Response to Reviewers' Comments (JoVE55313R1)

Merging Ion Concentration Polarization between Juxtaposed Ion Exchange Membranes for Blocking Propagation of the Polarization Zone

by Minyoung Kim, Hyunjoon Rhee, Ji Yoon Kang, Tae Song Kim, and Rhokyun Kwak

Reviewer #1:

1. The authors have not reviewed the recent advancement in the field of concentration ion polarisation in microfluidics. The fabrication technique presented here has been reported by J Han group at MIT almost 10 years ago. Recent works use paper as microporous material to replace the micro channel and can precent the instability at the concentration/depletion interface: D.T. Phan et al., Sample concentration in a microfluidic paper-based analytical device using ion concentration polarization, Sensors and Actuators B, Vol. 222, No. 1, 2016, pp. 735-740.

Response 1: Thank you for your insightful comment. We agreed that the fabrication technique presented here (i.e. microflow patterning) was developed almost 10 years ago. Various other techniques have been developed to integrate ion selective materials in microfluidics, however, microflow patterning is still the most easy-to-use method. It has been widely used until recently [Cheow et al., *Anal. Chem.*, 2014, **86**, 7455-7462; Cho et al., *Nanoscale*, 2014, **6**, 4620-4626; Choi et al., *RSC Adv.*, 2015, **5**, 66178-66184; Ouyang et al., *Anal. Chem.*, 2016, **88**, 9669-9677].

Our group also has experiences to build ICP preconcentrators in paper-based microfluidic systems [Han et al., *Lap Chip*, 2016, **16**, 2219-2227; Hong et al., *Anal. Chem.*, 2016, **88**, 1682-1687]. As the reviewer 1 addressed, the microporous structures of the paper may prevent the instability by suppressing electroconvective vortices in ion depletion zones [Rubinstein et al., *Phys. Rev. Lett.,* 2008, **101**, 236101; Kwak et al., *Phys. Rev. Lett.,* 2013, **110**, 114501]. However, the sizes of the paper channels are generally about 0.5~5 mm, which is much bigger than the conventional microfluidic channels. This wider paper channel with random fiber networks makes irregular motions of the preconcentrated plugs, instead of the smooth curved shapes in microfluidic channels (Fig. R1). This has been inevitable in paper-based ICP preconcentrators, because the minimum feature size of wax patterning and paper cutting (i.e. fabrication methods to build paper channels) is about few hundred micrometers. We added this explanation in the revised manuscript (Page 9).

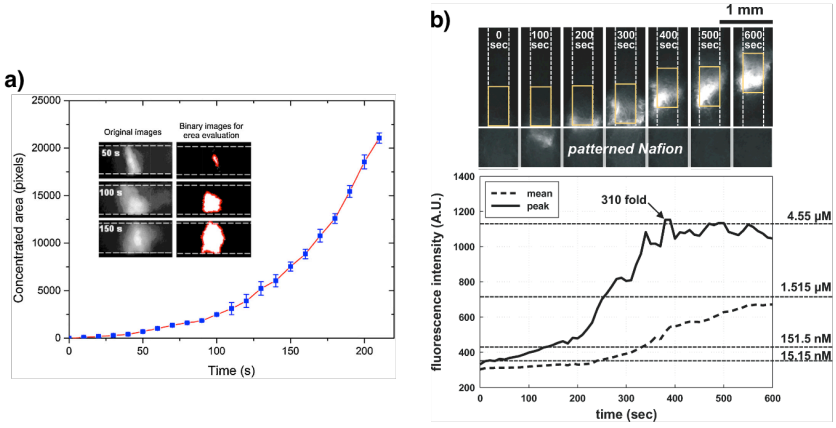

Figure R1. Irregular preconcentrated plugs in paper-based ICP preconcentrators adopted from a) Phan et al. [*Sens. Actuators B* 2016, **222**, 735-740], and b) Han et al. [*Lap Chip*, 2016, **16**, 2219-2227].

Reviewer #3:

1. Usually, the increase of the concentration using ICP is due to the continuous supply of the sample from the reservoir against the ion depletion zone. In the "merged" ICP presented in the manuscript, however, there is no continuous supply of the molecules from the reservoir. It looks like only the molecules confined between the two Nafion membranes get concentrated since there is no continuous supply of molecules from the anodic reservoir. Therefore, the increase is only 4-5 times. However, in Fig. 8, the authors show a 10,000-fold increase which is difficult to understand how. Also, this protein concentration result doesn't correspond to the results presented in Fig. 7 where there is hardly no preconcentration of the dye in 100 mM buffer solution. However, FITC-albumin showed a 100-10,000 fold increase in 1x PBS.

2. in the Discussion section: The first paragraph needs a clarification under which conditions 10,000-fold increase was achieved. In Fig. 7, at 100 mM and pH 3.7, there was hardly any preconcentration achieved. However, in Fig. 8, there was 10,000-fold increase achieved. Why? Any hypothesis?

Response 1-2: As we addressed in the original journal paper [Kwak et al., *Anal. Chem*., 2016, **88**, 988−996], the wall of the PDMS channel is negatively charged, and this generates electro-osmotic flow (EOF) between two Nafion membranes under an electric field (Fig. 2a; i). The EOF delivers targets toward the interface of the depletion and enrichment zones continuously, and its speed is proportional to the applied voltage. As a result, targets are delivered faster at high voltage, resulting faster preconcentration (Fig.5 in the manuscript and Fig. R2). We added these detail descriptions in the caption of Fig. 5 (Page 7).

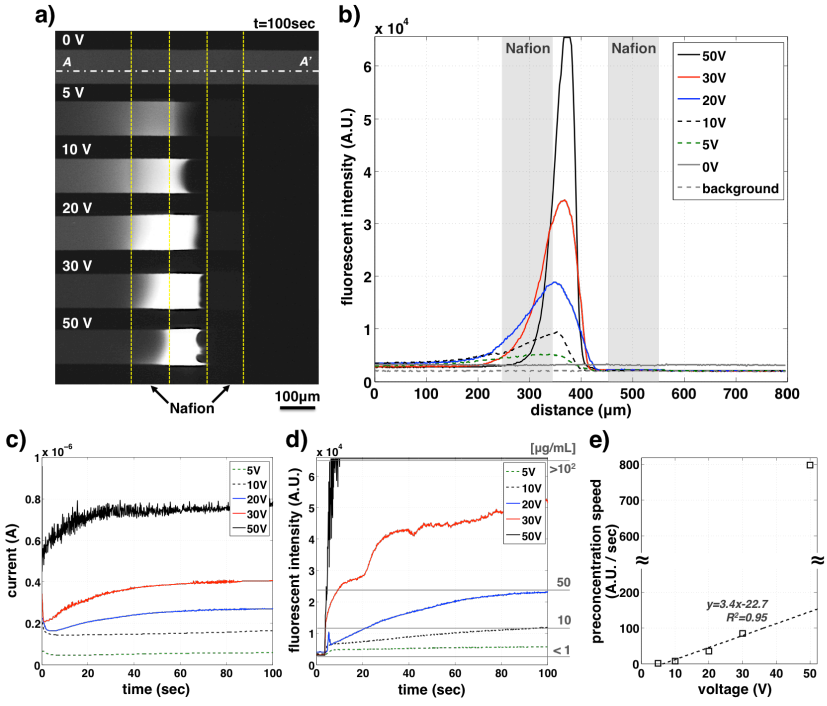

Figure R2. Spatiotemporally defined preconcentration in various operating voltages (5-50V). **a)** Fluorescent images after 100 sec operation, and **b)** fluorescent intensity profiles along *A-A*'. Yellow dotted boxes indicate the location of Nafions. **c)** In these tests, currents are also fully recovered the initial values. **d)** The peak fluorescent intensities are traced during 100 sec with the reference lines, which indicate the actual concentration of fluorescent dyes (1-100 μg/mL of Alexa Fluor 488 in 1mM KCl solution). e) In addition, preconcentration speeds are calculated from the linear regime of the peak intensity-time curves in (d). Under 30V, the preconcentration speed is linearly proportional to the applied voltage (the dotted line is the linear fitting curve), while the speed is much faster at 50V. This figure and contents are adopted from Kwak et al. [*Anal. Chem*., 2016, **88**, 988−996].

Again, we're sorry to drop important information from the original paper [Kwak et al., *Anal. Chem.*, 2016 **88**, 988−996]. As the reviewer 3 addressed, the preconcentration was hardly achieved in higher ionic strength (Fig. 7). Therefore, to facilitate the preconcentration of FITC-albumin in 1x phosphate buffer saline solution, we doubled the width of the Nafion pattern (200 μm) and used a narrower PDMS channel (width: 50 μm). In this way, ICP phenomenon can be enhanced by broadening the ions pathway but by reducing the absolute amount of ions in the channel. We added these detail descriptions in the caption of Fig. 8 (Page 8).

3. A discussion about the effect of the distance between two Nafion membrane is missing in the discussion section. If you increase the distance between two Nafion membranes, can you expect a higher concentration factor since more molecules trapped in between can be preconcentrated? Would the location of the spatiotemporally predefined region be also the same in front of the enrichment zone?

Response 3: As mentioned in the previous response, target molecules can be delivered by EOF, and then the faster EOF at the higher electric field accelerates the preconcentration speed. Therefore, if we increase the distance between two Nafion membranes d, the electric field E decreases under the same applied voltage V ($E=V/d$), resulting the decrease of the preconcentration speed (Fig.R3). We added this discussion in Page 8.

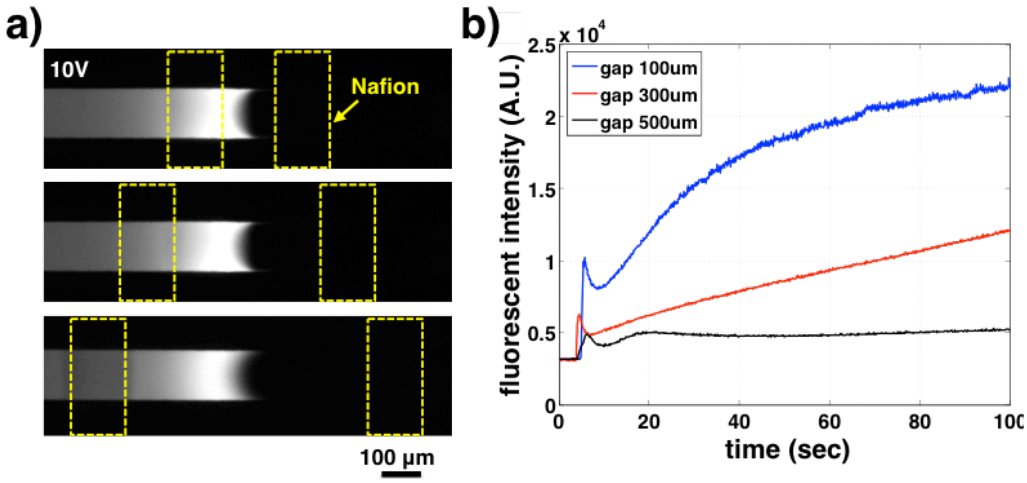

Figure R3. a) Merged ICP preconcentration with various inter-Nafion distances (100, 300, and 500 μm) under 10 V. Yellow boxes indicate the location of the patterned Nafions. b) The peak fluorescent intensities at the applied voltage 10V. The preconcentration plugs are always between the Nafions. However, longer inter-Nafion distances induce weaker preconcentration performance. This figure and contents are adopted from the Figure S2 in Kwak et al. [*Anal. Chem.*, 2016, **88**, 988−996].

4. It is claimed that the location of the plug is spatiotemporally predefined using the merged ICP. What is the longest preconcentration time tested using the merged ICP device? 20 min. are not really a long time for preconcentration. Even using the conventional ICP, a more or less stable plug at the same location can be achieved for ~20 min.

Response 4: Thank you for your valuable comment. Indeed, the longest preconcentration time in our experiments with the merged ICP platform is 20 min (Fig.8). In the FITC-albumin test, we decided that 20 min operation was enough because we achieved 10,000-fold already, and the general operating time in previous works also around few ten minutes [Cheow et al., *Anal. Chem.*, 2014, **86**, 7455-7462; Ouyang et al., *Anal. Chem.*, 2016, **88**, 9669-9677].

We agreed that our experiments could not cover the cases with longer operating times, and addressed this limitation in in the caption of Fig. 8 (Page 8).

5. on page 5, under 1.3.4): Should the PDMS mold be detached right after filling the Nafion resin in the channel? Or is it better to wait a little bit in order to minimize the loss of Nafion stuck on the PDMS surface?

Response 5: In our experiment, the PDMS mold was detached right after filling the Nafion resin. We recommend the detachment time within one minute after filling the resin, but it is not necessary. If we detach the mold few minutes later, we may obtain the thicker Nafion patterns, but the pattern would has a concaved shape; the thickest point would occur at the edge of the pattern because of the capillary effect. We added this content in the Protocol 1.3.4.

6. in the figure caption of Fig. 2: Why is there no increase of the ion concentration in the conventional platform without the ion depletion zone? Is it because there is not enough EOF generated in the channel since the Nafion is grounded and not the right outlet that is floating? What if the right outlet is grounded like the membrane? Would there be a concentration increase in front of the Nafion membrane in conventional ICP? In Fig. 6a, however, there is an increase of the concentration in the form of a plug using the conventional ICP platform. The statement seems to be contradictory. Please clarify this.

Response 6: We addressed that there is no increase of the "ion" concentration in the conventional platform without the ion "enrichment" zone. In this platform, one Nafion pattern rejects ions in the channel, generating only ion depletion zone where ion concentration drops. In this situation, there is no source of ions, so no increase of the ion concentration occurs. This is also directly observed in previous works [Kim et al., *Nanoscale*, 2012, **4**, 7406-7410] (Fig.R4). Also, in Fig. 6a, ion concentration did not increased even the fluorescent dyes were preconcentrated. It is noted that the concentration of a fluorescent dye (and target biomolecules) is not representing the concentration of ions.

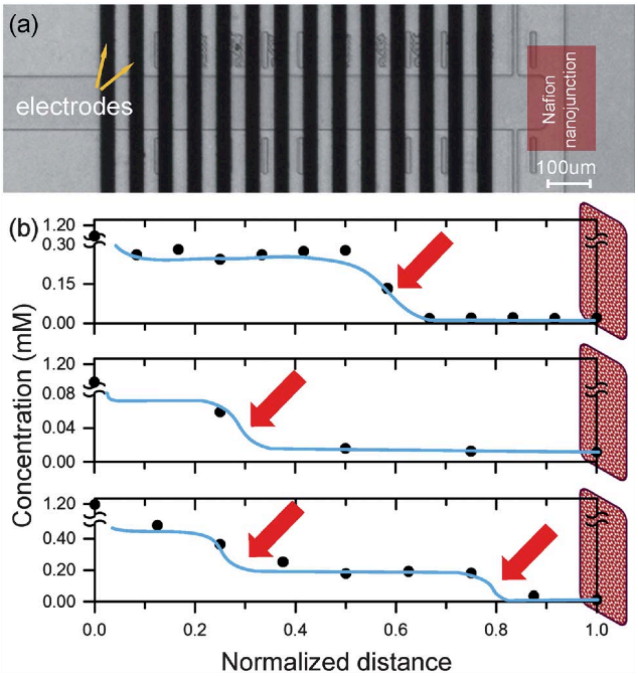

Figure R4. (a) Straight and single micro–nano–microchannel device with microelectrode pairs (black bars) for conductivity measurement experiments. (b) Conductivity drops as a function of normalized distance between ICP interface and Nafion membranes. The y-axes have a break since the concentration steeply decreased right inside ICP layer. The three measurements have different points (denoted by arrows) where the stepwise changes of the electrolyte concentration occurred with multiple vortices in the ion depletion zone. This figure and contents are adopted from Kim et al. [*Nanoscale*, 2012, **4**, 7406-7410].

7. in the figure caption of Fig. 5: why is the depletion shock only observed when the second Nafion membrane is present? A reference would be great if one would like to know more about this depletion shock.

Response 7: Thank you for your valuable comment. We believe that there would be a similar depletion shock in the conventional platform, but it would not make the spike in the peak intensity curves (Fig.5h and Fig.6h).

At high voltage, the flat depletion zone is developed in a moment, and propagates as the ions are rejected through the ion selective membrane continuously. The propagation of the flat depletion zone is reminiscent of the shock propagation, so Mani et al. called this dynamics as the deionization (or depletion) shock [Mani et al., *Phys. Rev. E*, 2011, **84**, 061504].

According to our observations, the fluorescent dyes were accumulated on the shock boundary as it pushes the dyes (Fig.4c). In the conventional platform, this initial accumulation was not identified in the peak intensity curves (Fig.6h); because the fluorescent intensity was keep increasing as the dyes was preconcentrated. However, in the new platform with two Nafion patterns, the initial accumulation was somewhat dispersed when the depletion shock met the ion enrichment zone. As can be seen in Fig.4, the width of the peak at 0.8 sec was wider than that at 0.4 sec. This is probably because the left side of the left Nafion pattern (Fig. 2a) was electrically floated, and the accumulated dyes could spread out. Indeed, in Fig.5g, the slope of the dye concentration was steeper on the right side of the intensity peak that on the left side. We added this content in the caption of Fig. 5-6 (Page 7).

8. in Fig. 6: the ticks on the x- and y-axis are missing in d), e), f). (see Fig. 5d, e, f)

Response 8: We added the ticks on the axis in the revised manuscript.

9. in Fig. 7: why are the yellow dotted boxes are used here while in all other figures, just the dotted lines are used? Use the dotted lines to indicate the Nafion membrane in order to be consistent. What is "0 distance"? In the figure caption of Fig. 7, it says that 10,20, 50 and 100 V were used to map the location of peak intensity. Are the graphs in Fig. 7b) and 7c) based on the results at 50V?

Response 9: Thank you for your specific comment. We changed the word “dotted boxes” to “dotted lines” in Fig. 7. The 0 distance in Fig. 7a represents the origin of the x-axis in Fig. 7b-c, which is on the right edge of the left Nafion membrane.

Next, the data in Fig. 7b-c shows the peak intensity locations and folds under four different voltages (10, 20, 50, and 100 V). For one case (6 cases: 1, 10, 100 mM and/or pH 3.7, 7, and 10), there were four data points corresponding to the four voltage conditions. At higher voltage, we have higher peak intensify fold in all cases. We revised the caption of Fig. 7 for more clear description (Page 7).

10. in Fig. 8: the unit for x-axis [sec.] is missing. Please indicate the location of the Nafion membranes using yellow dotted lines.

Response 10: We added the unit for x-axis, time (sec), and indicated the location of the Nafion membranes with yellow dotted lines.

11. Violent vortices -> strong vortices

Response 11: We changed the word in the revised manuscript.

12. Can you please add a short discussion about how this technique can be applied to enhance the detection sensitivity of a biosensor immobilized with capture molecules in the Discussion section? Plasma bonding of the PDMS chip with a glass slide will compromise the immobilized capture molecules.

Response 12: Thank you for the insightful comment. Indeed, plasma bonding can compromise immobilized capture molecules. Therefore, various protocols and devices have been developed to immobilize molecules after microfluidic channels are built through plasma bonding. There are three representative methods: i) adding target molecules and capture molecules together, and preconcentrating both of them together [Sarkar et al., *Lab Chip*, 2011, **11**, 2569-2576], ii) loading microbeads which holds immobilized molecules [Cheow et al., *Anal. Chem*., 2010, **82**, 3383-3388], and iii) building one bifurcated channel to immobilize capture molecules near the Nafion membrane while the main channel is closed by a value [Liu et al., *Lab Chip*, 2010, **10**, 1485-1490]. We added this discussion in Page 9.