Structure of a single-chain Fv fragment in complex with the first 17 amino acids of htt exon1

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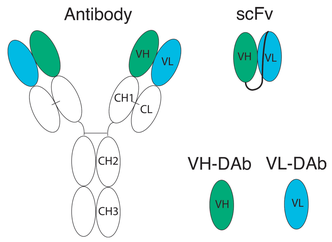
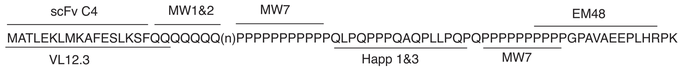
Intrabodies

Intra-cellular antibodies (intrabodies) provide a protein-based approach to neutralizing the pathogenic characteristics of toxic mis-folding of proteins. Intra-bodies are small, recombinant antibody fragments that target antigens intra-cellularly using their Fv variable regions (the areas responsible for antibody specificity). The figure below illustrates the antibody protein and the binding regions that have been further engineered into intrabodies, such as the scFv fragments and the single domain antibodies VL and VH. These constructs retain a high specificity and affinity for target epitopes. This, along with their size and the absence of the potentially inflammatory Fc region, make them powerful tools with which to target a wide range of pathways affected by pathogenic intracellular proteins.



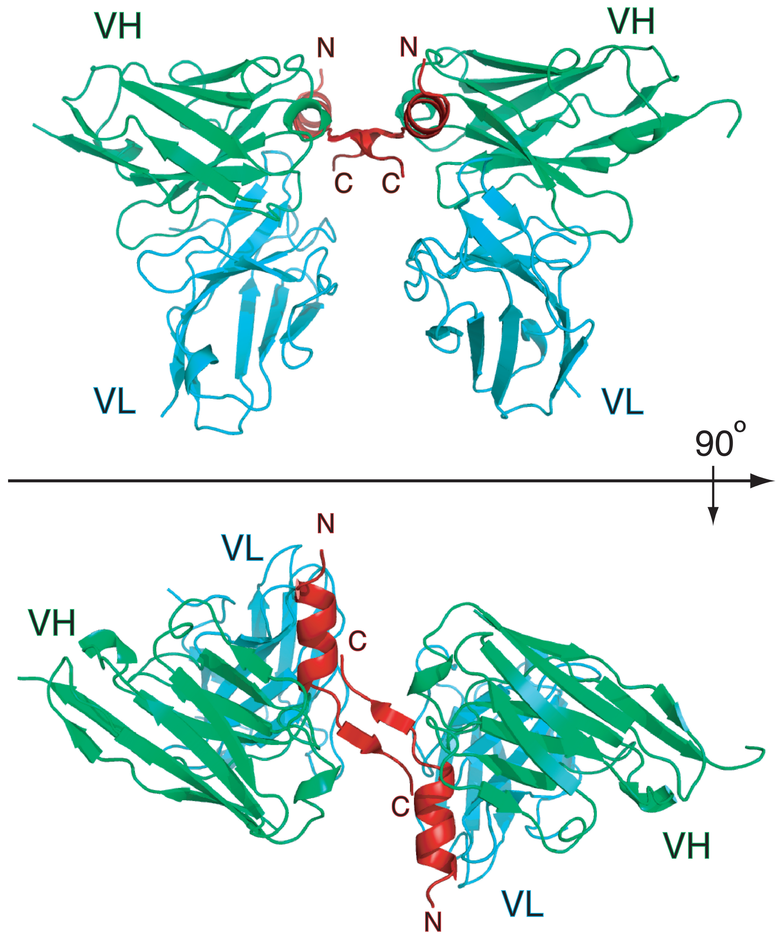
scFvC4 and other anti-HTT intrabodies

In several models of HD, only the expression of HTT exon 1 with an expanded polyQ stretch (mHTT exon 1), is sufficient to cause HD-like pathology. Therefore, a variety of recombinant antibodies against the translation product of HTT exon 1 (mapped on the mHTT sequence figure below) have been derived from phage or yeast surface-display libraries, as well as from hybridoma cell lines. Historically, intrabodies have been directed toward 3 separate regions of HTT exon 1: The N-17 AA, which form a highly conserved amphipathic alpha helix; the polyQ tract, which is the site of HD mutation; and the proline rich region which is located at the C-terminal end of the polyQ.



The first intrabody to successfully counteract *in situ* length dependent mHTT exon 1 aggregation and toxicity was scFvC4. This intrabody was derived from a naïve human spleen scFv phage-display library by panning with a peptide comprising the N-terminal amino acid residues 1–17 of HTT. Critically, scFvC4 preferentially binds to soluble mHTT N-terminal fragments, and has only a weak affinity to endogenous full length HTT.

Crystal structure of the scFvC4 htt $^{(1-17)}$ complex



Conclusions

The crystal structure of scFvC4 in complex with the first 17 amino acids of htt exon1 shows that the first 15 residues are in intimate contact with primarily the VH domain of the intrabody. This differs from another intrabody, VL12.3, which does not bind the first 5 residues of htt, and which also binds AAs 16 into the first part of the polyQ. Intrabodies may therefore differentially affect the kinetics of post-translational modifications of the htt peptide at Thr-3 and Ser-16. These have been implicated in subcellular localization and toxicity. The co-crystal with scFvC4-PEST (See abstract #60), which has stronger anti-HTT effects, could therefore be of particular interest.

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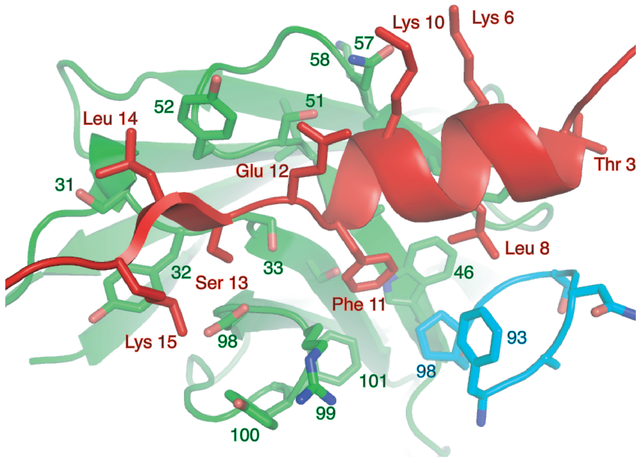
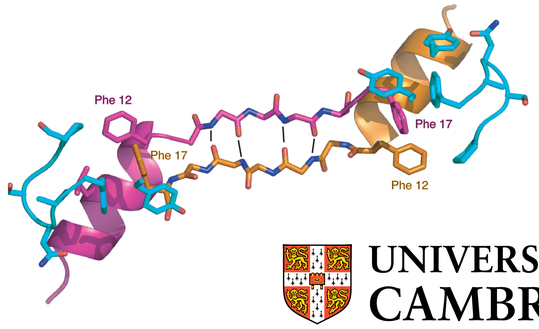
The scFvC4::htt$^{(1-17)}$ interaction

The residues of the scFvC4's VH domain form the main site of interaction with the htt$^{(1-17)}$ peptide. All three CDR loops of the VH domain are directly involved in the interaction with the peptide. By contrast, only six residues of the VL domain interact with the htt peptide; these are all located in the CDR3 loop. The htt peptide adopts a partly helical (residues 3-11) and partly extended (residues 12-17) conformation when bound to the scFv C4.



The htt$^{(1-17)}$ putative dimer interaction

In the crystal structure, the scFvC4::htt $^{(1-17)}$ complexes were observed to be bonded in pairs (central figure). This bond is formed between residues 12 to 16 of both htt$^{(1-17)}$ peptides, which associate to form an anti-parallel β-sheet, involving a strong hydrogen bond network between the main-chain atoms of residues 14-16. In addition, the aromatic side-chain of Phe 17 of each htt$^{(1-17)}$ peptide is buried in a hydrophobic pocket on the adjacent complex. This pocket is formed between the aromatic residues of the VL domain's CDR3 loop, and the side-chain of Phe 12.



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