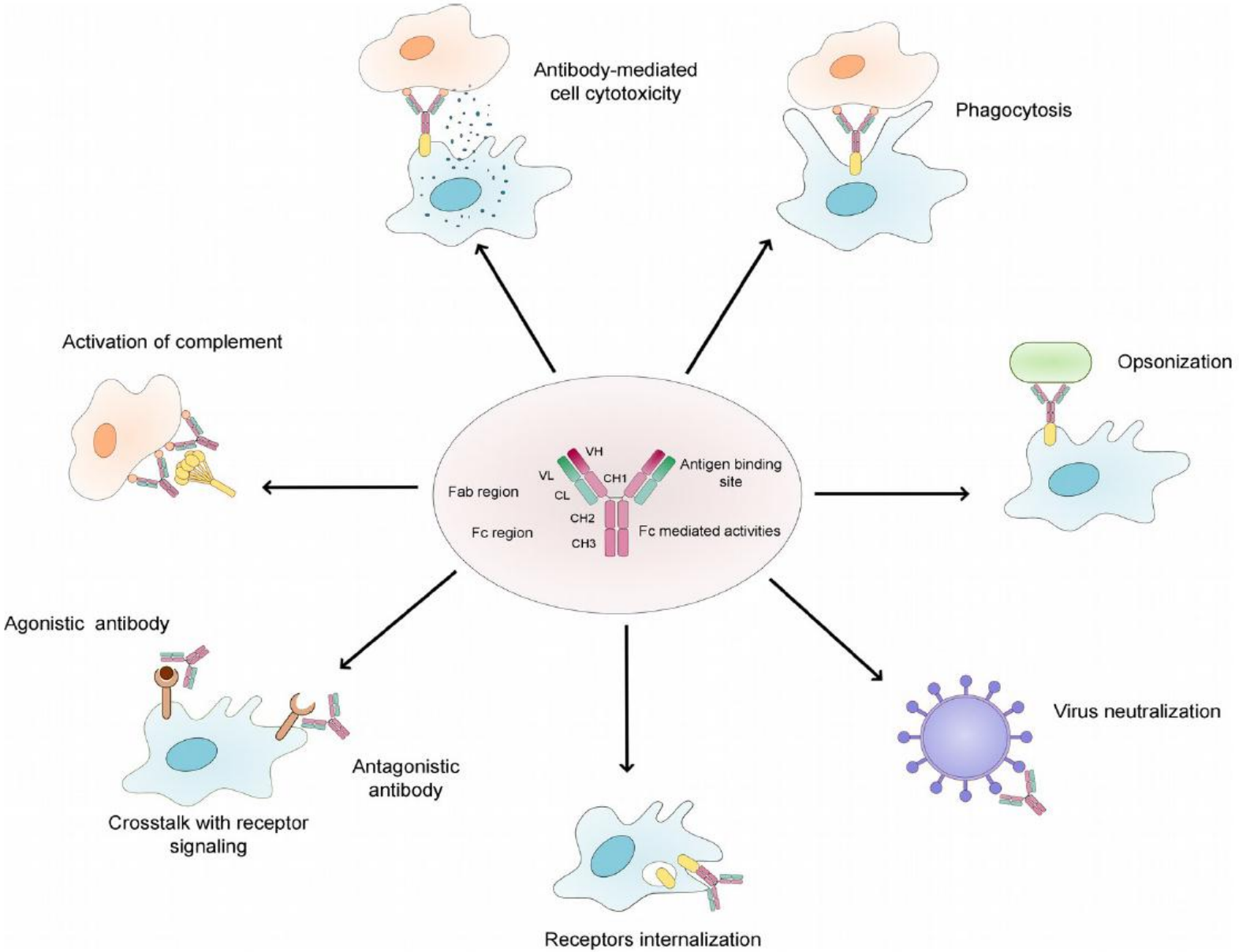
# *Bispecific antibodies with native chain structure*

**11.03.2014**

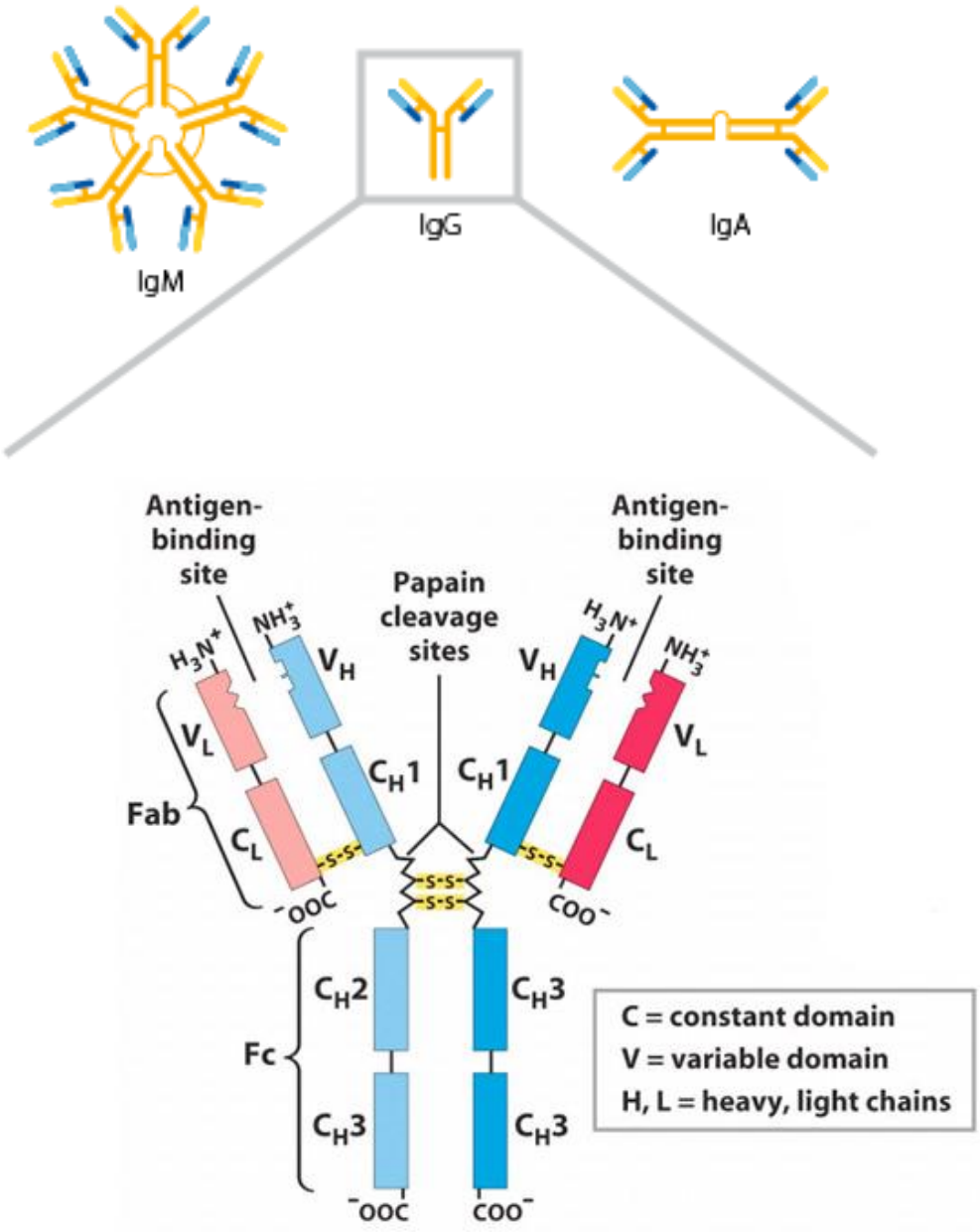
**Kristin Fritsch**

# Natural function of antibodies




*I. Spasevska, Biosciences Master Reviews, 2014*

# IgG Antibody




http://www.ebioscience.com/knowledge-center/antigen/immunoglobulin/structure.htm

# The organisation of the immunglobulin - genes

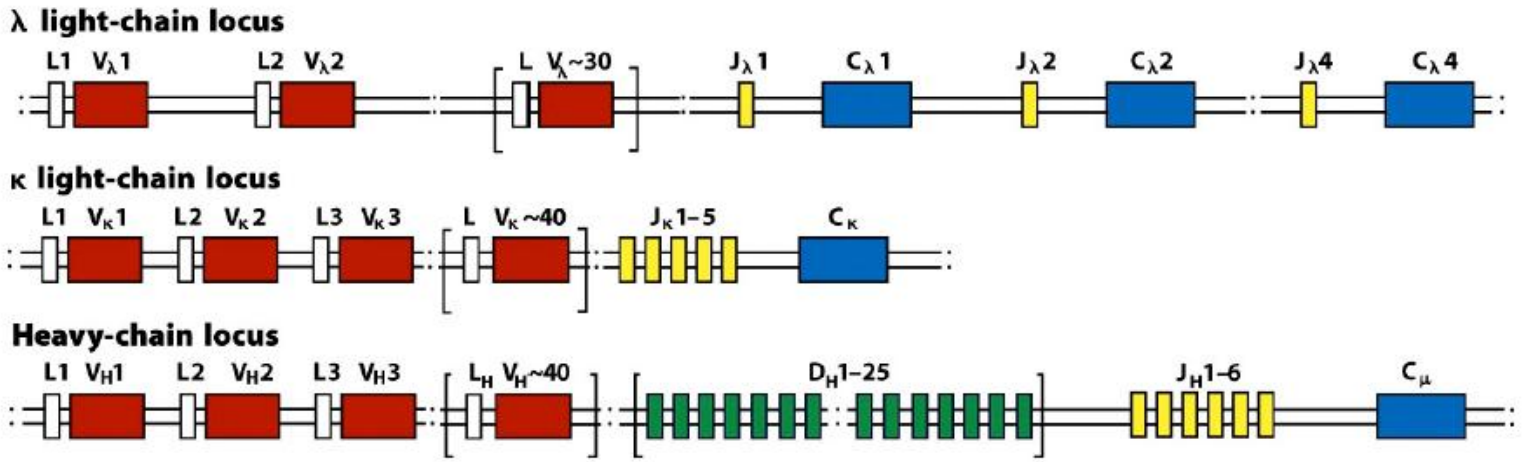



Figure 4-4 Immunobiology, 7ed. (© Garland Science 2008)

**Number of functional gene segments in human immunoglobulin loci**

| Segment | Light chains | | Heavy chain |
|---|---|---|---|
| | κ | λ | H |
| Variable (V) | 40 | 30 | 40 |
| Diversity (D) | 0 | 0 | 25 |
| Joining (J) | 5 | 4 | 6 |

# VDJ Recombination

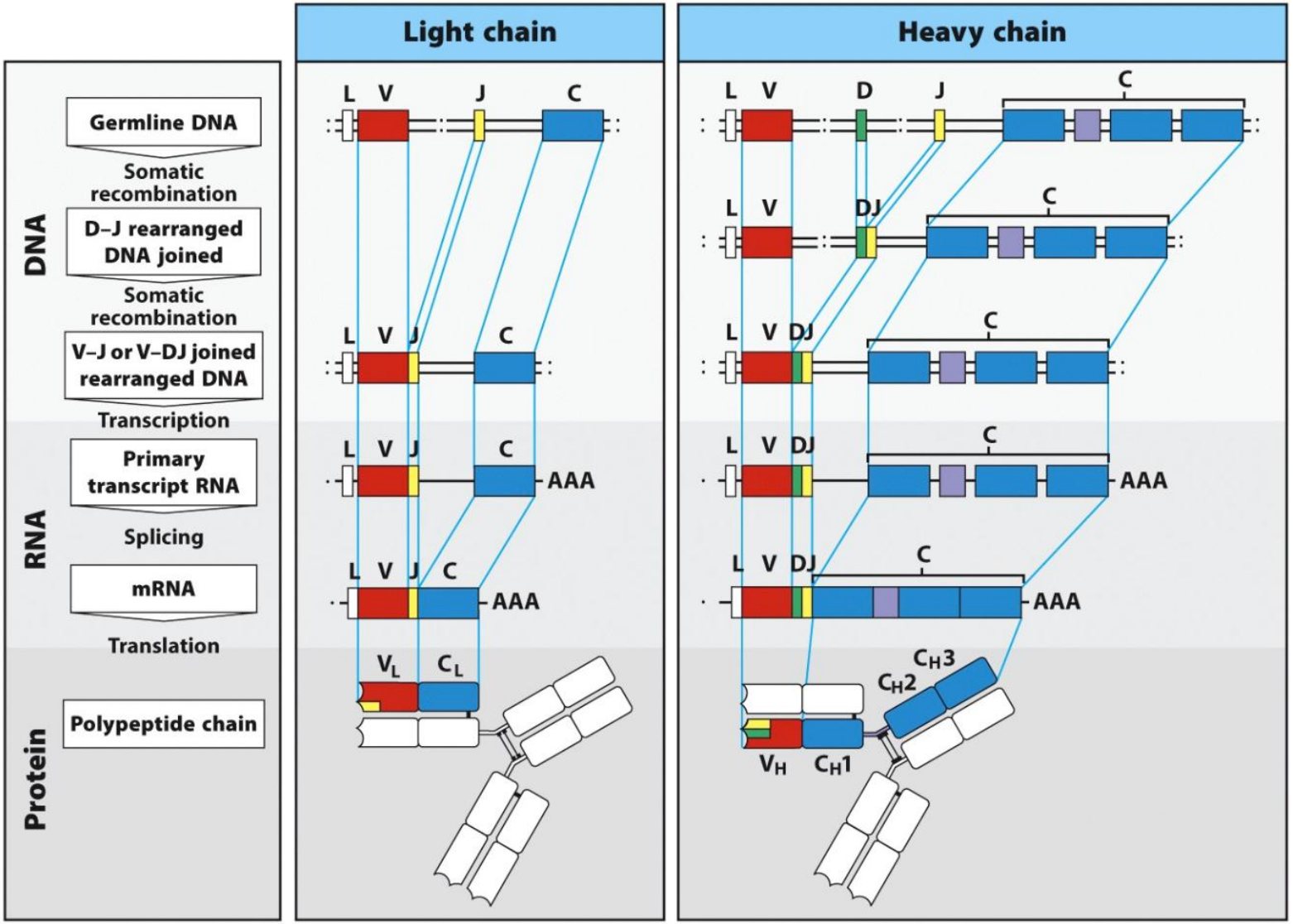
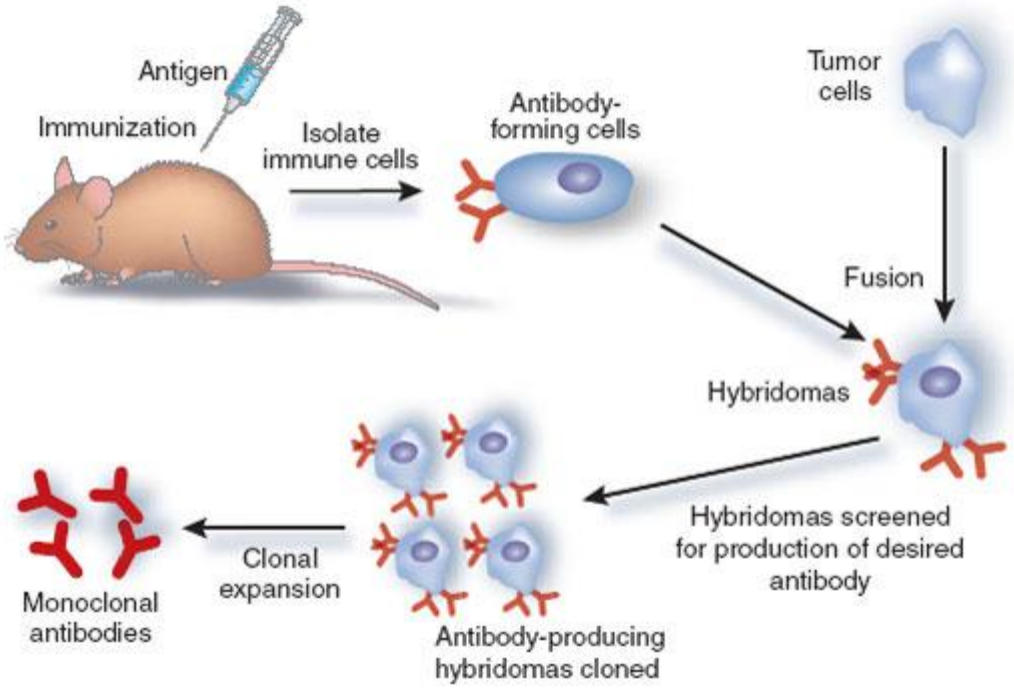



Figure 4-2 Immunobiology, 7ed. (© Garland Science 2008)

# *Monoclonal antibody*

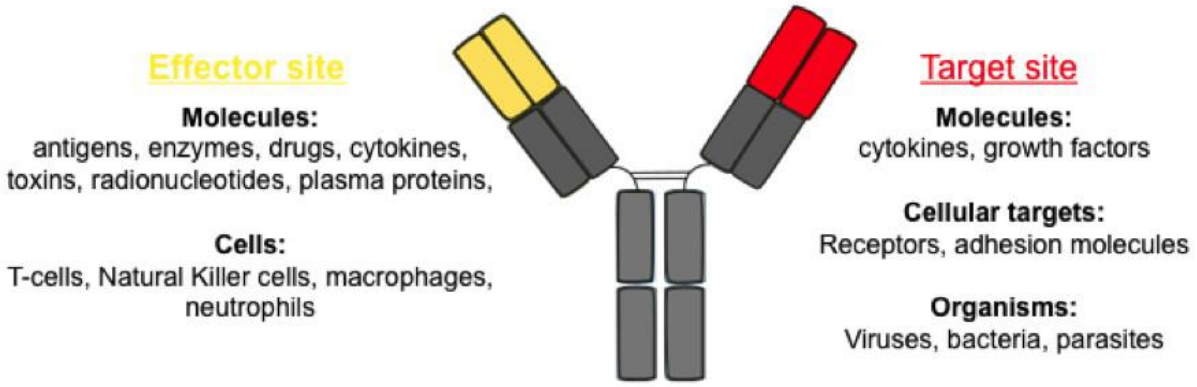
- Specificity for a single target antigen
- Can bind two copies of the same antigen molecule
- Most widely used form of cancer immunotherapy (have not been as successful as expected)




*S W Michnick & S S Sidhu, Nature chemical biology 2008*

# Bispecific antibodies (bsAbs)

- Single antibodies with specificity for two different target antigens




- Classes of bispecific antibodies:
  - Immunoglobuline-G (IgG)-like bsAbs
  - Small bsAbs

- Production:
  - Quadroma technology (fusion of two hybridoma cells)
  - «Knobs into Holes» approach (single amino acid substitution in oppisite CH3 domains)
  - CrossMab approach (combination of «Knobs into Holes» approach with modification of heavy and light chain in one hand)
  - Dual-Variable-Domain Immunoglobulin approach (combination of variable domains of two pre-existing mAbs with different specificities)

*I. Spasevska, Biosciences Master Reviews, 2014*

# Bispecific antibody

Cancer Immunology Research

*Priority Brief*

## Human Regulatory T Cells Kill Tumor Cells through Granzyme-Dependent Cytotoxicity upon Retargeting with a Bispecific Antibody

Bryan D. Choi[1,2], Patrick C. Gedeon[1,3], James E. Herndon II[4], Gary E. Archer[1], Elizabeth A. Reap[1], Luis Sanchez-Perez[1], Duane A. Mitchell[1,2,5], Darell D. Bigner[2,5], and John H. Sampson[1,2,5]

Chemistry & Biology
Article
Cell PRESS

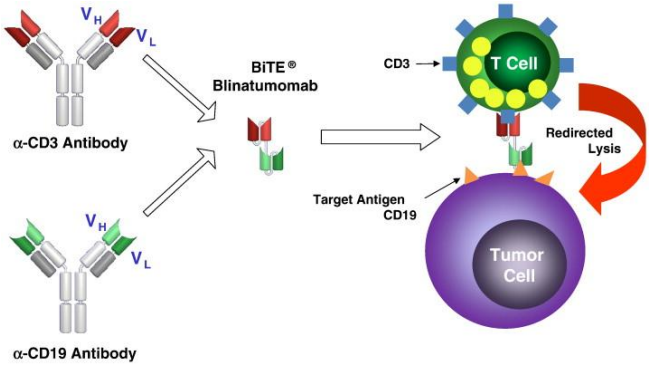
## Development of Bispecific Molecules for the In Situ Detection of Protein-Protein Interactions and Protein Phosphorylation

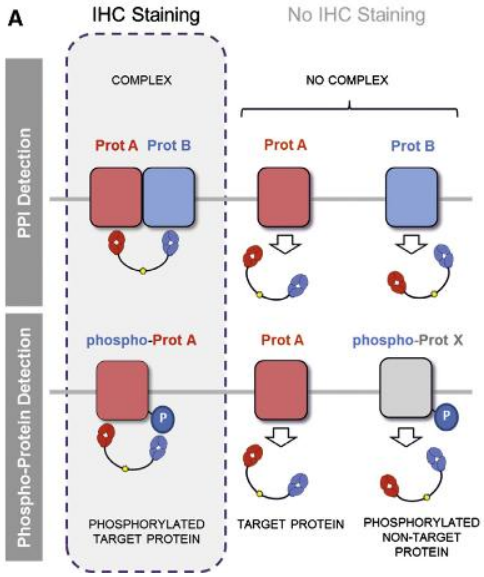
Jan van Dieck,[1,4] Volker Schmid,[1,4] Dieter Heindl,[2] Sebastian Dziadek,[2] Michael Schraeml,[2] Michael Gerg,[2] Petra Massoner,[2] Alfred M. Engel,[2] Georg Tiefenthaler,[1] Serhat Vural,[2] Simon Stritt,[1] Fabian Tetzlaff,[1] Monika Soukupova,[2] Erhard Kopetzki,[1] Birgit Bossenmaier,[1] Marlene Thomas,[1] Christian Klein,[3] Alfred Mertens,[1] Astrid Heller,[1] and Michael Tacke[2,*]
[1]Pharma Research and Early Development, Roche Diagnostics GmbH, 82377 Penzberg, Germany
[2]Roche Professional Diagnostics, Roche Diagnostics GmbH, 82377 Penzberg, Germany
[3]Pharma Research and Early Development, Roche Glycart AG, 8952 Schlieren, Switzerland
[4]These authors contributed equally to this work
*Correspondence: michael.tacke@roche.com
http://dx.doi.org/10.1016/j.chembiol.2013.12.018

## Influence of pegylation and hapten location at the surface of radiolabelled liposomes on tumour immunotargeting using bispecific antibody

A. Rauscher [a], M. Frindel [a], C. Maurel [a], M. Maillasson [a,b], P. Le Saëc [a], H. Rajerison [a], J.-F. Gestin [a], J. Barbet [a], A. Faivre-Chauvet [a], M. Mougin-Degraef [a,*]

[a] Centre de Recherche en Cancérologie Nantes–Angers (CRCNA), UMR 892 - Inserm/6299 CNRS/Université de Nantes, Institut de Recherche en Santé de l'Université de Nantes, 8 quai Moncousu, BP 70721, 44007 Nantes Cedex 1, France
[b] Plateforme Interactome & Puces à Protéines Biogenouest; Institut de Recherche en Santé de l'Université de Nantes, 8 quai Moncousu, BP 70721, 44007 Nantes Cedex 1, France




blood
2014 123: 554-561
Prepublished online December 5, 2013;
doi:10.1182/blood-2013-09-527044

## Cellular determinants for preclinical activity of a novel CD33/CD3 bispecific T-cell engager (BiTE) antibody, AMG 330, against human AML

George S. Laszlo, Chelsea J. Gudgeon, Kimberly H. Harrington, Justine Dell'Aringa, Kathryn J. Newhall, Gary D. Means, Angus M. Sinclair, Roman Kischel, Stanley R. Frankel and Roland B. Walter

# Problems with bispecific antibodies

- Alteration of the native antibody geometry with ist well-known stability and solubility properties

- Bispecific antibody formats using antibody fragments (domain antibodies, scFvs,diabodies) commonly require extensive engeneering to stabilize the variable domains outside the naive Fab context

- Some bispecific antibodies are tetravalent, but many clinical applications require monovalent antigen recognition to avoid unwanted agonism

- Production: separat production of monoclonal antibodies and then recombining them using protein engineering and biochemical methods (time consuming and requires generation of two master cell lines)

- Random pairing of light and heavy chains ( non-functional bsAbs)

- High immunogenicity

- Low efficacy and stability

LETTERS

nature biotechnology

# Bispecific antibodies with natural architecture produced by co-culture of bacteria expressing two distinct half-antibodies

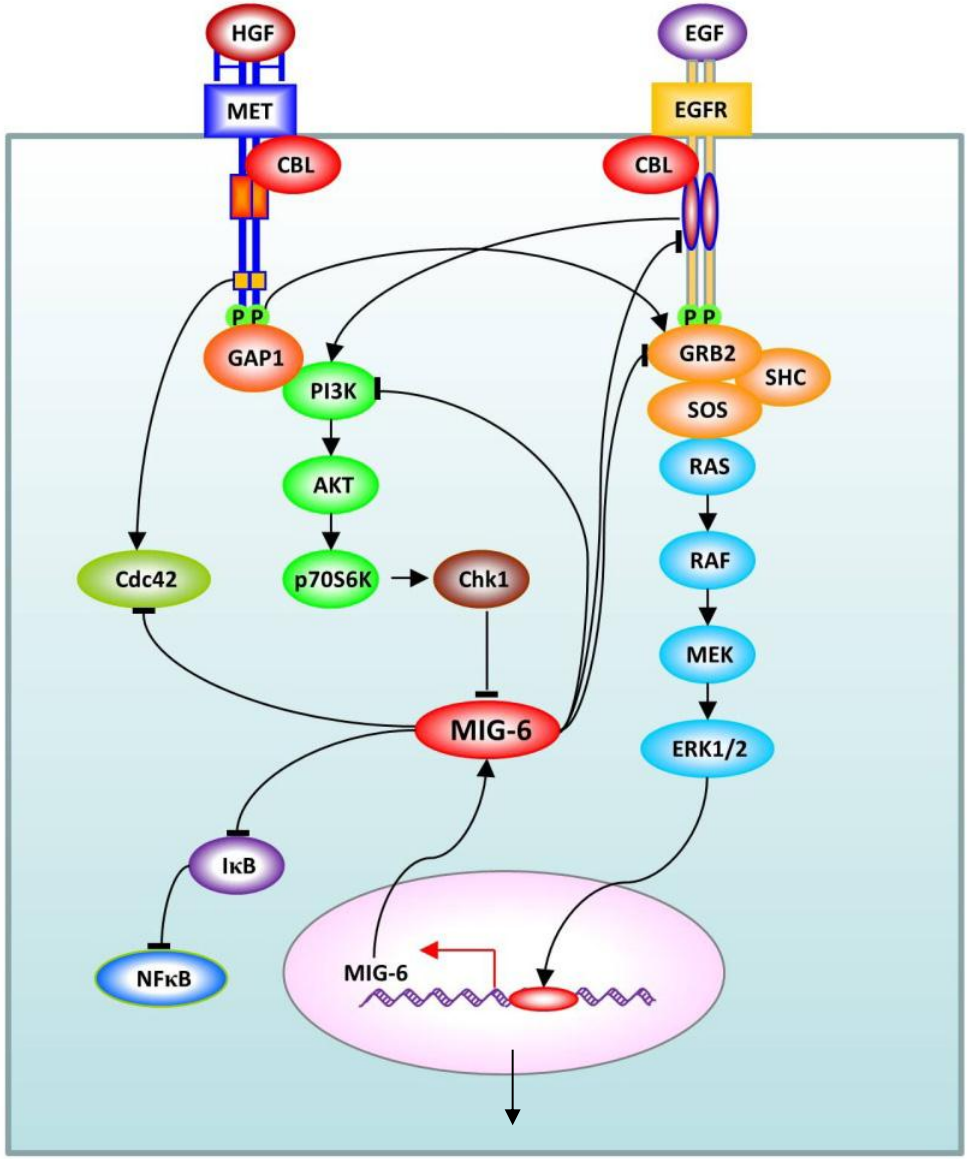
Christoph Spiess[1,6], Mark Merchant[2,6], Arthur Huang[1,5], Zhong Zheng[2], Nai-Ying Yang[2], Jing Peng[2], Diego Ellerman[3], Whitney Shatz[3], Dorothea Reilly[4], Daniel G Yansura[1] & Justin M Scheer[3]

August 2013

# aim

Efficient generation of nonimmunogenic, stable bispecific anfibodies with a natural IgG architecture that is able to simultaneously block signaling through MET and EGFR

# MET and EGFR drive growth of cancer cells

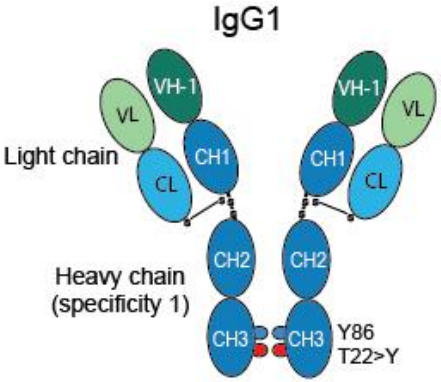



**proliferation**

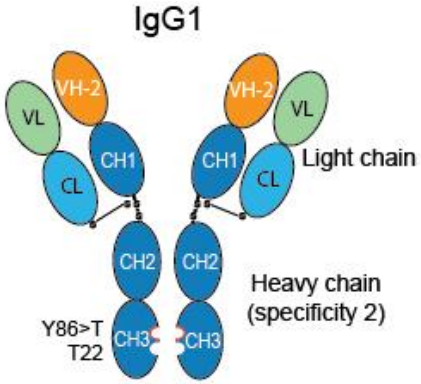
*Yue Cheng, Future Aspects of Tumor Suppressor Gene*

# Knobs-into-holes heterodimerization technology

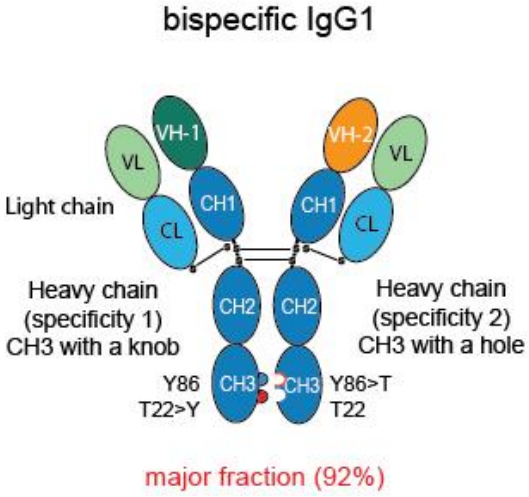
= heterodimerization of antibody heavy chains specific for different antigens




Amino acid change creating a knob on the CH3 of the heavy chain




Amino acid change creating a hole on the CH3 of the heavy chain

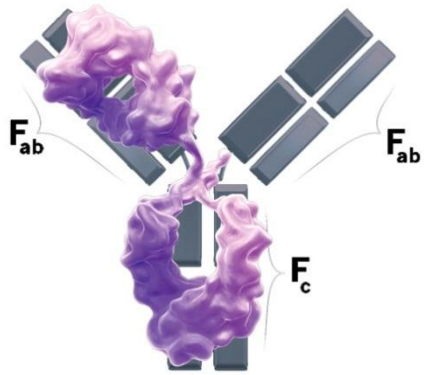


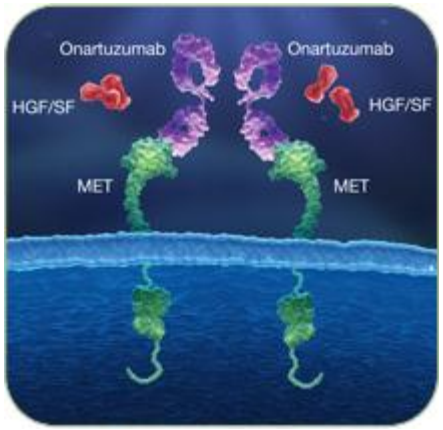

problem: light chains have no preference for one of the two heavy chains

*http://www.imgt.org/textes/IMGTbiotechnology/Knobs-into-holes_IgG.html*
*Ridgway et. al., Protein Eng, 1997*

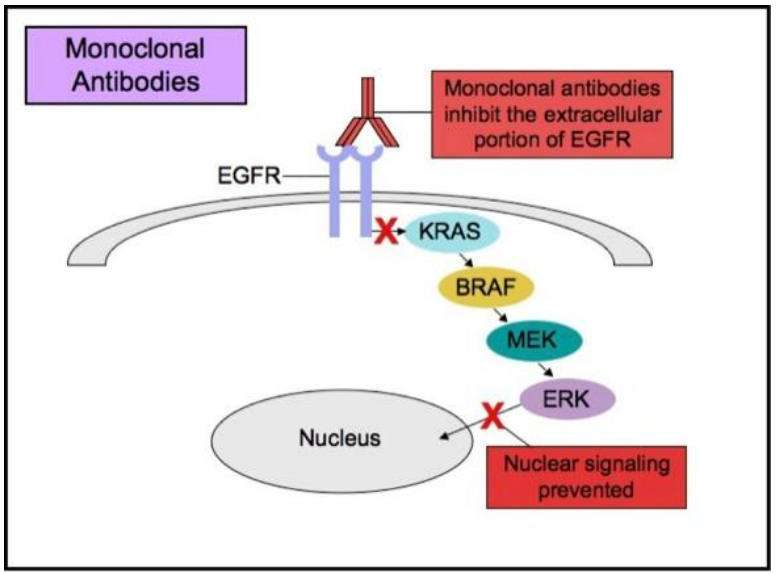
# Knobs-into-holes heterodimerization technology

MET-specific monovalent antibody



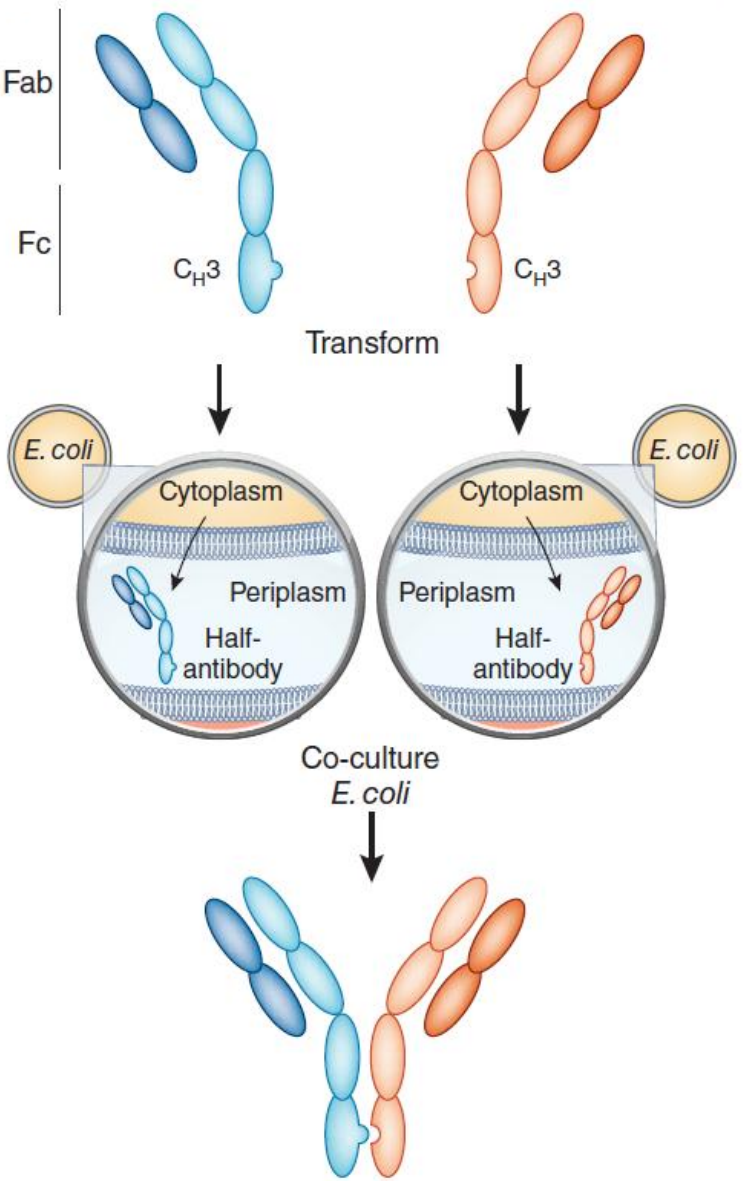

EGFR-specific antibody D1.5




*http://www.biooncology.com/pipeline-molecules/onartuzumab*

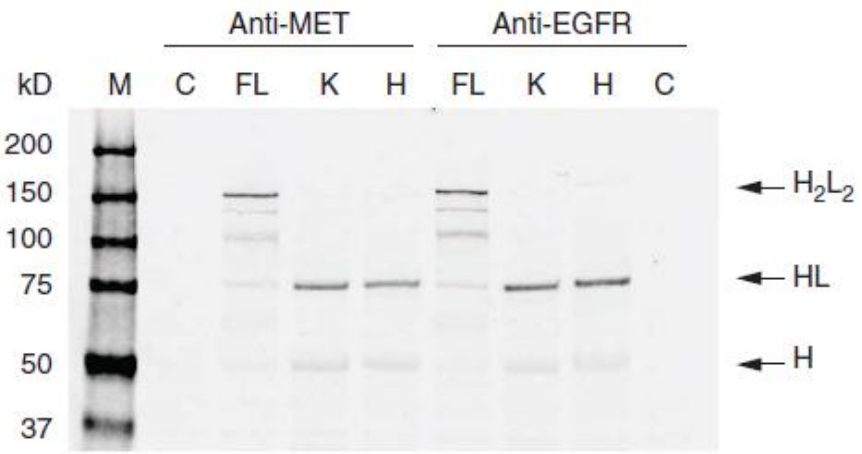
*http://www.affiliatedpath.com/OurPractice/molecular/Colon_Pathways/colon.html*

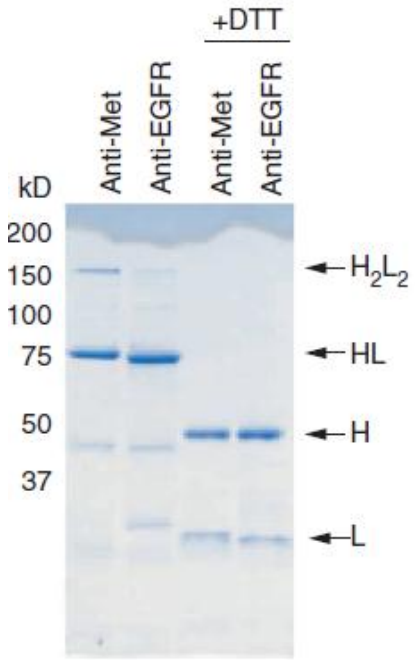
# Expression of human bispecific antibodies in *E.coli*

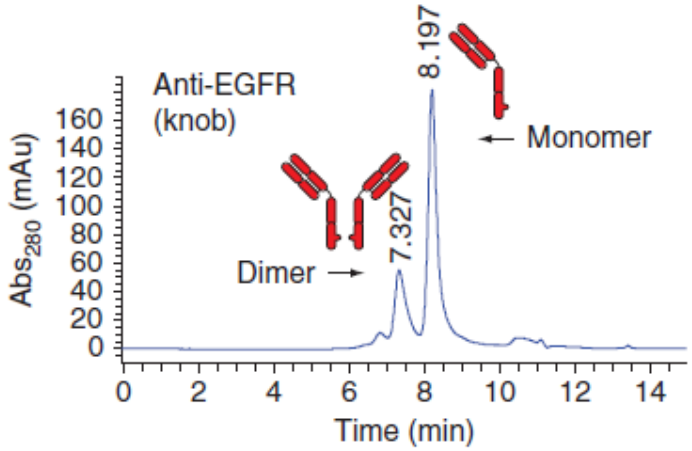


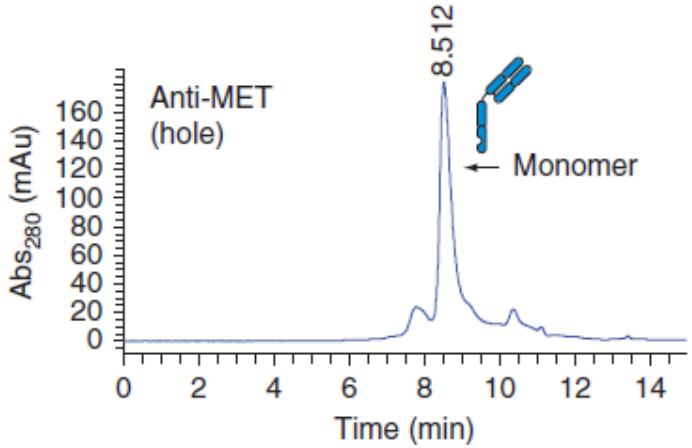

*R.Rouet & D.Christ, Nature Biotech, 2014*

# Production of knob and hole half-antibodies in *E.coli*

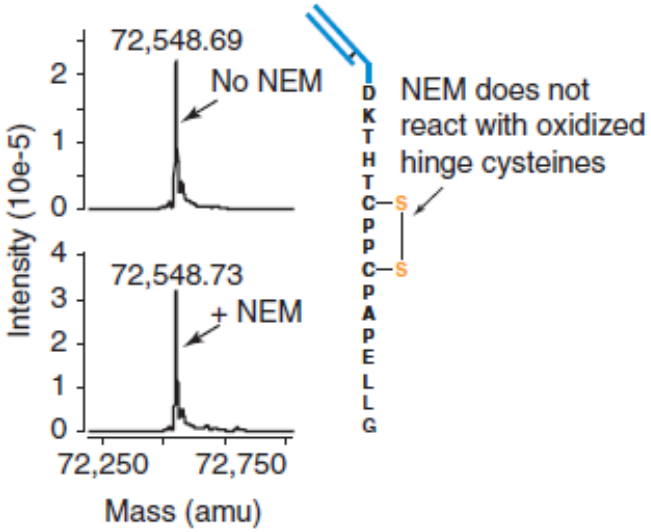


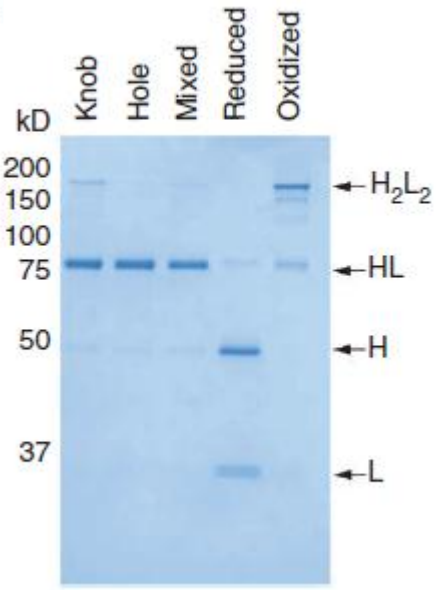



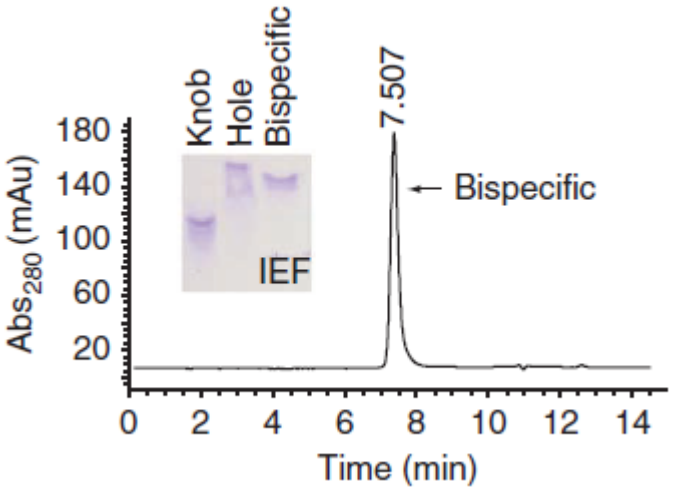



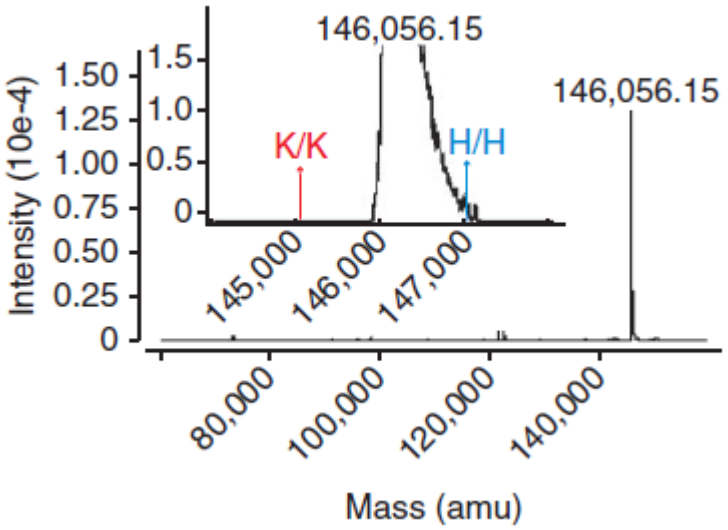



# *In vitro* assembly of knob and hole half-antibodies into intact bispecific antibody

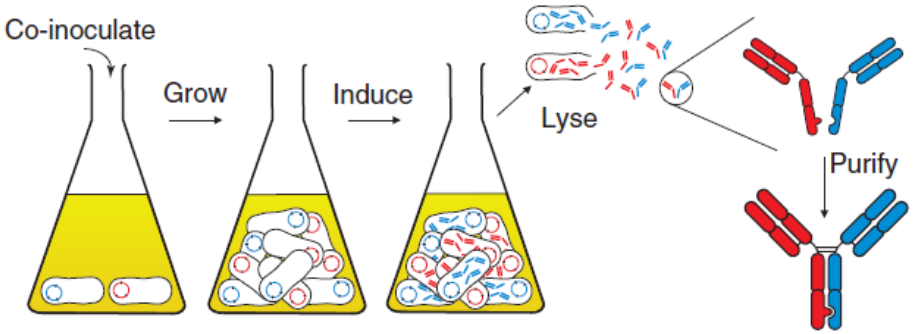


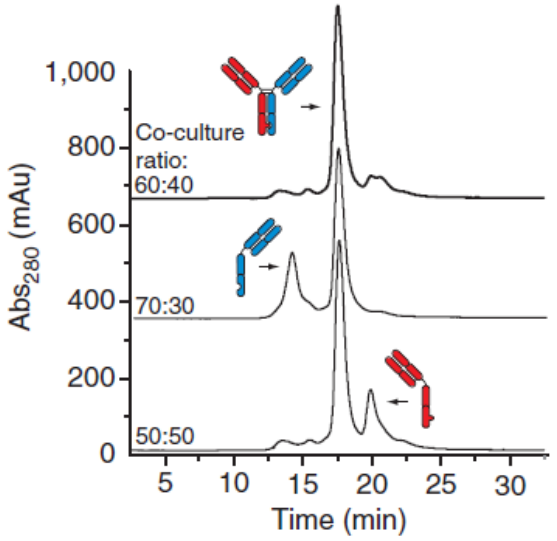



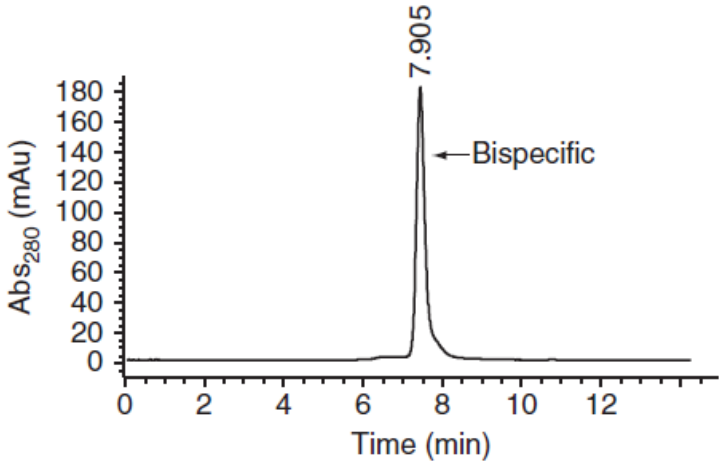



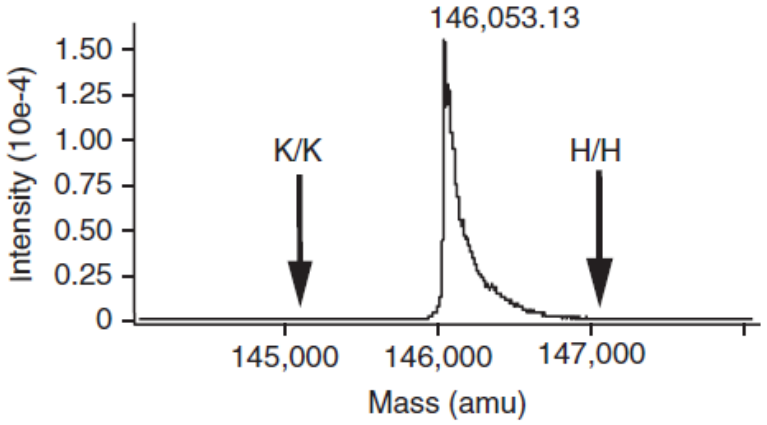



# Making bispecific antibodies using bacterial co-culture

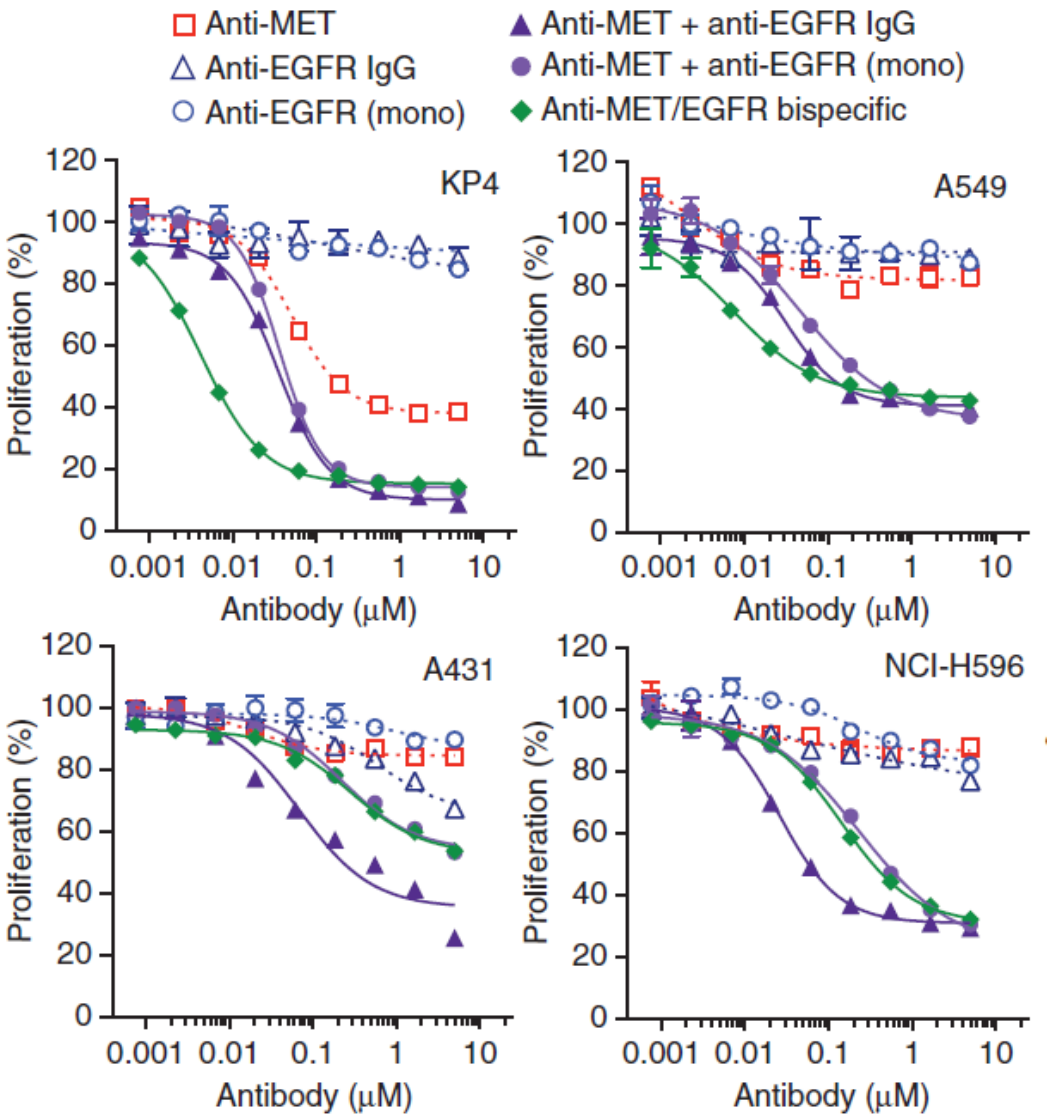


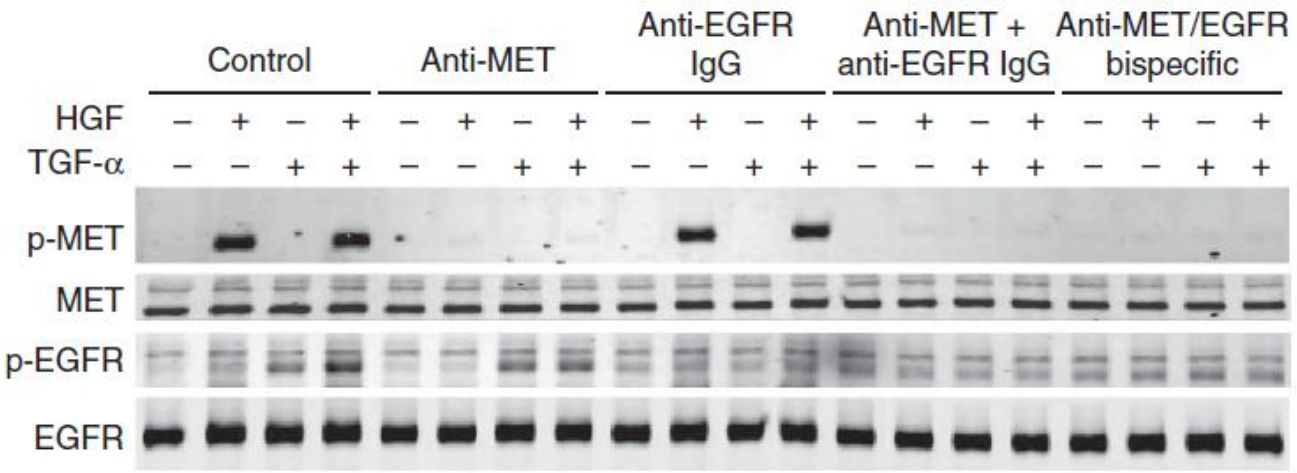



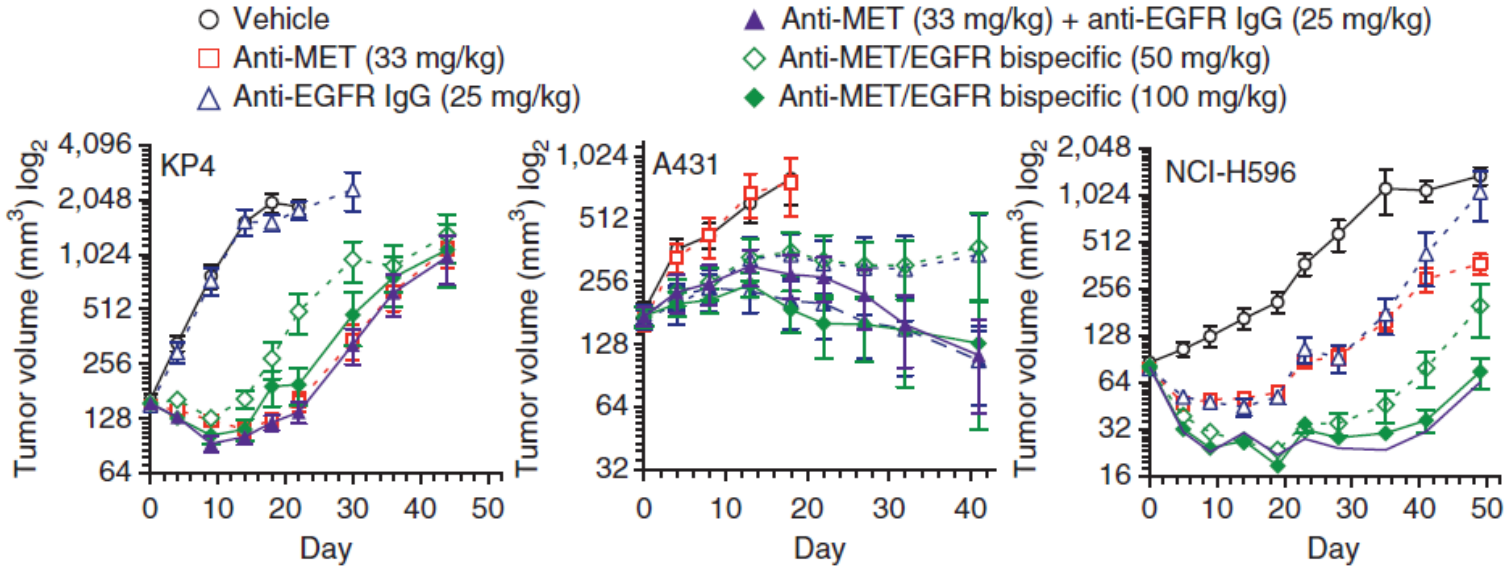
# Characterization of the MET-EGFR bispecific antibodies in vitro



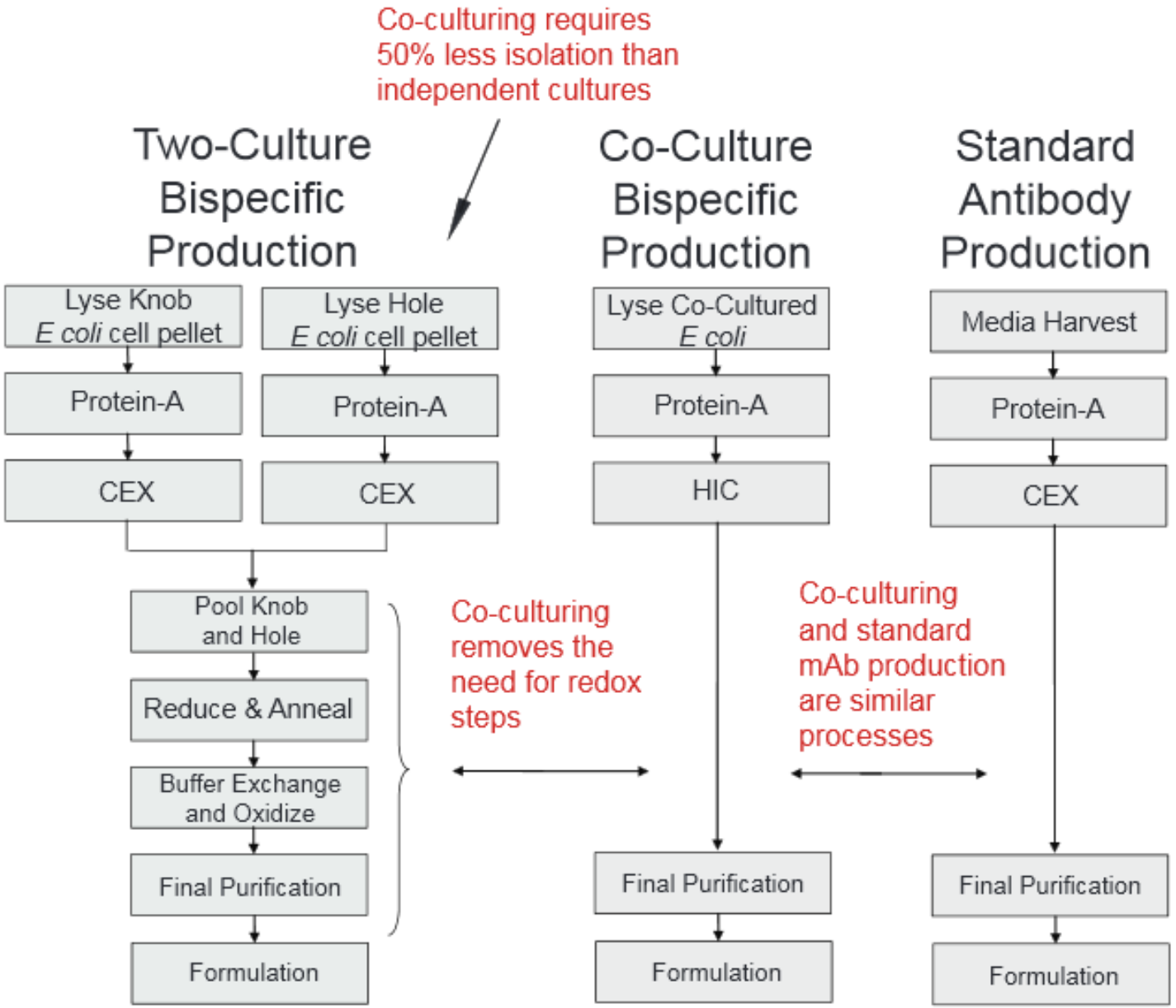

**KP4** - ductal pancreatic cancer cell line
(production of HGF in autocrine manner)
**A549** - non-small-cell- lung carcinoma cell line
(expression of wildtype MET and EGFR)
**A431** - epidermoid cell line
(harbors EGFR amplification)
**NCI-H596** cell line
(harbors loss of exon 14 in MET)

# Characterization of the MET-EGFR bispecific antibodies in vitro and in vivo



# Comparison of methods for monoclonal and bispecific antibody production



# conclusion

- Half-antibody bacterial co-culture approach has many advantages over existing approaches
  - Simple
  - Can be used with any two existing antibodies
  - Eliminates the need for a common light chain
  - Requires no reengineering of antibody functional domains
  - The resulting bispecific antibody maintain the native architecture of a typical antibody

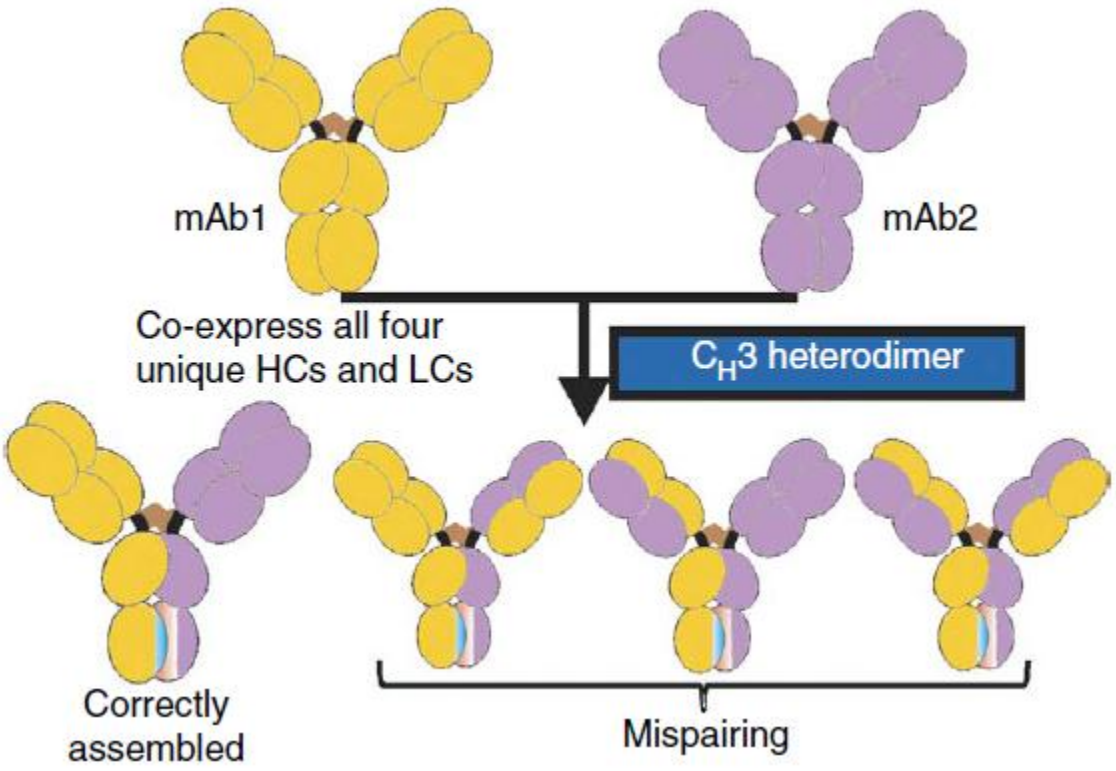
nature biotechnology

# Generation of bispecific IgG antibodies by structure-based design of an orthogonal Fab interface

Steven M Lewis[1,4], Xiufeng Wu[2,4], Anna Pustilnik[2], Arlene Sereno[2], Flora Huang[2], Heather L Rick, Gurkan Guntas[1], Andrew Leaver-Fay[1], Eric M Smith[2], Carolyn Ho[2], Christophe Hansen-Estruch[2], Aaron K Chamberlain[2], Stephanie M Truhlar[2], Elaine M Conner, Shane Atwell[2], Brian Kuhlman[1,3] & Stephen J Demarest[2]

February 2014

# Expression of human bispecific antibodies in mammalian cells



# aim

Design of an orthogonal IgG heavy chain – light chain interface using molecular modeling, feedback from X-ray crytallography and human-guided design

# $C_H1/C_L$ interface redesigns

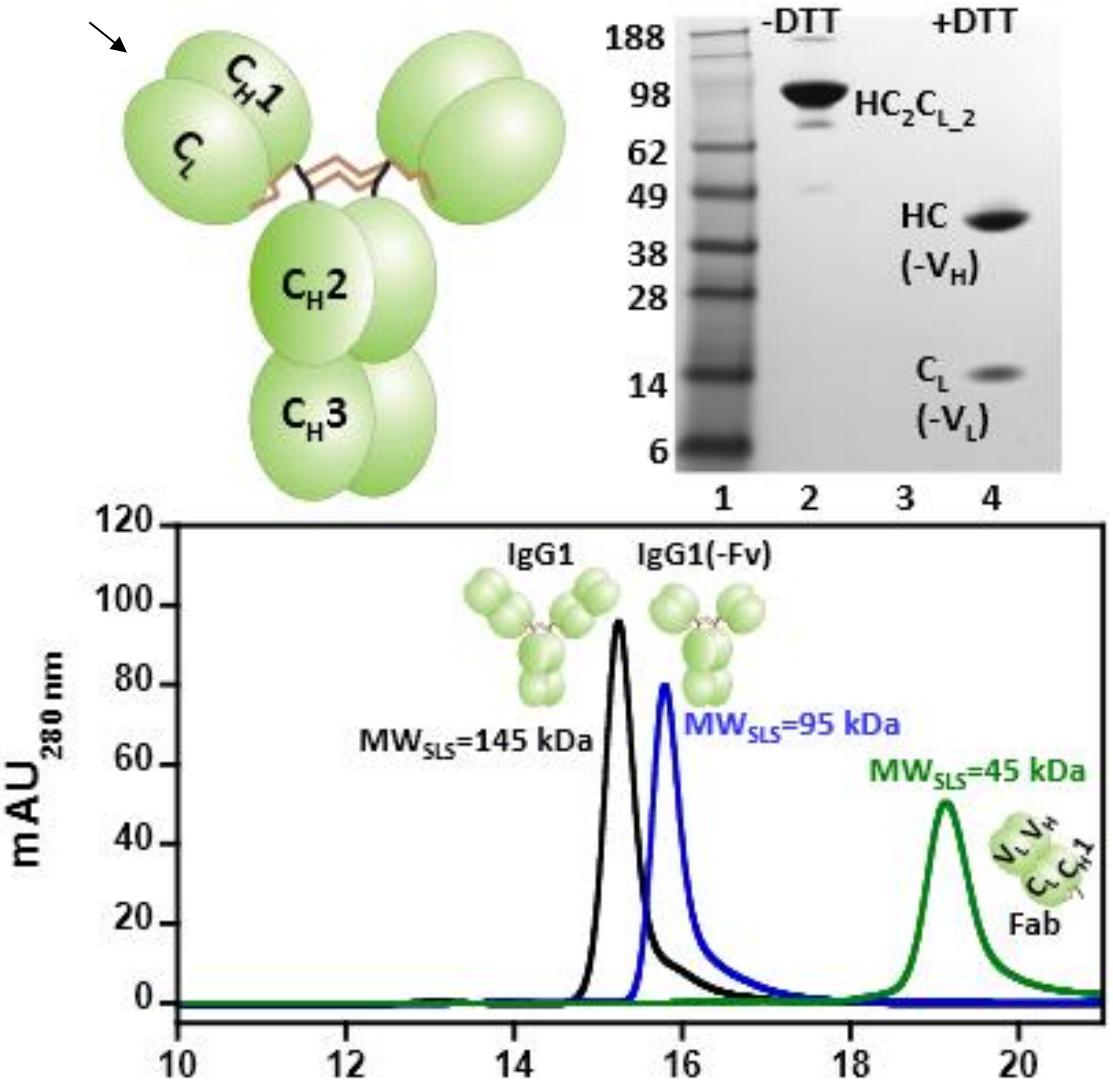
Screening for mutant $C_H1$-$C_L$ that disvavors binding to wildtype $C_H1$-$C_L$ with Rosetta

↓

Identification of 20 mutants that favour mutant-mutant pairing over mutant-wildtyp pairing

# IgG1(-Fv) protein was used for screening the $C_H1/C_L$ interface redesigns

lacks $V_H$ and $V_L$ domains



# $C_H1/C_L$ interface redesigns

Screening for mutant $C_H1$-$C_L$ that disvavors binding to wildtype $C_H1$-$C_L$ with Rosetta

↓

Identification of 20 mutants that favour mutant-mutant pairing over mutant-wildtyp pairing

↓

Transient transfection in HEK293F cells

↓

Identification of 10 mutants that were expressed at levels similar to that of the WT IgG1(-Fv)

↓

Transfection as mismatched heavy chain – light chain pairs with WT heavy chain or light chain

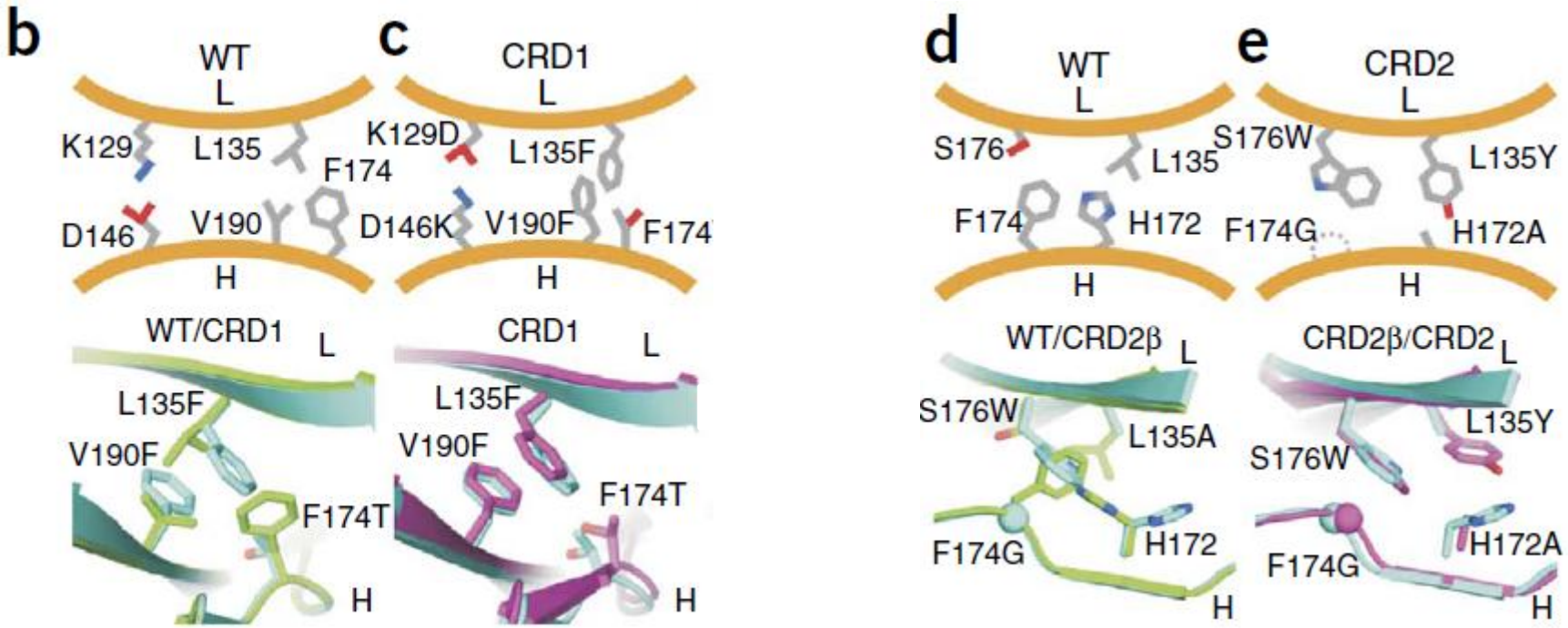
↓

Identification of 3 mutant proteins that had mutant-mutant pairs that were more stable then mismatched mutant – WT pairs

↙ ↘

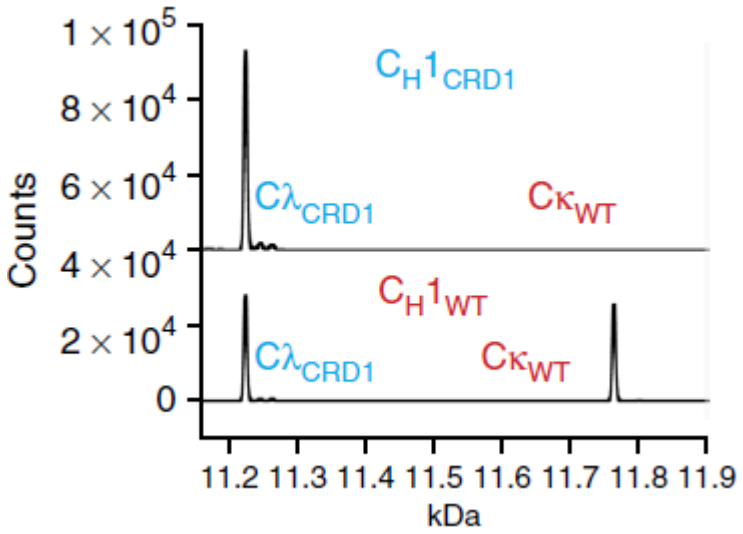
CRD1 CRD2

# Schematic structure models of designed CRD1 and CRD2

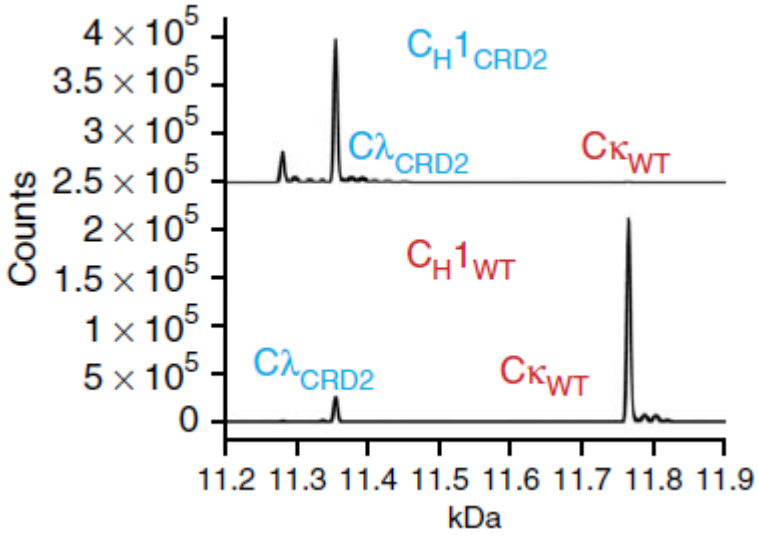


# Competition assay

CRD1

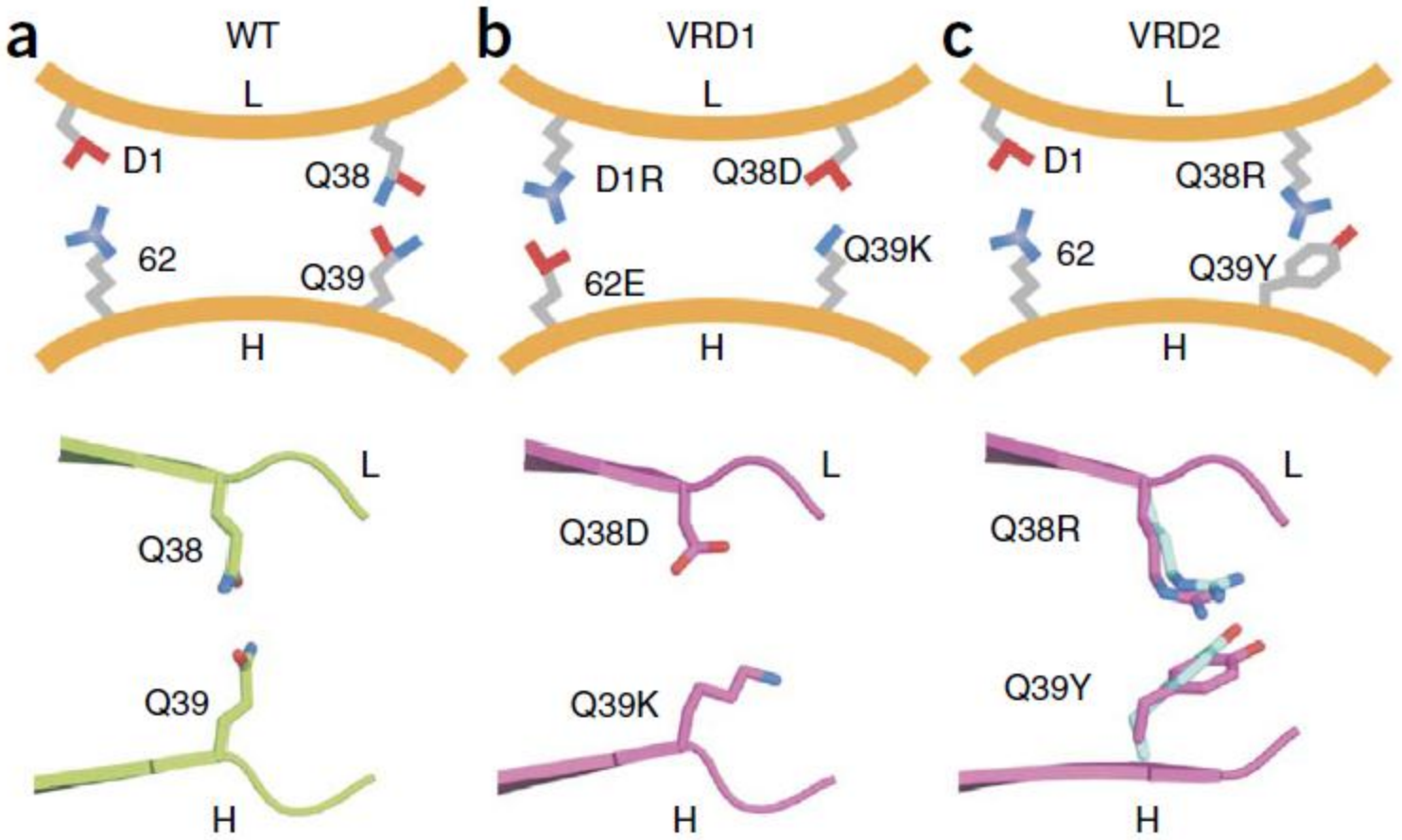



CRD2



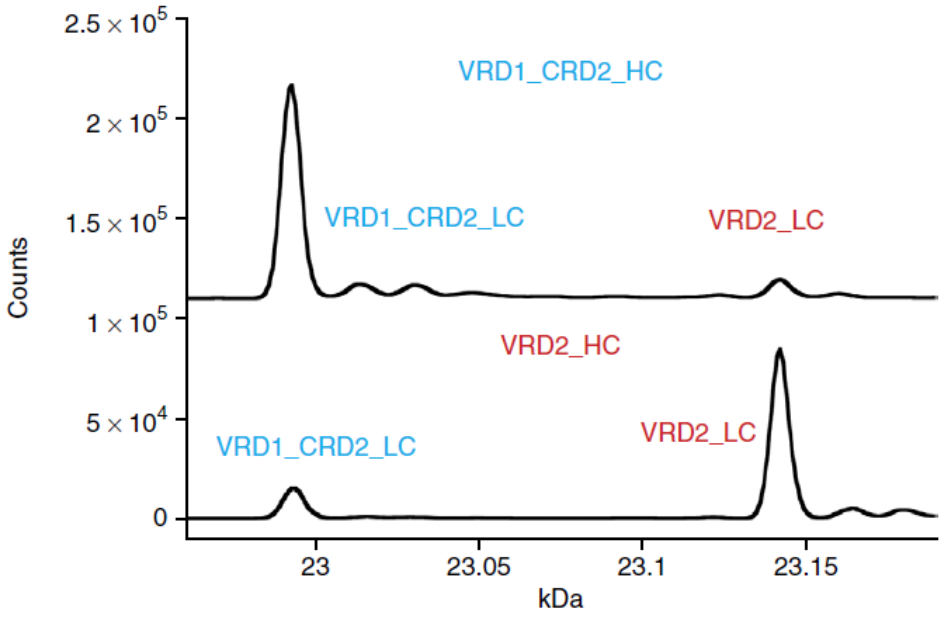

→ CRD2 was better then CDR1 in terms of expression, stability and specificty

# Schematic structure models of designed VRD1 and VRD2



# Competition assay

| LC1 | LC2 | HC | %Assembly[a] (LC1/HC) | %Assembly[a] (LC2/HC) | Expression (μg/mL) |
|---|---|---|---|---|---|
| **$C_H1/C_L$ Specificity Designs in IgG-lacking $V_H/V_L$** | | | | | |
| $C\lambda_{CRD1}$ LC | $C\lambda_{WT}$ LC | $CH1_{WT}$ HC | 36 | 64 | 24 |
| $C\lambda_{CRD1}$ LC | $C\lambda_{WT}$ LC | $CH1_{CRD1}$ HC | 96 | 4 | 18 |
| $C\lambda_{CRD1}$ LC | $C\kappa_{WT}$ LC | $CH1_{WT}$ HC | 58 | 42 | 28 |
| $C\lambda_{CRD1}$ LC | $C\kappa_{WT}$ LC | $CH1_{CRD1}$ HC | 100 | 0 | 61 |
| $C\lambda_{CRD2}$ LC | $C\lambda_{WT}$ LC | $CH1_{WT}$ HC | 7±3 | 93±3 | 29±18 |
| $C\lambda_{CRD2}$ LC | $C\lambda_{WT}$ LC | $CH1_{CRD2}$ HC | 99.7±0.3 | 0.3±0.3 | 46±30 |
| $C\lambda_{CRD2}$ LC | $C\kappa_{WT}$ LC | $CH1_{WT}$ HC | 5±5 | 95±5 | 47±27 |
| $C\lambda_{CRD2}$ LC | $C\kappa_{WT}$ LC | $CH1_{CRD2}$ HC | 100±0.1 | 0±0.1 | 60±30 |
| **$C_H1/C_L$ Specificity Designs in IgG with $V_H/V_L$** | | | | | |
| $VL_{WT}C\lambda_{CRD1}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 6 | 100 | 87 |
| $VL_{WT}C\lambda_{CRD1}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{CRD1}$ HC | 15 | 100 | 104 |
| $VL_{WT}C\lambda_{CRD2}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 50 | 50 | 29 |
| $VL_{WT}C\lambda_{CRD2}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{WT}CH1_{CRD2}$ HC | 79 | 21 | 28 |
| $VL_{WT}C\lambda_{CRD2}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 78 | 22 | 31 |
| $VL_{WT}C\lambda_{CRD2}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{CRD2}$ HC | 79 | 21 | 19 |
| **$V_H/V_L$ Specificity Designs** | | | | | |
| $VL_{WT}C\lambda_{WT}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 18 | 82 | 69 |
| $VL_{VRD1}C\lambda_{WT}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 10 | 90 | 73 |
| $VL_{VRD1}C\lambda_{WT}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{VRD1}CH1_{WT}$ HC | 61 | 39 | 108 |
| $VL_{VRD1}C\kappa_{WT}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 40 | 60 | 132 |
| $VL_{VRD1}C\kappa_{WT}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{VRD1}CH1_{WT}$ HC | 69 | 31 | 112 |
| $VL_{VRD2}C\lambda_{WT}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 39 | 61 | 94 |
| $VL_{VRD2}C\lambda_{WT}$ LC | $VL_{WT}C\kappa_{WT}$ LC | $VH_{VRD2}CH1_{WT}$ HC | 56 | 44 | 105 |
| $VL_{VRD2}C\lambda_{WT}$ LC | $VL_{VRD1}C\kappa_{WT}$ LC | $VH_{VRD1}CH1_{WT}$ HC | 23 | 77 | 95 |
| $VL_{VRD2}C\lambda_{WT}$ LC | $VL_{VRD1}C\kappa_{WT}$ LC | $VH_{VRD2}CH1_{WT}$ HC | 71 | 29 | 71 |
| **Combination of $V_H/V_L$ and $C_H1/C_L$ Specificity Designs** | | | | | |
| $VL_{VRD1}C\lambda_{CRD2}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 15±18 | 85±18 | 42±11 |
| $VL_{VRD1}C\lambda_{CRD2}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{VRD1}CH1_{CRD2}$ HC | 73±12 | 27±12 | 55±26 |
| $VL_{VRD2}C\lambda_{CRD2}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{WT}CH1_{WT}$ HC | 30±24 | 70±24 | 49±17 |
| $VL_{VRD2}C\lambda_{CRD2}$ LC | $VL_{WT}C\lambda_{WT}$ LC | $VH_{VRD2}CH1_{CRD2}$ HC | 84±10 | 16±10 | 51±28 |
| $VL_{VRD1}C\lambda_{CRD2}$ LC | $VL_{VRD2}C\kappa_{WT}$ LC | $VH_{VRD2}CH1_{WT}$ HC | 26±23 | 74±23 | 53±29 |
| $VL_{VRD1}C\lambda_{CRD2}$ LC | $VL_{VRD2}C\kappa_{WT}$ LC | $VH_{VRD1}CH1_{CRD2}$ HC | 73±6 | 27±6 | 73±41 |
| $VL_{VRD1}C\lambda_{CRD2}$ LC | $VL_{VRD2}C\lambda_{WT}$ LC | $VH_{VRD2}CH1_{WT}$ HC | 9±1 | 91±1 | 75±4 |
| $VL_{VRD1}C\lambda_{CRD2}$ LC | $VL_{VRD2}C\lambda_{WT}$ LC | $VH_{VRD1}CH1_{CRD2}$ HC | 89±4 | 11±4 | 76±16 |



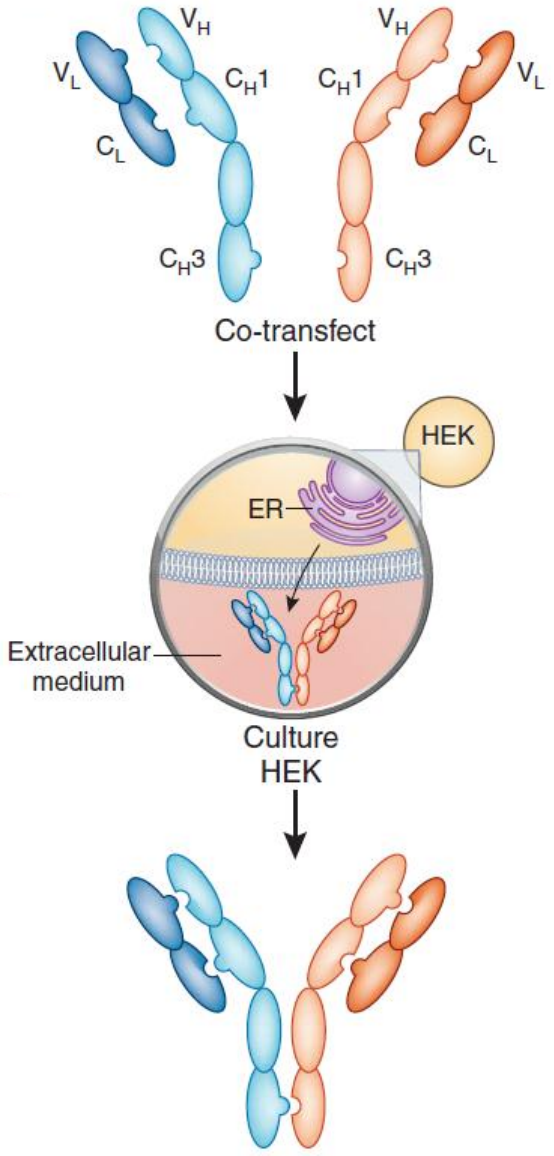

→ VRD1 and VRD2 bound more efficiently to their cognate mutant heavy chain and less efficiently to WT heavy chain

→ For further studies mutant VRD1_CRD2 is used

# Generation of bispecific IgGs

- IgG1 BsAbs:
  - HER2 x EGFR (paired pertuzumab and matuzumab)
  - cMet x Axl (paired anti-c-Met mAb and anti-Axl IgG1 YW327.6S2)
  - EGFR x cMet (paired matuzumab and anti-c-Met mAb)
  - EGFR x LTBR (paired matuzumab and anti-LTBR IgG BHA10)
  - $HER2^{P}$ x $HER2^{T}$ (paired pertuzumab and trastuzumab )

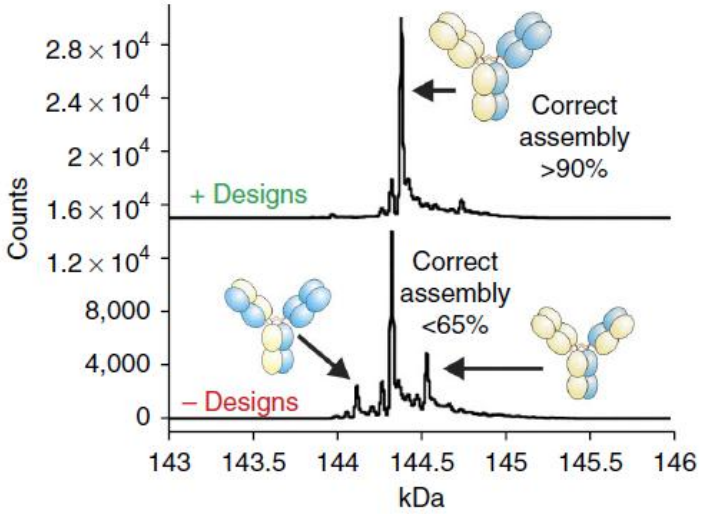
- Knobs-into-holes heterodimerization technology



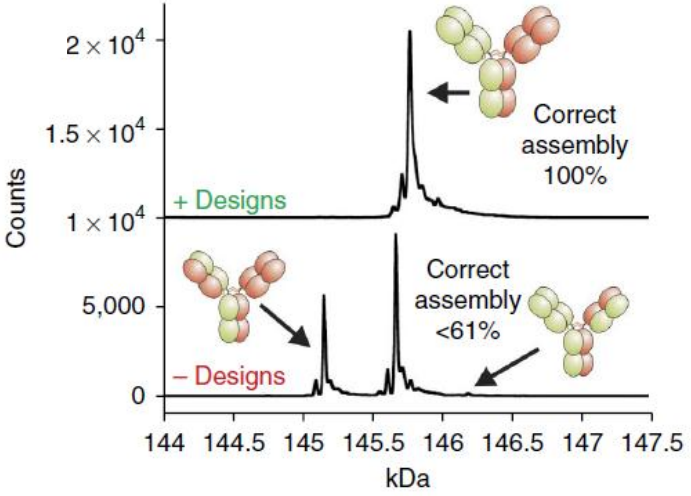

*R.Rouet & D.Christ, Nature Biotech, 2014*

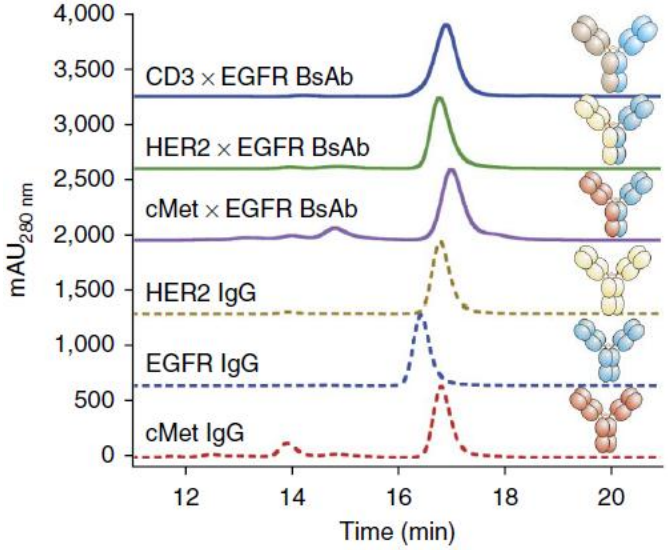
# Generation of bispecific IgGs

HER2 x EGFR IgG1 proteins




cMet x Acl IgG1 proteins

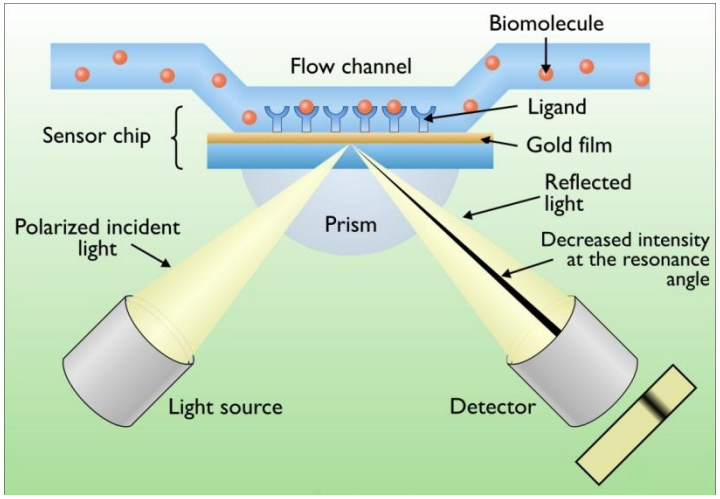


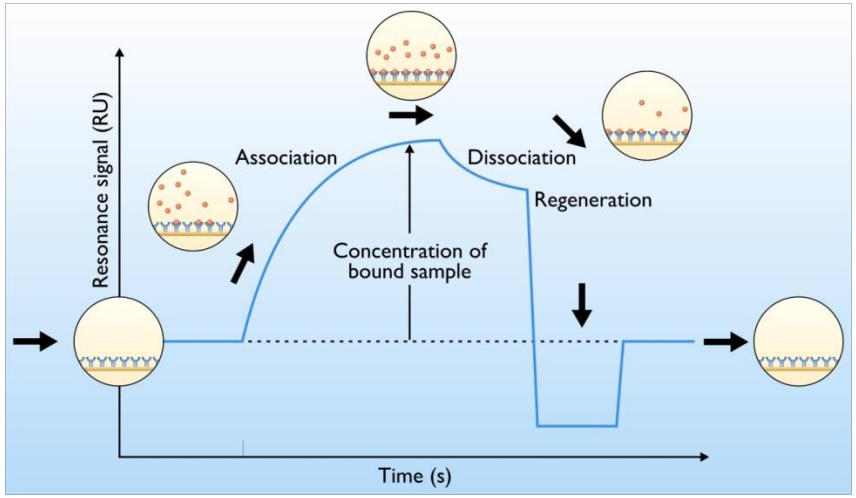




→ monometric

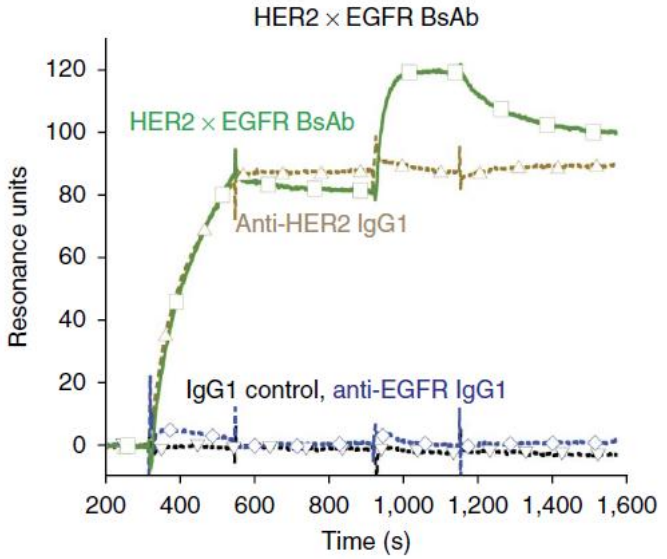
# Bispecific antigen binding behaviour

## Surface plasmon resonance (SPR)

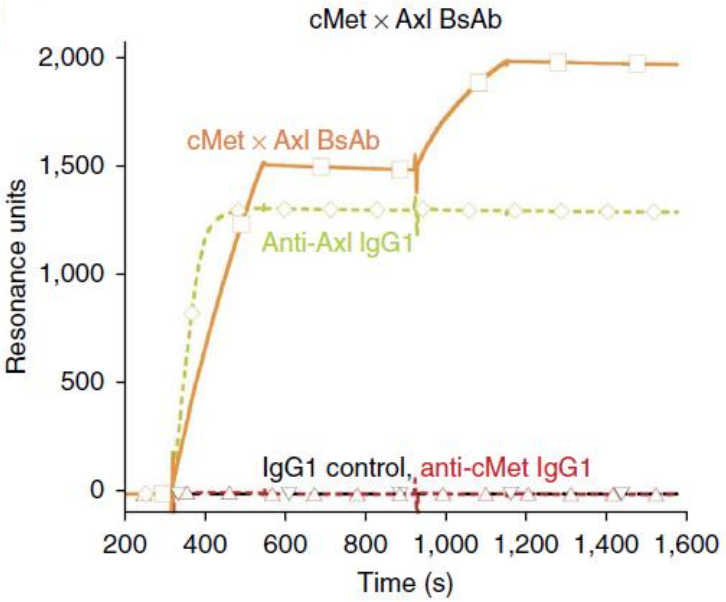



HER2/ EGFR antigen

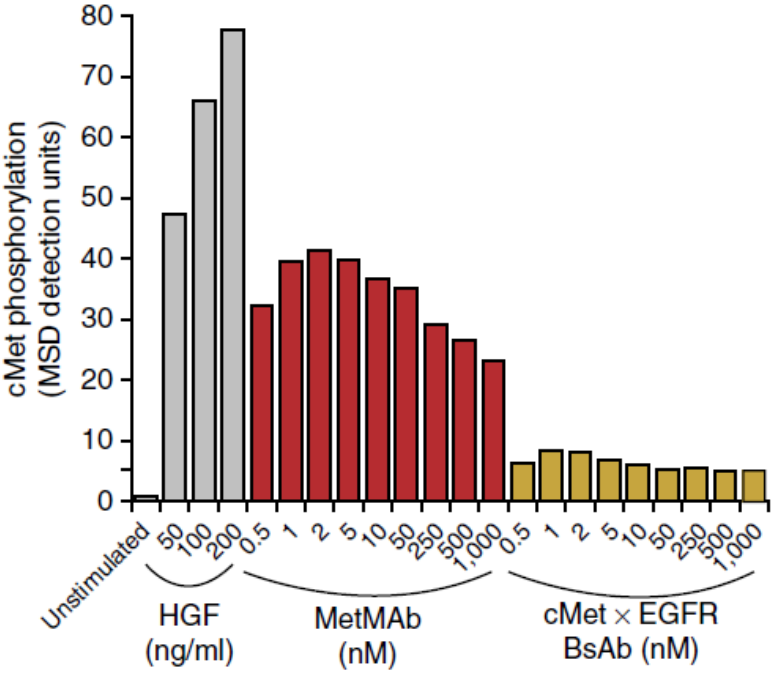


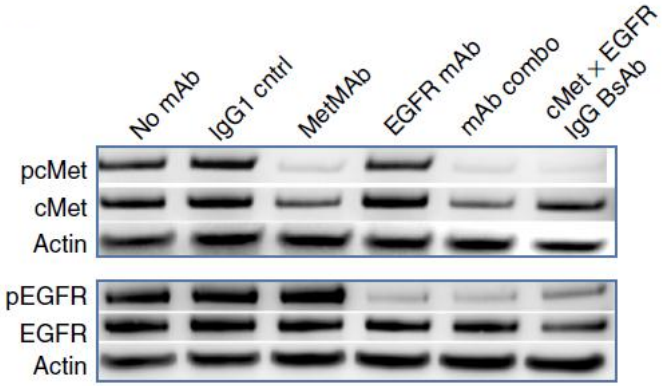

Axl/ cMet antigen




*http://elte.prompt.hu/sites/default/files/tananyagok/practical_biochemistry/ch08s07.html*

# Function of IgG BsAbs

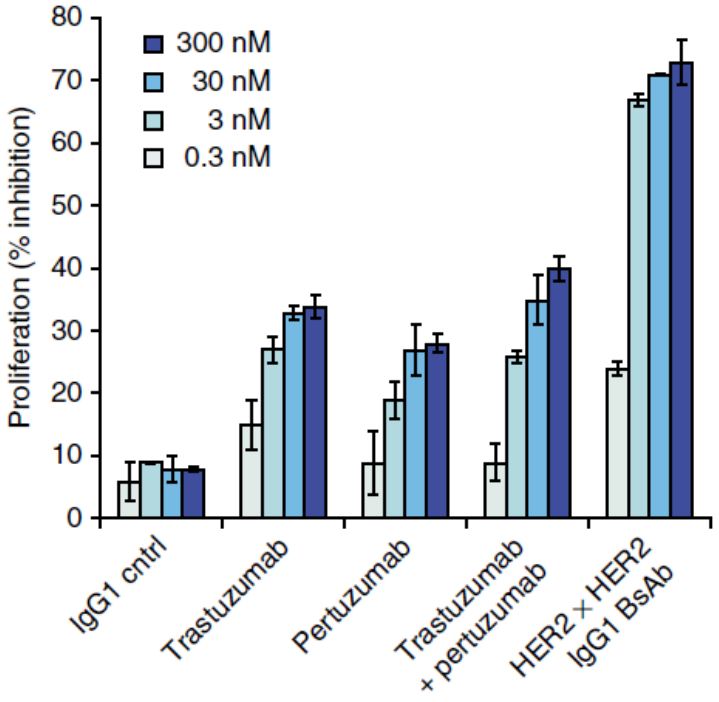



→ BsAbs inhibit phosphorylation of target receptor

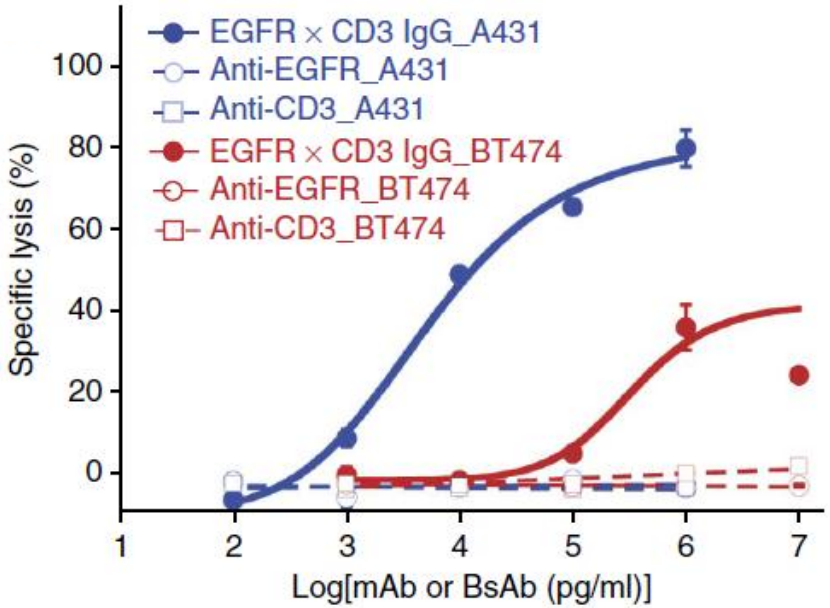
# Applications of bispecific IgGs

Her2 positive N87 gastric cancer cells




→ Her2xHer2 bsABs can reduce serum-induced proliferation

A431 - high EGFR expression
BT474 – low EGFR expression




→ EGFRxCD3 BsAB can enable T cells to kill tumor cells

# conclusion

- Indentification of mutations that prevent heavy – light chain misspairing, when expressed in the same mammalian cell, by using combination of computational design, site-directed mutagenesis ans X-ray christallography

- Combination of orthogonal heavy chain – light chain interface designs with the CH3 domain heterodimer strategy facilitated expression of correctly assembled IgG BsAb

- Further experiments and clinical studies in humans are needed to determine if these mutations increase the immunogenicity of the BsAbs

# Thanks for your attention

http://www.tebu-bio.co.uk/minisite/220/Discover_our_Reagents!.html