Artificial Polyvalent Antibodies

-Discrimination and Specificity
-Applications in cancer treatment?

T.R.C. Boyde, 18th September 2007
ACB Southern Region Meeting,
Royal Marsden Hospital

My topic is how to make and use new antibody constructs which have enhanced binding strength and discriminatory power. In the next slide are some of the words that will be employed in this presentation with changed emphasis or a distinct new meaning.

Underlying the whole topic are the concepts of discrimination and selectivity, which we wish to use as quantitative measures and must therefore distinguish from ‘specificity’ because that word is reserved identify the epitope or target with which a given site or ligand will react; not for comparison of binding strength.

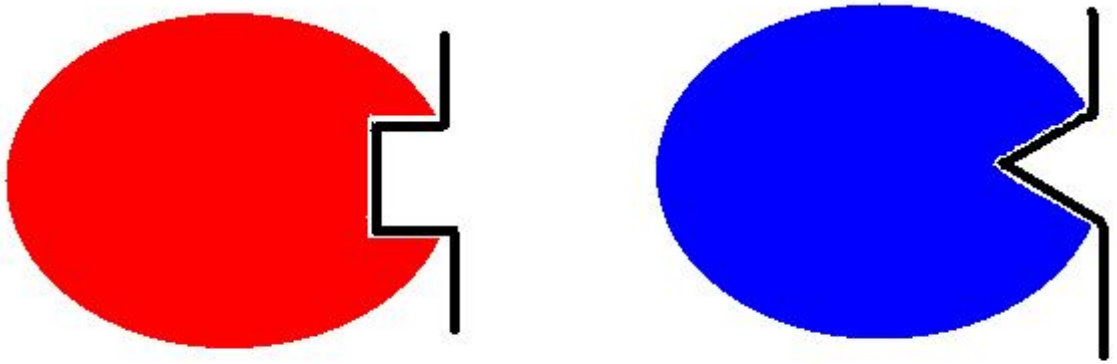
Words

- *binding site, epitope*
- *ligand, target*
- *specificity,*
- *selectivity = discriminatory power*
- *discrimination*
- *homopolyvalent, heteropolyvalent*
- *adduct, hybrid*

Binding units or sites

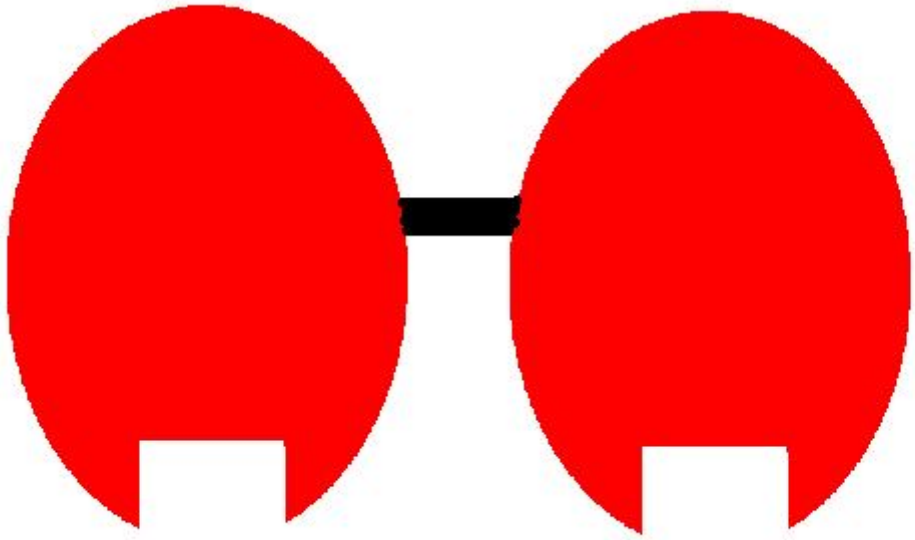
-of two different specificities

Individual AB units bind with specificity to their target epitopes and in the case illustrated will obviously discriminate between them



Bivalency and discrimination

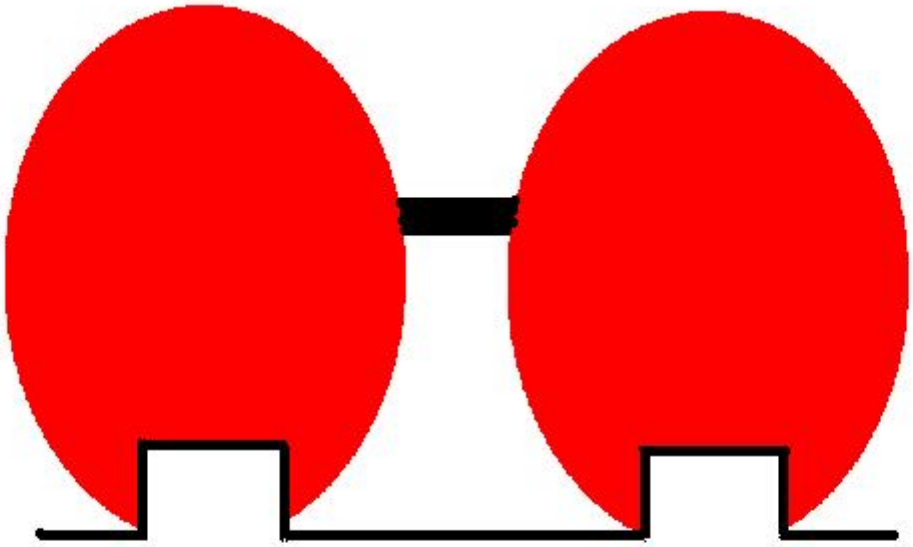
an artificial homobivalent construct -



What may not be quite so obvious is that a bivalent construct such as this, even a homobivalent construct ….

Bivalency and discrimination

…. will show improved discrimination for a homobivalent target as compared with a target bearing only the corresponding monovalent epitope



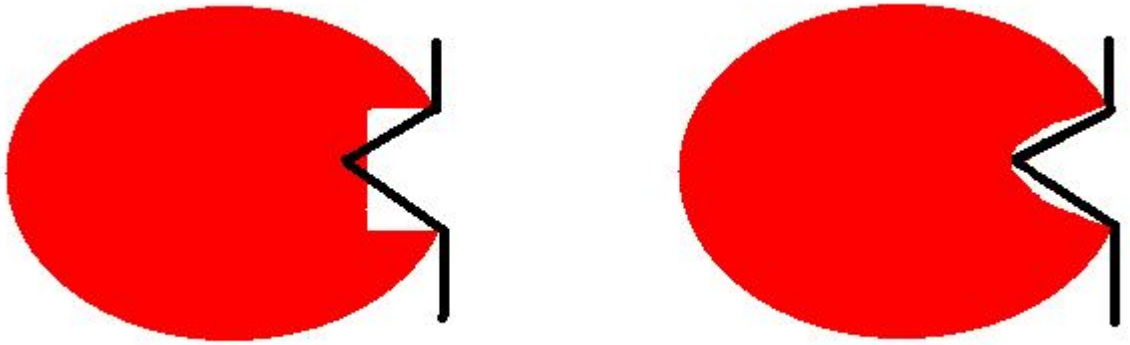
That is, a homobivalent ligand is capable of picking out its bivalent or polyvalent target from a background of monovalent.

It took twenty years to appreciate fully the importance of this insight, which eliminates an apparent mathematical problem over symmetry as between homo- and hetero-bivalency. There is more detail in the companion paper on “Enhanced Discriminatory Power … “

Here let us examine what happens when the fit of binding site to epitope is less than perfect so that specificity is not absolute and discrimination also less than perfect.

Specificity vs discrimination

badly fitting epitope binds with less affinity

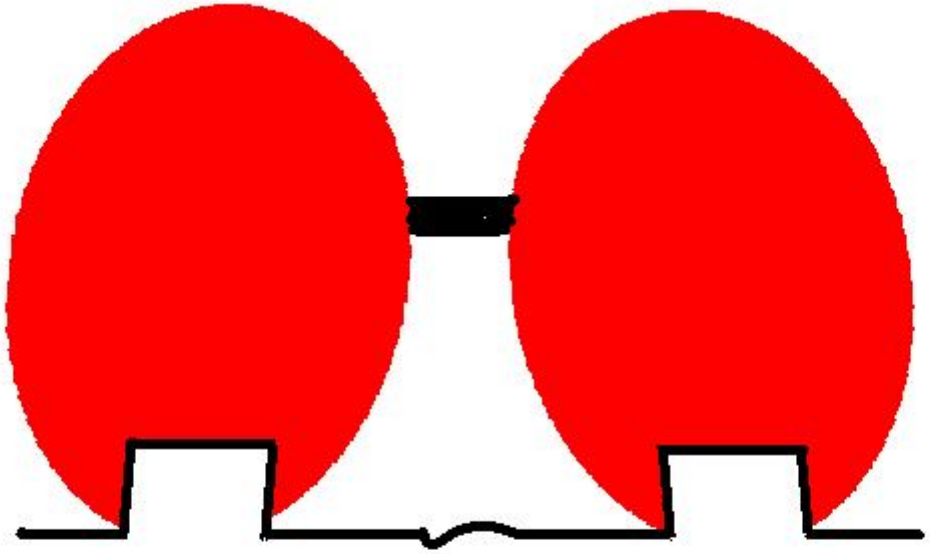


That last picture showed a triangular epitope projecting too far to be accommodated within the binding site of this type of binding unit. It can be accommodated if the binding site is able to change shape slightly, but the fit is both imperfect and achieved only with strain, leading to lower overall binding energy. Therefore:

1] Specificity is not absolute, the individual site will react to a detectable extent with whatever can adapt to the binding surface without causing too much distortion.
2] The differences are quantitative. A more precise way of speaking about antibody ‘specificity’is that antibodies discriminate between targets more or less well.

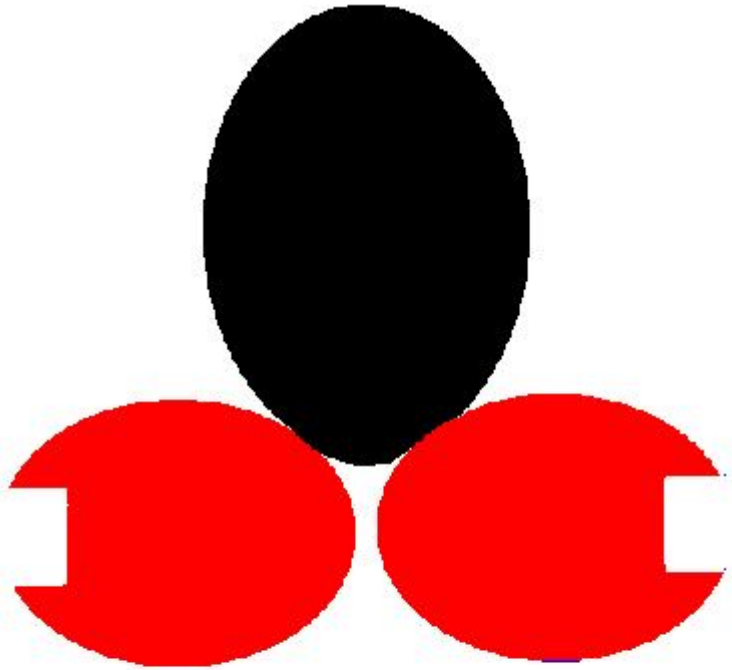
Specificity vs discrimination

If individual epitopes are more widely spaced than the binding units, again there is strain and loss of binding affinity.



The shape of a natural antibody

is quite different again



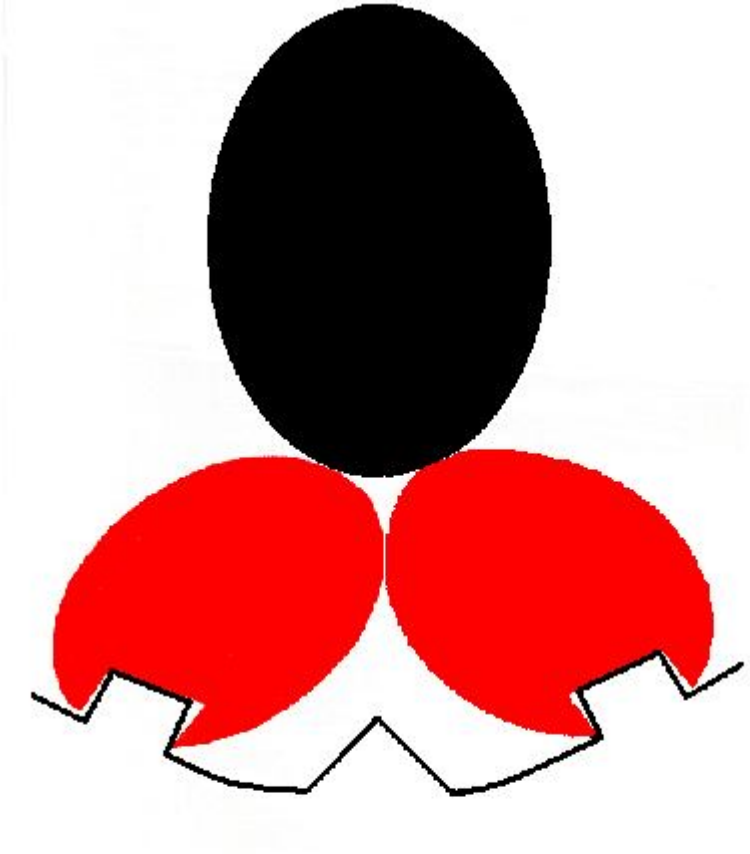
- so that for two binding sites to bind simultaneously, antibody and target have to distort. At least this is a common case.

The living organism makes use of this business of forcing of the antibody into an uncomfortable shape in order to induce effects elsewhere than the binding site, like killing a bacterium, or sending signals.

We have no need to discuss these knock-on effects, and it is clear enough that if our interest is only in high binding strength and high discrimination we should avoid molecular distortion of this kind.

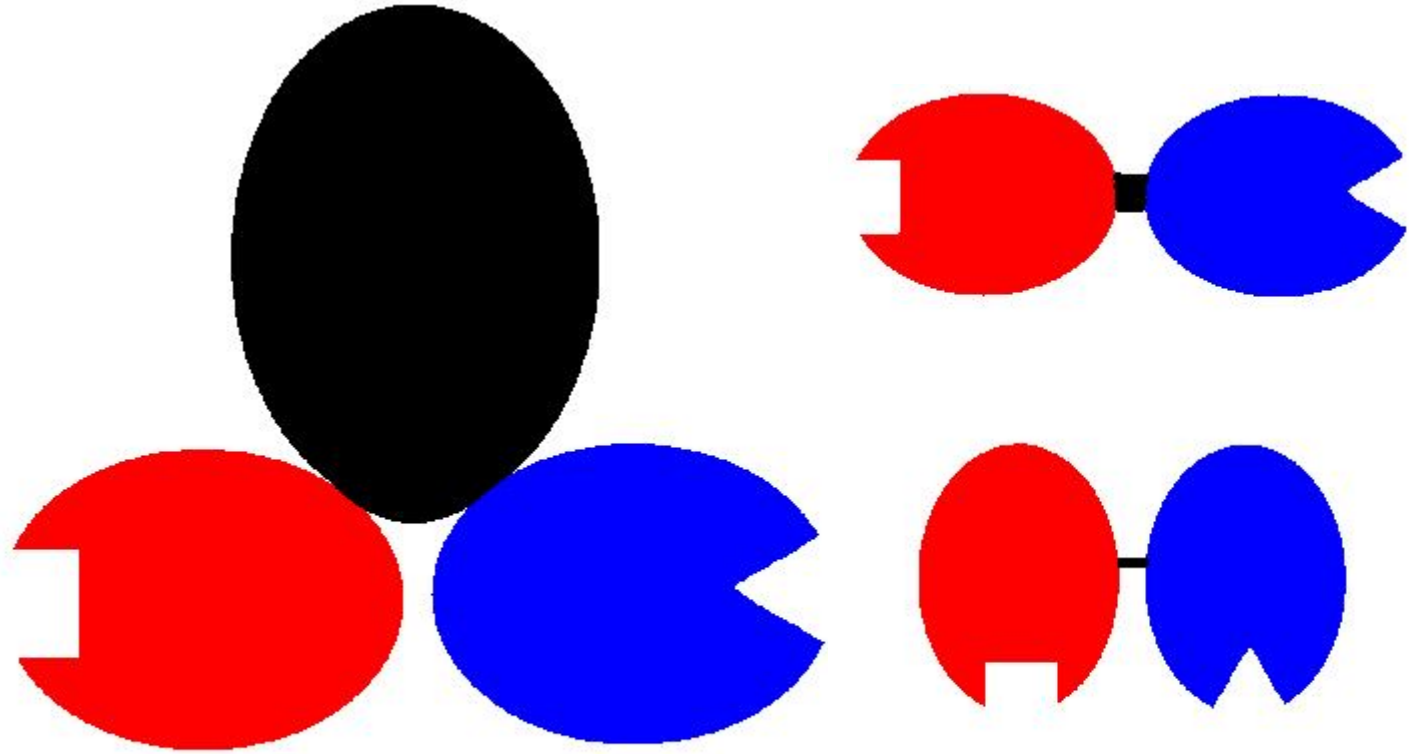
The shape of a natural antibody

both target and antibody may have to distort



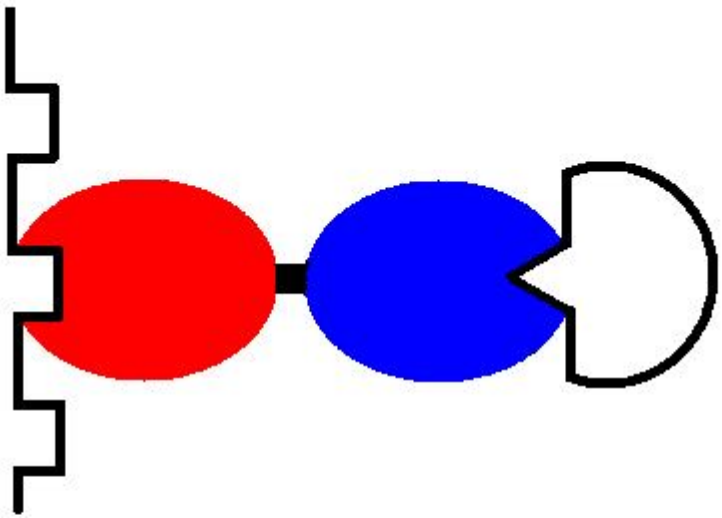
Artificial heteropolyvalency

- of many kinds already exists



Artificial heteropolyvalency

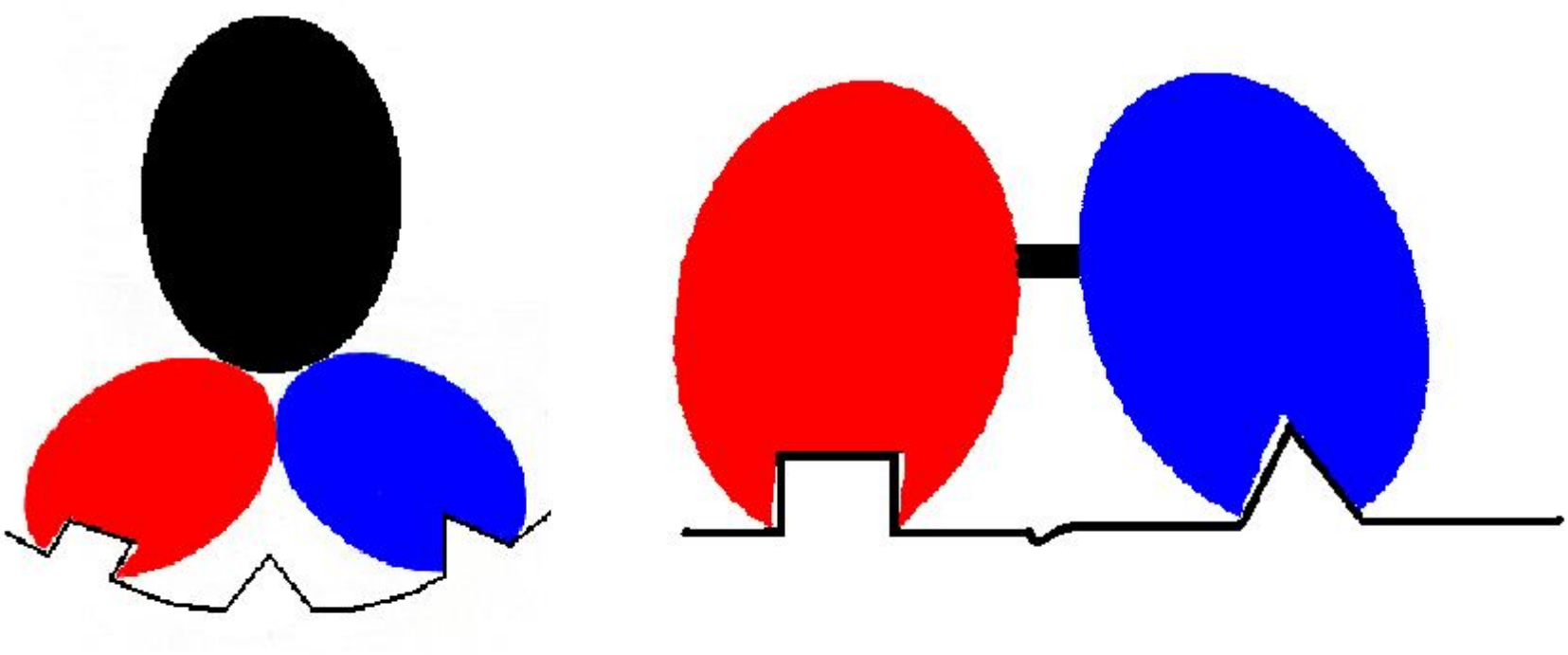
‘diabody’ connecting two distinct targets



And this is the dominant use - to bring different things together by binding separately to each rather than binding cooperatively to a single target with increased affinity and discrimination

Artificial heteropolyvalency

binding with distortion



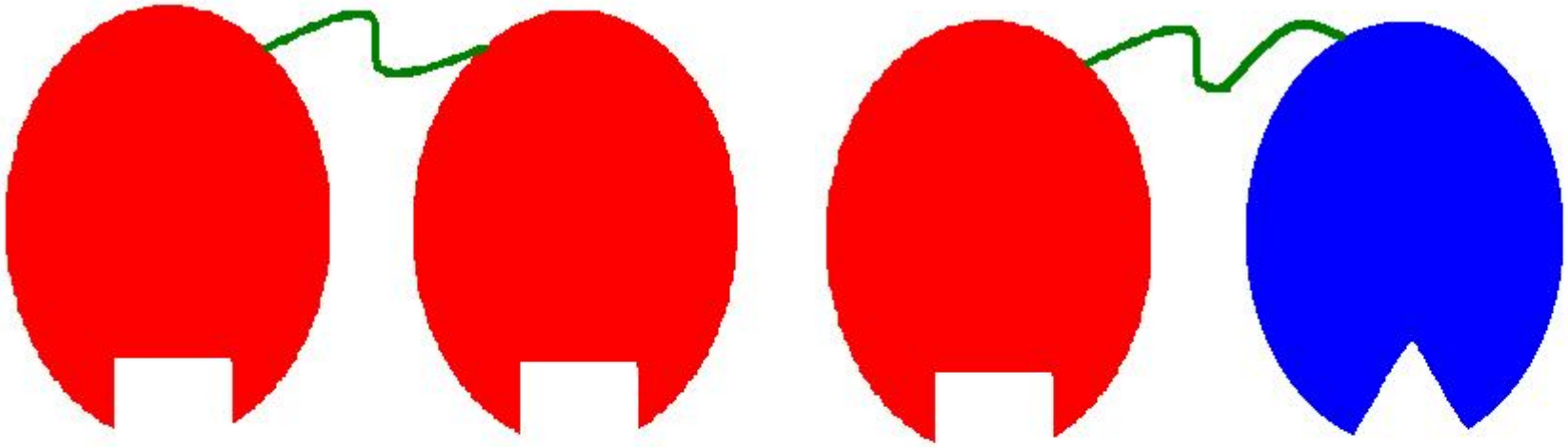
The history goes back more than 40 years, and the first were deliberately made to resemble natural antibodies structurally, like the first example here. The second is a so-called ‘diabody’ and the third shows two Fab’s artificially linked with no Fc.

Clearly, with a diabody, it is impossible for both binding sites to be able to react with the same target, and this shows up in the affinities when it has been tried.

The other kinds of heterobivalent hybrids are subject to similar distortion problems, upon binding to rigid targets, just like homobivalent antibodies or constructs.

Flexible linker, why not?

homo- or hetero-polyvalent



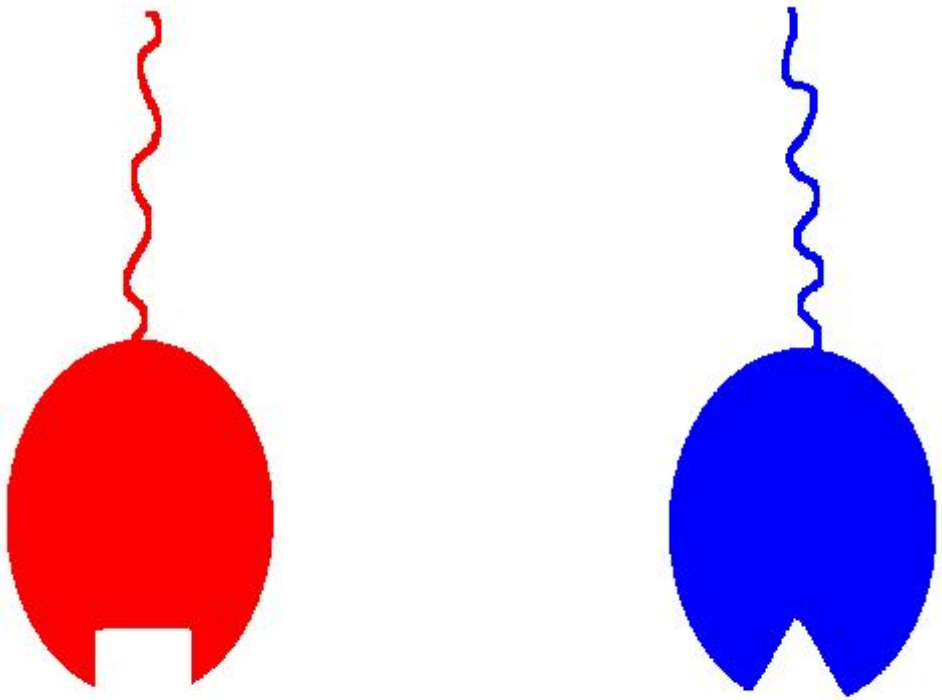
We may expect that the distortion problem will be removed and the true extent of additional affinity and discrimination will be revealed. However, there may instead be a “floppiness” problem; that is, loss of overall binding energy due to suppression of conformational entropy upon binding. There has been entirely proper controversy on this question and that too has taken the best part of twenty years to sort out in a fully satisfactory manner.

The results of actual polyvalent binding experiments are quite good provided the linkers are not made of simple, single-bonded chains. Algebra and key references are given in the companion paper

In this paper our concern from now is only with heteropolyvalent hybrids having flexible linkers.

Nucleic acid linkers

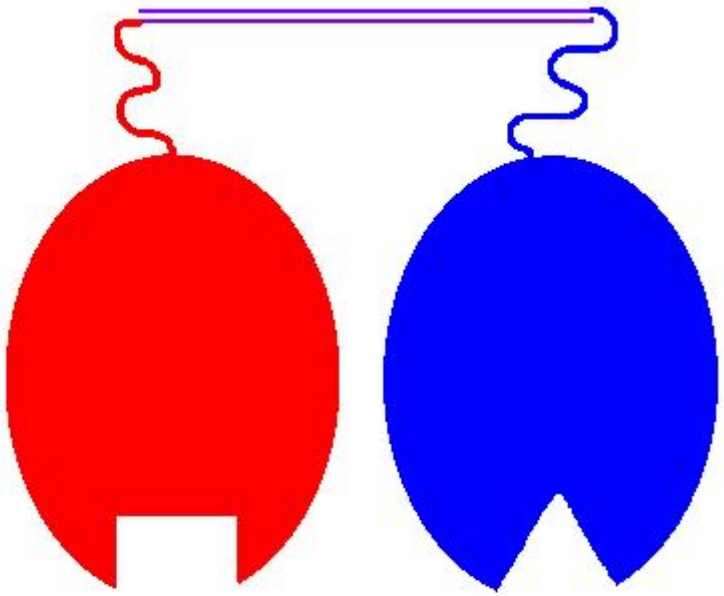
‘adducts’ with single-strand DNA tails



Such flexible linkers can in fact be made easily by attaching a nucleic acid strand to each AB unit. We call these things ‘adducts’.

Nucleic acid linker

duplex forms between complementary segments



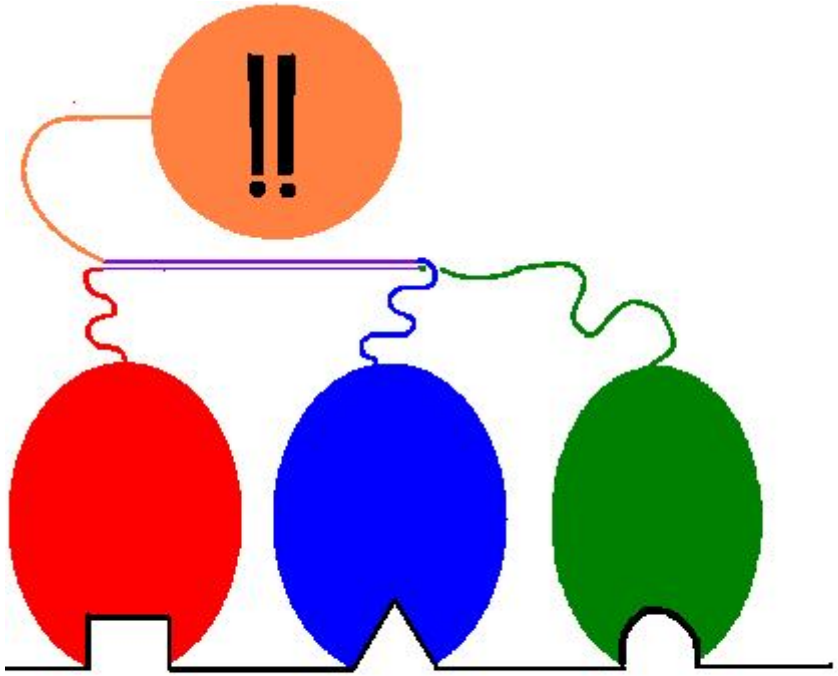
When the two adducts are merely mixed together in solution, a duplex forms. The bond between the AB units can be as strong as desired and single-stranded segments as long as needed, simply by correct choice of the nucleic acid sequences.

Plainly, the distance between epitopes on a target now matters much less, and such a hybrid could easily bind to a target bearing the corresponding epitopes even on opposite sides.

Nucleic acid linkers

three or more AB units, no problem

add functional components, no problem



Three or more AB units can be brought together just as easily as two, and effector molecules added to the assembly when needed, e.g. a toxin to kill a cancer cell or a reporter molecule for use in diagnosis. The method of tying the bits together shown in this picture is not the most adaptable, merely the easiest to draw.

The advantages of polyvalency should be clearer now than appears from the simple bivalent case. 1] This hybrid ligand binds very strongly because the interaction involves three different AB units all of which can react without strain. 2] It binds only to **this** target because both ligand and target possess three different kinds of binding entity. The power to discriminate one target from another is vastly increased. 3] We have erected an ***entirely new specificity*** identified by the presence simultaneously of all the chosen epitopes.

Mathematical symmetry demands that a homopolyvalent construct must show the same effects on binding, discrimination and specificity as the heteropolyvalent one, provided that we are comparing like with like; that is, the desired target itself is polyvalent. But then of course it selects only for multiple copies of type of epitope - a Good Thing in microbiology, less good for some other purposes.

Nucleic acid advantages

*easily prepare and purify adducts
*rapid choice and assembly of adducts
*choose according to the particular target

*use artificial or natural binding units
*smaller binding units to penetrate tissues
*assembly even *in situ*, within the body

*potentially good immune tolerance
*enzyme resistant forms possible

Applications in cancer treatment?

Relevant characteristics of cancer:

*Microscopy may mislead as to the **biochemical** signature

*Personal cancer signature: **personal** treatment

*Very few **cancer** 'stem cells' serve to renew the growth

*Many genetic changes as the growth develops

- Therefore, exceedingly high discriminatory power is required both in the testing system and in the treatment
- Also the treatment molecule must be assembled very quickly, because otherwise the patient may be dead

We follow the naïve notion of simply killing the cancer cells, each and every one, including the rare ‘cancer stem cells’ now known to exist in at least some instances.

Identification of useful targets is far more difficult than we used to think. What the pathologist classifies as a certain type of cancer may not be uniform in respect of the underlying functional and genetic changes: tumours that look alike under the microscope are likely not the same in respect of potential target epitopes or means of treatment.

We propose to identify the characteristic surface epitopes of the cancer cells of an individual patient and then assemble an appropriate attack molecule. The process needs to be quick and no

other means of doing it quickly enough has been described.

Applications in cancer treatment?

Barriers

Individualised treatment is not open to formal evaluation as presently understood

Therefore pharmaceutical companies find difficulty if taking this on board

Our hybrids are not susceptible to testing and approval prior to patient treatment, in the manner now thought obligatory in leading countries, simply because each individualised hybrid is a new, unique compound in the sense understood by a chemist, and should therefore be subject to the full programme of procedures, which takes many years and would be impractical even if the patient were Bill Gates or Warren Buffett.

Ethics must be revisited: we are looking here at a higher plane of ethical conduct. In practical terms, the treatment of an individual patient will require unconditional indemnity and also the cooperation of

brave and broadminded colleagues.

Applications in cancer treatment?

?

tom@boyde.com

Association of Clinical Biochemists 18th September 2007
Royal Marsden Hospital, Fulham Rd, Chelsea, London
Biochemical Management of Breast and Prostate Cancer

Presentation by Professor T.R.C. Boyde Abstract

Artificial polyvalent antibodies. Enhanced discrimination and additional specificities.

The presence of several binding sites on a single antibody molecule enhances discrimination for a polyvalent antigen and no doubt has evolutionary advantage. Similarly, joining together antibody fragments such as Fab or scFv, synthetically, offers an opportunity to combine intrinsic specificities and thus obtain a new, combined specificity for the previously chosen target together with very high affinity and discriminatory power, provided that the links between the fragments are suitable. Affinity advantage is lost if the binding sites cannot all adopt a good orientation for binding (linker rigidity and length) but also if the linkers are too flexible (entropy loss upon immobilization). Best results in model experiments have been obtained with reactants precisely tailored to match each other or in a more generally useful way by using linkers based on polypeptide or polynucleotide chains. The former are genetically engineered while quick and adaptable synthetic routes exist for the latter, given a prominent sulphydryl or carboxyl group on each antibody fragment.
Applications will be found in diagnosis and therapy.
This work differs from the established use of bi-specific antibody constructs for cross-linking independent targets