The design of proteins that switch folds upon stimulation

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Structural changes in proteins

- Energy of a fold switch
- Stimulation
- Oligomerization
- Ligand-binding
- Structural rearrangement

- Double-finned energy landscape, two (or more) energy minima
- Energy wells of a protein switch are not deep.
- Large stability values (< -5 kcal/mol) prevent switching between alternative states.

- Fold switch
- Two alternative folds exist in equilibrium, each one being stabilized in different conditions.
  - Relative populations of both folds vary depending on the medium conditions.
  - Switching stimulus will reversibly shift the equilibrium towards the alternative fold.

- Fold switch
- Fold A
- Fold B

- Equilibrium of a fold switch
- Defined by the secondary structure and functional properties of the protein.

Methods

Rational design
- Merge the sequence patterns of two different known structures.
- Rationally designed sequences contain structure determinants that allow for one or more single fold.

Computational design
- Computational algorithms calculate the energy of a large number of sequences to adopt the targeted folds.
  - Fold determinants can be fixed in the sequence.
  - Different conditions can be computationally tested for each structure in order to find the sequence that can adopt two low energy folds each one in a different pH context.

Phage display screening
- Sequence: Two starting sequences (A and B).
- Mutagenesis of sequence A towards homology with sequence B. The goal is to achieve the highest homology while maintaining the original A fold.
- Screening: Phage display. Column with immobilized antigen.

Ig-based screening
- Sequences: Through directed evolution and inserted into the scFv linker of an antibody.
- Screening: Column with immobilized antigen. Two-step selection.

Lymphotactin
- Stimuli: changes in temperature and salt conditions.
- The hydrophilic core and tertiary interactions are restructured.
- Quaternary contacts and binding affinities are not maintained.
- Secondary structure is rearranged into new motives that involve a different set of residues.

Designed switching proteins

G,G2
- Two highly homologous sequences derived from G1 and G2 that maintain their original folds.
- Increasing degree of homology, as much as 98% (only one different residue) but each sequence has its original, different fold.

Phages
- The central α-helix becomes longer, involving more residues.
- Rearrangement of α-helices into β-sheets.

Fold switch
- N and C termini become structured, β-sheet fold.

Applications

Moonlight proteins
- Two functions encoded in one sequence.
- Each fold allows one single function, the other one remains latent.

G,G2 encoded active sites for HSA and IgG binding:
- 3α fold → binds HSA, does not bind IgG.
- αβ fold → binds IgG, does not bind HSA.

Drug delivery
- Stimulus-responsive proteins are good tools for specific targeting.

Homo-oligomers
- Monomers change their structure and properties, and may disrupt the tripeptide Lys-Phe-Gly has been used to successfully target tumour cells.

References