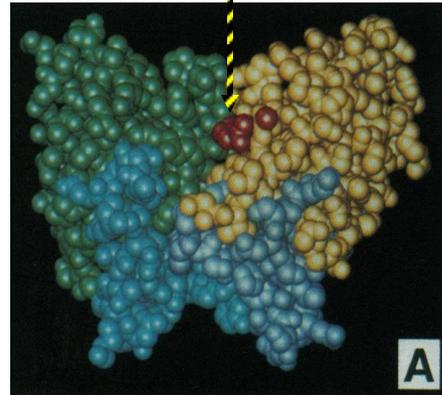


ANTIBODY-LIKE NEW MOLECULES.

PHAGE DISPLAY AND SINGLE-CHAIN ANTIBODIES. A technique for making antibody-like fragments from an actual human V_L and V_H repertoire, with or without immunization. Although there are variants, the basic procedure is this: First, all rearranged V_H and V_L gene segments are cloned from a mixture of B cells, based on unique splice signals present at the 5' and 3' end of these sequences. These are then cloned randomly into cassettes that insert one V_L , then a flexible spacer, and then one V_H segment. Done at a sufficient scale, every V_L in the repertoire will have a chance to combine with every V_H ; so that even though there is no new V(D)J recombination, there is more diversity than in the average person's repertoire (where, though every V_H can, in theory, combine with every V_L , in practice that doesn't happen.) The rearranged V_L - spacer- V_H constructs are inserted into a filamentous bacteriophage that is used to infect *E. coli*, and they are expressed on the bacterial cell surface as a "single-chain Fv." Fv is a combination of only the variable domains of a light and a heavy chain. The phage library can then be tested for binding or adherence to any particular antigen; binding phage are isolated, grown up in *E. coli*, and the phage rescued so that the Fv can be used as-is, or the sequence information used to create full human monoclonal antibodies by reverse engineering.

In this x-ray crystallographic view¹, the framework of V_H is in green (upper left) and of V_L in beige (upper right); the CDRs of V_H are below left, and in slightly lighter blue, of V_L on the lower right. The short brown sequence is what's visible of the linker. This Fv has naturally folded into an effective configuration.



¹ A recombinant immunotoxin containing a disulfide-stabilized Fv fragment.
Ulrich Brinkmann, Yoram Reiter, Sun-Hee Jung, Byungkook Lee, and Ira Pastan. Proc. Natl. Acad. Sci. USA 90:7538-42 (1993)

scFv (SINGLE-CHAIN VARIABLE REGION) ANTIBODIES are constructs of the variable domains of both H and L chains engineered into a single chain and expressed in *E. coli*. They are easy to make and bind well, but *in vivo* their half-life is measured in minutes and they do not seem to be promising drug candidates without significant further modifications.

New bi-specific products have been made by coupling two scFv back-to-back. An interesting one combines an scFv anti-CD3 and an scFv anti-CD19 to make a bifunctional reagent that couples killer T cells via their CD3 to CD19+ lymphomas, thus strengthening the killer cell's binding to its target.

AFFIBODIES are not antibodies but randomly-mutated versions of Staphylococcal protein A. Different ones may bind a variety of different proteins. Affibody (Bromma, Sweden) makes them for some commercial applications.

APTAMERS (RNA, DNA, or modified bases) are 15-20 bases long, fold into stable shapes, and if you make enough of them (4^{20} possible with a 20-mer) you may find one that binds to whatever you want. These have been around a while but for some reason have not yet become the Big Thing, though as proteomics gets more important they may become valuable tools. Developed by CU Boulder's Larry Gold, now at his company SomaLogic in Boulder.

NANOBODY, a term used by the developer Ablynx, is usually a single-domain antibody's combining sites, cloned from a camelid (camel, llama, dromedary). These animals have no light chains, so all the diversity is at the end of the H chain. They have an unusually long flexible CDR3. A cassette containing the CDRs and not much else is cloned and expressed, resulting in a very small but high affinity nanobody (about 12-14 kD, compared to the 50 kD of an Fab fragment). These can often be used where Fab doesn't work due to size-related steric hindrance. For example, one was used to stabilize the β_2 -adrenoceptor for structural studies. Rasmussen, SGF et al. [Nature 469:175 \(2011\)](#). In 2015 the membrane lipid kinase Vps34 complex [was studied](#): "Because the complex resisted crystallization attempts, we screened 15 different nanobodies [made in llamas] against the complex, and one of them enabled crystallization."