100 μL of the supernatant were transferred to new wells for signal measurement. The above experiment was performed in triplicate.

Figure S4 demonstrates two assay calibration curves with and without TAE wash of immunocomplexes, respectively. It is shown that 1 × TAE containing 0.05% Tween 20 does not impact the outcome of immunoassay and its potential negative effects can be ignored. Figure S4

also shows that the maximum loading concentration of antigen on the bead surface is around 10 nM.

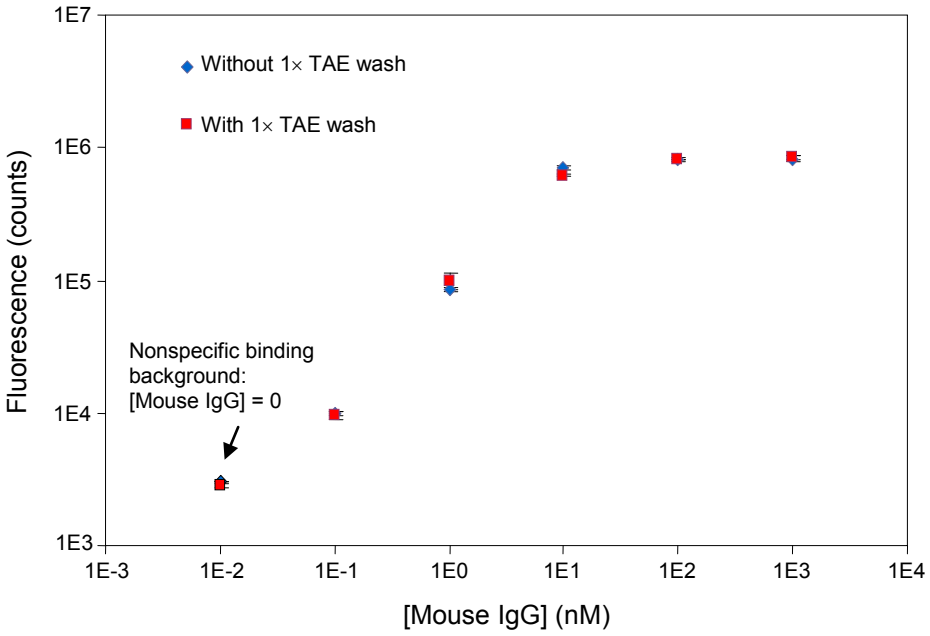

Figure S4. The calibration curves of immunoassay on mouse IgG with the optimized concentrations of detection antibody and enzyme – the first curve (in blue mark) presents the data of the assay in which immunocomplexes are not washed with TAE buffer; the second curve (in red mark) shows the data of the assay in which immunocomplexes are washed with TAE buffer