Versatility of Modular Antibodies:

From Rapid Format Switch to Fast Screening of Libraries and Bispecifics

Francisco Ylera, PhD

October 26, 2023

Agenda

- Bio-Rad Custom Antibodies
  - Who we are
- SpyTag Technology
  - Versatile, efficient protein ligation
- TrailBlazer™ Platform
  - Modular antibody assembly platform
- SpyDisplay Technology
  - Improved phage display selection technology
- SpyLock Technology
  - Reversibly inhibitable SpyCatcher for the generation of bispecific antibodies
- The Pioneer™ Library
  - New state of the art antibody library

BIO-RAD

Antibody Experts

20 years of experience with animal-free phage display custom antibodies

Antibodies by Design
A Division of MorphoSys

2004 HuCAL GOLD®

AbD serotec
A Division of MorphoSys

2006 HuCAL PLATINUM®

AbD Serotec®
A Bio-Rad Company

2013

BIO-RAD

Today **PIONEER ANTIBODY DISCOVERY PLATFORM**

We recently moved to new, bigger premises near Munich, Germany.

- Custom in vitro antibody development since 2004
- Developed **more than 58,000 antibodies** for customer projects
- Deep understanding of human antibody libraries and antibody selection methods
- Provider of SpyTag/SpyCatcher reagents

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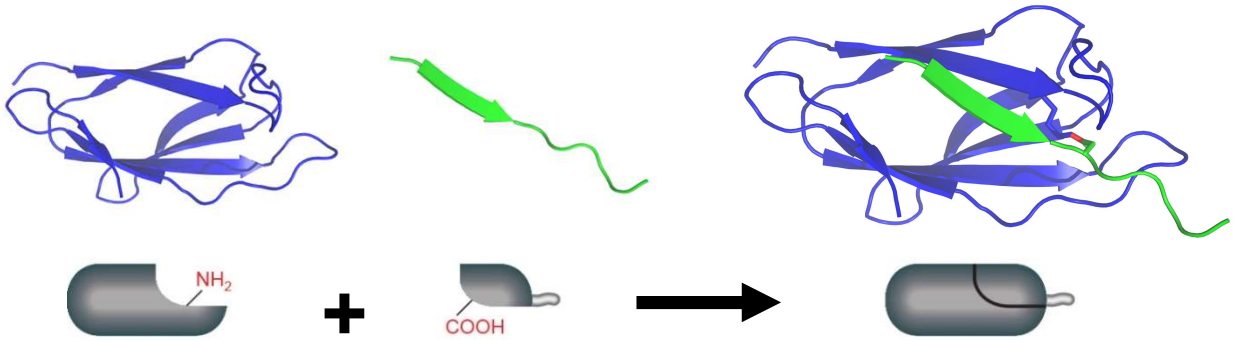
SpyTag Technology*

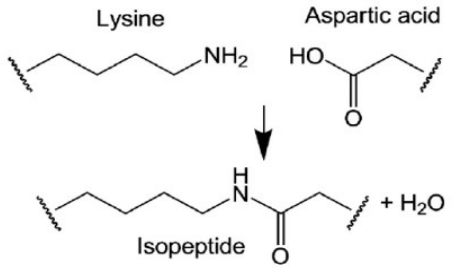


SpyCatcher
113 aa, 12 kDa
No cysteine

SpyTag
13 aa
Reactive at N- or C-terminus or internal

Ligated proteins

- Spontaneous (autocatalytic) reaction
- Covalent isopeptide bond formation, irreversible
- Fast, quantitative reaction
- pH 5–8, temperature 4°C to 37°C
- Unaffected by buffer conditions, Ca^{2+}/Mg^{2+} not needed
- Unaffected by detergents
- Reaction also occurs inside cells (in vivo)



Zakeri B et al. (2012). PNAS 109, E:690–697.

*Bio-Rad has an exclusive license for SpyTag technology in combination with antibodies

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Background

SpyTag/SpyCatcher developed by the Howarth Lab at the University of Oxford (published 2012[1])

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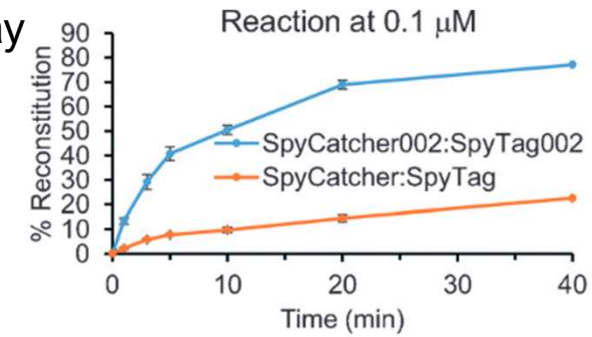
- ~10 times faster than version 1
- Fully compatible with version 1



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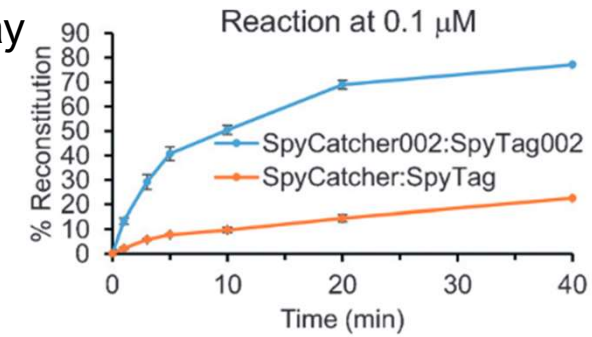

Optimized version (SpyTag3/SpyCatcher3) developed by rational design (published 2019[3])

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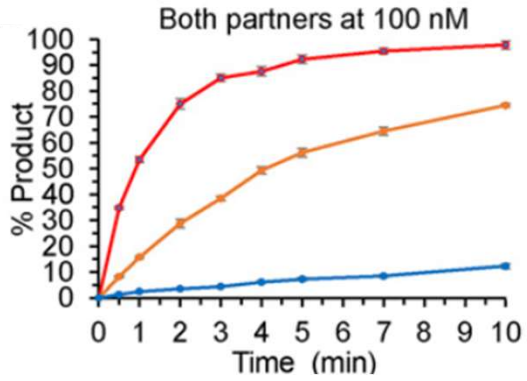
- Fully compatible with version 1 and 2



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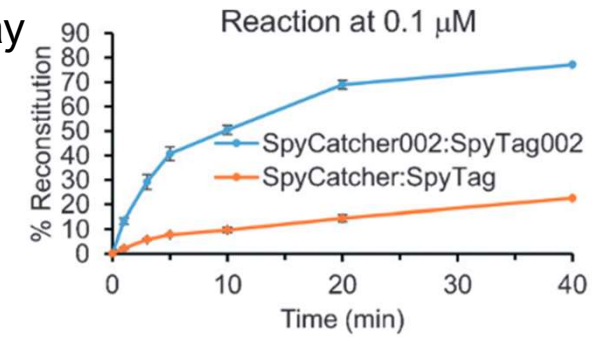

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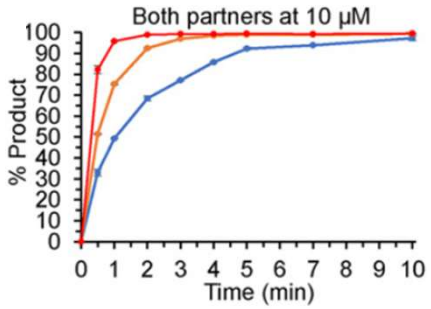

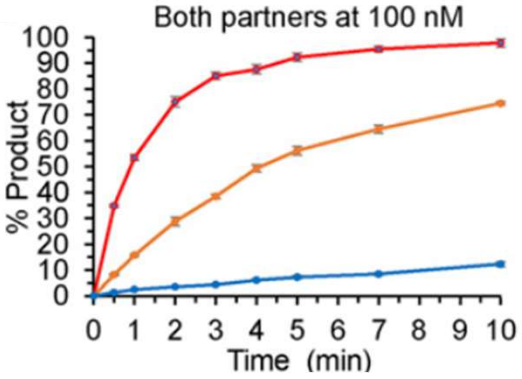

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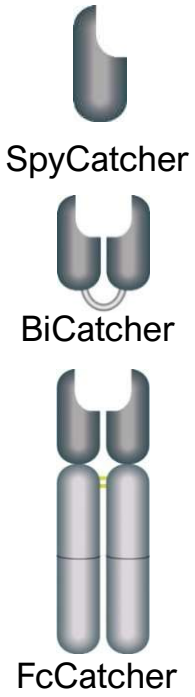
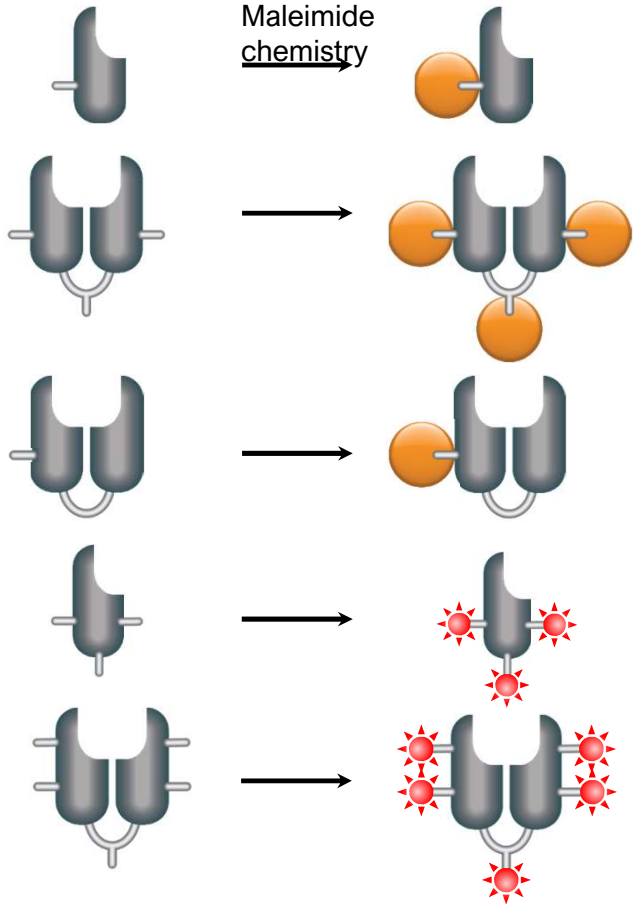
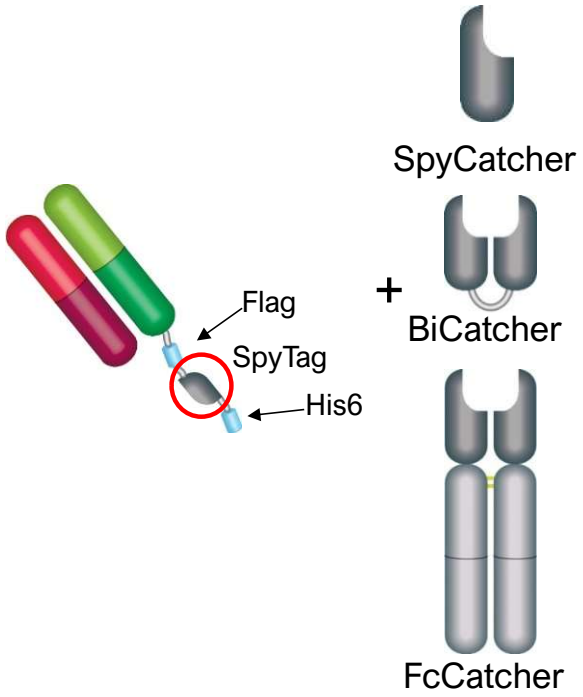
Development of Catcher Constructs



- Cysteines introduced into SpyCatcher
- Site-specific labeling
- Fixed degree of labeling
- High batch-to-batch consistency



One Antibody, Multiple Formats in an Instant



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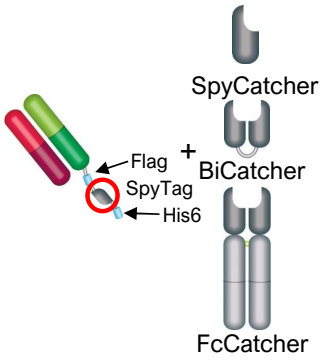

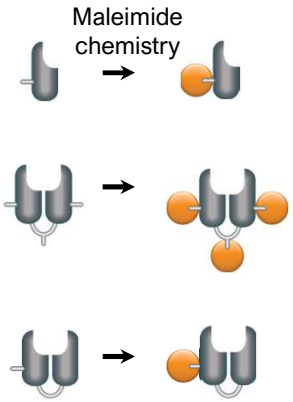

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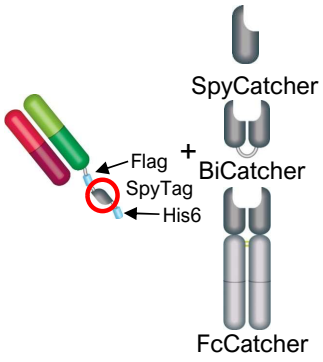

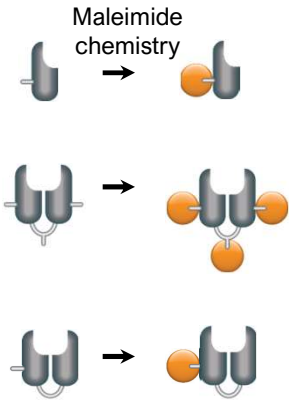

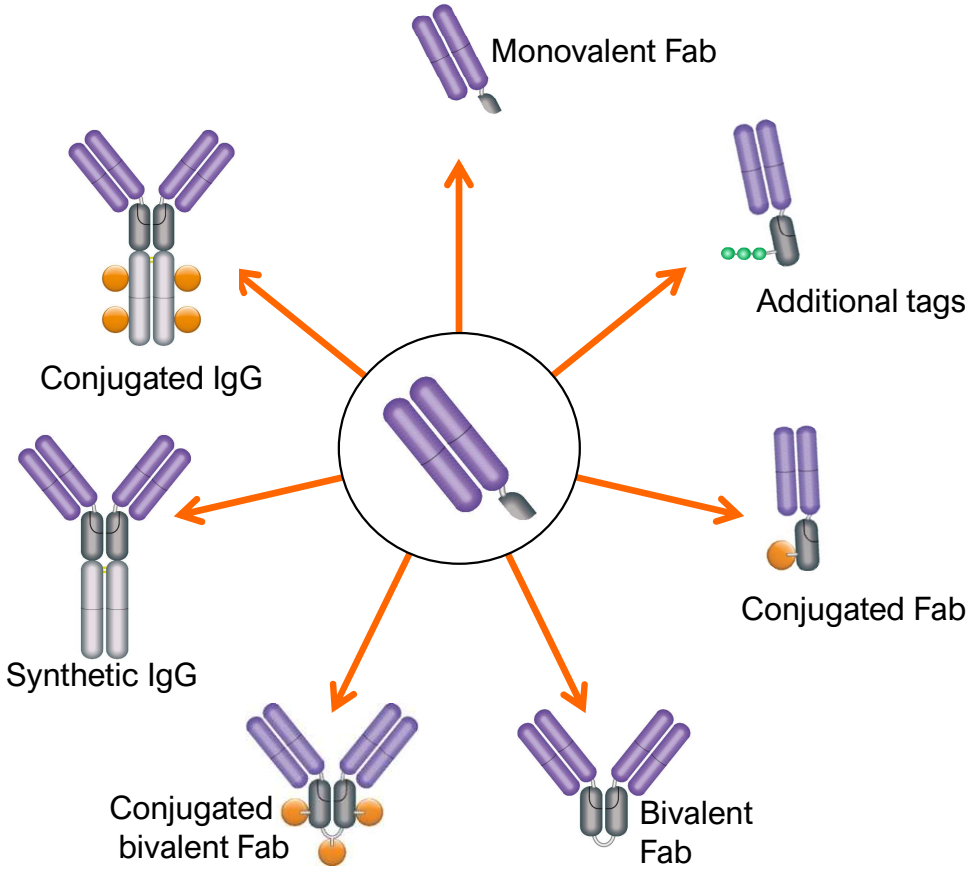

TrailBlazer

- Simplest protocol
- 1 hr reaction
- No purification
- Fully scalable
- Improved performance
- Accelerated workflow
- Economical

Hentrich et al. (2021). Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. Cell Chemical Biology 28, 1–12.

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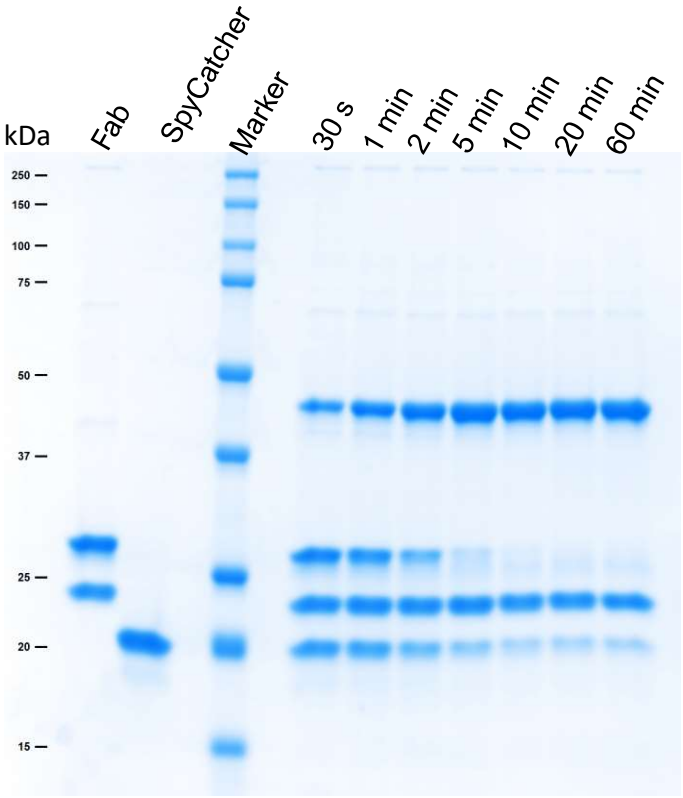
SpyTag Fab + SpyCatcher Coupling Reaction

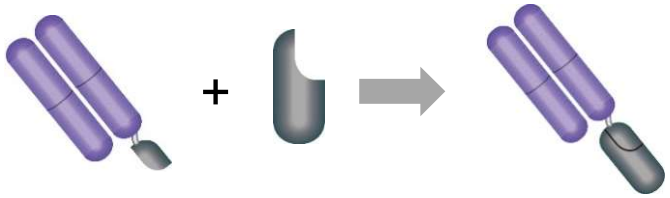


Fab-HC-SpyCatcher

Fab HC (with SpyTag)
Fab LC
SpyCatcher

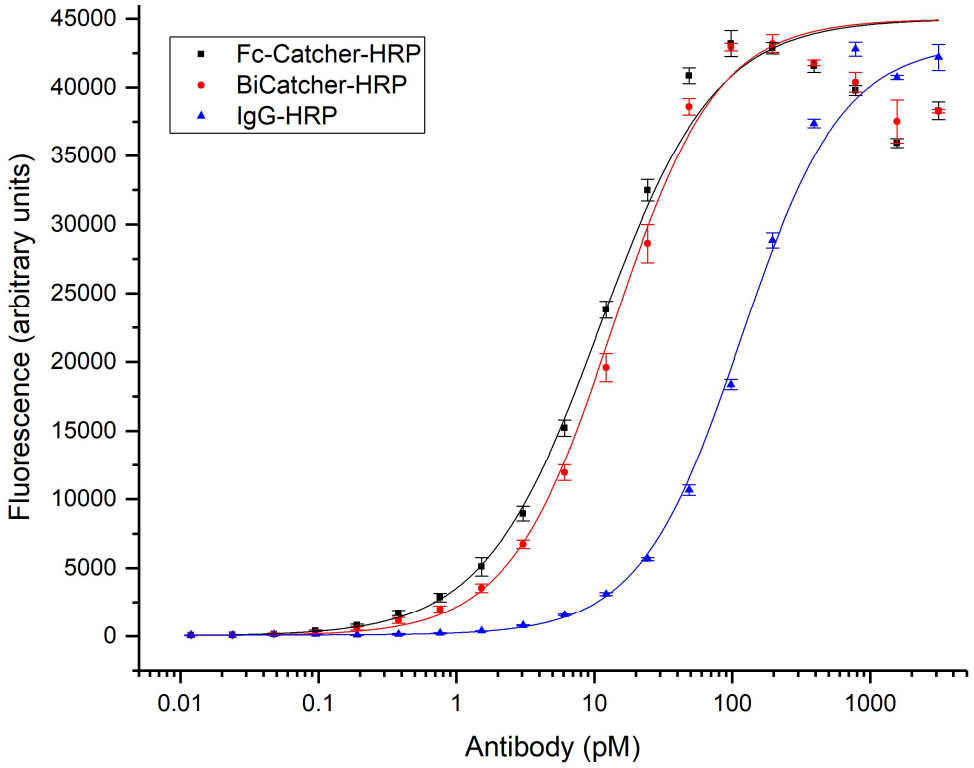
Coupling at room temperature
Ratio Fab:Catcher = 1:1.25
4 µM Fab + 5 µM SpyCatcher

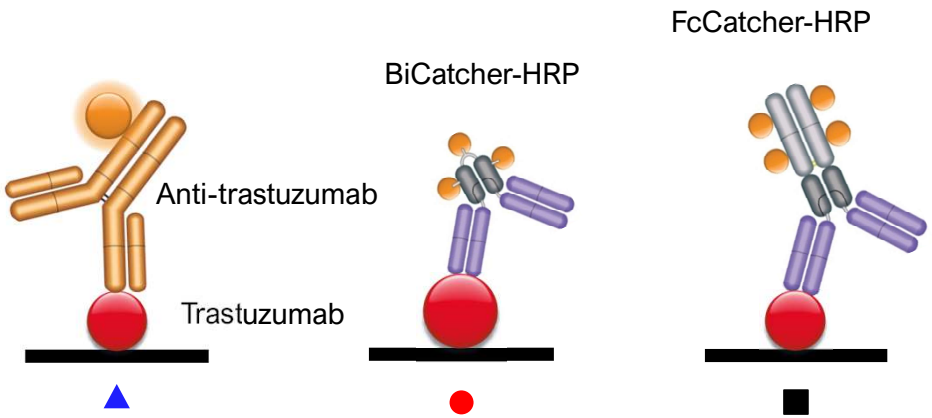
Nonreduced samples;
3 µg Fab loaded per lane



Assay Performance

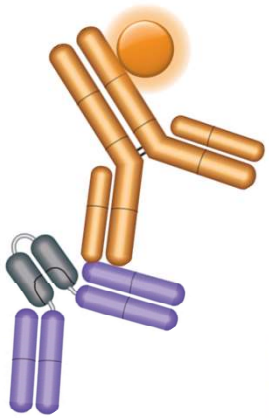
Site-Directed Labeling Leads to Better Performance



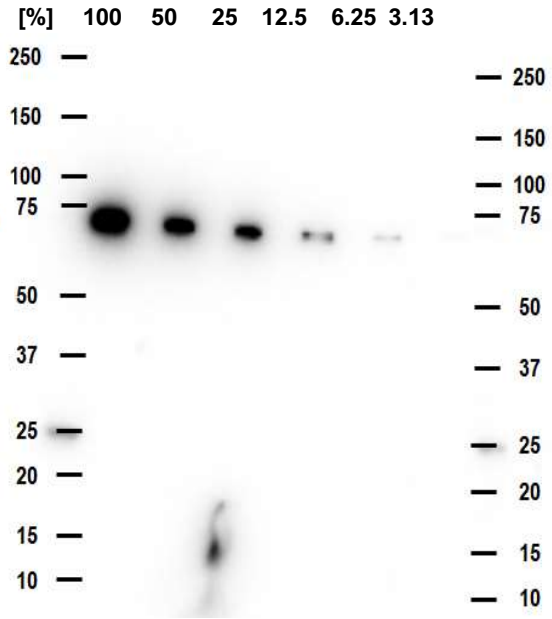


- No modification of the antibody binding site
- Better sensitivity with site-directed conjugation

Direct Detection is Better than Secondary Antibody Detection

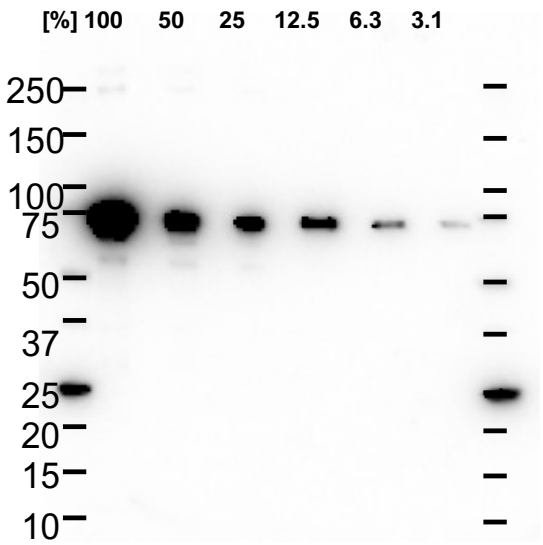


Anti-HSPA5 Fab-BiCatcher detected with anti-F(ab')$_2$-HRP

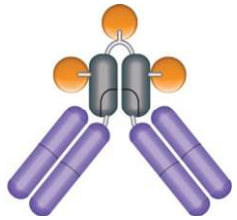


Fab-BiCatcher 10 nM
2.5 s exposure time

Anti-HSPA5 Fab-BiCatcher-HRP direct detection



Fab-BiCatcher-HRP 10 nM
1.5 s exposure time



- Increased sensitivity
- Faster

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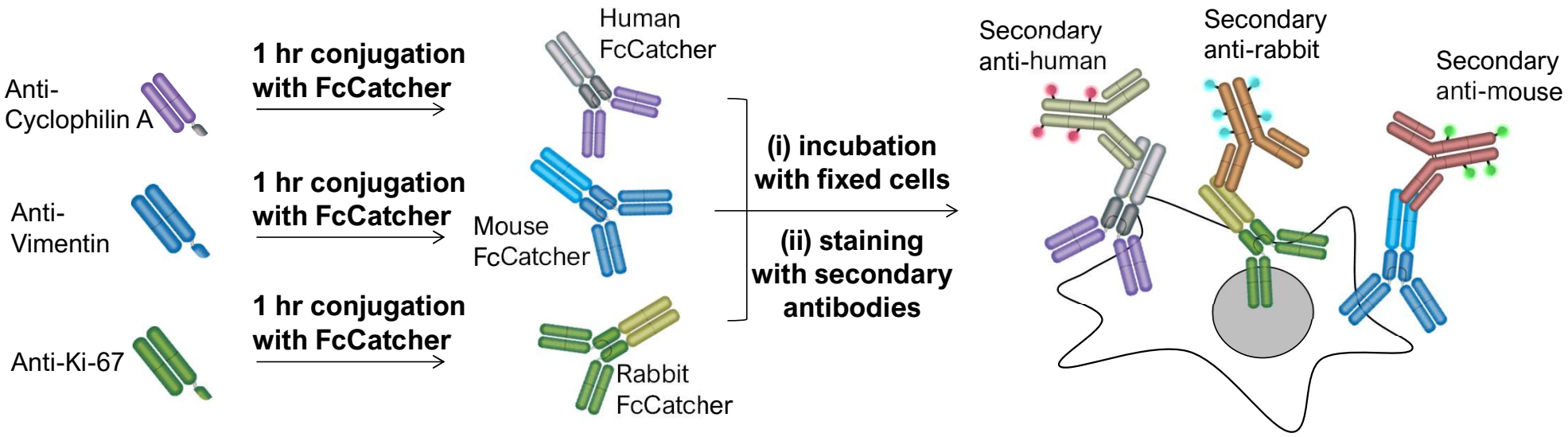
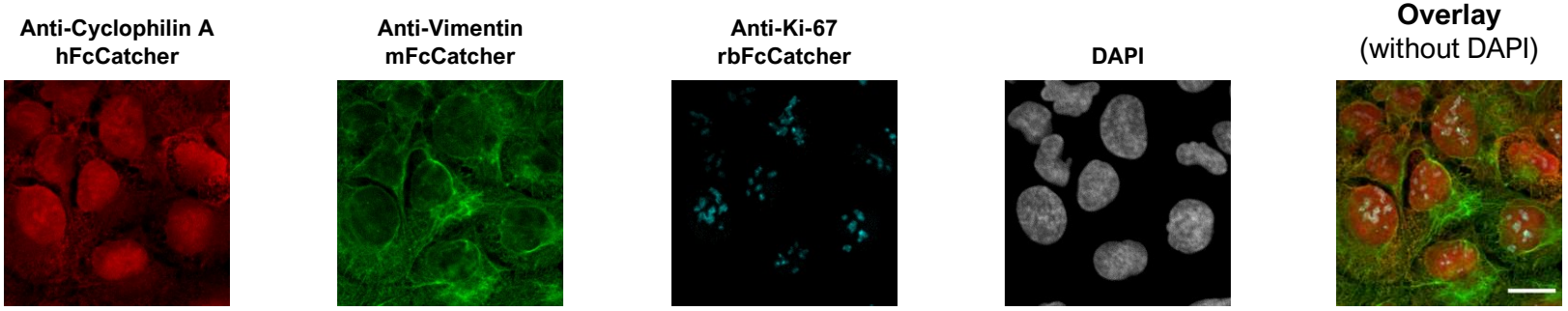
Multiplex Immunofluorescence





U2OS cells

bar = 20 µm

Three different Fabs conjugated with three FcCatchers were simultaneously incubated with the fixed cells, and, after washing, three different fluorescently labeled secondary antibodies were incubated simultaneously with the sample.

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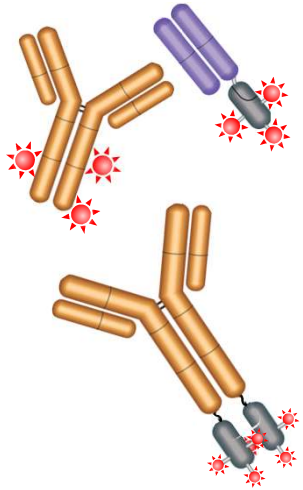
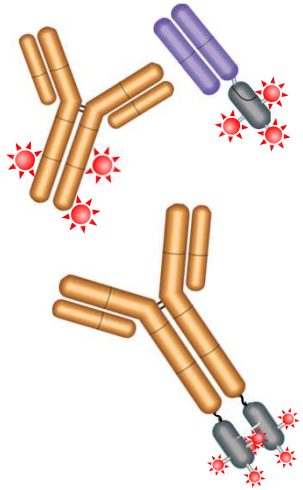
Fluorescence Catchers

- Coupling of Fabs and IgGs to SpyCatcher DyLight or BiCatcher DyLight conjugates



Fluorescence Catchers

- Coupling of Fabs and IgGs to SpyCatcher DyLight or BiCatcher DyLight conjugates
- Better performance than conventionally labeled IgG (Alexa Fluor, catalog product)



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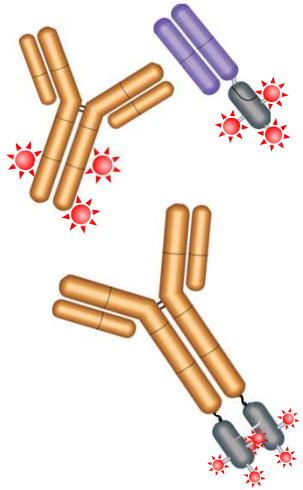

Staining of PBMC with anti-CD3 and flow assay at best stain index (12.5 nM for D488, D550, 6.25 nM for D650)

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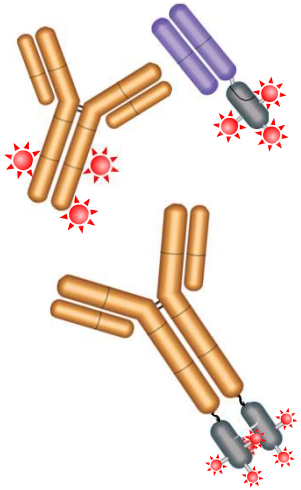

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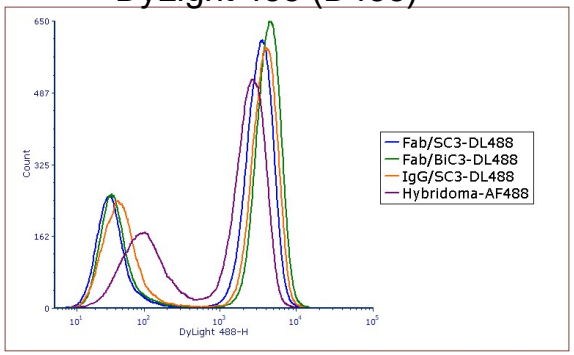
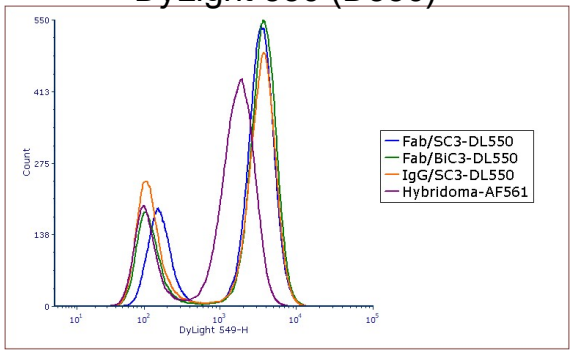
DyLight 488 (D488)



DyLight 550 (D550)



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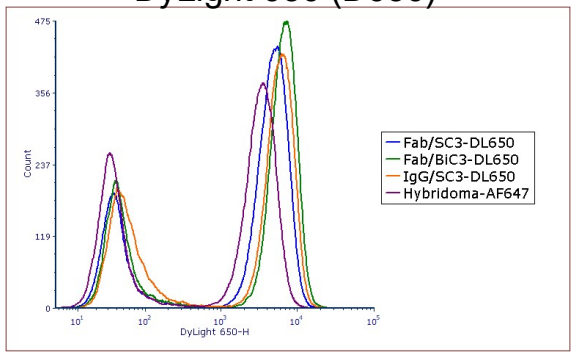

Recently launched for custom antibody generation projects

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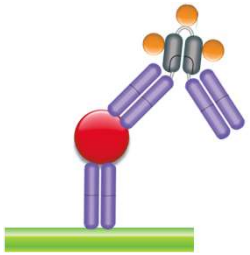
More Assay Options

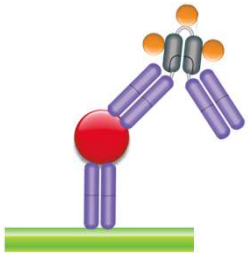
- Accelerating the identification of sandwich pairs through simple site-specific labeling

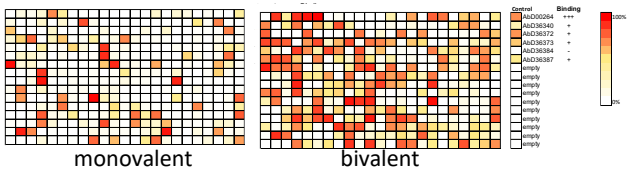


More Assay Options

- Accelerating the identification of sandwich pairs through simple site-specific labeling

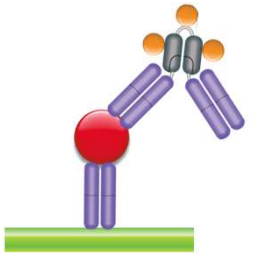
- Screening antibodies in mono- and bivalent format in parallel

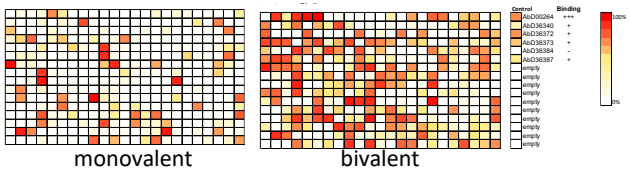


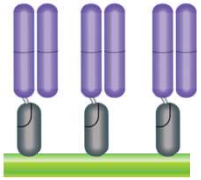


More Assay Options

- Accelerating the identification of sandwich pairs through simple site-specific labeling
- Screening antibodies in mono- and bivalent format in parallel
- Oriented immobilization on SpyCatcher surfaces (e.g., coated plates, bead etc.)







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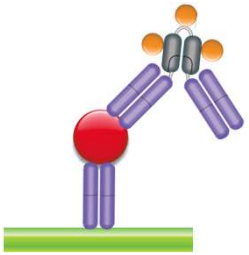
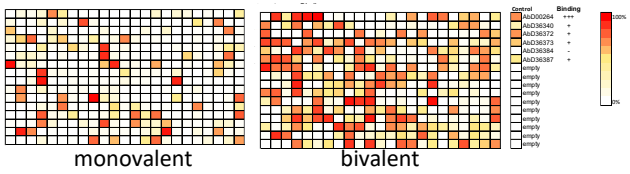
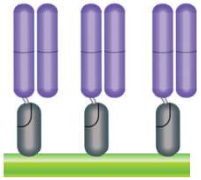
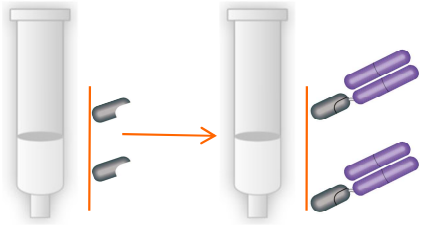
More Assay Options

- Accelerating the identification of sandwich pairs through simple site-specific labeling
- Screening antibodies in mono- and bivalent format in parallel
- Oriented immobilization on SpyCatcher surfaces (e.g., coated plates, bead etc.)
- Simple generation of affinity columns by immobilization of antibodies on SpyCatcher resin









SpyDisplay

Phage Display Using SpyTag

Phage Display Advantages

Most established and robust system for protein selections

- Large libraries of >10^{11} possible
- Fast protocol

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Naïve synthetic antibody library

- Defined germline genes, codon-optimized
- Large library size compared to natural repertoire
- Suitable for:
  - Low immunogenic antigens e.g., conserved molecules, immunosuppressants
  - Toxins
  - Vesicles, viruses, cells
  - Protein complexes
- Human antibodies

Phage Display Advantages

Most established and robust system for protein selections

- Large libraries of >10^{11} possible
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<u>Naïve synthetic antibody library</u>

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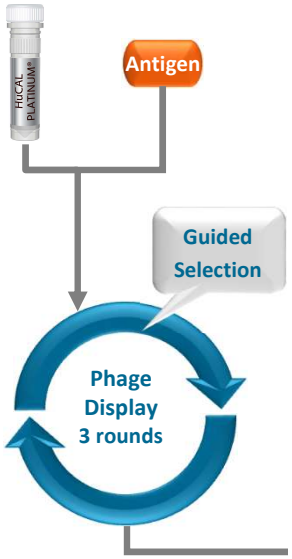
  - Low immunogenic antigens e.g., conserved molecules, immunosuppressants
  - Toxins
  - Vesicles, viruses, cells
  - Protein complexes
- Human antibodies

<u>In vitro method</u>



BIO-RAD

Phage Display Advantages

Most established and robust system for protein selections

- Large libraries of >10^{11} possible
- Fast protocol

<u>Naïve synthetic antibody library</u>

- Defined germline genes, codon-optimized
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- Guided selection:
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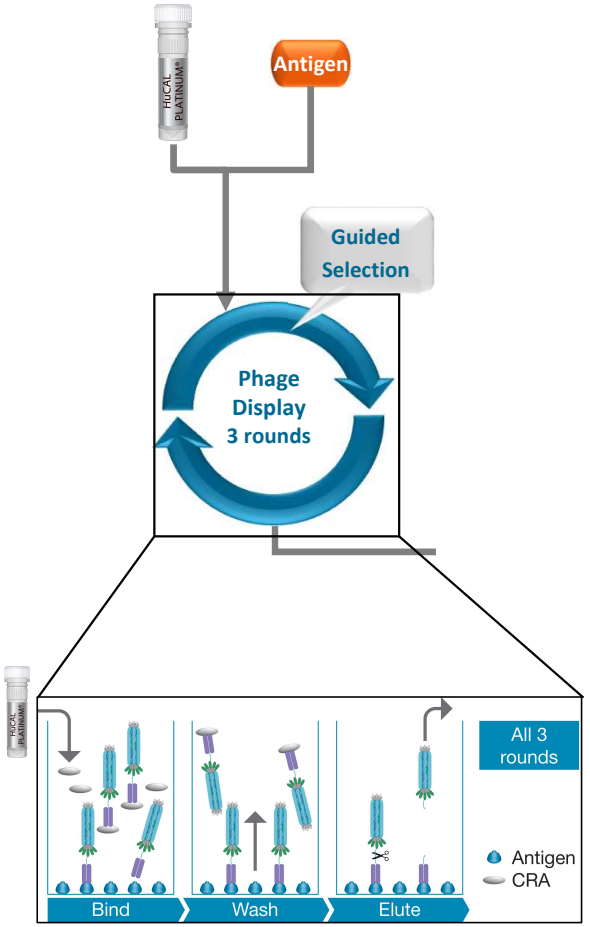

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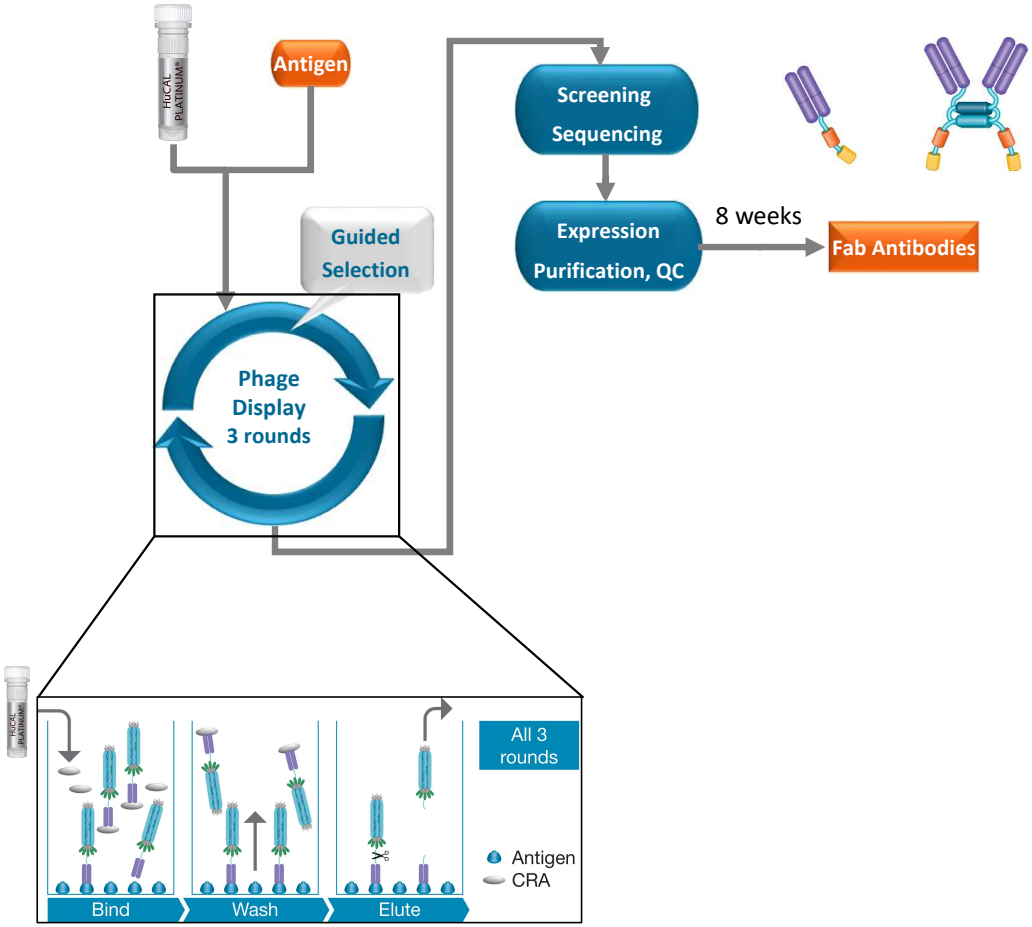
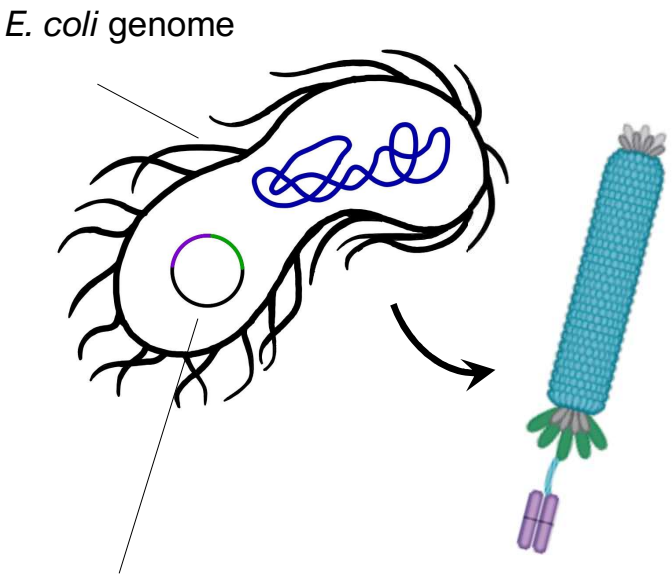
<u>In vitro method</u>

- Guided selection:
  - Block with closely related antigens
  - Alternate antigens between rounds for cross-reactivity
  - Adjustable panning conditions (buffer, salt, etc.)
- Fast — antibodies in 8 weeks (even 4 weeks possible)
- High success rate

Affinity improvement possible (affinity maturation)



Conventional Phage Display

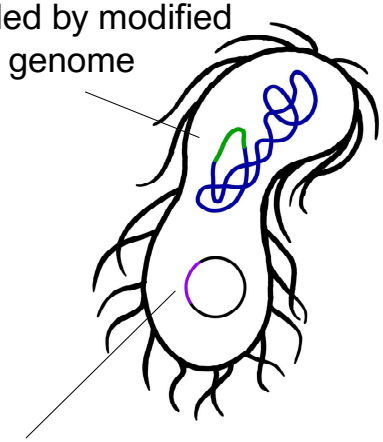


- Phagemid encodes genetic Fab-pIII fusion protein
- Helper phage infection introduces DNA for other phage proteins
- Wt pIII is incorporated faster into the phage resulting in:
  - Monovalent display of Fab on phage
  - Many phage without Fab

BIO-RAD

SpyDisplay

SpyCatcher-pIII fusion encoded by modified *E. coli* genome



Fab encoded by phagemid, same as expression vector

Helper phage encodes other phage proteins

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BIO-RAD

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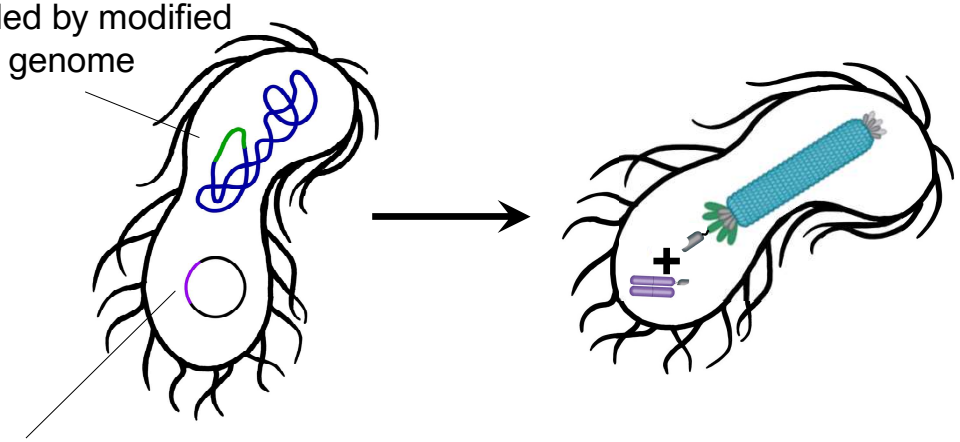

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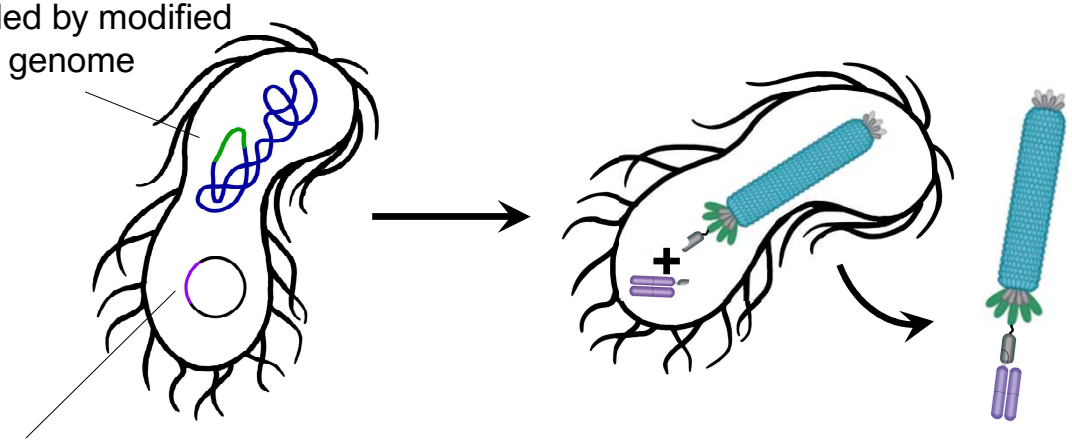

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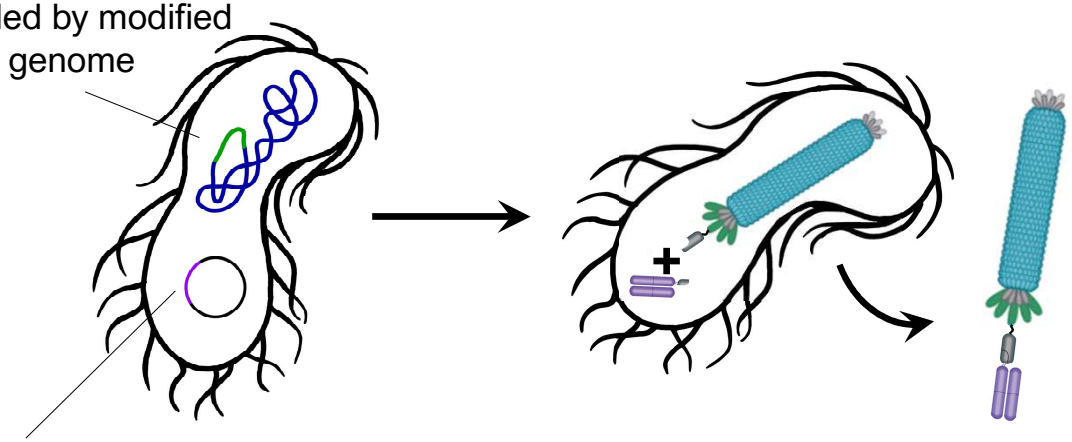

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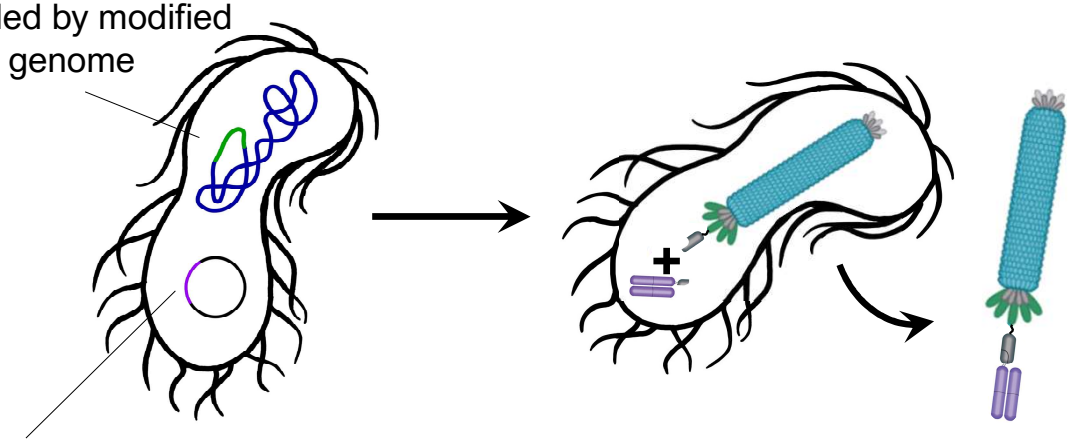

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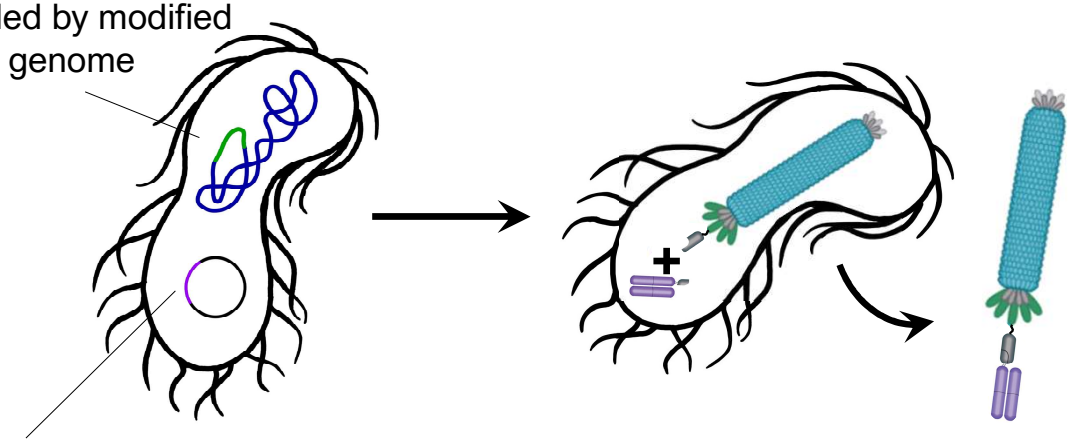

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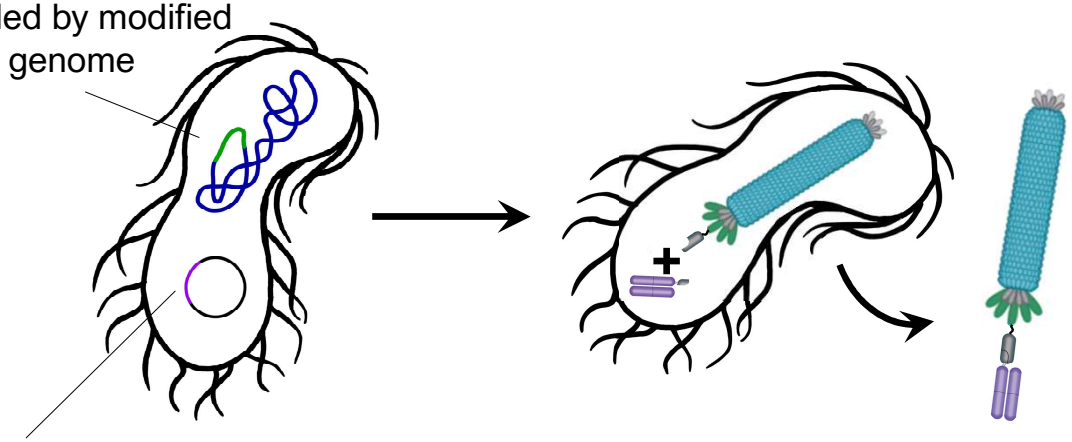

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- Resulting antibodies carry a SpyTag, compatible with the TrailBlazer Platform
- Monovalent display for selection of high-affinity antibodies
- Polyvalent display possible for antibodies against difficult targets

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Additional Advantages

N-terminal display of proteins:

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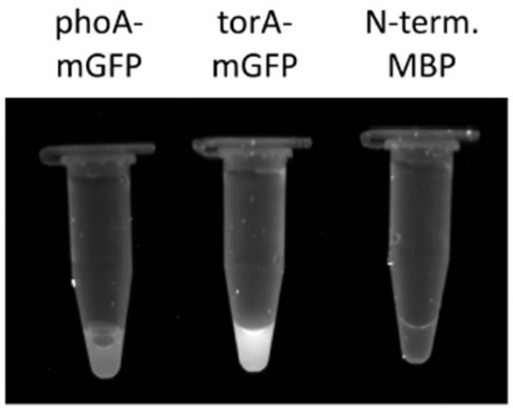


5 x 10^{11} cfu/ml

GFP only efficiently matures its fluorescence when expressed in the cytoplasm:

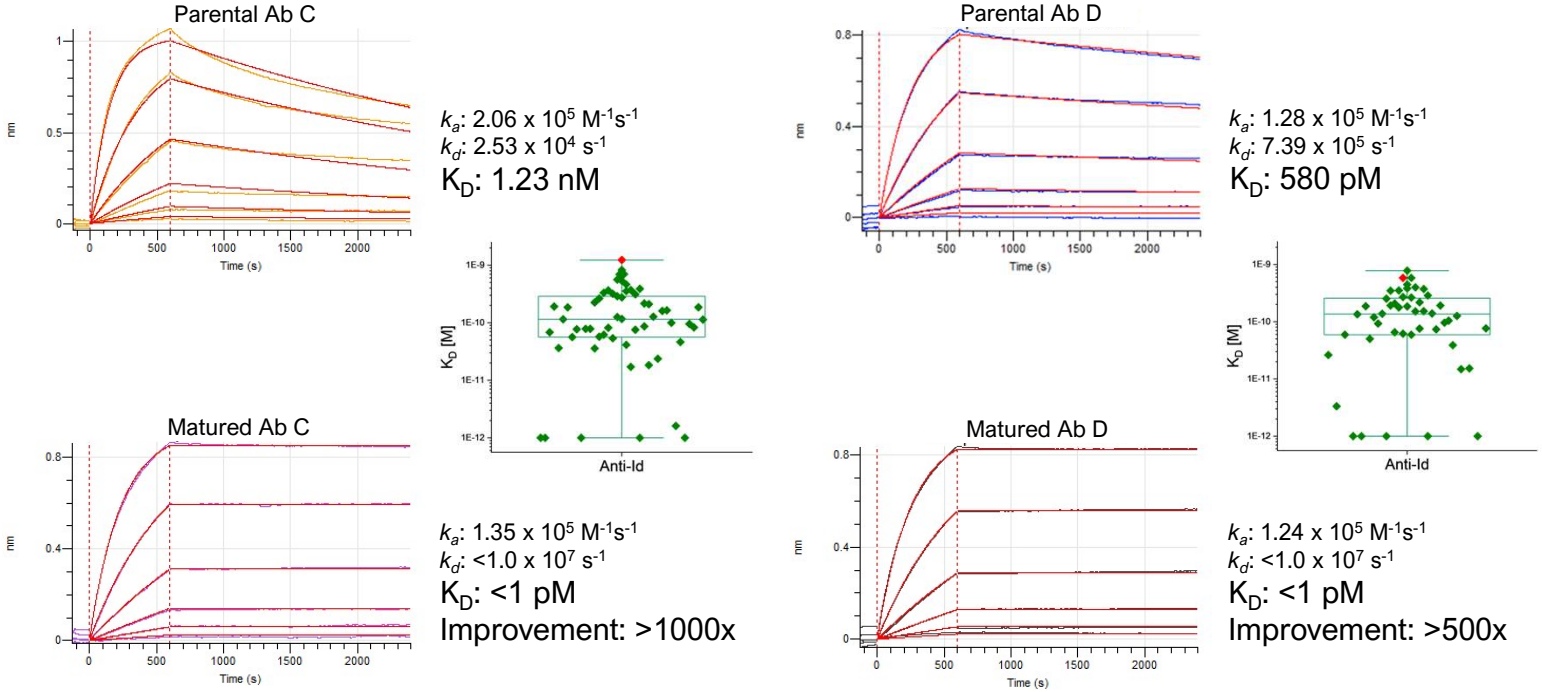
- phoA signal sequence (Sec) leads to phage with weak fluorescence
- torA signal sequence (TAT) leads to phage with strong fluorescence

Kellmann et al. (2023). SpyDisplay: A versatile phage display selection system using SpyTag/SpyCatcher technology. mAbs,15, 1-13

SpyDisplay Enables Selection of Ultra-High Affinity Antibodies

Affinity maturation with SpyDisplay selections

BLI sensograms of parentals and best matured binders

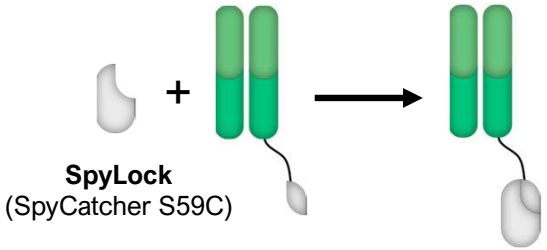


SpyLock

Rapid Generation of Bispecific Antibodies

SpyLock: Reversibly Inhibitable SpyCatcher

SpyCatcher S59C (= **SpyLock**) is fully catalytically active



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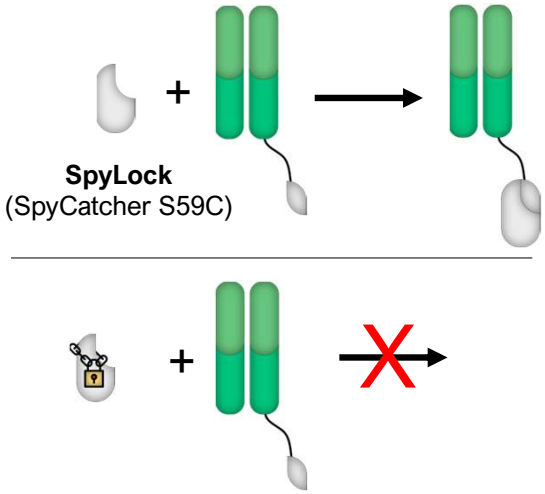
Locked SpyCatcher:

- Reacting the introduce cysteine with a disulfide bond-forming reagent

→ Inhibition of SpyCatcher reactivity to SpyTag



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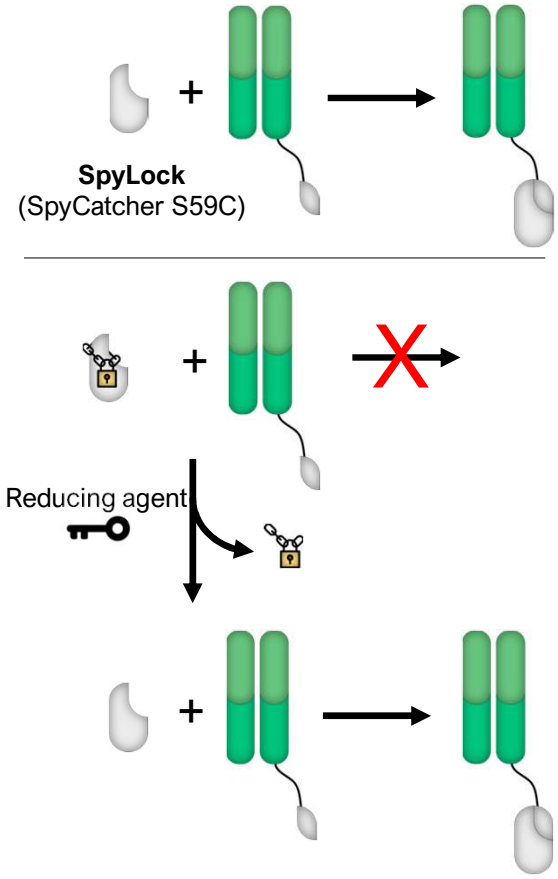

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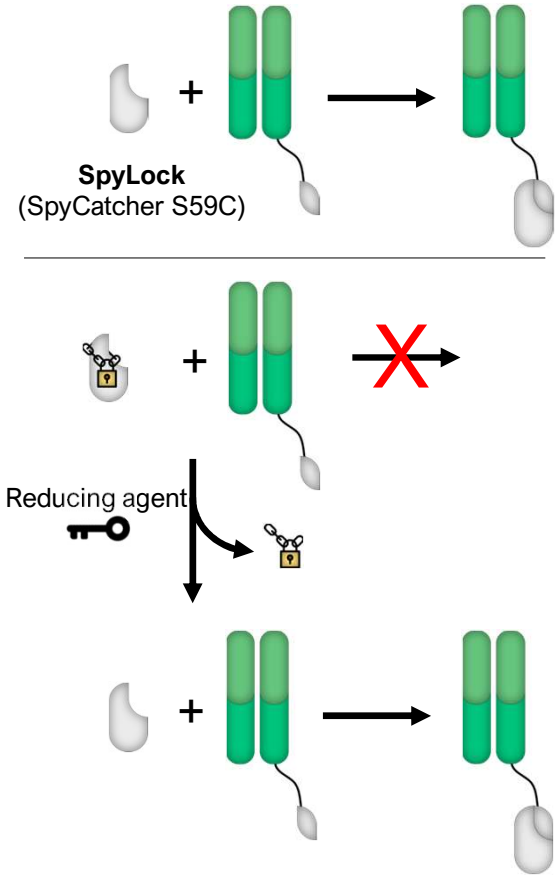
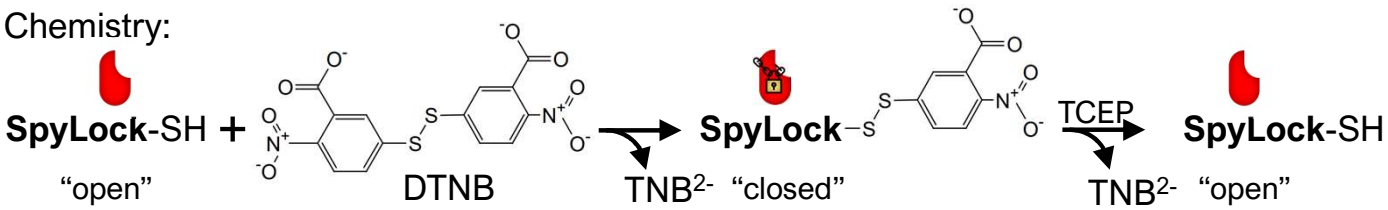


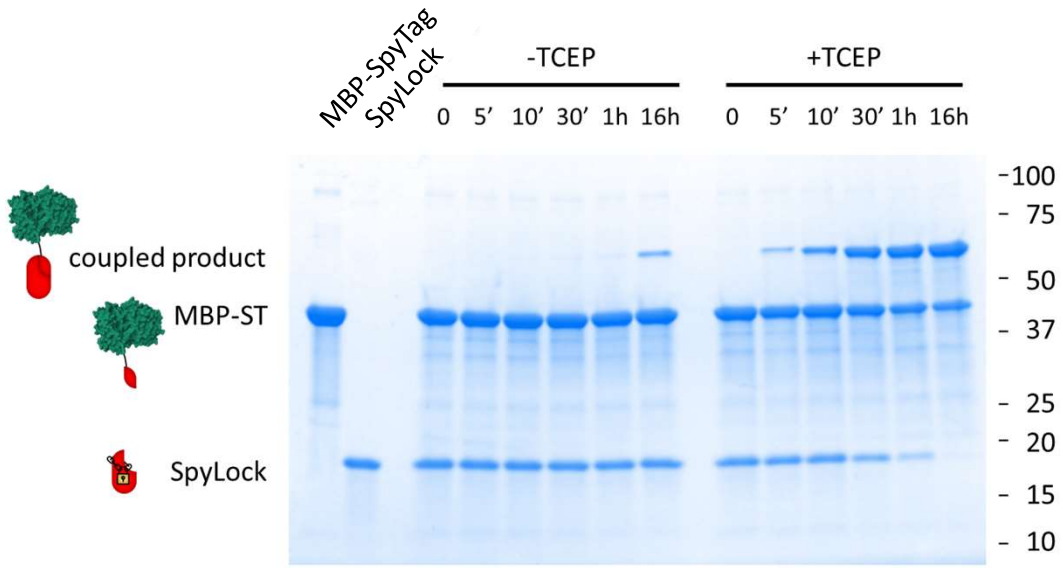
Chemistry:



SpyLock: Reversibly Inhibitable SpyCatcher

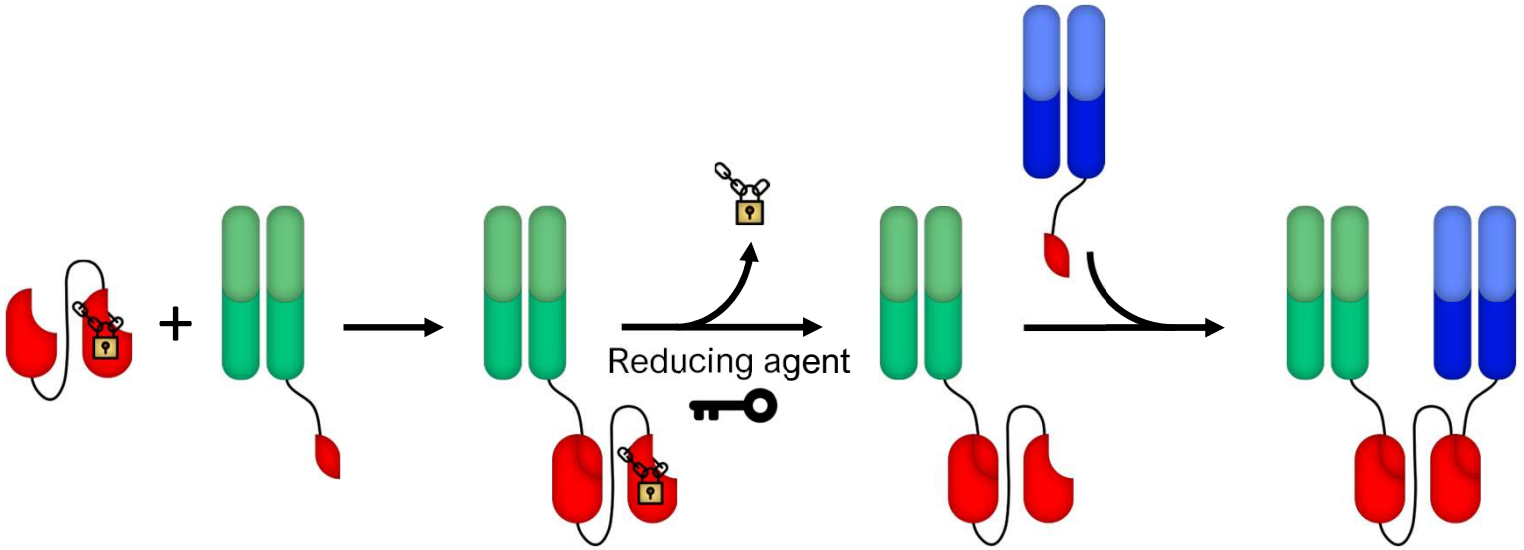
Locked SpyLock without reducing agent (TCEP) does not react with MBP-SpyTag

Upon cleavage of the disulfide bond with TCEP, full reactivity towards SpyTag can be restored



SpyLock for Bispecific Antibodies

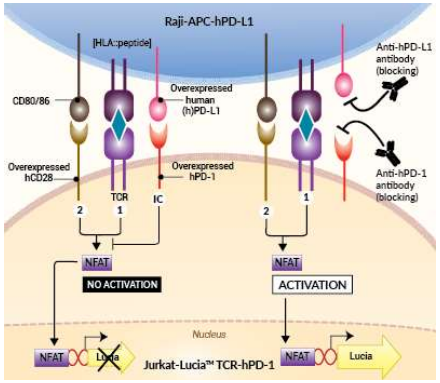
A novel, fast, and scalable method to generate bispecific antibodies for screening:



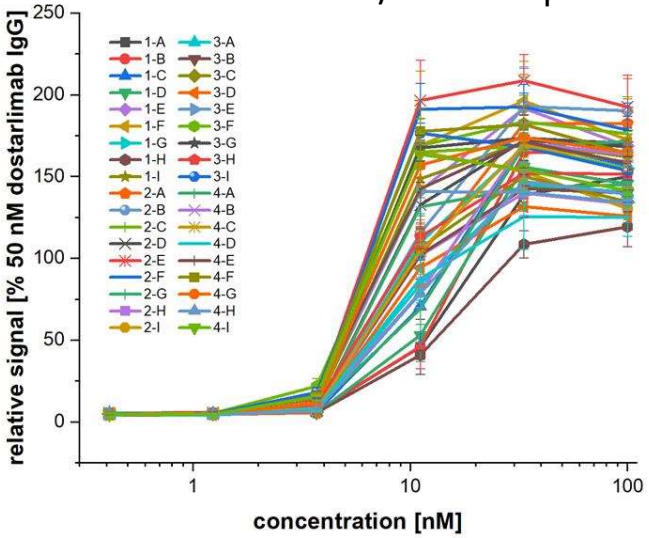
BIO-RAD

Benefits

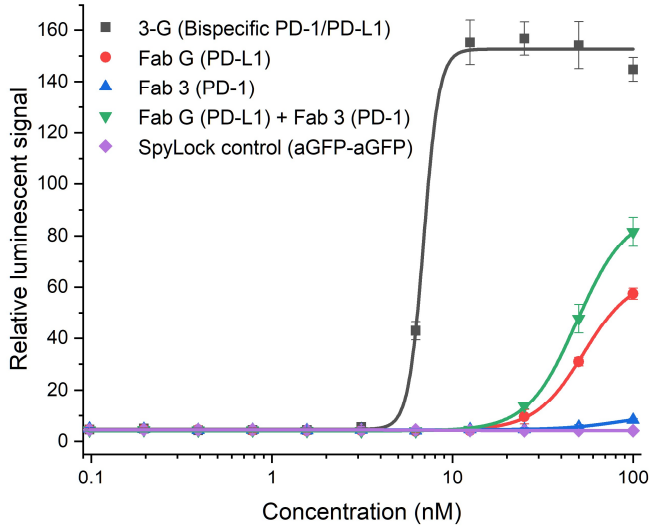
- Generation of bispecific antibodies with high yield and purity (>90%)
- Rapid generation of a large number of bispecific antibody combinations
- Rapid economic screening for the most interesting antibody pairs



Functional cell assay screening of anti-PD-1/PD-L1 bispecifics



Bispecific versus monospecific abs



The Pioneer Library

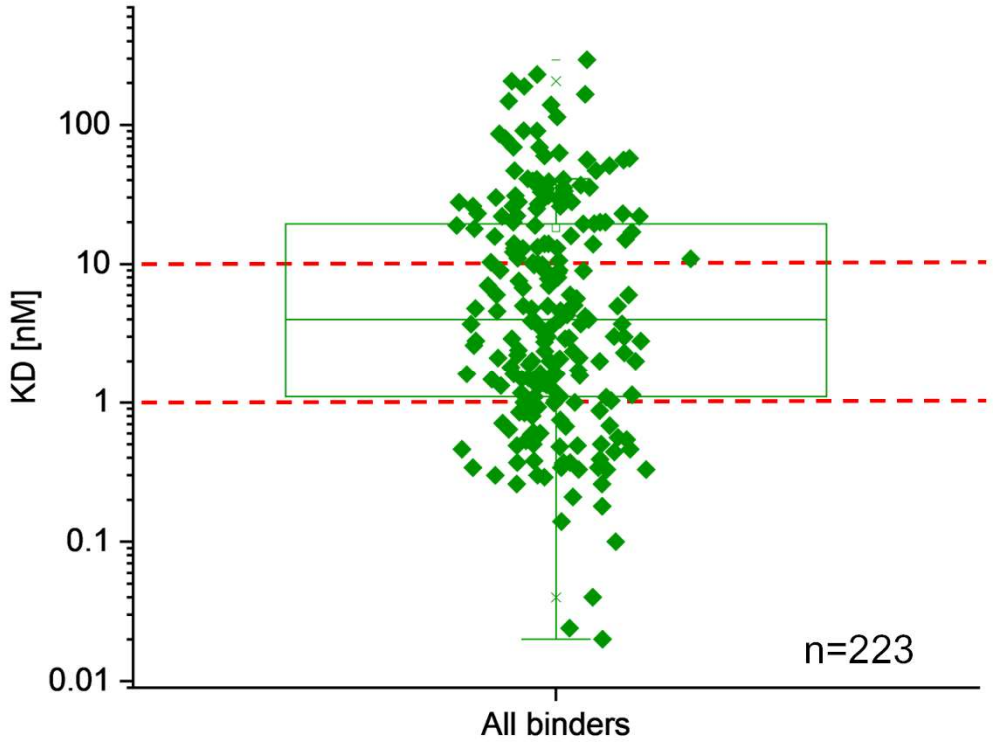
Pioneer Library

- A new, state-of-the-art Fab phage display library:
  - One of the largest functional phage display antibody libraries ever made
  - Optimized for phage display
  - Optimized for the generation of therapeutic lead candidates
    - Selected framework genes
    - CDR design is close to nature
    - Optimized for good antibody developability
- Uses SpyDisplay for selection
- Compatible with the TrailBlazer Platform for faster and more versatile screening and characterization
- Rapid access to bispecific antibodies through SpyLock technology

BIO-RAD

Pioneer Delivers Great Affinities

Test selections on 4 protein antigens (Ox40, IL6, GFP, IgG anti-Id)



23% of antibodies have affinities **< 1 nM,** which in most cases makes further affinity maturation superfluous.

BIO-RAD

Generation of Lead Candidates Against TIGIT

- TIGIT: immune receptor involved in T cell regulation with a potential role in cancer immunotherapy, over 30 clinical trials ongoing
- Generation and characterization of Pioneer antibodies and comparison to antibodies in clinical phase 3
- Great sequence diversity → antibodies against eight bins identified for a small protein (~16 kDa)
- Antibodies with excellent properties and developability directly out of the library without further optimization

Antibody	Monovalent KD [nM]	IgG Tm1 [°C]	IgG Tm2 [°C]	SEC	SI-BLI	Hydro-phobicity HIC	Poly-reactivity	Stability (4 weeks, 37°C)	EC_{50} in cellular assay [µg/ml]	Germline homology VL	Germline homology VH
Vibostolimab	0.3	65.2	78.2	99%	0.32	6.33	+	+	0.9	90%	82%
AbD54577	0.2	64.6	73.6	98%	0.31	5.73	+	+	0.8	92%	88%

BIO-RAD

Summary

TrailBlazer Platform

- Rapid change of antibody valency, species, or isotype
- Rapid site-specific labeling with prefabricated Catcher conjugates
- Rapid access to many more assay set-ups

SpyDisplay selection technology

- Fast and efficient selection technology, well suited for high-affinity antibodies
- New selection options (N-terminal display or TAT pathway)

Pioneer Antibody Discovery Platform

- One of the largest functional antibody phage display libraries
- High hit rate, great antibody diversity with excellent affinities
- Very good antibody developability
- Fast selection, screening, and characterization through SpyDisplay and TrailBlazer technology

SpyLock technology

- Rapid generation of bispecific antibodies with high purity and yield
- Ideal for screening for the best antibody pairs

Availability

- Spy-, Bi-, and FcCatcher are available for custom antibody generation projects and through our catalog (for research use only)
- Biotin and HRP conjugates are available in the catalog
- DyLight conjugates are available for custom projects, catalog will follow next year
- More conjugates will be launched in the future
- Bio-Rad has an exclusive license for SpyTag technology for antibody applications

Please inquire for non-research licenses

Email: antibody_tech_uk@bio-rad.com

Visit: bio-rad-antibodies.com/spycatcher

BIO-RAD

Visit our website at bio-rad-antibodies.com for more information.