Synthekine

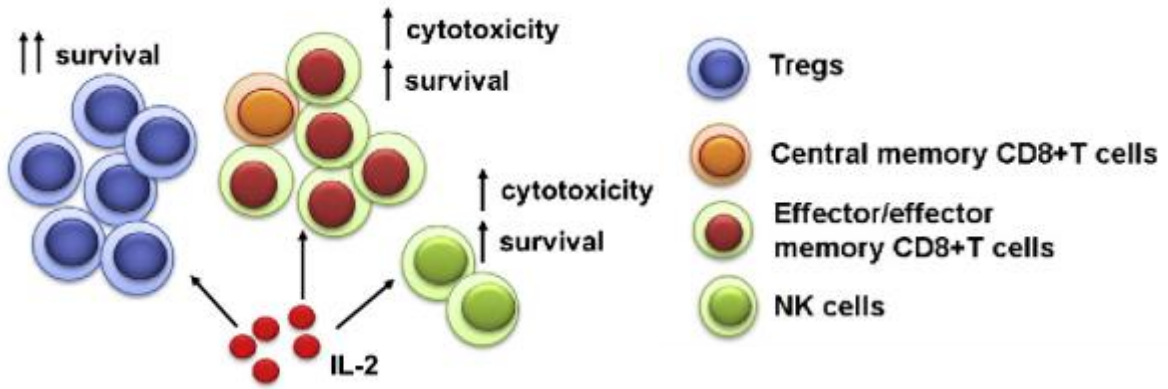
Surrogate Cytokine Agonist (SCA): unlocking natural and novel cytokine signals with VHH-based therapeutics

Sandro Vivona, PhD
Senior Director of Biochemistry and Biophysics
Synthekine, Inc.
PEGS May 2023

Cytokine therapeutics: limitations and potential

- **Approved drugs:** IL-2, type I IFNs, EPO, HGH, G-CSF
- **In clinic:** IL-2 muteins, IL-12, IL-22, IL-18, IL-10
- **Cytokine agonism within the immune system is pleiotropic**

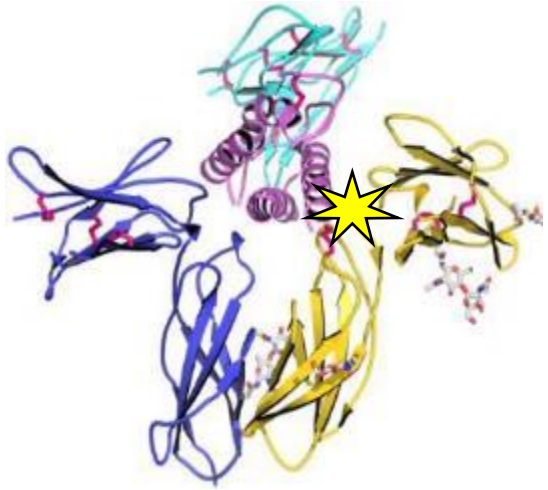
  leads to both positive and negative effects → partial agonism to decouple efficacy and toxicity



For example, the broad effects of IL-2 on multiple cell types limit therapeutic potential of wild type molecule

Partial agonism of engineered cytokines can elicit unique therapeutic properties

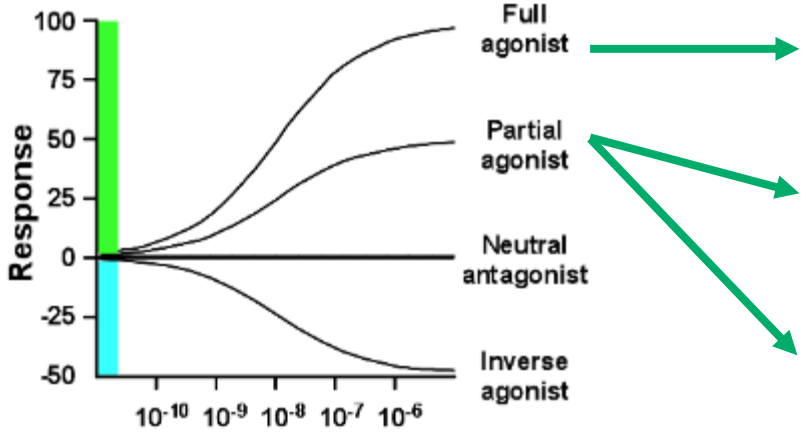
Structural information on the cytokine ligand / receptor interaction



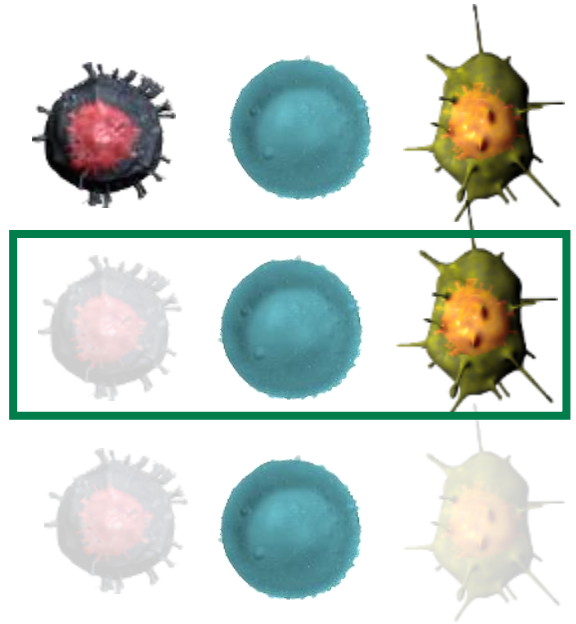
Modify receptor affinity

Cytokine “muteins”

Engineering of wild type cytokine to alter response



Selection of optimized lead based on signaling in specific cell type(s)



- ***Engineered cytokine partial agonists enable targeted activity on cell subtypes with high receptor expression***

A wealth of examples from the Garcia Lab

Stanford University

hhmi Howard Hughes Medical Institute

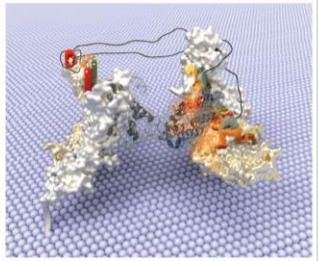
RESEARCH

RESEARCH ARTICLE SUMMARY

IMMUNOLOGY

Structure-based decoupling of the pro- and anti-inflammatory functions of interleukin-10

Robert A. Saxton, Naotaka Tsutsumi, Leon L. Su, Gita C. Abhiraman, Kritika Mohan, Lukas T. Henneberg, Nanda G. Aduri, Cornelius Gati, K. Christopher Garcia*



Engineering IL-10. The structure of the IL-10 receptor complex is shown on a cell surface with "engineers" depicted as welders making alterations to IL-10 at the receptor-binding interface that was the focus of Saxton et al.

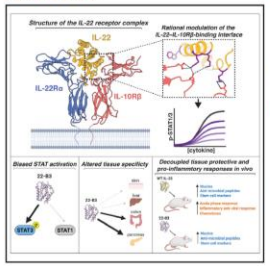
Saxton et al., Science 371, 1223 (2001) 19 March 2021

Article

Immunity

The tissue protective functions of interleukin-22 can be decoupled from pro-inflammatory actions through structure-based design

Graphical abstract



Authors

Robert A. Saxton, Lukas T. Henneberg, Marco Calafiore, Leon Su, Kevin M. Jude, Alan M. Hanash, K. Christopher Garcia

Correspondence

kcgarcia@stanford.edu

In brief

Saxton et al. engineer a high-affinity interleukin-22 (IL-22) super-agonist that enables structure determination of the IL-22-IL-22Rα-IL-10Rβ ternary complex. IL-22 receptor agonists designed based on these structural insights elicit activation of STAT3 but not STAT1 and promote epithelial protection without inducing loc inflammation.

Highlights

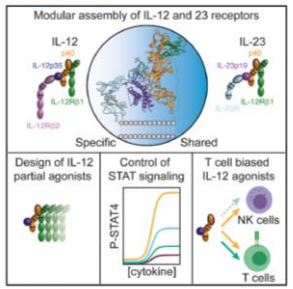
- 2.6-A-resolution structure of a stabilized IL-22 receptor ternary complex
- Structure-based design of STAT3-biased IL-22 receptor agonists
- Biased IL-22 variant 22-B3 elicits tissue-selective STAT3 activation *in vivo*
- 22-B3 uncouples the tissue-protective and pro-inflammatory functions of IL-22

Saxton et al., 2021, Immunity 54, 660–672
April 13, 2021 © 2021 Elsevier Inc.
https://doi.org/10.1016/j.immuni.2021.03.008

Cell

Structural basis for IL-12 and IL-23 receptor sharing reveals a gateway for shaping actions on T versus NK cells

Graphical abstract



Authors

Caleb R. Glassman, Yamuna Kalyani Mathiharan, Kevin M. Jude, ..., Christoph Thomas, Georgios Skiniotis, K. Christopher Garc

Correspondence

kcgarcia@stanford.edu

In Brief

Structures of the IL-12 and IL-23 recepto complexes guide design of T-cell-biase IL-12 agonists with reduced cytokine pleiotropy to support anti-tumor immunity without inducing toxicity.

Highlights

- Crystal structure of the complete IL-23 receptor complex
- Cryo-EM maps of the complete IL-12 and IL-23 receptor complexes
- The p40 subunit of IL-12 and IL-23 is a common gateway for induction of STAT signaling
- T-cell-biased IL-12 agonists elicit anti-tumor response without inducing toxicity

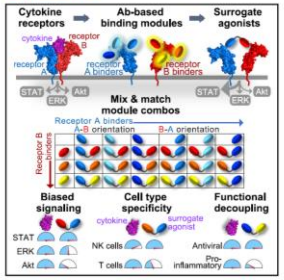
Glassman et al., 2021, Cell 184, 983–999
February 18, 2021 © 2021 Elsevier Inc.
https://doi.org/10.1016/j.cell.2021.01.018

CellPress

Cell

Facile discovery of surrogate cytokine agonists

Graphical abstract



Authors

Michelle Yen, Junming Ren, Qingxiang Liu, ..., Ralph S. Baric, Leon L. Su, K. Christopher Garcia

Correspondence

kcgarcia@stanford.edu

In brief

A discovery platform for functio diverse cytokine surrogates.

Highlights

- A platform to expand and diversify cytokine biology with modular surrogate agonists
- IL-2 surrogates reveal signaling plasticity and biased activities on T and NK cells
- Type-I IFN surrogates are potent antiviral agents with reduced cytotoxic properties
- IL-2/10 surrogates drive non-natural receptor hetero-dimerization on T and NK cells

Yen et al., 2022, Cell 185, 1414–1430
April 14, 2022 © 2022 Elsevier Inc.
https://doi.org/10.1016/j.cell.2022.02.025

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RESEARCH

IMMUNE ENGINEERING

Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes

Jonathan T. Sockolosky,[1,2] Eleonora Trotta,[2*] Giulia Parisi,[2*] Lora Picton,[1] Leon L. Su,[1] Alan C. Le,[3] Akanksha Chhabra,[3] Stephanie L. Silveria,[3] Benson M. George,[4,5,6] Indigo C. King,[7] Matthew R. Tiffany,[8] Kevin Jude,[1] Leah V. Sibener,[1,9] David Baker,[3] Judith A. Shizuru,[3] Antoni Ribas,[4,10] Jeffrey A. Bluestone,[2,10] K. Christopher Garcia[1,2,11,12]†

Interleukin-2 (IL-2) is a cytokine required for effector T cell expansion, survival, and function, especially for engineered T cells in adoptive cell immunotherapy, but its pleiotropy leads to simultaneous stimulation and suppression of immune responses as well as systemic toxicity, limiting its therapeutic use. We engineered IL-2 cytokine-receptor orthogonal (*ortho*) pairs that interact with one another, transmitting native IL-2 signals, but do not interact with their natural cytokine and receptor counterparts. Introduction of *ortho*IL-2Rβ into T cells enabled the selective cellular targeting of *ortho*IL-2 to engineered CD4$^+$ and CD8$^+$ T cells in vitro and in vivo, with limited off-target effects and negligible toxicity. *Ortho*IL-2 pairs were efficacious in a preclinical mouse cancer model of adoptive cell therapy and may therefore represent a synthetic approach to achieving selective potentiation of engineered cells.

Adoptive transfer of tumor-reactive T cells has evolved into a clinically useful therapy capable of inducing antitumor immunity in patients (*1*, *2*). However, the broad application of adoptive T cell transfer (ACT) therapies to treat cancer has several limitations, including the production of sufficient quantities of cells for infusion and the failure of transferred T cells to persist and remain functional in vivo. In the clinic, the concomitant administration of the T cell growth factor interleukin-2 (IL-2) improves the survival, function, and antitumor activity of transplanted T cells (*3*, *4*). However, the use of IL-2 to potentiate ACT is complicated by the pleiotropic nature of IL-2, which induces both immune stimulatory and suppressive T cell responses as well as potentially severe toxicities (*5*). This is governed by the interaction between IL-2 and the IL-2 receptor (IL-2R), which consists of α, β, and γ subunits (*6*). IL-2Rβ and the common γ-chain (IL-2Rγ) together form the signaling dimer and bind IL-2 with moderate affinity, whereas IL-2Rα (CD25) does not signal but increases the affinity of IL-2 for the binary (βγ) IL-2 receptor to sensitize T cells to low concentrations of IL-2.

The activity of IL-2 as an adjuvant to ACT is dependent on the balance between activation of transplanted and endogenous T cell subsets bearing natural IL-2 receptors, as well as host responses that cause dose-limiting toxicities. Strategies to overcome these limitations could improve T cell immunotherapy (*7*, *8*). Recognizing the need for new approaches that afford precise targeting of IL-2–dependent functions to a specific cell type of interest, we devised a strategy to redirect the specificity of IL-2 toward adoptively transferred T cells. This method, based on receptor-ligand orthogonalization, uses a mutant IL-2 cytokine and mutant IL-2 receptor that bind specifically to one another but not to their wild-type counterparts (Fig. 1A).

We focused on the murine IL-2/IL-2Rβ interaction to enable in vivo characterization in syngeneic mouse models. The IL-2Rβ chain was chosen as the mutant receptor because the β chain is required for signal transduction and can bind IL-2 independently. We devised a two-step approach to engineer orthogonal IL-2/IL-2Rβ pairs informed by the crystal structure of the IL-2 high-affinity receptor complex (*6*) (Fig. 1B). First, point mutations of the IL-2Rβ chain were identified from inspection of the interface between IL-2 and IL-2Rβ that abrogated binding to wild-type IL-2 (Fig. 1, C to E). The IL-2Rβ hotspot residues His^{134} and Tyr^{135} make numerous contacts with IL-2 that contribute a majority of the binding free energy between IL-2 and IL-2Rβ (*6*) (Fig. 1E). A double mutant IL-2Rβ [His^{134} → Asp (H134D) and Tyr^{135} → Phe (Y135F)], referred to herein as *ortho*IL-2Rβ, lacked detectable binding to IL-2 (Fig. 1D), even in the presence of CD25 (fig. S1) (*7*, *9*).

Next, we used yeast display-based evolution to mutate, and thus remodel, the wild-type IL-2 interface region that was opposing (or facing the site of) the IL-2Rβ mutations in the crystal structure, in order to create a molecule that bound to *ortho*IL-2Rβ but not to wild-type IL-2Rβ. IL-2 residues in proximity to the *ortho*IL-2Rβ binding interface were randomly mutated and were chosen on the basis of a homology model of the mouse IL-2/IL-2Rβ complex (Fig. 1E) derived from the crystal structure of the human IL-2 receptor complex (*6*). A library of ~10^9 unique IL-2 mutants was displayed on the surface of yeast (fig. S2) and subjected to multiple rounds of both positive (against *ortho*IL-2Rβ) and negative (against IL-2Rβ) selection (figs. S2 and S3). This collection of yeast-displayed IL-2 mutants bound the *ortho*IL-2Rβ, but not wild-type IL-2Rβ, and retained CD25 binding (Fig. 1D). Sequencing of yeast clones from the evolved IL-2 libraries revealed a consensus set of mutations at IL-2 positions in close structural proximity to the *ortho*IL-2Rβ mutations (fig. S4). Interestingly, a Gln^{70} → Asn (Q70N) mutation was highly conserved across three independent mutant IL-2 yeast libraries, whereas all other IL-2 positions used a restricted but not specific mutational signature. We found that IL-2 mutations Q70N, Met^{23} → Val (M23V), and Asp^{20} → Leu or Met (D34L/M) appear to form a small nonpolar pocket to compensate for the IL-2Rβ Y135F mutation, whereas Gln^{126} → Thr, Ser, Lys, or Gln (Q126T/S/K/Q) and Glu^{67} → Tyr or His (E67Y/H) mutations present a polar or charged surface to compensate for the IL-2Rβ H134D mutation (Fig. 1F).

Because of the affinity-enhancing effects of CD25 expression on the interaction of IL-2 with the binary (βγ) IL-2 receptor (*10*), IL-2 mutants with negligible binding to IL-2Rβ alone may still form a functional signaling complex on cells that also express CD25 (*6*). Therefore, we used a yeast-based functional screen to further triage IL-2 mutants that bound specifically to the *ortho*IL-2Rβ and signaled selectively on T cells that express the *ortho*IL-2Rβ (Fig. 1G and fig. S5), and produced recombinant forms of select IL-2 mutants (*ortho*IL-2) for characterization (figs. S6 to S8).

We focused our efforts on two *ortho*IL-2 mutants, IG12 and 3A10. *Ortho*IL-2 IG12 and 3A10 share the consensus Q70N, M23V, and D34L mutations but differ at positions Glu^{20}, Gln^{126}, Glu^{61}, and Arg^{81} (Fig. 1I). *Ortho*IL-2 IG12 and 3A10 bound the *ortho*IL-2Rβ with an affinity comparable to that of the wild-type IL-2/IL-2Rβ interaction and displayed little to no detectable binding to wild-type IL-2Rβ (Fig. 1H and figs. S7 and S8) but differed in their ability to activate IL-2Rβ signaling in CD25-positive wild-type

Sockolosky et al., Science 359, 1037–1042 (2018) 2 March 2018

Synthekine

Synthekine three-pronged approach to engineering cytokines

Developing potentially paradigm-changing programs using three distinct protein engineering platforms

Cytokine Partial Agonists Platform

- Engineered from cytokine structural insights
- Published in Science, Cell, and Immunity
- ***Ph1 dose escalation ongoing for lead program***

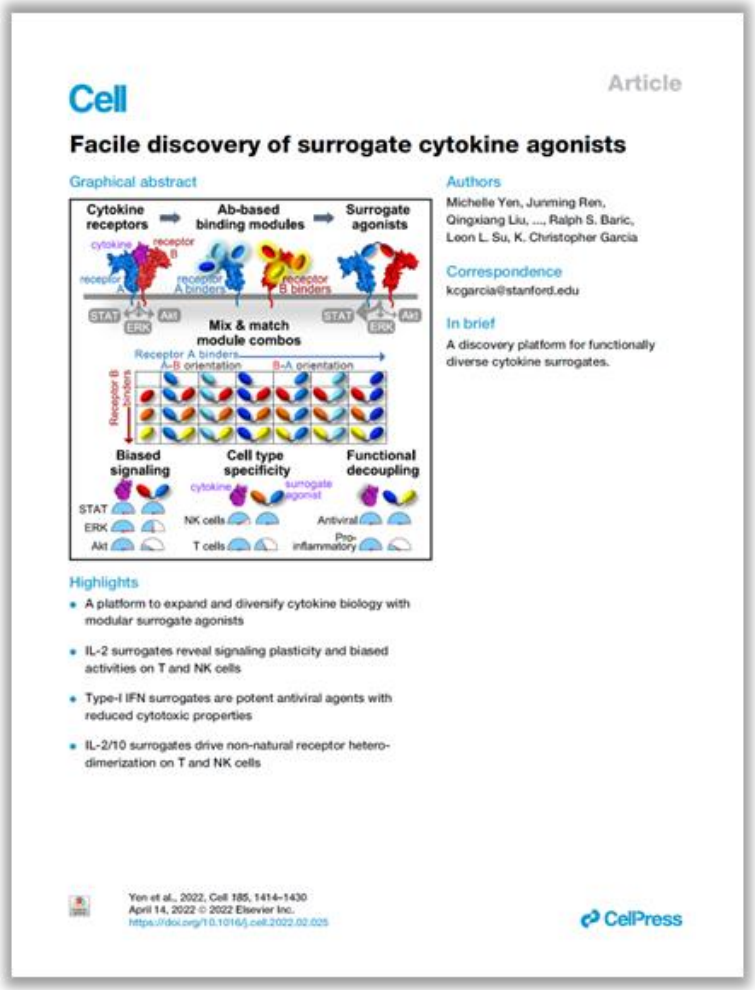
Orthogonal Cytokine Cell Therapy Platform

- Engineered cytokine receptor expressed on CAR-T and other ACTs
- ***Ph1 enrolling for lead program***

Surrogate Cytokine Agonist Platform

- Novel cytokine engineering approach using surrogate binders
- ***Collaboration with Merck and robust internal pipeline***

Pursuing partial agonism via Surrogate Cytokine Agonists (SCA)



Cell

Article

Facile discovery of surrogate cytokine agonists

Graphical abstract

Authors
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In brief

A discovery platform for functionally diverse cytokine surrogates.

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- A platform to expand and diversify cytokine biology with modular surrogate agonists
- IL-2 surrogates reveal signaling plasticity and biased activities on T and NK cells
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Garcia Lab

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Surrogate Cytokine Agonist (SCA) Platform

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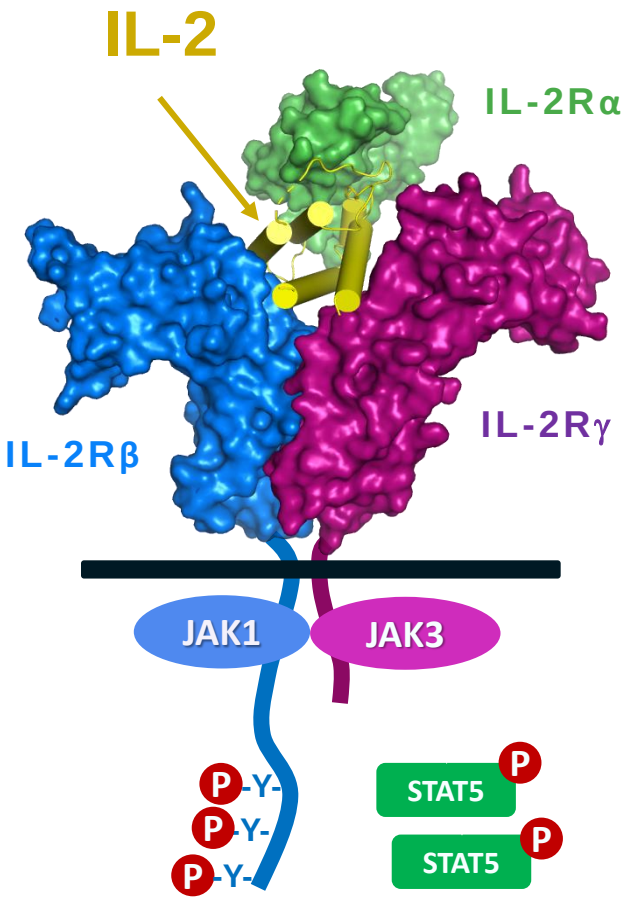
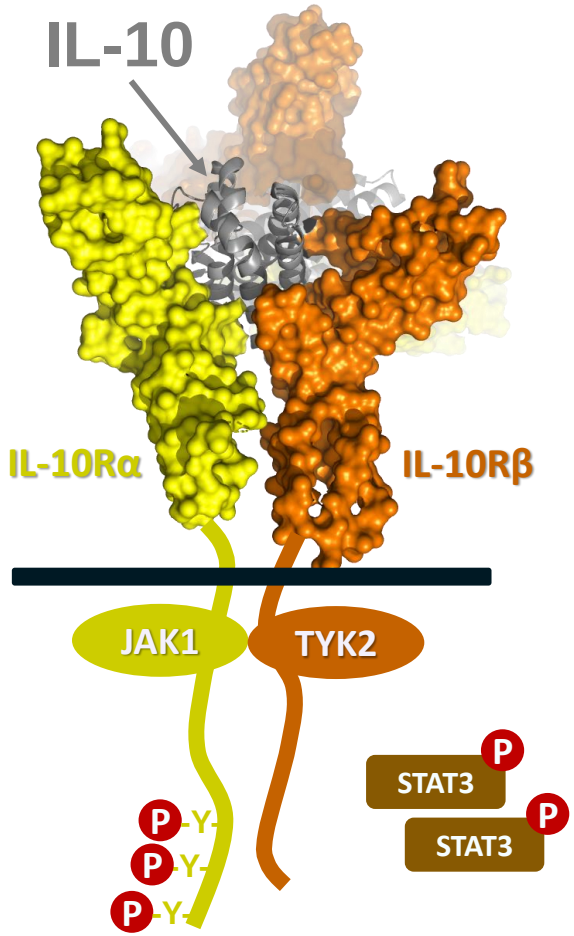
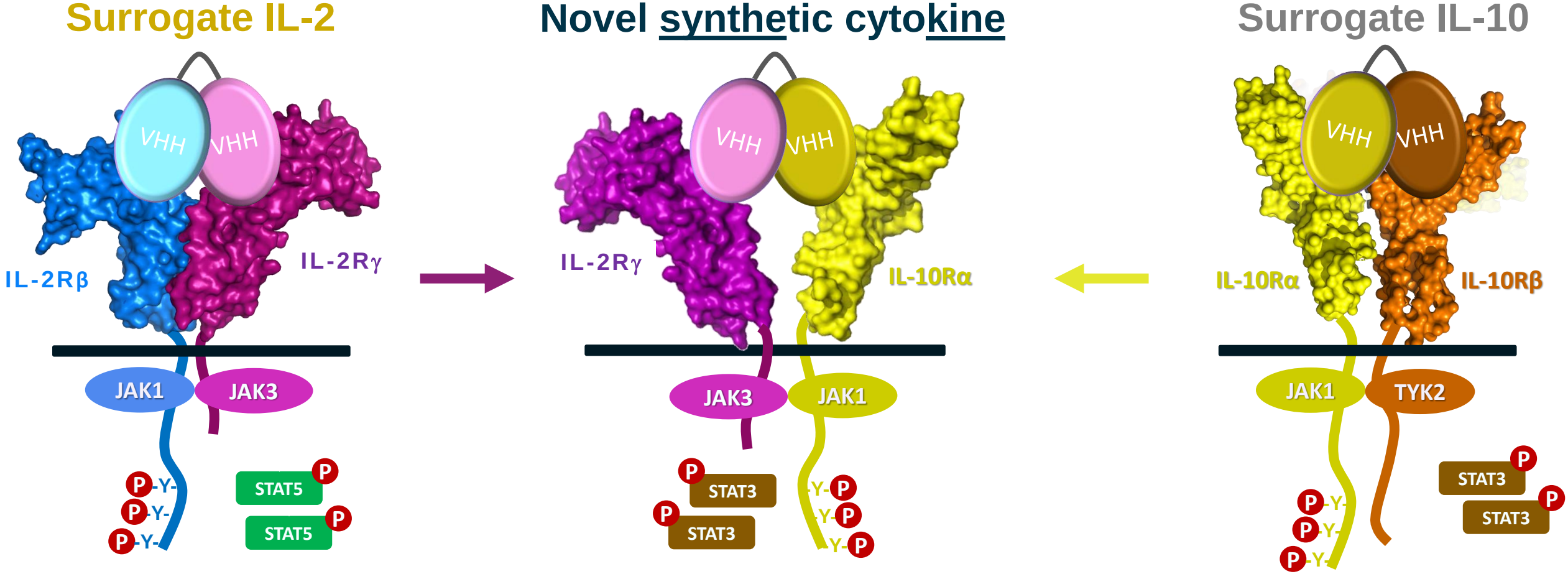
Synthekine

Cytokines enable pairing of a limited set of “natural” receptors





SCAs enable both natural as well as novel receptor pairing



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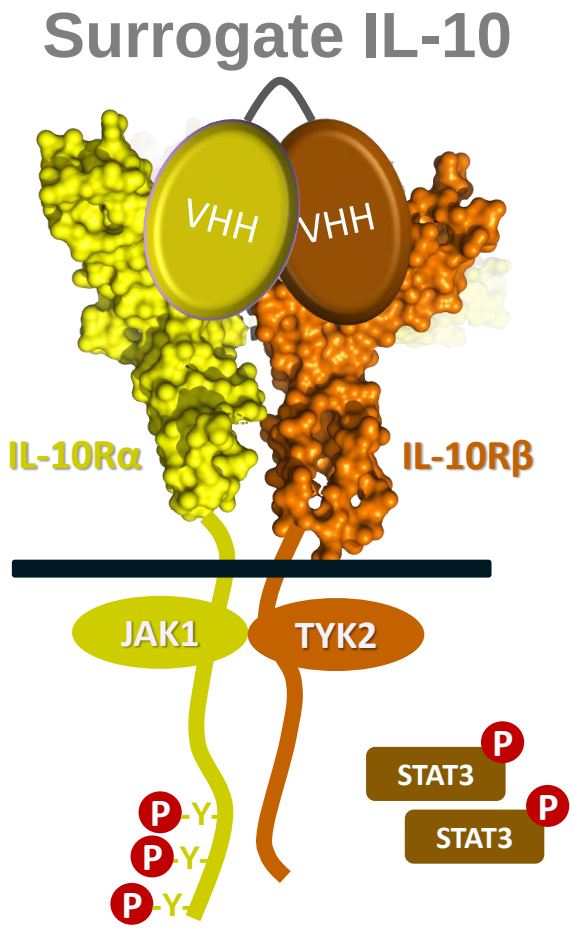
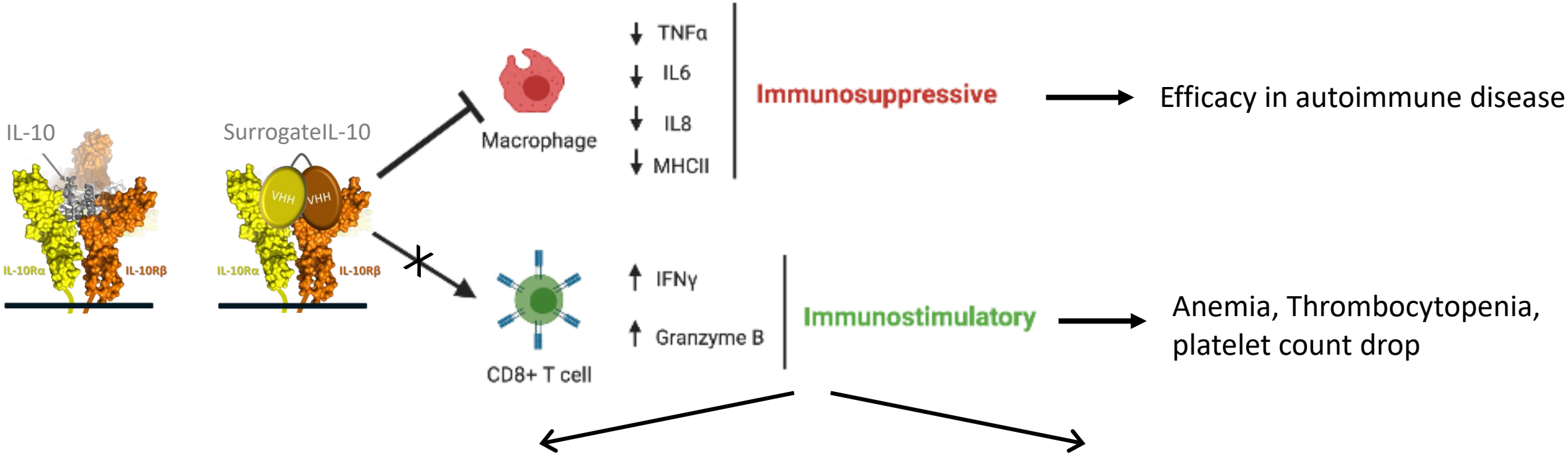
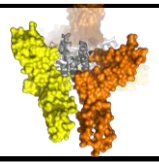


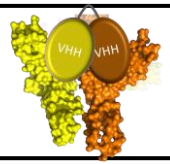
Our approach to developing best in class IL-10 for autoimmune and inflammatory disease





IL-10 Partial Agonists

- *Garcia lab solved complete IL-10.IL-10R1.IL-10R2 complex*
- *Based on the structure, IL-10 partial agonists were designed to weaken IL-10Rb interaction and decouple immunostimulatory from immunosuppressive functions*



IL-10 SCA

- *SCA Discovery is Structure-agnostic*
- *Partial agonism can be achieved via screening*
- *Activity is tunable through scaffold engineering*
- *Antibody-like development properties*

“Med Chem” approach to discovery of SCA at Synthekine

Discover library of binders
- Llama immunization
- Yeast/phage display with cytokine receptor
- Sequence and sort binders (VHH)

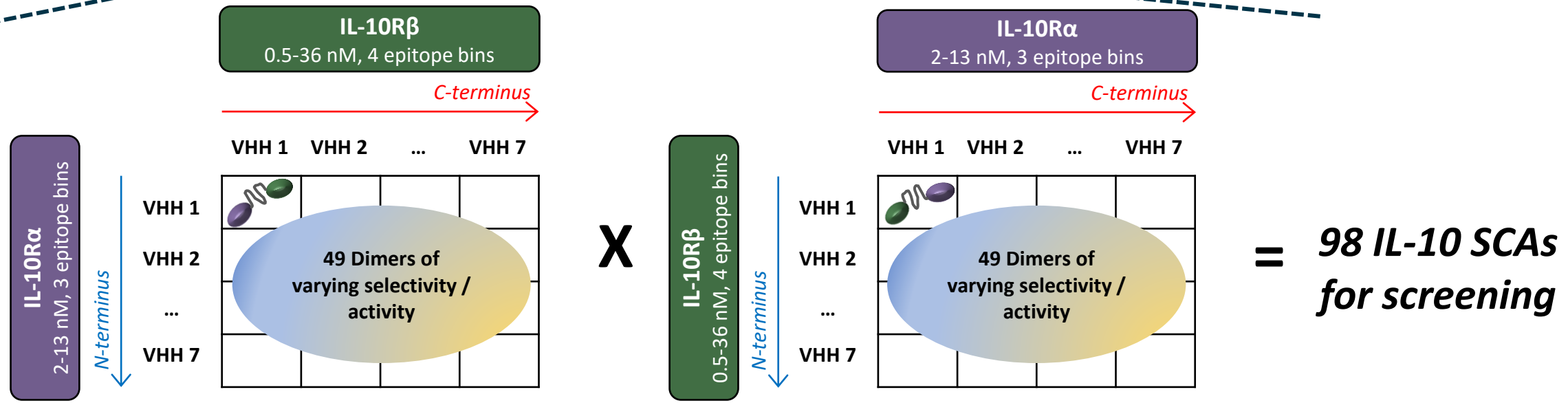
Generate panels of dimers
- Produce panels containing an all-by-all matrix of VHH dimers
- Express/purify protein

In vitro screening for desired signaling activity or cell type bias

Tune activity
- linker engineering
- Valency (Fc)

Half life extension

Advance to in vivo PoC



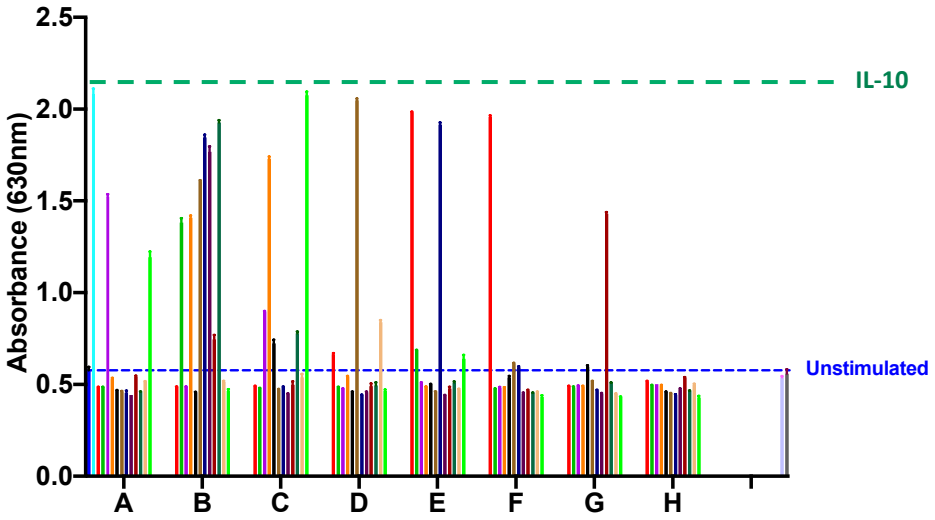
= ***98 IL-10 SCAs for screening***

Synthekine

IL-10 SCAs demonstrate broad range of signaling and cell type bias

Initial Screening: STAT3 Reporter Assay

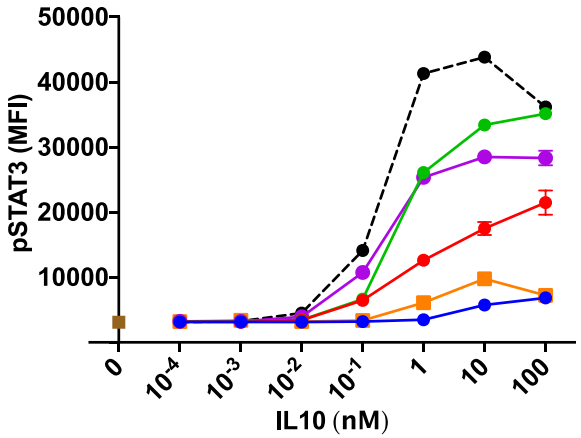
- **Panel of 98 IL-10 VHHs**
- **Several (~20-25%) generate Stat3 signals**



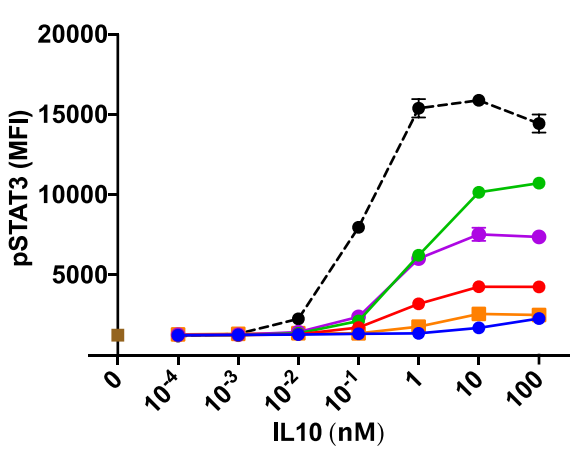
Evaluation of Hits: pSTAT3 Signals in PBMC-Derived Cell Populations

- Broad range of signaling on cells:
  - Recapitulation of IL-10 signaling
  - Partial agonists with reduced EC50 and Emax vs. IL-10
- Bias of IL-10 VHHs towards certain cell types observed

Monocytes pSTAT3



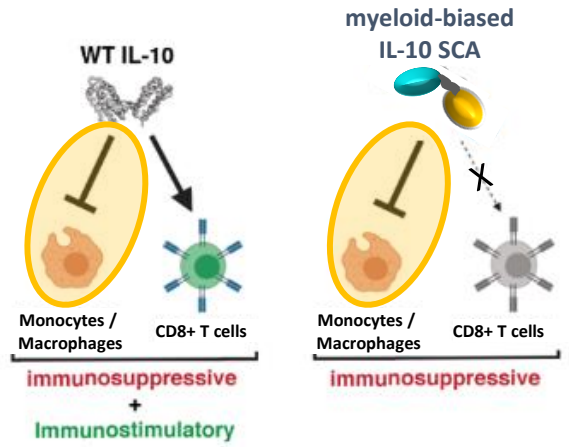
CD8 T cells pSTAT3



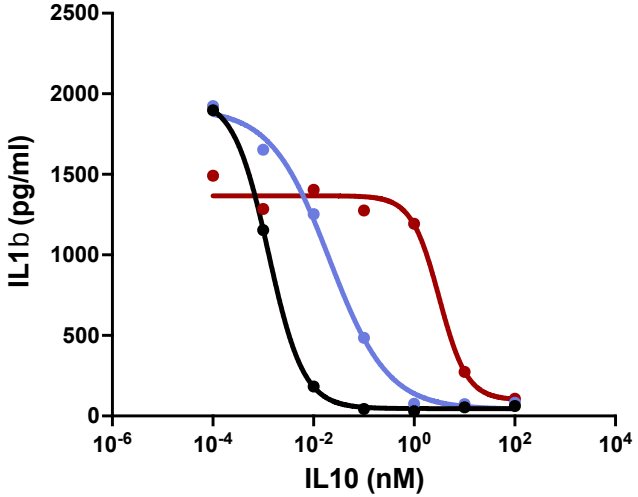
Synthekine

IL-10 SCA delivers partial agonism and shows biased activity in vitro

Goal: Retain immunosuppressive effects



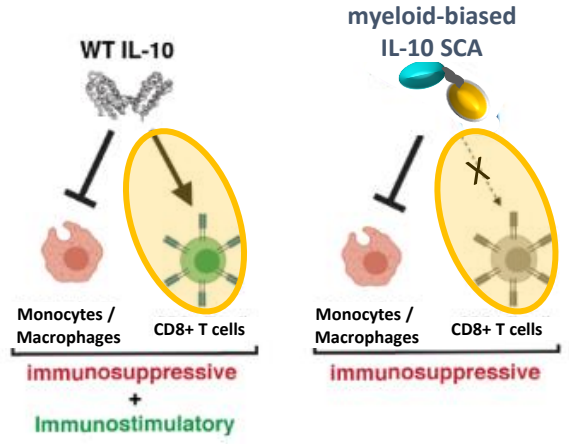
LPS-Activated Monocyte Cytokine Secretion



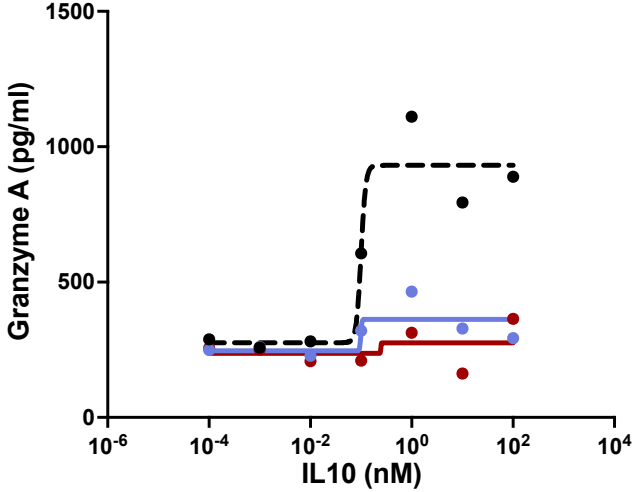
Similar results for the IL-10 surrogate suppressing LPS-induced:

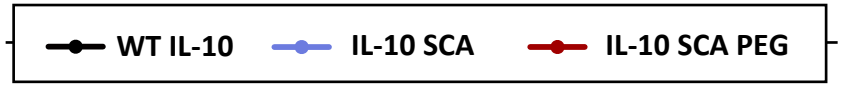
- ***IL-6***
- ***TNFα***

Goal: Uncouple immunostimulatory effects



Stimulated CD8 T-Cell Blasts Cytokine Secretion





Similar results for the IL-10 surrogate producing equally less:

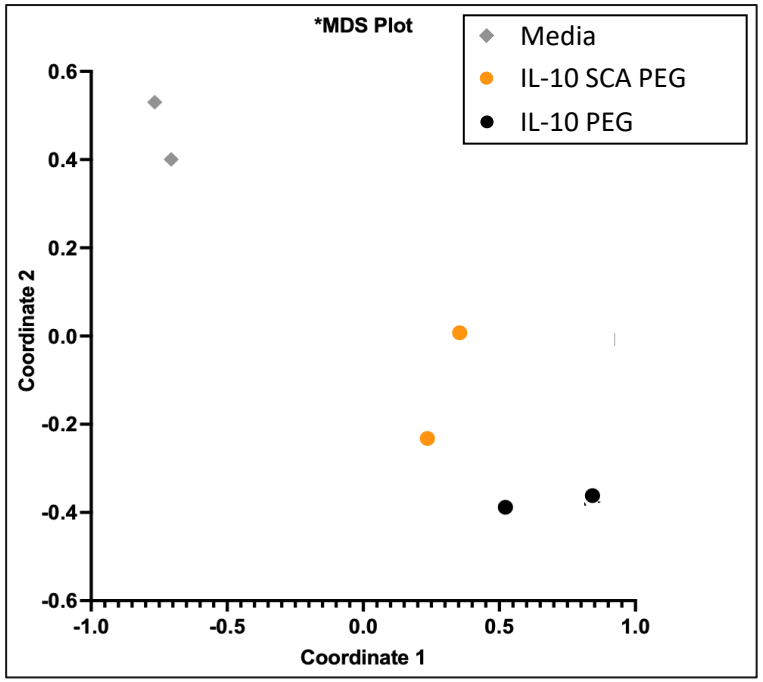
- ***IFNγ***
- ***Granzyme B***

Synthekine

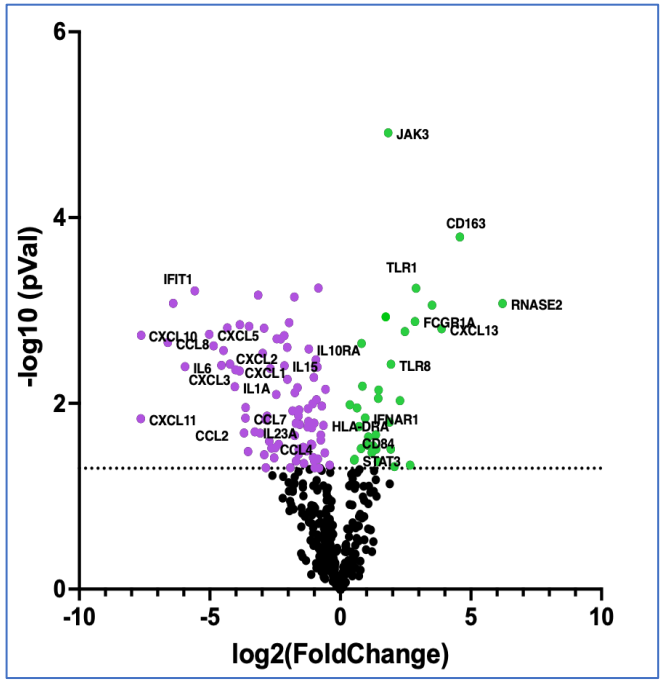
IL-10 SCA gene activation on Monocytes is consistent with IL-10

- IL-10 SCA modulates many of the same genes as IL-10
- IL-10 PEG and IL10-SCA PEG are active on human monocytes and modulate gene expression

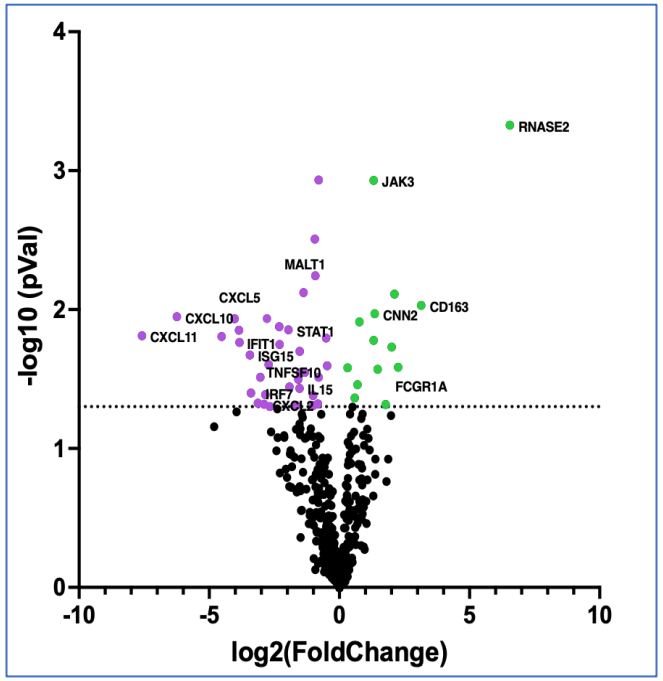
Multidimensional scaling analysis



IL-10 PEG



IL-10 SCA PEG



*Volcano plots of molecules compared to media alone treated monocytes

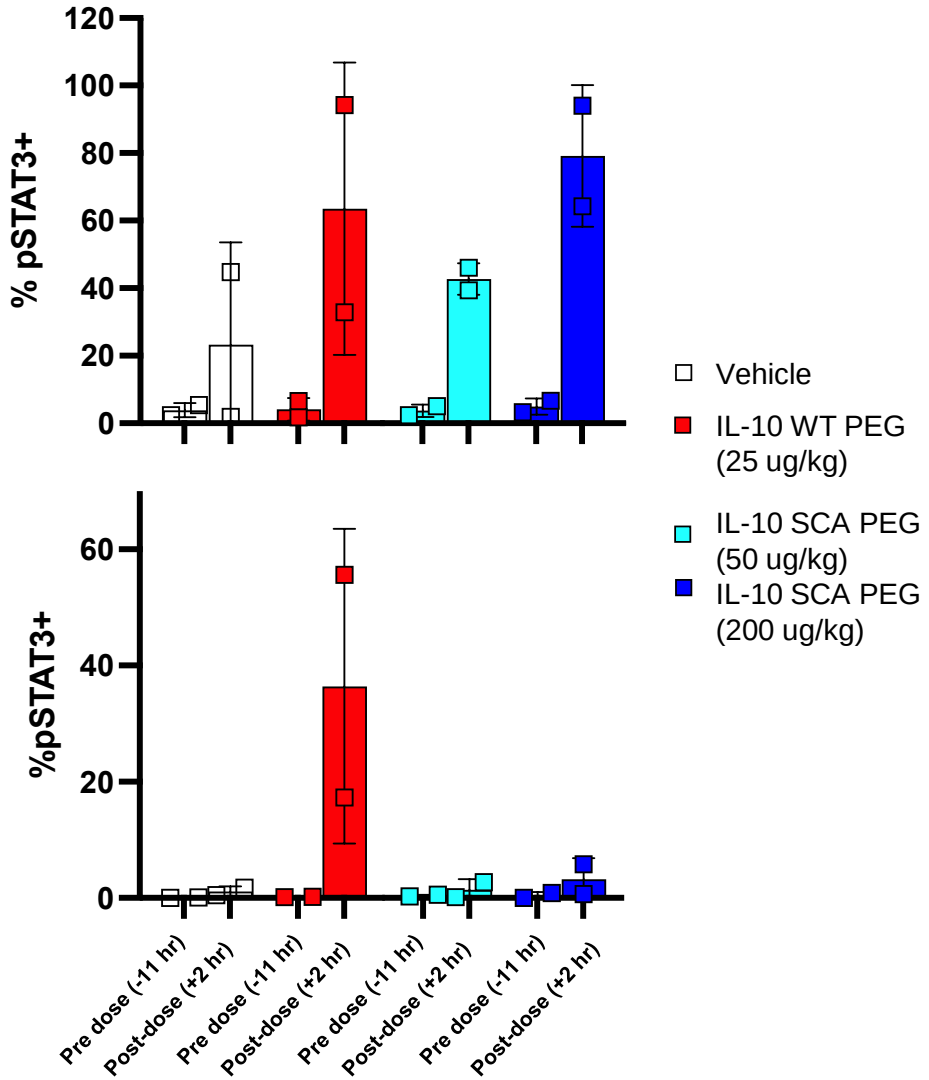
- Human Monocytes treated with Emax concentration of IL-10 PEG (10 nM) and IL-10 SCA PEG (1 uM) for 6 hours
- Cell lysates were sent to Nanostring Technologies for RNA isolation and analysis using the Myeloid innate immune panel (780 genes)

Synthekine

IL-10 SCA PEG shows selective STAT3 induction on monocytes and high and durable exposure in cyno

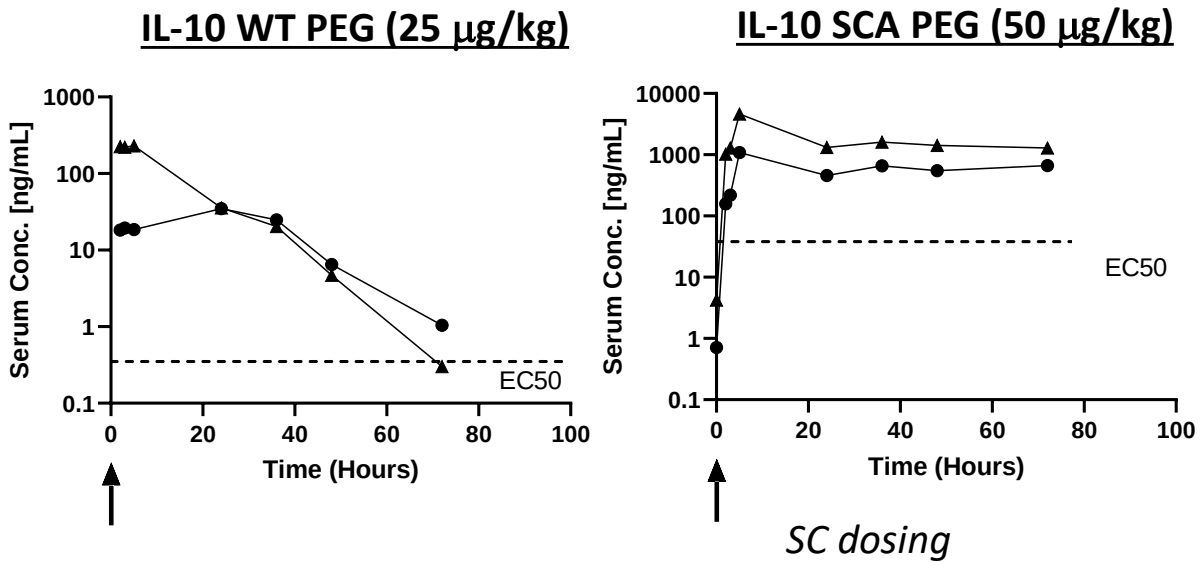
Monocytes

CD8 T cells



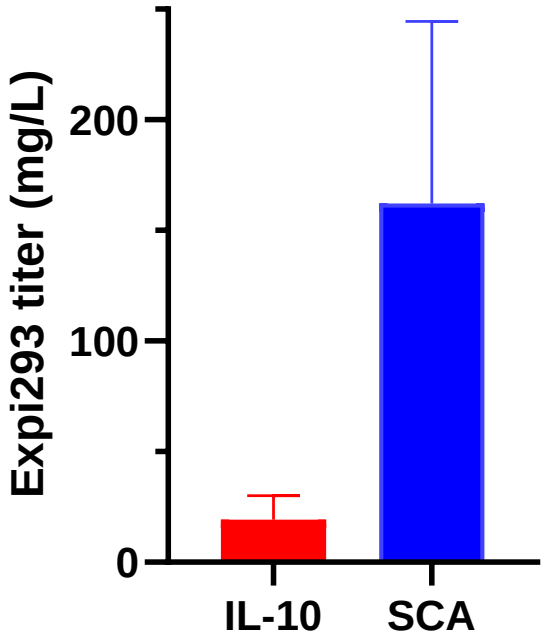
Phospho-flow of two cynos (1M, 1F) per treatment arm

- PEGylated IL-10 SCA showed preferential signaling on monocytes at both the high and low concentrations
- IL-10 SCA PEG have a stable PK profile

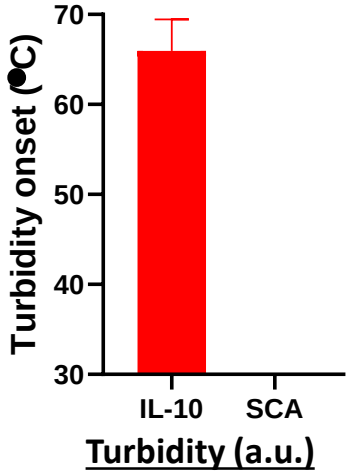


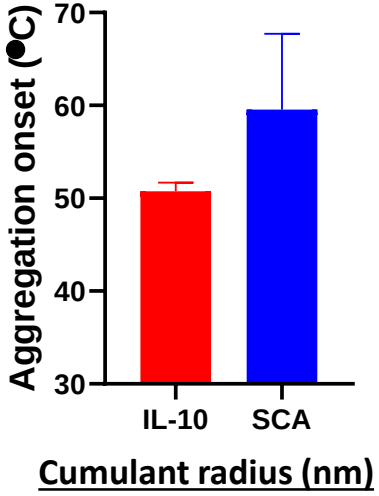
IL10-SCA shows superior expression yields and thermostability compared to IL10

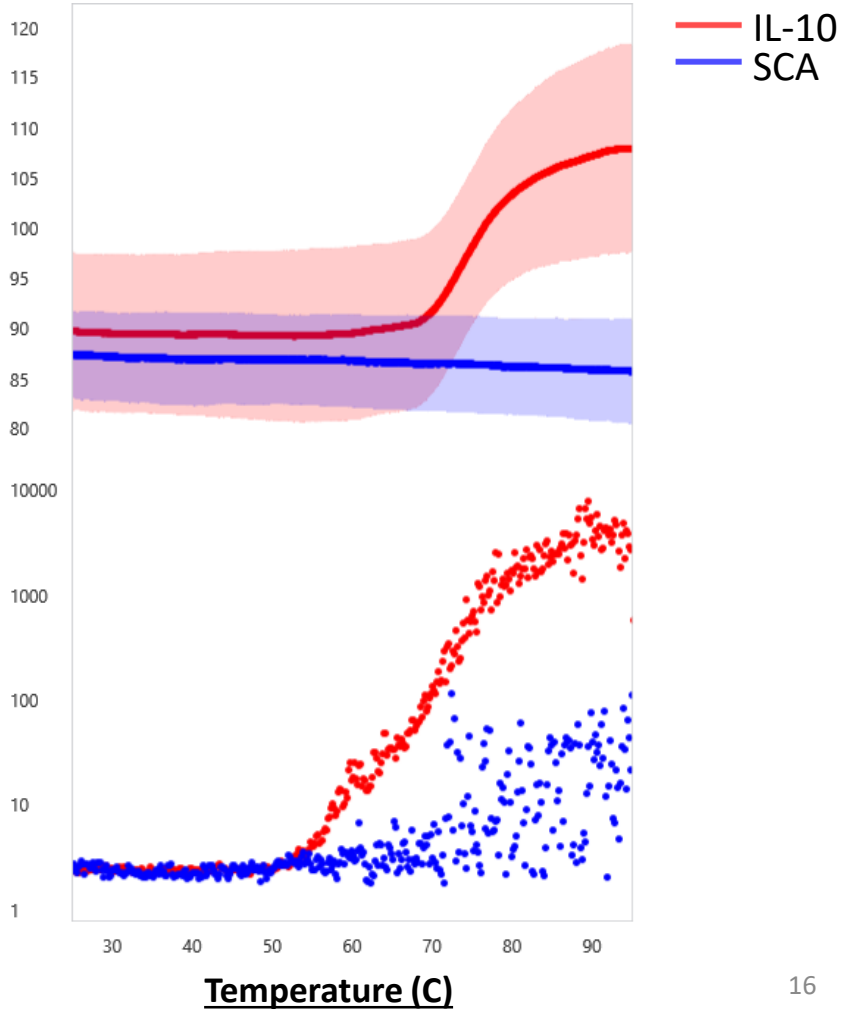
SCA expression yields 9x higher than IL-10



SCA shows increased thermal stability compared to IL-10

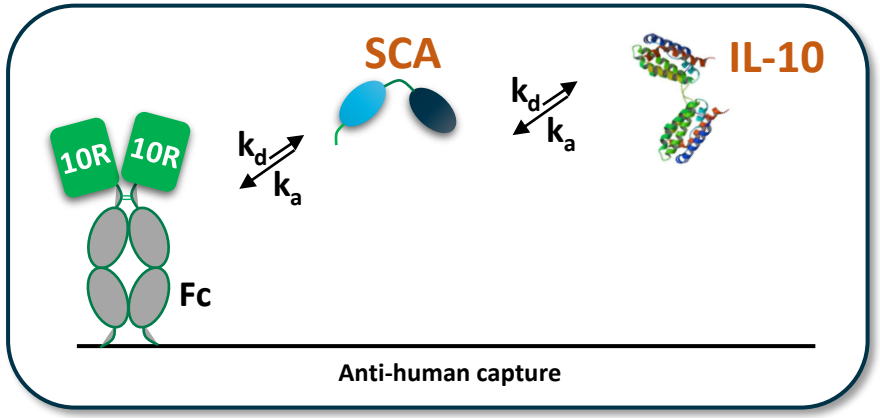




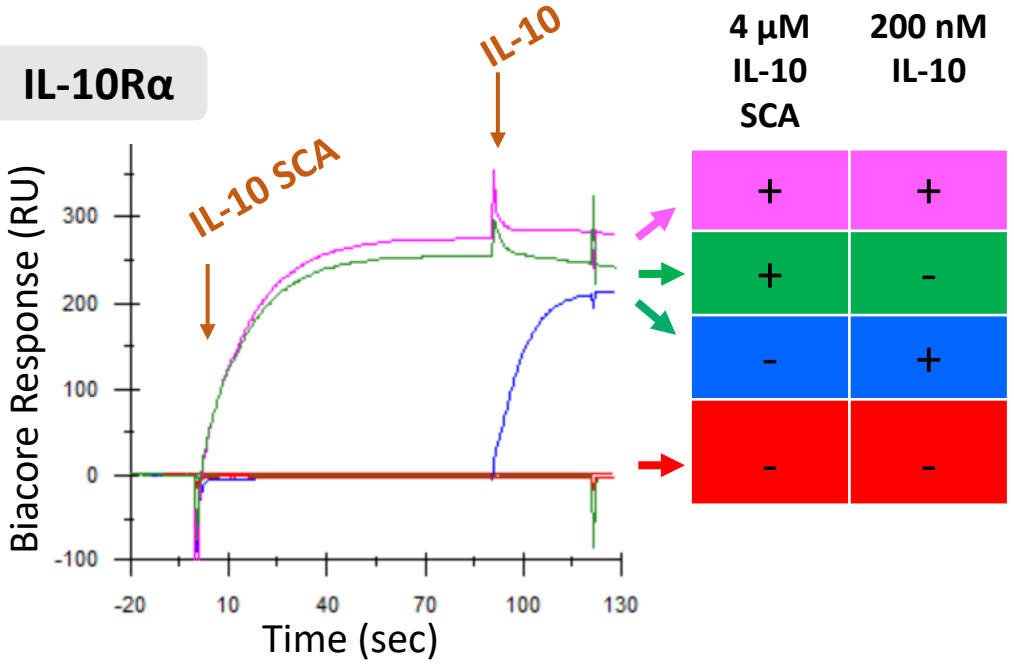


Synthekine

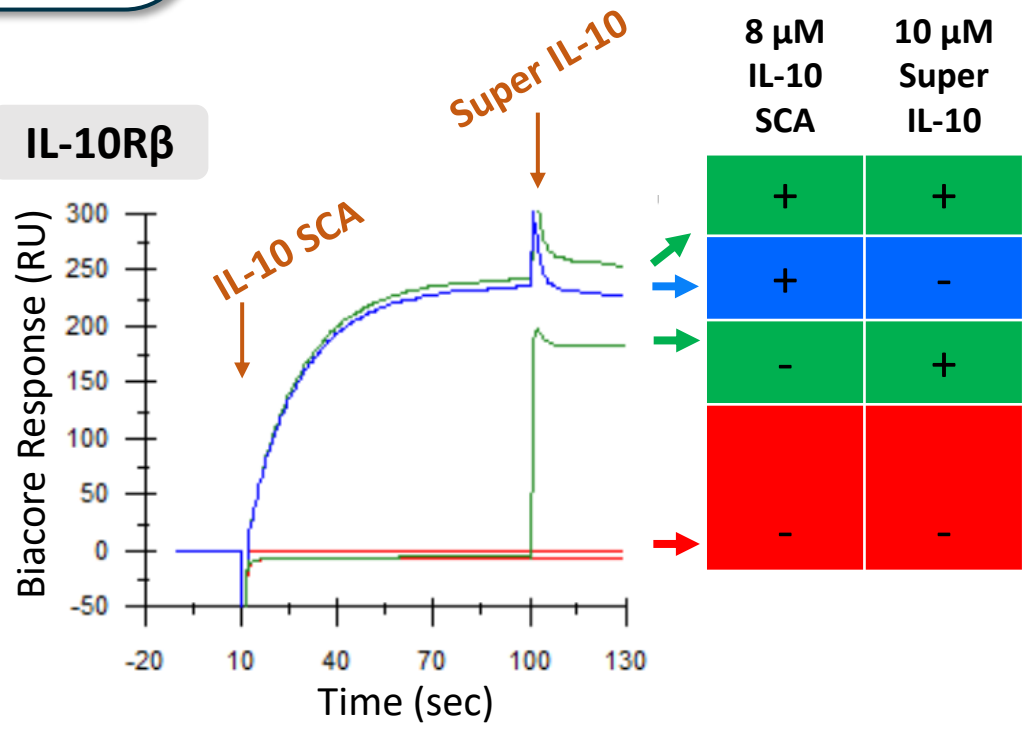
IL-10 SCA is competitive with IL-10 for binding to the IL-10 receptors



IL-10Rα



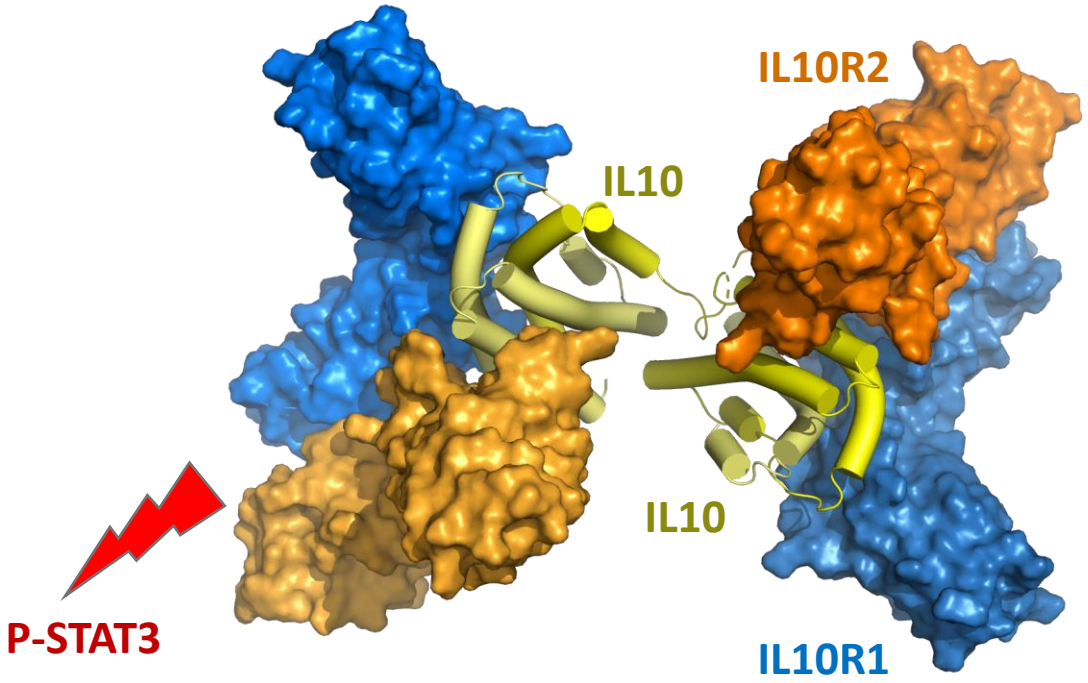
IL-10Rβ



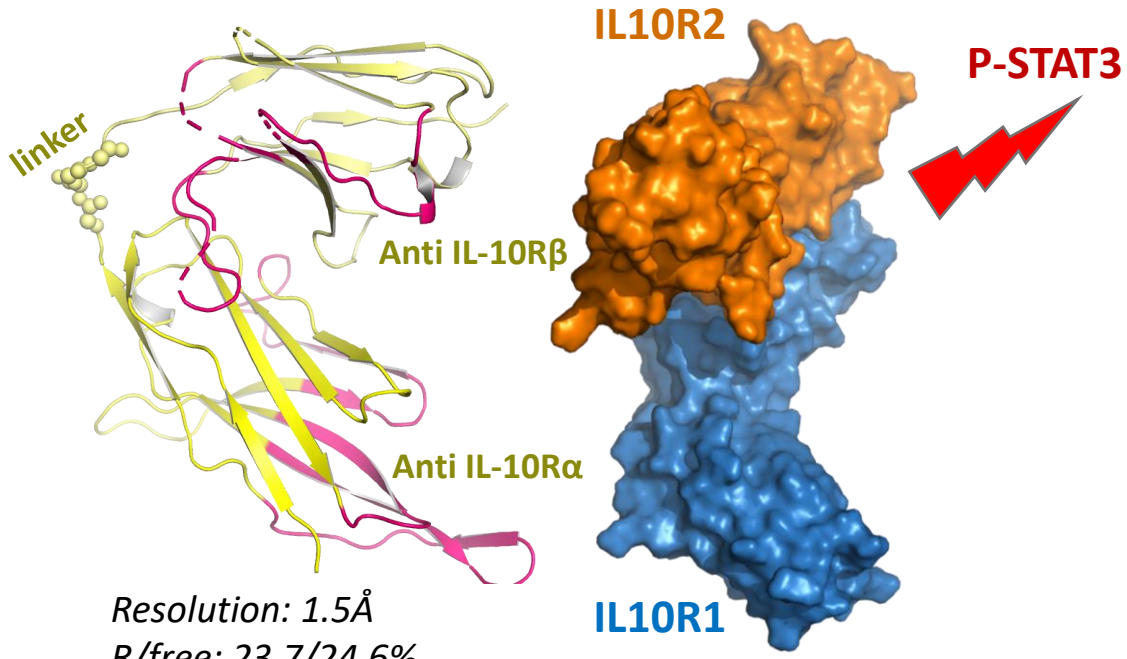
Synthekine

IL-10 SCA crystal structure reveals a compact conformation that mimics a cytokine

IL10 dimeric complex



IL10 SCA monomeric complex



Resolution: 1.5Å
R/free: 23.7/24.6%

Conclusions

1. Surrogate cytokine agonists (SCAs) composed of two receptor binding VHH can mimic cytokine signaling
2. Combinatorial screens of VHH pairs can identify molecules with a range of agonist activity
3. An IL-10 SCA achieves myeloid-biased IL-10 partial agonism *in vitro* and *in vivo*
4. Our IL-10 SCA displays improved development and pharmacokinetic properties over IL-10