Syngene
Putting Science to Work

Design and discovery of a bispecific conditional TNF-R agonist

Nordics Discovery Symposium, 2024 Sept 13th

Ralf Guenther, AVP & Head Large Molecule Discovery Syngene Intl.

Syngene has been a partner of choice for global clients, providing contract research, development, and manufacturing business solutions

450+
clients

>2 Mn
Square feet of Infrastructure

8500+
Global workforce

200+
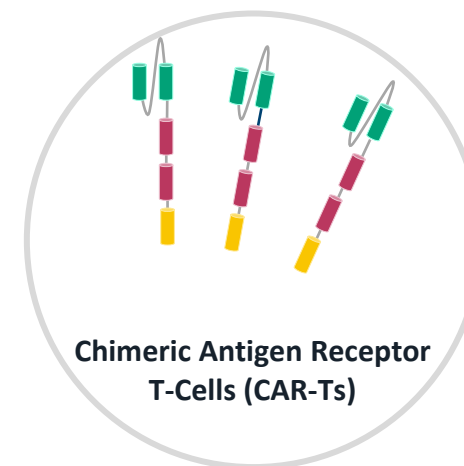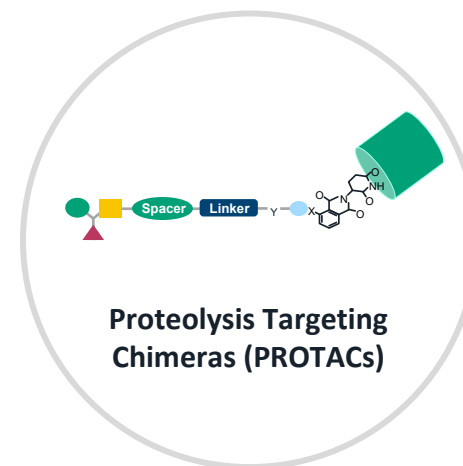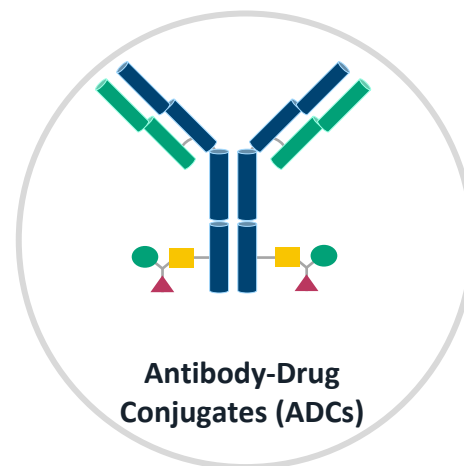client quality audits*

>35 regulatory audits*
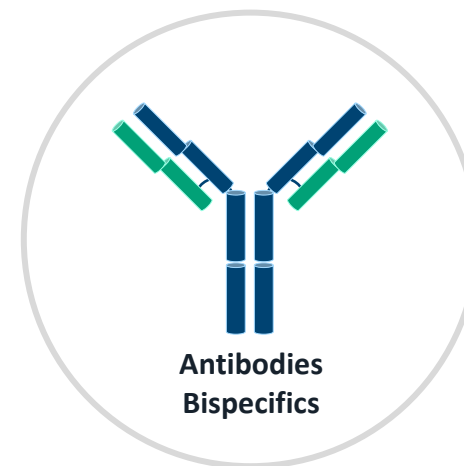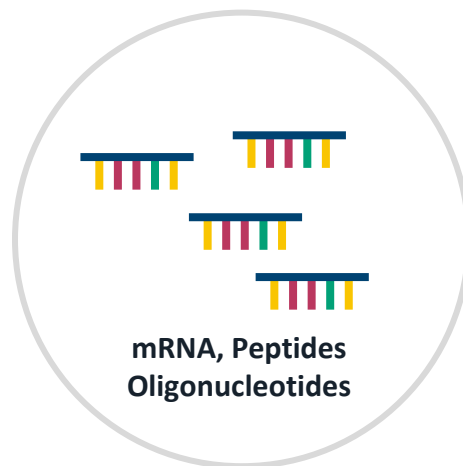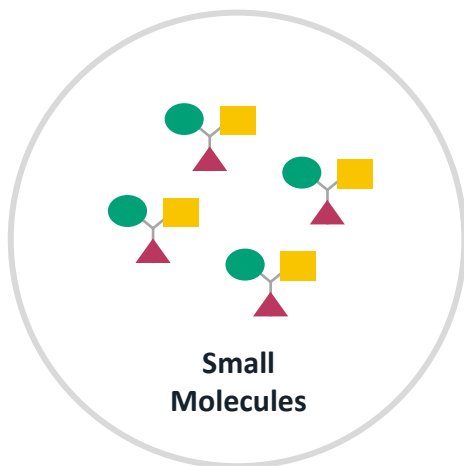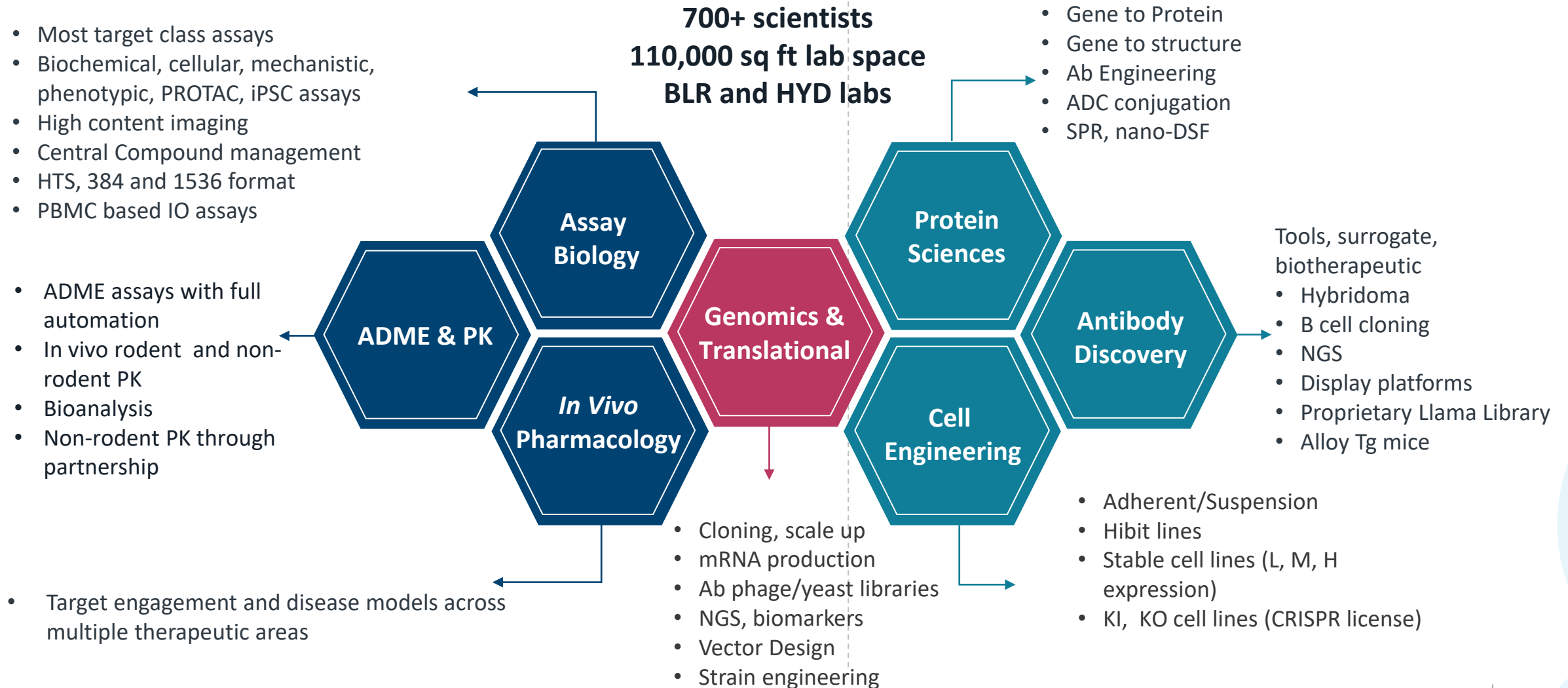
~$4 Bn
market cap

450+
patents
IP belongs to client

30
years of trust

The future of drug discovery lies in applying the most appropriate modality, and we work on all of them

Small Molecules

mRNA, Peptides Oligonucleotides

Antibodies Bispecifics

Antibody-Drug Conjugates (ADCs)

Proteolysis Targeting Chimeras (PROTACs)

Chimeric Antigen Receptor T-Cells (CAR-Ts)

The Biology team seamlessly supports drug discovery in a modality-agnostic fashion

700+ scientists
110,000 sq ft lab space
BLR and HYD labs

- Most target class assays
- Biochemical, cellular, mechanistic, phenotypic, PROTAC, iPSC assays
- High content imaging
- Central Compound management
- HTS, 384 and 1536 format
- PBMC based IO assays

Assay Biology

- Gene to Protein
- Gene to structure
- Ab Engineering
- ADC conjugation
- SPR, nano-DSF

Protein Sciences

- ADME assays with full automation
- In vivo rodent and non-rodent PK
- Bioanalysis
- Non-rodent PK through partnership

ADME & PK

Genomics & Translational

Antibody Discovery

Tools, surrogate, biotherapeutic
- Hybridoma
- B cell cloning
- NGS
- Display platforms
- Proprietary Llama Library
- Alloy Tg mice

In Vivo Pharmacology

Cell Engineering

- Target engagement and disease models across multiple therapeutic areas

- Cloning, scale up
- mRNA production
- Ab phage/yeast libraries
- NGS, biomarkers
- Vector Design
- Strain engineering

- Adherent/Suspension
- Hibit lines
- Stable cell lines (L, M, H expression)
- KI, KO cell lines (CRISPR license)

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Case:
Design and discovery of a bispecific conditional TNFR agonist

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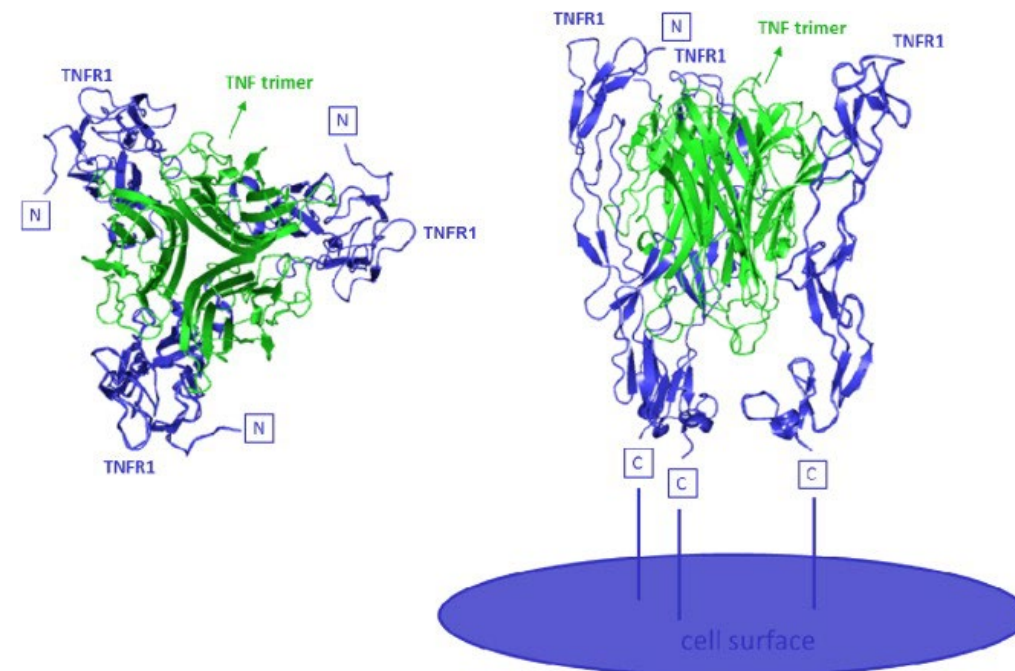# Tumor Necrosis Factor Receptor Superfamily (TNFRSF)

The tumor necrosis factor receptor superfamily (TNFRSF) is a protein superfamily of cytokine receptors characterized by the ability to bind tumor necrosis factors (TNFs) via an extracellular cysteine-rich domain.

Tumor Necrosis Factor (TNF) is a cytokine involved in systemic inflammation and is part of the body's immune response. TNF cytokines interact with TNF receptors (TNFRs), which are crucial in mediating cell survival, proliferation, differentiation, and apoptosis.
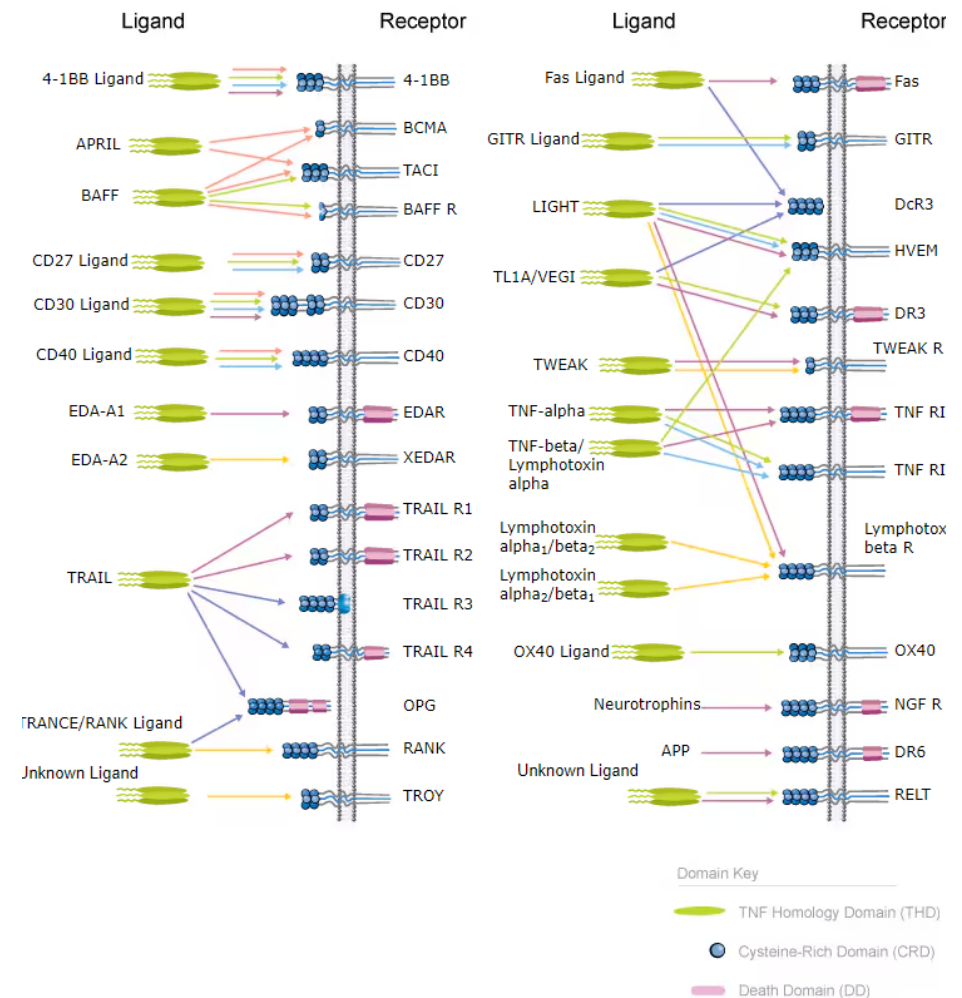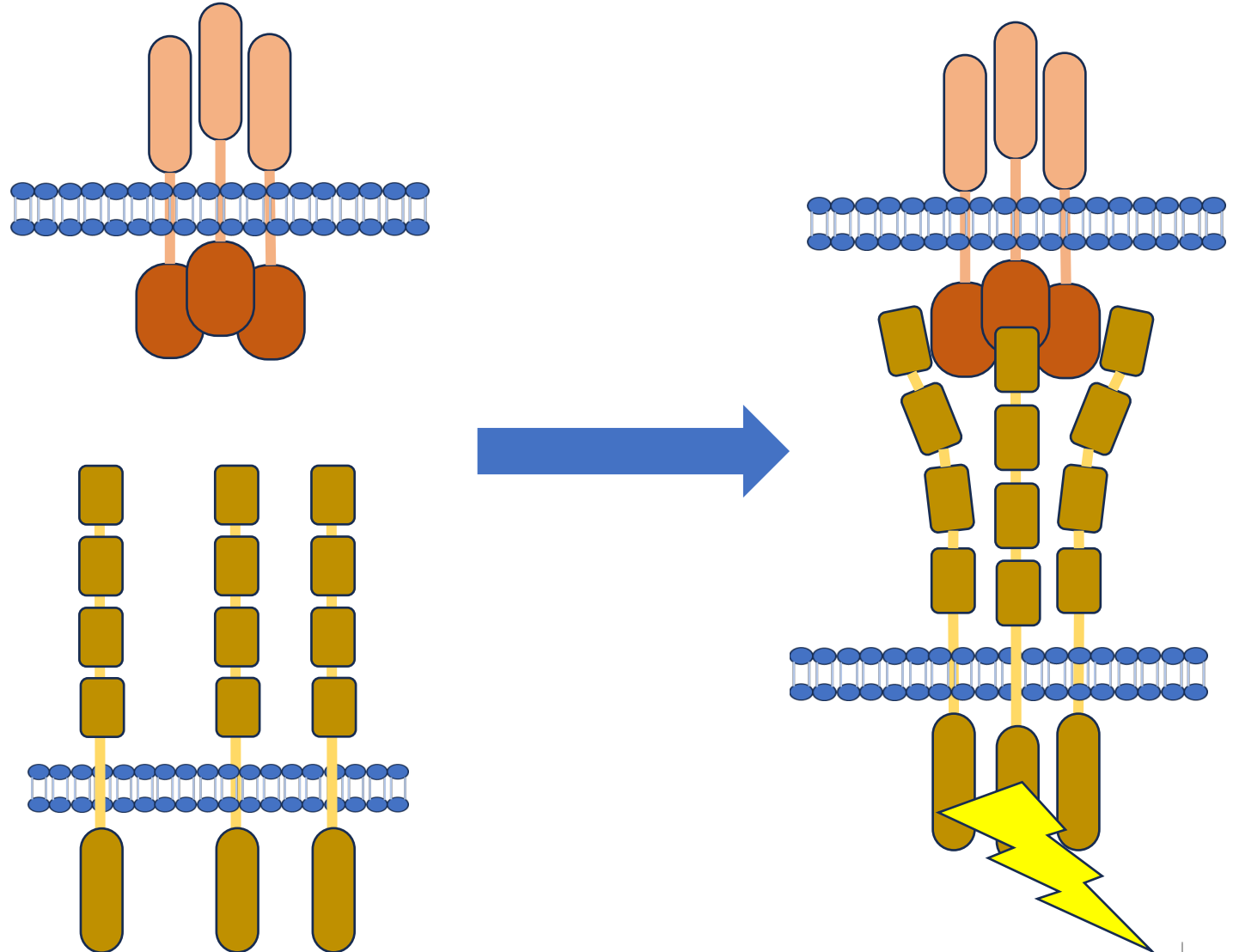
TNF-R agonistic antibodies

Tumor Necrosis Factor (TNF) receptor agonist antibodies represent a promising frontier in the treatment of cancer. These antibodies, by activating TNF receptors, can incite a robust anti-tumor immune response, leading to targeted destruction of cancer cells. TNF receptor agonist antibodies bind specifically to TNFRs, mimicking the action of natural TNF. This binding induces receptor trimerization, which is essential for downstream signalling. The activation of TNF receptors can lead to systemic inflammation and potential toxicity, which leads to a low maximum tolerated dose compromising efficacy.
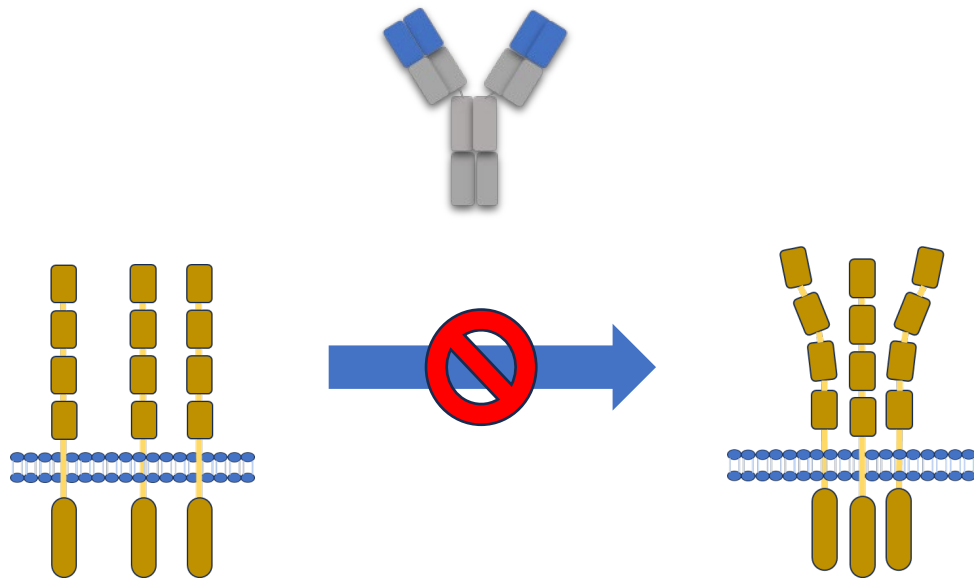
Molecular interaction of Receptor and Ligand

- Type I transmembrane glycoprotein
- Consists of extracellular cysteine-rich domains, a transmembrane domain, and cytoplasmic domain
- Active trimeric conformation upon binding of trimeric ligand

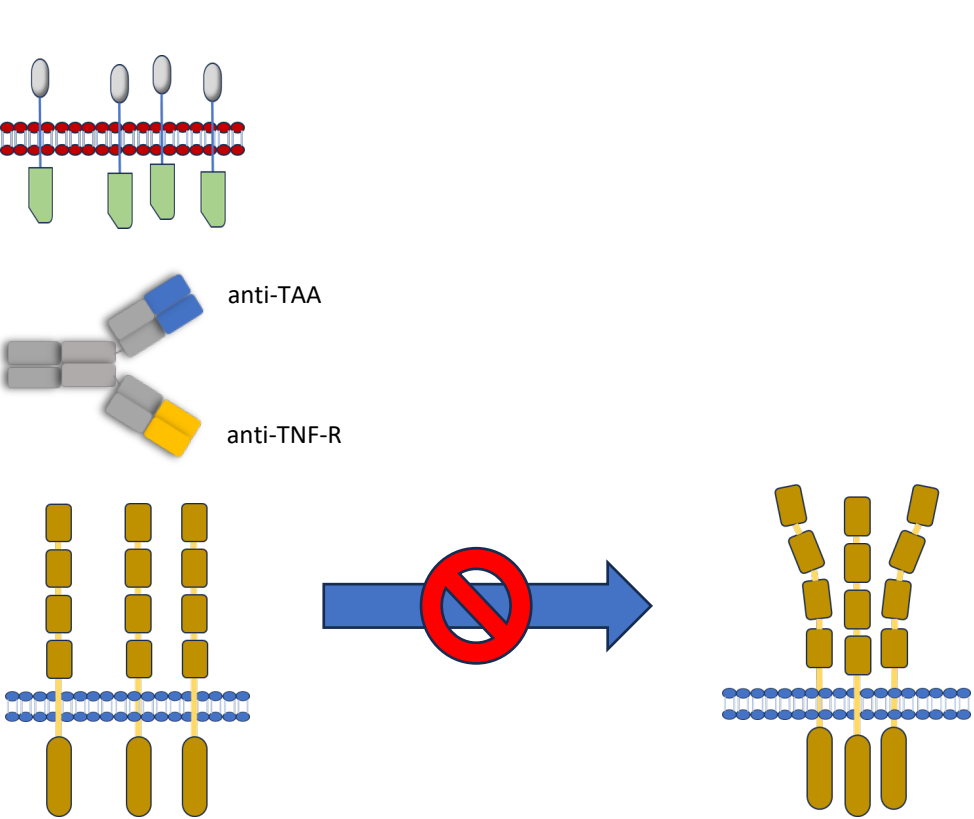# TNF-R agonistic Antibody

Fc gamma receptors

Conversion into a trimeric active conformation by a bivalent antibody is not working!
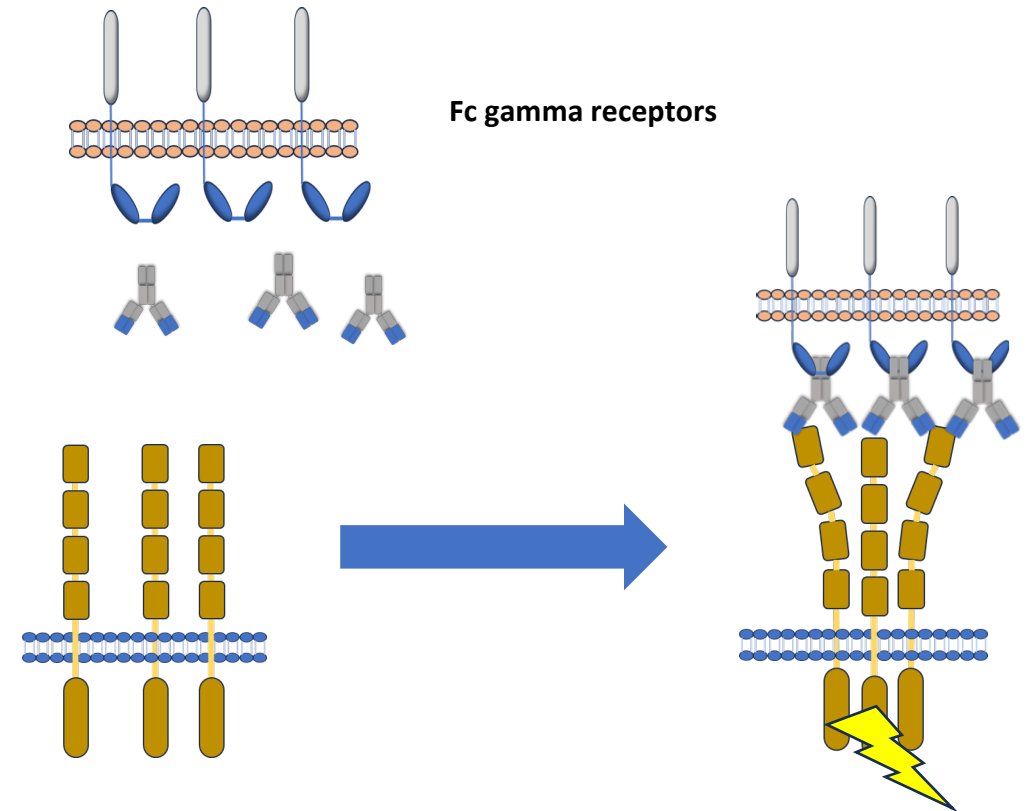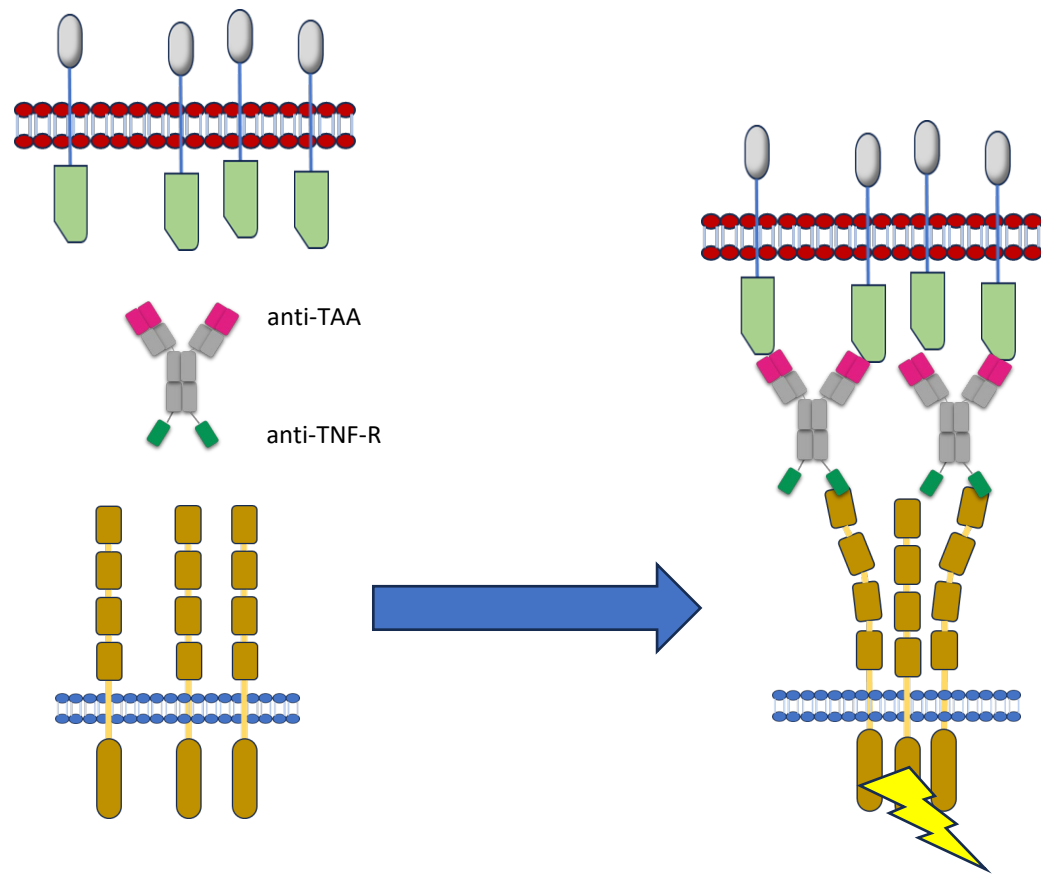
Conversion into a trimeric active conformation by FcgR clustered antibodies!

FcgR driven activation of TNF-Rs leads to a systemic activation causing severe side effects and reduce the therapeutic window.

Conditionally-active TNF-R agonistic Antibody

anti-TAA

anti-TNF-R

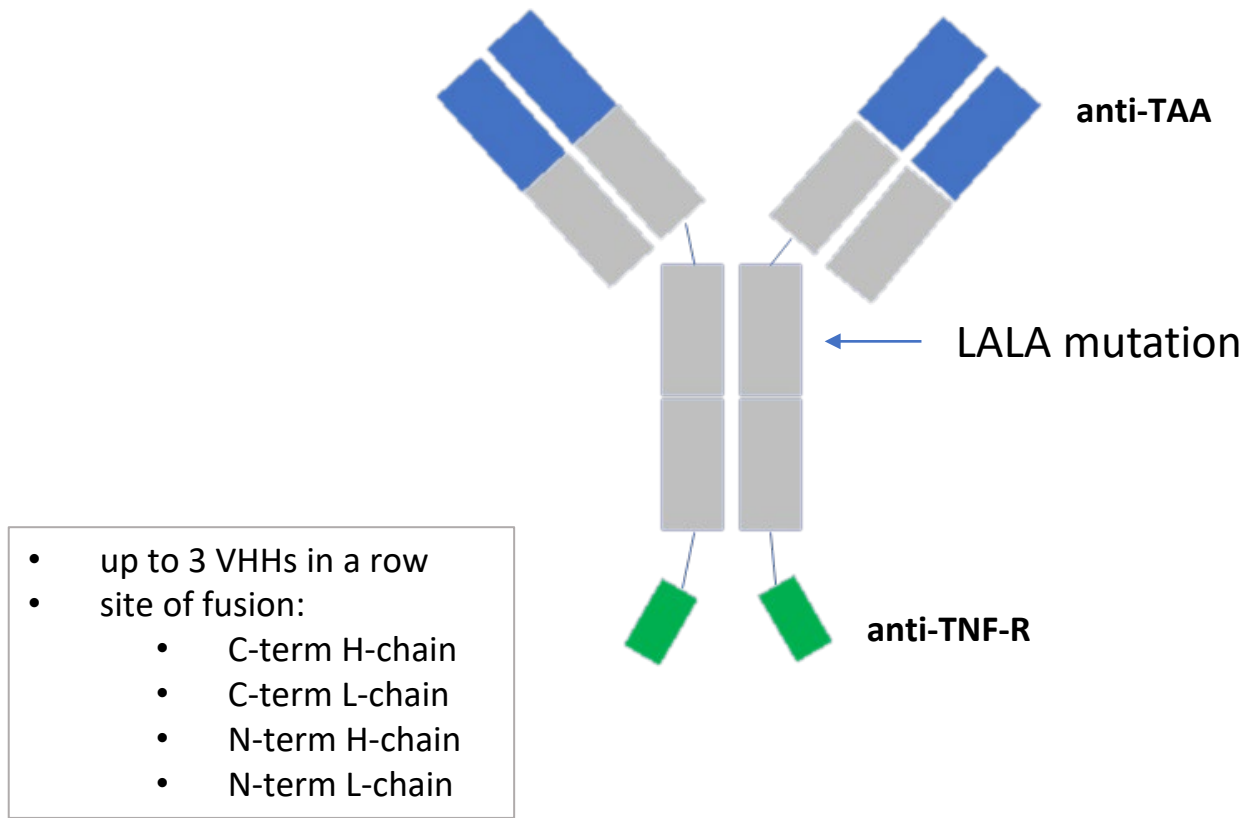Conversion into a trimeric active conformation by a bivalent bispecific antibody is not working!

anti-TAA

anti-TNF-R

Conversion into a trimeric active conformation by a **multivalent bispecific** antibody!

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FlexMab Format

Fusion of IgG and VHH

anti-TAA

LALA mutation

anti-TNF-R

- up to 3 VHHs in a row
- site of fusion:
  - C-term H-chain
  - C-term L-chain
  - N-term H-chain
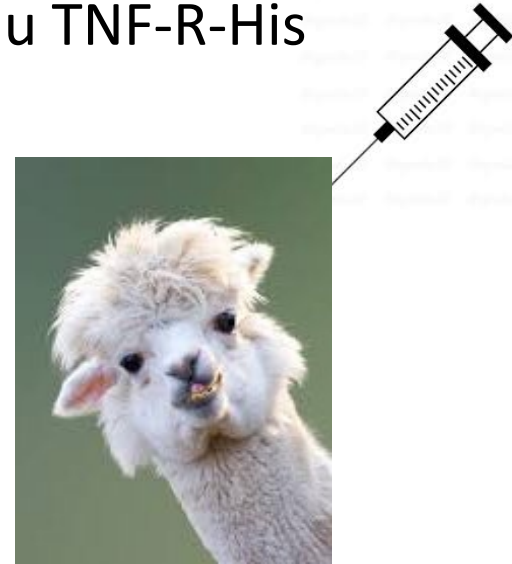 - N-term L-chain

Antibody Discovery

- hu TAA-His
- mu TAA-His
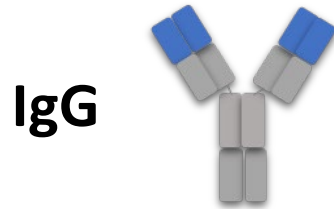

Transgenic mouse producing
fully human antibodies

➢ B-cell cloning
➢ Yeast Surface Display

- hu TNF-R-His
- mu TNF-R-His

➢ Yeast Surface Display

Antibody Discovery – early hit identification

IgG

Input 300-500 unique antibodies

- binding to human and mouse target protein
- binding to human and mouse target pos. cells
- affinity determination
- epitope binning

Output ~25 unique antibodies

VHH
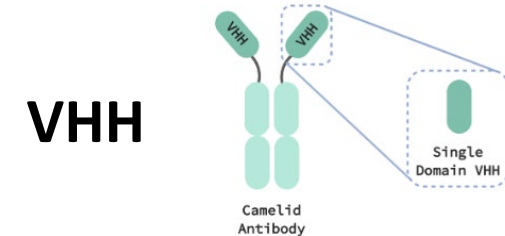
- **Input 150 - 200 unique VHHs**

- binding to human and mouse TNF-R protein
- binding to human and mouse TNF-R pos. cells
- affinity determination
- domain binning
- epitope binning
- ligand competition

Output ~10 unique VHHs

Antibody Discovery – bispecific hit identification
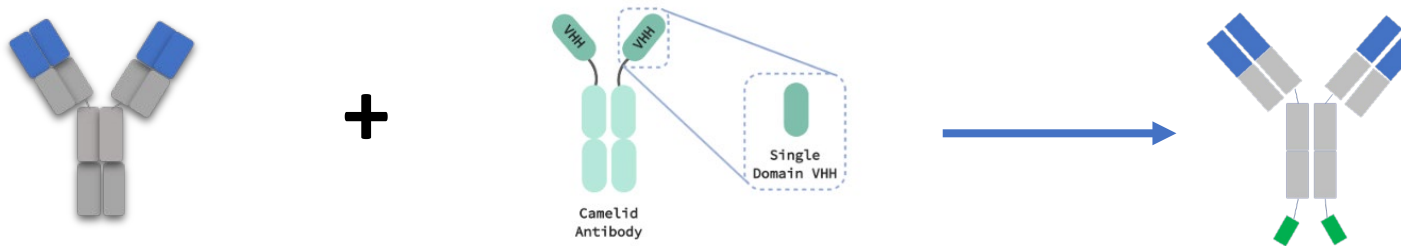
Functional assessment – TAA-dependent effector cell activation

High effector cell activation marker

Low effector cell activation marker

TAA

TNF-R
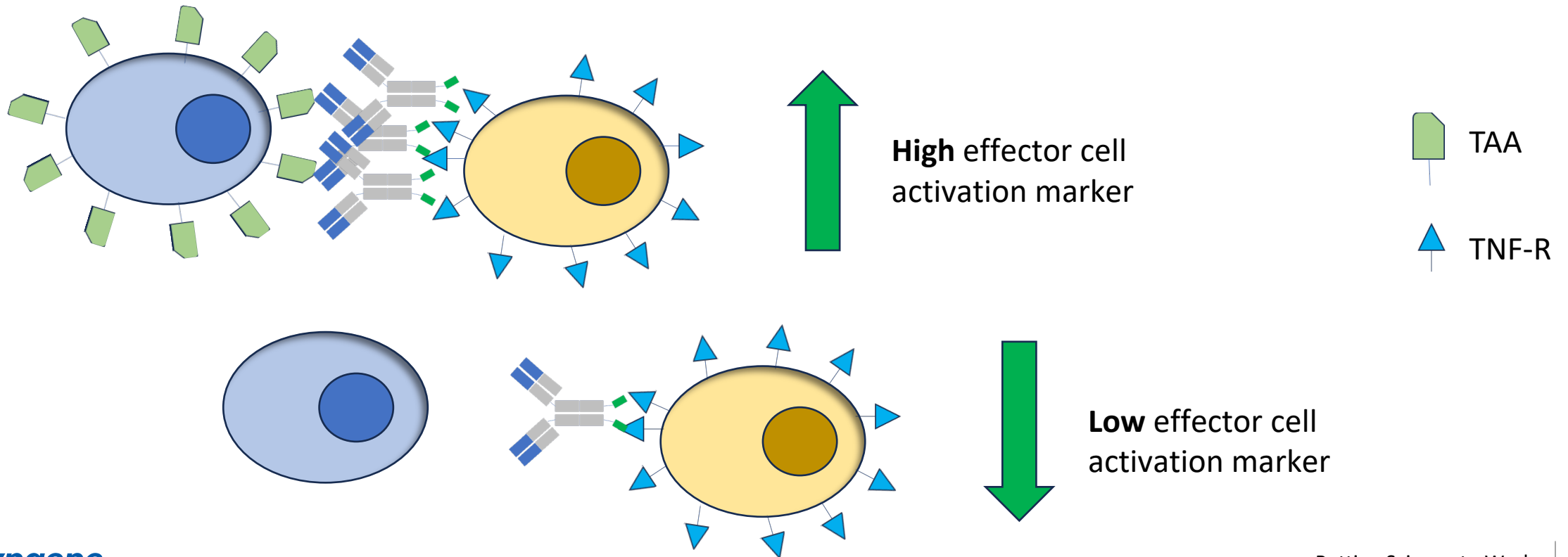
Lead Optimization & Selection

Tumor bearing TAA pos.

Tumor bearing TAA neg.
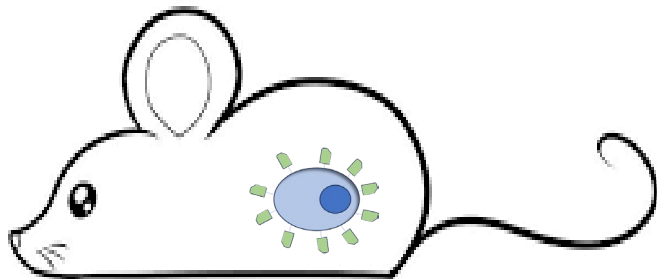
Immunocompetent mice
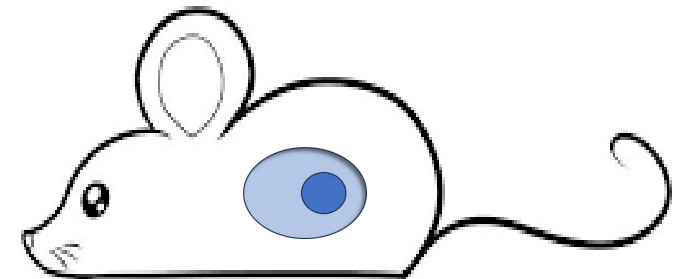
- tumor regression
- effector cell activation
- no elevated systemic cytokines

- tumor outgrowth
- no effector cell activation
- no elevated systemic cytokines

Lead Optimization & Selection

- ✓ SO of IgG and VHH

- ✓ Humanization of VHH

- ✓ Early developability assessment

- ✓ PK assessment in huFcRn-mice

- ✓ off-target binding assessment

Lead
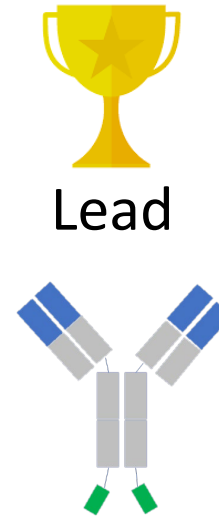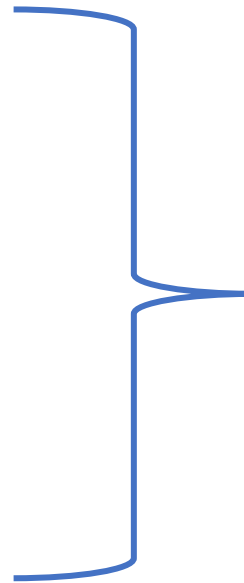
Syngene's Service Solution

It all starts with target selection and validation…..

Target Dossiers and Target Proposals, biochemical pathway elucidation, genomics analytics, drug repurposing

Gene/protein expression in tissues by qPCR, RNAseq, HT-Westerns, IHC, Mass Spec., biomarker analysis in normal vs. disease samples (rodents, humans): Multiplex platforms, ELISAs, Nexgen Sequencing.

siRNA studies, CRISPR/Cas9/ knock-out or knock-in in cell-lines

CAR T-cell engineering and testing *in vivo*

Lentiviral gene delivery systems in primary cells

Design of specific tool compounds and antibodies to test in vitro and in vivo

Transgenic animal evaluation *in vivo*

Our Cell Engineering team delivers highly Diverse and complex KI/KO/reporter cells for target validation and Screening Assays

Syngene Cell Engineering Capabilities

- Overexpression (Stable/Transient) in cell lines & Primary cells (Viral-based, Transposon-based, Electroporation-based).

- Knockout and Knockin (CRISPR-Cas9) including simultaneous multiple KO and KI including in difficult-to-transfect cells HiBiT engineering for PROTACs

- Knockdown (siRNA, shRNA) in cell lines & primary cells

- Reporter cell lines

- Genome-wide screening for target identification

Syngene has procured a license for CRISPR-Cas9 through ERS Genomics

Our expert protein sciences team of ~200 scientists produces ~3000-5000 customized proteins per year

Experience with a wide spectrum of protein classes

High-end protein analytics

Structural Biology

Protein Mass spec

Immunogens for Antibody campaigns

Antibodies: mono-bi/tri specifics, ADCs, fusion proteins

Enzymes for Assays

Proteins for Structural Biology

ADC conjugations

Conjugated reagents for Bioassays

HT and automated purification systems

Expression systems: Mammalian, Insect, Yeast, *E. coli*

Syngene

Gene to Protein Platforms

Mammalian Platform	E. coli Platform	Pichia Platform	BVES Platform

Purification

- Protein A/G Affinity Chromatography
- Gel filtration Chromatography
- Ni-NTA Affinity Chromatography
- Ion Exchange Chromatography
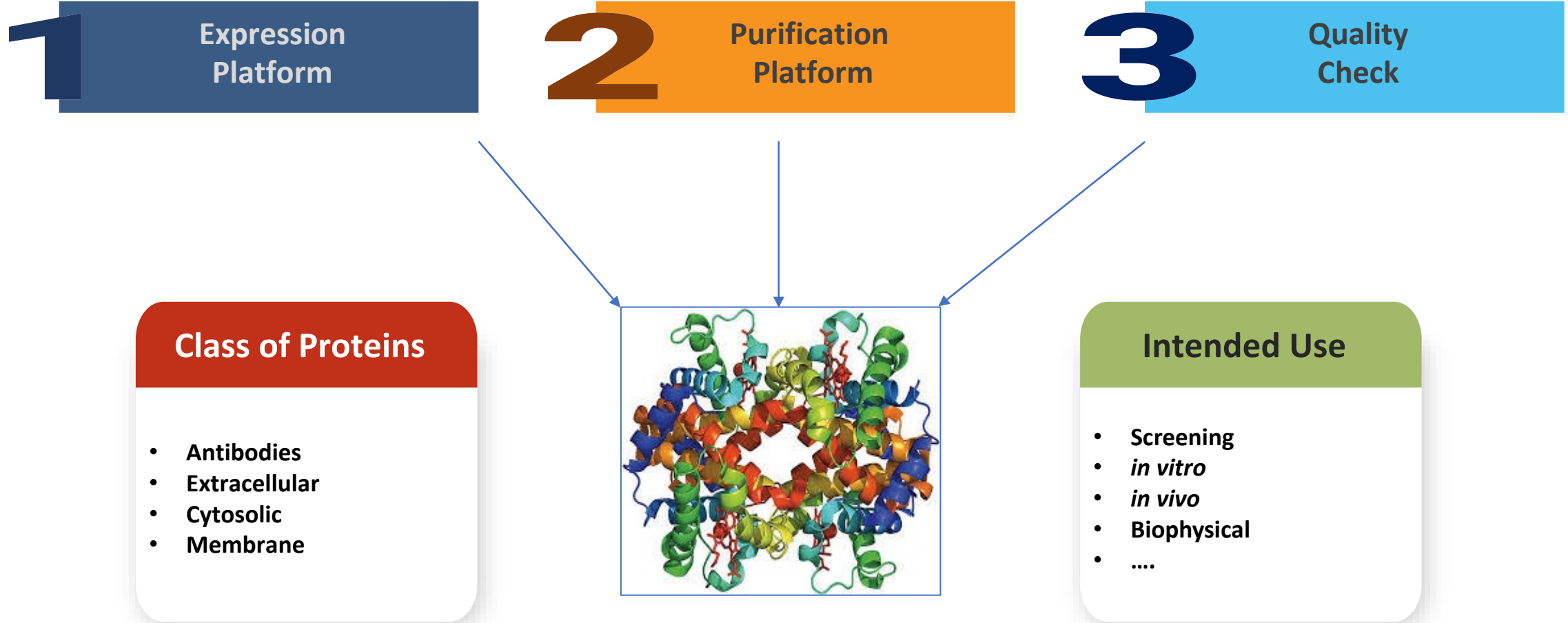- Hydrophobic Interaction Chromatography

Std. Q C

- Quantification: A280
- SDS-PAGE (reduced, non-reduced)
- Analytical SE-HPLC: Purity >95%
- Endotoxin: as specified
- Protein Identification by Mass Spec

Ext. Q C

- Quantification: A_{280}
- SDS-PAGE (reduced, non-reduced)
- Analytical SE-HPLC: Purity >95%
- Endotoxin: as specified
- Protein Identification by Mass Spec
- Analytical HIC
- Thermal Stability by NanoDSF
- Charge variants by Analytical IEX
- Binding by BLI/SPR
- EC_{50} Determination
- LCMS

Syngene

Customized Process Adaption

1 | **Expression Platform**

2 | **Purification Platform**

3 | **Quality Check**

Class of Proteins

- **Antibodies**
- **Extracellular**
- **Cytosolic**
- **Membrane**

Intended Use

- **Screening**
- ***in vitro***
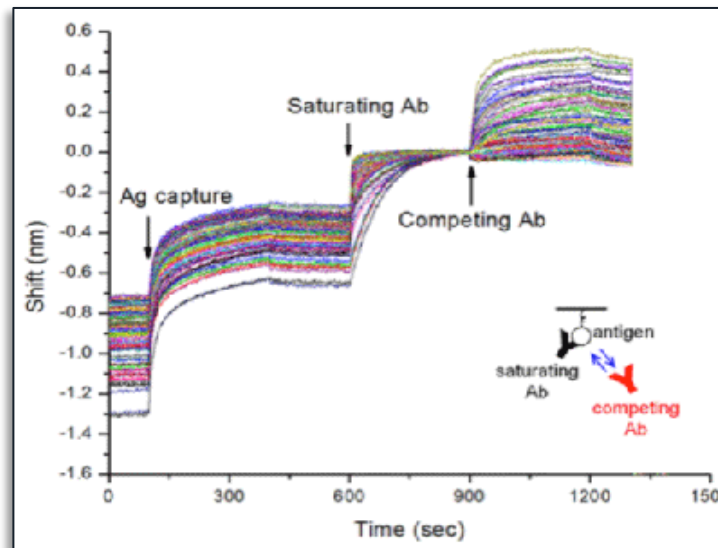- ***in vivo***
- **Biophysical**
- **....**

Biophysical assays help selection of molecules based on binding kinetics and stability
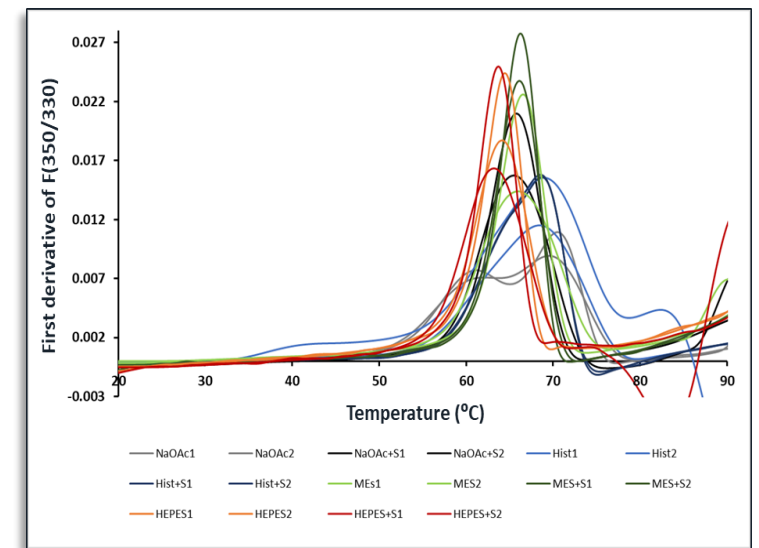
SPR for binding kinetics
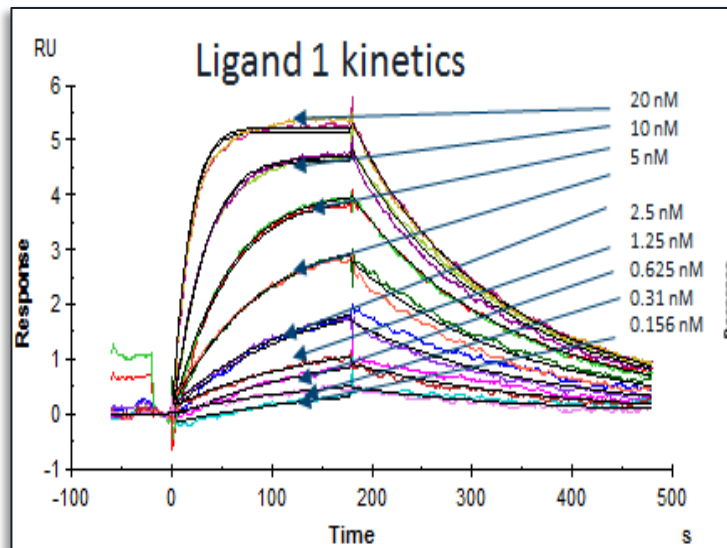
Octet for epitope binning of antibodies

Nano-DSF for thermal and buffer stability studies

Antibody Discovery:
Leveraging multiple platforms for biotherapeutic and tool antibodies

Protein Sciences and cell engineering Teams
Immunogen generation
Ab expression/purification
Structural Biology
Cell line generation

Immunizations
DNA, RNA, protein, cells, subtractive

Molecular Modeling
Structure-guided design
Immunogenicity prediction/mitigation
Humanization
Developability risk assessment/mitigation

Hybridoma

Single B cell cloning

Display platforms
Phage
Yeast

Proprietary
Llama library

Naïve/Immune

Naïve

Binding
ELISA,
Flow
SPR

Functional
Agonist/Antagonist
Engager
Tools/IHC

Stability Testing
Nano DSF

PK (bioassays)
Efficacy

Partnership with Alloy Tx for Tg mice with human Ig
Proprietary cell lines and bispecific platforms (WIP)

We have a breadth of capabilities to support your ADC research…
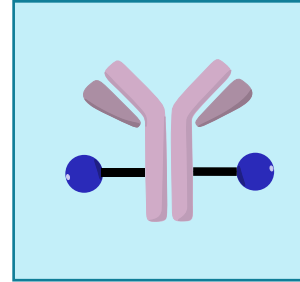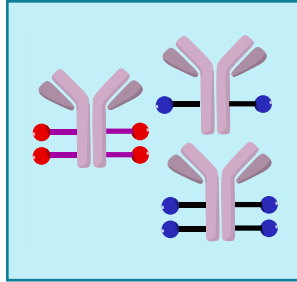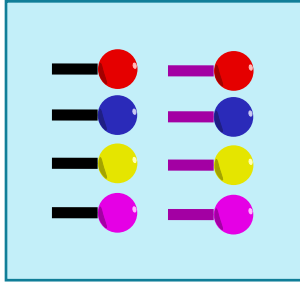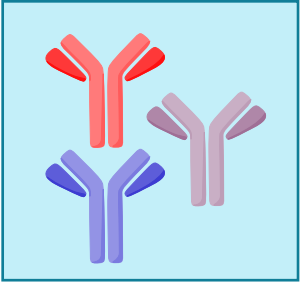
Antibody discovery	ADC Chemistry	Bio-conjugation	Assay Biology	DMPK and In vivo
Hybridoma	Designing and synthesizing of payload-linkers	Lysine-based	*In vitro cancer cell killing, immune modulation*	Linker-payload: LC-MS
Single B cell		Site-specific		ADC: Bioassay
Display platforms		Enzymatic		In vivo efficacy

GMP manufacturing of antibody, linker, payload
GMP conjugation: being built

Our structural biologists solve apo- and ligand-bound structures to evaluate binding mode of small and large molecules

Reagent Generation (Antibody, Proteins and Nucleic acids)

Crystallization Trials (mosquito robot) and Screening

Crystal Optimization and Cryo-protectant screening

X-ray diffraction data collection at Synchrotron (Austraila, France)

Structure solution and Refinement

Structure analysis and Interpretation
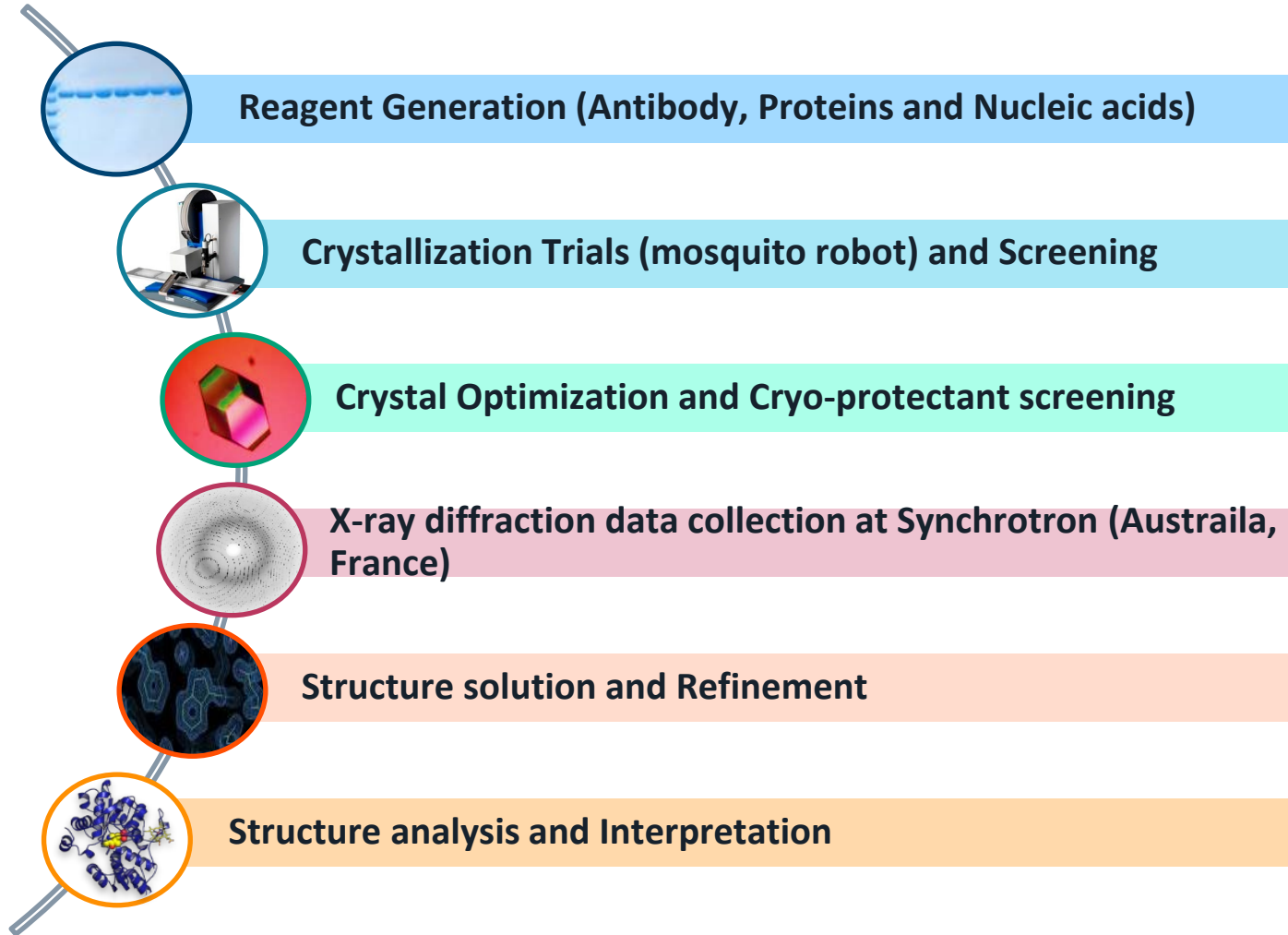
Productivity in 2022	
High quality crystals (Apo + Complex)	**95**
Datasets collected	~ 450
Structures solved/refined	63
Resolution Range	1.40 to 3.50 Å
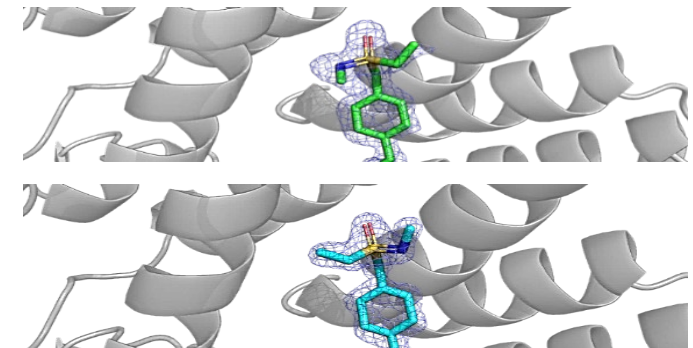
Our Assay Biology Team evaluates potency of test molecules in in vitro systems and evaluates mechanistic pathways

- Team of 160 + Masters and PhD level scientists
- 5:1 MSc: PhD ratio
- Average experience post degree: 1-12Y

- State-of-the-art infrastructure
- Streamlined process for sample submission to data & quality management

- Assay experience in Biochemical and biophysical
- Cell based, *ex-vivo* translational, biosimilarty
- Microbiology

- Multiple target classes (GPCR's, enzymes, NHRs, transporters and ion channels)
- Multiple modalities (Small molecule, Large molecule, ASO, Protacs etc.)

- Multiple assay formats [96-, 384-w]
- Assay platforms- TR-FRET, HTRF, FP, Lum, BRET, FL, RA
- Mechanistic assays (mRNA/protein) for target engagement

- Assay optimization and validation capabilities
- Target validation [CRISPR-cas; siRNAs]
- Strong capabilities in immunophenotyping for immuno-oncology

We have expertise in primary cell assays including iPSC platforms to prosecute Immunology and IO programs

~400 banked PBMCs ready-to-use in assays including on the *Incucyte* platform

Antigen specific T- cell responses
- Recall responses using flu, CMV, TT or similar peptides.
- Neoantigen screening using DC-T cell cultures and peptide stimulation.
- One way or two-way MLR assays using PBMCs or DCs-proliferation and cytokine analysis

Assays for CTL- Tumor Killing (INCYCYTE PLATFORM)
- FACS and Incucyte based target cell killing assays.
- PBMCs or T-cells as effector cells.

Effector Function Assays ADCC, CDC and ADCP
- FACS or CTG based CDC assays using human sera.
- Flow, BADTA and Incucyte based ADCC assays using NK cells
- Flow and Incucyte based ADCP assays using monocyte derived M1/M2 macrophages.
- ADCC and ADCP reporter assays.

Immune cell stimulation Cytokine Release Proliferation assays
- Flow cytometry analysis for all immune cell phenotyping
- Subset analysis with 14-17 color panels using NOVOCYTE, INTELLICYTE, QUANTEON
- Cytokine: MAGPIX, Bioplex, ELISA
- Proliferation/apoptosis/cell cycle analysis: CTG, CFSE, PI, BrDu

Functional Assays for Tregs, MDSCs, Macrophages
- Tregs: Sorted T-reg cells with CD4+CD25– responder T cells and bead-bound anti-CD3/anti-CD28.
- MDSC: Arginase activity, ROS production.
- Macrophages: M1 and M2 macrophages from human PBMCs or mouse BM. Suppression and phagocytosis assays.
- Mast cell and neutrophil activity from human PBMCs

iPSC Platform
- iPSC reprogramming and characterization from PBMCs,
- Generating knock outs in iPSCs using CRISPR
- Differentiation of iPSCs into T cells
- Functional assays using differentiated cells
- Generation of CAR-T and CAR-NK cells using primary cells and functional validation.

Approved protocols in place for donor blood samples (healthy & diseased) from local hospitals
*Also experienced in handling cyno PBMCs for immune mediated killing assays*

Our In vivo pharmacology team helps verify target engagement and efficacy in disease models

- **67,000Sq ft state-of-the-art vivarium, AAALAC accredited, GLP-certified, IACUC Approved.**

- **Target engagement and efficacy models. All models validated with reference compounds**

- Invivo Pharmacology: Virtual Tour
- List of Validated Animal Models: Website Link

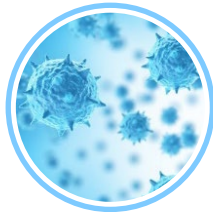**Evaluated NCEs, Antibodies, Bispecific molecules, ADCs, PROTACs, Peptides, SiRNA, Oligos & Cell therapy**

Therapeutic Area Expertise
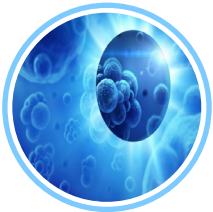
Oncology immuno-oncology
- Xenograft
- Syngeneic
- PDx
- Orthotopic, Metastatic
- Humanized models

Inflammation, Autoimmune
- Acute - Mechanistic
- Chronic – Disease
- Hypersensitivity
- Fibrosis

Metabolic Disorder
- Type 1, 2 Diabetes
- Diabetes complications
- Obesity
- Dyslipidemia
- Atherosclerosis
- NASH

Cardiovascular Diseases
- Myocardial Infarction
- Cardiac Hypertrophy
- Hypertension
- Thrombosis
- Hemodynamic
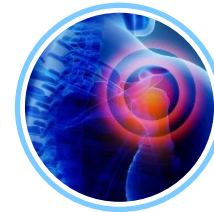
Pain Models
- Neuropathic pain
- Nociceptive pain
- Inflammatory pain
- Diabetic pain
- Post operative pain
- CRPS

Neuroscience
- Cognition & Alzheimer's
- Parkinson's
- Depression
- Stress & Anxiety
- Sleep and Circadian Rhythm

Systemic models
- Renal
- Pulmonary
- GI
- Musculoskeletal
- Ophthalmology

17000sqft dedicated space

~55+ Scientists

All efficacy models are supported by **histopathology, biomarker analysis, PK-PD correlation, Imaging** *and relevant* **mechanistic assays.**

Experienced scientists with ability to set up diverse efficacy models as per program/client needs
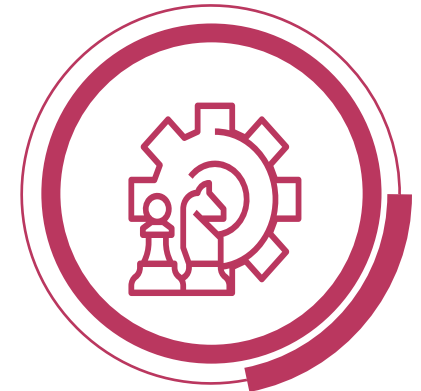
Syngene

In summary

We have a successful track record of delivering scientific excellence

We have the scale, talent, experience and expertise to create value for you

We place partnership and communication at the heart of how we work with clients

We can be your strategic partner

Syngene

Putting Science to Work

www.syngeneintl.com