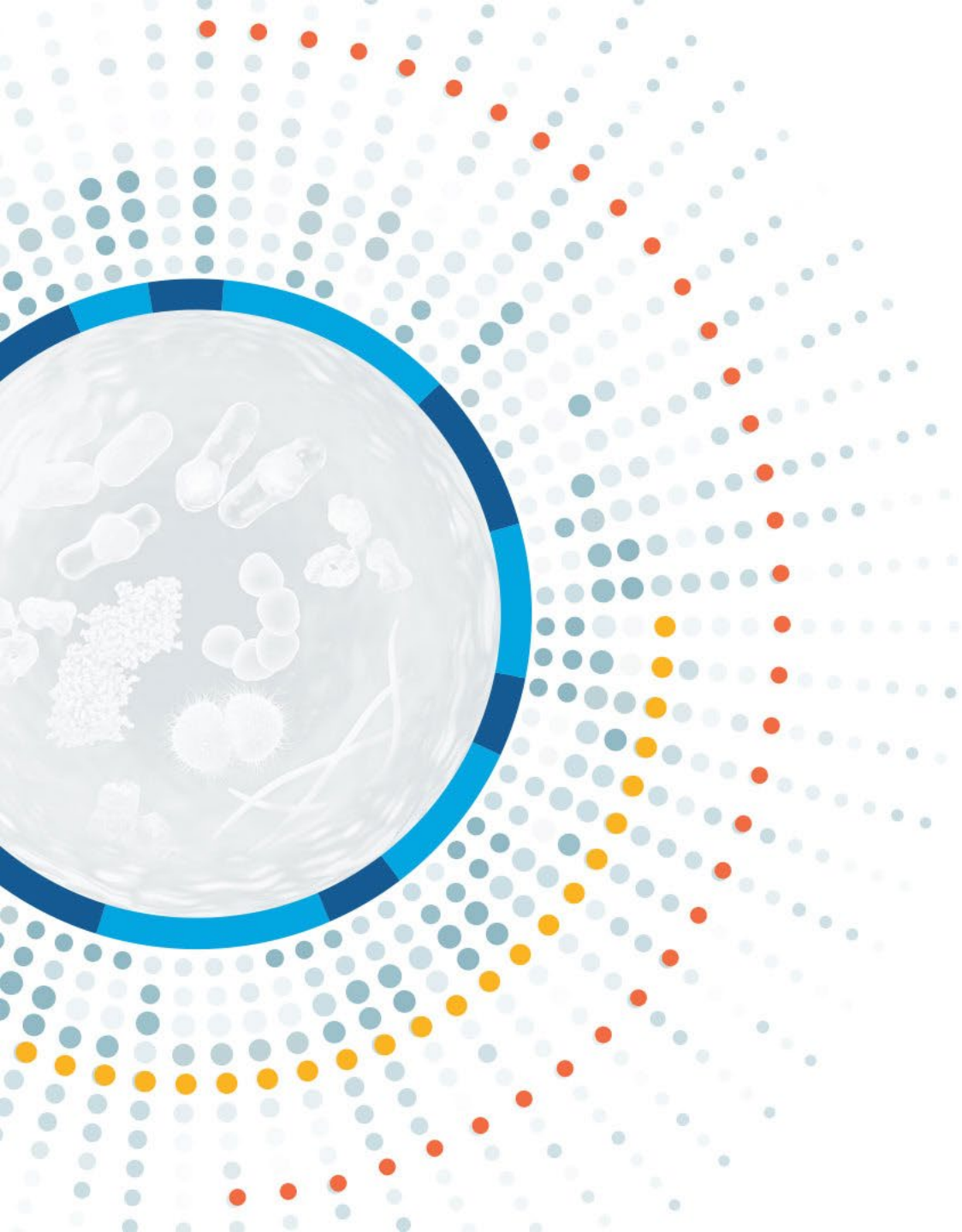
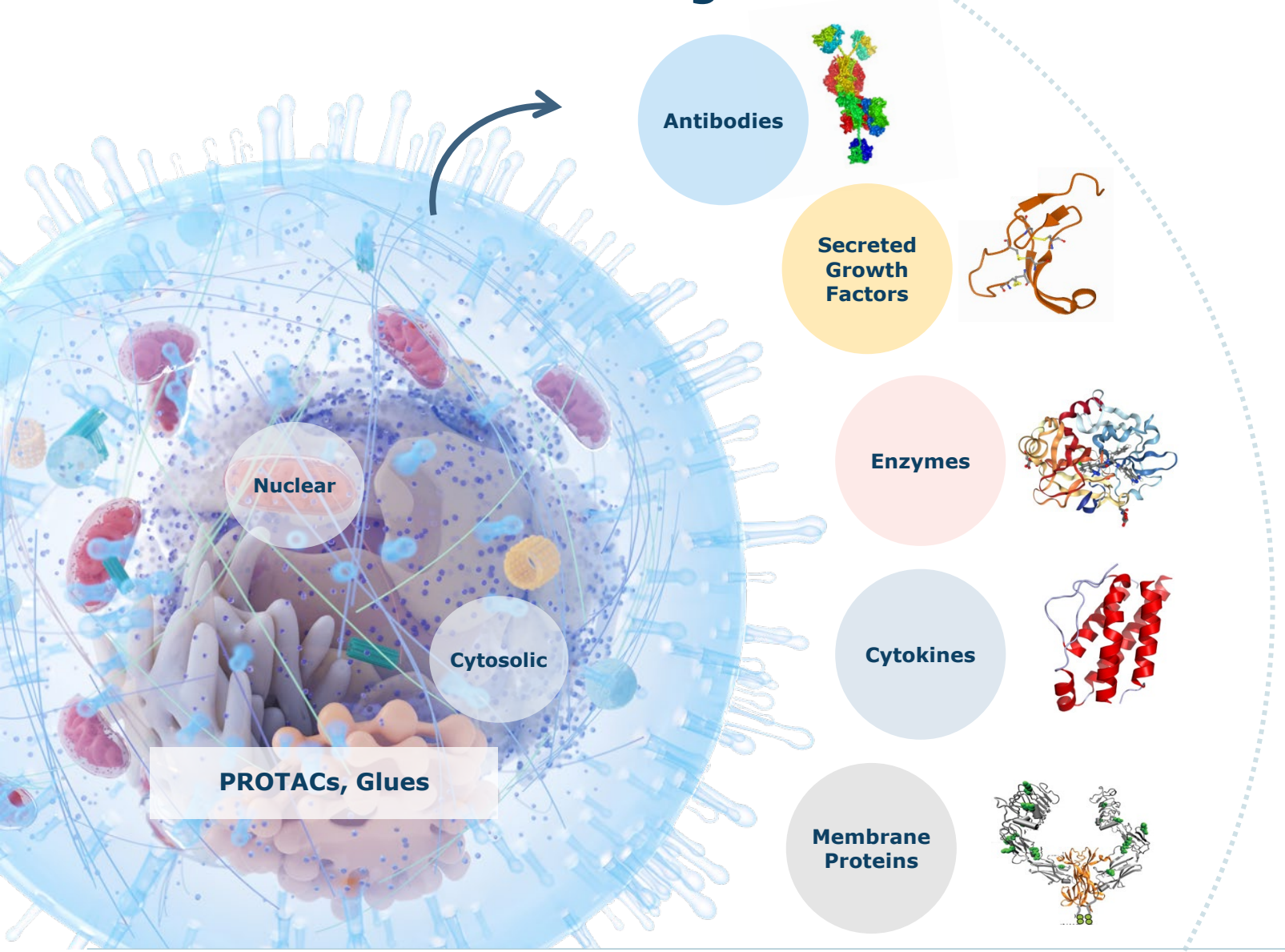


# Extracellular Protein Targeted Degradation with Novel ATACs (ASGPR Targeting Chimeras)

9th Drug Discovery Strategic Summit (DDSS)

May 9 - 10, 2022, Boston, MA

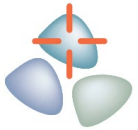
# What If We Could Degrade Extracellular Proteins?




- First generation degraders target intracellular proteins
- Yet almost 40% of human proteins are extracellular or membrane-bound
- Multiple classes and hundreds with established role in pathogenesis of diseases
- Degradation of extracellular proteins would dramatically expand the "degradome"

https://www.proteinatlas.org/humanproteome/tissue/secretome

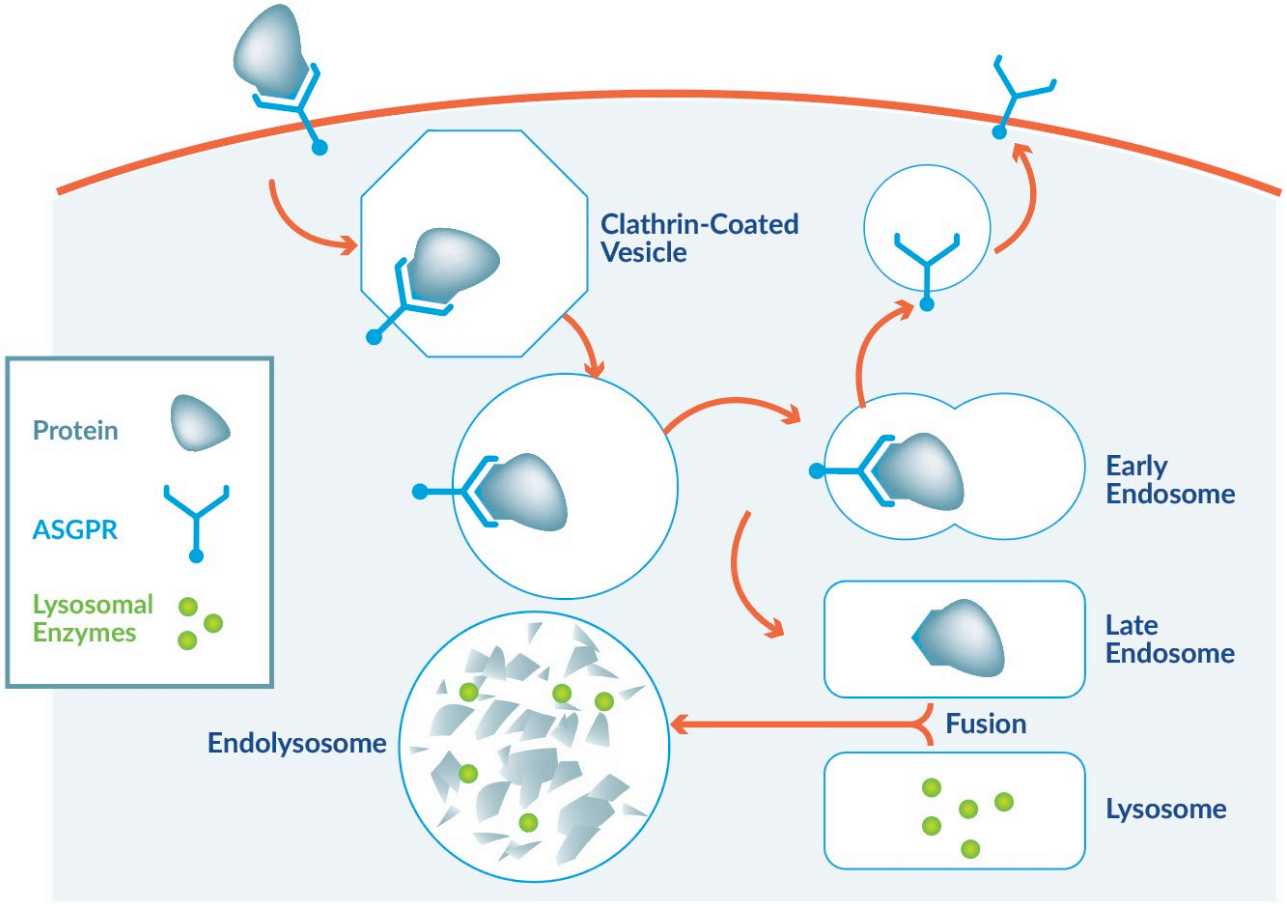
# Novel Applications for ATAC Extracellular Protein Degraders

| | | |
|---|---|---|
|  | **Drug Historically Undruggable Targets** | • Leverage ligands that bind but do not have – or need to have – functional activity to degrade previously undruggable targets |
|  | **Degrade Very High Concentration Proteins** | • Degrade very high concentration proteins that would otherwise require infeasibly or unattractively large doses of neutralizing mAb |
|  | **Selectively Target Relevant Proteins** | • Degrade specific protein types or subtypes responsible for disease, while leaving other related proteins unaffected |
|  | **Rapid Onset of Action** | • Rapidly degrade pathogenic protein to drive faster clinical benefit for patients in crisis or in acute need |
|  | **Remove Pathogenic Complexes** | • Degrade protein complexes or the necessary component elements of protein complexes causing diseases |
|  | **Oral Degraders** | • Use small molecule ASGPR ligands + small molecule protein binders to create oral ATACs for proteins currently targeted by injectable biologics |

# ASGPR Used in Body's Natural Cellular Degradation Machinery

- ASGPR used in natural cellular machinery for extracellular degradation (like E3 ligases in intracellular degradation)
- Cell surface receptor mediates the endocytosis and degradation of various endogenous glycoproteins in endolysosome
- Highly expressed on hepatocytes (~1M receptors per cell in humans)
- Endocytosed and recycled from endosome back to plasma membrane every ~15 minutes

**Natural Endocytosis and Degradation of Endogenous Proteins via ASGPR**



# Unique Platform for Designing, Building, and Developing ATACs

## Avilar ATAC Technology Platform

### Novel ASGPR Chemistry

- Novel, small molecule, high affinity ASGPR ligands designed using X-ray crystal structures
- Enable simplified chemistry, maximally efficient use of ASGPR, low dose, path to oral

### Modular Assembly

- ASGPR ligands deployable in various ATACs targeting different proteins
- Design of linkers, protein targeting ligands, and formulation to optimize degradation

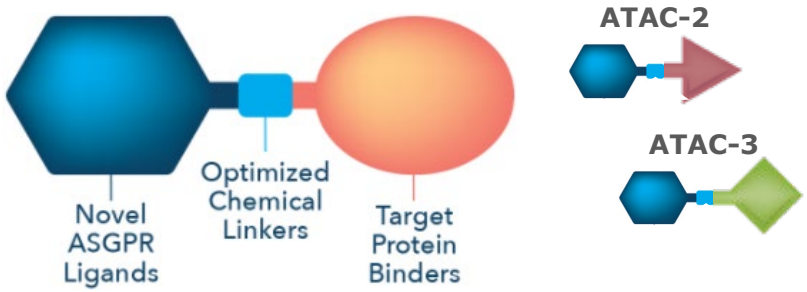
### Proteome Mapping

- Used to catalogue extensive technical & commercial info about extracellular proteins
- Advanced insights for pipeline target selection, protein ligand design, ATAC development

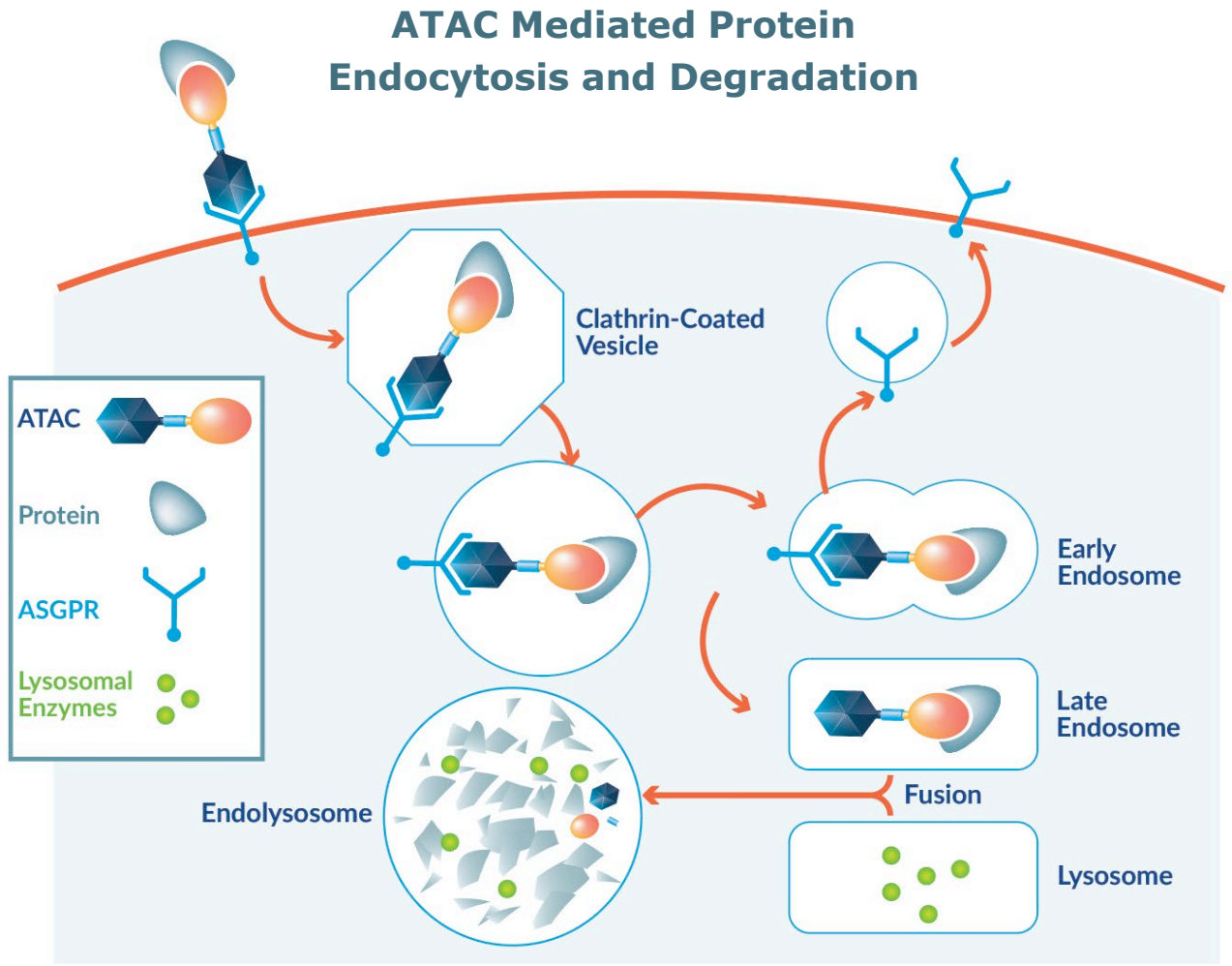
### Extracellular Degradation Modeling

- Advanced modeling integrates critical factors driving ATAC degradation PK/PD
- Predictive simulation data to efficiently drive optimization and translation

# ATACs Harness ASGPR Pathway to Degrade Extracellular Proteins



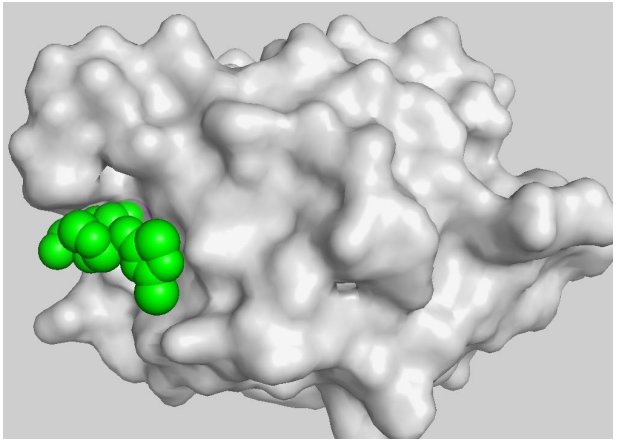

- Bi-functional molecules comprising ASGPR binder, optimized linker, and binder to a target protein
- Shuttle target protein from circulation to endolysosome for degradation
- Modular: optimized ASGPR binders and linkers deployed in synthesis of ATACs with diverse protein targeting binders

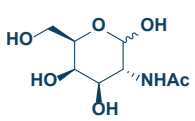


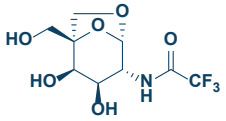
# Proprietary ASGPR Ligands with Significantly Improved Affinity

- Novel high affinity monosaccharides with superior properties versus historical GalNAc analogs
- Synthesized hundreds of monosaccharide ligands
- >100 ligands with $K_D \leq 1{,}000$ nM; ~40 ligands $K_D \leq 100$ nM
- >20 X-ray structures of ASGPR/ligand complexes
- Approximately 2,000-fold increase vs. GalNAc & >60-fold increase in affinity vs. bicyclic bridged ketal

**X-Ray Structure-Guided ASGPR Ligand Design**





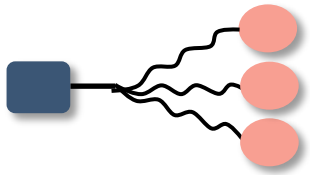



| Compound ID | GalNAc | bicyclic bridged ketal* | AVI-1 | AVI-2 | AVI-3 |
|---|---|---|---|---|---|
| ASGPR SPR Binding: $K_D$ (nM) | 52,800 | 1,650 | 720 | 210 | 24 |
| Increase in Affinity (X Fold) | 1 | 32 | 73 | 251 | 2200 |

*Liras, S. et al. U.S. Patent 9,340,553, May 17, 2016 (priority May 19, 2014)

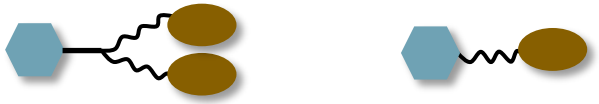
# Novel ASGPR Chemistry Enables Differentiated ATAC Design

**Previous Degraders Display Three GalNAc Monosaccharide ASGPR Motifs**



- GalNAc requires multivalency (avidity) to enhance receptor binding beyond the low-μM level to achieve efficient hepatocyte uptake*

**Bidentate ATACs** **Monodentate ATACs**



- Modular construction of pM-binding ATAC
- Translates to efficient hepatocyte uptake
- Structurally streamlined
- Simplified chemistry and low MW

**Target Protein-Binding Motif**

Ab

peptide or small molecule

**Linker**

Varying rigidity, length and branching

**ASGPR-Binding Motif**

GalNAc, ↓affinity

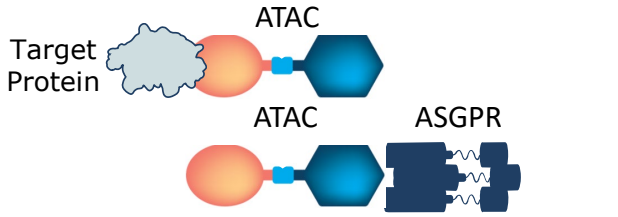
Novel, ↑affinity

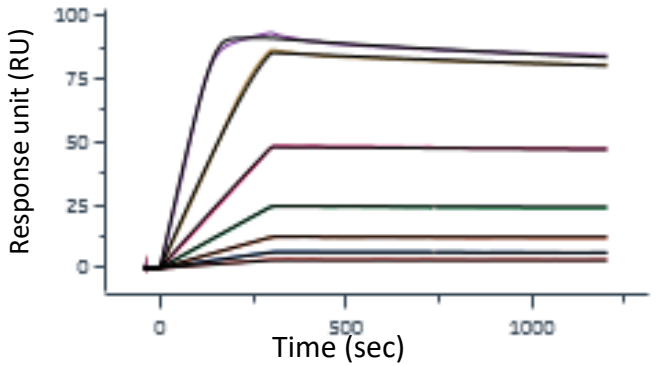
- Avilar's novel monosaccharides* enable structurally & functionally differentiated ATAC degraders

*Saulnier, M. G. et. al. ASGPR-Binding Compounds for the Degradation of Extracellular Proteins WO2021155317 (2021)

# Key Stage Gates for ATAC Discovery and Translation

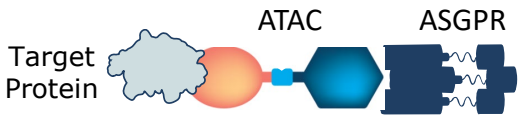
## ATAC Binding

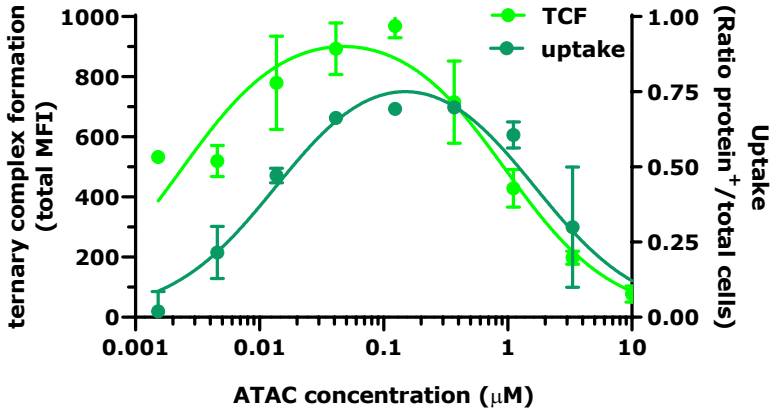



- Direct measure of ATAC-Target Protein and ATAC-ASGPR binary complex formation
- Surface plasmon resonance (SPR) measures ATAC affinity and kinetics

## Ternary Complex Formation & Cellular Uptake

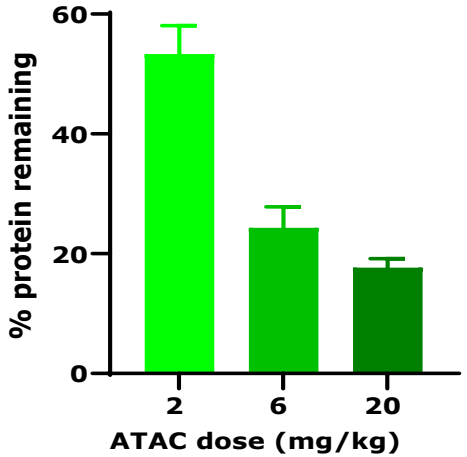



- Direct measure of ASGPR-ATAC-Target Protein ternary complex formation (TCF) and uptake in hepatocytes
- Fluorescent intensity measurement in the surface and within the cell
- Uptake/degradation in hepatocyte endolysosome measured by Western Blot (data not shown)

## *In Vivo* Degradation

**Degradation of Extracellular Protein Following Single Dose ATAC (Rat Model)**

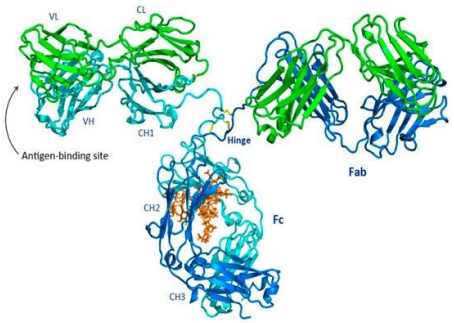



- Protein degradation measured 4 hrs after single dose ATAC injection
- Protein detection in plasma by ELISA
- Illustrative example from multiple rat, guinea pig, NHP studies showing ATAC mediated *in vivo* degradation

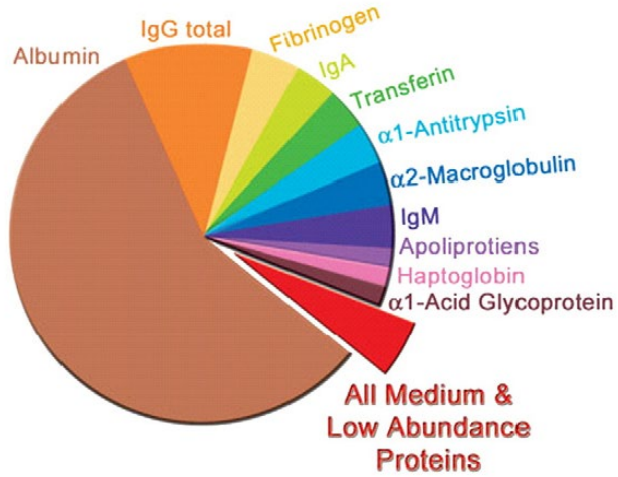
# Platform PoC Studies with ATACs Targeting IgG

- ATACs designed to target and degrade IgG
  - Second most abundant protein
  - Most common type of antibody
- IgG properties
  - High plasma concentration: 1.06 g/kg total body IgG = 74.2 g total in 70 kg human = 508 μmol**
  - Long half-life: $t_{1/2}$ = 21 days
  - Resynthesis: 32 mg/kg/day = 2.2 g/subject/day = 15 μmol/day; ~3% of total body IgG/day
  - MW ~146 kDa
- IgG biology
  - Binds cell surface receptors on many cell types to trigger phagocytosis or cellular cytotoxicity*

**Ribbon Representation of an Intact IgG***



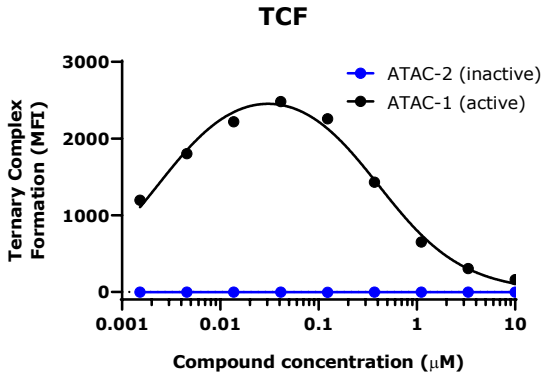

**Overview of the Major Human Plasma Proteins*****

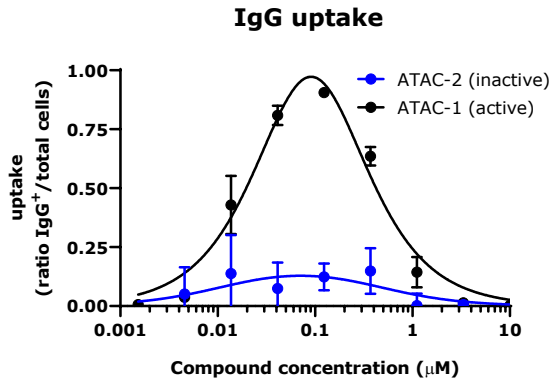


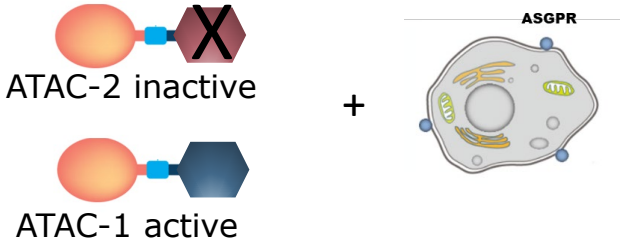

*Nezlin, The Immunoglobulins (1998), ** Waldmann et.al. Prog. Allergy (1969), ***Kratz et al. J. Control. Release (2012)

# IgG-ATAC Function Dependent on ASGPR Binding

## ASGPR-inactive control IgG-ATAC (ATAC-2) does not engage with ASGPR (absence of TCF and uptake)

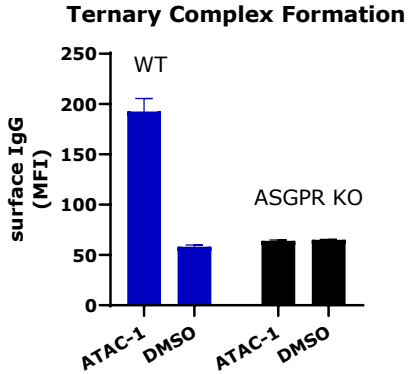


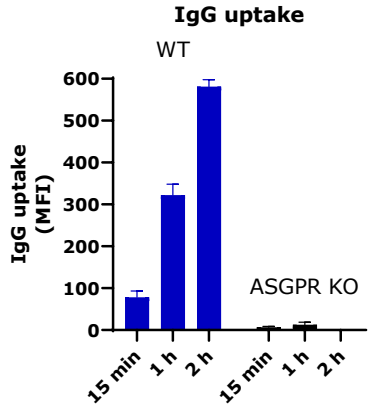




ASGPR requires ATAC with a functional ASGPR ligand for TCF and uptake

## IgG-ATAC AVI-1 does not engage with HepG2 cells lacking ASGPR (absence of TCF and uptake)



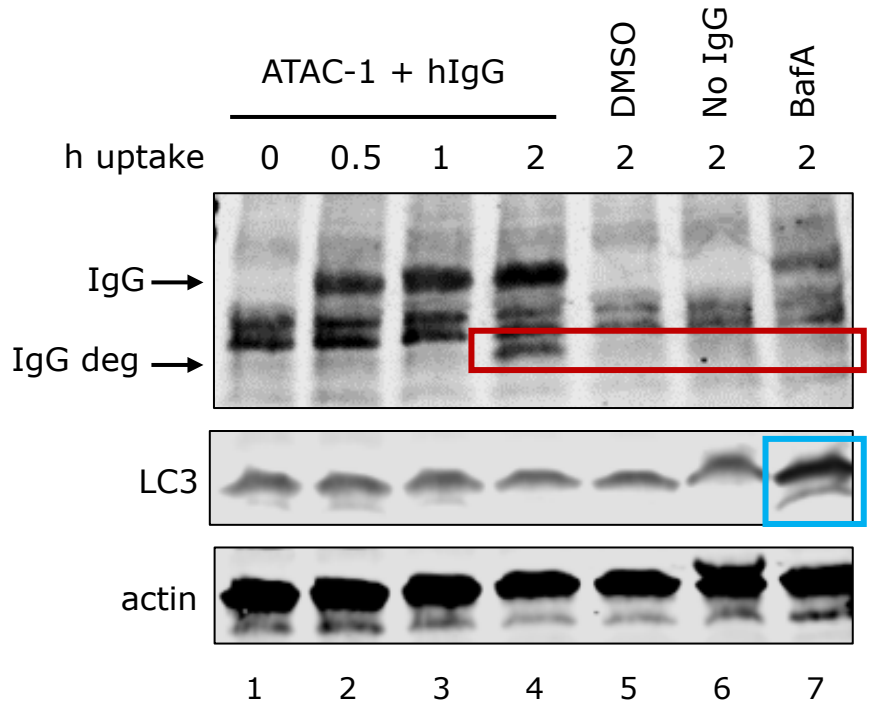


ATAC requires cells with a functional ASGPR receptor for TCF and uptake

Study design: Ternary Complex Formation (TCF) assays: HepG2 cells (WT or ASGPR-KO) were incubated with fluorescently labeled human IgG and ATACs or DMSO on ice for 1 h. Cells were washed and surface IgG was measured by flow cytometry (MFI). Uptake assays: HepG2 cells (WT or ASGPR-KO) were incubated with fluorescently labeled human IgG and ATACs or DMSO at 37°C for 6 h. Cells were washed and surface + internal IgG was measured by fluorescence microscopy (ratio of IgG+ cells/total cells)

# IgG Degradation in Rat Hepatocytes Requires Lysosomal Function

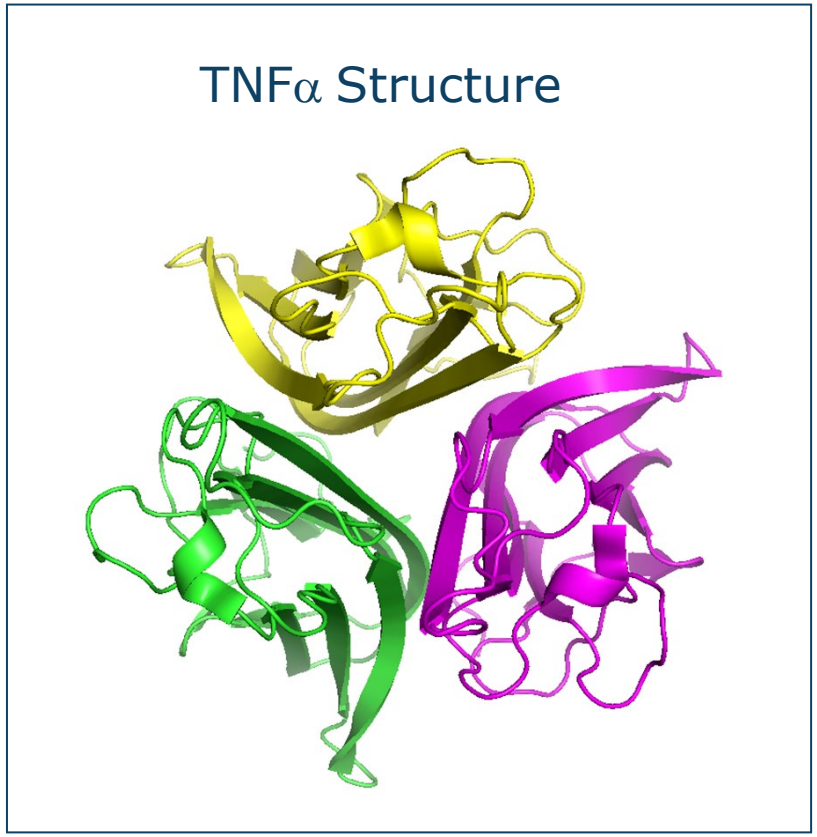
- Uptake of hIgG requires presence of IgG-ATAC
  - hIgG taken up over time into HepG2 cells in presence of ATAC-1 (IgG, lanes 1-4), but not in its absence (DMSO, lane 5)
  - Non-specific IgG antibody-reactive background bands observed in HepG2 cells in absence of hIgG (lane 6)
- IgG degradation after uptake is dependent on endolysosomal function
  - IgG degradation fragment appears ~ 2 h after IgG uptake begins (lane 4, red box)
  - Pre-treatment with lysosomal function inhibitor Bafilomycin A blocks endolysosomal pathway progression (confirmed by endosomal marker LC3 accumulation, blue box)
  - Baf A treatment prevents IgG degradation (no IgG degradation fragment after 2 h with IgG and ATAC-1, compare lanes 4 and 7, red box)
  - IgG degradation kinetics consistent in HepG2 cells and rat hepatocytes




- Study design: Primary rat hepatocytes are pre-incubated +/- lysosomal inhibitor BafA for 2 h and then incubated with ATAC-1 and hIgG for 0-2 h
- Cells are washed, lysed and IgG is detected by western blot with anti-IgG antibodies. Actin is a protein loading control. LC3 is a marker for endolysosomal pathway function.

# Platform PoC Studies with ATACs Targeting TNFα

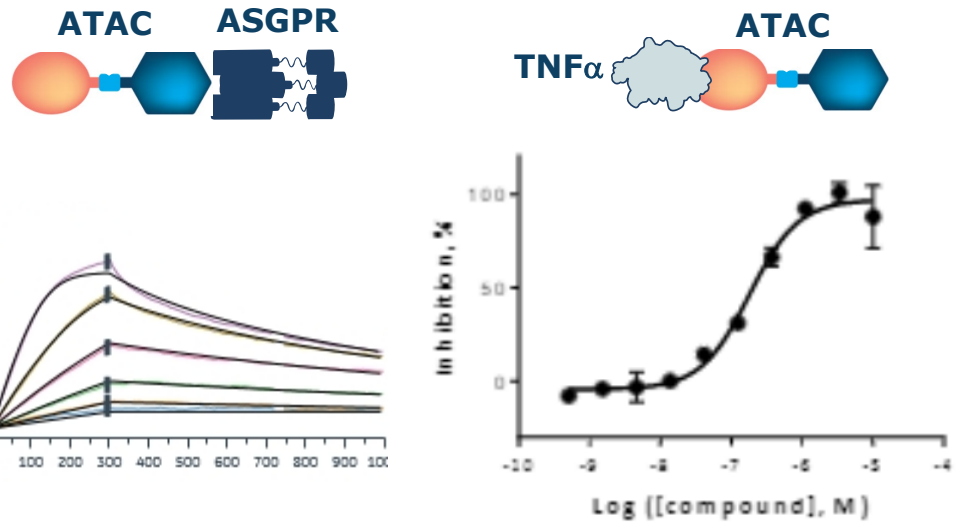
- ATACs designed to target and degrade TNFα
- Small molecule ligand used as protein binder
- TNFα properties
  - Low plasma concentration and short half-life extracellular protein
  - 2-20 pg/mL in healthy people and up to 5000 pg/mL in sepsis patients' serum*
  - Soluble TNFα MW: 17 kDa, and assembles as a 51 kDa trimer
- TNFα biology
  - Homotrimeric cytokine that binds two receptors, TNFR1 and TNFR2
  - Engagement with TNFR1 and TNFR2 initiates signaling cascades that result in inflammatory responses and control of apoptosis




*Damas P. et. al. Crit. Care Med. 1989. Eck, M.J. et. al. J Biol Chem 1989

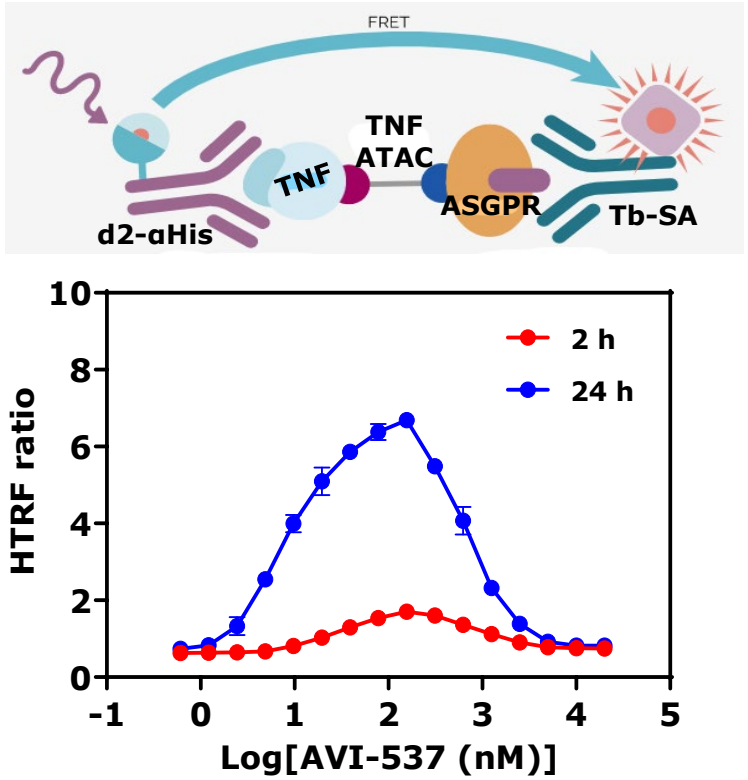
# TNFα ATAC Binary and Ternary Complex Formation Confirmed

## ATAC-4 Binds to ASGPR and TNFα




| Biochemical/Cellular Attributes | ATAC-4 |
|---|---|
| ASGPR $K_D$ (nM) | 0.72 |
| ASGPR $k_d$ ($s^{-1}$) | 1.6 x 10 $^{-3}$ |
| TNFα FP $IC_{50}$ (nM) | 180 |

## Time-Dependent Ternary Complex Formation of TNFα, ATAC-4 and ASGPR



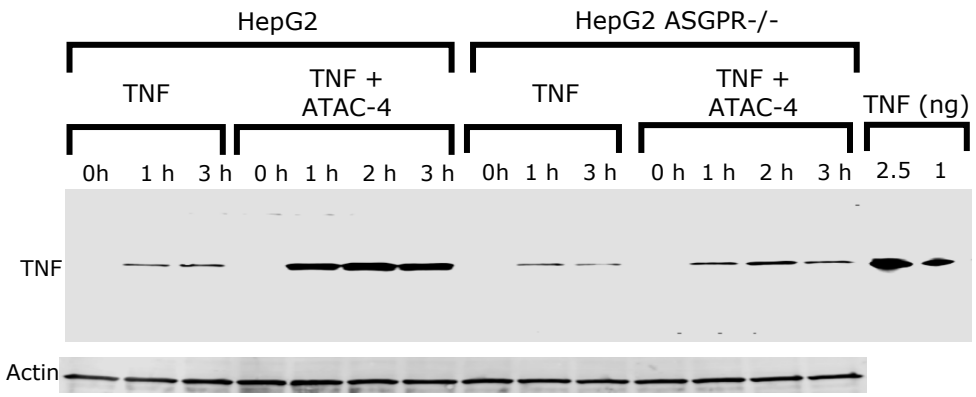

TNFα ternary complex formation assay (FRET)

# ATAC-Mediated TNFα Uptake and Degradation in HepG2 Cells

- Uptake of TNFα mediated by ATAC-4 is observed in HepG2 cells
- Significant uptake of TNFα requires the presence of an ATAC
- ASGPR is required to achieve significant ATAC-medicated TNFα uptake in HepG2 cells (no uptake in ASGPR knock-out cells)

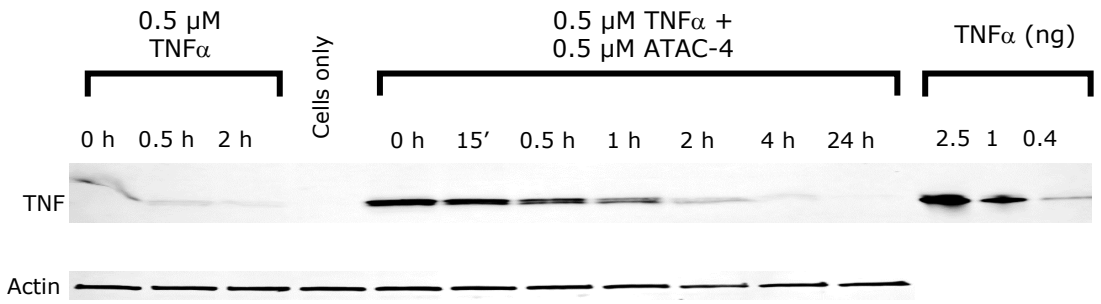
- Degradation of TNFα requires the presence of ATAC-4
- Half-life of TNFα degradation upon uptake by ASGPR is 15-30 min
- TNFα is fully degraded after 4h

## TNF Uptake in HepG2 Hepatocytes




Study design: 0.5 µM ATAC-4 and 0.5 µM human TNFα were pre-incubated for 18h to allow formation of stable complexes. ATAC-4/ TNFα complexes or TNFα alone were incubated for 0-3h on HepG2 cells. Cells were washed and lysed for Western blot analysis. TNFα and actin were detected with anti-TNFα and anti-actin antibodies, respectively, and 800cw-conjugated secondary antibody.

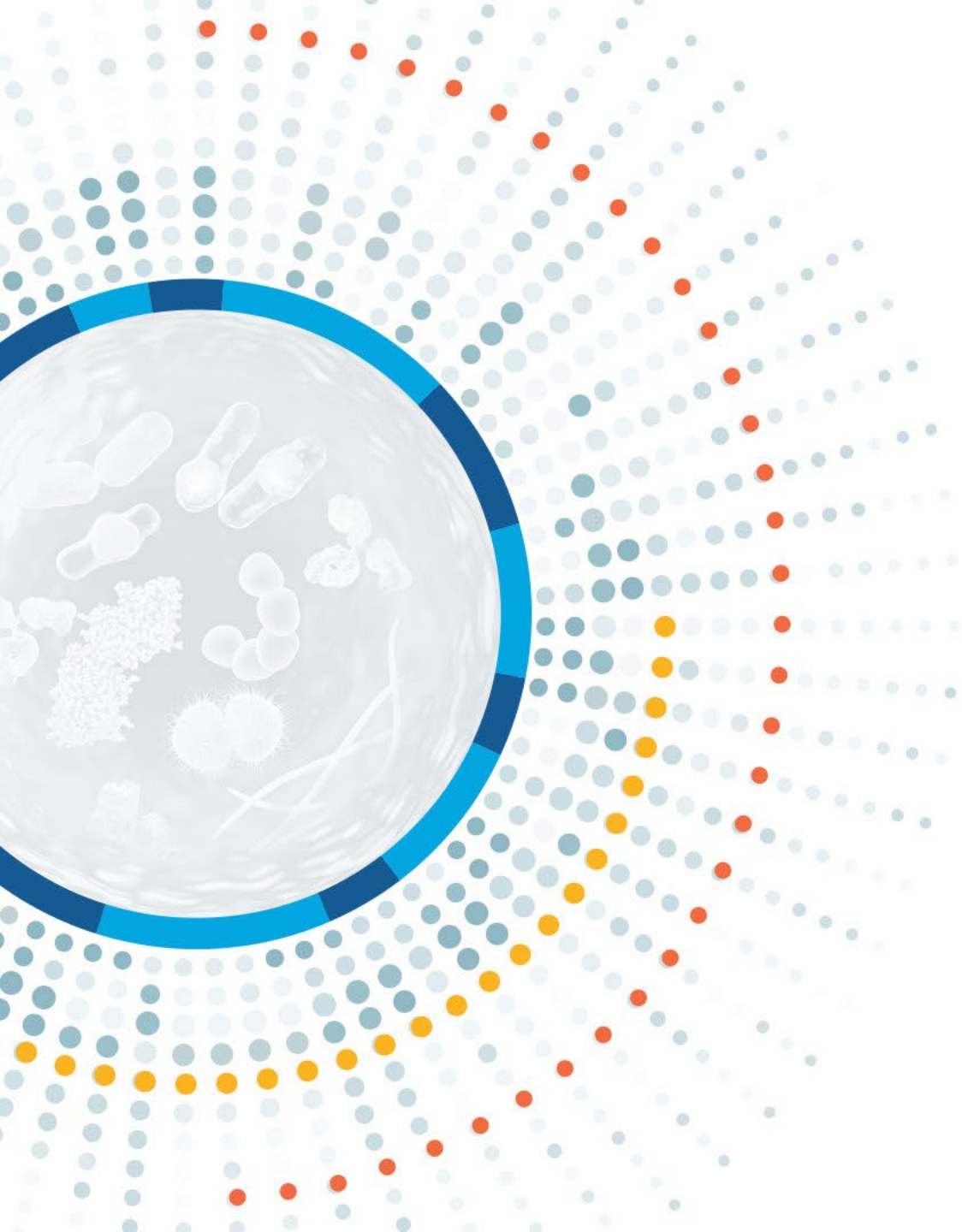
## TNFα Degradation in HepG2 Hepatocytes




Study design: ATAC-4 and human TNFα were pre-incubated for 18h to allow formation of binary complexes. ATAC-4 / TNFα complexes or TNFα alone were incubated for 2h on HepG2 cells. Cells were washed, incubated with cell media without reagents for 0-24h and subsequently washed and lysed for Western blot analysis. TNFα and actin were detected with anti-TNFα and anti-actin antibodies, respectively, and 800cw-conjugated secondary antibody.

# Summary

- Avilar created a unique platform for designing and creating ATACs as novel extracellular protein degraders
  - Library of proprietary, small molecule, high affinity ASGPR ligands
  - Optimized linkers, customized PK/PD modeling, and proteome mapping
- To exemplify our ATAC platform, we designed ATAC molecules to target and degrade two extracellular proteins with different concentration and kinetic properties:
  - IgG: high plasma concentration and long half-life
  - TNFα: low plasma concentration and short half-life
- For ATAC platform POC, we demonstrated *in vitro* ligand binding, ternary complex formation, cellular uptake, and degradation of IgG and TNFα



# Extracellular Protein Targeted Degradation with Novel ATACs (ASGPR Targeting Chimeras)

9th Drug Discovery Strategic Summit (DDSS)

May 9 - 10, 2022, Boston, MA