Cell-Free Production of Antibody Fragments for High-Throughput Analyses

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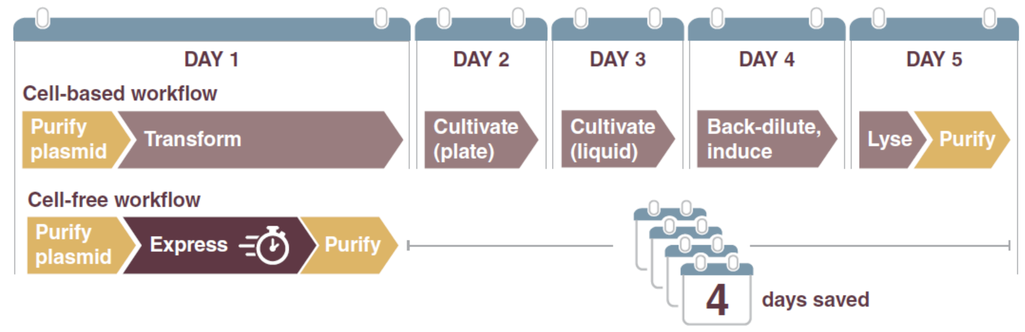
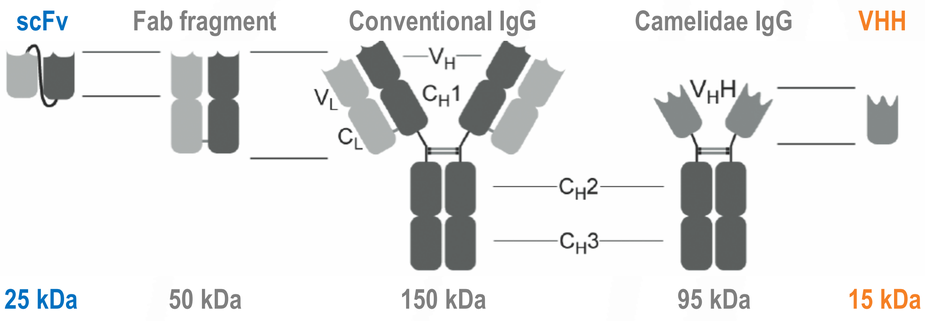
Introduction & Workflow

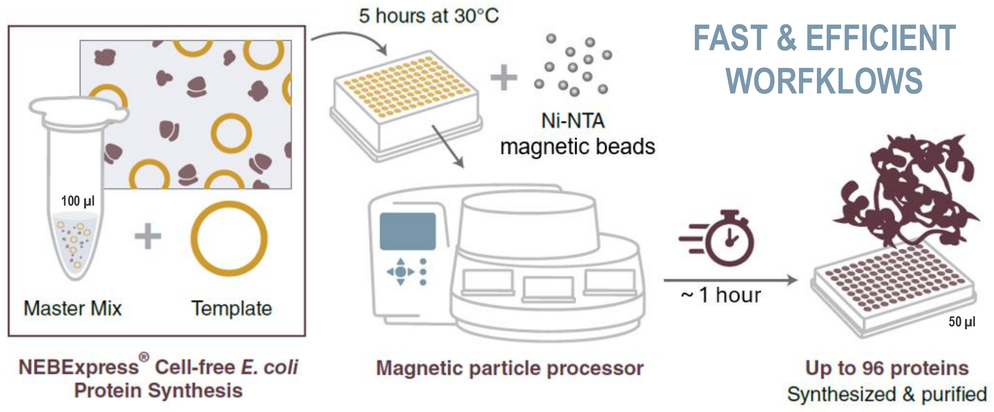
Single-chain antigen-binding fragments (e.g. VHH, scFv) offer several advantages over traditional therapeutic antibodies, including smaller size, enhanced solubility and thermal stability. Here we demonstrate the utility of our cell-free protein synthesis (CFPS) platform, NEBExpress® Cell-free *E. coli* Protein Synthesis (lysate), for the rapid production of antibody fragments in quantities suitable for direct analysis or further purification. CFPS workflows are fast, scalable and cost-efficient, and can be seamlessly integrated with our high-throughput DNA assembly methods to enable the rapid screening of diverse binder libraries. Additionally, the defined nature of these systems allows for straightforward optimization to improve expression of challenging constructs.



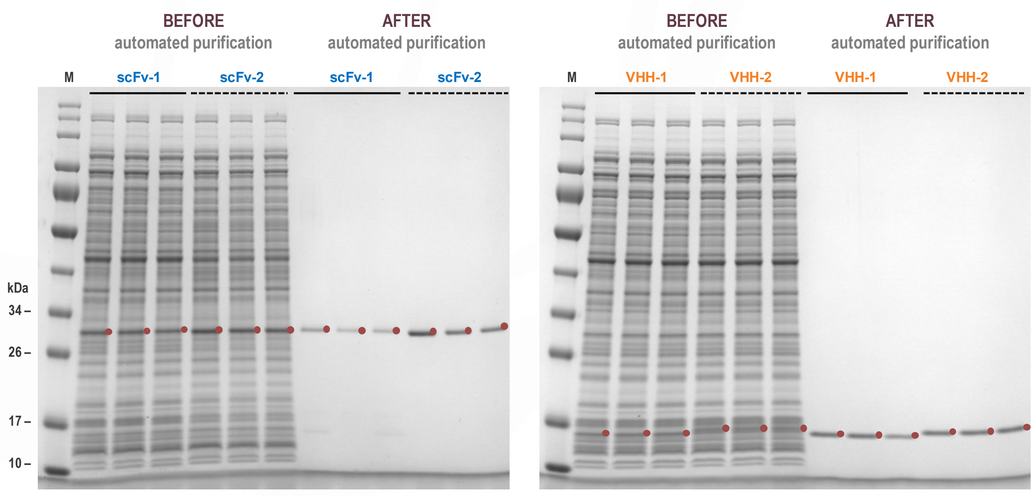


Single-chain benefits…

- improved solubility
- higher stability
- greater modularity
- increased permeability
- robust production



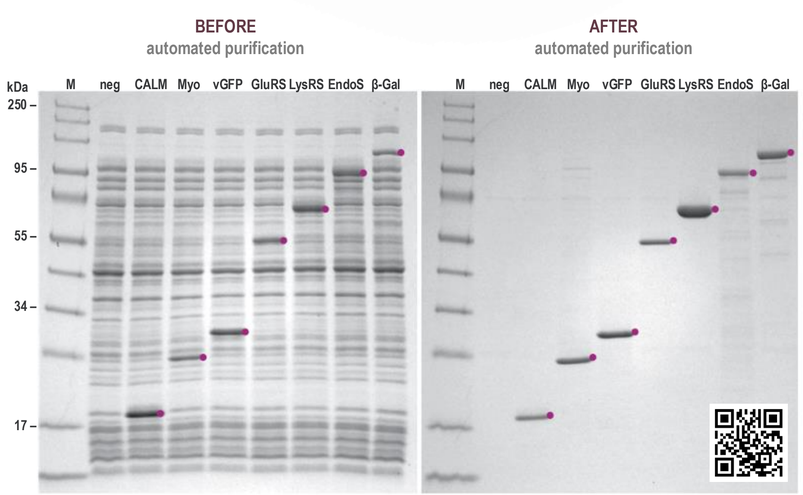
Automated Purification with NEBExpress® Ni-NTA Magnetic Beads



Protein	Name	MW (kDa)
αGFP VHH-1	VHH-1	13.7
αGFP VHH-2	VHH-2	14.6
αHER2 scFv-1	scFv-1	27.6
αHER2 scFv-2[2]	scFv-2	27.6

CLEAN & REPRODUCIBLE PURIFICATIONS

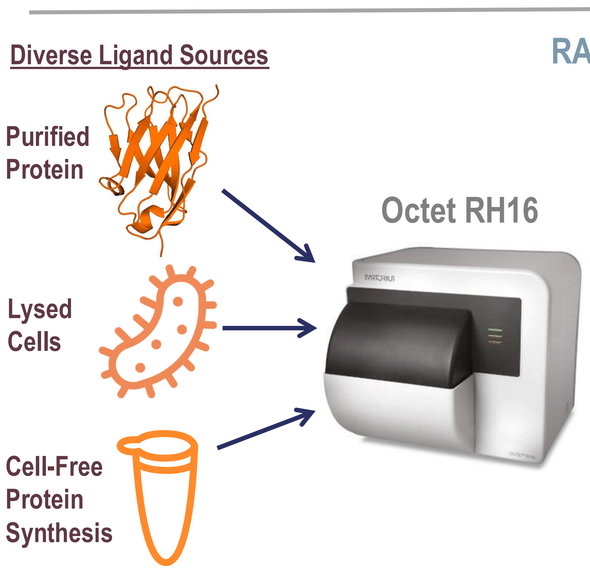
Yield	3.5 µM, 50-150 ng/µl
Purity	~90%



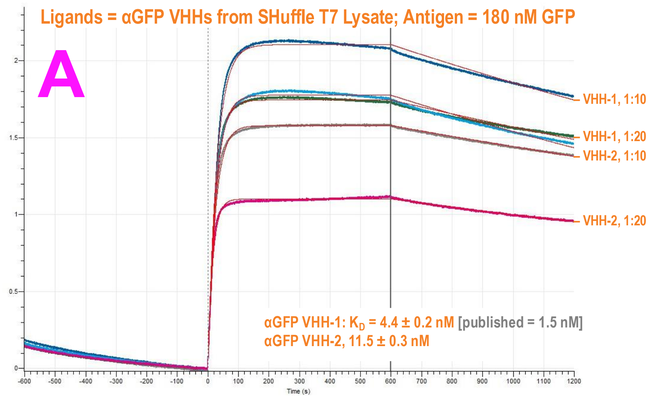
Protein	Name	MW (kDa)
–	neg	–
Calmodulin	CALM	16.7
Myokinase	Myo	25.5
YFP, Venus	vGFP	26.9
Glutamate-tRNA Ligase	GluRS	54.9
Lysine-tRNA Ligase	LysRS	75.0
Endoglycosidase	EndoS	89.0
β-galactosidase	β-Gal	100

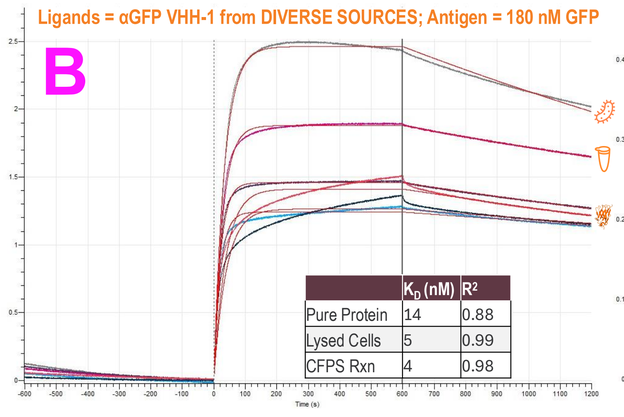
Yield	2 µM, 50-250 ng/µl
Purity	~90%

Functional Characterization via Bilayer Interferometry



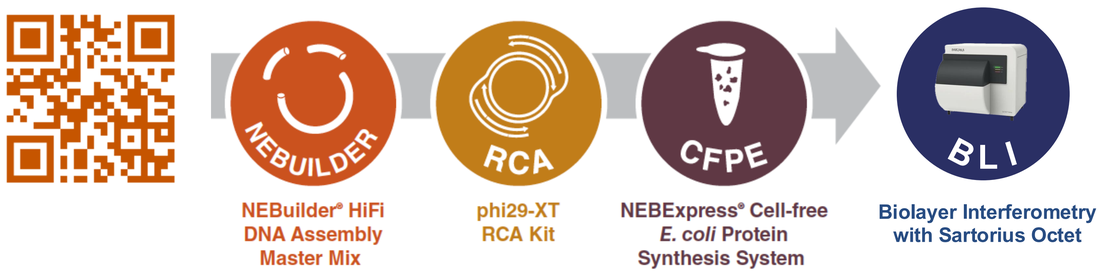
RAPID & HIGH-THROUGHPUT COMPATIBLE ASSAYS





	K_D (nM)	R^2
Pure Protein	14	0.88
Lysed Cells	5	0.99
CFPS Rxn	4	0.98

Comprehensive Cell-free Workflows for BLI Analyses

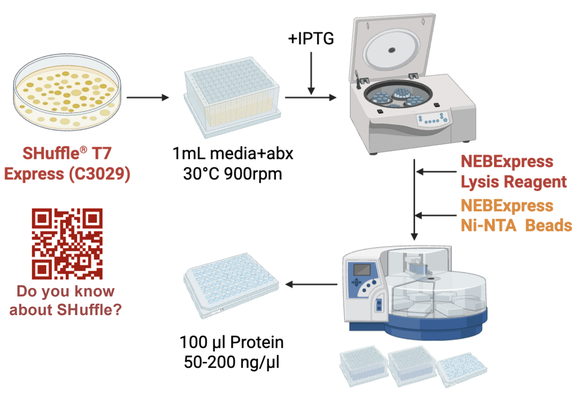


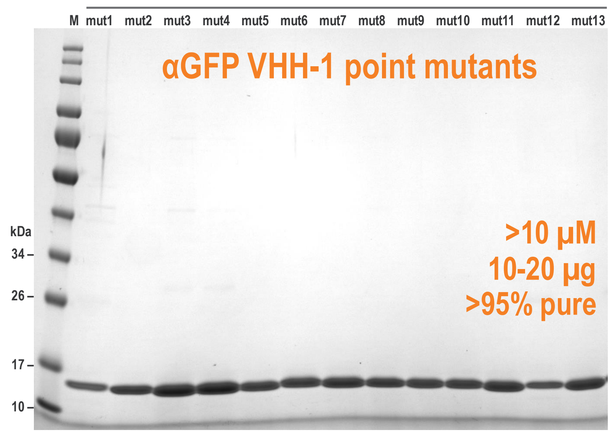
NEBUILDER ASSEMBLY REACTION COMPONENTS	STANDARD RXN (µl)	MINIATURE RXN (µl)
DNA Insert(s)	varied	1
Vector	varied	0.5
2X NEBuilder Master Mix	10	2.5
Nuclease-free water	up to 20	1
Total Volume (µL):	**20**	**5**

PHI29-XT RCA REACTION COMPONENTS	STANDARD RXN (µl)	MINIATURE RXN (µl)
Bacterial Colony/CTRL Vector	varied	0.5
Phi29-XT Reaction Buffer (5X)	4	1.0
dNTP Mix (10 mM)	1	0.5
Exo-Resistant Primers (500 µM)	1	0.5
Nuclease-free water	up to 18	2
Phi29 XT Enzyme	2	0.5
Total Volume (µL):	**20**	**5**

NEBEXPRESS CFPS REACTION COMPONENTS	STANDARD RXN (µl)	PURIFICATION RXN (µl)
Template (Plasmid, PCR, RCA)	varied	Varied
Protein Synthesis Buffer (2X)	25	50
NEBExpress S30 Extract	12	24
T7 RNA Polymerase	1	2
RNase Inhiibitor, Murine	1	2
RNase-free Water	up to 50	up to 100
Total Volume (µL):	**50**	**100**

High-throughput Purifications from Cellular Inputs





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Sample	Mutation	Effect	BL21 K_D (nM)	SHuffle K_D (nM)	CFPS K_D (nM)
01	C24A	disulfide	9	16	19
02	S35A	contacts GFP	5	9	7
03	R37A	contacts GFP	5	7	9
04	Y39A	contacts GFP	12	15	16
05	A42A	control, wt	2	23	6
06	E46A	contacts GFP	6	16	13
07	W49A	contacts GFP	1	9	8
08	R59A	contacts GFP	nf	nf	338*
09	V66P	unknown	3	11	4
10	C98A	disulfide	3*	11	8
11	N99A	unknown	62*	4*	125*
12	N101A	contacts GFP	38	191	227*
13	G103A	contacts GFP	2	9	7
14	F104A	contacts GFP	2	9	8
15	E105A	contacts GFP	17*	26	29
16	wt	plasmid control	1*	8	9

*1:1 model fit R^2<0.95
nf: model fit R^2<0.60

E