Рекомбинантные Антитела для диагностики и терапии

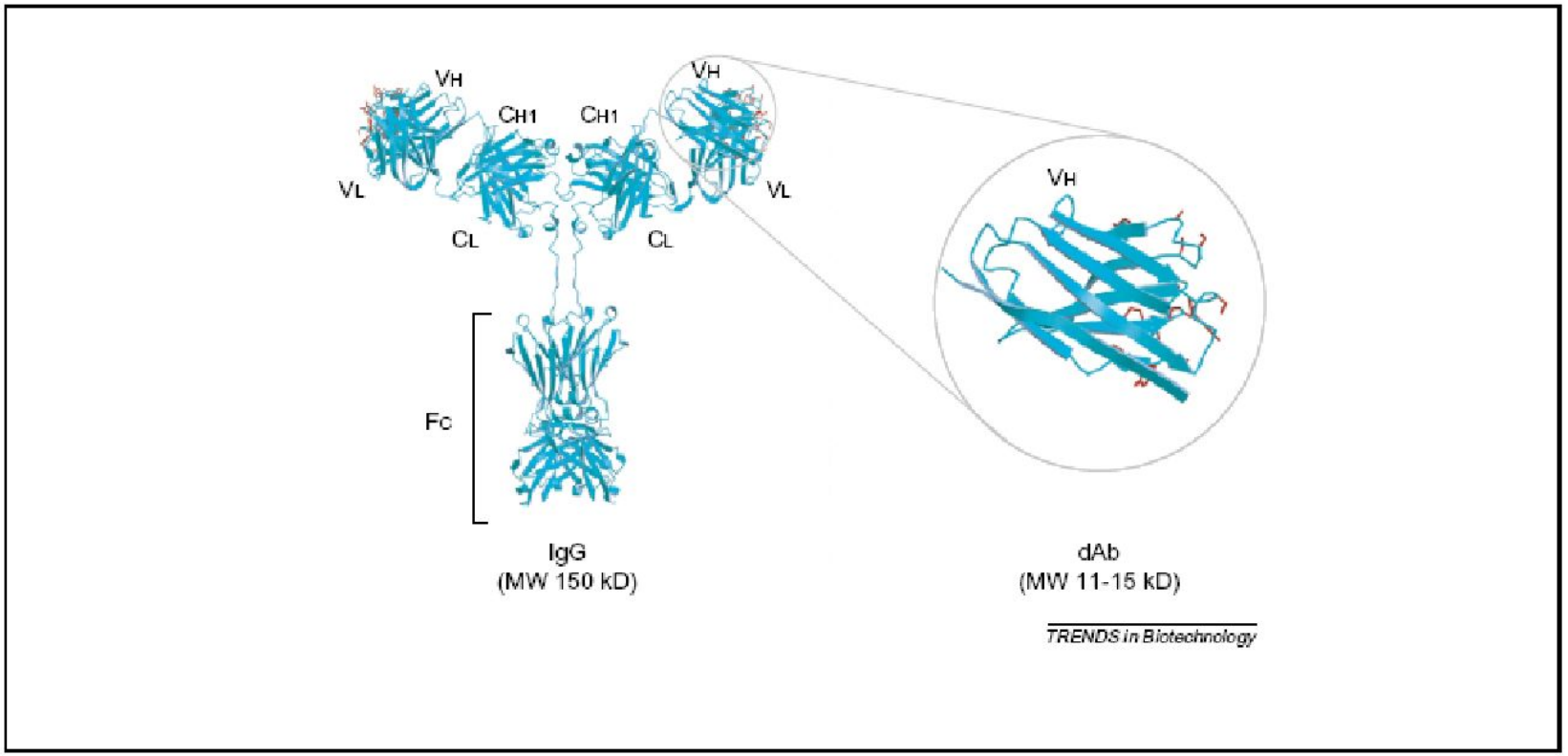


Figure 1. A human IgG molecule has both variable and constant regions. An IgG contains two variable regions (each composed of a V_H and V_L domain) that confer antigen-binding specificity on the antibody, and an Fc fragment in the constant region that recruits the effector functions of the immune system. Conventional recombinant antibody fragments contain one antigen-binding V_H–V_L pairing. At ~57 kDa, a Fab fragment comprises a V_H–C_H1 polypeptide disulphide-bonded to a V_L–C_L polypeptide. At ~27 kDa, a scFv fragment contains only the V_H domain fused to the V_L domain via a polypeptide linker. By contrast, the domain antibody, or dAb, of 11–15 kDa is either an isolated antibody V_H domain [2], as shown here, or an isolated antibody V_L domain [15]. Each dAb thus contains three of the six naturally occurring complementarity determining regions (CDRs) from a V_H–V_L pairing. The side chains of the CDRs are highlighted in red.

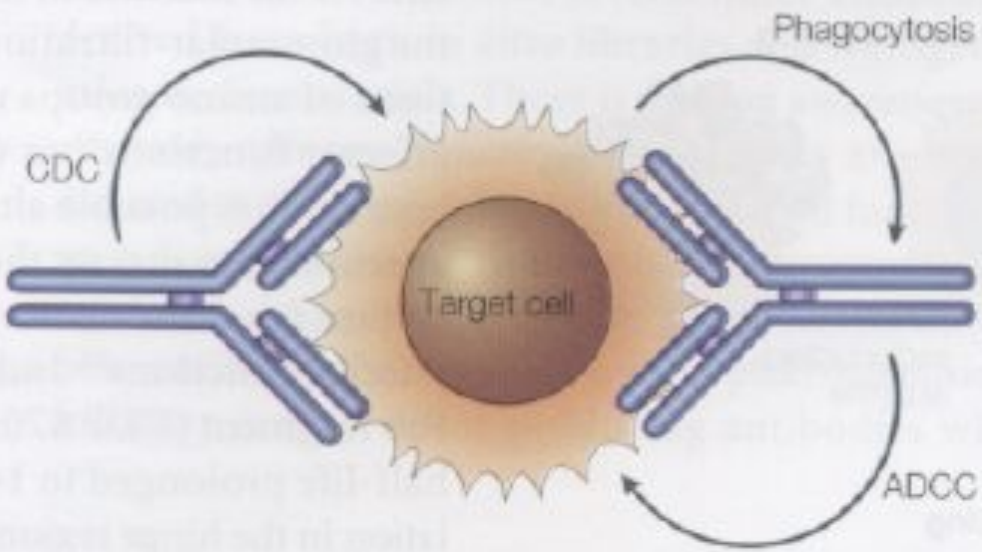
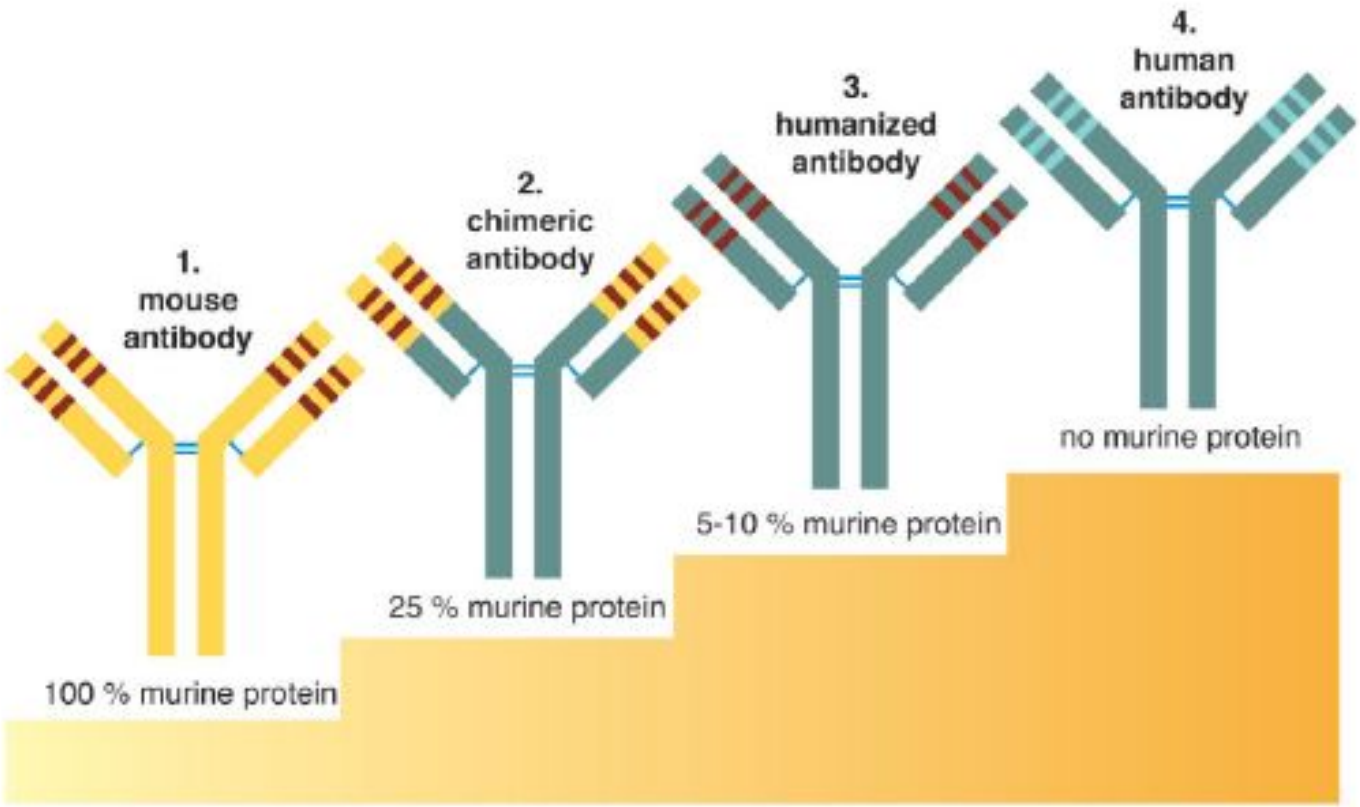


Figure 4 | **Natural immunoglobulin effector functions.** The classical complement pathway is initiated by the binding of C1 to immune complexes (that is, antibody-coated cells or antigens) and eventually leads to cellular lysis and elicits inflammatory responses for the ultimate elimination of the target cell. This process is called complement-dependent cytotoxicity (CDC). The Fc-receptor-mediated effector functions are associated with the recruitment of cellular effectors leading to either phagocytosis or antibody-dependent cellular cytotoxicity (ADCC). These mechanisms are accepted to be the processes that operate when naked antibodies are used as therapeutic agents.


1.
mouse
antibody
2.
chimeric
antibody
3.
humanized
antibody
4.
human
antibody
100 % murine protein
25 % murine protein
5-10 % murine protein
no murine protein

Schematic presentation of whole IgG and different fragments

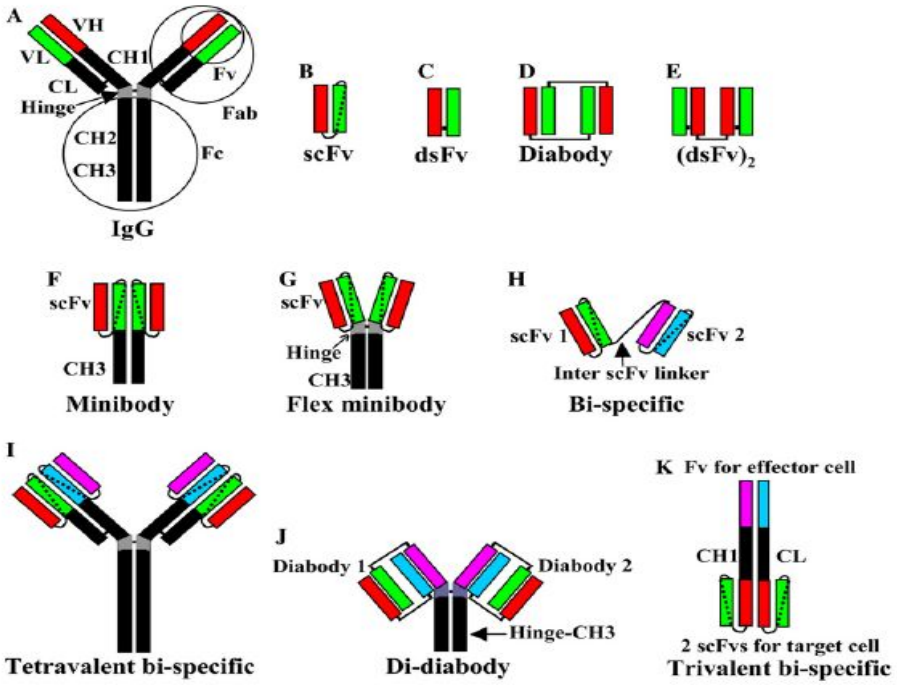


Fig. 1. Schematic representation of a whole IgG molecule (A) and different fragments that have been engineered (B–K). The diabody shown in (D) is a homodimer but can also be made in a heterodimer form when V-domains from two different antibodies are used.

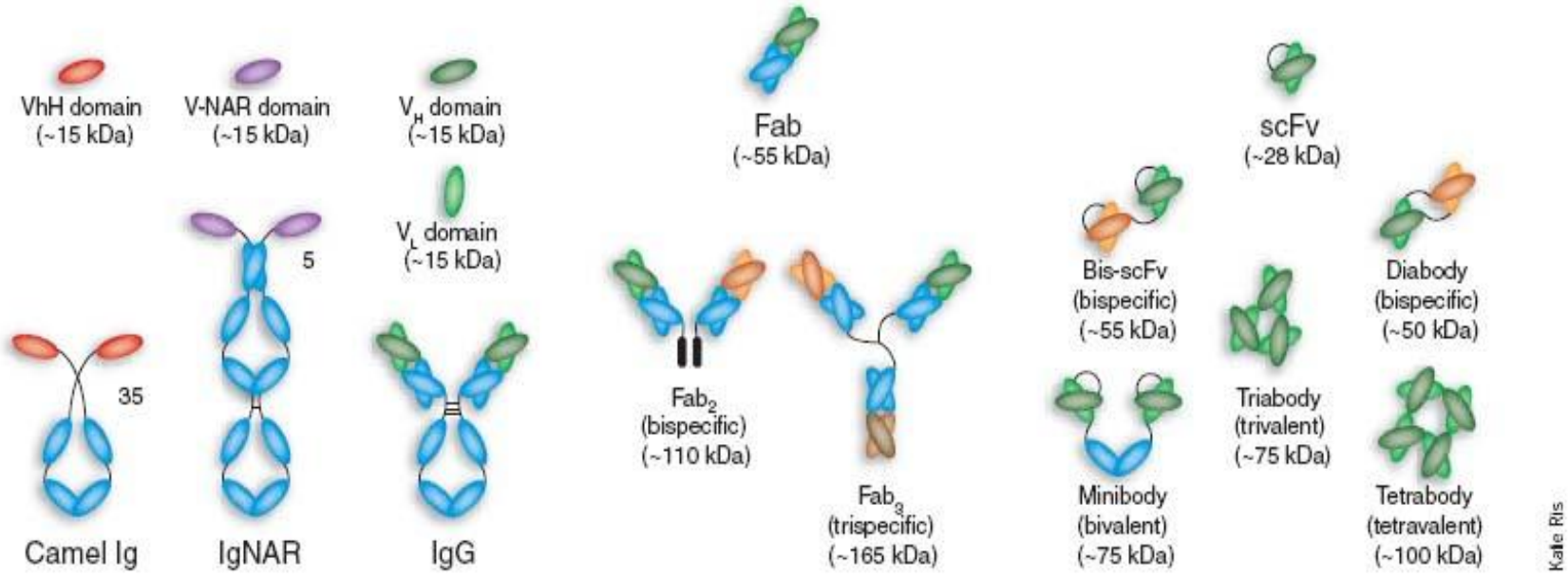
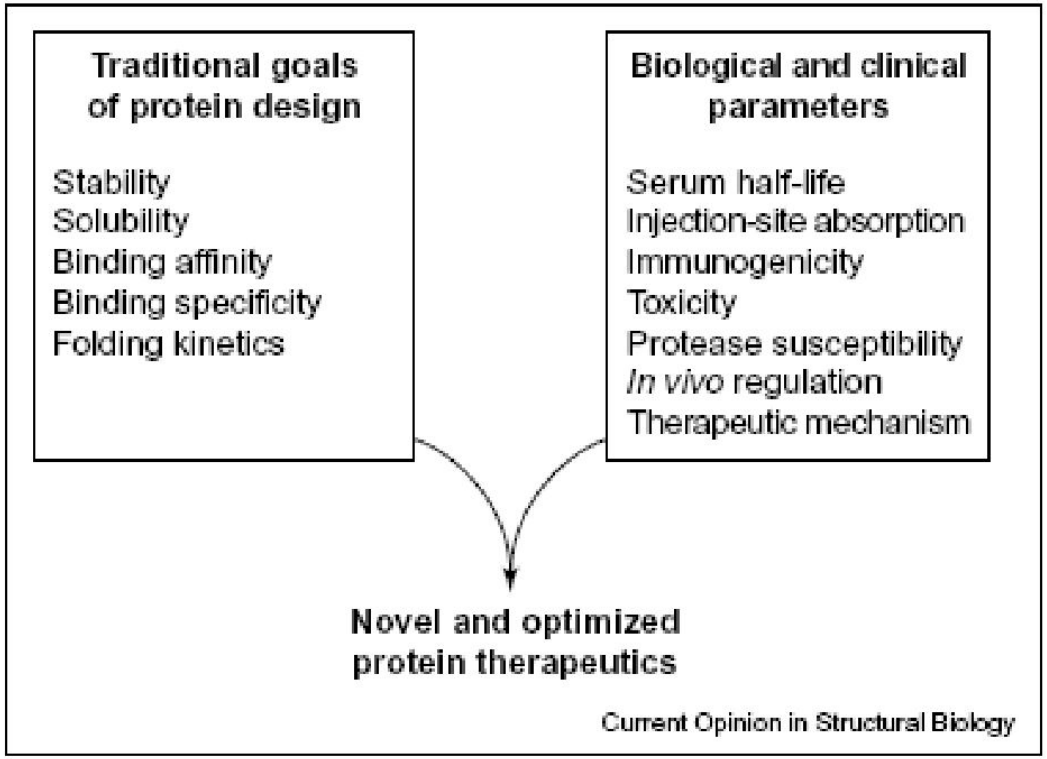
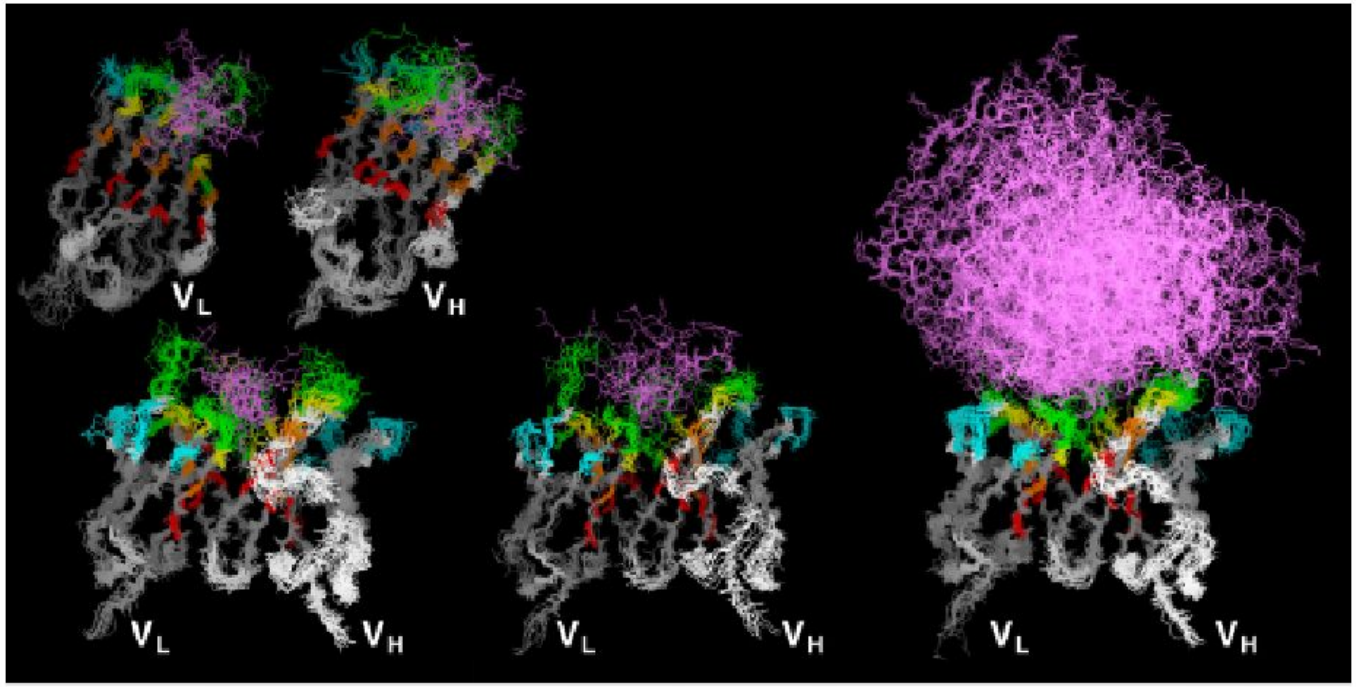


Figure 1 Schematic representation of different antibody formats, showing intact 'classic' IgG molecules alongside camelid VhH-Ig and shark Ig-NAR immunoglobulins. Camelid VhH-Ig and shark Ig-NARs are unusual immunoglobulin -like structures comprising a homodimeric pair of two chains of V-like and C-like domains (neither has a light chain), in which the displayed V domains bind target independently. Shark Ig-NARs comprise a homodimer of one variable domain (V-NAR) and five C-like constant domains (C-NAR). A variety of antibody fragments are depicted, including Fab, scFv, single-domain V_H, VhH and V-NAR and multimeric formats, such as minibodies, bis-scFv, diabodies, triabodies, tetrabodies and chemically conjugated Fab´ multimers (sizes given in kilodaltons are approximate).

Figure 1



The design of protein therapeutics integrates the traditional goals of protein design, shown on the left, with biological and clinical parameters, shown on the right.

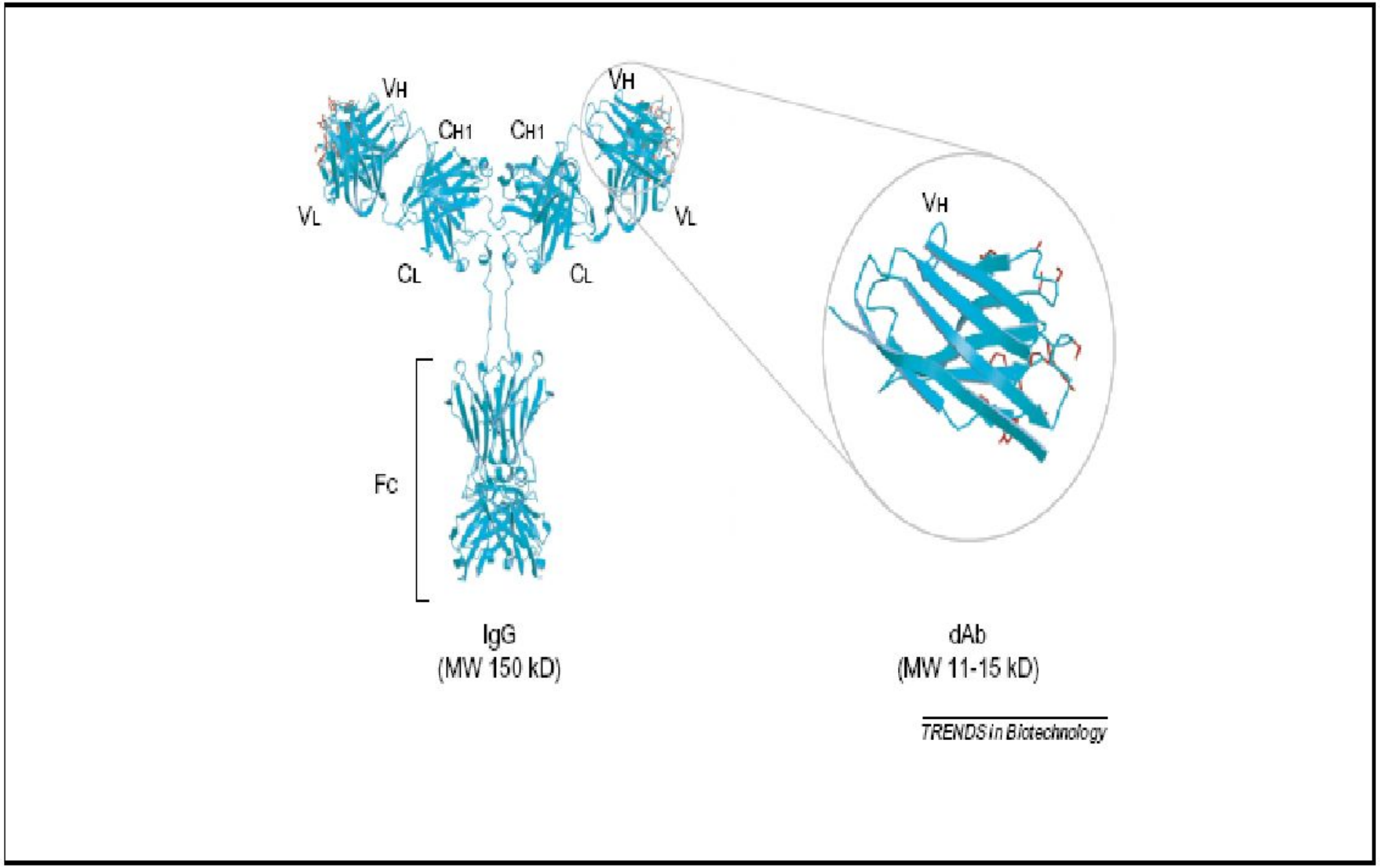


A: Hapten Binders
27 Structures representing
15 different Antibodies

B: Peptide/Oligomer Binders:
10 Structures
8 Antibodies

C: Protein Binders:
22 Structures
14 Antibodies

Fig. 1. Antibodies binding haptens, oligopeptides and oligosaccharides or proteins. Superposed crystal structures of antibody–antigen complexes were sorted into three classes according to the type of antigen. The antigens are colored pink. Structurally variable residues within the CDRs of the antibodies are shown in green, those at the N-terminus, to the N-terminal side of CDR-1 and within the outer loop in cyan. The structurally least variable residues whose Cα-positions were used for the least-squares superposition of the antibody fragments are shaded gray. Residues within the inner (dimer interface) β-sheet of V_L and V_H whose side chains contribute both to the dimer interface and, depending on the antigen, to antigen binding, are shown in yellow, orange, and red, depending on depth within the hapten binding cavity. It can be seen that there is extensive binding to residues which formally belong to the framework, either in the dimer interface region for hapten binders or binders of peptides (which frequently use a side-chain in a hapten-like binding mode), and to the outer loop in protein binders. Hapten binders commonly form a deep, funnel-shaped binding pocket enlarged by a long CDR-L1 and open CDR-H3 conformation, while protein binders preferentially utilize a relatively flat antigen binding surface characterized by a short CDR-L1 and a closed CDR-H3 conformation. This is one of the main reasons why the immune system uses different frameworks, rather than different CDRs all on the same framework. It also highlights the points to consider in loop grafting.



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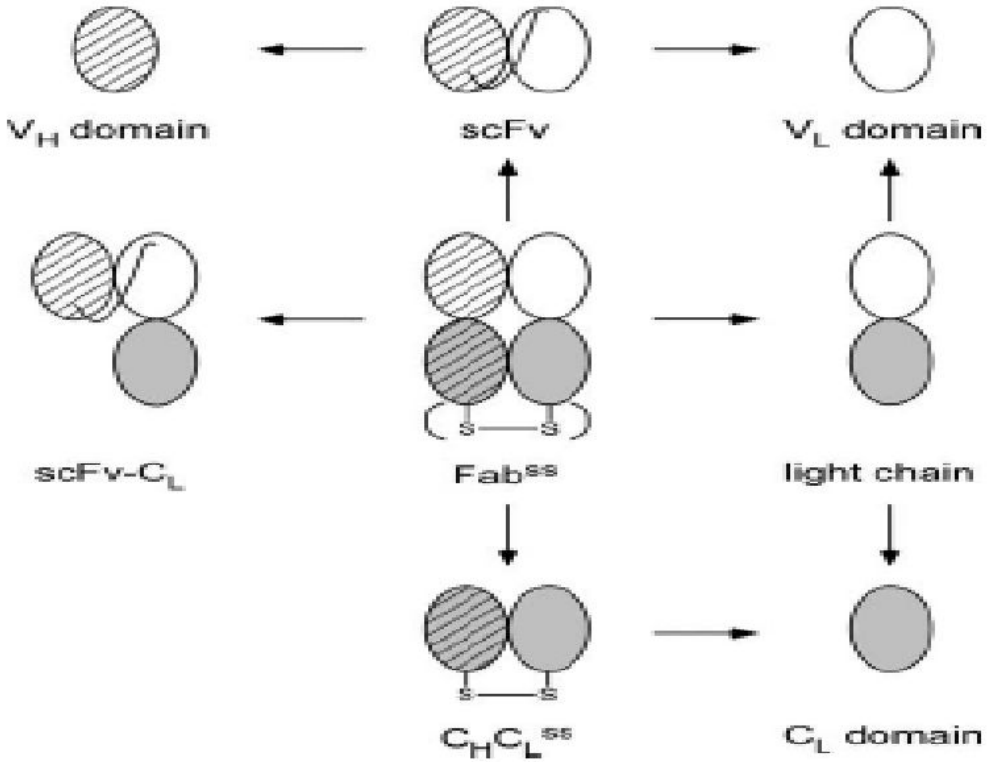


Figure 1. Dissection of the Fab fragment. All the antibody domain assemblies that were investigated are depicted. The investigation of the isolated C_H1 domain and the Fd fragment (V_H and C_H1) was not possible due to degradation and aggregation, respectively. Fab fragments with and without the C-terminal interchain disulfide bond were studied. This was not possible for the C_HC_L assembly. Because of the weak interaction across the interface, only the disulfide-bonded version resulted in a homogenous protein preparation. With these different assemblies, it was possible to untangle the contributions of the intrinsic domain stability and interface stabilization to the overall stability of the Fab fragment. The denaturation curves of two isolated V_L domains with low (V_L^w) and with high (V_L^s) stability, and three isolated V_H domains with very low (V_H^w), medium (V_H^m) and high (V_H^s) intrinsic stability were determined.

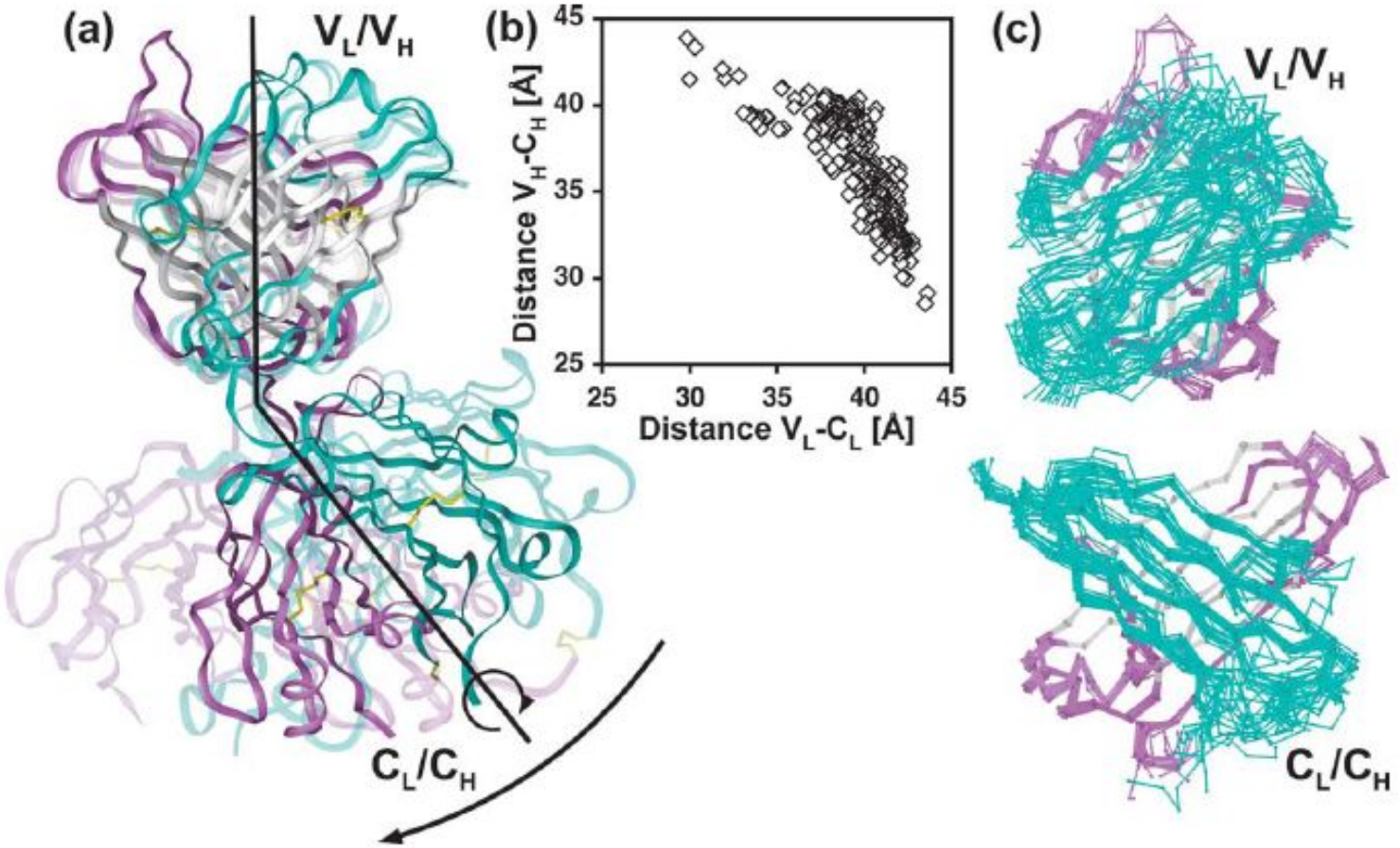
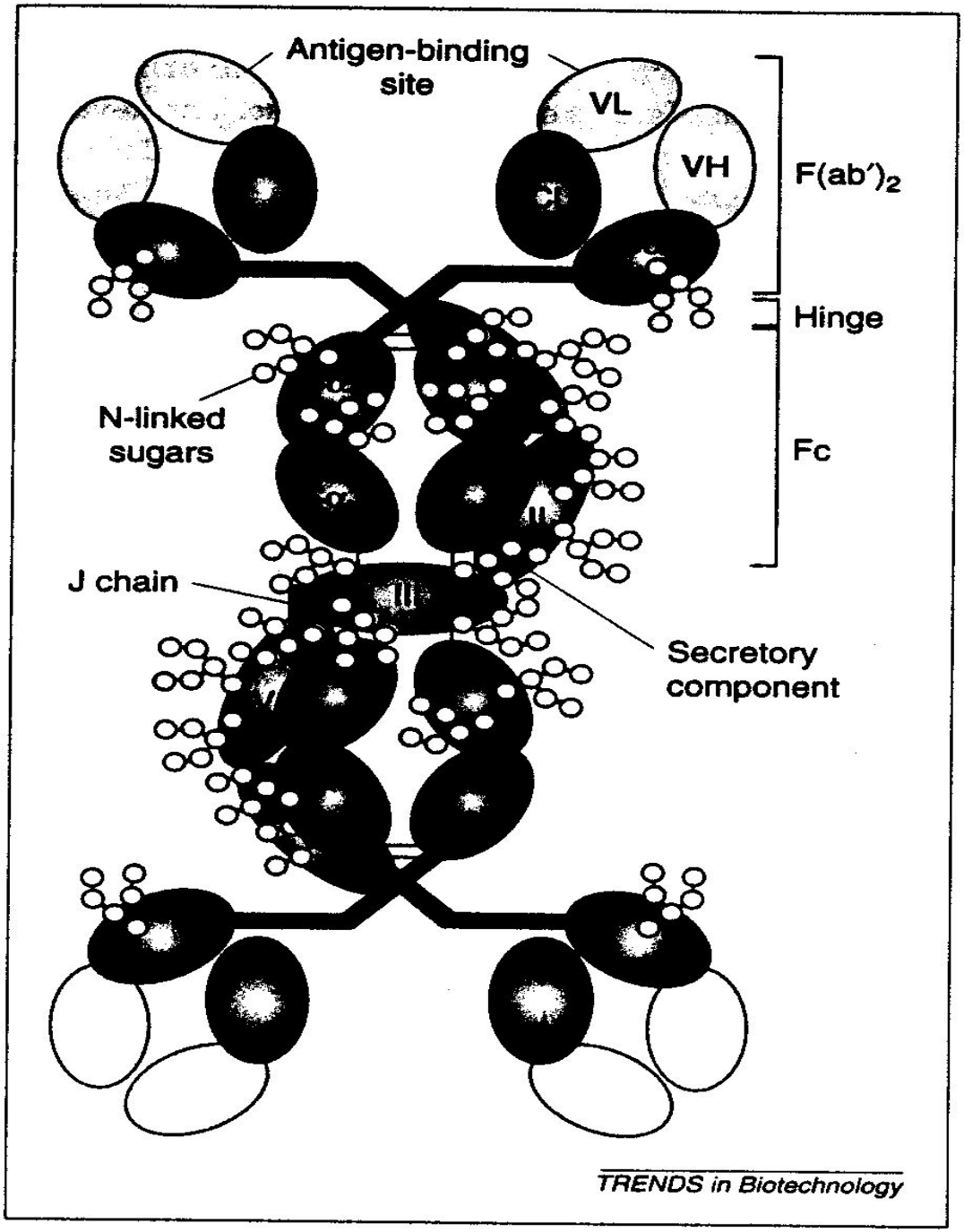


Figure 7. Flexibility of the relative orientations of the domains within the Fab fragment. (a) Three Fab fragments were superimposed by a least-squares fit of the structurally least variable C^{α} positions within the Fv part (indicated in white) to illustrate the flexibility of the variable/constant domain interface. (b) Plot of the distance between the centers of gravity of V_H and C_H1 *versus* the distance between the centers of gravity of V_L and C_L. Only the structurally conserved C^{α} positions (indicated by a gray background in Figure 6) were used for the calculation of the center of gravity. The observed anti-correlation is explained by a twist around the C_H1/C_L pseudo 2-fold axis. (c) Limited flexibility of the V_H/V_L and of the C_H1/C_L interface, demonstrated by structurally aligning the V_L and C_L domains, respectively. From the comparison of (a) and (c), it becomes apparent that V_H/V_L and C_H1/C_L are each moving as a unit.


Antigen-binding site
VL
VH
F(ab′)2
Hinge
Fc
N-linked sugars
J chain
Secretory component
TRENDS in Biotechnology

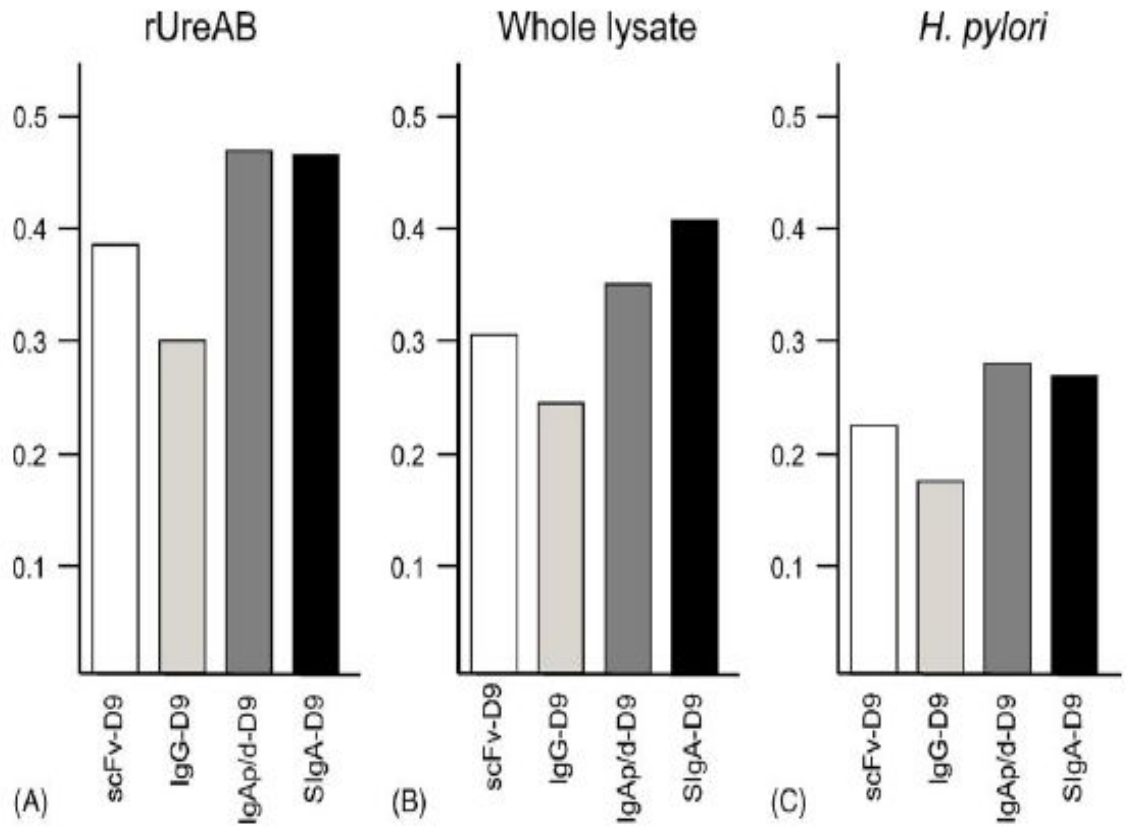


Fig. 4. Qualitative binding capacity of recombinant scFv polypeptide, and IgG-D9, IgAp/d-D9 and SIgA-D9 Ab examined under nondenaturing conditions. Wells of ELISA plates were coated with rUreAB (A), *H. pylori* whole lysate (B), or *H. pylori* (C) as indicated in Section 2. Due to the use of different primary and secondary Ab, bars are indicative of the relative binding capacity, but do not reflect the absolute affinity for the Ag of the different FvD9-bearing molecules assayed. Background values (range 0.01–0.03) obtained in the absence of recombinant Ab were substracted from the data presented.

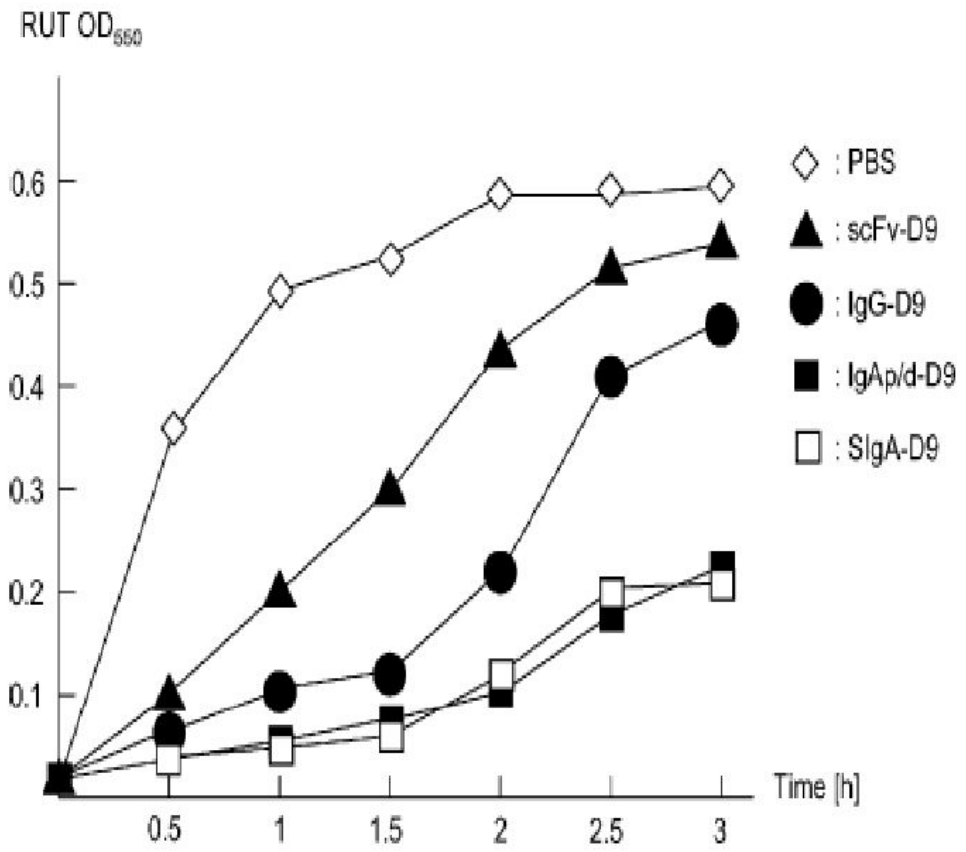


Fig. 5. Measurement of urease activity associated with *H. pylori* strain 69A in the presence of IgAp/d-D9, SIgA-D9, IgG-D9 Ab and scFv polypeptide comprising the same variable region. The mixture of antibodies and bacteria was incubated for 1 h prior to the addition to the urea substrate, and OD_{550} resulting from the conversion into ammonium ions is measured during 3 h at 30 min intervals. At usual end-points for the assay (2.5–3 h), IgAp/d-D9 and SIgA-D9 Ab exhibit the best capacity to inhibit urease activity associated with *H. pylori*. One representative experiment out of three performed is shown.

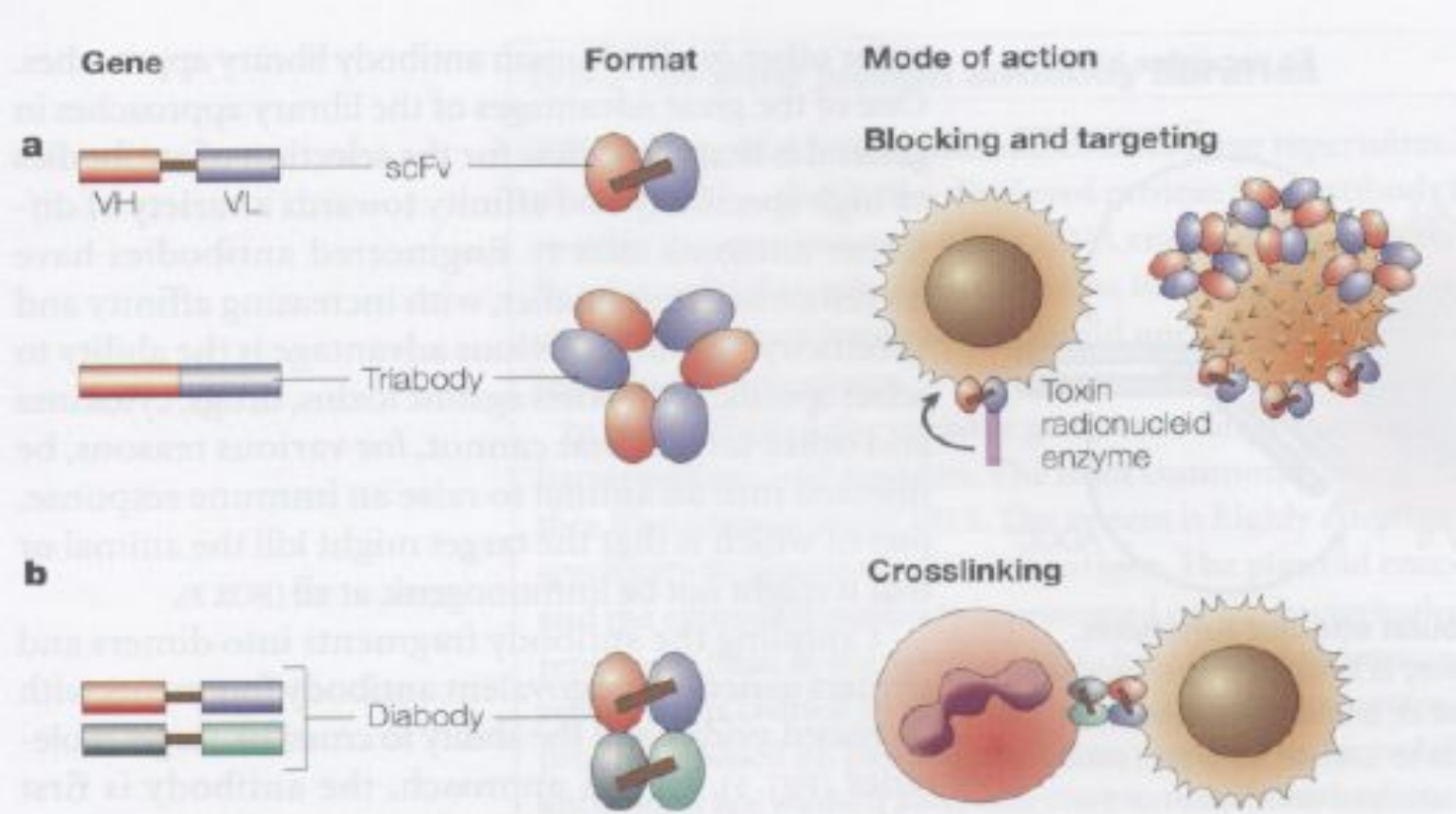


Figure 5 | **Different formats of antibody fragment. a** | Single-chain Fv fragments (scFvs) are mostly monomeric when the peptide linker (in red) between the V_H and the V_L domains is about 12–15 amino acids. When the linker is shorter than three amino acids or is totally deleted, the fragments tend to make trimeric fragments or even tetrameric formats. Multivalent antibodies gain avidity and such molecules are therefore perfect for blocking viral infection or receptor–ligand interactions, for example. **b** | If the linker is about five amino acids long, then the variable domains tend to dimerize. When equipped with variable domains with different specificities, such dimeric scFvs can make bispecific fragments called diabodies.

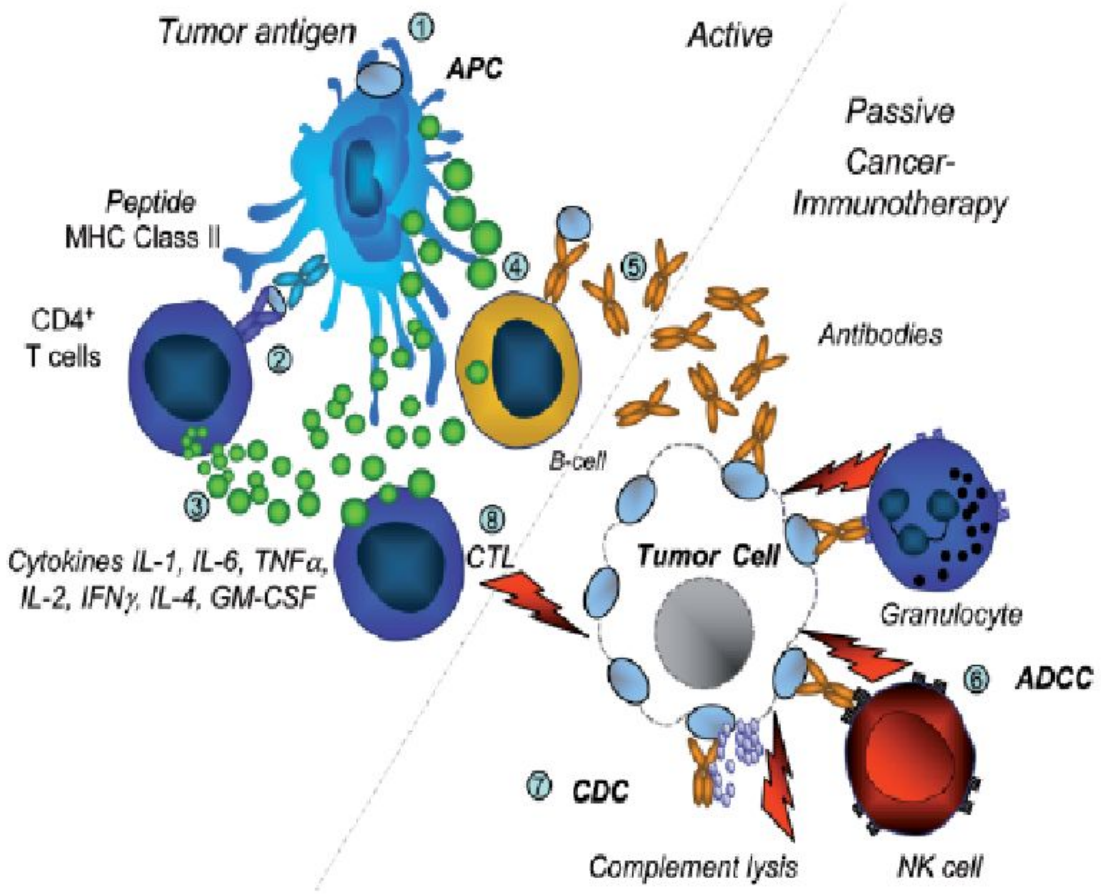
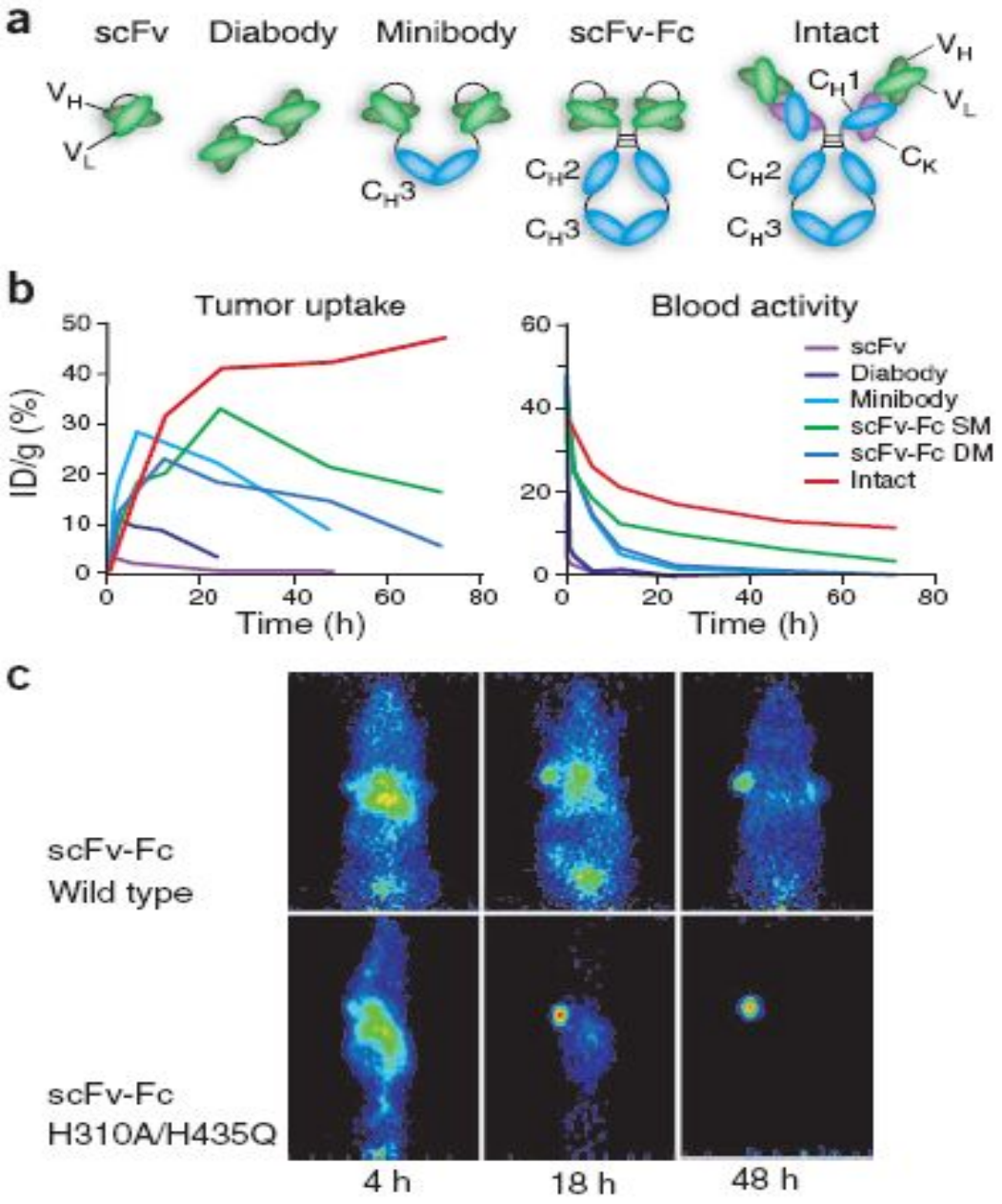


Figure 1. Cancer Immunotherapy. The whole spectrum of passive and active cancer immunotherapy is summarized. Active cancer immunotherapy comprises tumor antigen uptake by APCs (1), epitope (peptide) presentation to $CD4^+$ T cells (2), cytokine release (3), B cell activation (4), and antibody production (5), leading to lysis of tumor cells including different (passive) alternatives like ADCC (6), CDC (7) or unspecific attack by cytotoxic T lymphocytes (8).


a
scFv
Diabody
Minibody
scFv-Fc
Intact
V_H
V_L
C_H3
C_H2
C_H1
C_K
b
Tumor uptake
Blood activity
ID/g (%)
Time (h)
scFv
Diabody
Minibody
scFv-Fc SM
scFv-Fc DM
Intact
c
scFv-Fc
Wild type
scFv-Fc
H310A/H435Q
4 h
18 h
48 h

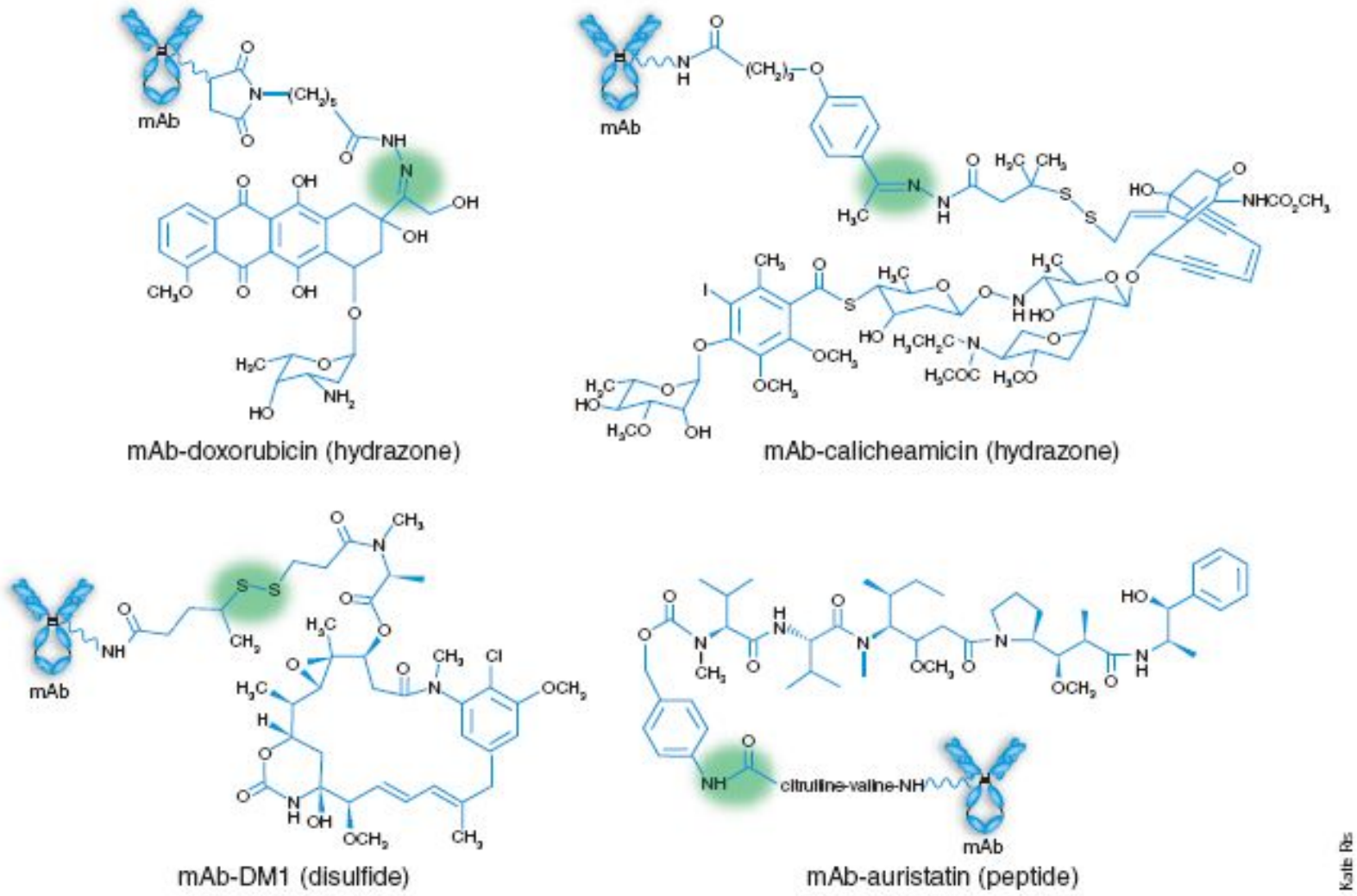
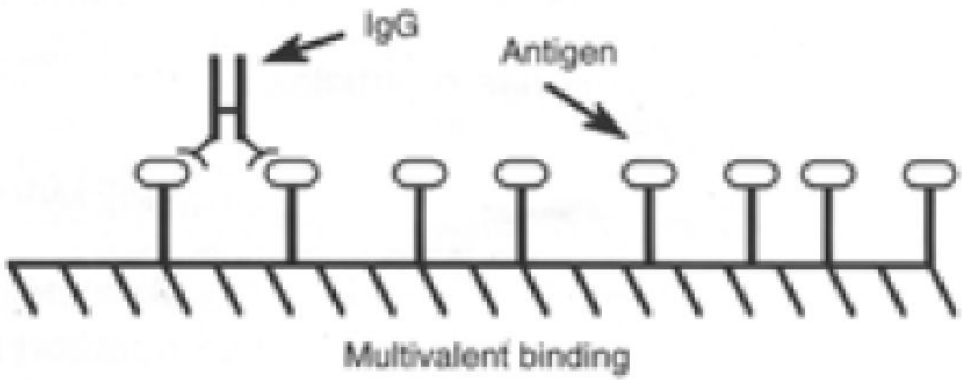


Figure 2 Chemical structures of some advanced mAb-drug conjugates. The linkers used for each drug are indicated in parentheses, and the labile bonds leading to drug release are shaded. For the examples shown, hydrazones release drug under acidic conditions within the lysosomes of target cells, disulfides undergo intracellular reduction and the peptides are enzymatically hydrolyzed by lysosomal proteases.

Box 3. Affinity and avidity

As defined in Box 1, the affinity constant K_a describes the 'strength' of the interaction between two binding partners in solution. Whenever one of the binding partners is present in multiple copies on a support that prohibits free diffusion (e.g. antigens on a cellular membrane or on a microtitre plate), the observed affinity constant K_a^{obs} corresponds to the true K_a constant only if the binder in solution is monovalent. Under the same conditions, a multivalent binder (e.g. an IgG) displays a higher apparent affinity (the 'functional affinity', or avidity) by virtue of rebinding effects and chelate binding. This apparent affinity constant is not a universal thermodynamic constant but is dependent on the antigen density on the solid support. While rebinding effects and multivalent binding are to be avoided to obtain 'true' affinity constants, avid interactions are the basis of several important biological and technological processes, for instance the stable binding of multivalent antibodies to cells exploited in flow cytometry. Avid 'apparent' affinity constants can be measured by a variety of methods, such as detecting cell binding by radio- or fluorescently-labeled antibodies with methodologies similar to those listed in Box 2.

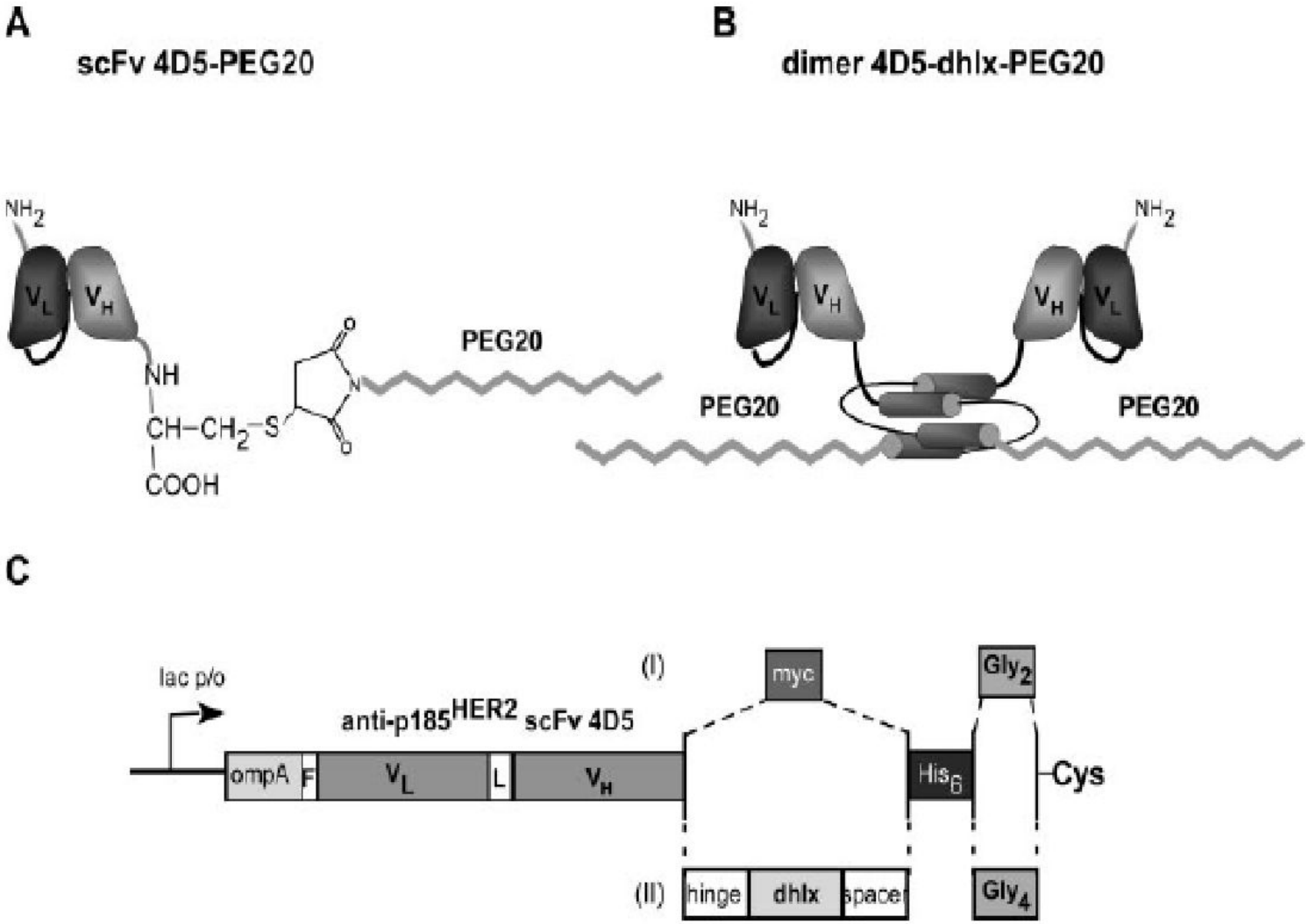


Binding Site Barrier Hypothesis

Anti –erbB2 scFv
Affinity from 10-7 to 10-9
Activity in cellular test not incriesed

Binding activity from 2 to 400 nM
Diabody and tumor targeting better for 2 nM

Anti mesothelin SS antibody (scFv) 0,8 nM 10 fold active that 11 nM,
But another mutant with 0,2 nM affinity have low activity that 0,8 nM


A
scFv 4D5-PEG20
B
dimer 4D5-dhlx-PEG20
NH2
VL
VH
NH
CH–CH2–S
COOH
PEG20
C
lac p/o
anti-p185HER2 scFv 4D5
ompA
F
L
(I)
myc
Gly2
His6
Cys
(II)
hinge
dhlx
spacer
Gly4

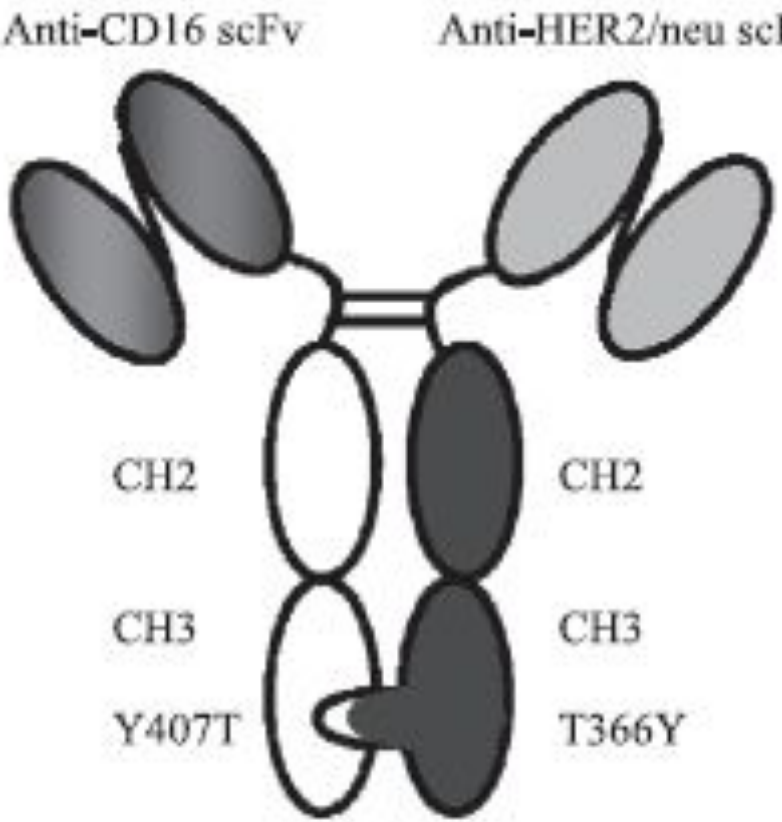


Fig. 1. Schematic diagram of the BsAb using "knobs-into-holes" CH3 mutations. CH2 and CH3, second and third constant regions of human IgG1; T366Y and Y407T, mutations in CH3 for the knob and hole. Two chains are heterodimerized by "knobs-into-holes"-engineered CH3 domains. Two naturally occurring hinge region disulfide bonds are indicated by horizontal lines.

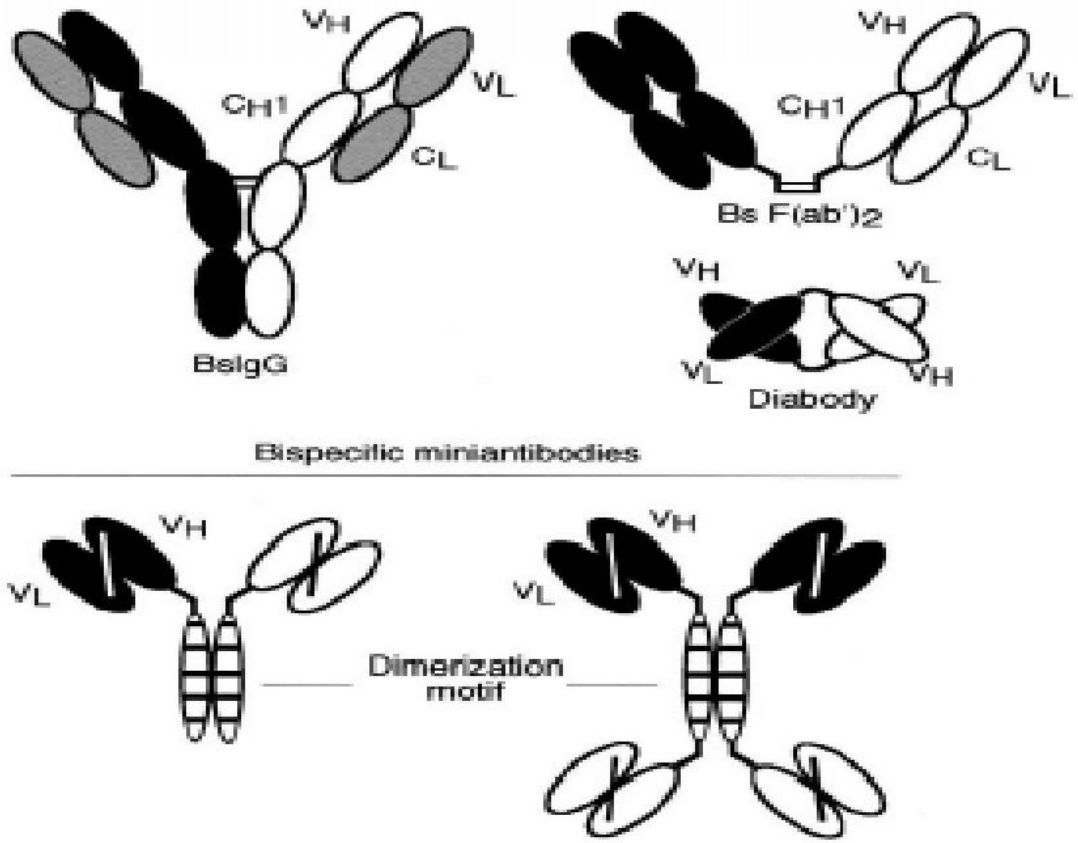


Fig. 3. Bispecific antibody formats that are particularly well suited for clinical development (see Section 4). The two different antigen-binding specifities are indicated by black and white shading, whereas the common Lchain of the BsIgG is colored grey. A linker connecting the carboxy terminus of V_L to the amino terminus of V_H is indicated as a bold white or black line for the miniantibodies. Additional BsAb formats have been reviewed by Carter et al. (1995), Plückthun and Pack (1997) and Hudson (1999).

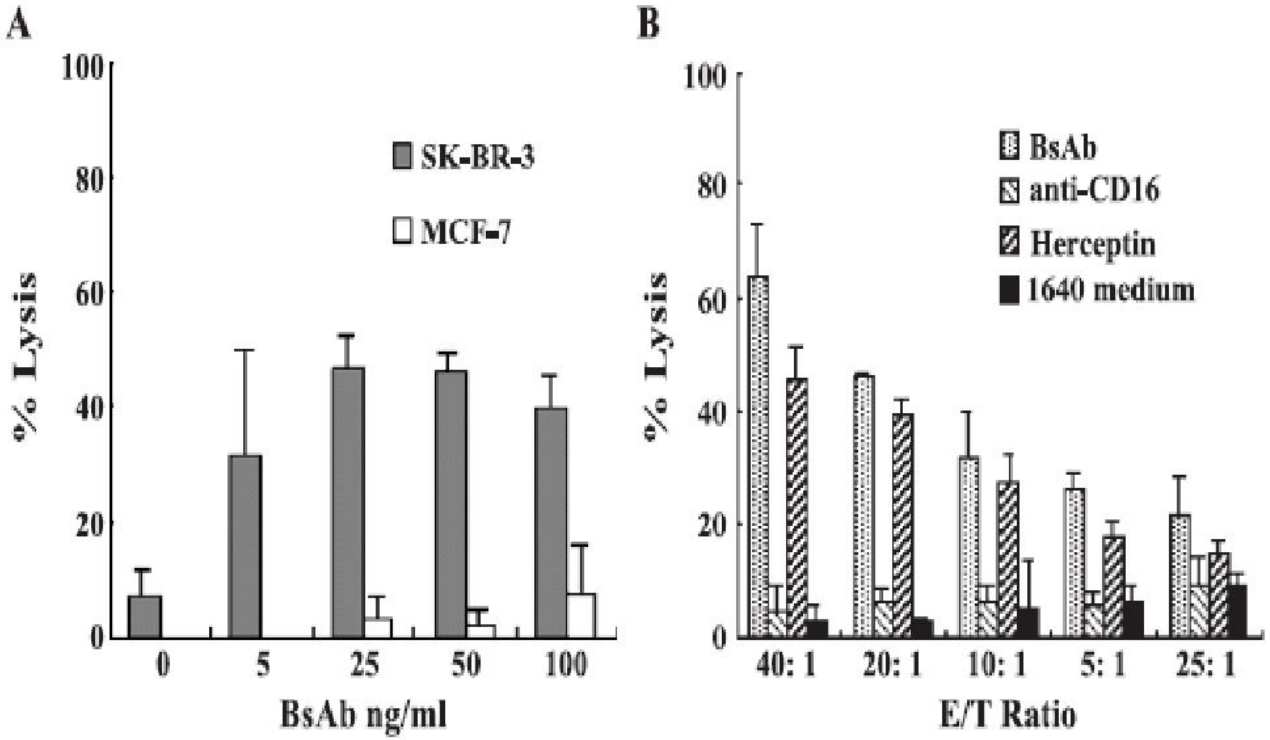


Fig. 6. Cytotoxicity assays of the BsAb using PBMC as effector cells. (A) E:T ratio 20:1; at different concentration of the BsAb, the assays were tested against SK-BR-3 and MCF-7 cells. (B) At antibody concentration of 25 ng/ml, and various E:T ratios, the assays were tested against SK-BR-3 cells. Three complete sets of experiments were done for both assays. For each experiment, the cytotoxicity assays were done in quadruplicate. The error bars are standard errors of three complete sets of experiments.

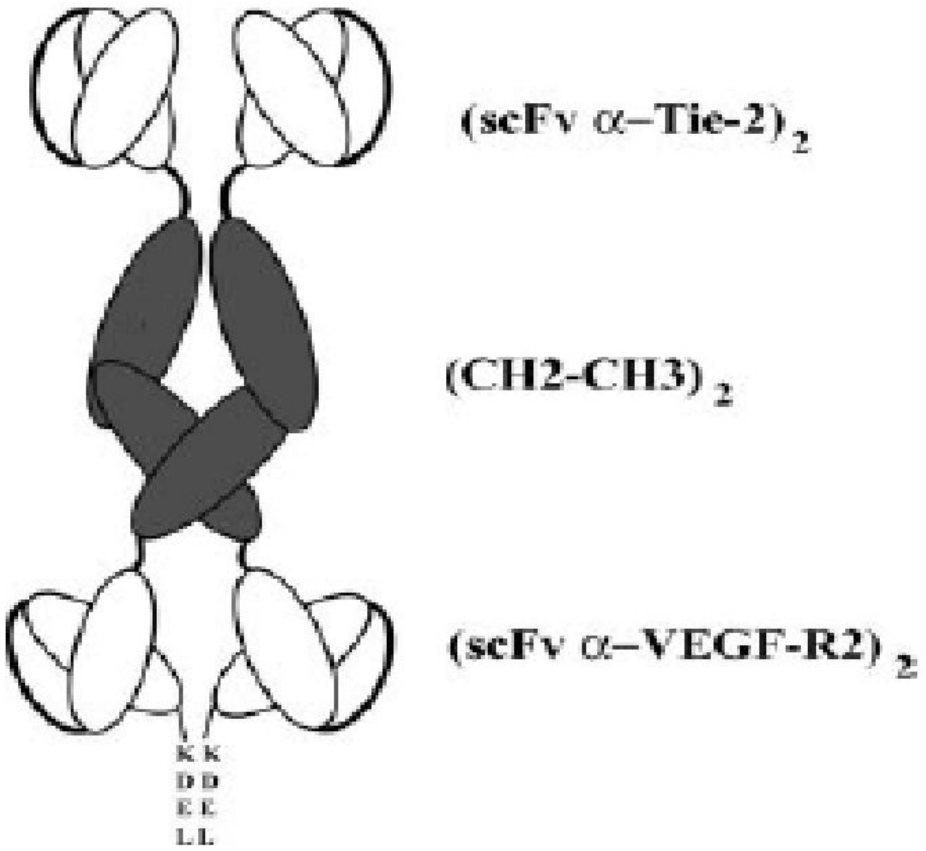


FIG. 1. **A bispecific, tetravalent intradiabody.** Two scFv directed to Tie-2 and VEGF-R2, respectively, were linked through the second and third heavy chain constant domains of human IgG1 to form a 150-kDa dimer. Through the homophilic interaction of CH3, the N-terminal and C-terminal scFv modules are displayed bivalently. The N-terminal scFv module binds Tie-2, and the C-terminal scFv module binds VEGF-R2. An ER retention signal (KDEL) was appended C-terminally.

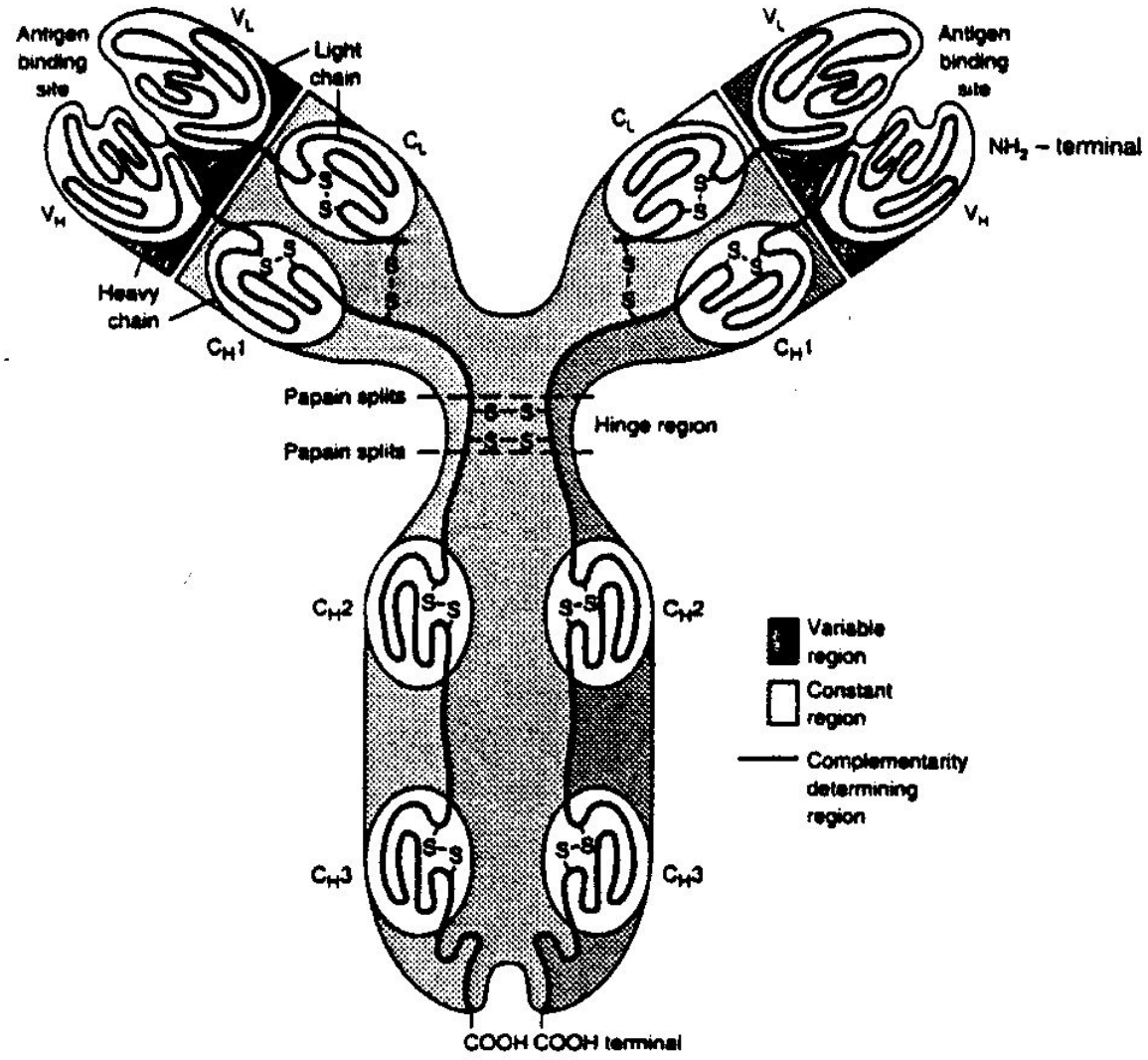
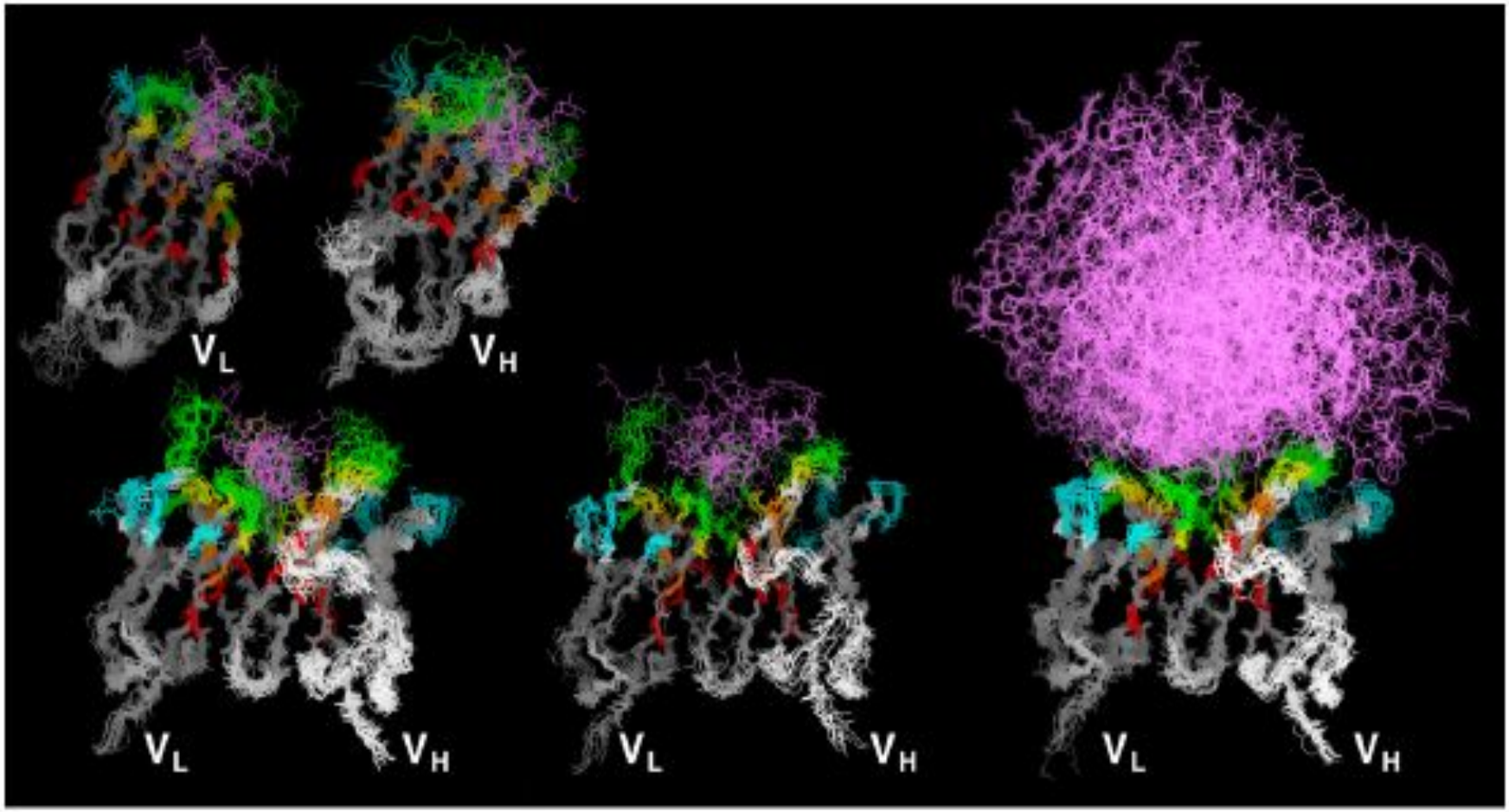


Fig. 1. Model of the IgG antibody molecule. The variable domains of the H (heavy) and L (light) chains are denoted as V_L and V_H respectively. The antigen binding site is comprised of $V_H + V_L$ and corresponds to the Fv fragment. The Fab fragment consists of $V_H C_H 1 + V_L C_L$



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27 Structures representing
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8 Antibodies

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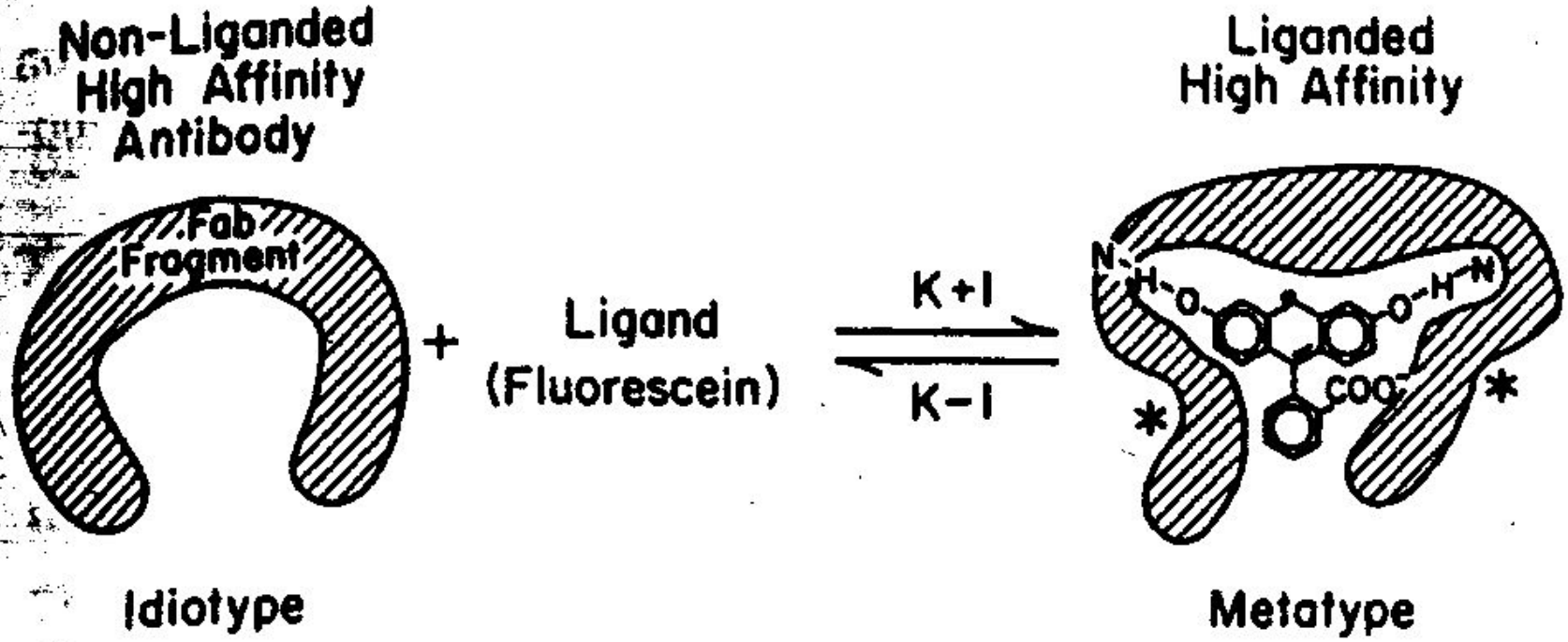


Fig. 2. Schematic representation of the idiotypic and metatypic states of a monoclonal antibody molecule. K_{+1} and K_{-1} denote the kinetic parameters of the rates of ligand association and dissociation respectively. These are also noted as k_1 and k_2. The fluorescein ligand is shown bound to the metatypic state. The * symbol denotes new antigenic determinants generated by ligand binding and the subsequent conformational changes

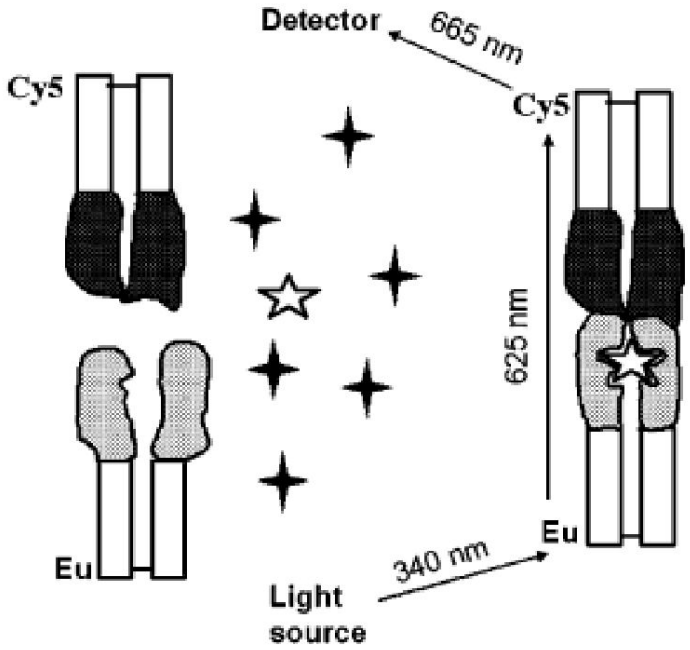


Figure 1. Schematic illustration of the principle of the one-step, noncompetitive FRET immunoassay for morphine. Eu-labeled anti-morphine and Cy5-labeled anti-IC Fab fragments are added into a saliva sample. FRET occurs only when two fluorophores are close to each other; i.e., anti-IC Fab is specifically bound to IC. The star represents the analyte, and dotted patterns represent variable regions of the Fab fragment.

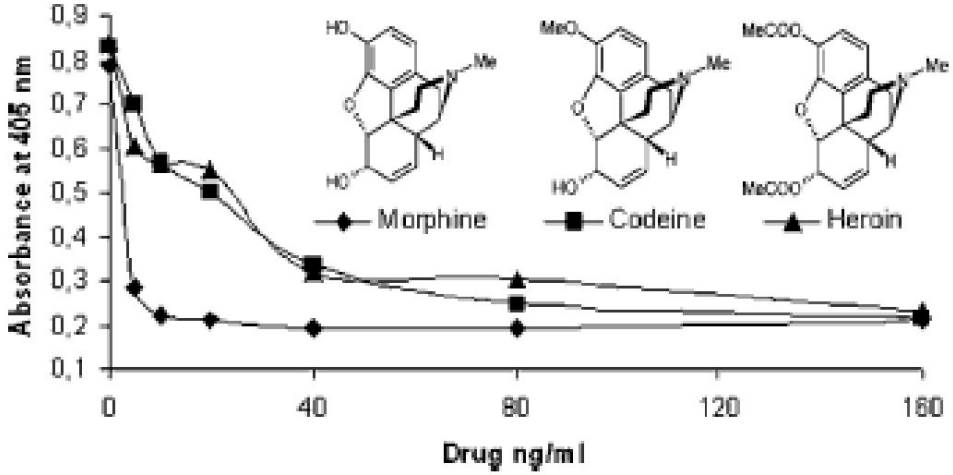


Figure 3. Cross-reactivity of the anti-morphine M1 Fab to codeine and heroin in a competitive ELISA. M1 Fab with various amounts of morphine, codeine, or heroin in PBS was added into morphine–BSA coated wells. After washings the bound Fab was detected with alkaline phosphatase conjugated anti-Fab antibody.

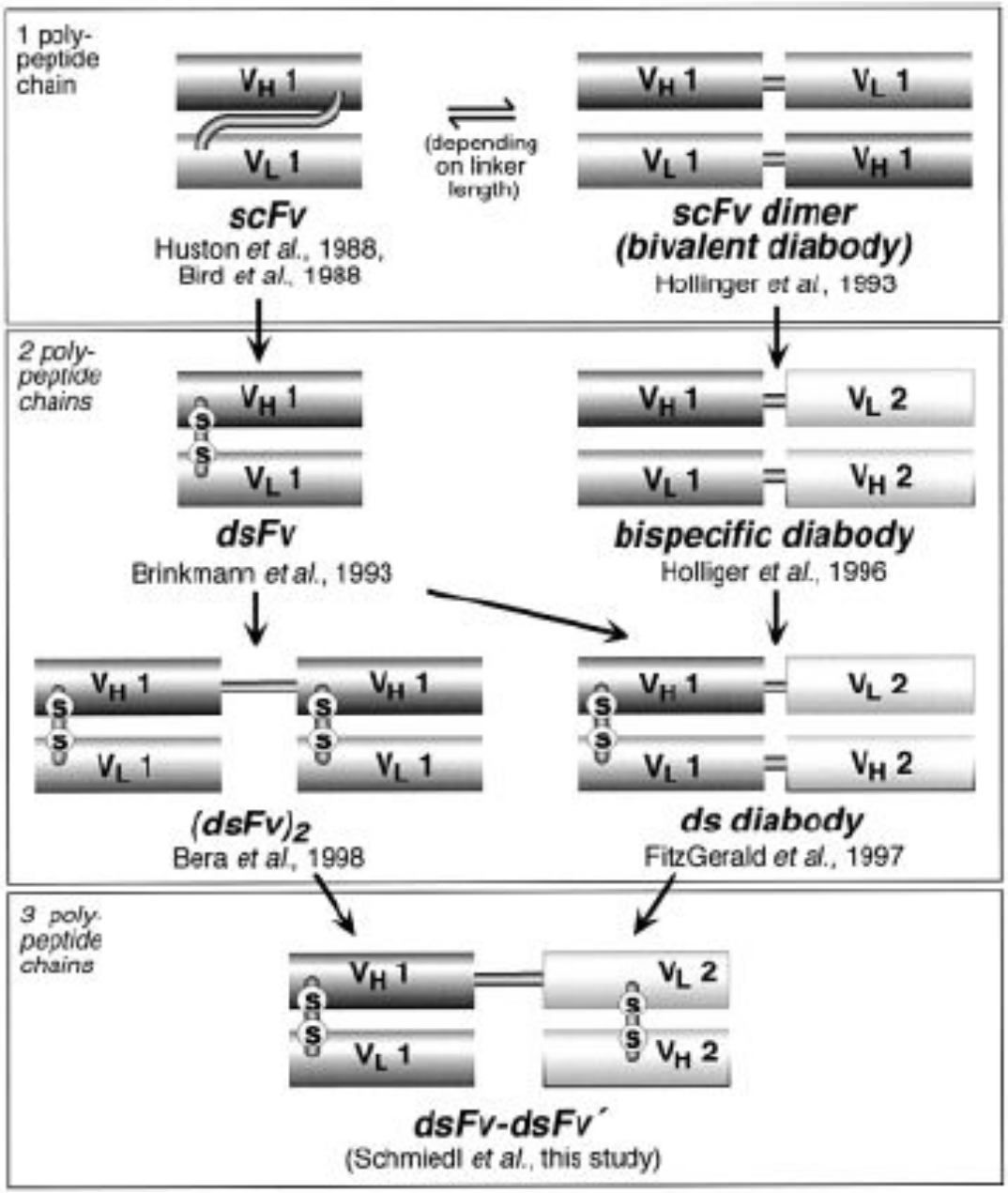
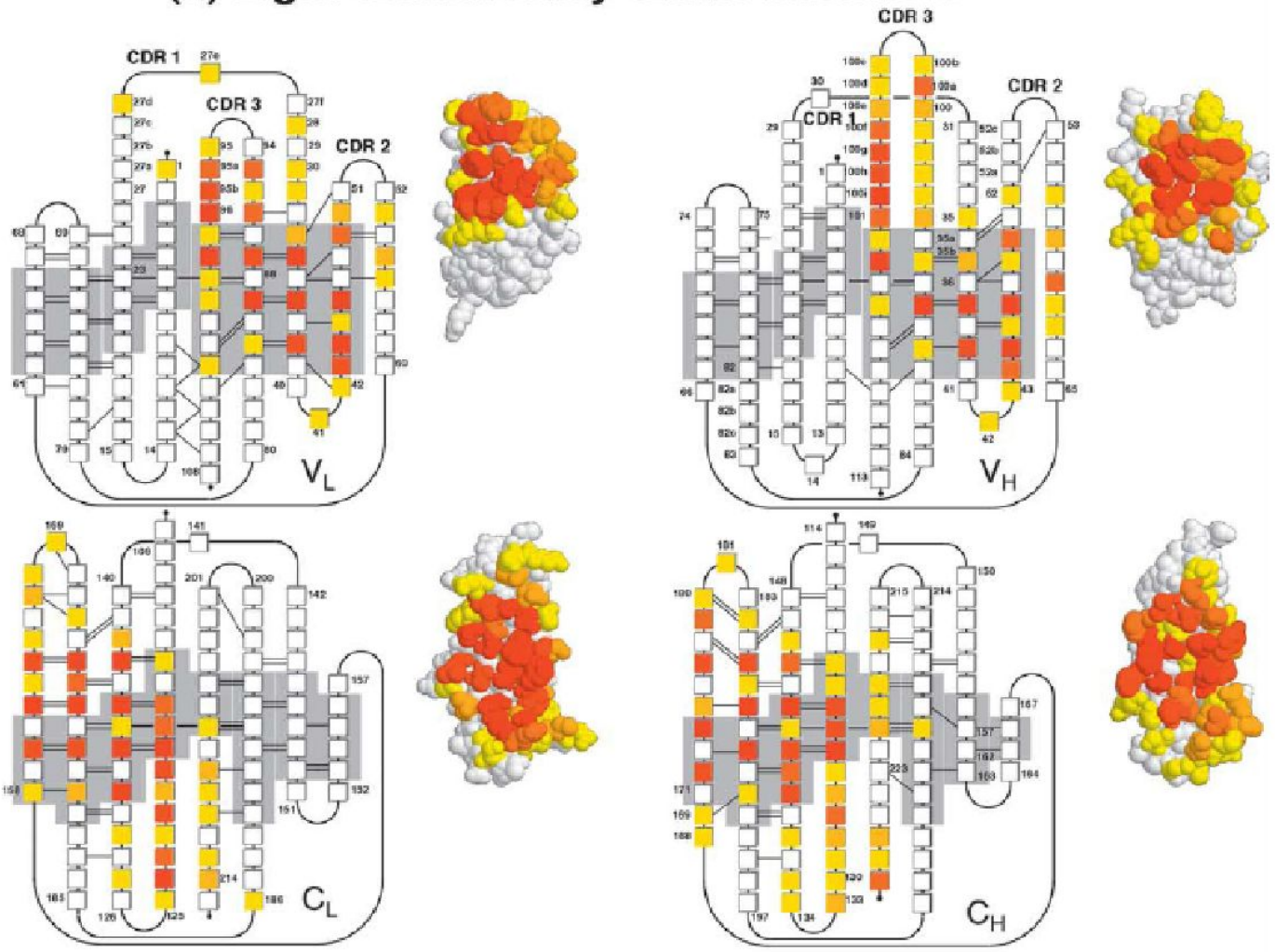


Fig. 7. Developments in the design of small bivalent and bispecific antibody constructs based on homologous fusions.

(a) Light Chain/Heavy Chain Interface:



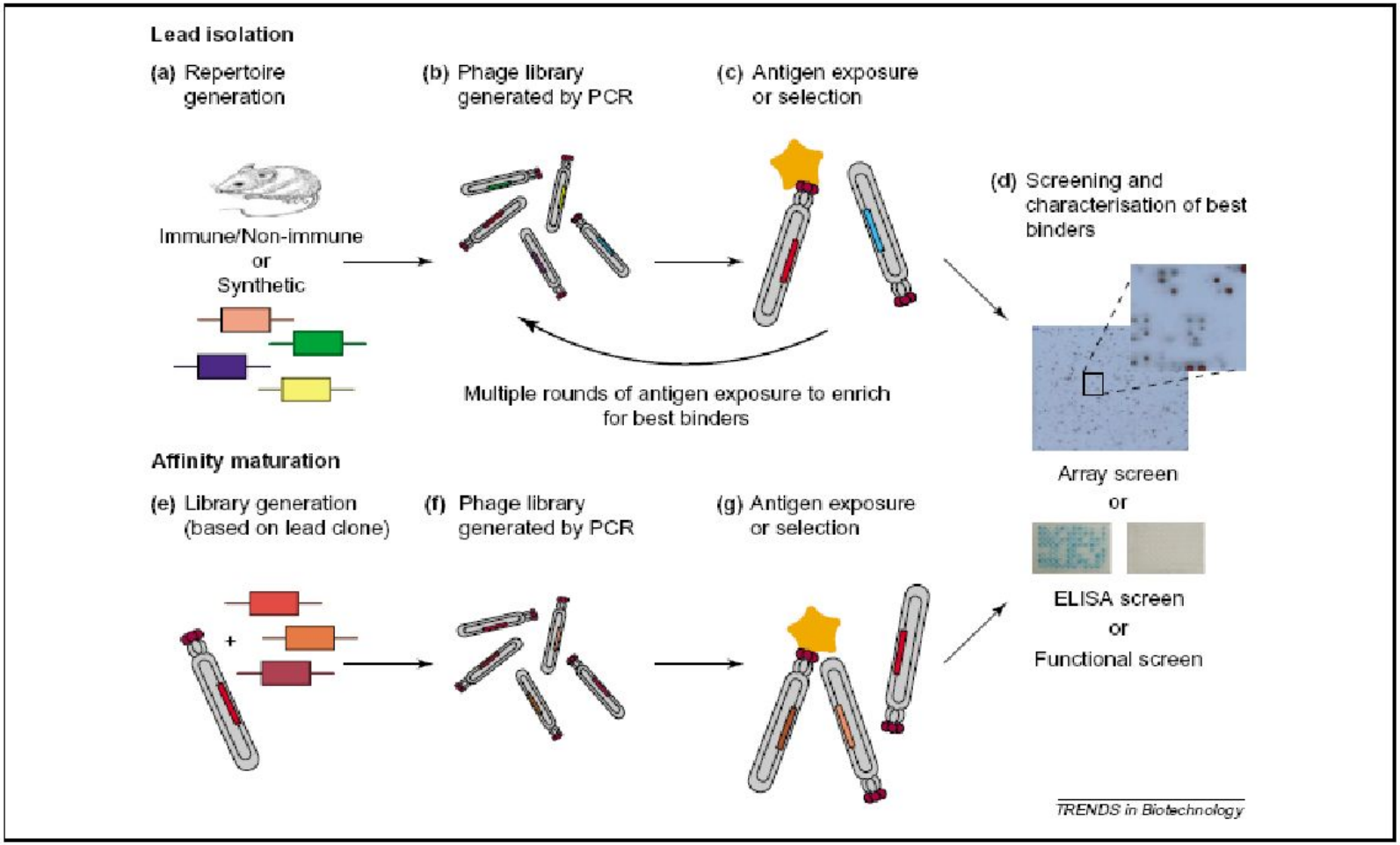


Figure 2. Isolation of antibody fragments by phage display involves **(a–d)** isolation of a lead antibody that binds antigen followed by **(e,f,g,d)** affinity maturation of the lead antibody resulting in identification of an antibody of high affinity. **(a)** A repertoire of antibody variable genes must first be cloned into a suitable phage or phagemid vector to allow **(b)** production of antibody-displaying phage. **(c)** Antibody-displaying phage are then exposed to antigen that is either adsorbed to plastic or presented in solution [46], and phage displaying antibodies that bind antigen are selected through washing and elution. Techniques aimed at improving the specificity and affinity of this selection step include preselection [71,72], the linkage of antigen recognition to phage infectivity [73] and trypsin-based elution [74]. **(d)** Lead antibodies are identified by binding assays such as ELISA or array screening, or more usefully by screening for biological activity in cell-based or receptor assays. **(e–g)** Expression and characterisation of individual antibodies can be followed by affinity maturation to optimise the best drug candidates. Maturation involves constructing further phage libraries based on the lead clone by using techniques including error-prone PCR [46], mutator strains of bacteria [47] to randomise the whole antibody gene, and CDR-targeted methods such as 'doped' oligonucleotide mutagenesis [48].

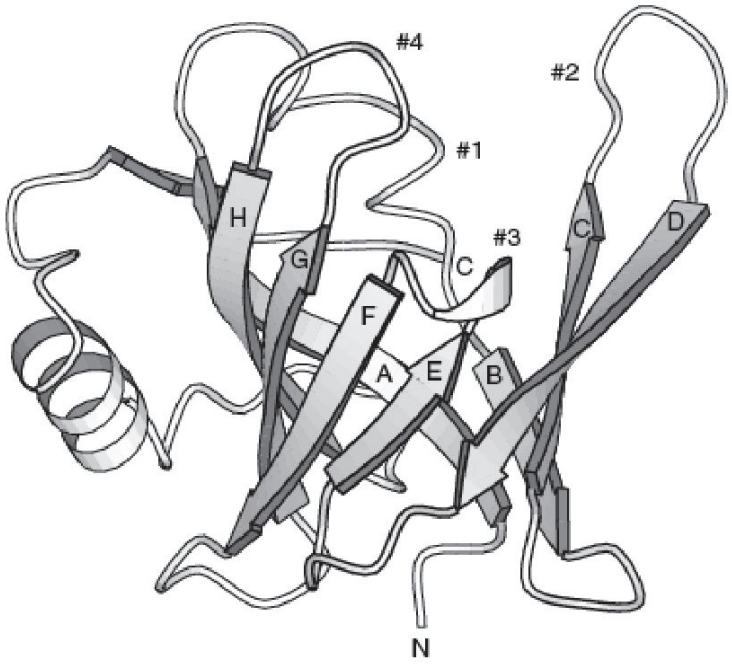


Figure 1. Schematic representation of the lipocalin fold. The ribbon diagram of Tlc (PDB code 1XKI) illustrates the central antiparallel β-barrel with eight strands (labelled A – H), to which an α-helix is attached (N and C termini of the polypeptide chain are labelled). The β-barrel supports four structurally variable and sometimes flexible loops at its open end (designated #1 – #4), which form the entrance to the ligand-binding site and can be engineered to achieve novel target specificities.
Tlc: Human tear lipocalin.

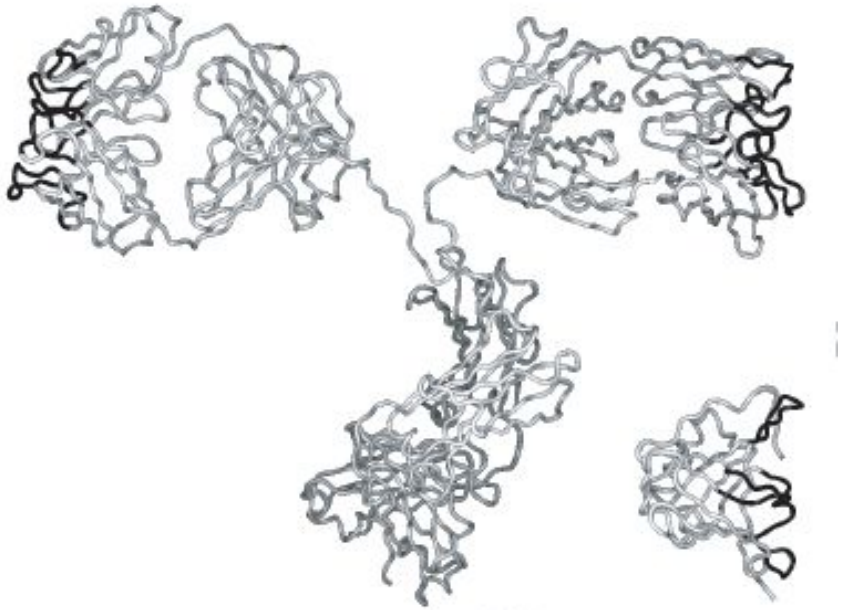


Figure 2. Comparison of two different families of binding proteins, immunoglobulins versus lipocalins. An antibody with its typical Y-shaped appearance (PDB code 1IGT), presenting a pair of identical antigen-binding sites at the two tips, is shown on the left. The much smaller lipocalin (PDB code 1BBP), which exhibits a single ligand-binding site, is depicted at the same scale on the right. The sets of hypervariable loops are coloured black for both proteins.

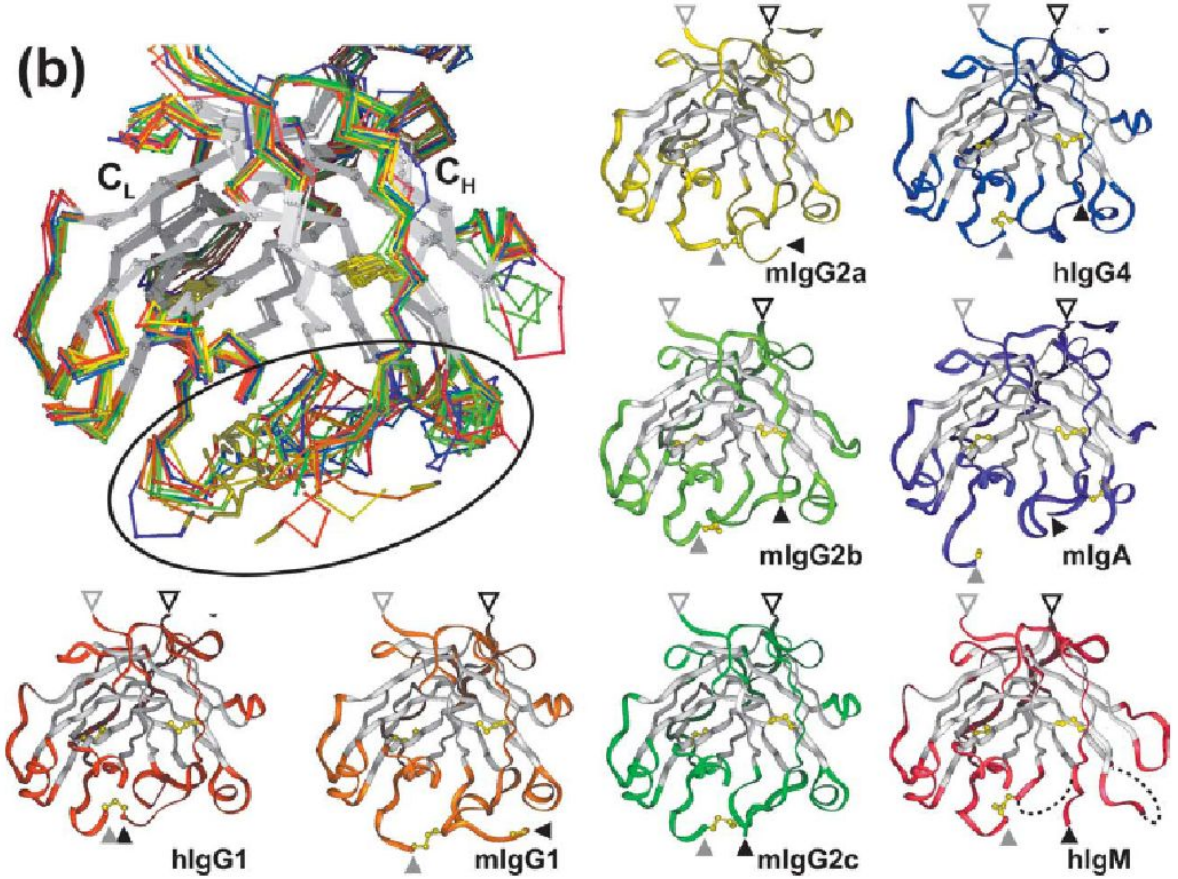
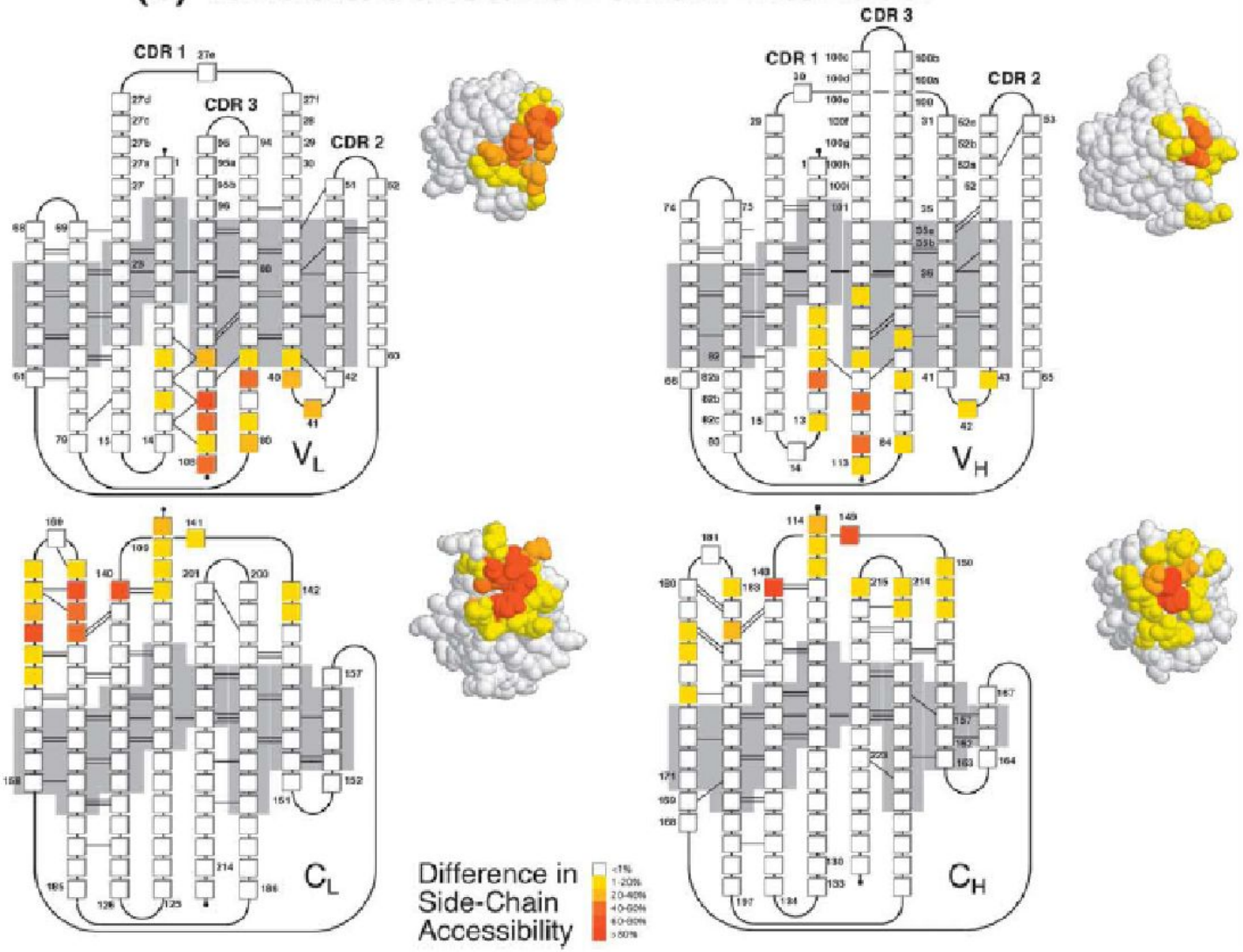


Figure 8. Flexibility of the interdomain disulfide bond. (a) A representation of a C_H1 and a C_L domain. The color code of the boxes representing the atom positions indicates the contribution of the side-chains of these residues to the C_H1/C_L interface as described in the legend to Figure 6. Continuous, thick black lines indicate different disulfide linkages that have been observed in Fab structures, broken lines indicate additionally possible disulfide bonds. For details, see the text. (b) Structural flexibility in the region containing the interchain disulfide bonds. An overlay of representative, well-resolved structures of the constant domains from Fab fragments from different immunoglobulin isotypes illustrates both

(b) Variable/Constant Domain Interface:



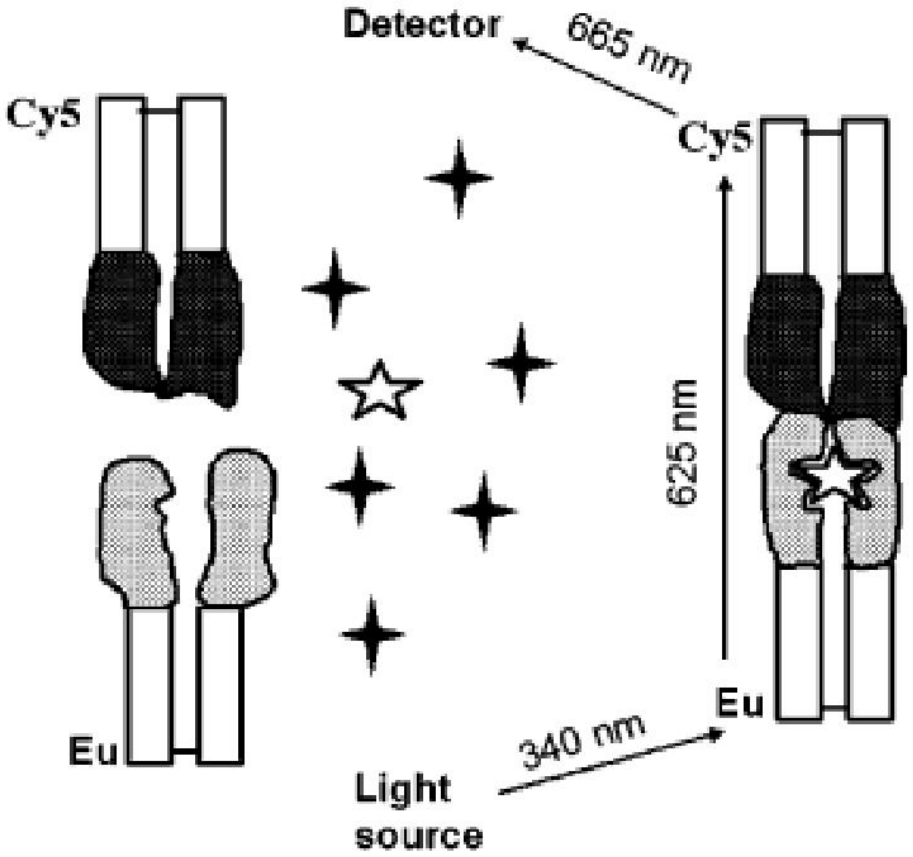


Figure 1. Schematic illustration of the principle of the one-step, noncompetitive FRET immunoassay for morphine. Eu-labeled anti-morphine and Cy5-labeled anti-IC Fab fragments are added into a saliva sample. FRET occurs only when two fluorophores are close to each other; i.e., anti-IC Fab is specifically bound to IC. The star represents the analyte, and dotted patterns represent variable regions of the Fab fragment.