



Set up and implementation of a platform for the production and quality control of biological drugs





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PIANO NAZIONALE
DI RIPRESA E RESILIENZA

WP2 Tasks

Task 2.1: Facility implementation for process development and the production of pre-clinical lots of biologics

Task 2.2: Quality control laboratory for biologics and *in vitro* testing

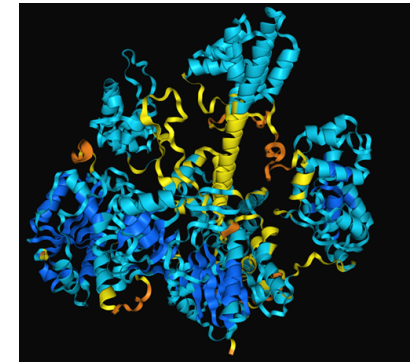
Task 2.3: Animal facility of selected mouse models for *in vivo* efficacy studies



PROTEINS

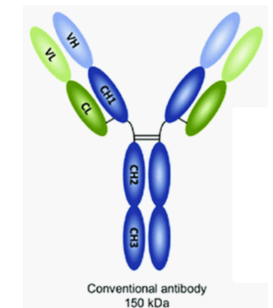
Recombinant Enzymes

Guanidinoacetate methyltransferase (**GAMT**)
Methionine adenosyltransferase (**MAT2A**)
Phenylalanine ammonia-lyase (**PAL**)
Human α -mannosidase
Ubiquitin-specific protease 2 catalytic domain (**Usp2**)
mini-Cas9 (**sRGN**)



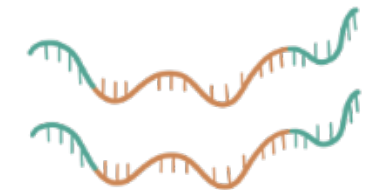
Recombinant Antibodies (Abs)

Full-length (Dia-T51, anti- β -1,3 glucans)
scFv (single chain-fragment variable):
scFv-3T (anti- β -1,3 glucans); anti-ICAM-1; anti-IGF-1;
anti-INSR



IVT modified mRNA

Guanidinoacetate methyltransferase (**GAMT**) mRNA
Ataxia Telangiectasia Mutated (**ATM**) mRNA



Innovative Biologics



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Recombinant protein expression workflow

Select/Design the end product
(amino acid sequence)



Choose expression system



Design expression clone
(DNA construct)



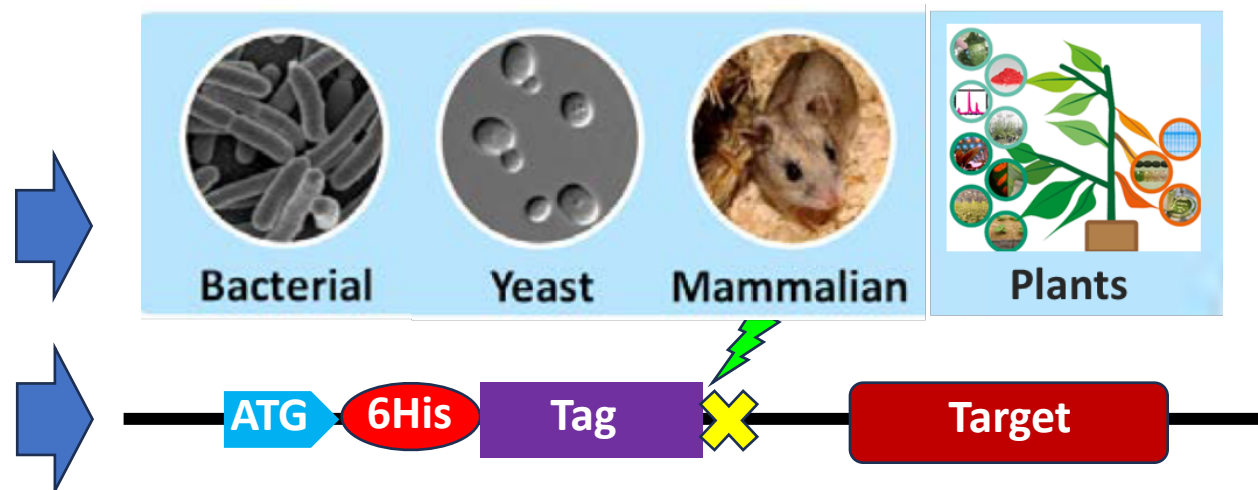
Express the protein



Purify the protein



Characterize the protein





Recombinant proteins generated

Bacteria

- GAMT *
- MAT2A
- PAL *
- USP2*
- sRGN
- UBB*
- Ub-GFP*
- Dia-T51*
- scFv-3T*
- scFv anti-IGF1*
- scFv anti-INSR*
- scFv anti-ICAM-1*

**Successfully
produced ***

Yeast

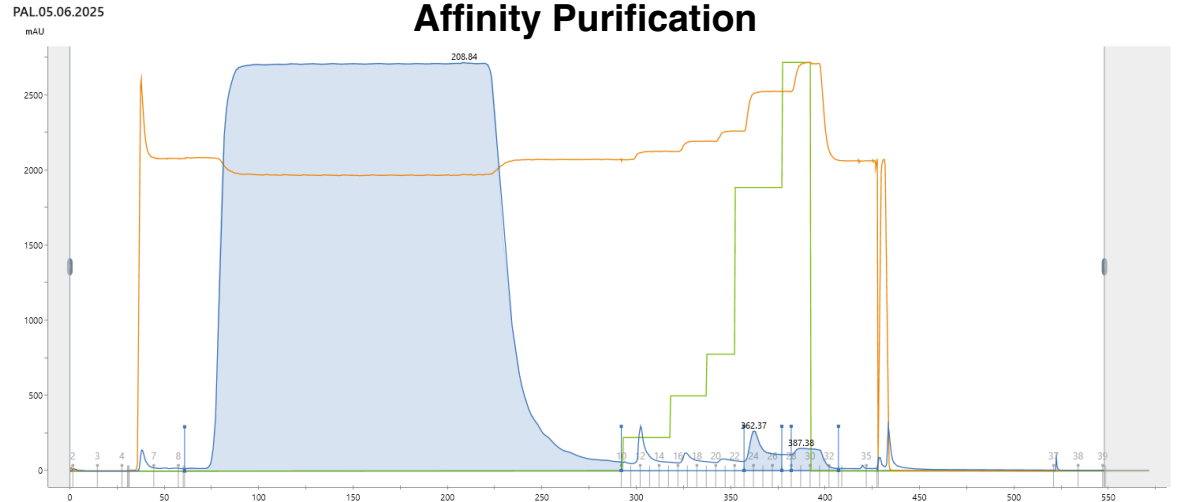
- MAT2A
- PAL *

Plants

- α -Mannosidase*

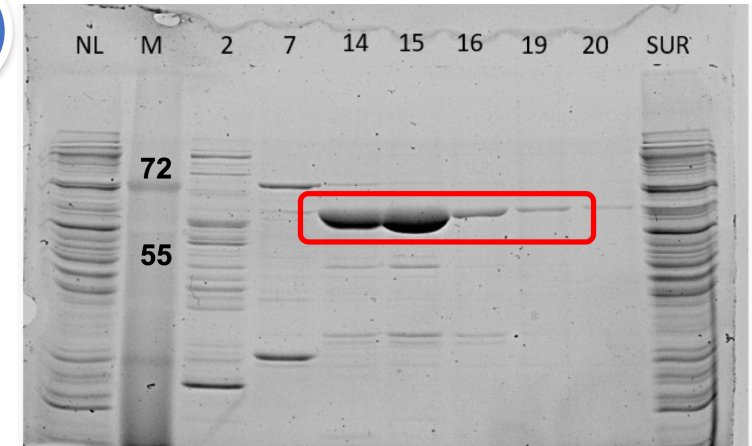
Phenylalanine Ammonia Lyase (PAL) expression in Bacteria

1



2

SDS-PAGE analysis



3

Activity Assay by Mass Spectrometry





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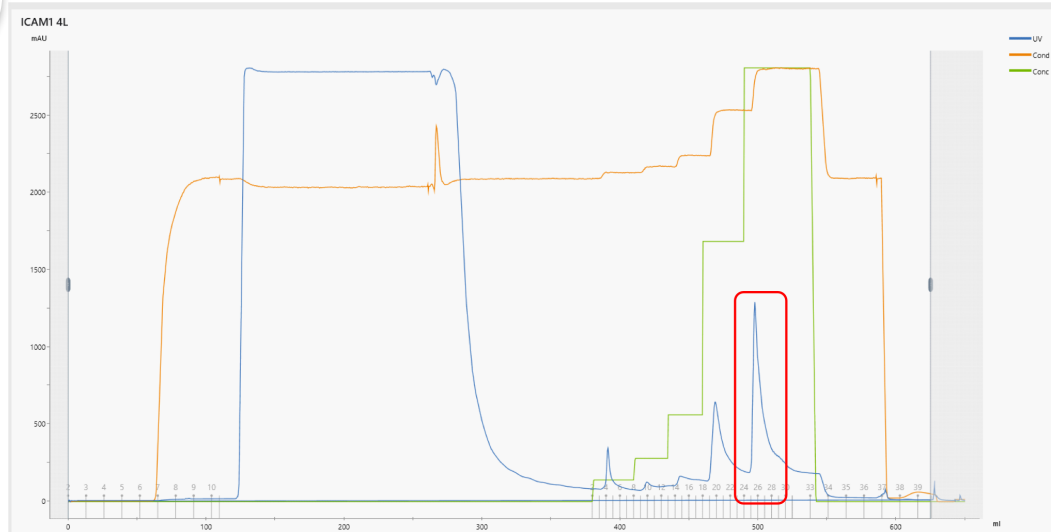


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Anti-ICAM-1 scFv expression in Bacteria

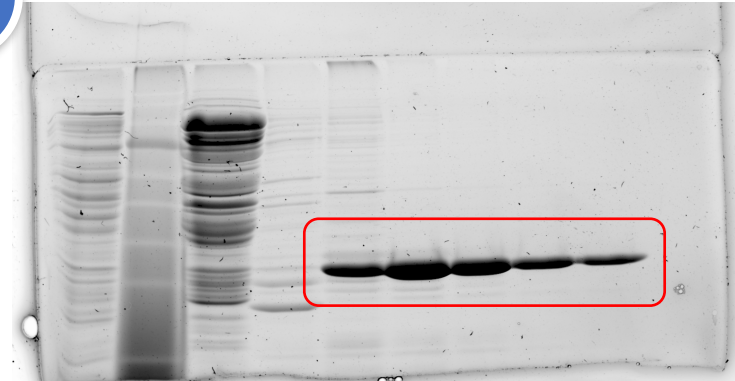
1

Affinity Purification



2

SDS-PAGE analysis



DAPI

Anti-HIS tag

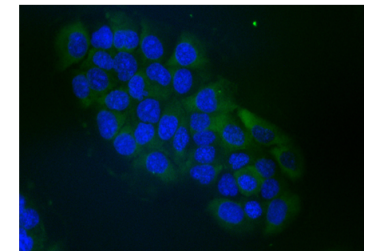
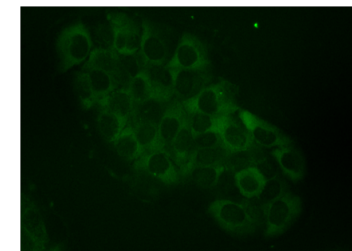
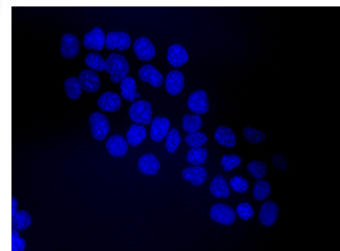
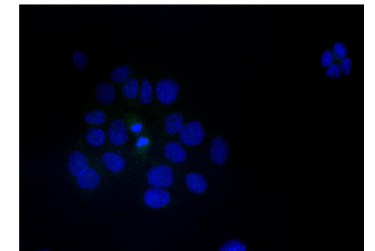
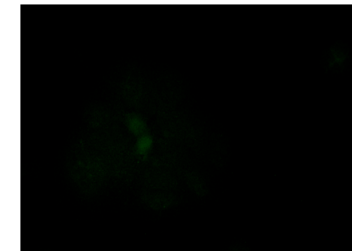
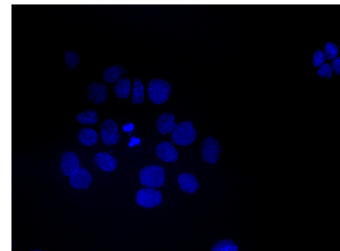
MERGE

Ctr -

3

Functional Assay by Immunofluorescence

Anti-ICAM-1





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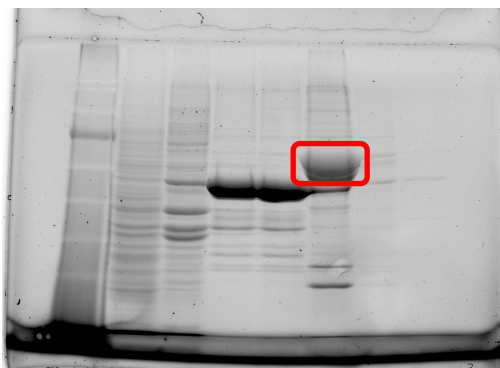
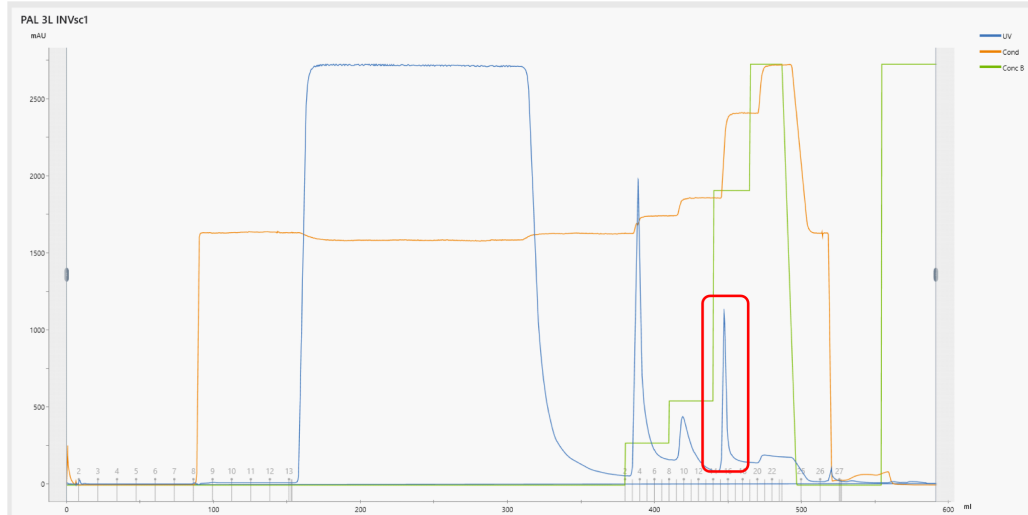


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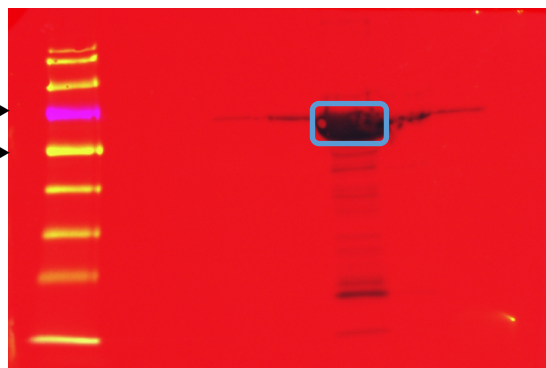
PAL and MAT2A expression in Yeast

1

PAL

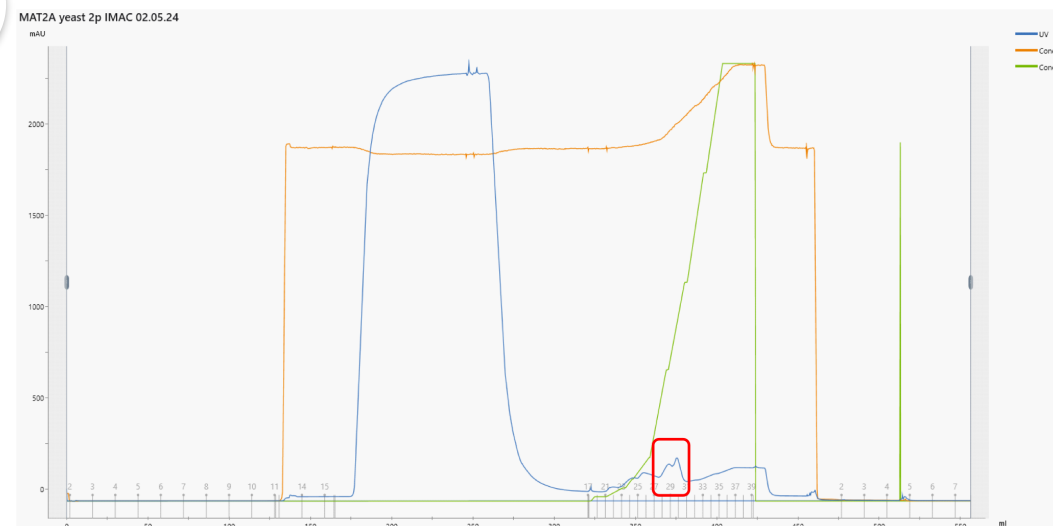


72
55

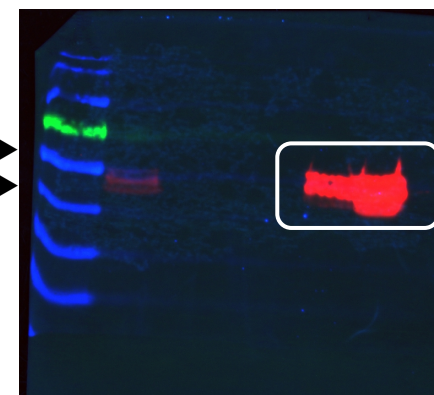


2

MAT2A



55
43





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Ongoing :

Patent application
Research article

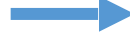
Plant-based biotechnology platform for a sustainable production of human recombinant α -mannosidase



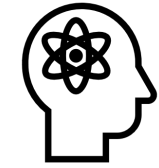
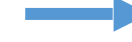
A rare **genetic disorder** caused by a deficiency of a human lysosomal enzyme. It affects approximately **1 in 500,000 living births**.



Plant as bioreactors
(*N. tabacum*)
for recombinant human
enzyme production

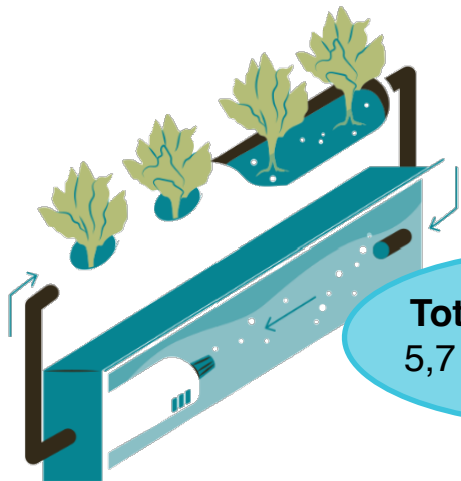


Culture system optimization
(Vertical Farming)
to improve scalability



**Development of new
innovative biosystems**
for a sustainable production

Vertical Farming – Hydroponic culture system



Total enzyme/plant:
5,7 μ g/g Fresh Weight

In collaboration with: Prof. Matteo Ballottari, Prof. Nico Betterle, Kristina Ljumovic Department of Biotechnology, University of Verona, Italy.

Enzymatic assay demonstrates promising results
for future therapeutic applications

Leaves sample	Enzyme
	Units/mg
Wild-type	0.046
Young transgenic leaves	0.47
Medium transgenic leaves	0.66
Old transgenic leaves	1.327

10x
30x

Ongoing :

Patent application
Research article

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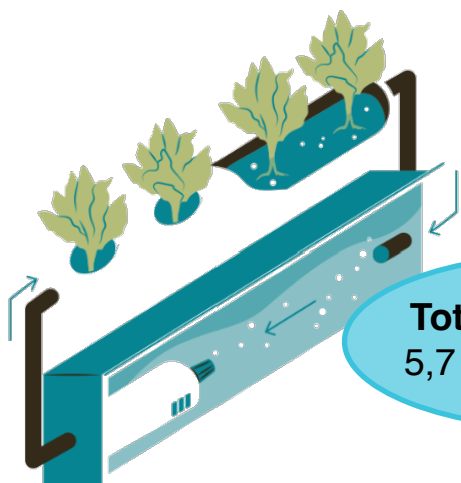


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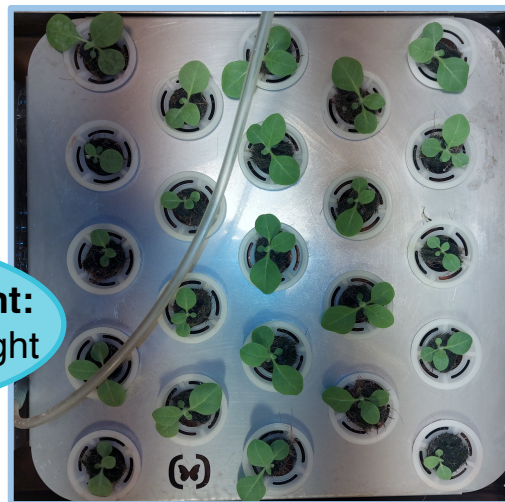


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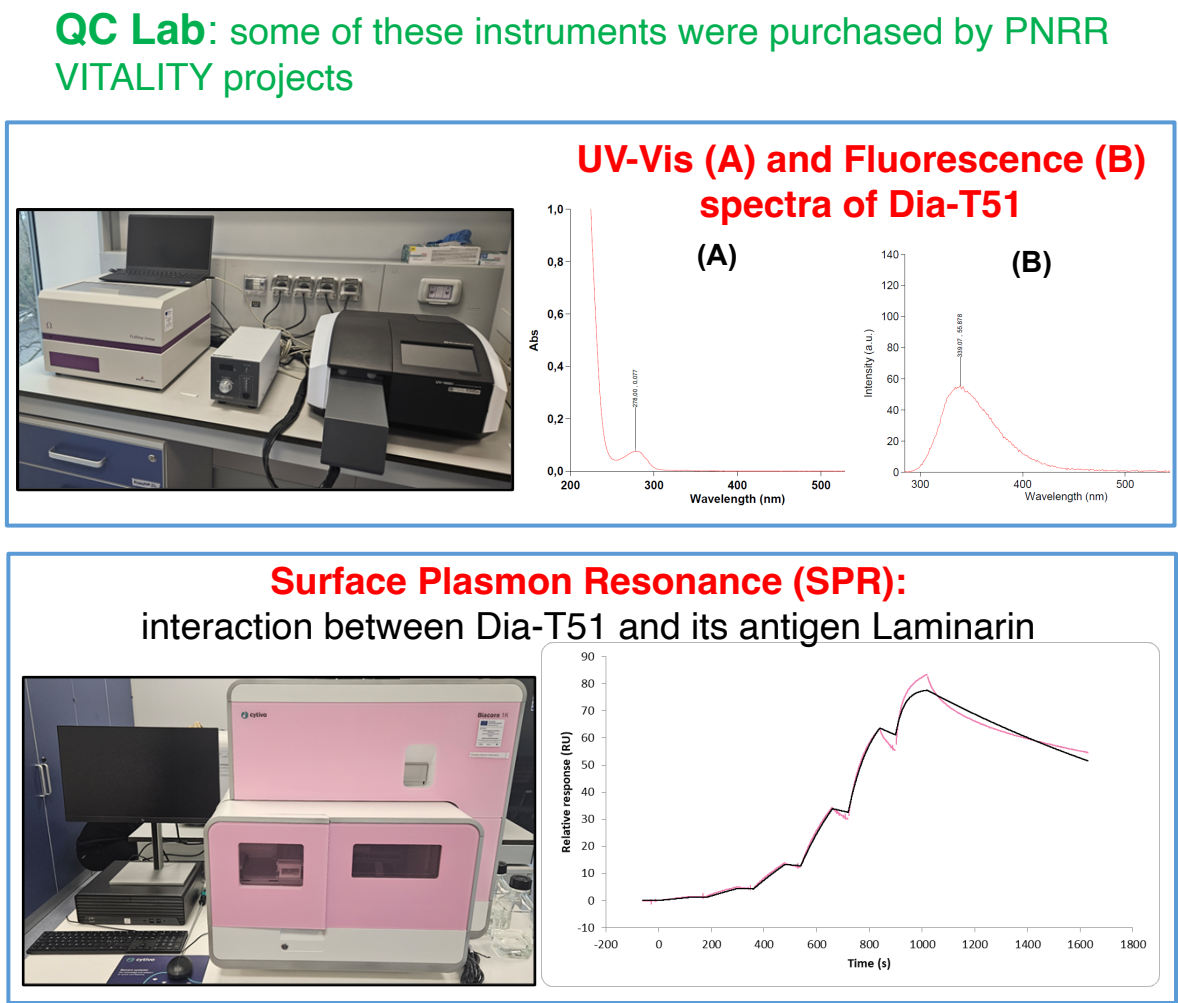
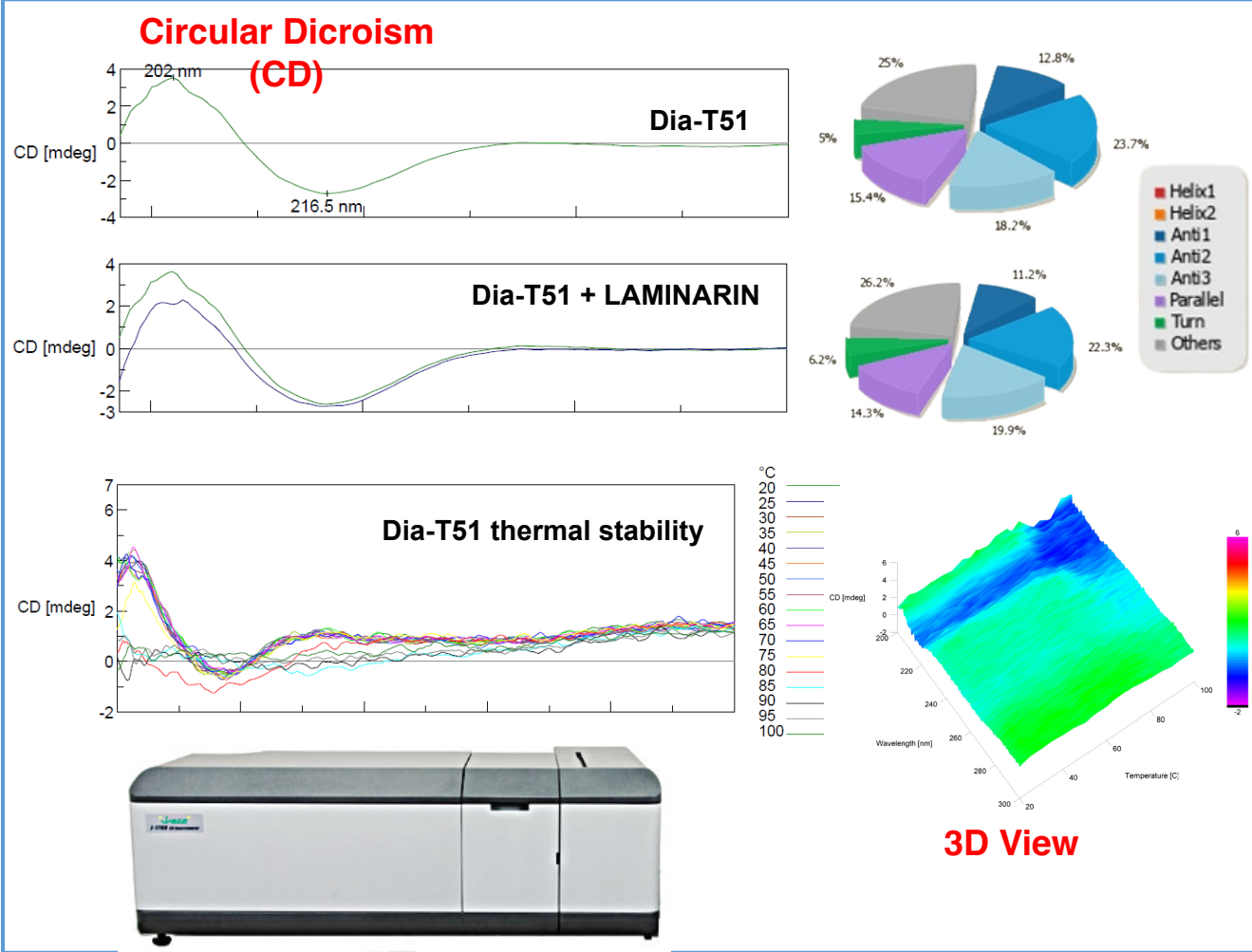
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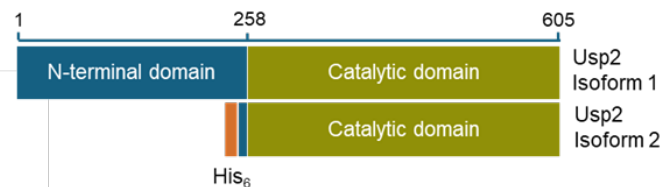
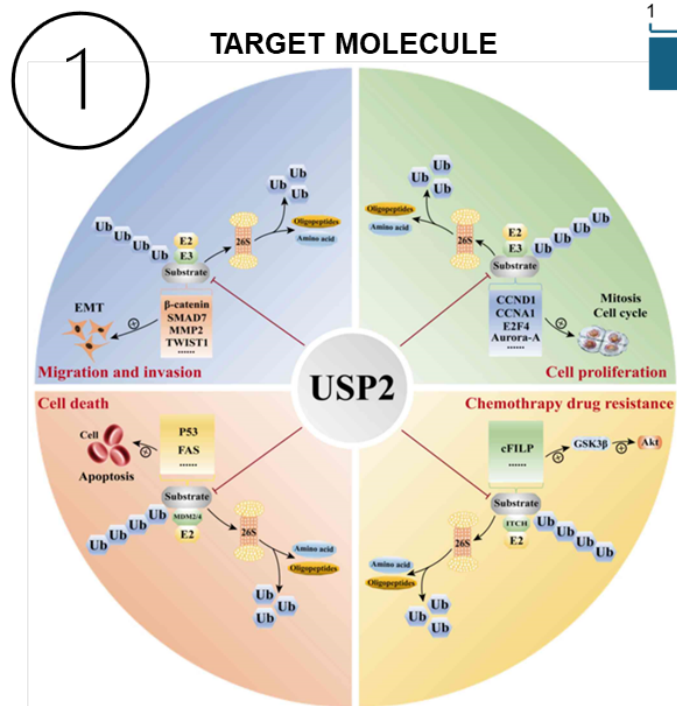


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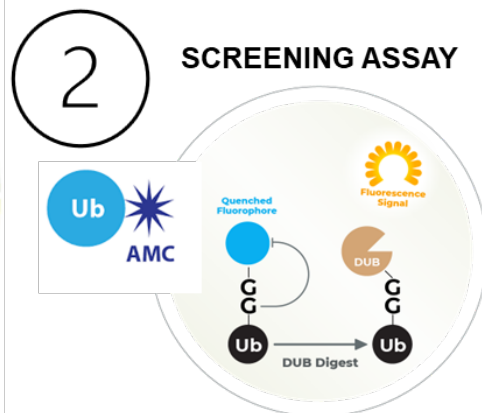


QC TESTS: BIOPHYSICAL CHARACTERIZATION OF RECOMBINANT PRODUCTS: the example of full-length antibody **Dia-T51**



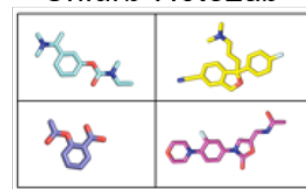


CLONING & EXPRESSION

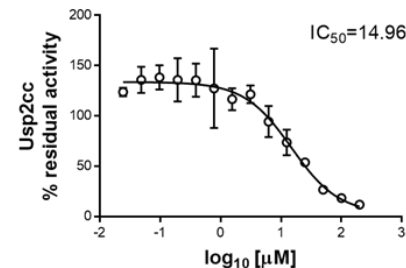


COMPOUND LIBRARY

Uniurb-HetsLab



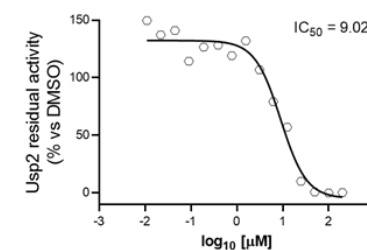
UR971



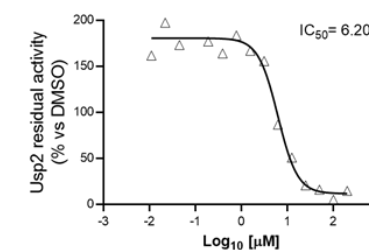
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UR971 SUB-LIBRARY

UR970



UR2701

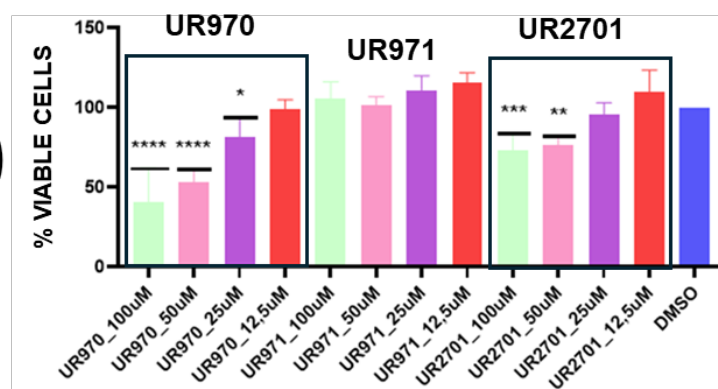


CYTOTOXICITY In HEK293T

UR970

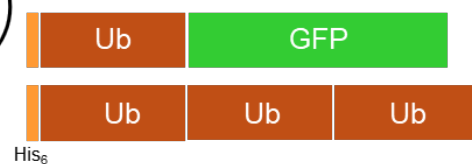
UR971

UR2701

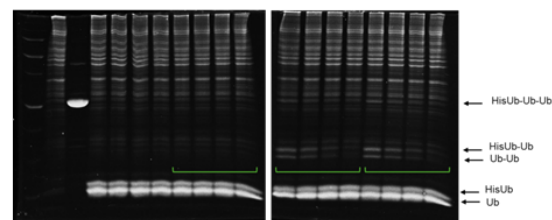
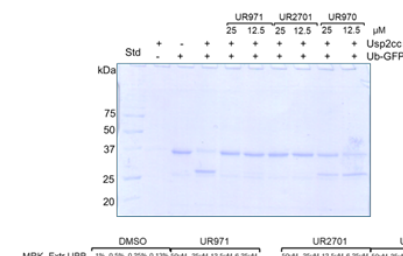
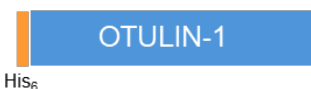


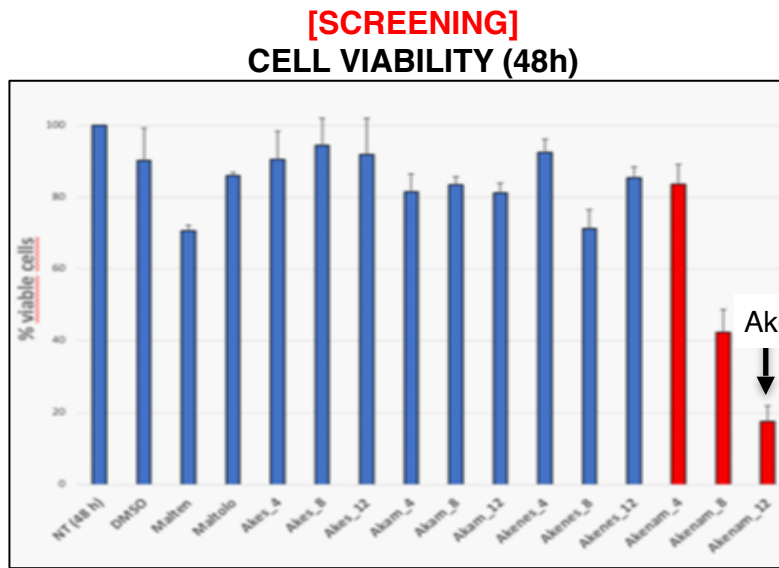
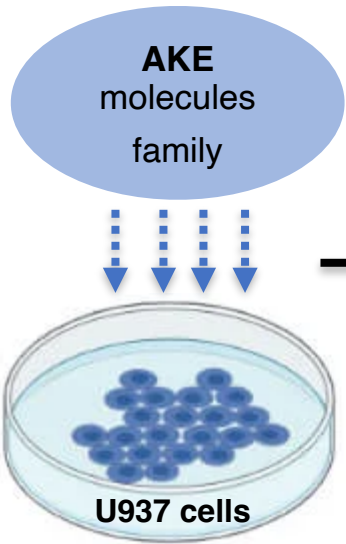
3

RECOMBINANT SUBSTRATES

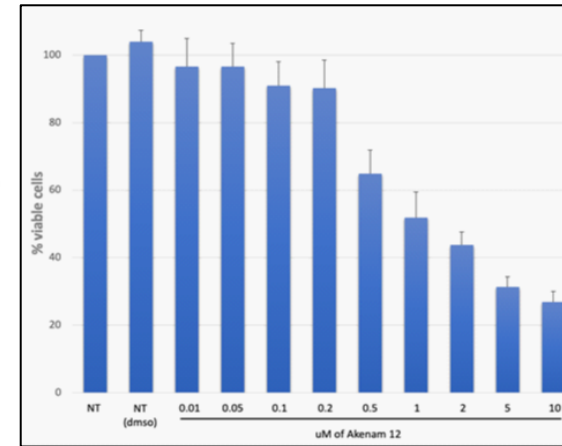


RECOMBINANT UNRELATED DUB

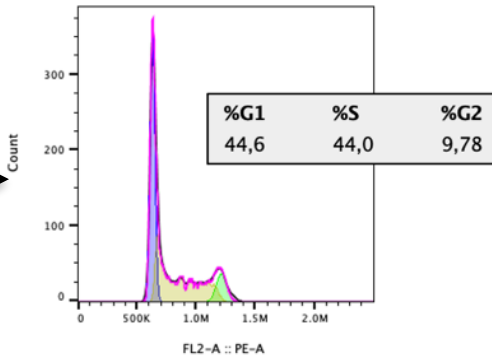




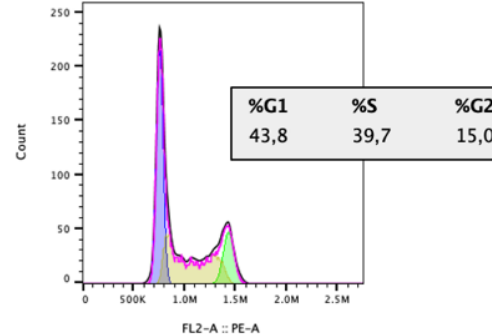
[DOSE RESPONSE: 0.01 – 10 μ M]
CELL VIABILITY (24h)



CELL CYCLE
DMSO (ctrl)



Akenam12 (0.5 μ M)

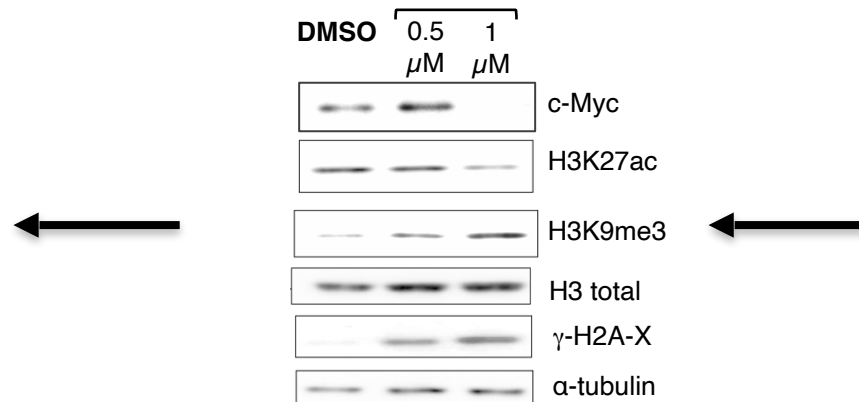


**CELL VIABILITY ASSAY IN CELL LINES OF
BOTH HEMATOPOIETIC AND SOLID
TUMORS**

Cell line	IC50 (24h)	IC50 (48h)
U937	0.76	0.65
K562	1.25	1.18
Jurkat	0.73	0.28
NB4	0.50	0.37
U373	6.67	1.62
HeLa	1.67	0.94

**SEARCHING FOR MOLECULAR
MECHANISMS**

WB ANALYSIS of Histones PTMs and cMyc



	DMSO	Ak 12_0.5uM
G1 % (mean)	44,5 $\pm$ 1,7%	43,8 $\pm$ 1,3%
S % (mean)	43,8 $\pm$ 1,5%	40,6 $\pm$ 1,2%
G2 % (mean)	9,2 $\pm$ 1,4%	14,1 $\pm$ 1%



Monoclonal Antibody Conjugation with stable metal complex

GOALS?

Targeted Delivery:

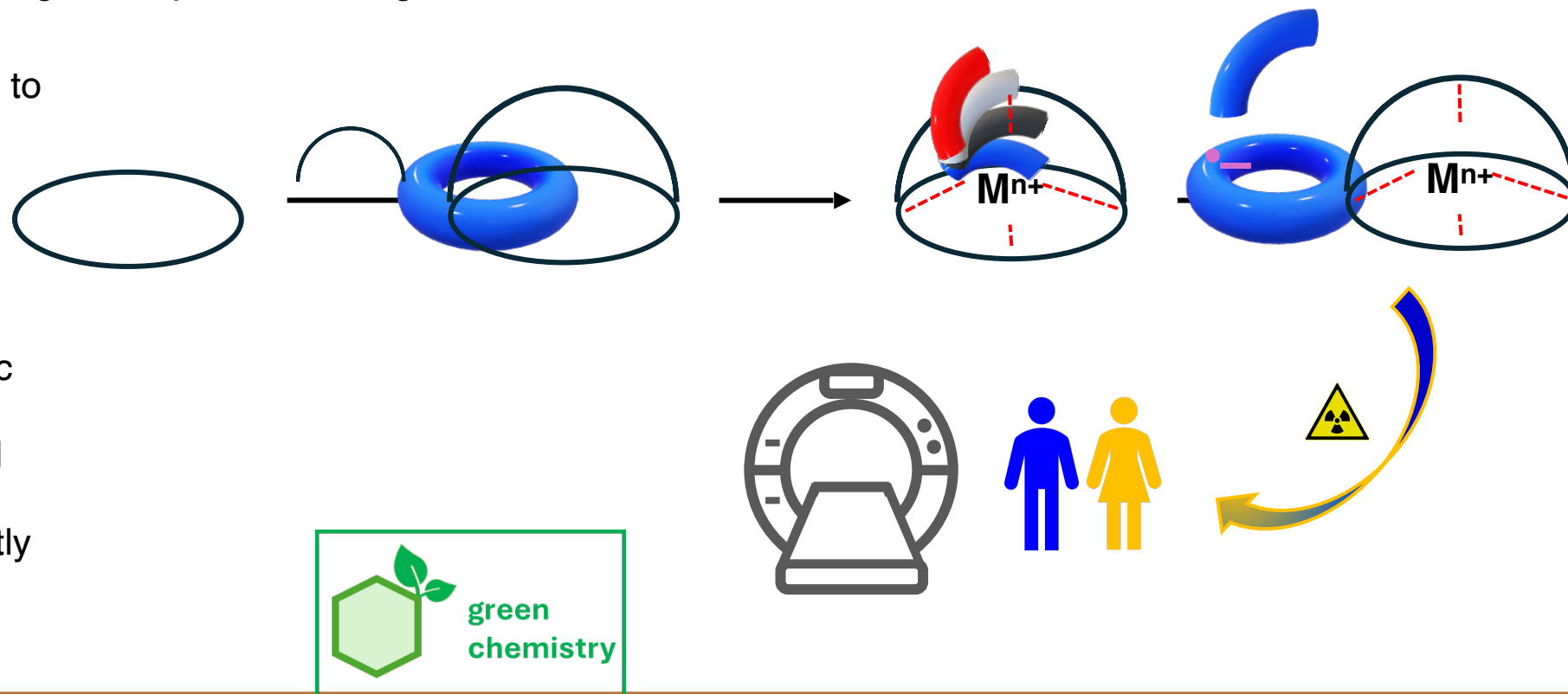
Antibodies to be used as specific delivery vehicles to deliver metal complexes (e.g., radiometals) to target sites like tumors, increasing efficacy and reducing side effects.

Imaging:

Radiometals conjugated to antibodies are used in diagnostic imaging techniques to visualize specific tissues or cells.

Therapy:

By carrying a therapeutic radionuclide or cytotoxic drug, the antibody-metal complex can deliver radiation or a drug directly to diseased cells.





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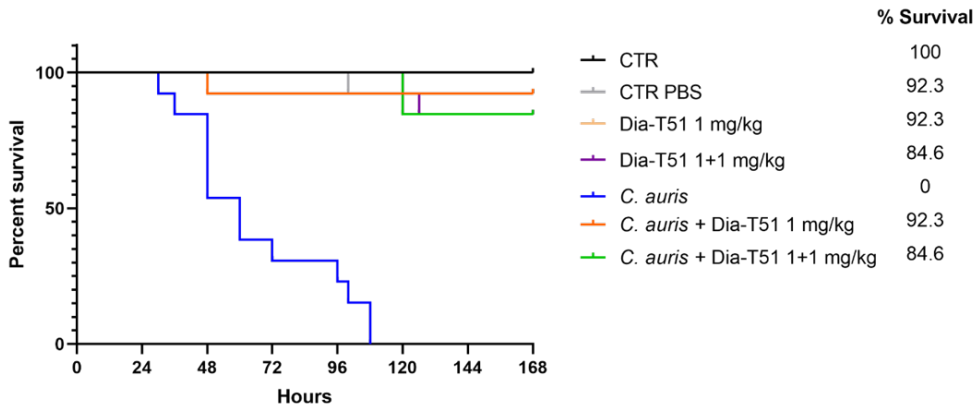
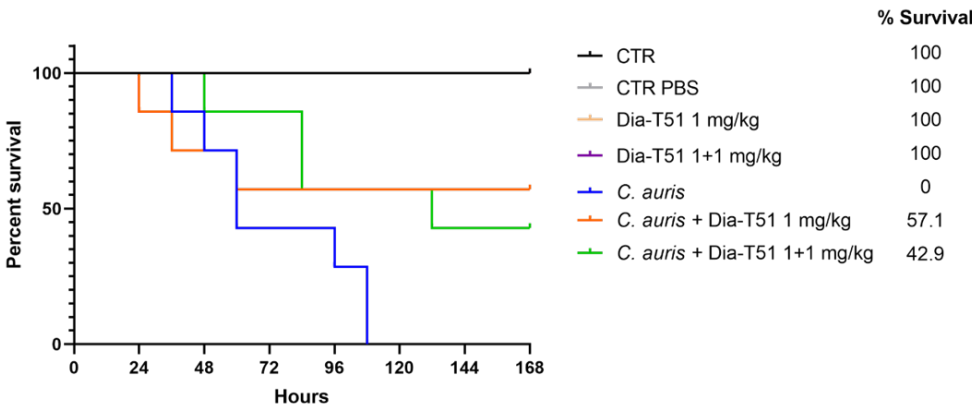
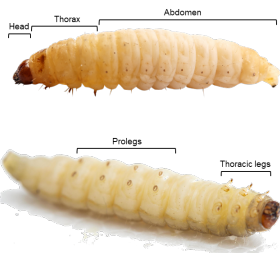


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In vivo studies of the antifungal humanized monoclonal antibody DIA-T51 ...



		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Pre-treatment	<i>C. auris</i>							
	Dia-T51 1 mg/kg + <i>C. auris</i>							
	Dia-T51 1 mg/kg + <i>C. auris</i> + Dia-T51 1 mg/kg							



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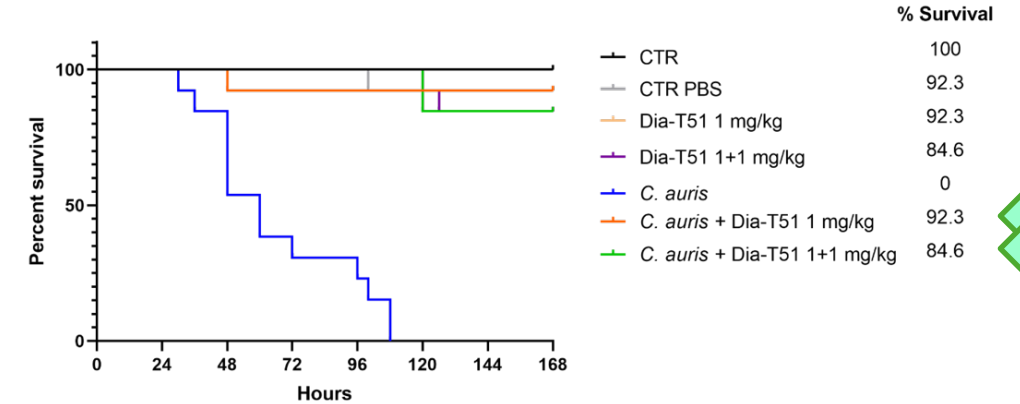
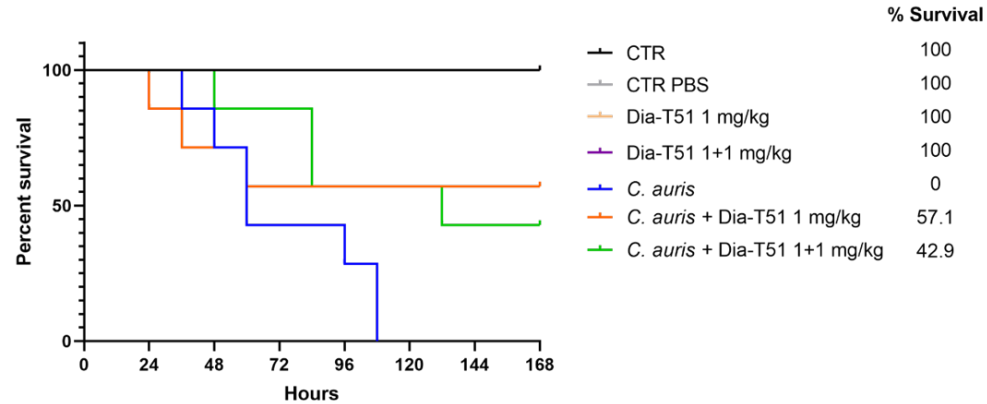
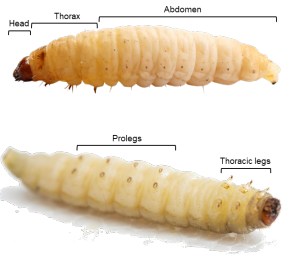


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Pre-treatment	<i>C. auris</i>							
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	Dia-T51 1 mg/kg + <i>C. auris</i> + Dia-T51 1 mg/kg							



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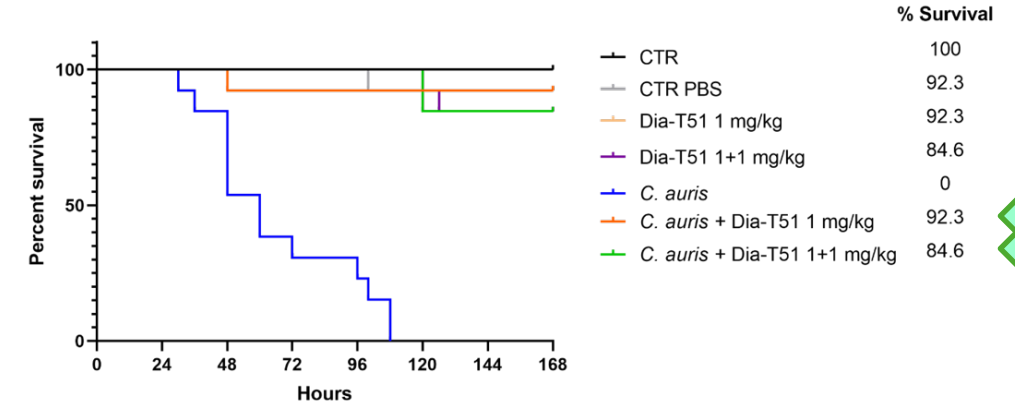
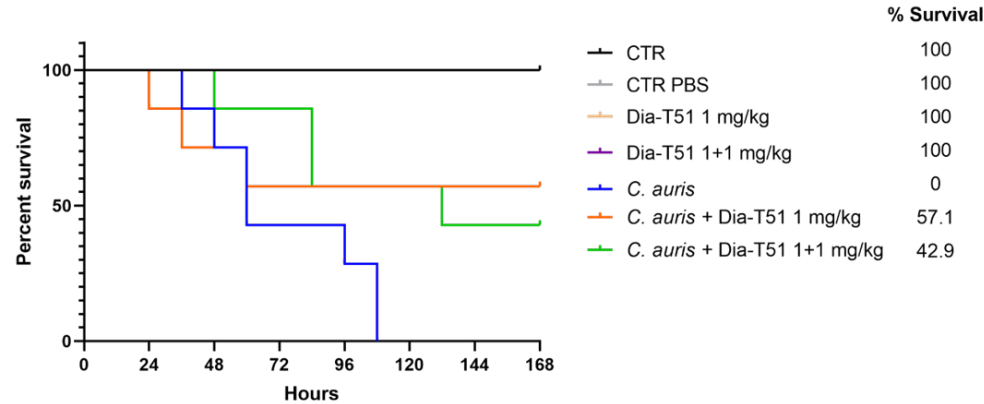
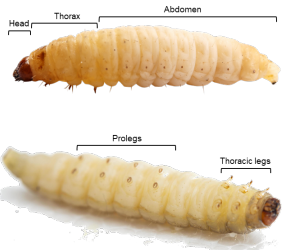


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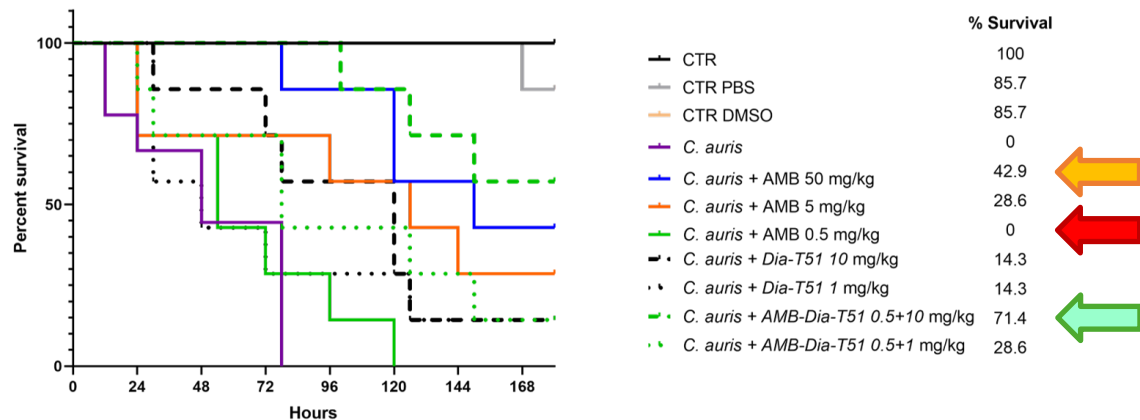


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In vivo studies of the antifungal humanized monoclonal antibody DIA-T51 ...



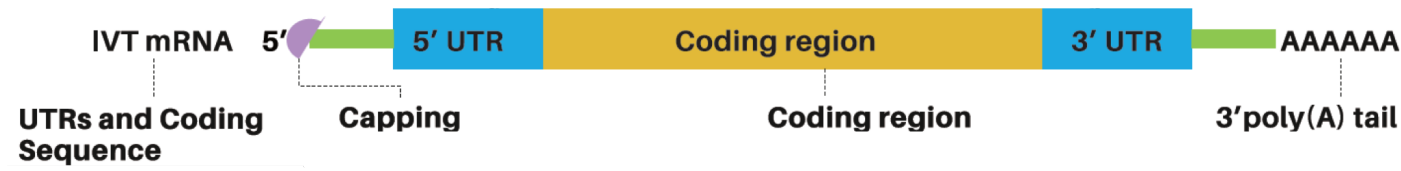
... and its synergy with AMPHOTERICIN B



	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
<i>C. auris</i>							
Pre-treatment							
Dia-T51 1 mg/kg + <i>C. auris</i>							
Dia-T51 1 mg/kg + <i>C. auris</i> + Dia-T51 1 mg/kg							



Modified mRNA to treat monogenic diseases



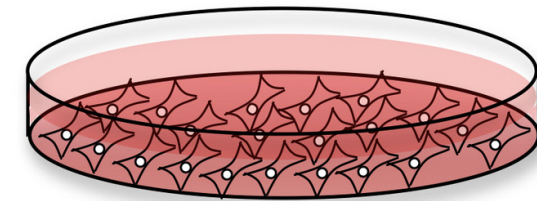
GAMT mRNA

- IVT-modified mRNA
- Mouse model of GAMT deficiency for *in vivo* studies



ATM mRNA

- CDS for ATM variants cloned in pMRNxp vector
- ATM-deficient cell lines for *in vitro* studies





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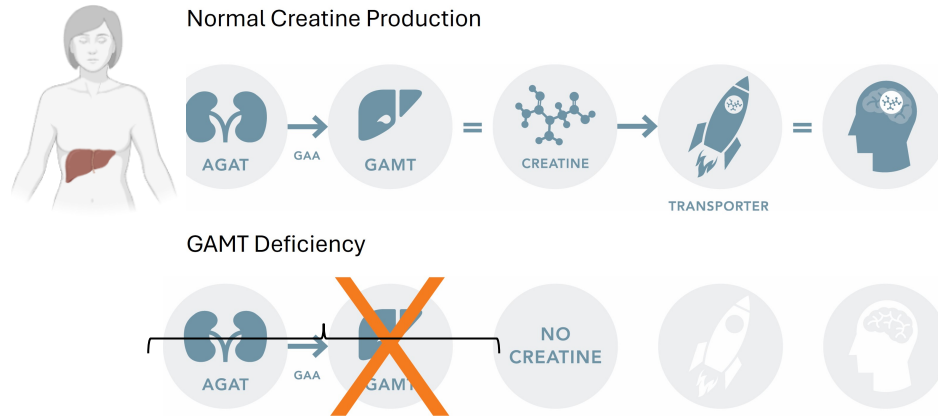


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RNA-based therapy for GAMT deficiency: *in vitro* studies

1

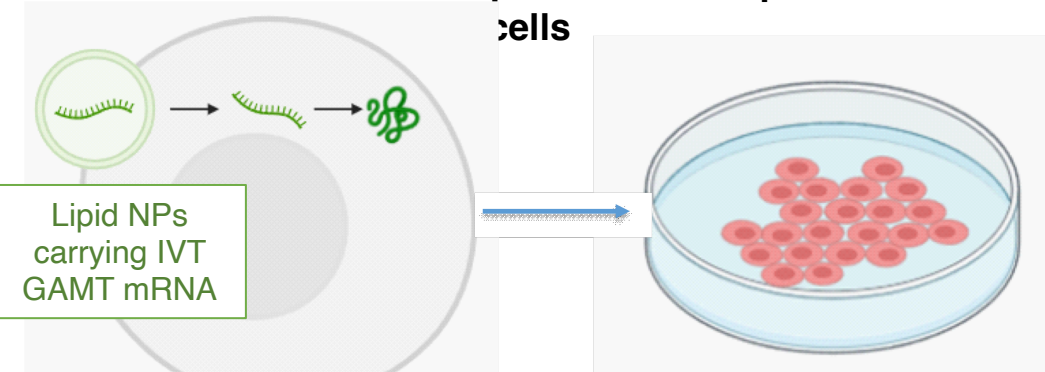
GAMT-mediated metabolism



IVT GAMT mRNA

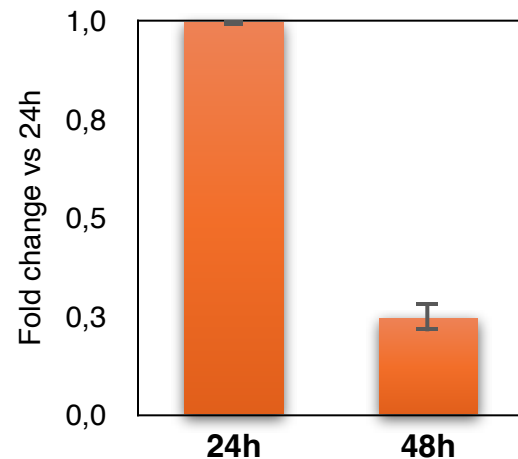
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GAMT mRNA lipofection in HepG2 cells



3

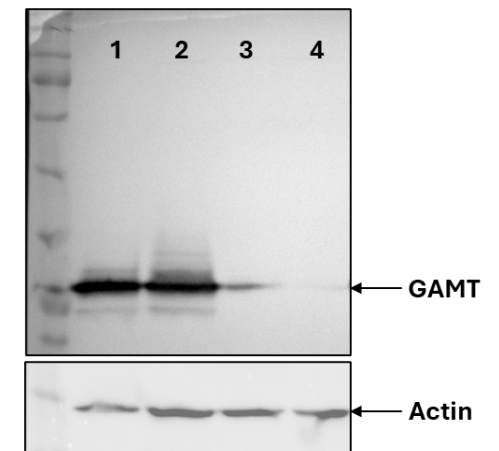
qPCR analysis



4

WB analysis

- 1: Transfected HepG2 24h
- 2: Transfected HepG2 48h
- 3: Control HepG2 24h
- 4: Control HepG2 48h

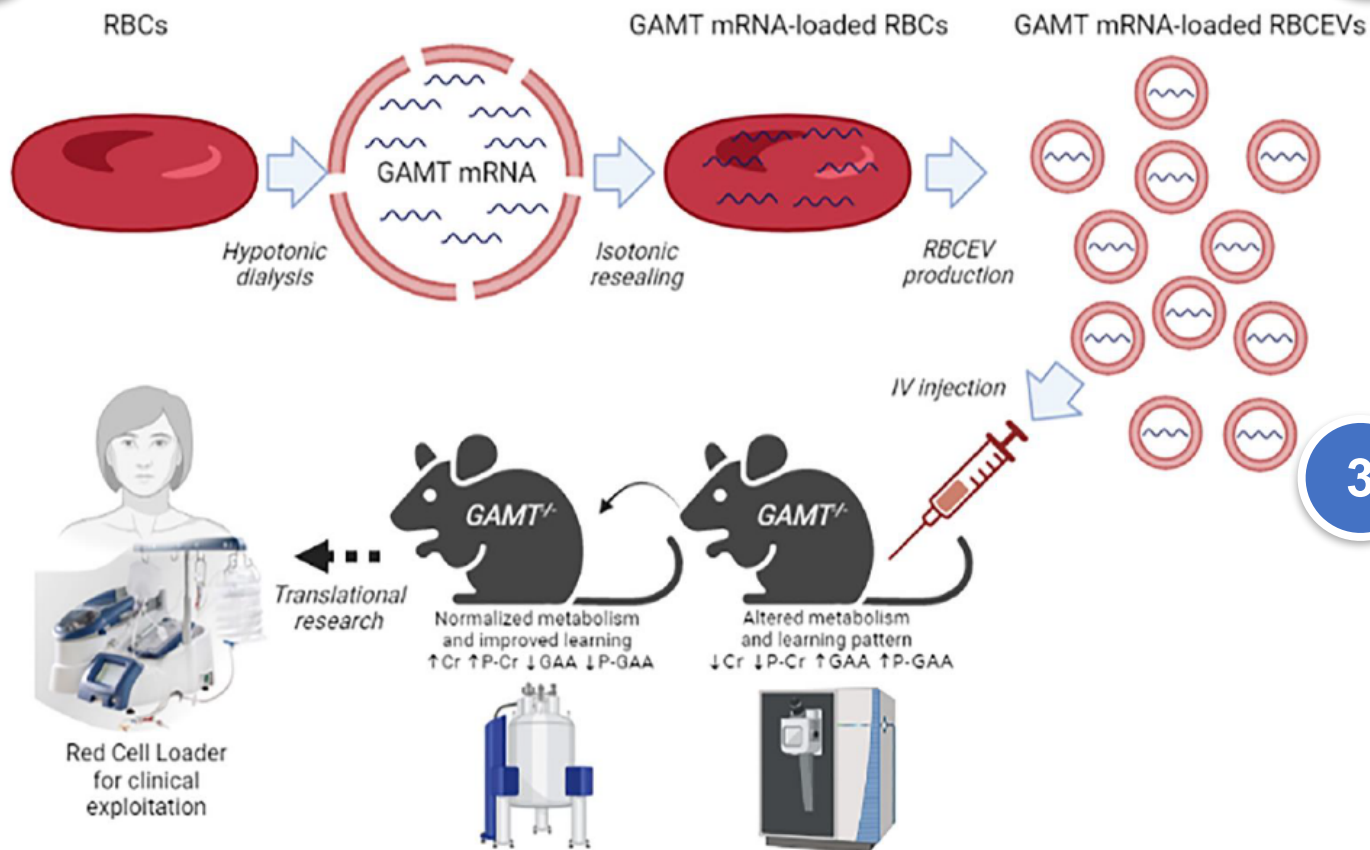




RNA-based therapy for GAMT deficiency: *in vivo* studies

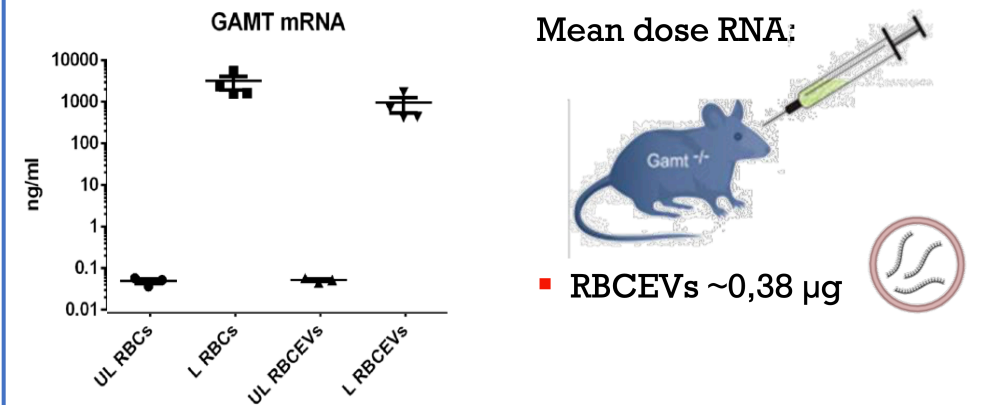
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PROPOSED STRATEGY



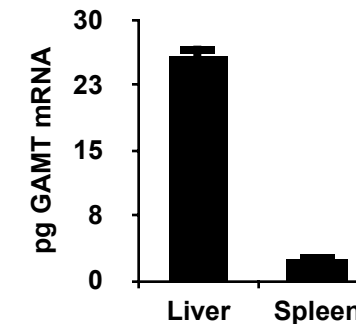
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Synthetic GAMT mRNA loading and *in vivo* administration

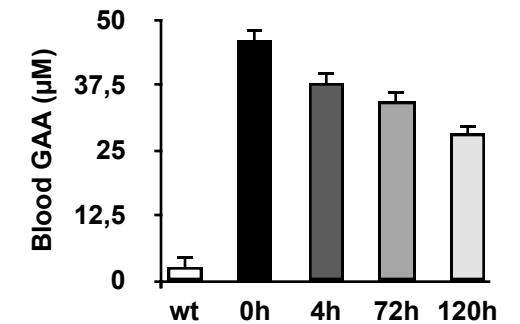


3

Biodistribution analysis



Metabolic analysis





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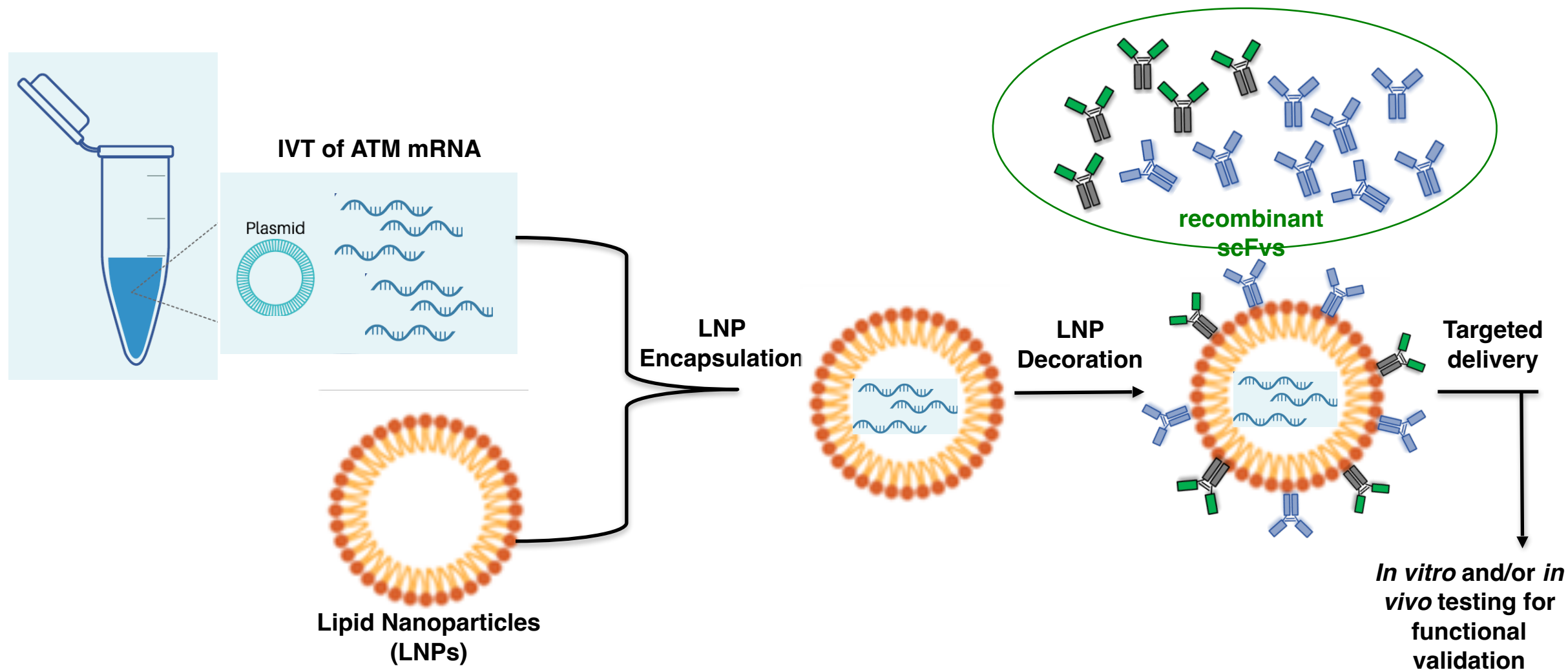


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mRNA-based therapy for ATM deficiency: *in vitro* studies





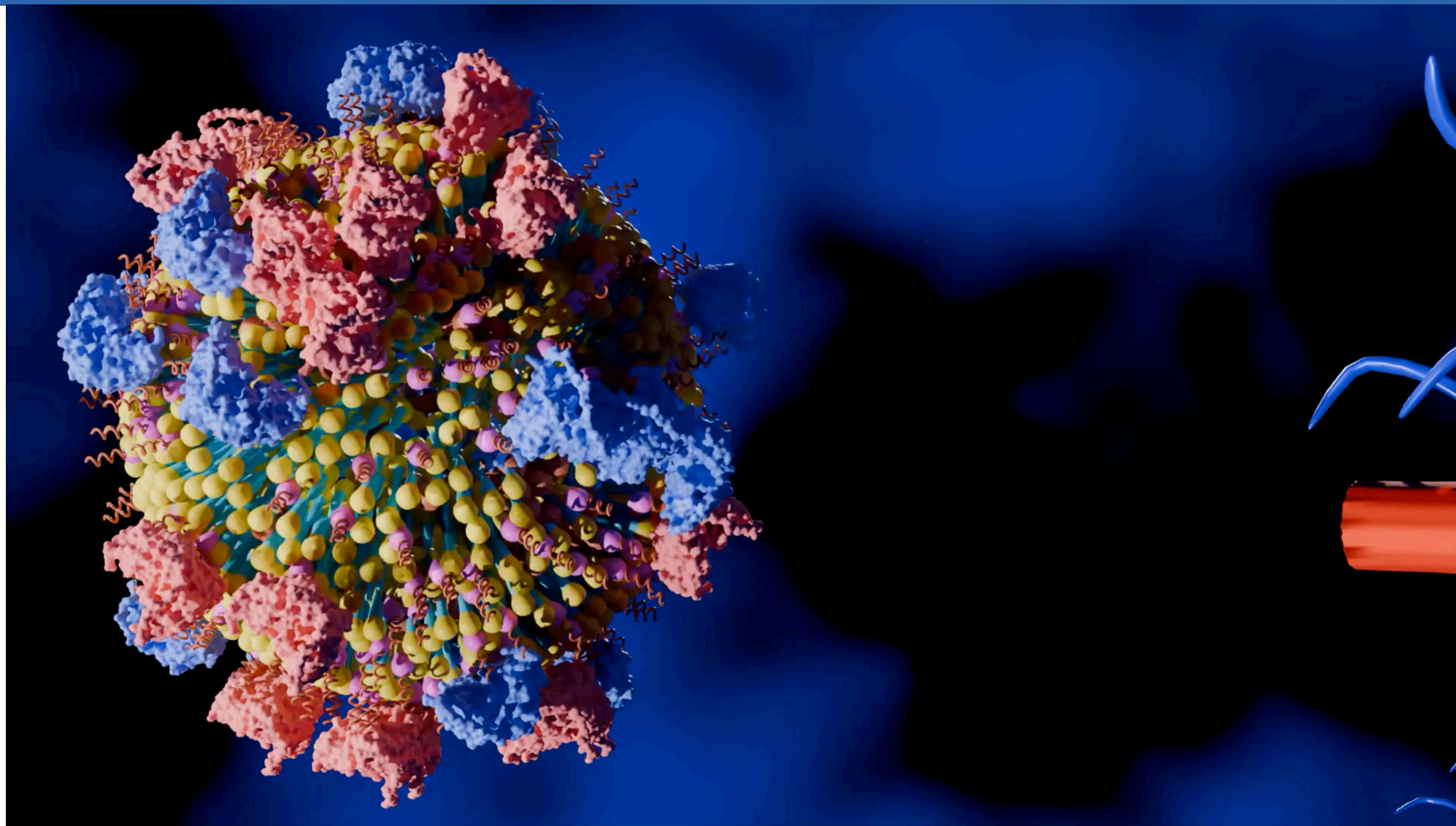
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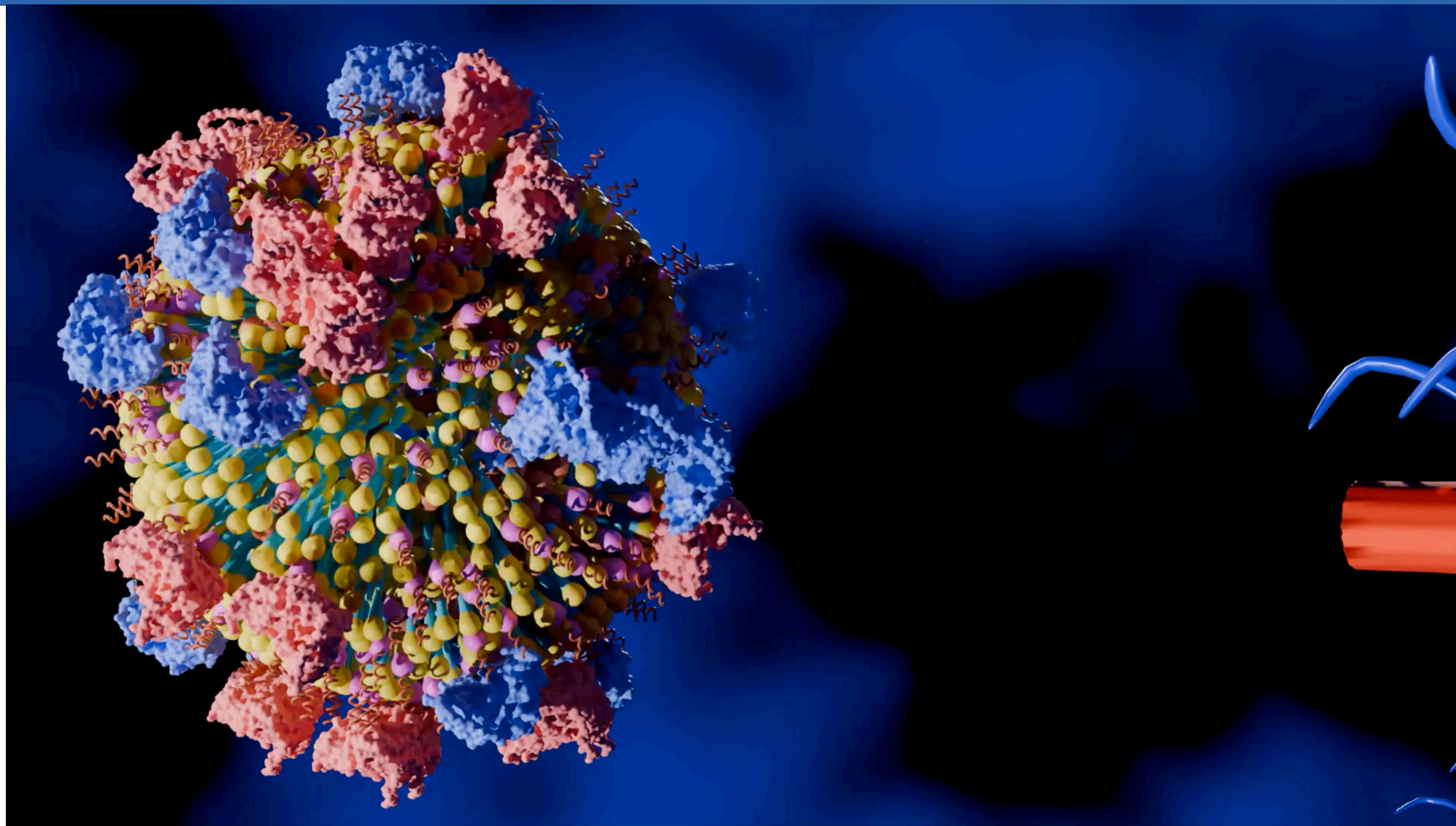
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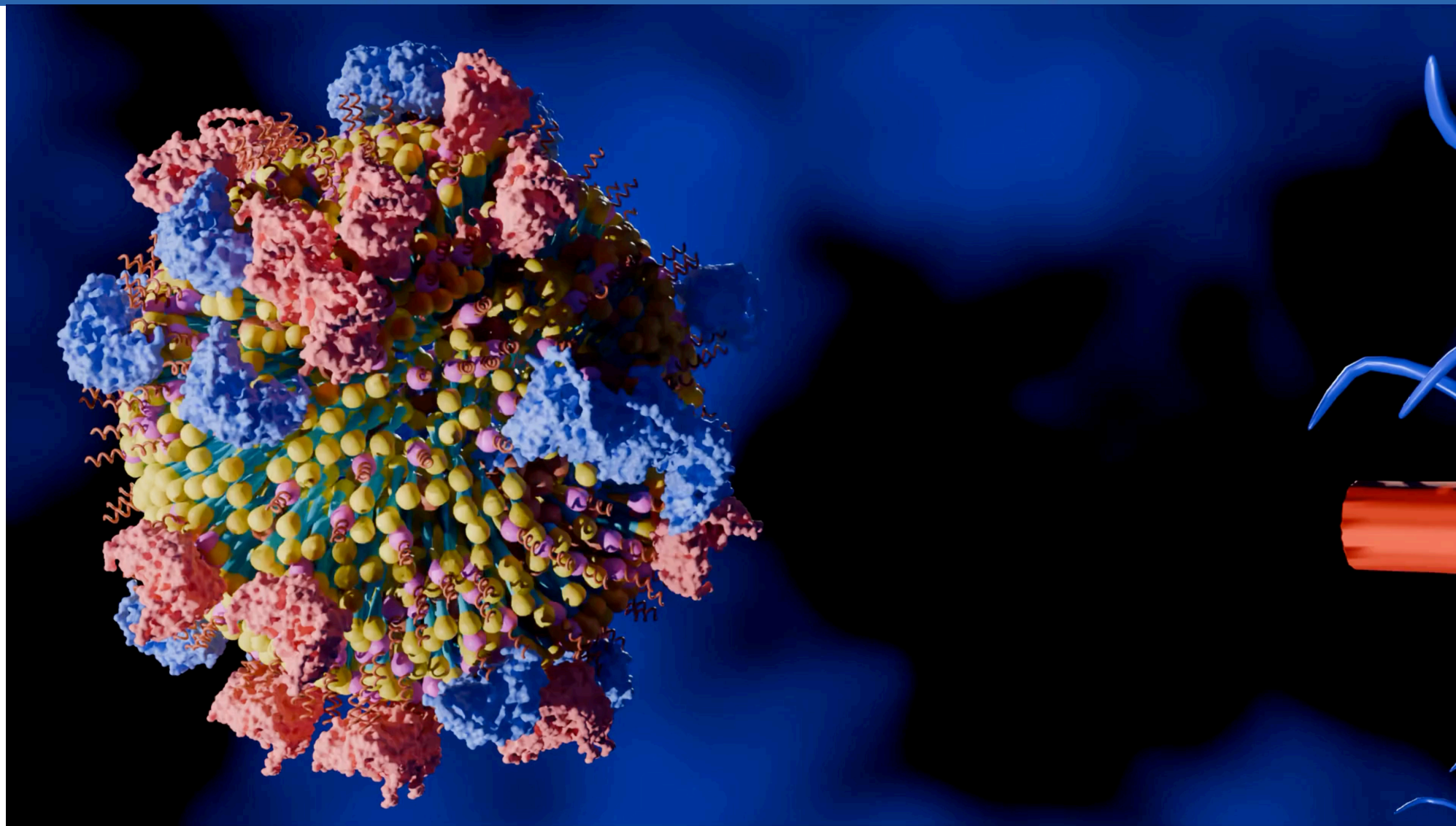
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