Malé vazebné proteiny: příslib pro nové biosenzory a terapeutika nové generace

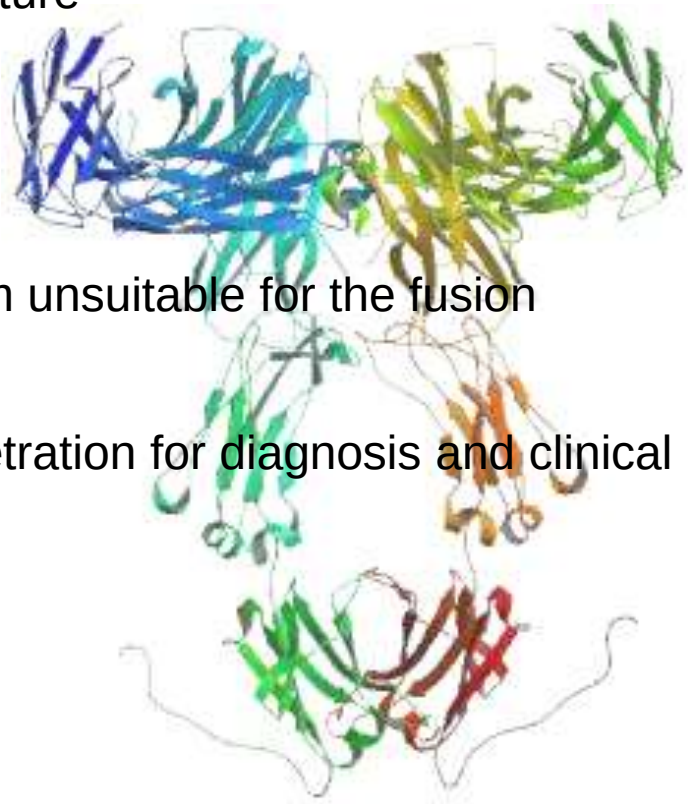
Laboratoř inženýrství vazebných proteinů
Biotechnologický ústav AV ČR

Petr Malý

Ligands or antibodies?

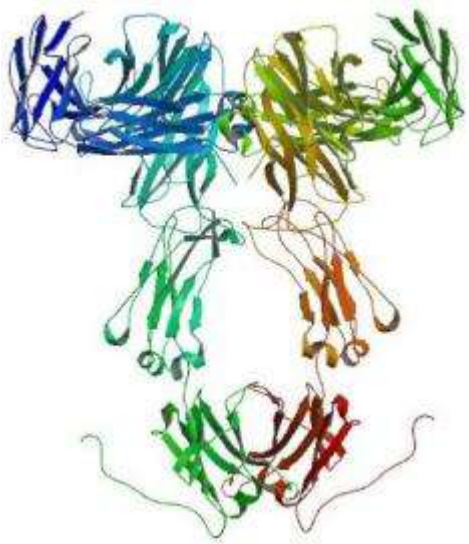
Antibodies are great tools, **BUT**:

- Large, multidomain assemblages with disulphide bonds and glycosylation
- Comparatively difficult and expensive to manufacture
- Sensitive to reducing intracellular environment
- Uncertain stability influences their shelf-life
- Complicated architecture of the gene makes them unsuitable for the fusion partners for biological research
- The large size of antibodies limits the tissue penetration for diagnosis and clinical therapy.



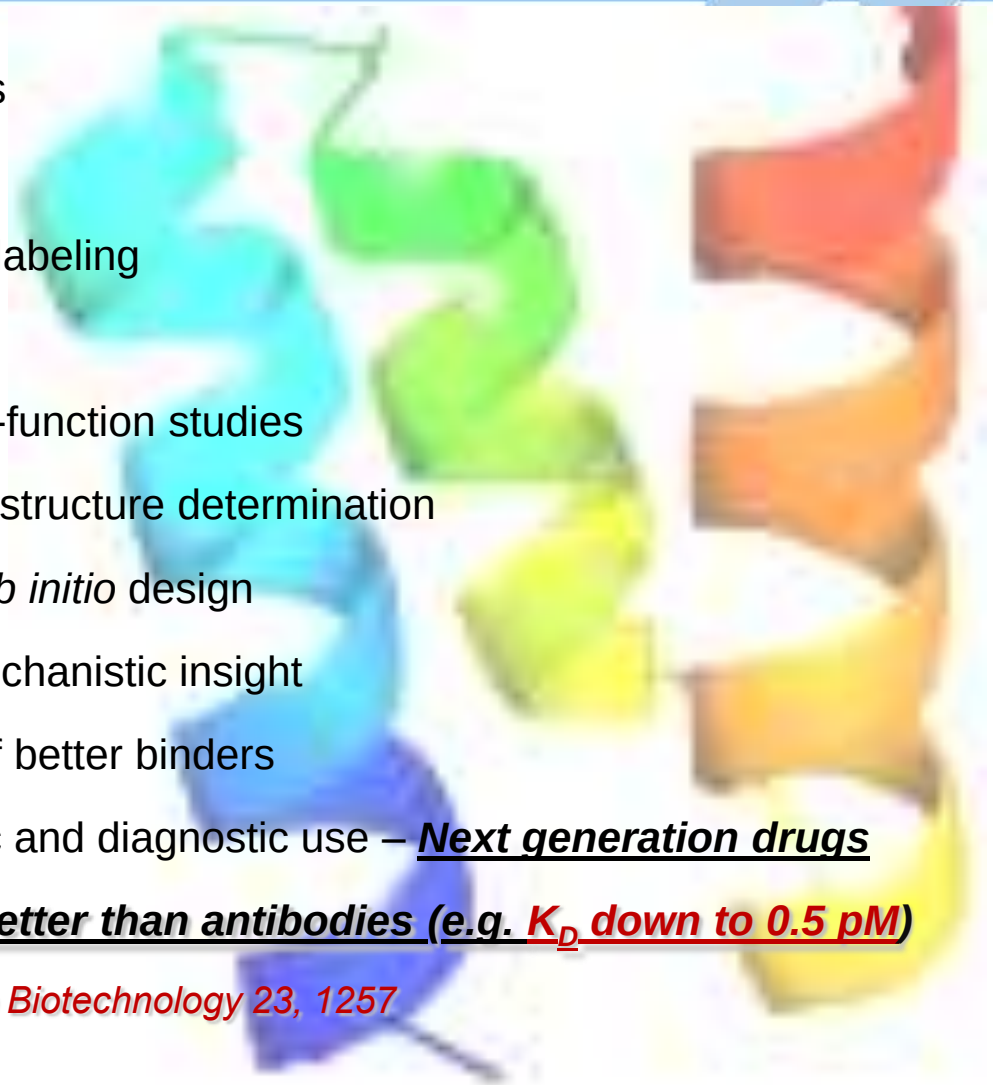
Binding proteins

	Antibodies	Receptor proteins	Small scaffold
Affinity	High	high	Can be high
Production	Not cheap	Problematic	Straightforward
Regeneration Stability	Problematic	Average	Excellent



IFNgRa

why recombinant ligands

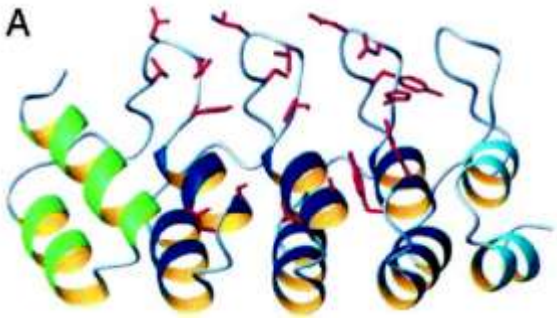


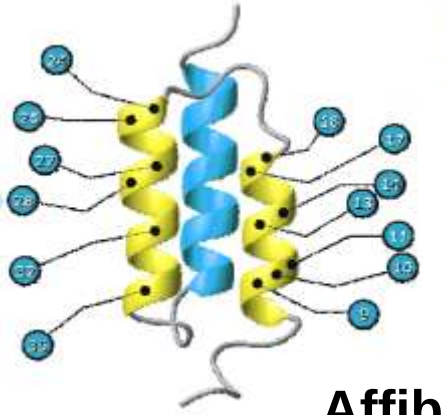
- Small, stable, robust and soluble proteins
- High thermal and hydrodynamic stability
- no disulphide bonds - free cysteines for labeling
- Bacterial expression *en masse*
- Amenable to crystallization and structure-function studies
- Amenable to protein NMR spectroscopic structure determination
- Amenable to rational improvement and *ab initio* design
- High potential of gaining fundamental mechanistic insight
- Amenable to high-throughput selection of better binders
- Extremely promising future in therapeutic and diagnostic use – ***Next generation drugs***
- Excellent research use –***can be doing better than antibodies (e.g. K_D down to 0.5 pM)***

Binz HK, Amstutz P, Plückthuhn A (2005) Nature Biotechnology 23, 1257

Examples of scaffolds

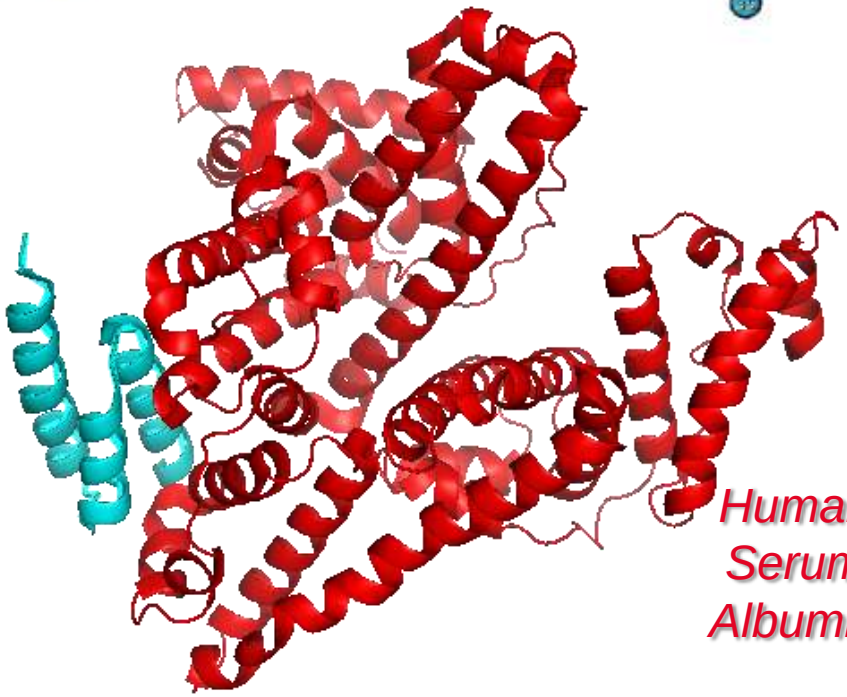
Anticalin





DARPin

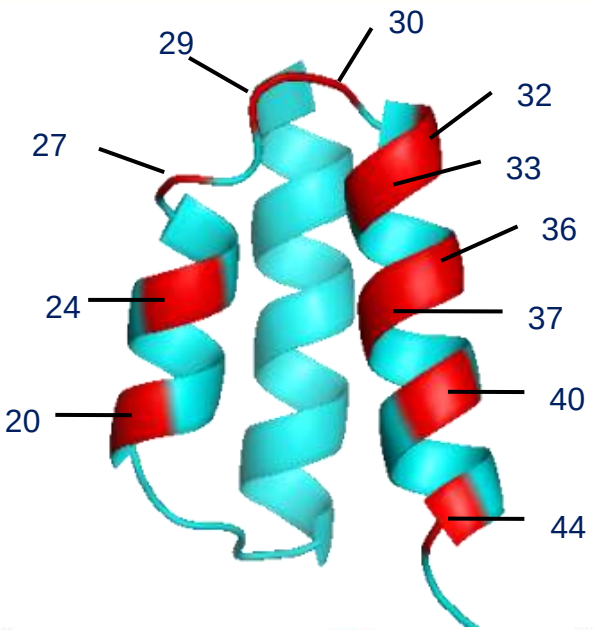
Affibody



ABD Albumin Binding Domain

Human Serum Albumin

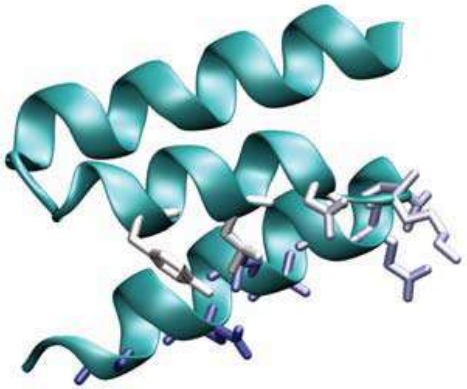
ABD library design



Randomization of selected positions

Library size of a high complexity

a)



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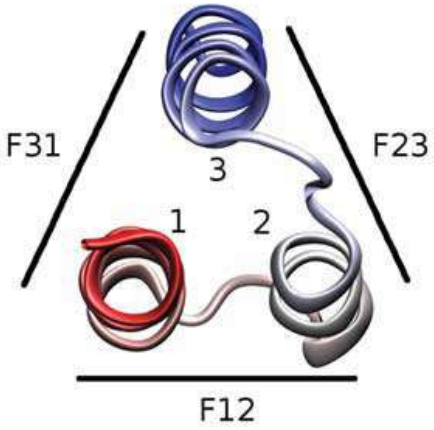
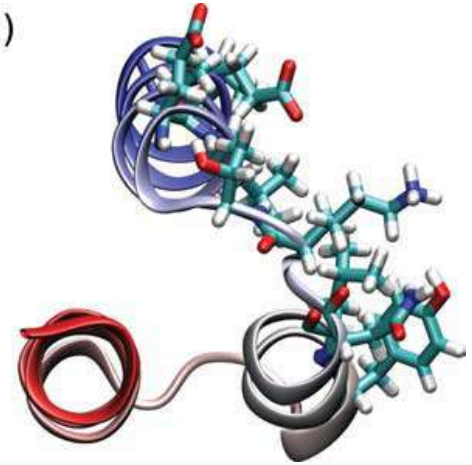
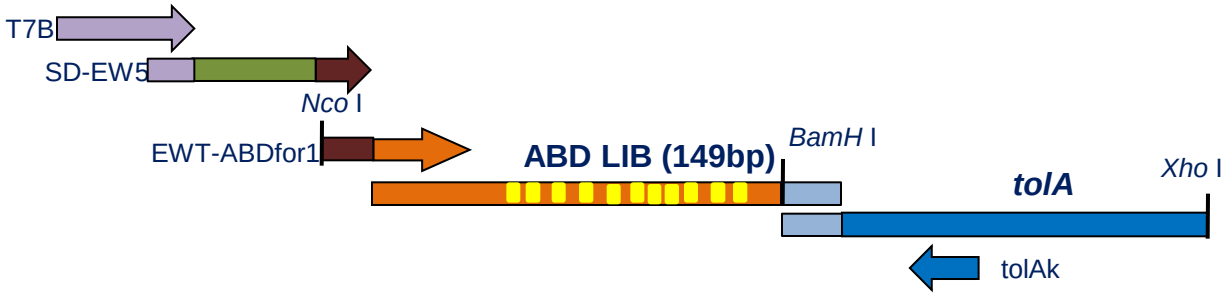


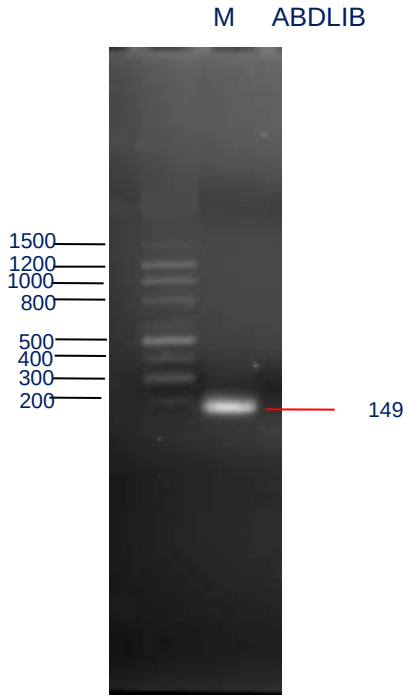
c)



Construct design and preparation

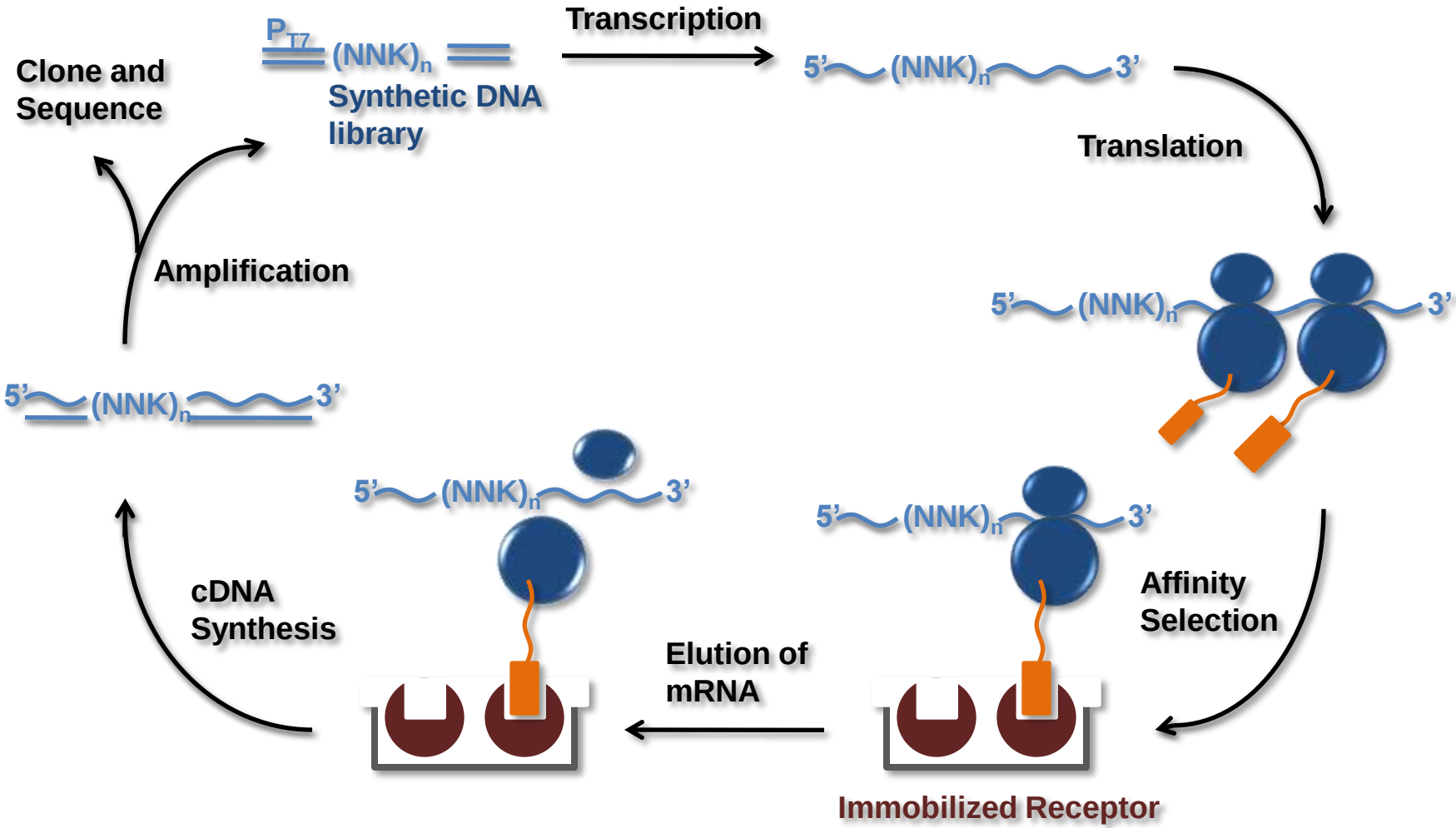
Library assembly using PCR



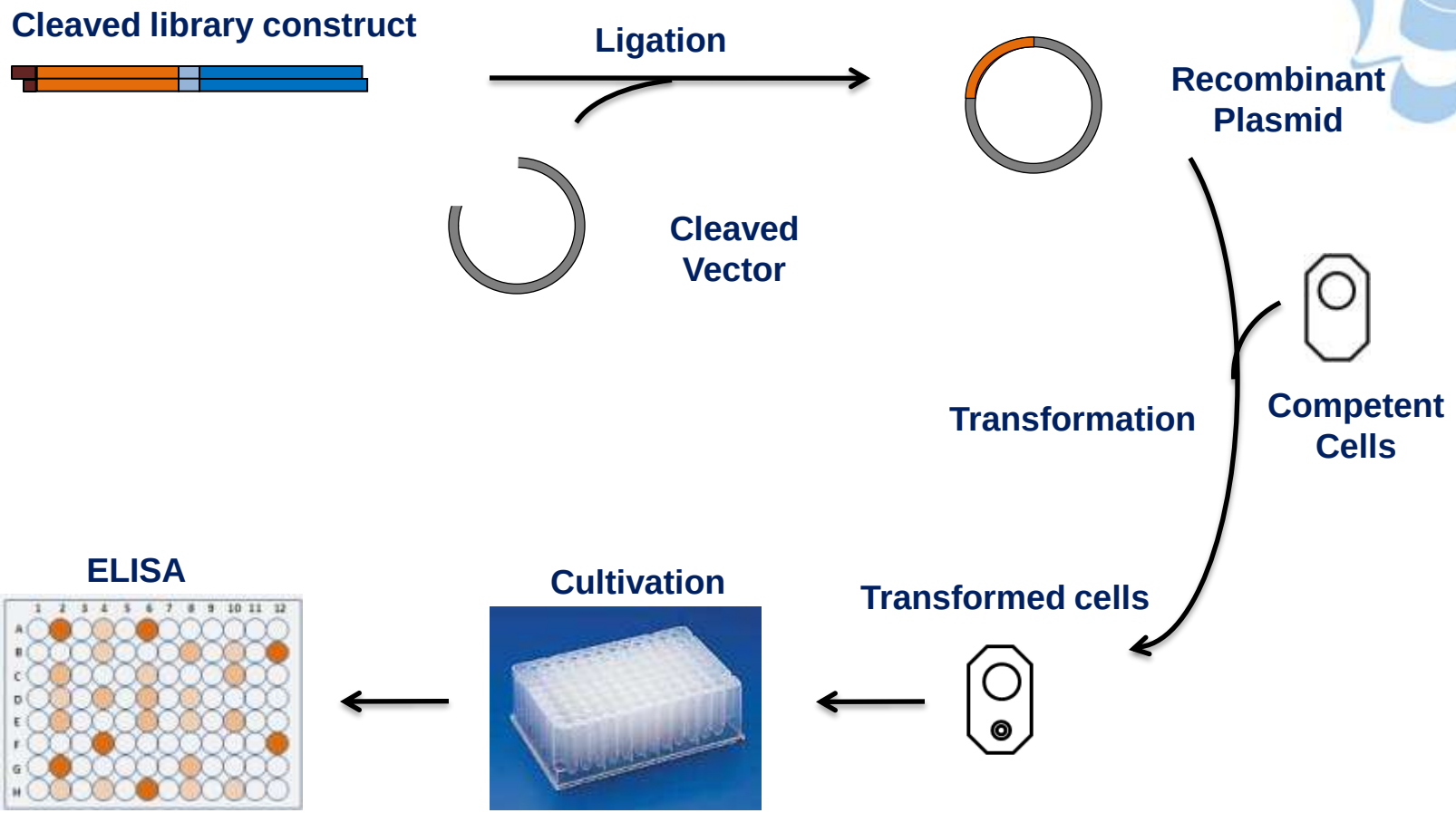


10^{16} codon variants

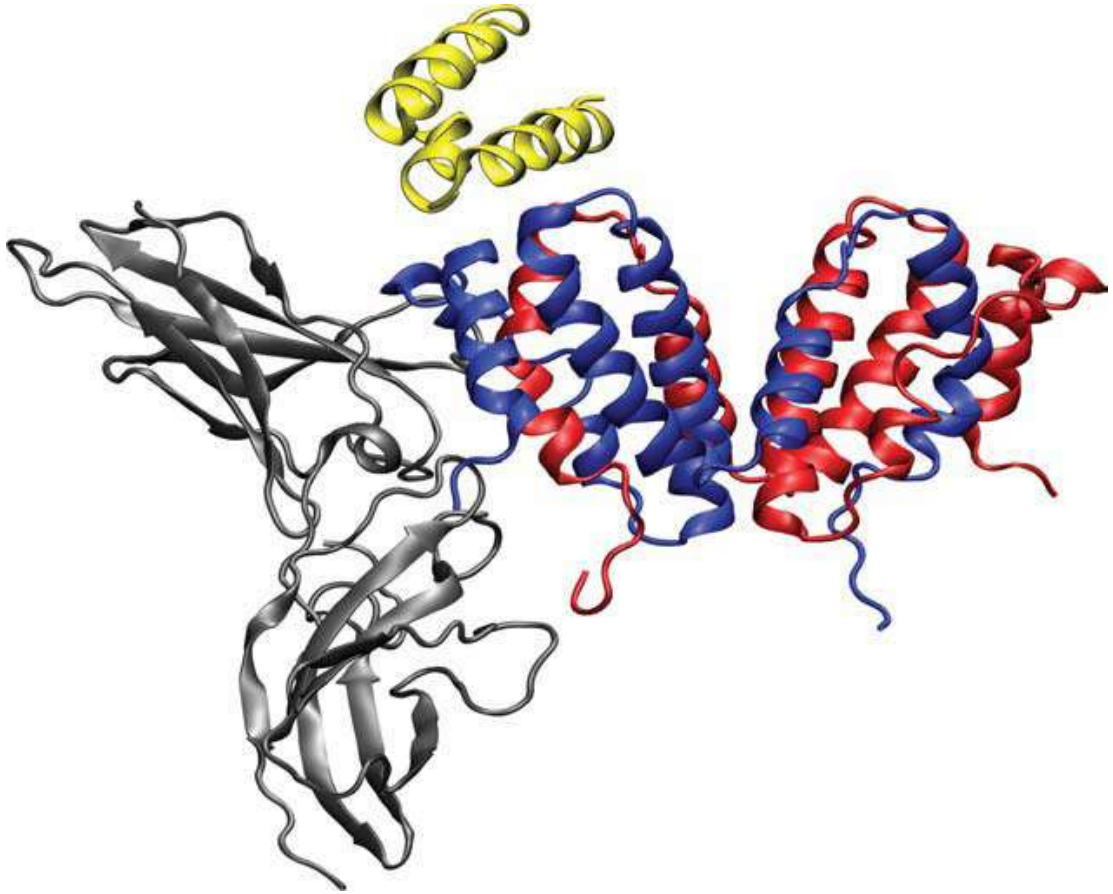
Ribosome display selection



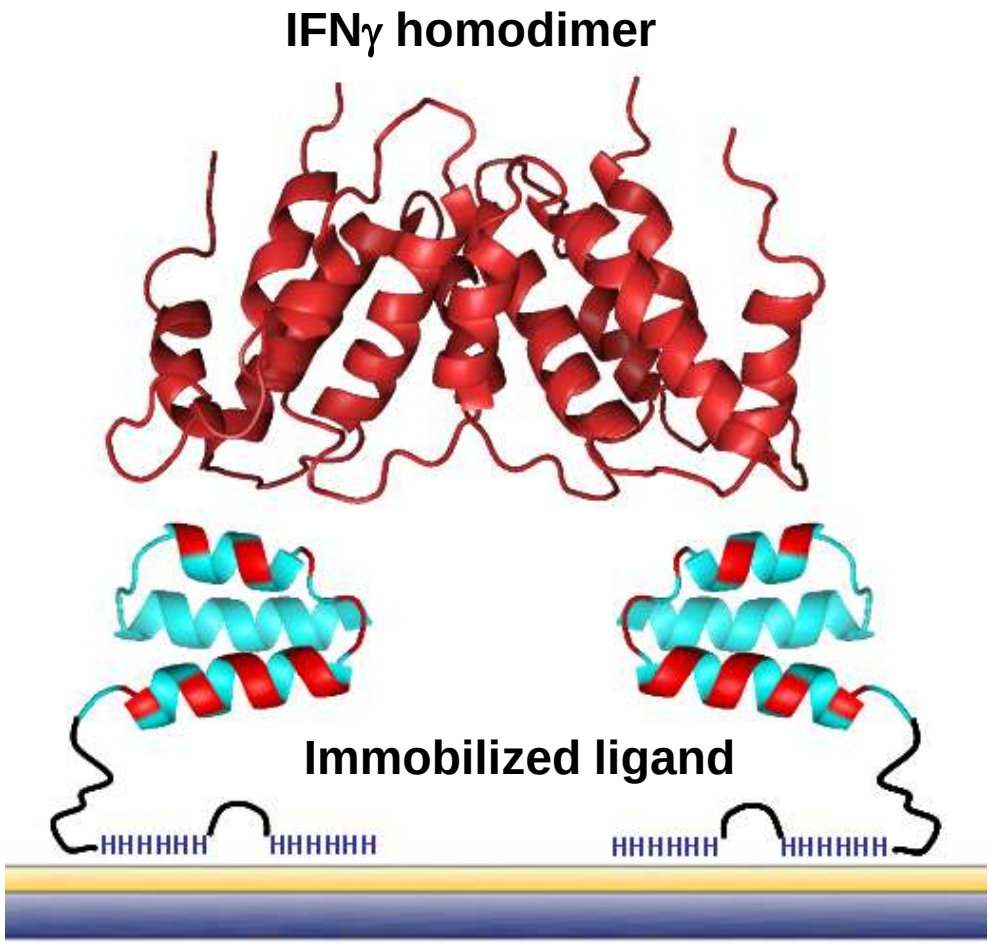
Screening of positive clones

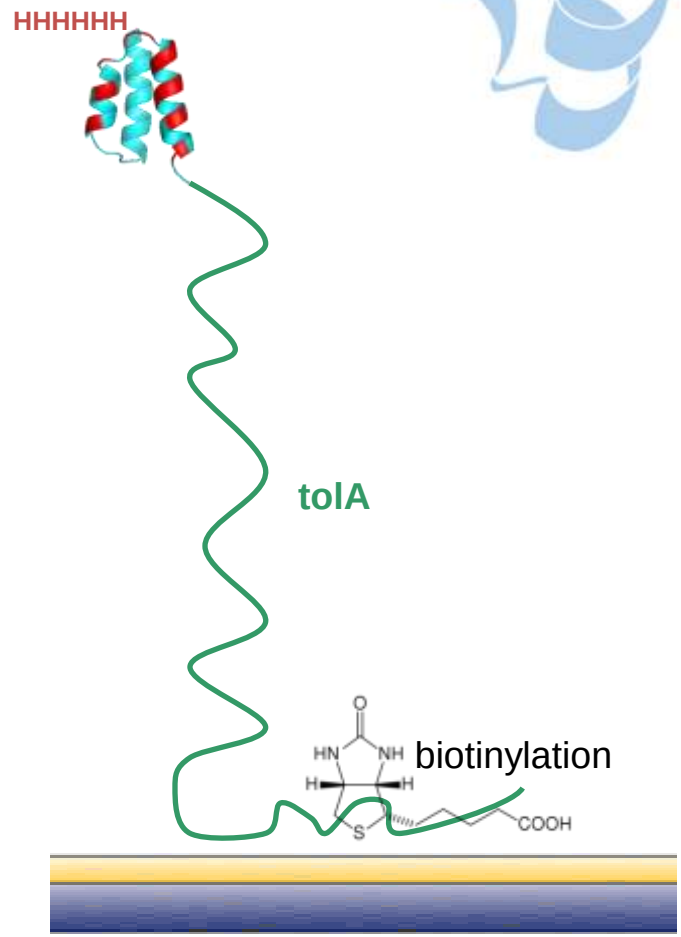


Novel ABD binders for IFNγ



Prototype of immunosensor for IFNγ





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