Revolutionizing Protein Synthesis: Emerging Technologies and Innovations

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Joint Graduate Seminar

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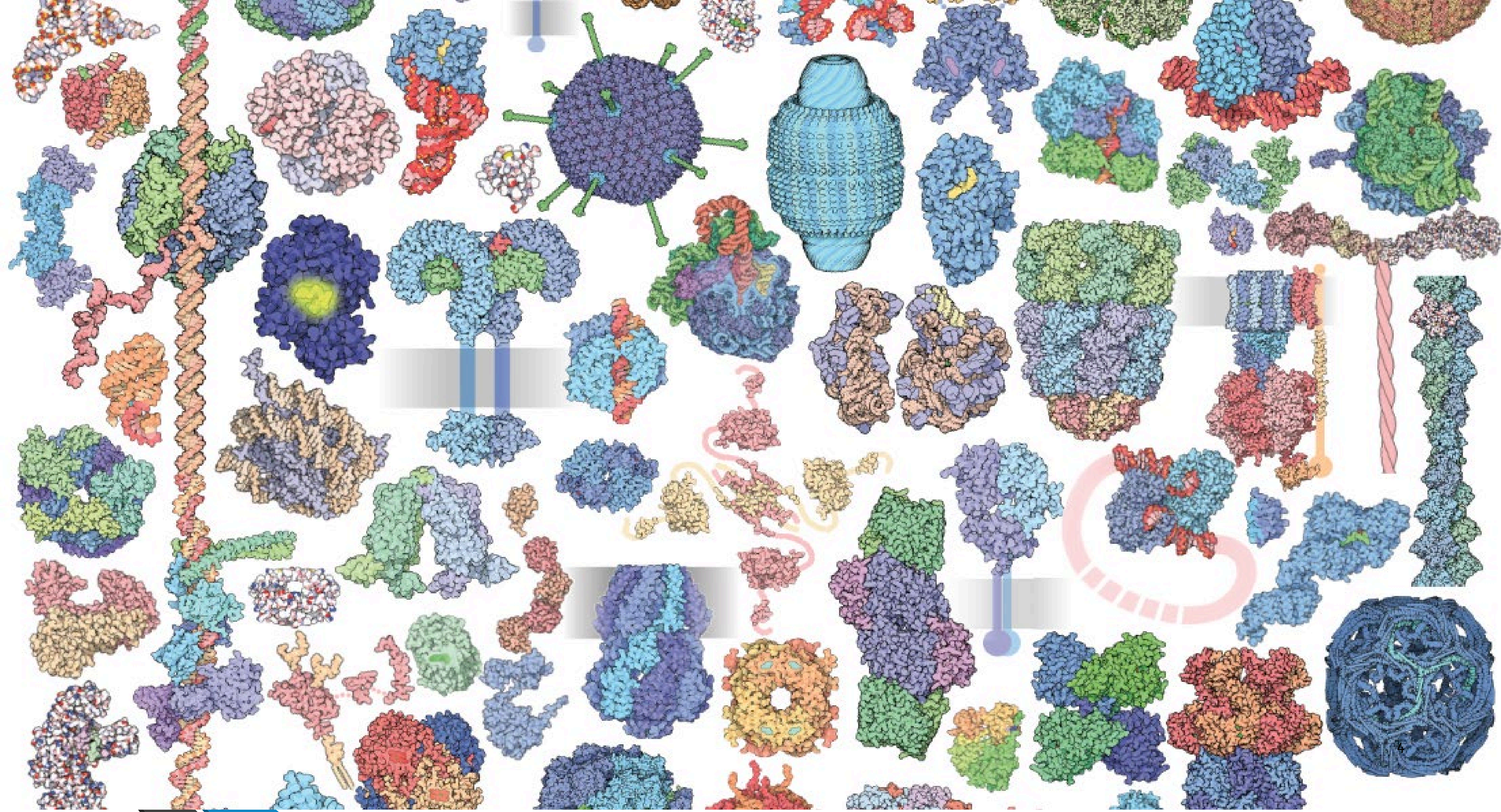
Outline

- Introduction
- Basics of Protein Synthesis
- Advancements in Synthesis Techniques
- Challenges and Future Directions



D.S. Goodsell and RCSB PDB (2015)

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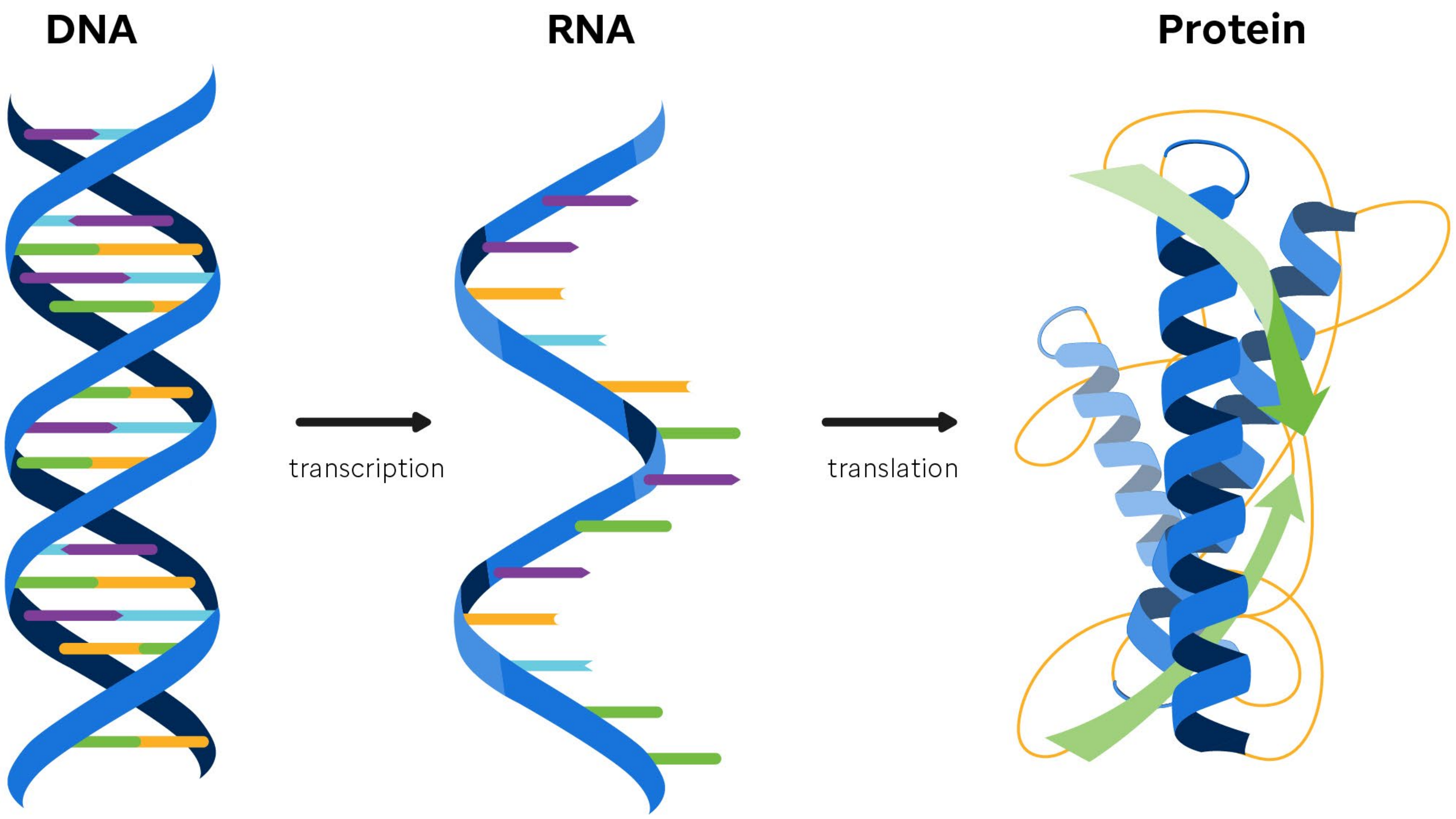
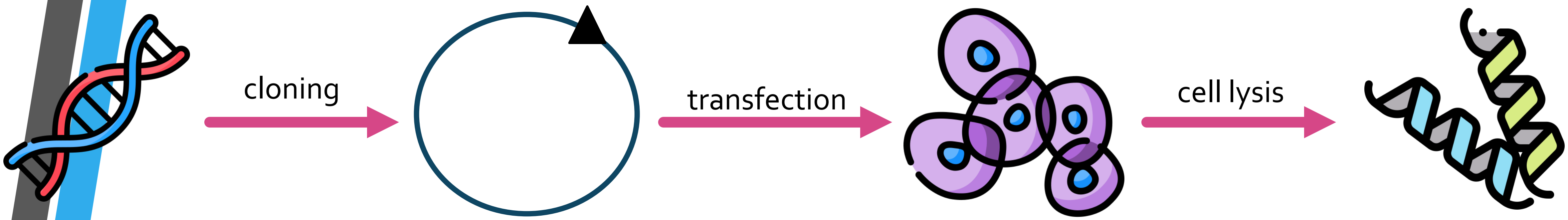
Central Dogma of Molecular Biology

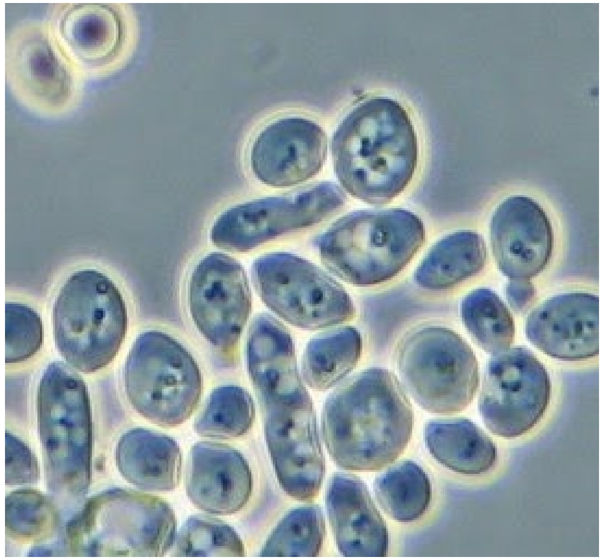


St. Jude Children's Research Hospital,(2022)

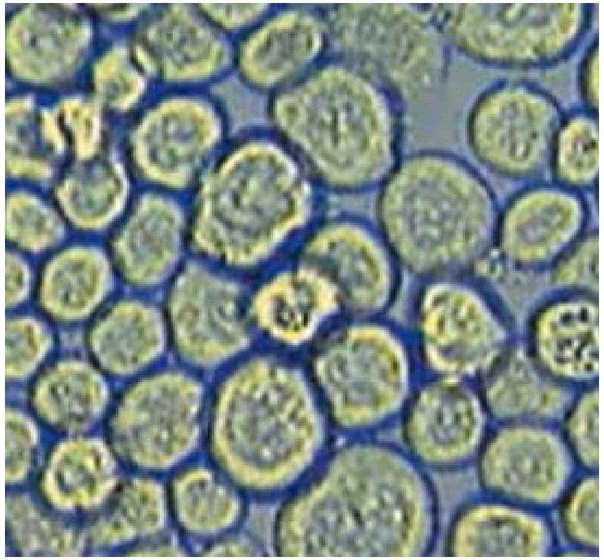
Recombinant protein expression systems



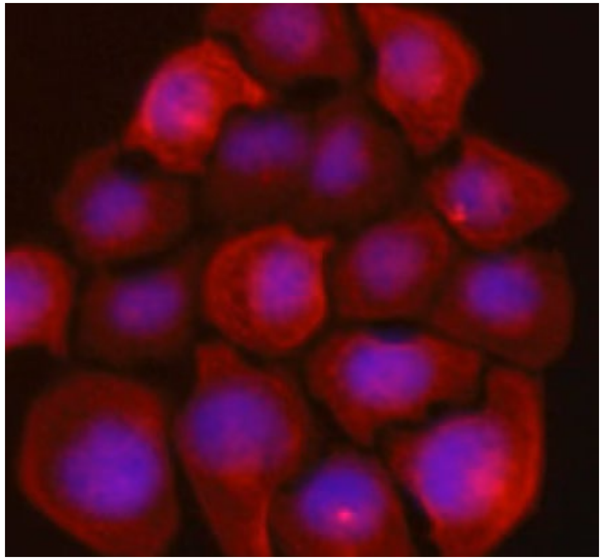
Bacteria
(10mg/L)



Yeast
(10g/L)



Insect
(900mg/L)

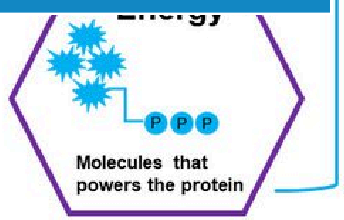


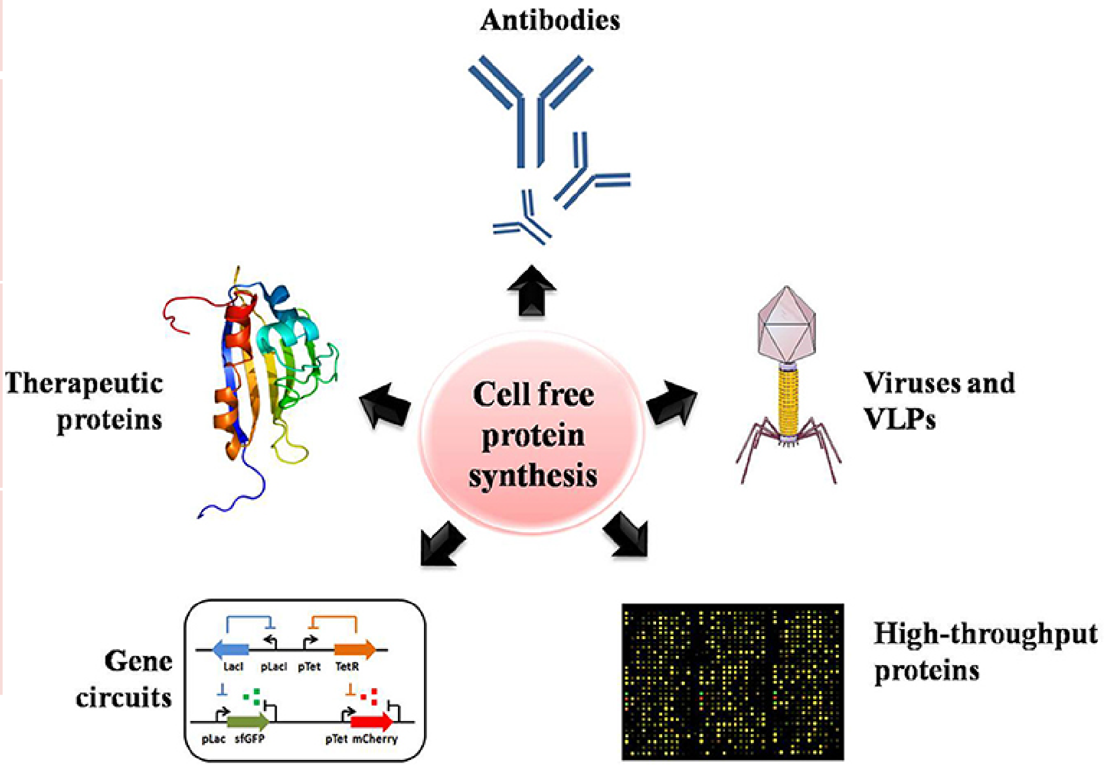
Mammalian
(1g/L)

Simple proteins ⟷ complex proteins

Cell-free Protein Synthesis (CFPS)

Rapid protein production	$$$$$
Open System	Low yield (50 µg/mL)
Easy to optimise	Lack of PTM
Express Toxic protein	Limited protein complexity





Maharjan A., et al, Biotechnology and Applied Biochemistry (2023)

Khambhati., et al, Front. Bioeng. Biotechnol. (2019)

Outline

- Introduction
- Basics of Protein Synthesis
- **Advancements in Synthesis Techniques**
- Challenges and Future Directions

BIOTECHNOLOGY and BIOENGINEERING

ARTICLE

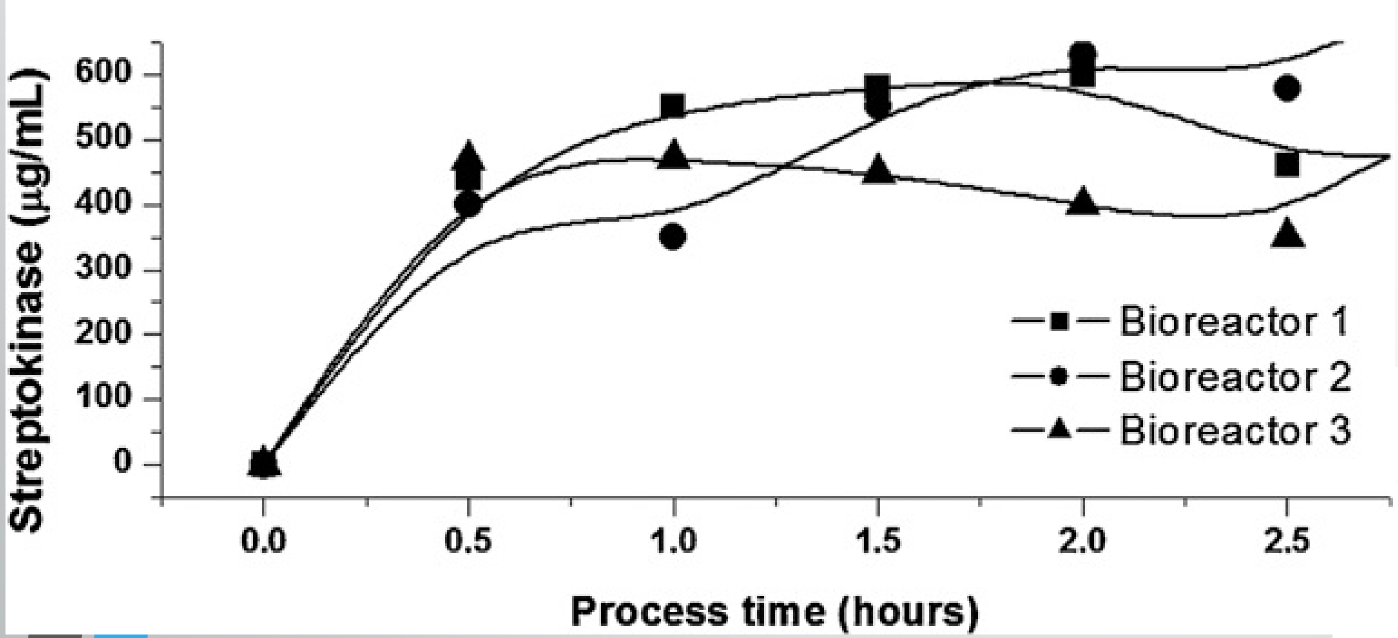
Cell-free production of a therapeutic protein: Expression, purification, and characterization of recombinant streptokinase using a CHO lysate

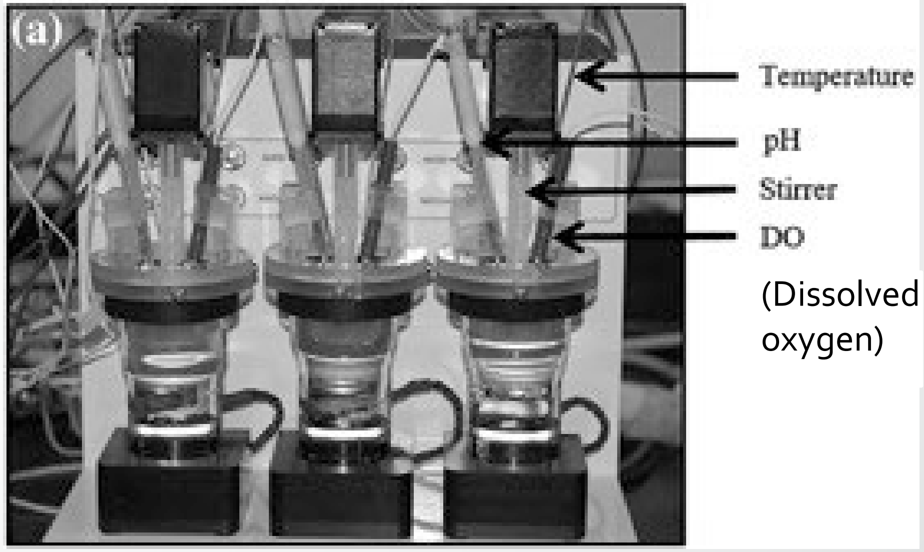
(SK) (Chinese hamster ovary)

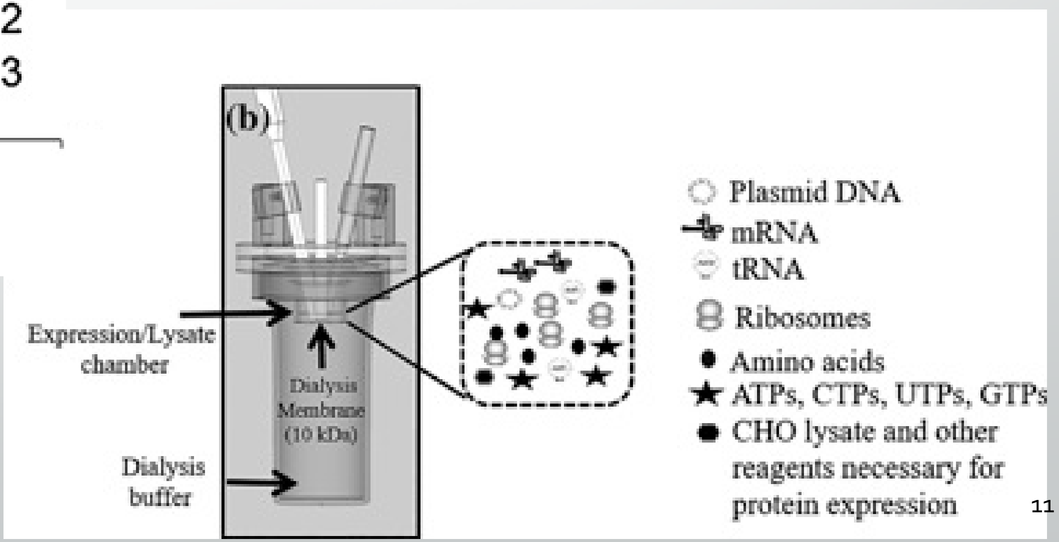
Kevin Tran, Chandrasekhar Gurramkonda, Merideth A. Cooper, Manohar Pilli, Joseph E. Taris, Nicholas Selock, Tzu-Chiang Han, Michael Tolosa, Adil Zuber, Chariz Peñalber-Johnstone, Christina Dinkins, Niloufar Pezeshk, Yordan Kostov, Douglas D. Frey, Leah Tolosa, David W. Wood, Govind Rao ... See fewer authors ^

First published: 26 August 2017 | https://doi.org/10.1002/bit.26439 | Citations: 32

Kevin Tran, Chandrasekhar Gurramkonda, and Merideth A. Cooper contributed equally to this work.







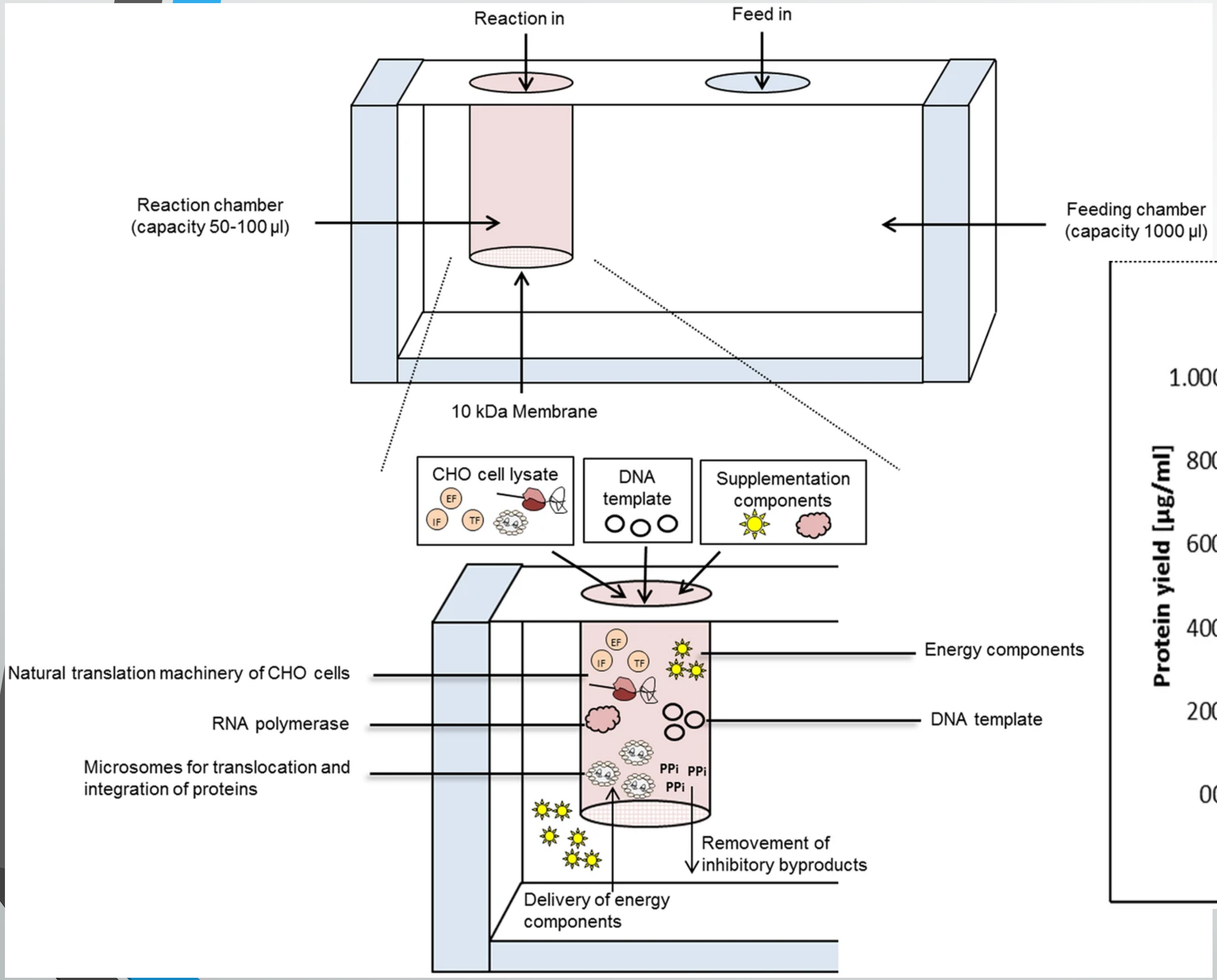
Tran K., et al, Biotechnology and Bioengineering (2017)

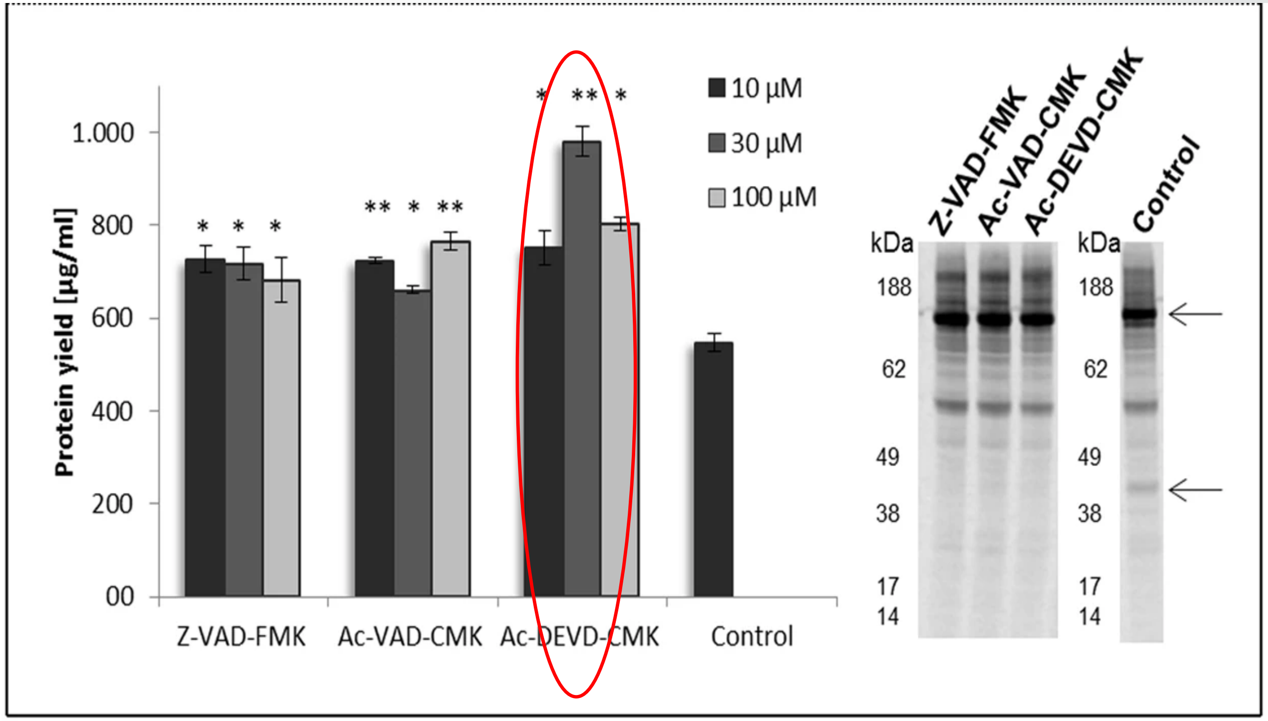
Article | Open access | Published: 15 September 2017

High-yield production of “difficult-to-express” proteins in a continuous exchange cell-free system based on CHO cell lysates

Lena Thoring, Srujan K. Dondapati, Marlitt Stech, Doreen A. Wüstenhagen & Stefan Kubick

Scientific Reports **7**, Article number: 11710 (2017) | Cite this article





Z-VAD-FMK: general caspase inhibitor
Ac-VAD-CMK: casp-1 inhibitor
Ac-DEVD-CMK: casp-3 inhibitor

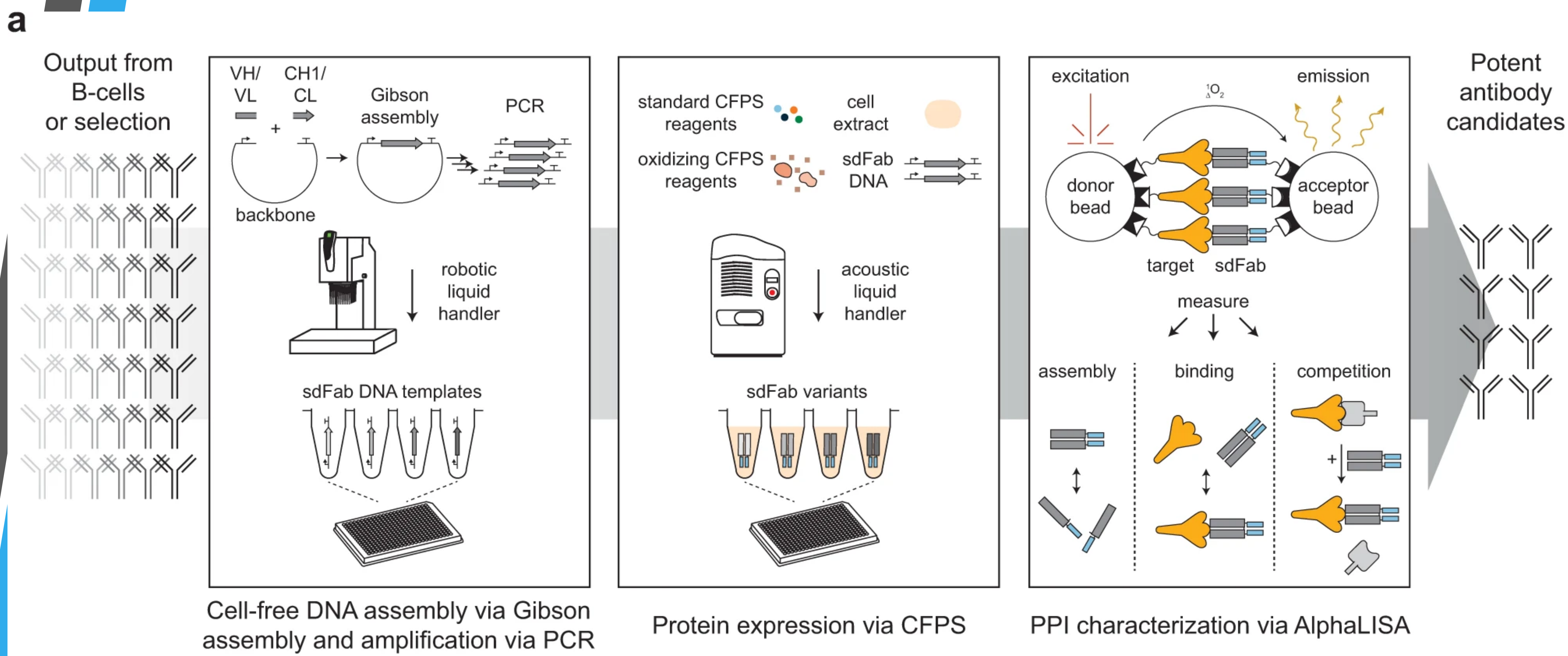
Thoring L., et al, Scientific Reports (2017)

Article | Open access | Published: 03 July 2023

A rapid cell-free expression and screening platform for antibody discovery

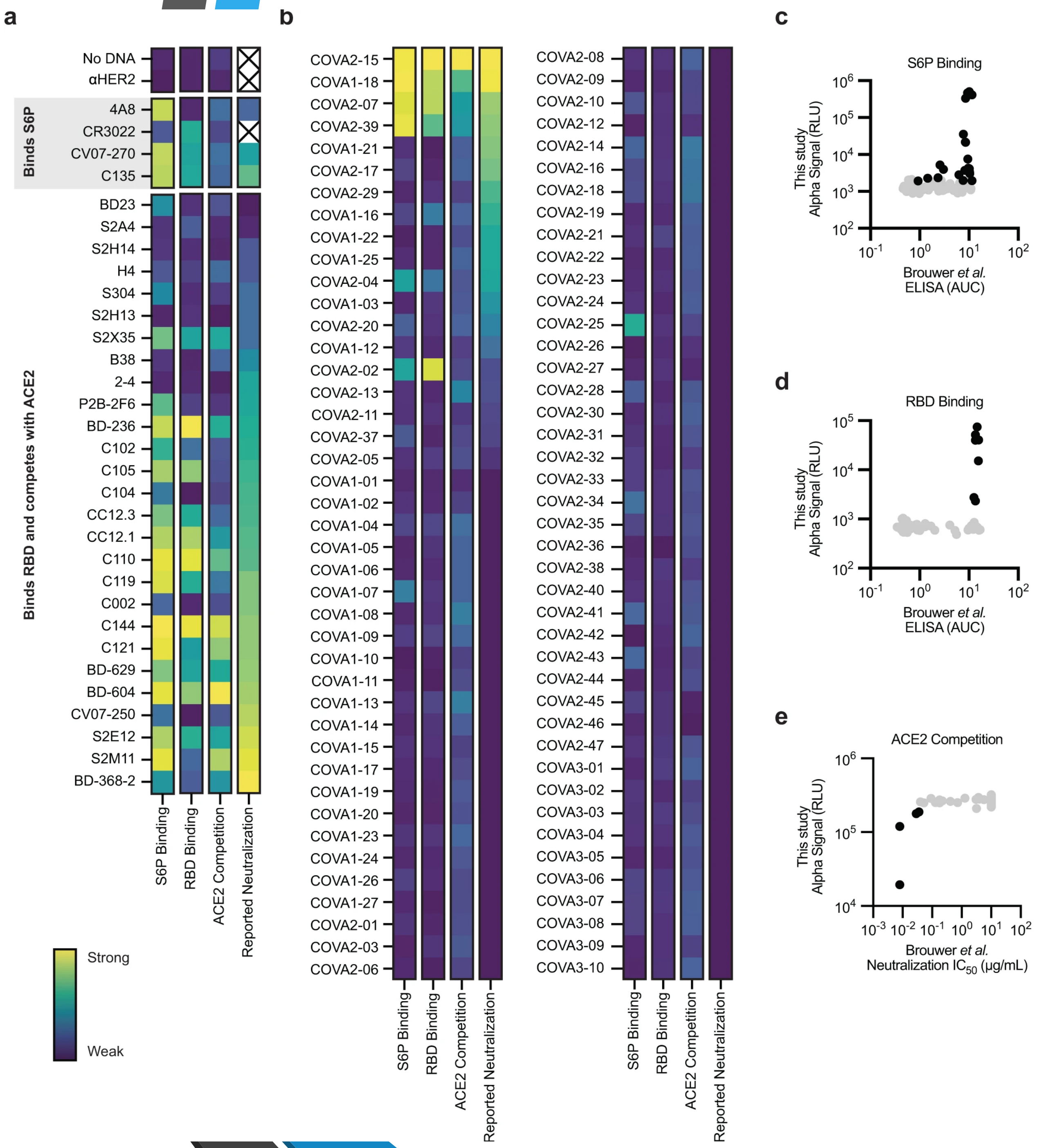
Andrew C. Hunt, Bastian Vögeli, Ahmed O. Hassan, Laura Guerrero, Weston Kightlinger, Danielle J. Yoesep, Antje Krüger, Madison DeWinter, Michael S. Diamond, Ashty S. Karim & Michael C. Jewett

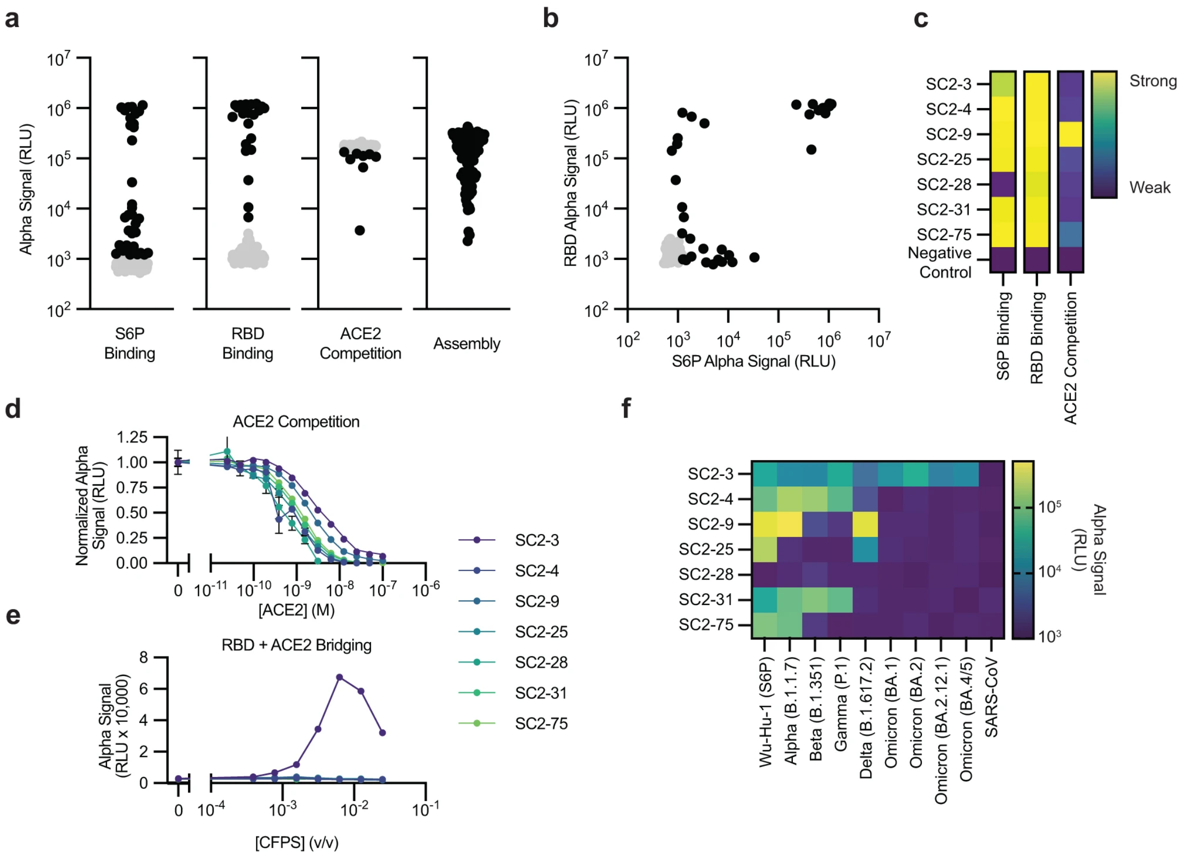
Nature Communications **14**, Article number: 3897 (2023) | Cite this article



VH: variable heavy chain
VL: variable light chain
CH1: Heavy chain constant
CL: Light chain constant
sdFab: synthetically dimerized antigen-binding fragment

Hunt A.C., et al, Nature Communications (2023)



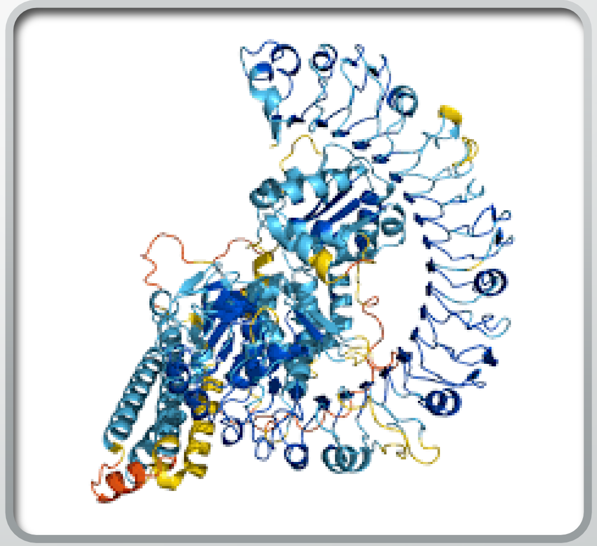


Whole workflow is completed in <24hours
By one researcher

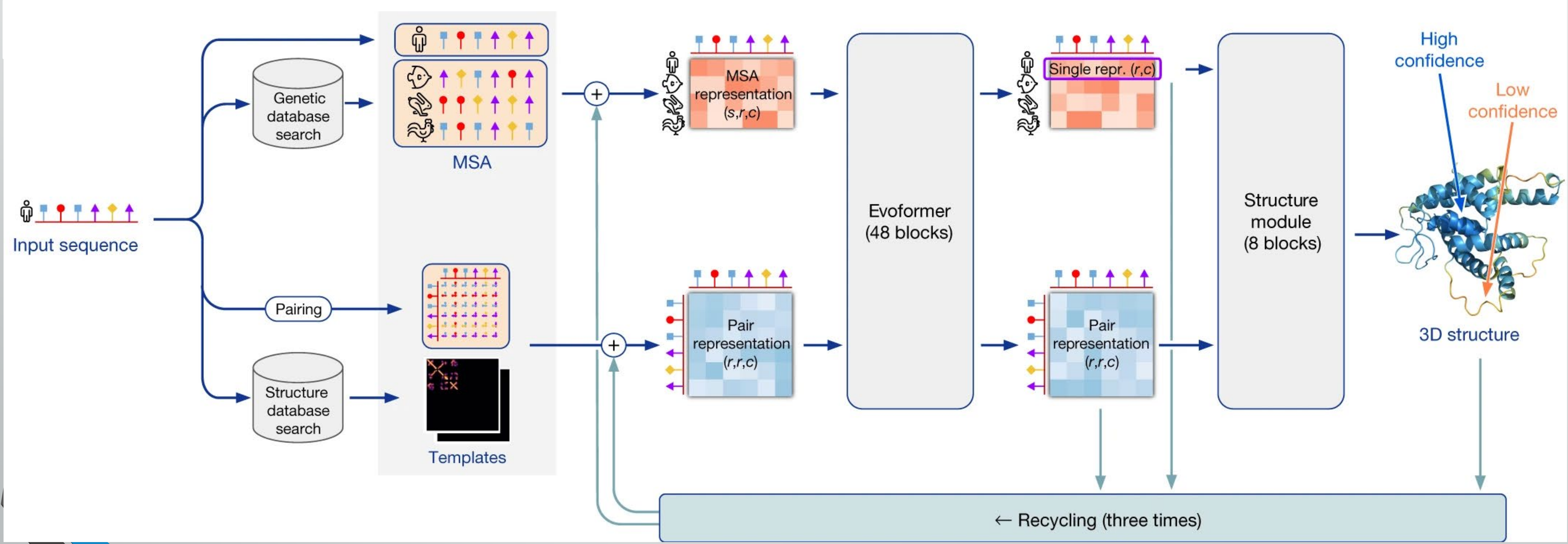
Hunt A.C., et al, Nature Communications (2023)

AI and machine learning

- 3-D>Protein folding problem
- Structure determined experimentally by:
- X-ray crystallography, cryo-EM, NMR
- >Expensive and time-consuming
- AlphaFold
- -AI developed by Deepmind



Multiple sequence alignments



Jumper J., et al, Nature (2021)

Application of AI in protein synthesis

- Impractical to synthesize all sequences or

investigate all functionally interesting variants

- Protein optimization to improve the efficiency of identifying desirable mutants through predictive modelling
- Machine learning can learn relationship between sequences and properties

1. Genomics
2. Protein structure and function
3. Protein design and evolution
4. Drug design

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Challenges and Future Direction

- CFPS Scalability and Cost-effectiveness
- Many proteins remain difficult to express
- AI focuses on protein designing

Future Direction

- ↑ cost-effectiveness
- Incorporate the AI-automated synthesiser to produce novel proteins
- Design and Synthesize of dream-up proteins

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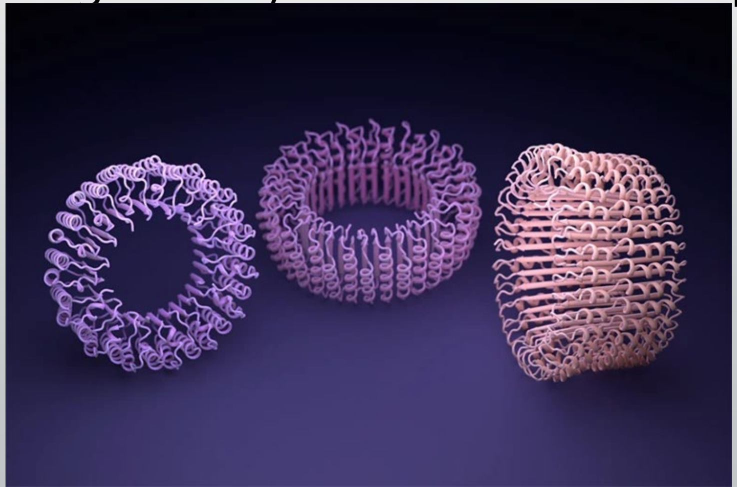

Ian C Haydon/UW Institute for Protein Design

Thank you!

Reference

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- Jumper, J., Evans, R., Pritzel, A. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589 (2021). https://doi.org/10.1038/s41586-021-03819-2
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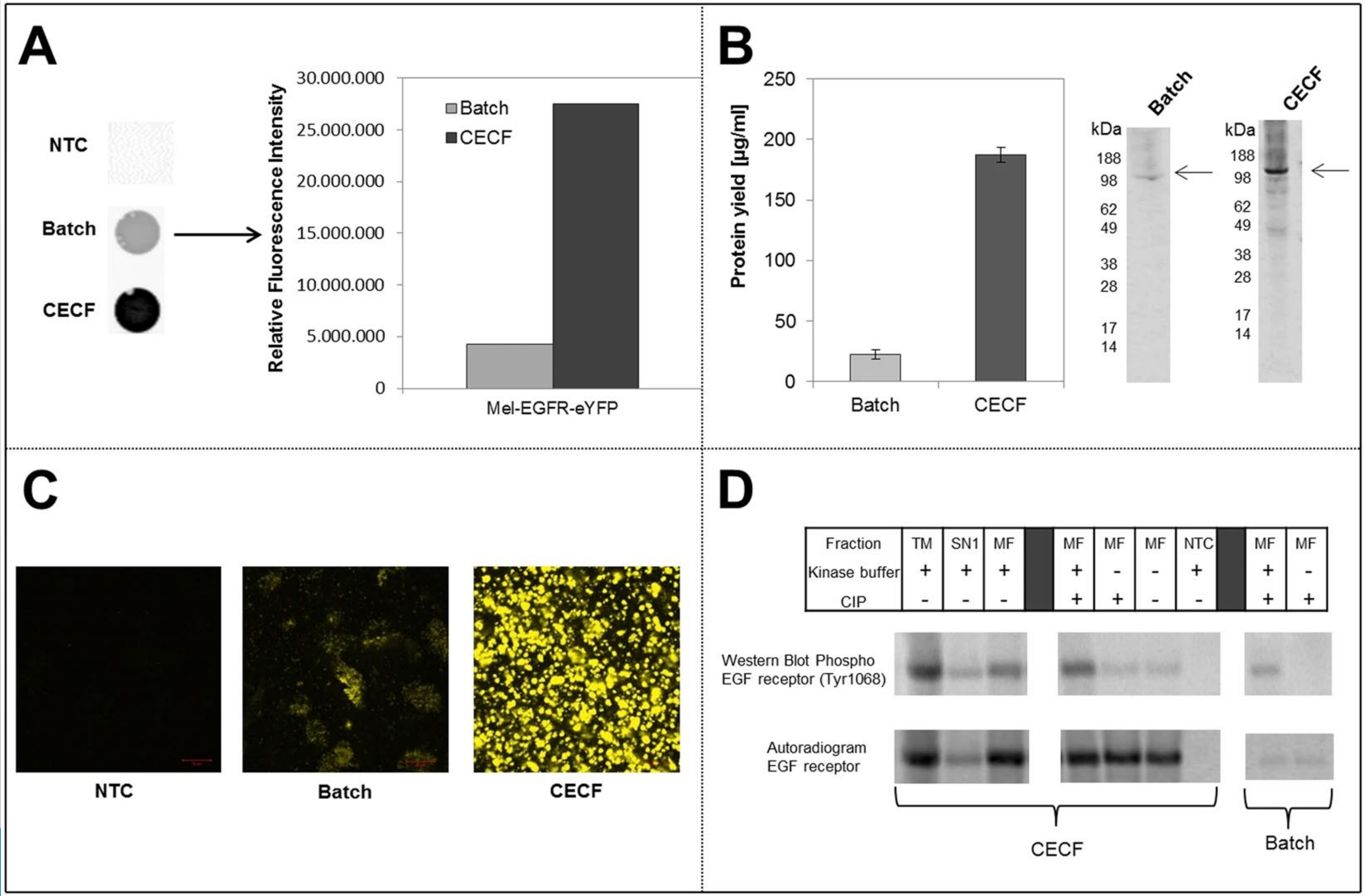
Supplementary (1)

Product	Cell extract	Titer (mg/mL)	Potential application	Reference
Single-chain antibody variable fragment against *Salmonella* O-antigen	Wheat Germ Extract	0.013	*In vivo* diagnostic and immunotherapeutic	Kawasaki et al., 2003
Urokinase protease	S30 extract (*E. coli* K12)	0.04	Treatment of thrombus	Kim and Swartz, 2004
Variant of human tissue-type plasminogen activator	*E. coli*	0.06	Treatment of acute ischemic stroke	Yin and Swartz, 2004
38C13B lymphocyte Id scFv	*E. coli* (Cytomim system)	0.043**	Lymphoma immunotherapy	Yang et al., 2005
Insulin-like growth factor I	*E. coli*	0.4	Central nervous system disorders (e.g., PMS, Rett syndrome)	Swartz, 2006
Murine granulocyte macrophage-colony stimulating factor (mGM-CSF)	*E. coli* (KC6)	0.854 ± 0.054*	Stimulator of systemic anti-tumor immunity	Goerke and Swartz, 2008
hGM-CSF		0.823 ± 0.060*	Cancer immunotherapy, healing chronic wounds	
Human granulocyte colony-stimulating factor (hG-CSF)		0.619 ± 0.068*	Cancer therapy	
Human Interferon alpha 2b (hIFNα2b)		0.692 ± 0.046*	Anti-cancer agent	
Murine scFv (Mvlvh)		0.519 ± 0.038*	Vaccines	
Human scFv (Hvlvh)		0.455 ± 0.007*		
Fusion protein with [bacterial immunity protein (im9)] Im9-hvlvh		0.441 ± 0.021*		
mGM-Im9-mvlvh		0.628 ± 0.056*		
mGM-Im9-hvlvh		0.591 ± 0.048*		
Human consensus interferon-alpha	*E. coli* (S30)	0.4	Anti-viral and anti-tumor agents	El-Baky et al., 2011
Human granulocyte-macrophage colony-stimulating factor (hGM-CSF)	*E. coli* (KGK10)	0.7	Cancer immunotherapy, healing chronic wounds	Zawada et al., 2011
Onconase	*E. coli* (PANOxSP system)	0.03 (>80% soluble)	Treatments of malignant mesothelioma	Salehi et al., 2016
Botulinum toxins	*E. coli* (RTS-100, RTS-500, and RTS-9000 HY kits)	1	Botulinum vaccine	Zichel et al., 2010
Streptokinase	HeLa and CHO cell lysates	0.50	Thrombolytic therapy	Tran et al., 2018
Crisantaspase	*E. coli* ClearColi	1	Cancer therapy	Wilding et al., 2019

**Total titer, **Soluble titer.*

Supplementary (2)



Supplementary (3)

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A Low-Cost, Thermostable, Cell-Free Protein Synthesis Platform for On-Demand Production of Conjugate Vaccines

Katherine F. Warfel, Asher Williams, Derek A. Wong, Sarah E. Sobol, Primit Desai, Jie Li, Yung-Fu Chang, Matthew P. DeLisa, Ashty S. Karim, and Michael C. Jewett*

Cite this: *ACS Synth. Biol.* 2023, 12, 1, 95–107
Publication Date: December 22, 2022
https://doi.org/10.1021/acssynbio.2c00392

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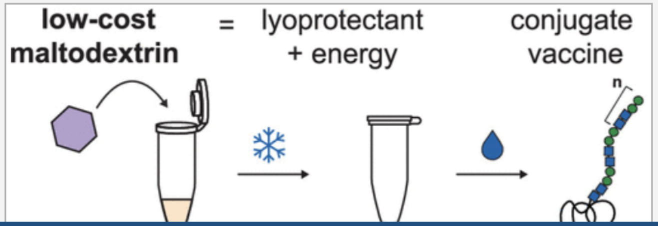
SUBJECTS: Addition reactions, Carbohydrates,

Abstract

Cell-free protein synthesis systems that can be lyophilized for long-term, non-refrigerated storage and transportation have the potential to enable decentralized biomanufacturing. However, increased thermostability and decreased reaction cost are necessary for further technology adoption. Here, we identify maltodextrin as an additive to cell-free reactions that can act as both a lyoprotectant to increase thermostability and a low-cost energy substrate. As a model, we apply optimized formulations to produce conjugate vaccines for ~$0.50 per dose after storage at room temperature



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Supplementary (4)