Rapid Design and Manufacture of Single Species, Site-Specific ADCs using Cell-Free Synthesis with nnAAs

From Concept to Clinic in 365 days ?

Trevor J. Hallam, PhD
Chief Scientific Officer

3rd World ADC Summit,
San Francisco
October 24th, 2012

ADCs – Exciting prospects but . . .

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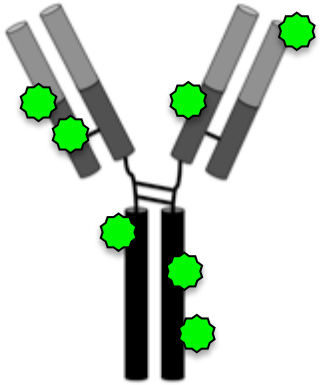
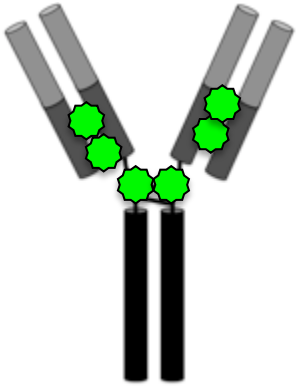
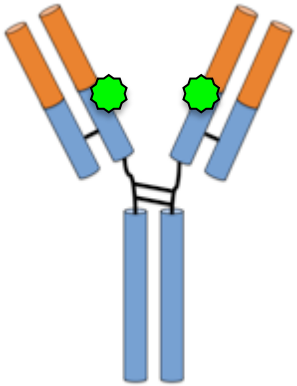


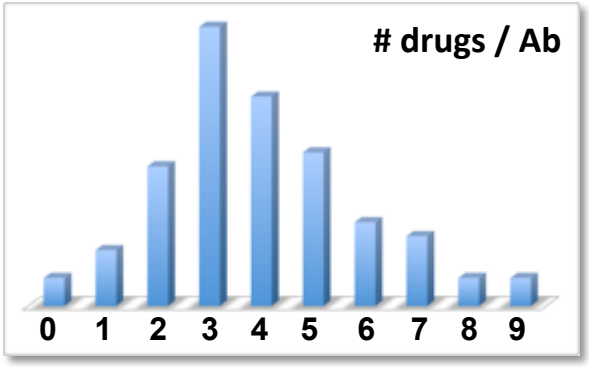


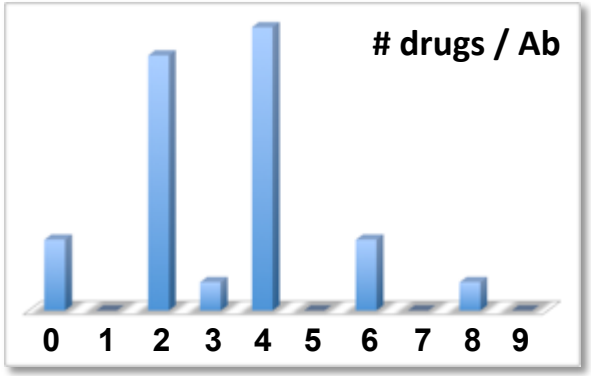


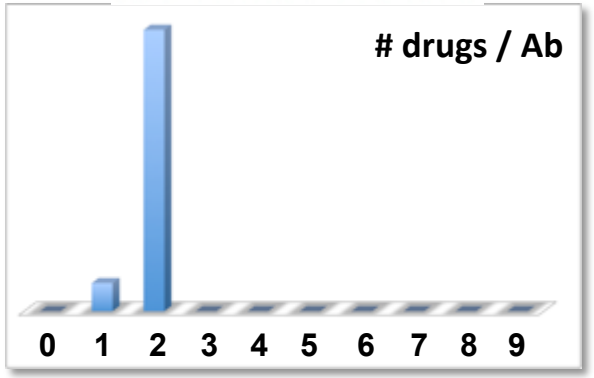
IMMUNOGEN, INC.

SeattleGenetics

Genentech
IN BUSINESS FOR LIFE







Random Lysine Conjugation

Cysteine Conjugation

Site Specific Cys Conjugation

Heterogeneity translates to poor PK, stability and efficacy

Production of Superior ADCs Using Non-Natural AA Incorporation

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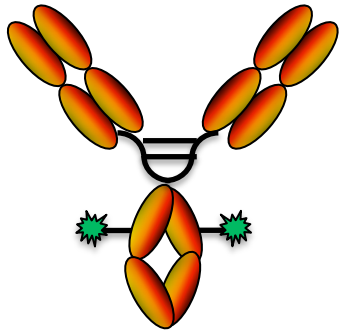
The Non-Natural Amino Acid Advantage

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



Anatomy of a Disruptive Protein Design and Manufacturing Solution…..

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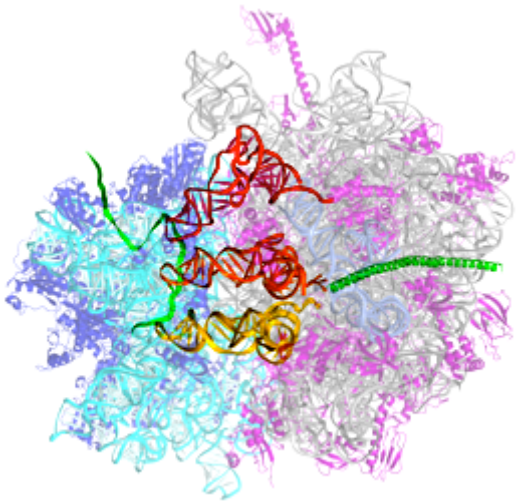
- Rapid Make-Test
  - Fast protein production (from DNA to g's/L in hours)
  - a 1-week cycle time for purification, characterization and testing
  - Micro-titre plate evaluation of 100's of variants in parallel
  - Parallel evaluation of optimal production conditions
- Chemical Diversity
  - Incorporation of non-natural amino acids
- Rapid Scale-up
  - Linearly scalable and predictable expression allows for seamless and rapid progression to specificity testing, efficacy models and GLP tox studies
- Same system for cGMP manufacture

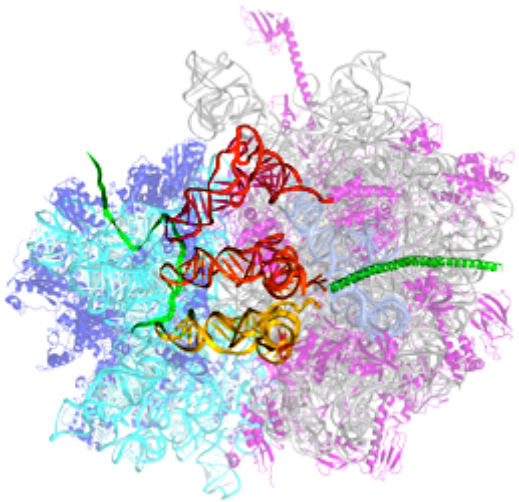
A Cell-Free Synthetic Biology Platform



Ribosome
(Catalyst)

(***E. coli***)

A Cell-Free Synthetic Biology Platform



Ribosome
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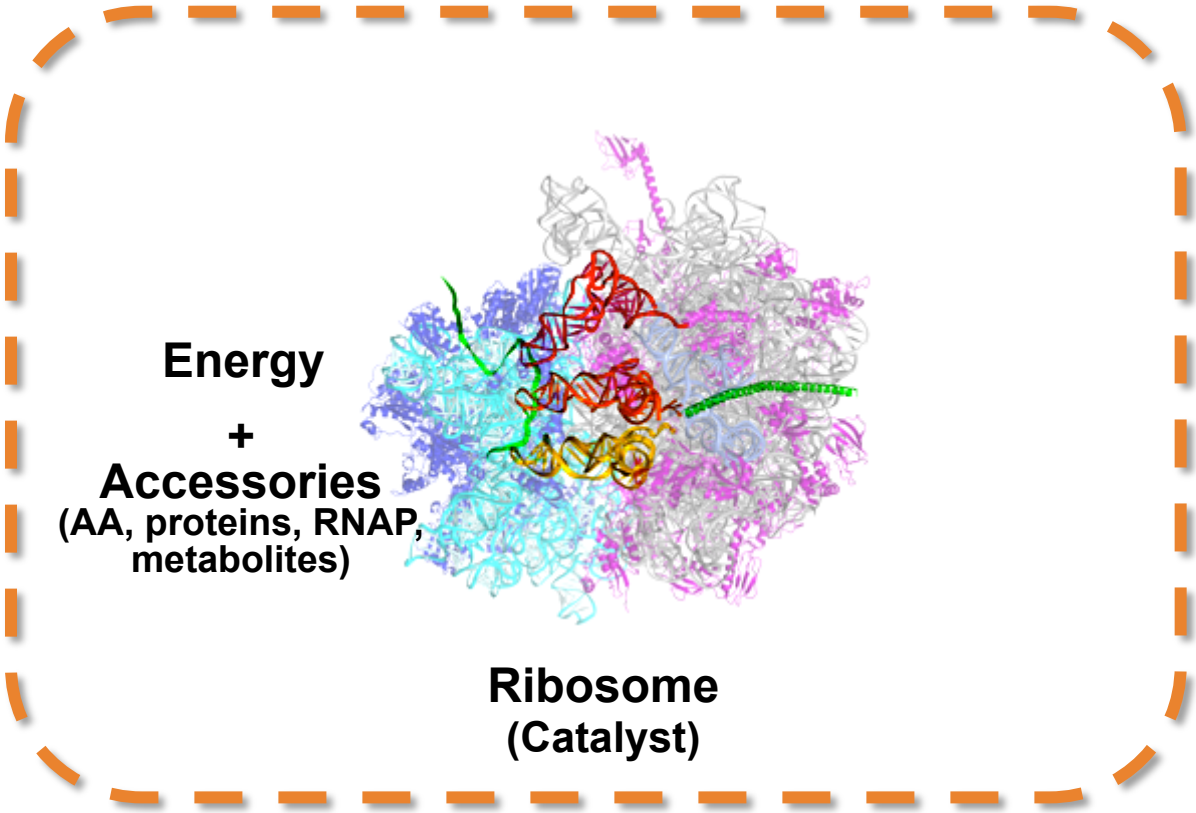
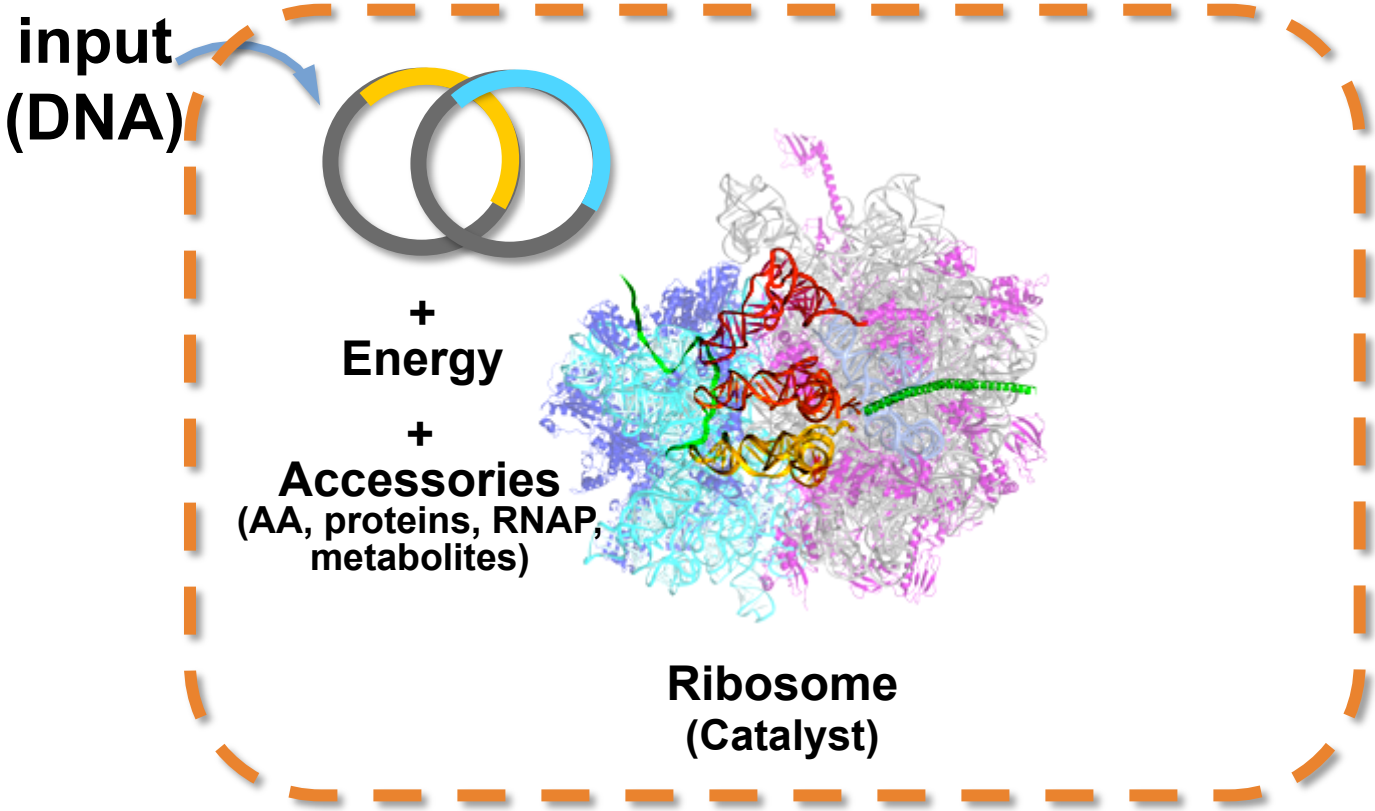


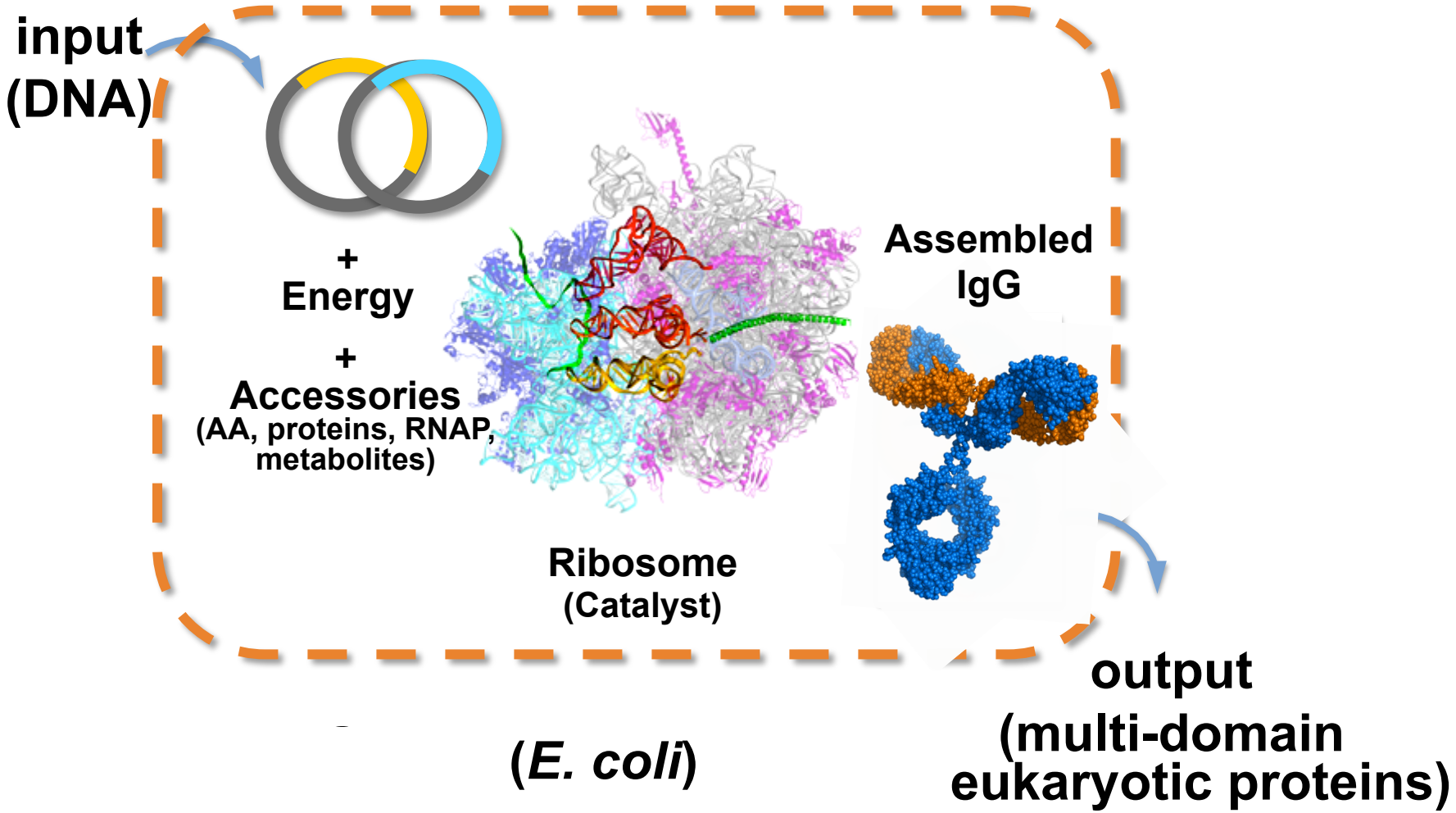
(*E. coli*)

A Cell-Free Synthetic Biology Platform



(*E. coli*)

A Cell-Free Synthetic Biology Platform



How versatile is cell free (CF) synthesis?

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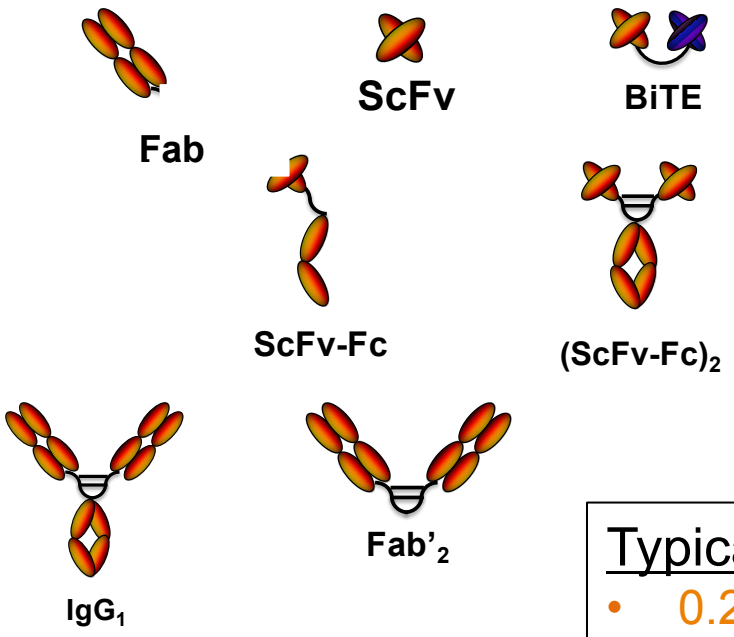
- Examples of Ab Fragments Produced by CF synthesis



- Other examples:
  - ✓ Growth factors
  - ✓ Immunotoxins
  - ✓ Proteases
  - ✓ Knottins
  - ✓ Cellulases
  - ✓ Hydrogenases

Typical Expression Levels:

- 0.25 -1 Gram / Liter (Pre-Optimized)
- 6-8 Hour Reaction
- 30% Extract

Translation of nnAA-Containing Proteins Enables Site-Specific Conjugation

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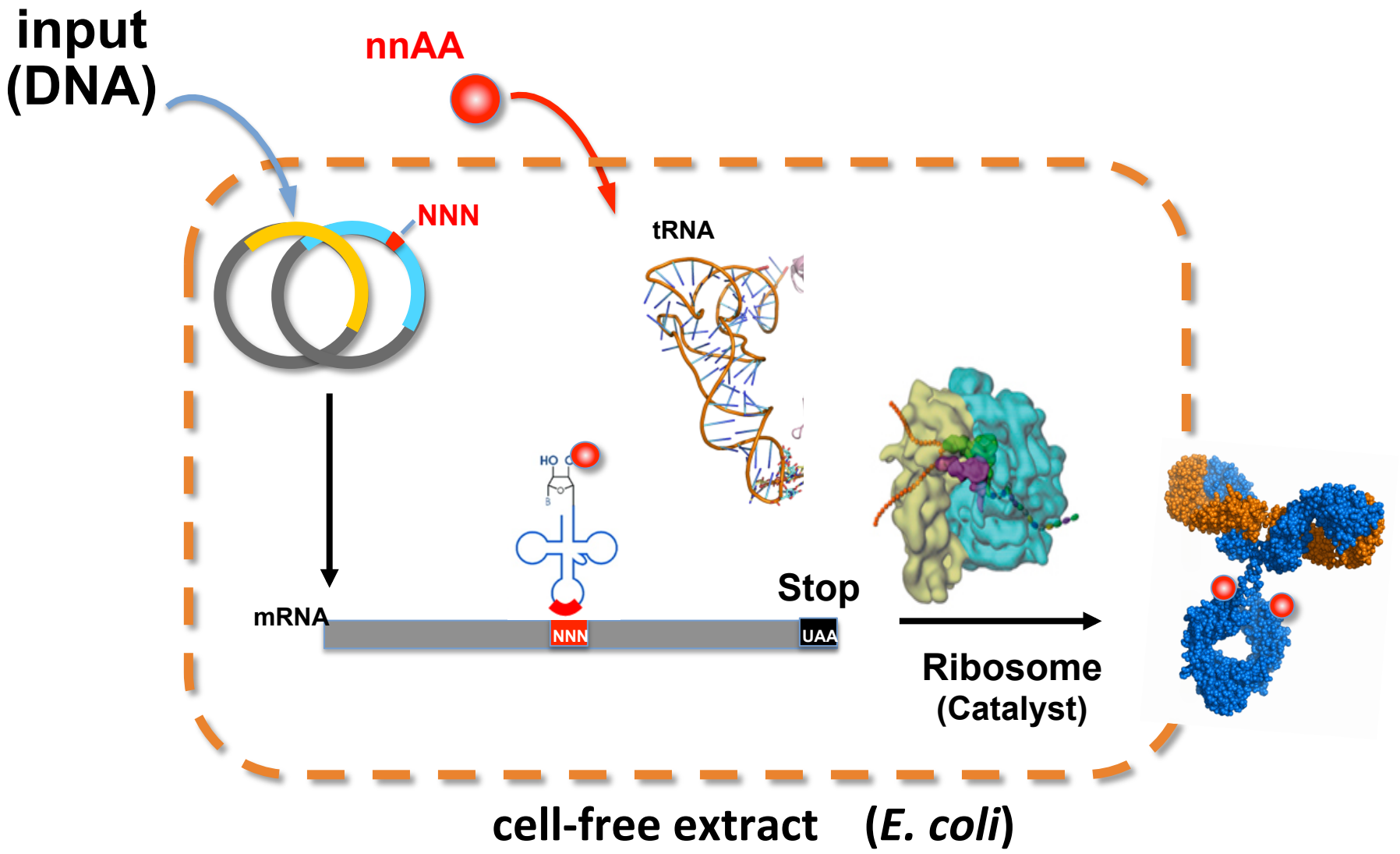
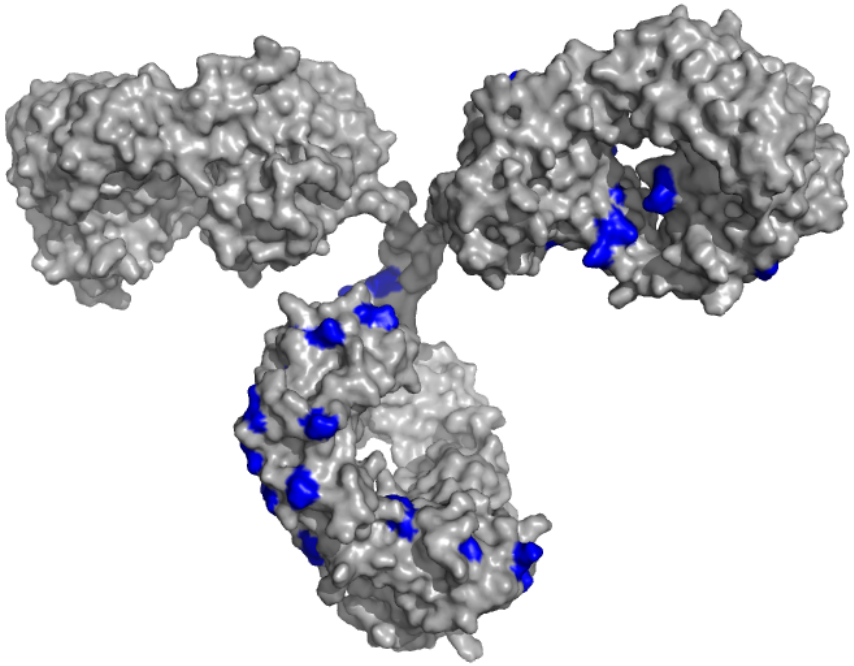


Data driven design: production of many variants in hours

- **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



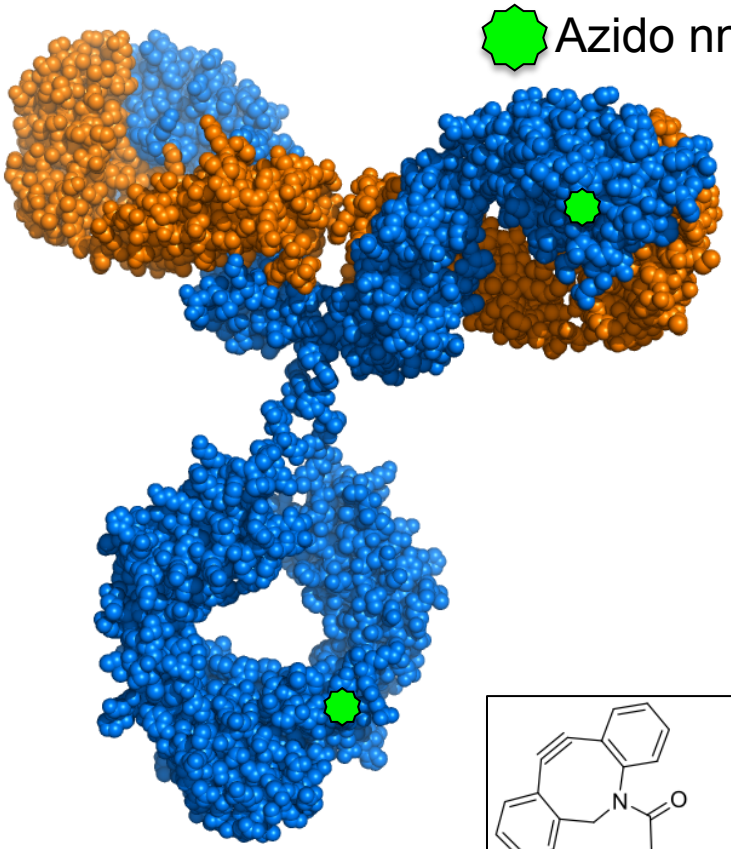
Surface Scan

TAG Screen

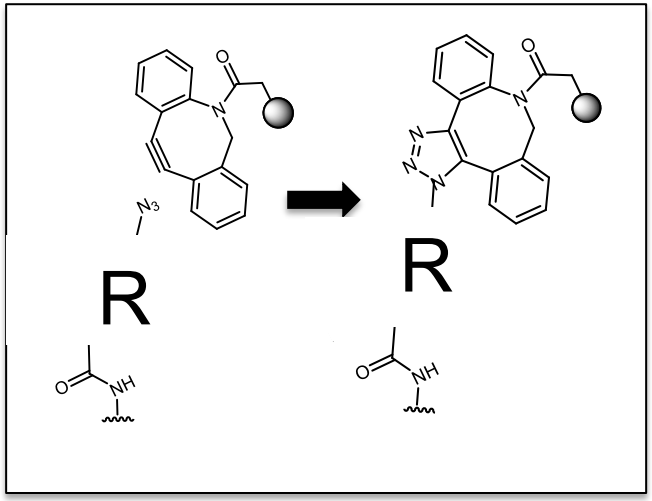
Light Chain: 111 Sites

Heavy Chain: 133 Sites

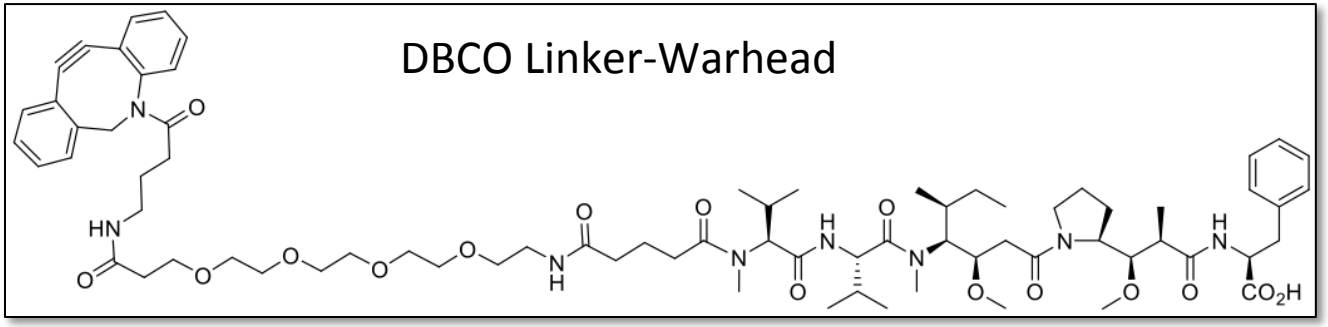
Sutro nnAA IgG with AB3627 Linker+Warhead



Cu Free Click
Conjugation Chemistry



DBCO Linker-Warhead



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

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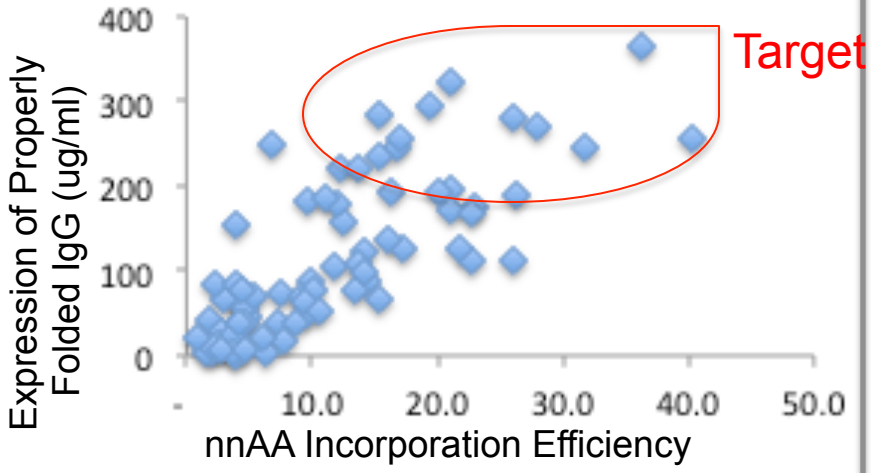
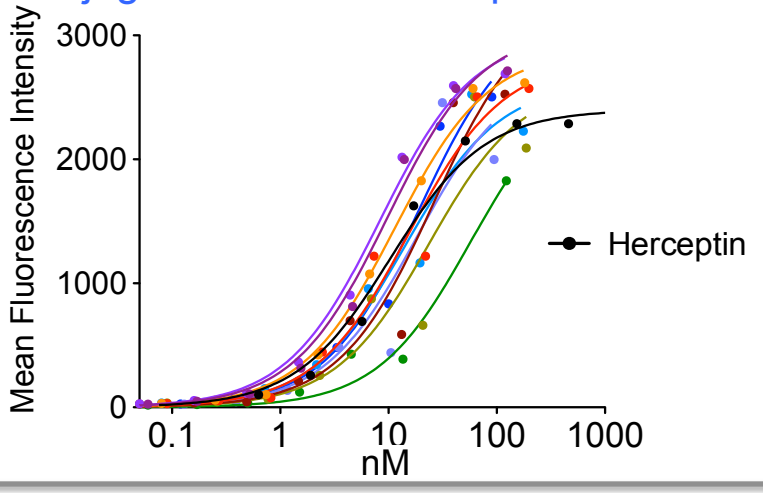
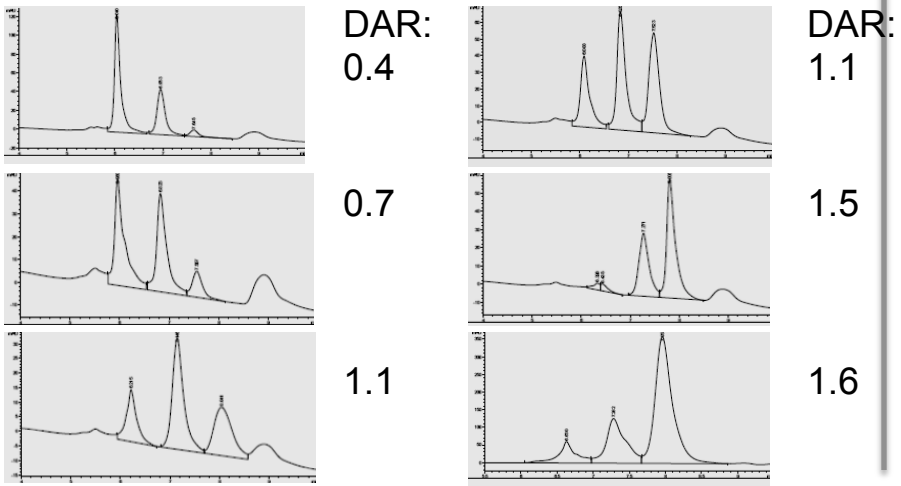
nnAA Incorporation and Expression



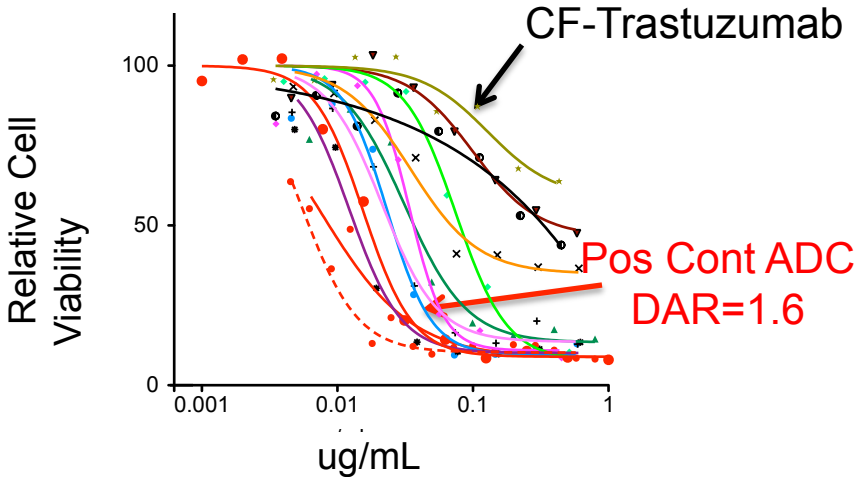
SKBR3 Binding Assay
Conjugated Variants Compared to Herceptin



Conjugation Efficiency (Drug/MAb Ratio)



Cell Killing Assay



Site-Specific Incorporation of MMAF Yields Potent Cytotoxic ADC

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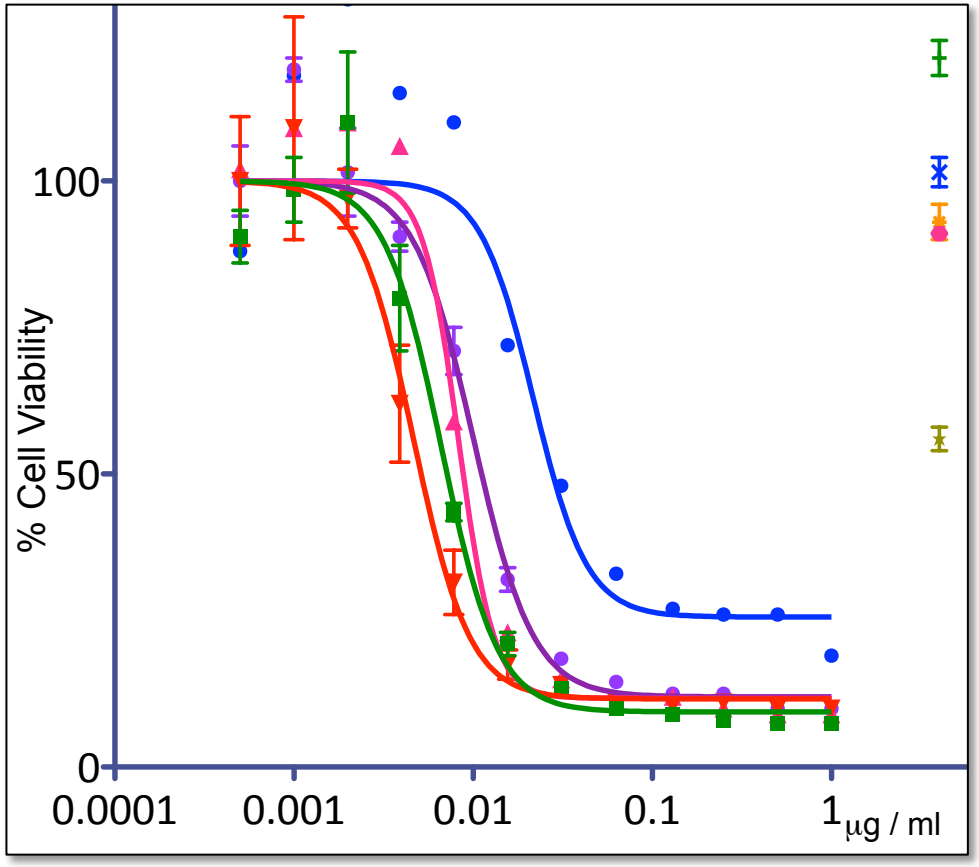
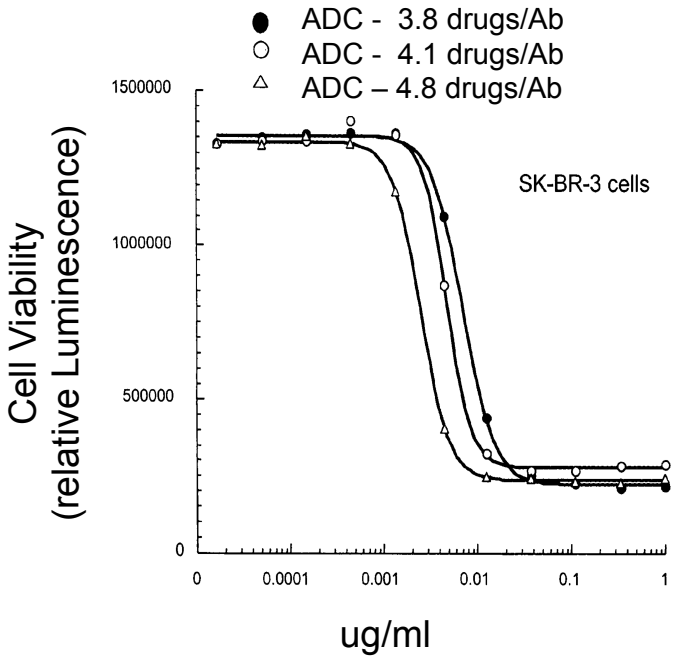
Cell Viability Assay SK-BR-3 cells at 120 hours



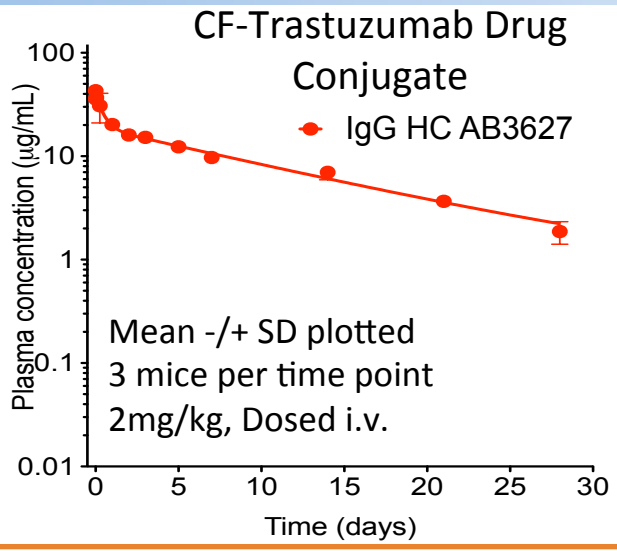
Published Data – Genentech patent

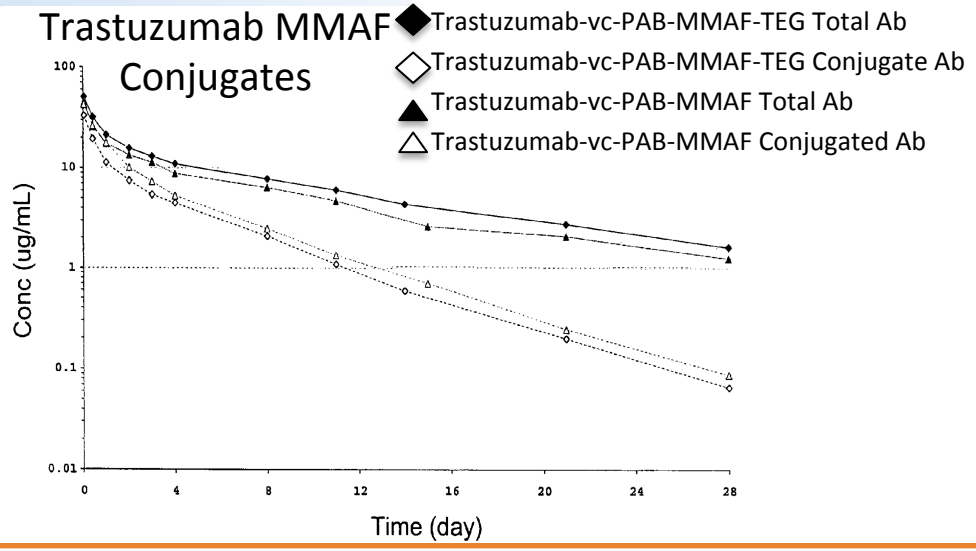
H-MC-MMAF [cys] (120 hours)
From: US Patent 7,994,135 B2 Aug 9, 2011; Fig 7



- Comparable IC50 to published ADC at equivalent IgG concentration
- More potent at equivalent drug concentration

Pharmacokinetics of Cell Free Produced ADCs are Comparable to Cell-Based derived ADCs



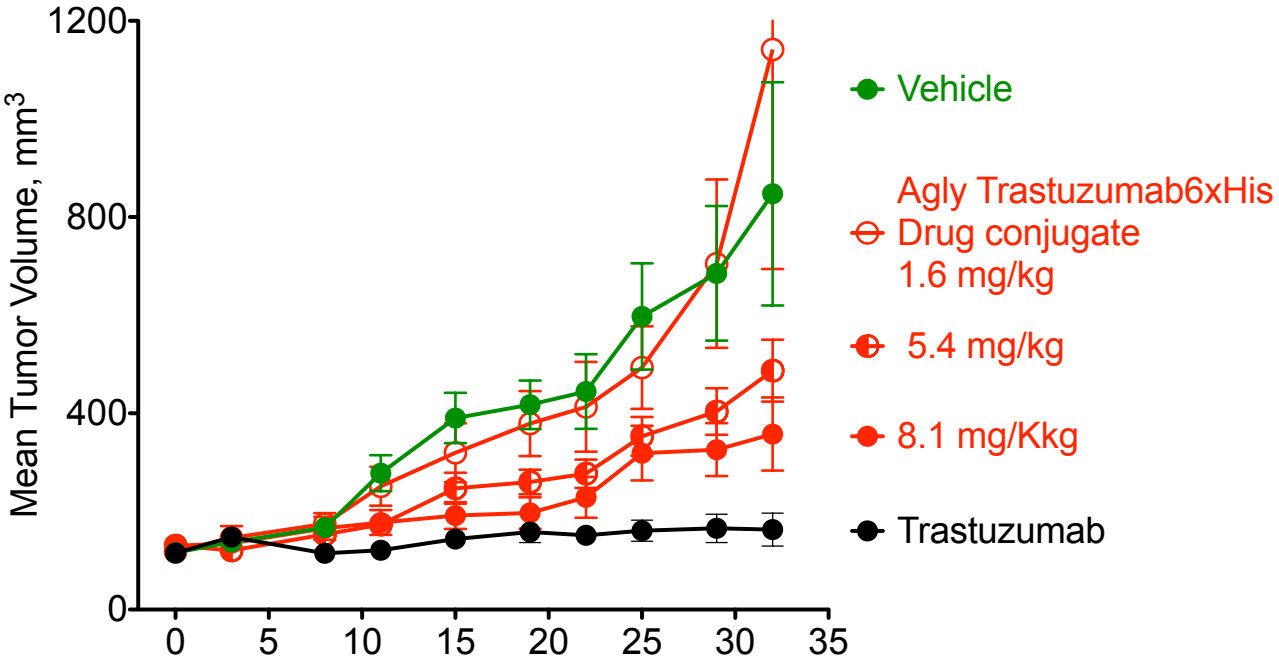


CF-Trastuzumab Drug conjugate pharmacokinetics are in good agreement with Trastuzumab- MMAF conjugate literature

	Sutro Data[a]	SeaGen Data[b]
	CF-Trastuzumab Drug Conjugate	Trastuzumab MMAF Conjugates
AUC_{inf} [day/µg/mL]	248	299
Clearance [mL/d/kg]	8.1	9
Half-life [d]	8	10

[a]5 mg/kg, Balb-c mice, Sutro [b]2mg/kg, Sprague-Dawley rats (US7994135B2, SeaGen patent 2011)`

Efficacy Study:
CF-Trastuzumab DC Dose Response



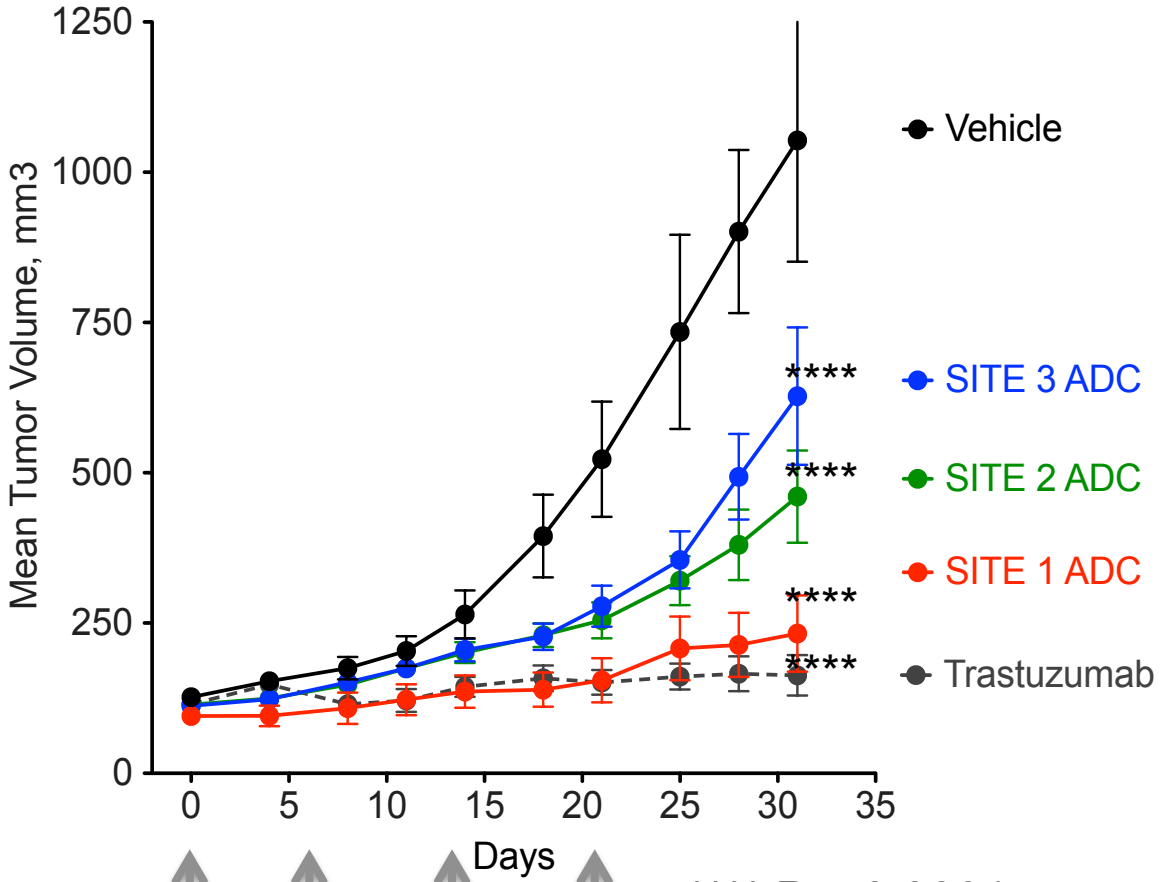
Trastuzumab
(i.p)

30 mg/kg

15 mg/kg

CF-Trastuzumab
Drug Conjugate (i.v)

Efficacy Study:
Agly Trastuzumab DC Dose Response



Aglycosylated Trastuzumab Drug Conjugates Sites 1, 2, and 3

(Single Dose 8mg/kg i.v; DAR=1.5)

Site Dependent Impact on Cell Killing Observed

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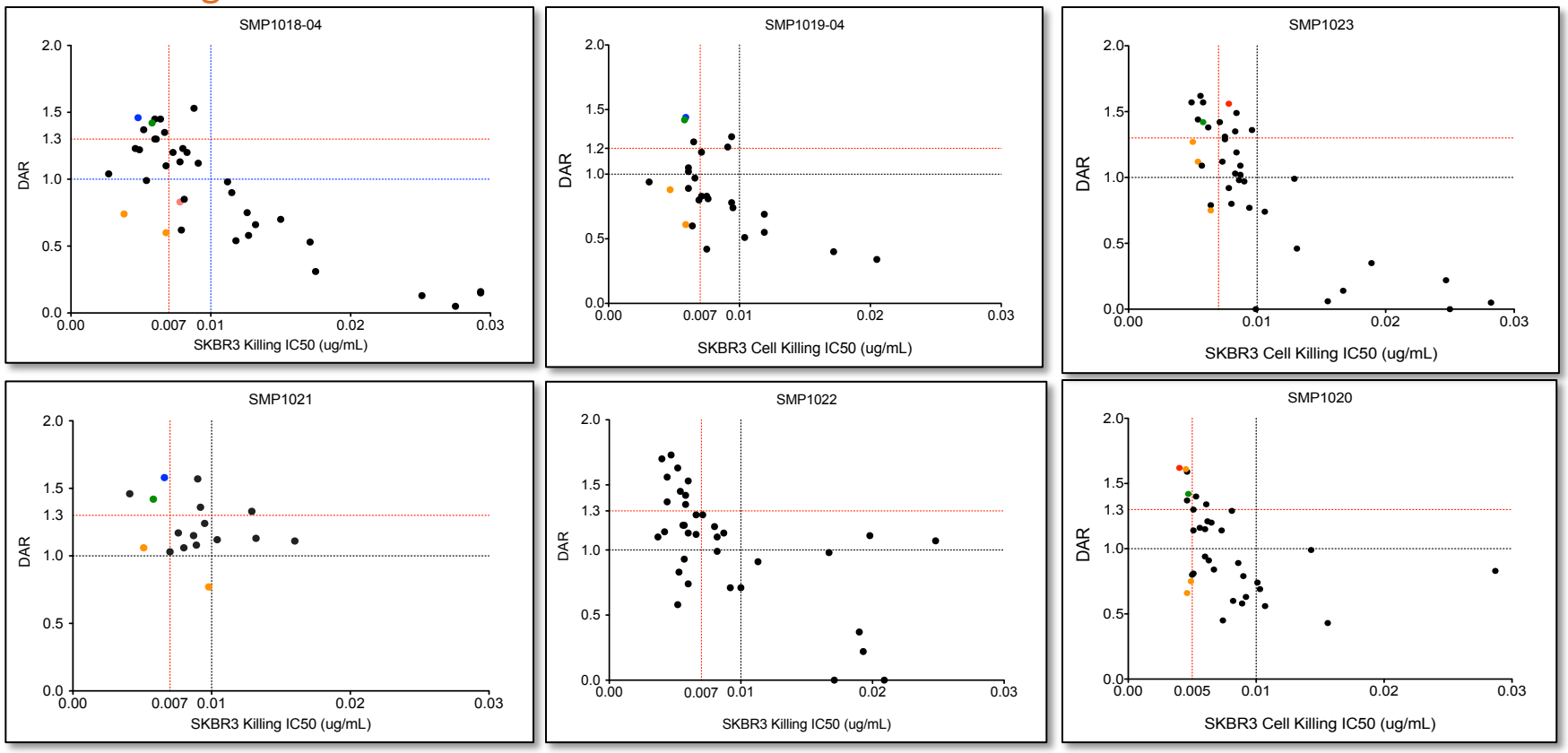
Cell Killing

DAR



TAG Scanning: DAR vs. Cell Killing

Efficient Integration of nnAAs and Conjugation Chemistry are key for manufacturing ADCs

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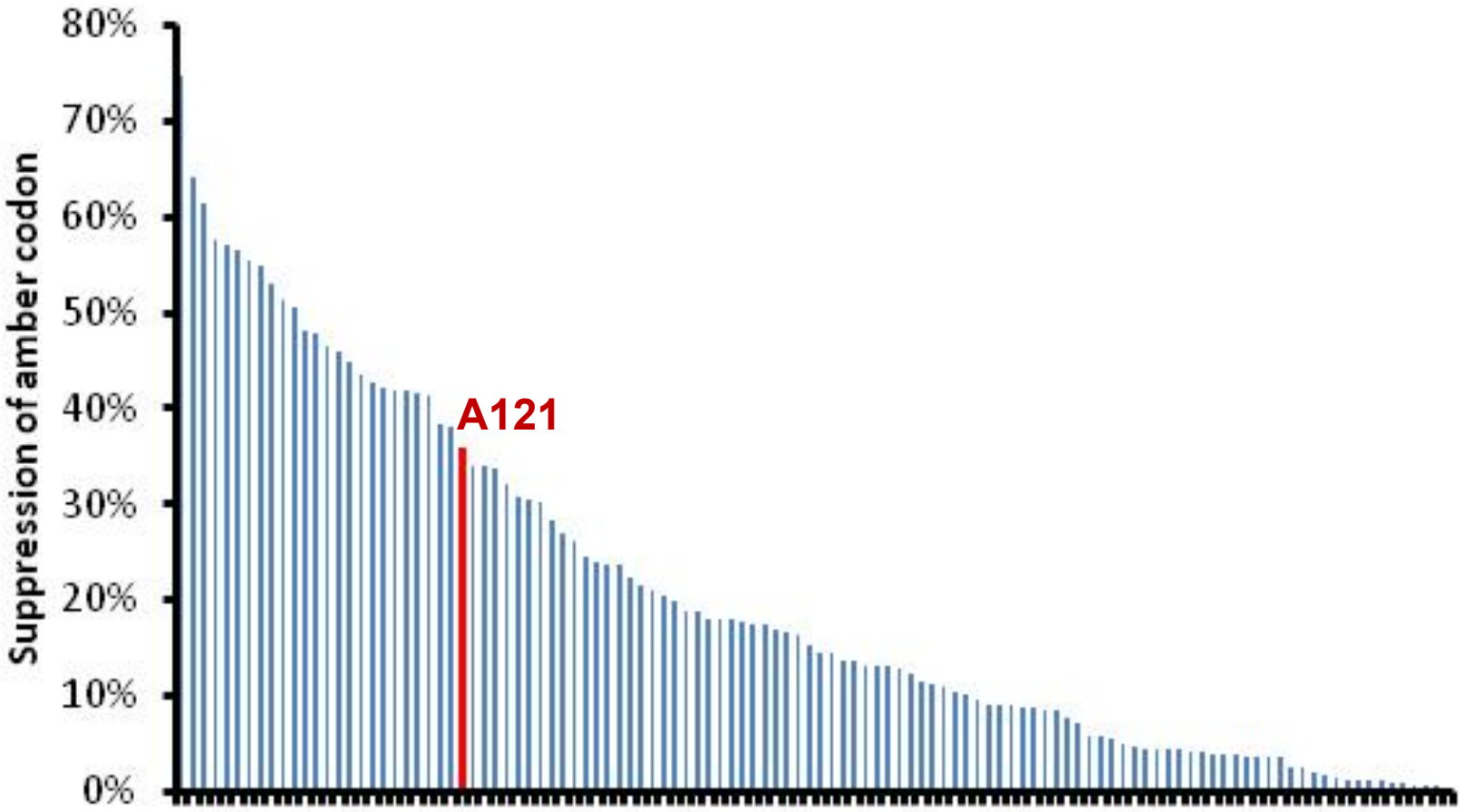
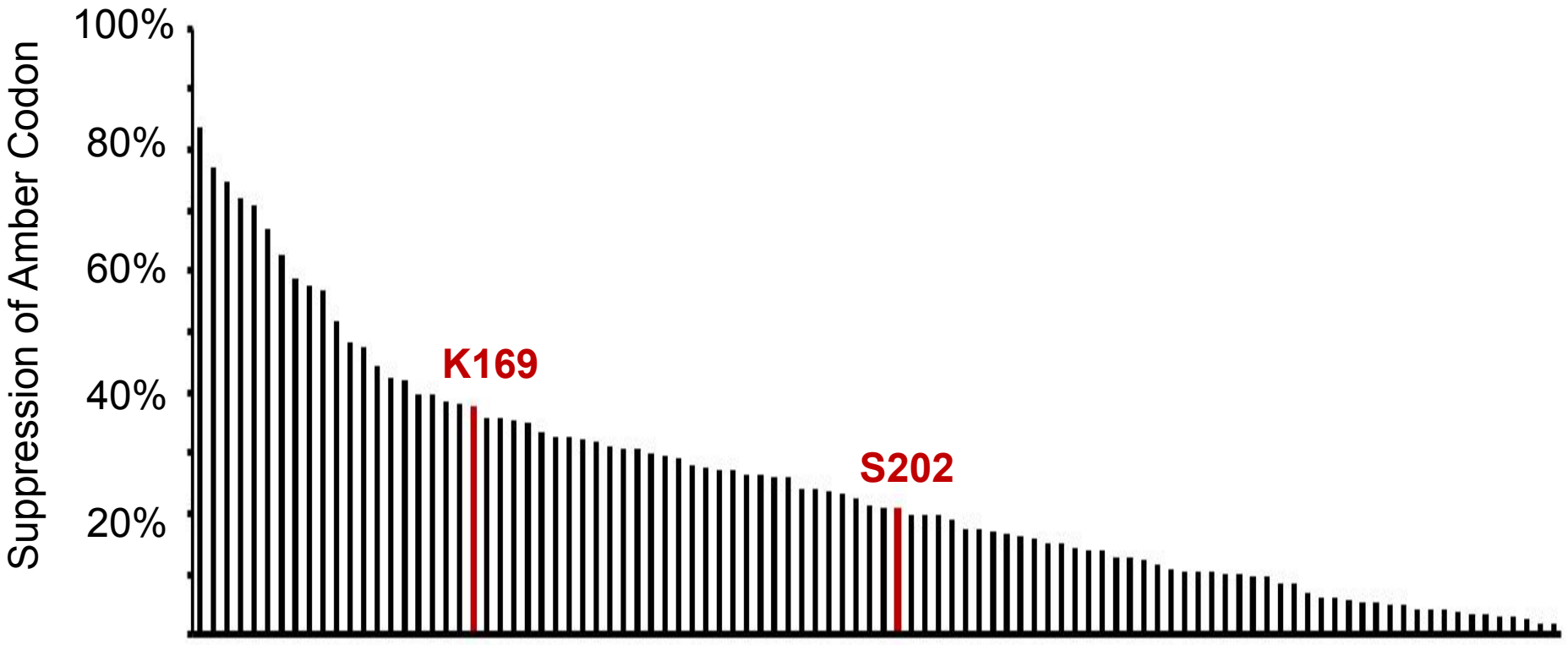
- nnAA Incorporation Efficiency
  - Site Dependence
  - RF1 Attenuation
  - Multiple nnAA Incorporation
- Conjugation Efficiency
  - Site Dependence
  - Improving Conjugation Kinetics

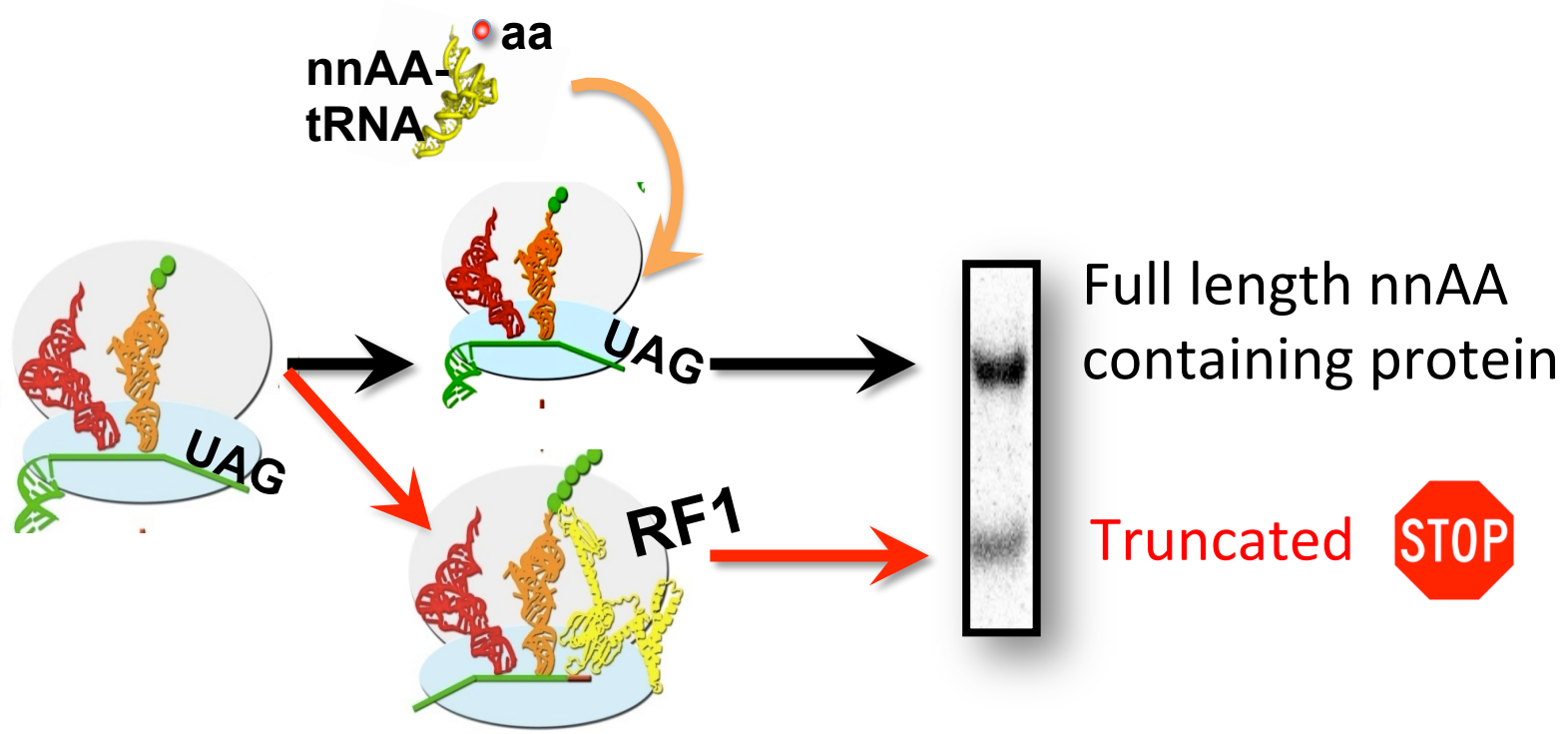
nnAA Incorporation Efficiency; Site Dependence in HC



nnAA Incorporation Efficiency; Site Dependence in LC



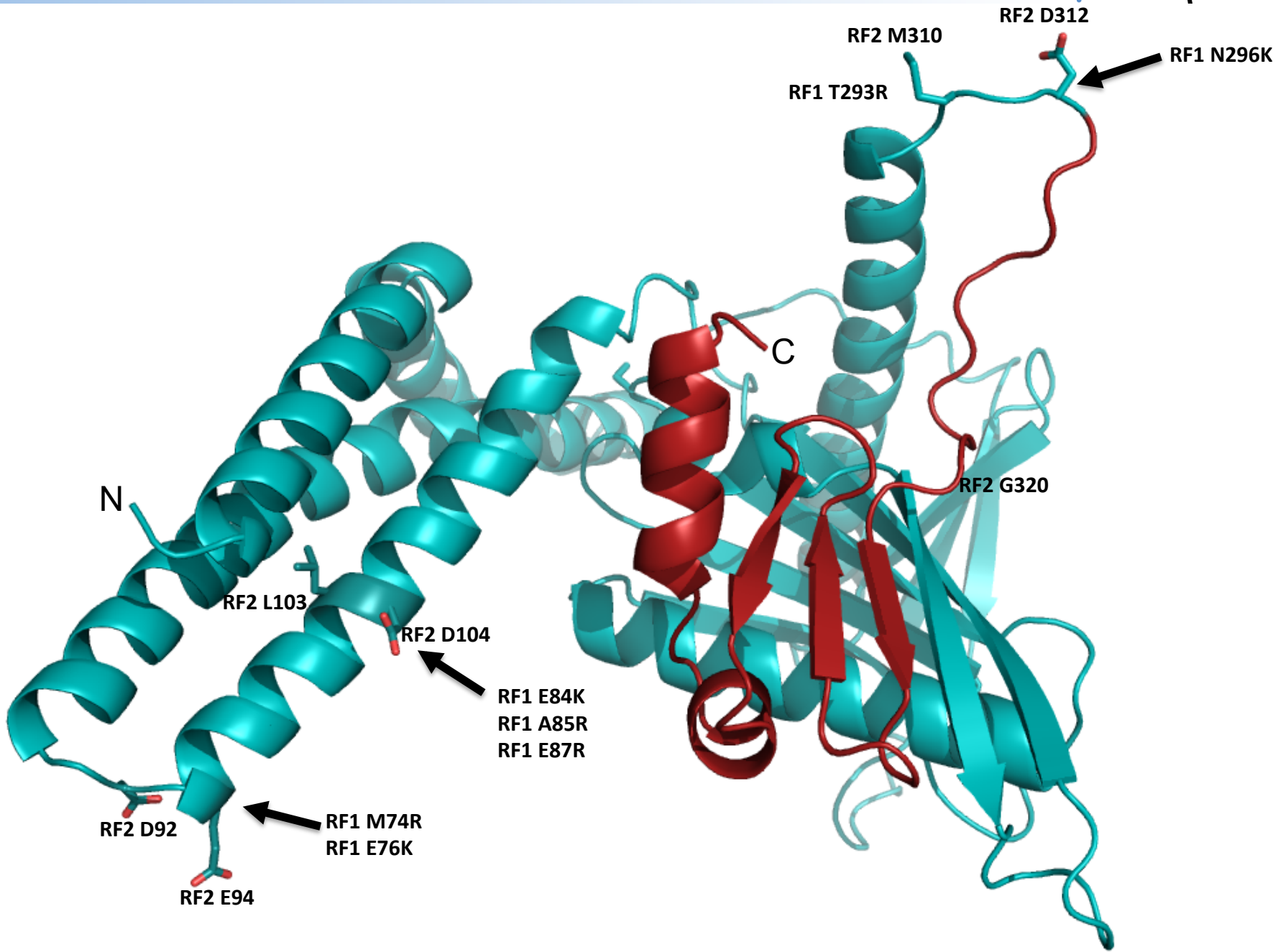
nnAA Incorporation Efficiency:



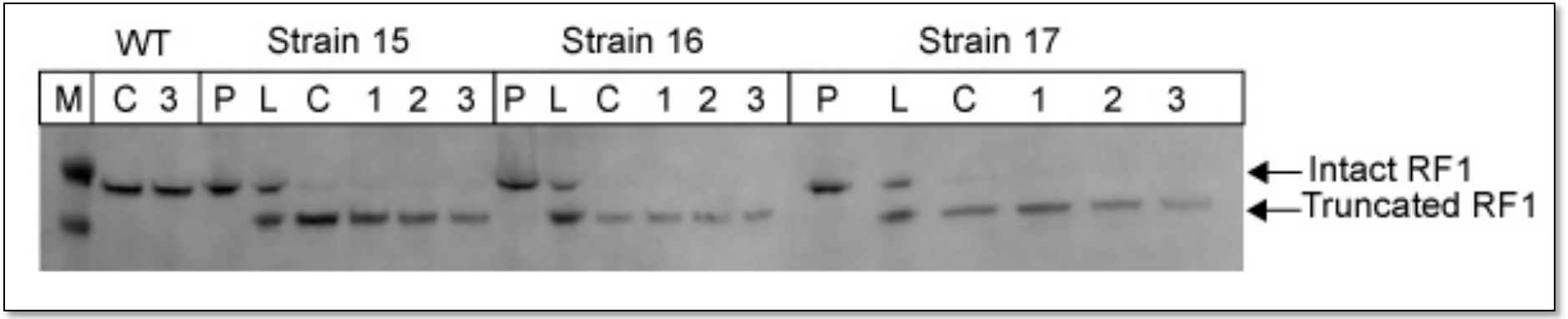
RF1 Attenuation

- ↑ nnAA Incorporation Efficiency
- ↑ Expression/yield

Design of OmpT-susceptible RF1



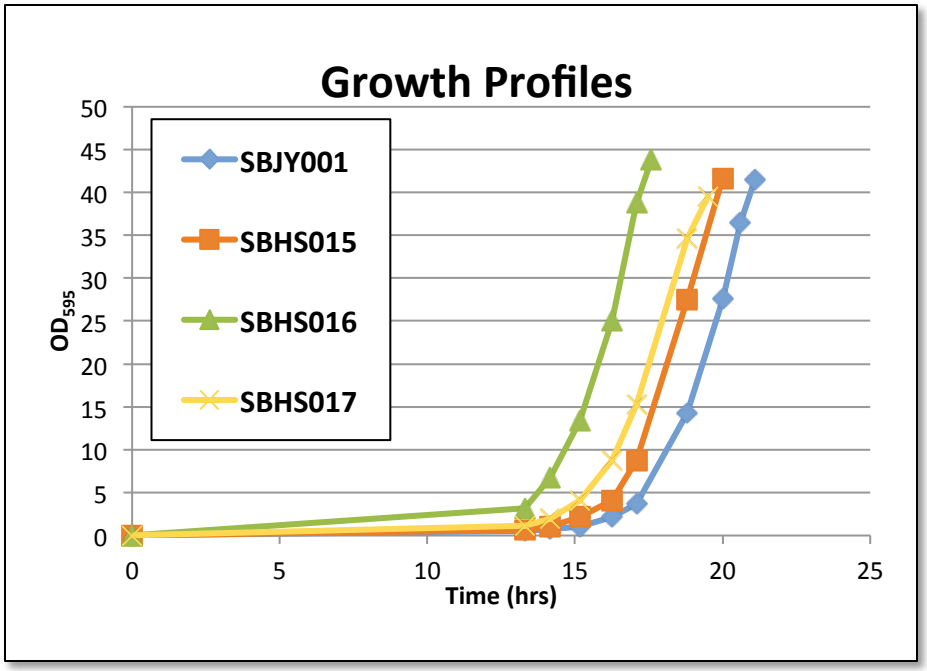
Protease Sensitive RF1 is inactivated during extract production

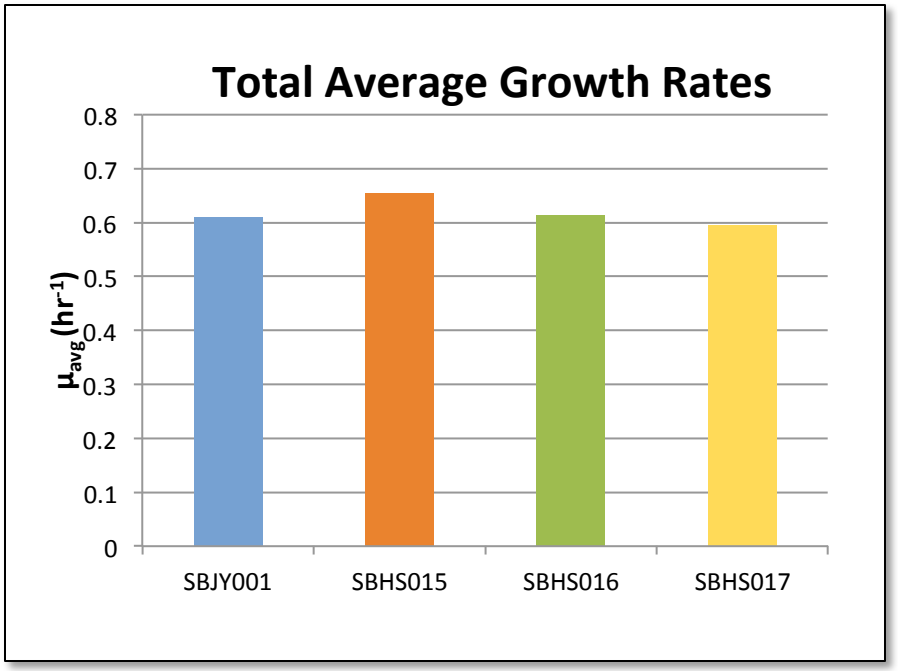


Abbrev.	Sample
M	Marker
P	Pellet
L	Lysate
C	Clarified
1	1 hour time point
2	2 hour time point
3	3 hour time point

De-compartmentalized Extract Cleaves RF1

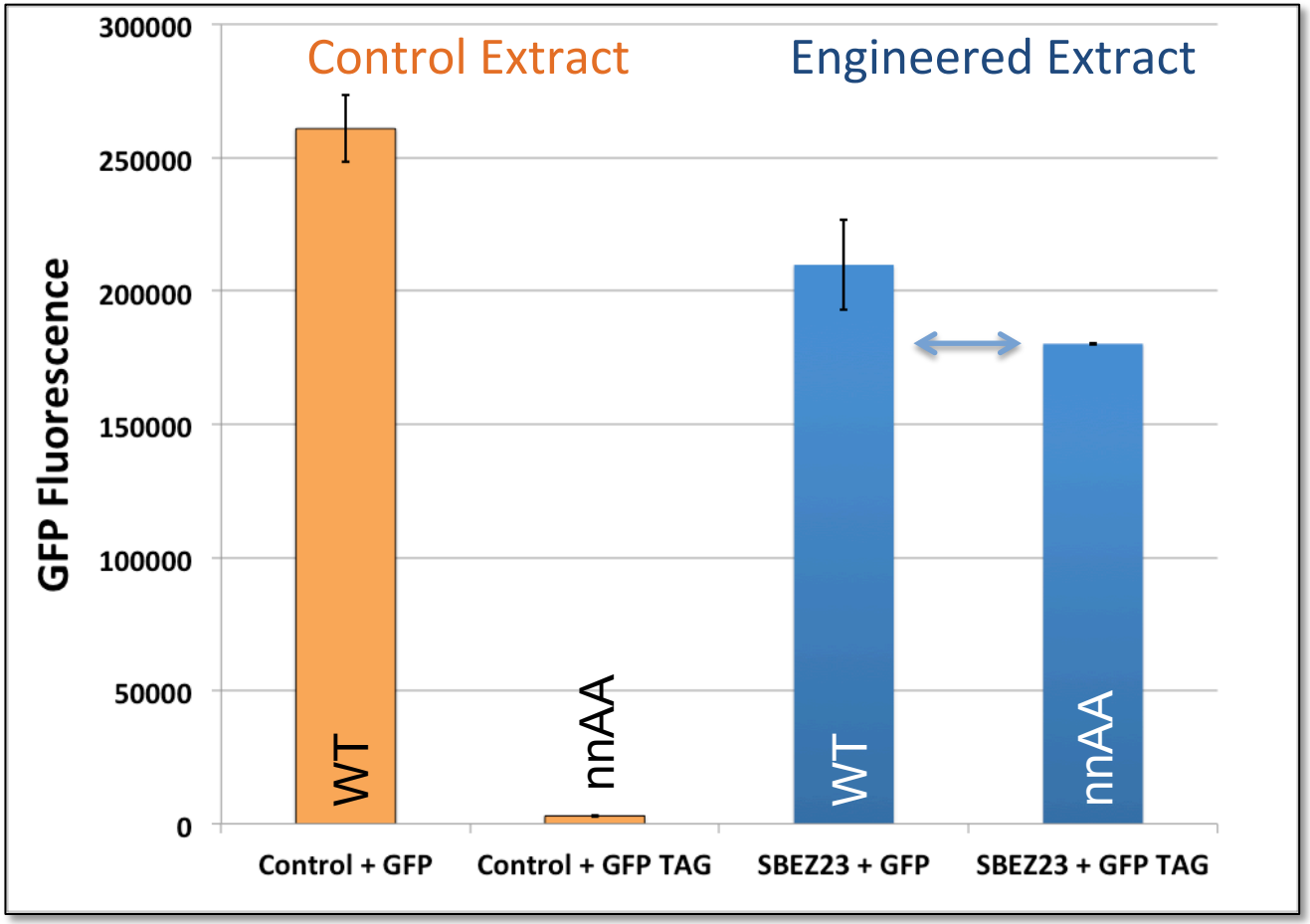
New Strains Have Similar Growth Rates To Control Strain





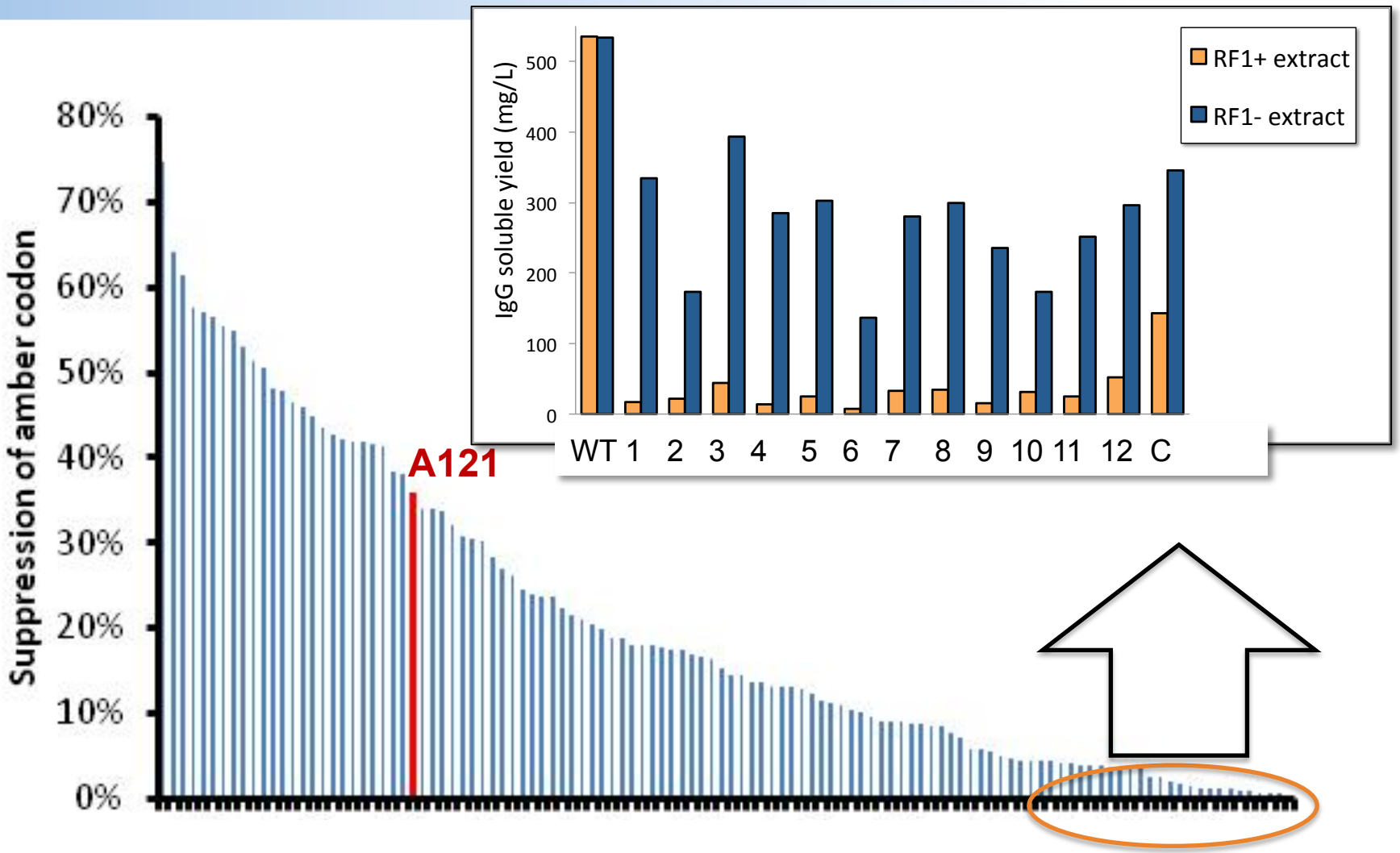
High Growth Rate is Historically Predictive of Good Extract Performance

RF1⁻ Strain Engineered Extract Boosts nnAA-Protein Production

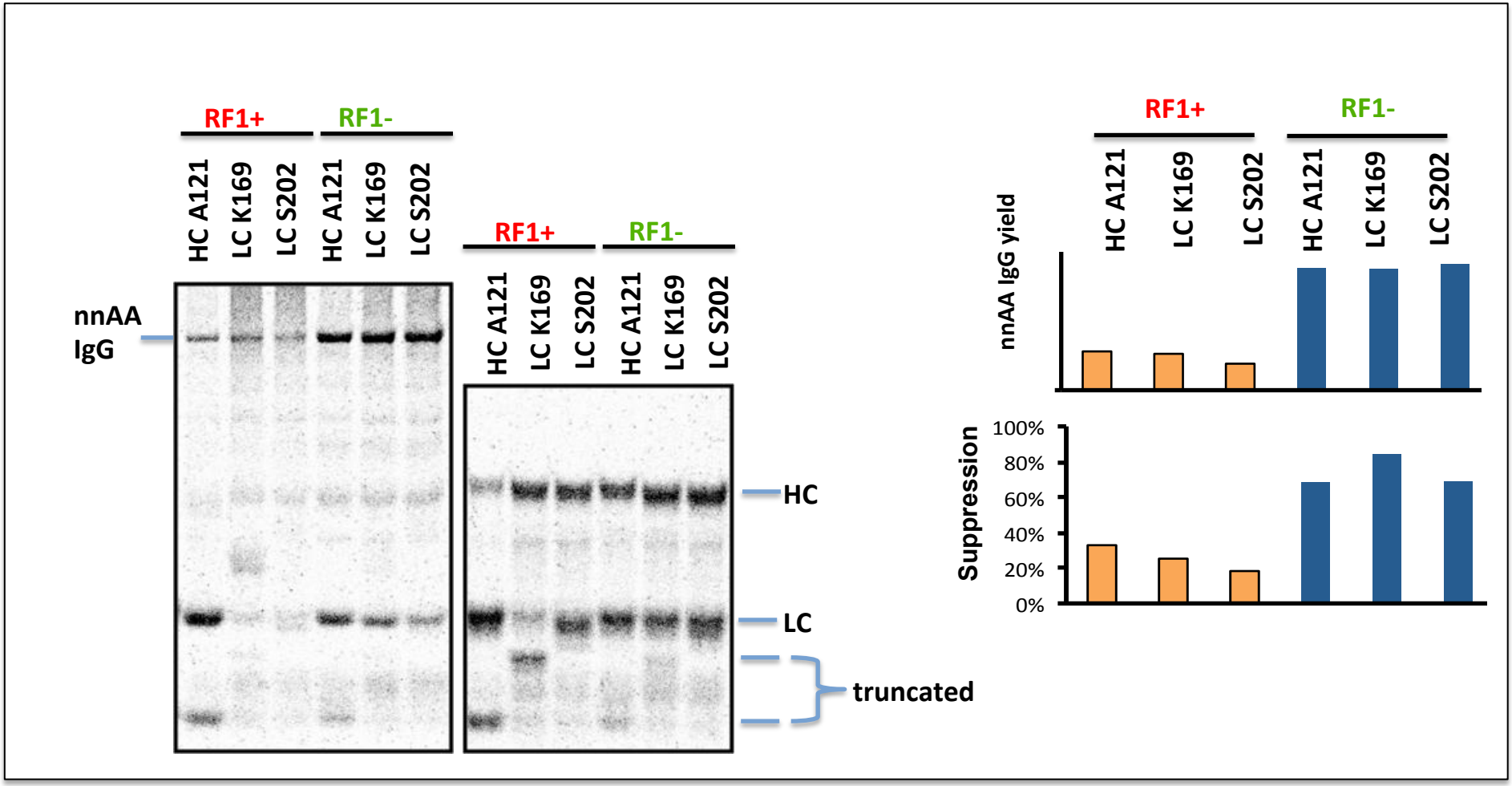


Strain: RF1 Attenuating Modification

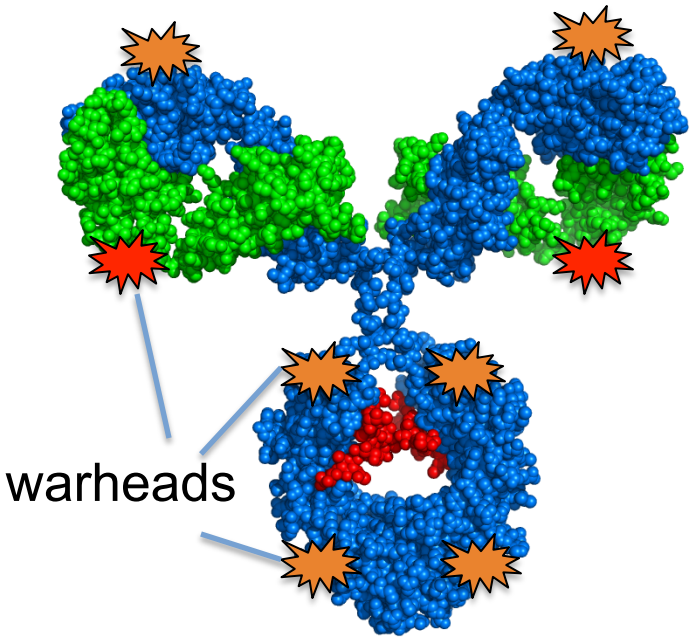
Suppression of amber codon in HC



Incorporation of p-Acetyl Phe into IgG at three selected sites



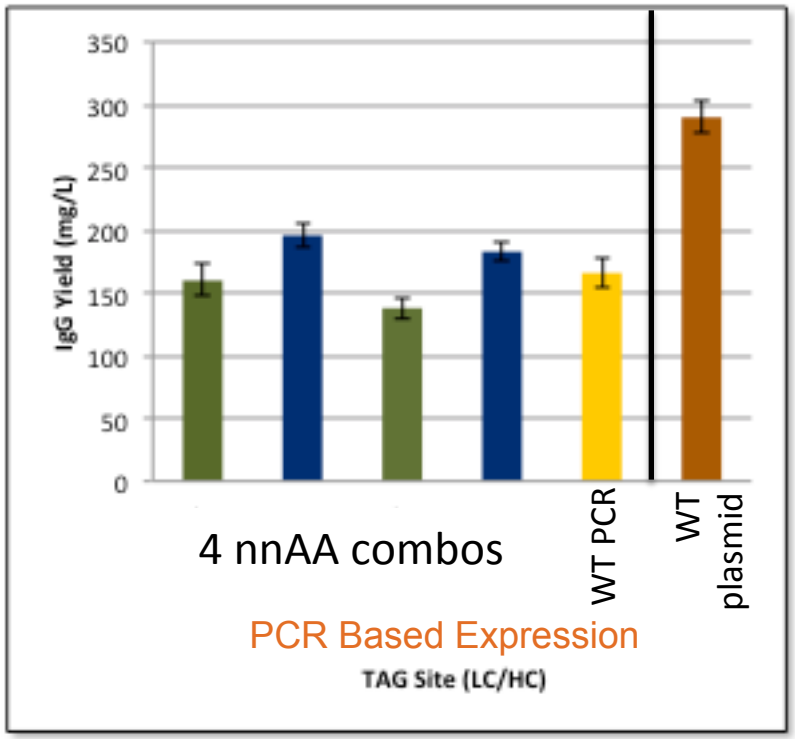
RF1 Depletion through Strain Engineering: Extract Can Produce IgG w/ Multiple nnAA's



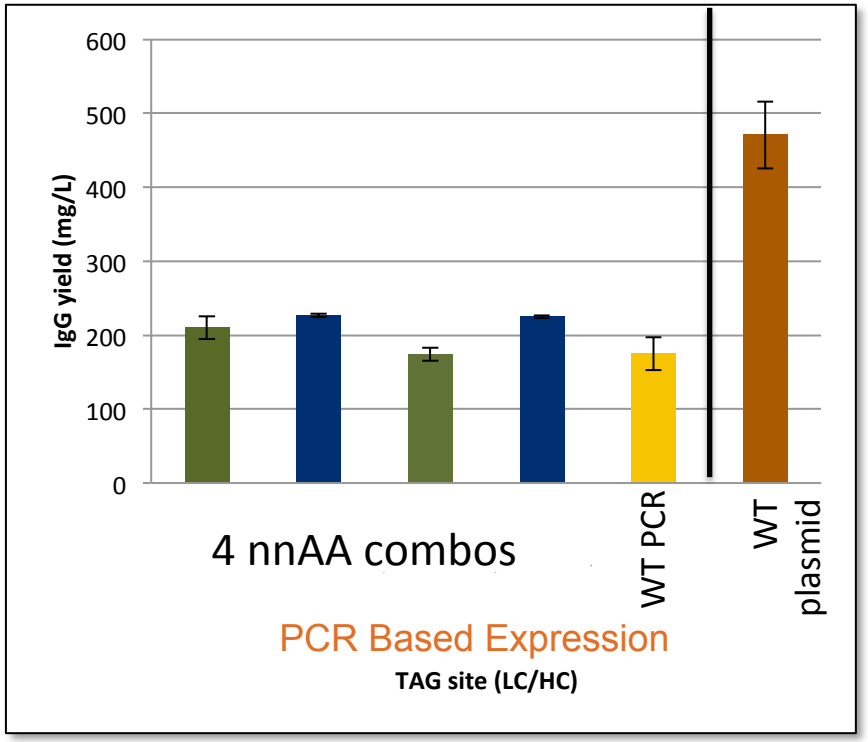
- Incorporation of multiple nnAAs in each IgG LC and HC
- Enables Multiple (4,6,8,10+) **site-specific combinations of warheads/IgG**
- Intractable using cell-based systems or wild-type extract due to significant accrued losses in yield
- Combinations of sites screened for:
  - nnAA Incorporation efficiency/expression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

4 nnAA IgG Yields Using Combinations of 2 LC and 2 HC Sites

- nnAA: nn-AA1
- Synthetase 3

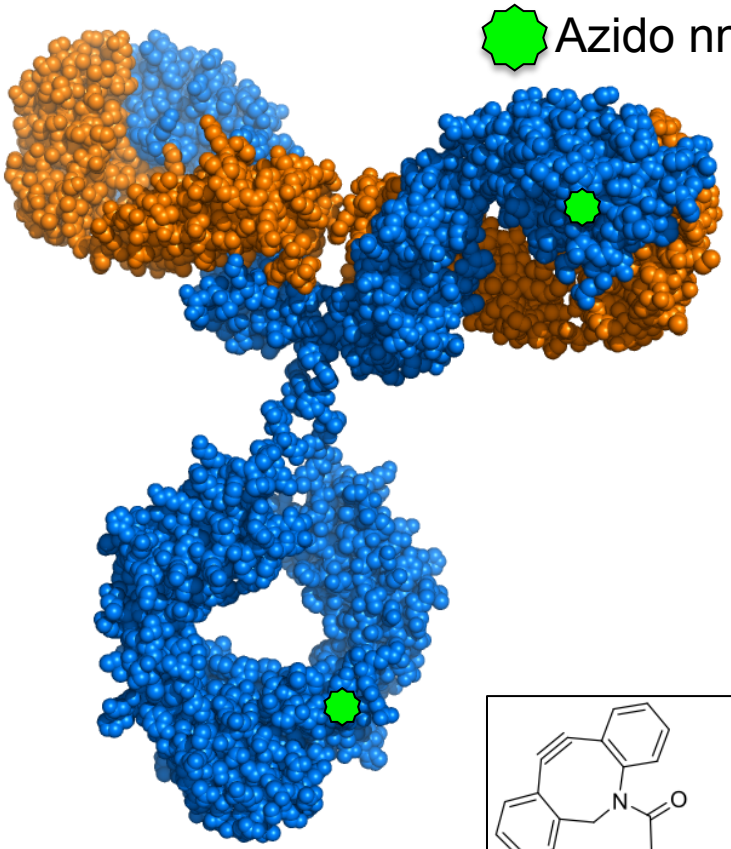


- nnAA: pN3nnAA
- Synthetase 1

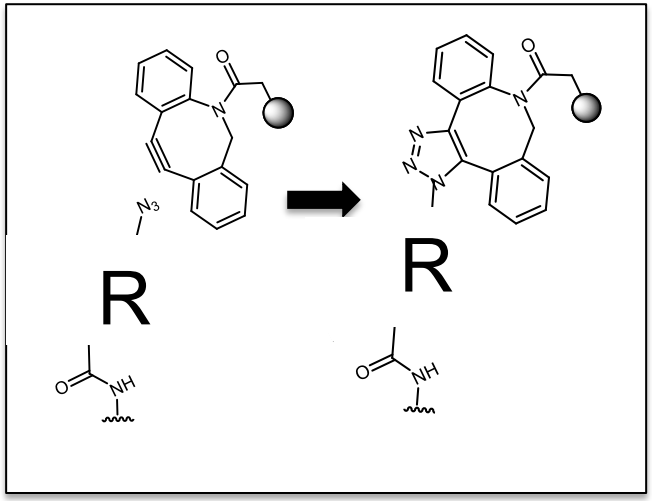


Several 4 nnAA combos (2LC+2HC) express similarly to WT

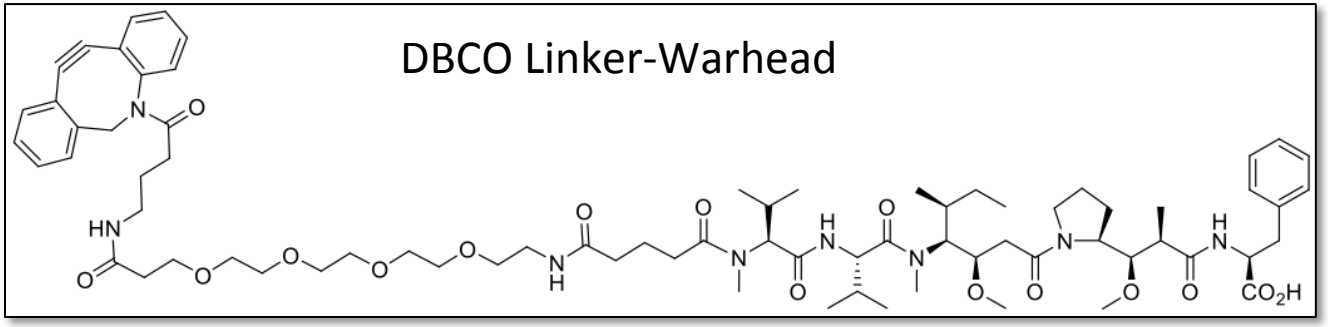
Conjugation Efficiency



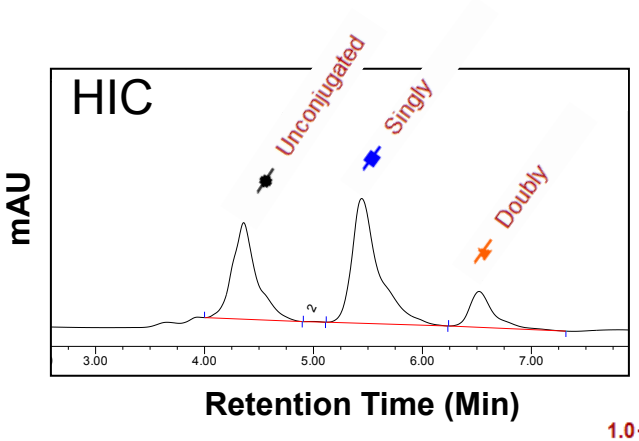
Cu Free Click
Conjugation Chemistry



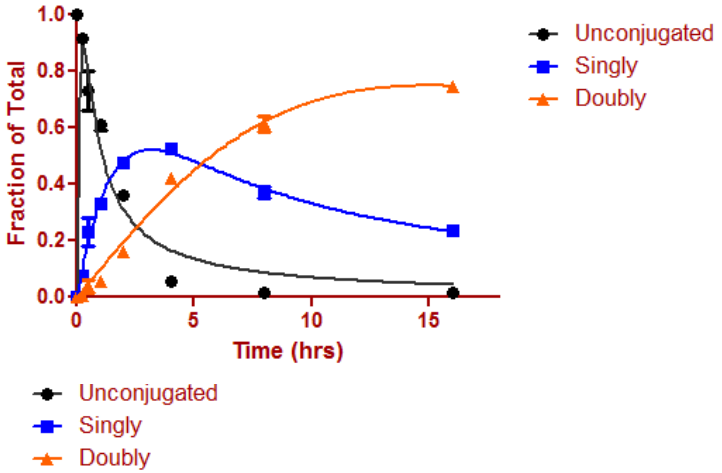
DBCO Linker-Warhead



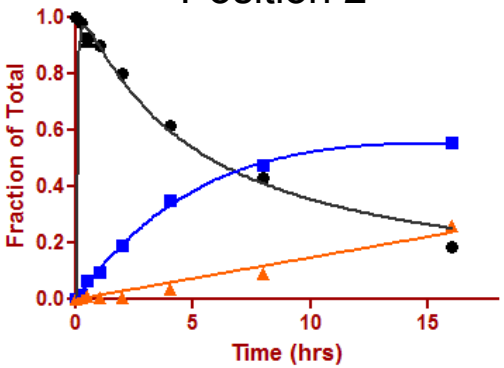
Position affects Kinetics of Conjugation



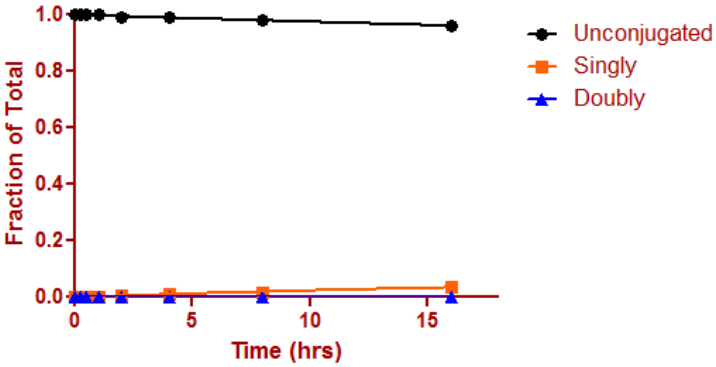
Position 1



Position 2



Position 3



FASTER CONJUGATION

Variant	$t_{1/2}$(Doubly) Hrs
Position 1	6.2
Position 2	39.7(est)
Position 3	ND

Conjugation Efficiency

Sutro Biopharma

PNAS

Synthesis of site-specific antibody-drug conjugates using unnatural amino acids

Jun Y. Axup[a,b], Krishna M. Bajjuri[c], Melissa Ritland[d], Benjamin M. Hutchins[a,b], Chan Hyuk Kim[a,b], Stephanie A. Kazane[a,b], Rajkumar Halder[a,b], Jane S. Forsyth[d], Antonio F. Santidrian[d], Karin Stafin[d], Yingchun Lu[e], Hon Tran[e], Aaron J. Seller[e], Sandra L. Biroc[e], Aga Szydlik[e], Jason K. Pinkstaff[e], Feng Tian[e], Subhash C. Sinha[c], Brunhilde Felding-Habermann[d], Vaughn V. Smider[c,1], and Peter G. Schultz[a,b,1]

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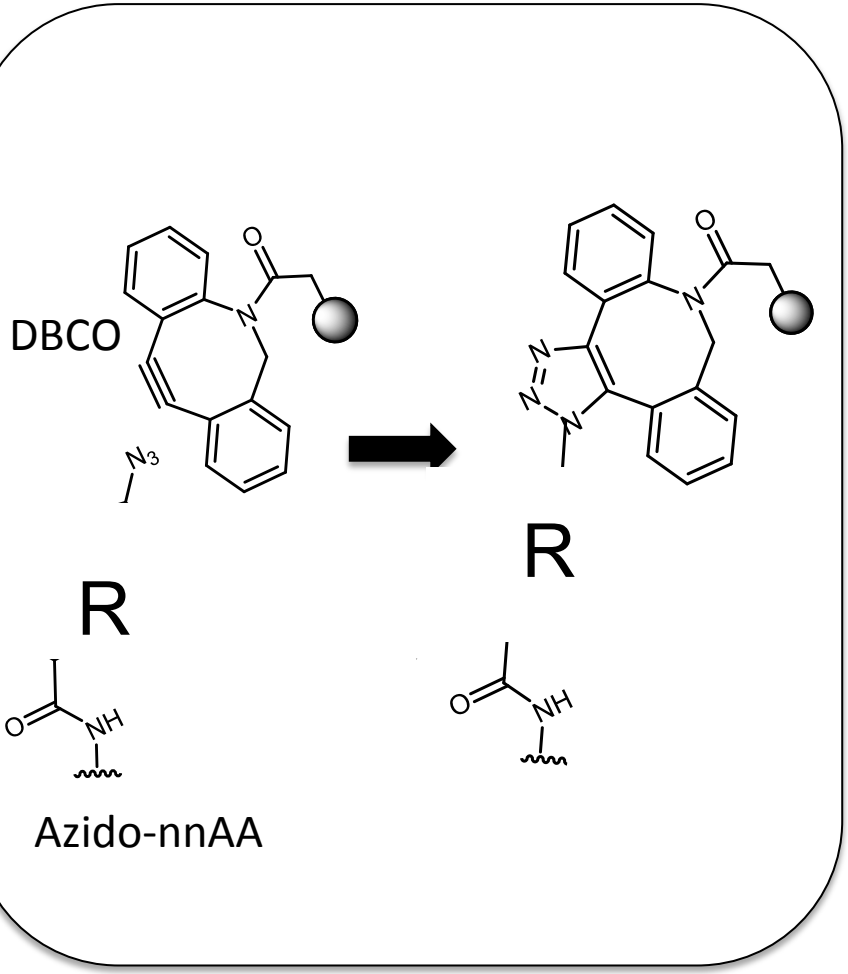
Contributed by Peter G. Schultz, July 20, 2012 (sent for review January 27, 2012)

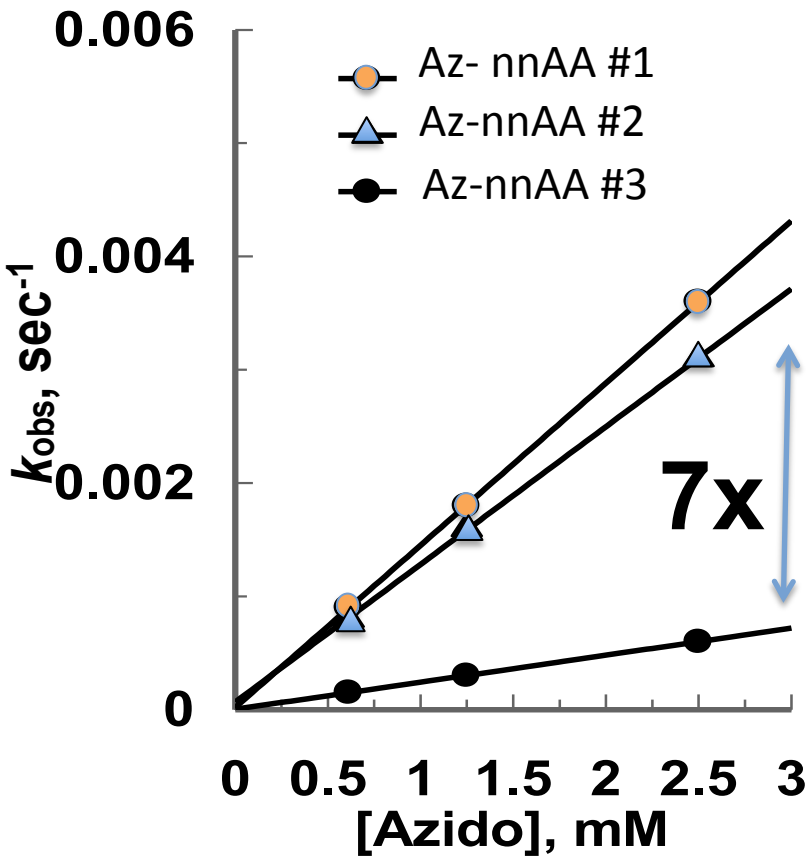
Materials and Methods Section

Oxime Ligation

Conjugation. Oxime ligation between pAcPhe on the antibody and alkoxy-amine-functionalized auristatin was carried out in 100 mM acetate buffer pH 4.5 with 100 μM (5 mg/mL) Fab and 3 mM AF (30-fold excess) for 1–2 d at 37 °C. **IgG was conjugated at 66.7 μM (10 mg/mL) and 1.3 mM AF (20-fold excess) for 4 d at 37 °C.**

Novel nnAAs with Boosted Conjugation Kinetics





New Chemistry offers Improved Kinetics, Flexibility

Top Sites: Nearly Complete Conjugation

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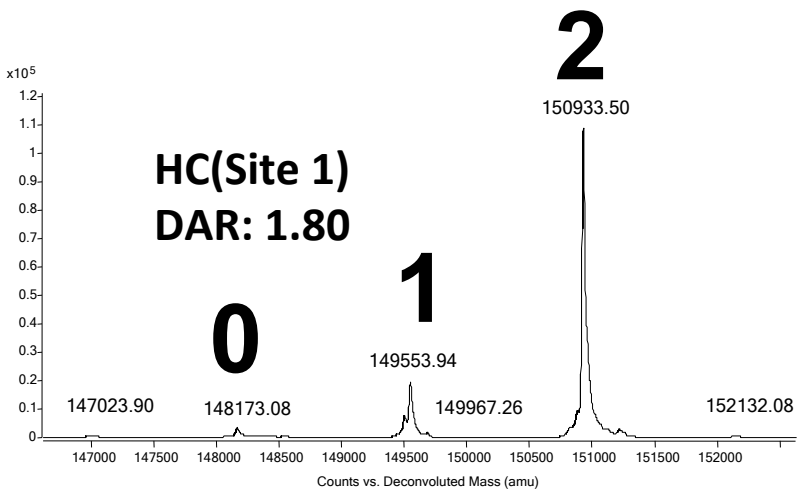
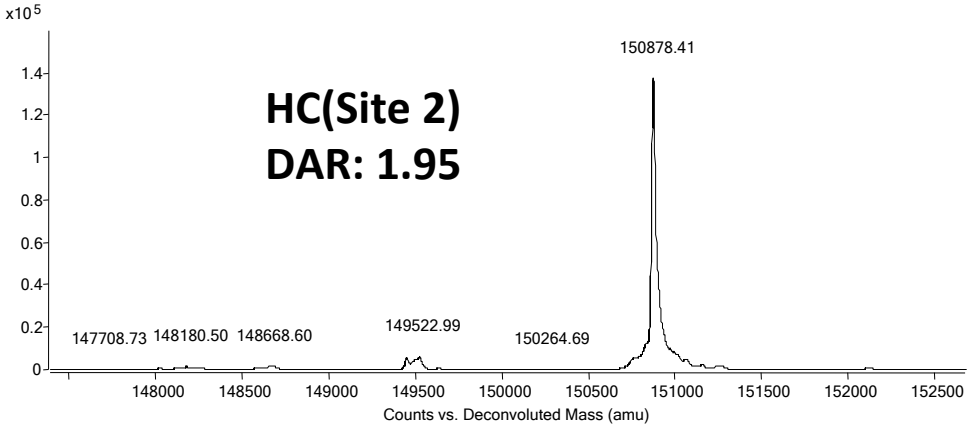
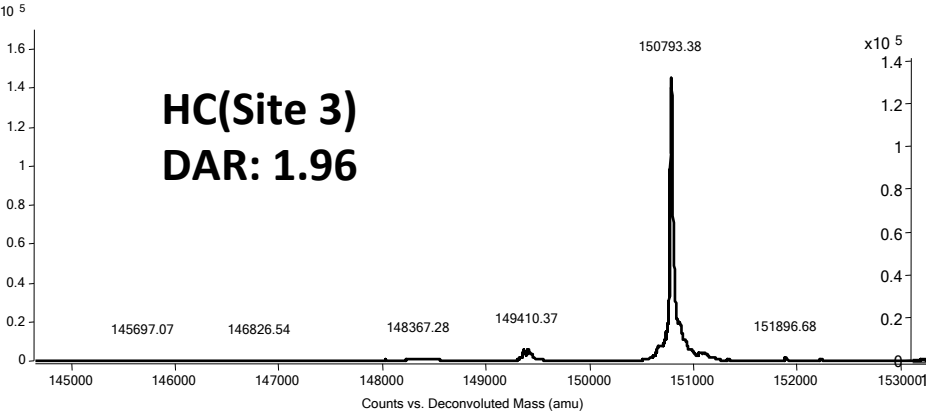
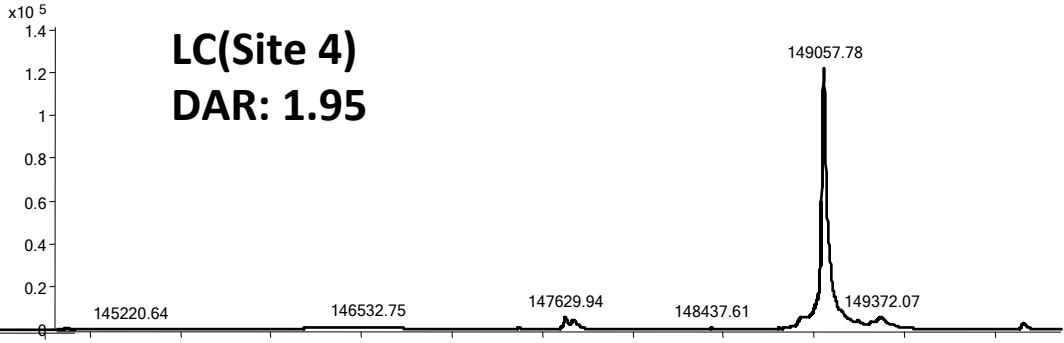
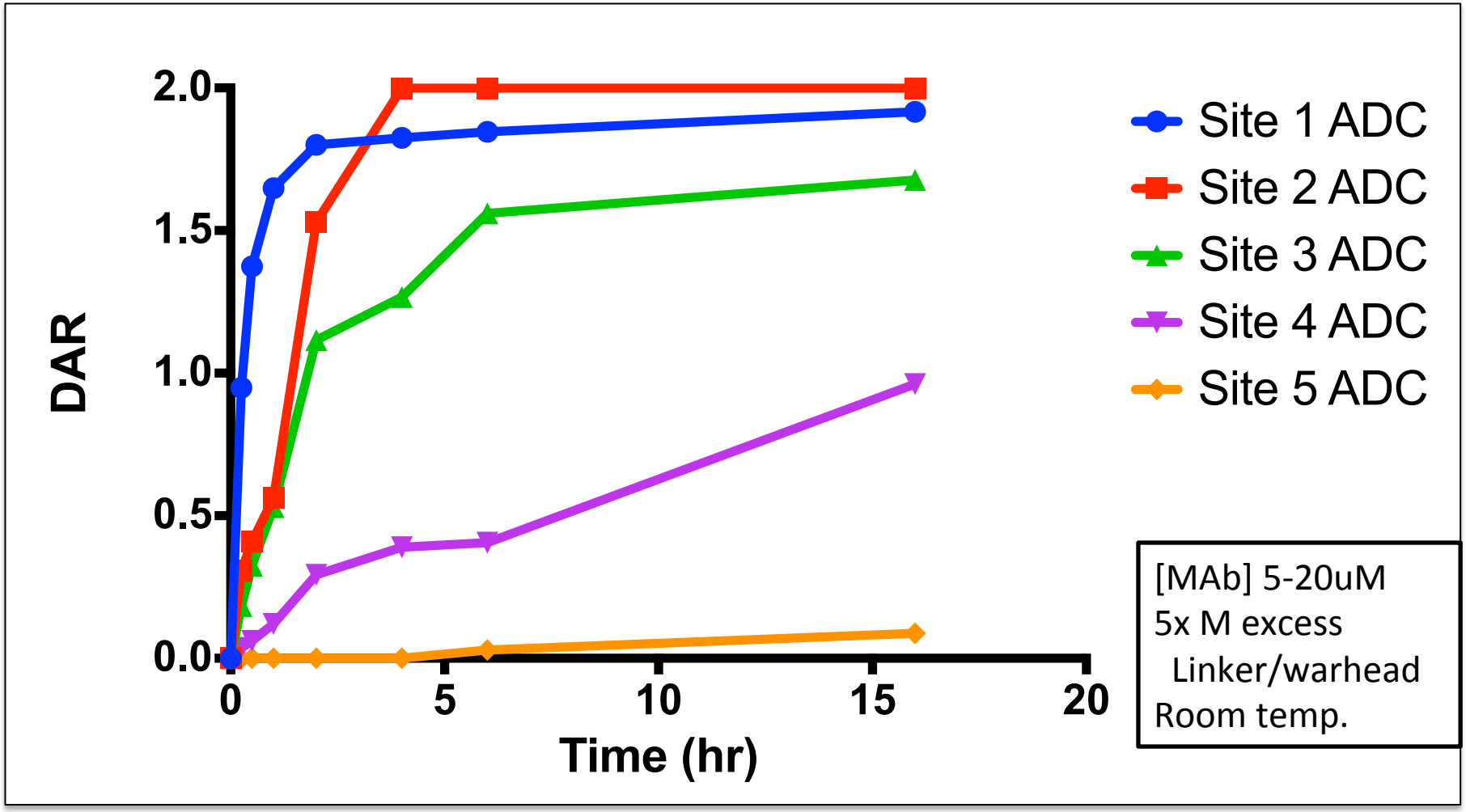








Some sites are completely conjugated in under 4 hours!

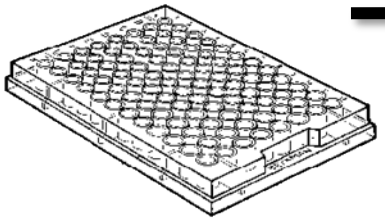


Cell-Free Manufacturing: GMP Facility a Critical Element

Sutro Biopharma

Rapid Production of Biotherapeutics

Sutro Biopharma



Synthetic DNA

DAYS

2 4 6 8

Sutro Technology

50 µg protein HTS plate format

5 g protein 5-L reactor

100 g protein 100-L reactor

- Direct linear scale-up from HTS to production scale
- Uses standard bioreactors & downstream equipment
- Minimal, rapid process development
- Gene sequence to drug substance in days

Sutro's technology enables Best-in-Class ADCs….

- Data-driven assessment of many potential ADC variants ensures optimized positioning for suppression, expression, conjugation efficiency, payload, cell killing, stability and pharmacokinetic properties

- Flexible and rapid scaling means pharmacodynamic and exploratory toxicology assessment studies can be front-loaded into the discovery phase

- Material for GLP Tox studies and clinical studies can be delivered in days/weeks from selection of Clinical Development Candidate

- 365 days?