Structural mechanism of tapasin-mediated MHC-I peptide loading in antigen presentation

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Loading of MHC-I molecules with peptide by the catalytic chaperone tapasin in the peptide loading complex plays a critical role in antigen presentation and immune recognition. Mechanistic insight has been hampered by the lack of detailed structural information concerning tapasin–MHC-I. We present here crystal structures of human tapasin complexed with the MHC-I molecule HLA-B*44:05, and with each of two anti-tapasin antibodies. The tapasin-stabilized peptide-receptive state of HLA-B*44:05 is characterized by distortion of the peptide binding groove and destabilization of the β2-microglobulin interaction, leading to release of peptide. Movements of the membrane proximal Ig-like domains of tapasin, HLA-B*44:05, and β2-microglobulin accompany the transition to a peptide-receptive state. Together this ensemble of crystal structures provides insights into a distinct mechanism of tapasin-mediated peptide exchange.

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component of the PLC. Thus, differences between the expression and function of TAPBPR and tapasin and allelic differences among MHC-I molecules with respect to tapasin dependence\(^{10,31,32}\) demand further exploration of the tapasin–MHC-I interaction. Specific unresolved details include the function of several loops of tapasin inferred from studies of TAPBPR\(^27\).

Here, we describe a crystal structure of a tapasin–HLA-B*44:05–β₂m complex (determined at 3.1 Å resolution) as well as structures of tapasin complexed with Fab fragments of two well-characterized monoclonal antibodies. These structures complemented by binding studies and NMR data, reveal details of tapasin function, emphasize the dynamic nature of the tapasin–MHC-I interaction, highlight differences and similarities to TAPBPR, and substantiate a revised mechanistic model.

Results

Interaction of tapasin with HLA-B*44:02 and HLA-B*44:05

HLA-B*44:02 and HLA-B*44:05 (B44:02 and B44:05 hereafter), represent tapasin-dependent and tapasin-independent MHC-I molecules, respectively, based on their cell surface expression in tapasin-deficient cell lines\(^{10,31,32}\). The two allelomorphs differ at a single residue in the F pocket region, Asp116 in B44:02 and Tyr116 in B44:05. When loaded with the same 9mer peptide (EEFGRCSFS) and NMR data\(^22,33,34\) suggest that increased dynamics of the F pocket region favor tapasin binding\(^35\), we asked whether increasing F pocket flexibility of the weaker binding B44:05 allelomorph would improve its affinity for tapasin. Using an approach previously applied to the TAPBPR–MHC-I interaction\(^28\), we replaced Thr73 in the α₁ helix of the B44:05 heavy chain with Cys and refolded it with human β₂m and a C-terminally truncated 6mer variant (EEFRC) of the original 9mer peptide. The F pocket is loosely conformed by inclusion of a GlyLeu dipeptide in the folding reaction\(^36\).

This strategy permits greater dynamics of the C-terminal portion of the peptide binding groove potentially allowing enhanced interactions with tapasin. As shown in Fig. 1b, c the kinetics of the interaction of tapasin with B44:05-T73C–6mer revealed an ~4-fold higher affinity than with the parental B44:05–9mer. This result is consistent with the view that F pocket dynamics influence tapasin interaction. Efforts to produce B44:02-T73C–6mer molecules in good yield were unsuccessful.

Structure of the Tapasin–B44:05-T73C–6mer complex

We obtained crystals of a complex of tapasin with B44:05-T73C–6mer (Table 1), and solved the structure by molecular replacement as described in Methods. The structure of the complex (Fig. 2) was refined to Rwork/Rfree of 28.4/30.8% (Table 1). For comparison we determined the crystal structures of unliganded B44:05-T73C–6mer (Supplementary Fig. 1a) and B44:05-T73C–9mer (Supplementary Fig. 1b) as well as tapasin bound to two well-characterized antibody Fab fragments.

Tapasin binds to a broad surface of the external aspect of B44:05 including the α₂-1 helix, the α₃ domain, and β₃m (Fig. 2a–c). The interface buries an area of 2494 Å², of which 1984 Å² is contributed by the peptide binding groove potentially allowing enhanced interactions with tapasin. The contacts of tapasin to B44:05 are mediated by loops that bind the MHC-I heavy chain and 510 Å² by β₃m domains. The tapasin N-IgV domain forms a glove-like surface that addresses the MHC-I platform and cradles the α₂-1 helix and supporting β7 and β8 strands and connecting loops (Fig. 2a and Supplementary Fig. 2). Superposition of the N-IgV domain of tapasin–B44:05 on the same preparations of tapasin and MHC-I molecules of the indicated interactions are shown below each panel as mean and as scatter plots. p values, calculated by two-tailed unpaired t-test as implemented in GraphPad Prism, are indicated. These data are representative of similar measurements performed with multiple preparations over a span of two years. Source data are provided as a source data file.
domain of tapasin–ERp57 (PDB 3FSU) reveals movement of the IgC domain (Supplementary Fig. 2c).

Several tapasin loops interact with the MHC-I platform: that bounded by residues Glu11 and Lys20, of which amino acids 12 to 19 are not visualized in electron density; a long strand/loop that extends from residues Glu72 to Lys84; and the hairpin loop from Glu189 to His195 (Fig. 3a–e). Superposition of the platform domain of bound and free B44:05-T73C–6mer reveals deformations of the groove of the tapasin-bound state that are incompatible with peptide binding (Fig. 3f, g). Specifically, the α1-helix is drawn away from the groove by about 3 Å, and the floor of the groove is displaced downward by 3 Å (Fig. 3a, b, f, g), leaving the peptide binding groove of the tapasin–B44:05-T73C–6mer complex without electron density representing peptide or the GlyLeu dipeptide (Supplementary Fig. 3b) employed in the refolding of B44:05-T73C–6mer. Electron density for peptide and dipeptide was clearly discerned in the unliganded B44:05-T73C–6mer (Supplementary Fig. 3a).

For the Glu11-Lys20 loop in tapasin–B44:05, where no intervening electron density is observed (but which is clearly visualized in tapasin complexed to the Fab of PaStal (Supplementary Fig. 3c), Glu11 interacts with Ile142 of the α2-1 helix of B44:05 (Fig. 3c). Lys20 is 3.9 Å from the O6i of the conserved Asn86 of B44:05, but still permits space for the N-linked glycan common to all MHC-I molecules (Supplementary Fig. 3d). The location of Glu11 and Lys20 precludes interaction of the connecting loop with the floor of the peptide-binding groove at the F pocket ruling out a scoop-like mechanism for displacing bound peptide. Additionally, the positions of B44:05 residues Thr80, Lys146, Tyr84, and Ile142 in the tapasin–B44:05 complex (see figs.) prevent access of the loop to the F pocket. Moreover, the distance from the side chain of either Glu11 or Lys20 to Ile95 on the floor of the groove is at least 16 Å. The structure is consistent with a hovering peptide trap model based on NMR studies on the HLA-A*02–TAPBPR complex. Additionally, Tyr84 of B44:05 directs its side chain towards the peptide binding groove, blocking access of the tapasin Glu11-Lys20 loop

Table 1 | X-ray data collection and refinement statistics

| PDBID    | B44:05-T73C-9mer 7TUC | B44:05-T73C-6mer 7TUC | Tapasin-B44:05-T73C-6mer 7TUE | Tapasin–PaStal (Fab) 7TU | Tapasin–PaSta2 (Fab) 7TUG | PaSta2 (Fab) 7TUH |
|-----------|------------------------|------------------------|-------------------------------|------------------------|------------------------|-------------------|
| Data collection |
| Space group | P2₁,2₁,2₁ | P2₁,2₁,2₁ | P6₁ | P2₁ | C22₁ | P2₁ |
| Cell dimensions |
| a, b, c (Å) | 50.75, 82.58, 110.89 | 49.17, 79.76, 107.21 | 116.87, 116.87, 131.14 | 117.42, 68.99, 142.98 | 101.09, 168.82, 108.66 | 49.10, 62.13, 128.46 |
| α, β, γ (°) | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 120.0 | 90.0, 108.42, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 |
| Resolution (Å)* | 38.70-1.25 (1.30–1.25) | 39.88-1.45 (1.50–1.45) | 55.03-3.11 (3.22–3.11) | 47.45-2.80 (2.69–2.80) | 46.04-3.90 (4.04–3.90) | 55.93-2.30 (2.38–2.30) |
| Rwork or Rmerge | 0.033 (0.413) | 0.071 (0.943) | 0.337 (0.978) | 0.087 (1.326) | 0.107 (1.416) | 0.109 (1.933) |
| /() | 11.6 (1.4) | 11.6 (1.1) | 3.2 (0.9) | 10.4 (1.0) | 7.5 (0.9) | 8.6 (0.78) |
| Completeness (%) | 95.4 (80.3) | 99.0 (97.0) | 99.8 (99.6) | 97.7 (95.3) | 95.2 (92.7) | 99.0 (98.8) |
| Redundancy | 2.0 (2.0) | 4.8 (4.1) | 5.8 (6.0) | 3.8 (3.7) | 3.5 (3.4) | 5.8 (5.8) |
| Rmerge | 0.033 (0.413) | 0.036 (0.509) | 0.149 (0.435) | 0.052 (0.821) | 0.065 (0.851) | 0.049 (0.866) |
| CC1/2 | 0.996 (0.555) | 0.998 (0.553) | 0.940 (0.587) | 0.998 (0.483) | 0.998 (0.477) | 0.997 (0.669) |
| Estimated twin fraction | 0.0 (none) | 0.0 (none) | 0.307 (h,-h,-k,-l) | 0.0 (none) | 0.0 (none) | 0.460 (h,-k,-l) |
| Refinement |
| Resolution (Å)* | 38.70-1.25 (1.30–1.25) | 39.88-1.45 (1.50–1.45) | 55.03-3.11 (3.22–3.11) | 47.45-2.80 (2.69–2.80) | 46.04-3.90 (4.04–3.90) | 55.93–2.30 (2.38–2.30) |
| No. reflections | 123417 (19335) | 74688 (3734) | 18395 (920) | 52731 (1998) | 8234 (409) | 34308 (1716) |
| Rwork/Rmerge (%) | 18.0/19.5 (26.5/26.1) | 19.2/21.3 (29.0/32.0) | 28.4/30.8 (31.6/34.2) | 23.7/27.6 (38.0/42.9) | 28.2/33.2 (35.1/38.3) | 21.8/24.0 (29.6/33.0) |
| No. atoms | 3650 | 3752 | 5630 | 11290 | 5304 | 6606 |
| Protein | 3172 | 3155 | 5630 | 11209 | 5290 | 6466 |
| Water + ligands | 454 + 24 | 583 + 14 | 0 | 53 + 28 | 14 | 140 |
| B-factor Wilson/Ave | 9.5/15.5 | 18.3/24.5 | 80.3/71.8 | 51.2/65.4 | 67.6/78.2 | 41.3/41.7 |
| Protein | 3172 | 3155 | 5630 | 11209 | 5290 | 6466 |
| Water + ligands | 454 + 24 | 583 + 14 | 0 | 53 + 28 | 14 | 140 |
| Ramachandran |
| bond length (Å) | 0.004 | 0.009 | 0.002 | 0.003 | 0.002 | 0.002 |
| bond angle (°) | 0.81 | 0.93 | 0.66 | 0.66 | 0.52 | 0.54 |
| Molprobity |
| clashscore | 1.92 | 7.29 | 12.7 | 8.8 | 3.75 | 7.03 |

(*Values in parenthesis are for highest resolution shell, †Values in parenthesis are the number of reflections for Rmerge)
The second B44:05-binding loop of tapasin, Glu72 to Lys84, a structural feature unique to tapasin and absent in TAPBPR, interacts with the α2-1 helix (including side chains Gln141, Arg145, and Glu148) of B44:05 (Fig. 3b, d). Residues Glu72 and Ser74 approach the α2-1 helix residues Ile142 and Arg145 (Fig. 3d), contributing to the displacement of the α2-1 helix. The third major tapasin loop, Gln189 to His195, protrudes beneath the β-sheet floor of the peptide binding groove to contact platform strands β6 and β7, also directing His190 towards Glu212 of the B44:05 α3 domain (Fig. 3e). In addition to these major interactions involving tapasin’s N–IgV domain, the IgC domain engages the membrane proximal domains of MHC-I through contacts with 16 residues of the α3 domain and 8 of β2m (Fig. 2d, f, Supplementary Table 1). This extensive interaction with β2m is of particular interest as it may weaken the β2m-dependent scaffolding of the peptide binding domain floor and prevent the well-documented cooperativity between peptide and β2m binding.

Consistent with this view, electron density corresponding to the 6mer peptide is lacking in the B44:05 groove in the tapasin complex (Supplementary Fig. 3b). The tapasin–B44:05-T73C–6mer complex provides a structural explanation for the behavior of mutants of both MHC-Hi and tapasin deleterious to the tapasin–MHC-I interaction (Supplementary Table 2).

**Fig. 2 | Overall structure of the Tapasin–B44:05–6mer complex.** a Ribbon representation of Tapasin (magenta), B44:05 heavy (α) chain (blue), and β2m light chain (lime). b Similar to a, but with B44:05 α and β2m in surface display. c Tapasin in surface display. d Interface residues of B44:05 α and β2m in surface display, residues colored and numbered. e Interface residues of Tapasin as indicated. f Rotated view of the membrane proximal trimer of B44:05 α3 domain (blue), β2m (lime), and Tapasin IgC domain (magenta). BSA Buried surface area.

**Structures of tapasin complexed with Fabs of PaSta1 and PaSta2**

To obtain additional mechanistic insight into the contribution of tapasin structure to function, we solved complexes of tapasin bound to Fab fragments of two well-characterized anti-tapasin monoclonal antibodies, PaSta1 and PaSta2 (Table 1, Fig. 4). PaSta1 binds all tapasin molecules, whether free or bound to MHC-I. In contrast, PaSta2 is unable to bind MHC-I-associated tapasin, indicating that it binds in the MHC-I binding site. Consistent with prior immunoprecipitation data, PaSta1 and PaSta2 clearly bind to distinct sites on tapasin when evaluated with purified proteins by SPR (Supplementary Fig. 4). Both antibodies bind tapasin with $K_D$ of 1 to 2 nM (Supplementary Fig. 4c, d). PaSta1 binds tapasin atop the N–IgV domain (Fig. 4a). This surface remains accessible in the tapasin–ERp57 complex (Fig. 4d). By contrast, PaSta2 contacts tapasin in the palm of the glove where MHC-I binds (Fig. 4b–d), interacting with both the N–IgV and the IgC domains at the tapasin hinge that joins the N–IgV domain to IgC (Fig. 4b). Although PaSta1 interacts with tapasin largely through the CDR3 of its H chain, PaSta2 predominantly exploits its L chain CDR1 for the interaction (Supplementary Figs. 5, 6). Comparison of these two Fab-complexed structures with that of tapasin–ERp57 (PDB 3F8U) and the tapasin–B44:05 structure reported here provides an opportunity to examine domain movements and loop flexibility in tapasin.

(Fig. 3c). The second B44:05-binding loop of tapasin, Glu72 to Lys84, a structural feature unique to tapasin and absent in TAPBPR, interacts with the α2-1 helix (including side chains Gln141, Arg145, and Glu148) of B44:05 (Fig. 3b, d). Residues Glu72 and Ser74 approach the α2-1 helix residues Ile142 and Arg145 (Fig. 3d), contributing to the displacement of the α2-1 helix. The third major tapasin loop, Gln189 to His195, protrudes beneath the β-sheet floor of the peptide binding groove to contact platform strands β6 and β7, also directing His190 towards Glu212 of the B44:05 α3 domain (Fig. 3e). In addition to these major interactions involving tapasin’s N–IgV domain, the IgC domain engages the membrane proximal domains of MHC-I through contacts with 16 residues of the α3 domain and 8 of β2m (Fig. 2d, f, Supplementary Table 1). This extensive interaction with β2m is of particular interest as it may weaken the β2m-dependent scaffolding of the peptide binding domain floor and prevent the well-documented cooperativity between peptide and β2m binding.

Consistent with this view, electron density corresponding to the 6mer peptide is lacking in the B44:05 groove in the tapasin complex (Supplementary Fig. 3b). The tapasin–B44:05-T73C–6mer complex provides a structural explanation for the behavior of mutants of both MHC-Hi and tapasin deleterious to the tapasin–MHC-I interaction (Supplementary Table 2).

**Fig. 2 | Overall structure of the Tapasin–B44:05–6mer complex.** a Ribbon representation of Tapasin (magenta), B44:05 heavy (α) chain (blue), and β2m light chain (lime). b Similar to a, but with B44:05 α and β2m in surface display. c Tapasin in surface display. d Interface residues of B44:05 α and β2m in surface display, residues colored and numbered. e Interface residues of Tapasin as indicated. f Rotated view of the membrane proximal trimer of B44:05 α3 domain (blue), β2m (lime), and Tapasin IgC domain (magenta). BSA Buried surface area.
Comparison of multiple structural models reveals dynamic domains and loops

We report here new structural models of tapasin based on X-ray diffraction data: tapasin–B44:05 and also two complexes with the antibodies PaSta1 and PaSta2. In addition to the experimentally determined model of tapasin–Erp5720, we also consider the computationally derived model of unliganded tapasin taken from the AlphaFold Database45. Comparison of these five structural models highlights the flexibility of the IgC relative to the N–IgV domain as tapasin interacts with different partners. The AlphaFold model, based largely on 3F8U, the tapasin–Erp57 heterodimer, serves as our unliganded standard. We compare the various models based on superposition of the N–IgV domains to visualize the potential for movement of the IgC domain, which is displaced by as much as 14.7 Å (for tapasin–B:44:05), 8.9 Å (for tapasin–Erp57) and 10.9 Å (for tapasin–PaSta2)(Fig. 3b). These apparent translations are accompanied by IgC rotation of 16 to 18 degrees.

In addition, the multiple snapshots show that several loops vary among the different structures, revealing their potential for dynamic movement and adaptability when interacting with ligands. The first loop of interest, residues Glu11-Lys20 (Fig. 3c), is almost completely modeled in the complex with PaSta1 where one of the copies in the asymmetric unit shows continuous electron density (Supplementary Fig. 3c). This loop of tapasin is not in direct contact with the PaSta1 Fab. Superposition of tapasin from the PaSta1 complex on the tapasin–B44:05-T73C-6mer structure provides an illustration of the likely location of the Glu11-Lys20 loop when tapasin interacts with MHC-I. The Glu11-Lys20 loop of tapasin (Fig. 6a, c) is shorter than the Lys21-Arg36 loop of TAPBPR, remains suspended over the binding groove, and the central Leu18 is ~16 Å from the floor of the F pocket. These loop residues of tapasin interact mainly with α2−1 and α1 helices of B44:05 (Fig. 6a, c). Tyr84, Ile143, Lys146, Thr80 and Glu76 of B44:05 form a barrier that restrains the tapasin loop from direct access to the F-pocket (Fig. 6c). In the tapasin–B44:05 structure, the side chain of
Tyr84 remains directed toward the F pocket of the peptide binding groove, and does not interact with tapasin, in contrast with the Tyr84-Glu102 interaction consistently seen in TAPBPR structures.27,28 These structural details are consistent with a peptide-trap mechanism30 and appear incompatible with the scoop-like mechanism originally proposed for the longer TAPBPR loop27 and extended to tapasin23,37. Examination of the AlphaFold model of tapasin, which is complete for the Glu11-Lys20 loop, leads to the same conclusion (Fig.6d).

The second region for comparison is the strand/loop involving β strand 4, loop S74 to Leu104 and β5 of tapasin. As compared to TAPBPR, this loop is some 13 residues longer (Fig. 6e), and makes several critical interactions with residues of the MHC-I α2-1 helix, including H-bonds from tapasin Glu72 and Ser74 to B44:05 Arg145 and additional contacts between Ser82 and Arg151 of the MHC α2-1 helix, and Ser82 and Ser131 of the MHC-I β8 strand. The tapasin structure also reveals the contact of Glu72 with MHC-I Arg145. By contrast, the homologous Glu102 of TAPBPR contributes to the reorientation of MHC-I residue Tyr84.

The third region for comparison is the hairpin Glu189 to His195 that dives under the MHC-I β-sheet floor of the peptide binding groove and destabilizes the bound peptide (Fig. 3e). Superposition of this loop (189-195) from all available tapasin structural models (Fig. 6f) reveals considerable plasticity, depending on the particular molecule bound to tapasin.

The fourth major region that shows conformational flexibility and clearly influences the structural integrity of the peptide binding groove is where the tapasin IgC domain interacts with both the MHC-I α3 domain and the β2m subunit (Fig. 2f, Fig. 6g–i). This interaction generates a trimer of the three Ig-like domains due to the movement of the IgC domain of tapasin by 16.3 Å (Fig. 6f), of the α3 domain by 9.1 Å (Fig. 6h), and of the β2m subunit by 9.8 Å (Fig. 6i). This distortion of the β2m interaction with the MHC-I α chain contributes to the displacement of the floor of the peptide binding groove (Fig. 3d). A movie to show these domain movements was generated by morphing these structural models (Supplementary Movie 1).

To evaluate the distortion of the β2m–B44:05 α chain interaction due to tapasin in a solution environment, we recorded NMR TROSY spectra of selectively methyl-labelled β2m, in free form and complexed with the B44:05–6 mer, as well as tapasin–B44:05–6 mer (Supplementary Fig. 7). The NMR spectra reveal significant chemical shift differences (CSDs) in β2m upon assembly with the B44:05 α chain, as well as line broadening upon addition of tapasin to B44:05–β2m. The substantial CSDs of β2m residues Leu54 and Trp60 upon addition of tapasin are consistent with the lack of mobility of the 55 to 62 loop of
Line broadening of specific resonances on exposure of B44:05–β2m to tapasin likely indicates exchange between B44:05–β2m and a tapasin-bound form at an intermediate (microseconds to milliseconds) NMR timescale (Supplementary Fig. 7e). Consistent with our crystallographic analysis, we observed a significant broadening for the methyl resonance of Leu54 located beneath the floor of the MHC-I groove induced by interactions with tapasin, suggesting a plausible mechanism for weakening peptide binding affinity through perturbations of the β2m–B44:05 α2 domain interface.

**Discussion**

This analysis of the interaction of tapasin with an MHC-I molecule adds considerable insight into the structural mechanisms that underlie peptide loading in the PLC. This extends our knowledge from multiple MD and structural studies of tapasin and the PLC as well as of TAPBPR to reveal a mechanism for tapasin-mediated peptide exchange which, although broadly comparable to that of TAPBPR, exploits structural features that are unique to tapasin. Several critical surfaces of tapasin engage MHC-I molecules both to stabilize peptide-receptive configurations and to destabilize peptide-bound ones. Tapasin exploits two major regions of the molecule for distinct purposes – the tapasin N–IgV unit grasps the peptide-binding domain of the MHC via the α2-1 helix and β-sheet floor, and the membrane proximal IgC domain repositions the MHC-I α3–β2m orientation. The N–IgV interaction may be visualized as a hand grasping the MHC-I platform domain with the index and middle fingers (connected by loop Glu11-Lys20) lying atop the α2-1 and α1 helices respectively (Supplementary Fig. 8a, d). Distinct from TAPBPR, this loop, visualized incompletely in the tapasin–MHC-I complex, but continuous in the tapasin–PaSta1 complex and in the AF tapasin model, is unable to approach the floor of the F pocket, thus favoring a trap model. The center of the palm (Supplementary Fig. 8b) (loop Glu72 to Lys84) engages the α2-1 helix, exploiting the strand/loop structure unique to tapasin, and is not found in TAPBPR. The thumb (loop Gln189-His195), exerting a function similar to that of TAPBPR, exploits structural features that are unique to tapasin.
of the equivalent loop of TAPBPR, probes the MHC-I floor of β strands 6, 7, and 8 (Supplementary Fig. 8b). The heel of the palm (tapasin IgC domain) pushes the MHC-I α3 domain and β2m resulting in distortion similar to but quantitively far greater than that observed in the TAPBPR–MHC-I structures27,28 (Supplementary Fig. 8c). On engagement of high affinity peptide, tapasin reverts to an unliganded state, and MHC-I, now compact and stably loaded, is released (Supplementary Fig. 8e).

The X-ray structures we describe here address several molecular states of an MHC-I molecule (B44:05) and of its associated chaperone (tapasin) in the course of PLC stabilization and peptide acquisition in the ER (Fig. 7). Immunoprecipitation studies indicated early association with β2m and calnexin and exposure of a unique epitope of the α1 domain 3,10 helix prior to integration into the full PLC49,50 (Fig. 7b, state 1). The structure of the B44:05-6mer (Fig. 7b, state 2), (PDB 7TUD), represents one likely substrate for tapasin binding. A tapasin conformation prior to MHC-I engagement (Fig. 7b, state 3) is observed in tapasin–B44:05–ERp57 (PDB 3F8U) and our two Fab complex structures (PDB 7TUF, 7TUG). The X-ray structure of the complex of tapasin–B44:05 captures a putative metastable, peptide receptive B44:05 conformation (Fig. 7b, state 4), (PDB 7TUE). This conformation in the tapasin–B44:05 complex is remarkable for the structural differences from the B44:05-6mer structure noted above–namely, the lack of detectable bound peptide, the distortion of the F pocket region, the movements of the α2-1 helix and distortion of the binding groove, and reorientation of β2m. On binding a high affinity peptide (Fig. 7b, state 5)(7TUC), the mature conformation of the pMHC-I complex is released. These structural snapshots support a refined model of the steps that contribute to peptide acquisition by MHC-I molecules in the PLC, and may contribute to an understanding of the variable tapasin

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**Fig. 6 | Loop movements and domain flexibility of tapasin.** Four X-ray determined structures (3F8U, 7TUE, 7TUF, 7TUG) and the AlphaFold model (AF-015533) were superposed. **a–d** illustrate the β1 and β2 strands of tapasin (Trp8 to Leu26) lying atop the HLA-B44 α2-1 and α2 helices (a, b, c, and d). a side view. b top view. c illustrates β1 and β2 (Trp8 to Leu26) from the tapasin–PaSta1(7TUF) complex. d AlphaFold model. As shown in the sequence alignment, only the tapasin structure from the PaSta1 complex (7TUF) and the AlphaFold computational model reveal a complete backbone through this region. The β1 and β2 strands clearly restrain (via Y84, T80, I142, and K146) the loop from descending into the B44:05 F pocket, and instead the loop provides a lid-like function. e shows the superposition of structures from Glu72 to Leu104 of tapasin for 7TUE, SOPI (TAPBPR-MHC), SWER (TAPBPR-MHC) and AF models of TAPBPR and tapasin. f superposition of β9 (Gly183-Arg188) and β10 (Leu196-Ala200) strands, and the hairpin loop (Gln189-His195) of the same structures. g, h, and i illustrate displacement of tapasin IgC, B44 α3, and β2m movement respectively. For comparisons shown in h and i, α1 residues 54-84 of unliganded B44:05 (7TUD) were superposed onto the same residues of tapasin–B44:05-T73C (7TUE) and displacement of α3 (h) and of β2m (i) was measured.
dependencies of HLA allelomorphs, informing our general understanding of antigen presentation.

**Methods**

**Plasmids**

DNA encoding human tapasin (UniProtKB–O15533) encompassing the signal peptide, residues Gly1 to Glu381 of the lumenal domain (numbered as in), a C cleavage site, a BirA biotinylation sequence, a 6X HIS tag, and a termination codon was inserted between EcoRI and ApaI sites of pMT/V5-His A vector (ThermoFisher Invitrogen, Carlsbad, CA, USA). A bacterial expression plasmid encoding HLA-B*44:05 was kindly provided by Dr. John Altman from the collection of the NIAID/NIH Tetramer Core Facility. A stop codon was inserted following Trp274 to prevent inclusion of the BirA biotinylation sequence in the translated protein. Additionally, the codon encoding Thr73 was replaced with a cysteine codon to yield a T73C mutant for refolding with cysteine-containing truncated peptides. Mutagenesis was performed with the QuikChange Lightning Multisite mutagenesis kit (Agilent, Santa Clara, CA, USA). Plasmid encoding human β2m was described previously.

**Protein expression and purification**

The tapasin expression construct was transfected into Drosophila Schneider 2 (S2) cells grown in Insect X-Press medium (Lonza, Walkersville, MD, USA) together with plasmids encoding puromycin and blasticidin resistance. Cells surviving selection on culture medium containing puromycin and blasticidin were expanded, and protein expression was induced with 1 mM CuSO4 for three to four days. Tapasin was purified from the culture media by affinity chromatography on a chelate agarose column (ABT, Madrid, Spain) followed by size exclusion chromatography on Superdex 200 (Cytiva, Uppsala, Sweden) in TRIS buffered saline (10 mM TRIS pH 8, 150 mM NaCl). SEC-purified tapasin was further purified by anion exchange chromatography on a Shodex QA-825 column developed with a gradient of 50 mM to 1 M NaCl in 25 mM TRIS pH 8. The BirA and 6X His sequences were removed by treatment with 3 C protease (Pierce, Rockford, IL, USA) overnight at 4 °C prior to crystallization trials.

**MHC-I (hβ2m HLA-B*44:05-T73C constructs)**

Bacterial expression and refolding of MHC-I was as described previously. Parental HLA-B*44:05 was refolded with hβ2m and the peptide EEGRAGSF, (DP α (46–54)). HLA-B*44:05-T73C was refolded with hβ2m and the truncated peptide EEGRCS with the inclusion of 2 mM Gly-Leu dipeptide (Bachem, Switzerland) during refolding, dialysis, and purification to stabilize MHC-I molecules. In addition, the parental HLA-B*44:05 was refolded with hβ2m and the full-length DP α peptide.

**Fig. 7 | Structures represent distinct states of MHC-I and tapasin in the peptide loading pathway.** a cartoons representative of (left to right) MHC-I bound to low affinity (LA) peptide, MHC-I in complex with tapasin (peptide receptive (PR) state), and MHC-I bound to high affinity (HA) peptide. b examples of distinct states of MHC-I–β2m and tapasin: 1, MHC-I immediately after biosynthesis, prior to engaging any peptide (represented by molecules immunoprecipitated by 64-3-749, which identifies the unfolded conformation of the α1 domain 3.10 helix); 2, MHC-I in conformed state bound to low affinity peptide (represented by our structure of HLA-B44:05-6mer (7TUD)); 3, tapasin in state not yet engaged with MHC-I (represented by tapasin complexed with ERp57 (3F8U), with PaSta1 (7TUF), or with PaSta2 (7TUG)); 4, the MHC-I–tapasin complex (represented by 7TUE); 5, MHC-I bound to high affinity peptide, released from tapasin complex (represented by 7TUC). c cartoon description of the tapasin–MHC-I complex as part of the PLC, based on1.
Antibodies
The expressed heavy (H) and light (L) chains of the anti-tapasin antibody producing hybridomas PaSta1 and PaSta224,25 were identified by RT-PCR amplification and sequencing of cDNA (Creative Biologics, Shirley, NY, USA). Genes encoding antibody H and L chains were cloned into pCDNA3.1 for secreted expression. H and L chain-encoding plasmids were mixed together at a ratio of 1:2, and transfected into Exp293F cells following manufacturer’s instructions (ThermoFisher Gibco, USA). Six days later culture medium was harvested, and antibodies were affinity purified on Protein G followed by SEC on a Superdex200·40 column (Cytiva, Uppsala, Sweden). For Fab preparation, sequences for a 6X HIS tag and stop codon were inserted following the second cysteine in the hinge region of the H chain using the Q5 Site Directed Mutagenesis Kit (New England Biolabs, Ipswich, MA, USA). Following transfection of Exp293F cells, Fab was affinity purified 6 days later from culture supernatants on a TALON metal affinity resin (Takara, Mountain View, CA, USA) followed by SEC on a Superdex200·40 column.

Surface plasmon resonance
All SPR experiments were performed at 25 °C on a Biacore T200 (Cytiva, Uppsala, Sweden) in 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.1 M AmSO4, 0.1 M Tris, pH 8.5; while crystals of B44:05–T73C alone and from 1
β
9mer, respectively. Diffraction data for complexes of PaSta1, tapasin–PaSta2, and for PaSta2 alone, were processed to 2.8 Å, 3.9 Å, and 2.3 Å respectively. The structures were solved by molecular replacement with Phaser18 using tapasin from PDB 3FSU as the search model. For the complex of tapasin and B44:05–T73C, the tapasin model was separated into two domains: N-Lig and IgC for molecular replacement search. For PaSta2, 2JYL with all CDR loops removed was used as the initial search model. For PaSta1, H chain (VH and CH1) of 6J15 and L chain (VL and CL) of 4ZSO were used as the initial search models, also lacking CDR loops. After molecular replacement solutions were found, the sequences of the model were replaced with those of PaSta1 and PaSta2, and all CDR loops were rebuilt manually. The initial models were subjected to several rounds of refinement with Phenix55 interspersed with manual building in Coot60. For refinement, refinements, as in the complexes of tapasin–PaSta1, tapasin–PaSta2 (3.9 Å), “reference” higher resolution models of tapasin (3FSU) and PaSta2 alone (2.3 Å) were used as restraints in Phenix60. The data of tapasin–B44:05–T73C–6mer (3.1 Å) in P62 revealed a pseudo twin (h, k, l) with a fraction of 0.307; the data of PaSta2 alone (2.3 Å) also was pseudo twinned (h, k, l) with a fraction of 0.460. For tapasin–PaSta2 (3.9 Å), B-factor sharpening of 100 Å2 was applied in order to highlight high resolution details, the average B-factor was reduced to 73.6 Å2. Rwork/ Rfree (%) values for final refined models of B44:05–T73C–6mer and B44:05–T73C–6mer and tapasin–B44:05–T73C–6mer are 18.0/19.5, 19.2/21.3, and 28.4/30.8 respectively. Data collection and refine ment statistics are summarized in Table 1. Graphics figures were generated with PyMOL. NMR-spectroscopy
NMR samples of free hβ2m, hβ2m–B44:05–T73C–9mer, hβ2m–B44:05–T73C–6mer for resonance assignments and chemical shift mapping experiments were prepared using the following isotope-selective labeling schemes: Ile methyl (Ile 1^13C6 in an otherwise U [15N, 1H, 2H] background), AllY methyl (Ala 1^13C6, Ile 1^13C6, Leu 1^13C6, β2 Val 1^13C5/1^13C4 in an otherwise U [15N, 1H, 2H] background), and IV (Ile 1^13C6, Leu 1^13C6, β2 Val 1^13C5/1^13C4 in an otherwise U [15N, 1H, 2H] background), using established protocols and reagents37,39. In all samples, the B44:05 heavy chain and peptide were at natural isotopic abundance, and the light chain hβ2m was isotopically labeled, as described previously for the hβ2m/HLA- A*02:01 system55. Samples were in the concentration range of 150 to 300 μM prepared in a standard NMR buffer (50 mM NaCl, 20 mM sodium phosphate pH 7.2, 10% D2O). Backbone and methyl resonances were assigned using a series of TROSY-based 2D and 3D experiments recorded at a 1 Hz field of 600, 700 MHz or 800 MHz at 25 °C, following a multi-pronged approach described previously for a similar system57. The structures were solved using an automated software, MAUS55. For tapasin chemical shift mapping experiments, we used a 1:1 tapasin: hβ2m–B44:05–T73C–6mer ratio prepared in the NMR buffer conditions. All NMR data were processed with NMRPipe and analyzed using NMRFAM- SPARKY16,17.
Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

X-ray structure factors and refined coordinates have been deposited in the Protein Data Bank (www.pdb.org) under accession numbers: 7TUC, 7TUD, 7TUE, 7TUF, 7TUG, and 7TUH for B44:05-T73C-9mer, B44:05-T73C-6mer, Tapasin-B44:05-T73C-6mer, Tapasin-PaSta1(Fab), Tapasin-PaSta2(Fab), and PaSta2 (Fab) respectively. NMR assignments for free hβ2m, hβ2m-B44:05-T73C-9mer, hβ2m-B44:05-T73-6mer, and tapasin with hβ2m-B44:05-T73C-6mer have been deposited into the Biological Magnetic Resonance Data Bank (http://www.bmrb.wisc.edu) under accession numbers 51097, 51098, 51099, and 51100. Expression vectors for recombinant proteins are available at request from the authors. Source data are provided with this paper.

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Author contributions
The studies were designed by J.J., P.C., M.G.M., N.G.S., D.H.M., and K.N. Experiments were performed by J.J., D.K.T., E.J.K., L.F.B., J.A., H.V.T., C.H.W., N.G.S., D.H.M., and K.N. The original draft was written by J.J., P.C., M.M., N.G.S., D.H.M., and K.N. All authors reviewed and edited to arrive at the final submitted manuscript.

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