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Targeting MHC-1 Deficient Cells With Red Blood Cell and MHC-1 Avoidance

Problem:

Which polypeptides are the most effective at binding to NKG2D molecules whilst ensuring red blood cell avoidance?

Hypothesis:

The peptide LYQMELRYLAL with the S enantiomer will have the highest natural killer cell to red blood cell binding ratio, due to length of the peptide increasing its binding potential, and the S enantiomer being found more commonly in drugs targeting MHC-1 deficient cells.

Variables:

- **Manipulated:** The construction of the peptides (i.e., LYQLRY, LYFLRY, etc.)
- **Responding:** The ability of the peptide to bind to 1T2Q (red blood cell) and 8TLZ (NK receptor)
- **Controlled:**
  - Temperature (37 °C)
  - Same solvent model (implicit water)
  - Program used for peptide design (PyMol/ChimeraX)
  - Program used for peptide binding (AutoDock Vina)
  - Same program versions used across trials
  - Same receptors used
  - Same binding location (i.e., I can't have one peptide bind at the top and the other at the bottom of the ligand)
  - Same protein preparation procedure
  - Only the target receptor, nothing else
  - Same simulation number
  - Same affinity output (ΔG vs Kd vs M)
  - Same selection criteria

Materials:

- Computer with Internet access
- ChimeraX (generate peptides/imaging)
- PyMol (R/S enantiomers)
- AutoDock Tools (prep peptides for Vina)
- AutoDock Vina (test docking)
- Protein Structures from PDB
- 3D printer with printing material to print peptide models

Procedure:

1. Identify NK cell activating receptor (NKG2D) using PDB.
2. Download receptor structure (PDB ID: 4S0U).
3. Open the receptor in AutoDock Tools.
4. Delete all water molecules.
5. Add polar hydrogens.
6. Compute Gasteiger charges.

7. Go to ligand and select *"choose molecule,"* then choose 4S0U.
8. Save receptor as a PDBQT file for AutoDock Vina.
9. Open ChimeraX.
10. Open tools → structure editing → build structure → peptide.
11. Enter the sequence (eg, LYQLRY).
12. Click build.
13. Save peptide.
14. In Pymol, make R enantiomer (use this command: transform_selection all, (-1,0,0, 0,1,0, 0,0,1, 0,0,0)).
15. Save molecule.
16. Repeat steps 9 - 15 for all peptides (I made 6).
17. Download a red blood cell membrane protein (glycophorin A) from PDB (PDB ID:1T2Q).
18. Prepare in AutoDock Tools, same as receptor (delete water, add polar hydrogens, compute Gasteiger charges, choose molecule).
19. Save as a PDBQT to test off-target binding.
20. Open AutoDock Vina.
21. Load NK receptor PDBQT as receptor.
22. Load peptide as ligand.
23. Define the grid box around the receptor binding site.
24. Run docking and record binding affinity (kcal/mol).
25. Repeat procedure with the RBC protein as the receptor.
26. Repeat steps 20 - 25 for all peptides and enantiomers.
27. Compare binding energies for the NK receptor vs RBC proteins.
28. Calculate the ratio of binding using this formula: $\frac{Kd2}{Kd1} = e^{\frac{\Delta G2 - \Delta G1}{RT}}$ (You want it to be ideally over 10:1, as most drugs have a similar ratio, if not higher)(you can convert to molar as well, if you want).
29. Now check for drug likeness using Lipinski's Rule of 5 (Molecular Weight < 500 Da, LogP < 5, ≤5 Hydrogen-bond donors, ≤10 Hydrogen-bond acceptors). This can be done using SwissADME or manually, though manually takes much longer.
30. Record observations.
31. If necessary, use AI to mutate the peptide and repeat steps 9-30 for a higher efficacy.

Observations:

Analysis:

The primary objective of this experiment was to computationally design a series of trial peptides to help treat MHC-1 deficiency in cancer cells by activating natural killer cells with the NKG2D receptor, whilst preventing red blood cell binding. This objective proposes a central problem among immunological treatments: how can immunological strength be enhanced while reducing unintended cytotoxicity?

The molecular docking program AutoDock Vina was used to estimate the binding strength of trial peptides to natural killer cell-associated receptors, as well as red blood cell surface proteins and MHC-1 molecules. The binding affinity values given by the simulations served as a comparative metric rather than an absolute indication of biological values.

The simulations revealed that the peptides exhibited a significantly higher overall binding affinity towards natural killer cell receptors, with a substantially lower binding strength towards red blood cell proteins and MHC molecules; the most effective peptide was 25 times stronger at binding to NKG2D than red blood cell proteins, and 28 times stronger than MHC-1 (There were peptides with greater differences, such as one with 50-fold binding affinity, but the most effective had the highest binding strength, not ratio). This shows preferential binding, something essential for the prevention of hemolytic behaviour and ensuring a lack of MHC-1 replacement, while also establishing that the trial

peptides could be an effective treatment. Furthermore, and most importantly, the difference in overall binding strengths exceeded the margin of error associated with docking programs, reinforcing the theoretical efficacy of these peptides.

However, molecular docking programs have many limitations in this field, foremost of which is the reliance on static protein structures for the molecular binding process. In vivo, protein flexibility, cell function, competing proteins and ligands could pose problems for the binding behaviour. For instance, there was an experiment done for HIV protease, where in silico experimentation was successful, but in vivo experimentation went poorly because of the flexibility not accounted for in silico, causing an inability to bind. Thus, while the in silico testing data support the project hypothesis that the in silico derivative of the parent polypeptide LYQMELRYLAL-S is effective, it must be used as predictive rather than definitive.

Selectivity is a key priority for any immunologically based treatment method, and was assessed in this project by comparing binding affinities across multiple protein binding sites, rather than a singular interaction. The peptides' 25-fold reduced affinity for MHC-1 and red blood cell molecules is significant for toxicity, as non-specific membrane binding is a common cause of red blood cell toxicity in peptide-mediated therapies, and while MHC-1 replacement is uncommon in molecular treatment methods, it could still have potentially life-threatening consequences. The lack of strong reactions for each of these implies that there will be little likelihood of any harm coming to either MHC-1 molecules or red blood cells.

These results align with the design choices, as the peptides were made to avoid high hydrophobicity and strongly cationic motifs, which promote non-specific membrane insertion, while balancing polarity and an even charge distribution. Moreover, the peptides are greatly dissimilar to MHC-1, preventing spontaneous insertion into the MHC-1 site.

Despite this, computational evidence is not enough to account for systemic exposure or acclimation in non-target tissues, as an immunological response to a foreign-body object or off-target binding could have dangerous side effects, such as prolonged disease or the rupturing of the cell membrane.

A drug-likeness analysis was performed using Lipinski's Rule of 5 (less than 5 hydrogen-bond donors and 10 acceptors, a molecular weight less than 500 Da, and a LogP of less than 5), and helped to evaluate whether the peptide falls into the acceptable physicochemical range for biological activity. Factors such as molecular weight (Da), polarity, hydrogen bond donors, hydrogen bond acceptors, and predicted solubility were calculated. The peptides generally adhered to guidelines, with the one major exception across all trials being a molecular weight of at least 850 Da, well above 500 Da, the recommended limit, limiting the ability to administer orally, thus requiring hypodermic or intravenous insertion.

However, ensuring drug-likeness comes with tradeoffs. A heightened binding efficacy usually requires a larger molecule with more hydrogen bond donors or acceptor molecules, or vice versa. The final peptide design represents a compromise between binding affinity and drug-likeness, rather than an extreme version of either.

Stereochemistry was analyzed by comparing the predicted behaviour of L and D enantiomers for the trial peptides. The docking results showed measurable differences between enantiomers, with L being much stronger at binding to NKG2D receptors vs red blood cells or MHC-1 molecules. This is significant because organic systems are inherently chiral, and a single enantiomer can exhibit higher binding affinity or lower toxicity than its inverse. Selecting an optimal stereoisomer is integral to enhancing selectivity toward natural killer cells while reducing off-target interactions. Nonetheless, computational evidence can’t account for biological context.

Natural killer cells are regulated by a balance of activation and inhibitory signals, with MHC-1 playing a major role within this balance. Cells that lack or downregulate MHC-1 molecules, barring sperm and red blood cells, are exceedingly more susceptible to natural killer cell-mediated therapies. It is by targeting pathways associated with these recognition processes that the designed molecule aims to enhance immune differentiation between normal and abnormal cells. The experiment supports the plausibility of this approach. The predicted binding of the peptides to the NKG2D receptor aligns with the known biology of natural killer cells, cementing the idea that computational design can align with immunological principles. However, immunological pathways are inordinately complex, and we can’t purely rely on computational design.

There were no major outliers in this project, as all of the D base peptides fell into the 7-14 times binding strength to NKG2D vs Hemoglobin A, while all of the L base peptides were in the 2-3 times binding affinity range. The modified peptides all had immensely strengthened binding affinity, being anywhere between 25-fold and 50-fold.

One major source of error was unnoticed until late into the experiment, but the LYQMELRYLAL was initially meant to have a methylated leucine, but due to an error that occurred in the design process, the program instead produced a peptide with methionine and glutamic acid. It ended as the most effective base peptide, and when I modified the peptides, I noticed the error, and continued with the peptide having the defect.

Conclusions:

To conclude, the experiment does not support the hypothesis that LYQMELRYLAL would be the most effective, although it was an in silico derivative of that peptide that was the most effective, with a binding ratio of 140 nM, an immensely high binding strength that would be effective for clinical treatments. Thus, it was a successful experiment, nonetheless, as I was able to create a rationally designed polypeptide that can target MHC-1-deficient cells associated with natural killer cell activation while minimizing interactions with MHC-1-presenting cells and red blood cells, having potential clinical applications. While the findings are predictive and subject to computational limits, they help to highlight the potential of structure-based design in medicine and immunotherapeutic research. This project helped to demonstrate both the promise and challenges of using computational biology to address biomedical problems, making it a strong foundation for further investigation.

Extensions:

This project can be extended to strengthen both the computational and application aspects of the results. On the computational side, molecular dynamics solutions could be added, as they could allow for viewing of ligand-receptor interactions over time, rather than statically, providing further insight. Adding additional targets, such as more natural killer cell receptors or off-target receptors, could further evaluate selectivity and off-target risk. Further exploration of molecular modifications could also help to optimize binding specificity and physicochemical properties. From an experimental standpoint, future extensions could include in vitro validation through natural killer cell activation and hemolysis assays, to test whether predicted selectivity translates into measurable biological impacts. Together, these extensions could help to bridge the gap between computational predictions and experimental validation.

Real World Applications:

The findings of this project have potential relevance to the early stages of immunotherapy and targeted drug development. A molecule capable of preferentially interacting with pathways associated with MHC-1 deficiency could contribute to strategies aimed at enhancing natural killer cell-mediated clearance of cancerous or virally infected cells. The potential for cancer treatment is vast. 70% of cancer is MHC-1 deficient in some manner, and this treatment method could be used alongside surgery for an alternative to chemotherapy and radiation therapy, while being much less harmful to your body. Additionally, it would have a much lower cost, as for $200 you can buy 5 mg, which would likely be enough, and bulk orders for hospitals would be much cheaper. While the current work is exploratory, it demonstrates how computational design can be used to narrow down candidate molecules before costly laboratory testing.