

# 新型蛋白表达平台

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# 全长跨膜蛋白表达

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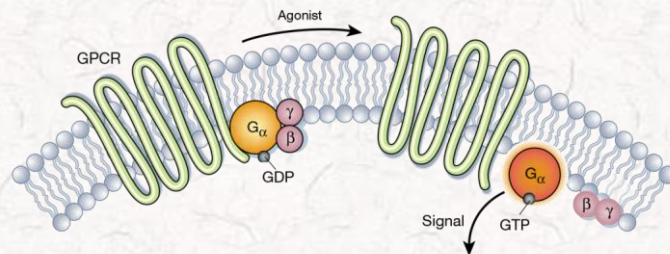
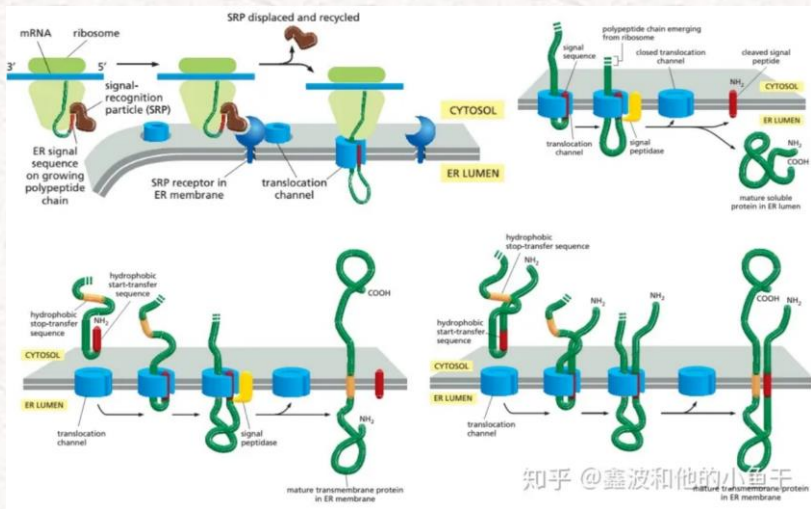
## VLP展示平台

VLP分类  
VLP产品

# 01

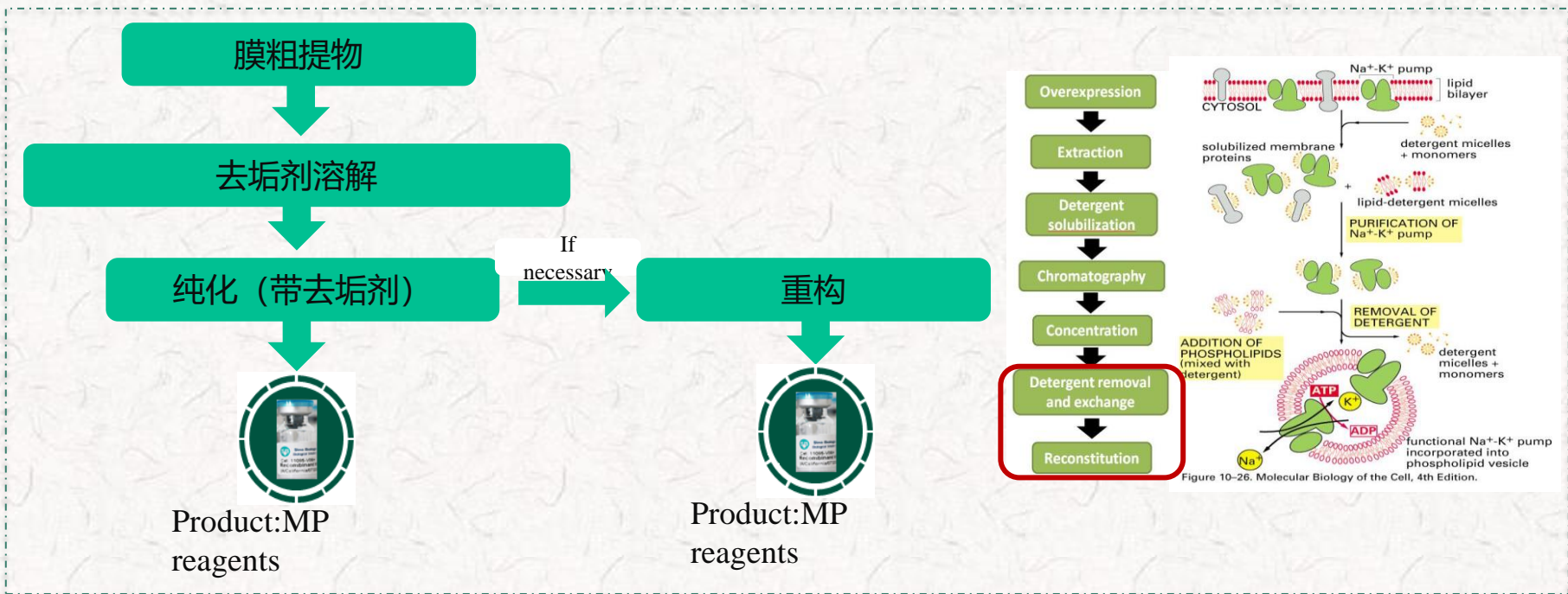
## 膜蛋白制备的常规方法

# 跨膜蛋白



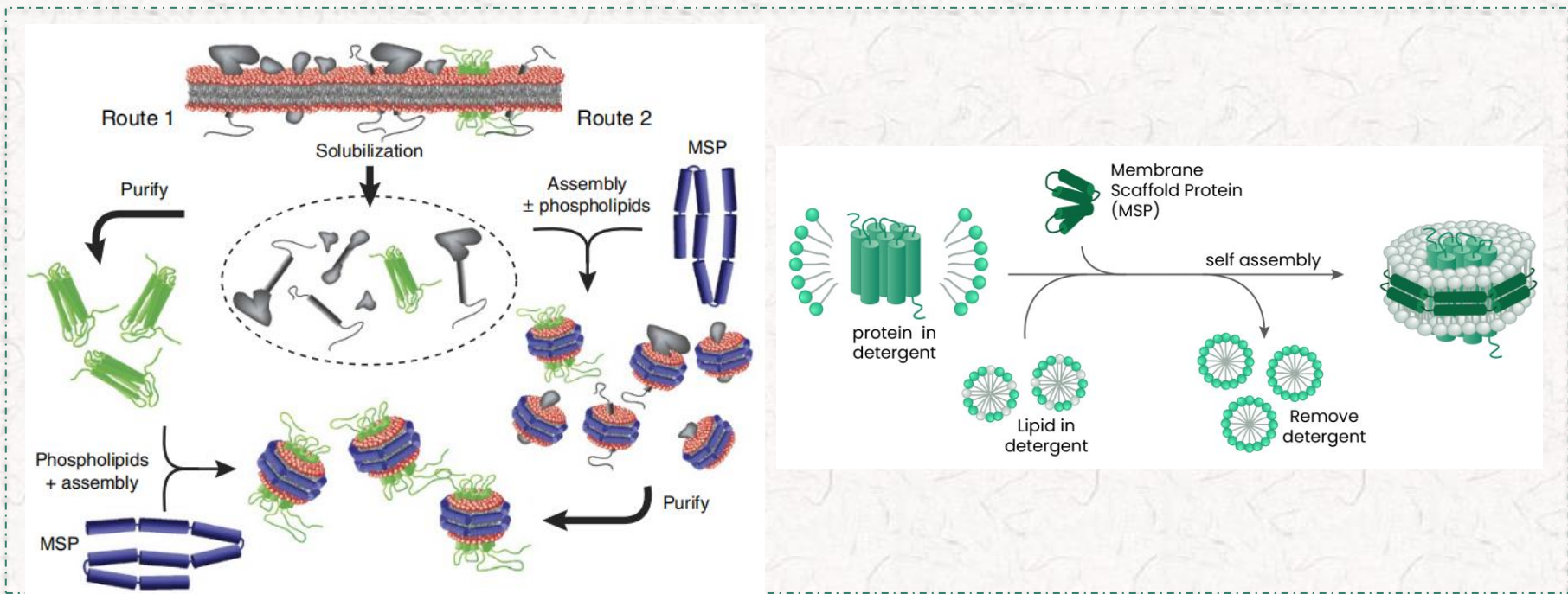
知乎 @ 鑫波和他的小鱼干

# ● 重组膜蛋白的常规制备流程





## ● 基于MSP蛋白的nanodisc

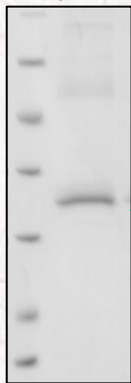


## ● 优劣势比较

	去垢剂	MSP nanodisc
脂质环境	非天然	类似天然
尺寸	不均一	尺寸均一
分散性	较差	单个分子分散
稳定性	不稳定，容易聚集	稳定，对温度不敏感
活性	容易失活	能更好的维持蛋白活性
难度	较难，需要摸索去垢剂	更难，还需要摸索重构条件

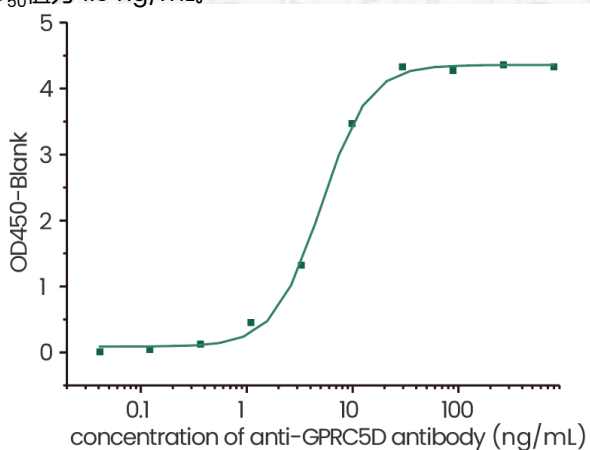
## ● 膜蛋白GPCR5D-Detergent产品

SDS-  
PAGE



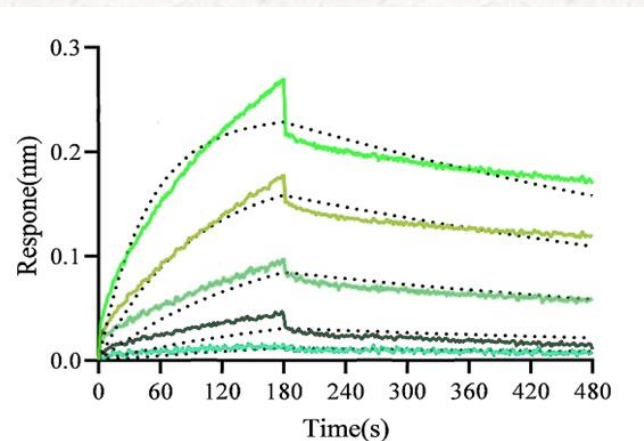
← GPCR5D

•经ELISA验证, Human GPCR5D-Detergent Protein (24447-H18H-DD) 可与抗GPCR5D抗体特异性结合,  $EC_{50}$ 值为4.0 ng/mL.



Immobilized Human GPCR5D-Detergent Protein (Cat:24447-H18H-DD) at 5  $\mu$ g/mL (100  $\mu$ L/well) on Nickel coated plate can bind Anti-Human GPCR5D Antibody, Human IgG1, the  $EC_{50}$  is 4.0 ng/ml (QC tested).

•经BLI验证, Human GPCR5D-Detergent Protein (24447-H18H-DD) 可与抗GPCR5D抗体特异性结合, 亲和力为**58.1** nM.



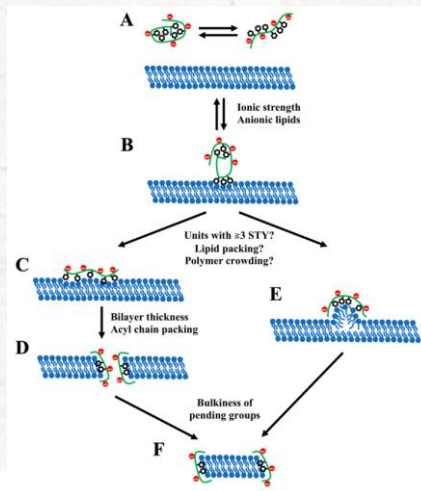
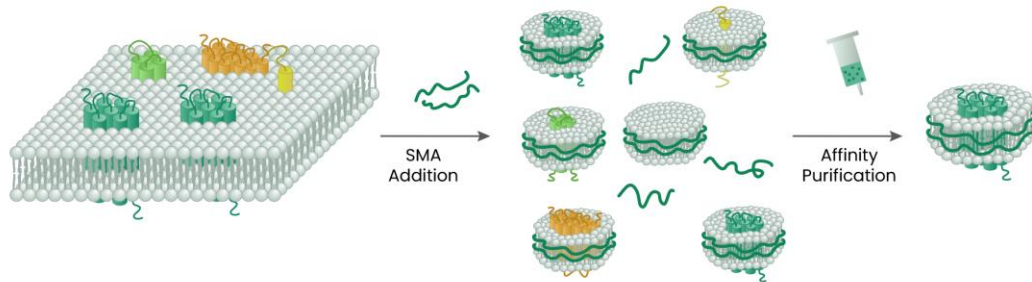
Loaded Anti-GPCR5D Antibody on Protein A Biosensor, can bind Human GPCR5D-Detergent Protein (Cat:24447-H18H-DD) with an affinity constant of 58.1 nM as determined in BLI assay (ForteBio Octet Red384) (Routinely tested).

# 02

## SMA-nanodiscs平台

## SMA-nanodiscs

- SMA (styrene maleic acid): “细长”两性分子，可自发内嵌到生物膜中，自主组装成水溶性nanodiscs的结构。
- SMA加入到膜抽提液中，直接从细胞膜上提取完整的膜蛋白，经一步亲和纯化即可得到高纯度的SMA-nanodiscs，最大限度的保证了膜蛋白的天然构象和活性。



## SMA类似物

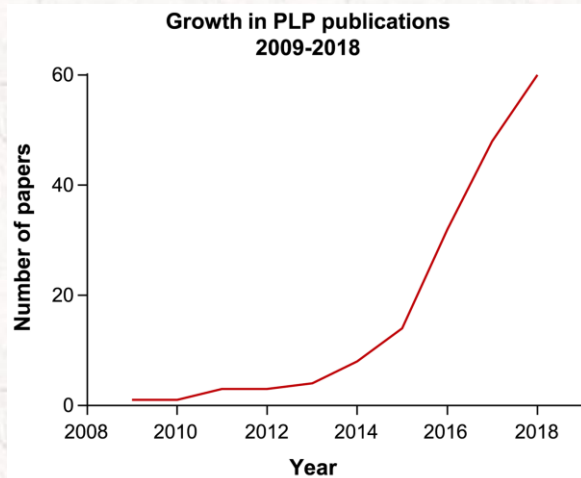
**Table 1 Selected alternative and functionalised amphipathic polymers, showing structures, size of nanodiscs and tolerance to divalent cations**

	SMA	DIBMA	SMI	SMA-QA
Chemical structure				
Optimum pH for nanodisc preparation	≥6.5	7.3, 8.4	5–7.8	2.5–10
Size of nanodiscs (nm)	10–13	18	6–11	30
Tolerance to divalent cations (mM)	5	35	>100	≤200
Key references	[7,15,46]	[37,47]	[36,37]	[47,48]

## SMA-nanodiscs技术进展

● 技术成熟：目前在膜蛋白制备中被广泛采用

● 技术适用性广：36次跨膜以下的膜蛋白均可用SMA-nanodiscs技术制备

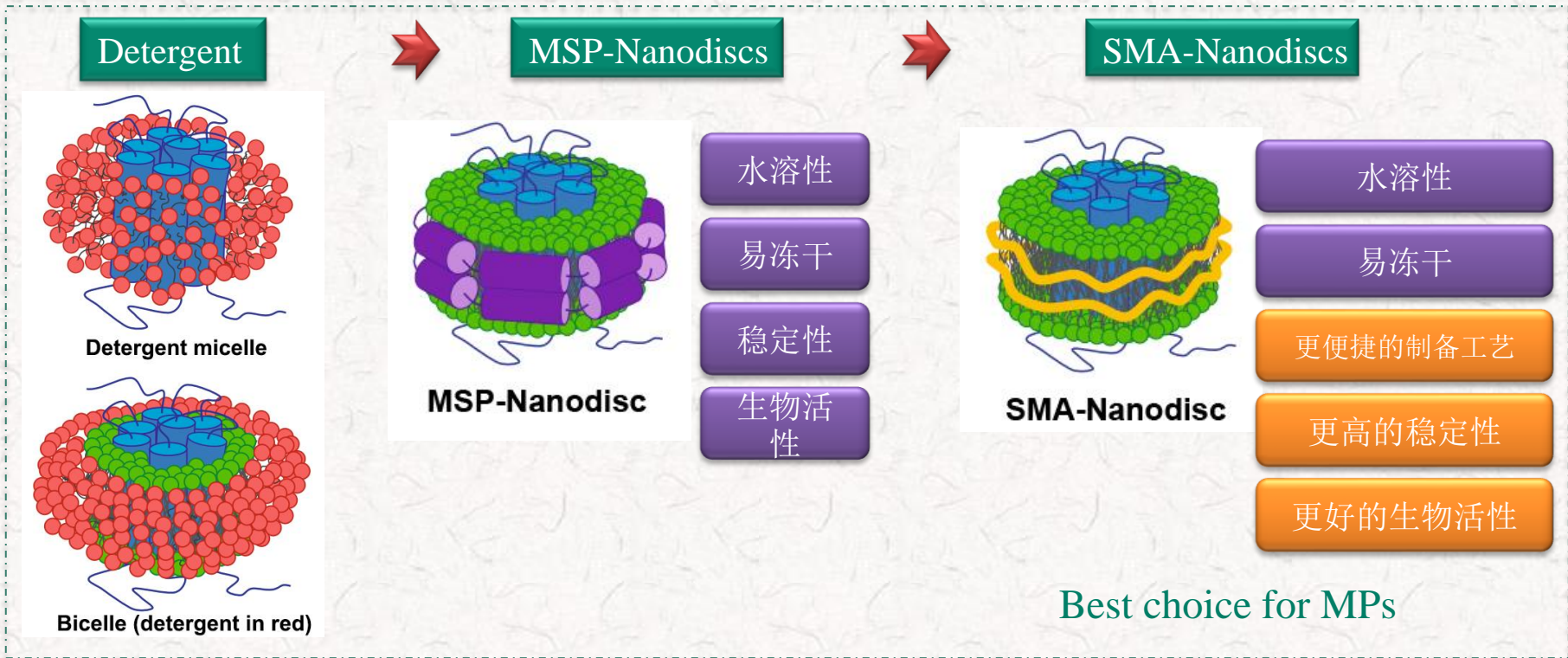


Growth of publications describing MPs purified using SMA-nanodiscs technology from 2008 to 2019.

Particle size of SMALPs with different incorporated proteins

Protein and native organism	No. of membrane-spanning segments	Estimated particle diameter
AcrB (trimer) (Postis et al. 2015) <i>Escherichia coli</i>	36 (3 × 12) helices	14 nm <sup>a</sup>
Respiratory complex IV (Long et al. 2013) <i>Saccharomyces cerevisiae</i>	26 helices <sup>b</sup>	12 nm
P-glycoprotein 1 (Pgp) (Gulati et al. 2014) <i>Homo sapiens</i> (expressed in <i>Trichoplusia ni</i> cells)	12 helices	10–15 nm <sup>c</sup>
Photosynthetic reaction center (Swainsbury et al. 2014) <i>Rhodobacter sphaeroides</i>	11 helices plus hydrophobic chromophores	13–14 nm
KcsA (Dörr et al. 2014) <i>Streptomyces lividans</i> (expressed in <i>E. coli</i> )	8 helices	10 nm
Bacteriorhodopsin (Knowles et al. 2009; Orwick-Rydmark et al. 2012) <i>Halobacterium salinarum</i> (solubilized after DMPC addition)	7 helices	12 nm
PagP (Knowles et al. 2009) <i>E. coli</i> (solubilized from DMPC liposomes)	8 strands	11 nm
PBP2 and PBP2a (complex) (Paulin et al. 2014) <i>Staphylococcus aureus</i>	Undefined <sup>d</sup>	18–24 nm <sup>d,e</sup>

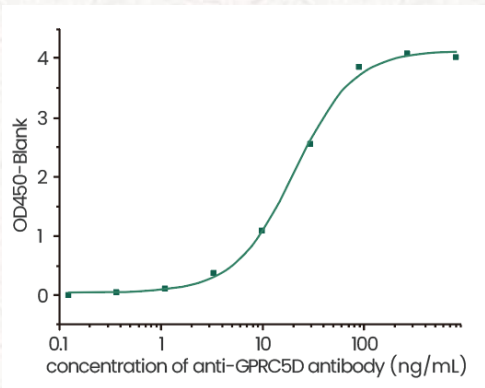
## ● SMA-nanodiscs技术优势



Best choice for MPs

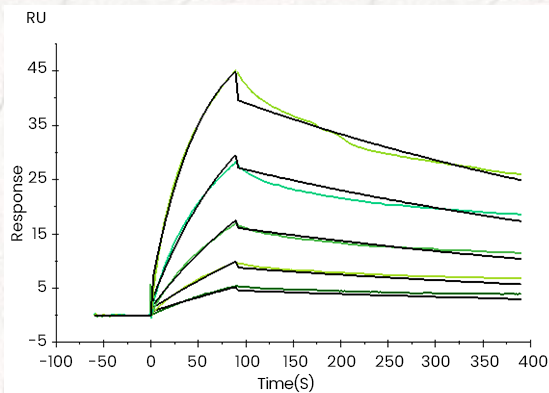
## ● 膜蛋白GPCR5D-Nanodiscs

•经ELISA验证, Human GPCR5D-Nanodisc Protein (24447-H18H-NA) 可与抗GPCR5D抗体特异性结合,  $EC_{50}$ 值为20.6 ng/mL。



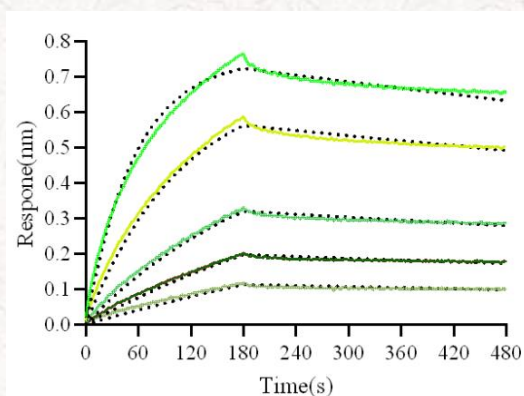
Immobilized Human GPCR5D-Nanodisc Protein (Cat: 24447-H18H-NA) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Anti-Human GPCR5D Antibody, Human IgG1, the  $EC_{50}$  is 20.6 ng/mL (QC tested).

•经SPR验证, Human GPCR5D-Nanodisc Protein (24447-H18H-NA) 可与抗GPCR5D抗体特异性结合, 亲和力为**9.6** nM。



Captured Anti-GPCR5D antibody on anti-Human IgG Fc via CM5 Chip can bind Human GPCR5D-Nanodisc Protein (Cat:24447-H18H-NA) with an affinity constant of 9.609 nM as determined in a SPR assay (Biacore T200) (Routinely tested).

•经BLI验证, Human GPCR5D-Nanodisc Protein (24447-H18H-NA) 可与抗GPCR5D抗体特异性结合, 亲和力为**0.78** nM。



Loaded Anti-GPCR5D antibody on ProA Biosensor, can bind Human GPCR5D-Nanodisc Protein (Cat:24447-H18H-NA) with an affinity constant of 0.78 nM as determined in BLI assay (ForteBio Octet Red384) (Routinely tested).

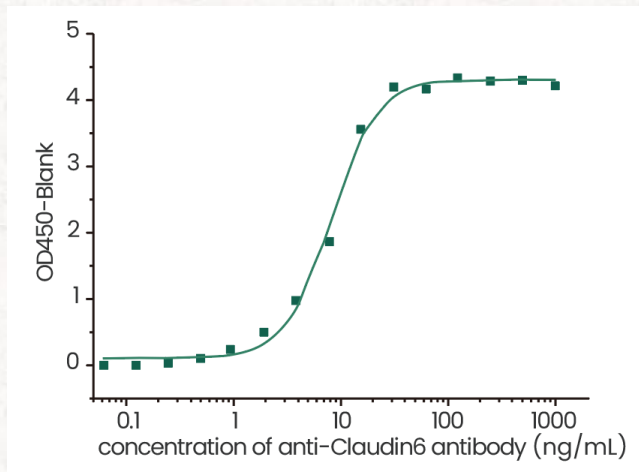
## ● 膜蛋白Claudin-6-Nanodiscs

### SDS-PAGE



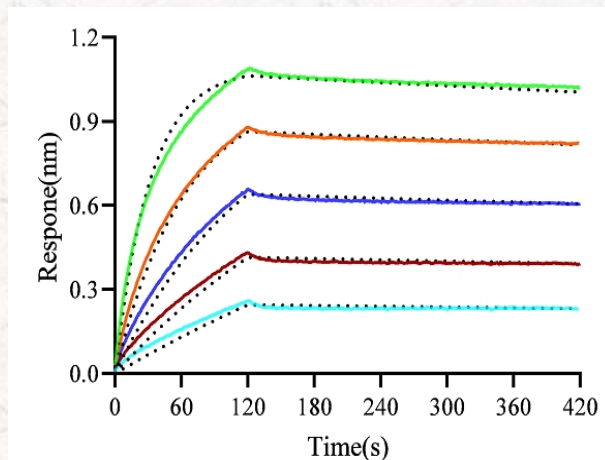
← Claudin 6

•经ELISA验证, Human Claudin-6 -Nanodisc Protein (16658-H61H-NA) 可与抗体特异性结合,  $EC_{50}$ 值为5.4 ng/mL。



Immobilized Human Claudin-6 -Nanodisc Protein (Cat: 16658-H61H-NA) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Anti-Human Claudin-6 Antibody, the  $EC_{50}$  is 5.4 ng/mL (QC tested).

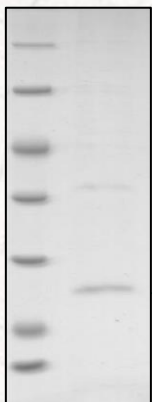
•经BLI验证, Human Claudin-6-Nanodisc Protein (16658-H61H-NA)可与抗体特异性结合, 亲和力为**0.536 nM**。



Loaded Anti-Claudin-6 antibody on ProA Biosensor, can bind Human Claudin-6-Nanodisc Protein (Cat:16658-H61H-NA) with an affinity constant of 0.536nM as determined in BLI assay (ForteBio Octet Red384) (Routinely tested).

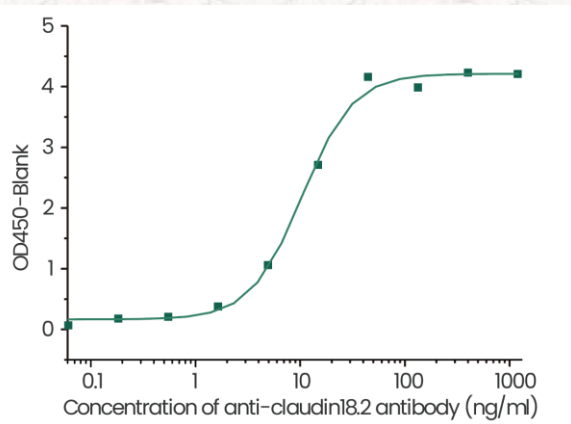
## ● 膜蛋白Claudin-18.2-SMA-Nanodiscs

SDS-PAGE



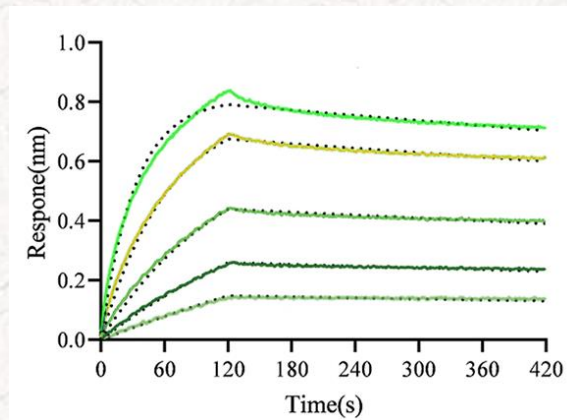
← Claudin 18.2

•经ELISA验证, Human Claudin-18.2-Nanodisc Protein (30025-H64H-NA) 可与抗体特异性结合,  $EC_{50}$ 的范围为 5-15 ng/mL



Immobilized Human Claudin-18.2-Nanodisc Protein (Cat:30025-H61H-NA) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Anti-Human claudin18.2 Antibody, Human IgG1, the  $EC_{50}$  is 5-15 ng/mL.

•经BLI验证, Human Claudin-18.2-Nanodisc Protein (30025-H64H-NA)可与抗Claudin 18.2抗体特异性结合, 亲和力为4.3 nM。



Loaded Anti-Claudin-18.2 antibody on ProA Biosensor, can bind Human Claudin-18.2-Nanodisc Protein (Cat:30025-H64H-NA) with an affinity constant of 4.3 nM as determined in BLI assay (ForteBio Octet Red384) (Routinely tested).

# 03

## VLP展示平台

● VLP分类

VLP

无囊膜

膜

囊

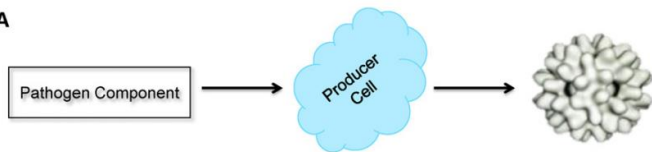
膜

病

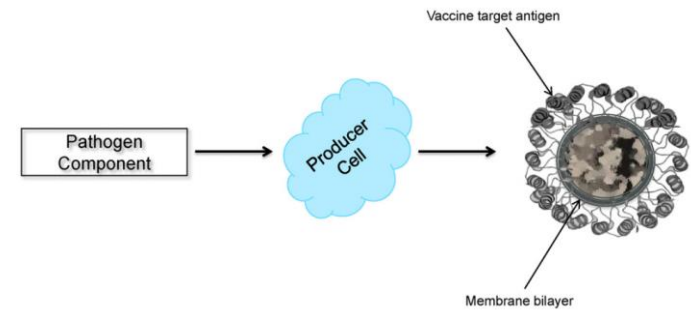
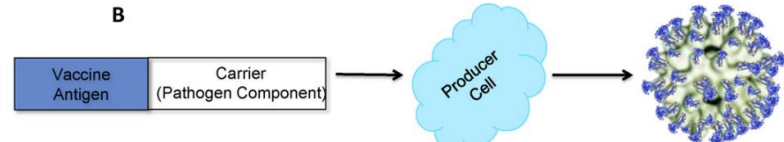
毒

组织  
细胞  
融合  
载体  
蛋白  
体与  
目的  
蛋白  
融合

A

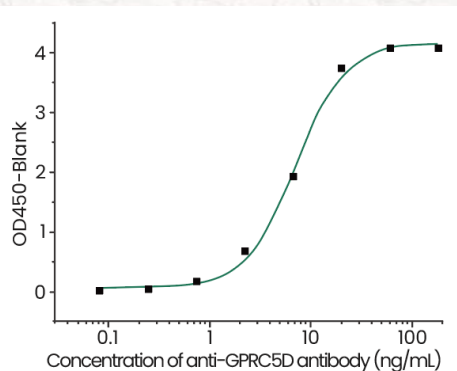


B



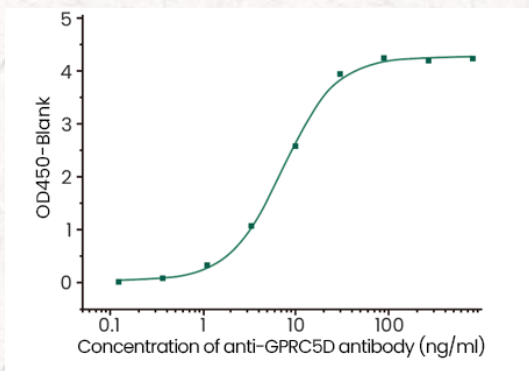
## ● 膜蛋白GPRC5D-VLP

•经ELISA验证,与抗GPRC5D抗体特异性结合,  
EC<sub>50</sub>范围为3-10 ng/mL。



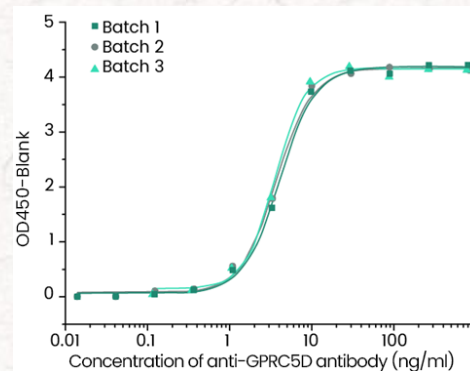
Immobilized Human GPRC5D-VLP (Full Length) Protein (Cat:24447-HNAH) at 5 μg/mL (100 μL/well) can bind anti-GPRC5D Antibody, the EC<sub>50</sub> is 3-10 ng/mL.

•经ELISA验证,与抗GPRC5D抗体特异性结合,  
EC<sub>50</sub>范围为4-12 ng/mL。



Immobilized Biotinylated Human GPRC5D-VLP (Full Length) Protein (Cat: 24447-HNAH-B) at 5 μg/mL (100 μL/well) on streptavidin precoated (2 μg/well) can bind Anti-Human GPRC5D Antibody, the EC<sub>50</sub> is 4-12ng/mL.

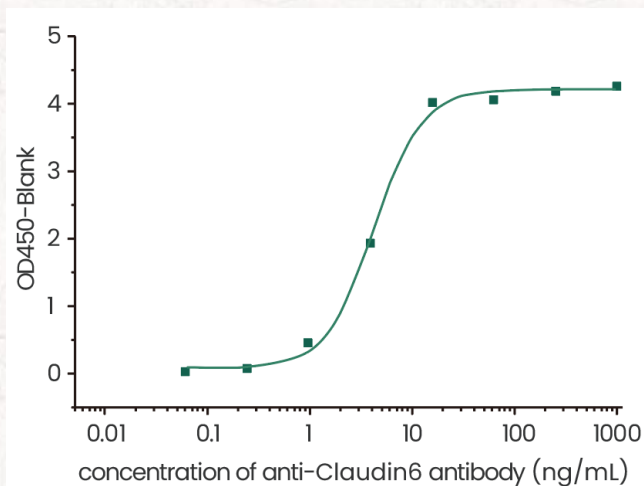
•ELISA检测: 批间稳定性高



Immobilized Human GPRC5D-VLP (Full Length) Protein (Cat:24447-HNAH) at 5 μg/mL (100 μL/well) can bind anti-GPRC5D Antibody.

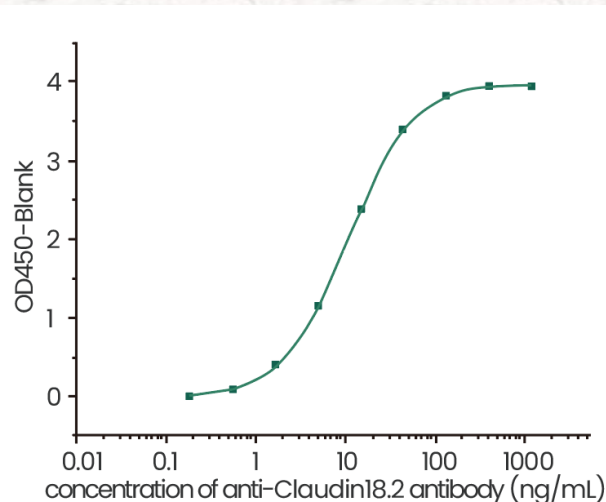
## ● 膜蛋白CLDN6/18.2-VLP

•经ELISA验证, **Claudin6-VLP** (16658-HNAH) 可与抗体特异性结合,  $EC_{50}$ 范围为2-10 ng/mL。



Immobilized Human CLDN6-VLP (Full Length) Protein (Cat: 16658-HNAH) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Anti-Human CLDN6 Antibody, Human IgG1, the  $EC_{50}$  is 2-10 ng/mL.

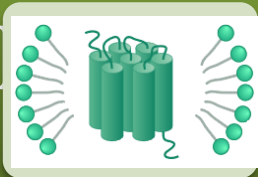
•经ELISA验证, **Claudin18.2-VLP** (30025-HNAH) 可与抗体药特异性结合,  $EC_{50}$ 范围为5-20 ng/mL。



Immobilized Human Claudin18.2-VLP (Full Length) Protein (Cat: 30025-HNAH) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Anti-Human claudin18.2 Antibody, Human IgG1, the  $EC_{50}$  is 5-20 ng/mL.

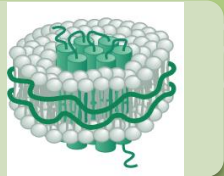


## ● 膜蛋白的展示平台比较



lipid bilayer展示平台

不稳定，动态平衡，蛋白易脱落



vesicle展示平台

非常稳定，可视为可溶性球蛋



nanoparticle展示平台

稳定，可类似为可溶性球蛋

● 稳定指数：膜蛋白展示平台比较

# 无细胞表达系统

1

## 无细胞表达系统介绍

- 无细胞表达系统概述
- 无细胞表达系统的工作原理
- 无细胞表达系统的特点

2

## 无细胞表达系统的应用与挑战

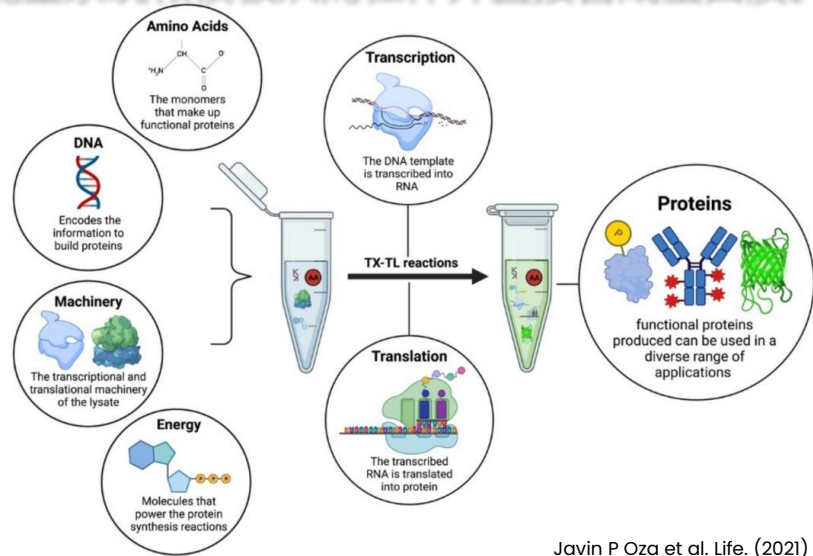
- 无细胞表达抗体片段
- 无细胞表达其他蛋白
- 基于无细胞表达的高通量筛选

# 01

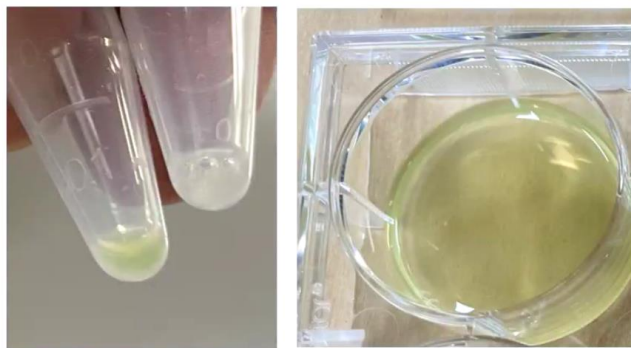
## 无细胞表达系统介绍

## ● 体外转录-翻译系统的基本概念

体外转录-翻译系统 (Transcription-Translation system, TXTL system), 即无细胞表达系统, 指将细胞内合成蛋白所需的核糖体等调控因子提取出来, 并辅以氨基酸及核苷酸等原料、能量系统和模板从而在体外直接合成蛋白质。

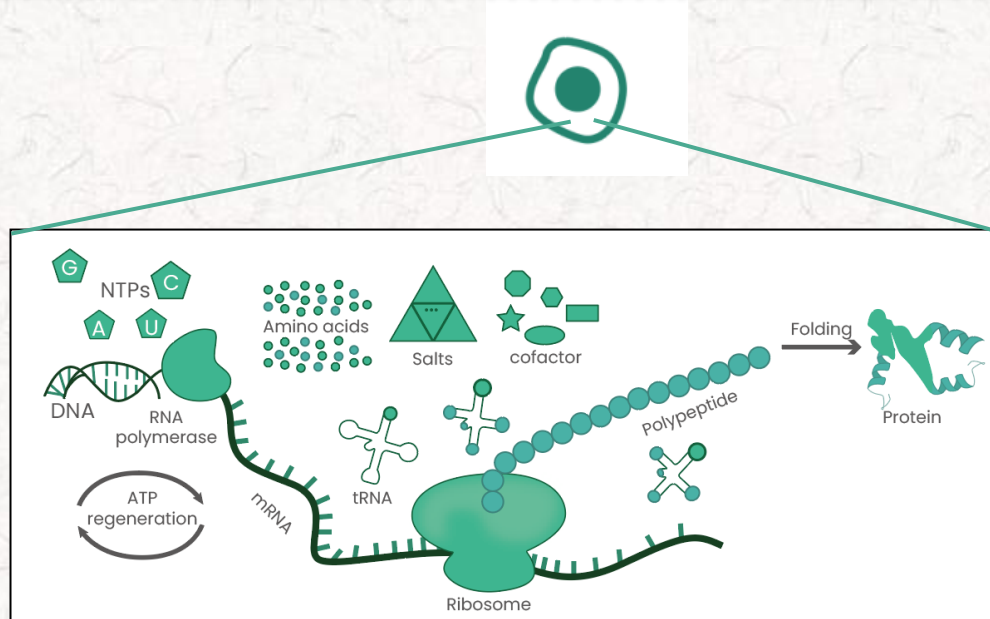
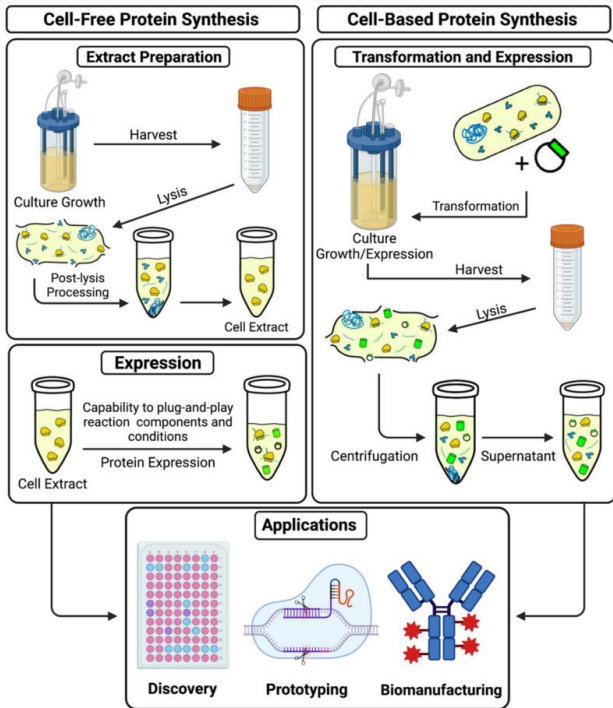


Javin P Oza et al. Life. (2021)



序列来源: sfGFP

# 无细胞表达系统的基本原理及工作流程

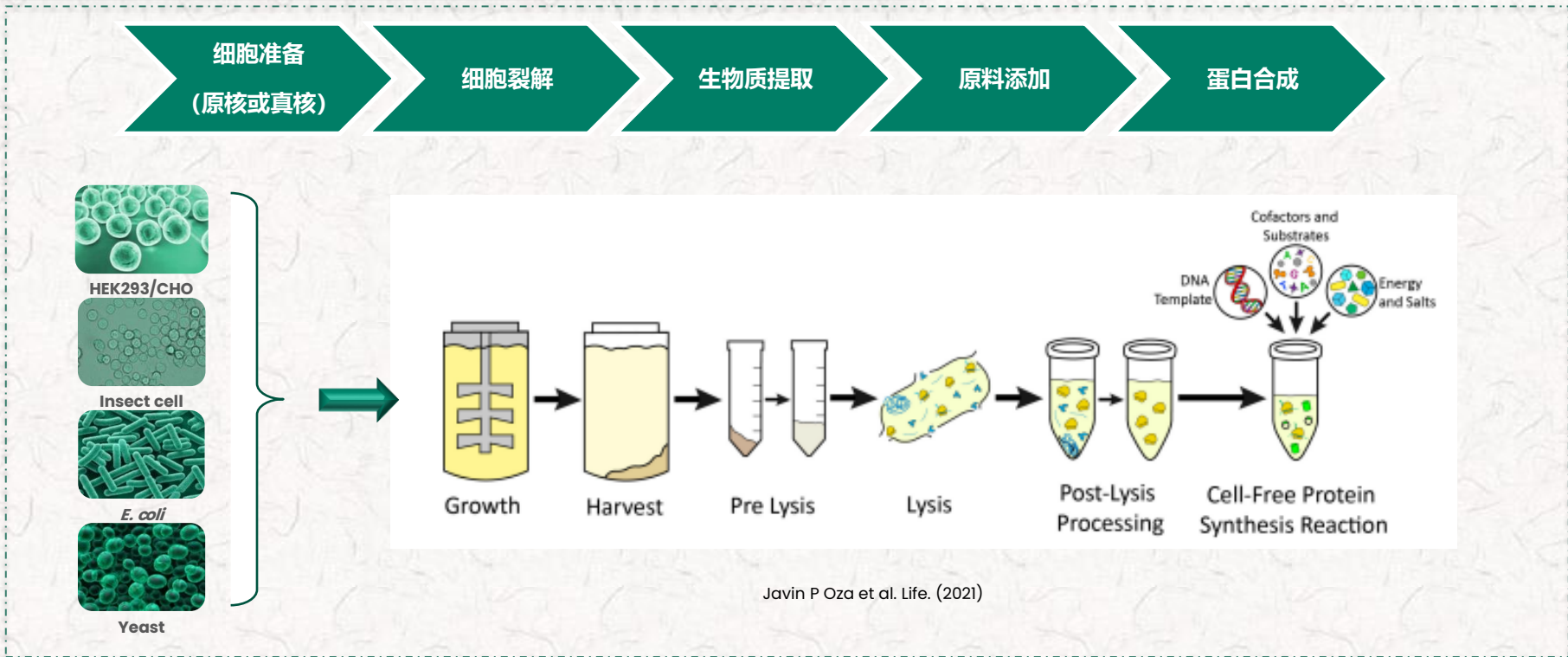


Javin P Oza et al. Life. (2021)

## 不同生物质来源无细胞系统的优劣势

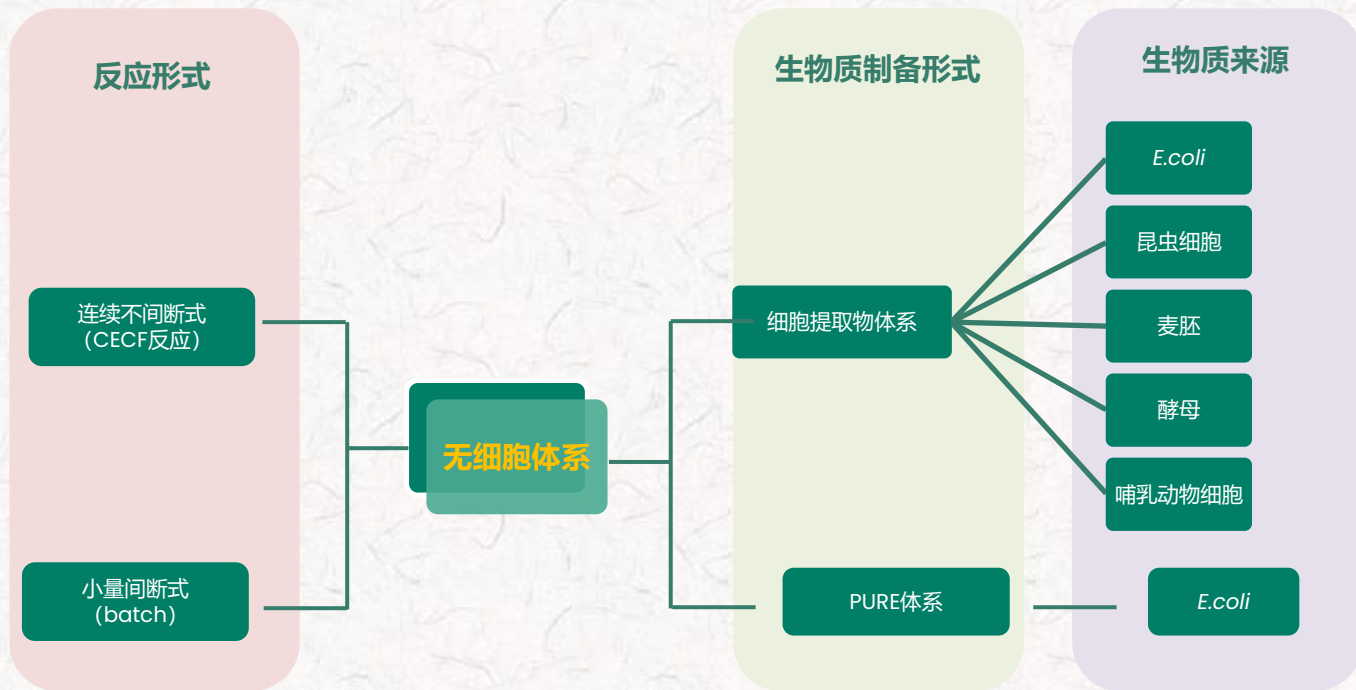
生物质来源	优势	劣势	应用
E.coli	<ul style="list-style-type: none"> <li>• 高效高产</li> <li>• 操作简便，成本低</li> <li>• 菌种多，可选择面较广</li> <li>• 遗传和转录翻译机制较为明确，易于改造</li> <li>• 载体所需元件少，构建方便</li> </ul>	<ul style="list-style-type: none"> <li>• 缺乏翻译后修饰系统</li> <li>• 缺乏内源性膜结构</li> </ul>	<ul style="list-style-type: none"> <li>• 大规模合成蛋白</li> <li>• 应用于ADC研发，用于表达VHH和ScFv</li> <li>• 为酶等蛋白引入非天然氨基酸</li> </ul>
酵母	<ul style="list-style-type: none"> <li>• 高效高产</li> <li>• 操作简便，成本低</li> <li>• 体系较为成熟</li> </ul>	<ul style="list-style-type: none"> <li>• 成本相较E.coli高</li> <li>• 无哺乳动物细胞的类似修饰</li> </ul>	<ul style="list-style-type: none"> <li>• 成熟的商品化体系</li> <li>• 重组蛋白的表达与生产</li> </ul>
麦胚	<ul style="list-style-type: none"> <li>• 高效高产</li> <li>• 提取物可以直接合成含二硫键蛋白</li> <li>• 折叠成功率较E.coli高</li> </ul>	<ul style="list-style-type: none"> <li>• 抽提物制备成本高</li> <li>• 无哺乳动物翻译后修饰</li> <li>• 产量较低</li> <li>• 无膜系统</li> </ul>	<ul style="list-style-type: none"> <li>• 部分疫苗和抗体的研发和制备</li> <li>• 蛋白芯片</li> </ul>
昆虫细胞	<ul style="list-style-type: none"> <li>• 抽提物制备简单</li> <li>• 可以进行部分翻译后修饰</li> <li>• 可以用于部分膜蛋白的表达</li> </ul>	<ul style="list-style-type: none"> <li>• 成本较高</li> <li>• 产量较低</li> </ul>	<ul style="list-style-type: none"> <li>• 膜蛋白的研发与生产</li> <li>• 教学体系，研究膜蛋白</li> </ul>
CHO	<ul style="list-style-type: none"> <li>• 背景明确，适用于医药研发与生产</li> <li>• 含微体</li> <li>• 具有翻译后修饰系统</li> </ul>	<ul style="list-style-type: none"> <li>• 成本较高</li> <li>• 产量较低</li> </ul>	<ul style="list-style-type: none"> <li>• 合成和筛选抗体及抗体功能性片段</li> <li>• 合成膜蛋白</li> <li>• 新型无细胞系统</li> </ul>
人源细胞（293、HeLa）	<ul style="list-style-type: none"> <li>• 背景明确，适用于医药研发与生产</li> <li>• 含微体</li> <li>• 具有人体的翻译后修饰系统</li> </ul>	<ul style="list-style-type: none"> <li>• 成本较高</li> <li>• 产量较低</li> </ul>	<ul style="list-style-type: none"> <li>• 生物制药研发</li> <li>• 教学体系，可研究病毒复制和翻译机制等</li> </ul>

## ● 无细胞表达系统的基本原理及工作流程



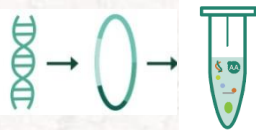
## ● 无细胞系统的分类

- 分类标准不同：按反应形式、生物质制备形式、生物质来源等



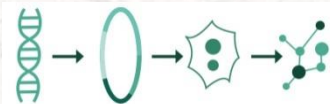
## 无细胞系统与基于细胞的表达系统的比较

### 无细胞体系



- **快速**灵活，从模板到纯化最快可以在6h完成
- **可控**性和调整性极强
- 无需活细胞存在，可以**耐受**对细胞有害的蛋白表达
- 单位体积**表达量**高，最高可达mg/ml级别，适用于高通量蛋白表达及筛选
- 初期材料成本较高，现已大幅度降低，成本对于应用已不再是问题
- **操作简便**，无需细胞相关操作只需配置反应体系
- 极其适合**自动化**和智能化流程
- 基本体系无法达到细胞体系现有的普适性，技术还需继续推进

### 基于细胞表达体系

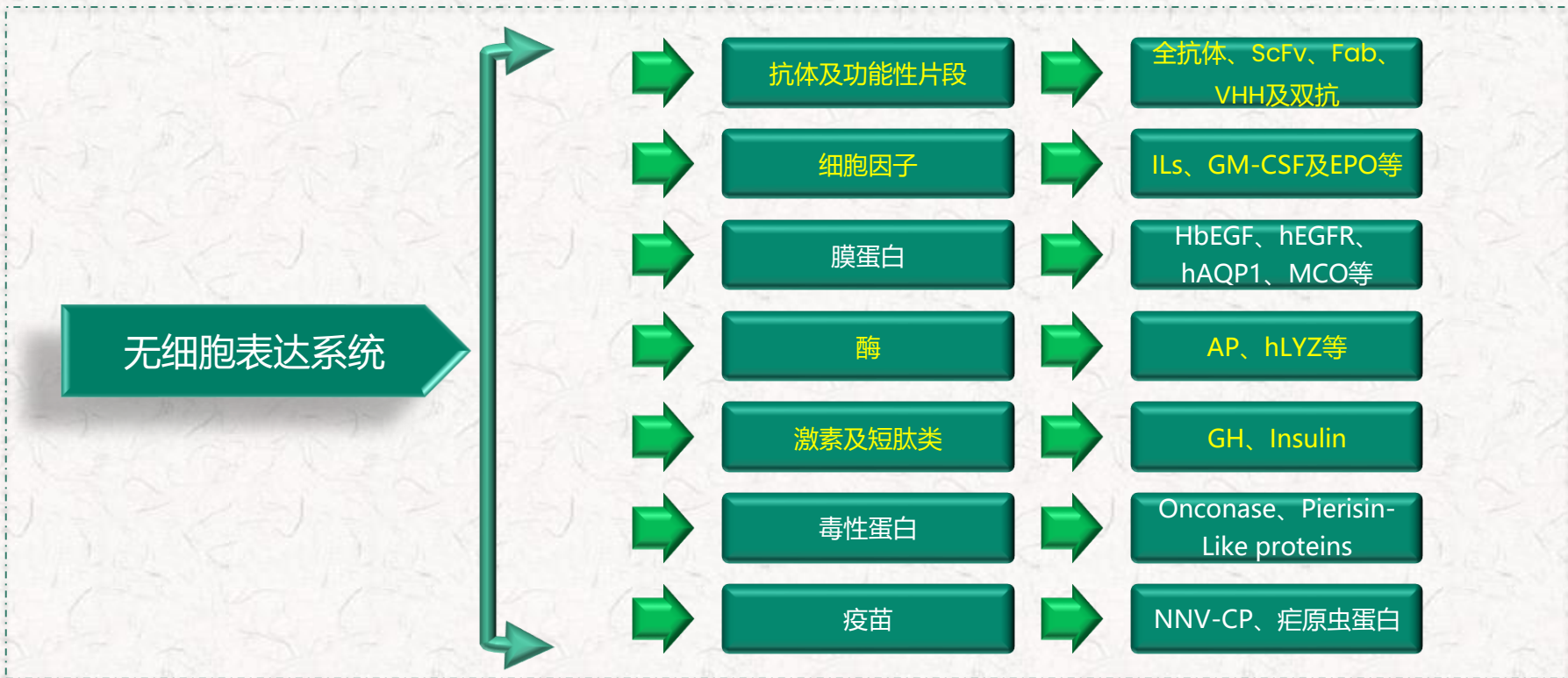


- **应用广泛**、**技术成熟**
- **元和和酵母**易于放大
- 仍为现如今蛋白表达的主要技术手段
- 适用于相应蛋白的大量表达及生产
- 由于细胞及细菌的生长周期限制，固有流程很难进一步缩短
- 不可控性
- 很难进行高通量蛋白表达
- 难以实现自动化和智能化

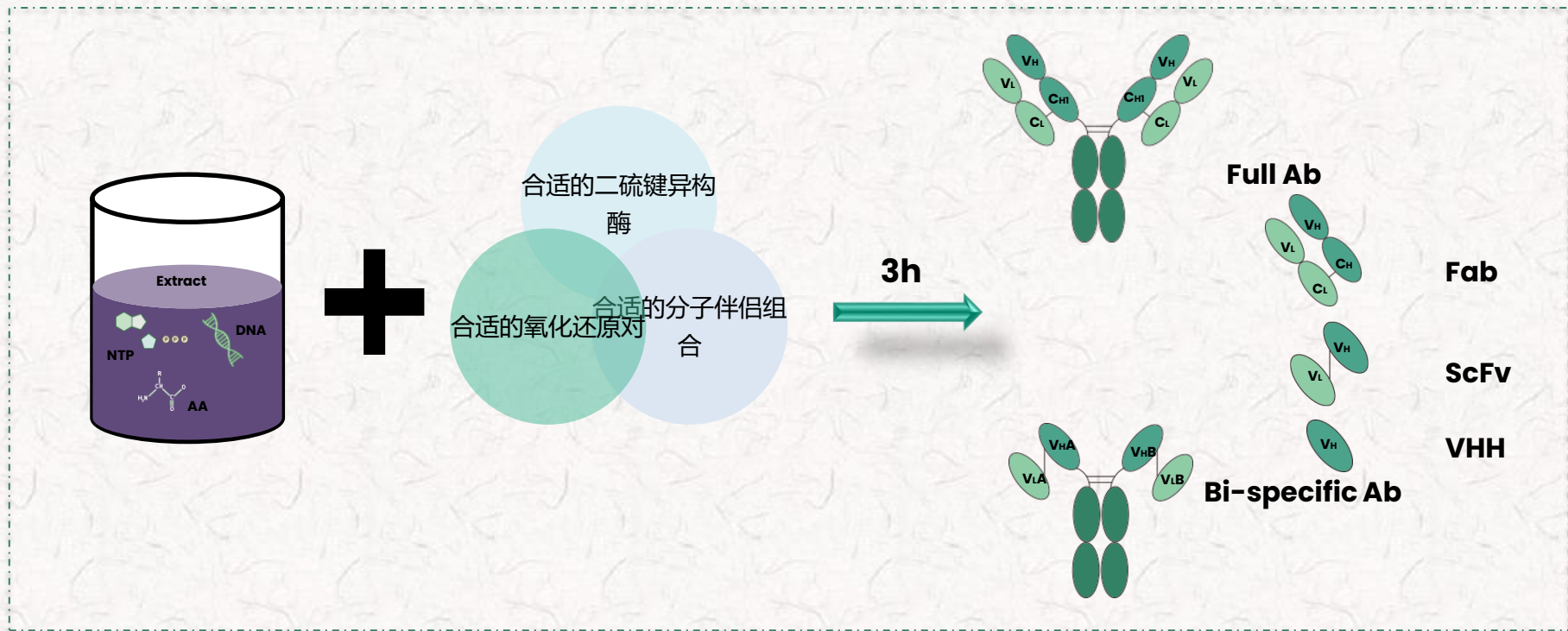
# 02

## 无细胞表达系统的机遇与挑战

## ● 无细胞系统在大量表达中的应用



## 无细胞系统在抗体及抗体功能性片段表达中的应用



## 无细胞系统在抗体功能性片段筛选中的应用

无细胞反应板

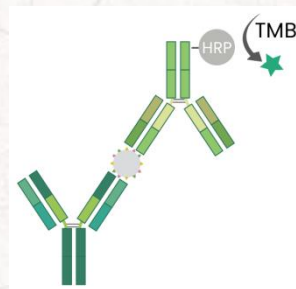
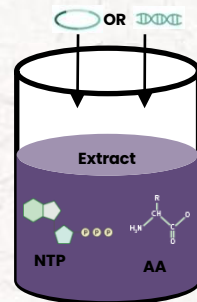
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

数小时 上清, 无需纯化

ELISA检测

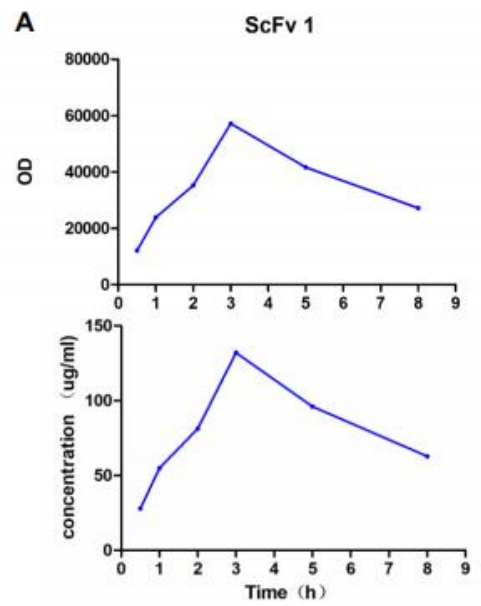
	1	2	3	4	5	6	7	8	9	10	11	12
A	●						●					
B			●									
C										●		
D				●								
E	●											
F								●				
G				●								●
H									●			

抗体片段模板



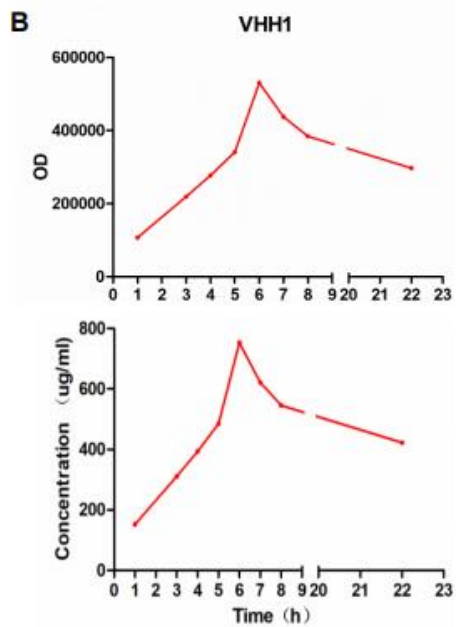
从模板到抗体片段的结合检测可以在1天内完成, 且适合高通量筛选

● 抗体功能性片段无细胞表达不同时间相合成曲线



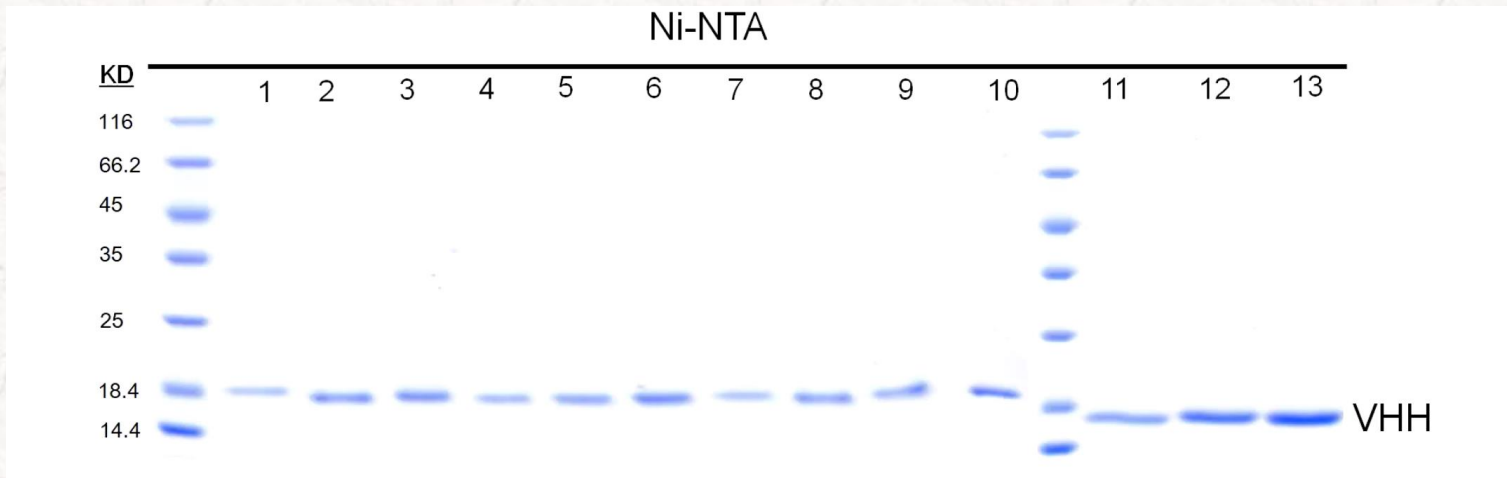
Batch反应 (100ul体系)

序列来源: 研发内部提供



CECF反应 (100ul体系)

● 无细胞系统VHH快速表达案例展示



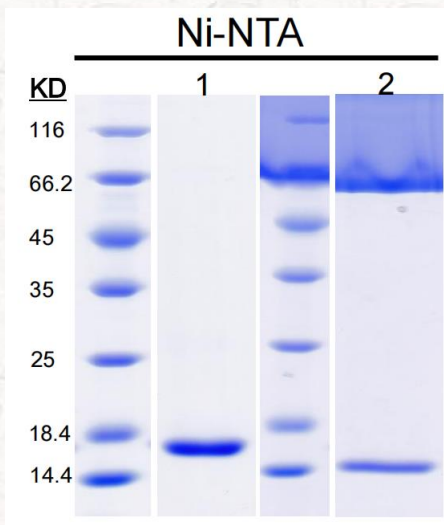
● 纯度大于85%，表达量大约300-800ug/mL，产量大于200ug/6mL

## ● VHH数据总结

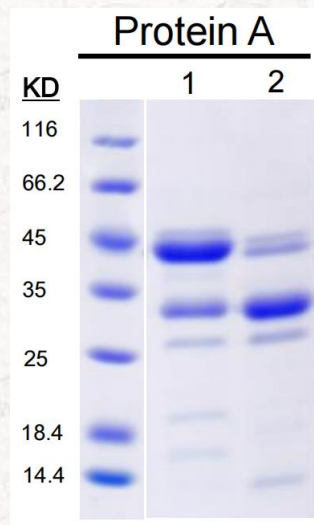
项目	总数	成功数量	成功率	总成功率
例1	1	1	100%	96.6%
例2	30	28	93.3%	
例3	23	23	100%	
例4	6	6	100%	

● 成功标准按照200ug, 85%SDS-PAGE纯度

- 无细胞系统可以表达细胞中较难表达的VHH

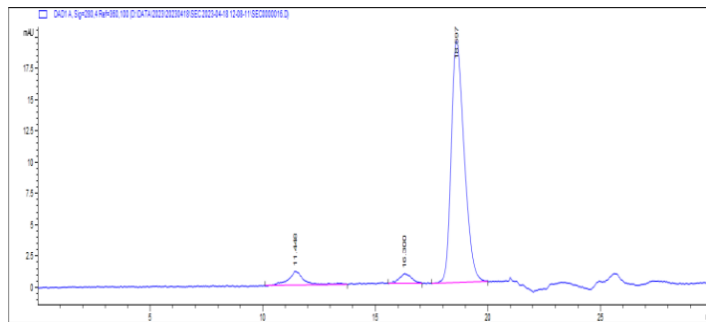
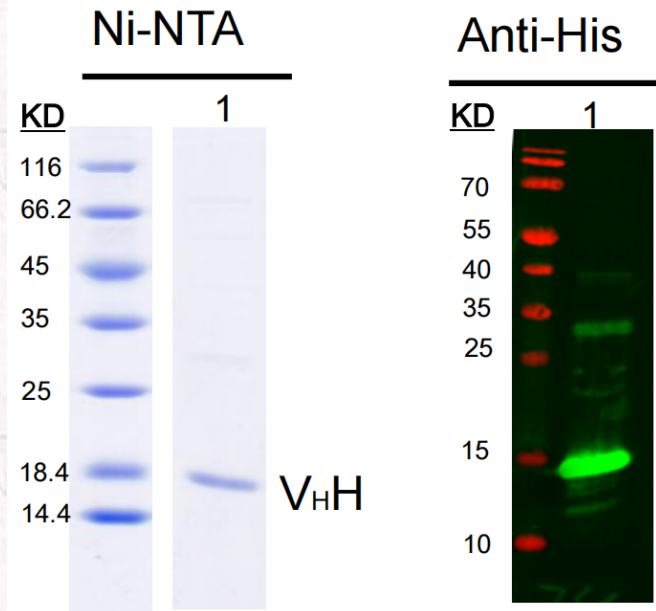


无细胞正常表达



293细胞表达纯度低

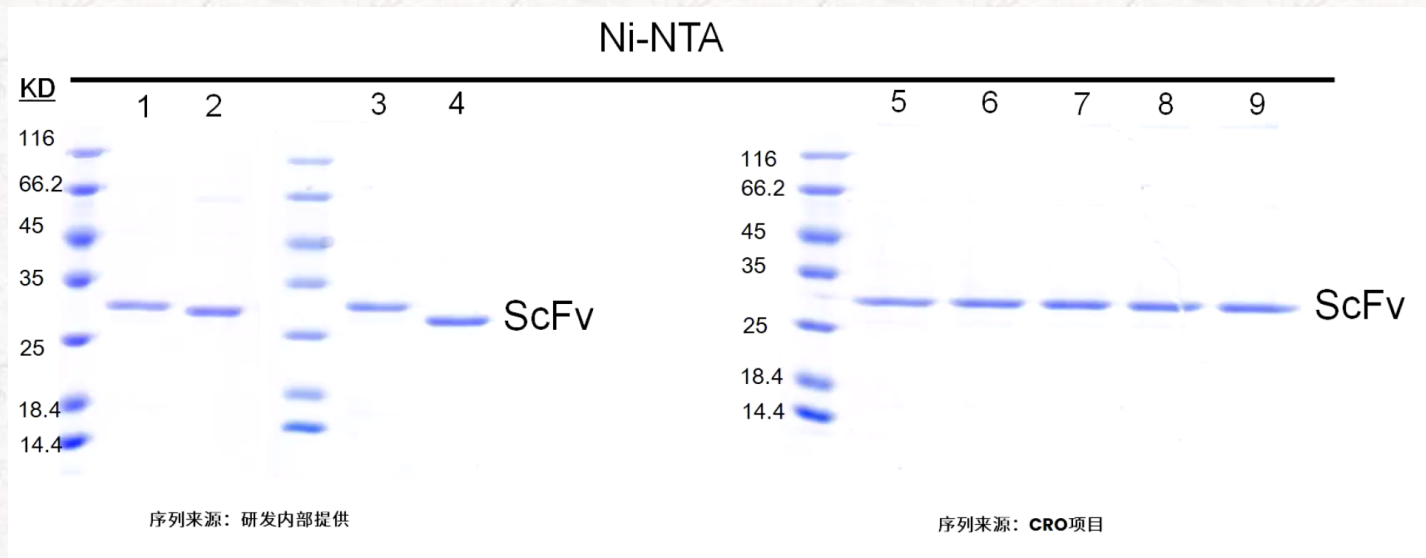
● 无细胞系统可以表达细胞中较难表达的VHH



#	时间	峰面积	峰高	峰宽	对称因子	峰面积 %	类型
1	11.448	60.1	1.1	0.7126	0.829	6.88	BB
2	16.3	31.6	8.10E-01	0.5368	0.842	3.615	BB
3	18.597	781.5	19.4	0.6223	0.678	89.505	BBA

● 其他公司原核表达失败项目

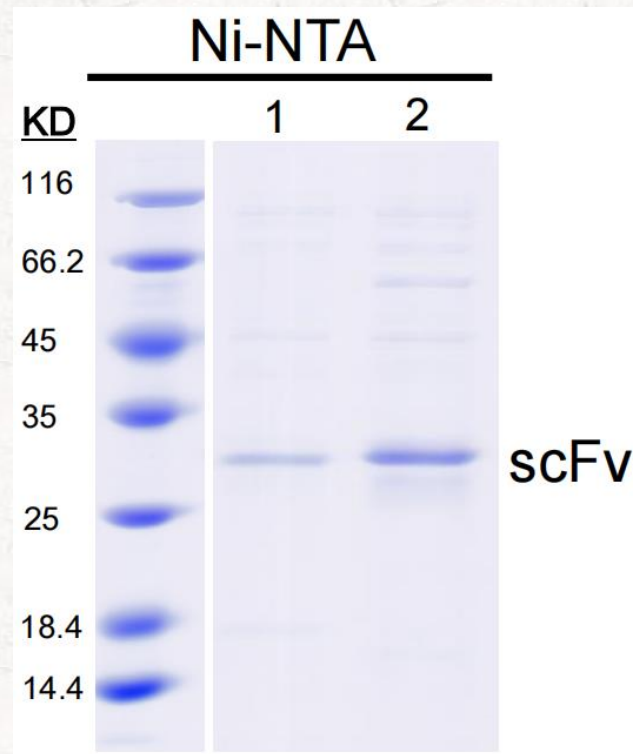
● 无细胞系统在ScFv快速表达的案例展示



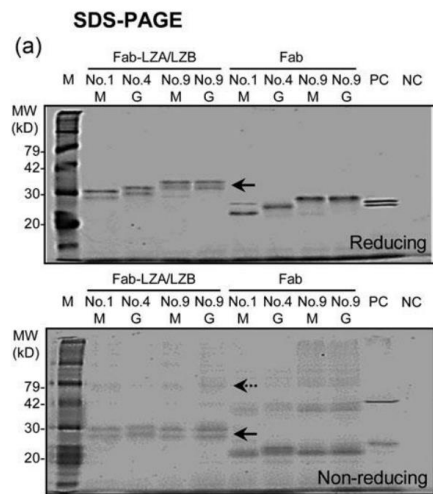
● 纯度大于85%，表达量约为150-500ug/mL，产量均大于200ug/6mL

- 无细胞系统可以表达细胞中较难表达的scFv

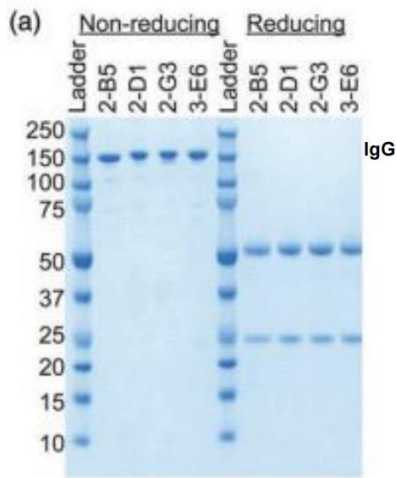
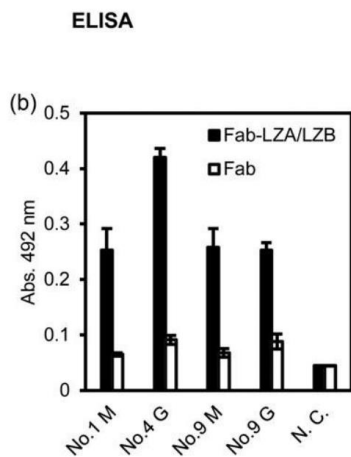
- 此两个scFv构建在HEK293细胞中均无法表达，而在无细胞系统中均可以有效表达纯化。



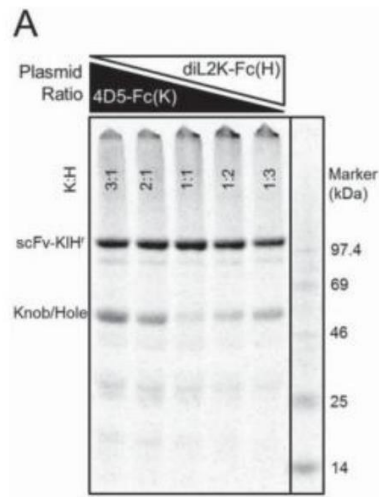
# 更长抗体片段表达



T Ojima-Kato et al. Protein Design Selection. (2016)

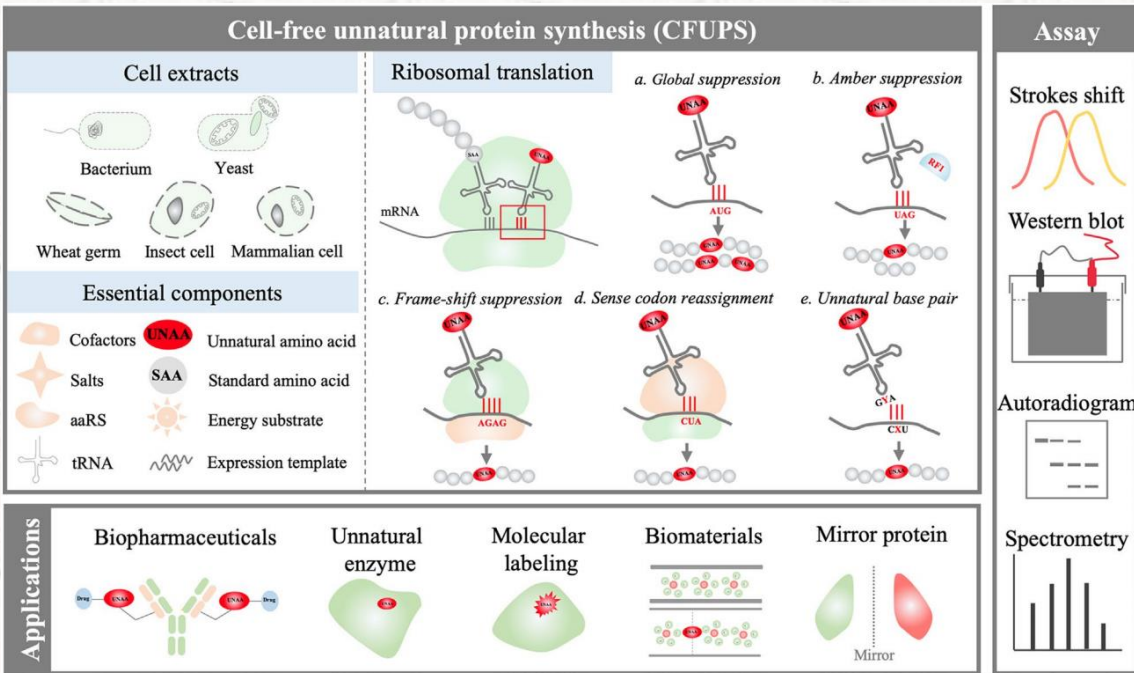


Dan Groff et al. mAbs. (2014)

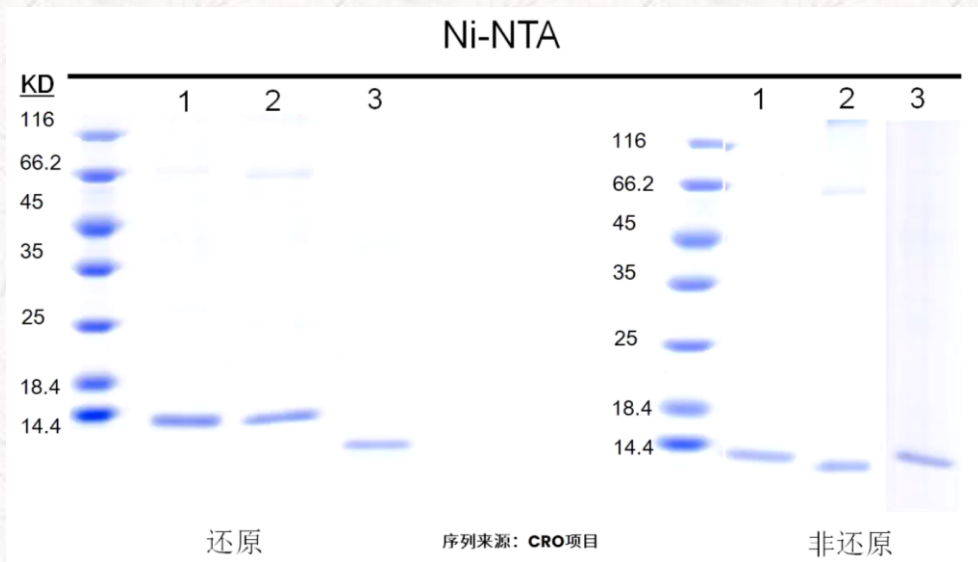


Yiren Xu et al. mAbs. (2015)

# 无细胞系统在非天然氨基酸引入中的应用

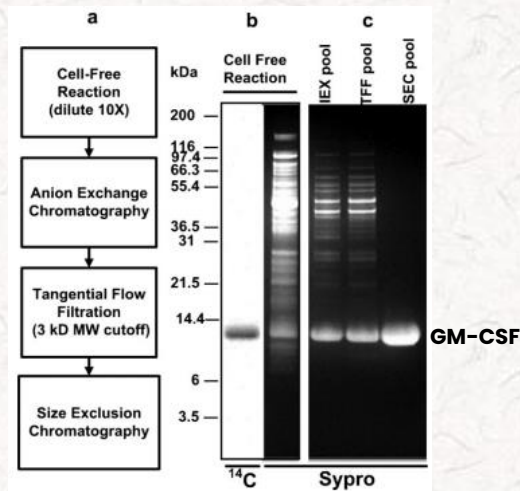
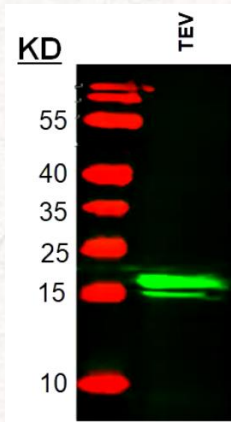
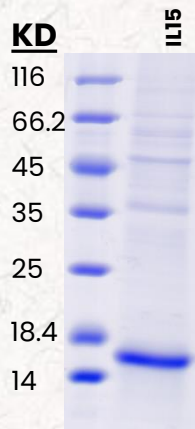


● 无细胞系统短肽快速表达案例展示



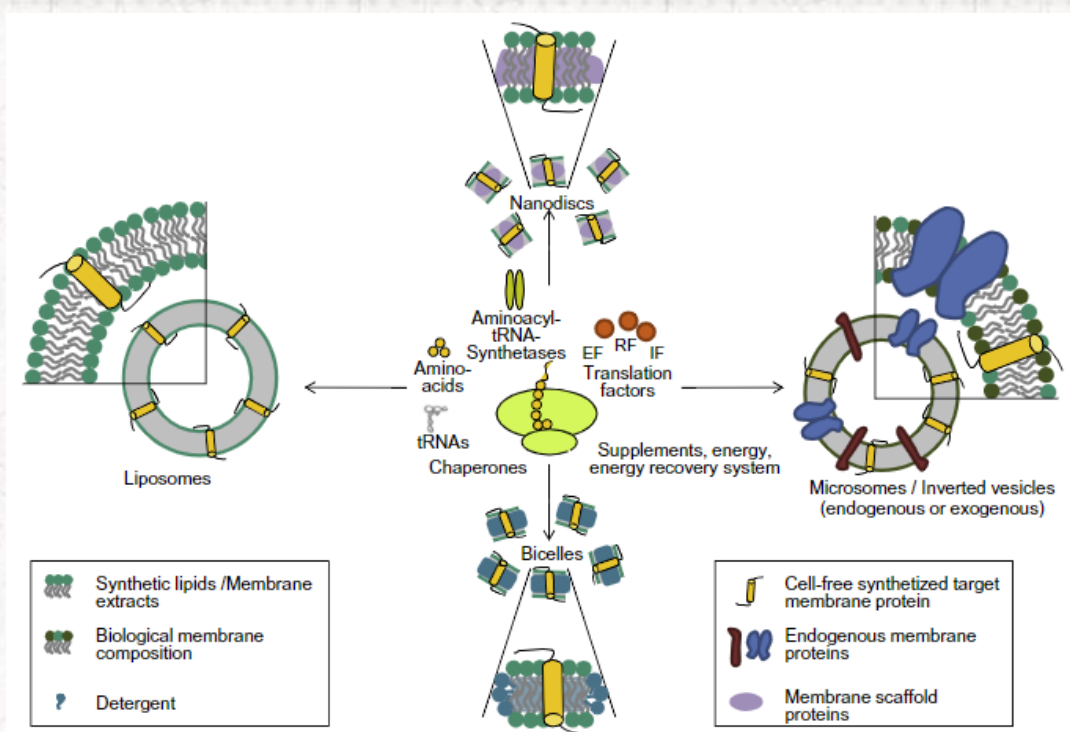
● E.Coli中均纯度大于80%，表达量约为50-100ug/mL，产量80-100ug/6mL

# 无细胞表达系统在细胞因子和蛋白酶表达中的应用



James F Zawada et al. BioTechnol Bioeng. (2011)

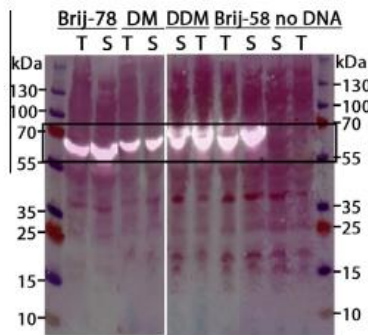
## ● 无细胞系统在膜蛋白表达中的应用



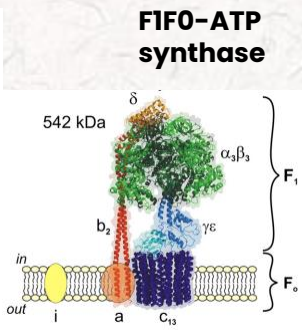
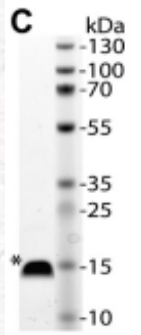
Rita Sachse et al. FEBS J. (2014)

# 无细胞系统在膜蛋白表达中的应用

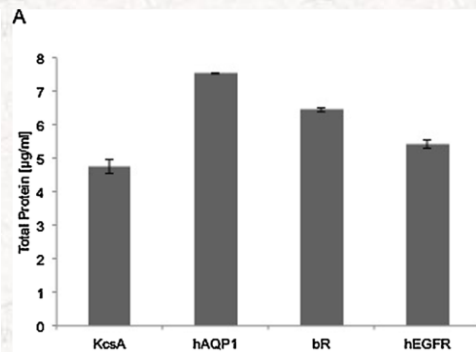
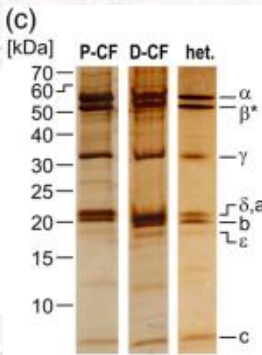
## NavSPIP



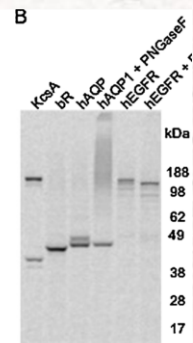
Gabriela et al. Protein Exaaaapr Purif. (2015)



Doreen et al. J Mol Biol. (2011)



Robert et al. J BioTechnology. (2015)

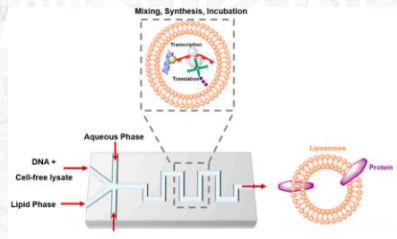


● 无细胞系统在高通量筛选中的应用

无细胞表达系统

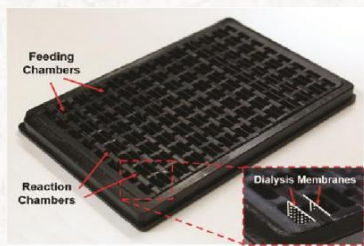
微流控  
检测

检测



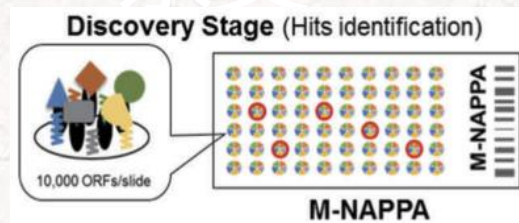
Damiati, Mhanna, et al. (2018)

各种条件  
及药物筛



Kirsten Jackson, et al. (2018)

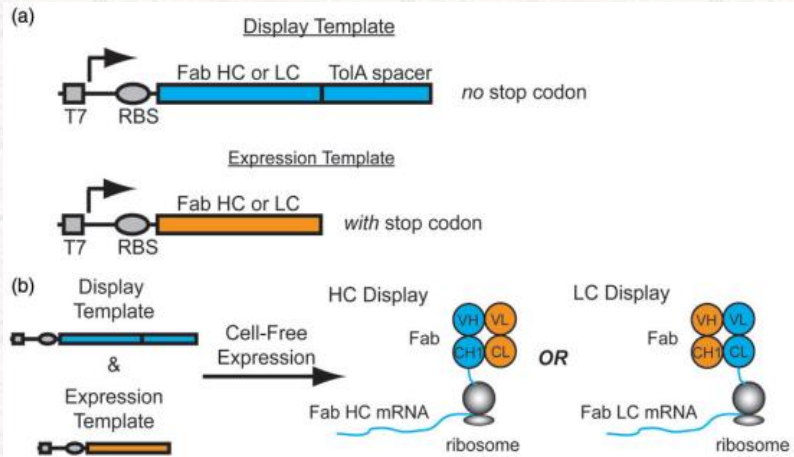
位表达及  
蛋白芯片



Karthikeyan K, et al. (2016)

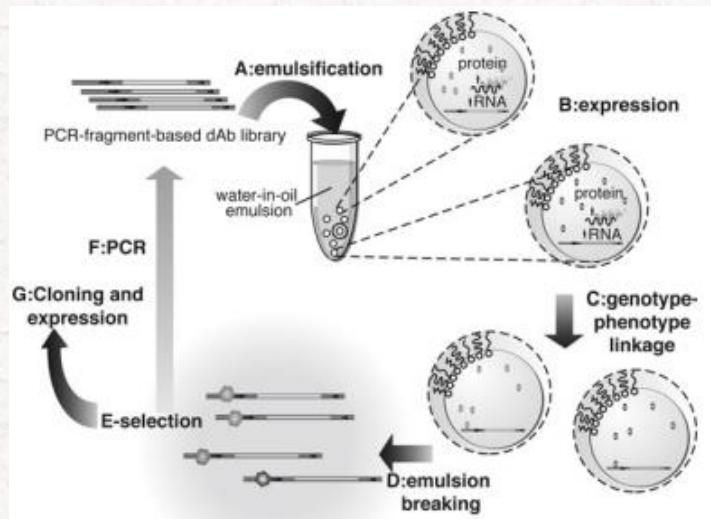
# 基于无细胞系统的展示技术

## Ribosome Display



Ryan L Stafford et al. Protein Design Selection. (2014)

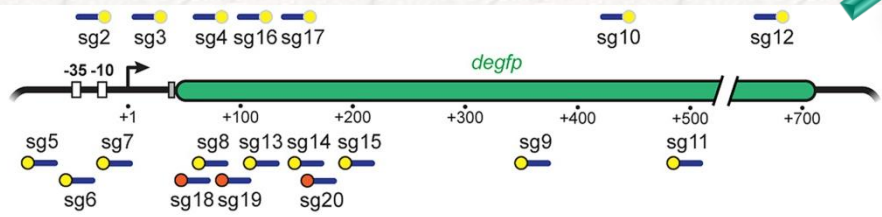
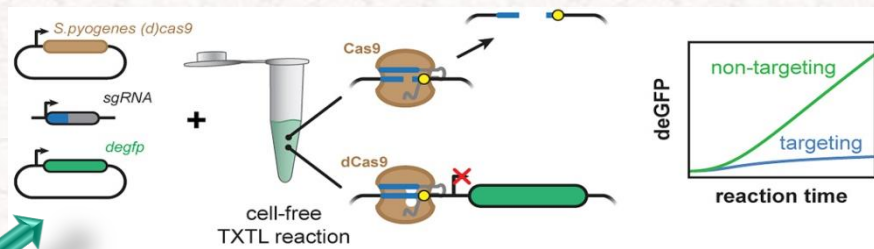
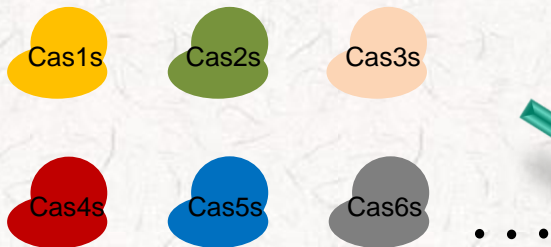
## In Vitro Compartmentalization Display



Armin Sepp et al. Methods in Mol Bio. (2012)

## 无细胞系统在CRISPR-Cas基因编辑系统中的应用

无细胞系统非常适合快速及高通量筛选gRNA、不同Cas和anti-CRISPR蛋白等相关因子



Ryan et al. Mol Cell. (2018)

● 无细胞表达系统面临的挑战

通用性

- 应用的广泛性和通用性还不如现有细胞系统

稳定性

- 批间一致性还需进一步优化
- 线性化模板的使用

成本

- Batch法成本相对较低，产量低，CECF产量高，

# THANK YOU

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Tel: +86-400-890-9989 Fax: +86-10-5095-3282

Website: [www.sinobiological.com](http://www.sinobiological.com)

Email: [order@sinobiological.com](mailto:order@sinobiological.com)