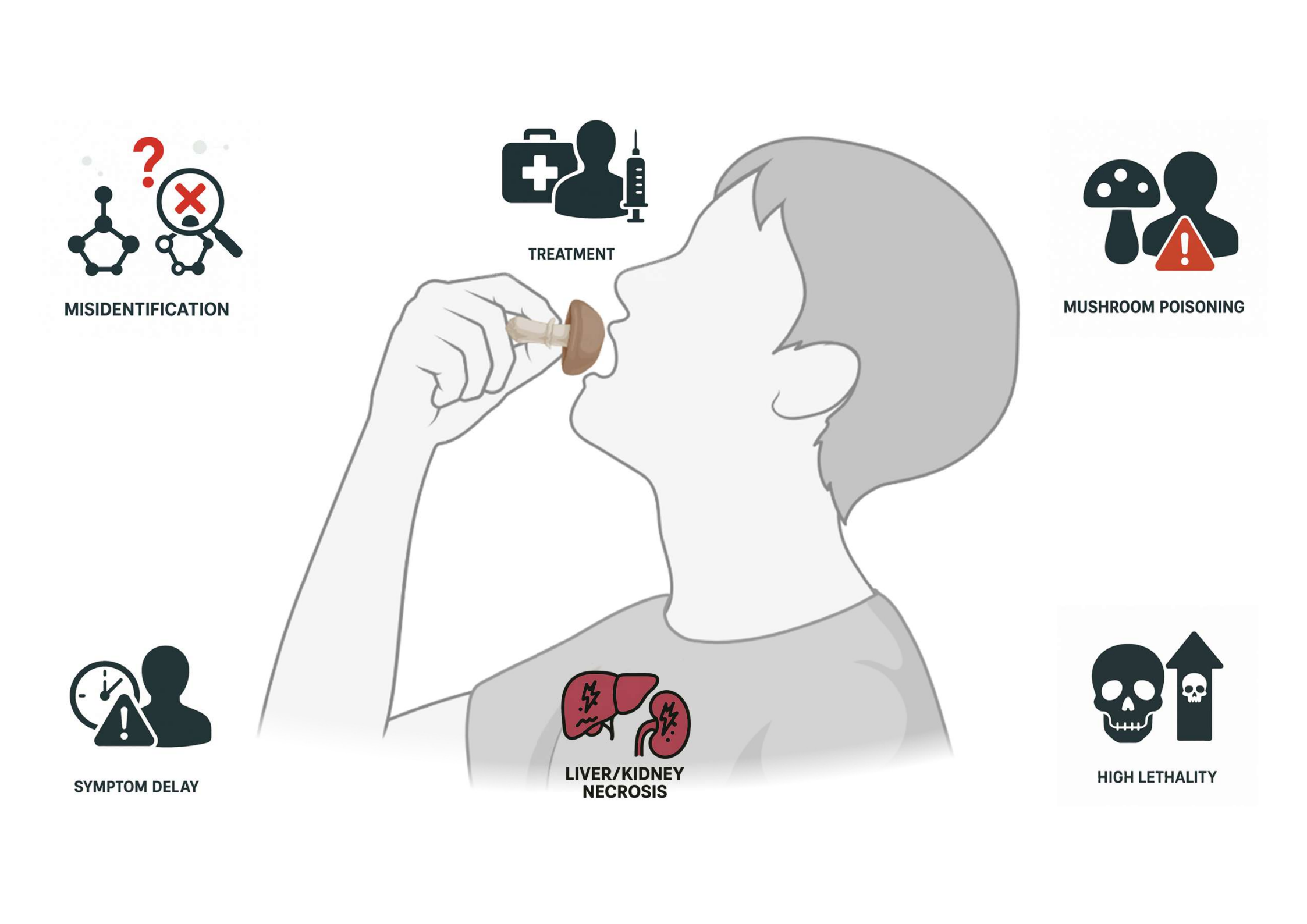

GEM
HAMBURG

WHO WE ARE?


DeathCapTrap
DeathCapTrap
DeathCapTrap

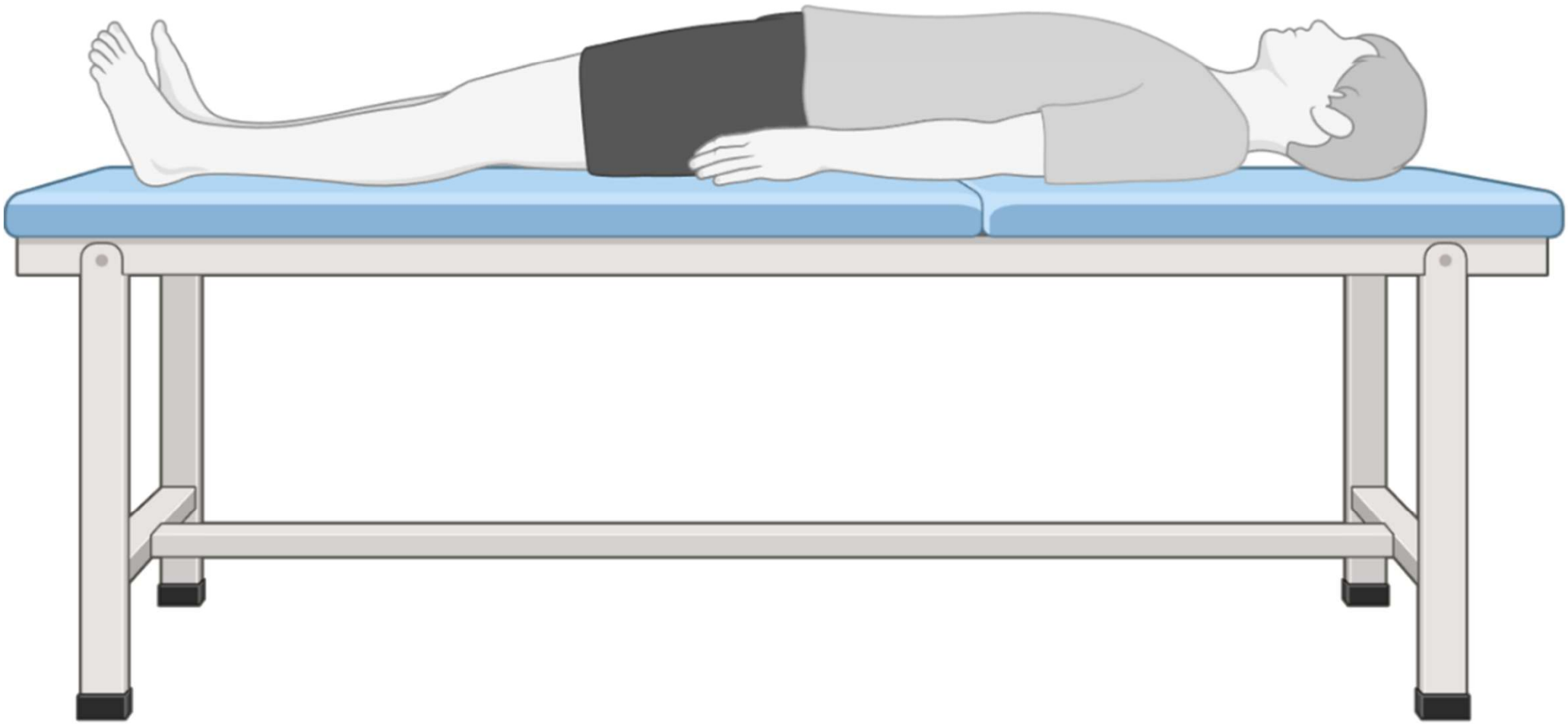
WHAT IS THE PROBLEM?

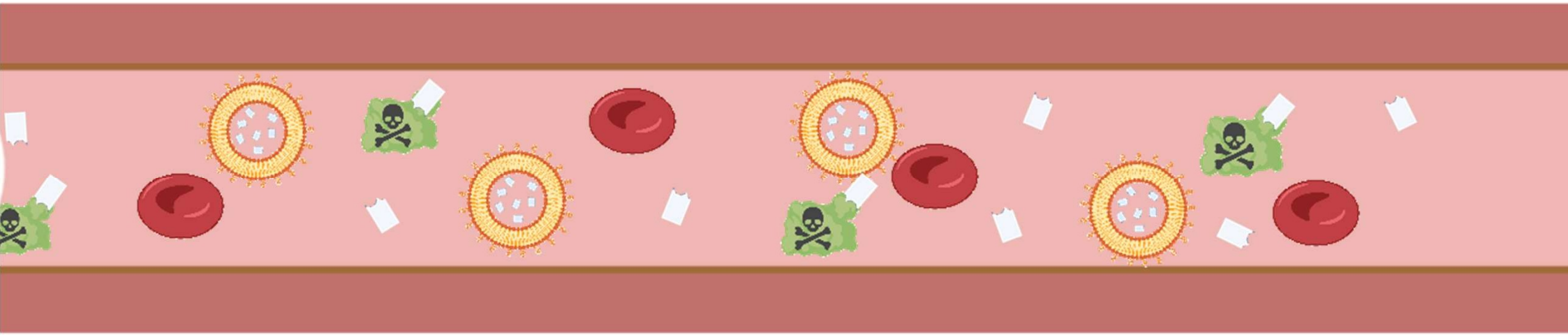

MISIDENTIFICATION
TREATMENT
MUSHROOM POISONING
SYMPTOM DELAY
LIVER/KIDNEY
NECROSIS
HIGH LETHALITY

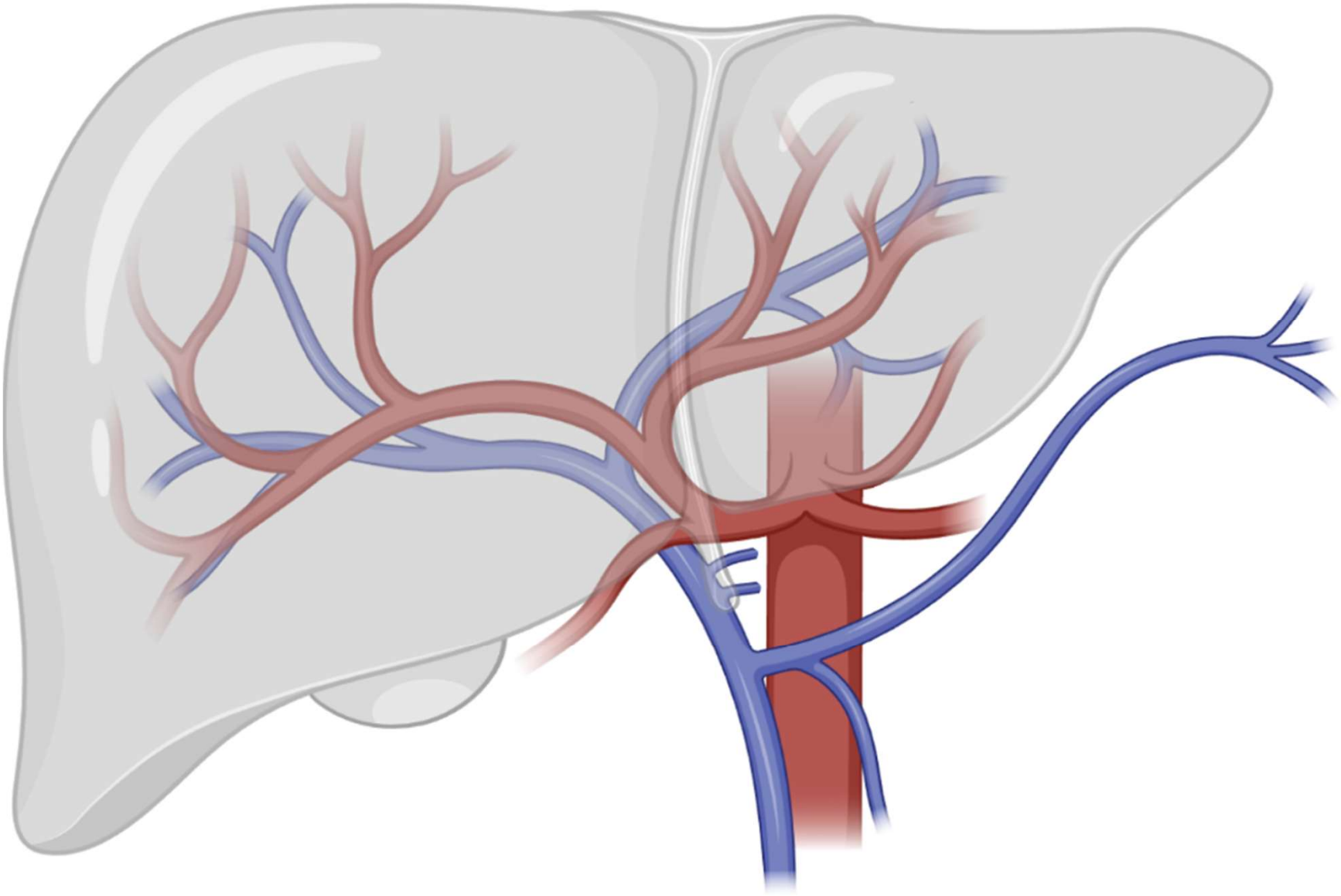
WHAT IS OUR SOLUTION?

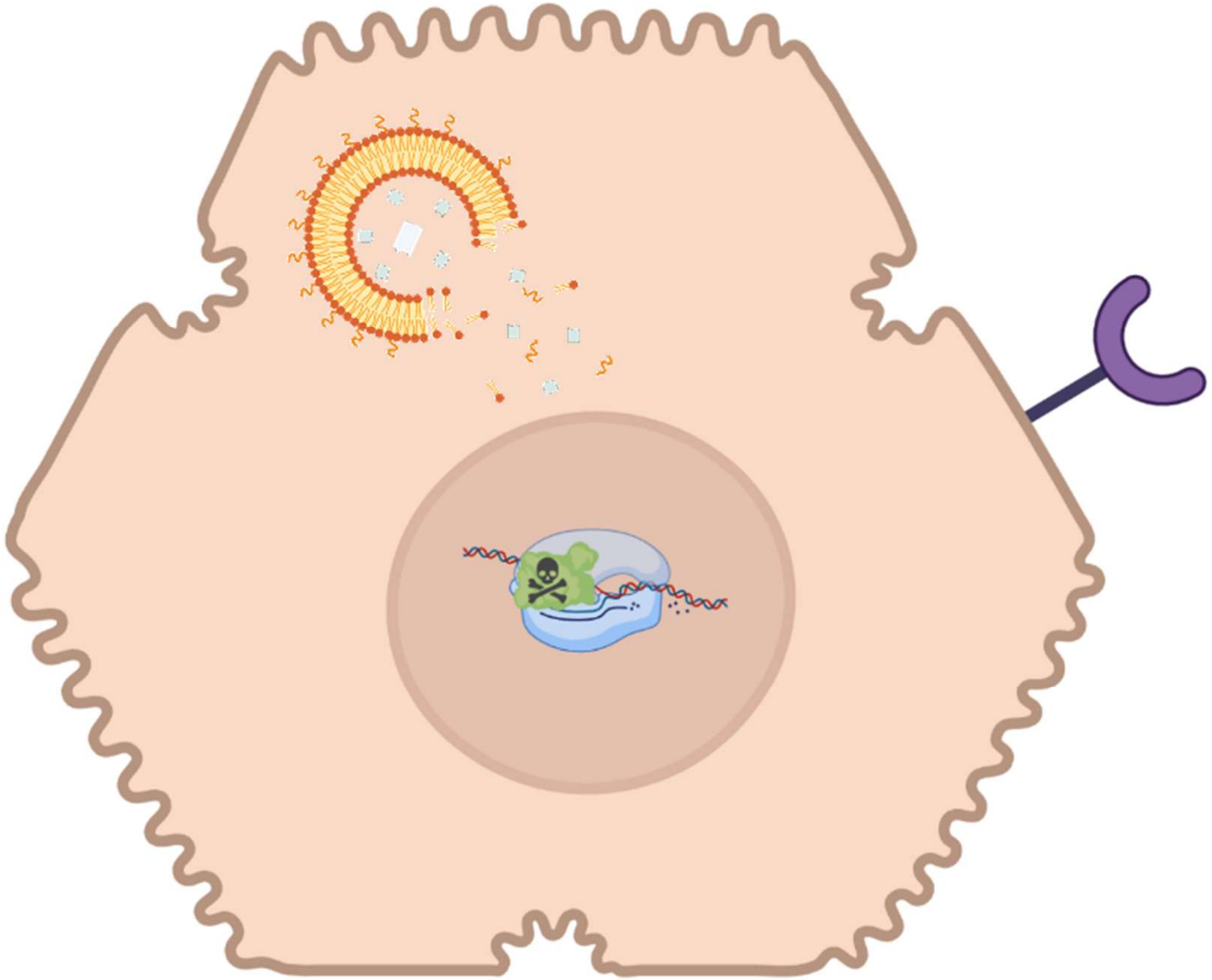
DeathCapTrap

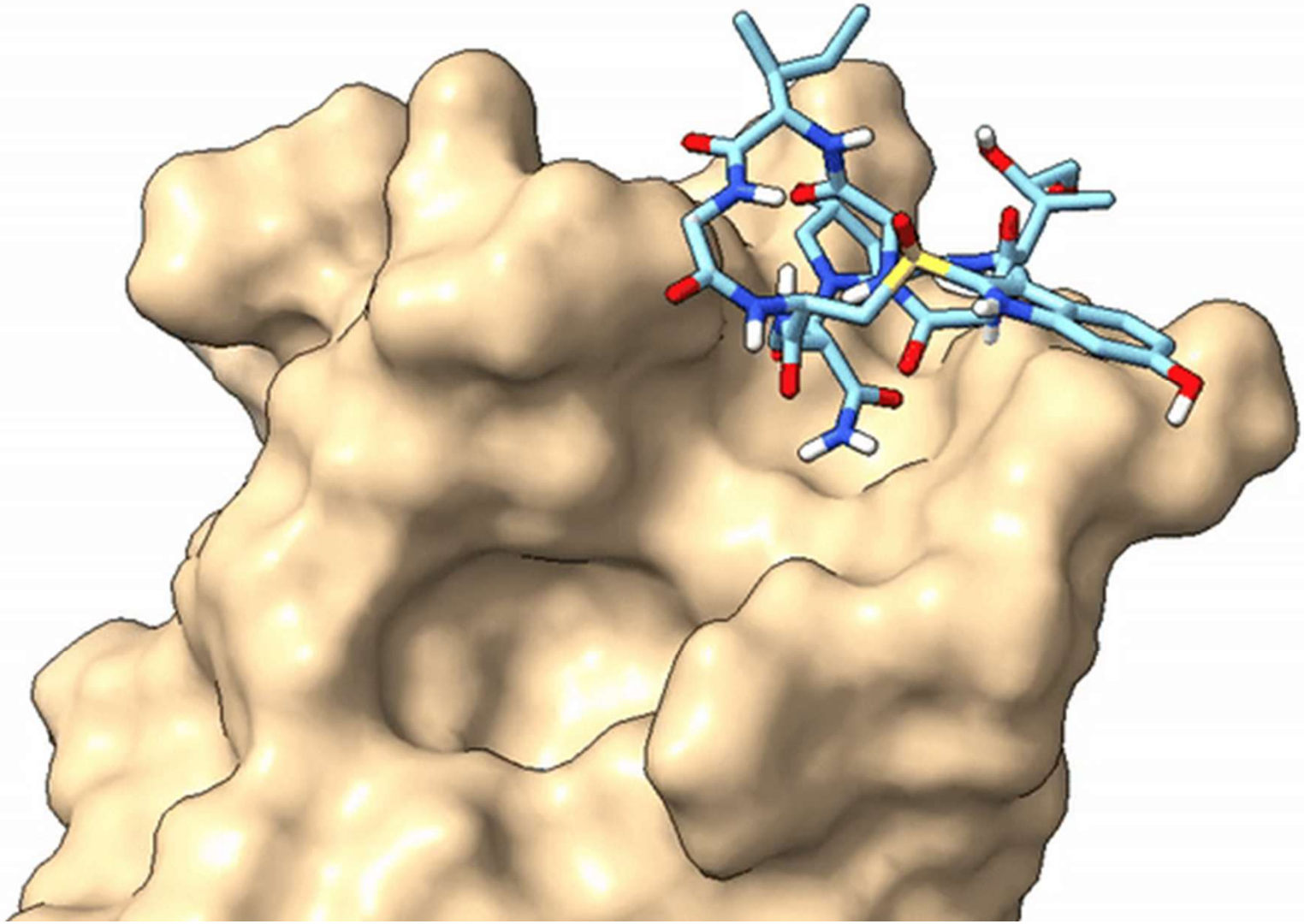
A liposomal nanobody complex for the treatment of death cap poisoning



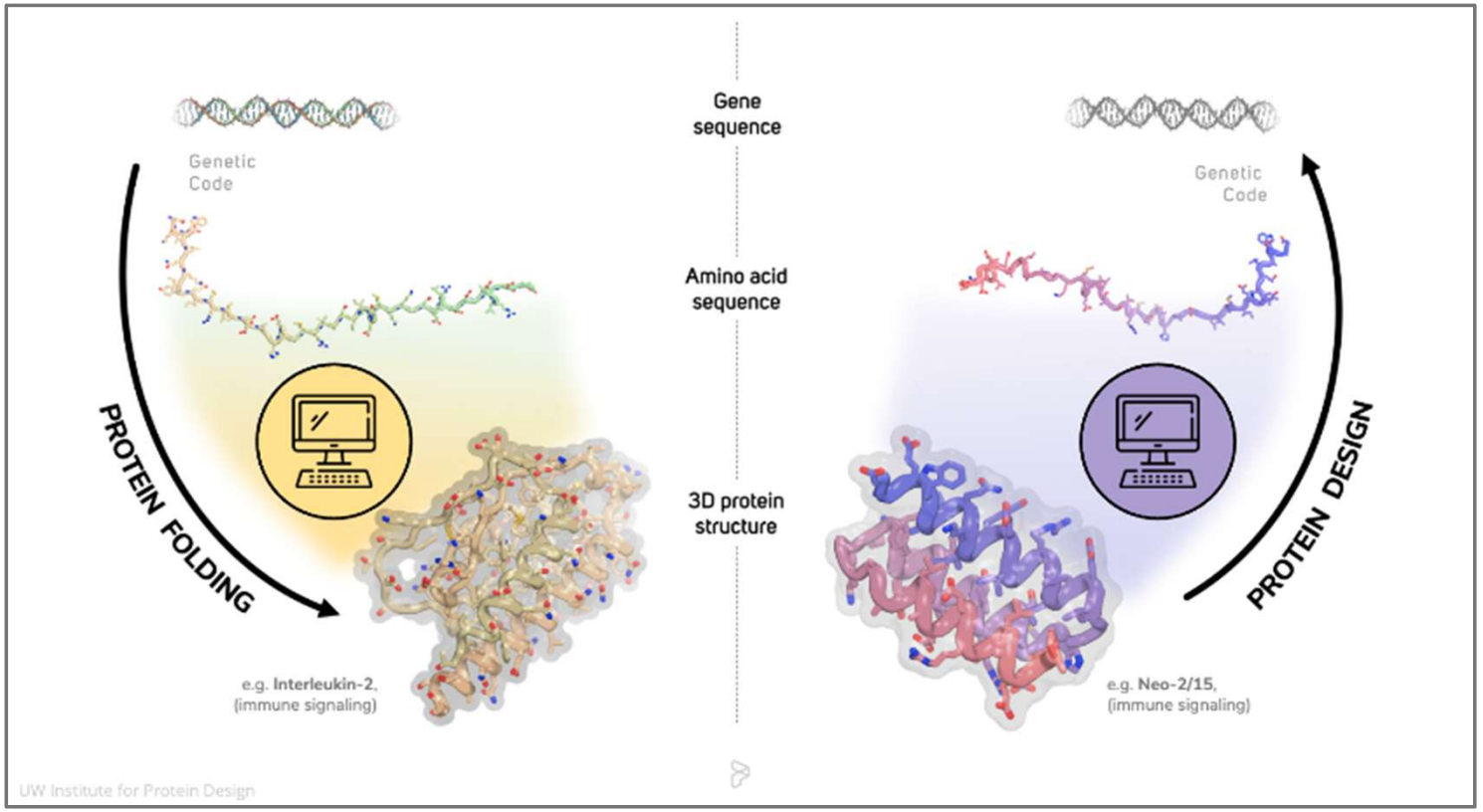




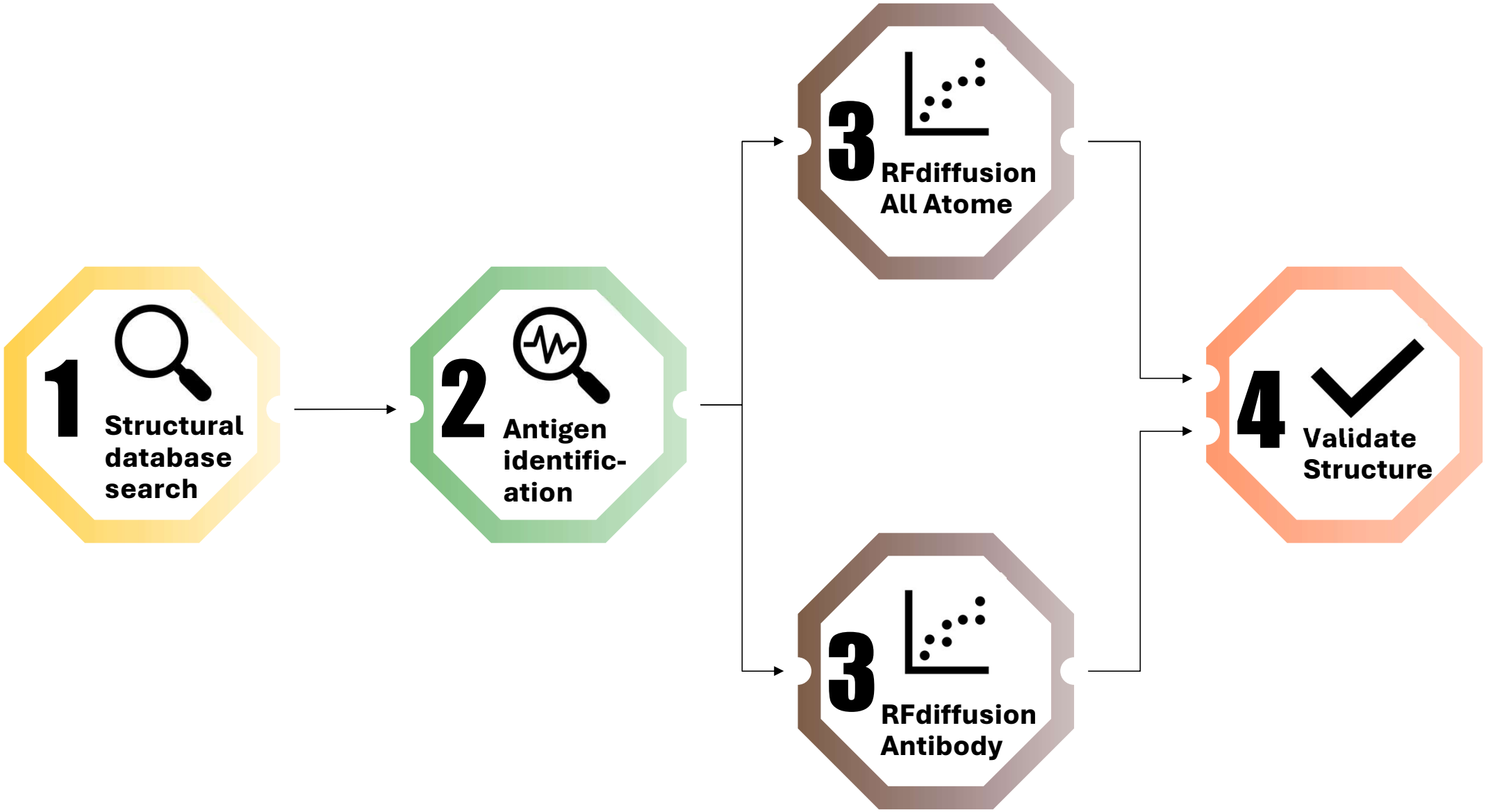




NANOBODY IN SILICO DESIGN



Adopted from University of Washington, Institute for Protein Design
https://www.ipd.uw.edu/2023/10/introducing-rosettafold-and-rfdiffusion-all-atom/


1
Structural database search
2
Antigen identific-ation
3
RFdiffusion All Atome
3
RFdiffusion Antibody
4
Validate Structure

Development of a therapeutic system for the treatment of α-amanitin poisoning using nanobodies designed in silico with diffusion-based artificial intelligence that bind α-amanitin and neutralize it

WHAT IS A NANOBODY?

Feature	Nanobodies	Conventional Antibodies
Structure	Single polypeptide chain	Multiple polypeptide chains
Size	~15 kDa	~150 kDa
Specifity	High	High
Stability	Highly stable	Less stable
Solubility	Very high	Moderate
Access	Can reach dense tissues & tumors	Limited by size
Expression	Easy to produce in bacteria/yeast	Requires mammalian cells
In vivo half life	Short	Long
Cost	Low	High

Definition: A nanobody is a small, single-domain antibody fragment derived from the unique heavy-chain antibodies naturally found in camelids.

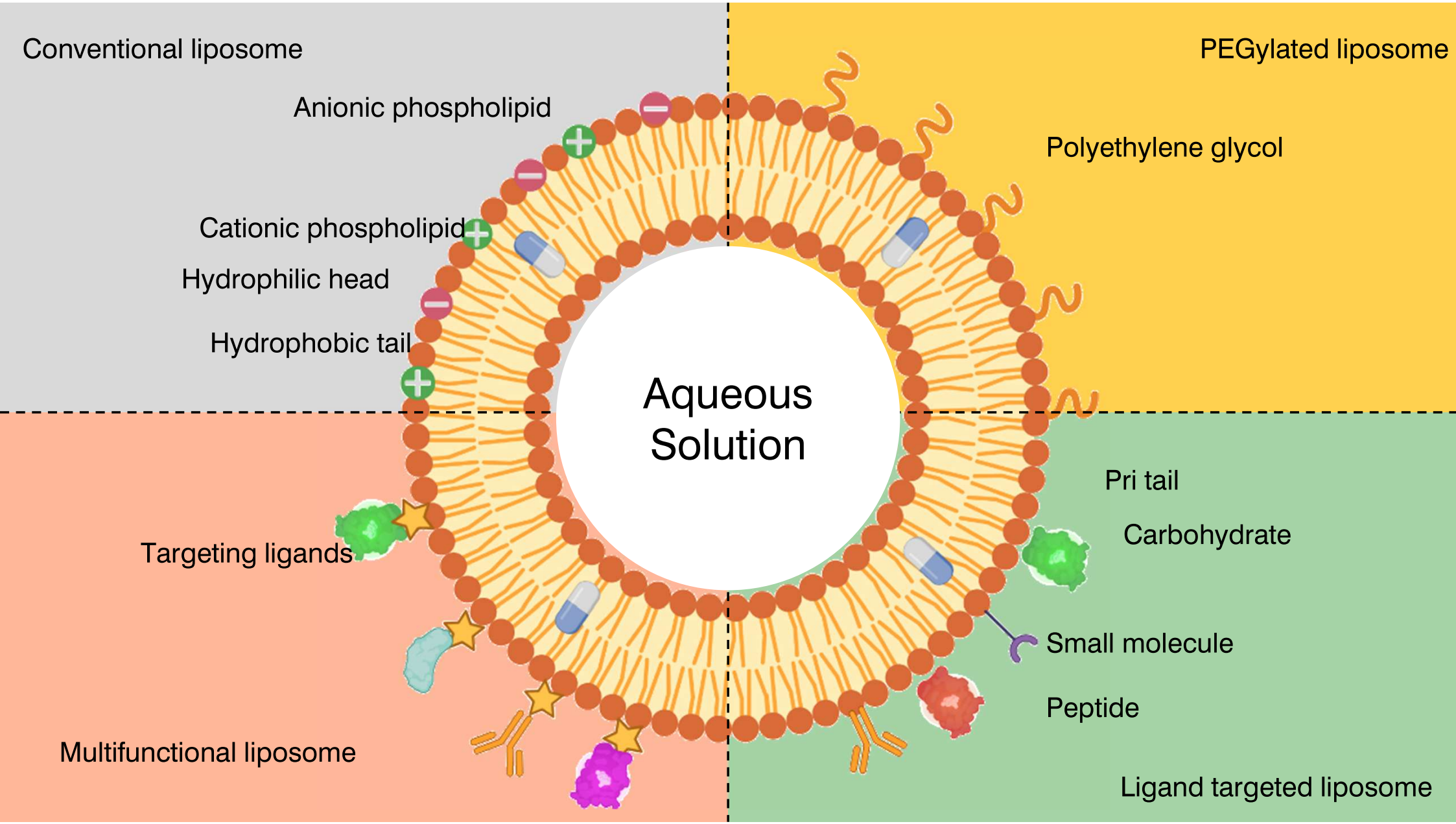
Key features:

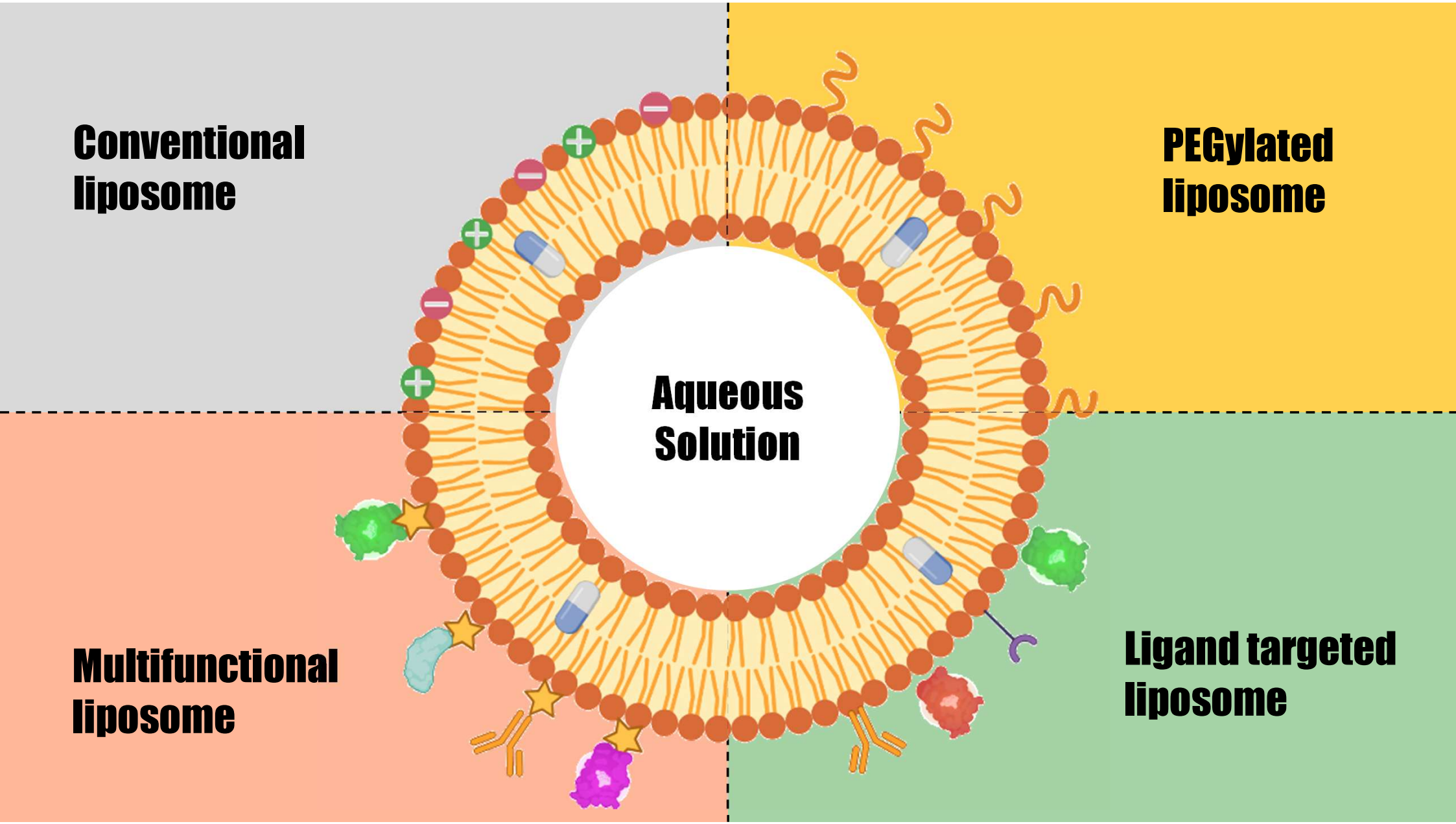
- Much s than conven antibod
- High st and
- ecificall ntige ike full-sized antibodies

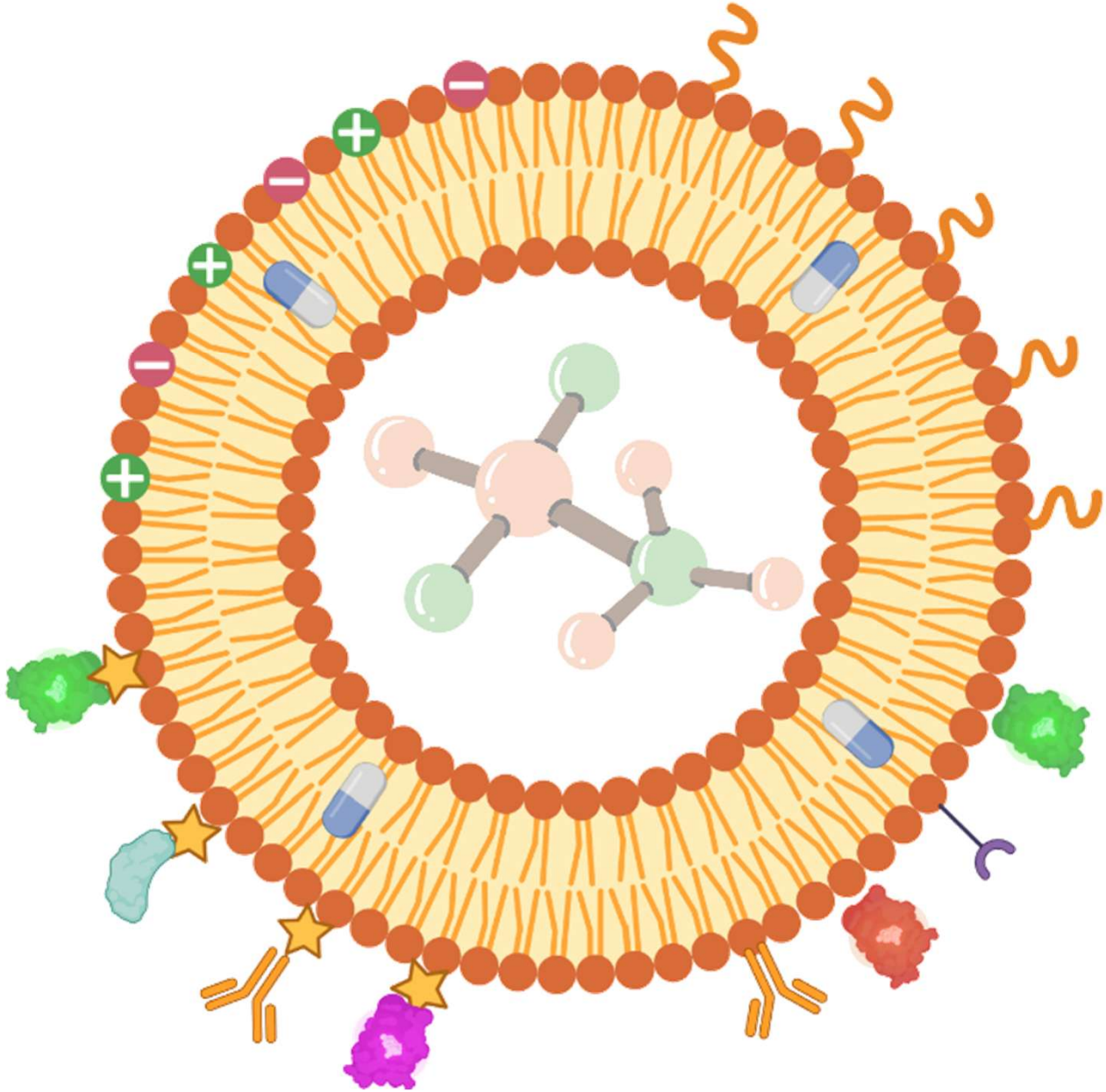
Advantages:

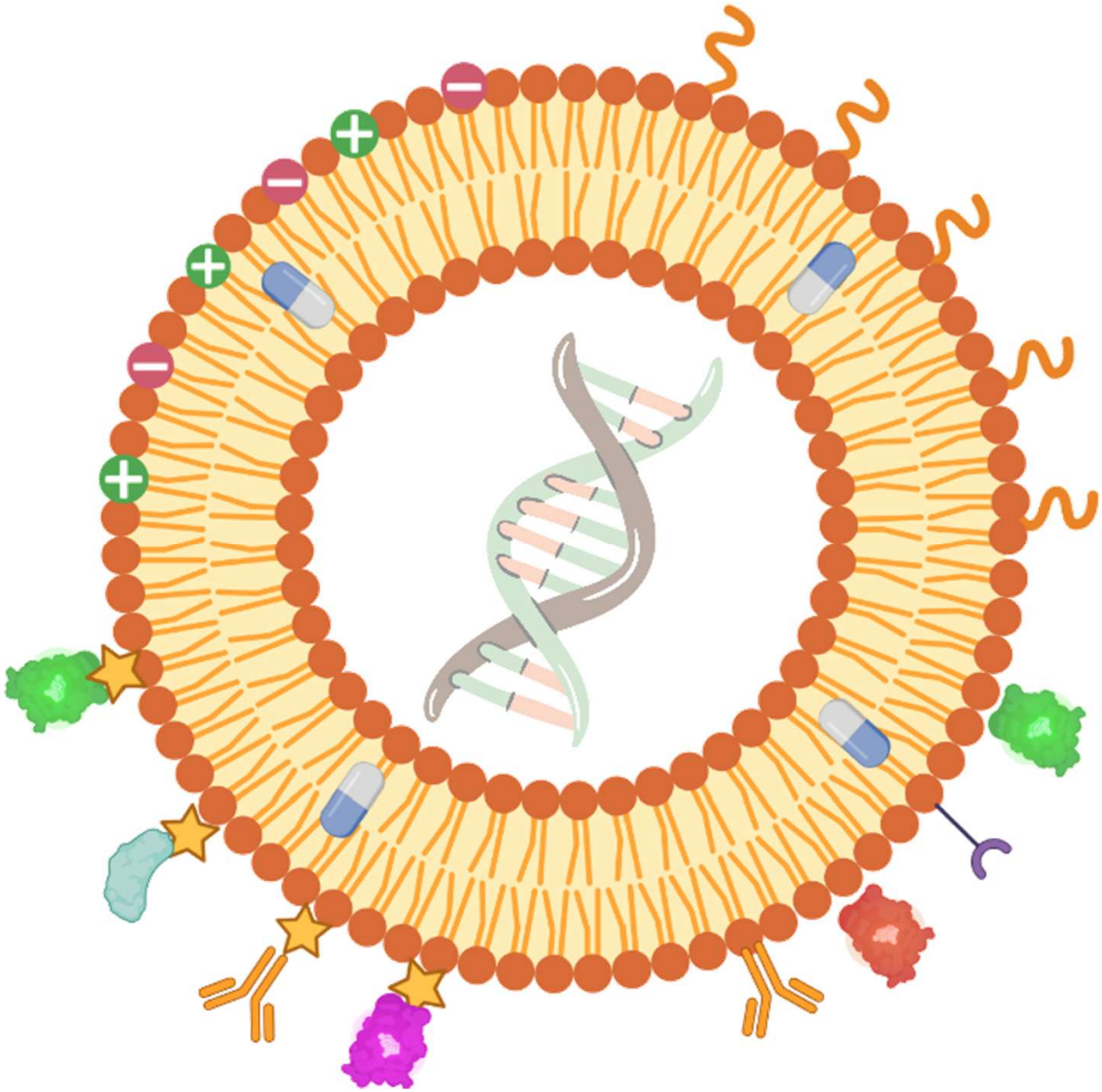
- er to produce
- er tissue etration
- robust under tions
-

WHAT IS A LIPID NANOPARTICLE?

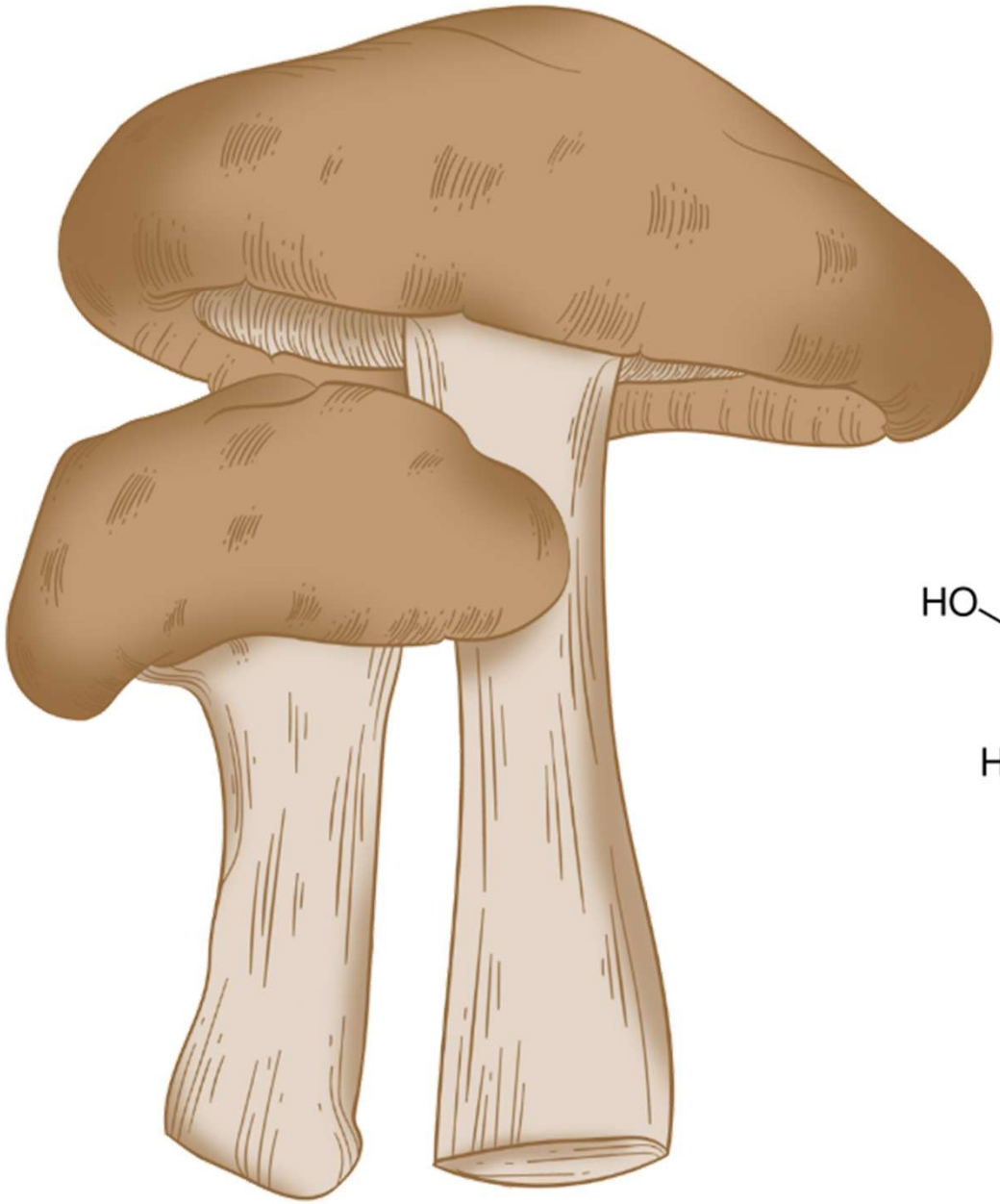

Conventional liposome
Anionic phospholipid
Cationic phospholipid
Hydrophilic head
Hydrophobic tail
PEGylated liposome
Polyethylene glycol
Aqueous Solution
Targeting ligands
Multifunctional liposome
Pri tail
Carbohydrate
Small molecule
Peptide
Ligand targeted liposome

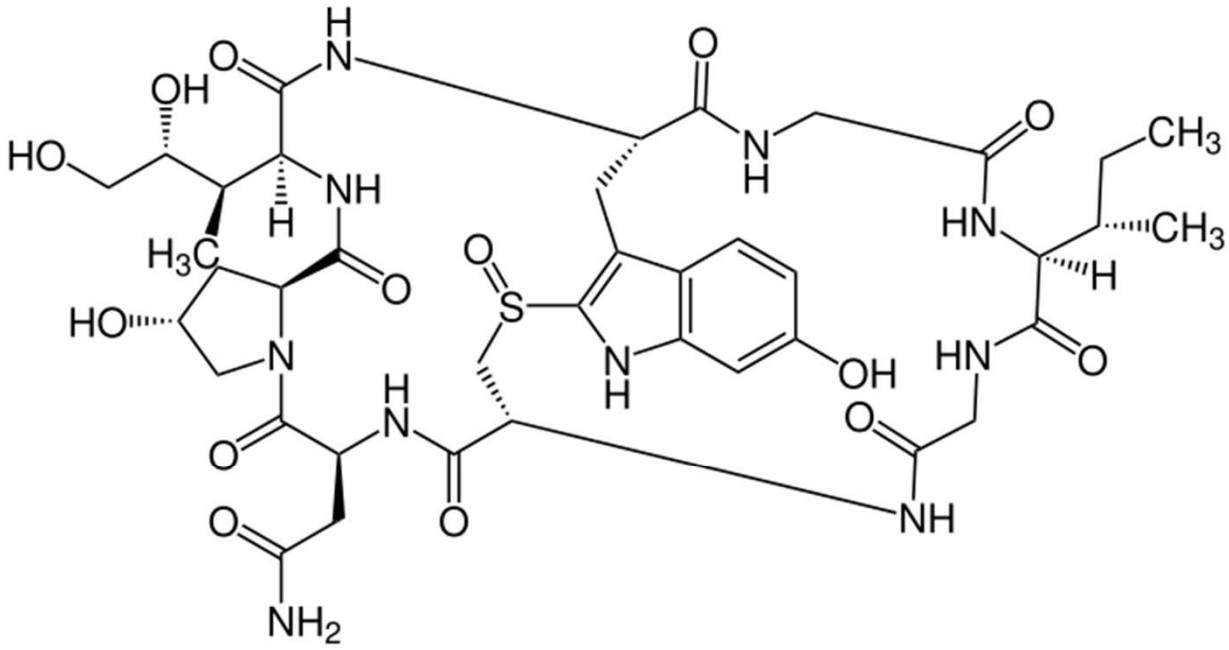

Conventional liposome
PEGylated liposome
Aqueous Solution
Multifunctional liposome
Ligand targeted liposome





WHAT EXACTLY IS α–AMANITIN?




HO
OH
O
H
N
NH
H
O
H_3C
HO
N
O
S
N
H
H
N
O
O
NH_2
O
N
H
O
HN
CH_3
CH_3
H
HN
O
OH
O
NH

1 Nanobody

2 Liposome

3 Library Screening

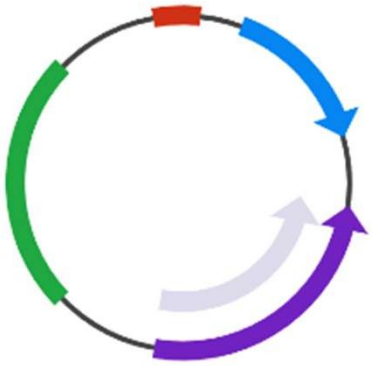
4 In silico

A BRIEF LOOK INTO OUR WORKFLOW...

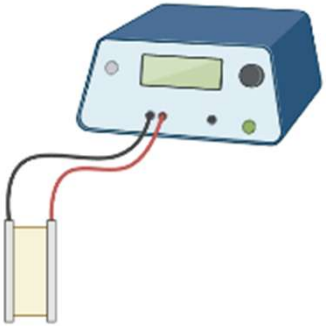
Nanobody 

OUR PARALLEL APPROACH

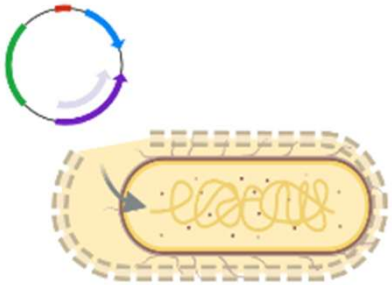
1 Plasmid construction and order



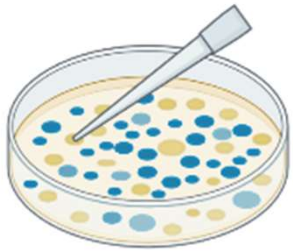
2 Production of competent E. coli (electroporation)



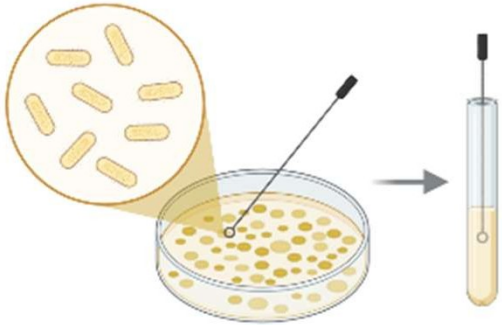
3 Transformation of competent E. coli (DH5a) for amplification of plasmids



4 Selection of positive colonies on LB plates

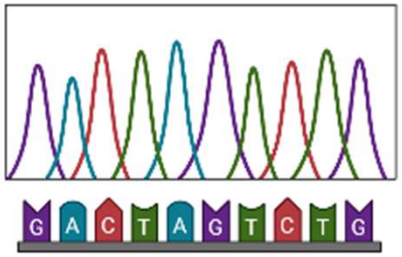


5 Liquid cultures of positive clones

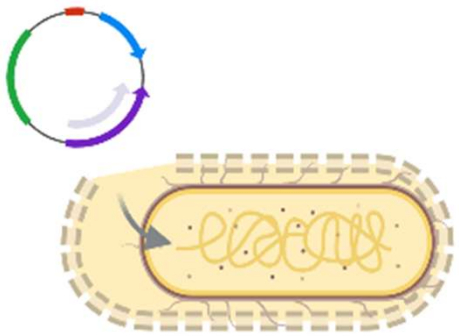


6 Purification of plasmid DNA

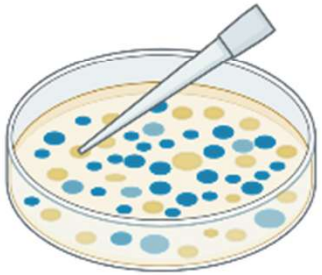
7 Sequencing to analyze the correct inserts



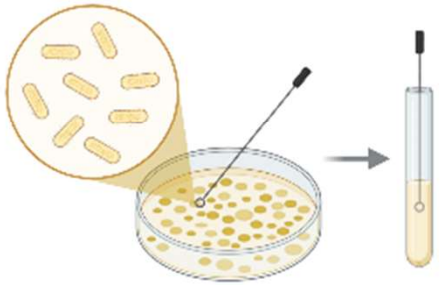
8 Transformation of competent E. coli (BL21) for protein production



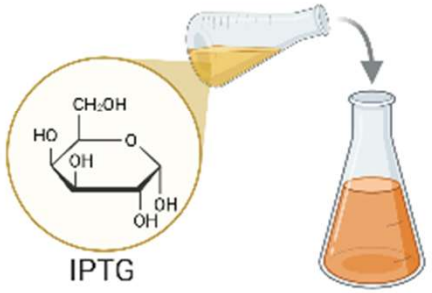
9 Selection of positive colonies on LB plates



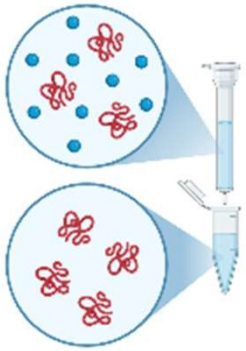
10 Liquid cultures of positive clones



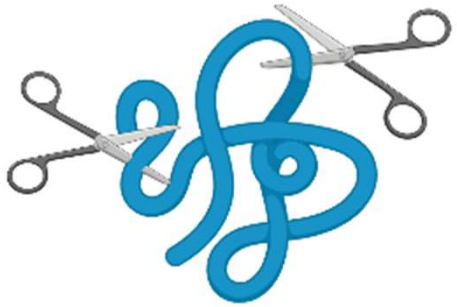
11 Induction of protein production by IPTG



12 Isolation of nanobodies



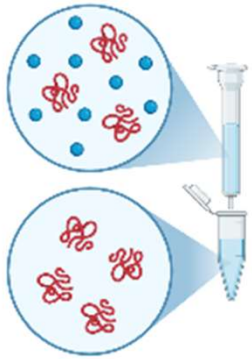
13 HRV3C cleavage (remove 5xD linker)



14 Dialysis to remove salts

15 TEV cleavage (remove His-Tag)

16 Purification of nanobodies

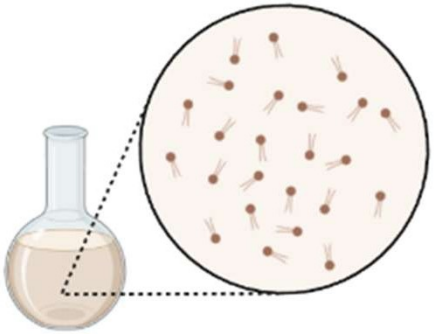


Liposome

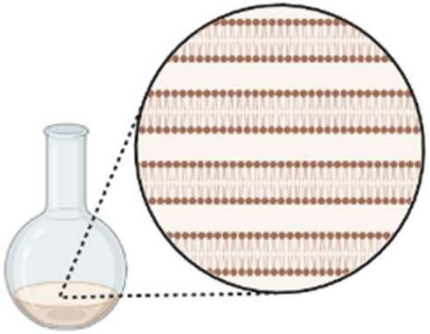
Nanobody

OUR PARALLEL APPROACH

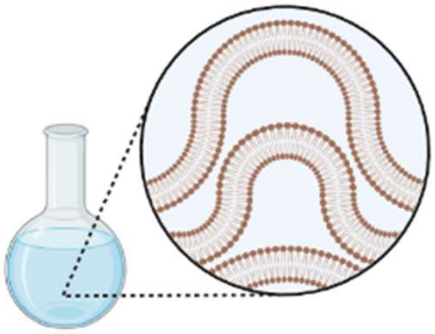
1 Lipids in organic solvent



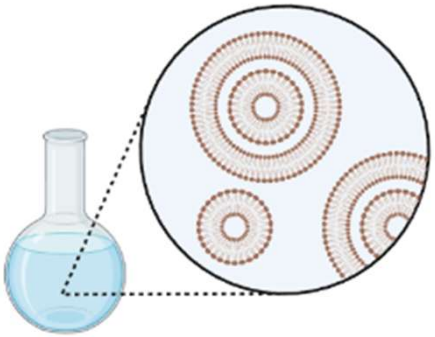
2 **Dried lipid film formation**: Organic solvent is completely evaporated by rotary evaporation



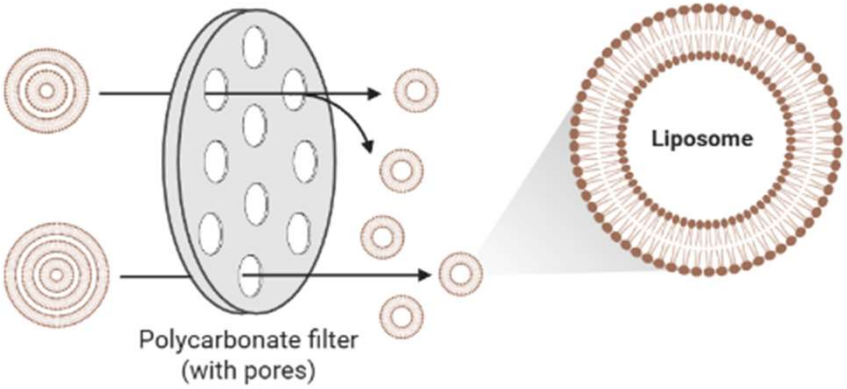
3 **Hydration**: Dried lipid film swells by adding aqueous medium



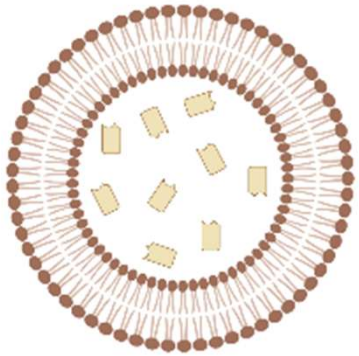
4 **Hydration and Agitation**: Lead to multilamellar vesicle formation



5 **Extrusion** of suspension through a polycarbonate filter with defined pore size to obtain unilamellar vesicles



6 **Loading** of Liposomes with Nanobodies



7 **Characterization** and **validation** of liposomes

OUR PARALLEL APPROACH

Liposome 

Library Screening 

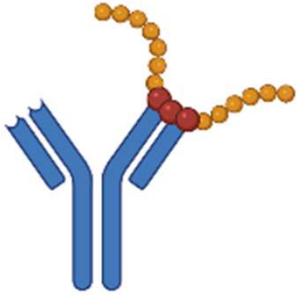
Nanobody 

1

Target Definition

2

Epitope Mapping & Paratope Planning

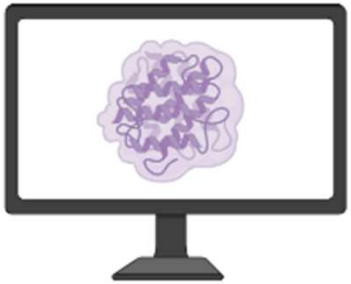


3

Diffusion-Based Sequence Generation

4

Structure Prediction

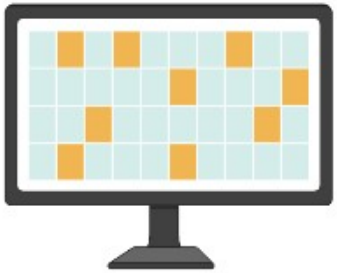


5

Affinity Docking

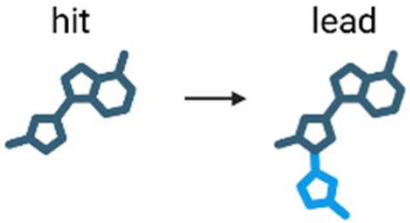
6

In Silico Screening / Filtering



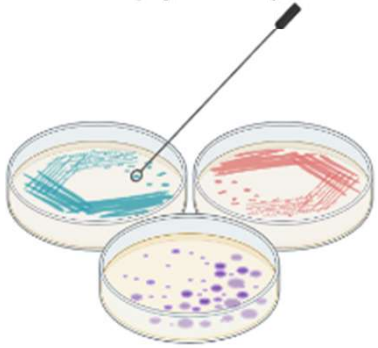
7

Candidate Selection & Optimization



8

Wet Lab Validation (optional)



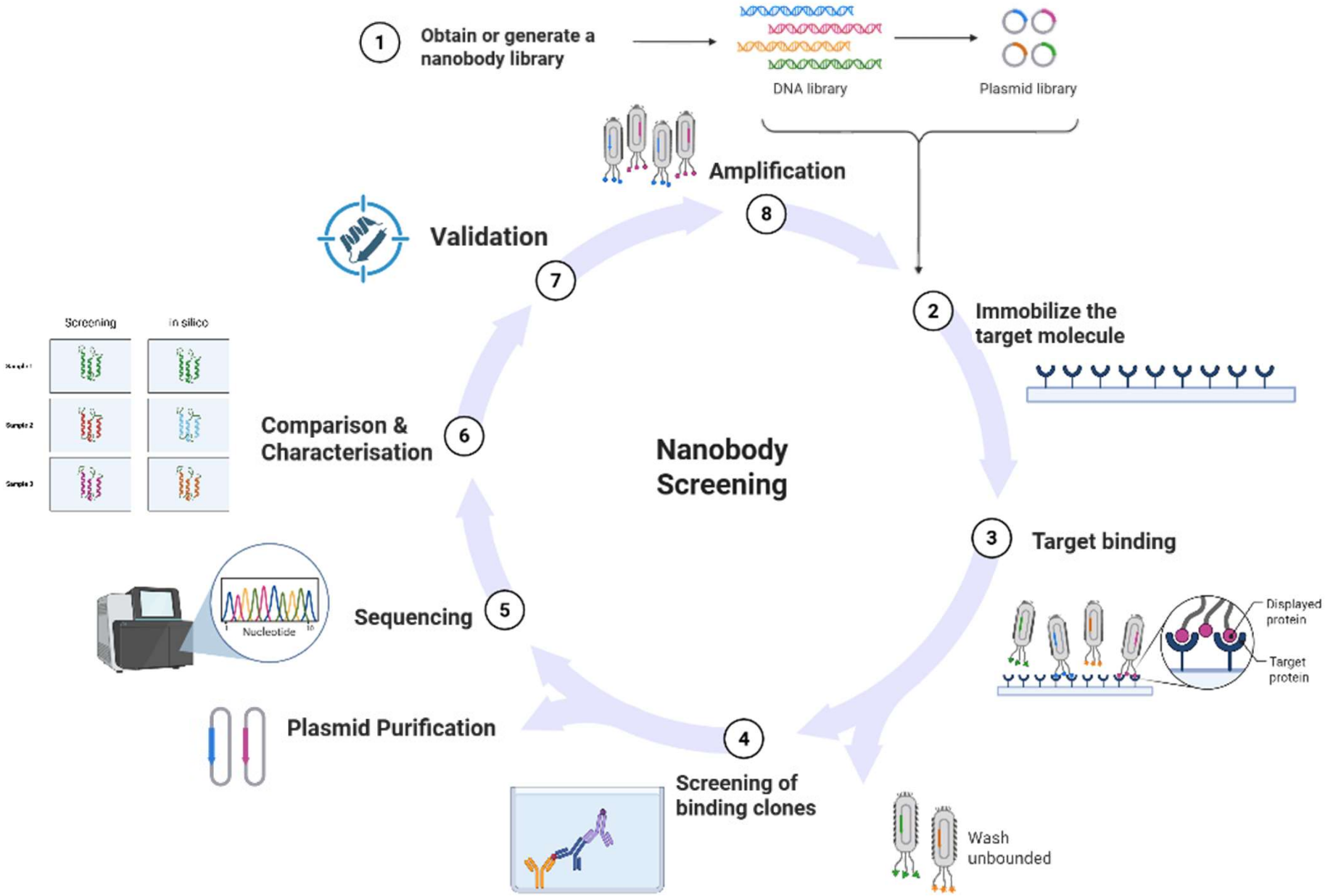
OUR PARALLEL APPROACH

Liposome 

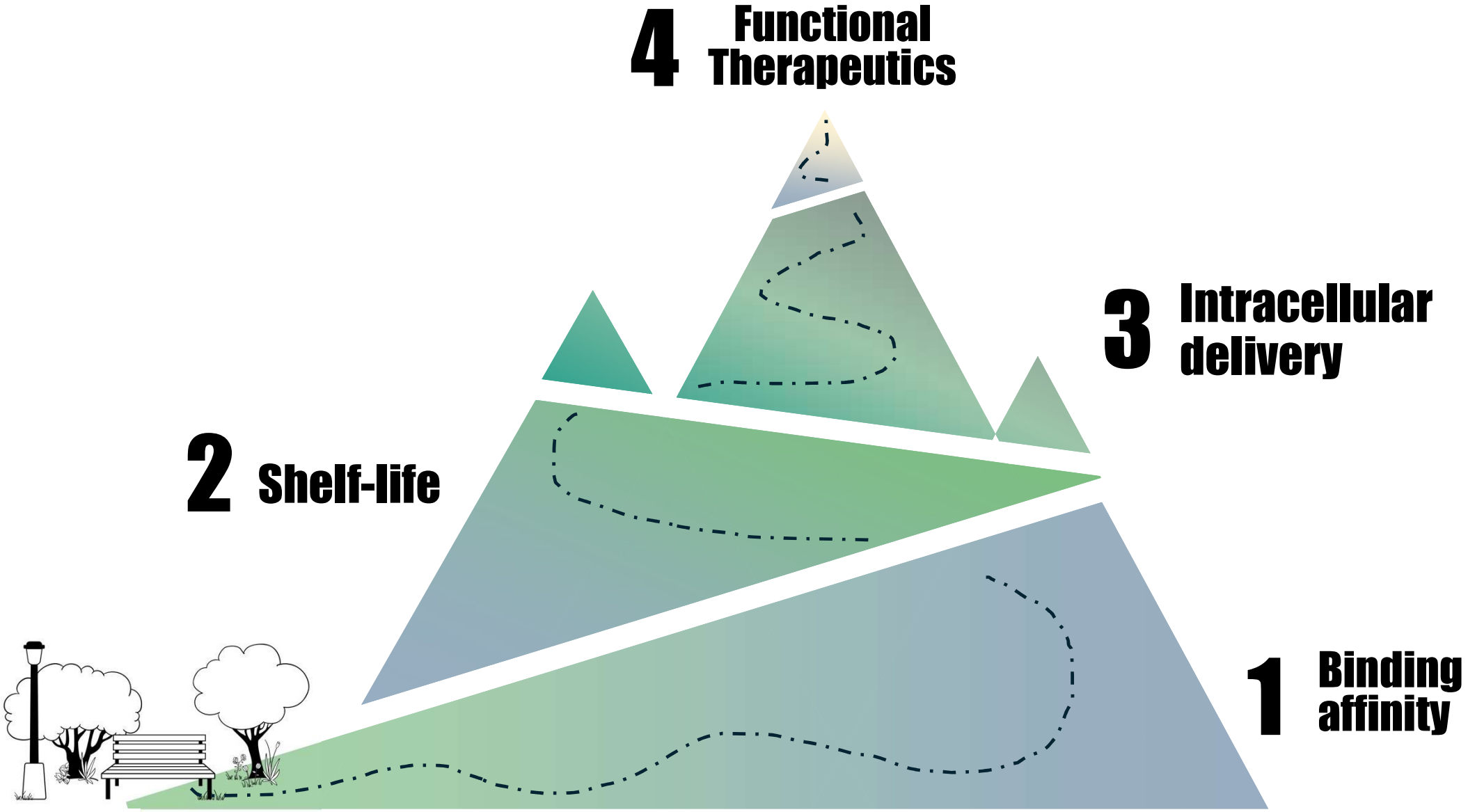
Library Screening 

Nanobody 

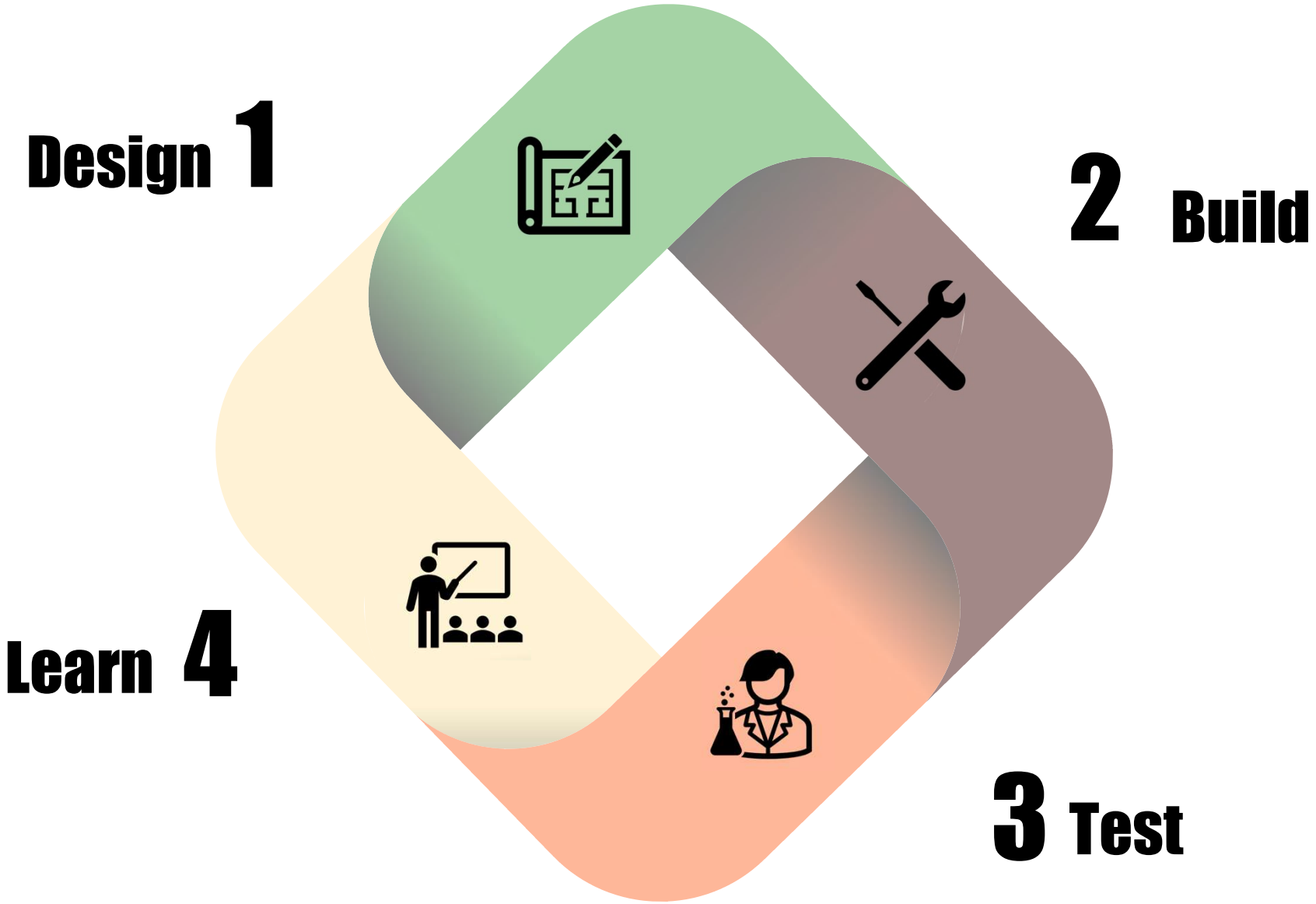
In silico design 


1
Obtain or generate a nanobody library
DNA library
Plasmid library
Amplification
8
2
Immobilize the target molecule
3
Target binding
Displayed protein
Target protein
4
Screening of binding clones
Wash unbounded
5
Sequencing
Nucleotide
Plasmid Purification
6
Comparison & Characterisation
Screening
In silico
7
Validation
Nanobody Screening

OUR CHALLENGES


4 Functional Therapeutics
3 Intracellular delivery
2 Shelf-life
1 Binding affinity

ENGINEERING SUCCESS


Design 1
2 Build
3 Test
Learn 4

OUTLOOK

Extend the treatment concept to other cell types (kidney cells)

Extend the treatment concept to other toxins (e.g. ricin, shiga toxin)

Load LNPs with additional drugs (e.g. polymyxin B and methylprednisolone)

THANKS TO OUR SPONSORS AND SUPPORTERS!

SOURCES:

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3. Kayes, T. & Ho, V. Amanita phalloides-Associated Liver Failure: Molecular Mechanisms and Management. *International Journal of Molecular Sciences* **25**, 13028 (2024).
4. Garcia, J. *et al.* An effective antidotal combination of polymyxin B and methylprednisolone for α-amanitin intoxication. *Arch Toxicol* **93**, 1449–1463 (2019).
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15. So erkennen Sie den Champignon im Wald. https://www.selbst.de/wiesen-champignon-43129.html (2022).