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Producing Homogeneous ADCs with Combination Warheads

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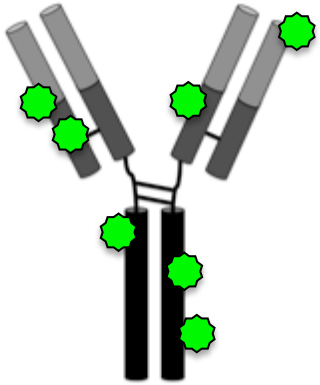
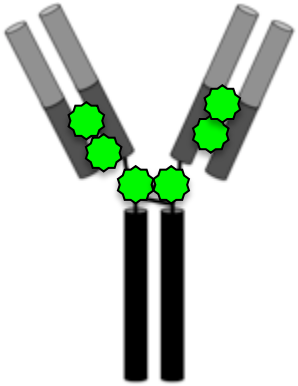
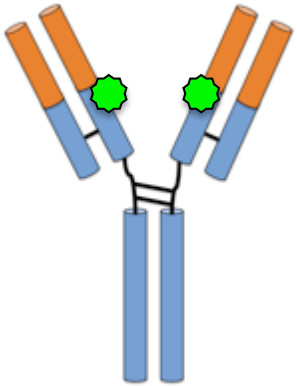
Chief Scientific Officer
October 15, 2013

ADCs – State of the Art. . .



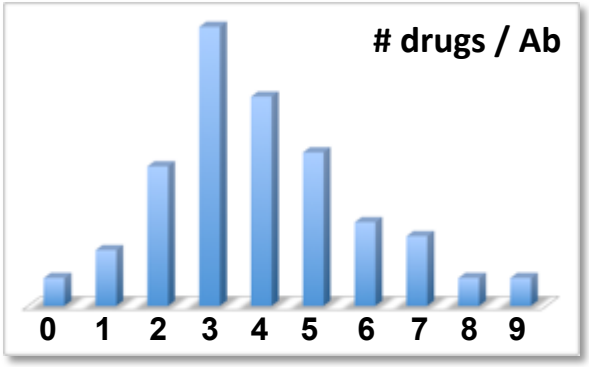


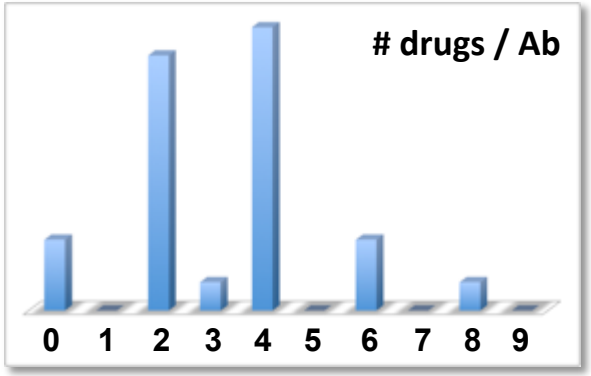


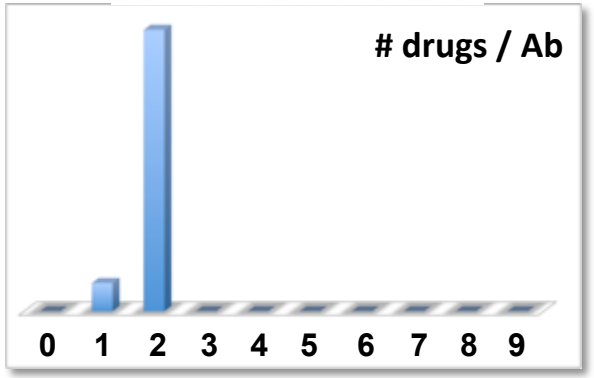
IMMUNOGEN, INC.

SeattleGenetics

Genentech
IN BUSINESS FOR LIFE







Random Lysine Conjugation

Cysteine Conjugation: S-S bonds

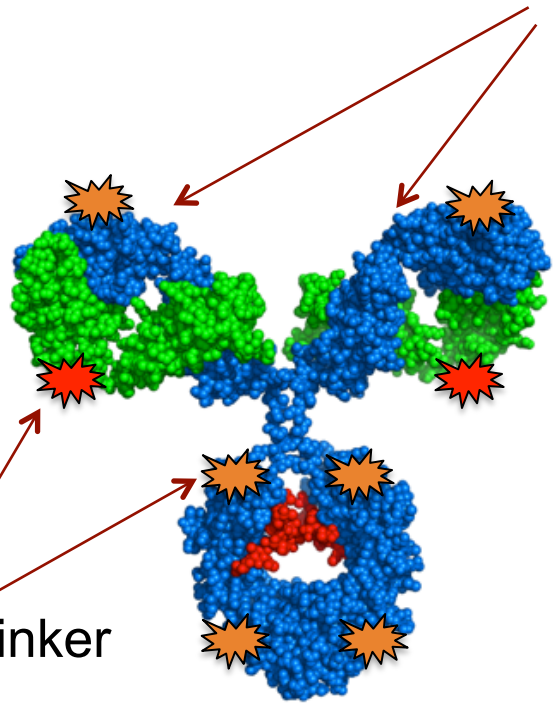
Site Specific Conjugation

Heterogeneity translates to sub-optimal PK, stability and efficacy

Success is a matter of Design

	Efficacy	Resistance	Safety
Antigen	Choice of Epitope Density Internalization rate	Tumor Heterogeneity (inter and intra Antigen mutation or shedding Therapy-induced changes Alterations in apoptotic pathways	Healthy tissue expression
Antibody / Scaffold	Tumor Distribution/Disposition Fc functionality and PK		Stability Non-specific Fc binding ADCC and CDC activity
Conjugation	**Specificity**		**Stability**
Linkers	Cleavable or non-cleavable Physicochemical props	Immunogenicity	Stability
Warheads	Potency Payload	Mechanism becomes redundant MDR/Pgp status Altered metabolism Immunogenicity	Bystander effect Systemic release

Next Generation ADC's? Homogeneous and Multi-functional….



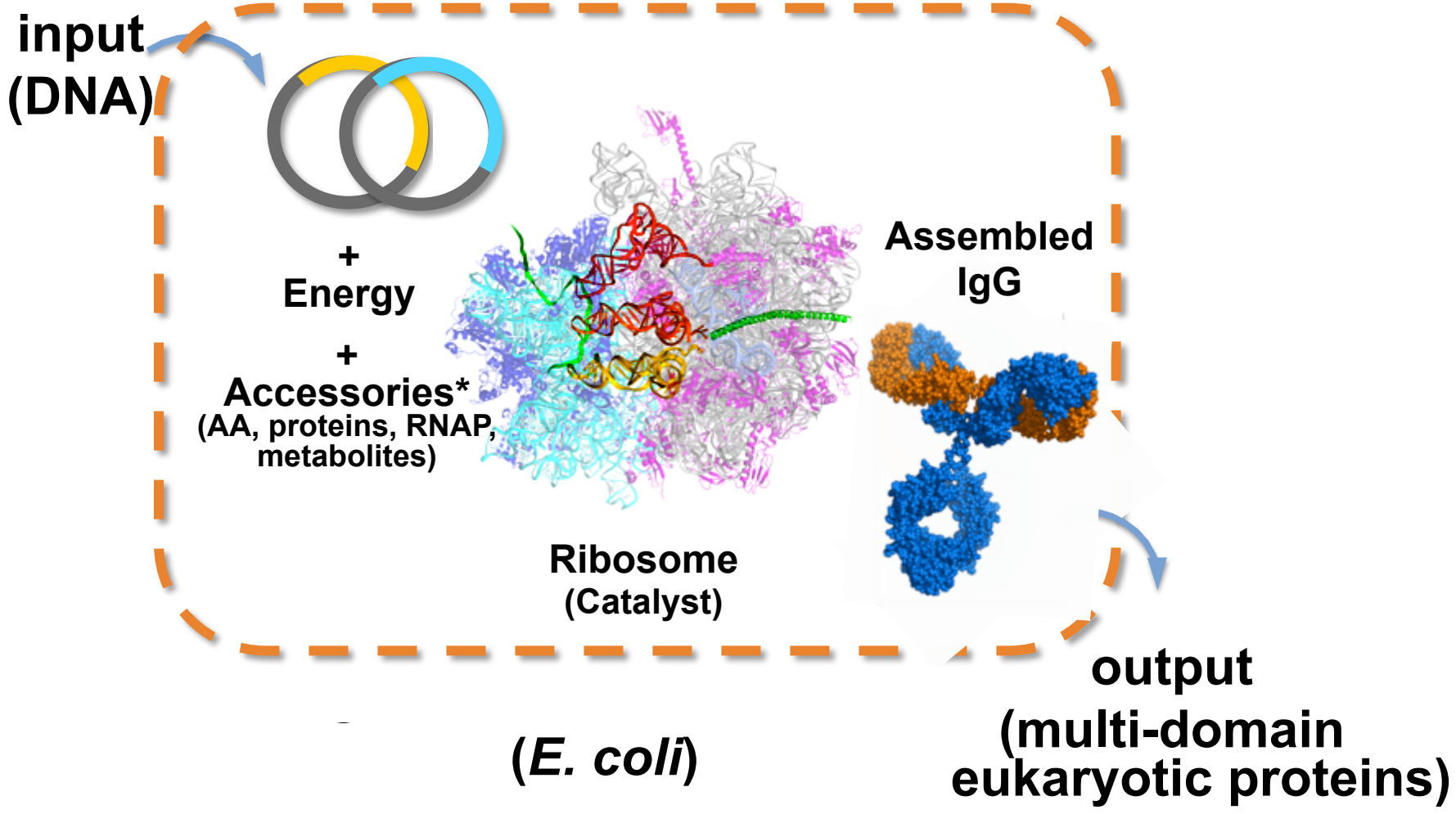
Single or Multiple Antigen Recognition

- Broaden target population, range of indications
- Enhance internalization
- Eliminate effects of heterogeneous antigen expression
- Overcome resistance due to treatment induced changes in antigen expression, shedding
- Leverage avidity effects to improve TI

Multiple Warhead-Linker Combinations

- Warheads with distinct mechanisms of action that are synergistic – lower payloads improves TI
- Eliminate effects of tumor heterogeneity due to variation in cell proliferation
- Overcome resistance due to toxin transport or metabolism

Sutro: A Cell-Free Synthetic Biology Platform



*To be incorporated into strain for commercial production

High Titers at Any Scale

Rapid Execution of Antibody Discovery Programs

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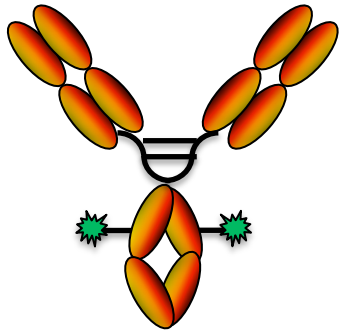
- Discover novel antibody fragments using ribosome display and cell free screening
- Express antibodies and fragments with cell free protein synthesis
- Reformat antibodies/ fragments in a whole host of different frameworks
- Choose the “Best Lead” based on in vitro and in vivo activity

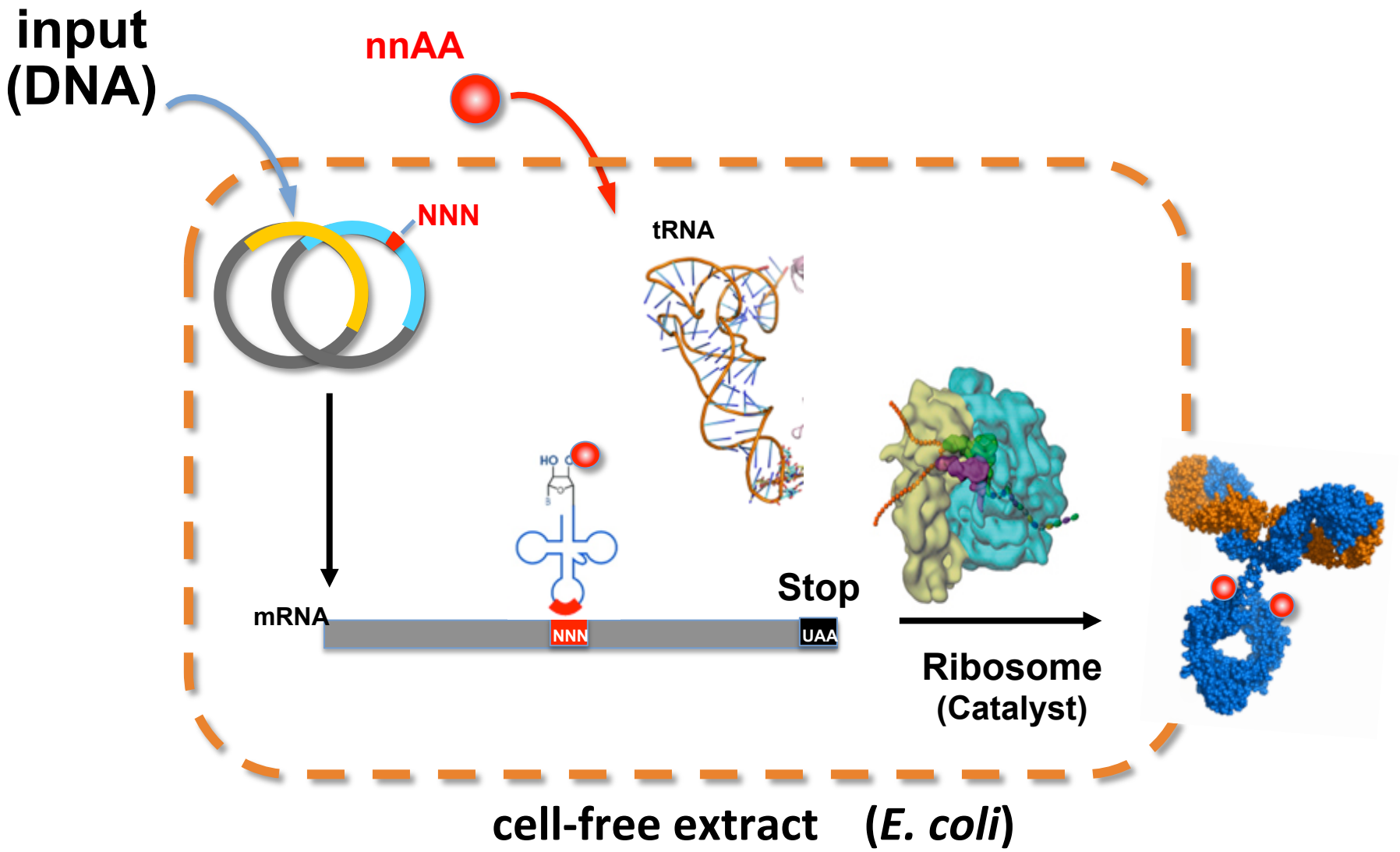
Production of Homogeneous ADCs Using Non-Natural AA Incorporation

The Non-Natural Amino Acid Advantage

1. **Controlled stability:** nnAA chemical space provides alternatives to cysteine or lysine for creating stable MAb~drug junction
2. **Homogeneity:** site-specific conjugation using orthogonal chemistries regulates number and location of drugs attached to Mab



Translation of nnAA-Containing Proteins Enables Site-Specific Conjugation

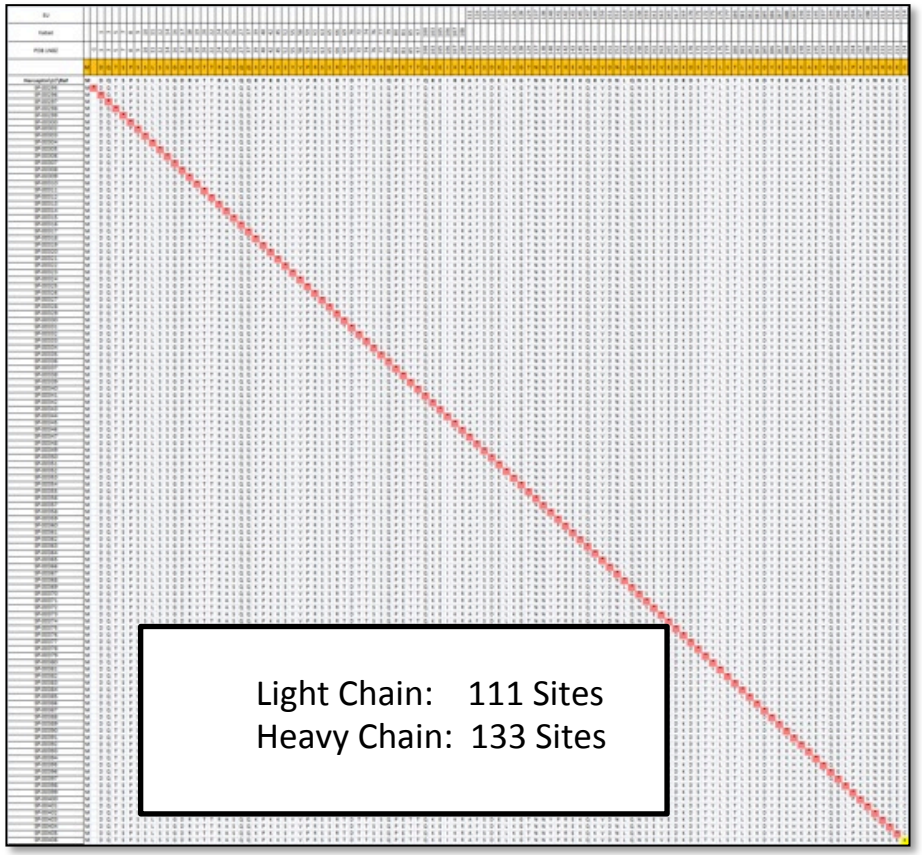


Data Driven Design:
Production of Many Variants in Hours

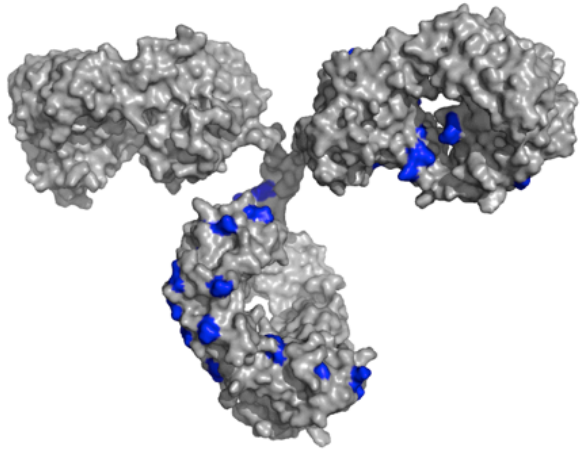
SP Number

Position

TAG site



- **Mutate Sites in IgG:** Choose nnAA sites using rational design, or just make all of them!
- **Produce nnAA IgG:** Incorporate nnAA at 100's of chosen sites
- **Conjugate:** Conjugate nnAA with appropriate chemistry
- **Purify:** Separate conjugated IgG away from unincorporated linker-warhead
- **Test:** Assay conjugated IgG's for binding and cell killing



Rapid Selection of Optimal Sites for Expression, Conjugation, Binding and Killing

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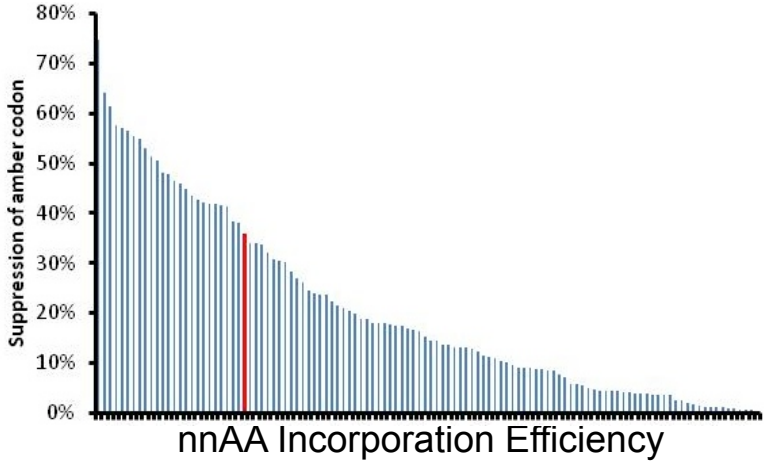
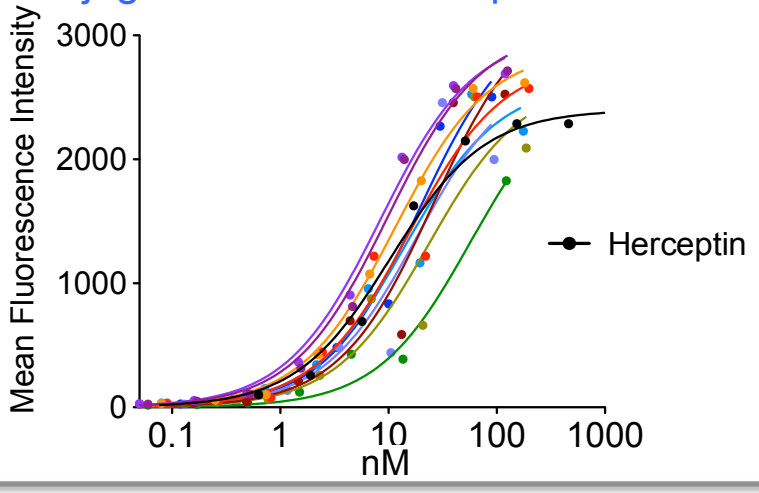
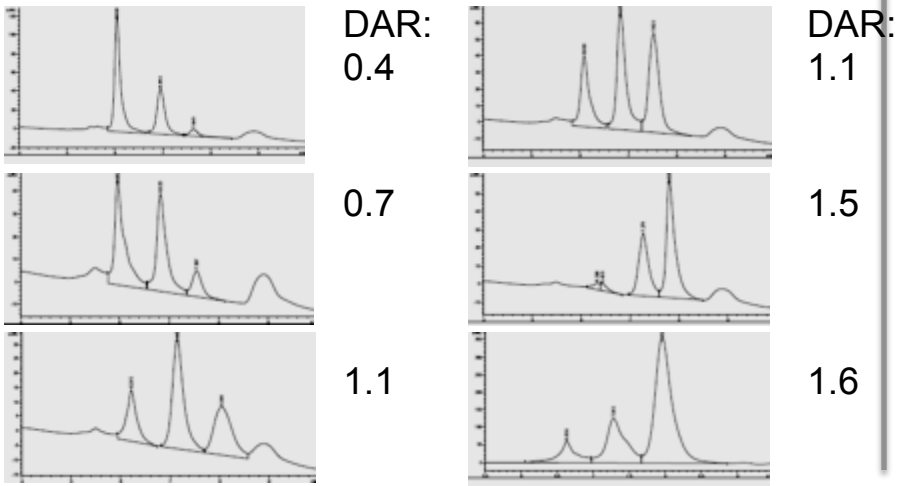
nnAA Incorporation and Expression



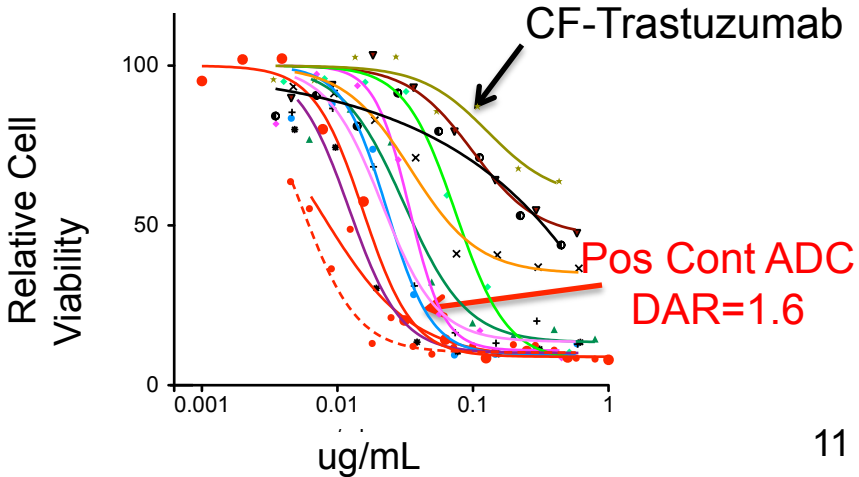
SKBR3 Binding Assay
Conjugated Variants Compared to Herceptin



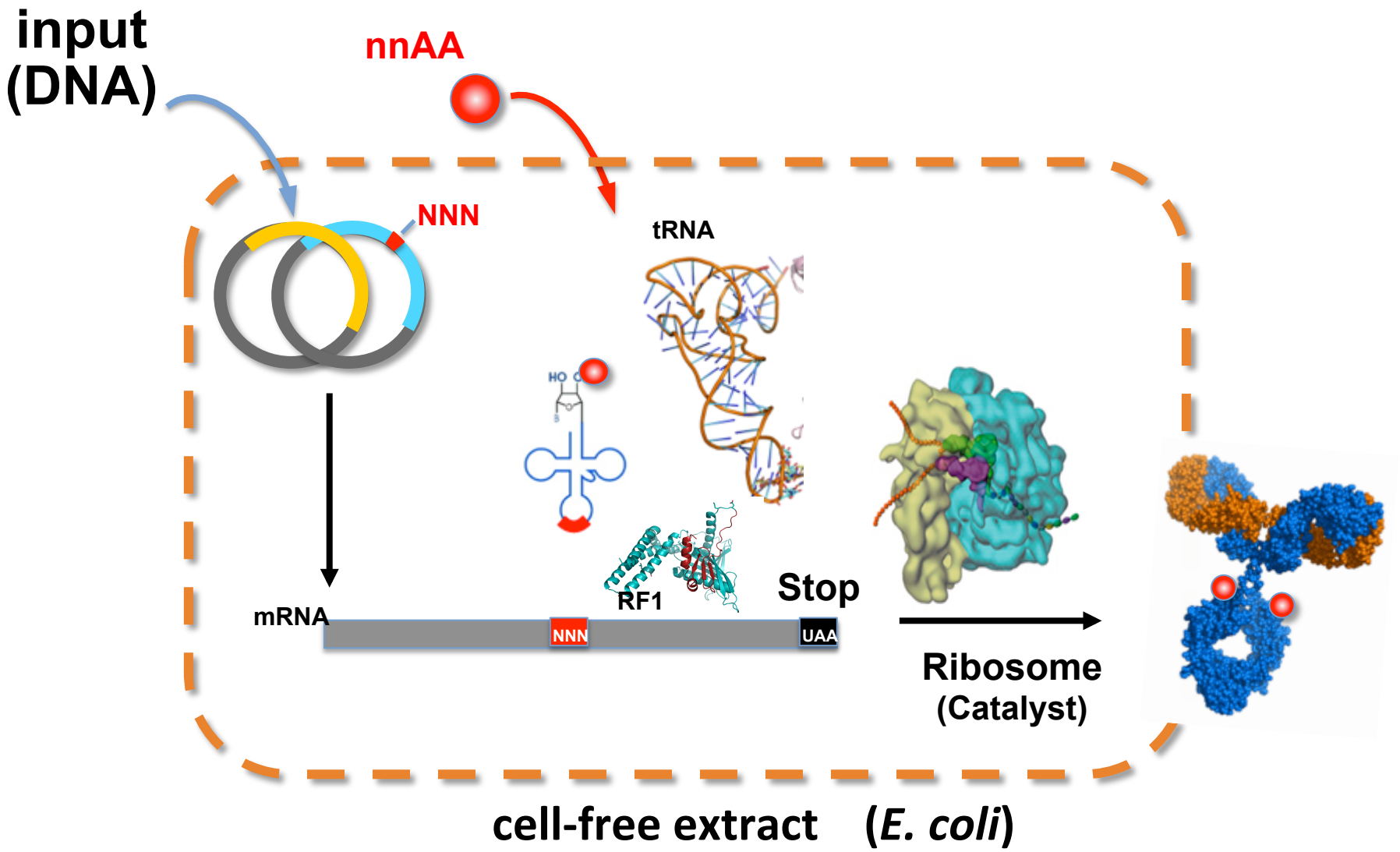
Conjugation Efficiency (Drug/MAb Ratio)



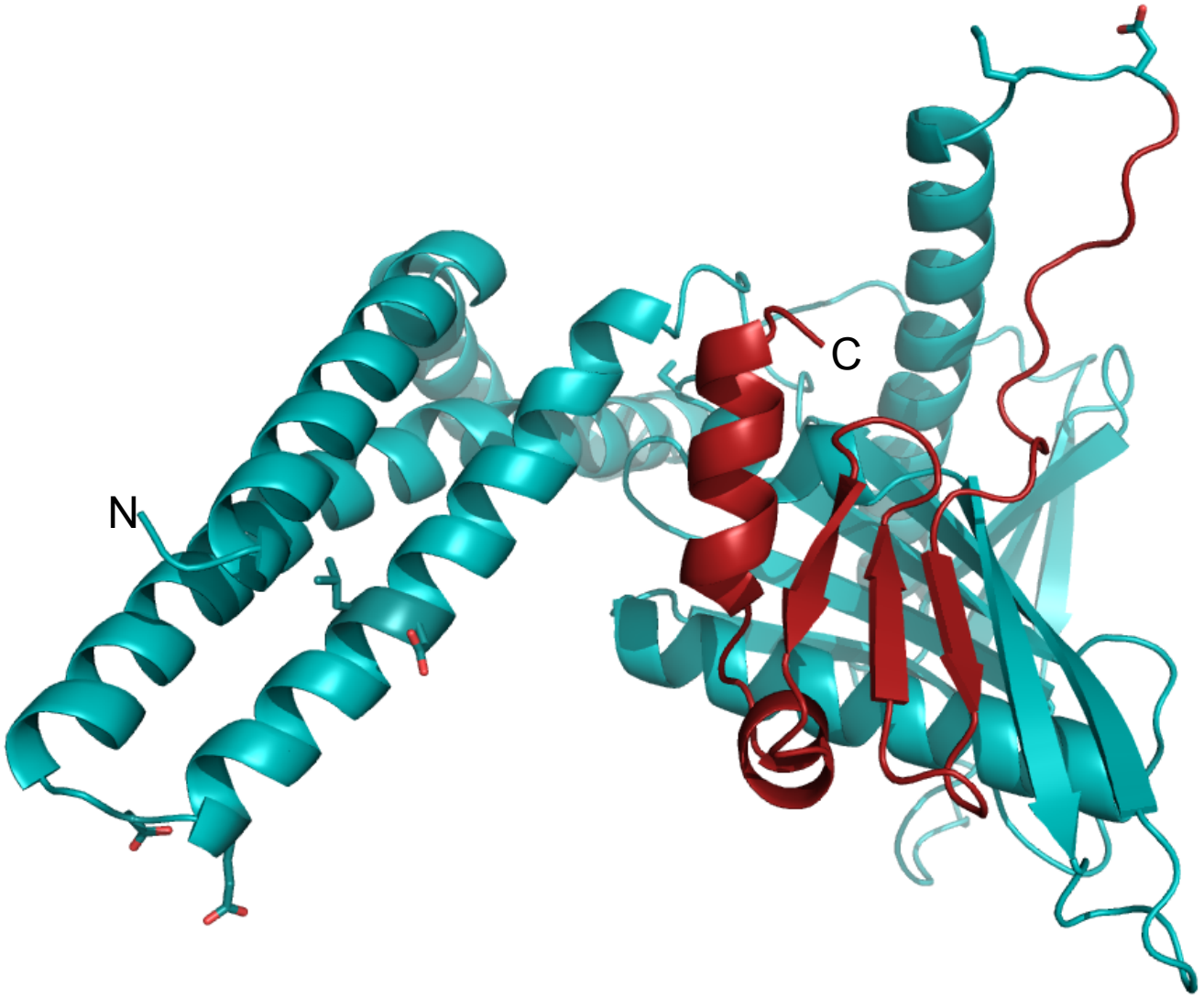
Cell Killing Assay



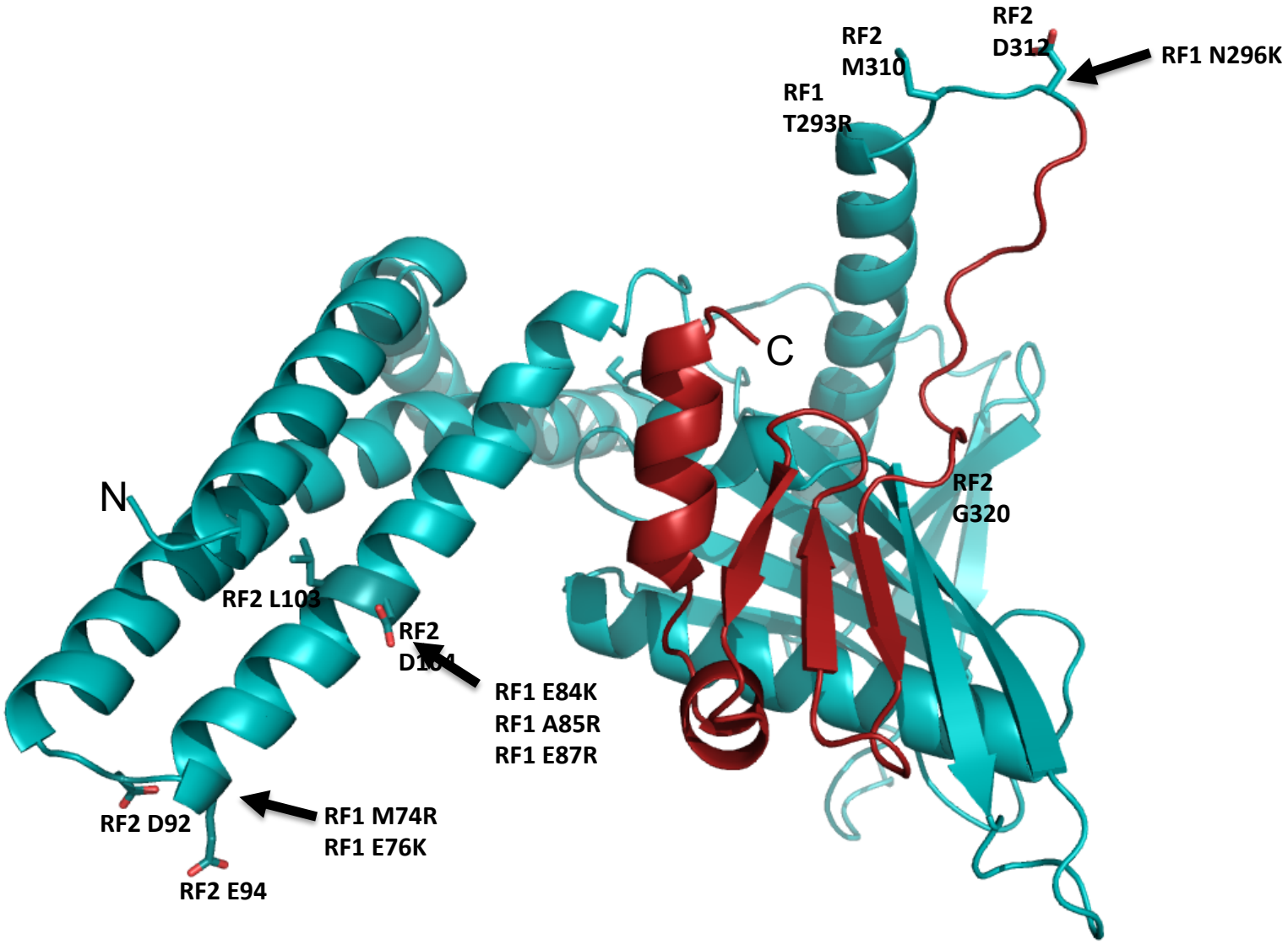
Translation of nnAA-Containing Proteins Enables Site-Specific Conjugation



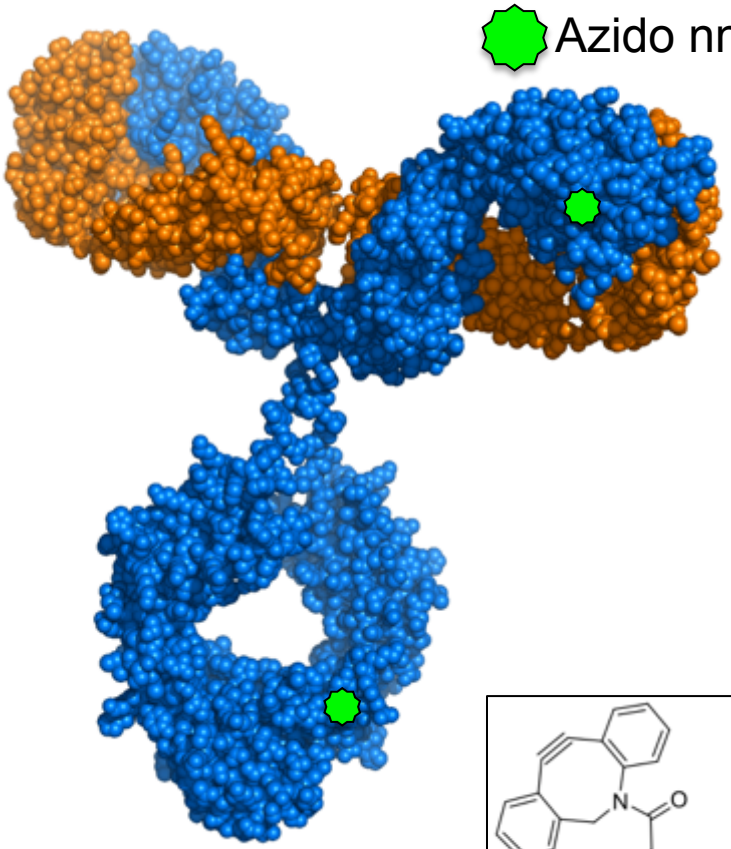
RF1



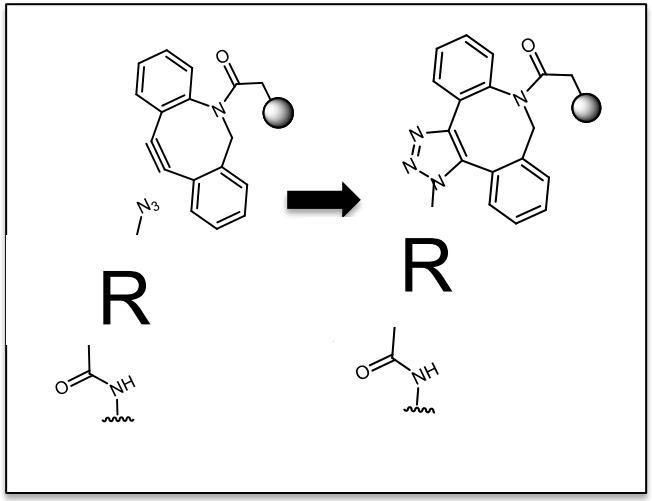
Design of OmpT-susceptible RF1

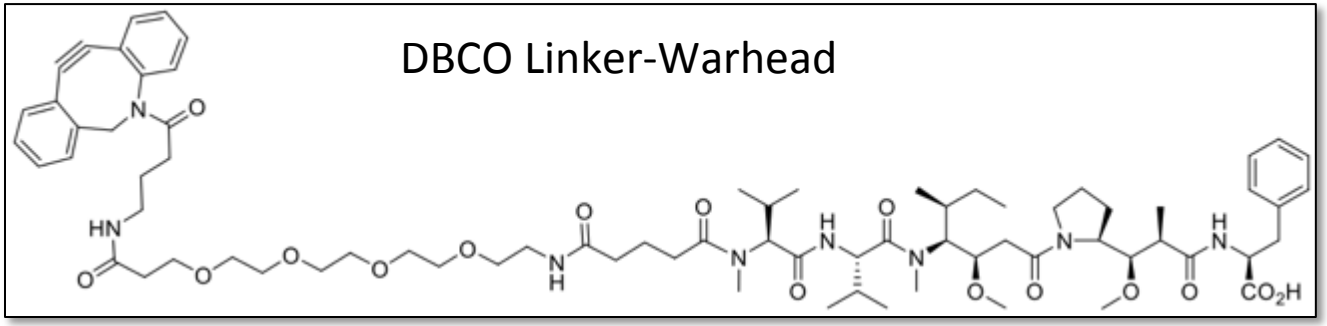


Conjugation Efficiency

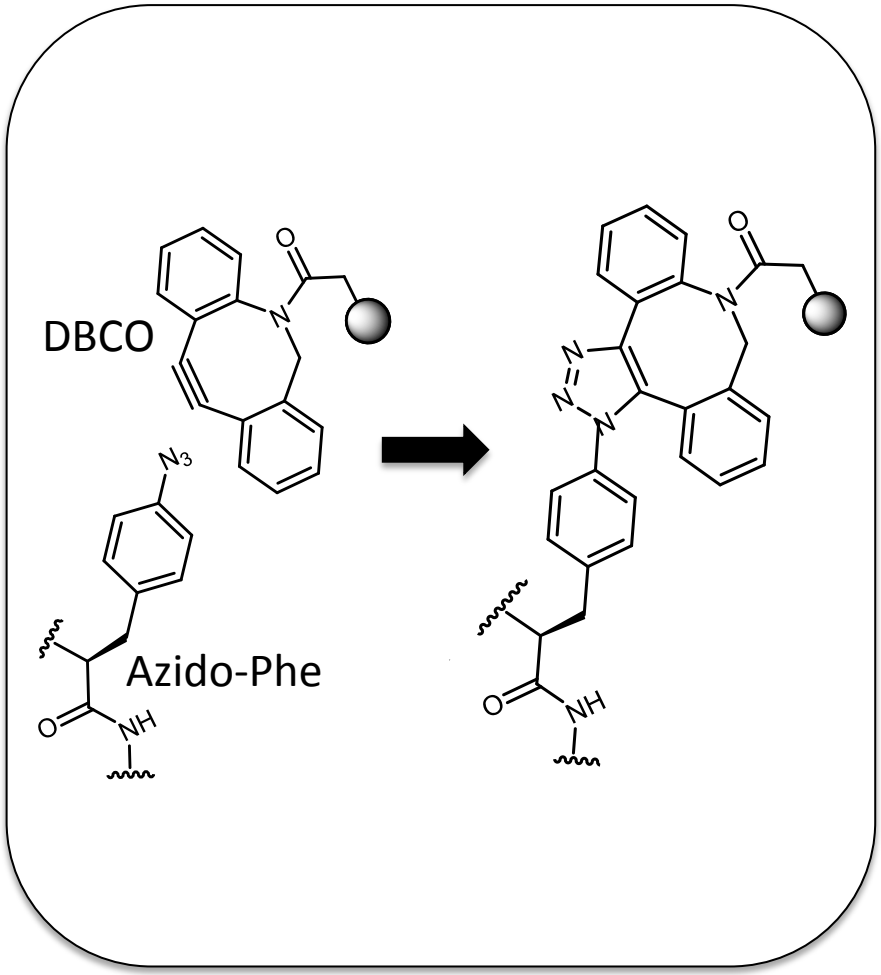


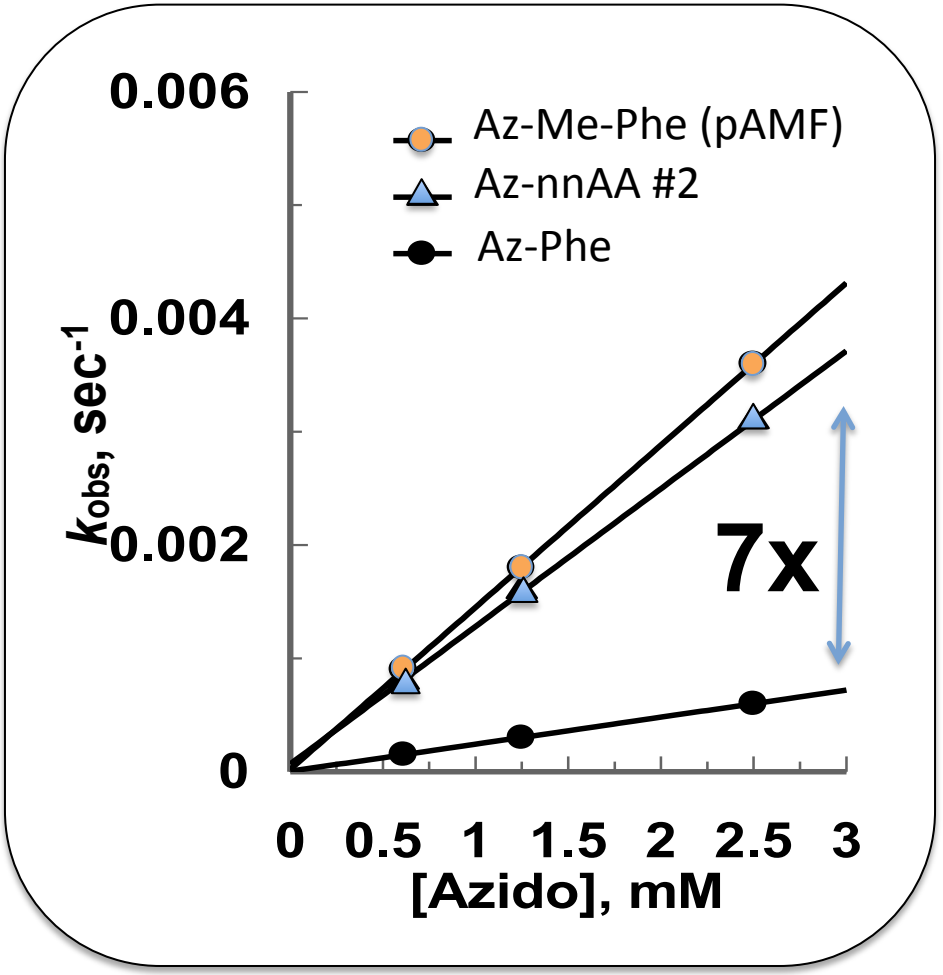
Cu Free Click
Conjugation Chemistry





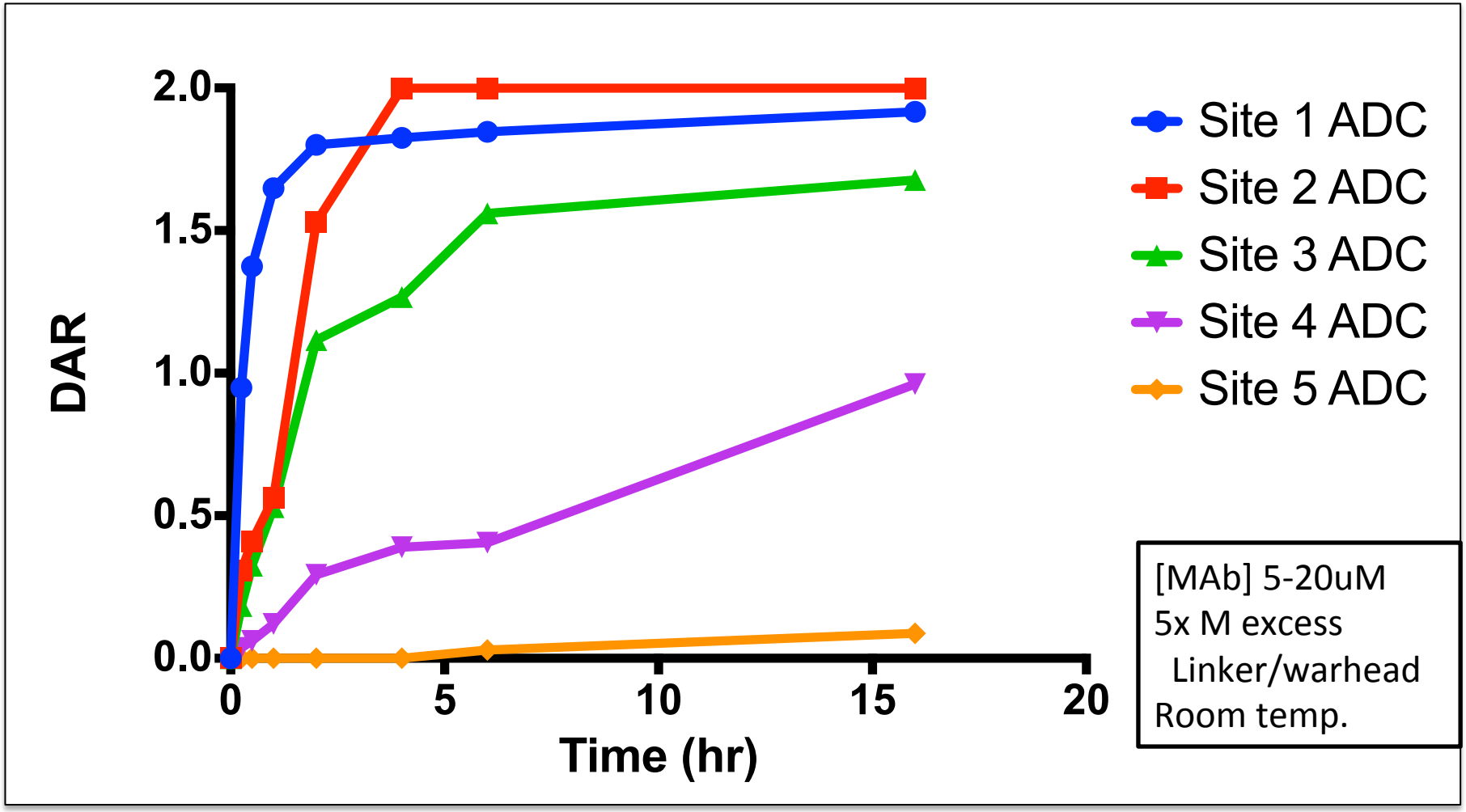
Novel Azido nnAAs with Boosted Conjugation Kinetics



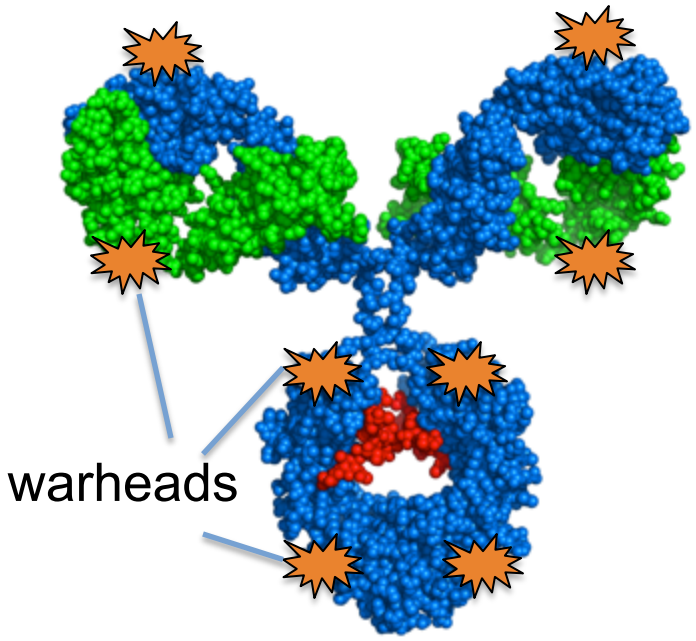


New Chemistry offers Improved Kinetics, Flexibility

Novel nnAAs with Boosted Conjugation Kinetics; Best Sites Completely Conjugated in Under 4 hrs



Multiple warhead payload; single species ADC



- Incorporation of multiple nnAAs in each IgG LC and HC
- Intractable using cell-based systems or wild-type extract due to significant accrued losses in yield
- Enables Multiple payloads (4,6,8,10+) of specifically positioned warheads/IgG
- Combinations of sites screened for:
  - nnAA Incorporation efficiency/expression
  - Conjugation Efficiency
  - Stability
  - PK/Potency in vivo

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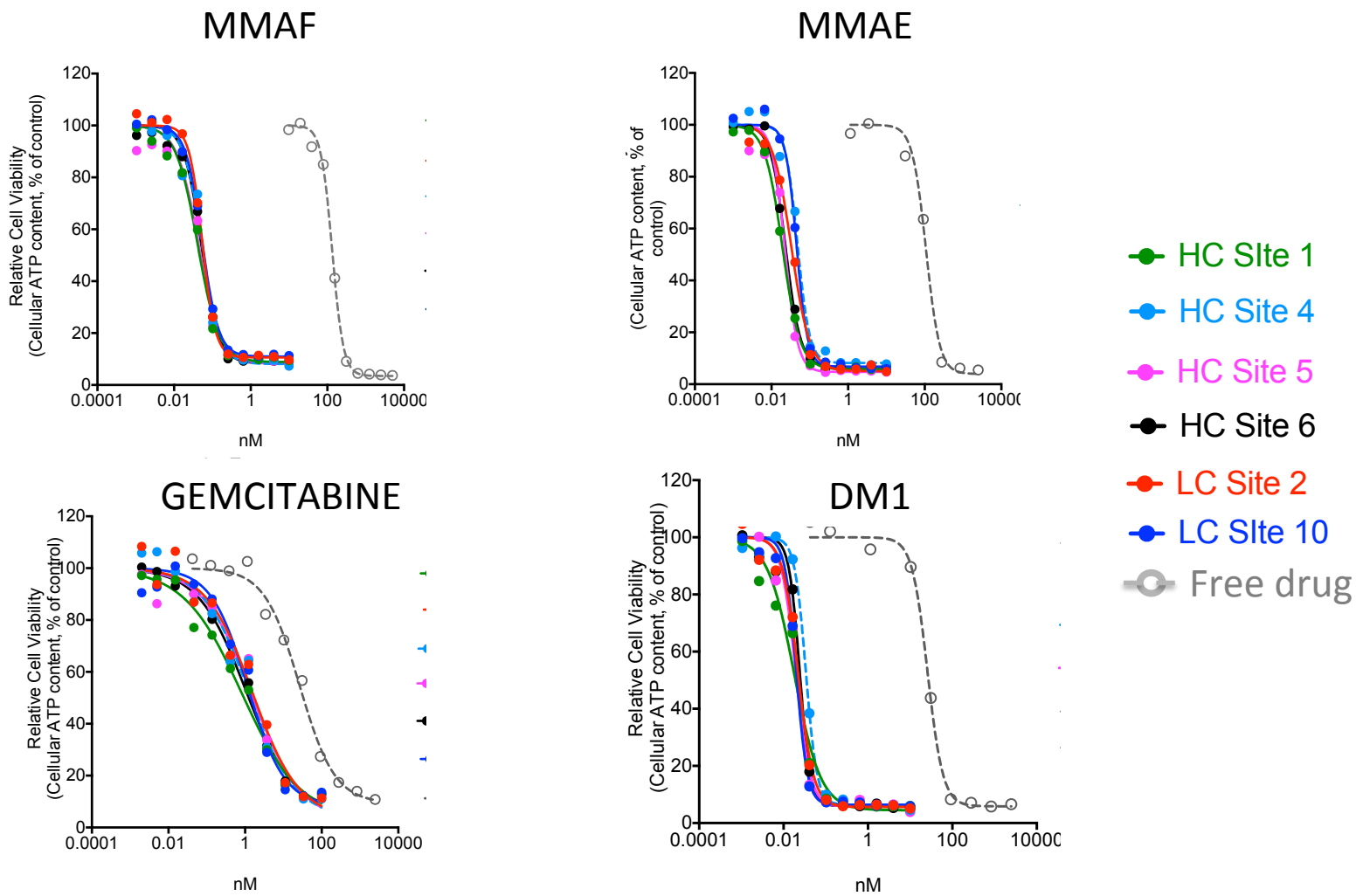
Learnings from ADC Specific Site Conjugation Variants

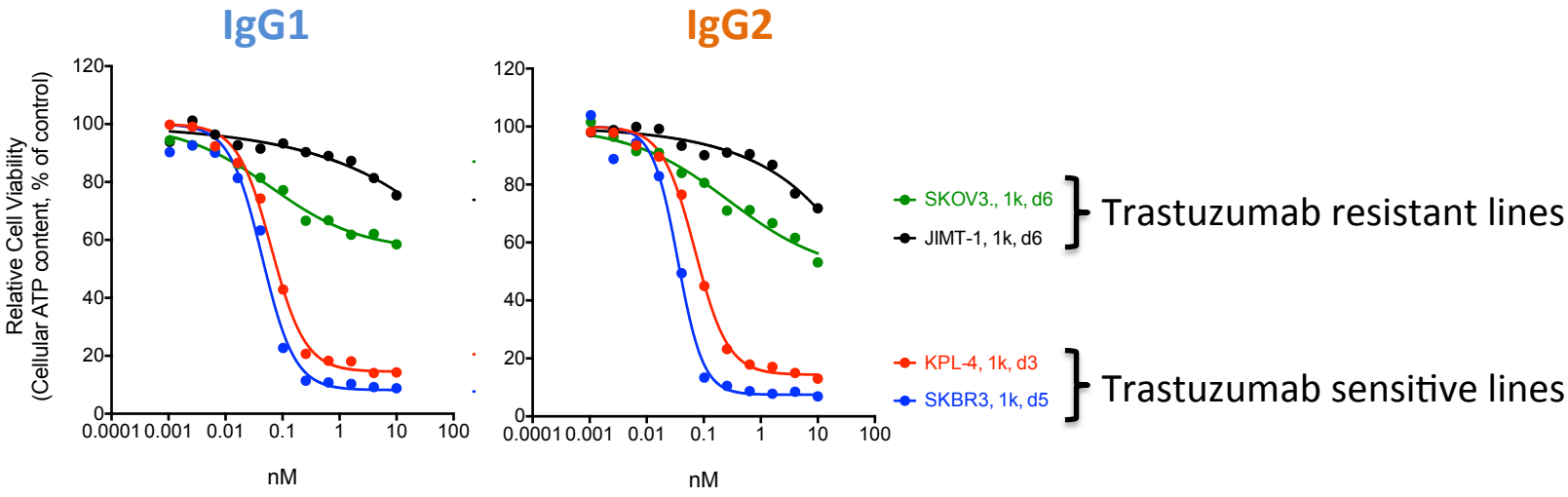
Site of Conjugation and Killing Activity With Different Cytotoxin Warheads

Trastuzumab-CF site specific ADC cell killing activity on SKBR3 cells

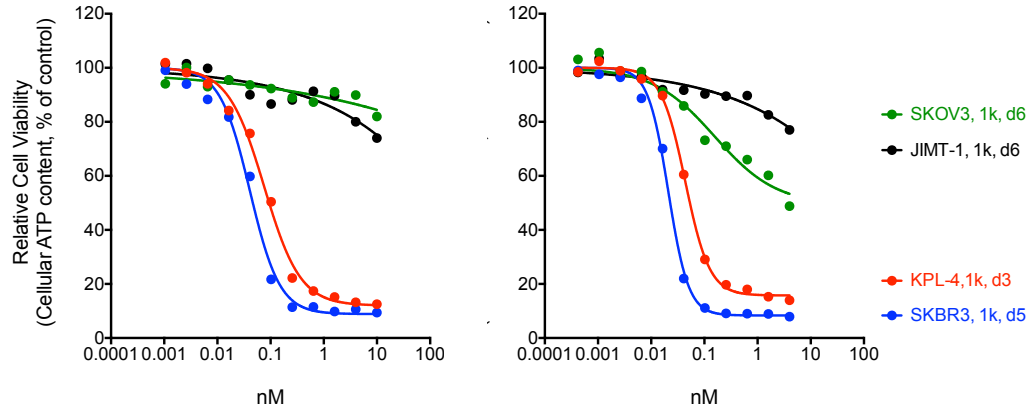


Trastuzumab-CF IgG1 and IgG2 Isotype Drug Conjugates Are Comparable

HC Site 5 ADC

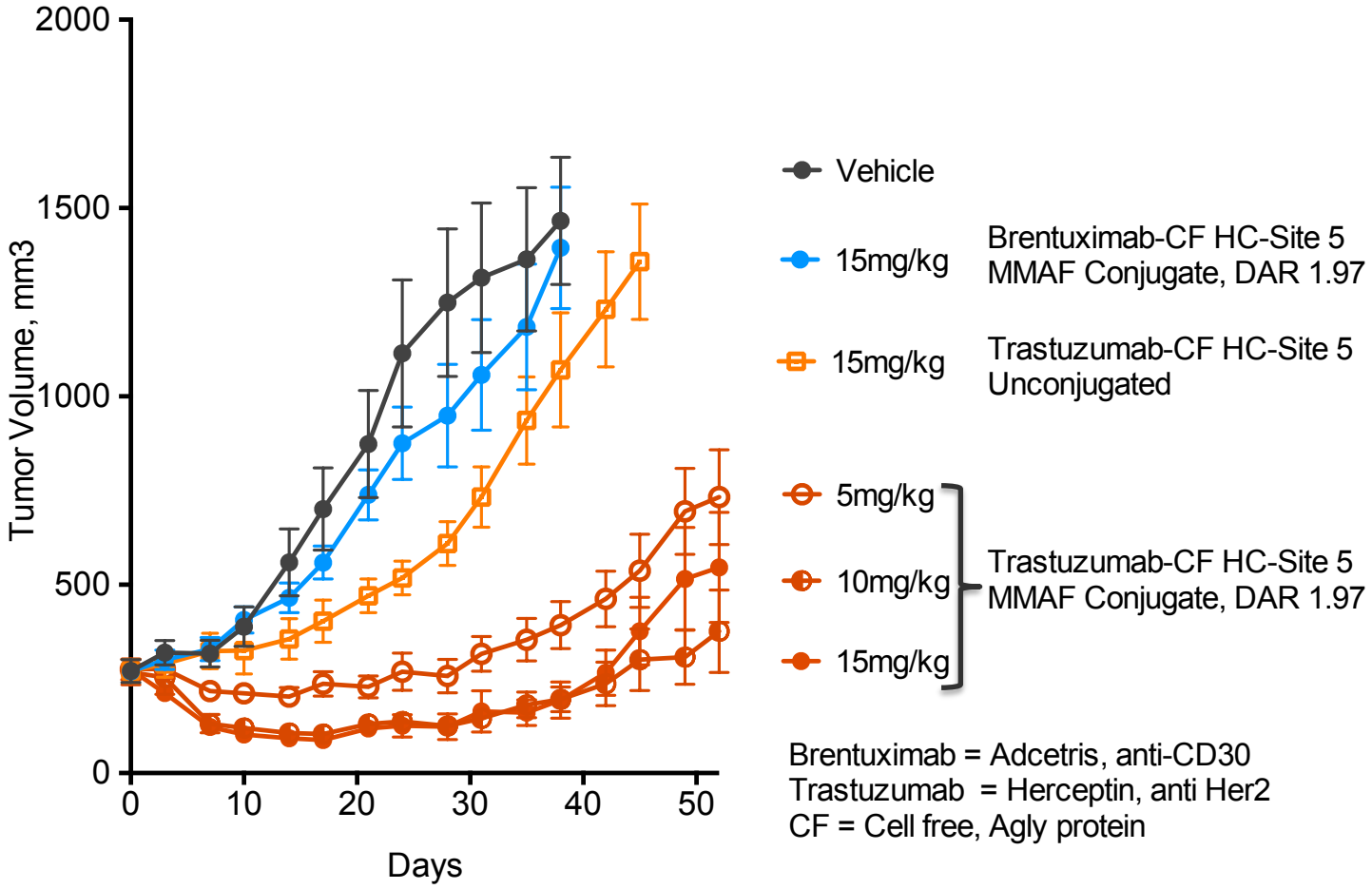


HC Site 1 ADC



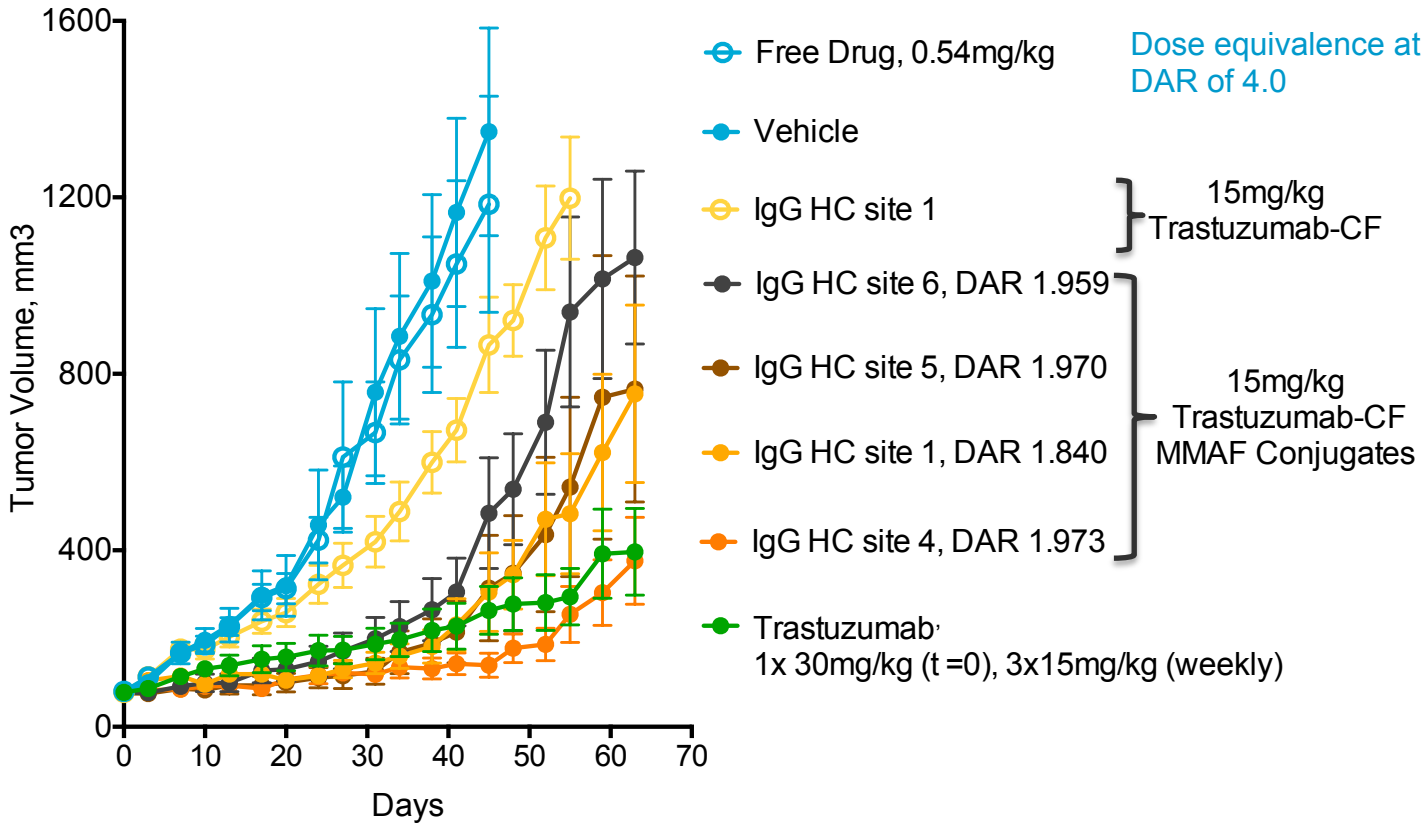
Trastuzumab-CF Single Site MMAF ADC	HC-Site	DAR	KPL-4 1k, d3	SKBR3 1k, d5
			IC50, nM	
IgG2	Site 1	1.5	0.11	0.053
	Site 5	1.9	0.071	0.035
IgG1	Site 1	1.5	0.064	0.04
	Site 5	1.9	0.076	0.04

Dose-dependent efficacy of a single conjugation-site homogeneous ADC



- n =10 each treatment group
- All treatments are single dose, i.v. @ t=0
- No significant weight loss observed in any treatment groups

Efficacy is Conjugation-Site Dependent



- n =10 each treatment group
- All treatments (except Trastuzumab) are single intra-venous dose @ t=0
- Trastuzumab multiple dose group dosed i.p
- No significant weight loss observed in any treatment group

Characterizing Multiple Site Specific MMAF ADC

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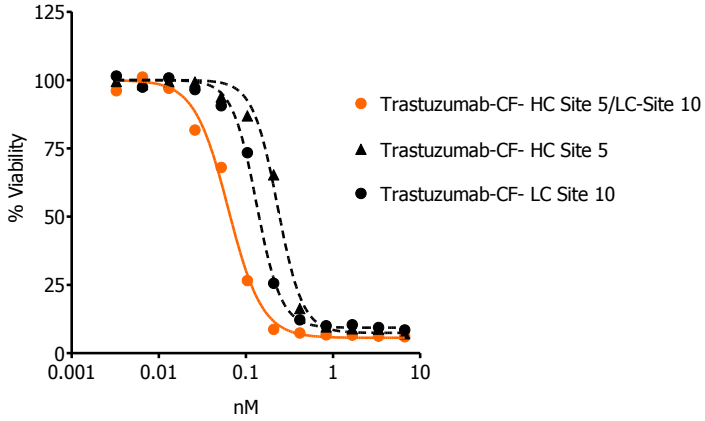
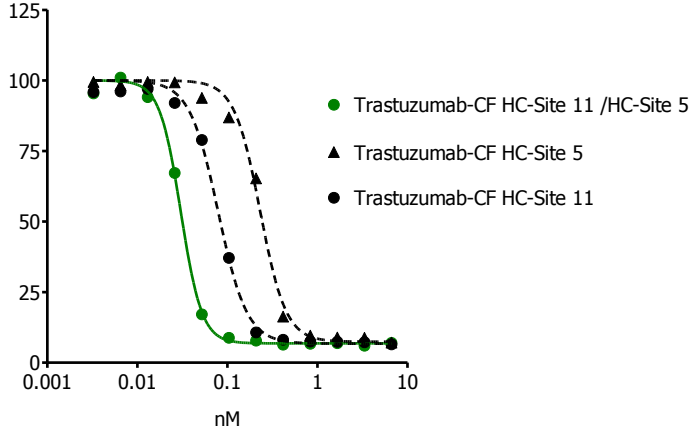
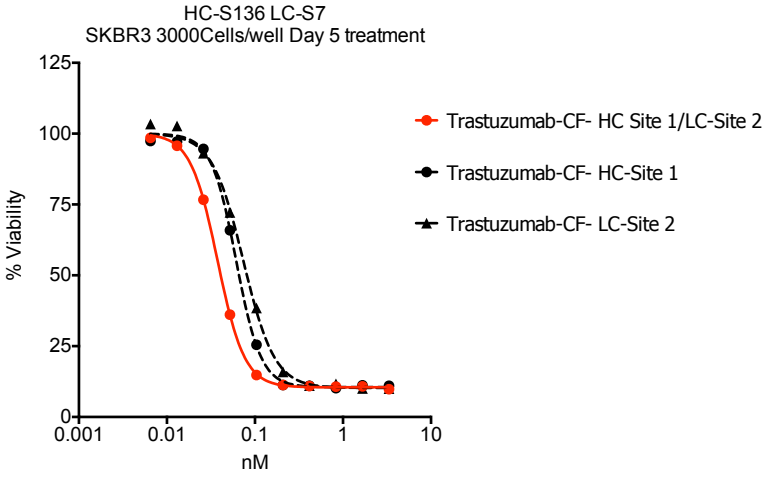
Multi-Site ADCs are more potent than single site ADCs

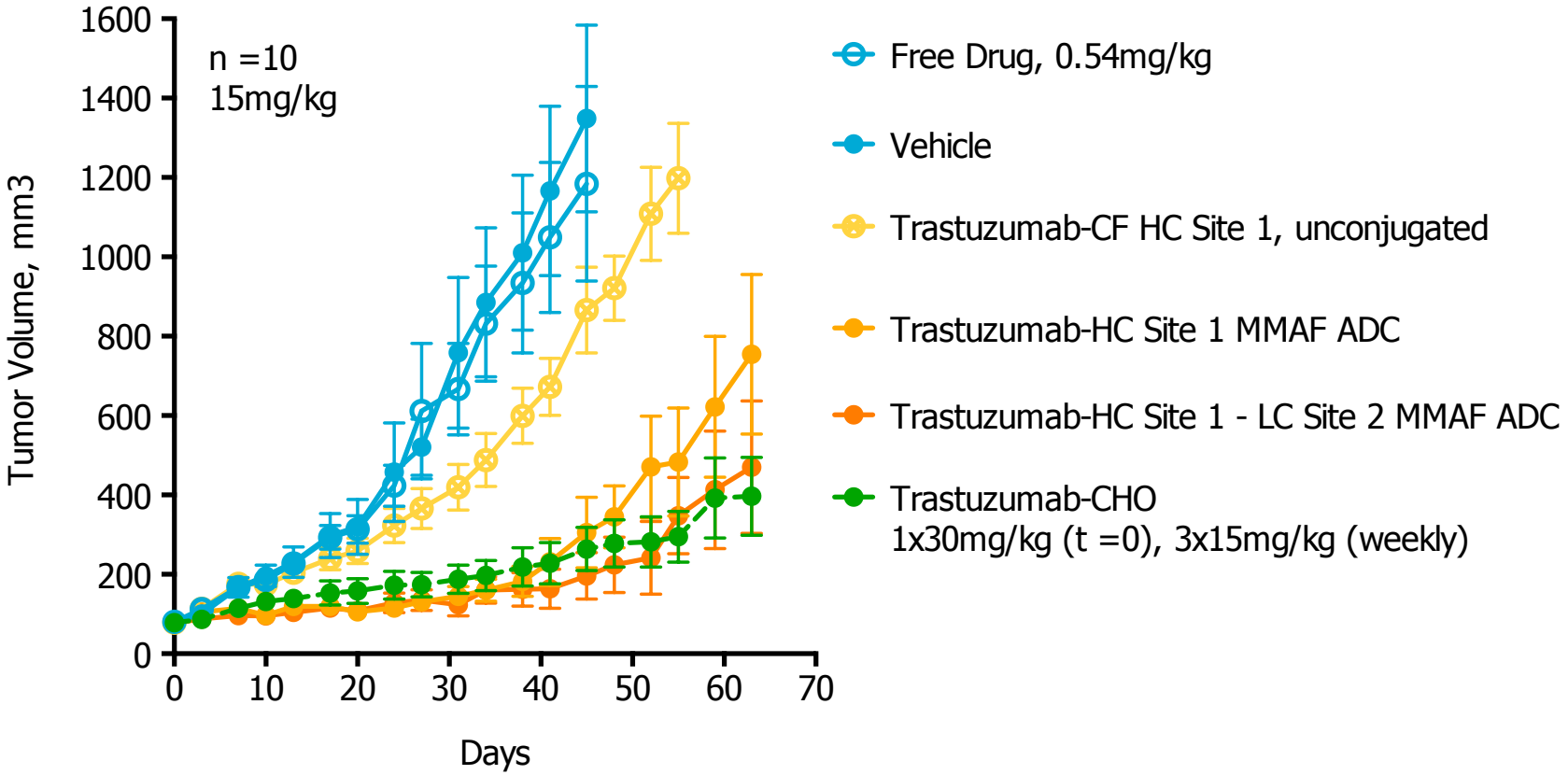






Trastuzumab-CF Single Site vs. Multi Site MMAF ADC	IC50, nM	DAR
HC - Site 5	0.23	1.8
LC - Site 10	0.13	1.5
HC - Site 5 - LC Site 10	0.06	4.0
HC - Site 5	0.23	1.8
HC - Site 11	0.08	1.4
HC - Site 5 - HC Site 11	0.03	3.8
HC-Site 1	0.06	1.9
LC-Site 2	0.07	1.5
HC Site 1 - LC Site 2	0.03	3.9

Multiple Site ADC is more Efficacious than Single Site ADC at Equivalent Dose



- KPL-4 Orthotopic breast cancer model
- ADC and Control, Single dose, i.v.
- Trastuzumab-CHO, multiple dose, i.p.

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Site Transfer across Scaffolds: IgG1 vs. scFv-Fc

Site Specific ADC: IgG1 and scFv-Fc Comparison *In vitro* Cell Binding and Killing

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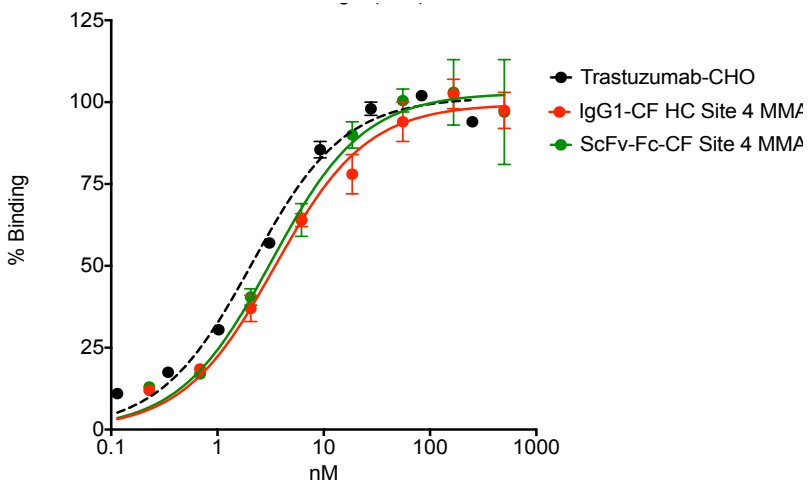
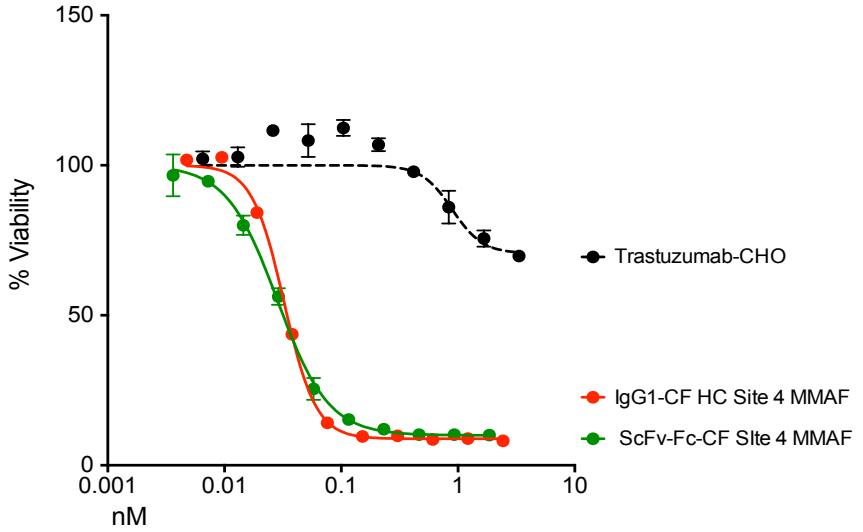
Cell Binding



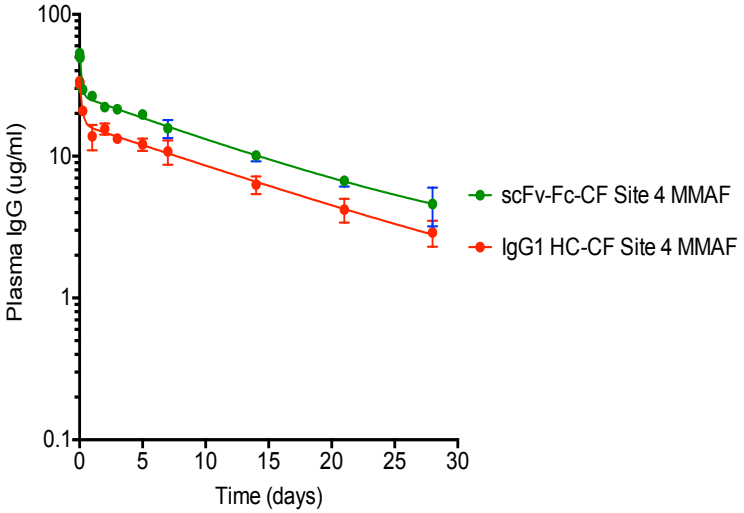
Cell Killing



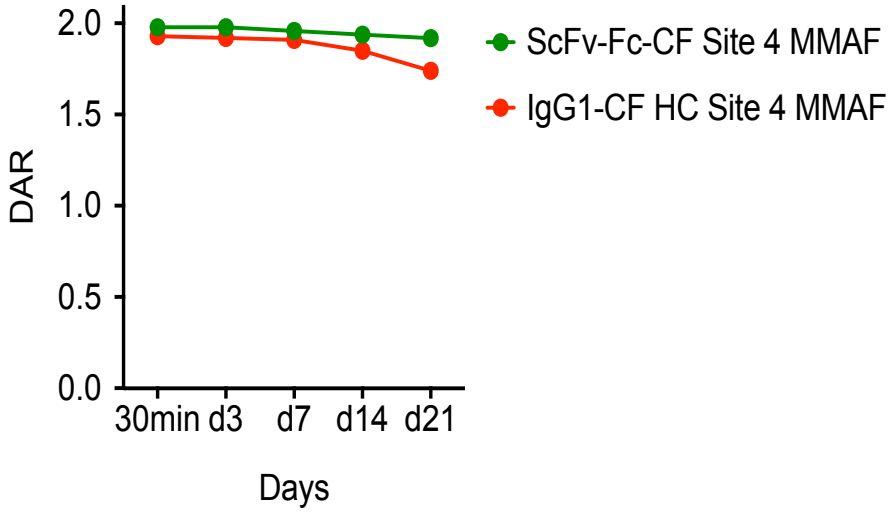
Site 4 MMAF ADC Scaffold	DAR	Cell Killing IC50, nM	Cell Binding Kd, nM
IgG1 HC-CF	2.0	0.034	2.3
ScFv-Fc-CF	2.0	0.06	5.0
Trastuzumab-CHO	NA	NA	2.0

Site Specific ADC: IgG1 and scFv-Fc Comparison PK and conjugate stability *in vivo*

PK Profile

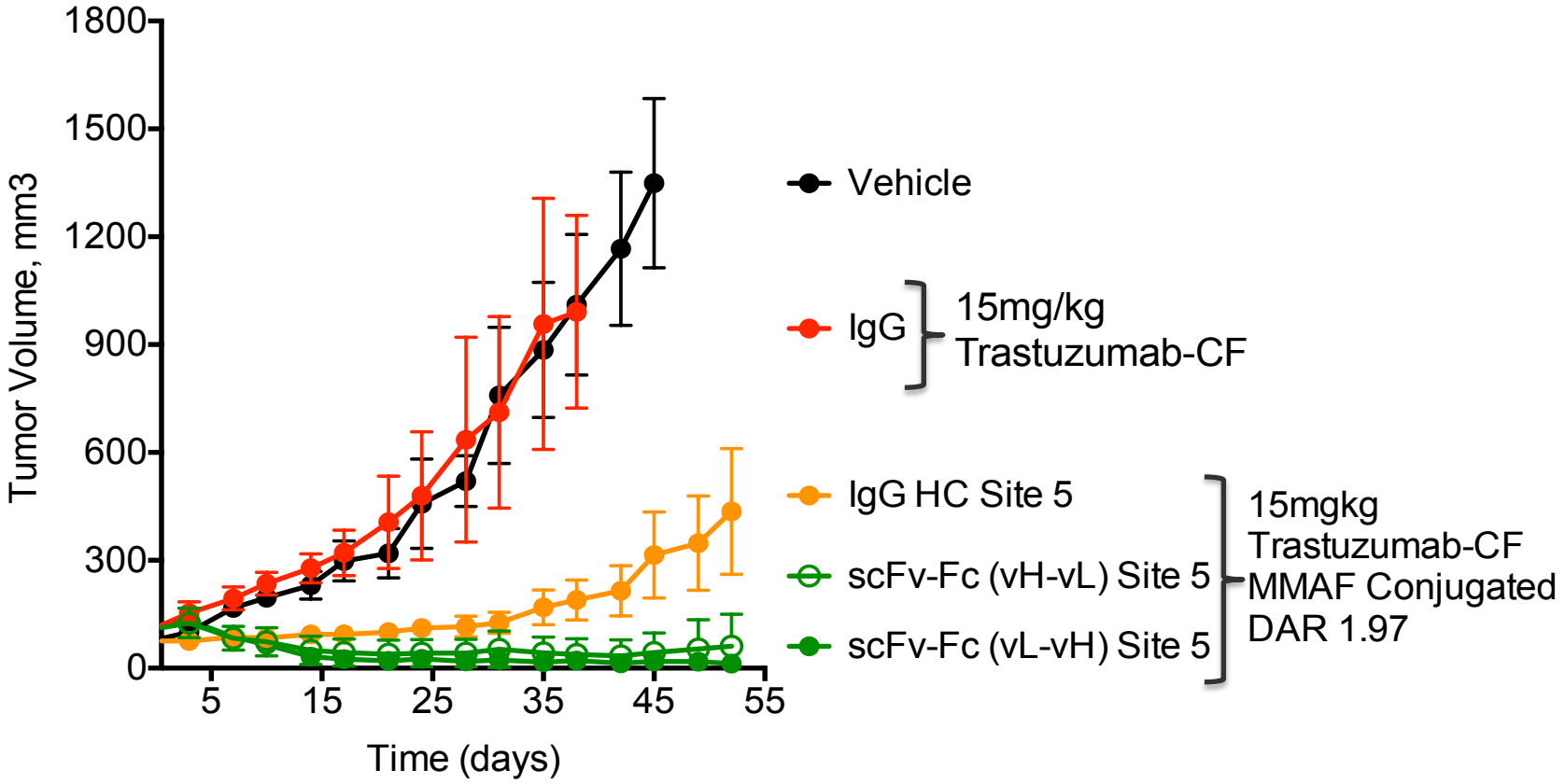


In vivo Stability of ADC



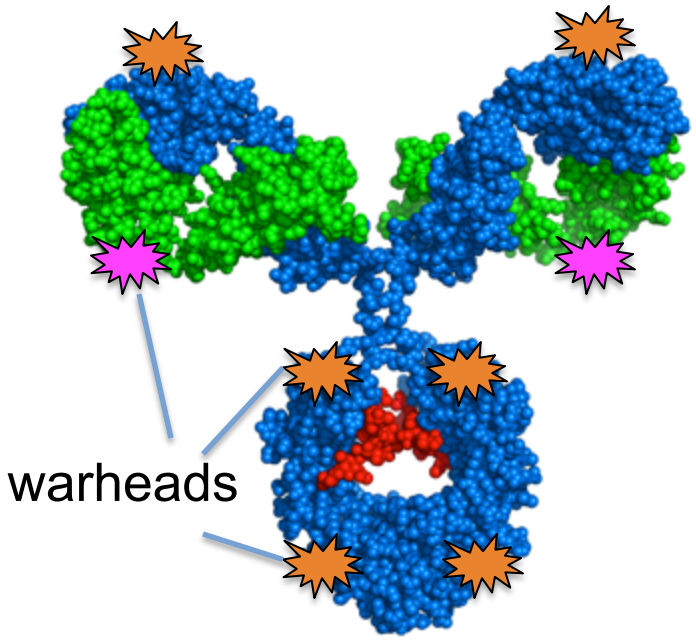
Site 4 MMAF ADC Scaffold	Terminal $t_{1/2}$ (day)	Cl (mL/day/kg)
IgG1 HC-CF	11.2	7.6
ScFv-Fc-CF	11.9	6.2

scFv-Fc Drug Conjugate Scaffolds are very effective



- n =10 each treatment group
- All treatments are single dose, i.v. @ t=0
- No significant weight loss observed in any treatment groups

Combination Warheads; single species ADC



- Incorporation of two different nnAAs in multiple copy number in IgG LC and HC
- Enables Multiple payloads (4,6,8,10+) of two different specifically positioned warheads/ IgG
- Combinations of sites screened for:
  - nnAA Incorporation efficiency/expression
  - Conjugation Efficiency
  - Stability
  - PK/Potency in vivo

Toward a dual nnAA incorporation system

The goal:

A system to incorporate 2 distinct nnAA's with mutually orthogonal chemistries (azide and tetrazine) into a single protein in a single CF reaction

The components*:

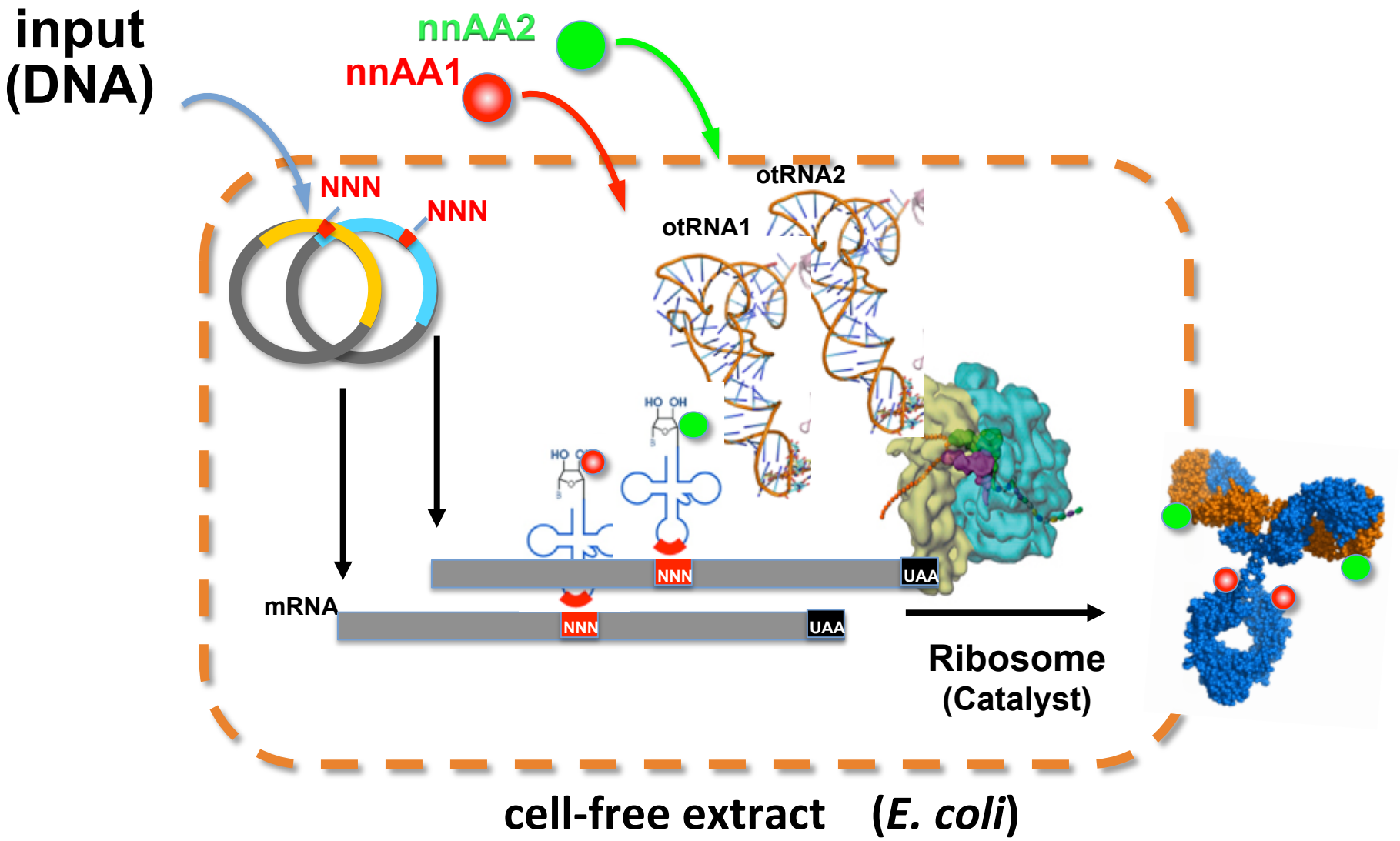
2 synthetases

2 nnAAs (azide and tetrazine)

2 tRNAs (can decode TAG, TAA, TGA, or 4-base codon)

*must be orthogonal to each other and to *E.coli*

Combination Warheads: Development of Dual Payload ADCs



Discovery of novel synthetases at Sutro

- 2000 synthetase variants from a rationally-designed library are expressed in individual CF reactions.
- Synthetase from aaRS expression reaction is added to GFP (K49TAG) reporter cell free reactions +/- nnAA.
- Desired synthetases will mediate amber suppression and GFP fluorescence in the presence but not absence of nnAA.

Rationally-designed MjYRS library

Library Positions

Tyr32 = Ala, Val, Leu, Thr

Leu65 = Ala, Leu, Ile, Val

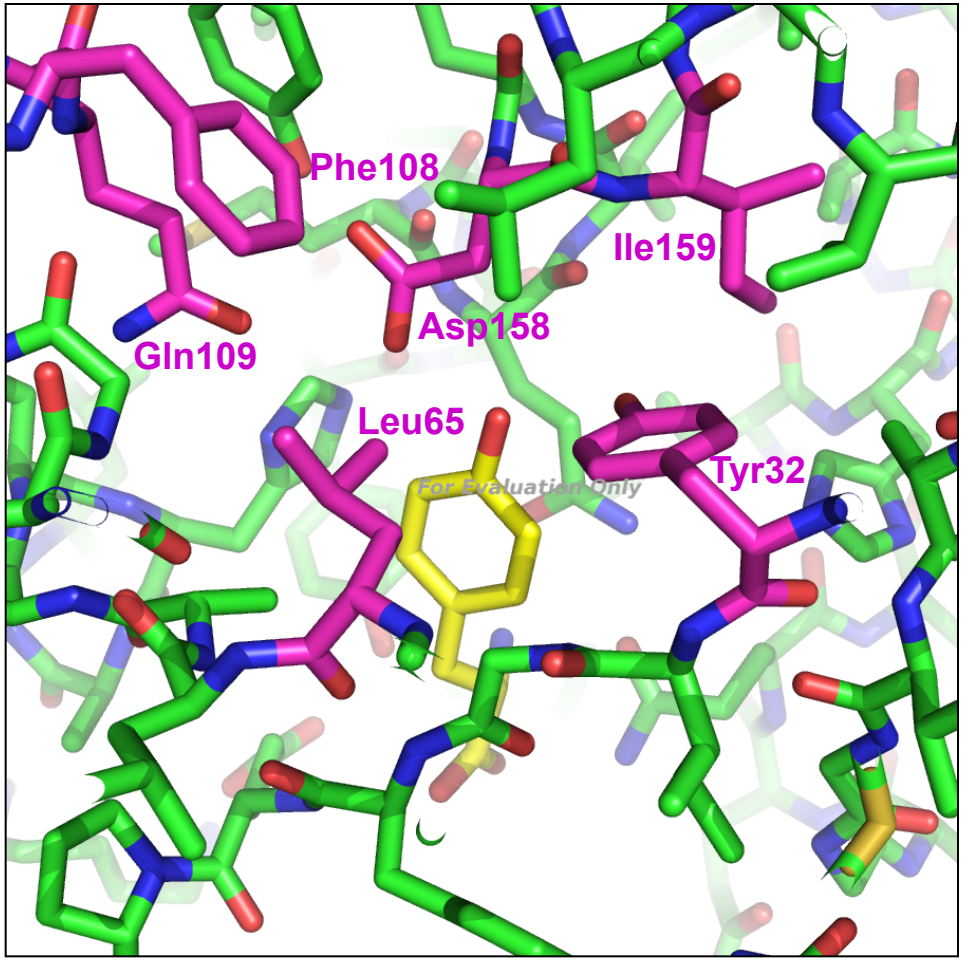
Phe108 = Phe, Tyr, Trp

Gln109 = Met, Leu, Ile, Gln

Asp158 = Ala, Gly

Ile159 = Ala, Gly, Val, Ser

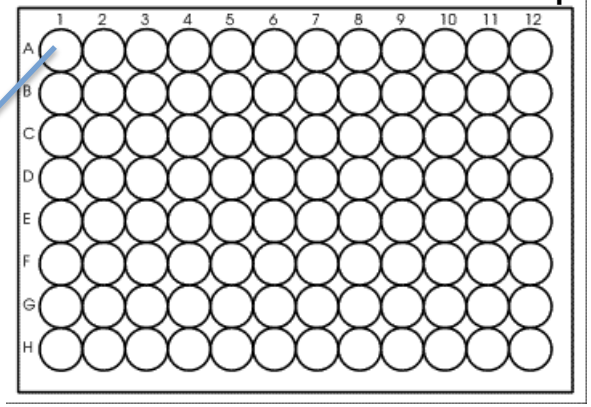
4x4x3x4x2x4 = 1536 unique variants



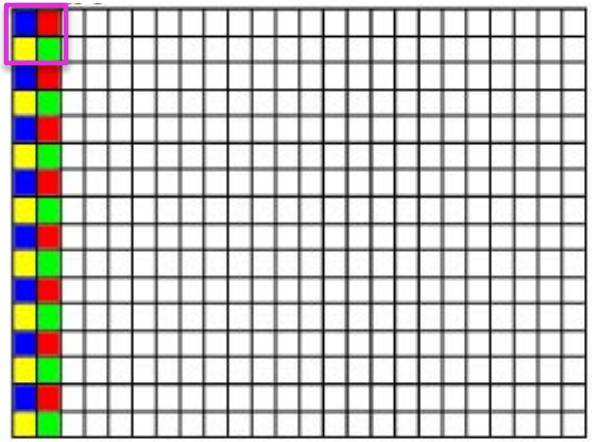
Testing ~2000 clones against 3 different nnAA's

Each aaRS variant can be tested under 4 conditions

CF1: 100ul 96W aaRS Expression



CF2: 20ul 384W GFP TAG Assay



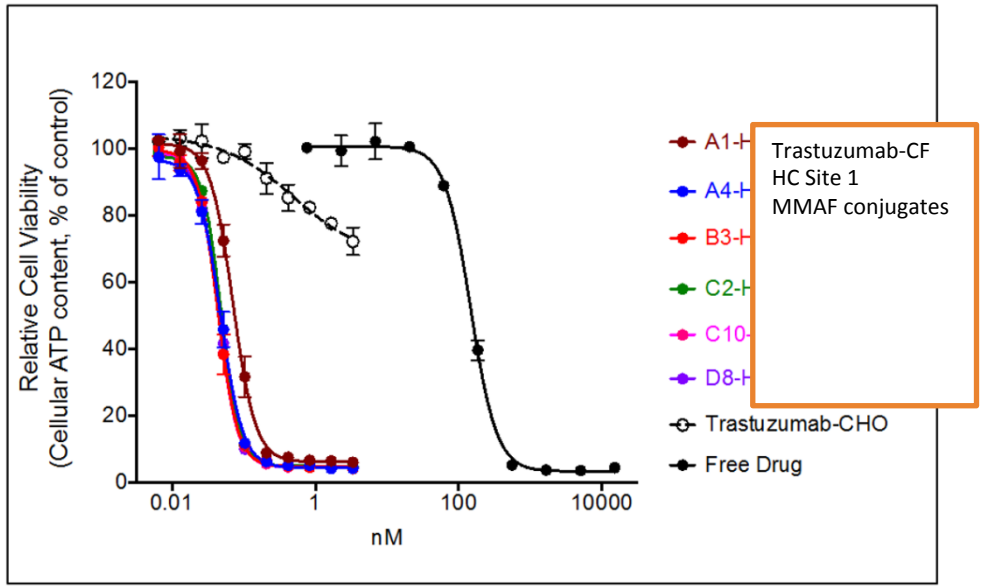
- No nnAA
- nnAA #1
- nnAA #2
- nnAA #3

Identification of a high fidelity, proprietary pAMFRS

A

pAMFRS variant	32	65	108	109	158	159	IgG HC 136-AB3627 DAR	EC50 (nM)
A01	T	A	Y	L	A	S	1.2	0.071
A04	V	A	W	M	A	G	1.5	0.047
B03	A	V	W	Q	A	G	1.9	0.043
C10	V	V	Y	Q	A	V	1.6	0.048
D08	T	V	W	Q	A	S	1.8	0.044
C02	V	V	W	I	A	S	1.5	0.044

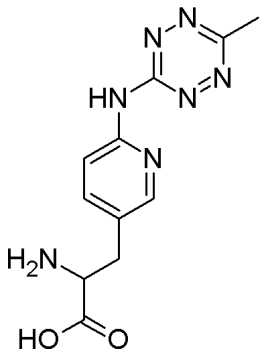
B



- pAMFRS variant B3 produces potent ADCs with DAR of 1.9
- Fidelity of pAMF incorporation >99.8% by peptide LC-MS/MS
- Novel amino acid sequence

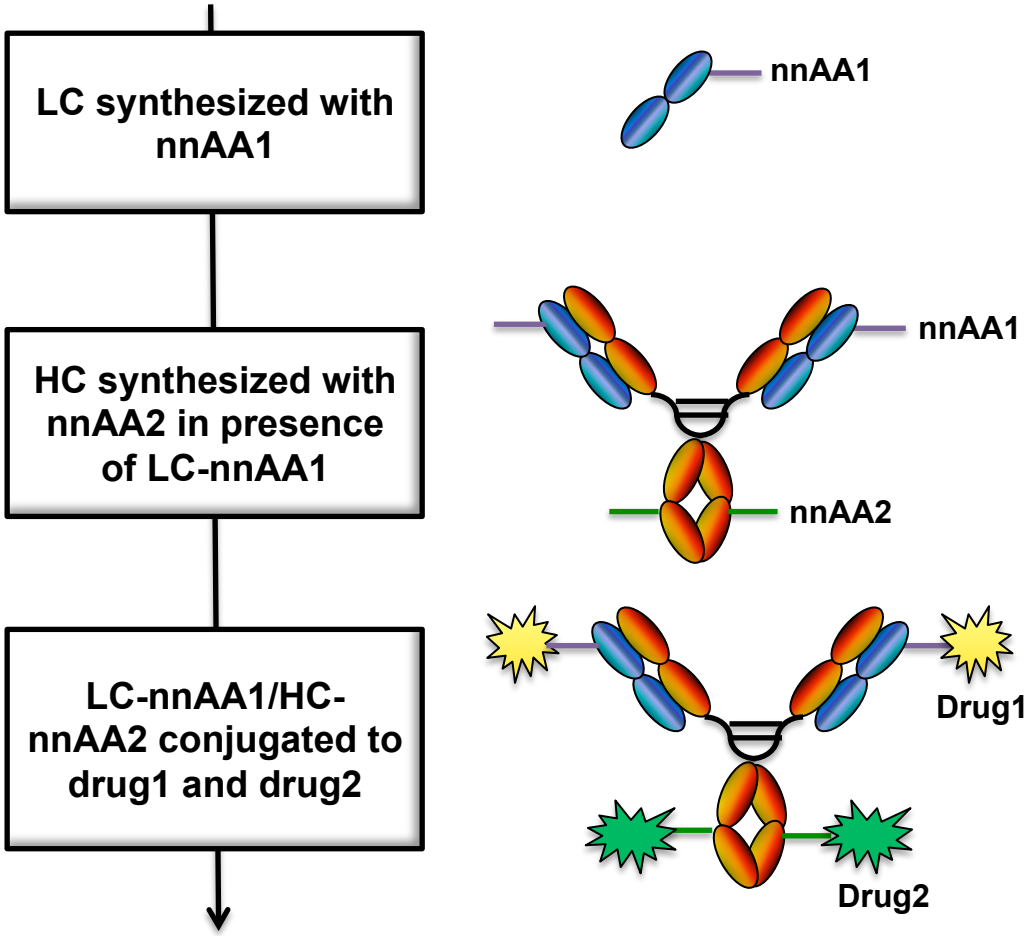
Tetrazine nnAA's to enable a second conjugation chemistry

- Tetrazine moiety enables retro Diels-Alder conjugation
- 30x faster than copper-free click, complete conjugation in minutes
- Compatible with and orthogonal to azide-click chemistry
- Pyridyl-derivative nnAAs are more soluble than phenylalanine derivatives

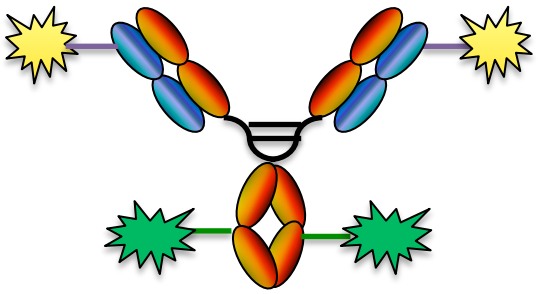


nnAA2

Dual Warhead ADC Concept



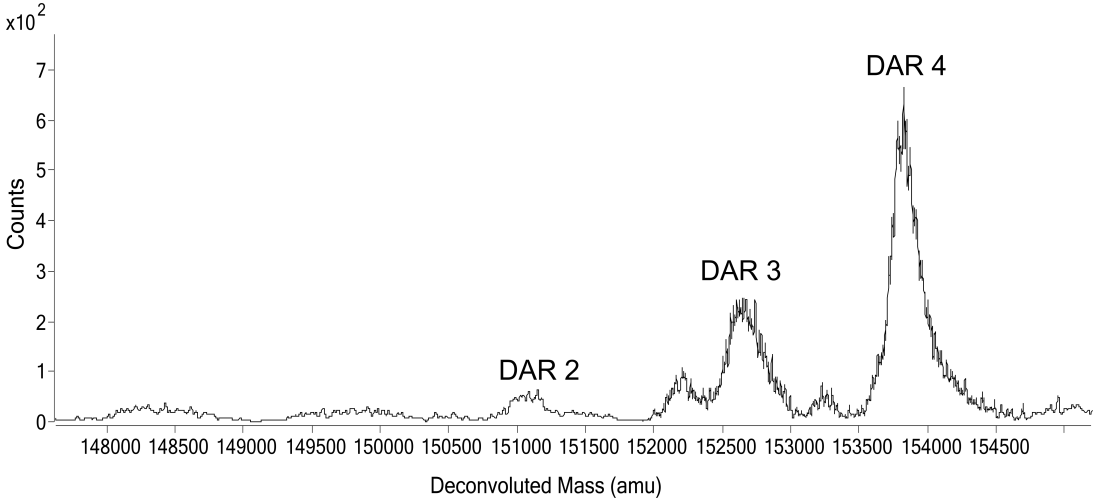
DAR Analysis of Combination Warheads



Drug1 on HC	Drug1 DAR	Drug2 on LC	Drug2 DAR	Total DAR
MMAF	1.8	SN-38	1.4	3.2
MMAF	1.8	PBD dimer	1.5	3.4
MMAF	1.9	Gemcitabine	1.6	3.6
PBD dimer	**1.9**	**MMAF**	**1.7**	**3.7**
Gemcitabine	2.0	MMAF	1.6	3.5

PBD Dimer/
MMAF Dual Warhead ADC

DAR=3.7



Homogeneous, Multispecific Antibody Drug Combination Conjugates

- Multiple warheads
  - to target synergistic mechanisms to improve safety
  - to simultaneously target potential resistance pathways
- Multiple epitope targeting
  - to increase internalization rate of an antigen
- Multiple antigen targeting
  - to increase apparent affinity to less densely expressed antigens
  - to give better specificity over normal healthy tissues
  - to address antigen expression heterogeneity of tumor