The design of proteins that switch folds upon stimulation

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June 2017

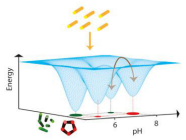
Structural changes in proteins

Folding-unfolding · Oligomerization · Ligand-binding · Structural rearrangement

Energy of a fold switch

Double-funneled energy landscape, two (or more) energy minimums.

Energy wells of a protein switch are not deep. Large stability values (< -5 kcal/mol) prevent switching between alternative states.



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Stimulus

- Δ T
- Δ pH
- Δ [protein]
- Ligand addition

Fold switch

Dramatic change of the whole protein fold – involving the secondary structure – due to alterations (stimuli) that destabilize one fold and stabilize the alternative one.

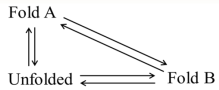
Folding of switching sequences is determined by non-local interactions.

Designed · **Natural**

Equilibrium of a fold switch

Two alternative folds coexist 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.



The transition between the two folds is possible without populating the unfolded state

Methods

Rational design

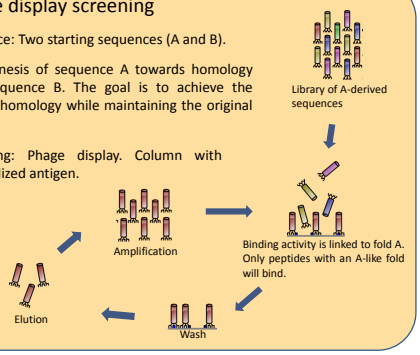
Merge the sequence patterns of two different known structures. Rationally designed sequences contain structure determinants that allow for more than one single fold.

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.

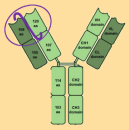


Computational design

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.

Ig-based screening



Sequences: obtained through **directed evolution** and inserted into the **scFv linker** of an antibody.

Screening: Column with immobilized antigen. Two-step selection.

1) **Negative:** Peptides whose conformation allows the binding of the antibody to fluorescein ⟶ **binding**

Stimulus ⟶ **conformational change**

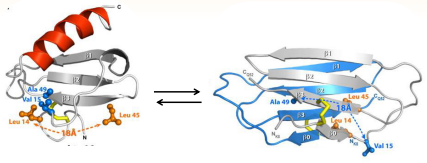
2) **Positive:** Peptides that have changed their fold will no longer allow the interaction ⟶ **elution**

Naturally-occurring fold switches

Lymphotactin

Stimuli: changes in temperature and salt conditions.

- The hydrophobic 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



Ltn-10
monomeric chemokine fold
3 β-strands and one α-helix
Binds to XCR1

Ltn-40
dimer, β-sandwich
4 β-strands and no helix
Binds to glycosaminoglycans

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Designed switching proteins

G_A/G_B

Two highly homologous sequences derived from G_A and G_B that maintain their original folds.

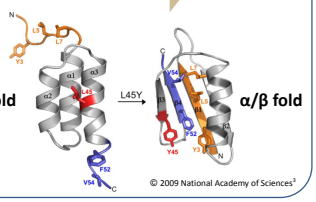
Increasing degree of homology, as much as 98% (only one different residue) but each sequence has its original, different fold.

3α ⟶ 4β + α

The central α-helix becomes longer, involving more residues

Rearrangement of α-helixes into β-sheets

N and C termini become structured, β-sheet fold



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Arc

Residues 9 to 14 of the Arc repressor protein:

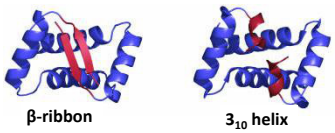
Q F N L R W
antiparallel β-ribbon

- Mutations N11L and L12N:

Q F L N R W
amphipathic 3_{10} helix

- Mutation N11L:

Q F L L R W
3_{10} helix and **β-ribbon** coexist
population of mixed structures



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ZiCo

Combination of two sequence patterns:

- Zinc finger (Hys_2Hys_2)
- Coiled coil (heptad repeat alternating polar and hydrophobic residues)

```
phpphpppphpppphpphpppphpppph  CC
YxCxxCxxxFxxxxxLxxHxxxxxHxx   ZF
YTHATHRKAFAKTART.FRHTRAT.FHAA ZiCo
```

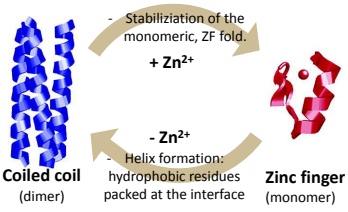
Chimeric sequence has the determinants of both folds

- Zn^{2+} binds to the four coordinating residues.
- Stabiliziation of the monomeric, ZF fold.

+ Zn^{2+}

- Zn^{2+}

- Helix formation: hydrophobic residues packed at the interface
- dimerization



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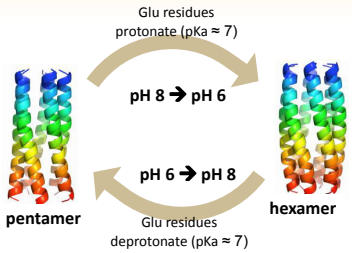
pHios

Oligomerization switch of high-order coiled coils.

Switch trigger: pH variation

- pH=8 ⟶ pentamer of parallel α-helix
- pH=6 ⟶ hexamer of antiparallel α-helix (trimer of antiparallel dimers)



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Applications

Moonlight proteins

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

G_A/G_B encoded active sites for HSA and IgG binding:

3α fold ⟶ - binds HSA
- does not bind IgG

α/β fold ⟶ - binds IgG
- does not bind HSA

Drug delivery

Stimulus-responsive proteins are good tools for specific targeting

Homo-oligomers —stimulus→ Monomers change their structure ⟶ Oligomers change their properties and may disrupt

The tripeptide Lys-Phe-Gly has been used to successfully target tumour cells *in vivo*.

Concentration-sensitive: Random coil self-assembly and vesicle formation —0.5 mg/mL → 5mg/mL→ β-sheet nanotube formation

pH-sensitive: Self-assembly, loaded structures —pH 7.4 → pH 6→ Disruption, drug release

Conclusions

- Switching proteins have more than one stable structure. They respond to small perturbations of the environment by undergoing a structural transition to the alternative fold.
- The development of new methods has made possible the design and screening of proteins with structural duality. Many switching proteins have been designed to date.
- Proteins with such features can be designed for concrete applications. Some have already been developed.
- Further development of design methods (mostly computational) will allow the creation of new proteins and their use in many applications.

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