Ultrasensitive, Multiplexed Detection of Cancer Biomarkers Directly in Serum by Using a Quantum Dot-Based Microfluidic Protein Chip

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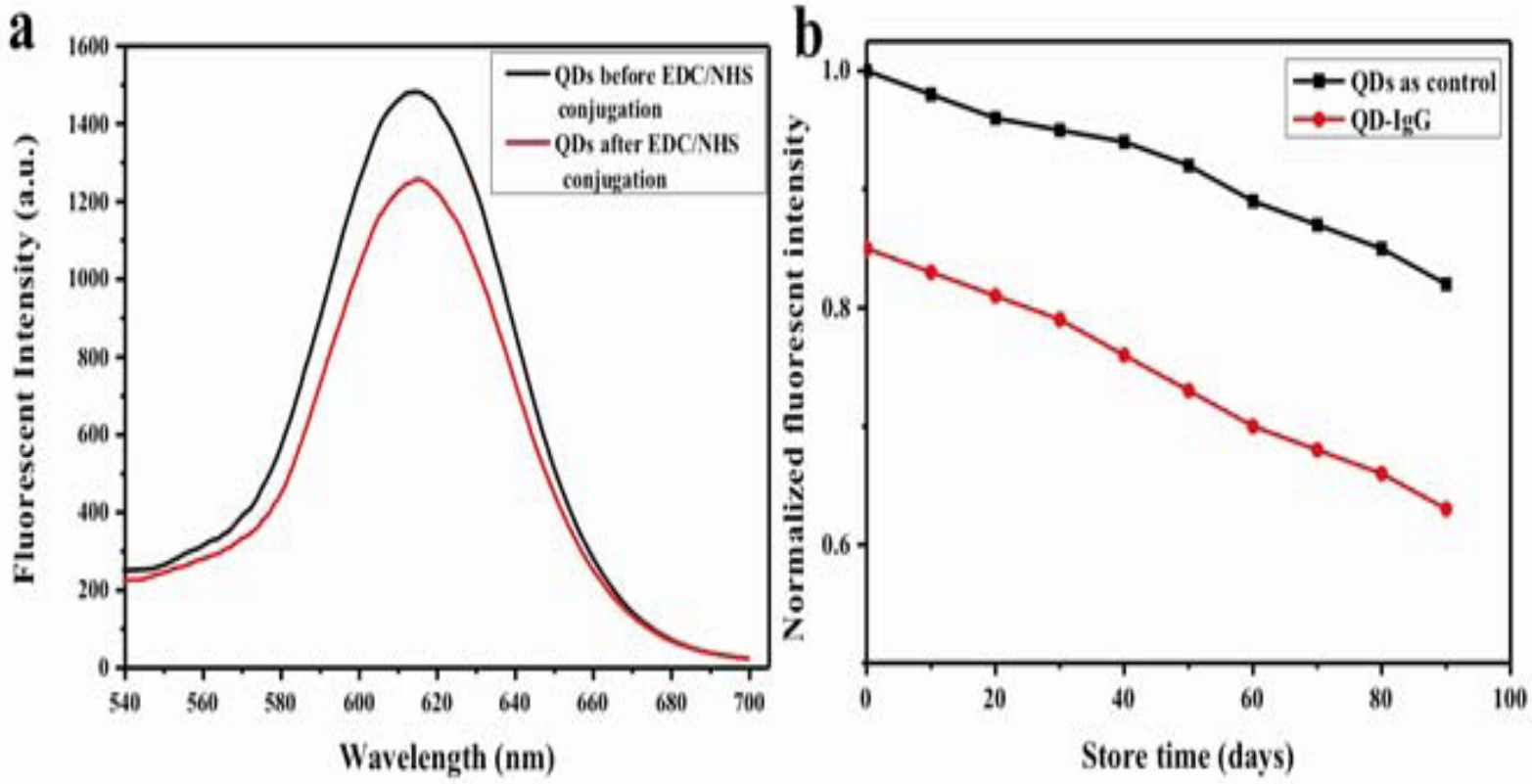
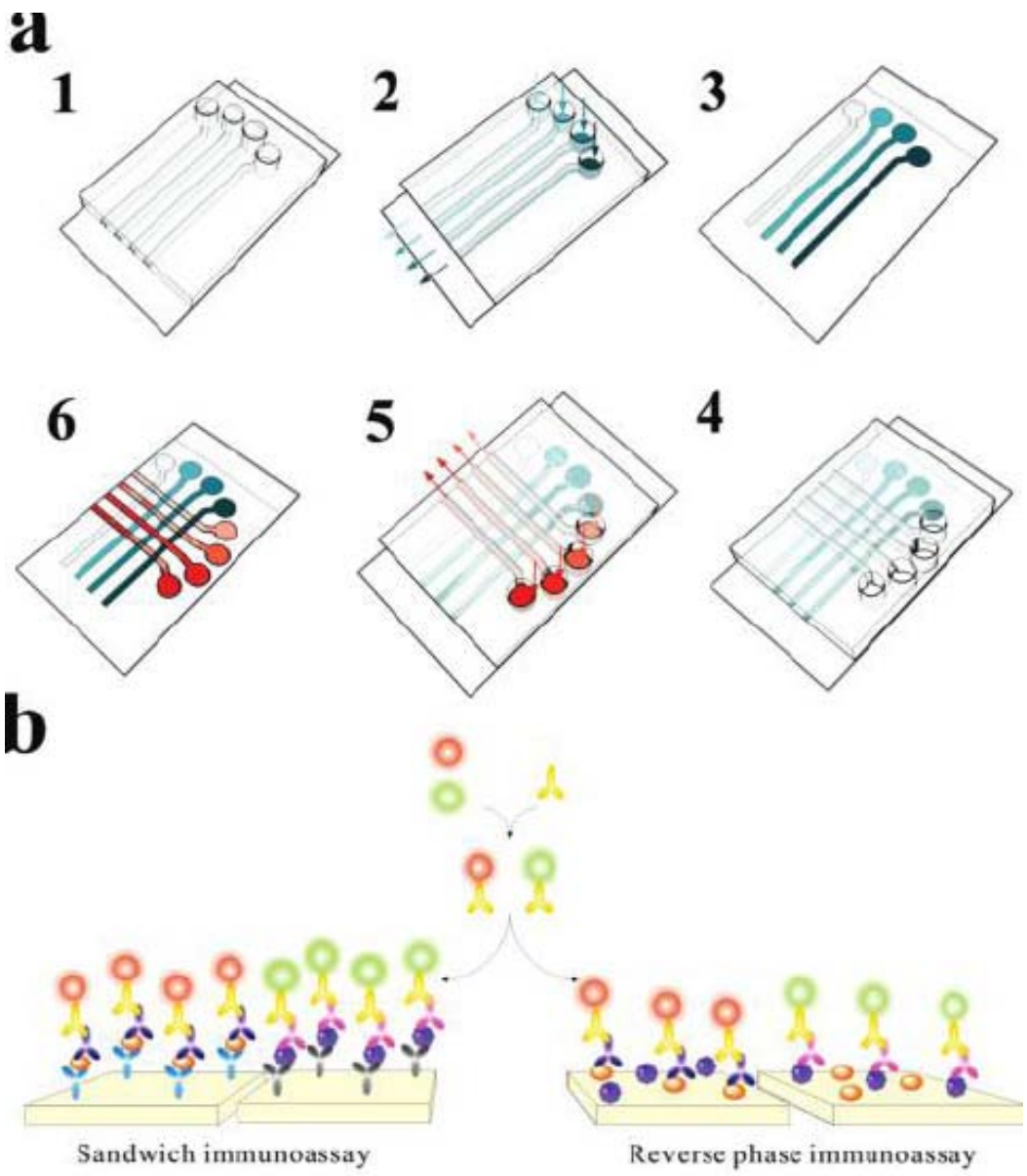
Introduction

1. Semiconductor quantum dots (QDs) and its fluorescent tuning have attracted intensive attention during the last two decades.
2. The QDs, which are synthesized in organic phase, often possess a high photoluminescent quantum yield (PLQY 6085%) and narrow size distribution.
3. QDs that are directly prepared in the aqueous phase (aqQDs) possess excellent aqueous dispersibility because their stabilizers (*e.g.,* thiols) are naturally water-dispersible and can be used for biological studies directly.
4. The QD-based bioconjugates were already applied into ELISA, Western blotting, and microarray for protein assays.
5. However, all of these techniques are based on a static solid/liquid interface reaction. In such systems, QDs are less active than a small molecular dye because of steric hindrance and are prone to deposit on surfaces.
6. To address this challenge, microfluidic chips based on the manipulation of a continuous liquid flow through microfabricated channels are introduced in this work.
7. Herein this group report the synthesis of antibody-conjugated aqQDs with strong photoluminescence and robust stability and an aqQD-based microfluidic protein chip for ultrahigh sensitive, selective, and multiplex detection of cancer biomarkers.

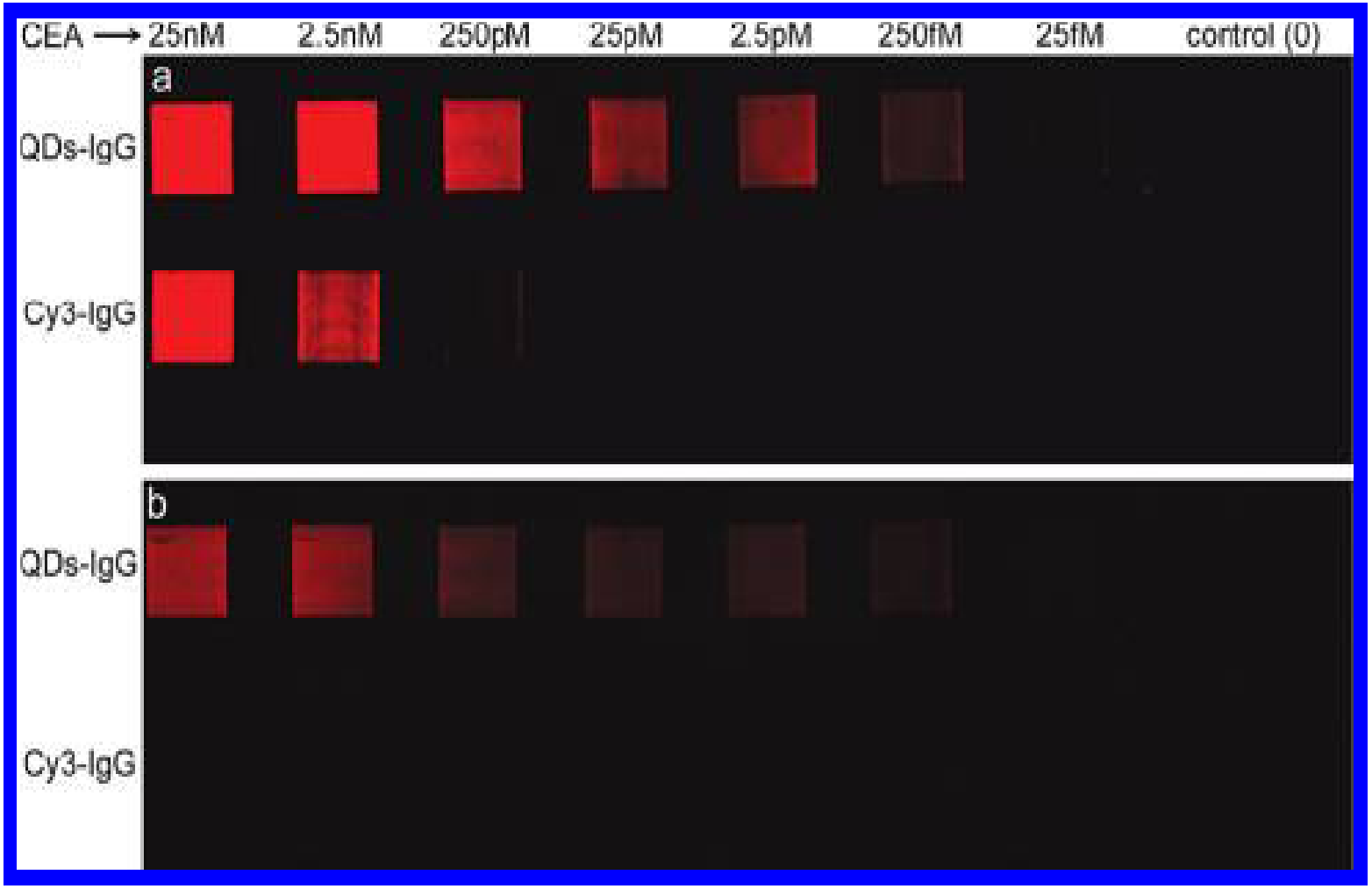
Preparation of QD-IgG Conjugates (anti-mouse igG)

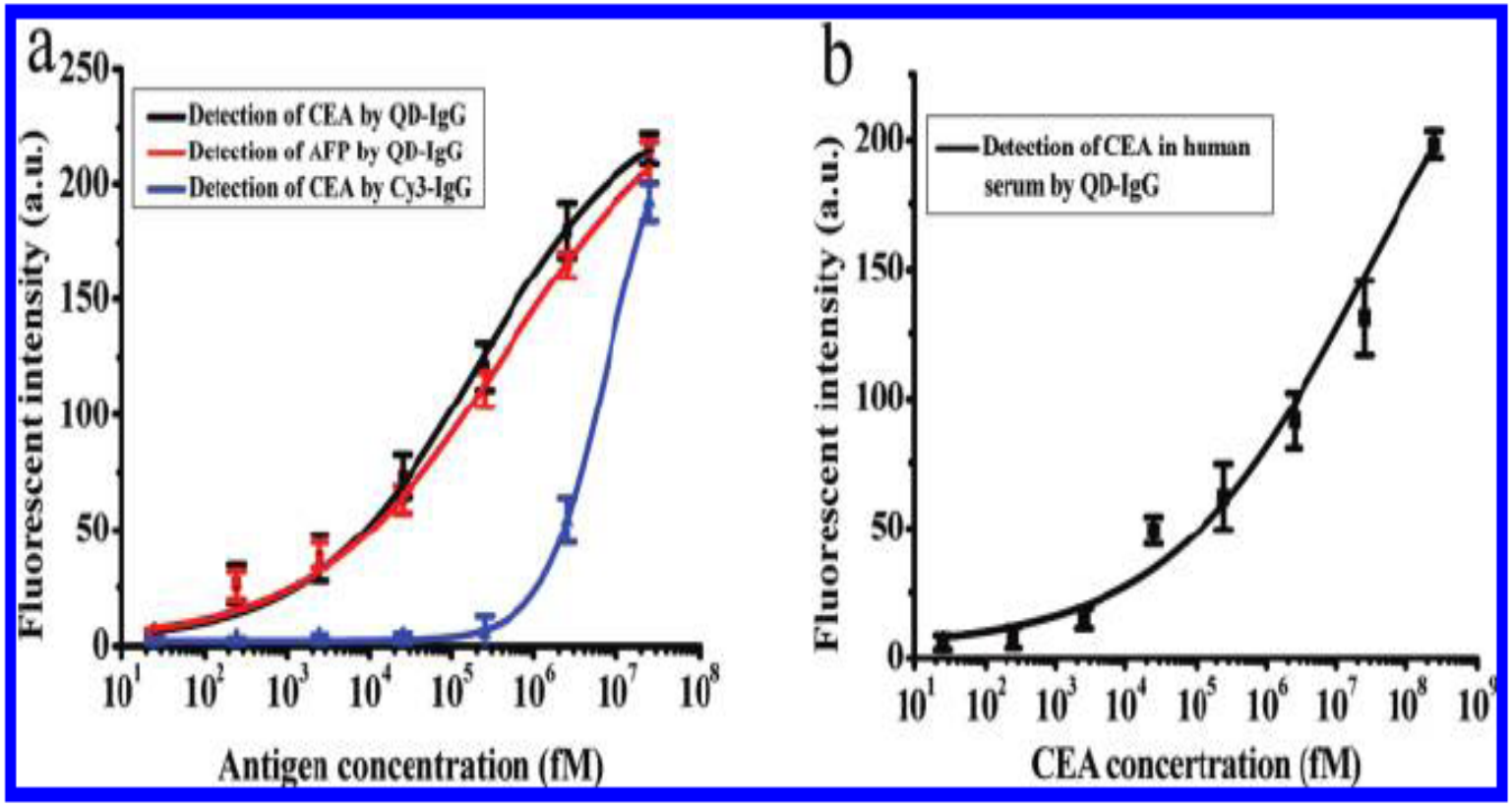
First, waterdispersed QDs (in 150 mM PBS, pH 7.3) were activated with EDC/ NHS. To 60 L of QDs (12 M), add 17.25 L of EDC (33.4 mM in H_2O) and 16.25 L of NHS (70.9 mM in H_2O). The reaction solution was incubated at 25 C for 15 min with gentle shaking. EDC is easily hydrolytic and should be dissolved in water immediately before use. Then 27 L of goat anti-mouse IgG (13.3 M) in PBS buffer was added to the activated QDs. The molar ratio of QD/EDC/NHS/IgG was calculated as 1:800:1600:0.5.

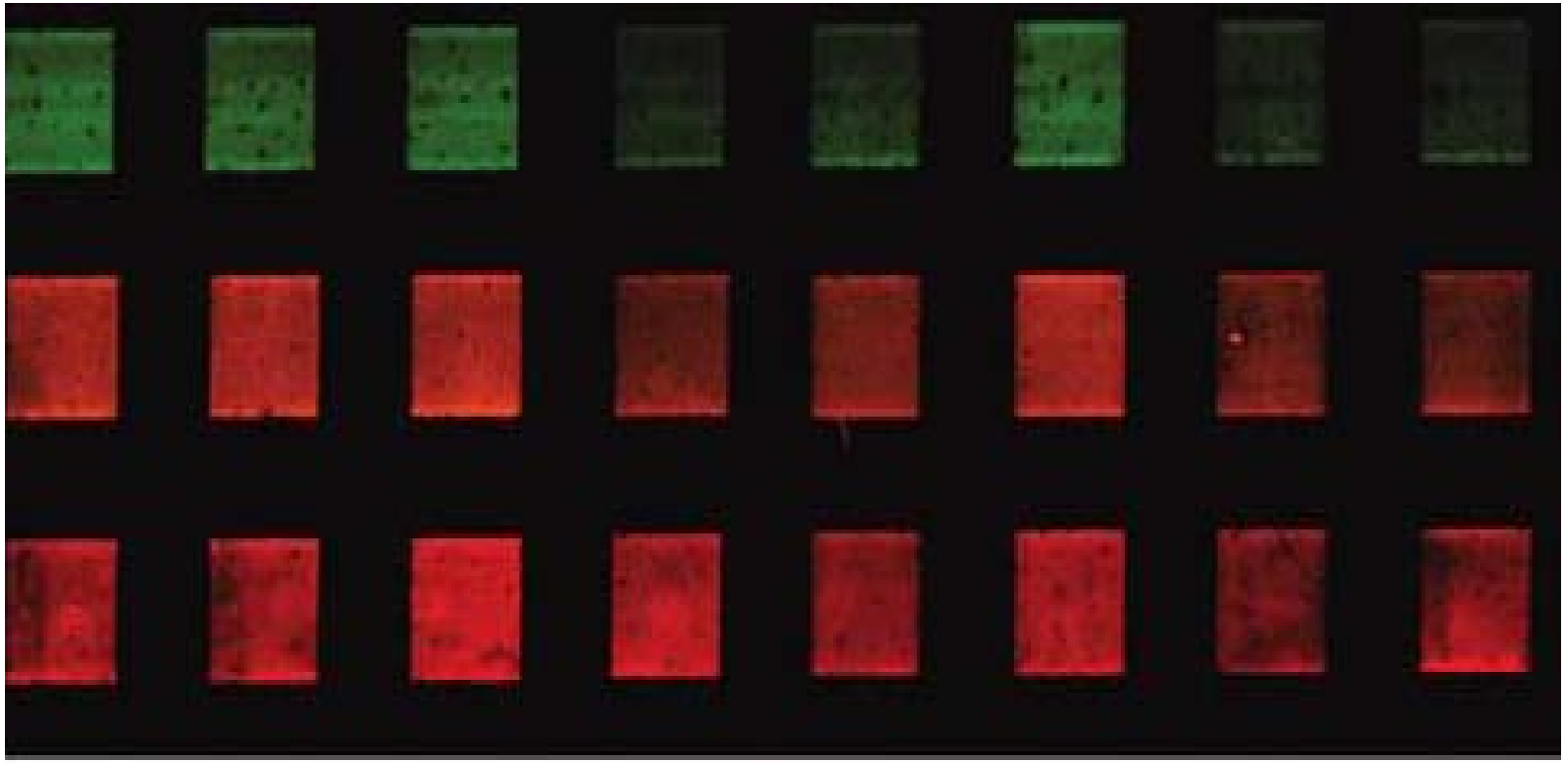

a
Fluorescent Intensity (a.u.)
Wavelength (nm)
QDs before EDC/NHS conjugation
QDs after EDC/NHS conjugation
b
Normalized fluorescnt intensity
Store time (days)
QDs as control
QD-IgG


a
1
2
3
6
5
4
b
Sandwich immunoassay
Reverse phase immunoassay

Carninoma embryonic antigen, alpha-fetoprotein




a
Fluorescent intensity (a.u.)
Detection of CEA by QD-IgG
Detection of AFP by QD-IgG
Detection of CEA by Cy3-IgG
Antigen concentration (fM)
b
Fluorescent intensity (a.u.)
Detection of CEA in human serum by QD-IgG
CEA concertration (fM)



Summary

1. **The present study has successfully conjugated QDs with a secondary antibody.**

2. **This bionanohybrid exhibits excellent performance as a fluorescent probe in protein assays.**

3. **The QD-IgG conjugate, combined with a microfluidic protein chip, improved the detection limits of cancer biomarkers up to 250 fM, which represents a sensitivity improvement up to 4 orders of magnitude as compared to organic dyes (*e.g., commercially* available Cy3-Abs conjugates).**

4. **Besides, multicolored imaging was realized and potentially useful for multiplexed detection in proteomic studies. This study thus opens a new path for practical clinical and proteomic applications of protein chips.**

Thanks