BCH 608

Developing Therapeutic Proteins by Engineering Ligand–Receptor Interactions

Review

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Developing therapeutic proteins by engineering ligand–receptor interactions

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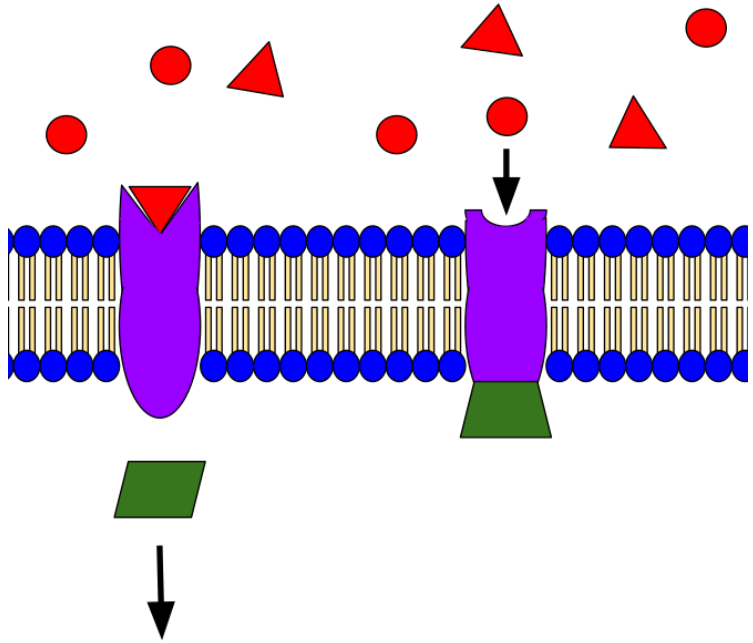
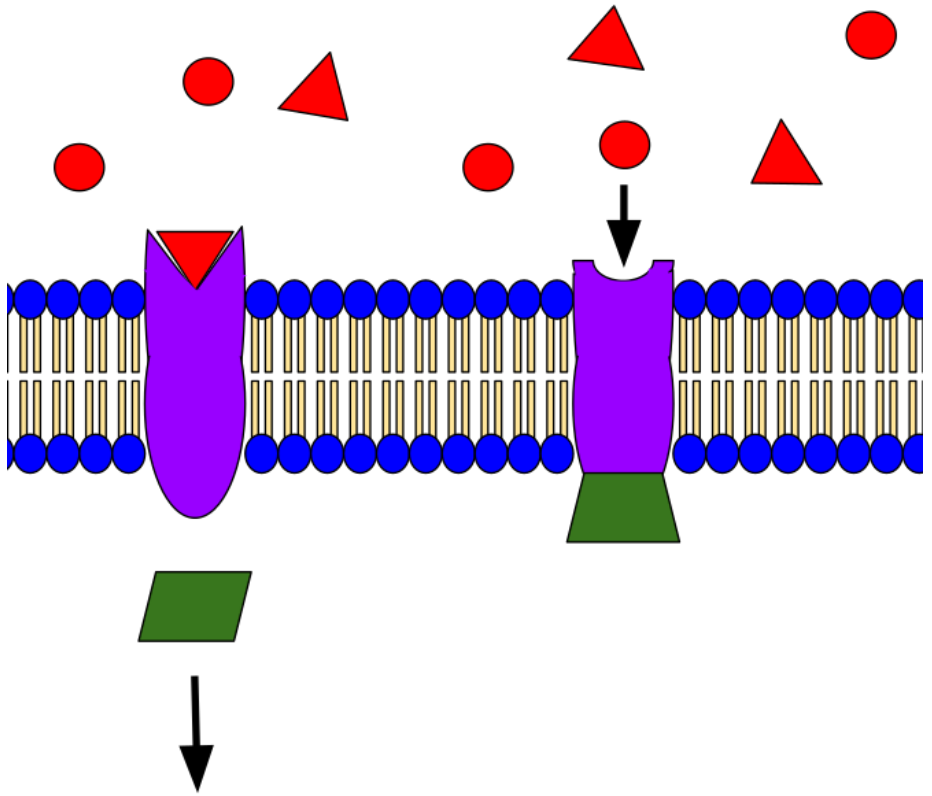
Outline:

- Ligand–Receptor Interactions
- Monoclonal Antibodies
  - Monoclonal Antibodies Limitations
- Therapeutic protein engineering strategies
- Engineering protein-based agonists
  - On the basis of ligand–receptor binding affinity.
  - On the basis of ligand–receptor trafficking.
  - On the basis of sequence variation
- Engineering protein-based antagonists
  - To bind to and antagonize receptors.
  - Soluble receptors to neutralize ligand activity.
  - Soluble receptors to inhibit cell-surface receptor activity.
- Factors to consider when playing with nature
- Conclusion



Ligand–Receptor Interactions

- Ligand–receptor interactions are tightly controlled to regulate signaling pathways.



Ligand–Receptor Interactions

1. The remarkable specificity of protein–protein interactions.
2. The success of protein-based therapeutics.

❖ Has demonstrated the potential to reduce a range of disorders by

Targeting specific ligand–receptor interactions.

Monoclonal Antibodies

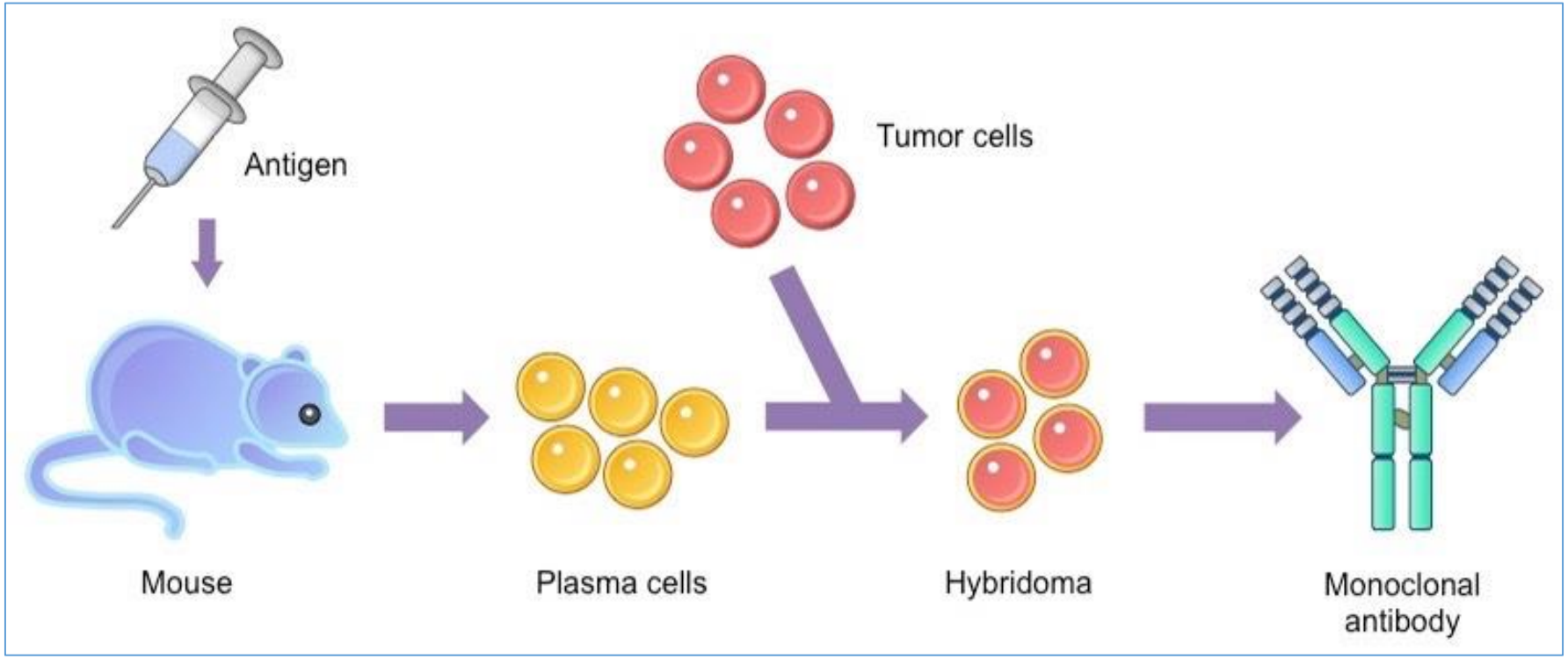


Figure: Monoclonal Antibodies Production

Monoclonal Antibodies Limitations

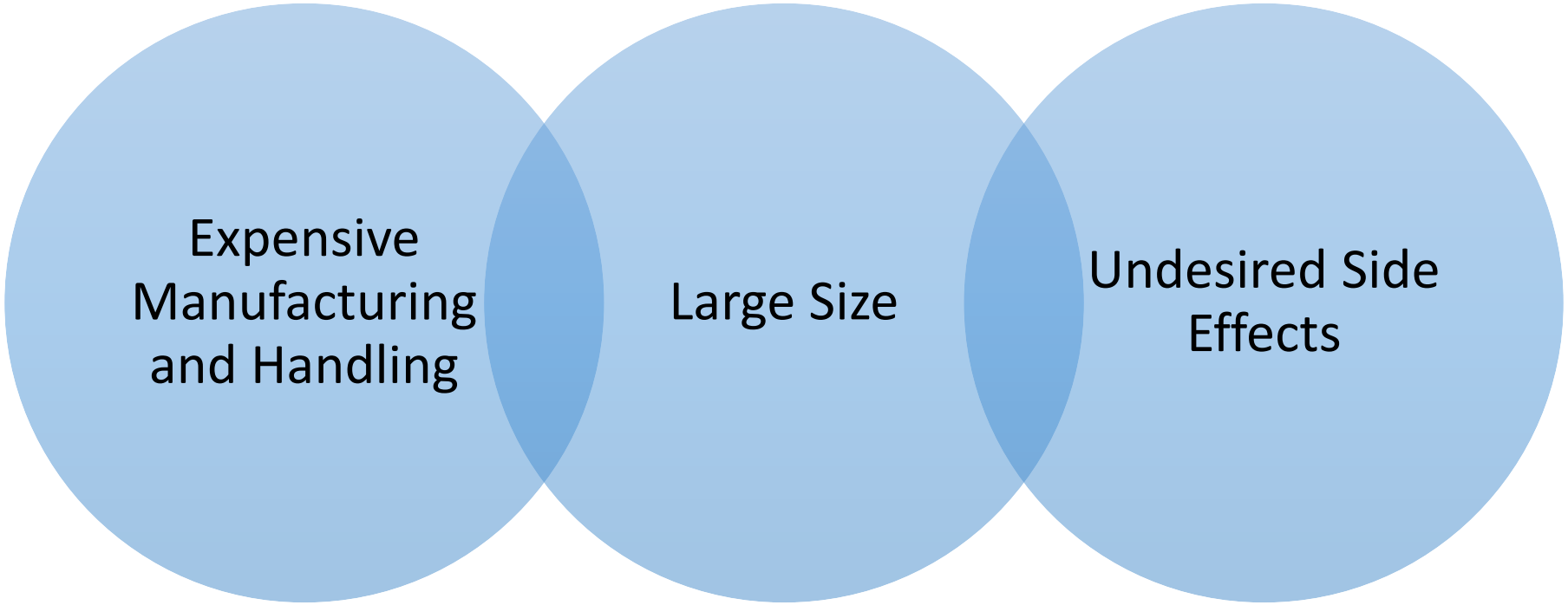
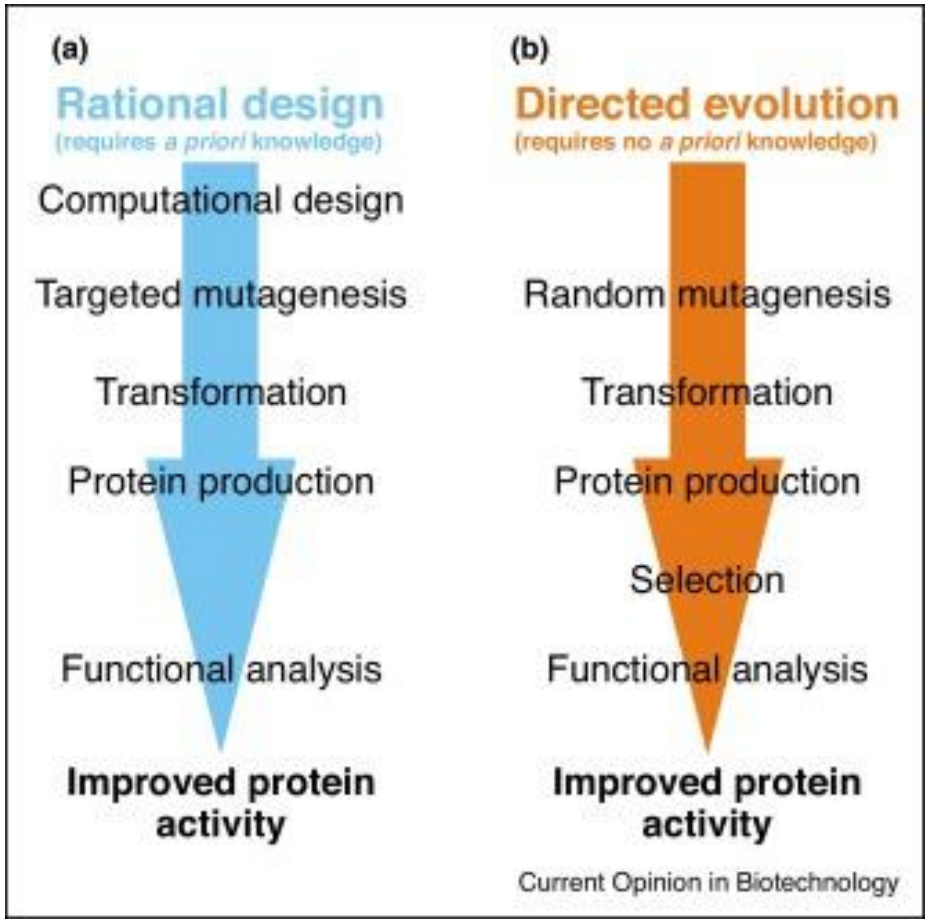


Figure. Monoclonal Antibodies Limitations.

Therapeutic protein engineering strategies:

- Rational and Directed evolution approaches have been used to engineer proteins with desired properties such as altered binding affinity, or increased stability and levels of recombinant expression.



Agonists and Antagonists

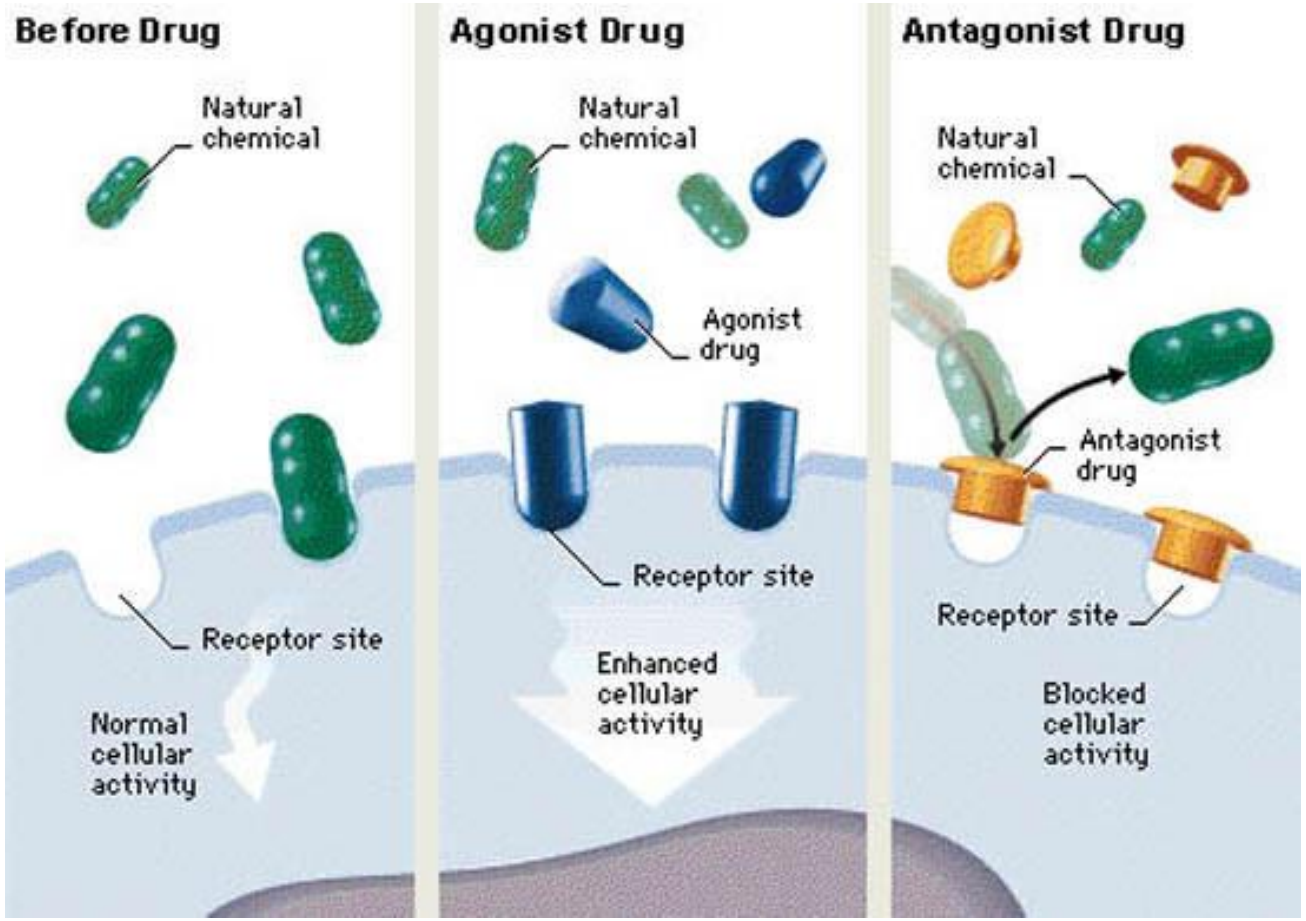


Figure. Receptor Interactions- Agonists and Antagonists.

Engineering protein-based agonists:

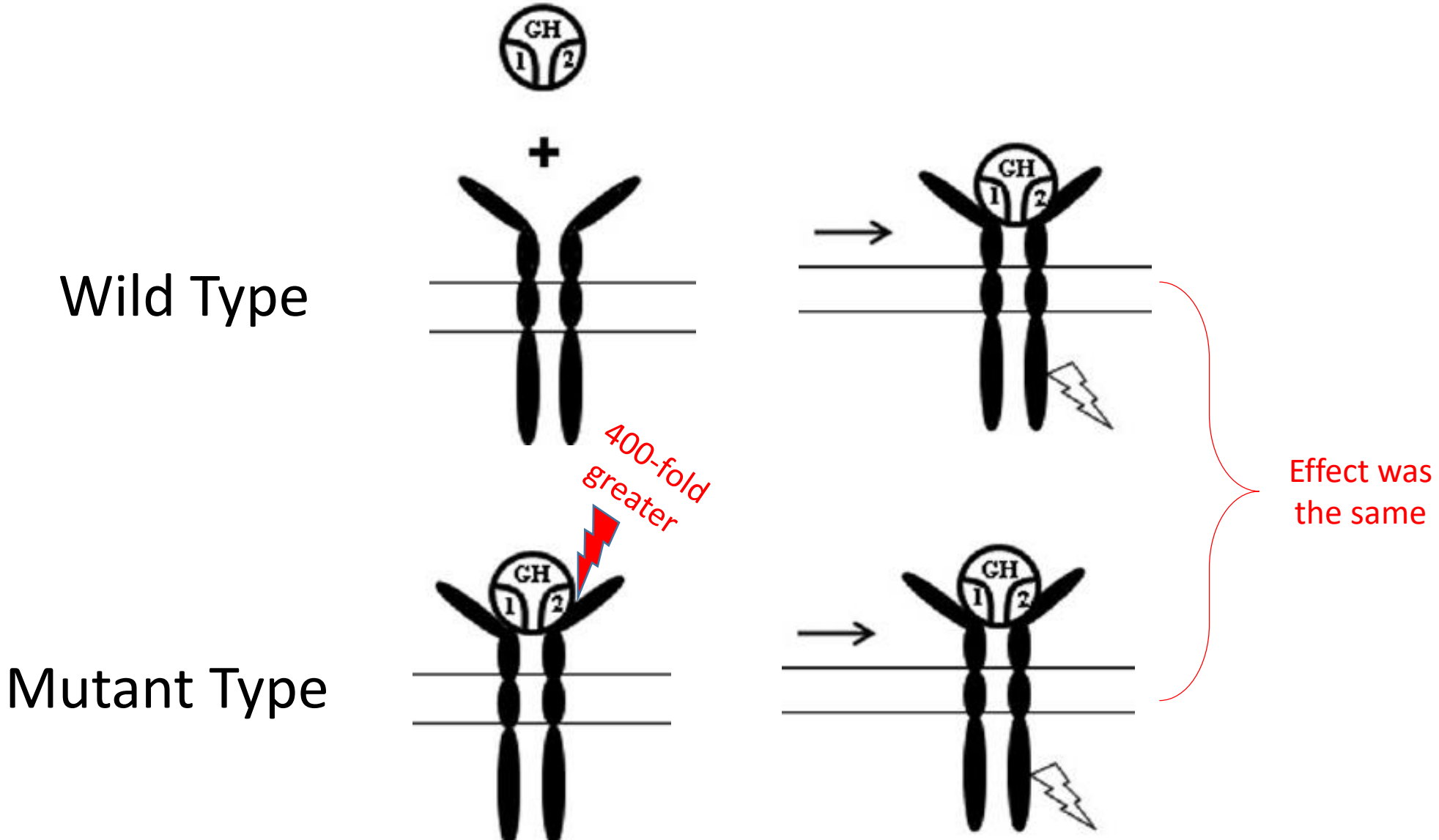
1. Engineering agonists on the basis of ligand–receptor binding affinity.
2. Engineering agonists on the basis of ligand–receptor trafficking.
3. Engineering agonists on the basis of sequence variation.

Ligand-Receptor Binding Affinity

- Introduce mutations in a ligand, with the aim of enhancing its receptor binding affinity.

Ligand-receptor binding affinity might not correlate with biological activity

Ligand-Receptor Binding Affinity



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Ligand–Receptor Trafficking

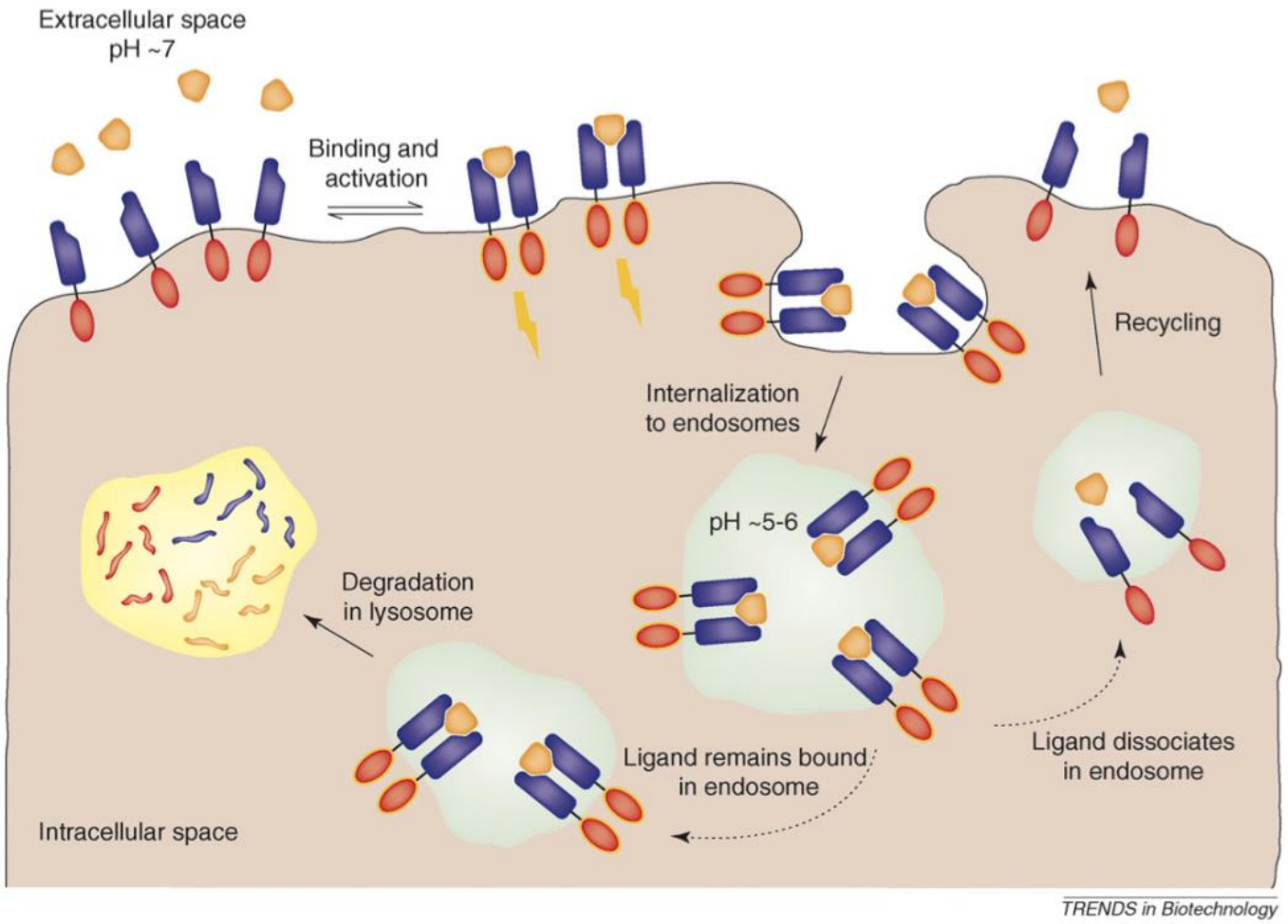


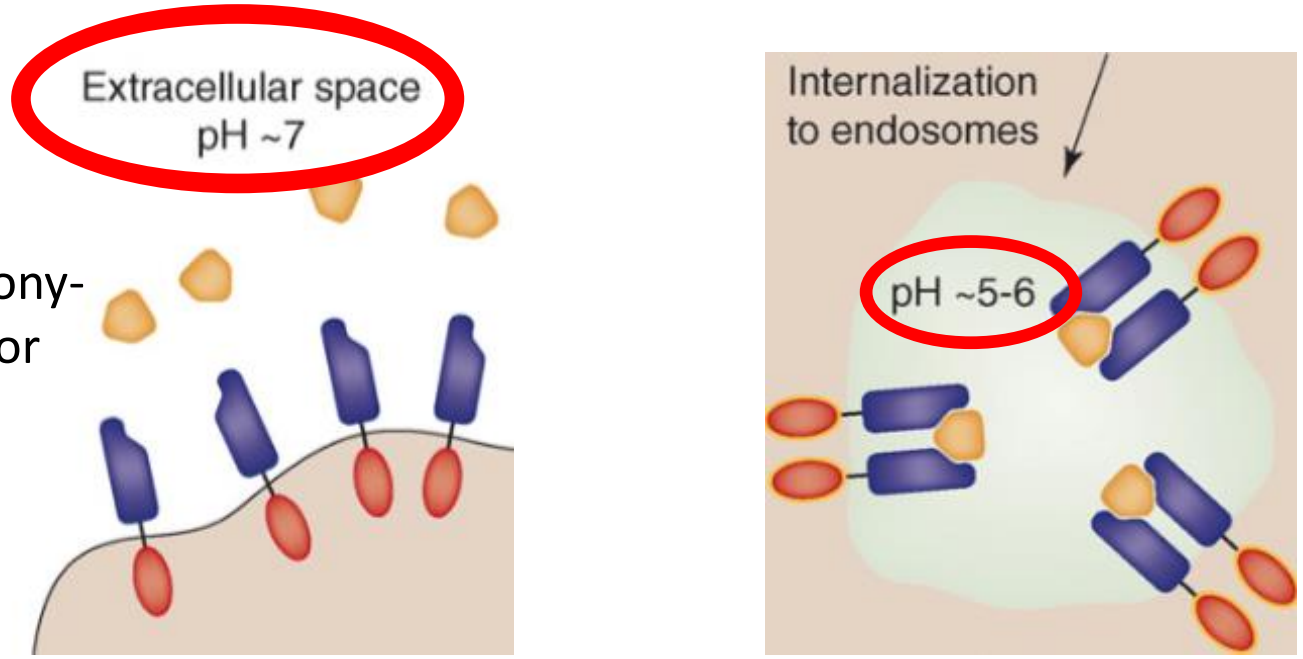
Figure 1. General model illustrating ligand–receptor trafficking.

Ligand–Receptor Trafficking

- ligand–receptor complexes that remain bound and active are favored for degradation, whereas those that easily dissociate are favored for recycling.
- This presents an obvious problem for those trying to develop effective agonists, because an engineered ligand with very high affinity might be degraded rapidly, thereby diminishing its potential activity.

Ligand–Receptor Trafficking

granulocyte colony-stimulating factor (GCSF)



Engineering protein-based agonists:

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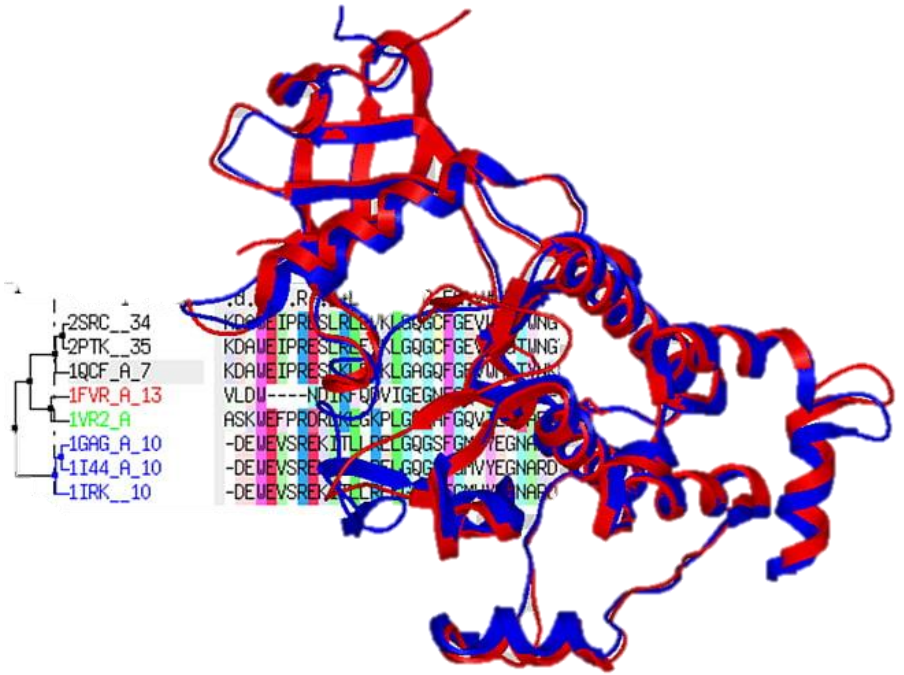
Sequence Variation

- Another approach to engineering agonists involves introducing mutations found in families of natural protein variants that are similar in structure or sequence.



Sequence Variation

- Fibroblast growth factor 1 (FGF1)
- Wild type FGF1 has low thermodynamic and proteolytic stability.
- Using homology (comperative) models, FGF1 alignment of 140 sequences.
- The resulting mutants had thermal denaturing temperatures up to 27 ℃ higher and exhibited improved proteolytic resistance

Engineering protein-based antagonists:

1. Engineering ligands to bind to and antagonize receptors.
2. Engineering soluble receptors to neutralize ligand activity.
3. Engineering soluble receptors to inhibit cell-surface receptor activity.

Engineering Ligands that Antagonize Receptors

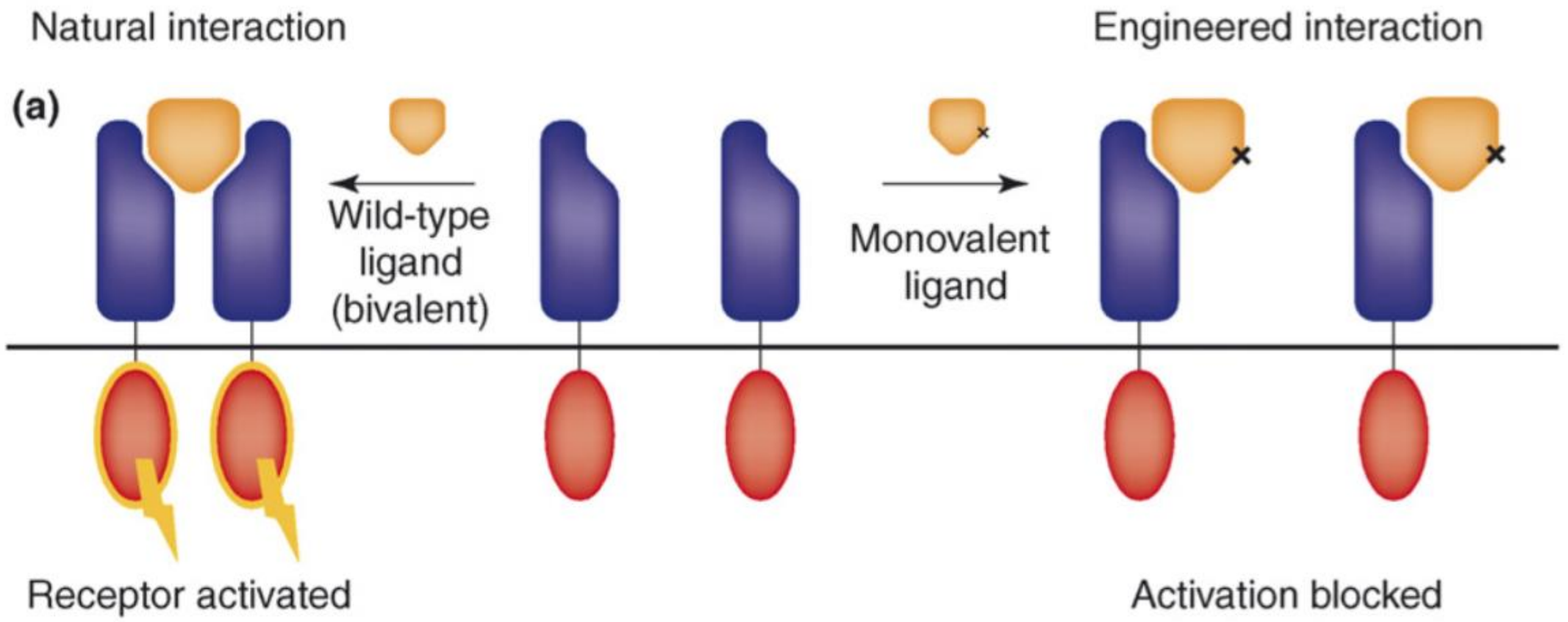
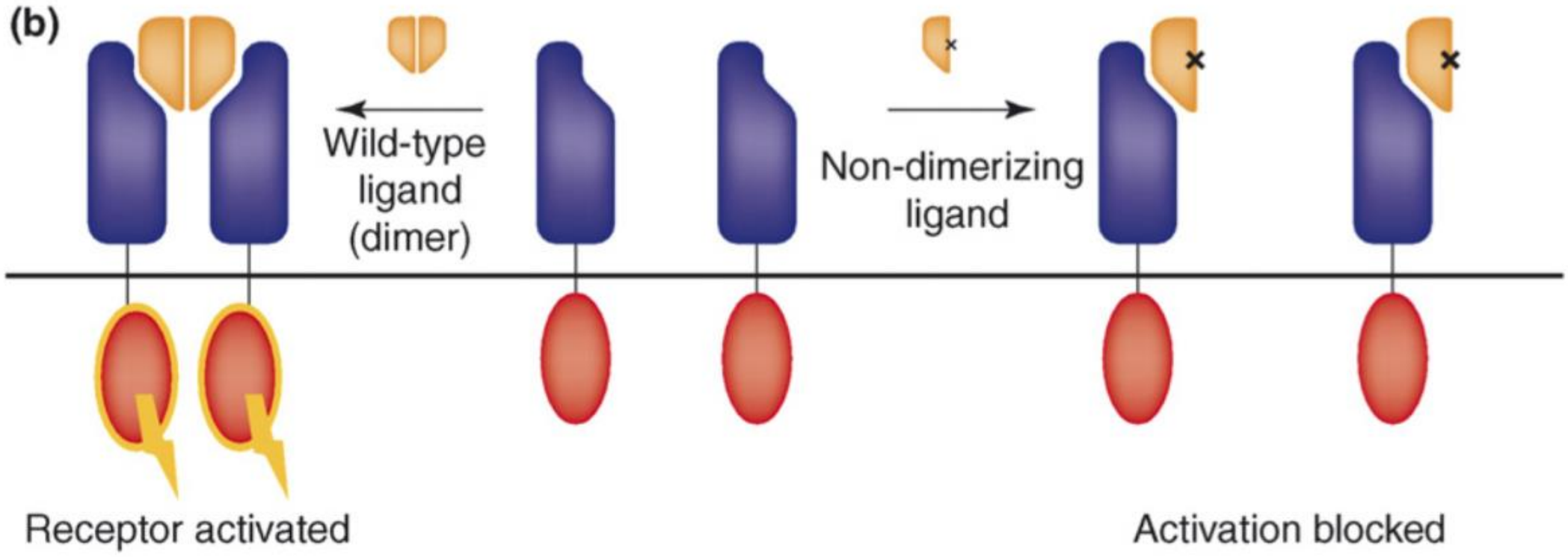


Figure. General strategies for developing antagonists by engineering ligand–receptor interactions.

Engineering Ligands that Antagonize Receptors



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Engineering soluble receptors to neutralize ligand activity

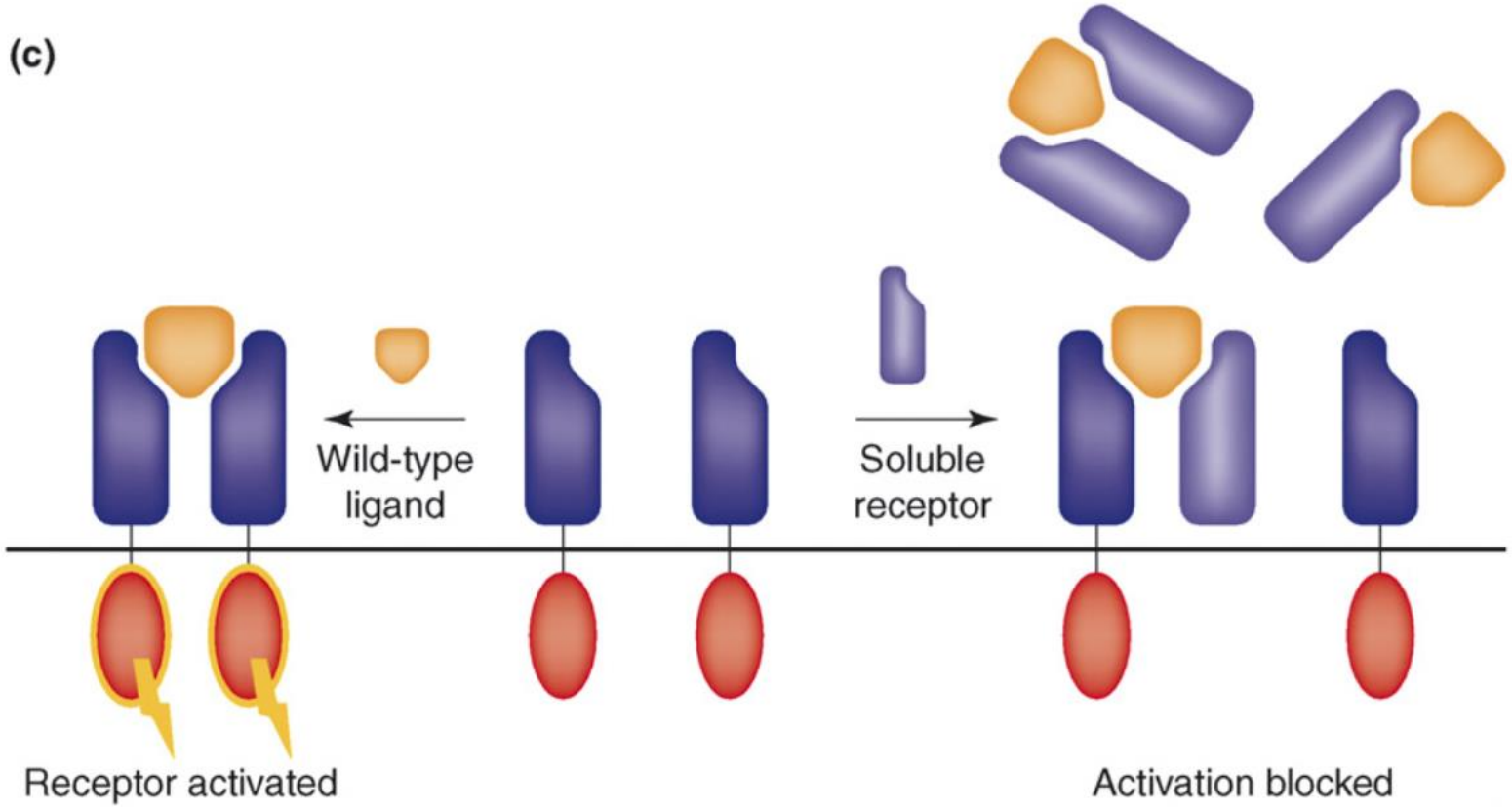

Figure 2. General strategies for developing antagonists by engineering ligand–receptor interactions.

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Engineering soluble receptors to inhibit cell-surface receptor activity

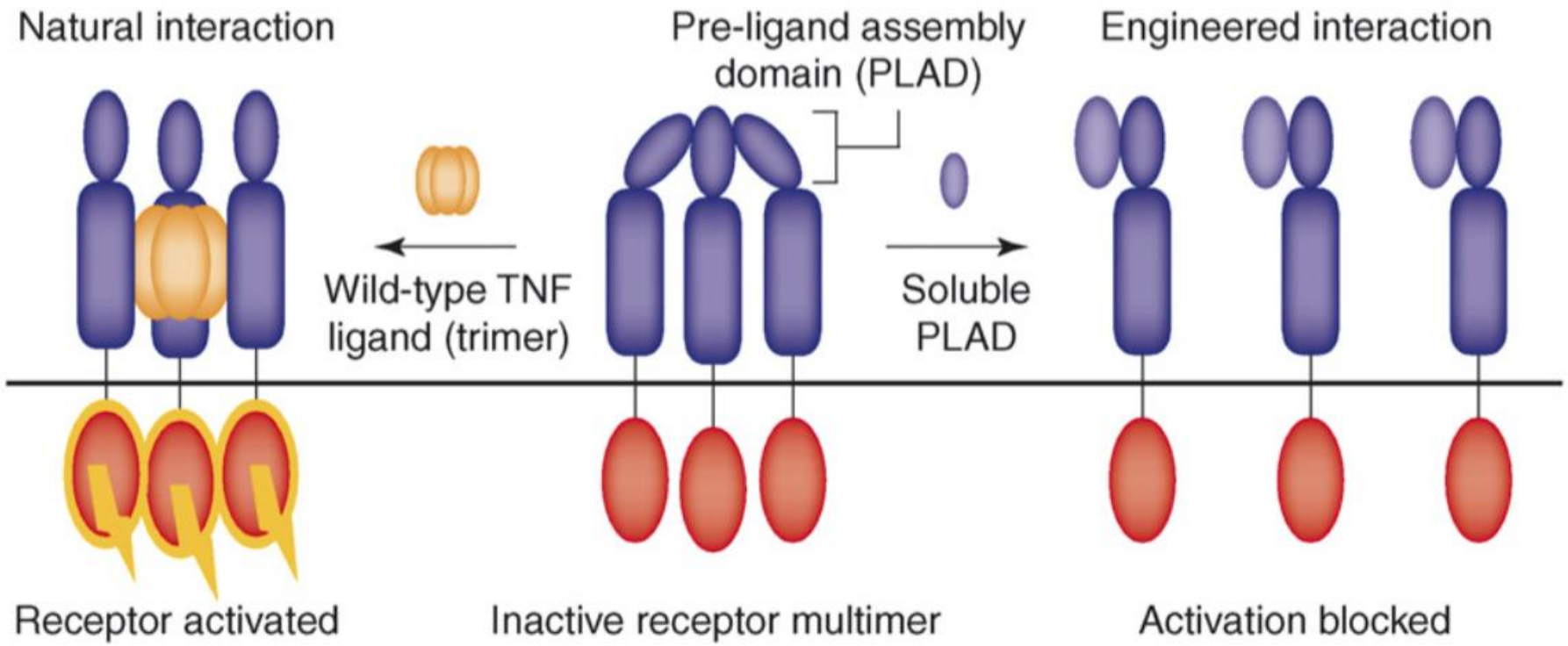
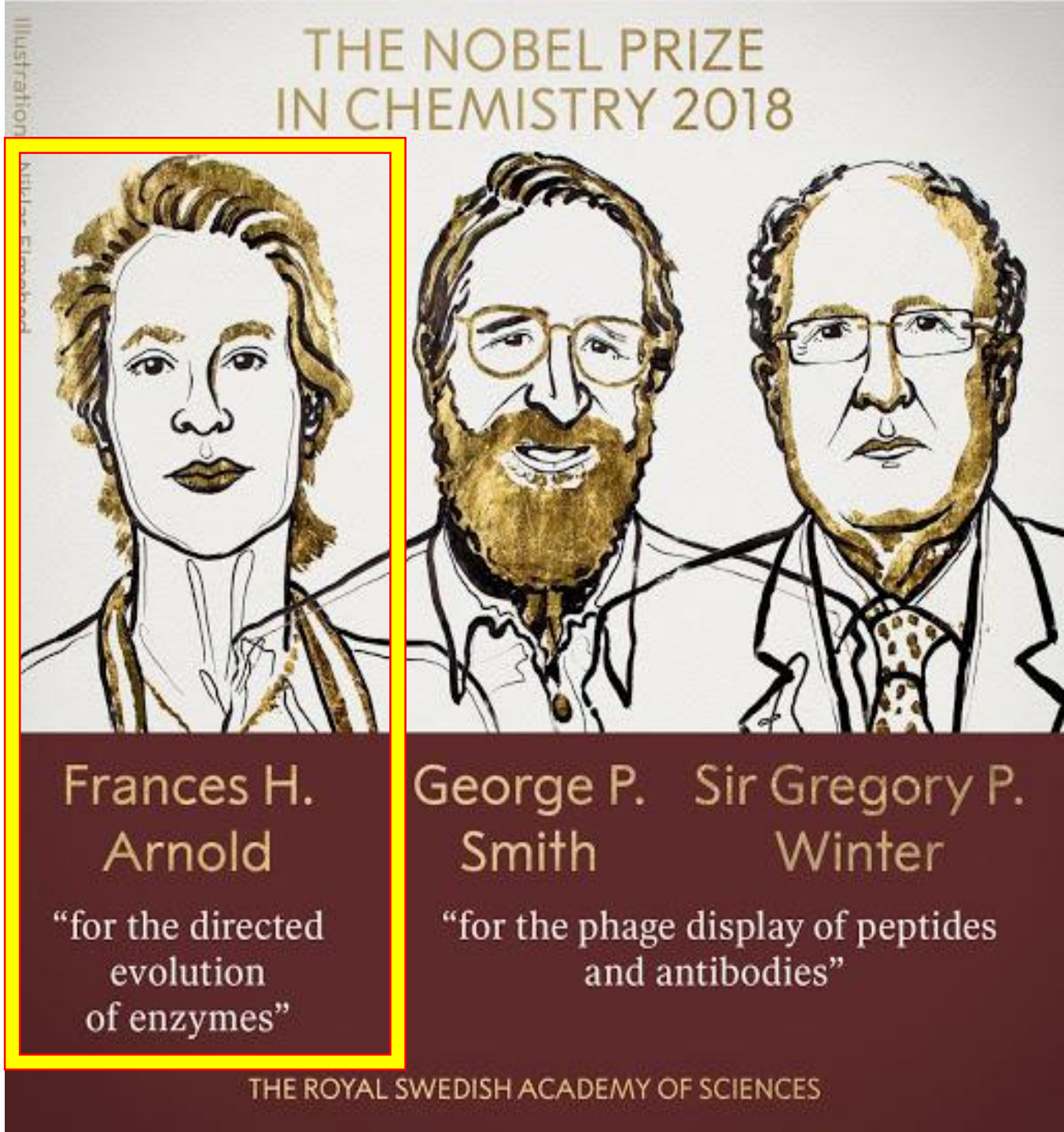


Figure 3. Provisional model showing the method of action of the soluble PLAD domain of the tumor necrosis factor receptor (TNFR-I or TNFR-II).

Factors to consider when playing with nature

1. The monomeric form of a dimeric ligand might show severely diminished binding affinity for its receptor.
2. Ligand binding affinity can be significantly decreased when the receptor extracellular domain is removed from the cell membrane.
3. Receptors with clinical relevance are generally complex, multidomain proteins and can suffer from low levels of recombinant expression.

Directed evolution can be helpful in overcoming each of these limitations



In conclusion

- As our understanding of biological systems continues to expand, direct engineering of ligand–receptor interactions will be increasingly used as a complement to, or in place of, antibody-based approaches.