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# Monovalent single-chain antibodies and bivalent miniantibodies from *E coli* : Structure and properties

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During the last few years, bacterial expression, mutagenesis and screening methods have made important contributions to our abilities to engineer antibodies for diagnosis and therapy [1, 2].

Of relevance for diagnostics might be a new methodology to obtain bivalent or bispecific "miniantibodies" in *E coli* [3]. In these contructs, single-chain Fv fragments [4, 5] (consisting of only the variable domain of the light chain and the one from the heavy chain connected by a peptide linker) are connected *via* a hinge peptide to an amphipathic helix motif. This way, two of the single-chain Fv fragments associate *via* their C-termini. This already happens *in vivo* in the producing *E coli* cell, from which completely functional dimers can be isolated. The higher functional affinity ('avidity') of these fragments even allows assaying antibodies with low intrinsic affinities, *eg* against sugar residues.

Two alternative molecular designs have been tested [3]. In the first, the single chain fragment ($V_H$-linker-$V_L$) was fused to the flexible upper hinge region of mouse IgG3. This was followed by one helix taken from the 4-helix bundle designed by Eisenberg *et al* [6]. This helix was either taken as such (construct scHLX) or extended by a small hydrophilic peptide ending in a cysteine residue (scHLXc) in order to covalently link two helices. Ultracentrifugation measurements are consistent with a dimer formation *in vivo*. From the arrangement of the charged residues on the helix, an antiparallel association is anticipated. The best stability and a surface binding identical to a whole antibody was however obtained with a design containing two helices connected by a short turn, which thus should be able to form a true four-helix bundle (Pack and Plückthun, unpublished).

In the other molecular design, instead of the helix from the 4-helix-bundle design, a helix from a parallel coiled-coil structure was used [7]. Specifically, we fused the leucine zipper peptide from the yeast transcription factor GCN4 to the $scF_v$ fragment. Again, the helix was either taken as such (scZIP) or extended with a short peptide ending in a cysteine (scZIPc). Interestingly, the avidity effect is not quite as pronounced as with the 4-helix-bundle design.

All of these designs are based on single-chain antibodies [4, 5], in which the two variable domains are connected by a peptide linker. By using NMR spectroscopy of an Fv fragment and of a single-chain Fv fragment, labelled with $^{15}$N-glycine and/or $^{15}$Nserine or $^{15}$Nammonium chloride, it could be shown that the structure of the unlinked and the linked Fv fragments are identical [8]. The linker is flexible, makes extremely few contacts to the variable domains, and the linker residues are not experiencing different environments but are probably mainly exposed to solvent [8]. Furthermore, the linker does not contribute much to stability [9]. It appears as if the linker is a very flexible part of the molecule which adapts to the surroundings.

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