PepTalk Pipeline 2: Recombinant Protein Therapeutics
Tuesday, 14 January 2014

Structural variation among antibody Fc fusion proteins for human therapy

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Overview

- FDA-approved mAb products
- Fusion protein
  - Design/Structure
  - Protein A binding
- Structural variation of approved fusion proteins
- Fc fusions in development
- Summary

FDA-approved mAb products

FDA-approved mAbs and derivatives

* Fab or (Fab')$_2$ antibody fragment

**Antibody-drug conjugate

† First mAb approved under animal efficacy rule

‡ Pending

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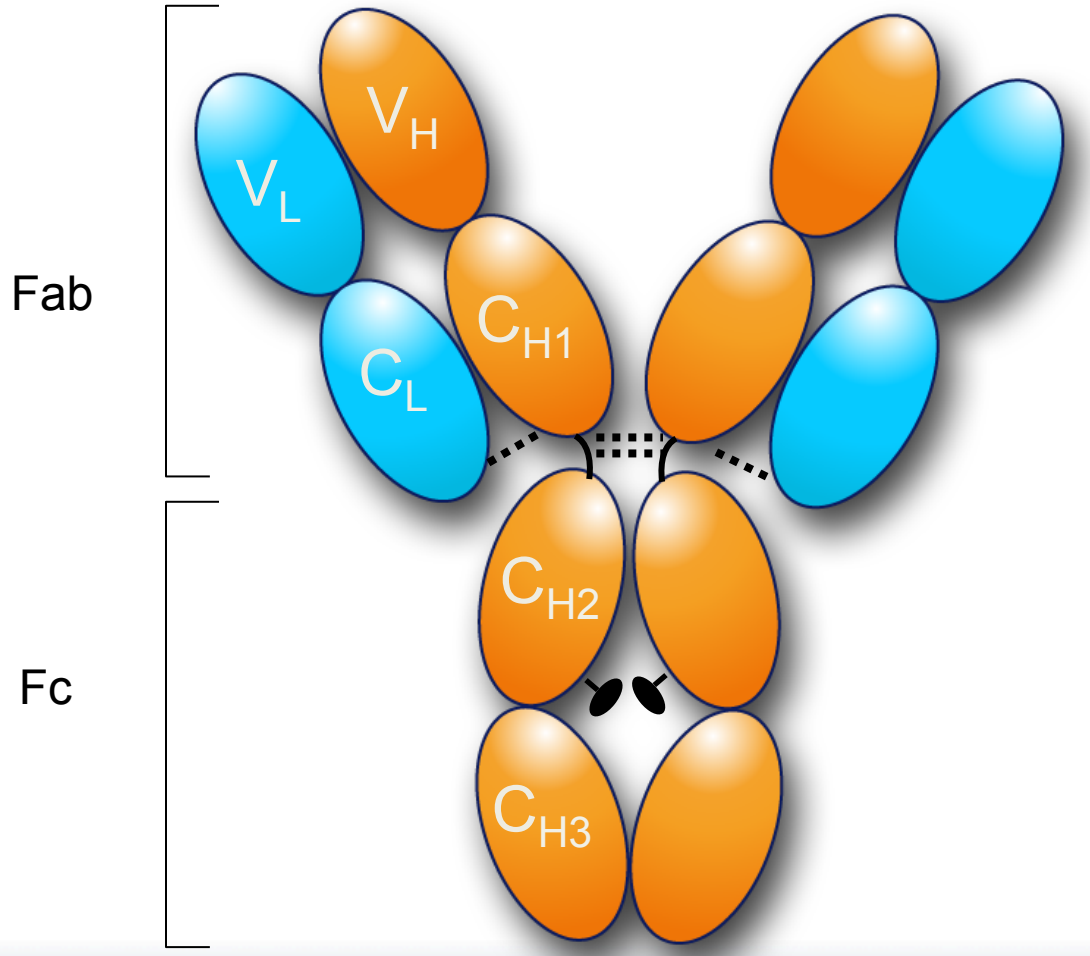
Fc-fusion protein design and structure

Schematic structure of a monoclonal antibody (mAb)



- Oligomer contains four polypeptide chains
- Crosslinks within and between chains, created by oxidized cysteine residues, stabilize structure
- mAbs are glycoproteins; contain attached sugar chains

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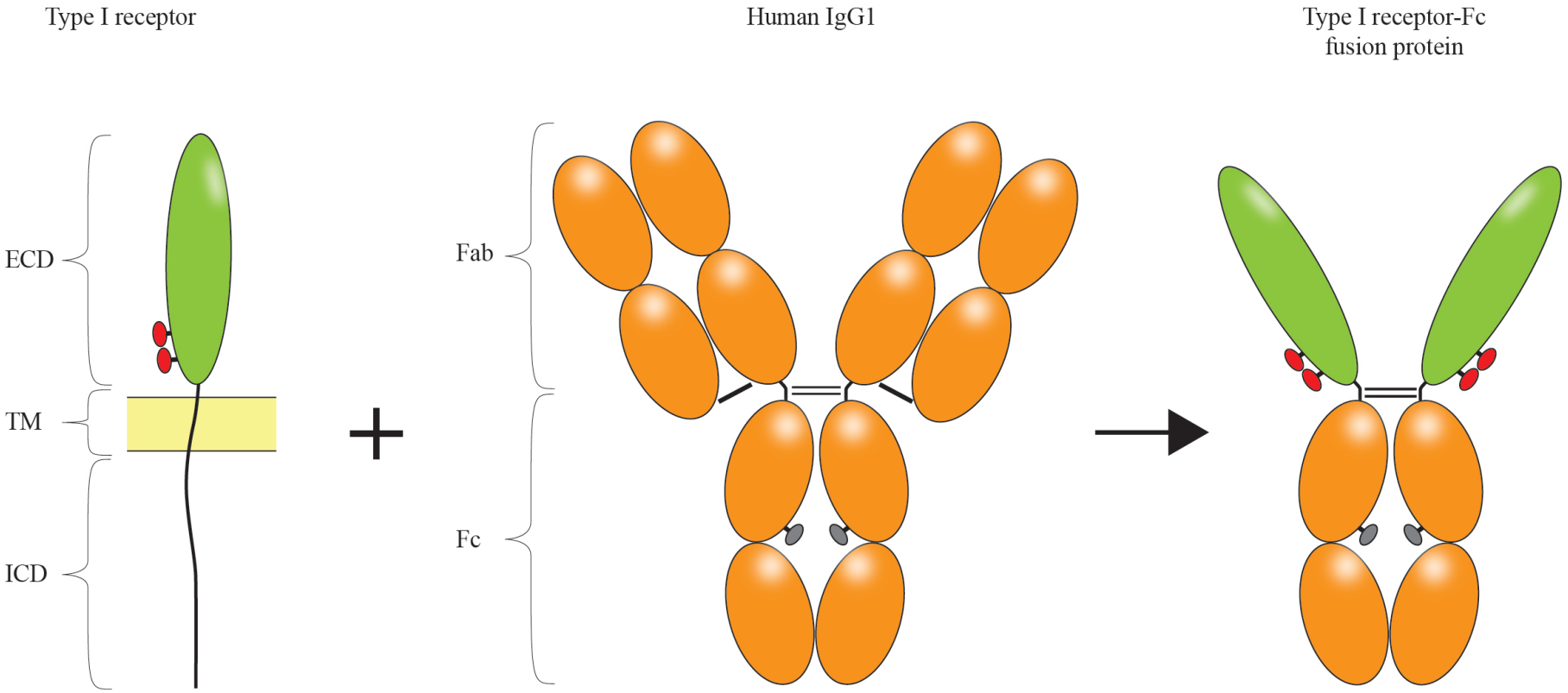
Fusion protein design



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Fc fusion protein: Key structural features

- Homodimer
  - Contains two copies of ligand binding domain
    - Receptor ECD
    - Cytokine
    - Enzyme
    - Peptide
- IgG Fc
  - Retains effector functions
    - ADCC
    - Complement activation
    - Half-life extension
    - Protein A binding
- Fully human
  - Do not require humanization
- High affinity
  - Cytokine traps (Eylea Kd 0.5 pM)

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Structural features (cont'd.)

- Ligand binding domain
  - Replaces Fab (V_L-C_L, V_H1-C_H1)
- Fused into Ig hinge
  - Hinge serves as flexible "spacer" between two parts
    - Ligand binding domain-DKTHTCPPCP-Fc
    - Ligand binding domain-EPKSCDKTHTCPPCP-Fc
- Natural signal sequence
- Acid stabile

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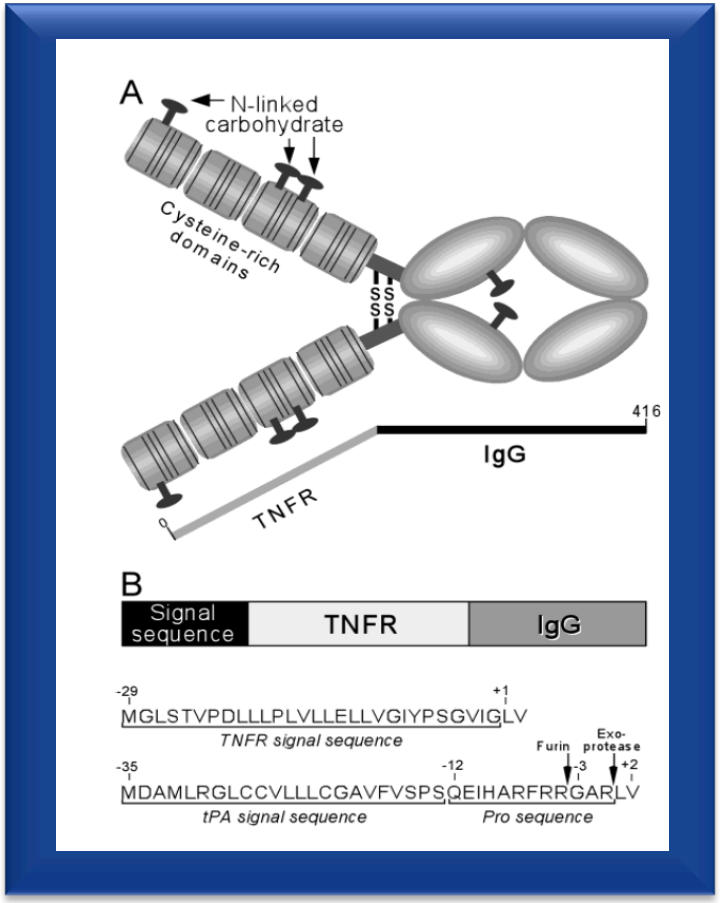
Example: TNFR1(p55)-Fc



MW: 130 kDa

Kohne *et al*., (1999) *J. Cell. Biochem.* **75**, 446-461

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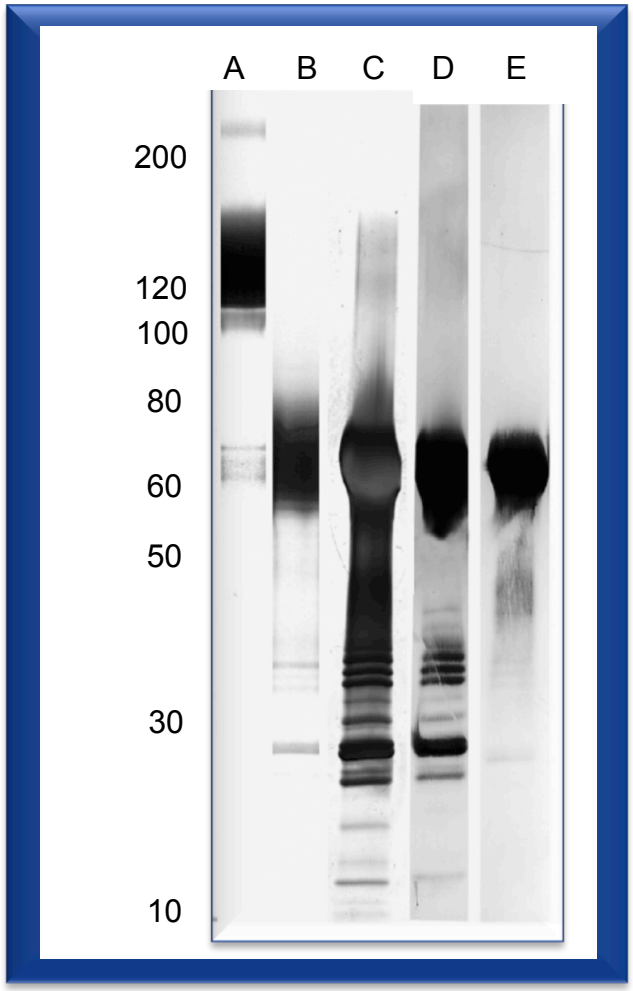
TNFR1-Fc is expressed as a homodimer

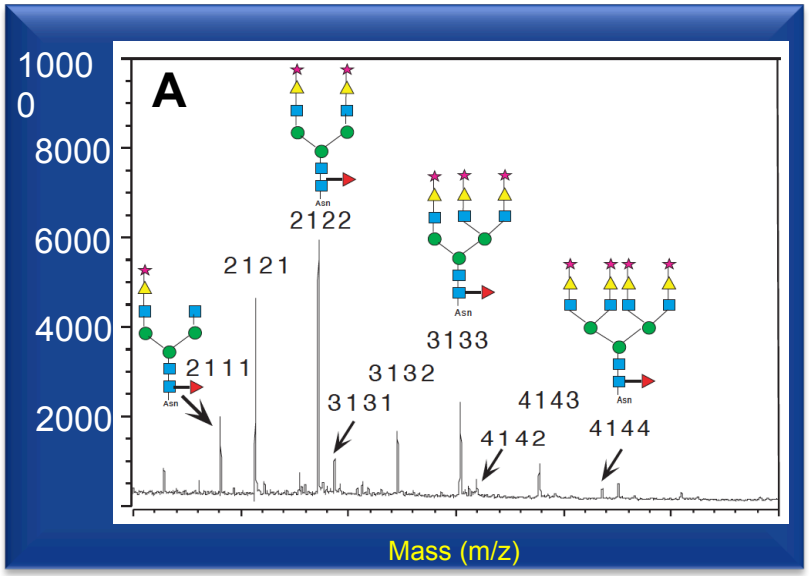


SDS-PAGE analysis of TNFR-IgG. 20 ug, Coomassie stained (A) non-reduced, (B) reduced; 5 ug reduced (C) silver stained, (D) anti-huIgG blot (E) anti-TNFr blot.

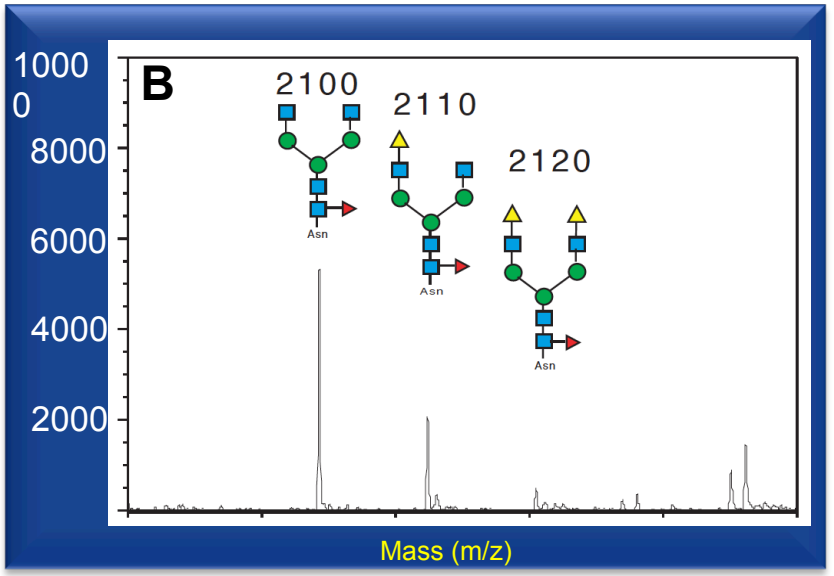
Keck *et al*., (2008) *Biologicals* **36**, 49-60

The TNFR1-Fc domains are glycosylated differently

TNFR1 domain



Fc domain



MALDI-TOF MS in (A) negative ion and (B) positive ion mode of oligosaccharides on TNFR-IgG produced by CHO cells.

Individual oligosaccharides are designated using a four-digit code that specifies the antennarity, number of fucose residues, number of galactose residues, and number of sialic acids present on a complex N-glycan containing trimannose core structure.

Weikert et al., (1999) *Nat. Biotechnol.* **17**, 1116-1121

Sialic acid Galactose N-acetylglucosamine Mannose Fucose

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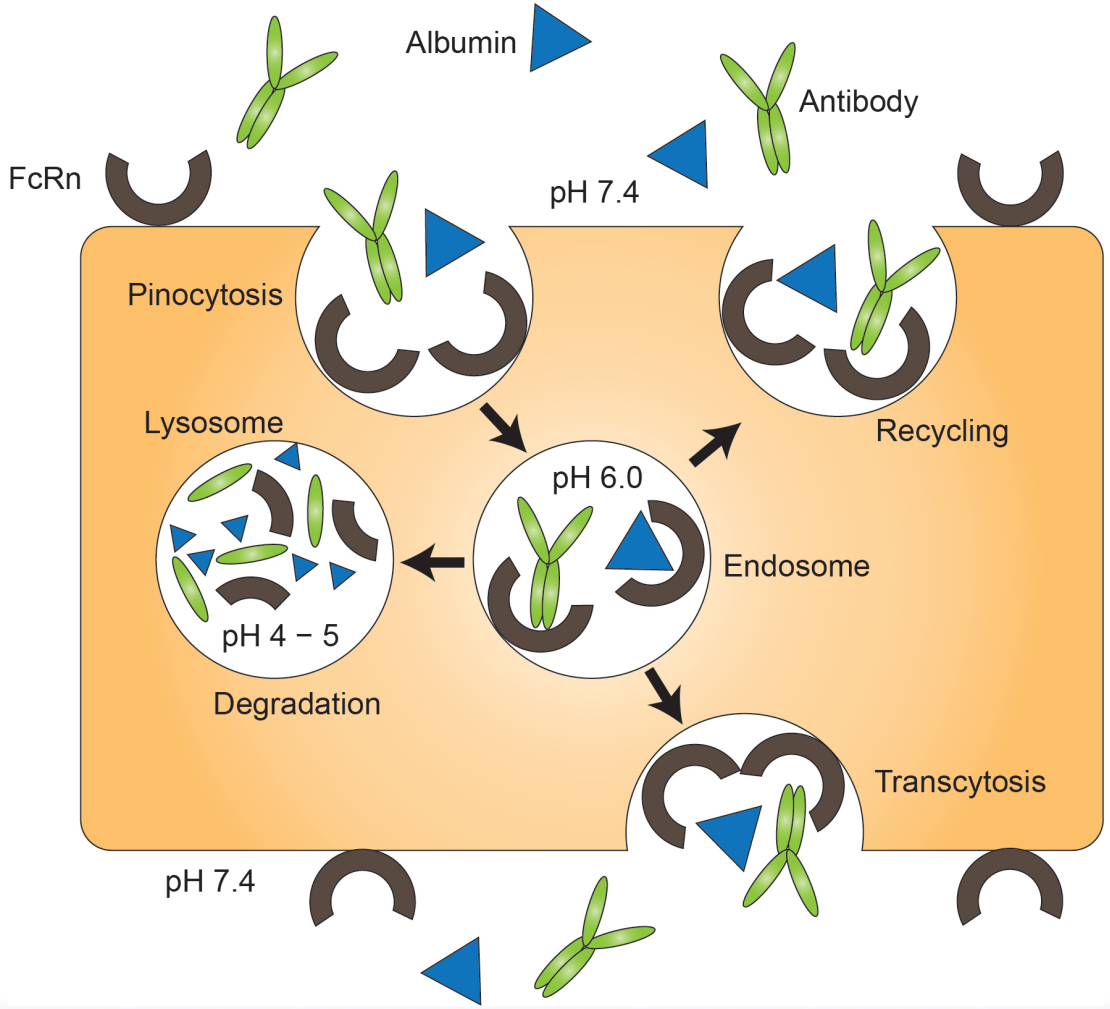
Stability of Fc fusion proteins is due to recycling via FcRn



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Binding capacity of Fc-fusion proteins on Protein A

Observation

- Compared to mAbs, Fc fusions generally have lower dynamic binding capacity on protein A
- Why?

mAbs and Fc-fusion proteins were compared for binding to Protein A

Table I. Properties of molecules used in this study.

Molecule name	MW (kDa)	Type of molecule
A	144.3	Antibody (IgG2)
B	102.4	Fc-fusion of IgG1
C	92.1	Fc-fusion of IgG1
D	152.2	Fc-fusion of IgG1
E	146.6	Antibody (IgG1)
F	102.4	Fc-fusion of IgG1
G	144.0	Antibody (IgG2)

Ghose *et al., Biotechnol. Bioeng.* **96**, 768-779 (2007)

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Dynamic binding capacity: Breakthrough curves MabSelect® Protein A

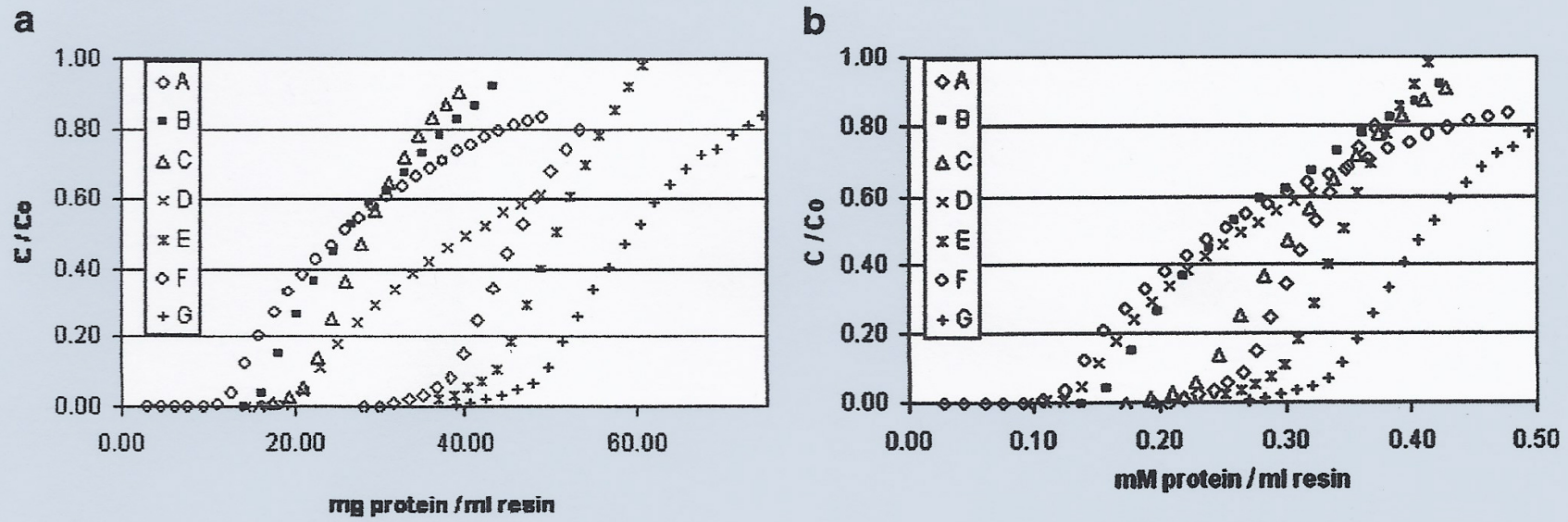

Figure 1. Breakthrough curves on MAbSelect® Protein A media at 5 min residence time. Column loading expressed in terms of (a) mg antibody/mL of resin (b) mM antibody/mL of resin.

Ghose *et al., Biotechnol. Bioeng.* **96**, 768-779 (2007)

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Conclusion from breakthrough curves
Order of dynamic binding capacity

E=G > C=A > F=D=B

A	mAb
B	Fc fusion
C	Fc fusion
D	Fc fusion
E	mAb
F	Fc fusion
G	mAb

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Dynamic binding capacity correlates *inversely* with molecular size

Fc fusions B, D, and F:

- Lowest DBC
- Largest hydrodynamic (Stokes) radius

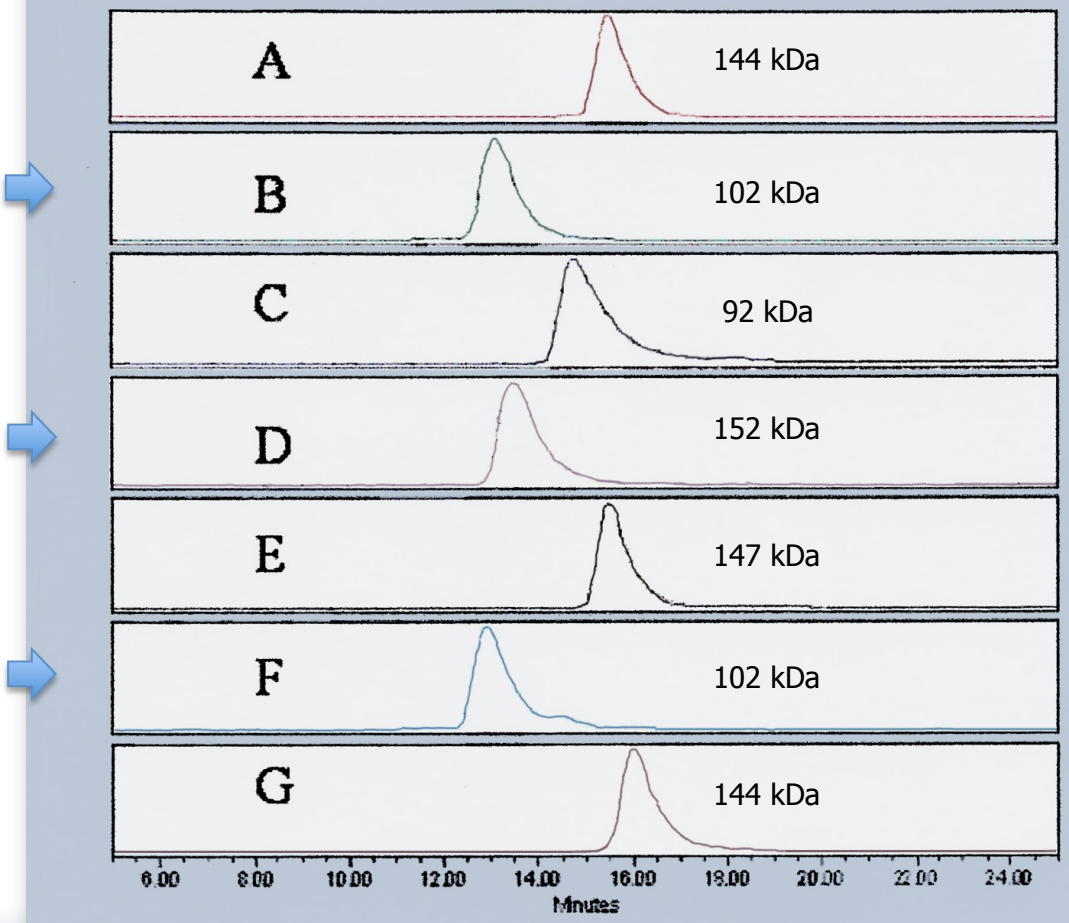

Figure 3. SEC chromatograms.

Ghose *et al.*, *Biotechnol. Bioeng.* **96**, 768-779 (2007)

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Dynamic binding capacity correlates *directly* with stoichiometry of binding to Protein A

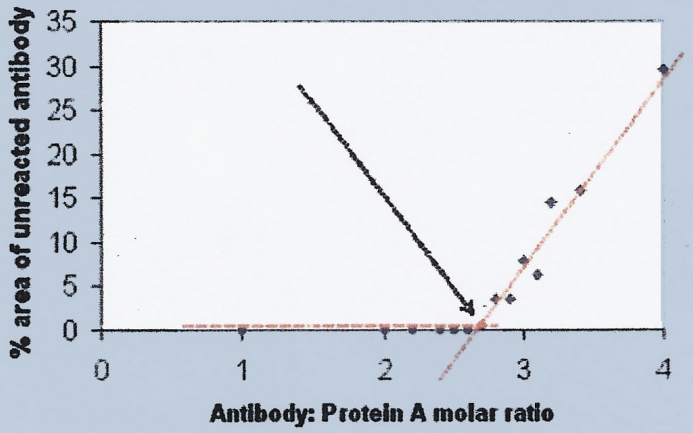

Figure 4. Calculation of solution binding stoichiometry between Protein A and a monoclonal antibody or Fc fusion protein.

Table IV. Solution binding stoichiometry data for the various molecules with Protein A.

Molecule name	Number of molecules bound/molecule of Protein A
A	2.54 ± 0.08
B	2.62 ± 0.04
C	2.4 ± 0.04
D	2.82 ± 0
E	3.05 ± 0.05
F	2.6 ± 0.09
G	3.1 ± 0.05

Ghose *et al.*, *Biotechnol. Bioeng.* **96**, 768-779 (2007)

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Summary: Dynamic binding capacity correlates with size/stoichiometry

ID	Type	Dynamic binding capacity	MW (kDa)	Size (Stokes radius) from SEC	Stoichiometry from solution binding
A	mAb	Intermediate	144	Small	Low
B	Fc fusion	Low	102	Large	Low
C	Fc fusion	Intermediate	92	Intermediate	Low
D	Fc fusion	Low	152	Large	Intermediate
E	mAb	High	146	Small	High
F	Fc fusion	Low	102	Large	Low
G	mAb	High	144	Small	High

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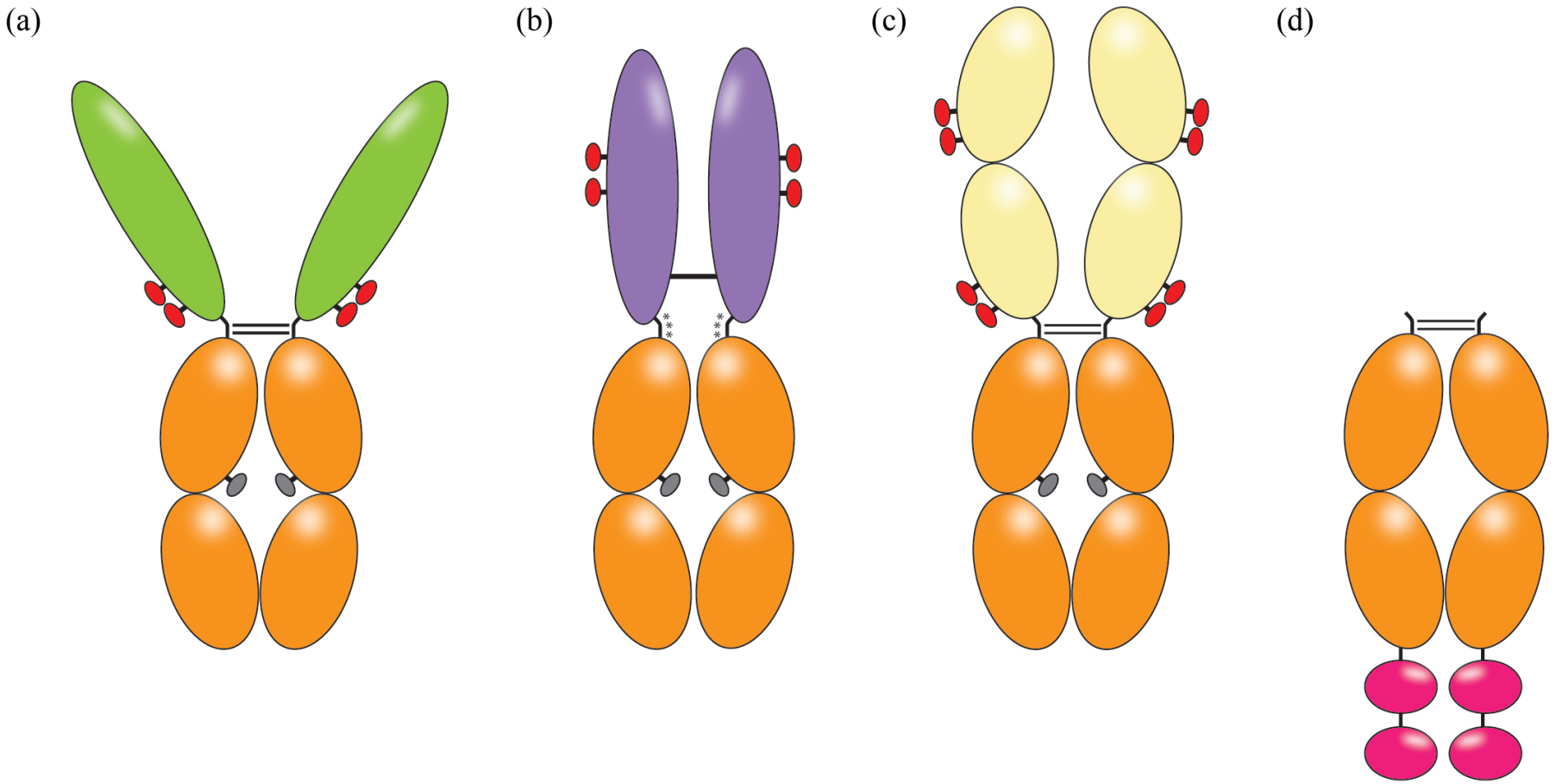
FDA-approved Fc fusion protein therapeutics

Approved Fc-fusions

Product	Molecular construct	Ligand and clinical effect	Expression system	Company	FDA approval
Amevive (alefacept)	LFA3-IgG1 Fc	Binds CD2; inhibits T-cell proliferation in psoriasis; transplant rejection	CHO	Astellas/BiogenIdec	2003*
Enbrel (etanercept)	TNFR2-IgG1 Fc	Binds to soluble and membrane TNF; for treatment of RA and plaque psoriasis	CHO	Amgen/Immunex	1998
Orencia (abatacept)	CTLA4-IgG1 Fc	Binds CD80, CD86, inhibits T-cell costimulation In RA	CHO	Bristol-Myers Squibb	2005
Nulojix (belatacept)	CTLA4-IgG1 Fc	Binds CD80, CD86, inhibits T-cell costimulation in transplant rejection	CHO	Bristol-Myers Squibb	2011
Eylea (aflibercept)	VEGFR1-VEGFR2-IgG1 Fc	Binds VEGF-A and B, PlGF; for treatment of wet age-related macular degeneration	CHO	Regeneron/Sanofi-Aventis	2011
Zaltrap (ziv-aflibercept)	VEGFR1-VEGFR2-IgG1 Fc	Binds VEGF-A and B, PlGF; for treatment of colorectal cancer	CHO	Regeneron/Sanofi-Aventis	2012
Arcalyst (rilonacept)	IL-1R – IgG1 Fc	Binds and neutralizes IL-1; for treatment of cryopyrin-associated periodic syndrome	CHO	Regeneron/Sanofi-Aventis	2008
Nplate (romiplostim)	Thrombopoietin-binding peptide–IgG1 Fc (peptibody)	Acts as agonist on thrombopoietin receptor to stimulate production of platelets in refractory immune thrombocytopenia	*E. coli*	Amgen	2008

*Withdrawn 2011

Structural variation: Approved Fc fusions



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Structural variation (cont'd.)

- Group (a)
  - Bivalent
    - Alefacept
    - Receptor antagonist
- Group (b)
  - Monovalent
    - Abatacept, belatarcept, etanercept
    - Receptor antagonists

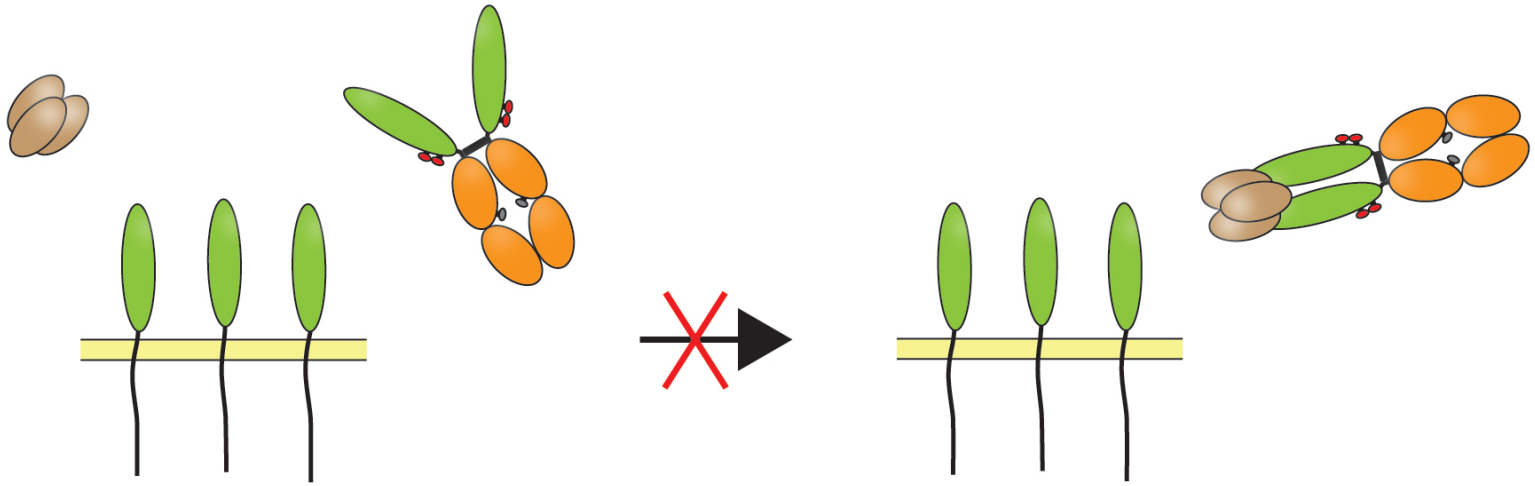
Structural variation (cont'd.)

- Group (c)
  - Traps: monovalent
    - Rilonacept, aflibercept, *ziv*-alfibercept
    - Receptor antagonists

- Group (d)
  - Peptibodies: multivalent
    - Romiplostim
    - Receptor agonist

Group b: Etanercept is monovalent

Signaling



No signaling

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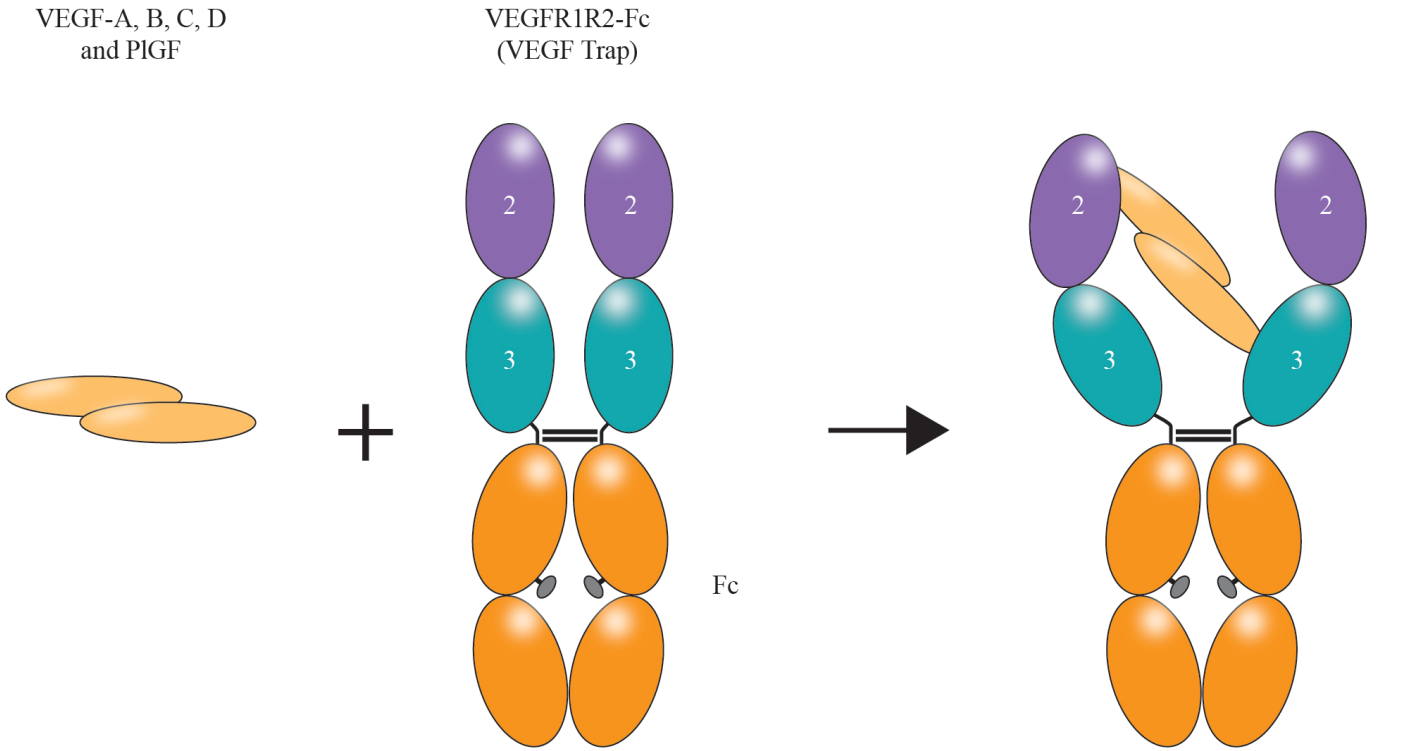
Group c: Aflibercept is monovalent



	K_D
VEGFR1	10 – 30 pM
VEGFR2	100 – 300 pM
VEGF Trap	~ 0.5 pM

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Novel Fc fusions in development and biosimilars

Selected therapeutic Fc-fusion proteins in clinical testing

Product	Molecular construct	Ligand and clinical effect	Expression system	Company	Clinical phase
rFVIII-Fc	FVIII-IgG1 Fc	Blood clotting factor; replacement therapy for hemophilia A	HEK-293	BiogenIdec	Approval pending
rFIX-Fc	FIX-IgG1 Fc	Blood clotting factor: replacement therapy for hemophilia B	HEK-293	BiogenIdec	Approval pending
trebananib (AMG386)	TIE2 mimetic peptide–IgG1 Fc (peptibody)	Targets and binds to Ang1 and Ang2, preventing interaction of angiopoietins with TIE2 receptor; antiangiogenic	E. coli	Amgen	Phase III
blisibimod (A-623, AMG 623)	BAFF-IgG1 Fc	Binds to BAFF and inhibits receptor interaction, decreasing B cell survival. May be effective in SLE and RA	CHO	Anthera/Amgen	Phase III
Dulaglutide (LY2189265)	GLP1 peptide analog-IgG1 Fc	Mimics effects of GLP1 on insulin resistance and VLDL production	CHO	Lilly	Phase III
CNTO 528/CNTO 530	Erythropoietin mimetic peptide-IgG1 (CNTO528) and -IgG4 Fc (CNTO 530) (Mimetibody)	Binds and activates erythropoietin receptor, stimulating erythrocyte production	CHO	CentocorJ&J/Edison	Phase I
APG101 (Apocet^{TM})	CD95-IgG1 Fc	Blocks CD95 ligand, reducing cancer cell migration in malignant glioma	CHO	Apogenix	Phase II

Heterodimeric fusion proteins

- Long-acting recombinant blood factors
  - rFVIII-Fc
  - rFIX-Fc
- Half-life enhancement
  - Monomeric structure improved PK, PD more than dimeric structure
    - 3-4 fold increase in $t_{1/2}$ over rFIX
- Produced in HEK293 cells
  - γ-Carboxylation required for bioactivity
  - 9:1 Fc:FIXFc plasmid ratio optimal for purification of heterodimer

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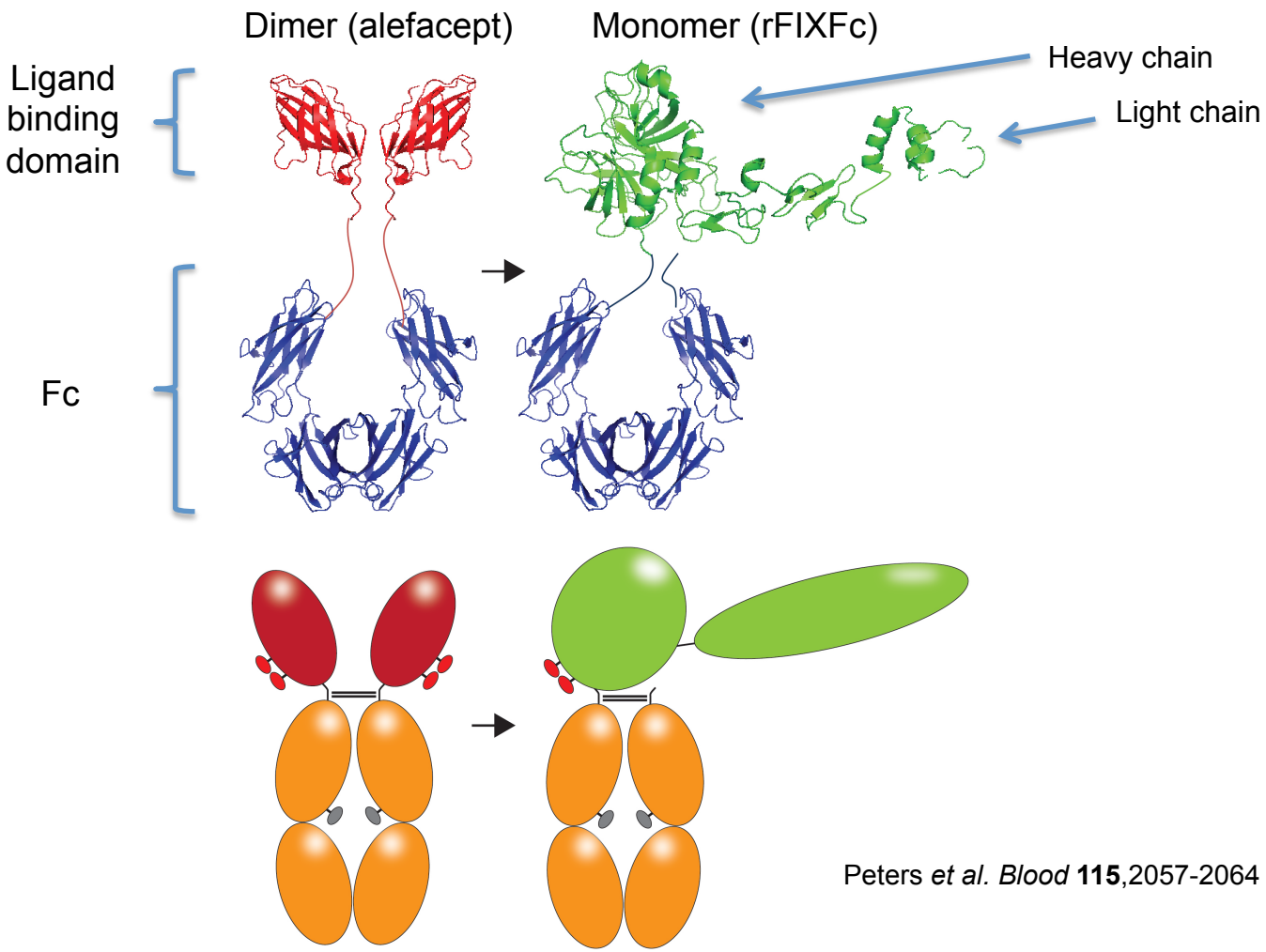
Dimeric vs. monomeric



Peters *et al. Blood* **115**,2057-2064 (2010)

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Fc fusion proteins as biosimilars

- Etanercept
  - Approved in 1998
  - 15 years market exclusivity
  - Candidate for biosimilar development
- Patent protection
  - Patents set to expire Oct 2012
  - In Nov 2011, new US patent (#8,063,182) issued to Roche (licensed exclusively to Amgen) extended patent protection for Amgen in US until 2028
- Etanercept biosimilars will be introduced in EU and markets outside the US

Conclusions

- Fc fusion proteins are an important new class of biotherapeutics
- Structural derivatives of mAbs; manufactured using processes that are based on the mAb production platform
  - SpA DBC correlates inversely with molecular size and directly with stoichiometry of binding
- Versatility of protein types in fusion partners
- Typically homodimers; can be functionally monovalent (abatacept, etanercept, traps), bivalent (alefacept), multivalent (romiplostim)
- Of 42 FDA-approved mAbs, 8 are fusion proteins, 8 more in clinical trials
- Biosimilars are coming

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Further reading: March 2014

Title: Therapeutic Fc Fusion Proteins

Editors: S.M. Chamow, T. Ryll, H.B. Lowman and D. Farson

Publisher: Wiley-Blackwell

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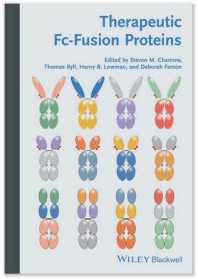

Therapeutic Fc Fusion Proteins

Steven M. Chamow (Editor), Thomas Ryll (Editor), Henry B. Lowman (Editor)

ISBN: 978-3-527-33317-2

400 pages
March 2014, Wiley-Blackwell

Description

Edited by three pioneers in the field, each with longstanding experience in the biotech industry, this is the first book to cover every step in the development and production of Fc-fusion proteins -- from choosing the right design on a molecular level to batch optimization during production to quality control.
The whole of the second part is devoted to case studies detailing the most important commercially available products and includes a chapter on promising new developments for the future.
An invaluable resource for professionals already working on Fc-fusion proteins and a must for researchers and students entering the field.

Author Information

Steven Chamow, Ph.D.
Steven Chamow has developed biopharmaceutical products for 24 years. During his career, he has contributed to the development of three marketed products (Avastin, Natrecor, Vectibix). He served in executive roles at several companies including Intradigm Corporation, a private biopharmaceutical company focused on developing RNAi therapeutics (acquired by Silence Therapeutics), Genitope Corporation, and Abgenix, Inc., (acquired by Amgen) where he built the company?s process sciences department and co-lead the design and construction of Abgenix? award-winning production facility in Fremont, CA (recently sold by Amgen to Boehringer-Ingelheim to become its first North American production facility). Before Abgenix, he served as Director of Biopharmaceutical Development at Scios, Inc. (acquired by J&J), and as a scientist and senior scientist in process development at Genentech, Inc. (acquired by Roche). Dr. Chamow was educated at the University of California (UC Santa Cruz, B.A. in biology; UC Davis, Ph.D. in

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