A 329 BACTERIAL EXPRESSION AND CHARACTERIZATION OF ANTIGEN BINDING FRAGMENTS OF THE ANTIBODY McPC603, A. Plückthun, J. Stadlmüller, A. Skerra, R. Glockshuber, and B. Steipe, Max-Planck-Inst. f. Biochemie, Genzentrum, D-8033 Martinsried, FRG.

The phosphorylcholine binding antibody McPC603 is particularly well studied and the crystal structure of its Fab fragment with hapten bound is known. Thus, the Fv and Fab fragment of this antibody are convenient model systems for quantitatively investigating binding interactions and transition state stabilization (i.e. catalysis) by systematic modification of the antigen binding site and the hapten. We have developed an expression system with which fully functional Fv or Fab fragments can be expressed in *E. coli*. Both chains are co-expressed and co-secreted into the periplasm of E. coli, with correct signal-processing, disulfide formation, and chain association. The Fv and Fab fragment can be purified to homogeneity in a single step by hapten affinity chromatography. The binding constant of the hapten to the Fv fragment was found to be identical to that of the whole antibody. Also, the variable domains were expressed as fusion proteins with ß-galactosidase, precisely cleaved with the protease factor Xa, and refolded *in vitro* to give a functional Fv fragment. The association constant between the VH and VL domains was determined by crosslinking and fluorescence experiments. Furthermore, we could show that VL dimerizes with itself with an association constant similar to that of the heterodimer, but VH does not. The binding of the hapten stabilizes the Fv fragment considerably. We also showed that the recombinant Fv fragment of McPC603 possesses catalytic activity toward the hydrolysis of suitable carboxylic acid derivatives. This constitutes the first catalytic antibody for which a three-dimensional structure and a convenient expression and mutagenesis system is available.

reprinted from:
A. Plückthun, J. Stadlmüller, A. Skerra, R. Glockshuber and B. Steipe, Bacterial expression and characterization of antigen binding fragments of the antibody McPC603. J. Cell. Biochem. 13A, 92 (1989).

A 330 THE DE NOVO DESIGN OF HELICAL PROTEINS, Lynne J. Regan and William F. DeGrado, C.R.&D., The Experimental Station, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

The question of how the primary amino acid sequence of a protein determines its 3-dimensional structure is still unanswered. Our approach to this problem involves the *de novo* design of model peptides and proteins that should adopt desired 3-dimensional structures. Characterization of these novel proteins (produced by solid-phase peptide synthesis or by expression in *E. coli*) allows us to assess our design successes and failures.

Our model proteins are idealized versions of the naturally occuring 4-helix-bundle motif found in proteins such as myohaemerythrin. A step-wise approach allowed peptides corresponding to the helices to be optimised. Connecting loops were added subsequently and finally the full-length model protein was designed. Structural features of the model protein were probed by circular dichroism, guanidine hydrochloride denaturation and the introduction of disulfide bridges to link segments of the protein modelled to be appropriately positioned. Further NMR and x-ray crystallographic analyses are in progress.

The model 4-helix bundle protein is extremely stable, with a free energy of folding of approximately -20 kCal mol^{-1}. Thus a stable framework is provided onto which we are adding functional groups. The first designs are for ligand binding sites, specifically for metals. Subsequently we hope to add to these designs to include catalytic activity.

A 331 DNA BINDING STUDIES OF ACRIDINE AND ANTHRACYCLINE DRUGS CONJUGATED TO PEPTIDES AND PSEUDOPEPTIDES, Robert T.C. Brownlee, Anastasis Eliadis, Robert J. Hook, Don R. Phillips and James A. Reiss, Departments of Biochemistry and Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia.

Synthetic drug conjugates derived from adriamycin and acridine have been prepared by condensing short polyamide or polymethylene linkers with 14-bromodaunomycin by means of an ester group. The precursor linkers and acridine derivatives were characterised by H-1 and C-13 NMR spectra, mass spectra and elemental analysis. The derivatives have been purified by HPLC and characterised by FAB mass spectrometry. The biophysical analysis of these new compounds together with a previously reported series of acridines conjugated to the netropsin/distamycin class of peptide analogues are reported. Viscometric analysis of solutions of calf-thymus DNA in the presence of drugs, dissociation rates of the drug/DNA complexes using a SDS drug sequestration technique, and DNA-footprinting and DNA transcription experiments were carried out. Footprinting studies of the acridine linked netropsin and distamycin ligands were determined on a 180-mer restriction fragment by inhibition of DNase I, and transcription inhibition experiments were carried out on an *E. coli* RNA polymerase system using a 497 base-pair DNA template. In general, bifunctional binding of these conjugates to DNA were observed with enhanced binding (100 to 1,000-fold) and increased base-pair specificity associated with recognition by the intercalating chromophore. In particular, the acridine-linked netropsin/distamycin conjugates behaved as true bifunctional mixed ligands for DNA showing enhanced preference for A-T rich sites in which co-operativity between the peptide residue and the intercalator occurred in the recognition of DNA. The acridine-anthracycline conjugates show promise as bifunctional ligands for DNA, and the presence of the peptide-like linkage should provide for increased specificity of recognition.