ON-CHIP WESTERN BLOTTING: IN-SITU RENATURATION OF SDS-PROTEIN COMPLEXES UNIFIES SODIUM DODECYL SULFATE (SDS) SIZING & BLOTTING IN ONE MICRODEVICE

Chenlu Hou[1] and Amy E. Herr[1,2,3*]

[1]University of California, Berkeley Electrical Engineering and Computer Science, USA and
[2]University of California, Berkeley Bioengineering, USA
[3]University of California, Berkeley – UCSF Graduate Program in Bioengineering, USA

ABSTRACT

Western blotting readily identifies specific proteins amidst complex biological backgrounds[1, 2]. Nevertheless, immunoblotting suffers from tremendous labor-intensive and time-intensive requirements [3]. The slab-gel assays require 1-2 days for completion with multiple hands-on "blotting" steps and yield semi-quantitative information. Recently, our group has introduced new approaches for completing Western blotting. The microfluidic integration strategies introduced and used allow rapid results reporting, full assay automation, and limited sample consumption (1-10 uL). Our integration strategies use spatial, temporal, and spatiotemporal modulation of separation mechanisms in fully electrophoretic systems. The present study reports on recapitulation of immunoaffinity in previously sized proteins, using novel in-transit electrophoretic removal of SDS from SDS-protein complexes. Early results show both the length- and timescales for protein 'renaturation' are compatible with on-chip operation. Further, substantial binding affinity is recapitulated using this streamlined and promising approach.

KEYWORDS: Electrophoresis, polyacrylamide gel, integrated microsystems, proteomics, immunoblotting.

INTRODUCTION

Western blotting comprises a ubiquitous suite of techniques central to measurements spanning clinical to life sciences. Recently, our group introduced unified microsystems for integrating native protein separations with subsequent antibody-based blotting[4-7]. Central to the integrated functionality of the approach is use of a two-dimensional chamber patterned with different cross-linked polymers. The spatial arrangement of the polymer features underpins the separate function of each assay stage: separation, transfer, and probe with antibody. While powerful, our 2D chamber approach has focused on native polyacrylamide gel electrophoresis (PAGE) as a first assay stage. The rationale for native PAGE is our interest in analysis of protein isoforms, where native PAGE can be quite useful. Owing to that focus, our work to date has not addressed a major functional limitation: integration of true protein sizing (i.e., SDS-PAGE) with subsequent immunoblotting.

A primary challenge is post-sizing recapitulation of antibody binding affinity (here, loosely termed 'renaturation') of SDS-protein complexes. In this first report, we surmount this challenge using rational design of the electrophoretic transfer between a protein sizing stage and an antibody blotting stage to demonstrate SDS-PAGE on-chip Western blotting. Our as-of-yet unreported design strategy, introduced here, is a major advance & forms the foundation for widely applicable on-chip Western blotting.

EXPERIMENTAL

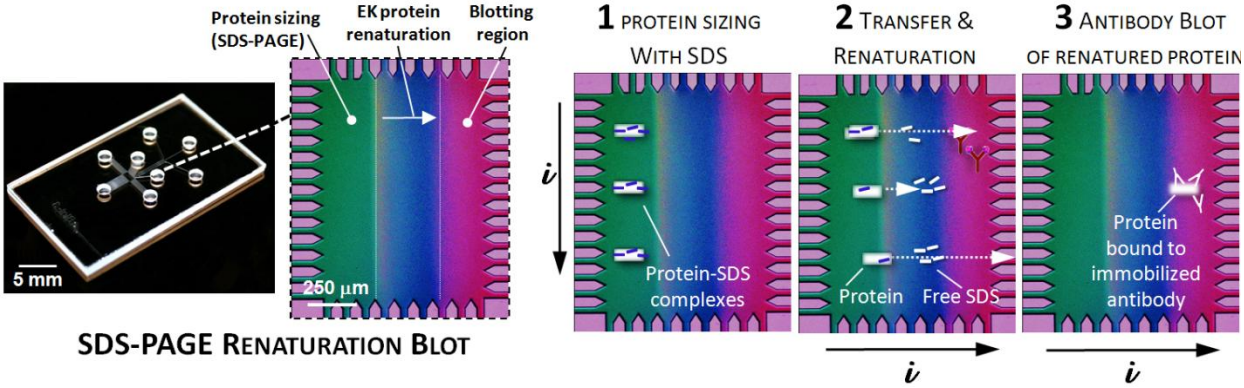
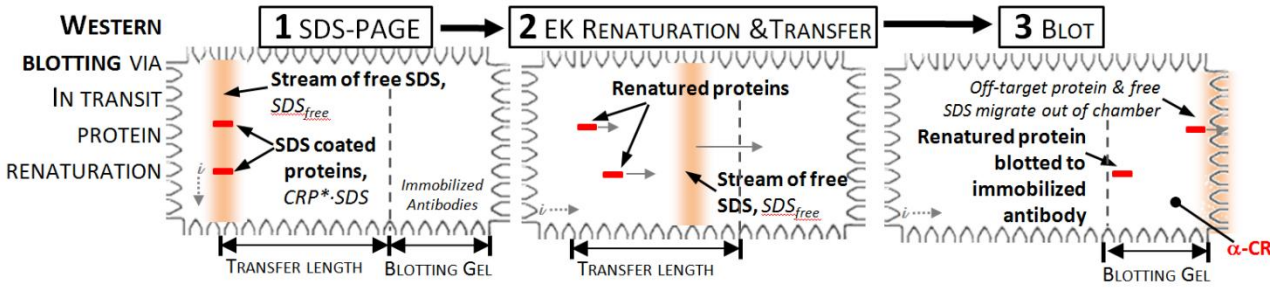


Figure 1. Integrated SDS-PAGE & antibody blotting in a microchamber patterned with polyacrylamide gels uses electrophoretically assisted protein renaturation (SDS dissociation) in transfer (step 2) between sizing (step 1) and blotting to an immobilized antibody (step 3).

Our assay design goal is to achieve high-resolution sizing of SDS-protein complexes then remove SDS thereby allowing 'renatured' proteins to regain binding affinity for the blotting gel, which houses immobilized antibodies (Figure 1). We model 'renaturation' as:

$$\frac{\partial[CRP^*]}{dt} = -k_{on}[SDS][CRP^*] + k_{off}[SDS \cdot CRP^*]$$

Where CRP* is our model protein, SDS-CRP* is the SDS-decorated protein, and k_{on} and k_{off} are the on and off rates, respectively. To guide our understanding of SDS dissociation from SDS-protein complexes during electromigration to the blotting gel, we simulated (Matlab) a non-dimensionalized reaction-diffusion-electromigration model, Eq (1)-(3) of Figure 2.



Reaction-diffusion-electromigration description:

$$\frac{\partial c_1}{\partial \bar{t}} = \frac{D_1}{E\mu_2 L}\frac{\partial^2 c_1}{\partial \bar{x}^2} - \frac{\mu_1}{\mu_2}\frac{\partial c_1}{\partial \bar{x}} + \frac{k_{on}L}{E\mu_2}C_2C_3 - \frac{k_{off}L}{E\mu_2}C_1 \quad | \text{ Eq (1)}$$

$$\frac{\partial c_2}{\partial \bar{t}} = \frac{D_2}{E\mu_2 L}\frac{\partial^2 c_2}{\partial \bar{x}^2} - \frac{\partial c_2}{\partial \bar{x}} - \frac{k_{on}L}{E\mu_2}C_2C_3 + \frac{k_{off}L}{E\mu_2}C_1 \quad | \text{ Eq (2)}$$

$$\frac{\partial c_3}{\partial \bar{t}} = \frac{D_3}{E\mu_2 L}\frac{\partial^2 c_3}{\partial \bar{x}^2} - \frac{\mu_3}{\mu_2}\frac{\partial c_3}{\partial \bar{x}} - \frac{k_{on}L}{E\mu_2}C_2C_3 + \frac{k_{off}L}{E\mu_2}C_1 \quad | \text{ Eq (3)}$$

c_1: SDS-CRP* complex conc.; c_2: free CRP*; c_3: free SDS; L: transfer distance; μ is mobility using the same sub-script indexing.

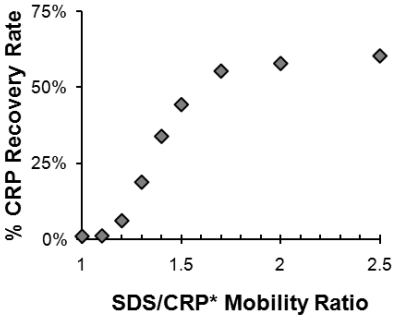


Figure 2. In-transit protein renaturation harness differential mobility of SDS and protein species to drive SDS-protein complexes to dissociation (simulation results at right), thereby recapitulating protein-antibody binding and allowing passive integration of sizing + blotting.

The model suggests that formation of free CRP* (c_2) is not only favored by isolation of CRP* from SDS (to minimize c_2c_3) but also enhanced by increasing the transfer duration. Thus, simulation points to an elegant integration strategy: harnessing the differential electrophoretic mobility between species to achieve protein renaturation during the transfer between sizing and blotting gels. This approach obviates the need for active renaturation functionality such as flow-based sample dilution or filtering thus eliminating crippling de-separation phenomena.

RESULTS AND DISCUSSION

Critically, experimental studies (Figure 3) are informed by the model: (1) the duration of electrotransfer and (2) the composition of the transfer gel matrix. Firstly, during transfer between the sizing and blotting regions of the device, decreasing the electric field increases the duration of SDS dissociation and correspondingly increases recovery rate (i.e., binding function). Design tradeoffs are currently being optimized, as increased duration of electrotransfer results in concomitantly longer total assay duration. Secondly, regarding the composition of transfer gel matrix, decreasing gel pore size in the lateral transfer region improves recovery rate by increasing relative mobility differences between analytes (Δμ/μ). Large mobility differences preclude free SDS from rebinding to protein (for high SR). Further, smaller pore-sizes increase migration time, as is important to the first aspect considered, the duration of the electrotransfer.

Experimental results in Figure 3 show SDS-PAGE (reduced) of BSA* and CRP* with a high SR = 2.6 at just t = 22 s of separation time. At t = 22 s, electro-transfer to the blotting region and electrophoretically assisted protein renaturation in transit are initiated. CRP* was successfully blotted with a binding efficiency of 55% (negative controls showed no binding) and a total automated assay time of ~3 minutes.

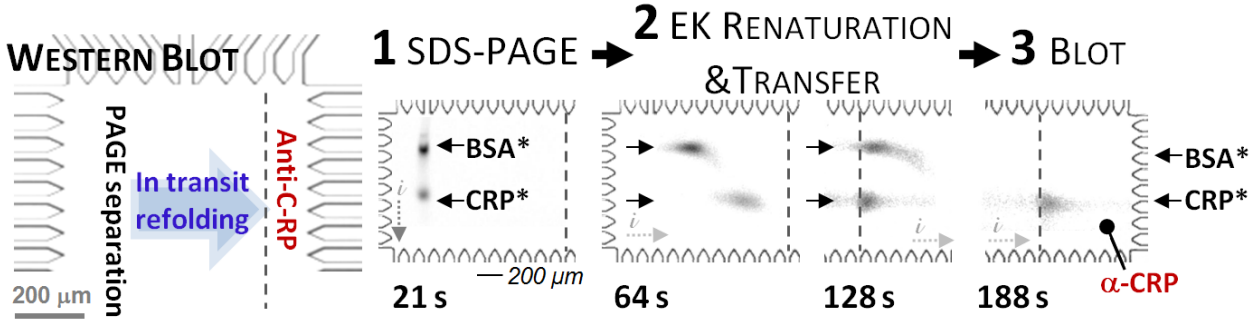


Figure 3. Experimental support for in-transit renaturation feasibility. Inverted fluorescence micrographs show proteins are sized via SDS-PAGE (1), SDS is removed using differential mobility strategy described (2), and target (CRP) blots on CRP antibody decorated gel while negative control (BSA) does not. Conditions: 8% separation gel with SDS in run buffer, $E_{transfer}$ = 75 V/cm.

CONCLUSION

In-transit recapitulation of antibody-binding capability for SDS-protein complexes has been demonstrated as a viable approach for integrating SDS-based protein sizing with subsequent antibody blotting (i.e., a heterogeneous immunoassay). Both the required time and lengthscales for the renaturation process are compatible with the assay and device design specifications reported previously by our group for integration of native PAGE with blotting. Substantial further work is needed, including enhancement of the capture efficiency over the reported ~50% antibody capture. While preliminary, the electrophoretic approach requires no additional sample handling or matrix functionalization over our previous reports. We see the demonstrated functionality as a key and promising component of future microfluidic Western blotting assays.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. M. He and the entire Herr Lab at UC Berkeley. The authors acknowledge financial support from the UC Discovery program and the Industry-University Cooperative Research Program (IUCRP). Partial infrastructure support was provided by the QB3 Biomolecular Nanofabrication Center (BNC). C.H. was a U.S. National Science Foundation g research fellow. A.E.H. is an Alfred P. Sloan research fellow in chemistry.

REFERENCES

[1] H. Towbin, T. Staehelin, and J. Gordon, "ELECTROPHORETIC TRANSFER OF PROTEINS FROM POLYACRYLAMIDE GELS TO NITROCELLULOSE SHEETS - PROCEDURE AND SOME APPLICATIONS," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, pp. 4350-4354, 1979.

[2] W. N. Burnette, "“Western Blotting”: Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose and Radiographic Detection with Antibody and Radioiodinated Protein A," *Anal Biochem*, vol. 112, pp. 195-203, 1981.

[3] B. T. Kurien and R. H. Scofield, "Protein blotting: A review," *Journal of Immunological Methods*, vol. 274, p. 1, 2003.

[4] M. He and A. E. Herr, "Polyacrylamide Gel Photopatterning enables Automated Protein Immunoblotting in a Two-dimensional Microdevice," *Journal of the American Chemical Society*, vol. 132, pp. 2512-2513, 2010.

[5] M. He and A. E. Herr, "Automated microfluidic protein immunoblotting," *Nature Protocols*, vol. 5, pp. 1844-56, 2010.

[6] S. Tia, M. He, D. Kim, and A. E. Herr, "Multianalyte on-chip native Western blotting.," *Analytical Chemistry*, vol. 83, pp. 3581-8, 2011.

[7] M. He and A. E. Herr, "Microfluidic Polyacrylamide Gel Electrophoresis with In-situ Immunoblotting for Native Protein Analysis," *Analytical Chemistry*, vol. 81, pp. 8177-84, 2009.

CONTACT

*Amy E. Herr, tel: +1-510-666-3396; aeh@berkeley.edu