From Design to Delivery

ADCs through Cell-Free

SUTRO BIOPHARMA

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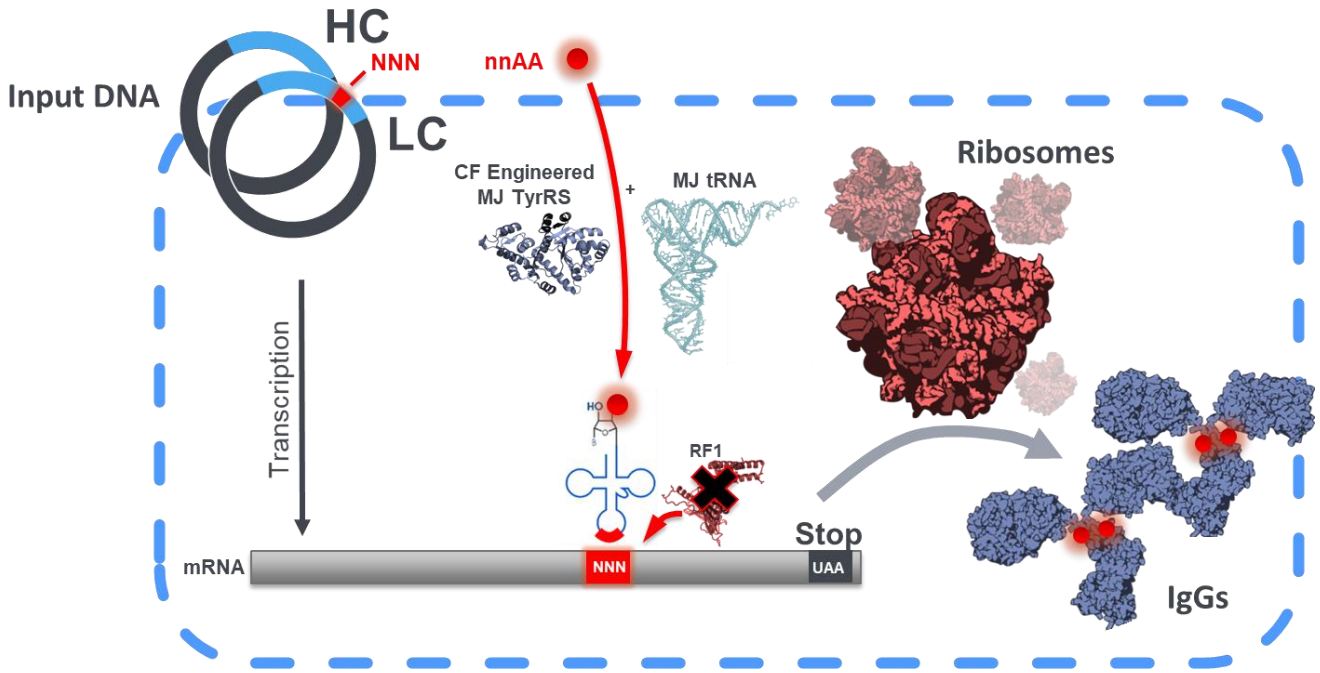
Cell-Free Primer in a Nutshell

- **Crude *E. coli* cell lysate**
  - Supplies ribosomes and translation factors
  - Can be stockpiled in advance
- **Provided Energy source, AAs, RNAP and Plasmid(s)**
- **Product expression occurs in the bulk mixture**
  - No cellular membranes/envelope barriers
  - No cellular responses to fight
  - Composition/conditions can be adjusted and controlled to suit product



Efficient nnAA incorporation

- No cell envelope barrier to nnAA transport
- No competition with HCP expression

Advantages of nnAA

- Enables specific and efficient conjugations
- Potential to incorporate multiple different nnAA with orthogonal chemistries allowing multiple, different linker payloads on a single ADC

One Extract for all Products

Antibody

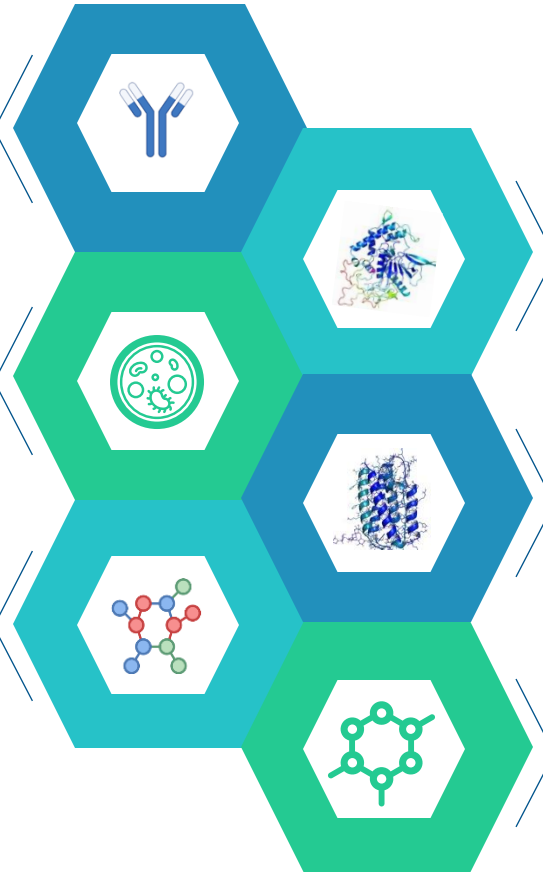
- Bispecific antibodies
- Monoclonal antibodies
- Antibody drug conjugates
- Antibody radioisotope conjugate

Antigen/Vaccine

- CRM197
- Pfs25
- *P. gingivalis* proteins
- RBD of SARS-CoV-2 spike protein

Peptide Fusion

- Integrin-binding knottin-Fc



Protease

- Recombinant plasminogen activator

Cytokine

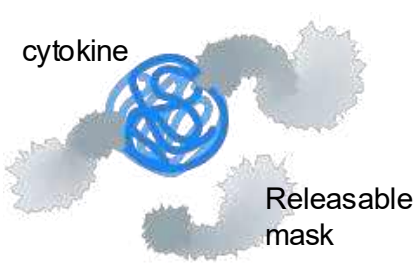
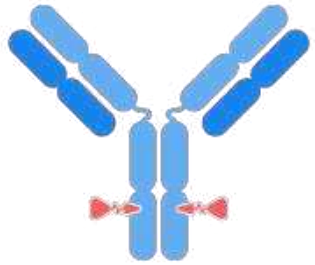
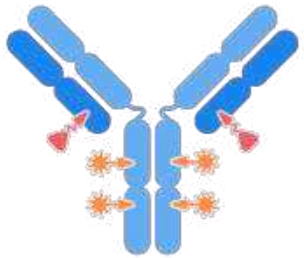
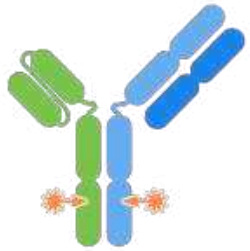
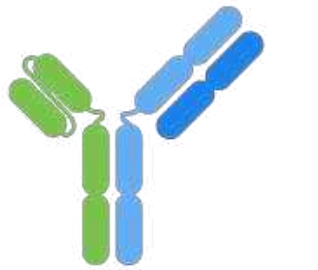
- GM-CSF, G-CSF
- IL-2
- IL-18
- IFN

Peptide

- Ceratotoxin

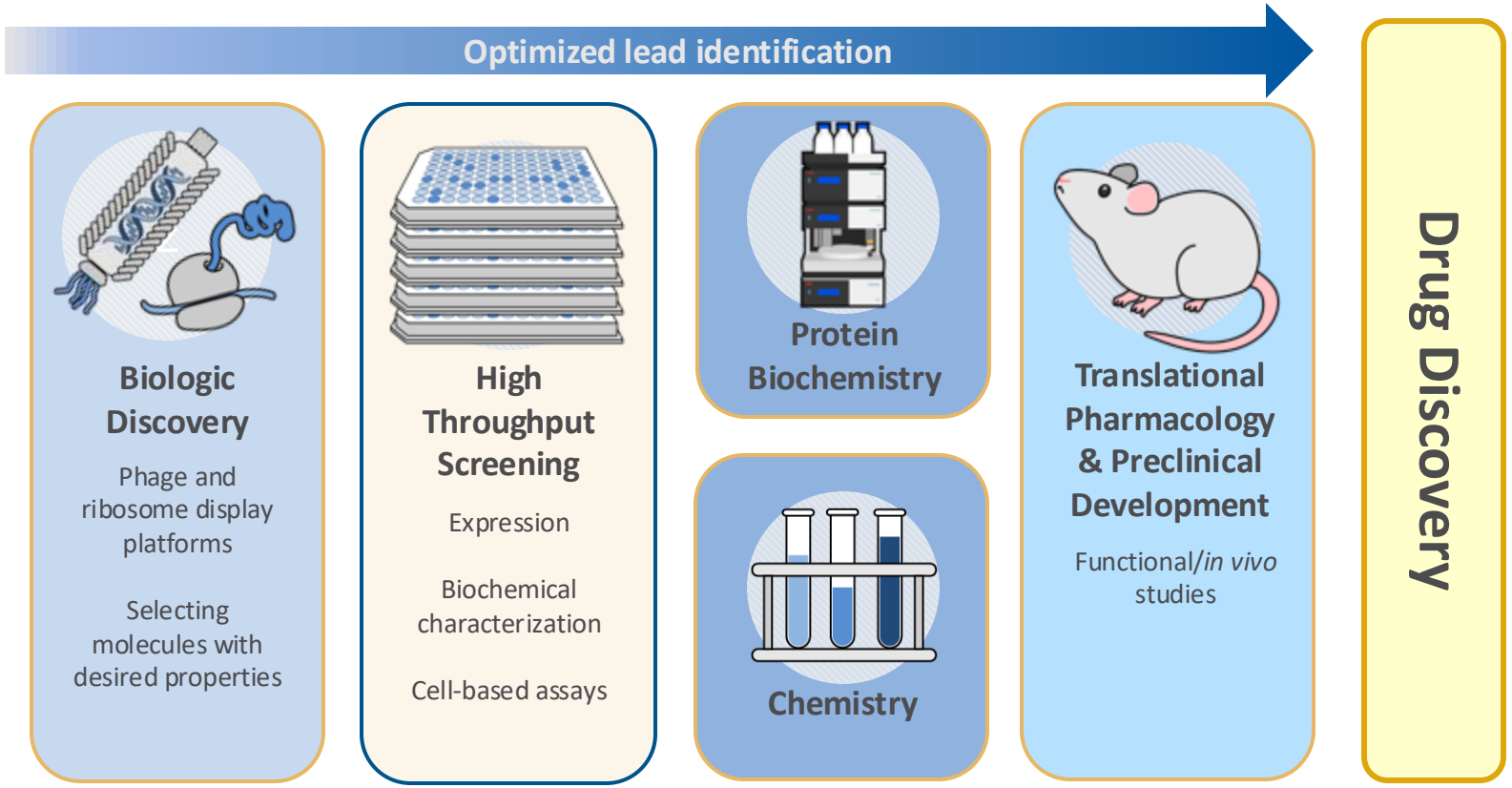
GM-CSF = granulocyte-macrophage colony-stimulating factor, IL-2 = interleukin-2,
Pfs25 = Pfs25, a postfertilization mosquito-stage surface expressed malaria antigen

Drug Discovery Platform Can Enable Multiple Modalities

	Cytokine Derivative	Conjugated Antibody			Bispecific Antibody	
Modality	*Prodrug Cytokine Derivative*	*ADC or ISAC*	*iADC / Dual Payloads*	*Bispecific ADC*	*Immune Cell Engager*	
Target	Tumor Selective Mask	Tumor Antigen	Tumor Antigen	Dual Tumor Antigens	Tumor or Stromal Antigen	Immune Cell Engager
Structure						
Drug Properties	Prodrug cytokine targeting functional cytokine to tumor	ISAC: Immune-stimulating ADC: targeting novel payloads	Site-specific dual drug conjugate with complementary modalities (TME modulator +/- immune modulator)	Enhanced tumor targeting of cytotoxic payloads	Optimized format and affinity Improved specificity for optimized therapeutic window	

iADC = immunostimulatory ADC, ISAC = immune-stimulating antibody conjugate

Rapid Biologic Discovery Enabled by Rapid Make / Test Cycle



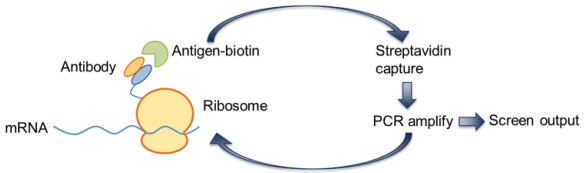
- Cell-free protein synthesis and site-specific conjugation are rapid and robust, allowing the production and characterization of numerous **mAb, DAR, linker and payload variants** in weeks for discovery.
- Capable of producing sufficient materials for *in vivo* studies, even in the early stage, including NHP toxicity studies.

Cell-free expression for Highly Efficient Antibody Discovery and Built-in Manufacturability

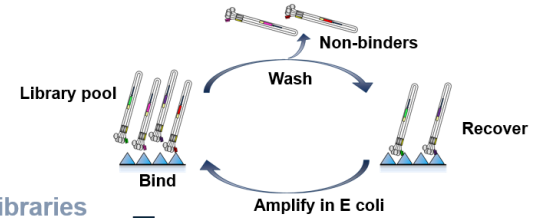
Candidate identification with **built in manufacturability**

Synthetic common LC libraries:
- Codon optimized for CF expression
- Sequence liabilities minimized for favorable biophysical properties
- Clinically validated framework(s) pre-screened for robust CF expression

Ribosome display (preferred platform):
- Built in bias for variants that express and fold better in CF
- Minimal library construction time, large library size (10^{12})
- Selection output translates directly into CF-based screening



Phage display:
- Enables alternative selection methods or targets not as accessible with ribosome display
- Produced in *E. Coli* cultures



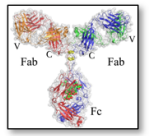
Immune Libraries
rabbit, mouse, chicken

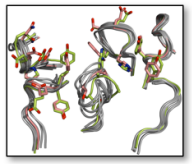
Humanize with CF-favorable frameworks & affinity mature candidates with best CF developability profiles

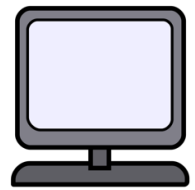
Pan immune library for binders to target antigen

Candidate refinement to minimize liabilities

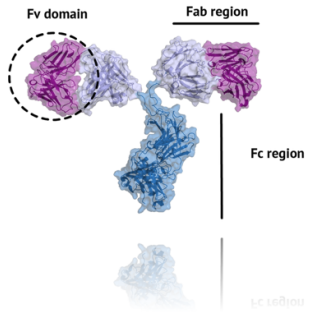
X-ray structure database → **Homology model** → **Molecular simulation** → **Model Fv surface properties potentially relating to:**
- Off-target clearance in vivo
- Aggregation
- High viscosity
- Extremes in pI
- Purification matrix interactions







Dynamically assess protein surface property when solvent exposed under variable temperature over time



In silico analysis

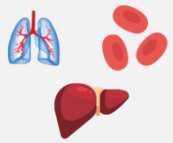
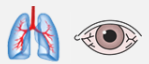
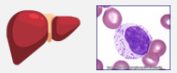
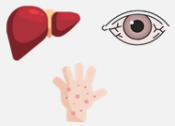
+

Sequence analysis

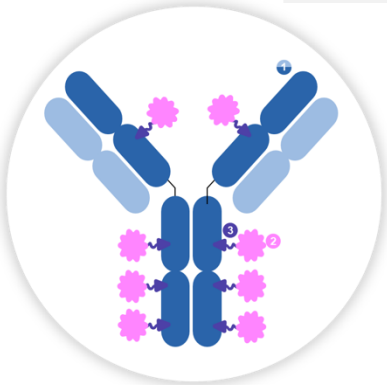
Potential liability motifs:
- *High risk* : always re-engineer
- *Low risk* : prefer to avoid; assess experimentally and determine next steps

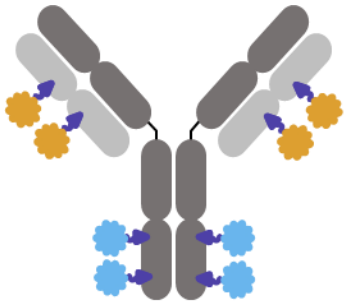
High throughput screen of 100s to 1000s of potential sequences
Target to lead in weeks

Improved ADCs by Design

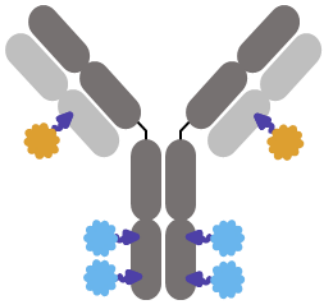
	ADC	mAB	mAb	Linker	Conjugation Chemistry	Payload
MOA inducing Tox	Untargeted Pinocytosis	Impaired FcRn recycling	FcγR uptake	Cleavage outside tumor	De-conjugation	Catabolism "Detox"
Toxicity Types						
Sutro Technology Improvements	Linker design & mAb eng.	Site specific conjugation	Lack of FcγR engagement	Linker design & chemistry, site selection	Click chemistry	Payload engineering

- Eliminates Fcγr and complement binding
- No ADCC / CDC activity
- Multiple payloads with complementary modalities in a single ADC with tunable and precise stoichiometry. Combination products delivered as single entities simplifying clinical evaluation
- Rigorous antibody selection with engineered site specificity for improved PK, lower toxicity, superior efficacy and ability to overcome payload resistance (location matters)

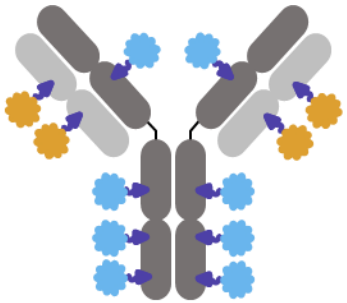




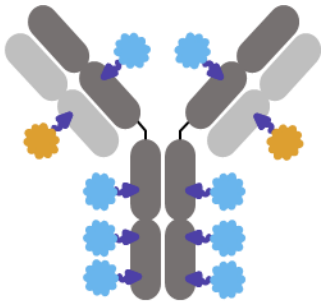
DAR 4+4



DAR 4+2

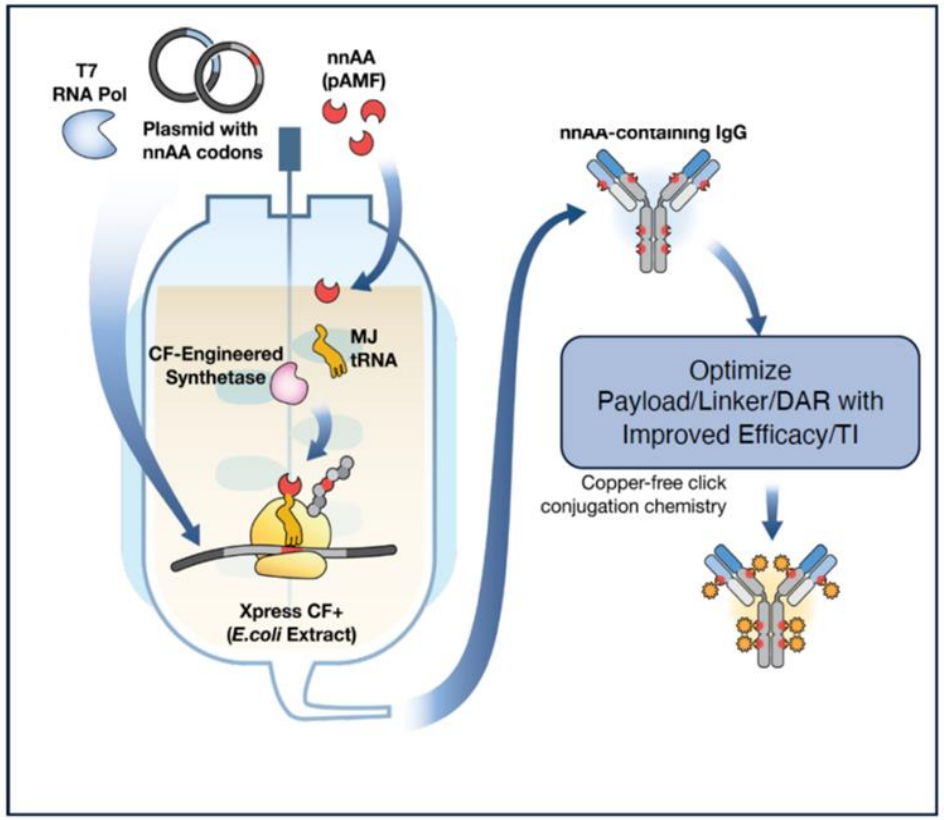


DAR 8+4



DAR 8+2

ADC Manufacturing by Cell Free Mab Production Simplifies CMC Development



- Cell free reaction ingredients can be manufactured at scale and stored and deployed in real time
- Eliminates cell line engineering and development for each product
- Ease of platform processes deployment for antibody manufacturing and linker-payload conjugation allow for simple and time-efficient tech transfers
- The process scales seamlessly from discovery-scale in multi-well plates to multi thousands of liters at manufacturing scale
- CMC modules are reproducible driving efficiency in regulatory submissions

Scalability of Cell Free Processes

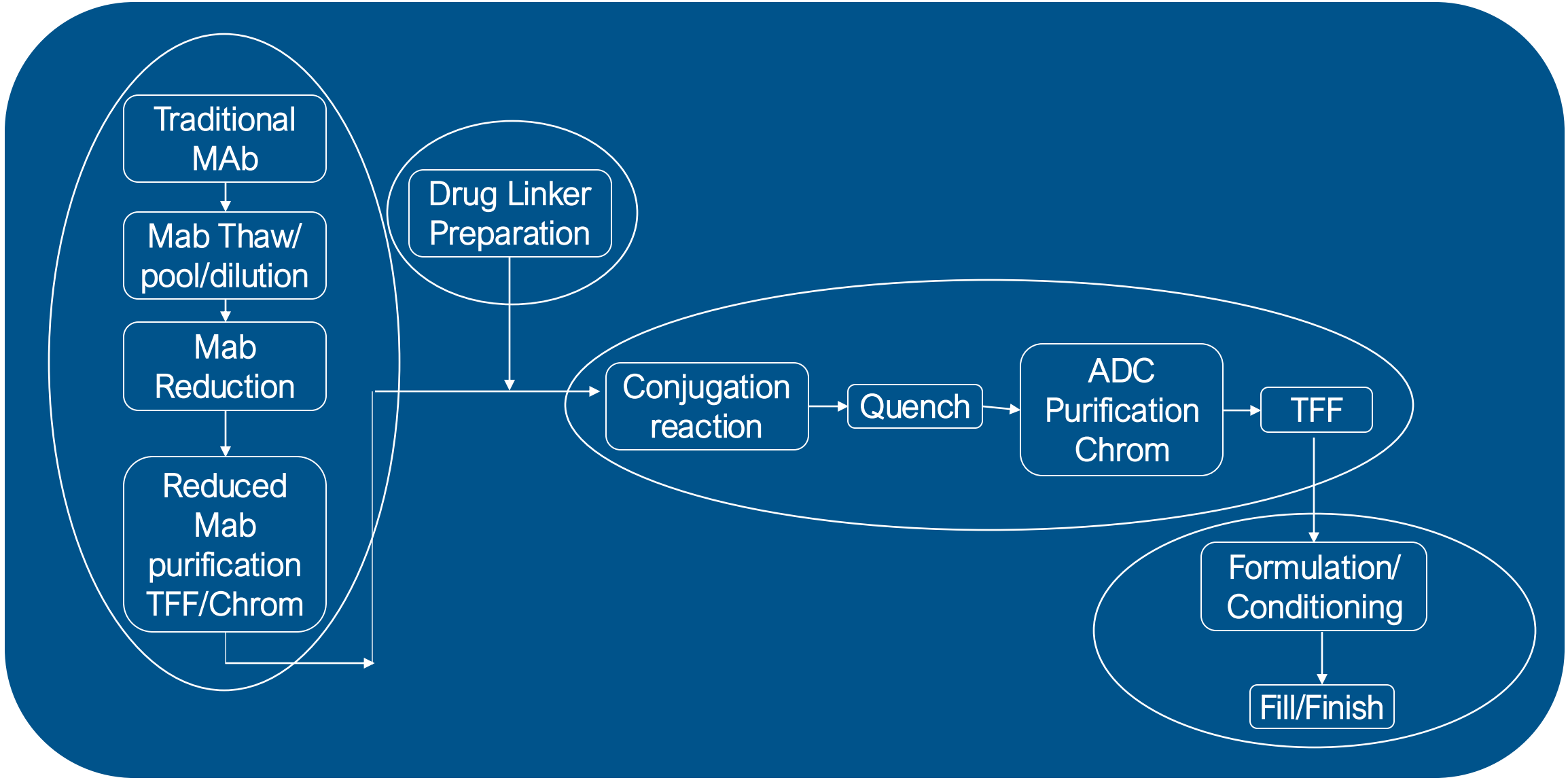
Scale-up **Manufacturing**

0.1 mL	1 mL	10 mL	100 mL	1 L	10 L	100 L	4000 L
Limited Control	Moderate Control	Full Control (Temperature, pH, dO2, feeding)					

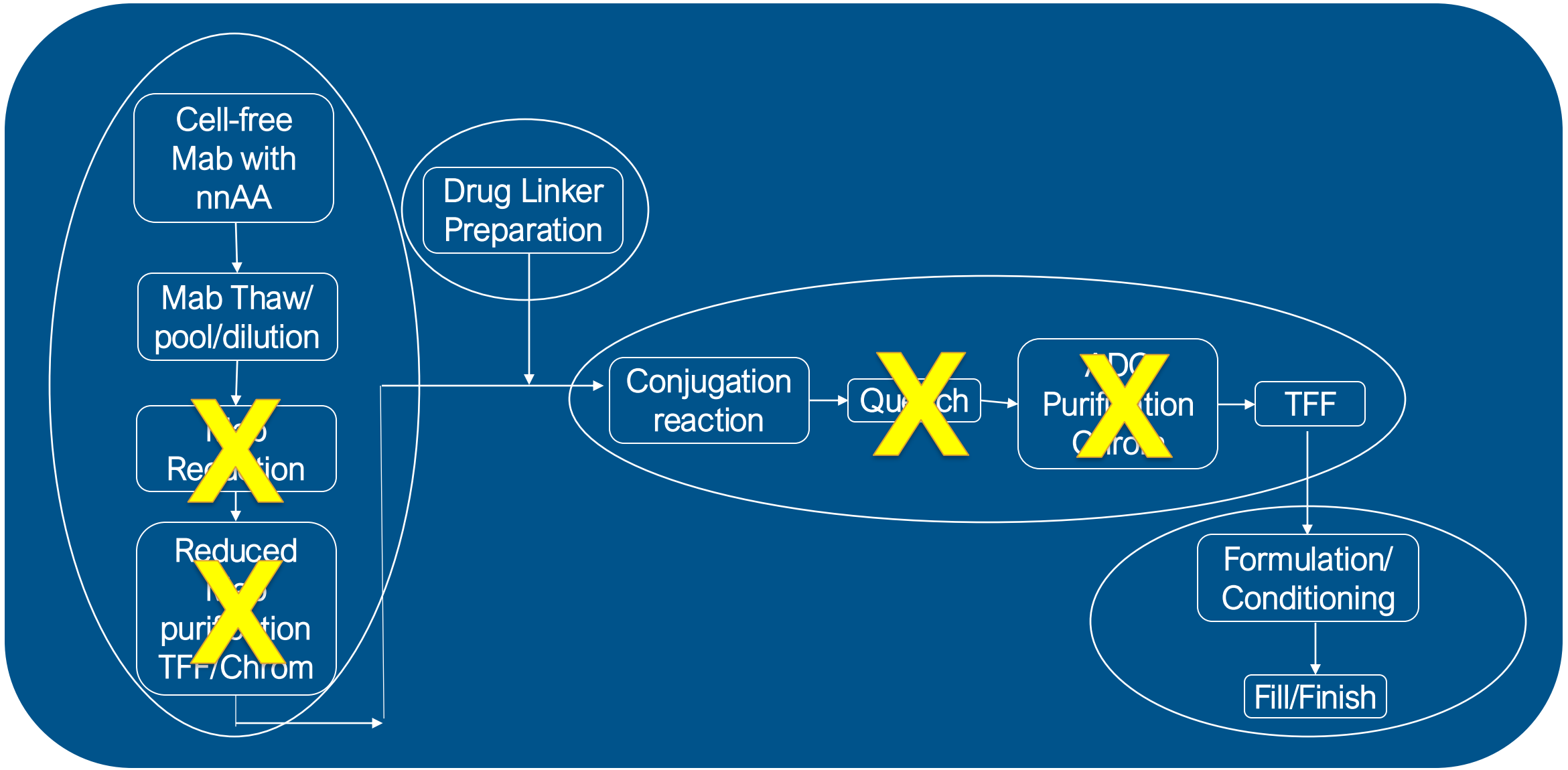
Multi-well Plates	mL-scale Bioreactors	0.2L Multi-Bioreactors	Scale-up Bioreactors 0.5 - 200 L	Manufacturing 1kL to 4kL

- Direct linear scale-up from HTS to production scale
- Minimal, rapid process development
- Uses standard bioreactors & downstream equipment
- Gene sequence to drug substance in days

ADC Manufacturing through Cell-Based Processes for MAbs



Efficiency of ADC Manufacturing from Cell-Free Mabs with nnAA



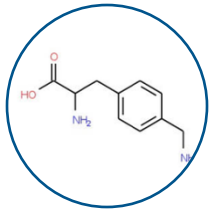
Improved Supply Chain Flexibility and Speed to Clinic enabled by Modular Manufacturing of ADCs

Product Agnostic Modules

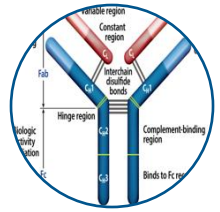
Off-the-shelf components for repeated use in many products

Cell Free Ingredients

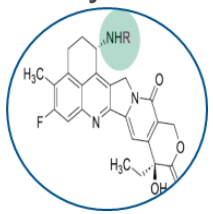
nnAA choice



PFLC choice



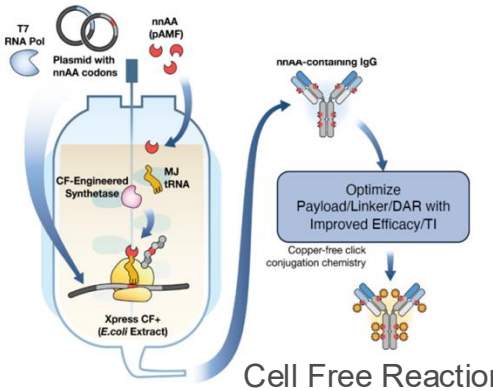
Linker Payload library



Product Specific

Real Time

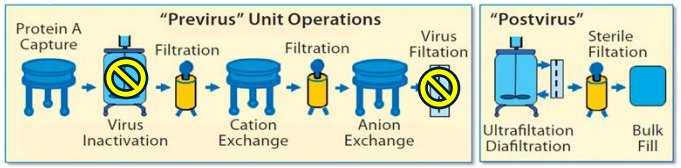
- ✓ Plasmid is the only product-specific ingredient
- ✓ Modular manufacturing using **off-the-shelf** components
- ✓ Cell free antibody production in less than one day
- ✓ Platform processes enable short tech transfer timelines
- ✓ Supply chain flexibility by building inventories of product agnostic modules to serve multiple products



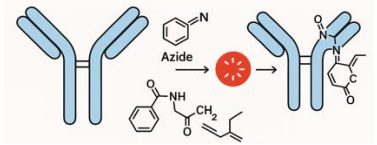
Cell Free Reaction

Simplified Purification and Conjugation Processes

Industry Standard Downstream Process



High efficiency / high yield
Click-chemistry based conjugation



Standard Fill Finish liq/lyo

Improved speed from development candidate to IND

Modularity Supports Flexible Supply Chain and CMC Concepts for Rapid and Scalable Supply from Clinical to Commercial Scale

Research

- Modular product design enables efficient selection among alternative product formats and modalities enabling the best candidates to be selected for pharmaceutical properties and manufacturability

Manufacturing

- Use of stockpiled off-the-shelf materials enables supply chain and manufacturing efficiency
- High similarity across the platform processes and analytics helps streamline tech transfer and scale up
- Modular and simple chemistry for conjugation across products facilitates reproducible, high-yield outcomes

CMC

- DMFs for core Cell Free Reagents and Linker-payload
- Standard product-specific Module 3 elements for each product, highly repeatable between product filings

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