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Sculpting the Immunological Response to Dengue Fever by Polytopic Vaccination

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Abstract

The twin challenges of immunodominance and heterologous immunity have hampered discovery of an effective vaccine against all four dengue viruses. Here we explore how the T cell competition and selection underlying these asymmetrical properties impede effective T cell vaccine design. The theory we develop predicts dengue vaccine clinical trial data well. From the insights that we gain by this theory, we propose two new ideas for design of epitope-based T cell vaccines against dengue: polytopic injection and subdominant epitope priming.

1 Introduction

Dengue virus infections are a serious cause of morbidity and mortality in most tropical and subtropical areas of the world [1–3]. Dengue virus causes an estimated 100 million cases annually [1]. The most serious form of the disease, dengue hemorrhagic fever, leads to the hospitalization of several hundred thousand patients each year, typically as a result of sequential dengue virus infections [1,4,5]. There are four dengue viruses. Immunization with one dengue virus is protective against future challenge with the immunizing virus. However, immunity built up after infection by one dengue virus protects only modestly or even negatively against reinfection by the other dengue viruses [6–8]. In particular, the risk of dengue hemorrhagic fever from one of the dengue viruses during a secondary infection appears to rise significantly if there was a previous primary infection from one of the other dengue viruses [4, 6, 9].

This 'original antigenic sin' implies that an effective vaccine for dengue must induce protective immunity against all four dengue viruses [1, 6, 7, 10]. To date, no such vaccine has been developed. The immunological epitopes of the four dengue viruses are closely related but differ in sequence, and it is believed that the T cell immunological response to each virus is largely to a single epitope [11, 12]. Indeed, in the epitope-based T cell vaccines that we consider, the response is directly solely to a selected set of virus-specific epitopes. Differences in the epitope sequences affect the quality of the effector response, and simultaneous exposure to the dominant epitopes from all four dengue viruses reduces the quality of the immune response to some of the viruses [1, 13].

In an effort to establish a connection between infection of mononuclear cells and pathophysiologic changes, some of the recent research has focused on the T cell response to the dengue viruses [6, 10, 13, 14]. In particular, there is an interest in quantifying low-avidity cross reactivity of T cells between the different dengue viruses, as such T cells are believed to lead possibly to an increased risk of dengue hemorrhagic fever [6, 7, 10]. Clinical trials of a four-component dengue vaccine [13] show an immunodominance effect in the T cell response. Why T cell immunodominance occurs so dramatically for dengue remains mysterious. Most studies suggest that CD8⁺ T cell receptors (TCRs) specific for dominant epitopes suppress response to other epitopes, due to resource competition, homeostasis, apoptosis, and reduction of viral load [15–17]. Immunization with non-structural proteins is viewed by some as a means to avoid possible antibody dependent enhancement [7]. While for NS1 the antibody response may contribute to protection, for the other non-structural proteins, it is assumed that a primarily T cell response will be induced [7]. The degree to which this T cell response will be beneficial or detrimental remains unclear. A quantitative understanding of immunodominance is hampered by the complex immunological interactions between the TCRs and the epitopes and by the complex competition and selection process among the TCRs. The focus of our paper is on these studies of induction of T cell-based immunity. While vaccine-induced antibodies may be needed in addition to T cells for protection against the dengue viruses, understanding the T cell response seems important for resolving the issues related to original antigenic sin and dengue hemorrhagic fever associated with dengue, both of which seem to be based in part on the T cell response.

We here explore the possibility of using polytopic, or multi-site, vaccination to induce an effective T cell immune response against all four dengue viruses. Note that multi-site vaccination means more than injecting a vaccine in multiple places, *e.g.* as is done with the tetanus vaccine in the stomach and buttocks. “Multi-site vaccination” as used here means injecting each component of a vaccine so that each component drains to a physiologically distinct lymph node. Moreover, we focus on multi-site vaccination for the *related* dengue viruses. We investigate whether injection of the epitopes from each of the four viruses in different physical locations sculpts a broader TCR response, by inducing TCR selection for each epitope in different lymph nodes. We determine whether polytopic vaccination reduces immunodominance and increases recognition of the four dengue viruses. Vaccination with individual epitopes at physically separated sites rather than with multiple epitopes at one site has been shown to reduce immunodominance in cancer vaccines [18]. Draining to a specific lymph node is especially pronounced in vaccination with antigen-bearing dendritic cells [19, 20]. We also consider whether subdominant epitope priming is an effective strategy for sculpting increased recognition of the subdominant dengue viruses within the TCR repertoire. Subdominant epitope priming has shown promise in LCMV and cancer experiments in mice [21–23].

In this study we develop a generalized NK [24, 25], or spin glass, theory of T cell original antigenic sin and immunodominance. The theory captures the stochastic nature of human immunity and realistic recognition characteristics between TCRs and virus at the sequence level. The theory models immune system dynamics to address the competition of cross-

active T cells to the four dengue viruses that leads to the selection of effector and memory T cells. This theory is a simplified virtual immune system and focuses on the competitive selection dynamics of CD8⁺ TCRs. In our consideration of CD8⁺ T cell vaccines, the additional contributions of the antibody, innate, and CD4⁺ T cell responses of the immune system are not considered. As will be shown, the theory is useful and predictive as a tool for the CD8⁺ T cell response. The theory allows investigation and determination of the fundamental qualitative and quantitative features that govern the interaction between an effective multivalent T cell vaccine and the variability of the virus. The theory complements and provide some guidance to the long and difficult process of experimental multivalent vaccine development.

2 Results

Specific Lysis

The theory captures the stochastic nature of human immunity and realistic recognition characteristics between TCRs and virus at the sequence level. Agreement within experimental error bars is observed between predictions from the theory and observations from clinical vaccine trials (see Figure 1). These trials measured specific lysis, a standard measure of the immunological response to a vaccine, which correlates well with vaccine efficacy [26]. We emphasize that this result is not a fit, as the model was calibrated to the altered peptide ligand data before this prediction was made.

Original Antigenic Sin and Immunodominance

We first discuss how cross-immunity to the four dengue viruses shapes the T cell repertoire differently under variant immunological histories. Figure 2a shows the response to a second antigen if the exposure to a first antigen exists (solid line) or not (dashed line) as a function of the difference between the first and second antigen, p_{epitope} . When the difference is small, the exposure to a first antigen leads to a higher clearance probability, Z (see Methods), than without exposure. For a large difference, the antigen encountered in the first exposure is uncorrelated with that in the second exposure, and so immune system memory does not play a role. Interestingly, the immunological memory from the first exposure actually gives worse protection, a lower Z , for intermediate differences than would no memory, which is original antigenic sin [8]. The epitopic variation for dengue lies in this range. During primary infection, T cell populations with higher affinity for the infecting virus are preferentially expanded and enter the memory pool. When exposure to another virus follows this first exposure, not only is the memory T cell population at a 100 to 1000 times greater concentration than the naive T cell population [27], but also the average binding constant of the memory T cell population may initially be higher than that of the naive population, as shown by the Round = 0 data in Figure 2b. For dengue, and other diseases, these memory T cells will, therefore, be selectively expanded, even though selection and expansion of the naive population would have produced superior binding constants of the effector T cells, as shown by the Round = 10 data in Figure 2b. That is, memory T cells are expected to perform better against cross-active viruses than would naive TCRs. But our theory shows that for dengue, from Figure 2b, this evolved behavior of the immune system is faulted, and naive TCRs can select for superior binding affinity.

We next address how the immune response to one or two dominant dengue viruses suppresses the response to the other, subdominant viruses. This immunodominance can be seen in Figure 1. The immune response is typically strong to only a few epitopes. The immune response to these dominant epitopes suppresses the response to the other, subdominant epitopes. This immunodominance is well captured by the generalized NK theory. Even in the absence of competition of the TCRs for resources, there is immunodominance in the immune response (Figure 2c, left column). This result shows that immunodominance stems from the heterologous nature of the immune system. It appears inevitable. Vaccination with only a single virus increases the immunodominance, as a result of original antigenic sin.

Sequential Dependence of Response

Priming with the subdominant epitope (Figure 2c, right columns, $i = 1$) sculpts a broader immune response and leads to reduced immunodominance in a secondary response than does priming with the dominant epitope (Figure 2c, right columns, $i = 4$). The improvement against the least-recognized dengue virus (Figure 2c red, $i = 1$ versus $i = 4$) is 77%, and the specific lysis values against each of the four dengue viruses are approximately equal with subdominant epitope priming Figure 2c $i = 1$). Epidemiological studies have suggested that the order of exposure to dengue virus infections is important [9]. CTLs induced by one dengue virus may recognize another dengue virus to a greater extent than the reverse, which increases the odds of dengue hemorrhagic fever [2,28]. We term this the ordering effect. Figure 2c shows the effect. Our theory suggest that there is nonreciprocal CTL cross-activity among the four dengue viruses. Initial exposure to the dominant dengue virus will suppress the available TCRs for a subsequent exposure to a subdominant dengue virus more quickly and harmfully than the reverse. Conversely, initial exposure to the subdominant epitope does not cause too much suppression against the dominant epitope because of the excess of high-affinity TCRs available for the dominant epitope. We expect that the increased TCR diversity induced by subdominant epitope priming will lead to a broader and more persistent immune response. Our theory (Figure 2c, right columns, $i = 1$) predicts and epidemiological studies [9] show that primary exposure to the subdominant DEN-2 followed by secondary exposure to the other dengue virus is the least likely infection sequence to produce severe dengue disease. Additionally our theory (Figure 2c, right columns, $i = 4$) predicts and epidemiological studies [9, 29] also show that primary exposure to other dengue viruses followed by secondary exposure to the subdominant DEN-2 is the most likely infection sequence to produce severe disease [9,29]. Our results direct that to construct and administer an optimal vaccine, the patient history and vaccine ordering effect must be considered.

Polytopic Injection

By physically separating the TCR selection and reducing the pressure on TCR resource competition within each lymph node, the TCR repertoire can be sculpted toward the subdominant epitopes, and so there is a reduction in immunodominance. We term this the polytopic effect. Figures 3 and 4c show the effect. Immunodominance and competition through space imply that to maximize protection and minimize pathologic heterologous immunity and to achieve a long-lasting immune response, an optimal dengue vaccine should induce a high concentration of high-affinity TCRs against all four dengue viruses [13–15,

30]. In other words, the immune system must be induced to expand TCRs against all dominant and subdominant viruses. Larger values of the parameter *mixing round* allow for a longer period of independent TCR selection during a typical 10-round immune response period and lead to less immunodominance, Figure 3. The parameter *mixing round* is relative to the T cell division time, which is typically 12–24 hours. It is seen that the results are not greatly sensitive to the exact value of this lumped parameter. Some T cells may leave the lymph node before the *mixing round*, and some may leave after the *mixing round*, but the average time to leave is *mixing round*, and the average behavior is shown in Figure 3. For the human immune system, the time for T cells to leave the lymph nodes plus the circulation time of the lymph system is in the range 6–10 days [27,31]. Within this entire physiological range, the polytopic effect is positive.

Sculpting the Immunological Response to a Dengue Vaccine

By combining polytopic injection with subdominant epitope priming, a vaccination protocol for sculpting the immune response to dengue is achieved, Figure 4d. This new protocol reduces immunodominance more fully than does polytopic injection or subdominant epitope priming alone, Figures 4c and 4b, respectively, both of which reduce immunodominance more than does a traditional four-component dengue vaccine, Figure 4a. Immunological memory and competition through time imply that response to a subdominant virus can be strengthened with prior exposure. Indeed, not only does patient history affect the response to vaccination, Figure 2a, but also a history can be imposed by a vaccination sequence.

3 Discussion

Original antigenic sin and immunodominance are two sides of one coin. They both stem from the competitive selection of cross-active TCRs. Original antigenic sin acts sequentially through time and arises from competition between memory and naive T cells. Because of original antigenic sin, prior exposure history is an important factor to the immune response. Immunodominance acts simultaneously through space on T cells competing for expansion against multiple, related strains or viruses. Because of immunodominance, relatedness of strains or viruses in a multi-component vaccine is an important factor to the immune response. From Figure 1a, we see that the competitive selection leading to original antigen sin occurs in the range $0.02 < p_{\text{epitope}} < 0.4$, in which dengue lies.

When a single-strain disease begins to mutate, the two sides of the heterologous immunity coin become connected. Original antigenic sin imparts to some of the mutants a selective advantage at escaping immune system control. Concomitantly, the dominant escape mutants skew the T cell repertoire toward themselves and away from the subdominant escape mutants [32]. These subdominant mutants can then proliferate. HIV and cancer are examples of such escape from the immune system [33, 34]. In both cases, immunodominance is a significant barrier to developing effective vaccines against these multi-strain diseases.

The strategies of polytopic vaccination and subdominant epitope priming apply to vaccination against disease strains or viruses expressing multiple epitopes. In this case, the optimal strategy would be to identify and vaccination against all of the significant epitopes in each strain or virus. While the epitope-based vaccines that we consider appear to be a

promising approach, polytopic vaccination and subdominant epitope priming will still reduce immunodominance in whole-strain vaccines if each virus or strain is administered separately.

The polytopic injection and subdominant epitope priming ideas appear to apply generically to multi-strain viral diseases. Competition due to limited partial cross-activity is the reason for original antigen sin. Immunodominance is a more general phenomenon, which persists for all values of p_{epitope} , and which also results from competition. Exactly this deleterious competition is why the separate selection that occurs in polytopic injection leads to less immunodominance and why subdominant epitope priming is useful to achieve a significant number of TCRs responding to the subdominant epitopes. Our theory captures realistic recognition characteristics between the TCRs and the virus, the primary and secondary dynamics due to TCR resource competition, and the stochastic nature of heterologous human immunity. Dengue was unique in our theory because the difference between the epitopes of the four viruses is roughly $p_{\text{epitope}} = 0.5/9$. Our theory suggests that our strategies can improve recognition of the least-recognized virus by a factor of four (Figure 4ad, red). Moreover, the immunodominance is nearly gone with these methods, as the specific lysis against each dengue virus is nearly the same (Figure 4d, $i = 1$, all color bars nearly equal). For virus or strains with larger differences, both subdominant epitope priming and polytopic injection continue to work effectively. When $p_{\text{epitope}} = 1.0/9$, such as a variant dengue strain or other multi-strain disease, our strategies can improve recognition of the least-recognized virus by a factor of eight (Figure 5ad, red). Moreover, the immunodominance is nearly gone in this case, as the specific lysis against each dengue virus is nearly the same. (Figure 5d, $i = 1$, all color bars nearly equal). The improvement of polytopic vaccination over single site persists to all values of p_{epitope} .

The results from our theory not only quantitatively reproduce and explain immunodominance, but also predict and explain original antigenic sin. From our theory of the ordering and polytopic effects, subdominant epitope priming followed by secondary polytopic injection of epitopes appears to be a promising vaccination strategy for dengue fever and other multi-strain diseases.

Methods

Generalized NK model

The generalized NK model for the T-cell response considers interactions between the TCR, MHC, and peptide epitope [25]. Parameters in the theory are shown in Table 1. The model returns the free energy of binding (U) as a function of the TCR amino acid sequence (a_j) and epitope amino acid sequence (a_j^{pep}).

The binding constant is related to the energy by

$$K = e^{a-bU}. \quad (1)$$

We determine the values of a , b in each instance of the ensemble by fixing the geometric average TCR:p-MHCI affinity to be $K = 10^4$ l/mol and minimum affinity to be $K = 10^2$ l/mol for the $N_{\text{size}} = 10^8/10^5 = 1000$ distinct TCRs that respond to one epitope [35,36]. This means that for the highest affinity TCR, K fluctuates between 10^5 l/mol and 10^7 l/mol for the different epitopes [37] (see Figure 6).

Specific lysis is a measure of the probability that an activated T cell will recognize an antigen presenting cell that is expressing a particular peptide-MHCI complex. It is given by [25]

$$L = \frac{zE/T}{1+zE/T}, \quad (2)$$

where E/T is the effector to target ratio. The quantity z is, therefore, the average clearance probability of one TCR:

$$z = \frac{1}{N_{\text{size}}} \sum_{i=1}^{N_{\text{size}}} \min(1, K_i/10^6). \quad (3)$$

TCR Selection Dynamics

The naive TCR repertoire is generated randomly from gene fragments. This is accomplished by constructing the TCRs from subdomain pools. Fragments for each of the L subdomain types are chosen randomly from 13 of the 100 lowest energy subdomain sequences. This diversity mimics the known TCR diversity, $(13 \times L)^M \approx 10^{11}$ [38]. Only 1 in 10^5 naive TCRs responds to any particular antigen, and there are only 10^8 distinct TCRs present at any one point in time in the human immune system [36, 39], so the primary response starts with a repertoire of $N_{\text{size}} = 10^3$ distinct TCRs. The quality with which these TCRs recognize the epitope can vary, as shown in Figure 6. A flow diagram of the TCR selection dynamics is shown in Figure 7.

The T-cell-mediated response is driven by cycles of concentration expansion and selection for better binding constants. The primary response increases the concentration of selected TCRs by 1000 fold over 10 rounds, with a rough T cell doubling time of 12–24 hours [27]. The diversity of the memory sequences is 0.5% of that of the naive repertoire [40]. Specifically, 10 rounds of selection are performed during the primary response, with the top $x = 58\%$ of the sequences chosen at each round. The T cell division may occur more rapidly than once per day, and the *mixing round* parameter is measured in the time scale of T cell divisions. In any case, 10 T cell divisions are necessary to achieve the the concentration expansion by a factor of 10^3 in the primary response, since $2^{10} \approx 10^3$. This process leads to 0.5% diversity of the memory repertoire, because $0.58^{10} \approx 0.5\%$. In vivo T cell tracking experiments have shown that T cells continue to interact with antigen in the lymph node up to 5 days after initial stimulation [41, 42], and examinations of draining lymph nodes have found that antigen capable of priming naive T cells is present up to 7 days post inoculation [43], both of which support the concept that the selection process may continue during each

T cell division. Although the exact mechanism of the T cell expansion during the primary immune response remains elusive, it is observed that the expansion of the T cells is non-linear [44], and that in most cases there is competition among the T cells for the presented antigen [45,46]. If memory TCRs are used in the secondary response, the top $x = 58\%$ of the sequences are chosen, and 3 rounds of selection are performed [44, 47]. This mimics the concentration factor of $10 \approx 2^3$ that occurs during the secondary memory response [27]. Conversely, if the naive TCRs are used in the secondary response, the dynamics is identical to that of the primary response. The total secondary response is the combined response, starting with a combination of memory and naive TCRs. As in [24], we take the fraction of the secondary response stemming from memory cells to be proportional to the average binding constant of the memory TCRs for the epitope (which is proportional to the clearance probability of the memory sequences, Z_m), and we take the fraction of the secondary response stemming from naive TCRs to be proportional to the average binding constant of the naive TCRs for the epitope (which is proportional to the clearance probability of the naive sequences, Z_n).

Parameters for Dengue

For each epitope, the sequence, model, and VDJ selection pools differ by p_{epitope} [24], where

$$p_{\text{epitope}} = \frac{(\text{non-conservative} + \frac{1}{2}\text{conservative}) \text{ amino acid differences in epitope}}{\text{total number of amino acids in epitope}}. \quad (4)$$

To generate results, an average over many instances of these random epitope sequences, models, and VDJ selection pools that differ by p_{epitope} is taken. In other words, the different dengue virus epitopes were chosen so that they differ by the requisite p_{epitope} . To compute average results, four new random epitopes were generated each time. The initial TCR repertoire is redetermined for each realization of the model—this must be done because the U^{sd} that defines the TCR repertoire is different in each instance of the ensemble.

For dengue, the nonstructural (NS3) protein is an attractive candidate for a subunit vaccine [11]. We consider the observed single conservative amino acid change in the epitope [12], and so $p_{\text{epitope}} = 0.5/9$. Qualitatively similar results are obtained for $p_{\text{epitope}} = 1/9$, as might occur for drift strains of dengue. We assume that for each dengue virus, only a single epitope is recognized by the immune system.

Polytopic Vaccination

For a polytopic vaccination, the different viruses are injected in different physical locations and evoke an immune response that evolves independently in different lymph nodes until *mixing round*, after which the lymph system is well-mixed. This is modeled by performing a response against each of the four viruses independently, with selection among 10^3 TCRs for each virus, until *mixing round*. At *mixing round*, 10^3 TCRs are randomly chosen from the 4×10^3 partially evolved TCRs. These 10^3 TCRs are then evolved from *mixing round* until round 10. In this way, we model the independent response in different lymph nodes against

each virus that occurs early on and the combined response in a typical lymph node against all viruses that occurs after the lymph system has mixed.

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46. It is not clear whether this competition occurs during each and every round of T cell division during the primary response. However, the general results of our model hold for any type of exponential expansion during the primary response of the T cells that best recognize the epitope.
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Fig. 1.
Specific lysis ratios for the least to the most dominant epitope of the four dengue viruses.
The error bars are given by standard error analysis of the experimental error bars.
Experimental data are from [13].

Fig. 2.

(a) Evolved clearance probability to a second virus after exposure to an original virus that differs in the epitope amino acid sequence by fraction p_{epitope} (solid line). The primary response in the absence of vaccine is shown (dashed line, independent of p_{epitope}). For dengue, $p_{\text{epitope}} = 0.5/9$ (circle). (b) During a secondary response for dengue, memory TCRs are initially better but eventually worse after selection than are naive TCRs ($p_{\text{epitope}} = 0.5/9$). This result explains the cause of original antigenic sin. (c) The immunodominance among the four viruses under different conditions. Left column: the immune response to each of the four viruses evolves independently during a primary response, and the immunodominance due to the heterologous nature of immunity is measured. That is, the response to virus i is measured after a primary vaccination against virus i . Middle columns: the i^{th} column shows the response to the four viruses after a primary vaccination against virus i . Note the immunodominance. That is, the response to virus j is measured after a primary vaccination against virus i . Right columns: initial primary response to virus i for the i^{th} column, followed by a secondary response as in the left column. That is, the response to virus j is measured after a primary vaccination against virus i and a secondary vaccination against virus j . In this figure, as in Figure 1 and subsequent figures, dominance order is defined by the primary response to single-site, four-component vaccination.

Fig. 3.

Predictions of the theory for a polytopic four-component dengue vaccine, $p_{\text{epitope}} = 0.5/9$, when the different dengue viruses are injected in different physical locations and evoke an immune response that evolves independently in different lymph nodes until *mixing round*, after which the lymph system is well-mixed.

Fig. 4.

$p_{\text{epitope}} = 0.5/9$. (a) Immunodominance for a four-component dengue vaccine. (b) Reduced immunodominance for a primary single-component dengue vaccine (virus i in the i^{th} column), followed by a secondary four-component dengue vaccine. (c) Reduced immunodominance for a polytopic four-component dengue vaccine, with each component administered to a distinct lymph node (*mixing round* = 9). (d) Reduced immunodominance for a primary single-component dengue vaccine (virus i in the i^{th} column), followed by a secondary polytopic four-component dengue vaccine (*mixing round* = 9). An improved response to the subdominant virus (red) provides the most immunological benefit in the case of dengue; this response is improved to $1.81\times$ in c) versus a) and to $4\times$ in d) versus a).

Fig. 5.

$p_{\text{epitope}} = 1.0/9$. (a) Immunodominance for a four-component dengue vaccine. (b) Reduced immunodominance for a primary single-component dengue vaccine (virus i in the i^{th} column), followed by a secondary four-component dengue vaccine. (c) Reduced immunodominance for a polytopic four-component dengue vaccine, with each component administered to a distinct lymph node (*mixing round* = 9). (d) Reduced immunodominance for a primary single-component dengue vaccine (virus i in the i^{th} column), followed by a secondary polytopic four-component dengue vaccine (*mixing round* = 9). An improved response to the subdominant virus (red) provides the most immunological benefit in the case of dengue; this response is improved to $4.3\times$ in c) versus a) and to $8\times$ in d) versus a).

Fig. 6.
(a) For the highest affinity TCR, the binding constant fluctuates between 10^5 l/mol and 10^7 l/mol. (b) Most binding constant differs within a factor of 10 for the 0.5% of antigen specific T cells selected to be memory T cells.

Fig. 7.
Flow diagram of the TCR selection dynamics in the Generalized NK model

Table 1

Parameter values for the Generalized NK model

Parameter	Value	Definition
TCR		
a_j		Identity of amino acid at sequence position j
M	6	Number of secondary structure subdomains
N	9	Number of amino acids in each subdomain
L	5	Number of subdomain types (e.g., helices, strands, loops, turns and others)
a_i	1 a_i L	Type of secondary structure for the i^{th} subdomain
K	4	Local interaction range within a subdomain
σ_{a_i}		Local interaction coupling within a subdomain, for subdomain type a_i
D	2	Number of interactions between subdomains
$\sigma_{i,j}^k$		Nonlocal interaction coupling between secondary structures
Epitope		
a_j^{pep}		Identity of amino acid of epitope at sequence position j
N	9	Number of amino acids in epitope
TCR-Epitope		
N_b	3	Number of hot-spot amino acids in the epitope
N_{CON}	3	Number of amino acids in TCR that each hot spot interacts with
σ_{ij}		Interaction coupling between TCR and epitope
σ_i^k		Interaction coupling between TCR secondary structure and epitope
Random couplings		
w		Gaussian random number with zero average and unit standard deviation
σ	$w_j + w/2$	Value of coupling for amino acid i of non-conservative type j