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The role of MHC I protein dynamics in tapasin and TAPBPR-assisted immunopeptidome editing
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The role of MHC I protein dynamics in tapasin and TAPBPR-assisted immunopeptidome editing

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Abstract

Major Histocompatibility Complex class I (MHC I) molecules are highly polymorphic, with allotypes differing in peptide binding preferences, and in their dependence upon tapasin for optimal peptide selection. The tapasin dependence of MHC allotypes is inversely correlated with their self-editing ability, and underpinned by conformational plasticity. Recently, TAPBPR has been shown to enhance MHC I assembly via a chaperone-like function, and by editing the peptide repertoire of some MHC I allotypes. Structural analysis has shown TAPBPR binding changes the conformation and dynamics of MHC I, with MHC protein dynamics likely to determine the prevailing TAPBPR function: generically enhancing MHC I assembly by stabilising highly dynamic peptide-empty MHC I; and by editing the peptide repertoire of highly dynamic MHC I allotypes.

An overview of MHC I peptide presentation

The proteolytic processing of intracellular antigens, and the presentation of the resulting peptides by MHC I molecules to cytotoxic T lymphocytes lies at the heart of protective immune responses to infection and cancer. The peptides are predominantly generated in the cytosol, and can be further trimmed following translocation into the endoplasmic reticulum (ER). These processes culminate in a diverse peptide pool being available for selection by assembling MHC I molecules.

Through a process known as peptide editing, or peptide filtering, a specialised peptide loading complex (PLC) ensures that high affinity peptides are presented by MHC I, without which MHC molecules are loaded with an unstable cargo [1]. In humans, the PLC constitutes two editing modules built upon the peptide transporter associated with antigen processing (TAP1/2) [2**]. Within each editing module tapasin tethers MHC I to TAP, and binds covalently to the oxidoreductase ERp57, while calreticulin simultaneously interacts with MHC I, ERp57 and tapasin [2**] and [3]. Of the PLC constituents, only tapasin edits the MHC I peptide repertoire [4-7].

Once furnished with peptide, MHC I dissociates from the PLC and progresses through the secretory pathway, where peptide-loaded MHC I (pMHC I) complexes encounter further peptide filtering mediated by the tapasin-like protein, TAPBPR [8]. With the assistance of calreticulin and UDP-glucose:glycoprotein glucosyltransferase, TAPBPR can return sub-optimally loaded or unloaded

molecules from the ER-Golgi intermediate compartment to the PLC for another loading attempt [9-12].

The dependence of MHC I allotypes upon tapasin for optimal peptide selection is negatively correlated with their self-editing ability

MHC I molecules are highly polymorphic, with allotypes differing in peptide binding specificities, and in dependence upon tapasin for optimal peptide selection [13,14]. Some MHC I allotypes efficiently self-select a relatively stable peptide repertoire even when tapasin is absent through genetic mutation, aberrant regulation, or when function is inhibited by viral subversion. Conversely, other MHC I allotypes, those with low self-editing ability, depend upon tapasin to select a stable peptide cargo. Modelling and biochemical evidence suggests that high self-editing ability is determined by an MHC I protein dynamic signature characterised by high conformational plasticity [15-20]. Thus, the negative correlation between self-editing and tapasin dependence suggests that catalysed peptide editing might also be explained by MHC protein dynamics [15,16]. Here, we review recent evidence supporting this hypothesis, focusing on structural and dynamic analysis of the TAPBPR-MHC complex, illustrating how TAPBPR, and by inference tapasin, might interact with conformationally dynamic MHC I molecules to achieve peptide editing.

TAPBPR binds to an intermediate MHC I conformation

In 2017, two groups published crystal structures of TAPBPR bound to MHC I [21**,22**] (Figure 1a). These structures show that TAPBPR makes extensive contacts with MHC I, with the concave N-terminal domain of TAPBPR cradling the α2 helix, and projecting a β hairpin underneath the peptide binding domain, while the C-terminal immunoglobulin-type domain of TAPBPR nestles between the α3 domain and β2-microglobulin (β2m). Binding to TAPBPR changes the conformation of MHC I compared with unchaperoned complexes (figure 1b), with the α2-1 and 2-2 sub-helices rolled outwards, some β strands of the peptide binding domain pulled downwards, and the α3 domain and β2m repositioned. Through extensive mutagenesis, McShan et al showed that TAPBPR binding to the α2 domain of HLA A*02:01 required the C101-C164 disulphide bond to tether the α2 helix to the floor of the peptide binding domain [23**]. The MHC conformation observed in these structures is novel, and it is likely that this peptide receptive intermediate structure is the preferred substrate for TAPBPR *in vivo*.

In the TAPBPR-bound state, MHC I rearranges key residues, including tyrosine 84 (Y84), which flips from where it would normally co-ordinate the C-terminus of the bound peptide and the opposing α2-1 sub-helix, to share a hydrogen bond with glutamate 102 (E102) of TAPBPR instead [21**,22**]. While this interaction might stabilise the complex, it is not obligatory: mutation of Y84 of MHC I does not adversely affect the ability of HLA A*02:01 to bind TAPBPR, or impair peptide loading of H2-K[b] molecules; while mutation of E102 of TAPBPR does not prevent HLA A*02:01 from being expressed at the cell surface [23**-25**].

In both TAPBPR-MHC structures there was no electron density within the peptide binding groove that might be attributed to a stably bound peptide even though in one of the structures a truncated peptide fragment was engineered to occupy the MHC A-pocket via a non-native disulphide bond [21**]. In the other structure, a loop region of TAPBPR was modelled to include a short α-helix that

entered the peptide binding groove (Figure 1a), extending from the tip of TAPBPR's luminal domain to protrude into the F pocket, a key determinant of peptide selection [5,16].

Mechanisms of tapasin and TAPBPR-assisted peptide exchange

Based on their structure, Thomas and Tampe proposed that the short α-helix within the loop region of TAPBPR might actively compete with peptide for binding to the MHC F pocket [22**], providing a possible mechanism for peptide editing involving TAPBPR and an intermediate MHC conformation. Supporting this possibility, Ilca et al found that mutating this region ablated the ability of TAPBPR to bind pMHC complexes and enhance peptide dissociation, and changed the immunopeptidome [26*]. Furthermore, McShan et al showed that mutagenesis of key residues of this region of TAPBPR was detrimental for binding pMHC complexes [23**].

However, this region of TAPBPR was not resolved in the structure by Jiang et al, and the veracity of its modelling remains uncertain [25** ,26*,27]. Furthermore, Sagert et al found that mutating this region did not affect the ability of TAPBPR to enhance peptide dissociation *in vitro* [28*]. McShan and colleagues recently provided evidence indicating the conformation and function of this region of TAPBPR might be reconsidered [25**] . Firstly, molecular dynamic simulations suggested that TAPBPR residues G24-R36 adopt various non-helical conformations, which alternate between pointing away and hovering above the peptide binding domain. Second, solution NMR experiments showed that the dynamics of MHC residues that would be in close proximity to the short α-helix, if it entered the peptide binding groove, were unchanged by truncation of the potential α-helix. Third, isothermal titration calorimetry and fluorescence polarisation experiments showed that this region of TAPBPR increased the affinity of peptides binding to MHC-TAPBPR complexes, contrary to what might be expected if this region competed with peptide for binding to MHC. McShan et al therefore concluded that this region of TAPBPR acts as a "peptide trap", hovering above the peptide binding groove, slowing the dissociation of peptide. These findings are compelling and suggest a similar function could be performed by the equivalent loop region of tapasin, which being shorter, is less likely to enter the peptide binding groove.

Another mechanism by which tapasin or TAPBPR might edit the immunopeptidome, which is consistent with the latest NMR data, is by catalysing the conformational change of MHC I molecules, as they transition between open and closed, peptide-bound and peptide-free states (figure 2) [16]. This catalysis might be achieved by tapasin, or TAPBPR, binding to and stabilising a high energy MHC I intermediate state. The NMR experiments conducted by McShan and colleagues, suggests that multiple points of contact may contribute to stabilise this intermediate, including the concave binding surface, membrane proximal Ig domain, as well as the β hairpin [23**,29**]. Such a mechanism would increase the flux of MHC I molecules through the peptide-loaded, closed state (McP), where the peptide binding affinity is sampled: with reopening of MHC I molecules that are loaded with low affinity peptides (MoP); while MHC I molecules loaded with high affinity peptides remain in the closed state (McP): thus defining the basis of peptide editing. This transition to the closed state (McP) requires peptides to form stabilising interactions at both ends of the peptide binding groove, leading to the displacement of TAPBPR, or tapasin, via negative allosteric coupling, and proceeds via the gradual peptide-dependent dampening of global MHC I protein dynamics distributed throughout the MHC I molecule [23**,29**].

MHC I allotype-specific variation in tapasin and TAPBPR-assisted peptide exchange

Computational modelling suggests that the key mechanistic step determining the ability of MHC I allotypes to self-select an optimal peptide repertoire is the rate at which the allotypes transition from an open, peptide-bound state to a closed, peptide-bound state (MoP to McP in figure 2) [16]. In this model, efficient self-selecting (tapasin-independent) MHC allotypes, with high conformational plasticity, have a lower energy barrier to breech in order to transition between these states, and consequently reach the peptide sampling (McP) stage faster, and thus have higher self-editing ability than poor self-selecting (tapasin-dependent) MHC allotypes (figure 2b). For allotypes with low conformational plasticity, this transition can only be achieved by binding to tapasin, hence their loading and cell surface expression is tapasin-dependent (figure 2c).

Nuclear magnetic resonance experiments have confirmed that TAPBPR binds different pMHC I allotypes via the same interaction surfaces [23**,29**]. In these studies, the authors compared the conformational dynamics of three TAPBPR-binding allotypes with the non-binding HLA A*01:01 allotype. The allotypes exhibited differences in their dynamic profiles, which was most pronounced at the α2 helix and α3 domain. A small proportion of the molecules of each allotype were in a highly excited state, likely to reflect open, unstable peptide-loaded MHC I intermediates (MoP). Notably the three TAPBPR-binding allotypes had a more prominent dynamic signature than A*01:01, suggesting TAPBPR selects highly dynamic pMHC intermediates, and in doing so promotes peptide binding and dissociation, and their transition to the native (McP) conformation in which peptide binding affinity is sampled.

TAPBPR has an additional chaperone function

In an impressive series of experiments McShan and colleagues took advantage of the comparatively high affinity with which TAPBPR binds MHC I to show that TAPBPR enhances pMHC I assembly via a "chaperone-like" ability to bind various MHC I allotypes early during their assembly, in addition to, or alongside, its allotype-specific editing function [23**,25**,29**]. These findings are consistent with those of Ilca et al, who observed recombinant TAPBPR bound a wider range of MHC I allotypes when incubated with whole cell lysates, containing assembling MHC I molecules, than when soluble TAPBPR was incubated with beads coated with purified pMHC I complexes [30*]. Recent evidence has shown that MHC I molecules are highly dynamic in the absence of peptide [20,29**], and the multiple contacts that TAPBPR makes with the MHC I heavy chain and β_2m are likely to stabilise the empty, highly dynamic molecule in a peptide-receptive state (figure 1).

Conclusion

In the last few years, we have learnt that the dynamic properties of MHC I allotypes determines their ability to self-select an optimal peptide cargo, with the self-editing ability being inversely correlated with the dependence on tapasin. The MHC I specific molecules tapasin and TAPBPR are likely to enhance peptide editing by decreasing the energy threshold required for MHC I molecules to adopt peptide exchange competent conformations, thereby increasing the frequency by which MHC I can sample its peptide cargo in a native conformation, so enhancing immunopeptidome editing. The recent TAPBPR-MHC crystal structures and NMR studies have revealed molecular features of these intermediate structures, and their dynamic attributes. While the function of the TAPBPR loop region that had been proposed to compete with peptide for binding to the MHC F pocket has been investigated in detail, the contribution of the other interaction surfaces has yet to be fully defined. Furthermore it is likely that allosteric communication between the multiple intermolecular contacts

is a key determinant: for example, stabilisation of the highly dynamic MHC I intermediate may involve the simultaneous engagement of the α1/α2 peptide binding domain and the α3 domain and β$_2$m, and their precise alignment within the MHC I:TAPBPR/tapasin complex.

Acknowledgements

Figure legends

Figure 1 TAPBPR binds to an open intermediate MHC I conformation

a) Model of the TAPBPR-H2-D^b crystal structure (PDB 5OPI) [22**], with TAPBPR coloured magenta, H2-D^b coloured green, β$_2$-microglobulin coloured blue. Selected structural features are labelled. The loop region containing a short α-helix varies in conformation in different studies: TAPBPR residues G24-R36 were modelled to adopt various non-helical loop conformations, which alternate between hovering above the groove and pointing away from the groove [25**]; or, as illustrated here, this loop region was modelled to include a short α-helix, which dived into the peptide binding groove [22**]; while this region was not resolved in the other TAPBPR structure [21**].

b) Comparisons of peptide-loaded (PDB 2F74) and TAPBPR-bound H2-Db (PDB 5OPI). The heavy chain is coloured green when TAPBPR-bound or yellow when unchaperoned. β$_2$-microglobulin is coloured blue when TAPBPR-bound or orange when unchaperoned. The panels show different views of the aligned structures, with regions differing in conformation being labelled.

Figure 2 MHC I peptide binding schematic

a) MHC I molecules transition between open and closed conformations, depending upon peptide occupancy [16]. Peptides bind to highly excited MHC I molecules that are in open conformations (labelled Mo*), with peptide binding dampening MHC I dynamics. Peptides may dissociate from open peptide-loaded MHC (MoP), or the peptide-loaded MHC I molecules transition to a stable, closed conformation, possessing low free energy (McP). The peptide binding affinity is most effectively sampled by MHC I molecules in their native, closed state (McP), with reopening of MHC I molecules loaded with low affinity peptides, and egress of MHC I molecules loaded with high affinity peptides through the secretory pathway. MHC I allotypes differ in their closing rates, with faster closing rates allowing the peptide sampling stage (McP) to be reached more quickly, leading to greater peptide exchange, than occurs for MHC I allotypes with slow closing rates.

b) MHC I allotypes differ in their ability to select an optimal peptide cargo without the assistance of tapasin. The intrinsic peptide selector function of MHC I allotypes is negatively correlated with their dependence upon tapasin, and is underpinned by the conformational plasticity of MHC I allotypes [16,17]. Tapasin-independent MHC allotypes (red curve), possessing high conformational plasticity, require less energy to transition between open and closed states, and consequently reach the predominant peptide sampling McP state more often, leading to high

self-selector ability, compared with tapasin-dependent allotypes (blue lines), possessing low conformational plasticity.

c) The MHC I specific molecules tapasin, or TAPBPR, bind to open MHC I intermediates (TMo*), generically enhancing MHC I assembly by stabilising the heavy chain:β_2-microglobulin heterodimer (chaperone function). The binding of tapasin, or TAPBPR, decreases the energy barriers for tapasin-dependent MHC I allotypes to transition between states (compare the red uncatalysed tapasin-dependent curve with the green tapasin catalysed curve), thus enhancing peptide filtering by catalysing the transition to the McP peptide sampling state. Furthermore, the binding of tapasin, or TAPBPR, to open peptide-loaded MHC I molecules (TMoP) may destabilise peptide binding, culminating in increased exchange of peptides (editing function): NMR studies have shown some MHC I regions, such as the α2-1 sub-helix, are highly dynamic when bound by TAPBPR, even when a peptide is bound [29**]; and that TAPBPR-binding allotypes have a more prominent dynamic signature than non-binders, suggesting TAPBPR selects highly dynamic pMHC intermediates to undergo peptide editing.

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** Outstanding interest.

The structure of the human peptide loading complex was resolved by single particle electron cryo-microscopy. This revealed two editing modules, linked by a synergistic network of intermolecular interactions, built above the TAP peptide transporter.

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21. Jiang J, Natarajan K, Boyd LF, Morozov GI, Mage MG, Margulies DH: **Crystal structure of a TAPBPR-MHC I complex reveals the mechanism of peptide editing in antigen presentation**. *Science* 2017, **358**:1064-1068.

** Outstanding interest.

One of two crystal structures of the TAPBPR-MHC complex. However, the short α-helical region that Thomas and Tampe modelled to enter the peptide binding groove was not resolved in this structure.

22. Thomas C, Tampe R: **Structure of the TAPBPR-MHC I complex defines the mechanism of peptide loading and editing**. *Science* 2017, **358**:1060-1064.

** Outstanding interest.

One of two crystal structures of the TAPBPR-MHC complex. In this structure, a short α-helix of TAPBPR was modelled entering the peptide binding groove, which the authors proposed might compete with peptide for binding to the empty F pocket.

23. McShan AC, Devlin CA, Overall SA, Park J, Toor JS, Moschidi D, Flores-Solis D, Choi H, Tripathi S, Procko E, et al.: **Molecular determinants of chaperone interactions on MHC-I for folding and antigen repertoire selection**. *Proc Natl Acad Sci U S A* 2019, **116**:25602-25613.

** Outstanding interest.

TAPBPR was shown to have broad MHC I allotype specificity in its chaperone-like function, but a restricted allotype specificity in its peptide editing function. The allotype specific peptide editing function was shown to follow binding of excited, highly dynamic peptide-loaded MHC molecules.

24. Hein Z, Uchtenhagen H, Abualrous ET, Saini SK, Janssen L, Van Hateren A, Wiek C, Hanenberg H, Momburg F, Achour A, et al.: **Peptide-independent stabilization of MHC class I molecules breaches cellular quality control**. *J Cell Sci* 2014, **127**:2885-2897.

25. McShan AC, Devlin CA, Morozov GI, Overall SA, Moschidi D, Akella N, Procko E, Sgourakis NG: **TAPBPR promotes antigen loading on MHC-I molecules using a peptide trap**. *Nat Commun* 2021, **12**:3174.

** Outstanding interest.

A variety of experimental techniques were used to show that a loop region of TAPBPR, that other groups had suggested entered the peptide binding groove and competed with peptide, hovers above the MHC peptide binding groove, where it slows peptide dissociation by acting as a peptide trap.

26. Ilca FT, Neerincx A, Hermann C, Marcu A, Stevanovic S, Deane JE, Boyle LH: **TAPBPR mediates peptide dissociation from MHC class I using a leucine lever**. *Elife* 2018, **7**.

* Special interest.

A loop region of TAPBPR, that other groups had suggested entered the peptide binding groove and competed with peptide, was shown to be important for recognition of pMHC I complexes, but not for recognition of unloaded, or sub-optimally loaded MHC I molecules, consistent with the findings of McShan et al 2019.

27. Margulies DH JJ, Natarajan K: **What have we learned about the dynamics of peptide loading from structures of TAP binding protein, related (TAPBPR)?** *The Journal of Immunology* 2018, **200**:99.10.
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* Special interest.

In this study, mutagenesis of a loop region of TAPBPR, that other groups had suggested entered the peptide binding groove and competed with peptide, did not prevent catalysis of peptide dissociation *in vitro*, suggesting that other parts of TAPBPR, besides this loop region, determine this functional property. The loop region was however, shown to help stabilise peptide-empty MHC I molecules, and was suggested to compete with peptides for binding to MHC.

29. McShan AC, Natarajan K, Kumirov VK, Flores-Solis D, Jiang J, Badstubner M, Toor JS, Bagshaw CR, Kovrigin EL, Margulies DH, et al.: **Peptide exchange on MHC-I by TAPBPR is driven by a negative allostery release cycle**. *Nat Chem Biol* 2018, **14**:811-820.

** Outstanding interest.

Nuclear magnetic resonance experiments showed TAPBPR binds conformationally dynamic regions of MHC I, and that some MHC I residues are highly dynamic when peptide empty molecules are bound by TAPBPR. TAPBPR transiently interacts with pMHC I complexes, with allosteric communication between non-overlapping peptide binding sites leading to dissociation of TAPBPR.

30. Ilca FT, Drexhage LZ, Brewin G, Peacock S, Boyle LH: **Distinct Polymorphisms in HLA Class I Molecules Govern Their Susceptibility to Peptide Editing by TAPBPR**. *Cell Rep* 2019, **29**:1621-1632 e1623.
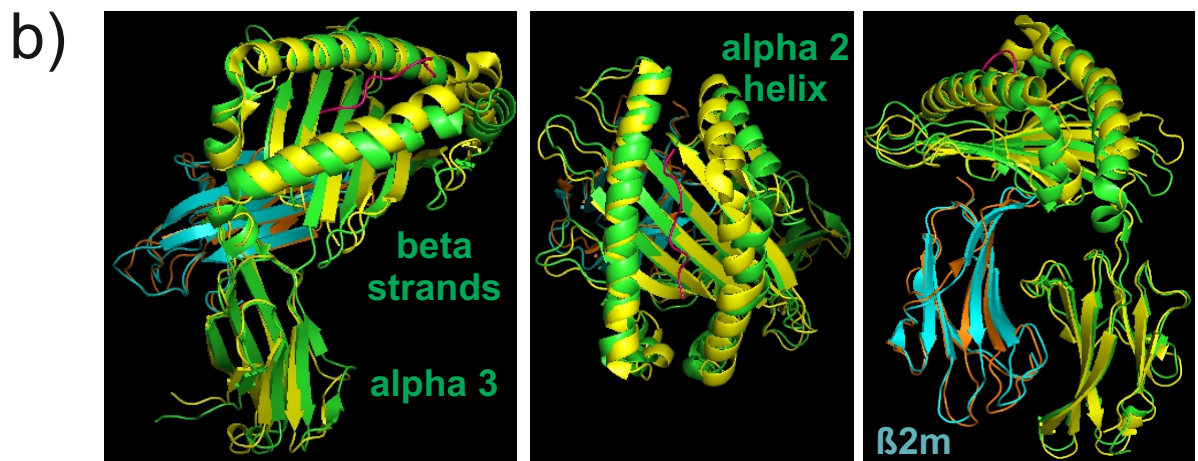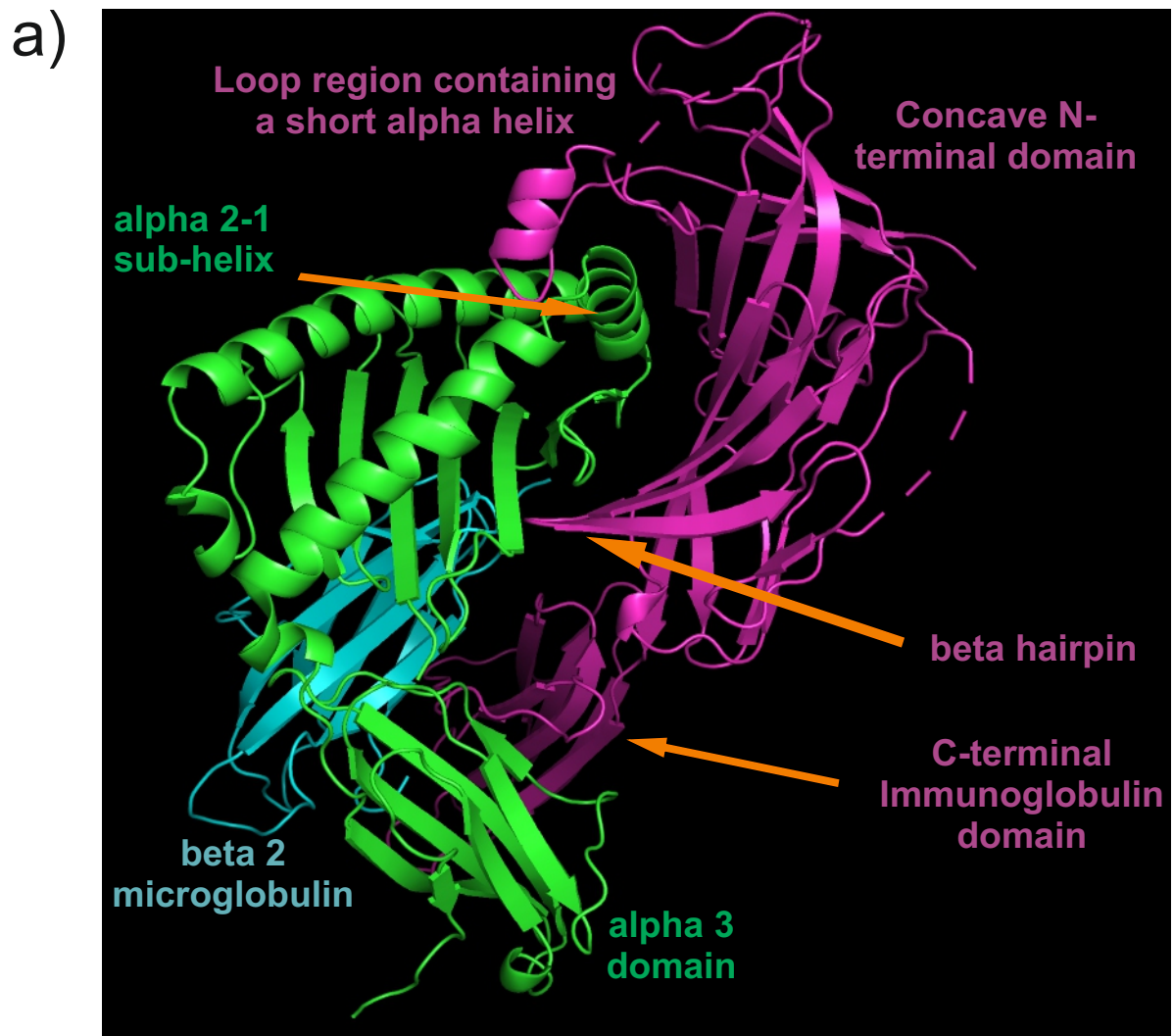
* Special interest.

The preference of TAPBPR to bind particular MHC I allotypes was investigated. Consistent with the broad chaperone-like function described by McShan et al 2019, TAPBPR bound a wider range of MHC I allotypes when incubated with whole cell lysates, compared with when TAPBPR was incubated with pMHC I complexes.
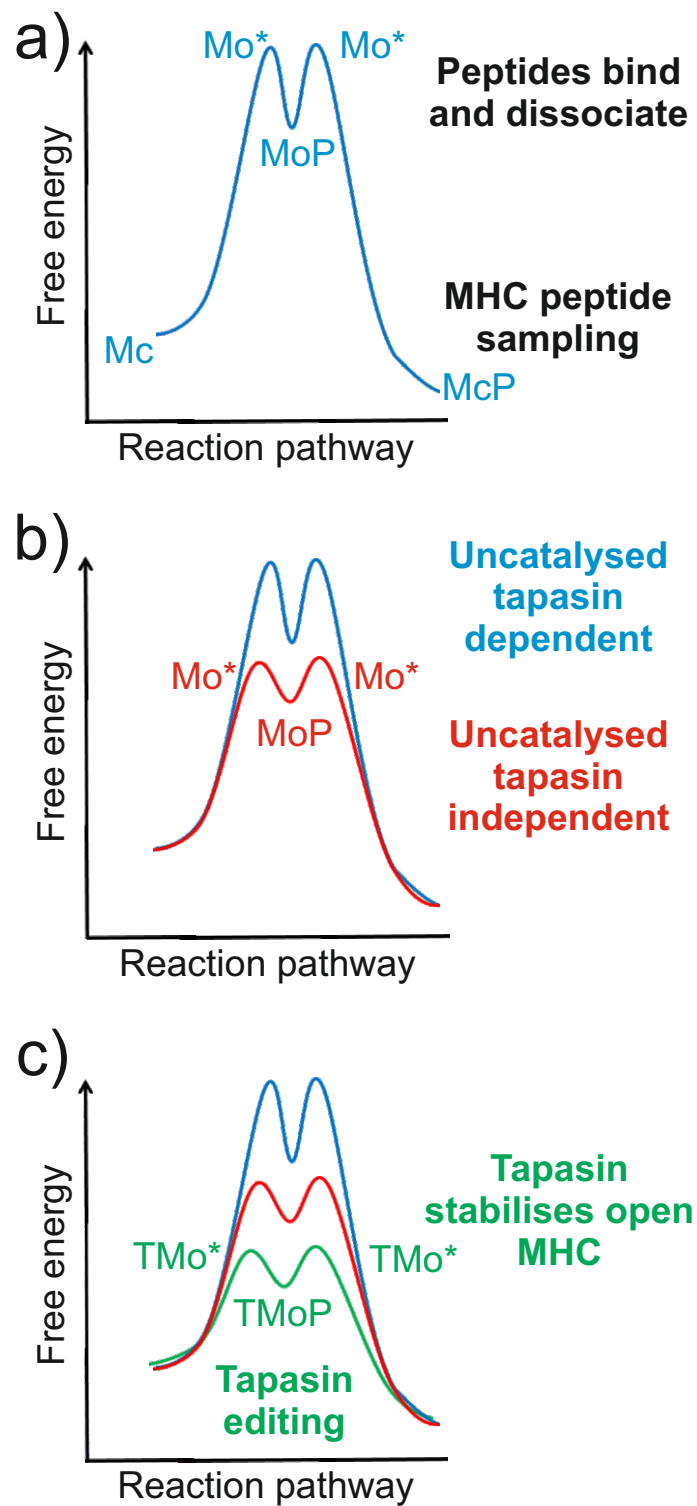
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure 1

a)

Loop region containing a short alpha helix

Concave N-terminal domain

alpha 2-1 sub-helix

beta hairpin

beta 2 microglobulin

C-terminal Immunoglobulin domain

alpha 3 domain

b)

alpha 2 helix

beta strands

alpha 3

ß2m

TAPBPR bound MHC   TAPBPR bound ß2m
Unchaperoned pMHC   Unchaperoned ß2m

Figure 2

a)

Free energy (y-axis) vs **Reaction pathway** (x-axis)

Mo*   Mo*
MoP
Mc
McP

Peptides bind and dissociate

MHC peptide sampling

b)

Free energy (y-axis) vs **Reaction pathway** (x-axis)

Mo*   Mo*
MoP

Uncatalysed tapasin dependent

Uncatalysed tapasin independent

c)

Free energy (y-axis) vs **Reaction pathway** (x-axis)

TMo*   TMo*
TMoP

Tapasin editing

Tapasin stabilises open MHC

Highlights:

- MHC I allotypes vary in their intrinsic ability to load peptides without assistance
- The self-editing ability of MHC I is negatively correlated with tapasin dependence
- This correlation can be explained by allelic variation in MHC I protein dynamics
- Recently the structure and dynamics of the TAPBPR-MHC complex have been analysed
- TAPBPR may provide a template for understanding tapasin-assisted peptide editing

Dear Professor Sadegh-Nasseri

We thank the editor and reviewer for their assessment of our manuscript. We have addressed the points individually below (shown in red text) and in the updated manuscript file.

Reviewer 1: The review by van Hateren & Elliott highlights the importance of protein dynamics in chaperone-assisted peptide editing during antigen processing and presentation. The review starts with a very brief summary of peptide presentation, before delving deeper into TAPBPR-mediated ligand exchange on MHC I and the impact of intrinsic flexibility in MHC I molecules on peptide selection.

This well-written review describes how differences in conformational plasticity of the various MHC I allotypes influence self-editing capabilities and facilitate peptide selection. For the most part, the authors succeed in condensing the current state of knowledge in the field. They also mention the different models and notions as well as some open questions. Thus, the review is suited for publication in Current Opinion in Immunology. However, van Hateren & Elliott should still address the following issues:

1) The classification of "Special interest" papers and publications of "Outstanding interest" might be reconsidered. In particular, references #2, #20, and #21 should also be marked as of "Outstanding interest" as these papers are the first to describe the structure of the fully-assembled native PLC and to elucidate the core principles of accelerated peptide editing, respectively.

Following the reviewers' comments, we have reconsidered the classification of the selected papers, and have now designated these papers as being of Outstanding interest. Please note that in response to the reviewer's point 5 (below) we have included an extra reference (new reference 3), and we have updated the numbering of the references accordingly.

2) The authors should avoid the word "cofactor" for tapasin and TAPBPR, as cofactors in biochemistry are typically non-protein molecules.

We have replaced the term "cofactor" in the revised manuscript.

3) Summary of reference #22: The authors should be very precise in stating which insights were provided by which publication. The fact that tapasin and TAPBPR possess dual functionality - as editor and chaperone - had already been shown before reference #22 was published.

 We have amended the text accordingly.

4) Summary of reference #27: The authors might want to add that this work also demonstrates that the loop region in TAPBPR contributes to the stabilization of chaperoned MHC I.

We have amended the text accordingly.

5) p.1, penultimate paragraph: The authors may consider removing "and (Fisette, O personal communication) as all information mentioned in this citation has been disclosed and published in reference #2. Indeed, all previous work based on MD simulations was misled in using rigid body fitting; and reference #2, #20, and #21 demonstrated the concept of plasticity and elaborated the structural rearrangement in MHC I chaperone complexes.

We have included an extra reference in our review: reference 3 Fisette et al, 2020, which complements the findings of reference 2 (Blees et al 2017). The work described in Fisette et al 2020 was not published when we initially submitted our manuscript in August 2020, and was therefore referred to as a personal communication.

Minor points:

1) p.1, last paragraph: The common spelling for UGGT is "UDP-glucose:glycoprotein glucosyltransferase" (note the colon).

Corrected.

2) p.2: "show that TAPBPR makes" instead of "show TAPBPR makes"

Corrected.

3) p.2: phrases like "β2-microglobulin (β2m) domains." and "β3 and β2m domains" sound as if β2m is a domain. As β2m is a self-sufficient protein, this should be re-phrased.

Corrected.

4) p.4: "(tapasin-independent)" and "(tapasin-dependent)" instead of "(tapasin independent)" and "(tapasin dependent)"

Corrected.

5) p.5: The authors should replace the word "adduct" with "complex, as "adduct" is a rather strictly defined term in synthetic chemistry.

Corrected.

6) p.5: The figure legends use capital letters for the subpanels, while the figures are labeled with lowercase letters.

Corrected.

7) p.5, figure legend 1b): "TAPBPR-bound" instead of "TAPBPR bound"

Corrected.

8) Fig. 1b): The authors might want to change "b2m" to "⬚2m"

Corrected.