Ampli: A Construction Set for Paperfluidic Systems

Elizabeth Phillips[ab‡] Anna K. Young[ab‡] Nikolas Albarran,[b] Jonah Butler,[ab] Kaira Lujan,[c] Kimberly Hamad-Schifferli,[d] and Jose Gomez-Marquez [ab]*

[a] Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge MA, USA 02139. E-mail: jfgm@mit.edu
[b] MIT International Design Centre, Massachusetts Institute of Technology, Cambridge MA, USA 02139
[c] Dartmouth College, Hanover, NH.
[d] University of Massachusetts-Boston, Boston, MA.
[‡] These authors contributed equally to this work.

E-mail: jfgm@mit.edu
265 Massachusetts Avenue, N52-373G, Cambridge, MA 02139, USA

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The design and fabrication of reconfigurable, modular paperfluidics driven by a prefabricated reusable block library, asynchronous modular paperfluidic linear instrument-free (Ampli) block, are reported. The blocks are inspired by the plug-and-play modularity of electronic breadboards that lower prototyping barriers in circuit design. The resulting biochemical breadboard is a paperfluidic construction set that can be functionalized with chemical, biological, and electrical elements. Ampli blocks can form standard paperfluidic devices without any external instrumentation. Furthermore, their modular nature enhances fluidics in ways that fixed devices cannot. The blocks' ability to start, stop, modify, and reverse reaction flows, reagents, and rates in real time is demonstrated. These enhancements allow users to increase colorimetric signals, fine tune reaction times, and counter check multiplexed diagnostics for false positives or negatives. The modular construction demonstrates that field-ready, distributed fabrication of paper analytical systems can be standardized without requiring the “black box” of craft and technique inherent in paper-based systems. Ampli assembly and point-of-care redesign extends the usability of paper analytical systems and invites user-driven prototyping beyond the lab setting demonstrating “Design for Hack” in diagnostics.

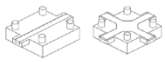
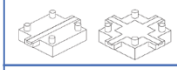
Paper analytical devices are a well established technology with a significant impact at the point of care[1–3].Their low cost has been facilitated by their large scale manufacturing using roll-to-roll and converting processes[4]. Paper analytical devices[5,6] have been used as biomarker assays[7],water quality testing[8], and many other applications[9,10]. However, in contrast to traditional microscopy[11] and traditional wet lab enabled assays[12–14], users are

unable to modify or design paper analytical devices on demand, as they are manufactured to be self contained[4,15]. Their development leads to a constant tradeoff between engineering for user-driven design and engineering for point-of-use[15–17]. Simplified methods of patterning[18,19], deposition[20] and analysis[21] have improved user-driven design in a laboratory but require significant mastery of the craft and technique of paperfluidic device fabrication. For instance, where a computer driven machine is used to trace or cut out patterns for channels users require knowledge of software such as CAD and access to an instrument such as a cutter plotter or wax printer. In methods where patterning has been simplified to be hand drawn[22] or stamp based[23] there are still additional steps such as layering, adhering, dimensioning, material selection, treatment, pipetting and drying of reagents that go beyond channel architecture. Additionally, handheld methods are still subject to the skill and manual dexterity of the operator. Reproducibility of a design by one user may vary according his or her skills compared to the original designer. This in turn will be affected by the clarity and complexity of a fabrication protocol. Practical limitations on sample volume (10-200 μL) require that device components are mm to cm in size[4]. Added sensitivity to pressure and oil contaminants mandates that the porous components be manipulated, overlapped, and layered with tweezers or mechanical guides. Unlike PDMS-based reusable modular devices[24,25] paperfluidic devices are "Write-Once/Read-Once"[26] systems. Despite the growth in paper analytical device publications[27] their manufacturing adoption is lagging. The focus on centralized manufacturing systems means that devices are less adaptable to the realities of the field. It has not previously been demonstrated that paper analytical devices can be constructed using a prefabricated modular platform. In addition to on-demand diagnostics, modular assay assembly could facilitate new forms of

paper assay programming for both hand-made and machine-assembled biochemical circuits reconfigurable for an array of assay conditions. One approach to extend the reach of paper diagnostics in a standardized way is to adopt the prefabricated modularity of electronic breadboards which accelerated redesigning of circuits in real time via picking and replacement of individual components[28]. The notion of modular prefabricated assembly is how a Lego construction kit assembled by an expert or a child is dexterity-proof and can have the same exact features and performance regardless of who builds it. Here, we have developed a library of prefabricated modular components that connect-and-react for the easy engineering of paper analytical devices. This parts library enables user-driven design on demand, without the need for subsequent fabrication equipment like laser cutters or 3-D printing. Each component, an Asynchronous Modular Paperfluidic Linear Instrument-free (Ampli) block, was designed to contain paper based fluidic elements pre-formed into shapes that passively store, transport, split, and mix reagents (Fig. 1a). When a string of Ampli blocks are connected to each other, they form a paperfluidic circuit. To connect a string of blocks together, individual blocks snap onto each other with upper and lower overlapping paper tabs. The resulting approach improves the performance of traditional paper diagnostic fabrication and enables new forms of analytical chromatographic flow. With a modular library of Ampli blocks we demonstrate multiple biochemical reagent combinations and system tuning via component level optimization of discrete elements. In addition, incorporation of third party parts permits a “Design for Hack” framework. Much like discrete element prototyping boards have permitted greater rapid experimentation than monolithic circuit boards, our library of prefabricated discrete paper elements could accelerate the

development of new paper-based analytical devices without the need for onsite machining.

A

Shapes	Function	Membrane	Typical Uses
	Sample Block	Chromatography	Wicks sample into the circuit
	Transport Block	Glass Fiber, Chromatography	Distributes single & multiway flow for chemistry functions; Spacers
	Conjugate Block	Glass Fiber	Loads and releases protein conjugates such as AgNP & AuNP
	Absorbent Pad	Chromatography	Absorbs excess liquid for constant flow. Serves as reaction end node.
	Protein Carrier	Nitrocelullose	Antibody testing zones
	Battery Block	Copper, Paper, Carbon, Magnesium	Generate current via salt bridges

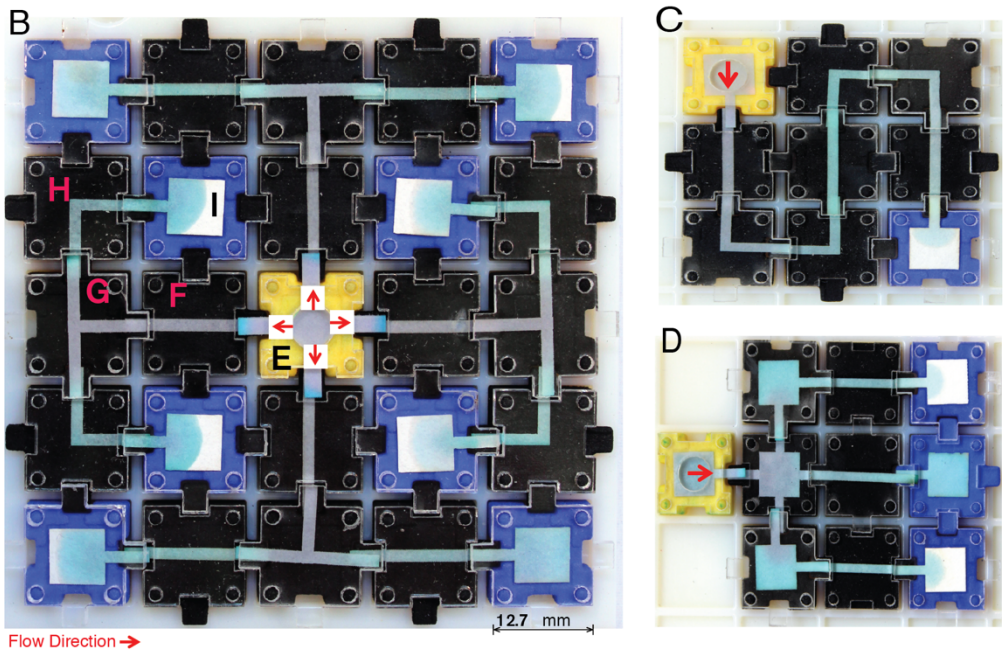


Table 1. Table estimating cost of blocks and reagents.

	TRADITIONAL	AMPLI 3-D PRINTED*	AMPLI DISPOSABLE*	AMPLI REUSABLE*
Nitrocellulose	$0.03	$0.01	$0.01	$0.01
Glass Fiber	$0.04	$0.01	$0.01	$0.01
Ligands or Reagents	$2.90	$2.90	$2.90	$2.90
Labels or Dyes	$4.50	$4.50	$4.50	$4.50
Housing	$0.32	$1.28	$0.03	$0.01
Total Cost	**$7.80**	**$8.71**	**$7.46**	**$7.44**
Total Cost Minus Reagents **Set of four blocks*	**$0.40**	**$1.31**	**$0.06**	**$0.04**

Figure 1. a) library of modular parts; b) image of setup with branched flow; c) image of setup for a single channel flow of customizable length; d) branched flow.

Library of Blocks. We constructed a library of pre-fabricated blocks containing various paperfluidic elements (Fig. 1a). Blocks were pre-fabricated by layering fluidic elements or reagent-embedded pads. Each block connects to another block via complementary slots. Blocks were designed in CAD and 3D printed in plastic to support one or more paperfluidic membranes covered by an acrylic protector. A small clearance between the acrylic protector and the membranes was created via inset slots on the blocks. No secondary flow between the acrylic tops and membrane was observed; liquid wicked through the sandwiched membranes at the same rate as when not covered by the protector (data not shown). Membranes were designed in CAD and laser cut in various shapes to allow for straight, curved, multiplex and end flow. The membrane type (paper or cellulose, nitrocellulose, glass fiber, etc.) is selected based on the block function. Standard fluid transport membranes were cut with untreated Whatman 1mm chromatography paper to make Transport Blocks (TB), Connection Blocks (XB), Sample Pad (SP) and Absorbent Pad Blocks (AP). Antibodies were printed onto nitrocellulose membrane to form Nitrocellulose Blocks (NC). Glass fiber membranes were treated with gold nanoparticle (AuNP)-antibody conjugates to form Conjugate Pad Blocks (CP). Chemical and biological reagents were spotted on the membranes with a pipette or automated dispenser before block assembly. (Suppl Info, S1).

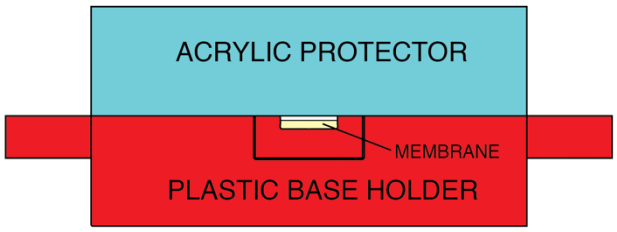


Figure 2. Cross section of block assembly.

Cost. One of the benefits of paper as an analytical platform is its low cost (Table 1). We designed our system as a prototyping interface that depending on the blocks' material

composition, could have the a) reusability of autoclavable glassware and metal instruments and b) the disposability of pipette tips (Fig 1e). In the case of reusability, the blocks' base and acrylic protector would be autoclaved after use and the sandwiched membranes would be replaced with new membranes. The overall costs of the blocks compared to paper alone is minimal compared to the cost of reagents both platforms as shown in the table. The lowest prototyped block was cast from Reynolds plastic resin at a cost of $0.0086 per block, and the 3-D printed versions used for most of our experiments were $0.32 a block. The lower end is well below the cost of an Eppendorf tube ($0.02).

Proof of Concept Paperfluidic Block Circuit. 25 pre-fabricated Ampli Blocks were arranged by hand in a 5x5 grid to demonstrate instrument-free assembly of a paperfluidic circuit (Fig. 1b). Each block connection takes 1 sec to place by hand. Different circuits were formed using SP, XB (Splitter and L-Turn) blocks. Additional circuits include S-channels and multiplex flow were fabricated (Fig. 1c-d). Colored dyes added to visualize flow indicated successful interconnections between each block with no leaks outside the paper membranes

Rapid Discrete Component Optimization

Realtime Reagent Swapping: A drawback in the development of traditional multi-reagent paper analytical devices is their Write-once/Read-once architecture[25]. Tweaks, such as changing reagents or modifying branches, often require complete re-fabrication of the entire structure and re-spotting of reagents. We demonstrate that Ampli blocks can overcome this drawback by enabling interchangeable discrete element assay fabrication (Fig. 3) starting with a colorimetric glucose assay. Three blocks in a row, SP, TB, AP were arranged and functionalized with simple proof of concept chemistry: SP (Glucose), AP (Glucose Oxidase (GOx and peroxidase), and in between, a swappable option of two TP blocks was created with 1.5%

potassium iodide (KI) or 5% o-toluidine. Blocks were arranged linearly and 40 µL 5% glucose into the SP resulted in flowing of the sample into the KI block, and then ultimately to the AP block with the GOx and peroxidase developer. This resulted in the appearance of a color, indicating successful reaction of the glucose with the GOx and peroxidase to make a colorimetric product, completing the reaction circuit. However, if the KI block was swapped with the block containing o-toluidine, the resulting colorimetric intensity in the AP block increased. Quantification of the color using ImageJ[26] shows an intensity increase of 2.7X. Control experiments with no glucose resulted in no color change in the AP block, demonstrating that previous color changes were a result of the glucose reaction and not migration of the slightly colored KI or o-toluidine into the AP block. This shows that instrument-free swapping allows the user to fine tune a colorimetric developer, in this case yielding a more intense signal simply by exchanging blocks from a library of options.

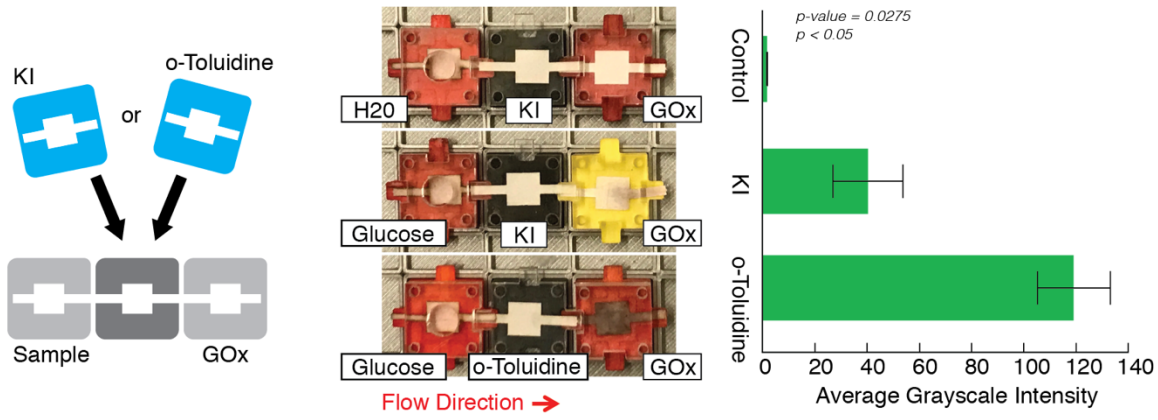


Figure 3. Glucose reaction circuit with swappable components shows ability to select and fabricate different reaction pathways. P-Value = 0.0275. Error values attributed to flow variations within paper lots.

Tuning Sequential Assay Paperfluidic Circuits. There are many other reactions that rely on intermediate chemicals to develop and accumulate[9,27,28] later to be revealed by a dyeing reaction step. This requires the ability to control the presence, absence, and quantity of multiple intermediates. These multi-step sequential reactions can benefit from real time

tweaking of the reaction time in between chemicals to increase overall colorimetric output. Next, we demonstrated that we could use this just-in-time feature to vary the reaction kinetics of a normally bulk solution assay that we incorporate into paper to detect the presence of nitrites, a common indicator of bacterial infection[29]. We used a simple proof of concept assay based on the well-known Brown Ring assay. Enhanced reactions like the Griess reaction are good alternatives but lack the presence of an intermediate that we can manipulate as a tuning example. If nitrites are present they react with sulfuric acid which forms a chemical intermediate[30]. This intermediate reacts with iron(II)sulfate to form a brown color. We translated the assay into 2 blocks, where one contained 0.5% sulfuric acid and the other 2% iron(II)sulfate. An SB was upstream of the block sequence. Production of the intermediate determines the amount of product available to react with the iron sulfate. In bulk solution, accumulation of the intermediate is constant because of solution mixing, and is able to react instantaneously with the iron sulfate. However, in a paper format of this reaction, careful attention has to be placed on the flow and accumulation of the intermediate because there is no bulk diffusion. We added spacer blocks in between the sulfuric acid and iron sulfate block to vary the length of the circuit, and thus the time in which in the intermediate complex is left to accumulate before reacting with the sulfuric acid. After forming circuits of 0, 1, and 2 spacers, the resulting brown intensity was present. It was determined that that 1 spacer was optimal (Fig. 4a). Next, we show we can modify this just-in-time feature to vary the reaction kinetics for an assay for the presence of isonicotinic acid, a metabolite of the anti-tuberculosis antibiotic isoniazid. This assay is a colorimetric test of urine for drug adherence[31]. If isonicotinic acid is present, then it reacts with Chloramine-T and potassium thiocyanate (KSCN), which forms a chemical intermediate. This intermediate

reacts with barbituric acid to result in a blue color. In this experiment, instead of placing spacers as a liquid fuse, we separated the barbituric acid step from the main circuit block and varied the time an intermediate was allowed to form before connecting this dyeing reagent. We translated the assay into 3 blocks, where one contained chloramine T, another contained KSCN, and then the final block contained barbituric acid (Fig. 4b-c). As in the nitrite reaction, accumulation of the intermediate on paper is required to calibrate the test for optimal color intensity. We allowed the intermediate to form after adding a 0.05 % isonicotinic acid solution at 0, 3 and 6 minutes before adding the barbituric acid block to the KSCN Chloramine-T circuit. Here, the resulting blue intensity was present, and increased with the time delay in connecting the barbituric acid block.

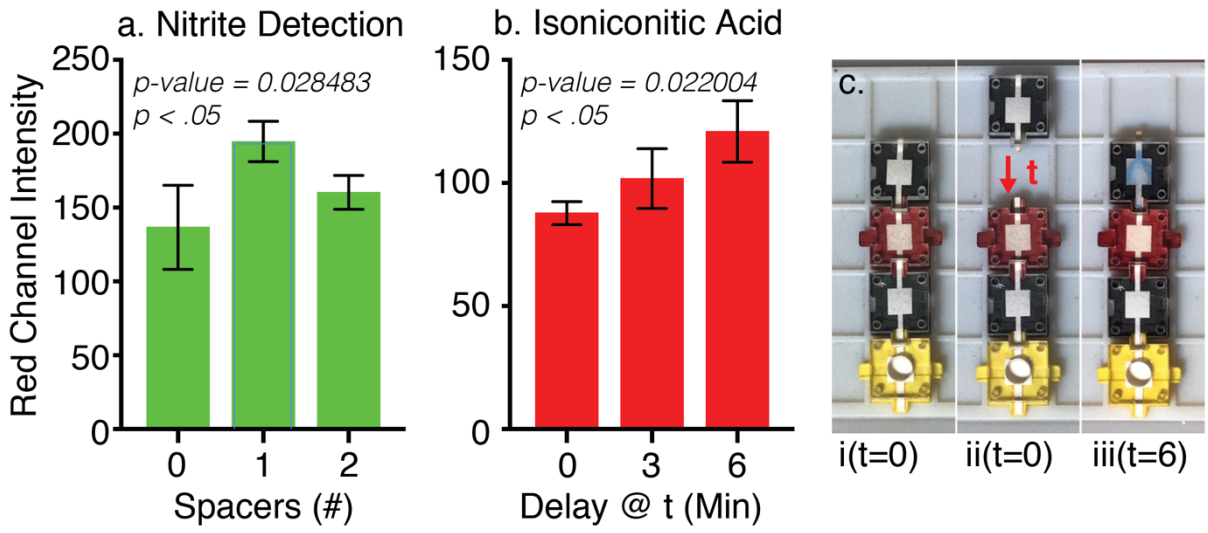


Figure 4. Variable timing and accumulation using modular flow: a. Spacer enabled variable circuit length for colorimetric intensity of nitrite detection (p-value = 0.028483) and b-c. variable delay at times 0, 3, and 6 minute connections for isonicotinic acid detection (p-value = 0.022004). Error bars show the standard deviation of three independent block setups which produce variations due to evaporation and flow differences due to minute paper membrane differences.

Construction and Expansion of Lateral Flow Immunoassays. Using these blocks, we demonstrated that a functional lateral flow immunoassay (LFI),[4] a well-established paper analytical device, could be constructed. Using a protocol for cancer biomarkers by Warren et

al[32], an LFI assay was translated into blocks. The blocks were linearly configured to detect a DNP-biotin peptide (Fig. 5a-c). For this we used a sample pad (SP), conjugate pad (CP), nitrocellulose carrier (NC) and an absorbent pad (AP). The paper in the CP block was loaded with AuNP streptavidin. The paper in the NC blocks were spotted with anti-DNP antibodies at the test line (left) and anti-streptavidin antibodies at the control line (right). DNP-biotin peptide was added to the SP block followed by a PBS wash (Suppl Info, S3).). A red test line appeared, indicating successful formation of a sandwich immunoassay with the DNP-biotin peptide and the AuNP-streptavidin. A red band also appeared at the anti-streptavidin control line, confirming proper flow of the AuNP-streptavidin and validating the test. NC block strips were analyzed with ImageJ at different DNP-biotin peptide concentrations, demonstrating a linear concentration dependence comparable to previously reported LFIs. The small SEM values, especially at lower dilutions, indicate that blocks can be robustly constructed into LFIs.

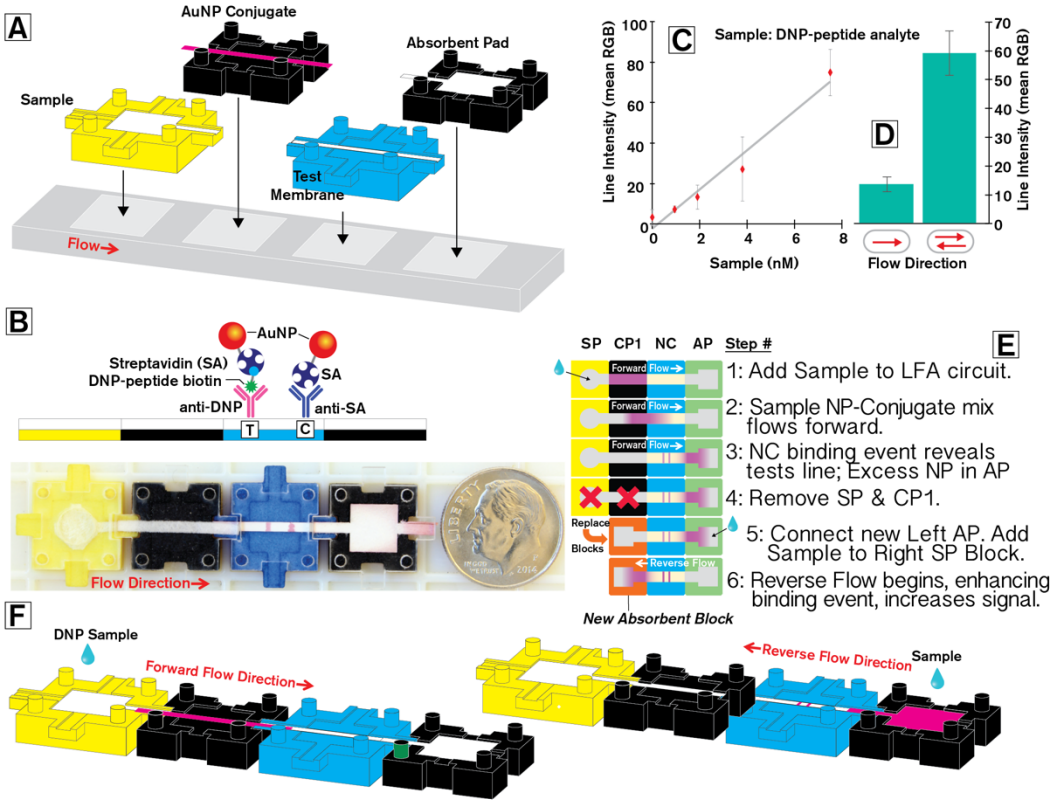


Figure 5. Modular assembly of lateral flow immunoassay and performance (A-C). Enhanced performance tuning using reversible flow with swappable absorbent pad blocks (D-F).

System tuning for dynamic flow control. Signal improvement in LFIs has been previously demonstrated by silver enhancement solutions[28], or approaches, such as isotachophoresis[33]. However, these require desktop lab equipment and specialized current and voltage controls. The success of these enhancement methods is dependent on the initial capture of sample and gold NP conjugate. However, some proportion of sample and gold NP conjugate often washes past the immobilized antibodies and is never captured, due to the weak capture affinity and/or poorly engineered flow time.

Conventionally, reverse flow in LFIs is seen an impairment to reliable signal. We demonstrate that our platform modularity permits flow reversal and thus a second capture opportunity to improve signal (Fig. 5). An LFI to detect a DNP analyte was constructed as

above. 10 min after introduction of DNP sample onto the SP, the SP and CP blocks were removed and replaced with a second AP block (Fig. 5e, Step 5), effectively reversing the flow gradient. Additional sample was applied to the first AP block which was already initiating flow in the reverse direction. After 10 min the test membrane was removed for analysis of signal intensity (Fig. 5d) showing a 4-fold signal improvement. This suggests that the AuNP-streptavidin conjugate was inadequately capturing sample leaving unbound AuNPs in the first AP block. To our knowledge, this is the first time reverse flow has been shown to purposely increase the intensity of an LFI test band. Flow reversal can both improve signal and elucidate which component (conjugated or immobilized antibody) is inadequately optimized for a single capture event. Furthermore, reverse flow can be useful for precious, limited access samples to minimize unreacted material.

Biological Multiplexing and Tweaking. We then demonstrated the robustness of our blocks using multiple biological reagents for detection of a cancer marker (Fig. 6). We constructed a multiplexed LFI by connecting three linear LFI (branches A-C) to a single sample block (SP, yellow). This multiplexed circuit replicated the architecture of prior urine based cancer reporters. To demonstrate tweaking, we added a fourth branch (D) with a transport block (TP) and a protein testing block assembled from a deconstructed urinalysis test (AP block, blue). To demonstrate specificity we applied 160 uL samples of DNP, FAM, or AF488 peptide analytes to the SP block and analyzed test strips line intensities in branches A-C after 20 min. A multiplexed LFI made a signal in the lane appropriate to the analyte with negligible background signal in the remaining lanes. Detection of other clinically relevant reagents, such as protein for urinalysis, may confirm the absence of cross reactivity or decrease in sensitivity. Using dynamic multiplexing of a paperfluidic circuit can be helpful to

ensure the diagnostic value of the multiplexed reagent system. In the case of the AF488-biotin peptide analyte, we observed a decrease in detection signal in the presence of protein. The multiplexed array with the high protein sample creates a false negative output but is flagged by the protein detecting block that provides a contraindication to the result. This just-in-time-assembly serves much like the presence or absence of a positive control band in single-plexed LFIs to confirm successful device usage. Additionally, this demonstrates the ease of commingling clinical and biological reagents in a single circuit without issues of cross contamination during the deposition process, since each block is striped and functionalized separately.

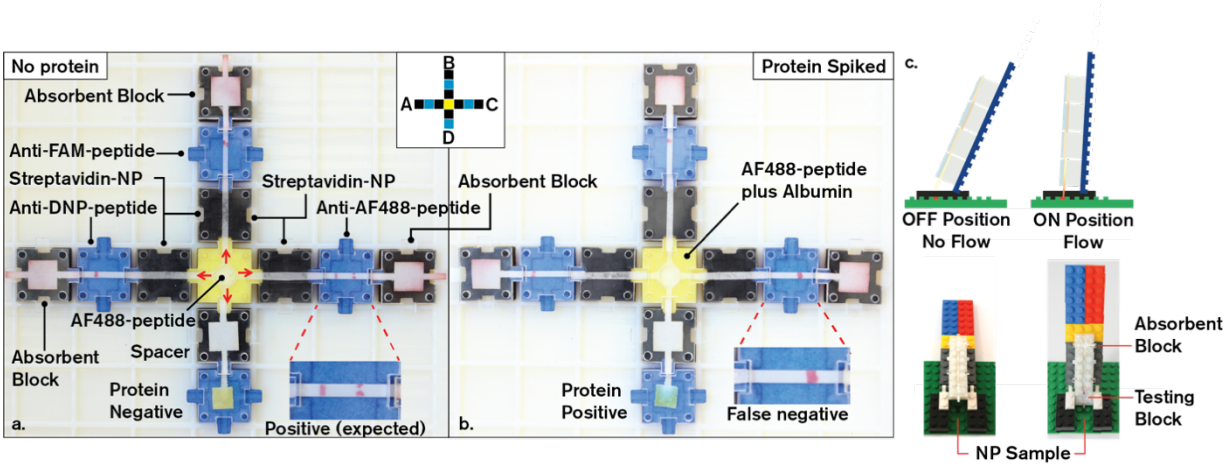


Figure 6. a-b. Multiplexed lateral flow immunoassay with swappable tweaking. c. Hybrid construction systems for variable angle flow using Lego blocks.

Design for Hack in Diagnostics. A successful toolkit for user driven design in life sciences should not be limited to its own parts ecosystem. Here we demonstrate how our block system can be used in conjunction with other construction set systems. Half-strip tests are precursors to lateral flow diagnostics typically run on a bench in a conical tube holder. They are a useful screening tool and typically hand made with nitrocellulose, paper and tape. A LFI block setup arranged into a NC block and an AP block can be used to create a variable

angle half-strip test to control chromatographic flow speed against gravity (Fig. 6). Lego blocks were used to create a pivot system arranges the assembly in an on or off position, permitting or preventing flow from a pooled sample source.

In summary, we have created a library of prefabricated primitive elements to enable the dynamic, instrument-free construction of just-in-time, semi-permanent and permanent paperfluidic paths resulting in precise component coupling and robust chromatographic flow. This approach to hands-on, modular paperfluidic programming could find applications in remote diagnostics, chemical synthesis, and new elements of a future biochemical breadboard environment. It is a step towards democratizing the manufacture and engineering of point-of-care devices beyond the lab to enable creative design from the end user.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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