

Thymic Selection of T-Cell Receptors as an Extreme Value Problem

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T lymphocytes (T cells) orchestrate adaptive immune responses upon activation. T-cell activation requires sufficiently strong binding of T-cell receptors on their surface to short peptides (p) derived from foreign proteins, which are bound to major histocompatibility gene products (displayed on antigen-presenting cells). A diverse and self-tolerant T-cell repertoire is selected in the thymus. We map thymic selection processes to an extreme value problem and provide an analytic expression for the amino acid compositions of selected T-cell receptors (which enable its recognition functions).

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The adaptive immune system clears pathogens from infected hosts with the aid of T lymphocytes (T cells). Foreign (antigenic) and self-proteins are processed into short peptides (p) inside antigen-presenting cells (APCs), bound to major histocompatibility (MHC) proteins, and presented on the surface of APCs. Each T-cell receptor (TCR) has a conserved region participating in the signaling functions and a highly variable segment responsible for antigen recognition. Because variable regions are generated by stochastic rearrangement of the relevant genes, most T cells express a distinct TCR. The diversity of the T-cell repertoire enables the immune system to recognize many different antigenic short pMHC complexes. Peptides presented on MHC class I are typically 8–11 amino acids long [1], which is enough to cover all possible self-peptides (the human proteome consists of $P \approx 10^7$ amino acids [2,3]) as well as many antigenic peptides. TCR recognition of pMHC is both specific and degenerate. It is specific because most mutations to the recognized peptide amino acids abrogate recognition [4,5]. It is degenerate because a given TCR can recognize several antigenic peptides [6].

The gene rearrangement process ensuring the diversity of TCR is random. It may thus result in T cells potentially harmful to the host, because they bind strongly to self-peptide-MHC complexes, or useless T cells which bind too weakly to MHC to recognize antigenic peptides. Such aberrant TCRs are eliminated in the thymus [7–10], where immature T cells (thymocytes) are exposed to a large set (10^3 – 10^4) of self-pMHC. Thymocytes expressing a TCR that binds with high affinity to any self-pMHC molecule are deleted in the thymus (a process called negative selection). However, a thymocyte's TCR must also bind sufficiently strongly to at least one self-pMHC complex to receive survival signals and emerge from the thymus (a process called positive selection).

Signaling events, gene transcription programs, and cell migration during T-cell development in the thymus [7–16]

have been studied extensively. Despite many important advances, how interactions with self-pMHC complexes in the thymus shape the peptide-binding properties of selected TCR amino acid sequences, such that mature T cells exhibit their special properties, is poorly understood. To address this issue, in Ref. [17] we numerically studied a simple model where TCRs and pMHC were represented by strings of amino acids (Fig. 1). These strings indicate the amino acids on the interface between TCRs and pMHC complexes, and it is assumed that each site on a TCR interacts only with a corresponding site on a pMHC. The binding interface of a TCR is actually composed of a region that is in contact with the MHC molecule and a segment that is in contact with the peptide. It is the latter part that is highly variable, while the former is more conserved. We shall therefore explicitly consider only the former amino acids but not the latter. Similarly, there are many possible peptides that can bind to MHC, and their

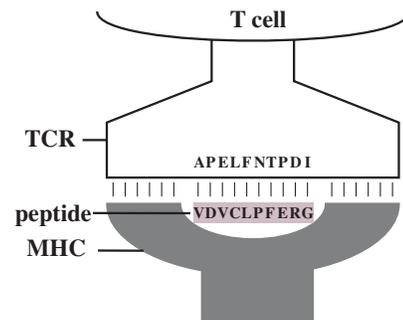


FIG. 1 (color online). Schematic representation of the interface between TCR and pMHC complexes. The segment of TCR that is in contact with peptides is highly variable and modeled by a string of N amino acids. The peptide is also modeled by a sequence of length N , and the binding energy is computed as a sum of pairwise interactions. We do not explicitly consider TCR sites in contact with MHC, as they are more or less conserved, and only assign them a net interaction energy E_c .

sequences are considered explicitly, whereas those of the MHC are not (there are only a few types of MHC in each individual human [1]). We could in principle add a few sites to the TCR and pMHC strings to account for any variability in the segments not considered.

Simplified representations of amino acids (e.g., as a string of numbers or bits) were employed earlier [15,16,18] in the context of TCR-pMHC interactions, mainly to report that negative selection reduces TCR cross-reactivity. In Ref. [17], we *numerically* studied the model in Fig. 1 (and described below) to qualitatively describe the role of positive and negative selection on the amino acid composition of selected TCRs. By randomly generating TCR and pMHC sequences, and implementing thymic selection *in silico*, we showed that selected TCRs are enriched in weakly interacting amino acids and explained how this leads to specific, yet cross-reactive, TCR recognition of antigen, a long-standing puzzle. In this Letter, we show that the model can be solved *exactly* in the limit of long TCR-peptide sequences. The resulting analytic expression for the amino acid composition of selected TCRs is surprisingly accurate even for short peptides and provides a theoretical basis for previous numerical results. Furthermore, we are able to obtain a phase diagram that indicates the ranges of parameters where negative or positive selection is dominant, leading to quite different bias in selection or function.

To assess the effects of thymic selection, as well as antigen recognition, we evaluate the free energy of interaction between TCR-pMHC pairs (for brevity, free energy will be referred to as energy). The interaction energy is composed of two parts: a TCR interaction with MHC and a TCR interaction with the peptide. The former is given a value E_c (which may be varied to describe different TCRs and MHCs). The latter is obtained by aligning the TCR and pMHC amino acids that are treated explicitly and adding the pairwise interactions between corresponding pairs. For a given TCR-pMHC pair, this gives

$$E_{\text{int}}(\vec{t}, \vec{s}) = E_c + \sum_{i=1}^N J(t_i, s_i), \quad (1)$$

where $J(t_i, s_i)$ is the contribution from the i th amino acids of the TCR (t_i) and the peptide (s_i) and N is the length of the variable TCR-peptide region. The matrix J encodes the interaction energies between specific pairs of amino acids. For numerical implementations we use the Miyazawa-Jernigan matrix [19] that was developed in the context of protein folding.

Immature T cells interact with a set \mathcal{S} of M self-pMHC complexes, where typically M is of the order of 10^3 – 10^4 . To mimic thymic selection, sequences that bind to any self-pMHC too strongly ($E_{\text{int}} < E_n$) are deleted (negative selection). However, a thymocyte's TCR must also bind sufficiently strongly ($E_{\text{int}} < E_p$) to at least one self-pMHC to receive survival signals and emerge from the

thymus (positive selection). A thymocyte expressing TCR with string \vec{t} will thus be selected if *the strongest interaction* with self-pMHC is between thresholds for negative and positive selection, i.e.,

$$E_n < \min_{\vec{s} \in \mathcal{S}} \{E_{\text{int}}(\vec{t}, \vec{s})\} < E_p. \quad (2)$$

Recent experiments [11] show that the difference between thresholds for positive and negative selection is relatively small (a few $k_B T$).

Equation (2) casts thymic selection as an extreme value problem [20], enabling us to calculate the probability $P_{\text{sel}}(\vec{t})$ that a TCR sequence \vec{t} will be selected in the thymus. Let us indicate by $\rho(x|\vec{t})$ the probability density function (PDF) of the interaction energy between the TCR \vec{t} and a random peptide. The PDF $\Pi(x|\vec{t})$ of the strongest (minimum) of the M independent random interaction energies is then obtained by multiplying ρ with the probability of all remaining $(M-1)$ energy values being larger— $[1 - P(E < x|\vec{t})]^{M-1}$, where $P(E < x|\vec{t})$ is the cumulative probability—and noting the multiplicity M for which energy is the lowest. The probability that TCR \vec{t} is selected is then obtained by integrating $\Pi(x|\vec{t})$ over the allowed range, as

$$P_{\text{sel}}(\vec{t}) = \int_{E_n}^{E_p} \Pi(x|\vec{t}) dx, \quad (3)$$

$$\text{with } \Pi(x|\vec{t}) = M\rho(x|\vec{t})[1 - P(E < x|\vec{t})]^{M-1}.$$

For $M \gg 1$, this extreme value distribution (EVD) converges to one of three possible forms [20], depending on the tail of the PDF for each entry. Equation (1) indicates that in our case, as each energy is the sum of N contributions, $\rho(x|\vec{t})$ should be a Gaussian for large N , in which case the relevant EVD is the Gumbel distribution [20].

To obtain an explicit form for $\Pi(x|\vec{t})$, we model the set \mathcal{S} of self-peptides as M strings in which each amino acid is chosen independently. The probability f_a for selecting amino acid a at each site is taken to be the frequency of this amino acid in the self-proteome. For a specific TCR sequence \vec{t} , the average interaction energy with self-peptides follows from Eq. (1) as $E_{\text{av}}(\vec{t}) = E_c + \sum_{i=1}^N \mathcal{E}(t_i)$, with $\mathcal{E}(t_i) = [J(t_i, a)]_a$, where we have denoted the average over self amino acid frequencies by $[G(a)]_a \equiv \sum_{a=1}^{20} f_a G(a)$. Similarly, the variance of the interaction energy is $V(\vec{t}) = \sum_{i=1}^N \mathcal{V}(t_i)$, where $\mathcal{V}(t_i) = [J(t_i, a)]_a^2 - [J(t_i, a)]_a^2$. For large N , we can approximate $\rho(x|\vec{t})$ with a Gaussian PDF with the above mean and variance. From standard results for the Gumbel distribution [20], we conclude that, in the limit of $M \gg 1$, the peak of the distribution $\Pi(x|\vec{t})$ is located at

$$E_0(\vec{t}) = E_{\text{av}}(\vec{t}) - \sqrt{2V(\vec{t}) \ln M}, \quad (4)$$

and its width is $\Sigma_0(\vec{t}) = \sqrt{\pi^2 V(\vec{t}) / (12 \ln M)}$. [Since the PDF $\rho(x|\vec{t})$ originates from a bounded set of energies, it

is strictly not Gaussian in the tails. Hence, once the extreme values begin to probe the tail of the distribution, the above results will no longer be valid. Indeed, in the limit when $M \sim \mathcal{O}(20^N)$, the EVD will approach a delta function centered at the M -independent value corresponding to the optimal binding energy.]

In the limit of long TCR-peptide complexes $N \gg 1$, we can exactly calculate the statistics of the amino acid composition of selected TCRs. To obtain a proper thermodynamic limit, we need to set $\{E_c, E_p, E_n\} \propto N$ and $\ln M \propto N$. The latter ensures that the peak of the distribution $E_0(\vec{t})$ is proportional to N and also results in a width $\Sigma_0(\vec{t})$ which is independent of N . (The relation $\ln M = \alpha N$ can be justified with the expectation that M should grow proportionately to the proteome size P , while $N \propto \ln P$ to enable encoding the proteome.) In this large N limit, the EVD is sufficiently narrow that the value of the optimal energy can be precisely equated with the peak $E_0(\vec{t})$, and Eq. (2) for the selection condition can be replaced with

$$E_n < E_c + \sum_{i=1}^N \mathcal{E}(t_i) - \sqrt{2 \ln M \sum_{i=1}^N \mathcal{V}(t_i)} < E_p. \quad (5)$$

Thus, for each sequence \vec{t} , we have to evaluate the ‘‘Hamiltonian’’ $E_0(\vec{t})$, and the sequence is accepted if this energy falls in the interval (E_n, E_p) . This is somewhat similar to the microcanonical ensemble in statistical physics, with the restriction of the energy to an interval rather than a narrow range only a minor elaboration (see below). From the equivalence of canonical and microcanonical ensembles for large N , we know that the probability for a sequence is governed by the Boltzmann weight $p(\vec{t}) \propto (\prod_{i=1}^N f_{t_i}) \exp[-\beta E_0(\vec{t})]$. Here $\{f_a\}$ indicate the natural frequencies of the different amino acids prior to selection, while the effect of thymic selection is captured in the parameter β which is determined by solving for the average energy.

The appearance of $\sqrt{2 \ln M \sum_{i=1}^N \mathcal{V}(t_i)}$ in the Hamiltonian initially appears as a complication that makes exact computation of the average energy from $\exp[-\beta E_0(\vec{t})]$ impossible. However, this apparent ‘‘coupling’’ is easily dealt with by standard methods such as Legendre transforms or Hamiltonian minimization [21]. This can be justified easily as follows: We need to solve a Hamiltonian $\mathcal{H}(U, V)$ which depends on two extensive quantities $U = \sum_{i=1}^N \mathcal{E}(t_i)$ and $V = \sum_{i=1}^N \mathcal{V}(t_i)$. The corresponding partition function can be decomposed as $Z = \sum_{U, V} \Omega(U, V) e^{-\beta \mathcal{H}(U, V)}$ but can be approximated with its largest term. Note that the same density of states $\Omega(U, V) \equiv e^{S(U, V)/k_B}$ appears, irrespective of the specific form of $\mathcal{H}(U, V)$. In particular, the choice $\mathcal{H}_0 = E_c + U - \gamma V - \ln M / (2\gamma) = E_c + \sum_{i=1}^N [\mathcal{E}(t_i) - \gamma \mathcal{V}(t_i)] - \ln M / (2\gamma)$ corresponds to a set of noninteracting variables, with

$$p(\vec{t}) \propto \prod_{i=1}^N f_{t_i} \exp\{-\beta[\mathcal{E}(t_i) - \gamma \mathcal{V}(t_i)]\}, \quad (6)$$

for which thermodynamic quantities (such as entropy) are easily computed. By a judicious choice of γ , we can then ensure that the same average energy appears for $\mathcal{H}_0(\vec{t})$ and our $E_0(\vec{t})$. Using Legendre transforms, which is equivalent to minimizing $\mathcal{H}_0(\vec{t})$ with respect to γ , one finds that the required $E_0(\vec{t})$ is obtained by setting $\gamma(\beta) = \sqrt{\ln M / (2N \langle \mathcal{V} \rangle_{\beta, \gamma})}$, where $\langle \dots \rangle_{\beta, \gamma}$ refers to the average with the noninteracting weight $e^{-\beta(U - \gamma V)}$.

Finally, the value of β has to be determined by constraining the average energy determined above to the range in Eq. (5), while maximizing entropy. Given the bounded set of energies, the inverse temperature β can be either negative or positive. The 20^N possible values for $E_0(\vec{t})$ span a range from E_{\min} to E_{\max} and a corresponding number of states which is a bell shape between these extremes with a maximum at some E_{mid} . If $E_{\text{mid}} > E_p$, we must set β such that $\langle E_0(\vec{t}) \rangle = E_p$. In this case, $\beta > 0$, positive selection is dominant, and stronger amino acids are selected. If $E_{\text{mid}} < E_n$, we must set β such that $\langle E_0(\vec{t}) \rangle = E_n$, $\beta < 0$, negative selection is dominant, and weaker amino acids are selected. For $E_n < E_{\text{mid}} < E_p$, we must set $\beta = 0$, and there is no modification due to selection.

Figure 2 depicts the variation of β as a function of $\ln(M)/N$ and threshold for negative selection E_n with $(E_p - E_n)/N = 0.5k_B T$. Consider TCRs that do not bind too strongly or weakly to MHC, as such TCRs are unlikely to be selected (e.g., $E_n - E_c = -21k_B T$). For the set of parameters that are relevant for thymic selection in a

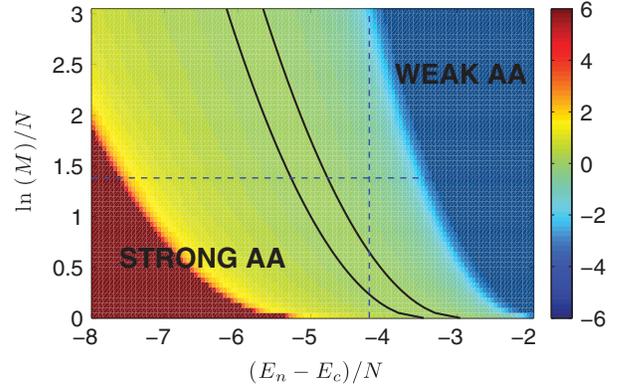


FIG. 2 (color). Color representation of the dependence of the inverse temperature β on the number of self-peptides $\ln M/N$ and the threshold for negative selection energy E_n/N with $(E_p - E_n)/N = 0.5k_B T$ in the limit of large N . The region between the black lines corresponds to $\beta = 0$, to the right (left) of which negative (positive) selection is dominant, and weak (strong) amino acids (AA) are selected. Note that, as $(E_p - E_n)/N$ goes to zero, the intermediate region disappears. The dotted lines indicate the relevant parameter values for thymic selection in a mouse (see text) that result in $\beta = -0.37(k_B T)^{-1}$.

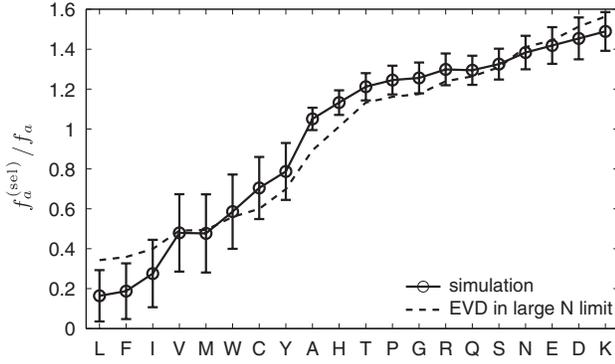


FIG. 3. Amino acid composition of selected TCR sequences, ordered in increasing frequency along the abscissa. The data points in black are obtained numerically with the parameters relevant to a mouse (see caption of Fig. 2 and text). The error bars reflect the sample size used to generate the histograms and differences for different realizations of M self-peptides. The dashed line is the result of the EVD analysis in the large N limit from Eq. (7), and the agreement is quite good. In both cases we have used the Miyazawa-Jernigan matrix J [19] and amino acid frequencies f_a from the mouse proteome [3,17].

mouse [17], i.e., $N = 5$, $E_p - E_n = 2.5k_B T$ and $M = 10^3$, we find $\beta = -0.37(k_B T)^{-1}$, which means that negative selection is dominant and weaker amino acids are selected. Also, $\gamma = 0.83(k_B T)^{-1}$ indicating a preference for amino acids with smaller variations in binding energy. With these parameters we can calculate the amino acid frequencies of selected TCRs as

$$f_a^{(\text{sel})} = \frac{f_a \exp\{-\beta[\mathcal{E}(a) - \gamma\mathcal{V}(a)]\}}{\sum_{b=1}^{20} f_b \exp\{-\beta[\mathcal{E}(b) - \gamma\mathcal{V}(b)]\}}. \quad (7)$$

It is important to ask if the above expression, exact in the limit of $N \rightarrow \infty$, has any relevance to the actual TCR-peptide complex with $N \sim 5-10$. We thus numerically simulated the case of $N = 5$ by generating a random set of 10^6 TCR sequences and selected them against $M = 10^3$ self-peptides. The selected TCRs were used to construct the amino acid frequencies depicted in Fig. 3. The dashed line in this figure comes from Eq. (7) with the same J and $\{f_a\}$. The agreement between the two is remarkable given the small value of $N = 5$ and may be indicative of small corrections to the $N \rightarrow \infty$ result.

Equation (7) thus provides an analytical expression that captures the characteristics of TCR amino acids selected against many peptides in the thymus. In accord with previous numerical results [17] and some available data from a normal mouse and human, it predicts (since $\beta < 0$) that TCR sequences are enriched in weakly interacting amino acids (small \mathcal{E}). This result was used previously [17] to explain their specificity. However, Eq. (7) further indicates the role of promiscuity of amino acids (captured by the parameter γ) which was not elucidated from the limited numerical data. Furthermore, the phase diagram in Fig. 2

indicates how upon raising the number of self-peptides there is a transition from preference for strong amino acids ($\beta > 0$, positive selection dominant) to weak amino acids ($\beta < 0$, negative selection dominant), which may be feasibly tested in future experiments, along the lines of Ref. [4].

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