Electronic Supplementary Material (ESI) for Lab on a Chip

Photopatterned free-standing polyacrylamide gels for microfluidic protein electrophoresis

*Todd A. Duncombe and Amy E. Herr**

UC Berkeley - UCSF Graduate Program in Bioengineering

342 Stanley Hall, Berkeley, California 94720, United States

*Corresponding Author, Email: aeh@berkeley.edu

SUPPLEMENTARY INFORMATION

Separation performance in *fs*PAGE improves with EOF suppression additives

To evaluate the impact of reservoir EOF suppression on the subsequent *fs*PAGE, we monitored the separation resolution (RS) of a protein ladder in Fig. S2. A protein ladder containing 500 nM BSA*, OVA*, and TI* was separated in a 20%T PAG with no EOF suppressor and with 0.5% Triton X-100 in the injection reservoir in Fig. S2A and S2B, respectively. Separations were compared in Fig. S2C when OVA* migrated 1 mm along the separation axis. For the *fs*PAGE separation with no EOF suppressor present, the BSA*-OVA* pair showed RS = 0.84 with the OVA*-TI* pair having an RS = 0.79. In comparison, when 0.5% Triton x100 was added to the reservoir, the BSA*-OVA* RS was enhanced by ~30% (RS = 1.11) as did the RS of the OVA*-TI* pair (~20% increase to RS = 0.94). The peak intensity of the injected sample zones also increased in the Triton x100 experiment: 42% for BSA*, 41% for OVA*, and 16% for TI* (RFU's of 2.55, 2.42 and 0.82 to RFU's of 3.61, 3.15, and 0.95 for BSA*, OVA*, and TI*, respectively).

Lower injection potentials result in less band distortion in discontinuous electrophoresis

During a discontinuous electrophoresis injection in *fs*PAGE an isotachophoretic stack migrates through the free-solution reservoir. The increased electric field – a result of the isothachophoretic stack – enhances EOF and results in significant band distortion when the protein sample is loaded into the PAG at 100 V/cm (Fig. 2B). EOF band dispersion persists even when an EOF suppressor additive is used (Fig. 2E). In Fig. S3 we demonstrate that the band distortion is related to the applied electric field, as would be expected for dispersive EOF. Further we show that band distortion is largely mitigated by using an injection potential of 12.5 V/cm. Of course, the major drawback of a reducing the injection potential is a slower injection.

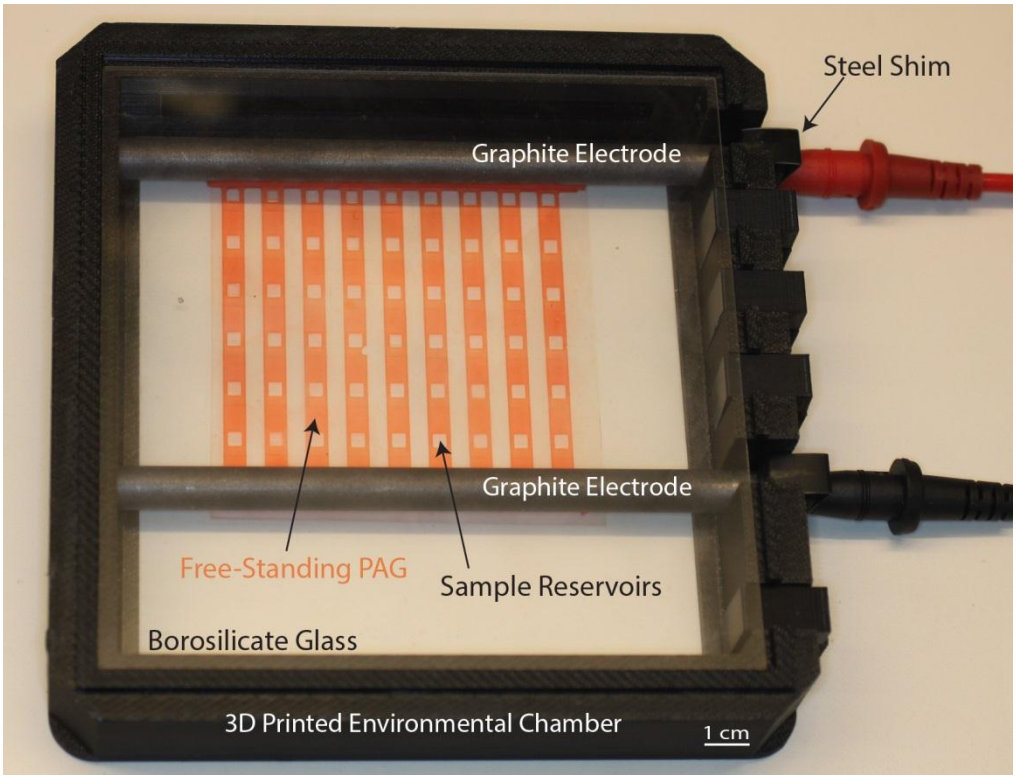

Fig. S1. We constructed an environmental chamber to minimize evaporation during *fs*PAGE. The 3D printed holder was designed in Solidworks (Waltham, MA) and 3D printed using a uPrint® sold by Stratasys (Eden Praire, MN). The .STl and .SLDPRT files are available on request. Graphite bar electrodes (#1702980) and M4 to banana plug connectors (#9007004) were purchased from Bio-Rad Laboratories. Steel shim stock with a 0.1 mm thickness was purchased from OnlineMetals.com (Seattle, WA). Borosilicate glass plates with 1 mm thickness were purchase from CBS Scientific (San Diego, CA). Electrode wicks (300 mm x 6 mm x 1 mm) manufactured by Serva (Heidelber, Germany) were purchased from Crescent Chemical Company (Islandia, NY) and were place between the *fs*PAG and the graphite electrodes.

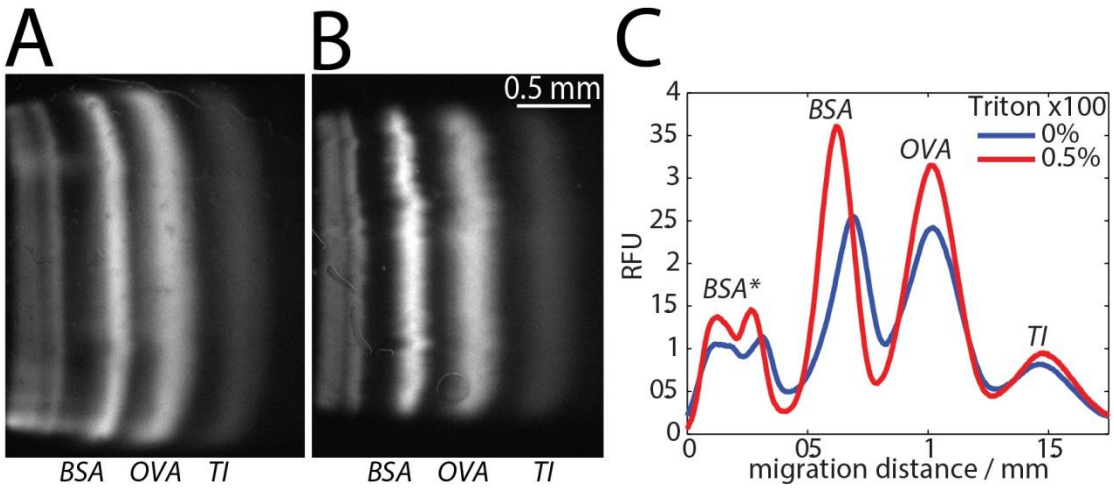

Fig. S2. The separation of a protein ladder in a 20%T *fs*PAG at 100 V/cm was performed (A) without an EOF suppressor and (B) with 0.5% Triton X-100. (C) The intensity plot profiles of the corresponding images, and intensity plots are aligned at the point where OVA* has migrated 1 mm, 40 seconds for the suppressor-less separation and 51 seconds for the 0.5% Triton x100 separation. The 0.5% Triton x100 increases the solution viscosity and reduces migration velocities by ~25%. In the separation with the EOF suppressor the protein peaks were both better resolved and larger than the separation without an EOF suppressor. As detailed in Fig. 2, the improvement is from a reduction of both EOF and protein adsorption in the reservoir.

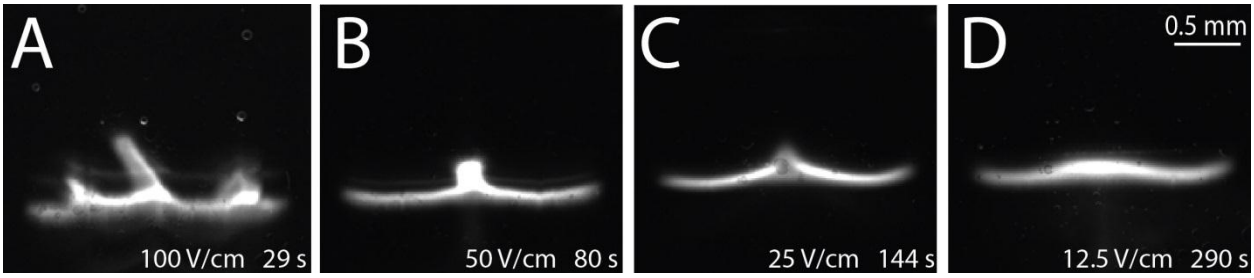

Fig. S3. Discontinuous electrophoresis injection of a 300 nM OVA* from a 1 mm (axial) by 2 mm (transverse) sample reservoir was performed at (A) 100 V/cm, (B) 50 V/cm, (C) 25 V/cm, and (D) 12.5 V/cm into a 20%T PAG. As expected with EOF induced dispersion, the band distortion was improved with a lower injection electric field. Consequently, the reduced injection potential also resulted in longer injection times: (A) 29 s, (B) 80 s, (C) 144 s, and (D) 290 s.