Supporting Information for

Microfluidic Platform for Efficient Nanodisc Assembly, Membrane Protein Incorporation, and Purification

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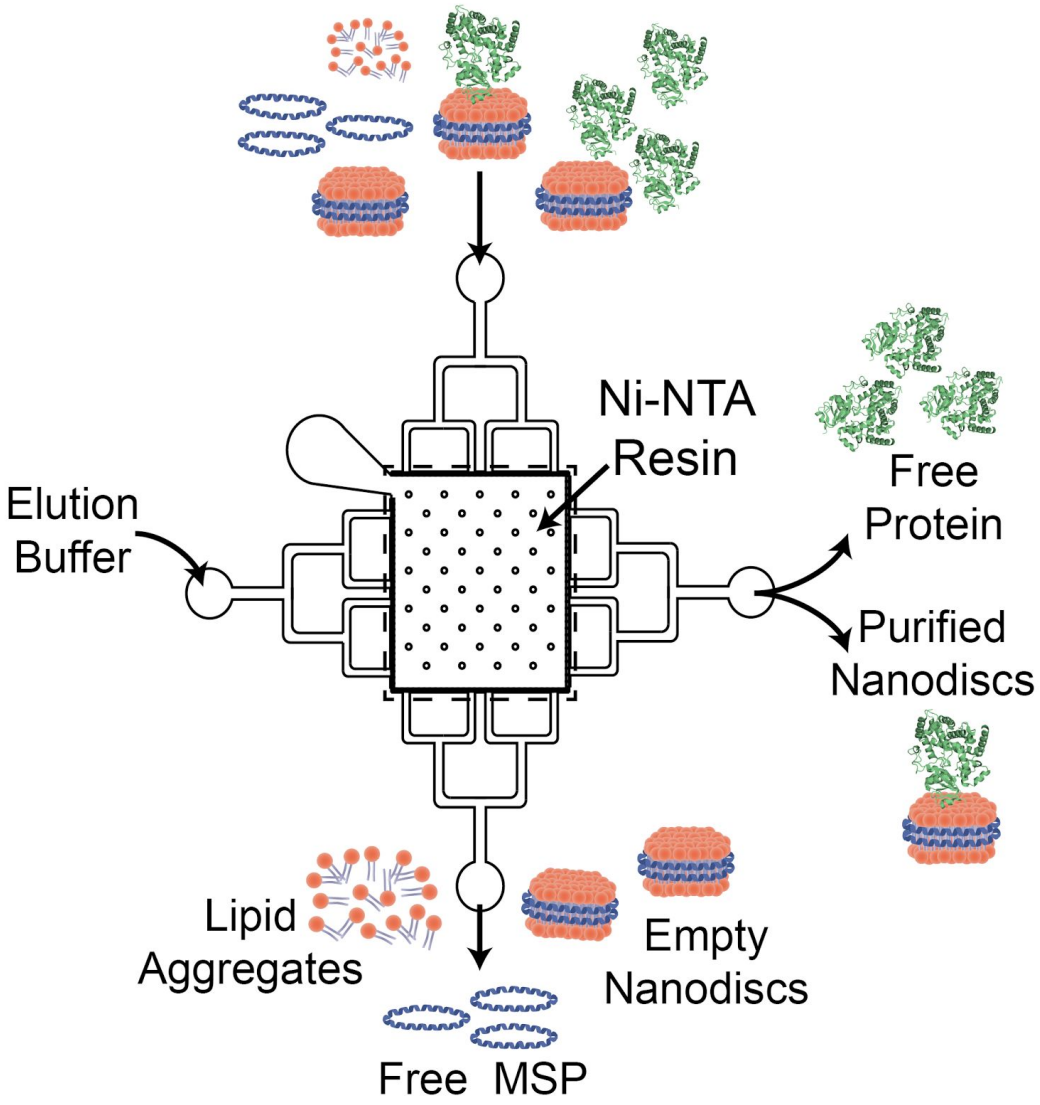
Table 1. Sample Nanodisc Preparation Sheet for Single Port Device

User Inputs	
Total Sample Volume (μL)	100
[MSP1E3D1] (μM)	125
CYP3A4 Stock Concentration (μM)	26.4
MSP1E3D1:CYP3A4 Ratio	10
POPC:MSP1E3D1 Ratio	120
[POPC] stock (mM)	35.4
Dried POPC (μL)	200
[POPC] after addition of detergent (mM)	50.0
Desired final [Cholate] (mM)	20
Desired [CYP3A4] (μM)	3.4
CYP3A4 Preparation Calculations	
nmol of CYP 3A4	0.3
Final [CYP3A4] (μM)	3.4
MSP1E3D1 Preparation Calculations	
nmol of MSP1E3D1	3.4
Final [MSP1E3D1] (μM)	34.0
Lipid Preparation Calculations	
nmol of POPC	408
Final [POPC] (μM)	4080
Reagents	**Volume (μL)**
100 mM Cholate into Lipid Film	141.6
SDB Buffer with 0.1% Emulgen 913	38.6
CYP3A4 Stock	12.9
MSP1E3D1 stock	27.2
50 mM POPC Stock	8.2
SDB Buffer	1.3
100 mM Cholate Stock	11.8

Table 2. Sample Nanodisc Preparation Protocol for a 3-Port Device

User Inputs	
Total Sample Volume (μL)	400
[MSP1D1] (μM)	175.0
DMPC:MSP1D1 Ratio	80
[DMPC] stock (mM)	36.9
Dried DMPC (μL)	200.0
Desired [DMPC] (mM)	50.0
Desired final cholate concentration	20.0
Desired final MSP concentration	50.0
MSP1D1 Preparation Calculations	
nmol of MSP1D1	20
Final [MSP1D1] (μM)	50
Lipid Preparation Calculations	
nmol of DMPC	1600
Final [DMPC] (μM)	4000
Instructions	**Volume (μL)**
0: 100 mM Cholate Stock into Lipid Film	147.6
1: MSP1D1 stock into Tube #1	114.3
1: SDB(-) Buffer into Tube #1	19.0
2: 50 mM DMPC Lipid Stock into Tube #2	32.0
2: 100 mM Cholate Stock into Tube #2	48.0
2: SDB(-) Buffer into Tube #2	53.3
3: SDB(-) Buffer Tube #3	133.3
Syringe ID	**Flow Rate (μL/min)**
Total device flow rate	30.0
MSP1D1 Syringe (1)	10.0
DMPC Lipid Syringe (2)	10.0
Buffer Syringe (3)	10.0



Scheme S1. Microfluidic purification of Nanodiscs by affinity chromatography.

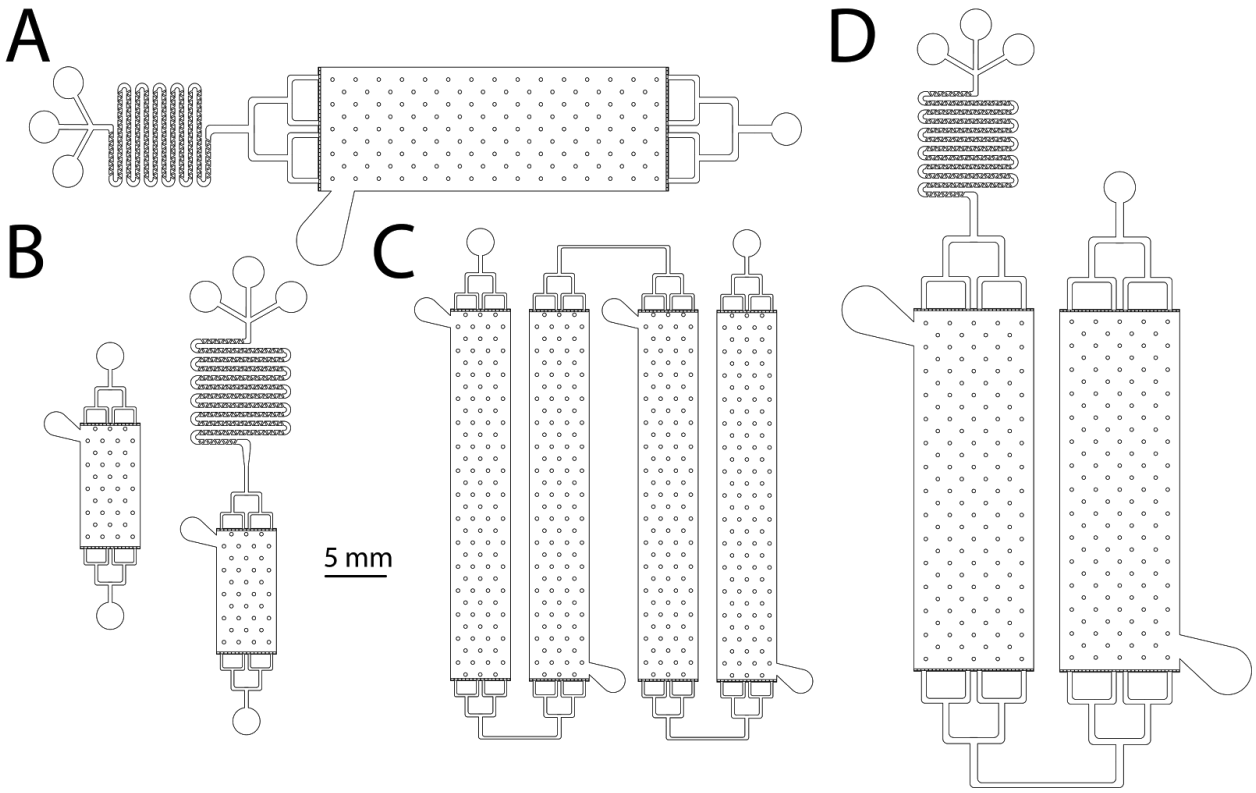


Figure S1. Additional Device Designs for Microfluidic Nanodisc assembly.

(**A**) A 3-port inlet device for on-chip reagent mixing with the same bed volume as the standard single port device is suitable for applications where exposure to lipid-solubilizing detergents may damage to membrane protein to be incorporated. The bed volume of both assembly and purification can be tuned to the desired application. (**B**) The smallest device designed was 10 µL, and this bed volume can be interfaced with either a single or multiport inlet. (**C**) This alternative design consists of a larger total bed volume of 120 µL with four beds. Each bed bed is packed individually and can be filled with either detergent removal resin or affinity purification resin. (**D**) Another large volume device (120 µL) consists of two packed beds interfaced with a multiport inlet for on-chip reagent mixing.

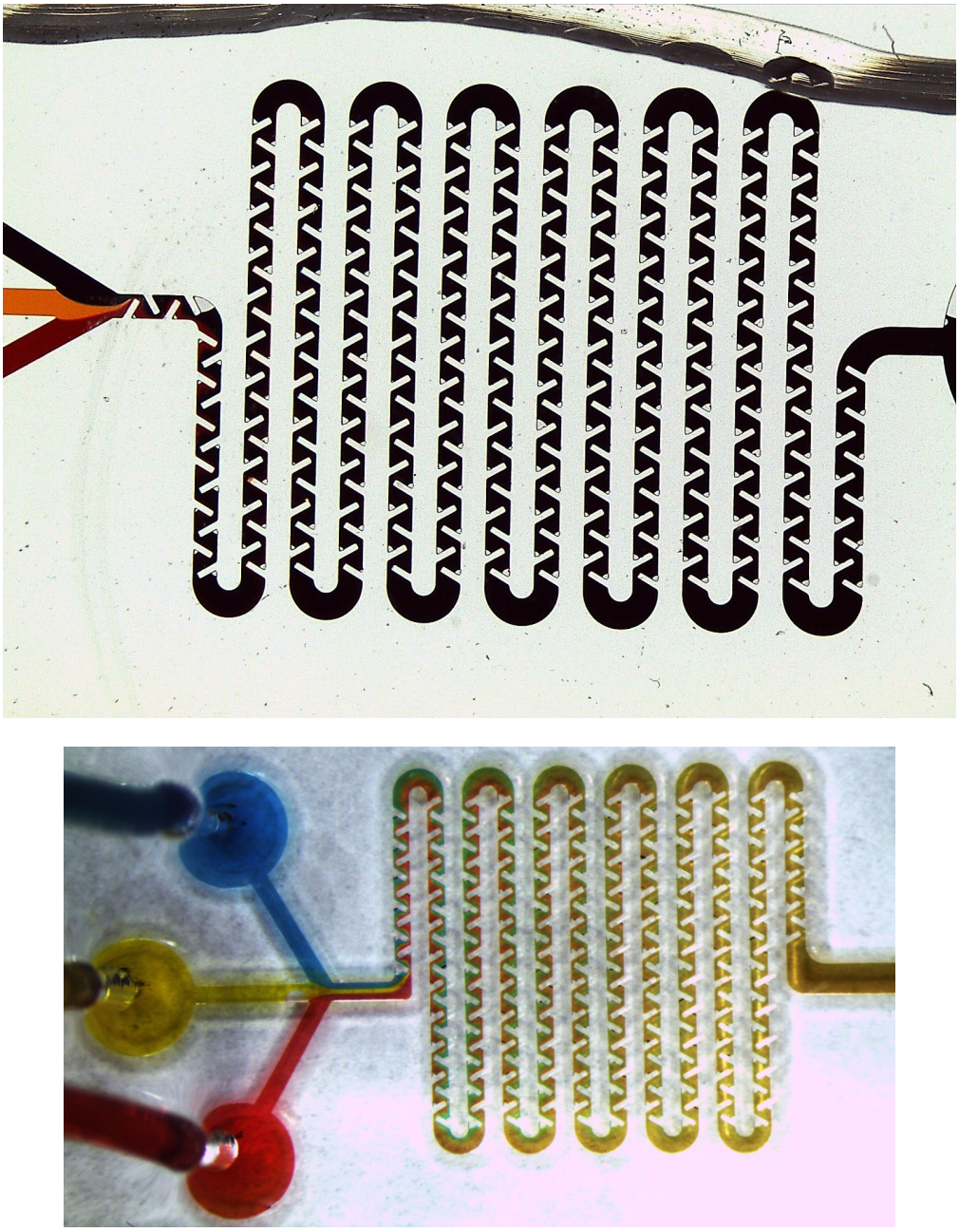


Figure S2. Flow Visualization of 3 Port Mixing Device.

The multiport device design used on-chip reagent mixing prior to Nanodiscs assembly upon detergent removal. The mixing channel featured alternating juts to encourage efficient mixing. Three different colors of food dye are fed into the device and complete mixing is clearly apparent.

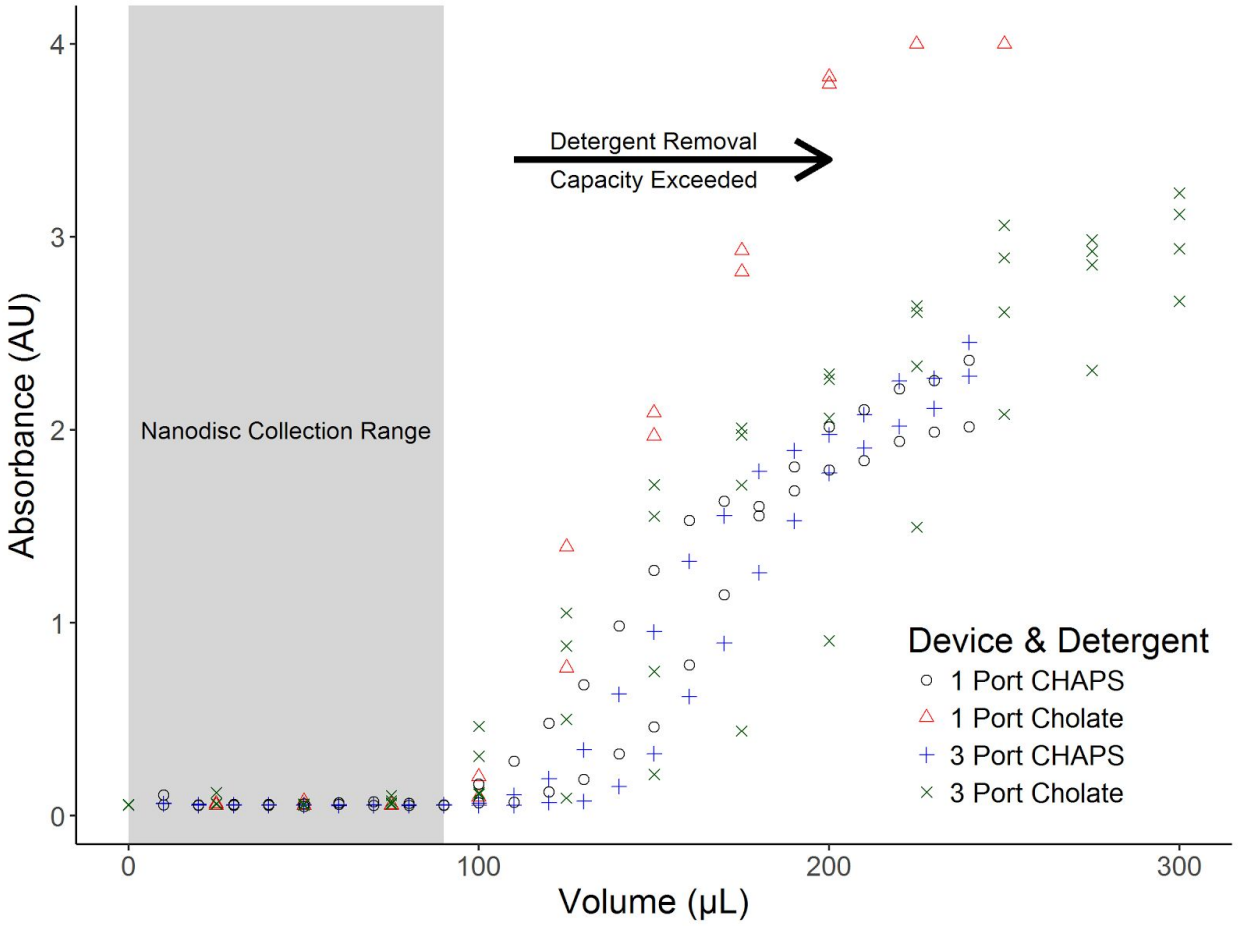


Figure S3. Detergent Removal Device Capacity.

Elution fractions collected from single port and multiport devices flowing 1% CHAPS (black and blue dots), 20 mM sodium cholate (red and green dots), and treated with concentrated sulfuric acid show increased absorbance once the detergent removal capacity is reached. This plot shows the detergent removal capacity for a device bed volume of 60 µL. The detergent removal capacity for both detergents is >90 µL, which corresponds to 1.5 µmol (0.9 mg) CHAPS and 1.8 µmol (0.78 mg) sodium cholate. The region shaded in grey represents the Nanodisc collection region. No Nanodiscs were collected from an assembly device above 90 µL of elution volume to ensure adequate detergent removal for samples fractions.

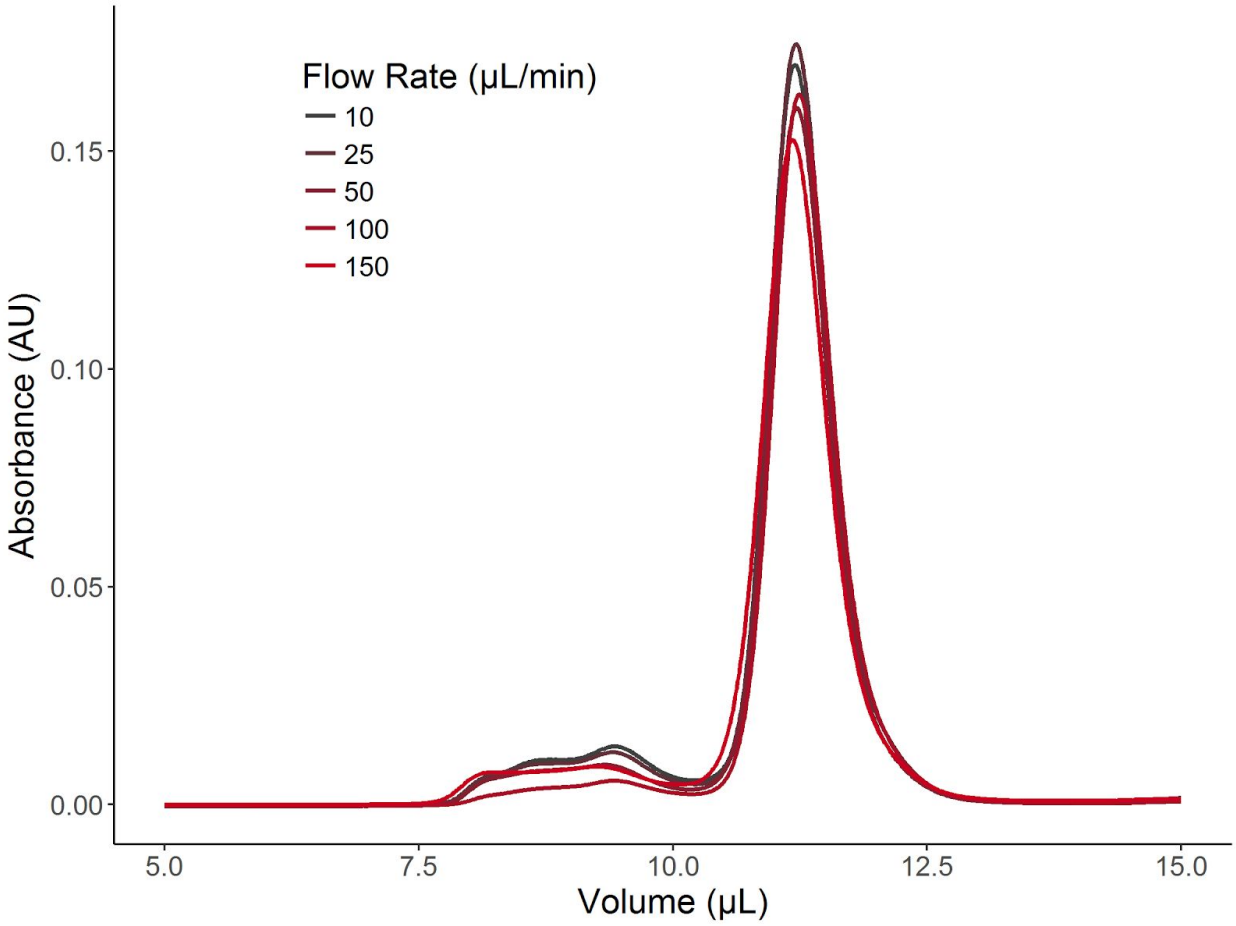


Figure S4. Assembly of Nanodisc at Various Device Flow Rates.

Size exclusion chromatograms (SEC) monitored at 280 nm for DMPC Nanodiscs with MSP1D1 formed at variable flow rates indicate minimal to no effect on overall Nanodisc assembly.

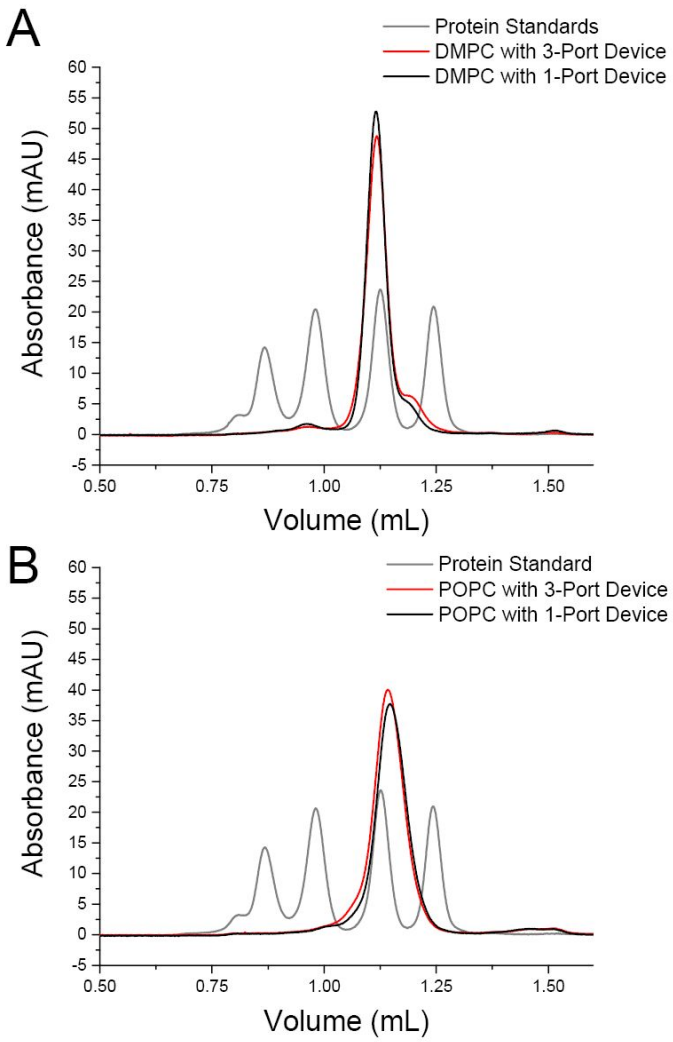


Figure S5. Comparison of Mixing versus No Mixing.

Nanodiscs formed with either multiport (3-port) or single port devices using MSP1D1 and sodium cholate as detergent both result in monodisperse Nanodiscs that co-elute when analyzed with SEC monitored at 280 nm. There was no observed impact on Nanodisc formation when prepared at RT using DMPC lipids (**A**) or at 4°C using POPC lipids (**B**).

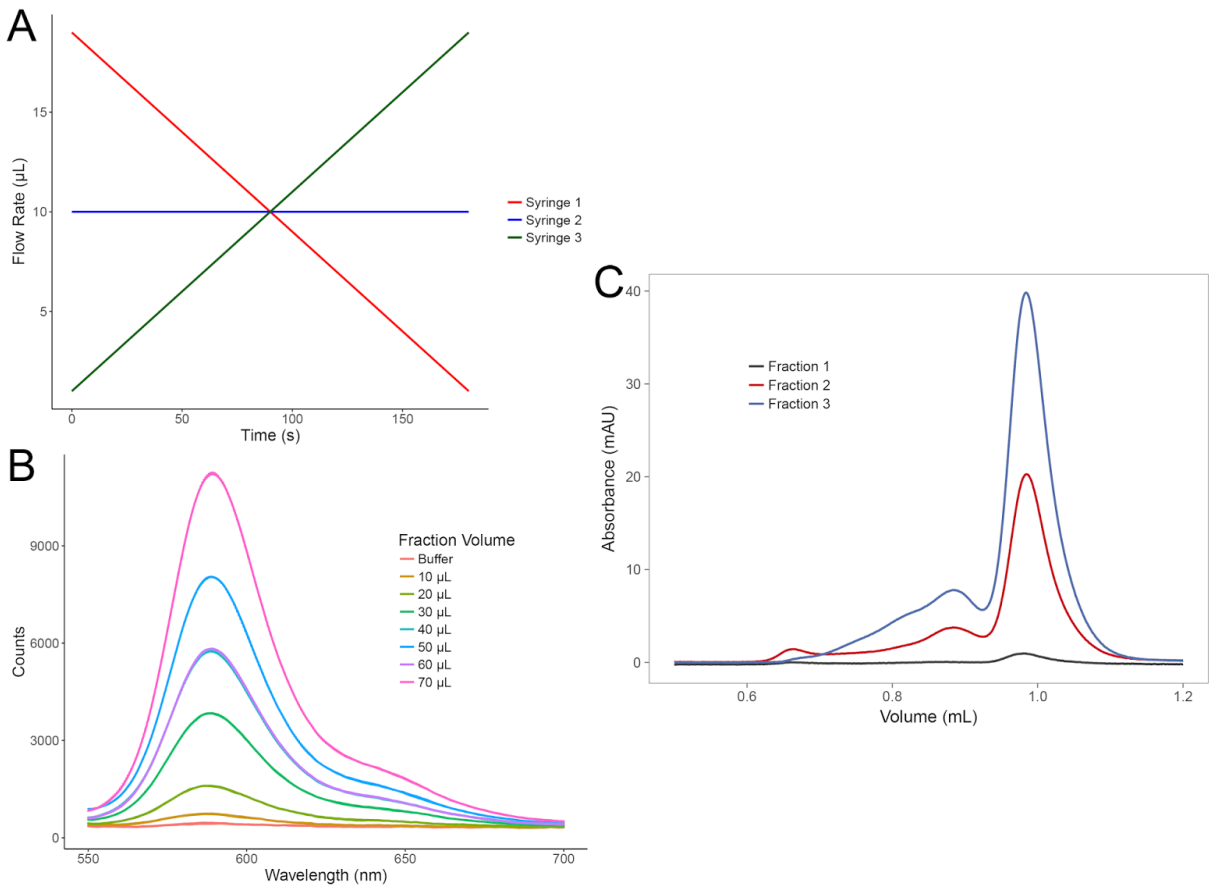


Figure S6. Microfluidic Gradient with Fluorescent Lipids.

(**A**) The flow rate for lipid-containing syringe was increased continuously at a rate of 0.1 µL/s^2 for the syringe containing DMPC with 0.05% Liss Rhod PE (Syringe 3) and -0.1 µL/s^2 for DMPC only syringe (Syringe 1). The syringe with MSP was held at a constant flow rate of 10 µL/min (Syringe 2). (**B**) The fluorescence with a maximum at 590 nm shows an increase in intensity as a function of flow rate. This corresponds to an increasing fluorescent lipid content and, thus lipid bilayer composition, over the course of the microfluidic gradient. (C)This increase in fluorescence over time measured at 590 nm is also seen in the SEC Nanodisc peak, showing incorporation of fluorescent lipids into Nanodiscs following the gradient.

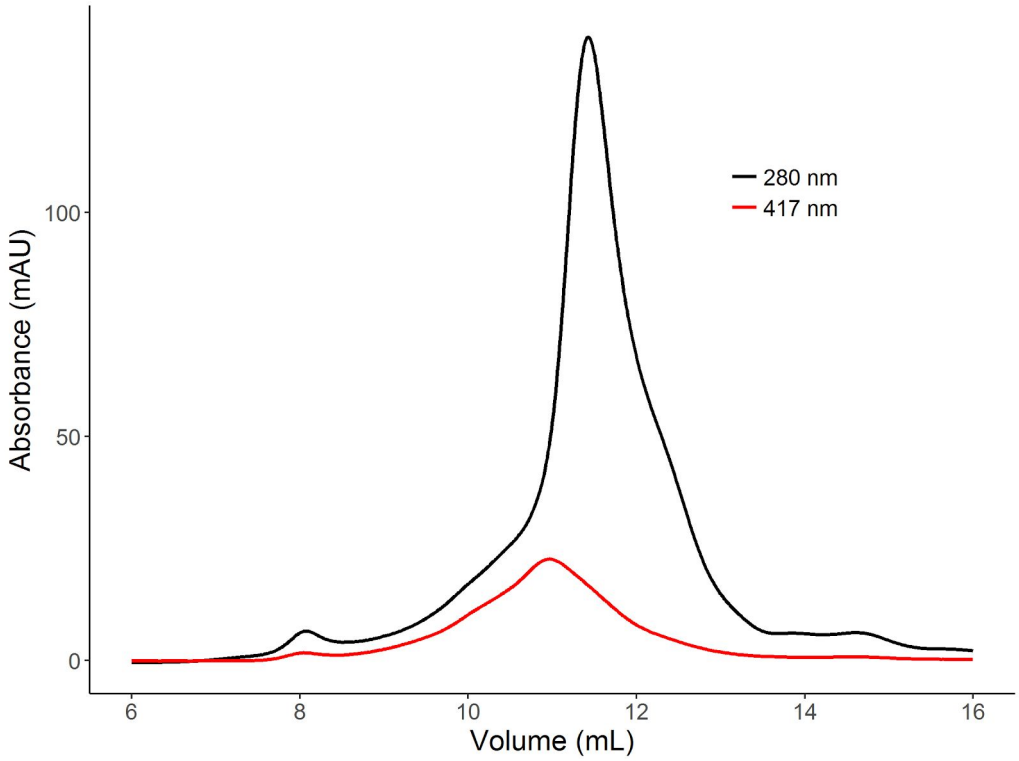
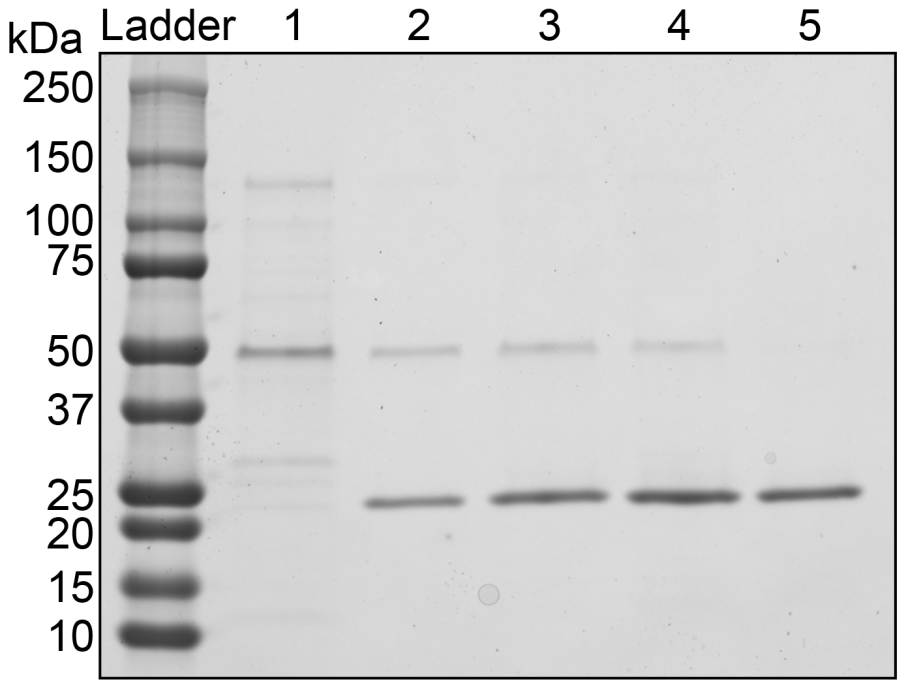


Figure S7. Incorporation of CYP3A4 into Nanodiscs with a 3-Port Assembly Device.

CYP3A4 incorporation into DMPC and MSP1D1 Nanodiscs using a 3 port assembly device measured at both 280 nm and 417 nm with SEC show incorporation of CYP3A4 into the Nanodiscs as indicated by the co-elution of the 417 nm and 280 nm peaks. No Nanodisc purification was performed prior to SEC analysis.



1. Free CYP3A4
2. Nanodiscs Filled with CYP3A4 after Affinity and SEC Purification
3. Nanodiscs Filled with CYP3A4 after Affinity Purification
4. Untreated Nanodisc Components
5. Empty Nanodiscs (without CYP3A4)

Figure S8. Polyacrylamide Gel Electrophoresis of Nanodiscs with incorporated CYP3A4. SDS-PAGE gel of DMPC and MSP1D1 Nanodiscs filled with CYP3A4 throughout the assembly and purification process stained with Coomassie Blue. Bands corresponding to CYP3A4 (57 kDA) and MSP1D1 (24.6 kDa) are present in all filled Nanodiscs and in Nanodisc components. Empty Nanodiscs only show the MSP band.