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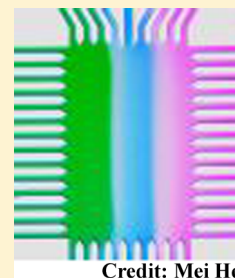
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Disruptive by Design: A Perspective on Engineering in Analytical Chemistry

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ABSTRACT: Perhaps paradoxically, we argue that the biological sciences are “data-limited”. In contrast to the glut of DNA sequencing data available, high-throughput protein analysis is expensive and largely inaccessible. Hence, we posit that access to robust protein-level data is inadequate. Here, we use the framework of the formal engineering design process to both identify and understand the problems facing measurement science in the 21st century. In particular, discussion centers on the notable challenge of realizing protein analyses that are as effective (and transformative) as genomics tools. This Perspective looks through the lens of a case study on protein biomarker validation and verification, to highlight the importance of iterative design in realizing significant advances over currently available measurement capabilities in the candidate or targeted proteomics space. The Perspective follows a podium presentation given by the author at *The 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences* (μ TAS 2012), specifically focusing on novel targeted proteomic measurement tools based in microfluidic design. The role of unmet needs identification, iteration in concept generation and development, and the existing gap in rapid prototyping tools for separations are all discussed.



Credit: Mei He

■ INTRODUCTION

“Information is the currency of the new biology”.¹ The keys to unlocking information for this “new biology” are analytical tools and methods. As such, analytical chemistry has powered a revolution in biology. Here, we take the perspective that analytical technology is the fusion of analytical chemistry and engineering. Enter engineering design. As outlined in Figure 1, the formal engineering design process is a framework for tackling problems. The design process progresses from identifying unmet needs to defining ideal specifications to concept generation in concept development to prototyping and testing. The process then finally aims to translate adoptable solutions out into the world.² Engineering design, especially in advancing instrumentation, has and will continue to play a vital role in advancing, inventing, and transforming analytical technology. We will focus on three major aspects of engineering design, as is relevant to analytical technology. First, to solve grand challenges (or really any challenge), the problem must be identified and defined. Second, iteration drives innovation and is a hallmark of design. Third, we provide a perspective on the importance of rapid prototyping in microanalyses, specifically the gap in rapid prototyping of bioanalytical separations. As a case study or “deep dive”, we focus this Perspective on the formal engineering design process and its role in tackling one prominent unmet measurement need: scrutiny of protein biomarkers of disease. We discuss highlights of needed performance specifications and current limitations, as well as a snapshot of well-suited microanalytical approaches. We then briefly reflect on analytical gaps that are likely to increase in importance. Throughout this Perspective, the engineering design process is used as a roadmap for needed, nascent, and established analytical technology. In the following sections, we use this roadmap to chart important landmarks and productive

paths and then to glimpse opportunities just beyond the horizon.

Understand the Need. The first and (perhaps) most important step of the formal engineering design process is immersion in the user environment (Figure 1). The goal of this early groundwork is identifying and understanding unmet needs. In the protein disease biomarker space, each year just a handful of proteins are approved by the FDA for inclusion in disease diagnostics.⁵ In spite of notable proteomic technology advances, a paradox has emerged. *Why have so few new protein biomarkers moved from proteomic discovery through the scrutiny of validation studies and been incorporated into clinical diagnostics?* The arguably lackluster impact of proteomics on clinical diagnostics has been attributed to a leaking, perhaps even gushing, protein biomarker “pipeline”.^{3,4} In the biomedical and basic sciences, proteins are of crucial interest owing to disease relevance. Proteins are hypothesized to reflect the state and response of numerous diseases. Yet, as captured in the analyses of Anderson et al.⁵ and Rifai et al.⁶ and more recent perspectives, the realized clinical utility of proteins as biomarkers of disease points to an important gap in the translation of information to actionable biomedical knowledge.

Compelling recent analyses posit that the leaking pipeline arises, in part, from the lack of reliable high-performance biomarker validation technology. Of course, numerous other factors are important, as the protein biomarker challenge is complex. While not the focus of this Perspective, critical shortcomings exist in a vast range of areas spanning from sample collection and handling (preparation)^{7,8} to regulatory hurdles.^{9,3,10} Lack of tools for protein biomarker validation, as

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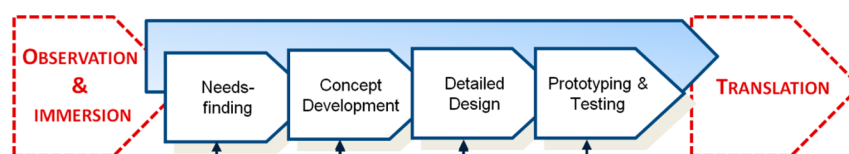


Figure 1. Engineering design provides a flexible framework for innovation in solving unmet and important challenges, regardless of the specific challenge. The design process proceeds from left to right, with substantial iteration between stages (blue arrows). Dashed arrows indicate points where substantial external interaction is beneficial. Early interaction with a wide range of stakeholders should include observation and immersion in use environments. Later stage interaction with external stakeholders includes product designers and developers, regulatory agencies, and lead users. Whiteley provides a fantastic discussion of end stage considerations.³ More detail on the engineering design process can be found in educational texts.^{2,4}

opposed to discovery of new biomarkers, presents a serious roadblock to clinical diagnostics development.^{11,12,7} Verification and validation studies are vital to assessing the diagnostic potential of proteins prior to development of diagnostics. Systematic scrutiny of promising biomarkers requires reproducible protein quantitation in complex biological fluids, standardized and quantitative outputs. The pivotal role that bioanalytical tools play in basic biomedical research is illustrated by the great strides proteomic science has made in protein biomarker discovery.⁶ Paradoxically, advances have yet to fully impact biomarker validation studies. In 2006, Rifai⁶ noted that:

the vast majority of these [protein biomarkers] are prosecuted no further than the original description of their disease association...The critical gap in biomarker development has been in winnowing candidate lists to find those few with performance characteristics that merit the effort and expense of full validation.

More recent analyses indicate that the gap is still wide open. Certainly, major advances have been made and are continuing to be made by protein measurement science. Nevertheless, the US National Institutes of Health (NIH) recently called for “disruptive” approaches to protein measurement tools, to transform the state-of-the-art from “good, but limiting”.¹³ Put succinctly, the NIH has levied a challenge that: “our ability to identify and quantify proteins in complex (e.g., clinical) samples is progressing steadily, but it is clear that orders-of-magnitude improvements in the associated technologies would enable substantial advances in a large range of biomedical research areas”.¹³

Mass spectrometry is a dominant and important approach in protein measurement science. The past decade has seen amazing advances in the analytical capabilities of mass spectrometry tools. Until recently, mass spectrometry was almost exclusively used in early stage discovery proteomics.¹⁴ In discovery proteomics, analytical technology scrutinizes as much of the protein repertoire as possible to identify new putative biomarkers. De novo protein leads are generated from this unbiased discovery proteomics approach. While obviously powerful, mass spectrometry has limitations, including: dynamic range, analytical sensitivity, throughput, and the need for intensive informatics-based analyses. The reproducibility of mass spectrometry is also of concern, as summarized by the assertion that: “the [protein] space to sample is so huge, that the mass spectrometer pulls out, every time, a slightly different subset [of proteins]”¹⁴ (bracketed words added). Of course, no one tool fits every measurement need.

Discovery tools act to narrow inquiry to a finite set of targeted proteins (e.g., unbiased mass spectrometry, DNA sequencing, and gene expression analysis). After initial identification of promising markers, study next focuses on a

targeted or candidate analysis. For example, once a pathway or set of proteins has been flagged as relevant, either owing to discovery efforts or the formulation of biological hypotheses, a truncated set of proteins can be prioritized for analysis, likely across numerous (hundreds to thousands of) samples. Immunoassays have been central to this so-called “targeted” or “candidate-based” proteomics. While targeted mass spectrometry is maturing and was named the 2012 *Method of the Year* by the journal *Nature Methods* (selective or multiple reaction monitoring),¹⁵ immunoassays remain a workhorse owing to exceptional detection sensitivity, scaling appropriate for a small set of proteins across hundreds to thousands of samples, and accessibility (i.e., instrumentation, data analysis strategies). In particular, enzyme-linked immunosorbent assays (ELISAs) have been a central tool for assessing biomarker candidates.

Owing to the growing importance of protein biomarker validation and verification, NIH has commented that innovation efforts should focus on “other technologies that hold promise for significantly improving proteomics capabilities, though they are currently less developed than MS”.¹³ The NIH’s vision of disruptive proteomics seeks to address specific gaps, including:⁹ (1) reducing the expense in technology and instrumentation and (2) removing bottlenecks arising from limited access to tools that are in high demand. Thus, advanced protein measurement tools optimized for targeted proteomics are emerging as a critical unmet need (Figure 1).

Setting the Bar through Specifications. Returning to the design process as a framework, we see that after needs identification we embark on the concept development phase (Figure 1). At this point, we set performance specifications, which are inherently quantitative. Specifications allow innovators to develop a clear picture of the performance required to fill a performance gap, thus formalizing how well a new instrument, assay, or device must perform to advance the state-of-the-art. In the protein biomarker space, instrument and assay gains are required to yield data sets that are both statistically powered and standardized.¹⁶

Statistically powered data translates into large data sets spanning a diverse set of conditions with replicates.¹⁶ Measurements must be robust (consistent) with appreciable sensitivity especially in complex samples.¹⁷ Measurable advances in biomarker validation can be realized, in part, by introducing automated protein assays capable of analyzing many hundreds, if not thousands, of samples in a single day. A further constraint, especially relevant to the biospecimen repositories so central to protein biomarker studies, is to minimize consumption of precious archived clinical samples. Consumption of 30 μL of archived sample for one analysis versus consumption of $<3 \mu\text{L}$ of archived sample for several

multiplexed analyses relaxes very real limitations on study design.

Standardization of results ensures the comparison of data generated in laboratories from around the world and is especially important when quantitation is involved. The capacity to make quantitative measurements across multiple targets is also attractive to these studies. Protein measurements focus on: isoforms, protein complexes and interaction kinetics, and protein conformation. These specific attributes dovetail with performance needs in quality control of biospecimen repositories which is a “national initiative to systematically address and resolve one of the most difficult problems that will drive 21st century cancer research: the limited availability of carefully collected and controlled, high-quality human biospecimens”.¹⁸

As a corollary, such performance is also relevant to systems biology approaches that researchers use to tease apart protein-mediated signaling networks across a spectrum of conditions. In each case, a need exists to establish tools that fit into a systems framework where “well-coordinated, global, curated data and knowledge base for proteomics findings is essential for broad dissemination and secondary analysis”.¹⁷

Western blotting also plays a role in validation studies, but slab gel formats suffer from performance limitations (Figure 2).

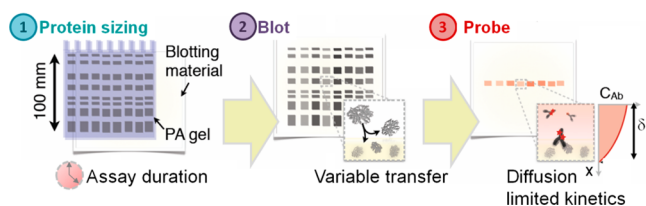


Figure 2. Western blotting assay consists of three major steps: protein sizing, blotting, and probing. When performed using a conventional polyacrylamide (PA) slab gel, each step of the assay suffers from losses stemming from fundamental transport limitations. The specific performance loss mechanism is highlighted here, for each assay stage. Adapted with permission from ref 19. Copyright 2012 National Academy of Sciences.

Owing to the time required for each assay step, as well as for transfer of materials from instrument-to-instrument, slab gel Western blots have substantially reduced throughput as compared to ELISA. Western blotting is needed, for example, if antibodies specific to the protein target (or form) under study do not exist, making inclusion of a separation step useful for teasing apart different protein forms present. Substantial effort is required to make the blot quantitative. Exciting new analytical formats, disruptive formats, are being introduced to

transform Western blotting to a quantitative and high-throughput option.

Recent efforts have centered on slab-gel and capillary separations,^{20,21} with instrument commercialization including capillary bundles that rely on both pressure-driven and electrophoretic transport.^{22,23} A summary of estimated performance specifications is provided in Table 1.

Bridging the biomarker validation gap, however, will likely require the capacity for scale-up, as well as quantitation. To allow for massively multiplexed formats, we have explored microfluidic design concepts as a means to introduce assays, device form factors, and interfacing hardware that is as simple as possible, while still yielding robust multistage function and separation performance.

Snapshot of New Concepts: Microsolutions to a Macrogap. Targeted proteomic assays would benefit tremendously from accessible data-rich, benchtop formats. Specifically, a Western blotting modality optimized to meet protein biomarker validation specifications could prove important to stemming the leaking protein biomarker pipeline and, perhaps, improve the performance of protein-based diagnostic assays. Western blotting is inherently a multistage assay: separation, blotting (immobilization), probing for readout, and several washing and incubation steps. We sought to introduce immunoblotting to accelerate putative protein biomarker validation throughput (number of samples assayed in a given time), while providing robust, quantitative information on the targeted proteins. Specifically, we identified a set of key design specifications where advancement would potentially make a large impact. These include: (1) Streamlined interfacing and control: A fully electrophoretic multistage immunoblot that would obviate the need for any external fluid routing hardware (pumps, valves, large fluid reservoirs). Macro-to-micro interfacing with minimal complexity underpins high assay scaling potential, beyond what is feasible with separation systems that require bulk fluid control and handling (storage reservoirs, pumps, valves, and tube for fluid delivery). (2) Compact and compatible device form-factor: Integration of all assay stages in one monolithic planar device architecture compatible with laboratory imaging equipment, archival storage, and subsequent reanalysis. A satisfactory constraint on overall device footprint and volume could be an ability to interface with existing standard life sciences laboratory imaging equipment (e.g., microarray scanners, gel scanners).

Microfluidic design, in particular, has proven useful in integrating multistage processes, including sample preparation. A pillar of benchtop protein analysis tools is multidimensional (or multistage) protein assays.¹¹ Multidimensional assays allow analysis of more than one physicochemical protein property by

Table 1. State-of-the Art in Western Blotting (Immunoblotting) Technology Performance Estimates and Requirements^a

assay	samples per run	through-put, h	detection sensitivity	molecular mass lower limit, kDa	ancillary equipment
Slab PAGE Western	12	~24	1 ng stain, pg HRP	1	high voltage power supply, gel imager
Microwestern arrays ²⁰	500	~24	1 ng stain, pg HRP	20	nanoplotter, high voltage power supply, imager
Capillary Western ²²	96	19	low ng	15	\$100k instrument: high voltage + pressure driven flow control, imager
Microfluidic Western ¹⁹	48	1	17 fg	20	high voltage, epi-fluorescence microscope

^aHRP: horseradish peroxidase.

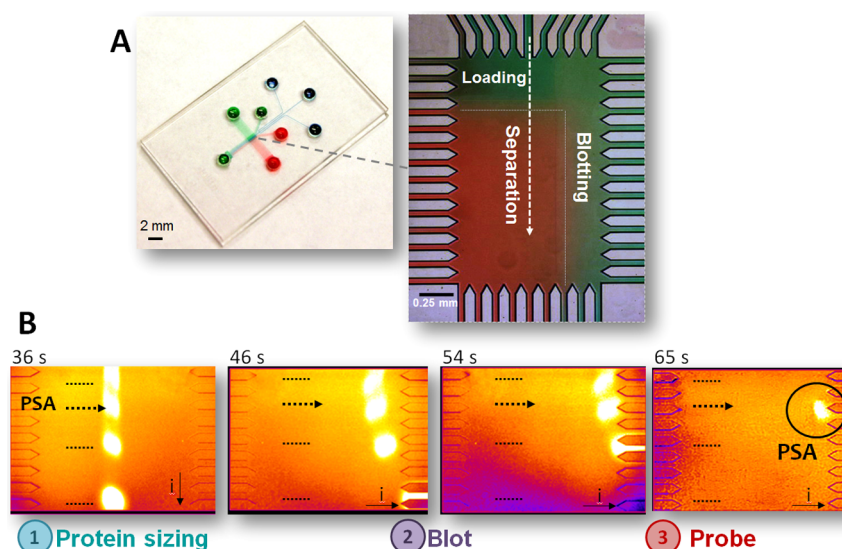


Figure 3. Microchamber patterned with spatially heterogeneous polyacrylamide gels integrates distinct immunoblotting steps in one device. (A) Image of microfluidic device with central microchamber (magnified in inset) flanked by microchannels and reservoirs. Inset shows different polyacrylamide gel regions in microchamber (colored with dye for visualization purposes). A loading region (large pore-size gel), a separation region (small pore-size gel), and a blotting region (streptavidin–acrylamide allows conjugation of biotinylated antibodies) comprise the microchamber immunoblot device. (B) Immunoblot of fluorescently labeled sample containing prostate specific antigen (PSA) is conducted in the chamber. Direction of current is indicated with “i” symbol and arrow, with elapsed separation time indicated for each image. Adapted from ref 25. Copyright 2010 American Chemical Society.

subjecting each sample to two or more separation processes. As compared to single-dimensional assays, multidimensional assays can offer a higher peak capacity or maximum number of resolvable peaks.¹² Such staged assays are especially relevant to proteins in complex samples, given the diversity of protein properties and the sheer number of proteins (many with similar characteristics).

Iterate to Innovate. After identifying the need and establishing concept requirements in the form of design specifications, we embarked on detailed design and prototyping/testing of the initial Western blotting concept (Figure 1): in a first iteration design, a microchamber flanked by a network of microchannels was initially explored as a chassis for integration of the multistep Western blot (Figure 3). Microchambers have played an intriguing role in advancement of free-flow electrophoresis and nanostructured sieving assays, two assays in which two-dimensions are needed.²⁴ Use of two spatial dimensions in microfluidic Western blotting would also be feasible. In our approach, a millimeter-scale microchamber was photopatterned with polyacrylamide gel regions, each having distinct physical and chemical properties.^{25,26}

A mask-based photopatterning process was adapted to achieve discrete gel regions in the chamber.²⁷ The polyacrylamide gel regions were designed to mimic both a miniature slab gel and a protein-blotting membrane. Again, with a focus on scaling up the assays, a purely electrophoretic approach was selected to eliminate the need for pumps and valves to complete the multistage assay: separation, capture (blotting), and probing for readout, as well as interim washing steps.

A cross-t injector at the top of the microchamber introduces an injection plug of sample into a separation gel that runs the length of the chamber center. This region of the microchamber supports PAGE, polyacrylamide gel electrophoresis. Adjacent to the PAGE separation axis is an antibody-conjugated “blotting gel” that runs the length of the separation axis (parallel). Blotting gels have been designed for immobilization of specific

targets (antibody-conjugate gel) and for nonspecific immobilization of charged species (charge patterned blotting gels). After separation, an electric field is applied orthogonal to the separation axis to mobilize charged species off the PAGE axis and into the blotting gel.

The integrated microchamber approach yielded substantially reduced total assay times, as the separation, transfer, and blotting stages each required only seconds to minutes. The ability to characterize the in-chip electrotransfer step (with fluorescently labeled protein markers) allowed characterization of losses and assessment of immobilization, both of which depend on transfer conditions including interaction time. Adaptation of the microchamber to probing with not one, but a set of distinct immobilized antibodies provides more information for each assay run and the potential for quantitative reblotting.²⁸

While the microchamber approach to integration of two-step assays is particularly relevant to integration of two unique separation stages, the need for biomarker validation requires the potential for assay scale-up. The total footprint of each microchamber-based immunoblot is small, compared to conventional Western blotting footprints. Nevertheless, the supporting network of microchannels and interfacing with electrodes is fairly bulky and would likely prove cumbersome when scale-up of the assays is a major goal.

Focusing on scalable immunoblotting, we chose to iterate in the design process (Figure 1) and explore the simplest possible microchannel architecture for protein electrophoresis, a straight channel connecting a terminal inlet and a terminal outlet reservoir. Ideally, the channel would offer a separation matrix (or bulk flow suppressor during electrophoretic separations), as well as an immobilization scaffold for subsequent probing. To define the separation, blotting, and probing stages, a functional hydrogel material was selected.²⁹ The switchable channel-filling polyacrylamide gel changes from a molecular sieving (or pH

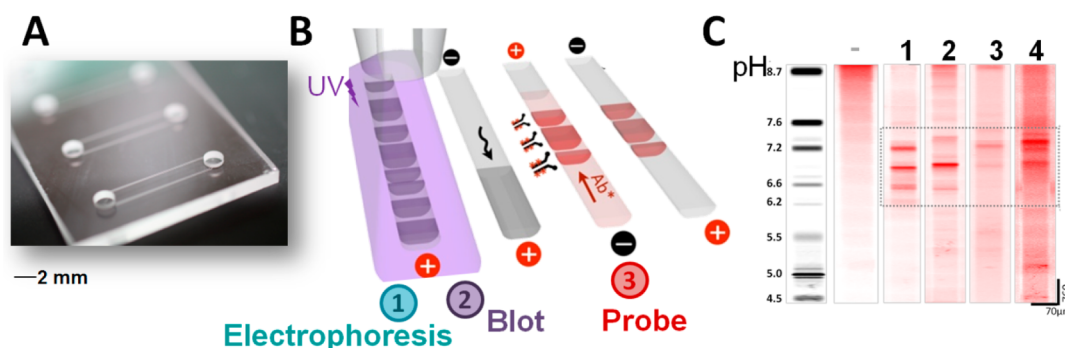


Figure 4. Single microchannel housing a photoactive polyacrylamide gel integrates immunoblotting steps by UV-based transformation of the assay from a homogeneous separation to a heterogeneous immunoassay. (A) Glass microdevice patterned with microchannels, each housing a polyacrylamide gel incorporating benzophenone methacrylamide. The photoactive gel yields photoinitiated covalent immobilization of proteins to gel after a brief UV exposure. (B) Immunoblot integrates electrophoresis in polyacrylamide gel with UV-initiated protein immobilization (blot). After electrophoretic “wash” of mobile material from separation channel, electrophoresis is used to drive probe (antibody) into gel housing immobilized protein separation output. (C) Fluorescence imaging of probed isoelectric focusing allows comparison of prostate specific antigen (PSA) isoforms in healthy human (–) and metastatic prostate cancer patient sera (patients 1–4). Adapted with permission from ref 29. Copyright 2012 National Academy of Sciences.

gradient support) matrix to an immobilization scaffold for the heterogeneous probing stage.

To realize a switchable gel, we utilized the commonly employed, and powerful, benzophenone chemistry to synthesize a photoactive version of the workhorse cross-linked polyacrylamide gels. This light-activatable volume accessible gel (LAVAgel) includes a benzophenone methacrylamide (BPMAC) monomer in a gel precursor solution that also contains bis-acrylamide cross-linker.²⁹ Brief exposure of a cross-linked LAVAgel to UV light (350–365 nm) covalently immobilizes species on the LAVAgel matrix. UV exposure promotes the carbonyl groups of the benzophenone methacrylamide monomer termini to an electrophilic triplet state³⁰ with the resultant hydrogen abstraction acting preferentially toward C–H bonds in target proteins. In this way, the LAVAgel yields a material that switches between functioning as a separation medium and, upon UV exposure, a protein-capture “membrane” for blotting and subsequent antibody probing. Inclusion of the photoswitchable gel in single, straight microchannels allows us to reach our goal of eliminating the need for physical transfer of sample between separation stage and blotting region, regardless of the assay format (e.g., slab-gel, microdevice).

Combining reactive channel-filling cross-linked hydrogels with solely electrophoretic transport confers key fundamental transport advantages. Importantly, limited-to-no bulk fluid flow is supported in the cross-linked gels. For interfacing, this means that no pumps or valves are utilized. In addition to affording switchable function, the reactive polyacrylamide gel offers assay performance advances over reactive channel surfaces, these include: (1) more reactive surface area: separated proteins are immobilized throughout the channel-filling volume of the photoswitchable gel (not simply on a surface layer), yielding effective analyte capture efficiencies; (2) pseudohomogenous reactions: use of electrophoretic transport through the nanoporous material reduces diffusion distances for two critical reaction steps (immobilization via benzophenone and probing of immobilized antigen with antibody).³¹

Two electrophoretic assays are particularly useful for protein measurements relevant to biomarker validation and have been the focus of our work: isoelectric focusing (IEF) to assay protein isoforms^{29,32} and protein sizing for analysis of

molecular mass.¹⁹ Given the importance of these bioanalytical tools, we developed single channel, planar microfluidic versions of each (Figure 4). To assess isoforms of prostate specific antigen (PSA), we detailed design and demonstration of a microfluidic probed isoelectric focusing assay in a planar microchannel format that does not require bulk flow control. Probed IEF has been developed in capillary-based systems showing appreciable potential but (in our assessment) remains bulky for scale-up due to the need for pressure-flow control hardware and use of capillary bundles in multiplexing. Thus, we introduced a microfluidic form factor well-suited to scale-up of probed IEF. Microfluidic design (i.e., fully electrophoretic operation and volume filling reactive gel) yielded significant performance advantages over the state of the art, with target antigen capture efficiencies ~100-fold higher than surface immobilization approaches. Important to scale-up, as well as analysis of precious samples, the microfluidic probed IEF assay consumed ~1 ng of probe antibody. In the PSA study, the probed IEF assay supported fully integrated measurement of endogenous PSA isoforms in both minimally processed human prostate cancer cell lysate (1.1 pg LOD) and crude sera from metastatic, castration-resistant prostate cancer patients.

Building on the knowledge gained through the probed IEF assay design and development, we again iterated in the design process (Figure 1) and explored the simplest possible microchannel architecture for protein electrophoresis and matured the microfluidic platform to support protein sizing as a first assay stage in immunoblotting. While the photoswitchable gel acted as a stabilizing support during IEF and as an immobilization scaffold, more function was required to realize microfluidic Western blotting (i.e., protein sizing in the first stage). Specifically, the gel must support additional functions: (i) defining a low dispersion injection “plug” even with no cross-t type geometry in the design and (ii) providing a molecular sieving function needed for PAGE. On the former point, we borrowed a canonical approach from slab-gel PAGE systems and employed a discontinuous pore-size and discontinuous buffer system. Consequently, the microfluidic Western blot relies on transient isotachopheresis followed by the transition to protein sizing as species migrate past a large-to-small pore-size discontinuity. Photopatterning facilitates definition of the location of such a pore-size discontinuity in

the microchannel, so we modified the LAVAgel to provide a photopatternable variant.

The microfluidic Western blot performance benefits from a 10-fold shorter separation distance than typically used for slab gel and capillary sizing, with sizing completed in under a minute. The blotting (photocapture) step required ~ 20 s and yielded exceptionally high capture efficiencies (~ 75 – 100%), rivaling those observed on conventional polymer blotting membranes. Compared to surface reactive capillary blotting approaches, the reactive hydrogel material demonstrated $\sim 10\,000$ -fold improvement in captured material ($\sim 0.01\%$ for green fluorescent protein for surface capture).²² In the antibody probing step, the microfluidic Western blot supported multiplexed analyte detection utilizing a cocktail of detection antibodies. The assay also performed well in analysis of human sera and cell lysate. Perhaps most important to our design goals, an initial demonstration of an array of microfluidic Western blots was readily realized in a device the size of a microscope slide. Here, in 24 min, 48 concurrent microfluidic Western blots were completed. Scale-up of the microfluidic Western blots to 96- and 384-plex versions is a current goal for our lab. Also important is design of the arrays for integration with standard liquid handling and microarray scanning equipment: the fewer intermediate steps requiring manual intervention or specialty equipment, the better. Taken together, we see microfluidic design as a cornerstone of achieving single-instrument functionality for Western blotting and other important multistage assays.

Our aim is that this early work establishes a scalable microfluidic framework for high-throughput targeted proteomics, as is relevant to personalized medicine through robust protein biomarker verification, to systematic characterization of new antibody probes for functional proteomics, and more broadly, to characterization of human biospecimen repositories. In particular, we are seeking to address a challenge recently levied by Hood et al. that instrument developers must “enhance throughput of proteomics assays so that reproducible, sensitive results can be obtained on many hundreds of specimens per day.”¹⁷ As pointed out by this group of thought leaders, high throughput assays not only will benefit disease biomarker studies scrutinizing proteins but also will be “useful for drug development, patient monitoring, epidemiological studies of populations, and complex time-course experiments tied to signaling pathways, biological networks, gene regulation, and disease phenotypes.” Certainly, Western blotting and other targeted proteomics approaches will not be able to alone address all questions in these spaces. However, Western blotting does and will continue to offer specific information in response to testable signaling pathway hypotheses and to vet protein marker discovery results.

Reflection and Translation. As we look to translation of the assay and devices outside of our lab to either collaborators or for further development (Figure 1), we see the limited availability of rapid prototyping methods for implementing one- and two-dimensional microscale separations as an important and largely unaddressed need. Through work to introduce sophisticated yet facile protein assays, we have identified a nested unmet need in rapid prototyping of such systems. As quoted by Francis Crick, Linus Pauling famously posited that “If you want to have good ideas you must have many ideas. Most of them will be wrong, and what you have to learn is which ones to throw away.”³³ In a similar spirit, engineering designers go by the mantra “fail early, fail often, fail cheap” to

determine which solution concepts to “throw away” and which to keep. An explosion in availability of rapid prototyping technologies from three-dimensional printing to soft lithography has substantially stimulated innovation in microfluidic assays.

While an impressively wide spectrum of assay types has benefitted from rapid prototyping, electrophoretic separations have not benefitted to the same degree. Mainly owing to ideal surface characteristics, the mainstay of microfluidic electrophoresis devices in our lab has been wet-etched glass. A survey of the literature also finds this to be more broadly the case. In tackling our design concepts and advancing to prototyping and testing, a slow concept-to-assay design cycle, as well as high cost, limited our ability to quickly assess ideas and approaches. This is particularly true in our assays that utilized either cross-linked polymers for molecular sieving or multistage device operation. Further, for any rapid prototyping approach, the so-called “resolution” of the prototype must fit the “resolution” of the operation. In other words, if a prototype format sacrifices performance in an area important to assay operation, then the prototyping approach is clearly not a good match for the problem.

For our work in polyacrylamide gel electrophoresis, recent effort has focused on introducing a format amenable to rapid prototyping, yet one that provides the performance demanded for protein (and other bimolecular) analyses. Chief considerations for the approach would be an ability to maintain a separation resolution competitive with similar electrophoretic assays in glass chip, capillary, or slab gel formats. Additional considerations include flexibility in separation architecture involving facile realization networks of separation channels (and other geometries) for one-, two-, or even three-dimensional separations; control over the injector geometry; versatility in the molecular sieving matrix characteristics; and the possibility of ready handling of select resolved peaks after separations. On the basis of these ideal metrics, our group has been devising photopatterned cross-linked gel structures that sit on top of a planar substrate layer in lieu of being housed inside enclosed channels.³⁴

The rapid prototyping approach utilizes free-standing polyacrylamide microstructures inspired by 3D hydrogels used to study cellular interactions. While still a fairly new approach for PAGE, a range of successful protein and nucleic acid separations have been demonstrated by our group in single stage assays with current focus on multistage separation processes. Briefly, the device prototyping workflow relies only on in-house processes. After mask design and mask printing, UV light is used with photopatterned polyacrylamide gels on a flat plastic or glass substrate. Photopolymerization of a gel precursor solution containing photoinitiator takes place while the precursor is sandwiched between the substrate and a glass lid, offset from the substrate with a spacer. After polymerization, the lid is removed, leaving free-standing polymerized structures on the substrate. The complete fabrication cycle is <10 min, with the entire “design-fabricate” cycle completed in well under 1 h. Notably, the approach does not require mold fabrication, as photopatterning alone is sufficient to define the x - y dimensions of the final hydrogel geometry.

The prototyping process is designed for adoption by engineers and analytical chemists seeking to quickly design and test electrophoresis assays, while the straightforward fabrication process uses materials and processes familiar to life scientists. As we seek to develop methods that are

translatable to nonspecialist laboratories, this aspect of the prototyping approaches fulfilling a major goal of much of our fabrication efforts. We look forward to this and other approaches emerging, hybridizing, and maturing to underpin rapid and effective innovation cycles.

CONCLUSIONS

Some have argued that the measurement needs in proteomics “dwarf the complexity” of the challenges encountered by the Human Genome Project.¹ Proteins (their conformations, forms, complexes, locations, and levels) are dynamic in a manner not found with genes. In fact, near consensus has been reached that a monumental challenge facing assay and instrument developers of the 21st century is development of protein analysis technology rivaling the performance of DNA sequencing tools. We look forward to seeing, and driving, the emergence and maturation of advanced protein measurement tools, especially in the targeted proteomics space. For example, we see targeted proteomic studies at the single cell level as a gap that is currently filled only by nonseparative methods (i.e., flow cytometry). Introduction of single cell Western blotting, quantitative tools for understanding protein interaction kinetics, and tools for assessing protein conformation and kinetics of state change would go far to provide insight and augment existing analytical platforms. Thanks in large part to advancing tools, a radical shift in life sciences-based inquiry is taking place: we are morphing from understanding components (e.g., proteins, genes) to understanding biology in the context of biological networks, bringing a holistic sensibility to the link between genotype and phenotype.

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Notes

The authors declare no competing financial interest.

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