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## Antibodies: specificity and promiscuity of ligand recognition

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To enhance our understanding of how recognition and specificity for different ligands can be accomplished by related antibodies, we are studying the evolution of ligand binding properties by site-directed mutagenesis. The most active catalytic Diels-Alder antibody known to date, 1E9, and the steroid binding antibody, DB3, derive from the same polyspecific germ line sequences and share 85% sequence identity. In spite of their close relationship, they fulfill very different specific tasks with high efficiency.
Through sequential amino acid exchanges, we changed the specificity of 1E9 to that of DB3. Binding studies reveal that just a few residues are predominantly responsible for achieving either an efficient catalysis of the Diels-Alder reaction or, when mutated, convert the 1E9 Fab into a strong steroid binder. We determined the structures of the 1E9 mutants as apo-proteins, as well as in complex with different steroids. The structures highlight that only two residues in the substrate binding site are necessary and sufficient for discriminating structurally diverse ligands. Additionally, the structures reveal that despite strong binding (nM Kds) several distinct binding modes are employed promiscuously in order to accommodate structurally different steroids in the engineered ligand binding sites.
The potential of antibodies as biocatalysts and the clinical utility of diagnostic and therapeutic antibodies have been the impetus behind the rapid development of antibody engineering. Our studies contribute to the advancement of this field by demonstrating how relatively minor changes can be rationally employed to modify antibody specificity and function.

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## Formation of amyloid fibres followed by X-ray diffraction in real time. Implications for the identity of bio-active species in conformational diseases like Alzheimer's disease

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Protein misfolding diseases, such as Alzheimer's disease (AD), Parkinson disease, the transmissible encephalophaty Creutzfeldt-Jakob's disease, familial amyloidosis and type II diabetes mellitus, are all associated with deposition of fibrous proteins in amyloid plaques. Amyloids of different proteins share a common structural motif, known as the cross-β structure. Increasing evidence indicates that the toxicity lies in soluble oligomeric species of amyloids rather than in the monomers or fibrils [1-3]. The structural nature of these oligomers is unknown.. We followed the *formation* of fibres by X-ray diffraction in real time. Aqueous solutions of amyloid peptides were placed in a capillary and allowed to evaporate slowly. This process takes several days. Our data reveal what stages occur in amyloid formation. This gave us insight in the precise structural nature of the toxic oligomeric species. These findings have implications for our understanding of the mechanism by which amyloids interact with protein molecules such as multi-ligand receptors [4] and antibodies.

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