Supplementary Information (SI) for Lab on a Chip.
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Supporting Information

Digitally Programmable Microfluidic Valving for Autonomous, High-Resolution Continuous Chromatographic Purification

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Movie S1. Operation of the ICV across sequential stages I1–I4. The ICV is positioned centrally, with four peristaltic pumps located at each corner. Each pump draws a distinct colored dye from its respective reservoir, corresponding to sample (S), wash (W), elution (E), and re-equilibration (R) buffers. These fluids are delivered into the ICV and subsequently directed to the four purification columns (C1–C4) located at the lower side of the setup. Under stepper motor actuation, the ICV rotates to align specific flow paths, cycling through four stages: stage 1 (I1 mode), stage 2 (I2 mode), stage 3 (I3 mode), and stage 4 (I4 mode). After completing stage 4, the valve returns to stage 1, establishing a continuous operational cycle. The resulting fluid routing at each stage is visualized by the distinct color patterns observed in the outlet tubing connected to C1–C4, reflecting the programmed buffer distribution. The video is displayed at 16× speed.

Movie S2. Continuous GFP protein purification using the MCPP system integrated with the CCV. The main panel shows the CCV module in operation alongside the temperature control unit, which maintains the system at 4°C to preserve protein stability during purification. GFP-containing eluates are continuously routed through the CCV into the designated product reservoir. The inset panel provides a magnified view of the product outlet, illuminated under blue LED light to enhance visualization of GFP's intrinsic green fluorescence. This fluorescence clearly demonstrates the uninterrupted collection of purified GFP over time, confirming the system's capability for automated, contamination-free, and continuous protein recovery.

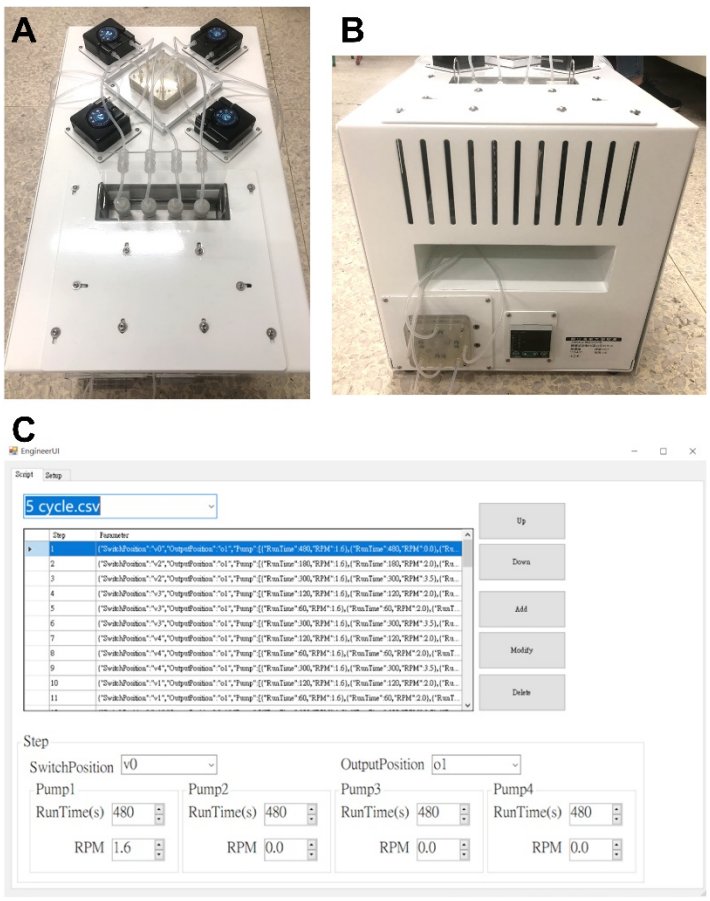


Figure S1. MCPP platform and control interface. (A) Top view of the MCPP platform showing the four peristaltic pump modules, ICV, tubing manifold, and valve/column connections. (B) Front view of the MCPP platform showing CCV and temperature controller. (C) Screenshot of the custom PC-based GUI used to execute stepper-motor actuation sequences. The GUI imports a CSV-based operation table and displays stepwise parameters including switch position, output position, and run time (and motor speed/RPM when applicable). Users can edit and reorder steps (e.g., up/down, add/modify/delete) and run the programmed schedule to synchronize pump actuation with ICV/CCV switching for deterministic buffer routing and time-gated fraction collection during continuous operation.

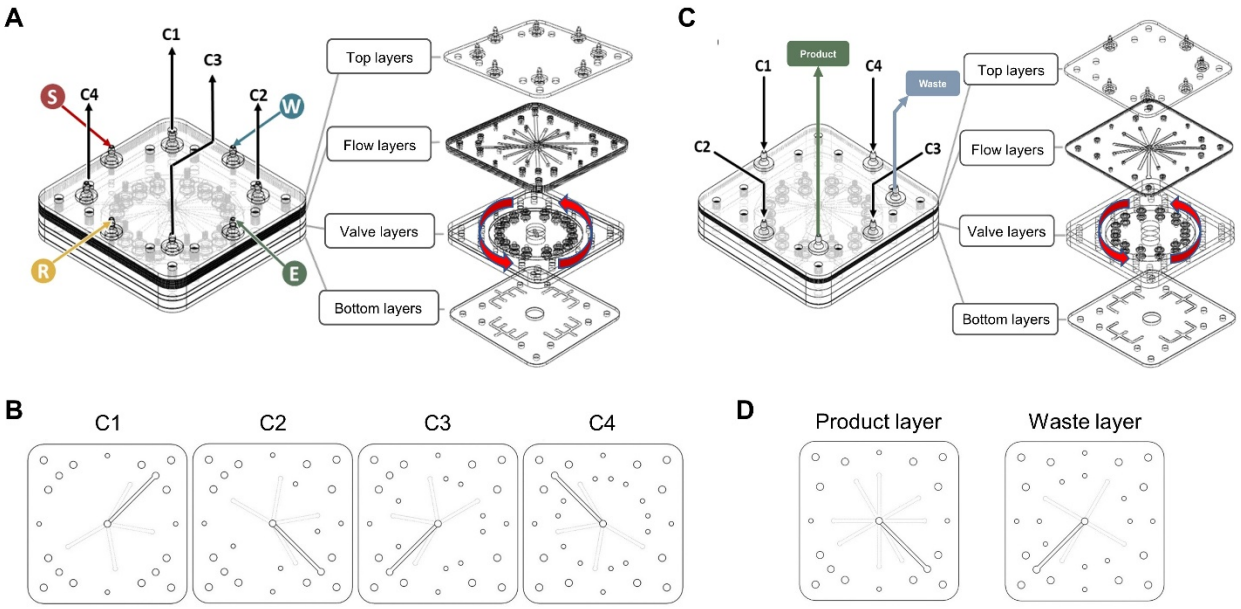


Figure S2. Structural design of the ICV and CCV. (A) Exploded and top-view schematic of the ICV, showing its four-layer architecture: the top layer receives fluid inputs; the flow layer radially distributes liquids; the valve layer contains a rotatable disc that defines flow paths; and the bottom layer channels fluids to specific purification columns (C1–C4). (B) Top view of the ICV flow layers. The flow layers comprise two interleaved sub-layers: the upper layer (solid lines) and the lower layer (lighter lines), illustrating the alternating flow pathway arrangement. The ICV flow layer is divided into four sections, each independently connected to its corresponding purification column (C1–C4). (C) Exploded and top-view schematic of the CCV, which shares the same four-layer construction but is configured to direct eluates into either the product reservoir or waste reservoir according to programmable flow switching. (D) Top view of the CCV flow layers, consisting of two sections. The two CCV outlet layers are independently connected to the product and waste reservoirs, enabling selective fraction routing based on preprogrammed collection logic.

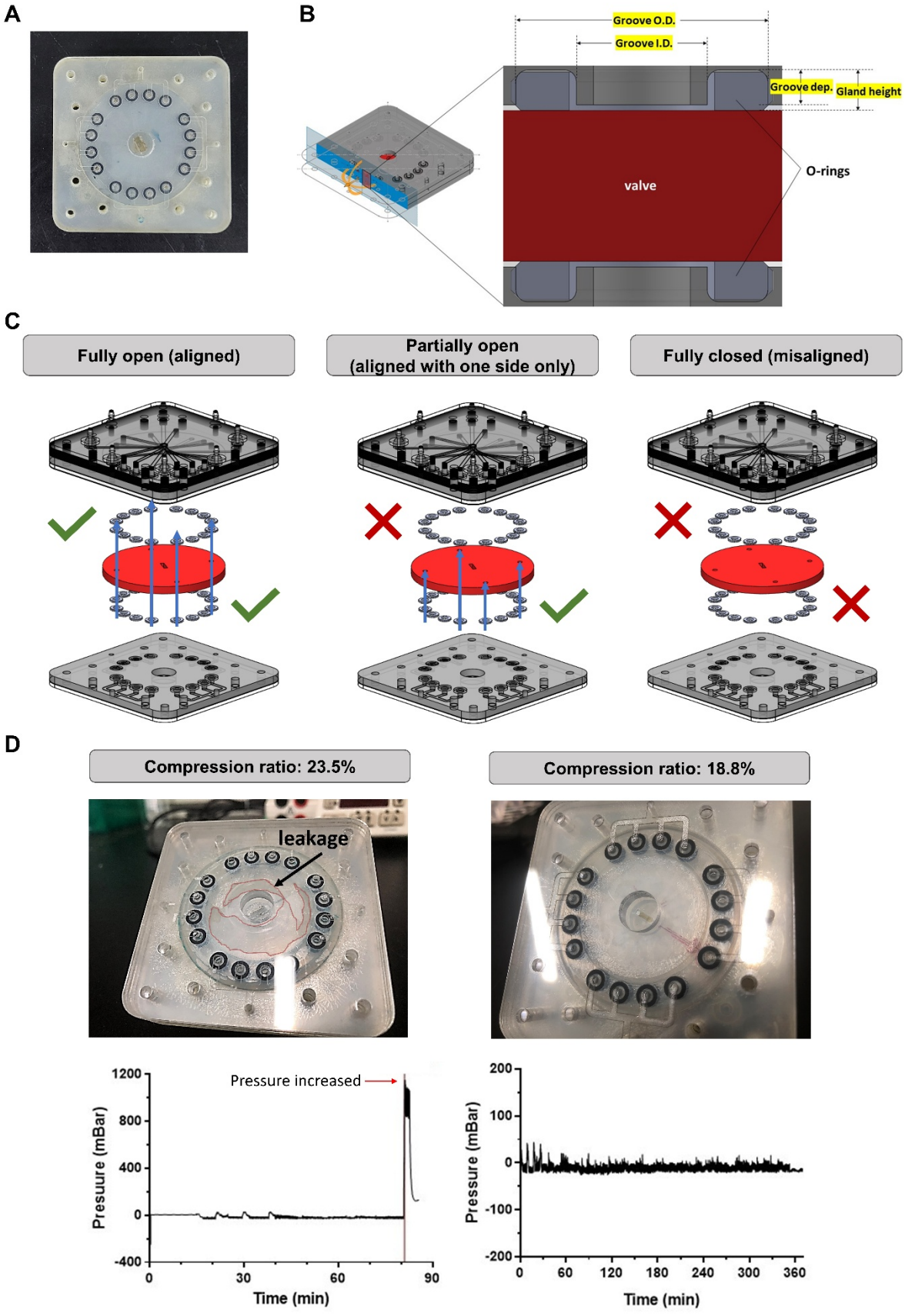
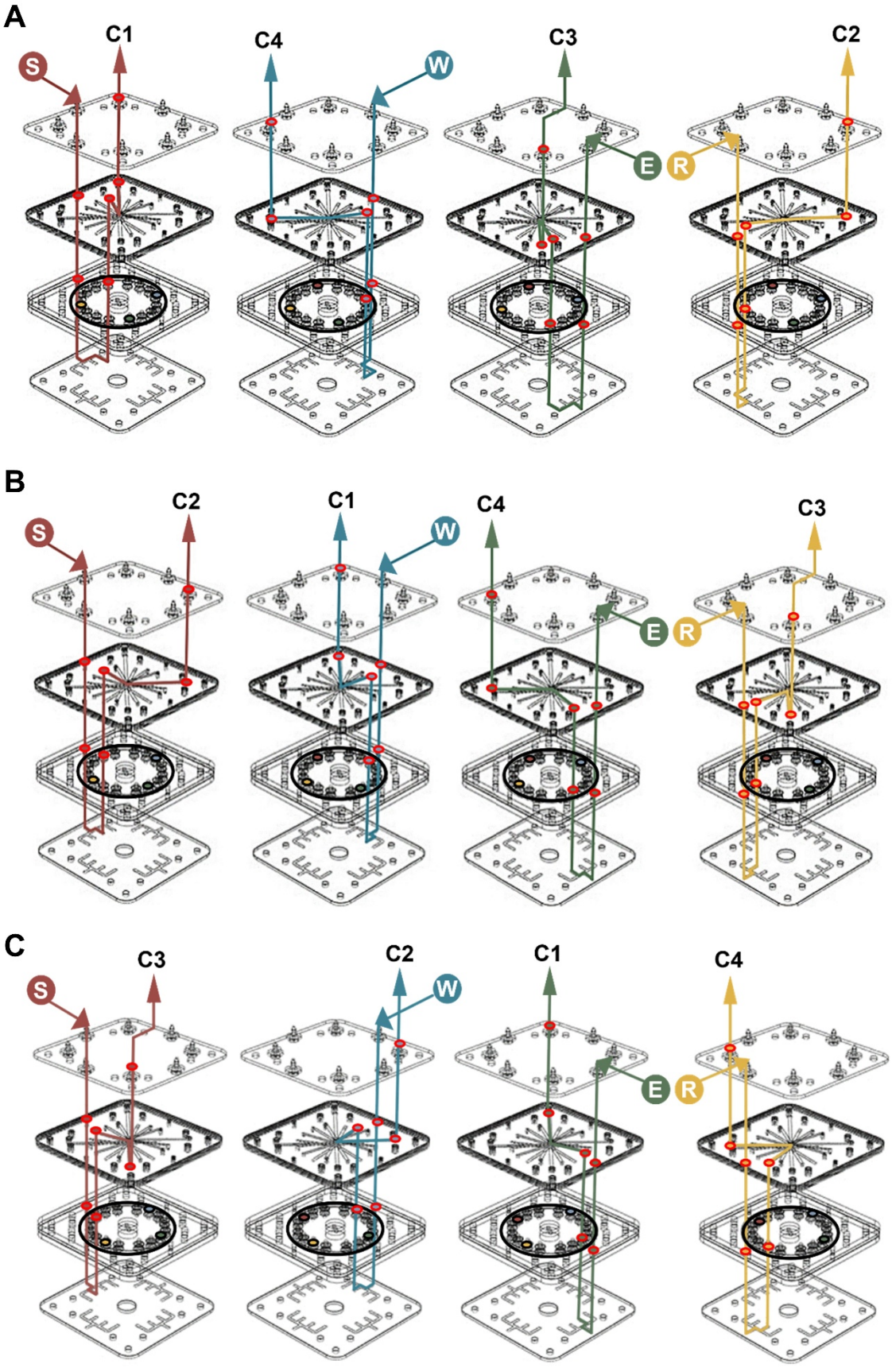

A
B
Groove O.D.
Groove I.D.
Groove dep.
Gland height
valve
O-rings
C
Fully open (aligned)
Partially open
(aligned with one side only)
Fully closed (misaligned)
D
Compression ratio: 23.5%
Compression ratio: 18.8%
leakage
Pressure increased
Presuure (mBar)
Time (min)
Pressure (mBar)
Time (min)

Figure S3. Structural and functional design of the valve layer and O-ring sealing mechanism. (A) Top view of the valve layer showing a rotatable disc with 16 evenly spaced hollow ports around its center. These ports define the alignment states for programmable flow routing. (B) Cross-sectional schematic of the multilayer structure, illustrating that vertical flow occurs only when a valve port is simultaneously aligned with both the upstream (flow layer) and downstream (bottom layer) channels. (C) Three representative states of the valve: fully open (aligned), partially open (aligned on one side only), and fully closed (misaligned). Blue arrows indicate the flow direction. (D) Enlarged view of a single valve port featuring an embedded O-ring. The O-ring is compressed between adjacent layers to form a leak-proof seal, preventing fluid leakage during rotation. The groove depth determines the compression ratio of the O-ring. The excessive compression can cause a sharp increase in device pressure, leading to leakage. A groove depth of 0.78 mm, corresponding to an 18.8% compression ratio, was ultimately adopted to ensure precise flow control and complete mechanical isolation between active and inactive channels.


A
C1
C4
C3
C2
S
W
E
R
B
C2
C1
C4
C3
S
W
E
R
C
C3
C2
C1
C4
S
W
E
R

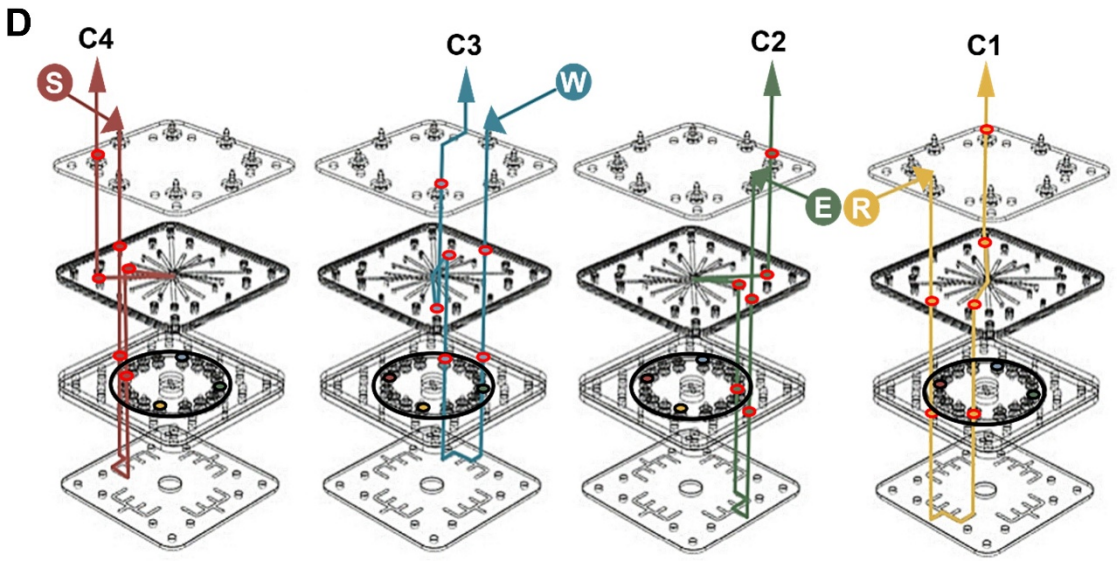
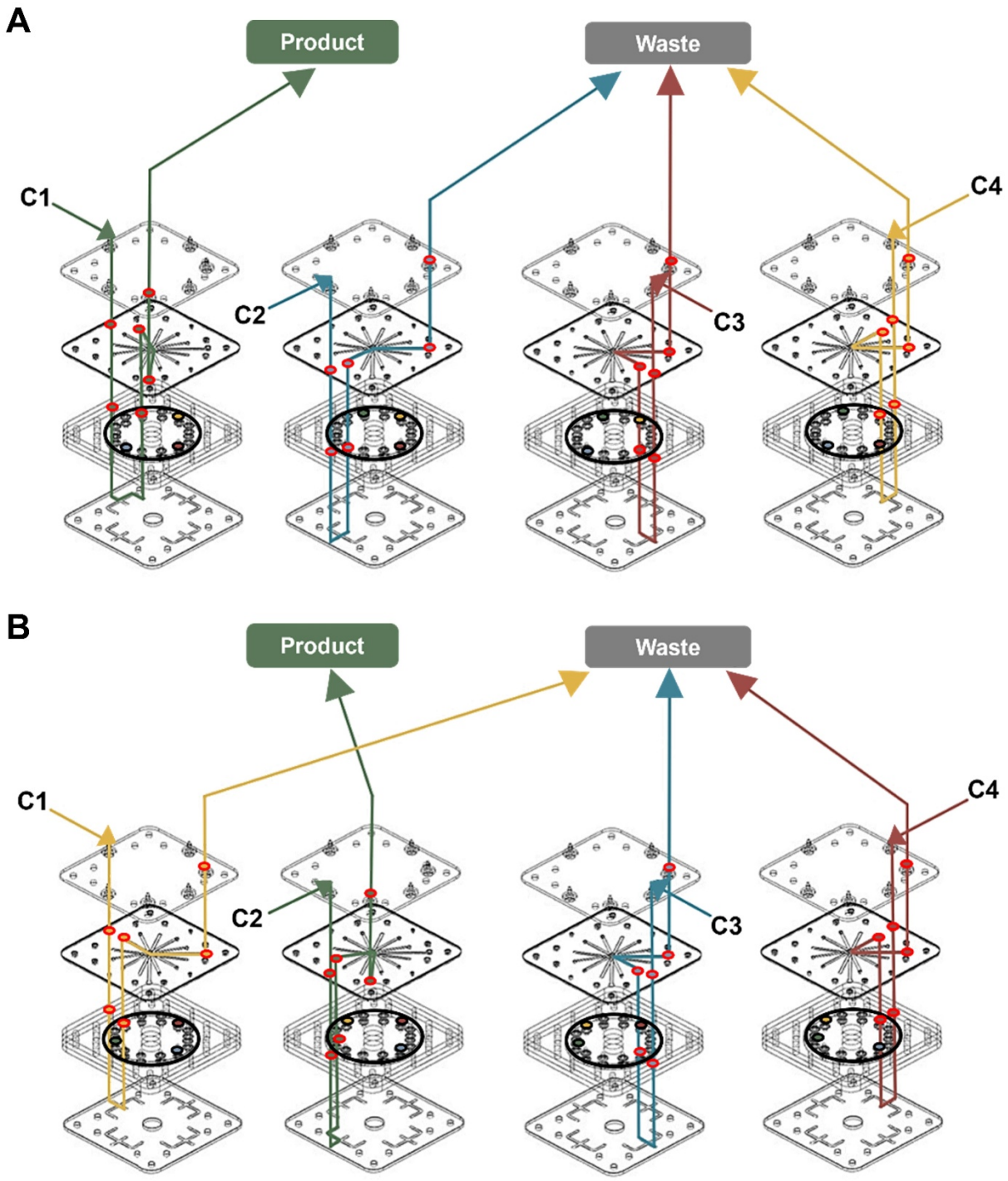


Figure S4. Fluid routing in ICV mode. (A) I1, (B) I2, (C) I3, and (D) I4 indicate the four inlet channels directing fluids to their respective final outlets. S, W, E, and R represent the sample, washing, elution, and re-equilibration buffers, respectively, while C1–C4 represent the purification columns. Red circles indicate the specific points where fluids pass through each layer of the ICV. The four buffers are introduced into the top layers, guided downward to the bottom layers, and then routed upward to the valve layers. In the valve layers, the rotational disc driven by a step motor selectively aligns ports to designated flow channels, enabling programmable buffer switching. Fluids are subsequently directed into the interleaved flow layers, which distribute them independently to the corresponding purification columns (C1–C4) via dedicated outlet channels.


A
Product
Waste
C1
C2
C3
C4
B
Product
Waste
C1
C2
C3
C4

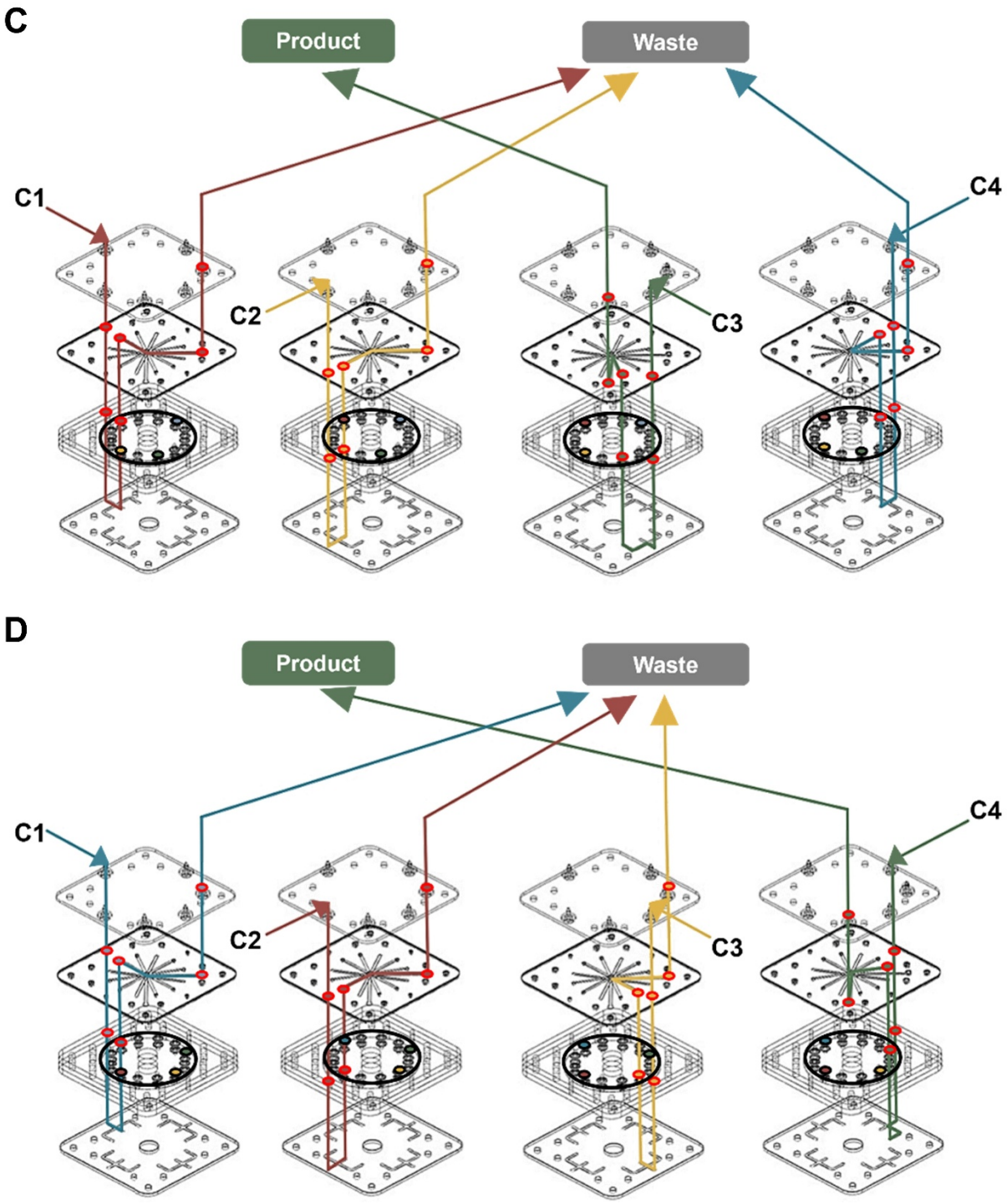
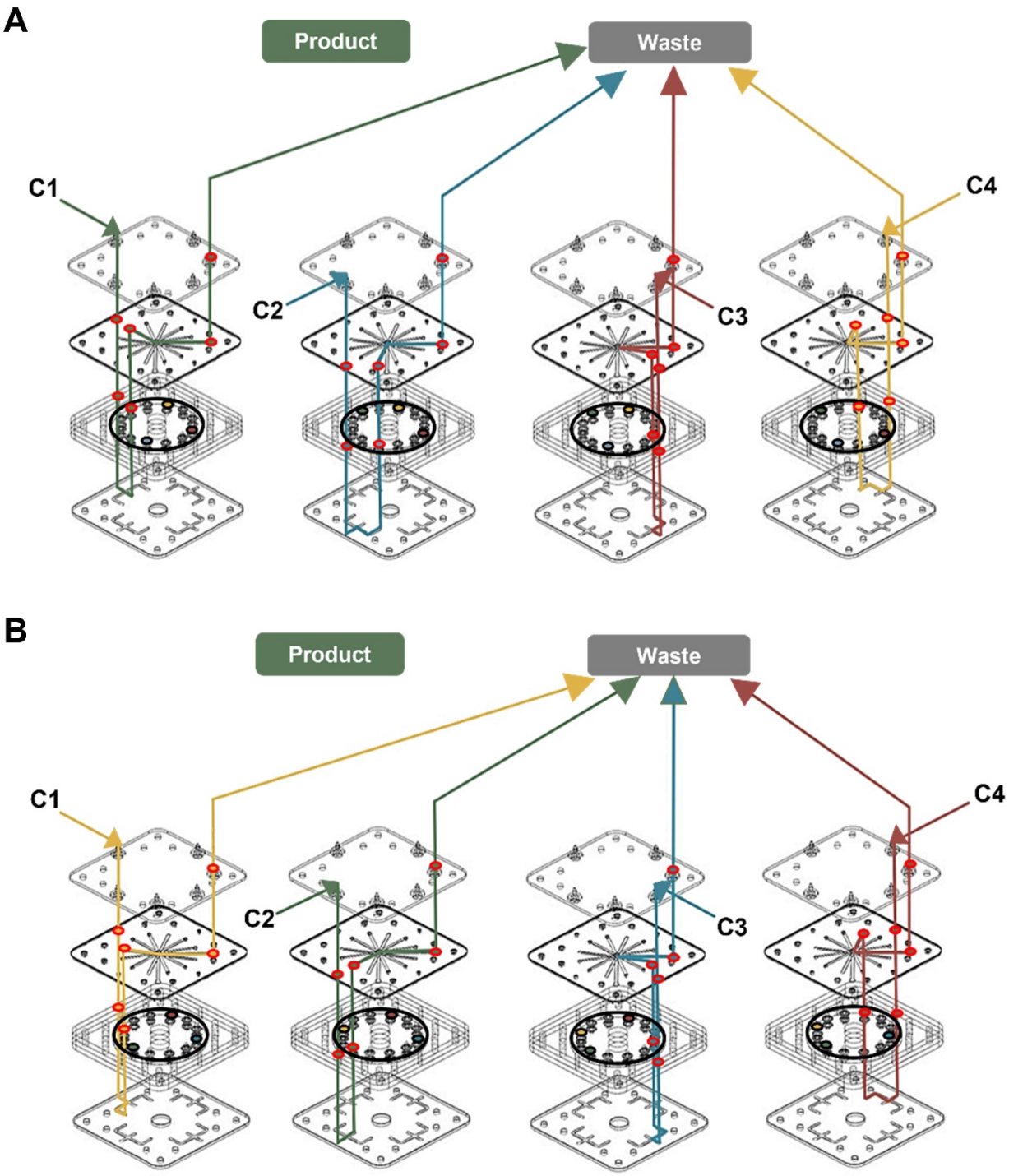


Figure S5. Fluid routing in CCV product mode. (A) P1, (B) P2, (C) P3, and (D) P4 indicate the four outlet channels directing eluates to their respective final product outlets. C1–C4 represent the purification columns, and red circles mark the specific points where fluids pass through each layer of the CCV. In product mode, eluates from each column enter the top layers, are guided downward through intermediate layers, and then routed upward to the valve layers. In the valve layers, the

rotational disc driven by a step motor controls the opening and closing of flow channels based on the preprogrammed fraction collection window, ensuring that only high-purity fractions are directed to the product outlet while lower-purity fractions are diverted to waste. The interleaved flow layers then channel the selected fractions to their designated product or waste outlets independently.


A
Product
Waste
C1
C2
C3
C4
B
Product
Waste
C1
C2
C3
C4

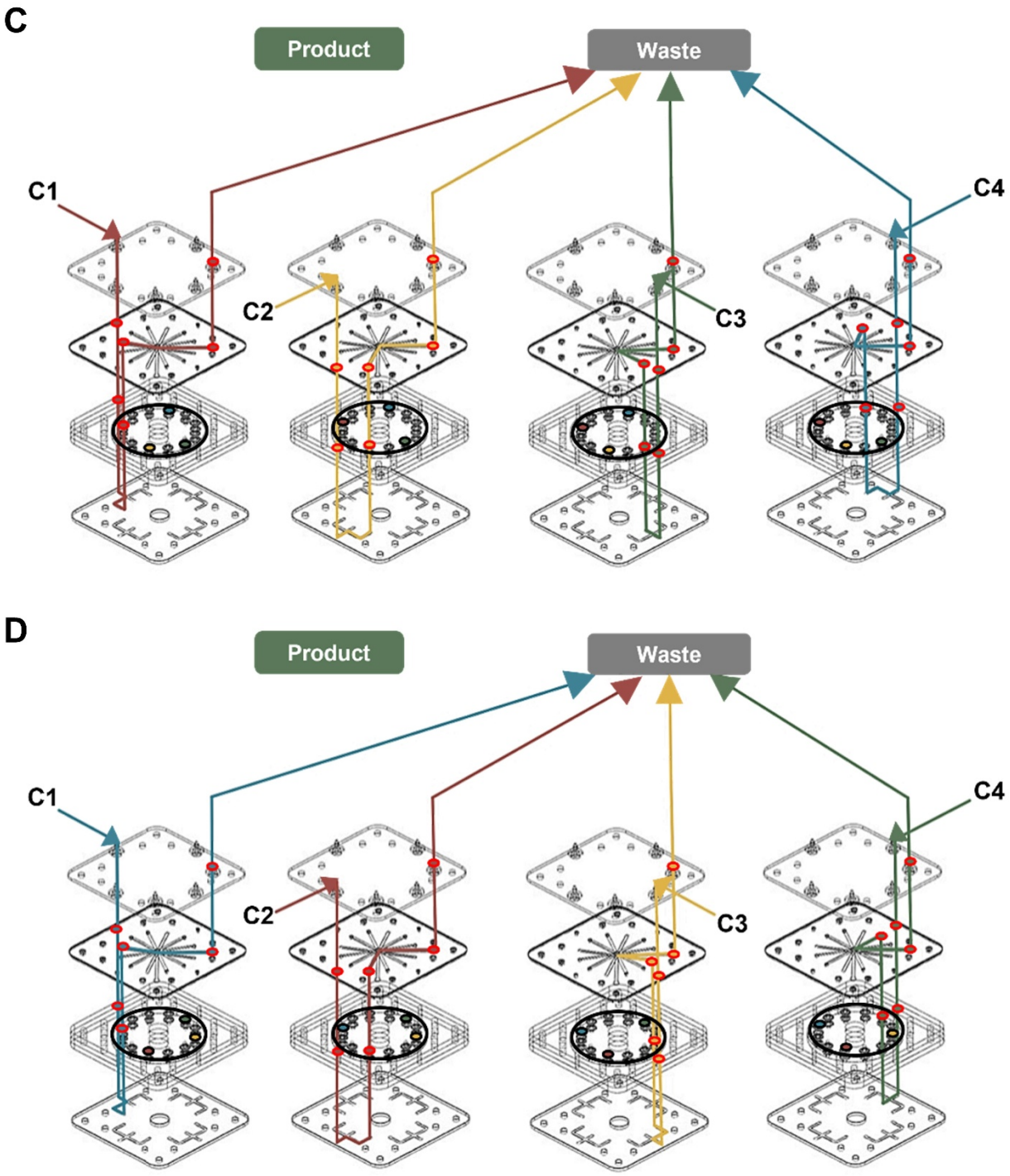


Figure S6. Fluid routing in CCV waste mode. (A) O1, (B) O2, (C) O3, and (D) O4 indicate the four outlet channels directing eluates to their respective final waste outlets. C1–C4 represent the purification columns, and red circles mark the specific points where fluids pass through each layer of the CCV. In waste mode, eluates from each column enter the top layers, are guided downward through intermediate layers, and then routed upward to the valve layers. In the valve layers, the

rotational disc driven by a step motor controls the opening and closing of flow channels according to the preprogrammed waste collection window, ensuring that lower-purity fractions are diverted to waste. The interleaved flow layers then channel the discarded fractions to their designated waste outlets independently.