An In-Depth Look into Affibodies

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Review

Affibody molecules: Engineered proteins for therapeutic, diagnostic and biotechnological applications

J. Löfblom [a], J. Feldwisch [b,c], V. Tolmachev [b], J. Carlsson [b], S. Ståhl [a,*], F.Y. Frejd [b,c]

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Structural basis for high-affinity HER2 receptor binding by an engineered protein

Charles Eigenbrot[a,b,1], Mark Ultsch[a], Anatoly Dubnovitsky[c], Lars Abrahmsén[d,1], and Torleif Härd[c,1]

[a]Department of Structural Biology and [b]Department of Antibody Engineering, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080; [c]Department of Molecular Biology, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, SE-751 24 Uppsala, Sweden; and [d]Affibody AB, Lindhagensgatan 133, SE-112 51 Stockholm, Sweden

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What are Affibodies?

- Staphylococcal protein A derivations
- Three-helical bundle structure
- Z-domain creation by mutations
  - 58 amino acids long
  - Central binding sequence includes 13aa

http://www.protein.pl/?name=repetitive_scaffolds

Löfblom, J. et al, 2010

The differences to Antibodies

Antibody	Affibody
Large	Small
High Abundance	Low Abundance
Disulphide Bonds	No Disulphide Bonds
Lower Heat Stability	Higher Heat Stability
Lower Affinity/Specificity	Higher Affinity/Specificity
Lower Chemical Stability	Higher Chemical Stability

Löfblom, J. et al, 2010

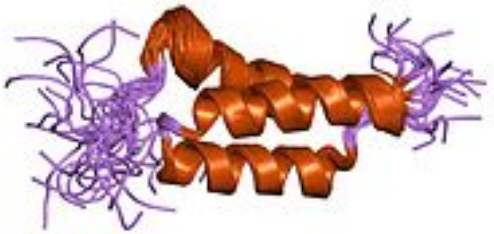
Affibody Synthesis Techniques

Usage of synthetic combinatorial libraries

Phage display technology

Microbial display-based systems

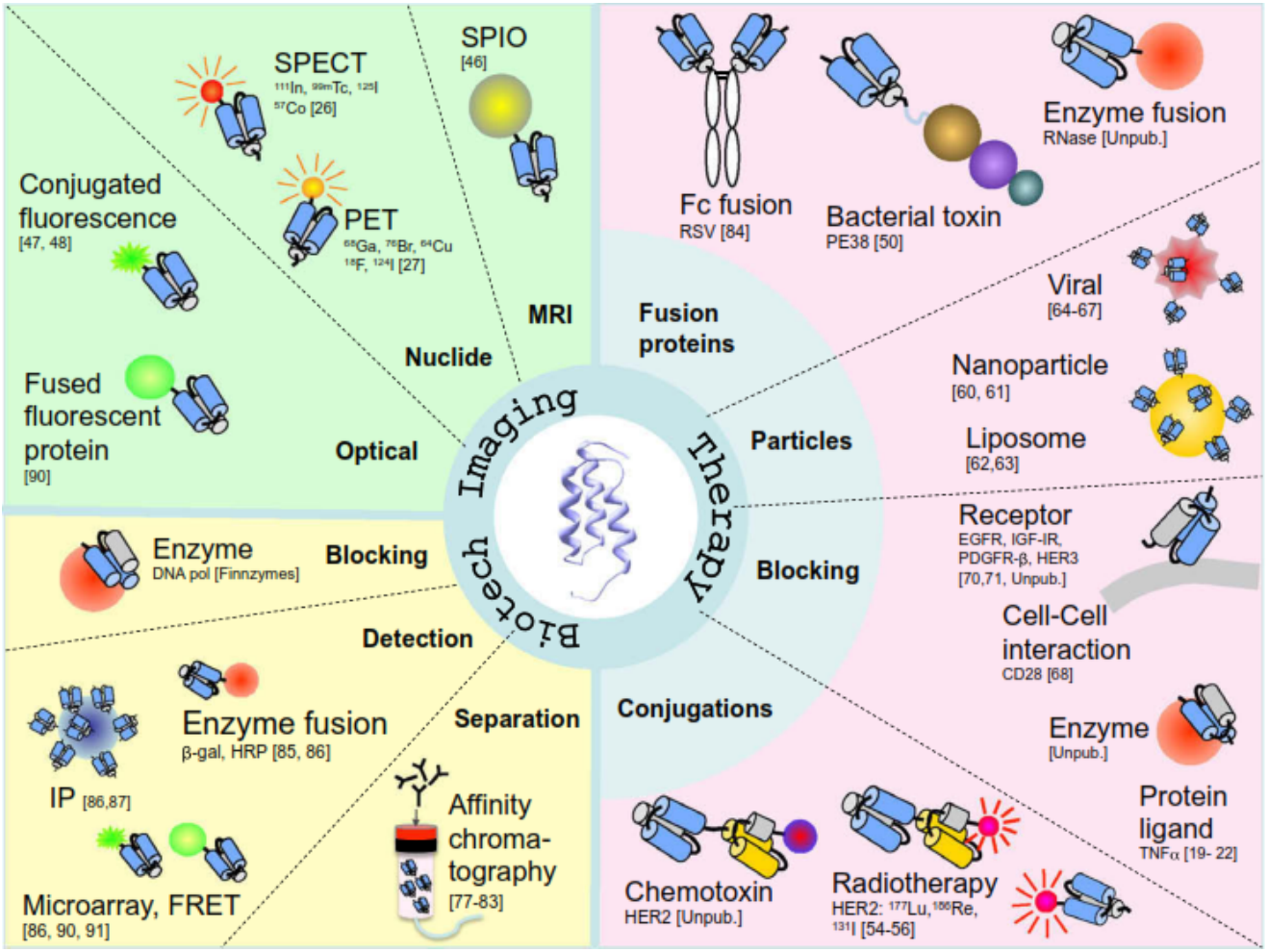
Applying microbeads



http://upload.wikimedia.org/wikipedia/commons/thumb/c/c9/PDB_1ss1_EBI.jpg/220px-PDB_1ss1_EBI.jpg

Löfblom, J. et al, 2010

Affibody Applications



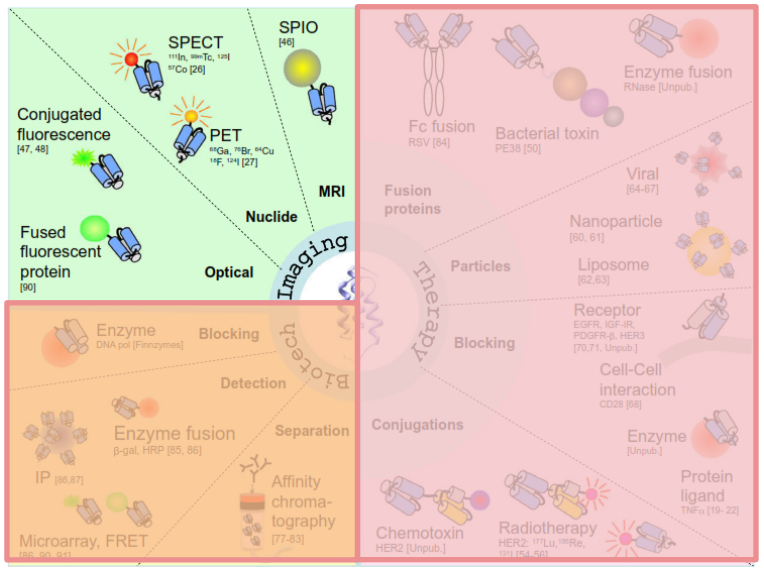
Löfblom, J. et al, 2010

Affibody Applications - Imaging

Most promising tracers

Site-specific radiolabeling

Help target tumors during MRI



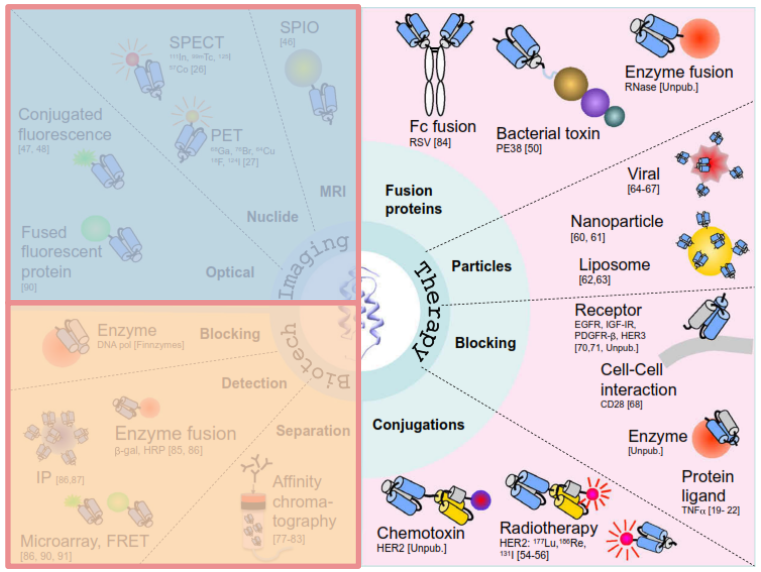
Löfblom, J. et al, 2010

Affibody Applications - Therapy

Targeting payloads

- Immunotoxins
- Radionuclides

Affibody bound to albumin increases therapy treatments



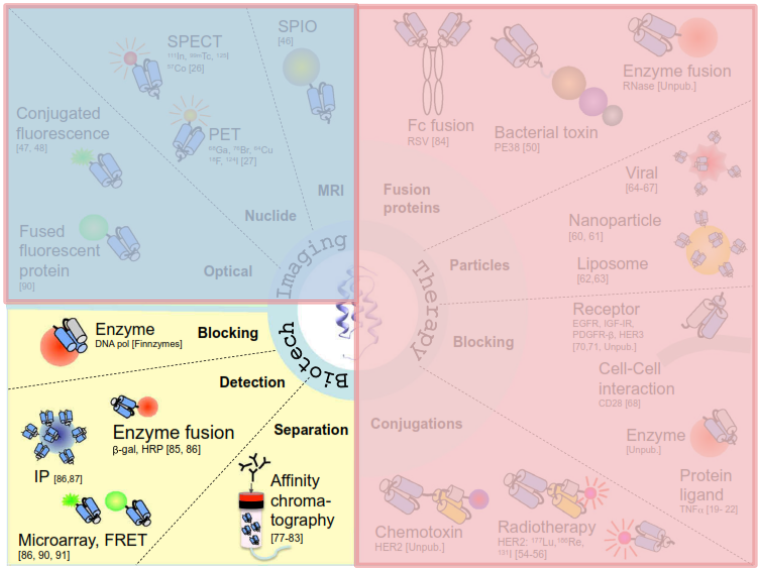
Affibody Applications - Biotechnology

Affibody chromatography

Immunoprecipitation

Fluorescence-based assays

Biosensors in FRET



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HER2 as a target for cancer therapy

HER2 (human epidermal growth factor receptor 2) as focus for SPACE-P

Orphan receptor

Multiple binding molecules

- mAb
  - Trastuzumab & Pertuzumab
- **$Z_{HER2:342}$ (ZHER2)**

Eigenbrot, C. et al, 2010

High affinity of HER2 to ZHER2

Affinity maturation

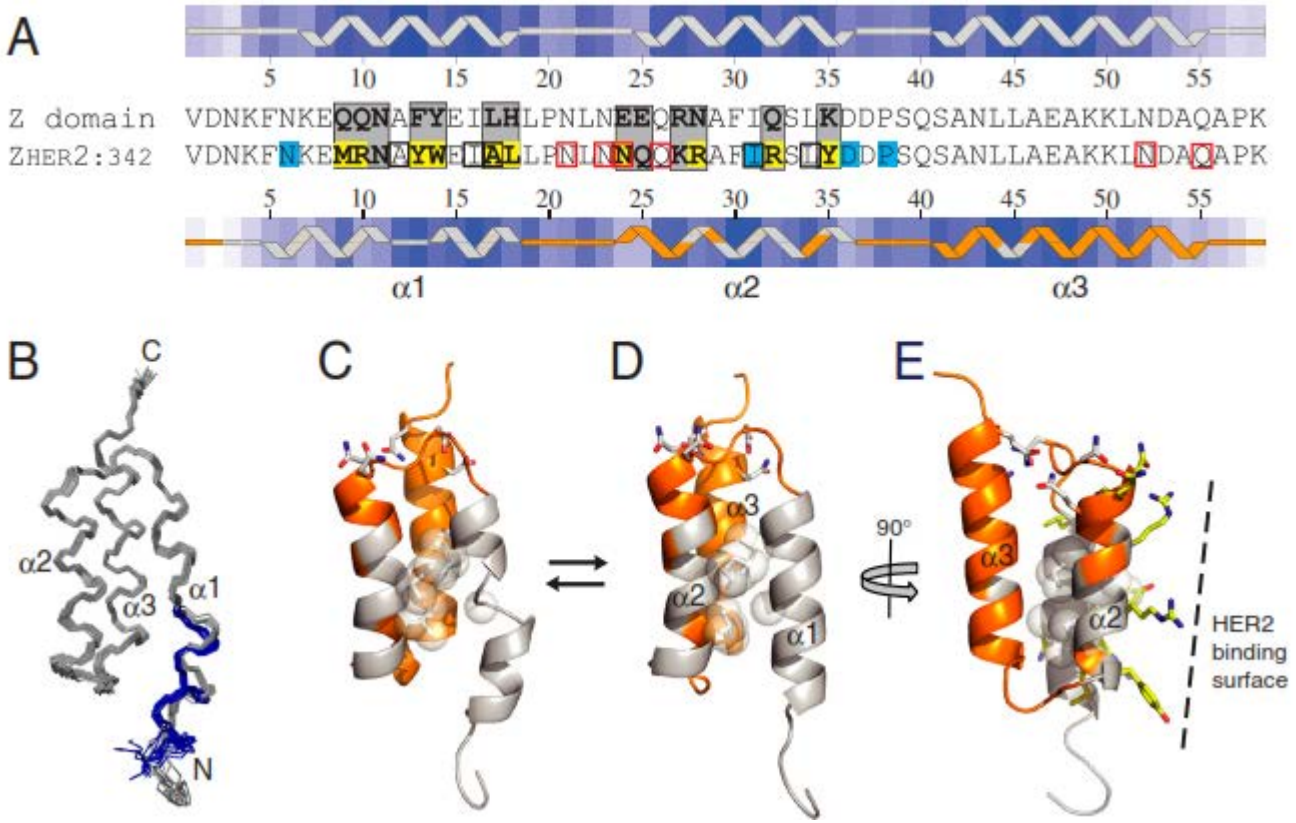
- Cycles of Protein Engineering
- K_D = 22pM

No competitive binding with other molecules

Acts as a tracer through molecular imaging

Eigenbrot, C. et al, 2010

ZHER2 sequence structure and dynamics



Eigenbrot, C. et al, 2010

Concluding Remarks

The HER2-binding affibody is thermodynamically stable and has a significantly high affinity towards HER2.

Two conformations due to helix 1 folding differences

Conformational dynamics may still increase affinity

Non-competitiveness has a significant advantage

Thank
you very
much