Computational Design of Protein-Protein Interactions

Initiating Apoptosis in Cancer Cells

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What’s to come...

• Computational protein design methods

• Using a purpose-built protein scaffold: Design of a cancer-specific toxin

• Intracellular delivery of a designer toxin: a new generation of antibody-drug conjugates?
ROSETTA:
A computable energy function for proteins
Protein structure prediction:
Finding the lowest energy on a conformational landscape
Protein design:
Finding the lowest energy on a sequence landscape
Viruses hijack apoptosis signaling: Epstein-Barr viral BHRF1 sequesters Bim
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Designed helical bundles to bind BHRF1: Guided *ab initio* protein design

(i) Bim-BH3
Guide topology (3LHP_S)
BHRF1

A target topology is chosen for building a new protein

(ii) Bim-BH3 (folding nucleus)
Designed Protein

Guide for distance constraints

The folding nucleus is extended by fragment assembly

(iii) Design is docked to BHRF1 and surrounding interface is designed

(iv) Process is repeated to generate many structural homologues
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- **Bim-BH3**
  - App. $K_D$ 12 ± 4 nM

- **BbpG2.D**
  - App. $K_D$ 60 ± 8 nM

- **BbpG3.D**
  - App. $K_D$ 70 ± 20 nM

- **BbpG4.D**
  - App. $K_D$ 81 ± 8 nM

- **BbpD04**
  - App. $K_D$ 58 ± 3 nM

- **BbpD07**
  - App. $K_D$ 60 ± 10 nM
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Guided *ab initio* protein design

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Sequence-fitness landscape:
Importance of fold and interface
Evolved variants have increased stability:
High affinity requires a rigid structure
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BINDI binds BHRF1 with high affinity and specificity
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Crystal structure of BHRF1•BINDI at 2.05 Å:
High accuracy of the computational design
BINDI is a “de novo” design:
New sequence and structure compared to the guiding scaffold
BINDI binds an expanded contact surface:
Molecular basis for specificity

- Total buried SASA: 2,738 Å²
- BHRF1: 1,312 Å²
- BINDI: 1,426 Å² (BH3 motif: 587 Å²)

- Total buried SASA: 2,004 Å²
- BHRF1: 908 Å²
- Bim-BH3: 1,096 Å²
Mitochondrial cytochrome c release:
BINDI preferentially initiates apoptosis in EBV+ cells
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Treating an EBV+ B lymphoma xenograft in mice:
An antibody-copolymer delivery system for BINDI
Treating an EBV+ B lymphoma xenograft in mice: A diblock copolymer for intracellular protein delivery
Treating an EBV+ B lymphoma xenograft in mice: Intracellular delivery of BINDI slows tumor growth
Treating an EBV+ B lymphoma xenograft in mice:
Intracellular delivery of BINDI prolongs survival
Redesign of BINDI: Specific targeting of other BCL2 proteins

BLI kinetics analysis: Mcl-1 interaction with MINDI
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