Linkerology®
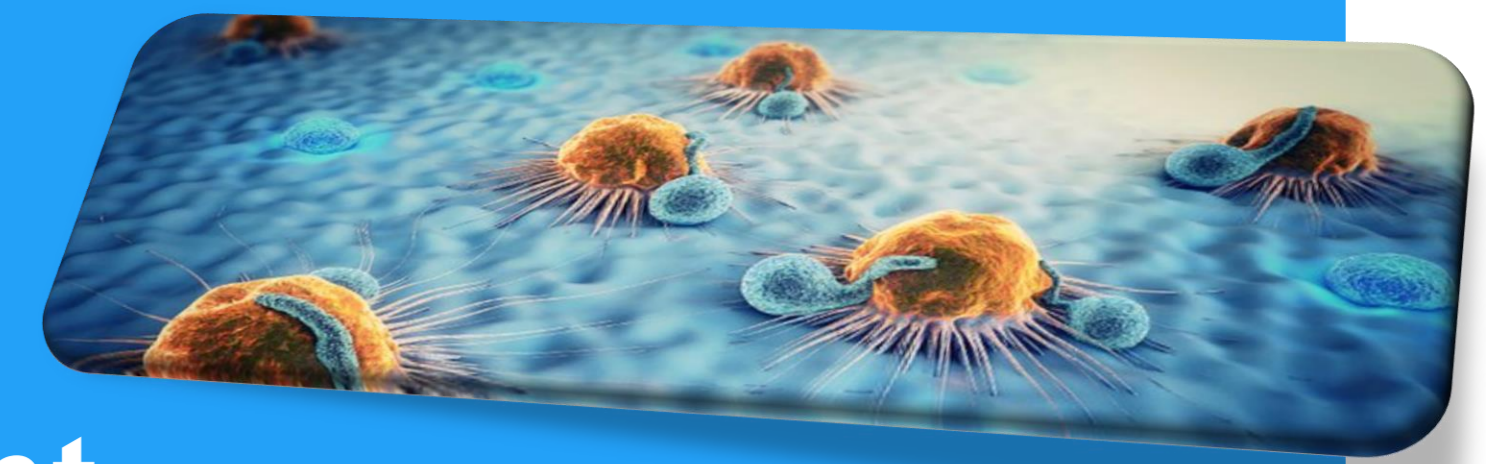
2023 # 06 – Bioengineering and Linker Attachment
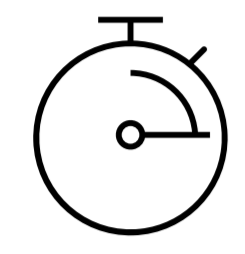
Method how Biomolecules can be Decorated with (Self-Immolative) Linkers

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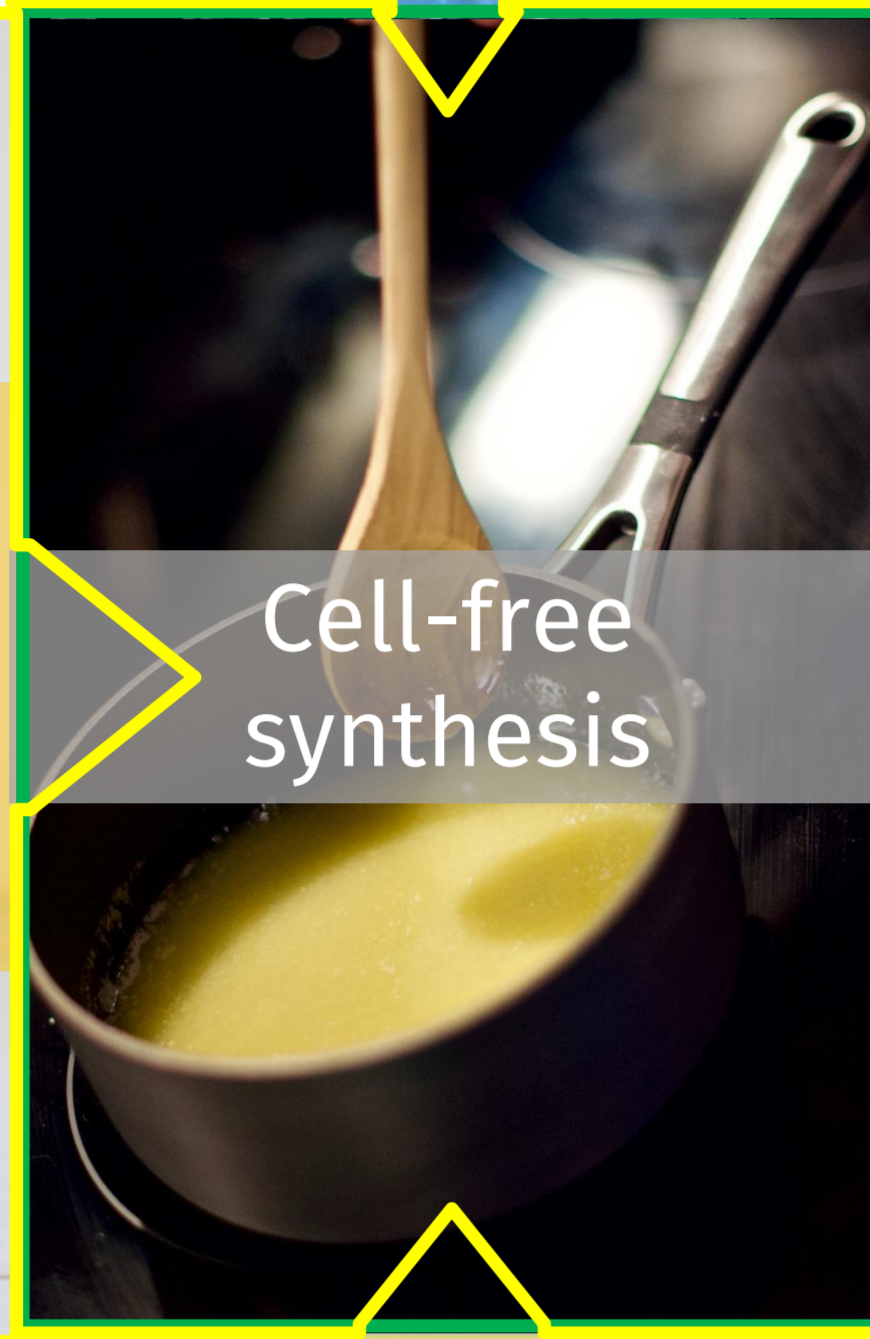## *Principle of Cell-free Reaction*

Short Reaction Time ~ 3 h

DNA → mRNA

CHO cell lysate
Ribosomes
IF – TF – EF
Aminoacyl-tRNA-synthetase

Cell-free synthesis

Amino acids Energy (ATP/GTP)

Unique Products
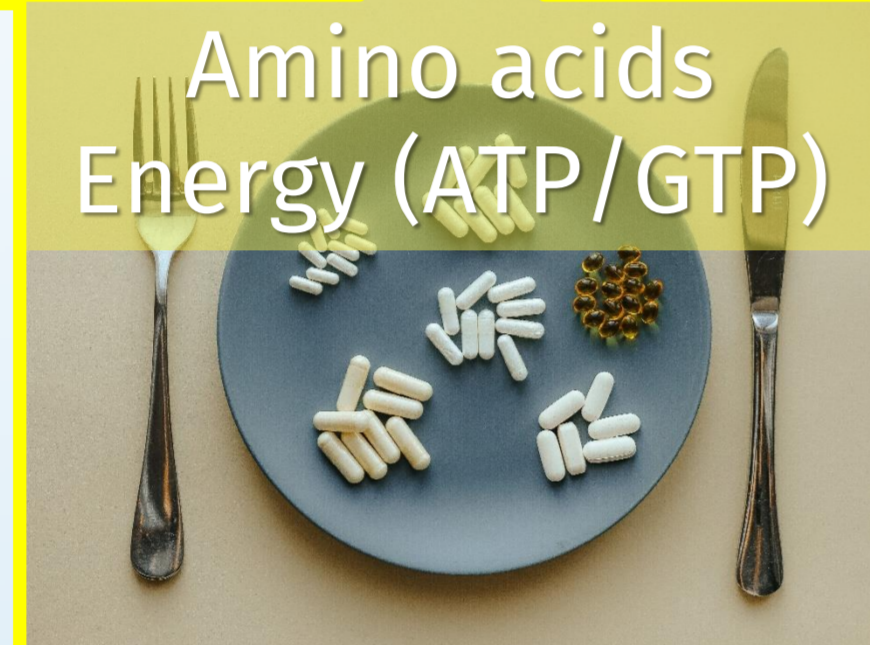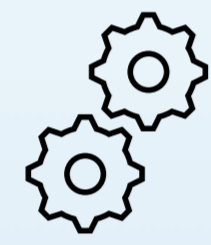
✓ Membrane proteins
✓ Glycoproteins
✓ Antibody fragments
✓ Toxic proteins
✓ Labeled proteins

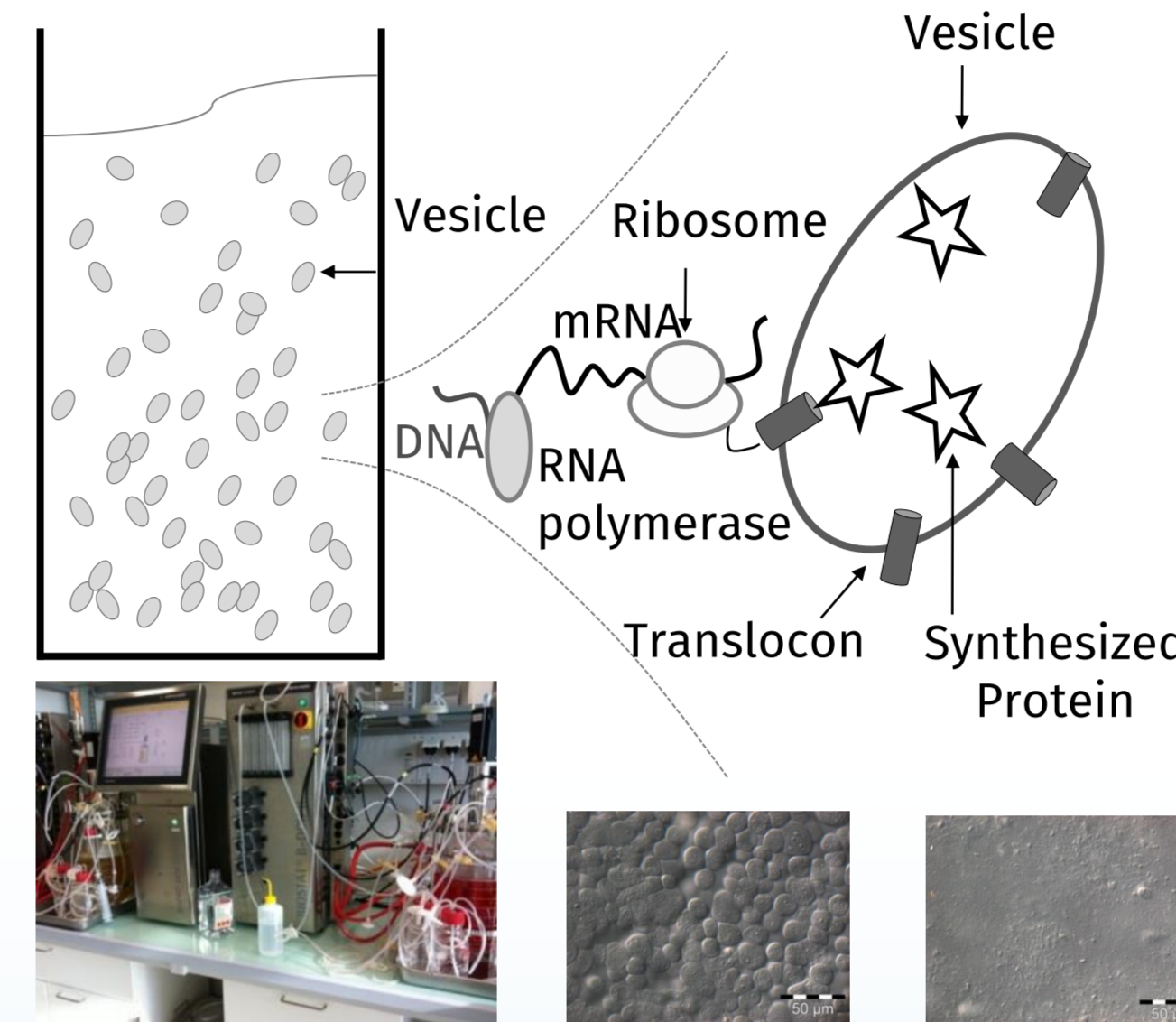**R&D & Production**

✓ Easy handling
✓ Scalability
✓ High yields

Flexibility & Versability
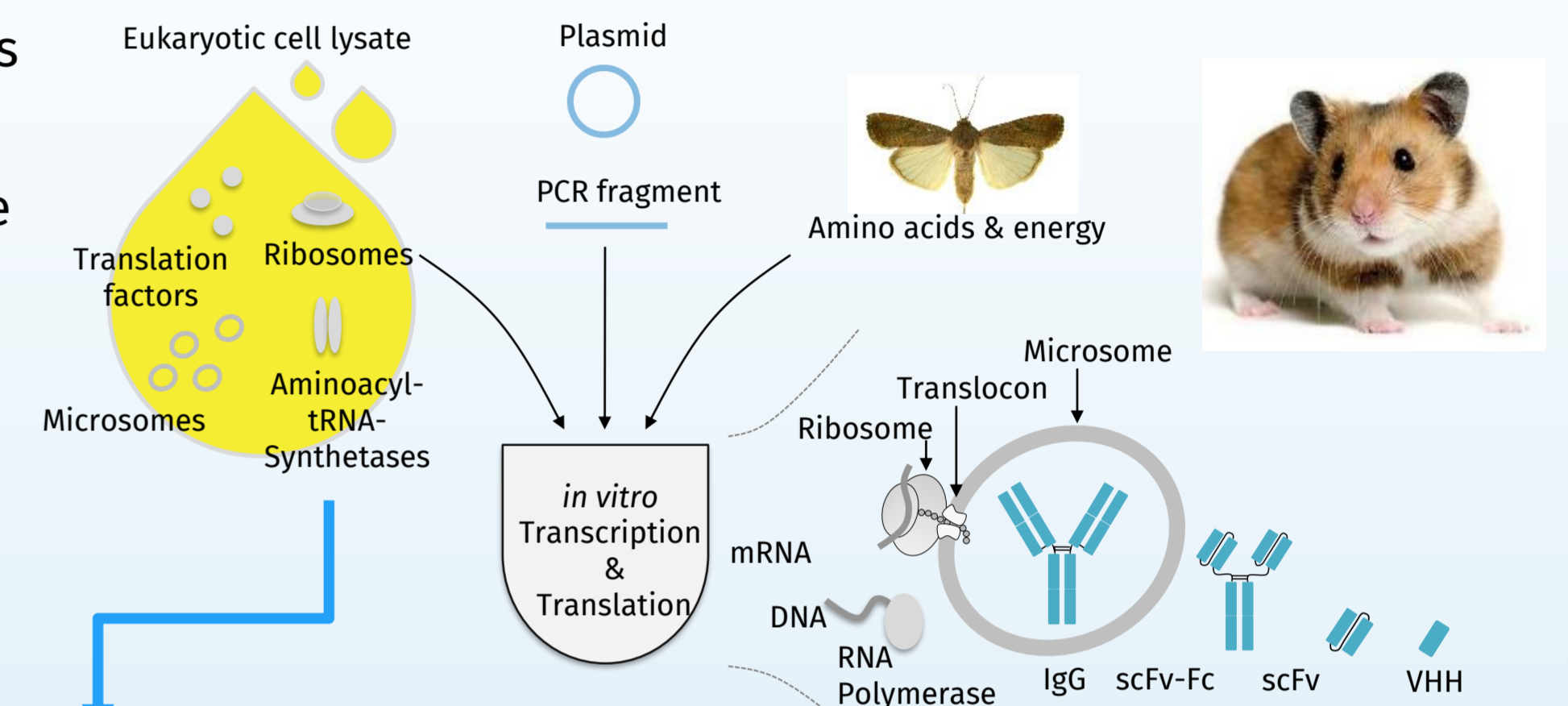
✓ HTS-compatibility
✓ Rapid domain screening
✓ Post-translational modifications in eukaryotes

Production Modi

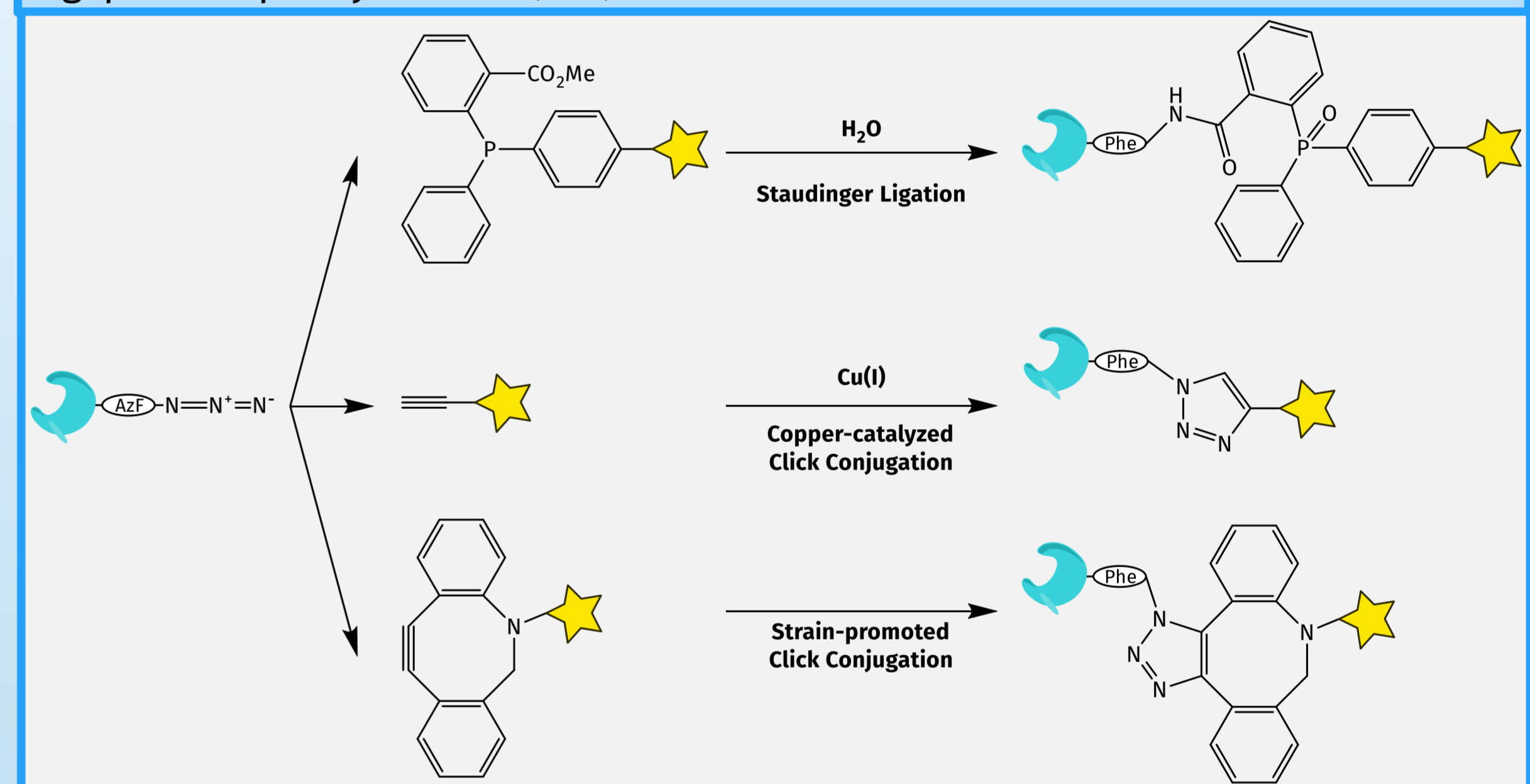### Batch Reactor

Reaction compartment

Vesicle
Ribosome
mRNA
DNA
RNA polymerase
Translocon
Synthesized Protein
Vesicle

Fermentation of mammalian cells
→ cell harvest
→ highly productive lysate

Dialysis Reactor

Reaction compartment
Reaction mixture:
Template
Lysate
RNA polymerase
Amino acids
ATP, GTP
Protease inhibitor
Target protein

Feeding compartment
Feeding mixture:
Amino acids
ATP, GTP
Protease inhibitor

Vesicle

Substrate, Energy
By-products

Eukaryotic cell lysate
Translation factors
Ribosomes
Microsomes
Aminoacyl-tRNA-Synthetases

Plasmid
PCR fragment
Amino acids & energy

in vitro Transcription & Translation

mRNA
DNA
RNA Polymerase

Microsome
Translocon
Ribosome

IgG   scFv-Fc   scFv   VHH

Engineering by side specific introduction of non-canonical amino acids e.g. p-azido-phenylalanine (AzF)

H$_2$O — Staudinger Ligation

Cu(I) — Copper-catalyzed Click Conjugation

Strain-promoted Click Conjugation

Linkerology® Tune your kinetics!

RL-3820-based **SLOWEST** — Drug

RL-3850-based **SLOW** — Drug

RL-3500-based **FAST** — Drug

RL-3550-based **FASTEST** — Drug
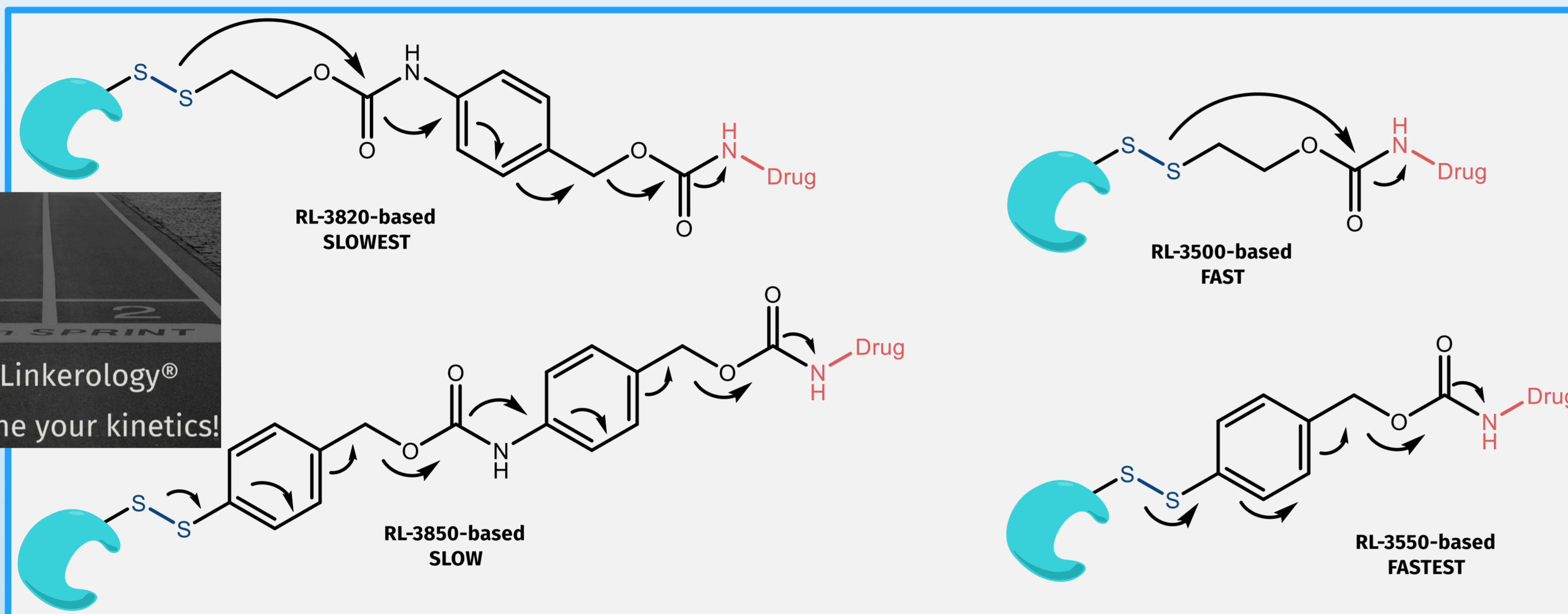
Linkerology® - Conceptual Overview

Carrier	Surface Treatment & Conjugation Chemistry	Cleavage	Fragmentation	Functionality of Natural Product
Metal surface	Affinity of sulfur to gold and silver	**Enzymatic hydrolysis:** • Val-Ala • Val-Cit • Phe-Lys • Gly-Phe-Leu-Gly • Ala-Leu-Ala-Leu • Cyclobutyl-Ala • Cyclobutyl-Cit • Glucuronic acid	*p*-Aminobenzyl *p*-Hydroxybenzyl	Primary & secondary amines
Metal oxide	Chelat formation		CO$_2$	H$_2$N—
Silicates	Affinity of silicon and oxygen			
Carbon: • Nanotubes • Fullerenes	Nitrenen addition via photoactivation of perfluoroarylazides		Oxathiolone X = NH, S	Tertiary amines
Plastic polymers: • Teflon • Polyethylene • Polystyrene • Latex	Ammonia or acrylic acid plasma followed by amide bond formation	**Reduction**		R$_1$ / N— / R$_2$ Alcohols Phenols
Biopolymers: • Peptides • Proteins • Antibodies • Single Chain • Nanobodies • Camelides • Oligonucleotides • Aptamers	Thioether formation with maleimide Disulfide bond formation Acylation of Amines His-Tag acylation Click conjugation (CuCAAC, SPAAC, IEDDA) Enzyme supported conjugation: HaloTag® CLIP-Tag™ SNAP-Tag® Sequence dependent conjugation (Sortase)	**Acidic hydrolysis**	Dimethylimidazolidinone	HO— Carboxylic acids

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