Antibodies as Drugs

- An emerging theme
- Historical breakthroughs
- Terms to know
- A look at antibody structure
- Production of monoclonal antibodies
- Antibodies as biopharmaceutical
- Autoimmune Disease
  - Rheumatoid Arthritis
- Variations and future directions
Emerging Themes

• Antibodies are naturally occurring
• Discovery of their innate properties hinted at great therapeutic potential
  – High-specificity in binding
  – Already present in the body
  – Can activate and couple components of the immune system
• Modification to structure and refinement in production methods have made antibodies a viable modern drug
At the turn of the 20th century:

- Emil Adolf von Behring
  - Developed serum therapy as an effective treatment against diphtheria and tetanus
  - For this, he received the first ever Noble Prize in Physiology or Medicine in 1901
  - The serum derived from immunized animals was latter shown to be effective because of the antibodies it contained
- Paul Ehrlich
  - Side-chain theory:
    - Toxins and antitoxins were chemical substances
    - Antitoxins were side-chains on cells that could bind with a toxin like a lock and key
  - Predicted autoimmunity or “horror autotoxicus”
  - Received the 1908 Nobel Prize in Physiology or Medicine for his work in immunity
More recently:

- **“Discovery” of antibody chemical structure**
  - Gerald Edelman and Rodney Porter, circa 1961
  - Received the 1972 Nobel Prize in Physiology or Medicine

- **Development of hybridoma technology**
  - Jerne, Kohler, and Milstein, 1975
  - Received the 1984 Nobel Prize in Physiology or Medicine

- **Production of the 1\textsuperscript{st} monoclonal antibody**
  - In 1986, OKT-3 was approved for use in organ transplant rejection
Important Terms

• **Antibody** – immunoglobulin secreted by B cells

• **Antigen (antibody generator)** – any substance capable of eliciting an adaptive immune response

• **Monoclonal antibodies (mAbs)** – antibodies secreted from a single B cell, have identical paratopes

• **Epitope** – region of the antigen recognized by an antibody

• **Paratope** – region of the antibody that binds the epitope
Antibody production

Monoclonal Antibodies
- Expensive production
- Long production time
- Large quantities of specific antibodies
- Recognize a single epitope on an antigen
- Production is continuous and uniform once the hybridoma is made

Polyclonal Antibodies
- Inexpensive production
- Rapid production
- Large quantities of nonspecific antibodies
- Recognize multiple epitopes on an antigen
- Different batches vary in composition
Polyclonal Antibody Production

1. Inject antigen into rabbit.
2. Antigen activates B cells.
3. Plasma B cells produce polyclonal antibodies.
4. Obtain antiserum from rabbit containing polyclonal antibodies.

https://opentextbc.ca/microbiologyopenstax/chapter/polyclonal-and-monoclonal-antibody-production/
Monoclonal Antibody Production

1. Injection of antigen into a mouse
2. Collection of spleen cells
3. Formation of hybrid cells
4. Selection and growth of hybrid cells
5. Separation of hybrid cells into clones
6. Screening for desired antibody
7. Chosen hybridoma is then grown to produce large batches of desired monoclonal antibody (mAB)
The Structure of an Antibody

- 2 identical light chains (~220 amino acids long)
  - Variable domain: \( V_L \)
  - Constant domain: \( C_L \)
- 2 identical heavy chains (~440 amino acids long)
  - Variable domain: \( V_H \)
  - 3 Constant domains: \( C_H1, C_H2, C_H3 \) (IgG, IgA, IgD)
  - 4 Constant domains: \( C_H1, C_H2, C_H3, C_H4 \) (IgM, IgE)
- Covalent, disulfide bonds between cysteine residues
- Flexible “hinge region” (rich in proline and cysteine residues, not in IgM/IgE)
- Immunoglobulins are glycoproteins
  - glycans associate with the Fc domain
  - glycans affect antibody function
The Structure of an Antibody

![Diagram of antibody structure](image)

- **Antigen binding site**
- **Variable region**
  - HV regions
  - Framework regions
- **Constant region**
  - Disulfide bonds
- **Light chain**
- **Heavy chain**
- **IgG**
- **Fab**
  - Proteolytic cleavage by papain
- **Fc**

BioOrg8-10
Isotypes have different heavy chains. They are represent classes of antibody.

Allotypes have the same constant regions with minor, but immunologic differences. Different individuals have different allotypes.

Idiotypes are antibodies that recognize different specific epitopes. Each idiotype is composed of several idiotopes or combining sites.
A Dynamic Binding Site

- The functional groups of the paratope (Fab) interact with the epitope (antigen)
  - Electrostatic forces (attraction between opposite charges)
  - Hydrogen bonding (Hydrogens shared between electronegative atoms)
  - Van der Waals forces (fluctuations in electron clouds around molecules oppositely polarize neighbouring atoms)
  - Ionic interactions (hydrophobic groups pack together to exclude water, involves Van der Waal's forces)

- The CDRs (Complementarity-Determining Regions) are necessary for antigen binding
- The tertiary structure of this region can contain pockets, undulating flatter surfaces, and even protrusions
- Small antigens typically bind in deep pockets
Hypervariable Loops

- Complementarity-Determining Regions (CDRs)
- Regions of increased amino acid sequence variability
- In each variable region,
  - 3 CDRs interspersed in between framework regions
  - Each CDR is between 5-10 amino acids long
<table>
<thead>
<tr>
<th></th>
<th>IgM pentamer</th>
<th>IgG monomer</th>
<th>Secretory IgA dimer</th>
<th>IgE monomer</th>
<th>IgD monomer</th>
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<tr>
<td>Heavy chains</td>
<td>µ</td>
<td>γ</td>
<td>α</td>
<td>ε</td>
<td>δ</td>
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<tr>
<td>Number of antigen binding sites</td>
<td>10</td>
<td>2</td>
<td>4</td>
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<td>2</td>
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<tr>
<td>Molecular weight (Daltons)</td>
<td>900,000</td>
<td>150,000</td>
<td>385,000</td>
<td>200,000</td>
<td>180,000</td>
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<tr>
<td>Percentage of total antibody in serum</td>
<td>6%</td>
<td>80%</td>
<td>13%</td>
<td>0.002%</td>
<td>1%</td>
</tr>
<tr>
<td>Crosses placenta</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
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<tr>
<td>Fixes complement</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Fc binds to</td>
<td>phagocytes</td>
<td></td>
<td>mast cells and basophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor</td>
<td>Main blood antibody of secondary responses, neutralizes toxins, opsonization</td>
<td>Secreted into mucus, tears, saliva, colostrum</td>
<td>Antibody of allergy and antiparasitic activity</td>
<td>B cell receptor Microbial sensing and immune activation</td>
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</table>
Engineered hapten-binding antibody derivatives for modulation of pharmacokinetic properties of small molecules and targeted payload delivery

- MCPC603 antibody
- Fab fragment
- hapten phosphorylcholine in the binding pocket
- haptens are very important and robust immunogens
- First antibody structures were those of hapten binders
- Expansion of antibody engineering technologies

Immunoijlogcal Reviews
Volume 270, Issue 1, pages 165-177, 10 FEB 2016 DOI: 10.1111/imr.12386
Antibody Pharmokinetics

- Antigen binding is reversible
  - Antigen (Ag) + Antibody (Ab) ⇌ AntigenAntibody (AgAb) [bound]
  - $K_{affinity} = \frac{[AgAb]}{[Ag][Ab]}

- For some therapeutic mAbs, the affinity must be balanced so that effective antigen binding occurs while tissue penetration is allowed
Cross-linking

- All antibodies are at least bivalent
  - Two paratopes can bind with two epitopes
- With 2 epitopes on a single antigen, cyclic or linear cross-linking can occur.
- Three or more epitopes on an antigen leads to formation of large three-dimensional lattices
Mechanisms of Action

1. Blocking action of molecular targets
   - Can work antagonistically by binding a receptor to prevent activation
   - Can also bind the antigen and prevent activation

2. “Magic Bullet”
   - Compound with target specificity is coupled with various effector groups
     • Toxins, radionuclei, enzymes, DNA

3. Signal molecules
   - Coupled to mediators of apoptosis, cell division, etc.
“Humanizing” Antibodies

- **Chimeric Antibodies**
  - Murine Fv + human Fc
  - Human anti-chimeric antibodies (HACA) still observed
- **Humanized Antibodies**
  - Murine CDRs + human framework and Fc
Identification of Human Antibodies

Pharmaceutical Antibodies

• The fastest growing segment of the biopharmaceutical market
  – $14 billion in sales for 2005
  – Expected to grow to $30 billion by 2010
• Today, 20 therapeutic mAbs are on the market in the US
• However, an estimated 500 antibody-based therapies are currently under development
# Nomenclature of Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Prefix</th>
<th>Target</th>
<th>Source</th>
<th>Suffix</th>
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<tbody>
<tr>
<td>-o(s)-</td>
<td>bone</td>
<td>-u-</td>
<td>human</td>
</tr>
<tr>
<td>-vi(r)-</td>
<td>viral</td>
<td>-o-</td>
<td>mouse</td>
</tr>
<tr>
<td>-ba(c)-</td>
<td>bacterial</td>
<td>-a-</td>
<td>rat</td>
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<tr>
<td>-li(m)-</td>
<td>immune</td>
<td>-e-</td>
<td>hamster</td>
</tr>
<tr>
<td>-le(s)-</td>
<td>infectious lesions</td>
<td>-i-</td>
<td>primate</td>
</tr>
<tr>
<td>-ci(r)-</td>
<td>cardiovascular</td>
<td>-xi-</td>
<td>chimeric</td>
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<td>-mu(l)-</td>
<td>musculoskeletal</td>
<td>-zu-</td>
<td>humanized</td>
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<td>-ki(n)-</td>
<td>interleukin</td>
<td>-axo-</td>
<td>rat/murine hybrid</td>
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<td>-co(l)-</td>
<td>colonic tumor</td>
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<td></td>
</tr>
<tr>
<td>-me(l)-</td>
<td>melanoma</td>
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<td></td>
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<tr>
<td>-ma(r)-</td>
<td>mammary tumor</td>
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<td></td>
</tr>
<tr>
<td>-go(t)-</td>
<td>testicular tumor</td>
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<td></td>
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<tr>
<td>-go(v)-</td>
<td>ovarian tumor</td>
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</tr>
<tr>
<td>-pr(o)-</td>
<td>prostate tumor</td>
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<td>-tu(m)-</td>
<td>miscellaneous tumor</td>
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<tr>
<td>-neu(r)-</td>
<td>nervous system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-tox(a)-</td>
<td>toxin as target</td>
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</tbody>
</table>

**variable**
Autoimmune Disease

• An immune reaction against self
• Mechanism unknown, arises out of a failure in immune regulation
• Examples:
  – Rheumatoid arthritis
  – Systemic lupus erythematosus
  – Multiple sclerosis (MS)
  – Insulin-dependent diabetes mellitus
  – And the list goes on…
Rheumatoid Arthritis

- Chronic, autoimmune disease characterized by:
  - Severe joint inflammation
  - Increased synovial fluid and thickened synovial membrane
  - Destruction of bone and cartilage in several joints
  - Elevated levels of pro-inflammatory cytokines
    - TNF-α, IL-1, IL-6
- Affects 1% of the US population
- Women are 3 times more likely to develop
- If untreated for 2+ more years, irreversible damage occurs
TNF-antagonists

**Infliximab**
- 25% murine; ~75% human
- Variable region of a mouse monoclonal anti-TNF antibody coupled to the constant region of a human IgG1
- Half-life of 8–10 days

**Adalimumab**
- 100% human peptide sequence and structure
- Full-length human IgG1
- Phage display technology resulting in human derived variable regions and human IgG1 constant regions
- Half-life of 10–20 days

**Etanercept**
- 100% human peptide sequences, but artificial construction
- Fusion protein made up of 2 soluble TNF receptor molecules (TNFR1I) fused with the Fc fragment from human IgG1
- Half-life of 3.0–5.5 days

**Chimeric Monoclonal Anti-TNF Antibody**

**Human Monoclonal Anti-TNF Antibody**

**Soluble TNF Receptor – Fc Fusion Protein**
Infliximab

- Remicade® by Johnson & Johnson
- Chimeric mAb
- Anti TNF-α
- Approved by the FDA in 1998
- Administered intravenously
- Designated for use in patients who did not respond to methotrexate
- Proven to slow the clinical and radiological progression of rheumatoid arthritis

Adalimumab

- Humira® by Abbott Laboratories
- Fully human IgG1 mAb
- Anti-TNF-α
- Approved by the FDA in 2002
- Available in 1 mL Humira pens and syringes for convenient use at home
Rituximab

- Rituxan® by Genentech
- Anti-B cell (CD20) antibody
- First approved in 1997 for use in B-cell lymphoma
- Given in combination with Methotrexate
- Directed for patients who do not respond to Anti-TNF treatments
- Indicates the rheumatoid arthritis has a B cell component to its pathology
Anti-Tumor Antibodies

Bevacizumab

VEGF: Vascular endothelial cell growth factor.

Diagram showing the interaction of Bevacizumab with VEGF, inhibiting angiogenesis, progression, metastasis, and survival.
Trastuzumab/HER2

A. Trastuzumab and HER2

B. HER1/EGFR, HER2, HER3, HER4

C. HER2 and Pertuzumab

D. HER1/EGFR, HER2, HER3, HER4

Inhibits HER2 forming dimer pairs
Suppresses multiple HER signaling pathways, leading to a more comprehensive blockade of HER signaling
Flags cells for destruction by the immune system
Antibody-Drug-Conjugates (ADC)

- Find ideal toxins to couple to antibodies
- selection of the right antibodies targeting the most appropriate antigens
- design of mAb/toxin
- linkers with ideal properties
- optimization of chemical strategy to covalently attach small molecule toxins to the chosen antibody in a directed, efficient, stable and reproducible fashion;
- controlling the release of the toxin from the ADC once it is delivered to the target cell
**ADC**

**Antibody-Drug-Conjugates**

Kadcyla uses a noncleavable linker to attach maytansine, a microtubule inhibitor, to the therapeutic anti-HER-2 antibody trastuzumab.

treatment of metastatic breast cancer

Adcetris uses a capthesincleavable linker to tether auristatin, a microtubule assembly inhibitor, to an anti-CD30 monoclonal antibody.

treatment of Hodgkin’s lymphoma (HL)

Ambrx: Engineered Incorporation of p-Ac Phe to Enable Site Directed Conjugation

- Expansion of the genetic
- incorporation of the nonnatural amino acid p-acetyl-phenylalanine
- ketone functionality that has orthogonol reactivity and allows for highly selective and precise conjugation

Chemistry & Biology 21, September 18, 2014
Radioimmunoconjugates

(a) $^{90}$Y-ibritumomab tiuxetan (Zevalin®)
Anti-CD20 monoclonal antibody
DTPA chelating moiety

(b) $^{131}$I-tositumomab (Bexxar®)
Anti-CD20 monoclonal antibody
Advancing therapeutic antibody sciences

- Brain shuttle antibody fusions
- Glycoengineered antibodies
- Antibody cytokine fusions
- Bispecific antibodies
- Antibody immunomodulatory conjugates
- Pre-targeted radio immunotherapy
Bispecific antibodies

THE ENGINEERING OF BISPECIFIC ANTIBODIES

**Antibodies**

Are Y-shaped proteins used by the immune system to identify and neutralize foreign and potentially damaging entities like bacteria, viruses and cancer cells. Engineered therapeutic antibodies have significantly improved treatment of cancer, viral infections and inflammatory diseases.

**“Quadroma”** First generation bispecific antibody (~1980)

Bispecific antibodies are generated by combining the light and heavy chains of two different monoclonal antibodies. The two antigen binding sites of the resulting antibody are aiming at different targets of interest.

**“Knob-into-hole”** Specific pairing of heavy chains (1997)

The “knob-into-hole” technology solves the problem of heavy-chain mispairing.

**“CrossMAb”** A technology developed by Roche (2007-2010)

The CrossMAb technology solves the problem of light chain mispairing.

An antibody is built within the cell by combining two identical “heavy chains” and two identical “light chains.”

The Quadroma approach produces a mixture of ten different antibodies. Purification of the desired antibody out of the mixture of potential products is very complex.

With specific pairing of the heavy chains, only one possible product is formed, reducing purification complexity.

By exchanging the “molecular bridges” between heavy and light chain, only specific interactions may take place in this region, selectively producing the desired bispecific antibody.

**Antibody**

Simple antibodies are fully symmetrical

**Bispecific antibodies**

Antigen binding sites (CDRs) specifically bind to the target of interest

Fc part - interacts with Fc receptors on immune cells

Fab region - Elassohide bonds

Hinge region - flexible region connecting the Fab regions

~1980

One out of ten

1997

One out of four

2010

Only one out of one
(A) A schematic of human IgG1. (B) The structure of CH3 domains of wild-type Fc (PDB: 3AVE). Key residues involved in CH3-CH3 interface are showed as sticks. Green, CH3A; cyan, CH3B. (C) Fc heterodimer engineering based on the knob-hole strategy (KiH). (D) Knob (A) and hole (B) chain formation was analyzed by non-reducing SDS-PAGE. The yield of Fc heterodimer was assessed by densitometry analysis and shown in C.

Enforcing correct heavy chain association by CH3-CH3 interface modification. Structural model of heterodimeric Fc (one CH3 domain as black line, the other as gray line) with (A) KiH mutations and S-S stabilization (1 knob mutation, blue; 3 hole mutations, red; one disulfide bridge, yellow). (B) charged residues located at the CH3-CH3 interface (Glu, Asp, red; Lys blue) that can typically be used to enforce heterodimerization by appropriate exchange as shown by Amgen and Chugai. (C) optimal variant (4 mutations, purple and magenta) obtained by Xencor. (D) schematic representation of (from left) the desired heterodimer and the two unwanted homodimers obtained by the KiH method (top) or use of electrostatic steering (bottom).
Design of CrossMAbs

Design of CrossMAbs with CH1-CL crossover: Top: Typical structures of VH, Vk and Vλ domains and their superposition in the sense that the Cα atoms of the β-sheets adjacent to the elbow region (displayed as spheres in the structures, gray in the sequences) match in space. Middle: the same applied to the CH1, Cκ and Cλ domains. Bottom: wildtype and designed CrossMAb sequences; β-sheets adjacent to the elbow regions are colored gray. The newly designed sequence is shown in red.
Overview of bispecific heterodimeric IgG antibodies with heterodimeric Fc-region. (A) Quadroma approach with isolation of the desired bispecific Triomab. (B) KiH approach with two different light chains based on in vitro assembly; the white circle indicates the lack of glycosylation due to expression in E. coli. (C) KiH approach with common light chain. (D) CrossMab$^{\text{CH1-CL}}$ based on KiH approach in combination with light chain crossover. (E) (SEED)body approach based on strand exchange between IgG and IgA CH3 domains. (F) LUZ-Y with C-terminal fusion of a leucine zipper to the heavy chain to ensure HC heterodimerization and common light chain. The leucine zipper can subsequently be cleaved off proteolytically.

C. Klein et al., mAbs Vol. 4, Iss. 6, 2012

alternating segments of human IgA and IgG C(H)3 sequences
Bispecific MAbs

Overview of the clinical stage CrossMAbs (2016): (A) Ang-2-VEGF CrossMAb vanucizumab (RG7112) for oncology, (B) VEGF-Ang-2 CrossMAb RG7716 for ophthalmology, (C) CEA TCB (RG7802) for CEA-positive solid tumors, (D) FAP-DR5 tetravalent CrossMAb RG7386. The black star depicts the P329G LALA (P329G, L235A, L234A) mutations to abolish FcγR and C1q binding, the green star Triple A (I253A, H310A, H435A) mutations to abolish FcRn binding. Constant heavy chain domains are colored in gray, constant light chain domains in white, variable heavy chains are colored uniformly, light chain domains are colored with a line pattern.
Interesting Variations

- Small antibody fragments (Fv or Fab) are also effective in blocking cytokines
  - Benefit: More readily penetrate tissue
- Coupling of antibody fragments to form dimers and tetramers
  - Increases avidity and cross-linking
- Engineered Diabodies
  - Two different antigen specificities
    - One against the target
    - The other against effectors
  - Can cross-link effector cells
scFv and multivalents

single-chain variable fragment (scFv)

tandem di-scFv

tandem tri-scFv

diabody

tri(a)body
Various bispecific antibodies (bsAbs) are currently in clinical development or are already approved for cancer therapy. The upper two lines depict immunoglobulin (Ig)-like bsAbs comprising an IgG Fc region, either as bivalent or tetravalent molecules. Furthermore, several small bsAb and bsAb fusion proteins have entered clinical trials. **Abbreviations:** BiTE, bispecific T cell engager; DART, Dual affinity retargeting; DNL, dock-and-lock; DVD-Ig, dual variable domain immunoglobulins; HSA, human serum albumin; kih, knobs into holes.

Roland E. Kontermann, Ulrich Brinkmann. [https://doi.org/10.1016/j.drudis.2015.02.008](https://doi.org/10.1016/j.drudis.2015.02.008)
Engineered hapten-binding antibody derivatives for modulation of pharmacokinetic properties of small molecules and targeted payload delivery

- delivery approaches with preformed complexes
- digoxigenin-binding IgG-derived tetravalent bispecific antibodies to target small compounds
- Cell surface: HER2, IGF1R, CD22, or LeY carbohydrate
- digoxigeninylated payloads
- antigen-specific targeted delivery of payloads to tumor cells
Characterisation

- Hydrophilic interaction chromatography (HILIC)
- Size-exclusion chromatography (SEC)
- Ion-exchange chromatography (IEX)
- High performance liquid chromatography (HPLC)
- Capillary electrophoresis sodium dodecyl sulfate (CE-SDS)
Roche pRED’s Complex Biologics

“traditional” mAbs represent only a small part of today’s portfolio
Nanobodies

- 1989 - Raymond Hamers
- Discovered in camels
- Completely lack the light chain!
- Same antigen affinity as their four-chain counterparts
- Structure makes them more resistant to heat and pH
  - May lead to development of oral nanobody pills
Affilins are genetically engineered proteins with the ability to selectivity bind antigens. They are structurally derived from one of two proteins, gamma-B crystallin or ubiquitin.

Small size, improved tissue permeability, heat stability up to 90 °C, stability towards proteases as well as acids and bases, pass through the intestine, are not absorbed into the bloodstream, renal clearance causes short plasma half-life.
Anticalins are artificial proteins (based on lipocalins) that are able to bind to antigens, either to proteins or to small molecules. They are not structurally related to antibodies, which makes them a type of antibody mimetic.

Lipocalins: storage and transport of hydrophobic and/or chemically sensitive compounds such as vitamins, lipids, and steroids found in many different organisms, including insects, bacteria, and humans.
Engineered Bispecific Antibodies

The zoo of bispecific antibody formats
Overview of bispecific antibody formats reduced to practice, grouped into molecules with symmetric or asymmetric architecture
Combinatorial diversity of bispecific IgGs

Overview of possible combinations to arrange heavy and light chains from two different antibodies, including strategies to overcome incorrect heavy chain and heavy-light chain pairing.
Overview of strategies to generate bispecific IgG molecules including strategies to force correct assembly of heavy chains and/or heavy-light chains, or utilizing postproduction purification or assembly strategies.
**CrossMab principle.** Starting from a conventional IgG antibody correct chain association in a bispecific heterodimeric antibody can be achieved using the KiH technology to enforce correct heavy chain heterodimerization in combination with domain crossover of light chain domains to enforce correct light chain association. The three possible light chain domain crossovers are depicted: Fab domain crossover on the left, VH-VL domain crossover on the right and CH1-CL crossover on the top.
Engineered hapten-binding antibody derivatives for modulation of pharmacokinetic properties of small molecules and targeted payload delivery
Bispecific Antibody (Fab)

HER2- and VEGF-binding dual acting antibody (DAF) High affinity antigen recognition of the dual specific variants of herceptin is entropy-driven in spite of structural plasticity. Variants of the antibody herceptin that interact with HER2 and VEGF at the antigen binding site shown as superposition of the complex structures with HER2 (PDB:3bdy) and with VEGF-A (PDB:3be1). The picture illustrates that HER2 (red) interacts mainly with the heavy chain of the antibody (dark blue), whereas VEGF-A (orange) interacts almost exclusively with the light chain (light blue). There are no significant interactions within the unrelated pairs HC-VEGF and LC-HER2. The Fab of 3be1 has been omitted for clarity since both Fabs exhibit an almost identical structure.
Strategies for bi-specific Abs

Schematic overview of the different strategies used to generate bispecific antibodies (bsAbs) derived from the antigen-binding sites of two different antibodies. Symmetric bsAb are generated by the assembly of antibodies with unmodified heavy chain constant regions, such as by the heterodimerization of heavy chains from two different antibodies or homodimerization of heavy chains extended by an additional binding site resulting in bivalent or tetravalent molecules. Using heavy chains modified to force heterodimerization (e.g., using a knobs-into-holes strategy) results in asymmetric bsAb. Alternatively, two different antibody fragments, such as scFv, can be fused to a non-immunoglobulin protein, such as albumin. Furthermore, two antigen-binding fragments can be directly fused, resulting in small bsAb molecules. Finally, bsAb can also be generated by chemical conjugation of two different antibodies.