Conformational analysis and rational design of tumor-associated altered peptide ligands

F. Ballabio[1], C. Paissoni[1], C. Camilloni[1]

federico.ballabio@studenti.unimi.it

(1) Università degli Studi di Milano

Introduction

Tumor-associated antigens (TAA) are short linear peptides that are exposed by Class I Major Histocompatibility Complex (MHC-I) in tumor cells but, usually, are not able to activate a strong immunogenic response. Achour and co-workers at Karolinska Institutet have been developing a strategy to design antigens, altered peptide ligands (APLs), able to induce a stronger immune response against tumors, evaluating new possibilities for immunotherapy(1). While it has been hypothesized a correlation between immunogenic response and MHC-I-APL increased stability, recent work refuted such correlation, finding increased response also without increased stability, thus making unclear how a design strategy should be implemented(2).

Our purpose is to investigate and characterize the conformational free energy landscape of multiple APLs in their free-state, MHC-I bound state and MHC-I-T-Cell Receptor (TCR) bound state, employing an optimal sampling strategy based on advanced molecular dynamics (MD) simulations. By characterizing, at atomistic resolution, the dynamics of multiple APLs, we aim to identify the key interactions responsible for tuning the molecular recognition mechanisms of known APL variants and the consequent immunogenic response. Eventually we will be able to propose a rational design strategy to further improve the immunogenic response and thus pave the way for a new generation of active APL.

Aims

Exploit the advantages of MD simulation to investigate structural and dynamical aspects of the role of APL variants in the pMHC-TCR recognition mechanism with particular focus on:

-Rationalization of the proline replacement in position 3 of known APL.

-Rational design of alternative APLs by evaluating replacements of amino acids in other positions.

-Investigation of long-range structural alterations induced by APL and their consequences for the overall immunogenic response.

Background

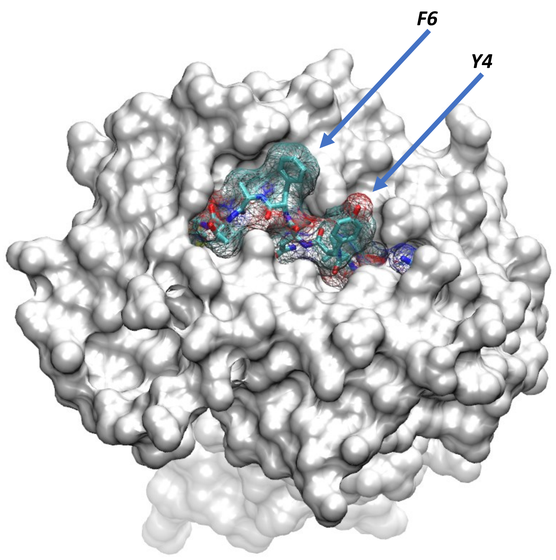


Fig.1 MHC-I HC surface and the peptide KAVYNFATM buried in the cleft (Licorice and wireframe). Peptide residues Y4 and P6 protrude from the surface.

All nucleated cells transport on their surface the heterotrimeric MHC-I complex, consisting of three not covalently bonded subunits: ~365 residues Heavy Chain, ~100 residues β2-microglobulin and an 8-10 residues linear, high variable, peptide.

The set of loadable peptides, the so-called epitope repertoire, processed both in physiological and pathological conditions, mirrors the status of the cell.

The surface [Fig.1] that results from the combination of MHC α1 and α2 domains and the peptide is the key for the interaction with the TCR of CD8+ Cytotoxic cells (CTL) that, with MHC, has a central role in adaptive immune responses against cancer and pathogens.

TCR consist of two chains α and β, each composed by a constant (C) domain and a variable (V) domain [Fig.2]. Both V domains have three hypervariable loops that define the antigen binding site. α and β CDR3s establish a cleft in which TAA residues, that can protrude from surface, interact with, while CDR2s and CDR1s conserved residues are often involved in non-covalent enthalpically driven interactions with MHC-I α1 and α2 α-helical domain anchor regions. CTLs are able to recognize with low avidity the endogenous MHC surface, and the "not self" loaded peptide acts as a discriminant factor that, by sufficiently transforming the pMHC interface, with the respect to the one encountered during negative selection, increases avidity recognition(3).

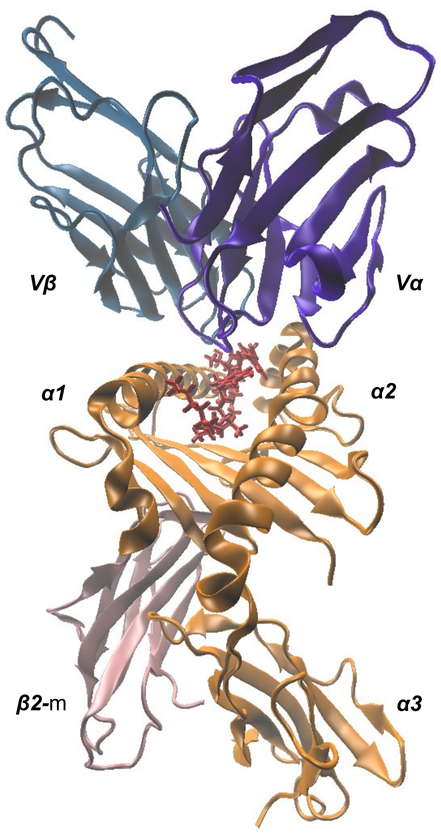


Fig.2 pMHC-TCR Complex

Most of the identified TAAs are poorly immunogenic (4). Several approaches in designing APLs with enhanced immunogenic capabilities have been tested: the most common consists in structurally conservative peptide substitutions of anchor residues, without compromising the molecular mimicry.

KAVYNFATM WT - agonist TCR affinity: 8.9 μM	KAV**A**NFATM Y4A – semi agonist TCR affinity: 54.5 μM	KAV**S**NFATM Y4S - antagonist TCR affinity: *NA*	KAV**F**NFATM Y4F - antagonist TCR affinity: ≃ 1 mM
KA**P**YNFATM V3P – agonist	KA**PA**NFATM V3P-Y4A - agonist	*NA*	KA**PF**NFATM V3P-Y4F – semi agonist

Table1 TAA and relative APLs summary

The analysis are focused on Lymphocytic Choriomeningitis Virus derived antigen gp33 (KAVYNFATM) and its derivatives. Y4F mutant is an highly efficient natural escape variant and, as well as designed Y4A and Y4S, have been characterized looking for induced conformational modifications that could affect TCR affinity(5,6). It exists an immunological hierarchy [Table1]: KAVYNFATM (WT) is a full agonist, Y4A a semi agonist and both Y4S and Y4F are not recognized. The only structural difference between WT and Y4F is the lack of the hydroxyl group. TCR can partially overcame the almost complete loss of the exposed side chain at position 4 (Y4A), whereas Y4S or Y4F lead to the completely loss of recognition. Crystal structures did not reveal any relevant conformational change that could explain the differences. Gp33 APL variants with p3P modification have been synthesized, conserving mimicry proprieties. In each of the complexes tested, p3P increased the thermostability of respective pMHCs with a significantly increased TCR affinity. Furthermore, V3P and V3P-Y4F resulted in significantly increased immunogenicity and converted the unrecognized Y4F into a semi-agonist. Vaccination of C57BL/6 mice with V3P-Y4F but not Y4F elicited a CTL response against the native escape variant Y4F.

Setup

Initially, peptide-only simulations have been performed, to optimize systems and protocol, to evaluate performances, scalability and relative computational efforts required, as well as to retrieve the first Free Energy data. Then the simulations have been extended on same peptide variants loaded in MHC and bound to TCR [Table2/Fig.3]. The calculations were performed on the Tier-0 HPCS Marconi, the largest supercomputer available in Italian Academic sector, hosted by SCAI department of CINECA, Casalecchio Di Reno (Bo).

All-atom, plain MD simulation details:

- Custom AMBER99SB force field.
- 4 points Transferable Intramolecular Potential water model.
- Dodecahedral box, protein centered at 1 nm from edge.
- Protein net charge neutralized with Na^+ and Cl^-, 0.018M.
- Minimization: steepest descent, conjugate gradient.
- Equilibration: position restraint (309°K, 25000 steps of 2 fs), Berendsen (1 bar, 2500000 steps of 2 fs).
- Simulation at NpT ensemble (250.000.000 steps of 2 fs, 500 ns).

Table2 unbiased simulation details

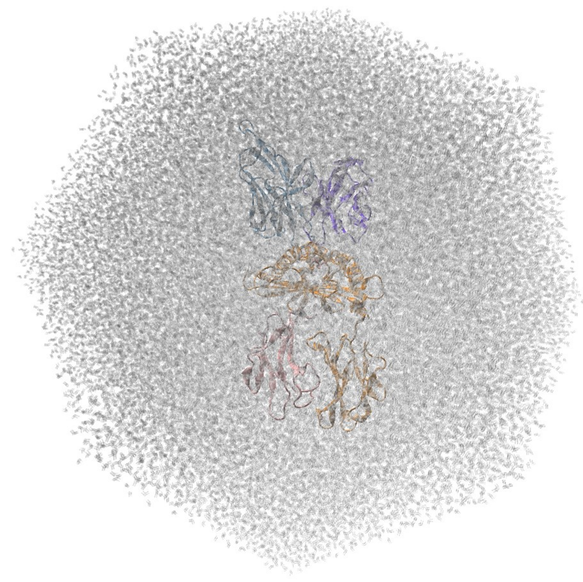


Fig.2 pMHC-TCR Complex in dodecahedral box filled with 62371 water molecules

Differently from the single peptide in water analysis, studying pMHC, pMHC-TCR and coreceptor CD8 complexes is e a greater effort in computational resources and analysis complexity, so the Collective Variables (CVs) choice will be of major importance. The MHC and TCR deposited structures used correspond to H-2Db and p14 respectively, but several differences can be found from structure to structure. The initial configuration must be consistent, all the MHC and TCR should have the same identical sequences and only the peptide one is allowed to change so, is necessary to add the missing residues, remove the unwanted crystallographic waters, except the relevant ones, adjust the protonation states and correct the cis-trans configurations. After this preliminary work, all the structures can be processed for the MD simulations.

In a first stage PBMetaD simulations will be employed to obtain Free Energy surfaces as a function of the same CVs used for the peptides, retrieving a systematic comparison of the MHC complex effect of the conformational freedom of the peptide variants. As an hypothesis, the increased affinity of p3P substitution can be due to the re-sculpting of the free energy, avoiding entropy loss upon binding. Comparing systematically the effect of the WT, V3P, V3P-Y4F, we expect to be able to pinpoint the specific contribution of the proline not only to the inherent conformational flexibility of the peptide but also on effects with MHC neighbor.

In a second stage we will focus on the role of position 6, designing mutations with the aim to predispose the peptide in its TCR binding configuration without altering its chemical properties, using D-amino acids or introducing N-methylation or Glycine substitution, either in position 6 or in adjacent positions. Once a modification will be identified with confidence, our collaboration with Achour group will allow to test the design both in vitro (crystal structure, stability and binding) as well as in mouse model to evaluate the immunogenic response. It is clear that a double design of position 3 and 6 could result in an extremely tunable APL.

In a third stage we will extend analysis further than pMHC-TCR complex: CD8 binds pMHC but far from the cleft. Different APLs may induce long-range conformational changes that can facilitate the binding with the co-receptor.

Preliminary Results

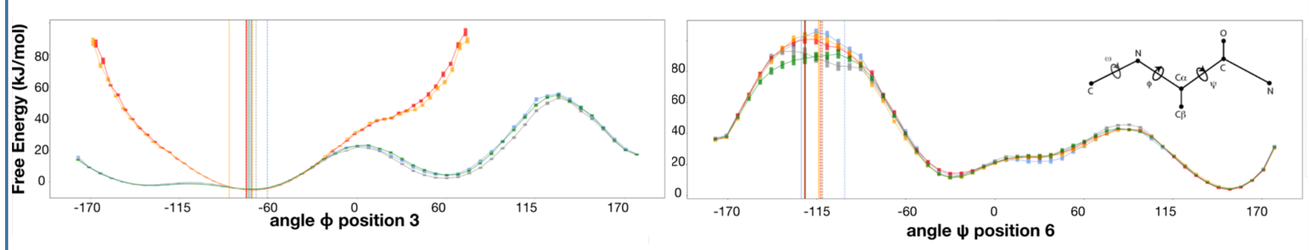
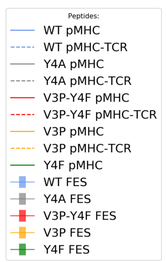


Fig.4 FES for position 3 and 6 of peptide in solution



For each APL a PBMetaD simulation was performed speeding up the backbone and sidechain dihedrals. Free Energy Surface (FES) of each CV have been obtained. As expected, comparing the FES profiles of the CV Φ3[Fig.2], it can be noticed the behavior of proline in the peptide, which imposes rigidity to flanking residues through its constrained phi angle: with valine in position 3 the situation is completely different as several minima in the relative FES can be observed. The crystallographic configurations can be used as reference by indicating the specific angle assumed in the crystal (vertical continuous line for pMHC complex and dashed for pMHC-TCR). For each CV the crystal configuration falls close to FES global minimum but Ψ6 behavior is totally different. The crystallographic Ψ6 APLs values in pMHC and pMHC-TCR complexes correspond to FES maximum for the peptide in solution, so the absolute less probable configuration for that CV in the system in free-state. We hypothesize that the peptide not only contributes to define the pMHC interface, but also that MHC has a role in imposing to the peptide specific conformations, such as in that case, the less probable. We propose that it could be an interesting candidate, beside the already know important role of p3P, for further analysis and rational design hypothesis.

References

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Dipartimento di Bioscienze

UNIVERSITAS STUDIORUM MEDIOLANENSIS