

Team meeting

Who: Whole Team

Where: Library

Description:

- As we have heard from Morganne we have good news about funding.- The bad news is that Uli's lab is unable to accommodate us as there isn't enough space especially now that they have to accommodate some people from Bimolecular labs due to the fire.

- Gram positive bacteria express the isopeptide bonds we are interested in in their pili - proteins with a lot of small domains covalently bonded to their cell's plasma membranes.- Their stalk domains contain isopeptide bonds between lysine and asparagine or aspartic acid residues (three types of amino acids which are small units that make up proteins)- All the membrane proteins in these bacteria have immunoglobulin like domains (good for us as we will be modelling immunoglobulin domains) as they have 'beta sandwich' fold- The isopeptide cross link can form either between two terminal strands - Uli thinks that might be easiest or anywhere in the middle too- although the insides of the proteins are hydrophobic, the protein doesn't unfold due to polar residues inside due to stabilisation from the covalent isopeptide bond- The bonds form autocatalytically - no enzyme activity has been detected- Only about 20 bonds have been crystallised but 1000ds are predicted

FbaB

- the hydrophobic environment around isopeptide bond is conserved (amino acids surrounding the bond are non-polar)- NMR studies show that isopeptide bonds are found in slightly different environments which is good - we can hope to create one where we want it to be even if the environment is slightly different- The isopeptide bond resists denaturing in acidic pH or high temperatures or hydrolytic digestion- There might be more families of proteins with this sort of bond but our body relies on turning proteins over so proteins that cant be hydrolysed would be a problem, not a problem in bacteria

- > theoretically introducing the bond should be possible in any protein that has a hydrophobic centre and we change three residues (distance between the three residues must be correct, it is conserved)

Antibodies

- > There is big interest in developing cheaper smaller systems to produce antibodies such as E. Coli by its tricky especially the sulfide bonds in antibodies propose a problemWe are able to produce single antibody domains relatively easily> Therefore a goal would be to get rid of sulfide bonds and replace them with autocatalytic isopeptide bonds then bacteria would be able to produce antibodies that would be just as stable> Antibodies with isopeptide bonds have a huge range of potential applications - for example these sort of antibodies could be used in biosensors in extreme conditions (as they would be able to withstand

them)

Note: protein engineering is very difficult as we don't fully understand all the laws that govern proteins and a single change in one residue can completely change something

Isopeptidisation

Idea : replace two Valines with Lysine and Asn and replace two cysteines with Ala and Asp (shown on slides)- Uli tried three other combos in this particular domain but had no success

Note: taking a domain out changes the environment and some bonds may disappear that have been present in the whole antibody

To be successful

> use computational approach by using modelling software such as Rosetta>

Another potential modelling software is Foldit> These programs don't know about isopeptide bonds however their developers may be willing to help us - Uli can give us some names of people to get in touch with> Rational design: just look at the structure and try to guess which residues we can replace - unlikely to yield results

Combinatorial approach - ('intellectually insulting') - very difficult, need to create a random library of the protein with 3 residues changed; often yields voids that need to be filled by tweaking surrounding hydrophobic residues - unsure how to create a library with just these residues randomly changed> Could look at evolution of the bond and learn how it emerged and what conditions favour it
> could fuse GFP (a fluorescent protein) to our protein, if our protein folds correctly, GFP will also fold correctly and emitted light will be the indicator of success = GFP based screening

- creating a shorter version of lysine would be useful for bond formation as lysine needs to be tacked away on the inside of the protein- Some other residues can also participate in the isopeptide bond but the bond forms slower- Another approach - try to catabolise the three residues with H-bonds first and then proceed onto isopeptide bonds but may be too time consuming- Could replace cysteine with Ser and Asp = could form a polar clamp to get rid of sulfide bond which is also resistant to high temperatures

- Antibody people are very interested in this project - could potentially get antibody plasmids from them. Otherwise they are not that expensive