Tumor selective cytotoxicity by TAA x CD3 bispecifics utilizing a 2:1 mixed-valency format

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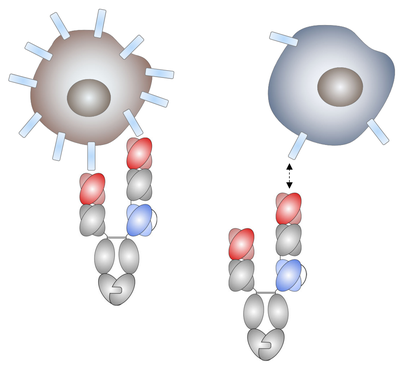
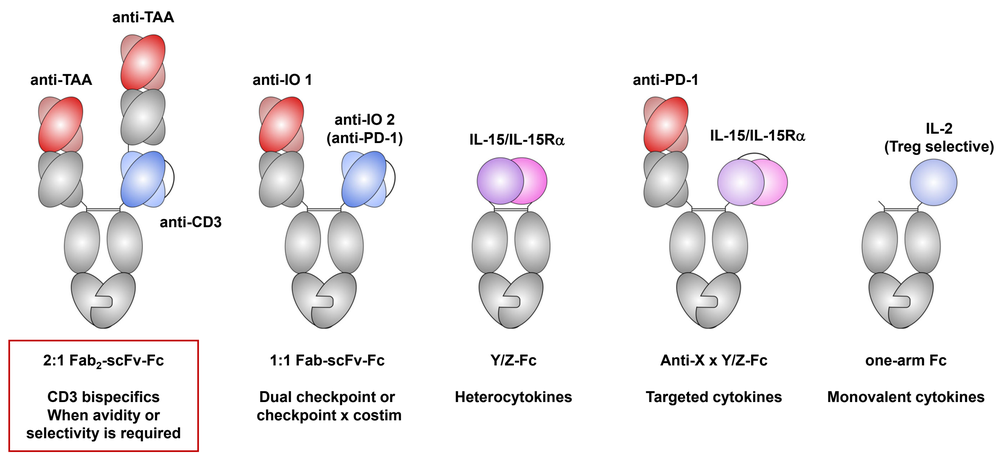
Introduction

- Tumor-associated antigen (TAA) x CD3 bispecifics have been shown to recruit T cells to mediate cytotoxicity against tumor cells.
- The pharmacodynamics and tolerability of TAA x CD3 bispecifics are impacted by multiple aspects of TAA biology such as tumor load, cell surface antigen density, and normal tissue expression.
- Using a bivalent/monovalent (2:1) mixed-valency format, we have engineered multiple examples of TAA x CD3 bispecifics that exhibit selective redirected T-cell cytotoxicity (RTCC) of high versus low antigen density cell lines that mimic tumor versus normal tissue, respectively.
- The selectivity exhibited by the 2:1 format potentially empowers TAA x CD3 bispecifics to address an expanded set of tumor antigen biologies.



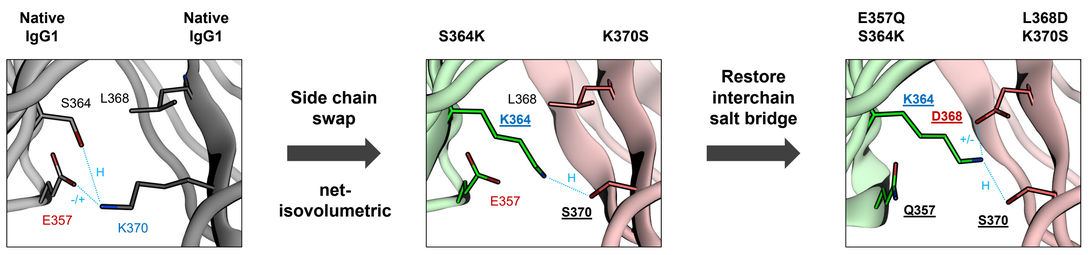
Heterodimeric Fc empowers next-generation bispecific formats with altered valencies



Stable and well-behaved heterodimeric Fc enables 2:1 Fab_2-scFv-Fc bispecific format

Novel set of Fc substitutions capable of achieving heterodimer yields over 95% with little change in thermostability

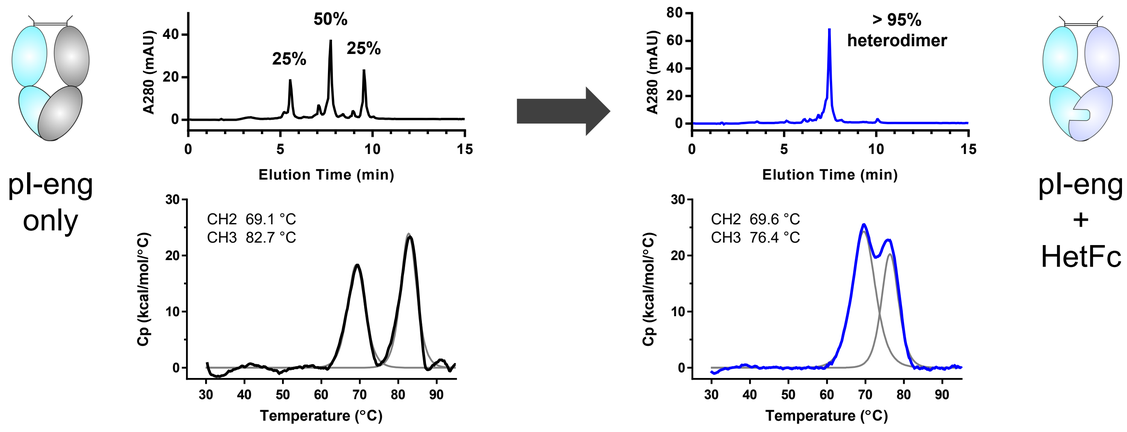
Structural models of CH3-CH3 interface built using MOE based on Protein Data Bank entry 3AVE



Engineered isoelectric point differences in the Fc region allow straightforward purification of heterodimer

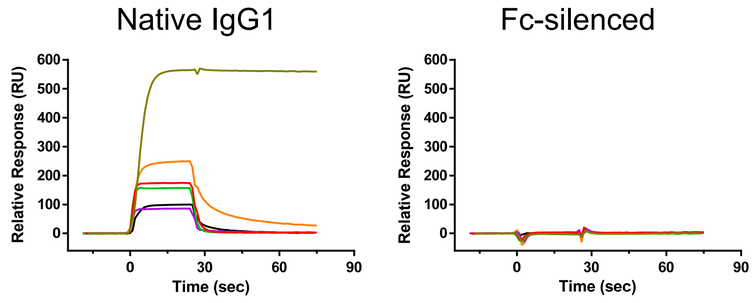
Isosteric substitutions used to minimize impact to tertiary structure

Distribution after standard protein A purification only (analytical IEX)



Thermostability (DSC) of purified protein after a cation exchange purification step

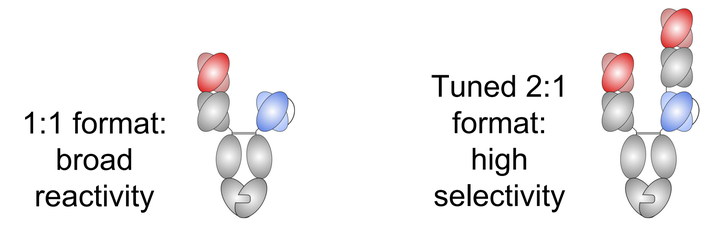
Hinge and CH2 substitutions abolish FcγR binding



FcγR (1 μM) binding measured by SPR (Biacore 3000), protein A sensor chip/antibody capture format

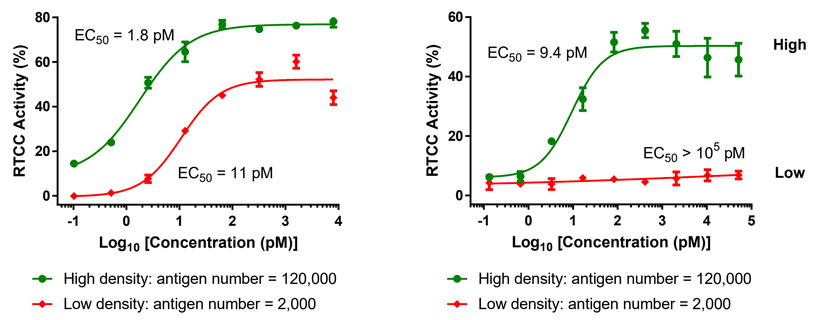
2:1 Fab_2-scFv-Fc format enables targeting of solid tumor antigens with low density on normal tissue

Tuning TAA valency and TAA/CD3 affinities enables selective cytotoxicity of cell lines mimicking cancer tissue vs. normal tissue (high/low antigen density)



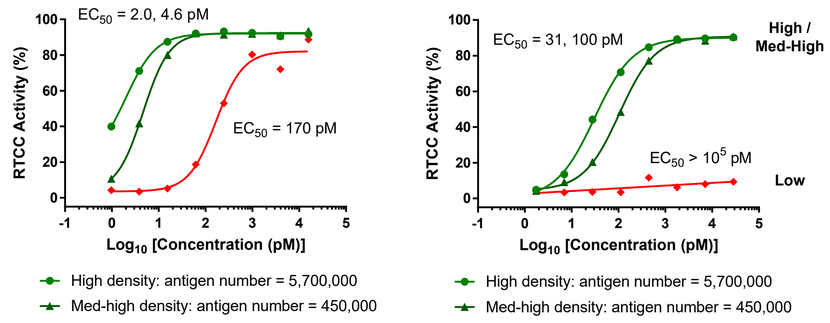
Solid tumor TAA-1

RTCC Activity by LDH (CytoTox-ONE)
10k target cells
100k purified human T cells
(E:T = 10:1)
24 hr incubation



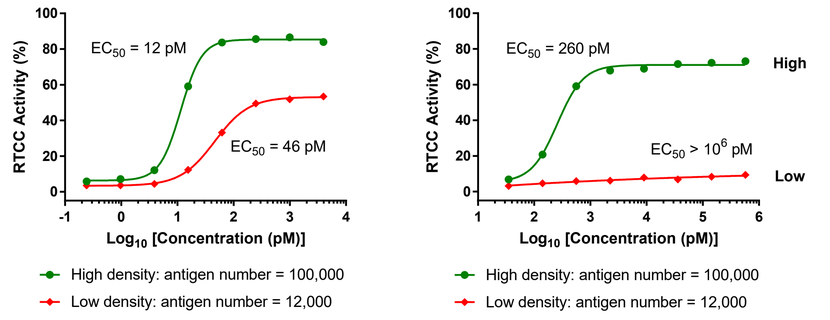
Solid tumor TAA-2

RTCC Activity by cell counts
(Zombie Aqua, BioLegend)
20k CFSE-labeled target cells
400k human PBMC
(E:T = 20:1)
48 hr incubation



Solid tumor TAA-3

RTCC Activity by cell counts
(Zombie Aqua, BioLegend)
40k CFSE-labeled target cells
400k PBMC
(E:T = 10:1)
48 hr incubation



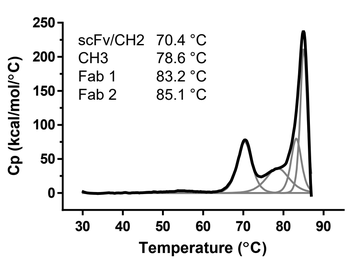
Tuned 2:1 bispecifics also have reduced interference from soluble antigen and reduced cytokine release

2:1 Fab_2-scFv-Fc CD3 bispecifics are stable, well-behaved, and easily purified

Research scale production is straightforward

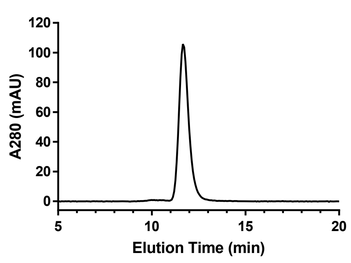
Antibody-like thermostability (DSC)

pI-eng + HetFc + Fc-silenced

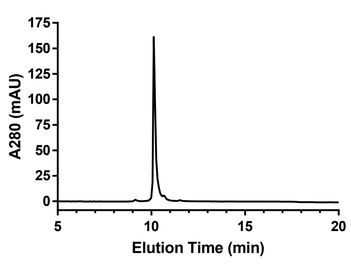


Favorable solution properties (SEC)

After standard protein A, cation exchange purification steps



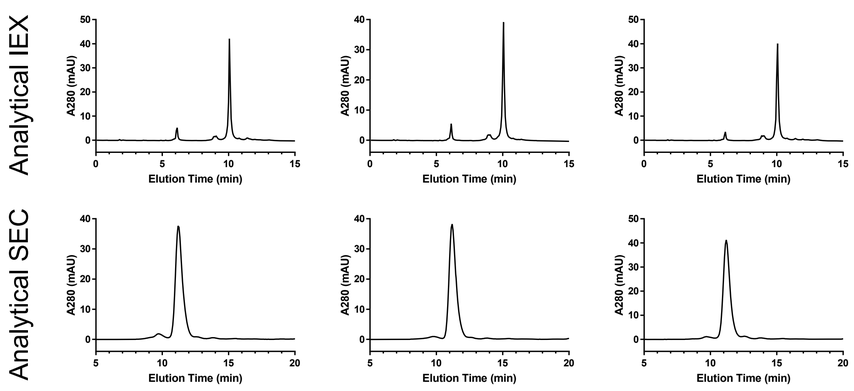
High purity bispecific (IEX)



Stable cell line development results in clones with high titer and high heterodimer prevalence

Top clones have shake flask yields of 1 - 2 g/L with ~ 90% heterodimer content

After only a standard protein A purification step



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