Validation of Commercial tool Antibodies

The Antibody Society Webcast series – Antibody Validation #10

Specific detection reagents: what's the future?

Simon L. Goodman
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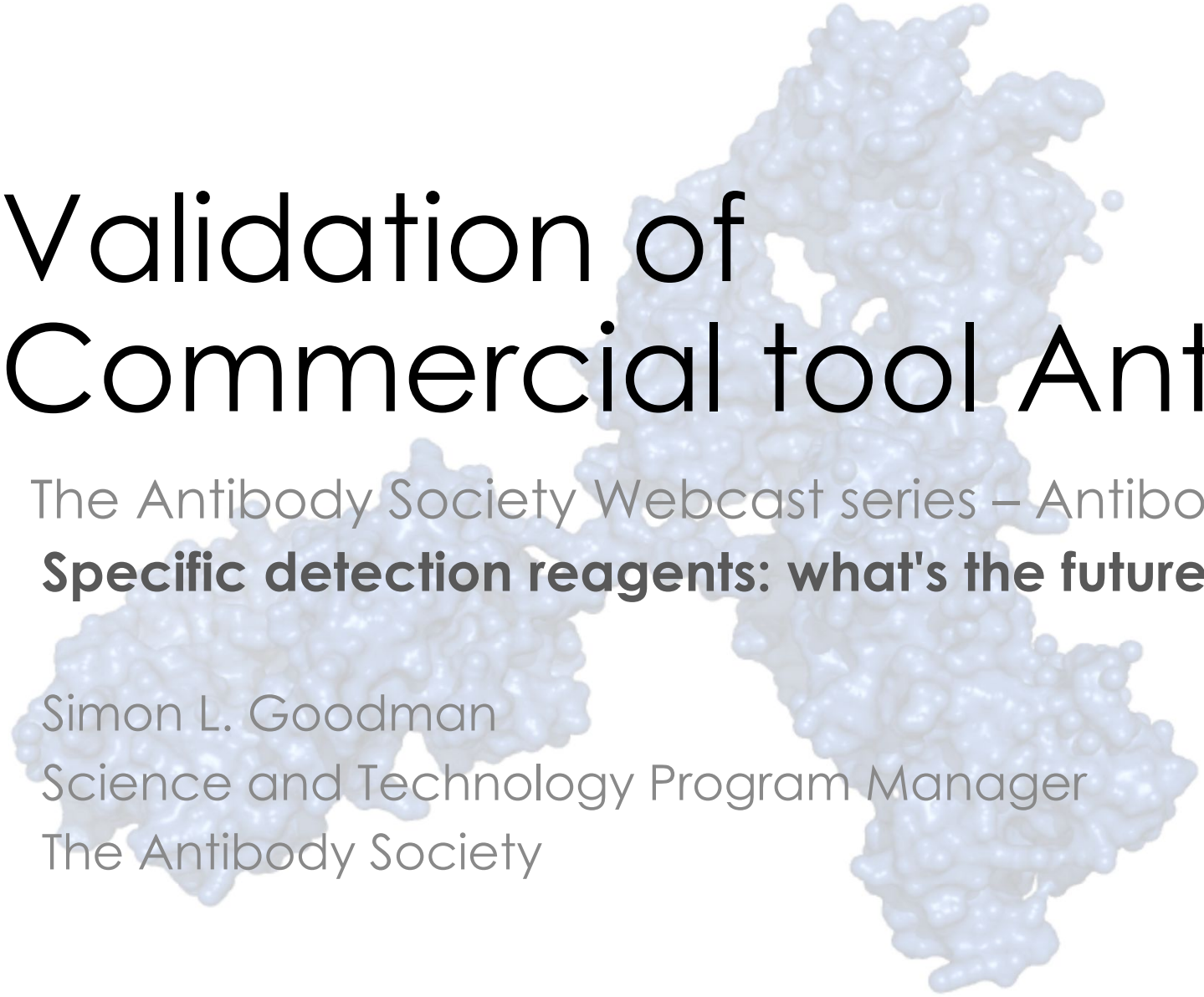
The Antibody Society



Antibody Validation: a 10-part series

1.	Andreas Plückthun	:	The different antibody formats
2.	Glenn Begley	:	Antibodies and the reproducibility crisis in biological science
	Cecilia Williams	:	The Erß story – is your antibody like this?
3.	Jan Voskuil	:	Beware the supplier OEM
	Andy Chalmers	:	Finding antibodies in the Antibody Databases
4.	Anita Bardowski	:	Which antibody are you looking for? The RRID
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Specific detection reagents: What's the future?

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Andreas Plückthun
University of Zurich

For biological research, we need specific binding reagents

Historically, these have always been antibodies

- Initially, antisera or polyclonal antibodies
- Later, antigen-purified polyclonal antibodies
- Then monoclonal antibodies
- Finally recombinant antibodies

We have heard at great length about the importance of quality control for specificity.

This requires application-specific testing.

This does not go away, no matter what we use as reagent!

So it is also true for this section!

For biological research, we need specific binding reagents

Historically, these have always been antibodies

- Initially, antisera or polyclonal antibodies
- Later, antigen-purified polyclonal antibodies
- Then monoclonal antibodies
- Finally recombinant antibodies

But the development of recombinant technologies has made us independent of using antibodies as binding reagents!

We only need two ingredients:

1. A "repertoire" or "library" of variants of a binding protein (like an antibody library)
2. A selection technology (like phage display or similar)

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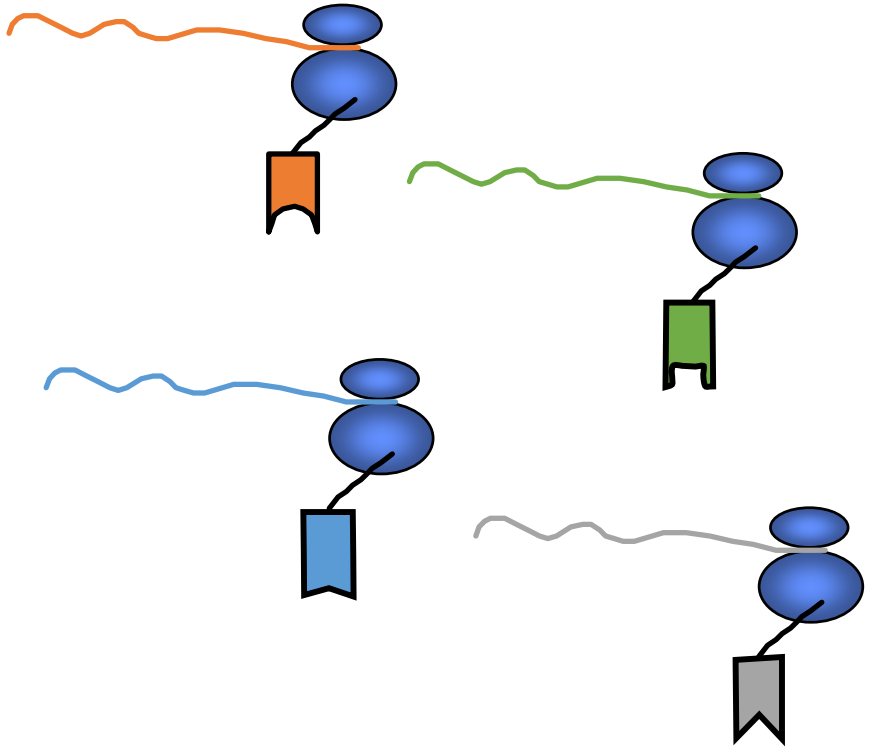
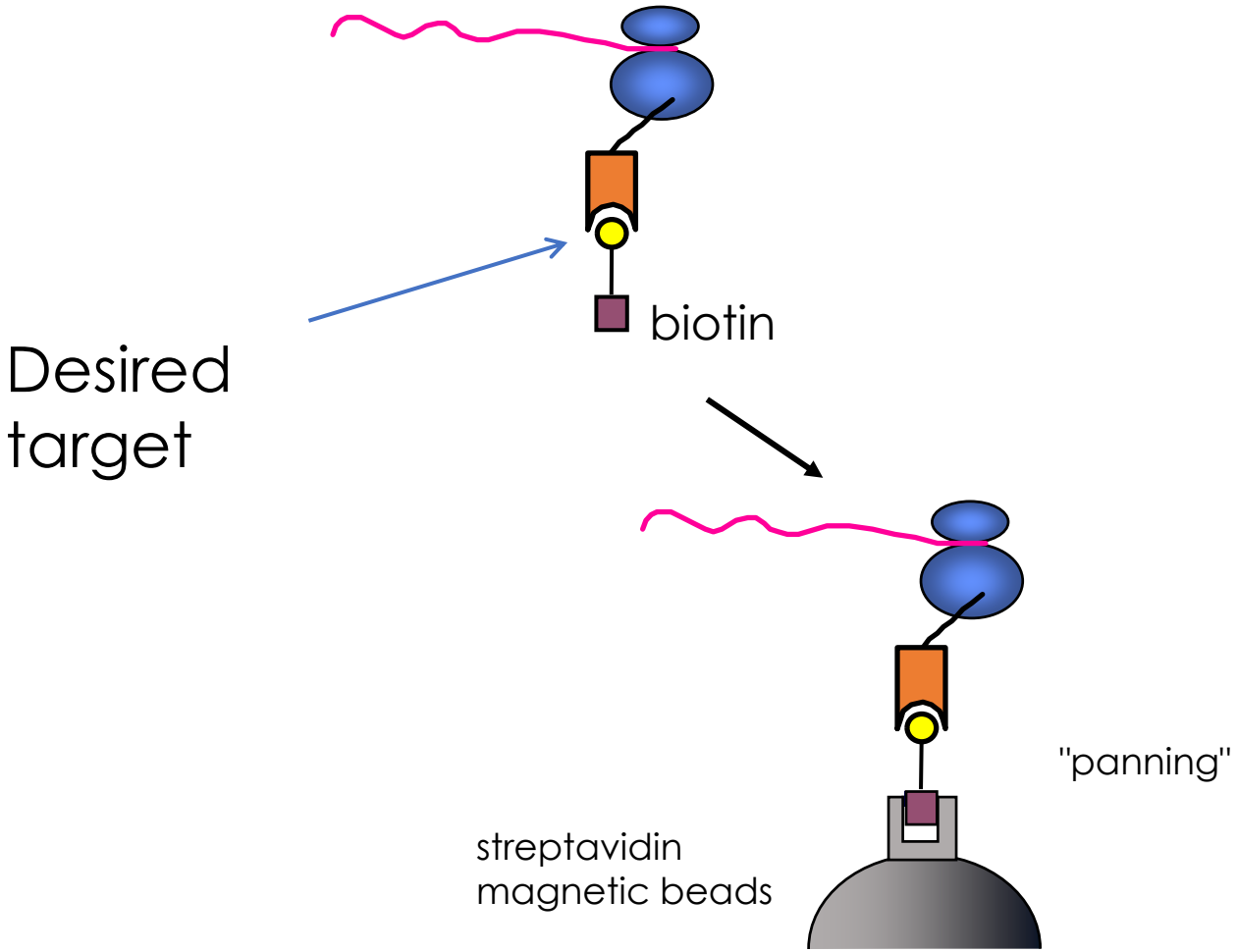
Two ingredients are needed: a library and a selection technology



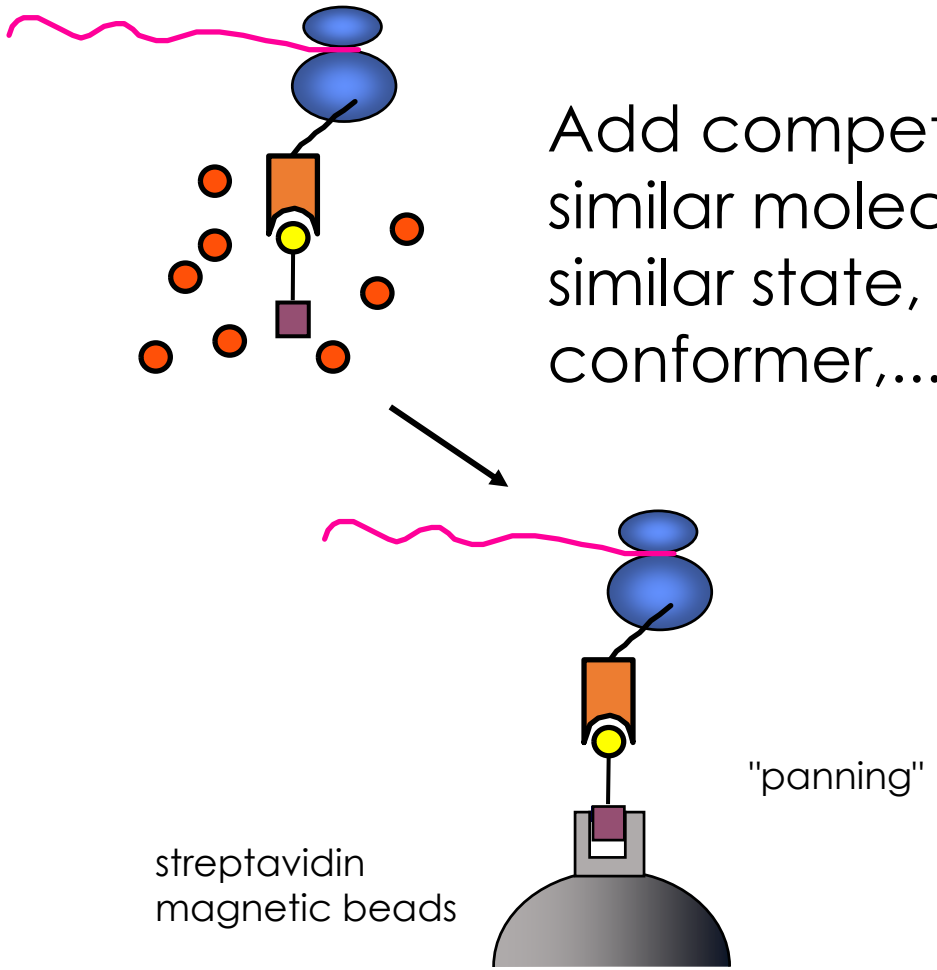
- Any binding protein can be converted into a library, by randomizing interaction surfaces
- A selection technology couples genetic variation to the protein phenotype
- Example shown: ribosome display

Selections from libraries allow direct selections for specificity



- Pulling our binders for the desired target from solution

Selections for specificity: a huge advantage for recombinant methods

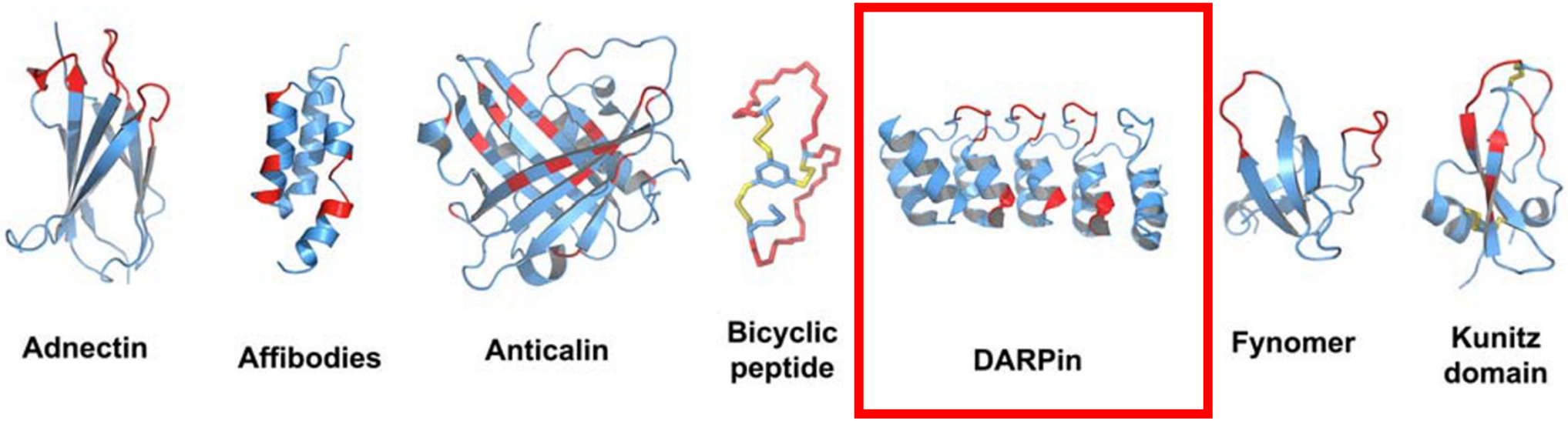


Add competitor to similar molecule, similar state, PTM conformer,...

"panning"

- Pulling our binders for the desired target from solution
- ... And counter-select against similar molecules shat should not be bound

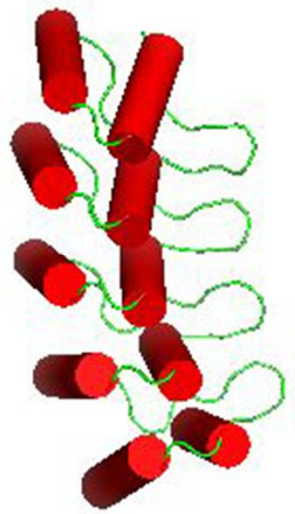
Non-antibody scaffolds: Examples of those which are in the clinic



- In principle, any protein can be used
- These examples are stable ones, which have shown properties good enough to use them in the clinic in human patients

Nature Biotechnology **35**, 602–603 (2017)

Specific binding by structural complementarity



Example of a co-crystal structure of a DARPin with its target

Picomolar affinity

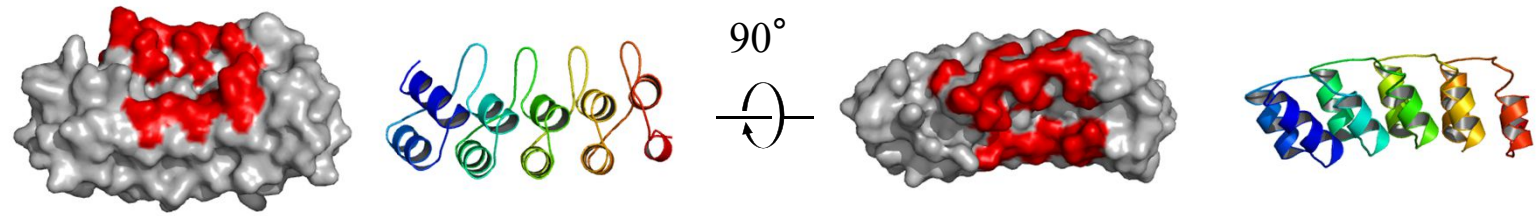
Very high stability

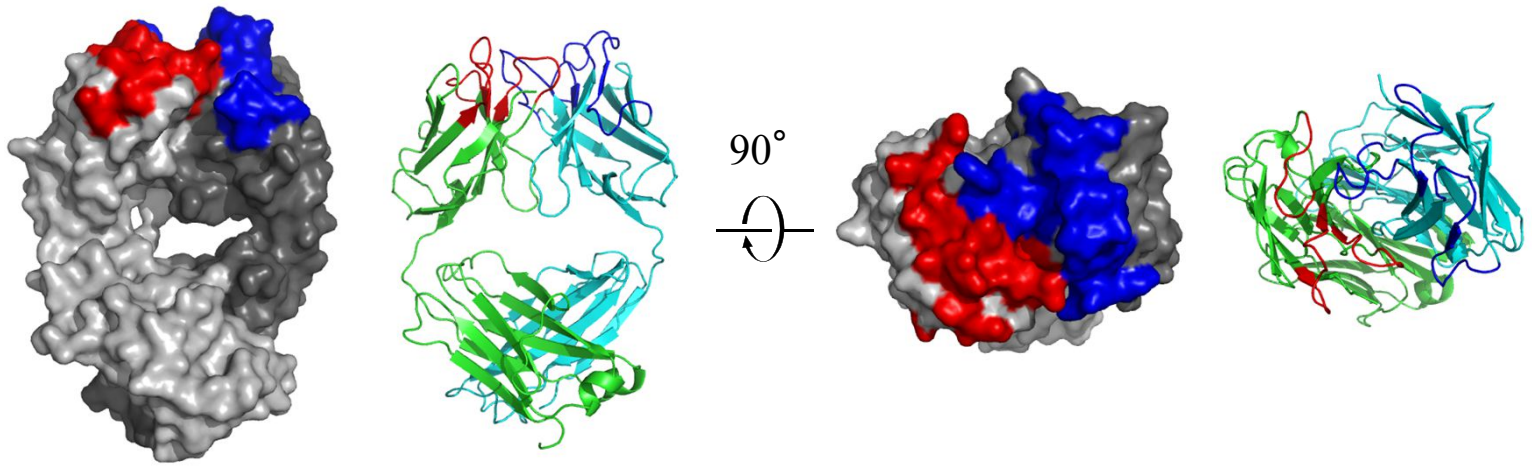
Very high production levels in bacteria

Annu. Rev. Pharmacol. Toxicol. ***55***, 489-511 (2015)

Specific binding by structural complementarity

DARPin



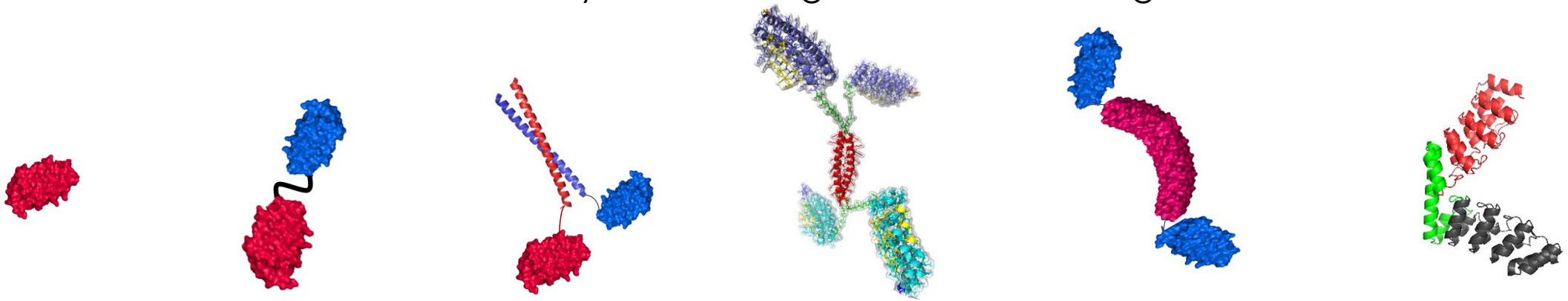


Fab fragment

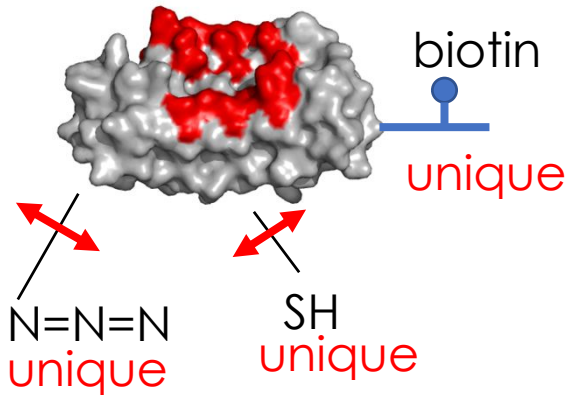
- Very similar interaction surface of a DARPin and a Fab fragment with its target

Making them multivalent, or chemically modified

Many different oligomerization strategies



Monovalent | Flexibly bivalent | Head-to-head or tail-to-tail bivalent | Tetravalent (up to 4 specificities) | Bivalent, with a rigid spacer | Bivalent, with a rigid angle



All of these can be easily produced in *E. coli*

Site-specific conjugation, freely choosable

These are obviously recombinant proteins

Lets focus on two different types of applications:

1. Taking advantage of them as proteins, which can be easily modified

2. Taking advantage of having their genes

Two types of applications

Applications requiring pure **protein**

– All applications discussed in the previous Webcasts, e.g.

ELISA, FACS, immunohistochemistry, Western blots, ...

– ...but also those where lots of protein is needed, which would be very expensive with antibodies

... and applications where you need the **gene**

– Expressing the binding proteins on the surface of a cell or a virus

– Expressing the binding proteins inside a cell

– Fusing the binding protein to other proteins: fluorescent proteins; enzymes; cytokines)

What about secondary reagents ?

Over the decades, secondary reagents have been developed that allow antibodies to be detected, in many applications.

These tend to rely on the constant domains, and species-specificity

This is not a limitation for recombinant reagents.

Recombinant molecules can all be "tagged", i.e. provided with a short peptide sequence. E.g.,

– his tag, FLAG tag, HA tag, ...
– spectrum of orthogonal detection tags

First, focus on applications requiring pure protein. Why would one ever use anything else but antibodies?

The only reason: enabling things that are hard to do with the current molecules.

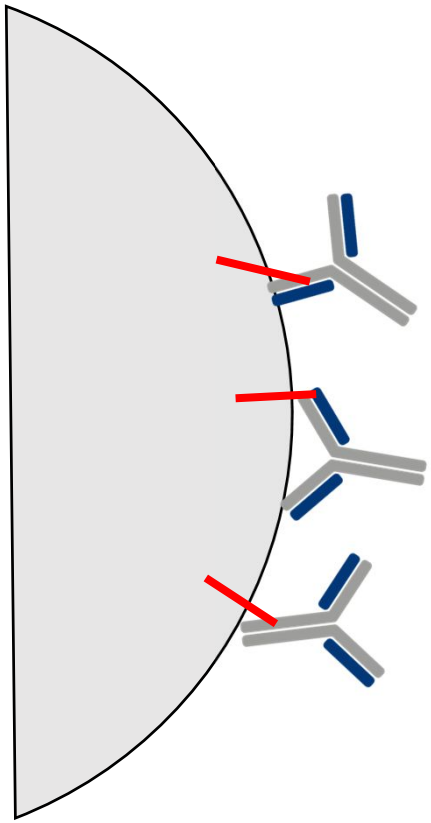
“But aren't antibodies "perfect" molecules for all applications?”

Maybe not.

– Antibodies are **expensive to make at large scale**. This is a big limitation for applications where large amounts (tens of mg) are needed, e.g.

– as immobilized "immuno"-purification agents

First, focus on applications requiring pure protein. Why would one ever use something else but antibodies?



- Expensive
- Randomly coupled

➔ immuno-affinity chromatography is rarely used

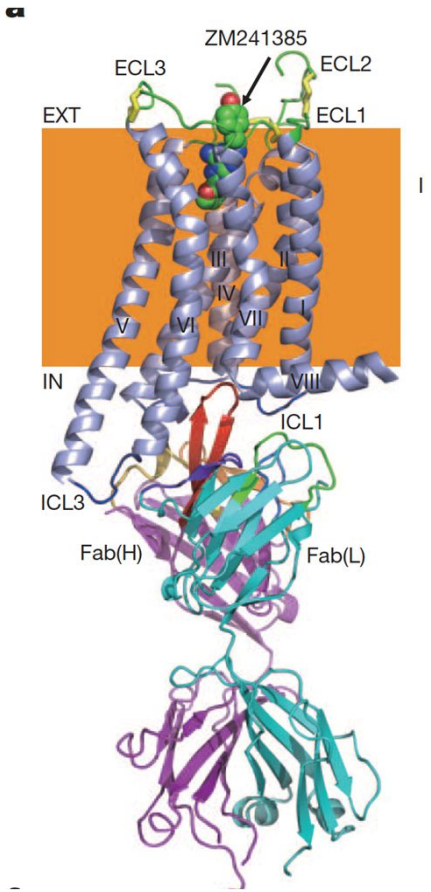
By contrast:

DARPins are an inexpensive, one-chain binding protein which is directionally immobilized

– Antibodies are **expensive at large scale**. A big limitation for applications where large amounts are needed

– e.g. as "immuno"-purification agents

First, focus on applications requiring pure protein. Why would one ever use something else but antibodies?



– Antibodies are **expensive at large scale**. A big limitation for applications where large amounts are needed

– as "immuno"-purification agents

– in structural biology as crystallization chaperones

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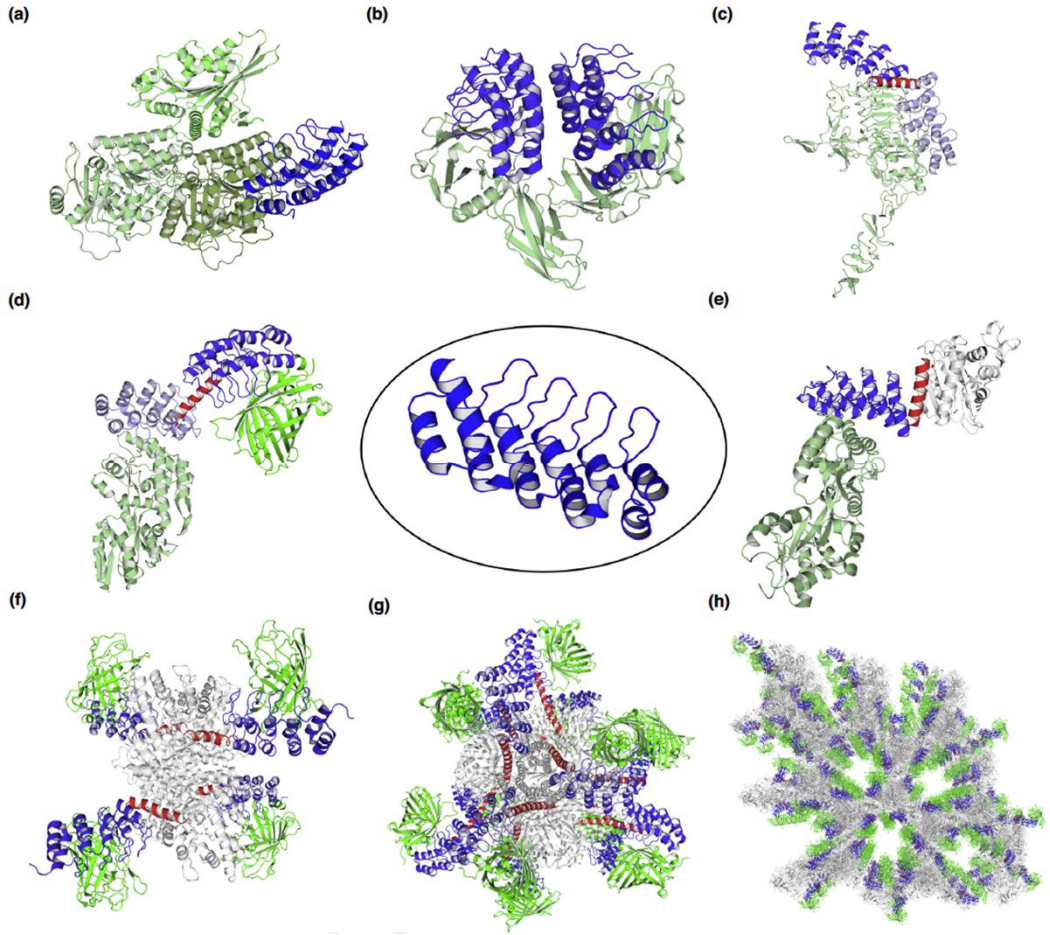
First, focus on applications requiring pure protein. Why would one ever use something else but antibodies?



- Antibodies are **expensive at large scale**. A big limitation for applications where large amounts are needed
- as "immuno"-purification agents
- in structural biology as crystallization chaperones

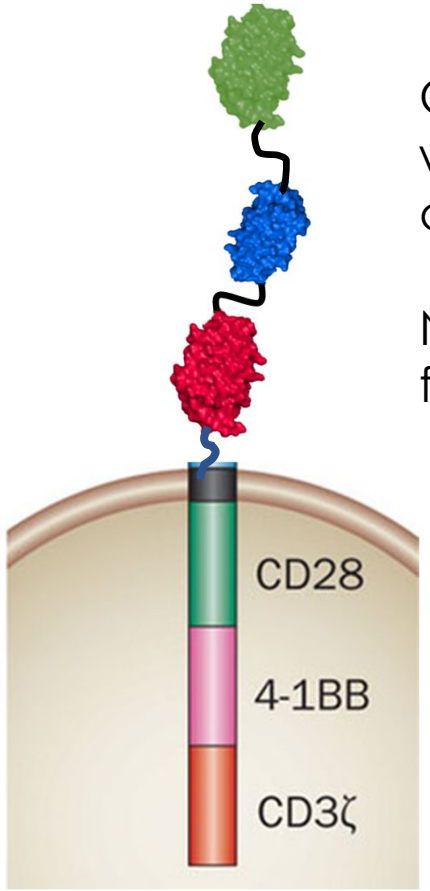
Focus on logistics: how to handle millions of specific binding agents?

- Hybridomas producing antibodies are expensive to store (as frozen cells).
- If a clone is lost, the antibody may be lost forever
- Traditional antibodies are not only undefined (as their sequence identity is not known) but can become extinct

Binding reagents based on scaffolds produced in bacteria solve these problems:

- Their genes sequences "immortalize" the reagents
- They are stored as sequence files
- Re-synthesis is on-demand, anywhere
- Expression is inexpensive
- The gene information enables subsequent production of novel constructs

What you can only do if you have the gene: (some examples) #1



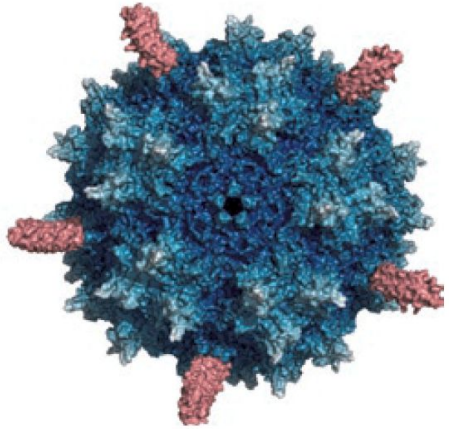
Chimeric antigen receptor with specificity against three different tumor antigens

Note: tandem antibody scFv fragments tend to aggregate

Expression of binding proteins on the surface of cells

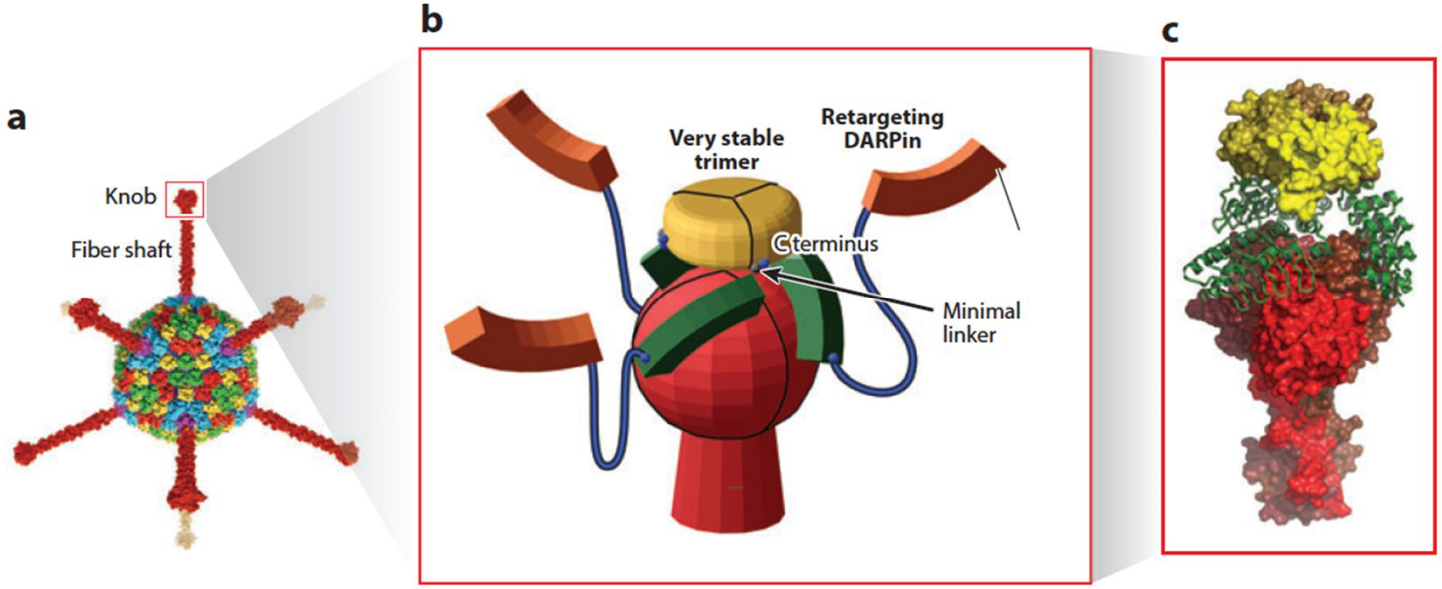
(example: T-cells)

What you can only do if you have the gene: (some examples)#2



Adeno-associated virus:
Genetic fusion

Adenovirus: adapter strategy



Redirecting a virus to achieve cell-specific infection in gene therapy

- As fusion proteins with virus coat proteins: many scFv fragments aggregate
- ➢ Both viral fusion-proteins and the adapter-strategy are very robust with DARPins

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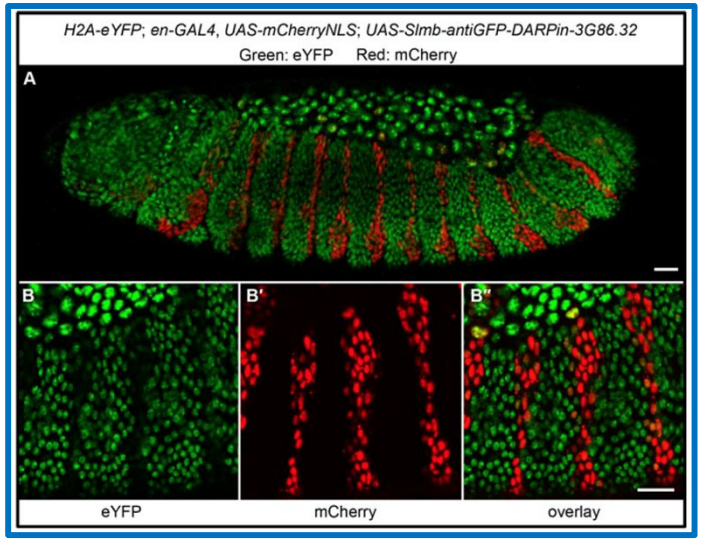
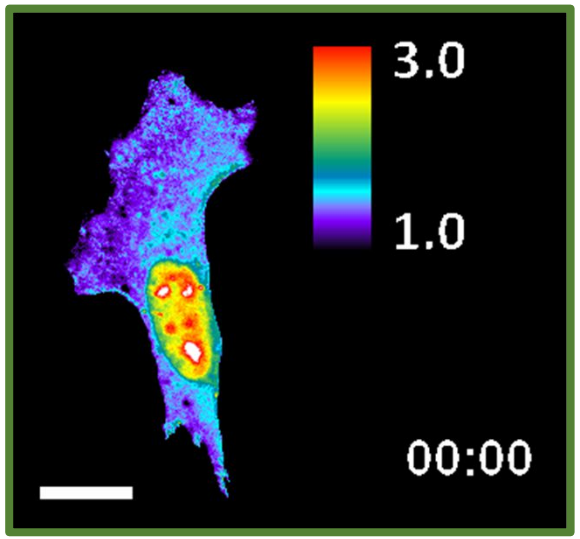
What you can only do if you have the gene (some examples) #3



Functional studies (e.g. induced degradation)

DARPins and targets are directly fused to different fluorescent proteins



A DARPin specific for phospho-ERK has been fused to a dye, whose fluorescence increases on binding phospho-ERK, but not ERK

Expressing binding proteins in the reducing cytoplasm

- Many antibody scFvs do not fold well and aggregate
- Most antibody scFvs do not fold well as fusions with fluorescent proteins: e.g. GFP

➢ Both problems are solved with DARPins

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If this is all so great, why can we not buy more of these reagents?

Many scaffolds are patented, but these patents will expire over the next few years.
The scaffolds have all been commercialized, but have been used almost exclusively for therapy.
The reason is that the profit margin is **much** greater for therapeutics than research reagents.

Importantly, therefore, the reason why we cannot yet buy many such affinity reagents is **purely commercial.**
Indeed, they are used for human therapy which emphasizes that they are **at least as specific as antibodies**, and are safe and efficacious.

What can we do if we want them today?

All these scaffolds have come out of academic labs.

Several of them offer centers for academic collaboration, where reagents can be made for particular scientific projects.

E.g., the University if Zurich provides access to the DARPin technology for academic collaborations

https://www.bioc.uzh.ch/research/core-facilities/high-throughput-binder-selection/

Conclusions (1)

- Binding reagents should be uniquely identified by a sequence (like genes and plasmids)
- Binders from synthetic libraries will always have a known sequence
- This makes them
  - Molecularly defined
  - Reproducible, comparable between researchers and labs
  - Immortal
  - Easy to be distributed (as data files) and expressed at any site in the world
- Of course, a quality control is still necessary

Conclusions (2)

- By having access to the gene, you have many additional advantages:
- The binder can be expressed
  - On the surface of cells (e.g. CAR-T cells)
  - On the surface of viruses (retargeted viruses for gene therapy)
  - In the cytosol or organelles of cells (as reporter or inhibitor)

Conclusions (3)

- Non-antibody scaffolds routinely provide binders of *at least* the same affinity and specificity as antibodies
  - Validated in clinical trials — extreme quality controls are applied
- In research applications, they are attractive:
  - They can be produced cheaply in quantity (high yield in E. coli)
  - So applications in structural biology and biotechnology (affinity chromatography) become very attractive
  - Easy conjugation and modification (one chain; often no cysteine)
  - They typically fold well in all environments and can be functionally expressed in the cytosol, or as fusions to many other proteins

Thanks for your interest!

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Presented by Andreas Plückthun
Produced and Directed by Simon L. Goodman
Production Manager Fran Breden
Written by Simon Goodman
https://www.antibodysociety.org/

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