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Department of Chemistry & Biochemistry

JoVE (Journal of Visualized Experiments)
1 Alewife Center, Suite 200
Cambridge, MA 02140
USA

August 25th, 2016

Dear Dr. Nguyen,

We are submitting the revised version of our manuscript: 55216_R0_071216.
All the changes made are tracked in the manuscript in red color. We are also submitting a new Figure 2 where we changed the figure part labels in lower case (a and b) to upper case 'A' and 'B', and a new Figure 3 where "GdDOTA" (in the original figure) in the axis title and legend has been changed to "Gd-DOTA" (in the new figure). In addition, please find below our response to the editorial and reviewers' comments and we would be happy to address any additional comments.

Thank you very much for your time.

Editorial comments: The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55216_R0_071216.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
2. Please include the DOIs for all references.
3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
4. Please correct the grammar regarding article usage. In particular, "Gd-sensor" should often be "the Gd-sensor". Such editing is required prior to acceptance.
5. Results: Please expand on the results. Two sentences is insufficient. What is the interpretation of the data presented? This can be moved from the figure legends, which should just be brief descriptions of what data is shown.

Our response: we have re-structured a few sentences and made all the necessary changes and corrections to comply with all the suggestions above. We have expanded on the result section. In addition, we have also added the issue numbers for the references.

Reviewers' comments: Reviewer #1:
Manuscript Summary: The overall manuscript clearly outlined the methodology for using an aptamer previously identified by the PI's laboratory in a fluorescence assay for unchelated Gd(III). While the approach is not overly novel, the method described in sufficient detail for researchers interested in utilizing this approach for Gd ion analysis, or similar analyses with aptamers for other target molecules. The anticipated results are reasonable and consistent with the detailed experimental description. As a whole, the manuscript is appropriate for publication in this journal.

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Major Concerns: None

Minor Concerns:

1. The primary concerns in the manuscript involve a variety of minor grammatical or formatting issues that occur with a high enough frequency to be distracting. These include unnecessary capitalization of element names (e.g. Sodium, Potassium, and Calcium, in lines 60-61) and spelled-out acronyms (Inductively Coupled Plasma-Mass Spectrometry in line 85 and SELEX in line 94), as well as unusual placement of superscripted reference numbers before the periods at the ends of sentences.

Our response: we have made all the necessary corrections on the capitalization of words. However, the unusual placement of superscripted reference numbers is in compliance with JoVE's formatting requirement, and therefore, we have made no changes to the location of the superscripts.

2. In the Protocol section, the details for emission wavelength are unnecessarily confusing (step 3.7 and subsequent references). Presumably, the emission maximum of the fluorophore in 528 nm, but the authors are noting that plate readers with less precise wavelength selection might need to just pick the wavelength rounded to the nearest multiple of 5 nm (although its not clear why they picked 525 nm vs. 530 nm). This issue probably warrants a brief clarification and rationale within the protocol.

Our response: we have clarified this fact in step 3.9 in the new manuscript.

Additional Comments to Authors: N/A

Reviewer #2:

Manuscript Summary: The manuscript describes the detailed procedure for quantitative detection of unchelated gadolinium (III) (Gd) ions in aqueous solution to address a safety issue caused by unchelated ions in GBCAs. The original research paper was published in Analytical and Bioanalytical Chemistry, which described the selection of DNA aptamers targeting Gd. Though the signal-on mode to detect target by combining fluorescently labeled aptamer and a quencher has been reported previously by other groups, the selection of Gd aptamer is novel and the assay described here is of applicable significance. But before the manuscript is considered for publication, I have a few comments below:

Major Concerns:

1. The construction of the fluorescence calibration curve and the detection of unchelated Gd3+ should be carried out simultaneously, not separately, to ensure the comparability of data between the calibration curve assay and detection assay.

Our response: we have re-formatted the protocol section, combining steps 3 and 4 in the original manuscript into one step (step 3 in the new manuscript).

2. As the authors stated, at above ~10 uM of Gd3+, fluorescence emission of the aptamer plateaus and it eventually decreases at even higher concentrations of the ion. The protocol suggested a preparation of 2 or 3 different concentrations of the sample (line 203). What about if the values being detected fall on the decreasing side of the peak? Will this result in a misinterpretation of the result? E.g mistakenly determining the ion at a lower concentration by assuming the dots fall on

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the increasing side of the peak? Should more dilutions of the sample be prepared? Or, is it very unlikely that the concentration of unchelated Gd in GBCAs will fall on the decreasing side of the peak?

Our response: we have included a note to guide the readers on how to avoid preparing solutions that may contain concentrations of Gd^{3+} which may be too high. This note is added under step 3.3 in the new manuscript. We have also made all the necessary changes to the protocol to incorporate this suggestion.

Minor Concerns:

1. Line 121, the company where the aptamer was ordered should be specified.

Our response: we did not make the changes as suggested by the reviewer. The original manuscript submitted to JoVE listed the company in the protocol, the company name was subsequently deleted from the protocol by the JoVE editor. Therefore, we decide to retain the changes made by the JoVE editor and not follow the reviewer's suggestion.

2. Line 130, "….water (molecular biology grade)" is repetitive as it has been mentioned in line 115.
3. Line 184, what is the incubation temperature? I assume it's room temperature.

Our response: we have incorporated the response to suggestions 2 and 3 above in the new manuscript.

Additional Comments to Authors: N/A

Yours truly,

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