Mathematical modeling and stochastic simulations suggest that low-affinity peptides can bisect MHC1-mediated export of high-affinity peptides into “early”- and “late”-phases

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ABSTRACT

The peptide loading complex (PLC) is a multi-protein complex of the endoplasmic reticulum (ER) which optimizes major histocompatibility I (MHC1)-mediated export of intracellular high-affinity peptides. Whilst, the molecular biology of MHC1-mediated export is well supported by empirical data, the stoichiometry, kinetics and spatio-temporal profile of the participating molecular entities are a matter of considerable debate. Here, a low-affinity peptide-driven (LAPD)-model of MHC1-mediated high-affinity peptide export is formulated, implemented, analyzed and simulated. The model is parameterized in terms of the contribution of the shunt reaction to the concentration of exportable MHC1. Theoretical analyses and simulation studies of the model suggest that low-affinity peptides can bisect MHC1-mediated export of high-affinity peptides into time-dependent distinct “early”- and “late”-phases. The net exportable MHC1 (eM1β(r)) is a function of the retrograde (rM1β(r))- and anterograde (aM1β(r))-derived fractions. The “early”-phase is dominated by the contribution of the retrograde/recyclable (rM1β ≅ 61%, aM1β ≅ 39%) pathway to exportable MHC1, is characterized by Tapasin-mediated peptide-editing and is ATP-independent. The “late”-phase on the other hand, is characterized by de novo PLC-assembly, rapid disassembly and a significant contribution of the anterograde pathway to exportable MHC1 (rM1β ≅ 21%, aM1β ≅ 79%). The shunt reaction is rate limiting and may integrate peptide translocation with PLC-assembly/disassembly thereby, regulating peptide export under physiological and pathological (viral infections, dysplastic alterations) conditions.

1. Introduction

The major histocompatibility complex (MHC), is a clustered group of cell surface proteins that participates in the adaptive immune response and is present in most vertebrates [1, 2, 3, 4, 5, 6, 7, 8, 9]. These genes, in humans (n = 240), are also known as human leukocyte antigens (HLA) and are present on the short arm of chromosome 6 (6p21.3 – 6p22.3) (Fig. 1a). In contrast, the minor histocompatibility proteins (MiHA) are smaller (9 – 12 aa) and occur in genes which exhibit polymorphisms [10, 11, 12, 13]. Proteins of the MHC have been ascribed roles in the endogenous- (MHC class I), exogenous- (MHC class II), and cross-processing pathways of peptide immunogens (Figs. 1b and 1c) [6, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. The ubiquitously present MHC class I proteins present peptides to CD8+ cytotoxic T-cells and are derived from cells infected with intracellular pathogens or undergoing dysplastic alteration(s) (Figs. 1a and 1b) [6, 17, 20, 23]. In contrast, immunogens derived from extracellular pathogens are internalized and processed by MHC class II proteins in the endoplasmic reticulum (ER) of professional antigen presenting cells (APC) en route to being presented to CD4+ helper T-cells (Figs. 1a and 1c) [24]. The miscellaneous MHC class III proteins partake in processing immunogens via the complement pathway(s) (C2, C4, B-factor) and function as cytokines (tumor necrosis factor-a, leukotrienes -A and -B) or heat shock proteins (Fig. 1a) [25, 26, 27, 28, 29]. The proteome fingerprint of an altered cell is distinct from neighboring cells and may serve as a molecular flag of infection or impending cellular alteration (Fig. 1) [14, 15, 16, 17, 18, 21, 22, 24].

The peptide loading complex (PLC), is a transient complex of several (n ≥ 5) proteins and functions to translocate and thence load cytosolic peptides onto newly synthesized MHC1 in the lumen of the ER [30, 31, 32, 33, 34, 35]. The molecular participants of the PLC comprise the

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transporters associated with antigen processing (TAP1/2), Tapasin, ERp57, one or more chaperone proteins, and the MHC class I protein(s) in complex with β2-microglobulin (M1β) [30]. The transmembrane (TM)-domain of Tapasin or TAP-associated glycoprotein, associates concomitantly with TAP1/2, ERp57 and M1β [30]. Tapasin is integral to PLC-assembly and has also been shown to facilitate peptide-editing, i.e., the competitive binding and exchange of peptides with incremental affinities for M1β (Fig. 2) [30, 31, 32, 36, 37, 38, 39, 40]. ERp57 or protein disulfide-isomerase A3 (PDI3) (EC 5.3.4.1), utilizes two catalytic (‘CGHC = [a, a’]) and lysine-rich binding (h, h’) domains along with several non-specific protein-protein interactions in forming an extensive contact surface with Tapasin [41, 42, 43, 44, 45, 46, 47, 48, 49, 50]. Whilst, the association of Tapasin and ERp57 is critical to the assembly of the PLC, the inclusion of M1β completes its formation [36, 41, 46, 47, 48, 49, 51, 52, 53, 54]. The role of a non-ERp57 Protein disulfide isomerase (PDI) has been shown to effect this process as well, although the mechanism by which it does so, and indeed the presence of as well has been the subject of much debate [50, 52, 55].

The simplicity of the canonical model of PLC (TAP1/2) : Tapasin : ERp57 : M1β : 4 : 4 : 4 : 4 : 4 notwithstanding, MHC1-mediated peptide export comprises several interleaved steps [35, 56]. These include: a) ubiquitin-proteasome system (UPS)-mediated intra-cellular protein degradation, b) peptide-affinity dependent PLC-disassembly and c) peptide editing (Fig. 2) [30, 31, 32, 56, 57, 58, 59, 60, 61, 62, 63]. Briefly, UPS-derived cytosolic peptides bind TAP1/2 and enter the ER lumen by an ATP-dependent and Tapasin-facilitated translocation across the ER membrane [57, 58]. PLC-disassembly is then triggered by the autocatalytic (C406ERp57 + C95Tapasin − C57ERp57 → C406ERp57 − C95Tapasin + C57ERp57) reduction of a disulfide (−SS−) linkage between Tapasin and ERp57 [41, 46]. A high-affinity peptide bound to M1β will result in immediate disengagement from the PLC with subsequent transport via the Golgi apparatus to the plasma membrane (antegrade pathway) (Fig. 2) [46, 59, 60, 61]. On the other hand, a low-affinity peptide, whence bound to M1β, is incorporated along with Tapasin into COPI-coated vesicles and recycled back to the ER (retrograde pathway) (Fig. 2) [46, 62]. The complex kinetics of MHC1-mediated export of high-affinity peptides results in several interesting empirical observations. These include redox regulation, protein-protein interactions and peptide editing (Fig. 2) [30, 31, 45, 55]. The latter is particularly relevant given that high-affinity peptides are present at concentrations much lower that low-affinity variants. Here, too, Tapasin is a major contributor although, the manner in which it does so is speculative [39, 40].

Although the underlying molecular biology of MHC1-mediated export of high-affinity peptides is well understood, mechanistic details (stochiometry, kinetics, spatio-temporal profiles) of the participating molecular entities are unclear. In this study, the regulatory influence of low-affinity peptides on MHC1-mediated adaptive immunosurveillance is explored via the shunt reaction and peptide editing [39, 40]. This is accomplished by formulating, implementing and analyzing a low-affinity peptide-driven (LAPD)-model of MHC1-mediated peptide export. The detailed theoretical analyses will be complemented by stochastic simulations of the model. Stochastic simulations, unlike ordinary (ODE) and partial-differential (PDE)-equation based kinetic modeling are accurate and unbiased in their approximation of the chemical master equation (CFME). However, this also implies that inferring meaningful information from the raw data post hoc will mandate considerable pre-processing. Initial simulations will be conducted to parameterize the steady state of the LAPD-model of exportable MHC1. The resulting datasets will be extensively parsed for time-step matched concentrations of the molecules and analyzed by regression (timestep--molecule. molecule--molecule) models. These data will then be utilized in later simulations to establish a temporal profile of the molecular entities and the complexes that they partake in.

The manuscript comprises a “Methods”-section, where a generic representation of a closed set of reactions and the numerical approximation of their solutions is given as a rationale for this study. Additionally, the section also highlights the modeling strategy deployed, tools and numerical methods needed to process the data that results from the simulations. This is followed by the “Results”-section where the definition and formulation of the LAPD-model, notations, derivations of the CME of net exportable MHC1 and preliminary results of the underlying molecular biology of
MHCI-mediated export of high-affinity peptides are given. Finally, these results are “Discussed” in context of the patho-physiological relevance of low-affinity peptides and Tapasin in the regulation of MHCI-mediated export of intracellular high-affinity peptides to the plasma membrane. A final section, “Conclusions” summarizes the main findings, limitations and future directions of this work.

2. Methods

2.1. Modeling strategy and rationale

The modeling approach adopted in this manuscript is the numerical approximation of a chemical master equation (CME) of net exportable MHCI by Gillespie’s stochastic simulation algorithm (SSA) [64, 65]. Here, the model is a closed well-mixed set of inter-dependent non-enzymatic reactions of the investigated molecular entities. This results in a system whose products are generated and utilized in accordance with their computed propensities.

Consider an arbitrary system of molar concentration of reactants (A-D) and reactions that they participate in. The reactions are non-enzymatic and paired (forward, backward) with rate constants (\( k_f, k_b \)) in terms of a dissociation (\( K_d \)) constant, i.e., \( K_d \approx \frac{k_f}{k_b} \).

The equations to determine the molar concentration of [A] at any instant of time are then,

\[
[A] = k_2([AB]) + k_4([AC]) - k_1([A][B]) - k_3([A][C])
\]

\[
[B] = k_2([AB]) - k_1([A][B])
\]

\[
[C] = k_4([CD]) - k_3([C][D])
\]

\[
[D] = k_4([CD]) - k_3([C][D])
\]

\[
[AB] = k_1([A][B]) - k_2([AB])
\]

\[
[CD] = k_3([C][D]) - k_4([CD])
\]
The solutions of these equations can be incorporated into the equation (1) along with the substitution,

$$k_3([A]([C]) = k_6([C]([D]) - k_5([C]([D])$$  \hspace{1cm} (7)

to generate a composite CME for $[A]$,

$$[B] = \int (k_2([AB]) - k_1([A][B])) \, dt$$
$$= k_2([AB]) - k_1([A][B]) + \zeta_{AB}$$  \hspace{1cm} (8)

$$[C] = [D] = \int (k_6([CD]) - k_5([C][D])) \, dt$$
$$= k_6([CD]) - k_5([C][D]) + \zeta_{CD}$$  \hspace{1cm} (9)

$$[A] = \int (k_3([AB]) + k_4([AC]) - k_1([A][B]) - (k_6([CD]) - k_5([C][D])) \, dt$$
$$= k_2([AB]) + k_4([AC]) - k_1([A][B]) - (k_6([CD]) - k_5([C][D])) + \zeta_{[A]}$$  \hspace{1cm} (10)

Here,

$[\cdot]$ : \textit{Molar concentration of reactant (mol L}^{-1}\text{)}

$k$ : \textit{Rate constant of reaction (mol L}^{-1} \text{ s}^{-1}\text{)}

$\zeta_{[\cdot]}$ : \textit{Arbitrary constant of the solution of the equation for reactant}

Equation (10), which is the net concentration of molecule “A” may then be directly approximated by simulation data.

2.2. Implementing a model of MHC1-mediated peptide export

2.2.1. Constraint-based model of exportable MHC1

MHC1-mediated export of high-affinity peptides is a critical process in the processing and presentation of intracellular immunogens to circulating T- and B-cells. Whilst, there are several excellent manuscripts on individual molecules, the manner in which these work as a cohesive unit in vivo, is speculative with several open research problems. This paucity of data is reflected in the absence of usable rate- and stoichiometric-constants of the molecular entities. Here, an \textit{ab initio} constraint-based derivation of the stoichiometric matrix of the molecular participants and the rate constants of reactions that they partake in is undertaken. The primary constraint utilized is the steady state of the modeled system both, at the outset and during simulation runs. This is an essential step in approximating the CME of net exportable MHC1. Other constraints include equivalent and non-limiting initial values of each molecular entity (zero-order kinetics) and bounded rate constants for any arbitrary pair of reactions. The latter are chosen such that the net flux is not more than 6-fold ($10^{-6} < \frac{\text{ flux }}{\text{ net flux}} \leq 1$) (Table 1). The rationale for this is that the SSA computes the propensity of occurrence of a particular reaction and any value in excess of this ratio may result in a particular state/reaction occurring more frequently which will bias the system. Additional constraints are based on empirical data, whence available. This includes the proportions of exportable MHC1 and low- and high-affinity peptides (Table 1). Since the objective of this work is to study the effect of low-affinity peptides on the shunt reaction and thence the exportable MHC1, the model is parameterized in terms of the same (Table 1). The model is implemented in R-3.1.2 and includes in-house coded scripts for controlling the runs, parsing, analyses and processing the resulting data (Table 1).

2.2.2. Simulations and numerical approximation of the CME of exportable MHC1

The CME of exportable MHC1 that is formulated \textit{vide infra}, is a complex mathematical expression of several molecular entities (ERP57, Tapasin, low- and high-affinity peptides, MHC1-$\beta_2$) and/or their complexes. The major focus of this manuscript is the temporal assessment of the molecules that influence MHC1-mediated export of high-affinity peptides to the plasma membrane. Since the time-step is randomly chosen, a comparative assessment is only possible by simulating a single time-step and thence inferring the number of molecules at that time-step [66]. The initial round of simulations is done to parameterize the steady state of the system. The linear models thus generated along with simulation data from the final

| S. No. | Name | Parameter | Quantity |
|-------|------|-----------|----------|
| 1     | Range of numerical values utilized for disassociation constants | $k_r$ | $10^{-n}, 1.00$ |
| 2     | Proportion of empirically observed MHC1 | $\frac{[M]}{[M/1]}$ | $\pm 5.00$ |
| 3     | Fold difference between low- and high-affinity peptides | $\log (\frac{[M]}{[H]})$ | $\pm 5.00$ |
| 4     | Rate limiting step (shunt reaction) | $k_{sh}$ | $30 - 35$ mol L$^{-1}$ s$^{-1}$ |
| 5     | Total number of reactions | $RXN$ | 30 |
| 6     | Index of molecular entity | $i$ | 18 |
| 7     | Number of in silico experiments | $j$ | 3 |
| 8     | Number of independent runs or observations | $h$ | 30 |
| 9     | Number of molecules of an entity per in silico experiment in an independent run or observation | $y_{ijkl}$ | — |
| 10    | Wall time of each independent run or observation | $—$ | 600 s |
| 11    | Time units | $rf$ | 100 |
| 12    | Degree of freedom | $df$ | 28 |
| 13    | Simulation time to assess temporal variation of molecular entity | $t$ | [60 s, 900 s] |
round of simulations will result in a time-based trajectory of each molecular entity. Clearly, this non-standard usage of the SSA mandates a detailed explanation which along with the relevant formulas and equations is outlined. This approach has been successively deployed previously and the data generated was used to glean insights into the vectorial chemotaxis of an advancing phagocyte [66].

Briefly, (h)-independent runs or “observations” comprise a single in-silico experiment (j) for an indexed set of molecular entities (i) (Table 1). A linear model is then used to associate the run-specific time-step \( \{ i_{jh} \in T \} \) with a numerical estimate of a specific molecular entity \( \{ y_{ijh} \in Y \} \),

\[
y_{ijh} \sim i_{jh}
\]

This will compute several coefficients such as estimates of standard error, t-value, and the probability of error \( (p_r > |t-value|) \) of the intercept (\( \lambda \)), slope (\( \theta \)) and the degree of freedom (\( df \)).

\[
\left\{ \begin{array}{l}
\lambda_{ij} \in R_+ \mid \lambda_{ij} = \lambda(y_{ij}) = \text{mean} (\lambda_{ij1}, \lambda_{ij2}, ..., \lambda_{ijh}) \\
\theta_{ij} \in R_+ \mid \theta(y_{ij}) = \text{mean} (\theta_{ij1}, \theta_{ij2}, ..., \theta_{ijh})
\end{array} \right\} \ y_{ijh} \in Y
\]

(11)

(12)

(13)

Since, the time-steps chosen by the SSA during each run are random the median value \( (i_j) \) for a set of \( (h) \)-runs is chosen,

\[
\left\{ i_j \in R_+ \mid i_j = \text{median} \{ i_{j1}, i_{j2}, ..., i_{jh} \} \right\} \ t_{jh} \in T
\]

These parameters are then utilized to compute the time-step invariant quantities of each molecule in a single in silico experiment,

\[
\left\{ \Delta y_{ij} \in R_+ \mid y_{ij} = (\{ i_j \} (\theta_{ij}) + \lambda_{ij}) \right\}
\]

The temporal variation of an arbitrary molecular entity is then calculated in triplicate \( (j \in \{1, 3\}) \) using the arithmetic mean (\( \mu \)) and standard deviation (\( \sigma \)) as numerical indices (Table 1). The aforementioned steps are summarized,

\[
\begin{pmatrix}
y_{ij1} & \cdots & y_{ijh} \\
\vdots & \ddots & \vdots \\
y_{ij1} & \cdots & y_{ijh}
\end{pmatrix}
\rightarrow
\begin{pmatrix}
\Delta y_{ij1} \\
\vdots \\
\Delta y_{ijh}
\end{pmatrix}
\rightarrow
\{ \mu (\Delta y_{ij}), \sigma (\Delta y_{ij}) \mid j \in \{1, 3\} \}
\]

The raw parameterization data (initial simulations) and the temporal variations of the molecular entities observed (final simulations) are included as supplementary material (Supplementary Tables 1-4, Supplementary Texts 1-4). Miscellaneous parameters such as console interval and time units are in accordance with the package guidelines (GillespieSSA) and previous work (Table 1) [66].

3. Results

Protein-protein interactions (PPI), are the molecular basis for PLC assembly/disassembly and may have a significant role in the export of high-affinity peptides by MHC1 to the plasma membrane [30, 31, 32, 33]. The PLC is a large macromolecular complex and is arguably the most important component of MHC1 (\( M \beta \))-mediated antigen processing and presentation of intracellular immunogens (Fig. 2). Similarly, the ternary complex of Tapasin and low-affinity peptide bound \( M \beta \) \( ( \text{PPI}_{\text{Tapasin-M}β\text{-P}}) \), represents the exchangeable fraction of MHC1 and is a key determinant of peptide editing (Fig. 2). Other notable complexes of physiological relevance include Adenosine triphosphate (ATP) with the transporters associated with antigen processing \( ( \text{PPI}_{\text{ATP}}\text{-T-AP}1\text{Z}) \) and the association between ERp57 and Tapasin \( ( \text{PPI}_{\text{ERp57-Tapasin}}) \) (Fig. 2).

3.1. Definitions and preliminary results of a model of MHC1-mediated export of high-affinity peptides

The low-affinity peptide-driven (LAPD)-model purports that low-affinity peptides \( (L_{p_n} \in L_p, n \in N) \) are significant determinants of the efficient and continuous export of high-affinity peptides \( (H_{p_m} \in H_p, m \in N) \) by the MHC1-\( β_2 \)-microglobulin heterodimer \( (M \beta) \). These definitions are derived from empirical data of the dissociation constants, \( K_d(\cdot) \), of these peptides in association with \( M \beta \).

\[
L_{p_n} \equiv K_d(M \beta L_{p_n}) \approx 1.0, \text{ Def. (1)}
\]

\[
H_{p_m} \equiv K_d(M \beta H_{p_m}) = 0.0, \text{ Def. (2)}
\]

This implies that higher-order complexes such as the peptide loading complex (PLC) that result from these interactions will exhibit a dual distribution,

\[
\{ \text{PLC}_z \in \text{PLC} \mid \text{PLC}_z \sim \text{PLC}_z H_{p_m} \lor \text{PLC}_z L_{p_n} \} \text{ Def. (3)}
\]

Here, \( \text{PLC}_z \) is an arbitrary indexed entity from the pool of cytosolic PLC (\( \text{PLC} \)) and can be bound to a high \( (\text{PLC}_z H_{p_m}) \)- or low \( (\text{PLC}_z L_{p_n}) \)-affinity peptide where, \( \{ z, m, n \} \in N \). The joint probability of the simultaneous occurrence of every PLC form \( (\text{Prob}(\text{PLC})) \) may be approximated by the probability mass function of the Binomial Distribution:

\[
p = \text{Prob}(\text{PLC}_z = \text{PLC}_z H_{p_m}) \equiv \text{Prob}(H_{p_m}) = 0.00001
\]

\[
q = \text{Prob}(\text{PLC}_z = \text{PLC}_z L_{p_n}) \equiv \text{Prob}(L_{p_n}) = 0.99999
\]

\[
X = \text{Prob}(\text{PLC}) \sim \binom{s}{r} \left(p^r \right) \left(q^{s-r} \right) = B(s, p)
\]

lim \( X = \lim_{s \to \infty} B(s, p) \rightarrow 0 \forall s > 1 \)

\[
s := \text{Combined pool of indexed PLC}_z \text{ forms}
\]

\[
r := \text{Occurrence of PLC}_z \text{ bound high-affinity peptide}
\]

\[
B := \text{Binomial distribution of cytosolic PLC}
\]
Table 2. Low-affinity peptide-driven (LAPD)-model of MHC1-mediated export of high-affinity peptides.

| Non-stoichiometric molar representation of molecular entities | Molecular Biology |
|---------------------------------------------------------------|------------------|
|                                                               | **k_{on} (mol L^{-1} s^{-1})** | **PT** | **PLCa** | **PLCd** |
| [Tapasin_TAP1/2] + [M1β] ↔ [Tapasin_TAP1/2,M1β,ERp57,Cx/Cr] | **k_1 = 1.00, k_2 = 10^{-4}** | - | + | - |
| [Lp_n] + [ATP,TAP1/2] ↔ [ATP,TAP1/2,Lp_n] | **k_3 = 1.00, k_4 = 5.00** | + | + | - |
| [Tapasin_TAP1/2,M1β,ERp57,Cx/Cr] + [ATP,TAP1/2,Lp_n] ↔ [PLC,Lp_n] + [ADP] | **k_5 = 1.00, k_6 = 10^{2}** | - | + | - |
| [PLC,Lp_n] ↔ [ERp57,Cx/Cr] + [ATP1/2] + [Tapasin,M1β,Lp_n] | **k_7 = 1.00, k_8 = 10^{-6}** | - | + | + |
| [Hp_n] + [ATP,TAP1/2] ↔ [ATP,TAP1/2,Hp_n] | **k_9 = 1.00, k_10 = 5.00** | + | + | - |
| [Tapasin_TAP1/2,M1β,ERp57,Cx/Cr] + [ATP,TAP1/2,Hp_n] ↔ [PLC,Hp_n] + [ADP] | **k_11 = 1.00, k_12 = 10^{2}** | - | + | - |
| [PLC,Hp_n] ↔ [ERp57,Cx/Cr] + [ATP1/2] + [Tapasin] + [aM1β] | **k_13 = 1.00, k_14 = 10^{-3}** | - | + | + |
| [Lp_n] + [Hp_n] + [ATP,TAP1/2] ↔ [ATP,TAP1/2,Lp_n] | **k_15 = 1.00, k_16 = 1.00** | + | + | - |
| [Tapasin,M1β,Lp_n] + [Hp_n] ↔ [rM1β] + [Lp_n] + [Tapasin] | **k_17 = 1.00, k_18 = 32.5** | + | + | + |
| [Tapasin] + [ERp57,Cx/Cr] ↔ [Tapasin_TAP1/2] | **k_19 = 1.00, k_20 = 10^{2}** | - | + | + |
| [M1β] + [Hp_n] ↔ [M1β,Hp_n] | **k_21 = 1.00, k_22 = 0.99** | - | + | - |
| [M1β] + [Lp_n] ↔ [M1β,Lp_n] | **k_23 = 1.00, k_24 = 0.99** | - | + | - |
| [TAP1/2] + [ATP] ↔ [ATP,TAP1/2] | **k_25 = 1.00, k_26 = 10^{4}** | + | + | - |
| [rM1β] + [Tapasin,M1β,Lp_n] | **k_27 = 1.00, k_28 = 1.01** | - | + | + |
| [Lp_n] + [Hp_n] + [ATP,TAP1/2] ↔ [ATP,TAP1/2,Hp_n] | **k_29 = 1.00, k_30 = 1.00** | + | + | - |

3.2. Theoretical analyses of the LAPD-model of MHC1-mediated high-affinity peptide export

The LAPD-model incorporates several physiologically relevant molecular details of MHC1-mediated export (Fig. 2, Table 2) [30, 31, 32, 33, 34, 35, 50, 55, 56, 57, 58]. These include: a) the proportion of high- and low-affinity peptides in the cytosol and the ER, b) ATP-driven translocation of ubiquitin-derived cytosolic peptides into the ER lumen through TAP1/2, c) assembly of the PLC with Tapasin, ERp57 and M1β, d) PLC-disassembly and the generation of exportable MHC1 (eM1β) via the anterograde (aM1β)- and retrograde/recyclable (rM1β)-pathways, e) Tapasin-mediated peptide editing of low- with high-affinity variants via the shunt reaction, and f) the contribution of the cis- and trans-faces of the Golgi apparatus (cGolgi, tGolgi).

The basic premise of this work is that exportable MHC1 (eM1β) is not a single entity, but is derived independently from the high- and low-affinity peptide forms of the PLC via the antero (aM1β)- and retro (rM1β)-grade pathways (Eqs. (16), (17), (18) and (19)),

\[ [eM1\beta] \propto [PLC] \]
\[ [eM1\beta] \propto [PLCHp + PLC_{LP}] \]
\[ [eM1\beta(t)] = \gamma \int_{t=0}^{t=N} \left( [PLC_{h}H_{p_n}(t)] + [PLC_{l}L_{p_n}(t)] \right) \]
\[ = \gamma \int_{t=0}^{t=N} \left( [PLC_{h}H_{p_n}(t)] + \int_{t=1}^{t=N} [PLC_{l}L_{p_n}(t)] \right) \]  

(20)

Case (1): Consider an arbitrary high-affinity peptide in complex with the MHC1-complex (M1βH_{p_n}). This interaction will abrogate the interaction between MHC1 and Tapasin (PLC_{h}H_{p_n} = PP1_{E}p57-Tapasin + M1βH_{p_n}) prior to autocatalytic reduction [53]. This results in the appearance of anterograde-derived M1β and readily exportable (aM1β) in the ER lumen and thence at the plasma membrane (eM1β) (Fig. 2) [59, 60, 61]. This can be represented as:
\[
PLC_z H_{\beta n} \rightarrow TAP1/2 + ERp57 + Tapasin + M1\beta H_{\beta n} \\
M1\beta H_{\beta n} \equiv r M1\beta
\]

Combining the aforementioned partial reactions and incorporating the observation that TAP1/2 is membrane bound we can rewrite this,

\[
PLC_z H_{\beta n} \rightarrow ERp57 + Tapasin + aM1\beta
\]

As indicated previously MHC1-mediated export of high-affinity peptides is characterized by protein-protein interactions and inter-molecular complex formation. Rewriting the left hand side of the above reaction as molar concentrations of the component complexes of PLC,

\[
\left[ PLC_z H_{\beta n} \right] = \left[ PPI_{ERp57-Tapasin} \right] \cdot \left[ PPI_{Tapasin-M1\beta H_{\beta n}} \right]
\]

(20.1)

Using these we can derive a numerical expression for the high-affinity peptide bound form of the PLC (\(PLC_z H_{\beta n}\)),

\[
(Abrogation, disassociation) = \left[ PPI_{ERp57-Tapasin} \right] \cdot \left[ M1\beta H_{\beta n} \right]
\]

(20.2)

\[
(Autocatalytic reduction) = \left[ ERp57 \right] \cdot \left[ Tapasin \right] \cdot \left[ aM1\beta \right]
\]

(20.3)

\[
\text{Combining equations (20.2) and (20.3)}
\]

\[
\left[ PLC_z H_{\beta n} \right] = \left[ ERp57 \right] \cdot \left[ Tapasin \right] \cdot \left[ aM1\beta \right]
\]

(20.4)

\[
\text{Solving equation (20.4)}
\]

\[
\frac{\partial (\log \left[ PLC_z H_{\beta n} \right])}{\partial \left[ PLC_z H_{\beta n} \right]} = (\log \left[ ERp57 \right] + \log \left[ Tapasin \right] + \log \left[ aM1\beta \right])
\]

(20.5)

\[
\frac{\partial (\log \left[ PLC_z H_{\beta n} \right])}{\partial \left[ PLC_z H_{\beta n} \right]} = (\log \left[ ERp57 \right] + \log \left[ Tapasin \right] + \log \left[ aM1\beta \right])
\]

(20.6)

\[
\frac{\partial (\log \left[ PLC_z H_{\beta n} \right])}{\partial \left[ PLC_z H_{\beta n} \right]} = (\log \left[ ERp57 \right] + \log \left[ Tapasin \right] + \log \left[ aM1\beta \right])
\]

(20.7)

\[
\frac{\partial (\log \left[ PLC_z H_{\beta n} \right])}{\partial \left[ PLC_z H_{\beta n} \right]} = (\log \left[ ERp57 \right] + \log \left[ Tapasin \right] + \log \left[ aM1\beta \right])
\]

(20.8)

\[
\frac{\partial (\log \left[ PLC_z H_{\beta n} \right])}{\partial \left[ PLC_z H_{\beta n} \right]} = \frac{1}{2} \left( \left[ ERp57 \right] + \left[ Tapasin \right] + \left[ aM1\beta \right] \right) + \frac{c}{2}
\]

(20.9)

\[
\text{Rearranging equation (20.9)}
\]

\[
\left[ PLC_z H_{\beta n} \right] = \frac{2}{\log \left( \left[ ERp57 \right] + \left[ Tapasin \right] + \left[ aM1\beta \right] \right) + \frac{c}{2}} \left[ ERp57 \right] \cdot \left[ Tapasin \right] \cdot \left[ aM1\beta \right]
\]

(21)

Here, \(c\) and \(c_{PLC_z H_{\beta n}}\) represent arbitrary constants of the solutions of the equations to establish molar concentration of \(PLC_z H_{\beta n}\).

**Case (2):** The presence of a low-affinity peptide in complex with the MHC1-complex (\(M1\beta \beta_{\beta n}\)) leads to the persistence of the corresponding PLC (\(PLC_z L_{\beta n}\)). The autocatalytic reduction or “reductive escape” of the ternary complex of bound Tapasin with \(M1\beta\) (\(PLC_z L_{\beta n} \equiv PPI_{Tapasin-M1\beta L_{\beta n}} + ERp57\)) is then the de facto primary reaction [53]. This results in the appearance of the Tapasin-driven recyclable \(M1\beta\) at COPI-exit sites of the ER [62].

\[
PLC_z L_{\beta n} \rightarrow TAP1/2 + ERp57 + PPI_{Tapasin-M1\beta L_{\beta n}} \\
PPI_{Tapasin-M1\beta L_{\beta n}} \xrightarrow{ER=GoToGolgi} PPI_{Tapasin-M1\beta L_{\beta n}}
\]

We can exclude TAP1/2 (membrane bound) and rewrite the combined reaction as,

\[
PLC_z L_{\beta n} \rightarrow ERp57 + PPI_{Tapasin-M1\beta L_{\beta n}}
\]

Rewriting the left hand side as molar concentrations of the component complexes of PLC,

\[
\left[ PLC_z L_{\beta n} \right] = \left[ ERp57 \right] \cdot \left[ PPI_{Tapasin-M1\beta L_{\beta n}} \right]
\]

(21.1)

In the presence of a random high-affinity peptide anywhere else along the pathway, the “shunt”-reaction is triggered. The ternary complex of Tapasin then rapidly dissociates to yield exportable \(M1\beta\).

\[
PPI_{Tapasin-M1\beta L_{\beta n}} \xrightarrow{H_{\beta n}} Tapasin + L_{\beta n} + M1\beta H_{\beta n} \\
M1\beta H_{\beta n} \equiv r M1\beta \equiv e M1\beta
\]

Rewriting the left hand side of “shunt”-reaction along with the generated exportable \(M1\beta\) (\(M1\beta H_{\beta n} \equiv r M1\beta\)),

\[
\left[ PPI_{Tapasin-M1\beta L_{\beta n}} \right] = \left[ Tapasin \right] \cdot \left[ L_{\beta n} \right] \cdot \left[ M1\beta H_{\beta n} \right]
\]

(21.2)

\[
\left[ PPI_{Tapasin-M1\beta L_{\beta n}} \right] = \left[ Tapasin \right] \cdot \left[ L_{\beta n} \right] \cdot \left[ r M1\beta \right]
\]

(21.3)

\[
\text{Combining equations (21.1) and (21.3)}
\]

\[
\left[ PLC_z L_{\beta n} \right] = \left[ ERp57 \right] \cdot \left[ Tapasin \right] \cdot \left[ L_{\beta n} \right] \cdot \left[ r M1\beta \right]
\]

(21.4)
Solving equation (21.4)

\[
\log \left[ \text{PLC}_\beta \right] = \log \left[ \frac{\text{ERp57}}{\text{Tapasin}} \cdot \left| L_p \right| \cdot [rM1\beta] \right] = \log \left[ \frac{\text{ERp57}}{\text{Tapasin}} \right] + \log \left[ L_p \right] + \log [rM1\beta]
\]

(21.5)

\[
d \left( \log \left[ \text{PLC}_\beta \right] \right) = \left( \frac{d \log \left[ \text{PLC}_\beta \right]}{d \left[ \text{PLC}_\beta \right]} \right) \frac{d \left[ \text{PLC}_\beta \right]}{d \left[ \text{PLC}_\beta \right]} = \left( \frac{d \log \left[ \text{PLC}_\beta \right]}{d \left[ \text{PLC}_\beta \right]} \right) \frac{d \left[ \text{PLC}_\beta \right]}{d \left[ \text{PLC}_\beta \right]}
\]

(21.6)

\[
d \left( \log \left[ \text{PLC}_\beta \right] \right) = \left( \frac{d \log \left[ \text{PLC}_\beta \right]}{d \left[ \text{PLC}_\beta \right]} \right) \frac{d \left[ \text{PLC}_\beta \right]}{d \left[ \text{PLC}_\beta \right]}
\]

(21.7)

\[
\frac{d \left( \log \left[ \text{PLC}_\beta \right] \right)}{d \left[ \text{PLC}_\beta \right]} = \frac{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{d \left[ \text{PLC}_\beta \right]}
\]

(21.8)

\[
\frac{1}{\text{PLC}_\beta \left[ L_p \right]} = \left( \int \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right) d \text{PLC}_\beta \left[ L_p \right]
\]

(21.9)

Rearranging equation (21.9)

\[
\text{PLC}_\beta \left[ L_p \right] = \frac{2}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)} + \frac{2}{C}
\]

(22)

Here, c and \(c_{\text{PLC}_\beta \left[ L_p \right]}\) represent arbitrary constants of the solutions of the equations to establish the molar concentration of \(\text{PLC}_\beta \left[ L_p \right]\).

Substituting equations (21) and (22) in equation (20),

\[
[eM1\beta] = \frac{2 \cdot \left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)} + \frac{c_{M1\beta}}{c} + c_{\text{PLC}_\beta \left[ L_p \right]}
\]

(23)

where \(c_{M1\beta}\) is the combined arbitrary constant of the solution of the equation to establish the molar concentration of exportable MHC1,

\[
c_{M1\beta} = c_{\text{PLC}_\beta \left[ L_p \right]} + c_{\text{PLC}_\beta \left[ L_p \right]}
\]

(23.1)

It is clear from Equation (23) that the final exportable form of MHC1 (\(eM1\beta\)) is complex and dependent on the concentrations of unbound low-affinity peptides, Tapasin, ERp57, antero (\(aM1\beta\))- and retro (\(rM1\beta\))-grade derived fractions of MHC1.

3.3. Elucidating the molecular biology of the LAPD-model of MHC1-mediated high-affinity peptide export

Comprehend the underlying molecular mechanisms that constitute MHC1-mediated high-affinity peptide export mandates formulating and simulating the CME of net exportable MHC1. The resulting data can then be used to numerically approximate the CME and infer biological function.

3.3.1. Formulating the CME of net exportable MHC1

Equation (23), suggests that exportable or high-affinity peptide bound MHC1 (\(eM1\beta\)) is generated concomitantly by the antero (\(aM1\beta\))- and retro (\(rM1\beta\))-grade pathways (\(eM1\beta = aM1\beta + rM1\beta\)). However, the origin and temporal variation of these proportions is unclear. Although multifacto-

rial, peptide editing and the production of \(rM1\beta\), along with \(de novo\) \(aM1\beta\)-generation are probably major contributors. In order to gain insights into these phenomena a time-dependent mathematical expression of exportable MHC1, i.e., the CME of net exportable MHC1 (\(\Delta \left[ eM1\beta(t) \right] \)) is formulated.

Using \(\left[ H_p \right](t = 0) = \left[ L_p \right](t = 0) \rightarrow 0\) and equations (16) and (17) to rewrite equation (23),

\[
[eM1\beta](t \rightarrow 0) = \frac{2 \cdot \left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}
\]

(24)

\[
[eM1\beta](t \rightarrow 0) = \frac{2 \cdot \left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}
\]

(25)

Simplifying equations (24) and (25) as partial fractions,

\[
[eM1\beta](t \rightarrow 0) = \frac{2 \cdot \left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}
\]

(26)

\[
[eM1\beta](t \rightarrow 0) = \frac{2 \cdot \left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}
\]

(27)

Rearranging and equating,

\[
\left[ eM1\beta(t \rightarrow 0) - [eM1\beta(t \rightarrow 0)] \right] = \gamma \cdot \left( \left[ L_p(t) \right] + [rM1\beta(t)] \right) - [aM1\beta(t)]
\]

(28)

where,

\[
\gamma = \frac{2 \cdot \left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}{\left( \log \left[ \text{ERp57} \right] + \log \left[ \text{Tapasin} \right] + \log \left[ L_p \right] + \log [rM1\beta] \right)}
\]

(28.1)

Rewriting equation (28)

\[
\left[ eM1\beta(t \rightarrow 0) \right] = \gamma \cdot \left( \left[ L_p(t) \right] + [rM1\beta(t)] \right) - [aM1\beta(t)]
\]

(29)

Clearly, the time-dependent CME of exportable MHC1 is influenced by the proportion of antero (\(aM1\beta\))- and retro (\(rM1\beta\))-grade derived fractions of MHC1. Equation (29), also highlights the non-trivial role that low-affinity peptides may have in the genesis of exportable MHC1. These analyses suggest that the LAPD-model may be the dominant operative mechanism in vivo, by which MHC1 exports high-affinity peptides to the plasma membrane of nucleated cells.
Fig. 3. Insights into the molecular biology of the LAPD-model of MHC1-mediated export. a), b) High- \((H_p, \in H_p)\) and low- \((L_p, \in L_p)\) affinity peptides are translocated across the ER membrane and result in differential forms of the PLC, i.e., \([PLC_\text{p} = PLC_\text{c} = PLC_\text{p} + PLC_\text{c} - H_p] \), and other intermediate complexes \((PP1_xERp-Tapasin, PP1_yTapasin-M1\beta_p)\). An important result is that while the concentration of unbound low-affinity peptides progressively declines, high-affinity peptides accumulate in the ER lumen. This progressive enrichment, is all the more relevant since low-affinity peptides are almost five orders of magnitude in excess at any given time. Additionally, this implies a temporal bisection of the MHC1-mediated export of high-affinity peptides into “early”- and “late”-phases, and c) Simulation studies of the LAPD-model of exportable MHC1. The CME of net exportable MHC1 \((\epsilon M1\beta)\), is a complex mathematical expression that involves interactions between Tapasin, Erp57, low- and high-affinity peptides, Tap1/2, and M1\beta. A key finding of the early-phase is that the shunt reaction \((PP1_yTapasin-M1\beta_p + H_p \rightarrow Tapasin + L_p + rM1\beta)\) (RXN 18) is rate limiting. The LAPD-model is parameterized \((k_{11} = 30 - 35 \text{ mol } L^{-1} s^{-1})\) on the basis of empirical data \((\epsilon M1\beta) \approx 5.00\). Abbreviations: \(aM1\beta\), anterograde-derived fraction of exportable MHC1; ER, endoplasmic reticulum; \(\epsilon M1\beta\), major histocompatibility I antigen in complex with \(\beta_2\)-microglobulin; \(rM1\beta\), exportable MHC1; MHC1, major histocompatibility complex I antigens; PLC, set of differential forms of the peptide loading complex; PPI, protein-protein interaction; \(rM1\beta\), retrograde-derived fraction of exportable MHC1; RXN, reaction.

3.3.2. The LAPD-model suggests that the recyclable fraction of MHC1 is a significant early contributor to exportable MHC1

Although PLC assembly/disassembly is bimodal, the theoretical results and simulation data from this study suggests that the contribution of the anterograde- and retrograde-pathways to exportable MHC1 is also distinctly biphasic (early, late). A key finding of the “early”-phase is that the shunt reaction Eqs. (21, 22), (21, 23) (RXN 18) is rate limiting. This data \((k_{11} = 10^{-6}, 50, n = 65)\) is robust for \(k_{11} \geq 10.00 \text{ mol } L^{-1} s^{-1}\) and can be parameterized \((k_{11} = 30 - 35 \text{ mol } L^{-1} s^{-1})\) \((\text{Fig. 3; Supplementary Table 3})\). This choice is based on the empirical observation \((\epsilon M1\beta) \approx 5.00\) and is corroborated directly by comparing the proportion of net exportable-MHC1 generated by the retrograde pathway \((1.64 \leq \frac{\epsilon M1\beta}{\epsilon M1\beta} \leq 4.87)\) Eqs. (36), (37), (38), (39) and (40) \((\text{Fig. 3, Table 3})\). Thus, while the retrograde/recyclable fraction is an “early”-contributor, the anterograde-derived fraction of exportable MHC1 contributes to the MHC1 net-mediated export of high-affinity peptides at “later” time points.

3.3.3. The LAPD-model of exportable MHC1 leads to enrichment of high-affinity peptides in the ER lumen

Cytosolic-derived peptides \((H_p, \in H_p)\) are translocated across the ER membrane in association with \(Tap1/2\) in an ATP-dependent step. Interestingly, and in complete contrast to their baseline levels, the concentrations of the unbound peptides exhibit an exponential increase Eq. (52) \((\text{Figs. 3a and 3b, Table 4})\). These findings are intriguing given that the initial concentrations of the PLC \((pPLC_C\rightarrow\epsilon M1\beta_p)\) and the concentration of the intermediate complexes is trivial Eqs. (53), (54) \((\text{Figs. 3a and 3b, Table 4})\). Additional findings include the relatively unchanged concentration of the low-affinity peptide bound form of the PLC and the progressive enrichment in the ER-lumen of the high-affinity peptide bound variant Eqs. (55), (56) \((\text{Figs. 3a and 3b, Table 4})\).

4. Discussion

The release of MHC1 from the PLC is a critical event in the export and presentation of endogenously derived intracellular peptides to the surface of nucleated cells. Since this occurs preferentially for high-affinity peptides, insights into the molecular mechanisms that regulate these steps may be relevant to disease progression.

4.1. Low-affinity peptides can regulate MHC1-mediated export of high-affinity peptides

The LAPD-model bisects MHC1-mediated export of high-affinity peptides into an “early”- \((t \leq 600 \text{ s})\) and “late”- \((t > 600 \text{ s})\) phase. The “early”-phase is characterized by the kinetics of disassembly and exchange, and is mediated by the shunt reaction \((\text{Figs. 3c and 4a})\). In fact, simulation data
suggests that more than 50% of $PPITapasin\rightarrow M$ that exits the ER via the retrograde pathway is potentially exchangeable with the high-affinity variant ($rM1\beta \approx 61\%$, $aM1\beta \approx 39\%$) Eq. (57) (Table 4). Interestingly, the data also suggests that the contribution by the retrograde pathway to the exportable MHC1 is self-limiting and will progressively diminish Eqs. (58), (59) (Figs. 3c and 4a, Table 4). This implies that the “shunt”-reaction mediated peptide editing that results from this exchangeable fraction is saturable Eq. (60) (Fig. 4a and 4c, Table 4). This is in part due to unbound Tapasin and low-affinity peptides being progressively depleted Eqs. (61), (62) (Figs. 4a and 4c, Table 4). Prior to the complete exhaustion of low-affinity peptides, equivalent quantities of unbound Tapasin, ERp57 ($PLC,LP_a\Rightarrow PPITapasin\rightarrow M\Rightarrow LP_a\Rightarrow [Tapasin,ERp57]$) and a progressively increasing concentration of untransported high-affinity peptides participates in the de novo assembly of $PLC,HP_a$ (Figs. 4b and 4c). The ensuing “late”-phase is then characterized by rapid disassociation of the PLC, linear increase in the concentration of $PLC,HP_a$ and the equivalent pairing of Tapasin and ERp57. Anterograde-derived MHC1, is therefore, a significant contributor to MHC1-mediated peptide export ($rM1\beta \approx 21\%$, $aM1\beta \approx 79\%$) at later time points Eqs. (56), (63) (Figs. 4b and 4c, Table 4).

4.2. Elucidating the role of Tapasin in MHC1-mediated high-affinity peptide export

Tapasin is a critical modulator in the MHC1-mediated export of high-affinity peptides. Since, Tapasin is absent from the final exportable MHC1 its role is indirect, i.e., of a facilitator and regulator [67]. However, the molecular mechanism(s) by which Tapasin accomplishes this is unclear. The LAPD-model suggests that the proportion of unbound- and bound ($PPITapasin\rightarrow M,PPITapasin\rightarrow LP_a$)Tapasin may influence MHC1-mediated high-affinity peptide export. Simulation data suggests that the concentration of unbound Tapasin varies inversely with $PPITapasin\rightarrow M,LP_a$, is nonlinear and skewed Eq. (64) (Fig. 4a, Table 4). This implies that Tapasin can partake in the de novo assembly and subsequent disassembly of the PLC in the presence of high-affinity peptides Eq. (65) (Fig. 4b, Table 4). The LAPD-model also lends support to the molecular plasticity of Tapasin and suggests a role in the “early”- and “late”-phases of MHC1-mediated export of high-affinity peptides to the plasma membrane. The presence of

### Table 3. Regression equations and assessment of robustness of time-dependent behavior of molecular entities in the LAPD-model of MHC1-mediated export of high-affinity peptides.

| Molecule (s) | Equation (37) | R² | Equation (30), (31) |
|-------------|---------------|----|---------------------|
| High-affinity peptide | $y = 6.2158 e^{0.0387t}$ | 0.9818 | (30) |
| Low-affinity peptide | $y = 2.6422 e^{-0.273t}$ | 0.9839 | (31) |
| $PPITapasin\rightarrow M$ | $y = 1.0389 ln(t) + 3.1197$ | 0.9581 | (32) |
| $PPITapasin\rightarrow LP_a$ | $y = 0.1511 ln(t) + 3.4803$ | 0.9751 | (33) |
| PLC,LP_a | $y = 3.32$ | 1.0000 | (34) |
| PLC,HP_a | $y = 0.1234 t + 3.4652$ | 0.9743 | (35) |
| rM1β | $y = -0.582 ln(t) + 4.1018$ | 0.9498 | (36) |
| $PPITapasin\rightarrow LP_a$ | $y = 0.5842 ln(t) + 4.9191$ | 0.9507 | (37) |
| eM1β | $y = 3.336 e^{-0.037t}$ | 0.9916 | (38) |

### Table 4. Temporal profile of molecular entities in the LAPD-model of MHC1-mediated export of high-affinity peptides.

| Equation/Expression/Equivalence | No. | Molecular entity $[y, \dot{y}]$ |
|-------------------------------|-----|--------------------------------|
| $1 \leq \dot{y}_{PLC,LP_a} \leq \dot{y}_{PLC,HP_a}$ | (52) | Equations (30), (31) |
| $\dot{y}_{PLC,LP_a} \leftarrow \dot{y}_{PLC,HP_a}$ | (53) | Equation (32) |
| $\dot{y}_{PLC,LP_a} \leftarrow \dot{y}_{PLC,HP_a}$ | (54) | Equation (33) |
| $\dot{y}_{PLC,LP_a} \leftarrow 0$ | (55) | Equation (34) |
| $\dot{y}_{PLC,LP_a} \leftarrow 0.1234$ | (56) | Equation (35) |
| $0.35 \leq \dot{y}_{PPITapasin\rightarrow LP_a} \leq 0.5$ | (57) | Equations (36), (37) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 0 \left( t \geq 0 \right)$ | (58) | Equation (36) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 3 + 0.0003 t^2 - 2 + 0.0034 t - 0.145$ | (59) | Equation (38) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 0 \left( t \geq 0 \right)$ | (60) | Equation (37) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 0 \left( t \geq 0 \right)$ | (61) | Equation (31) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 0 \left( t \geq 0 \right)$ | (62) | Equation (42) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 1.001$ | (63) | Equations (42), (43) and (44) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 0 \left( t \geq 0 \right)$ | (64) | Equations (37), (42) |
| $\dot{y}_{PPITapasin\rightarrow LP_a} \leftarrow 0 \left( t \geq 0 \right)$ | (65) | Equations (35), (42) |
a transmembrane-domain and the propensity to form extensive protein-protein interaction surfaces concomitantly with TAP1/2, ERp57, and MHC1 suggest that the role of Tapasin as a master regulator may be justified [38, 39, 40, 41, 46, 47, 48, 49, 50].

4.3. LAPD-model of MHC1-mediated high-affinity peptide export may offer insights into adaptive immunosurveillance

Intracellular immunogens that result from cytoplasmic infections and dysplastic cellular alterations of a host cell can elicit a spectrum of responses from circulating T- and B-cells. These include tolerance, apoptosis and autoimmune-mediated lysis of the affected cell. The LAPD-model posits that a critical mass of exchangeable MHC1 (rM1β ≈ 61%) is necessary to execute immediate export and present a novel peptide-immunogen to circulating T- and B-cells (Figs. 4a, 4c and 4d). The de novo PLC-assembly/disassembly that occurs subsequently will, in turn, generate sufficient exportable MHC1 (αM1β ≈ 79%) for a sustained immune response (Figs. 4b, 4c and 4d). The LAPD-model also offers plausible explanations into the empirically observed negative regulation by soluble Tapasin on MHC1-mediated export of high-affinity peptides [58]. In particular, soluble Tapasin might result in the complete abrogation of the “early”-phase, viz. peptide editing and the retrograde-derived exportable M1β (Figs. 4c and 4d). Furthermore, since unbound Tapasin is also a pre-requisite for the “late”-phase, molecular mechanism(s) which sequester Tapasin may severely dampen the magnitude of this phase as well (Figs. 4c and 4d). The findings presented, whilst, re-affirming the significance of Tapasin, also emphasizes the regulatory influence of low-affinity peptides, ERp57 and ATP (Figs. 4c and 4d). For example, dysplastic cellular development can saturate the ATP-driven translocation mechanism with low affinity peptides [36, 37, 38]. This will not only deplete ATP, but also ensure that Tapasin is perpetually in complex with M1β (Figs. 4c and 4d). Consequently, even if the proportion of high-affinity peptides was high the absence of ATP would render MHC1-mediated peptide export ineffectual and unavailable. A similar analogy might operate for ERp57 wherein, the absence/paucity of the mature protein may retard PLC-assembly/disassembly kinetics [46, 54]. Similarly, ATP-depletion is utilized by the human cytomegalovirus glycoprotein US6 to inhibit peptide translocation across the ER [6, 69, 70].

5. Conclusions

The adaptive immune response mandates that potential immunogens are presented in adequate quantities and in a sustained manner to circulating T- and B-cells. MHC1-based export of endogenous intracellular peptides is a complex interplay of molecules (high- and low-affinity peptides, TAP1/2, Tapasin, ERp57, ATP) and higher-order complexes. The LAPD-model, bisects MHC1-mediated export of high-affinity peptides into distinct
time-dependent “early”-, and “late”-phases. The total exportable MHC1 is initially dominated by the retrograde (recyclable)- and later by the anterograde (de novo)-pathway. Future models will explore cross-presentation of endo- and exo-genous proteins by MHC1 and MHC2, non-protein immunogen presentation, as well as perturbed antigen presentation in the pathogenesis of autoimmune diseases.

**Declarations**

**Author contribution statement**

Siddhartha Kundu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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