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Functional Analysis of Trail Protein Crystallographic Data: Selectivity and Apoptosis of Tumor Cells

There are several tumor necrosis factor proteins (TNF) responsible for signaling cell death (TNF-R1, DR3, DR4, DR5, and DR6). The binding of TNF related apoptosis-inducing ligand (TRAIL) to some of these receptors initiates the cell death pathway and causes apoptosis. TRAIL is a homotrimer which binds three like death receptors (Figure 1). This leads to trimerization of the death receptors and signaling for cell death. Currently known are four receptors of TRAIL: DR4, DR5, DcR1, and DcR2. DcR1 and DcR2 do not signal for cell apoptosis (or any other cell death). These receptors can compete for TRAIL with DR4 and DR5 and potentially act as a protection mechanism for target cells. TRAIL is widely expressed in the body, as are DR4 and DR5 receptors. Interestingly, only receptors of tumor cells are sensitive to TRAIL binding.

There are several residues which seem to have important roles in the function of TRAIL-induced apoptosis. Glu 147, Glu 151, Arg 154, and Asp 175 are all conserved in the structure of active DR5 receptors. In particular, Glu 147 appears to make a salt bridge with Arg 149 on TRAIL. When compared to other TNF family proteins, the loop containing Arg 149 on TRAIL is considerably longer (Figure 2). Mutagenesis of a 19 amino acid sequence of TRAIL, including that of Arg 149, to a shorter SSL sequence mimicking other TNF proteins, showed 95% reduction in binding with DR5. This shows the importance of the longer loop on TRAIL to facilitate this interaction.

Substitution with alanine for various surface residues (Gln 205, Val 207, Tyr 216, Glu 236, Tyr 237) also shows a reduction larger than 5 fold in apoptotic activity.

Substitutions at Gln 205, Tyr 216, Glu 236, and Tyr237 also showed a more than 5 fold decrease in binding affinity to DR4, DR5, and DcR2. In addition, substitution of Gln 205 and Tyr 216 yields a reduction of activity by more than 300 fold. Various other alanine mutations show a decrease in binding and apoptosis of 2-5 times. An interesting alanine mutation to note when thinking about possible drugs is that of Arg 158 and Tyr 237. In this case, DcR2 has the largest decrease in binding of the four receptors. Glu155, Arg158, and Tyr216 correspond to residues in other TNF structures that have been shown to be essential for binding. However, residues on other TNF proteins similar to Gln205, Val207, Glu236, and Tyr237 are not functional on these proteins. Interaction of these residues adds to a more tightly binding ligand and could be a contributing factor for the activity of TRAIL.

It was also discovered that TRAIL contains a zinc-binding site containing the Cys230 residues from each monomer (Figure 3). The site forms a distorted tetrahedral geometry with a solvent molecule. This site is otherwise inaccessible to solvent. Mutations of Cys230 to either alanine or serine caused a 20-70-fold reduction in apoptosis. When the metal is stripped from the protein, apoptotic activity decreases by 90-fold and a decrease in receptor binding is observed. Also, in the absence of metal, the protein forms dimers via disulfide bonds.

Two other consequences of non-metalated protein are a reduction in β-sheet content and tryptophan fluorescence compared to native protein. The loss of fluorescence is due to an increase in solvent exposure of Trp231 (one residue to the Zinc binding Cys 230) from a conformational change when the metal is removed. The only other TNF conserve tryptophan is Trp 154, which is buried in the monomeric core. Therefore, it should not be affected by zinc binding. It was also found that zinc depleted protein

melted at about 25°C lower than native ligand suggesting the stability contribution of bound zinc.

Overall, TRAIL does not bind by a few residue interactions, but by many weak interactions. This opens the possibility to engineer mutants which may only suffer small decreases in affinity to apoptotic receptors while significantly effecting binding to decoys.

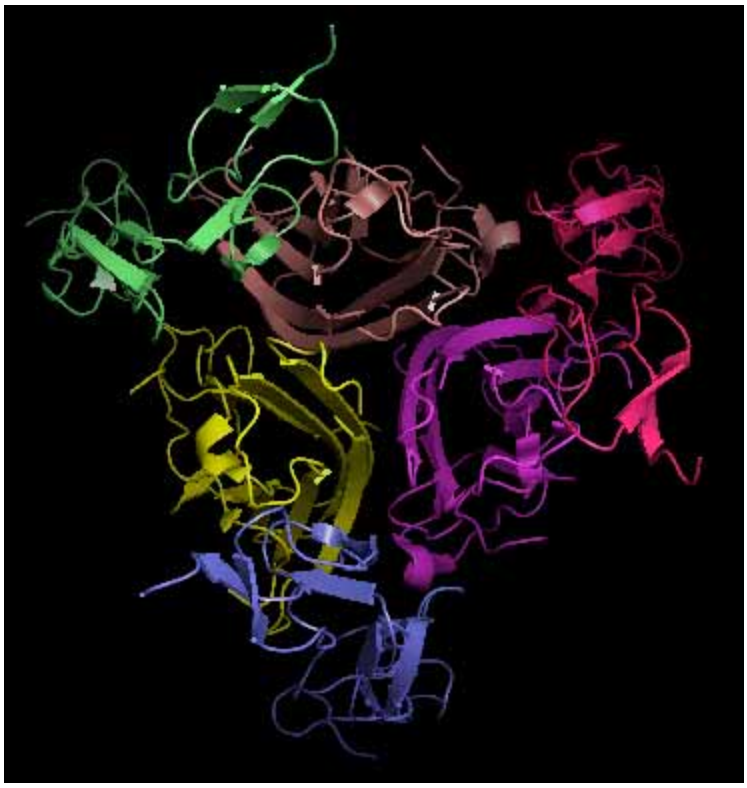

Figure 1: Trail Bound to three DR5 receptors.

Figure 2: Arg 149 residues from each monomer highlighted. Note the close proximity to the bound DR5 receptors.

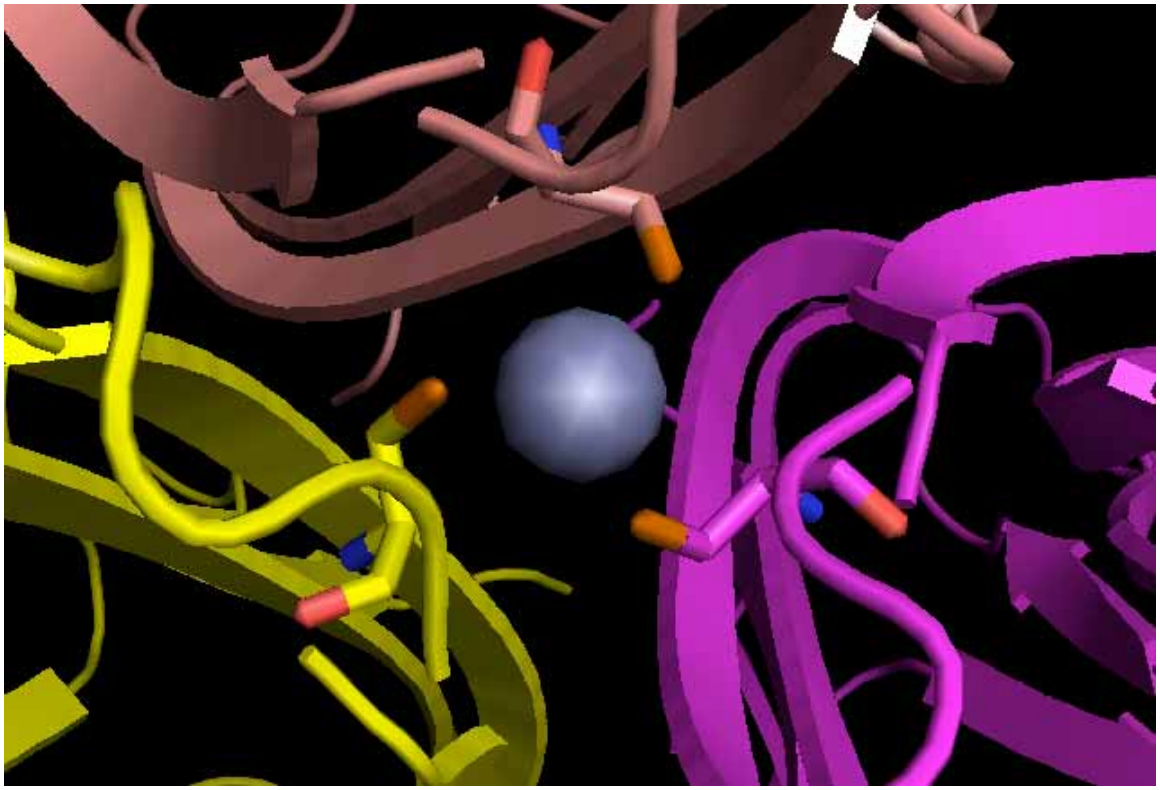

Figure 3: Zinc coordination site at the trimeric interface.

References

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