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Homology modeling and molecular dynamics simulations of MUC1-9/H-2K^b complex suggest novel binding interactions

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1 **Abstract**

2 Human MUC1 is over-expressed on human adenocarcinomas and has been used as a
3 target for immunotherapy studies. The 9-mer MUC1-9 peptide has been identified
4 as one of the peptides which binds to murine MHC class I H-2K^b. The structure
5 of MUC1-9 in complex with H-2K^b has been modeled and simulated with classical
6 molecular dynamics, based on the x-ray structure of the SEV9 peptide/H-2K^b com-
7 plex. Two independent trajectories with the solvated complex (10 ns in length) were
8 produced. Approximately 12 hydrogen bonds were identified during both trajecto-
9 ries to contribute to peptide/MHC complexation, as well as 1-2 water mediated
10 hydrogen bonds. Stability of complex was also confirmed by buried surface area
11 analysis, although the corresponding values were about 20% lower than those of the
12 original x-ray structure. Interestingly, a bulged conformation of the peptide's cen-
13 tral region, partially characterized as a β -turn, was found exposed from the binding
14 groove. In addition, P1 and P9 residues remained bound in the A and F binding
15 pockets, even though there was suggestion that P9 was more flexible. The complex
16 lacked the numerous water mediated hydrogen bonds that were present in the refer-
17 ence peptide x-ray structure. Moreover, local displacements of residues Asp4, Thr5
18 and Pro9 resulted in loss of some key interactions with the MHC molecule. This
19 might explain the reduced affinity of the MUC1-9 peptide, relatively to SEV9, for
20 the MHC class I H-2K^b.

21 **Keywords:** Class I MHC; H-2K^b; homology modeling; molecular dynamics; MUC1;
22 tumor

1 Introduction

Major histocompatibility complex (MHC) proteins bind small peptide fragments derived from pathogenic proteins and form peptide/MHC (pMHC) complexes (Raghuvan et al., 2008). MHC proteins are divided into two classes: class I (MHC-I) and class II (MHC-II). The MHC-I consists of a polymorphic transmembrane heavy chain and β 2-microglobulin, which are non-covalently associated (Zhang et al., 1998). The proteolysis of intracellular proteins by the proteasome produces the majority of peptides suitable for MHC-I binding. In most cases, peptides of 8-10 residues in length are found in the binding groove of MHC-I.

After the first crystal structures of pMHC complexes were available, (Bjorkman et al., 1987; Fremont et al., 1992; Matsamura et al., 1992) it was suggested that peptides bound to MHC-I with a canonical extended structure. MHC class I residues that form the binding groove are responsible for the specificity of the peptide selection. Six (out of 8-10) residues of the peptide sequence are [accommodated](#) within the A-F binding pockets of the MHC-I protein (Saper et al., 1991). Residues that do not participate directly in binding are believed to interact with the TCR.

Human mucin, MUC1, is a membrane-bound glycoprotein, expressed on the surface of epithelial cells. It is often overproduced and/or underglycosylated in adenocarcinomas (breast, ovary, colon, lung, kidney, etc) and is present in the serum of cancer patients. MUC1 is immunogenic in mice and in humans, with both humoral and cellular immune responses being induced by MUC1-based vaccine constructs (Tang et al., 2008b,a). MUC1 mucin partly consists of a variable number of tandem repeats region of the consensus sequence 1 PDTRPAPGSTAPPAHGVTS 20 which is repeated 40-80 times (Gendler et al., 1988). The majority of anti-MUC1 antibodies recognize sequences within the SA 1 PDTRPAP 7 region (Price et al., 1991; Xing et al., 1991, 1992; Burchell et al., 1989). The SAPDTRPAP (MUC1-9) 9-mer peptide was also found to be presented by MHC-I H-2K b and to be immunogenic (Apostolopoulos et al., 1997). MUC1-9 binds with low affinity to H-2K b (Apostolopoulos

51 et al., 1997) via a noncanonical mode and it was suggested that the C-terminus of
52 the peptide looped out of the peptide binding groove (Apostolopoulos et al., 1998;
53 Apostolopoulos and Lazoura, 2004).

54 Computer simulation of molecular dynamics is a well established method for
55 studying several aspects of biomolecular structure and function (Hansson et al.,
56 2002; Karplus, 2003; Aksimentiev et al., 2008; Tantar et al., 2008). In recent years
57 such computational approaches have been increasingly incorporated in drug design
58 (Galeazzi, 2009), in immunological research (Morikis and Lambris, 2004; Mallik and
59 Morikis, 2006; Stavrakoudis, 2010b) and also to peptide/MHC complexes (Omasits
60 et al., 2008; Knapp et al., 2009). Moreover, biomolecular modeling can complement
61 experimental studies (van Gunsteren et al., 2008) and can elucidate dynamics of
62 immunological synapse (Wan et al., 2008), allows to study the dynamics of a peptide
63 bound to antibody (Tatsis et al., 2009; Stavrakoudis, 2010a), could be used to model
64 disulphide peptide complexed proteins (i.e. C8 γ (Stavrakoudis, 2009)) or even more
65 excitingly to help in clinical decision making (Sadiq et al., 2008).

66 Modeling of the MUC1-9 peptide with both murine and human MHC class I,
67 H-2K^b and HLA-A2 respectively have been previously performed (Apostolopoulos
68 et al., 1998), based on a simulated annealing protocol and high temperature molec-
69 ular dynamics (Chelvanayagam et al., 1996). That work was a considerable progress
70 in our knowledge of peptide/MHC interactions in the MUC1-9 case and provided a
71 possible structural explanation of the antibody binding of MUC1 peptides presented
72 by the MHC molecules. However, modern progress in computational biophysics, ac-
73 companied with the big enhancement of available computer power, can be utilized to
74 further improve the computer-generated model of the MUC1-9 peptide complexed
75 the the MHC class I H-2K^b.

76 Here, we present a homology modeling and molecular dynamics approach of
77 MUC1-9 (SAPDTRPAP) in complex with MHC class I H-2K^b. Since the initial
78 conformation was modeled rather than taken from an x-ray structure, we chose

79 to perform two independent simulation runs, to obtain more robust results. Long-
80 run dynamics, inclusion of the whole MHC molecule and explicit representation of
81 solvent have been utilized in order to more accurately picture the MUC1-9 structure
82 and interactions with the MHC molecule. Such an approach has been suggested to
83 give more reliable results in MD investigations (van Gunsteren et al., 2008; Omasits
84 et al., 2008). Our results suggest that this was a beneficial approach in the current
85 study, and has given insights into the peptide binding mode of the MUC1-9.

86 2 Methods

87 Initial coordinates for the SEV9/MHC complex were downloaded from Protein Data
88 Bank (Berman et al., 2002), access code: 1kpv. The original peptide from Sendai-
89 virus, FAPGNYPAL was mutated to SAPDTRPAP, whilst the MHC molecule remained
90 untouched. The SEV9 peptide was selected from other candidates due to its homol-
91 ogy with the MUC1-9 peptide. Pro residue homology in positions P3 and P7 was
92 also crucial for selection. Since the backbone dihedral angle ϕ of Pro residue is
93 restrained, it is preferable to choose a peptide that has the same residue in these po-
94 sitions. Ideally, it would be perfect to also have alignment for position P9, however
95 there was no such option. Topology and force field parameters for all atoms were
96 assigned from the CHARMM22-CMAP parameter set (MacKerell Jr et al., 2004b,a).
97 It has been noted that addition of cross terms with CMAP potential improves the
98 system parametrization and helps to avoid undesired backbone helical transitions
99 (Buck et al., 2006; Stavrakoudis, 2008).

100 Hydrogen atoms were added with the VMD program (Humphrey et al., 1996) and
101 its autopsf utility. Protonation status of Histidine side chains were determined with
102 the REDUCE program (Word et al., 1999). The peptide/MHC complex was centered
103 in a rectangular box with dimensions $95.7 \times 88.3 \times 102.9 \text{ \AA}^3$. The box was filled with
104 TIP3P water molecules and neutralized with the addition of 26 Na^+ and 20 Cl^- ions

105 respectively, to approximate a 0.1 mM ion concentration. Crystallographic water
106 molecules (345) were also included in the model. The final system contained 24429
107 water molecules. Total number of atoms of the entire system were 80598.

108 Non-bonded van der Waals interactions were gradually turned off at a distance
109 between 12 and 14 Å (Yonetani, 2006). Long range electrostatics were calculated
110 with the PME method (Darden et al., 1993). Non-bonded forces and PME elec-
111 trostatics were computed every second step. Pair list was updated every 10 steps.
112 Bonds to hydrogen atoms were constrained with the SHAKE method allowing a 2 fs
113 time step for integration. The system was initially subjected to energy minimization
114 with 5 000 steps. The temperature of the system was then gradually increased to 310
115 K, with Langevin dynamics using the NVT ensemble, during a period of 3 000 steps,
116 by stepwise reassignment of velocities every 500 steps. The simulation was continued
117 at 310 K for 100 000 steps (200 ps). During minimization and equilibration phases,
118 protein backbone atoms (N, C^α, C', O) and oxygen atoms of crystallographic waters
119 were restrained to their initial positions with a force constant of 50 kcalmol⁻¹Å⁻².
120 The system was equilibrated for further 200 ps with the force constant reduced to
121 5 kcalmol⁻¹Å⁻². Finally, 400 ps of NVT simulation at 310 K was performed with
122 total elimination of the positional restraints. The simulation was passed to the
123 productive phase, by applying constant pressure with the Langevin piston method
124 (Feller et al., 1995). Velocities were re-initialized and two independent trajectories
125 were produced (trA and trB). Pressure was maintained at 1 atm and temperature
126 at 310 K. Results are based to a period of 10 ns of this isothermal-isobaric (NPT)
127 runs. Snapshots were saved to disk at 1 ps interval for structural analysis.

128 The initial structure of the SEV9/MHC complex (PDB code 1kpj) were also
129 simulated under identical conditions for comparative analysis (tr0 trajectory).

130 Trajectory analysis was performed with Eucb (Tsoulos and Stavrakoudis, 2009;
131 Stavrakoudis, 2009) and Carma (Glykos, 2006) software packages. Secondary struc-
132 ture analysis was performed with STRIDE (Frishman and Argos, 1995). Circular

133

134 data statistics (dihedral angles, etc) were calculated with appropriate corrections
135 (Agostinelli, 2009; Döker et al., 1999). Structural figures were prepared with Py-
MOL (www.pymol.org).

136 2.1 Buried surface area calculation

137 Calculation of buried surface area (BSA) was performed with the NACCESS pro-
138 gram (<http://www.bioinf.manchester.ac.uk/naccess/>), based on the formula:

$$BSA = S_p + S_a \otimes S_c \quad (1)$$

139 thus as the difference of the surface accessible area of the complex (S_c) from the
140 sum of the of surface accessible areas of the peptide (S_p) and MHC molecule (S_a)
141 respectively.

142 2.2 β -turn classification

143 β -turn classifications were based on geometrical characteristics of the backbone
144 conformation (Hutchinson and Thornton, 1994). Initially, a β -turn was accepted
145 if $d(G_i^a \otimes G_{i+3}^a) \approx 7 \text{ \AA}$ and $\angle G_i^a \otimes G_{i+1}^a \otimes G_{i+2}^a \otimes G_{i+3}^a < 90^\circ$, where d is the
146 distance and a is the dihedral angle between the corresponding atoms. Further
147 classification of the β -turn was based on hydrogen bond patterns and backbone
148 dihedral values of the $i+1$ and $i+2$ residues.

149 2.3 Instant water count number

150 155

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153

154

In water molecules in the peptide/MHC

orde

rite to interface the instantaneous water coordination number (N_c) approach (Petrone and

iden

tify Garcia, 2004). This method counts the water oxygen atoms within a range (typically

isola

ted 3.5 Å) of any water oxygen atom, which is actually the first hydration shell. The

(fro

m N_c can be found between 0 and 15, depending on the local structure of water. In

the

bulk the bulk water this number is always greater than 3, while in the protein interior

)

135

is 0 to 2. This implies that a water molecule has no other water neighbours and
157 it is inside the protein interior. The N_c is measured for all the MD trajectory and
158 isolated water molecules are identified if the N_c value is small for a prolonged period
159 of time. In the current study, a search of water molecule with $N_c \leq 1$ for at least
160 70% of the MD time has been performed.

161 2.4 MM-PBSA calculation of $\Delta G_{binding}$

The binding free energy of the association of two molecules ($A+B \rightarrow AB$) can be
estimated, according to the MM-PBSA approach (Kollman et al., 2000; Wan et al.,
2005), as:

$$\Delta G_{binding} = G^{AB} - G^A - G^B \quad (2)$$

where:

$$\Delta G^i = \langle E_{MM} \rangle + \langle G_{solv} \rangle - TS \quad (3)$$

162 In the above equations, $\langle \rangle$ denotes average value for a set of snapshots along a
163 molecular dynamics trajectory, while E_{MM} is the molecular mechanics energy of the
164 i^{th} molecule in the gas phase, namely the sum of internal bonded energy (comprising
165 bond, angle and dihedral terms), van der Waals and electrostatic interactions. G_{solv}
166 is the solvation free energy of the i^{th} molecule. This term can be estimated as the
167 sum of the electrostatic solvation free energy calculated by the Poisson–Boltzmann
168 equation and the non-polar solvation free energy calculated from the SASA.

Hence, the binding free energy is:

$$\Delta G^i = \langle \Delta E_{MM} \rangle + \langle \Delta G_{solv} \rangle - TS \quad (4)$$

169 The average properties can be computed directly from the MD trajectory snap-
170 shots. In the current study, the last 5 ns were used, assuming that equilibrium was
171 reached after the first 5 ns of the simulation. 5000 structures were utilized for the

172

SASA and E_{MM} calculations, while 50 structures (one every 100 frames) were used
173 for the calculation of the G_{solv}^{elec} with the APBS (Baker et al., 2001; Dolinsky et al.,
174 2004) software.

175 3 Results and Discussion

176 3.1 RMSF and RMSD analysis

177 Root mean square fluctuations (RMSF) of the C^a atoms of the MHC and peptide
178 chains, as well as the time evolution of the root mean square deviation (RMSD) of
179 the backbone atoms (N, C^a, C') of the MHC and peptide chains, during both MD
180 trajectories, trA and trB respectively, are shown in Figure 1.

181 In both trA and trB cases, RMSF profiles of chains A and B from the MHC
182 molecule were almost identical, which indicates the robustness of the study. RMSF
183 values were between 0.5 and 2.0 Å, which is quite common in similar MD studies of
184 protein complexes around equilibrium. Similarly, RMSD time series were also very
185 similar for chain A and B, with only a small exception of the trA trajectory: RMSD
186 values escaped from stationarity around 4ns in trA, and a small peak of RMSD 2.8 Å
187 was observed. In general, both trajectories were quite stable, Figure 1. Time series
188 of RMSD fluctuated around 1.5–2.0 Å for chain A and around 1.0 Å for chain B. If
189 we take into consideration the simulation temperature (310 K) these values are con-
190 sidered small, indicating the stability of the complex. Moreover, there is strong evi-
191 dence that the MHC molecule did not undertake significant conformational changes
192 upon mutation of the peptide residues (Fremont et al., 1992; Matsamura et al.,
193 1992). This is in accordance with other X-ray studies of the H-2K^b MHC class-I
194 molecule with different nonamer peptides in the binding groove. These observa-
195 tions corroborate our hypothesis that homology modeling coupled with molecular
196 dynamics simulations produces a reliable model of the MUC1-9/H-2K^b complex.

197 Peptide's RMSF values of C^a atoms showed an interesting [differentiation](#) between

198

199 trA and trB trajectories. While values of 0.5-1.0 Å were recorded for residues 1-7
200 in both cases, trA trajectory showed increased values of 1.5 and 2.0 Å for residues
201 8 and 9 respectively. In trB trajectory, RMSF remained close to 1.0 Å for all
202 residues. Values in the order of 2.0 Å are still considered relatively small, however,
203 the **differentiation** is notable. Since this fact was observed in only one of the two
204 trajectories, it could be **considered** as a relative random effect of the simulation.
205 On the other hand, it definitely indicates that the peptide binding to the MHC
206 groove is not so tight at the C-terminal region, as previously has been suggested
207 (Apostolopoulos et al., 1998; Apostolopoulos and Lazoura, 2004).

208 Peptide's RMSD time series of backbone atoms were very similar in both cases.
209 RMSD values ranged between 0.99 and 2.1 Å and averaged at 1.46 (0.16) Å for
210 trA, whilst the RMSD values ranged between 1.04 and 1.69 Å and averaged at 1.36
211 (0.09) for trB case. There is only a minor difference between these two profiles:
212 trB trajectory showed slightly smaller values with smaller standard deviation of the
213 time series. This is possibly due to increased fluctuation at the C-terminal end in
214 trA . However, as it was previously noted for chains A and B of the MHC molecule,
215 RMSD profiles of the peptide corroborate the stability of the trajectories and the
validity of the proposed model.

216 **3.2 Peptide backbone dynamics**

217 Backbone conformations play an important role in peptide/MHC binding (Barinaga,
218 1992; Matsamura et al., 1992). Here we present a detailed analysis of the peptide's
219 backbone conformation.

220 Figure 2 displays the distribution (Ramachandran map) of the backbone dihedral
221 angles φ ψ of peptide residues in the region 2-8. It is evident that, for most of the
222 residues, the backbone dihedral **angles** show very similar distributions in the trA and
223 trB trajectories. The only exception comes from the A1a8 residue. As it has been
224 noted, the C-terminal residues showed increased mobility (higher RMSF values),

199

225 and this is very well reflected in the distribution of its backbone dihedral angles.

226 Backbone dihedral angles of Ala2 and Pro3 residues were very well conserved
227 during both MD trajectories. For the great majority of the trajectory frames, all
228 dihedral values were found within 0° of the initial values.

229 Asp4's backbone ϕ - ψ dihedral angles were -120° and 153° respectively in the
230 initial structure. Contrary to the Pro3 case, Asp4 residue experienced a significant
231 move to its backbone ϕ dihedral angle. Time series of this angle fluctuated between
232 -30° and -122° and averaged at $-69^\circ(11^\circ)$. Only 35% of the trA frames and 55% of
233 the trB frames remained within 30° of the initial value. Similarly, backbone ψ angle
234 averaged at $-35^\circ(12^\circ)$. Thus Asp4 residue showed (in total) an approximately 100°
235 move in backbone dihedral angles. It could be considered that Asp4 represents a
236 first **differentiation** between the crystal structure of the reference peptide and the
237 MUC1-9 peptide studied here.

238 Thr5's backbone ϕ - ψ dihedral angles were 74° and 48° respectively in the initial
239 structure of the SEV9 peptide. A positive ϕ angle, although abnormal in other cases,
240 is not uncommon in peptide's conformation of other peptide/MHC complexes. For
241 example ϕ angle of residue Ser5 was found to be 60° in SRDHSRTPM (YEA9)
242 peptide (Apostolopoulos et al., 2002). During both trA and trB trajectories, the
243 sign of backbone ϕ dihedral angle of residue Thr5 changed quickly and the residue
244 adopted backbone ϕ angles close to -150° (Figure 2). Time series of Thr5's ϕ angle
245 averaged at $-151^\circ(22^\circ)$ in both trA and trB trajectories. Negative values of ϕ at
246 position 5 have also been observed in other crystal structures of peptide/MHC H-
247 $2K^b$ complexes. For example, in the SSYRRPVG I peptide from influenza A virus,
248 the ϕ angle of Arg5 was found to be -67° (PDB access code 1wbz) (Meijers et al.,
249 2005). The identical results obtained in both trajectories underline the robustness
250 of the found values for Thr5's ϕ angle. Backbone dihedral ψ of Thr5 averaged at
251 $162^\circ(65^\circ)$ and $160^\circ(49^\circ)$ in trA and trB trajectories respectively. Average values are
252 approximately 115° different from the initial value.

1100

253 Arg6's backbone ϕ and ψ dihedral angles were -59° and 107° respectively in the initial
254 structure. Similarly to Thr5, backbone dihedral angles were altered during MD
255 trajectories. Average values of ϕ angle were found to be $-129^\circ(14^\circ)$ and $-128^\circ(13^\circ)$
256 in trA and trB trajectories respectively. Average values of ψ angle were found to
257 be $153^\circ(17^\circ)$ and $151^\circ(12^\circ)$ in trA and trB trajectories respectively. Only 47% of
258 trajectories frames in trA and 30% in the trB retained backbone dihedral angles
259 within 30° of the initial values.

260 Residues Pro7, Ala8 and Pro9 showed minimal fluctuations of their backbone
261 dihedral angles. The presence of two proline residues left few space for maneuvers
262 in this part of the peptide's sequence.

263 The β -turn structure in the peptide's structure is an interesting finding of this
264 study. Hairpin and β -turn structures in peptides bound to MHC molecules have
265 been identified in case of MHC class II molecules (Zavala-Ruiz et al., 2004). However,
266 this happens to the peptide's region that is outside of the binding group. In the
267 current study, we have identified a very interesting case of β -turn in the central
268 region of the peptide, covering residues Pro3 to Arg6. This sequence has been
269 found in β -turn conformation for 50 and 77% of the simulation time, in the trA
270 and trB trajectories respectively. We did not observe any intra-peptide hydrogen
271 bond stabilizing this β -turn. Table 1 lists the values of backbone dihedral angles
272 as calculated for the central residues of the β -turn, Asp4 and Thr5 respectively.
273 Both trA and trB trajectories showed very close values of backbone ϕ and ψ dihedral
274 angles. These values differ from the initial values found in the crystal structure of the
275 SEV9 peptide. However, the common finding from the two independent trajectories
276 (trA and trB) corroborate the suggestion that a β -turn around the Asp4-Thr5
277 region exists, at least partially.

278 Bulged peptide structure have been identified in previous studies (Speir et al.,
279 2001; Tynan et al., 2005), however for longer peptide sequences, like the ILF-
280 PSSERLISNR peptide sequence (Speir et al., 2001). The presence of a bulged (turn)

1101

281 structure In the nonamer peptides bound In the class I MHC molecules has been
282 suggested to profoundly affect the recognition of the pMHC complex by the T-cell
283 receptor (Rudolph et al., 2006).

284 **3.3 Interactions between the peptide and the MHC**

285 The binding mode of nonamer peptides with the H-2K^b MHC class I molecule has
286 been investigated in the past. There are numerous studies in the literature (Mat-
287 samura et al., 1992; Fremont et al., 1992; Apostolopoulos et al., 2002; Meljers et al.,
288 2005; D. H. Fremont and E. A. Stura and M. Matsumura and P. A. Peterson and
289 I. A. Wilson, 1995) addressing the principles of peptide anchoring to MHC's bind-
290 ing groove. It is generally assumed that H-2K^b has six binding pockets, A to F,
291 that accommodate residues P1, P2, P3, P6, P7 and P9 of nonamer peptides (Matsamura
292 et al., 1992; Saper et al., 1991). Residues P4 and P5 do not make direct contacts
293 with the MHC molecule and protrude towards the solvent, hence their side chains
294 are available for interaction with the TCR. The charge groups of N- and C-terminal
295 residues make strong interactions with the MHC binding clefts (pockets A and F
296 respectively).

297 A general view of the peptide/MHC binding motif is shown in Figure 3, whilst
298 the peptide's orientation inside the MHC's binding is depicted at Figure 4.

299 Peptide's Ser1 (P1) was found to form two stable hydrogen bonds with the
300 MHC molecule. Its backbone atoms N and O were found in hydrogen bond state
301 with side chains of Glu63A and Tyr159A respectively. These hydrogen bonds were
302 conserved, in both trA and trB trajectories, for approximately 91 to 95% of the
303 simulation time (Table 2). The distance between Ser1:N and Glu63A side chain
304 oxygen atoms, in the initial structure, were found 4.6 and 5.8 Å for O^{c1} and O^{c2}
305 respectively, which indicates that this strong (charged) hydrogen bond between the
306 N-terminal group of the peptide and the side chain of Glu63A was formed during
307 the modelling process and was not present in the initial structure. Indeed, Glu63A's

308

309 side chain (atom O^{C1}) actually was to form a hydrogen bond with Ala2:N atom, In
310 the structure of the original peptide (Matsamura et al., 1992). The hydrogen bond
311 between Ser1:O and Tyr159A:O^N, on the other hand, was well formed in the initial
312 structure (distance 2.67 Å) and very well conserved in both MD trajectories (Table
313 2). Another hydrogen bond interaction between Ser1 and the MHC molecule was
314 present between the side chains of Ser1 and Tyr7A (or Tyr171A for short periods),
315 for approximately 95% of the simulation time. This is very interesting, since no
316 side-chain interactions have been observed in the x-ray structure of SEV9 peptide
317 (Fremont et al., 1992). Thus, overall two to three hydrogen bonds contributed to
318 peptide's binding. These results corroborate the importance of this binding pocket
319 in the peptide/MHC binding process.

320 Side chain of Glu63A (pocket B) accepted hydrogen bond from Ala2 Nitrogen
321 atom (position P2). This interaction was conserved for 93.5% (trA) or 98.6% (trB)
322 of the simulation time, and it was well formed in the initial structure (the distance
323 between Ala2:N and Glu63A:O^{δ1} was found 2.9 Å). This finding underlines the
324 importance of the Glu63A residue, since its negatively charged side chain formed
325 two stable hydrogen bonds with the peptide's backbone amide groups. Side chain
326 of Lys66A was found in hydrogen bond state with Ala2:O atom for over 90% of the
327 simulation time. The corresponding distance between Lys66A:N^ζ and Ala2:O atoms
328 in the initial structure was found 2.7 Å, indicating the existence of the hydrogen
329 bond. Moreover, side chains of Tyr7A and Tyr45A made hydrophobic contacts
330 with Ala2's aliphatic side chain. The above analysis for the Ala2 interactions is
331 almost identical with the x-ray structure of the SEV9 peptide (Fremont et al., 1992),
332 indicating the fact the preservation of the Ala2 residue in position P2 (binding
333 pocket B) contributed to the retaining of the same peptide/MHC interactions.

334 Pro3(P3) made important hydrophobic interactions with Tyr159A's side chain.
335 Average distance of their side chain centers were found 4.0 Å(0.6) or 4.2 Å(0.6)
during trA or trB MD trajectories respectively. For approximately 25% of the time,

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the two side chains were found in parallel orientation forming a stacking interaction.

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It is noted that Tyr159A's side chain donated a hydrogen bond to Ser1:O, hence

338

this MHC residue is considered to contribute significantly to peptide's binding. The

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original hydrogen bond between Pro's backbone oxygen atom and Asn70A's side

340

chain was found to be relatively weak during trA and trB MD trajectories: 12.7 and

341

27.2% of the frames respectively satisfied the hydrogen bond criteria.

342

Central residues Asp4 and Thr5 did not show any significant interactions with

343

the MHC's residues. Only Asp4's side chain was found hydrogen bonded to Arg62A's

344

side chain for limited period of simulation time, $\approx 15\%$. Both residues were exposed

345

outside of the binding groove.

346

Binding pocket C plays an important role in peptide recognition by MHC H-2K^b

347

molecules (Molano et al., 1998; D. H. Fremont and E. A. Stura and M. Matsumura

348

and P. A. Peterson and I. A. Wilson, 1995; Huard et al., 1997). Peptide's residue

349

Arg6 side chain at position P6, was found to form a strong hydrogen bond with

350

Glu24A side chain. Actually, these side chains remained hydrogen bonded the entire

351

time in both trA and trB MD trajectories. On the other side, there was no

352

backbone interaction with the MHC molecule. However, the ability of the MHC

353

molecule to bind different peptide sequences, since the original peptide has Tyr in

354

this position (Apostolopoulos et al., 2002), which is a canonical residue at this position

355

for MHC binding. Tyr6 (SEV9 peptide) to Arg6 (MUC1-9 peptide) mutation

356

led to some loss of hydrophobic interactions between peptide and MHC molecule, a

357

fact that might explain the reduced binding affinity of the MUC1-9 peptide, relative

358

to SEV9 peptide. However, the Arg6 remained inside the canonical C-pocket, unlike

359

the Arg6 residue in YEA9 peptide (SRDNSRIPM) which utilized the non-canonical

360

E binding pocket (Apostolopoulos et al., 2002).

361

Residue Pro7, at peptide's P7 position, had a weak backbone hydrogen bond

362

with Tyr117A's side chain. Occurrence was found 28% in trA and only 7% in trB

363

trajectories respectively. Given the fact that in crystal structures of peptides bound

364

365 in the H-2K^b molecule, no such hydrogen bond exist (Table 2), the result is not so
366 suprising. However, significant hydrophobic interactions with Trp147A and Trp133A
367 side chains were found to contribute in peptide/MHC interactions. For example, side
368 chain distances between Pro7 and Trp147A varied between 3 and 5 Å and averaged
369 at 3.6 Å (0.2). To a lesser degree, Leu156A and Tyr116A also made hydrophobic
370 contacts with side the chain of Pro7.

371 Position P8 was occupied by Ala8. The backbone carbonyl group of this residue
372 was found to be in hydrogen bond state with Trp147A's side chain. This is a well
373 expected interaction, as it has been found in the crystal structure of the original
374 peptide. A relatively weak hydrogen bond was also formed for part of trA trajec-
375 tory, between Ala8:N and Glu152A:Oc2. The corresponding distance in the initial
376 structure was found to be 5.8 Å.

377 Finally, residue Pro9 at position P9 (binding pocket F). The C-terminal car-
378 boxyl group was found to form two hydrogen bonds (Table 2) with Thr143A and
379 Lys146A side chains, for almost all of the simulation time, in both trA and trB tra-
380 jectories. The same interactions were also present in the x-ray sructure that served as
381 initial point for these calculations. However, the lack of amide hydrogen in proline's
382 structure resulted to the abolishment of a backbone hydrogen bond between peptide
383 and the MHC molecule. Thus, the Leu to Pro (SEV9 to MUC1-9 peptide) mutation
384 resulted in a small shift of the position of this residue. These subtle changes in
385 peptide's conformation have been shown (Hoare et al., 2008) to affect drastically
386 the MHC recognition and might explain to some extent the reduced affinity of the
387 MUC1-9 peptide when bound to class I H-2K^b. Pro9's side chain also made hy-
388 drophobic contacts with Val76A, Leu81A and Trp147A side chains. For at least 90%
389 of the simulation time, a pair of side chain heavy atoms from these residues were in
390 close contact (distance less than 4.5 Å) with a side chain heavy atom from Pro9.
391 These hydrophobic interactions further stabilized the peptide/MHC interactions,
and along with the hydrogen bonds strengthen the anchoring role of Pro9.

392

393 Overall, as it can be seen from Figure 6, there were approximately 12 hydrogen
394 bonds between the peptide and the MHC molecule, during both MD trajectories.
395 This number approximates very well the number of the reported (Fremont et al.,
1992) hydrogen bonds (11) between the peptide SEV9 and the MHC molecule.

396 3.4 Buried Surface Area

397 Buried surface area (BSA) is a good indicator of the binding of a ligand into a
398 protein (Olsson et al., 2008). Figure 7 shows the time evolution of BSA between
399 the peptide and the MHC molecule. BSA **fluctuated** between 666.9 and 1005.6 \AA^2
400 and averaged at 848.7(47.5) \AA^2 in the trA trajectory. In the trB case, BSA values
401 were found between 656.7 and 999.6 \AA^2 with mean value of 824.9(52.9) \AA^2 . As it
402 is can be drawn from the graphical **representation** of BSA time evolution, and from
403 basic statistical analysis, both trajectories showed similar profiles for the calculated
404 BSA of peptide/MHC interface. The difference of approximately 25 \AA^2 (3%) in the
405 mean values is very small and could be considered to be within expected error. In
406 a recent experimental re-investigation of BSA of protein x-ray structures (Novotny
407 et al., 2007) it was suggested that differences from 50 to 100 \AA^2 in BSA values
408 were expected as a measurement error rather than actual difference in BSA. These
409 findings corroborate our statement that the peptide/MHC complex was stable and
410 that the fluctuations in BSA time series are normal.

411 The BSA value in the x-ray structure of the SEV9 peptide was 1076 \AA^2 , while
412 the BSA value in the MUC1-9/MHC complex after restrained energy minimization
413 was found to be 937.5 \AA^2 . The loss of approximately 140 \AA^2 can be attributed to
414 minor conformational changes that occurred during MD run in order for the mutated
415 peptide to adapt to the binding groove of the MHC molecule. Considered, however,
416 that trajectories were obtained in 310 K, thus the spontaneous thermal moving of
417 the atoms resulted in somewhat reduced BSA values.

418 Thus, the difference of more than 140 \AA^2 in the BSA of the peptide/MHC in-

393

419 terface, in the SEV9 and MUC1-9 cases, is another indication of the lower binding
420 affinity that the MUC1-9 has to the H-2K^b molecule, relatively to the SEV9 peptide.

421 **4 Concluding remarks**

422 Homology modeling and molecular dynamics simulations have been used to assess
423 the structure of the SAPDTRPAP/H-2K^b complex. Results presented here indicate
424 that a stable complex is formed, based on the analysis of two MD trajectories.

425 MHC binding pockets A and F interacted closely with the N- and C-terminus
426 of the peptide which played an important role in stabilizing the complex. The
427 Buried Surface Area of the peptide/H-2K^b interface remained constant during the
428 simulation indicating the stability of the complex and its similarity to the initial
429 peptide/MHC complex.

430 Replacement of Leu with Pro at P9 position did not affect significantly the
431 MHC's binding of the peptide. The C-terminal carboxyl group was found to form
432 stable hydrogen bonds with the MHC molecule, and the non-polar side chain of Pro
433 residue made a number of close contacts with hydrophobic residues of the MHC's
434 F binding pocket. However, the peptide showed relatively increased mobility in the
435 C-terminal region, that may affect the strength of the MHC binding.

436 A main difference between MUC1-9's simulated structure and SEV9's x-ray
437 structure was the ϕ angle of Thr5. A significant transition from +74° to \approx -150°
438 occurred. Since it is well known that backbone conformation plays a very important
439 role in peptide/MHC recognition (Barinaga, 1992), it is expected that this confor-
440 mational transition would alter the MHC's binding affinity for the peptide, most
441 possibly downwards. Moreover, MHC H-2K^b molecules prefer hydrophobic residues
442 at position P6 (for nonamer peptides), even though MUC1-9 has Arg in this place.

443 This has resulted in a notable alteration of the backbone conformation of the
444 central part of peptide and the enhancement of the exposure of the Asp4-Thr5 re-

445

446 gion outside of the MHC's binding groove. For a considerable amount of simulation
447 time this bulged region (Fremont et al., 1992) adopted a β -turn conformation, how-
448 ever without the presence of the characteristic hydrogen bond. This had not been
449 noted in previous modeling studies (Apostolopoulos et al., 1998) and provides a new
450 framework for the peptide/MHC interactions.

451 Inclusion of explicit water molecules in the current study helped a lot to clarify
452 the role of the solvent in peptide/MHC interactions. Water mediated hydrogen
453 were found only sparingly and although existed, a clear contribution to the binding
454 process can not be attributed to this kind of interaction.

455 Leu to Pro mutation at position P9 resulted in slight movement of this residue
456 within the F binding pocket. However, this fact, along with the loss of a hydrogen
457 bond interaction of the Leu amide hydrogen might be enough reason for observing
458 the reduced affinity of the MUC1-9 peptide to H-2K^b binding.

459 All of the above observations reflected well in the reduction of the BSA between
the peptide and the MHC molecule, where a loss of 140 \AA^2 has been measured.
460 Finally, it seems that while the MUC1-9 peptide forms stable complex with the H-
461 2K^b molecule, it is clear that certain structural reorganization occurred and resulted
462 in reduced binding affinity.

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650 Tables

651 **Table 1** Backbone dihedral angles in the region Asp4-Thr5 of the MUC1-9 peptide, where
 652 a β -turn was found in the MUC1-9 peptide. Corresponding region of the SEV9 peptide is
 653 Gly4-Asn5. Column PDB lists the corresponding values from the crystal structure of the
 654 SEV9 peptide, with the residues Gly and Asn at positions 4 and 5 respectively. Averages
 655 values (and variances in parentheses) is given from trajectories tr0, trA and trB.

| Dihedral | PDB | tr0 | trA | trB |
|----------|--------|--------------|--------------|--------------|
| ϕ_4 | -119.9 | -138.3 (4.9) | -67.8 (1.1) | -69.4 (1.2) |
| ψ_4 | 153.2 | -173.3 (3.6) | -34.8 (1.3) | -35.4 (1.2) |
| ϕ_5 | 74.4 | 58.8 (0.9) | -151.3 (2.1) | -151.4 (2.2) |
| ψ_5 | 48.2 | 49.2 (1.1) | 161.7 (3.5) | 160.0 (3.9) |

656 **Table 2** Hydrogen bond interactions between the SEV9 and MUC1-9 peptides and the H-
 657 2K^b molecule. Percentage of frames is given, from trajectories tr0, trA and trB, that met
 658 the geometrical criteria for hydrogen bond interaction. Distance between donor-acceptor
 659 atoms are taken from the initial structure (PDB column).

| Donor | Acceptor | PDB (Å) | tr0 (%) | trA (%) | trB (%) |
|--------------------------------------|---------------------------------------|---------|---------|---------|---------|
| Phe _{1P} :N | Tyr _{59A} :O ^η | 4.13 | 32.4 | | |
| Phe _{1P} :N | Glu _{63A} :O ^{c1,2} | 4.61 | 95.6 | | |
| Ser _{1P} :N | Glu _{63A} :O ^{c1,2} | 5.83 | | 93.2 | 94.1 |
| Tyr _{159A} :O ^η | Ser _{1P} :O | 2.67 | 59.6 | 91.4 | 94.8 |
| Tyr _{59A} :O ^η | Ser _{1P} :O ^γ | 5.77 | | 77.7 | 81.9 |
| Ser _{1P} :O ^γ | Tyr _{7A} :O ^η | 5.82 | | 93.2 | 97.9 |
| Ser _{1P} :O ^γ | Tyr _{171A} :O ^η | 5.00 | | | 20.6 |
| Ala _{2P} :N | Glu _{63A} :O ^{c1,2} | 2.90 | 90.1 | 93.5 | 98.6 |
| Lys _{66A} :N ^ζ | Ala _{2P} :O | 2.74 | 76.6 | 97.3 | 90.0 |
| Asn _{70A} :N ^δ | Pro _{3P} :O | 3.63 | 63.4 | 12.7 | 27.2 |
| Arg _{62A} :N ^{η2} | Asp _{4P} :O ^{δ1,2} | 6.78 | | 19.0 | |
| Arg _{6P} :N ^{η1,2} | Glu _{24A} :O ^{c1,2} | 5.21 | | 92.4 | 97.2 |
| Tyr _{116A} :O ^η | Pro _{7P} :O | 4.11 | | 28.2 | 7.2 |
| Ala _{8P} :N | Glu ₁₅₂ :O ^{c1,2} | 5.87 | | 38.5 | 5.5 |
| Trp _{147A} :N ^c | Ala _{8P} :O | 2.86 | 11.7 | 70.4 | 98.5 |
| Leu _{9P} :N | Asp _{77A} :O ^{δ1,2} | 3.02 | 82.4 | | |
| Lys _{146A} :N ^ζ | Leu _{9P} :O ^{T2} | 3.00 | 95.2 | | |
| Tyr _{84A} :O ^η | Leu _{9P} :O ^{T2} | 2.84 | 39.3 | | |
| Thr _{143A} :O ^γ | Pro _{9P} :O ^{T1,2} | 2.68 | | 94.1 | 92.7 |
| Lys _{146A} :N ^ζ | Pro _{9P} :O ^{T1,2} | 3.00 | | 98.6 | 98.7 |

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660 **Figures**

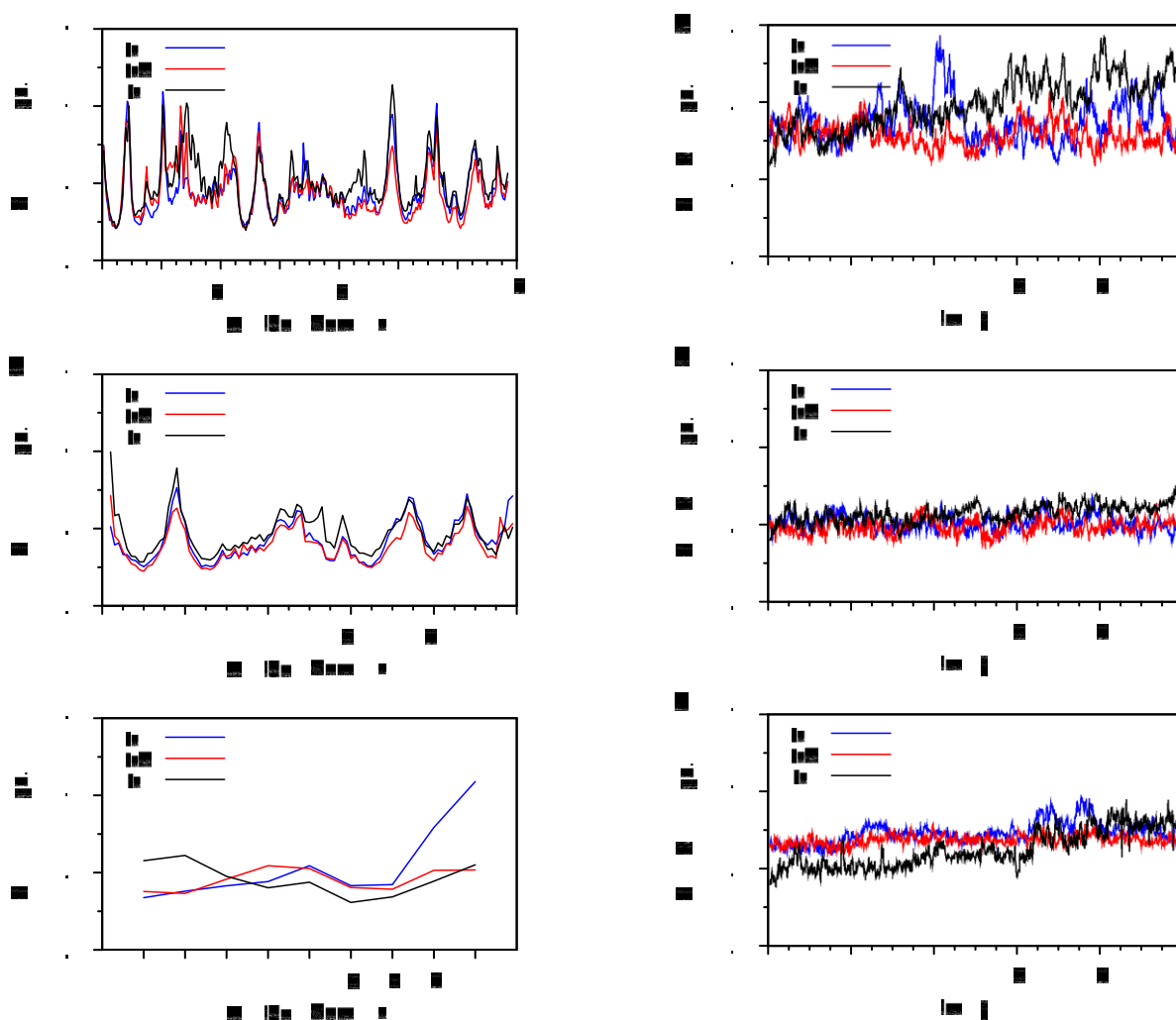


Figure 1: Root mean square fluctuation (left column) of C^{α} atoms and root mean square deviation (right column) time series of backbone atoms (N, C^{α} , C') of the pMHC complex after fitting the corresponding atom positions from MD trajectory to initial (X-ray) coordinates. Results from different trajectories (tr0, trA and trB) are indicated with different line colors. A) RMSF of MHC chain A, B) RMSF of MHC chain B, C) RMSF of MHC chain P (peptide), D) RMSD of MHC chain A, E) RMSD of MHC chain B and F) RMSD of MHC chain P (peptide).

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Figure 2: Ramachandran plot of backbone dihedral angles of the peptide. Horizontal axis is for ϕ and vertical axis is for ψ angle respectively. The plots represent probability density maps, z-axis is the percentage of frames found within 10° dihedral angle bin. The adjacent colour bar is used to identify regions of low (grey) versus high (blue) populations.

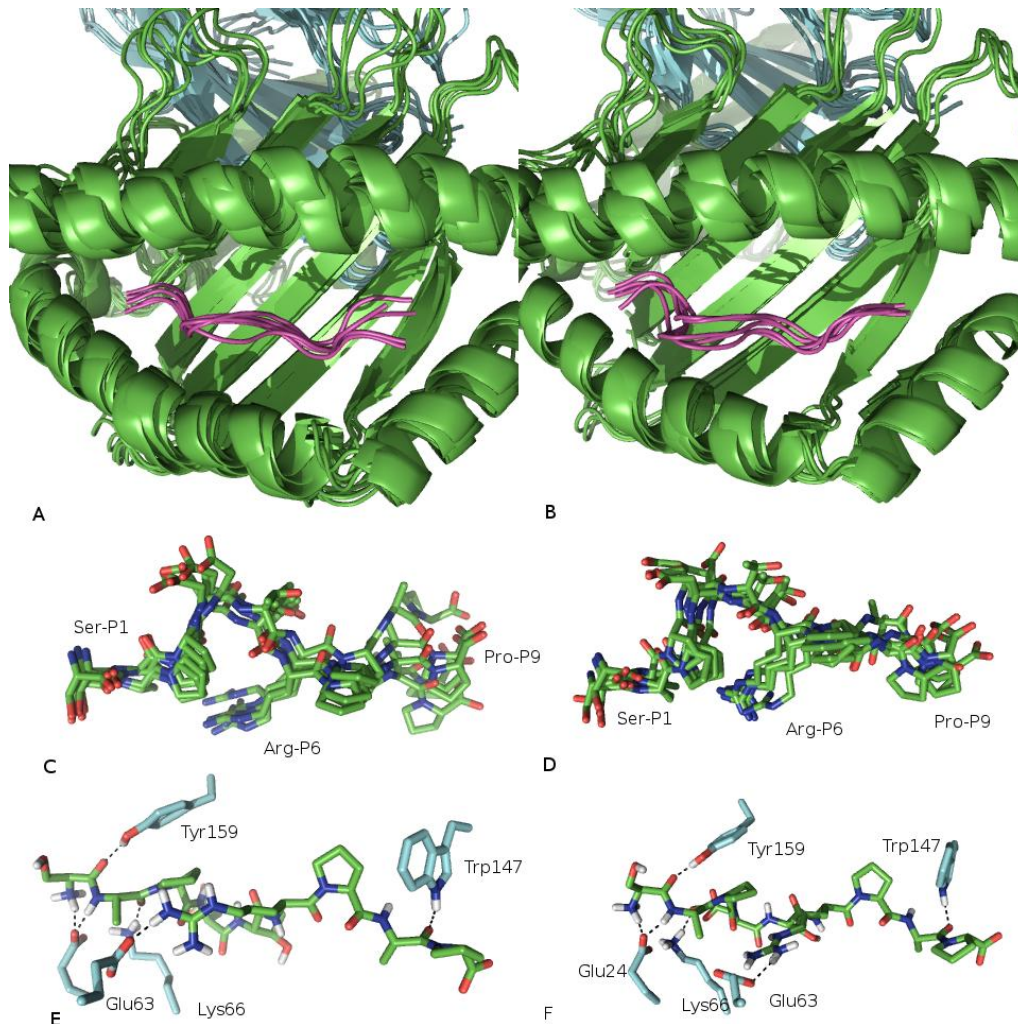


Figure 3: A) Ribbon representation of five selective structures of the MUC1-9/H-2K^b complex (one frame every 2 ns) from trA trajectory, B) Ribbon representation of five selective structures of the MUC1-9/H-2K^b complex (one frame every 2 ns) from trB trajectory, C) Stick representation of the peptide bound in the MHC groove from trA trajectory, D) Stick representation of the peptide bound in the MHC groove from trB trajectory, E) Important hydrogen bond interactions between the peptide and MHC molecule in the trA trajectory and F) Important hydrogen bond interactions between the peptide and MHC molecule in the trB trajectory. Hydrogens were omitted from stick representations. Structures have been fitted to the first frame using the backbone atoms.

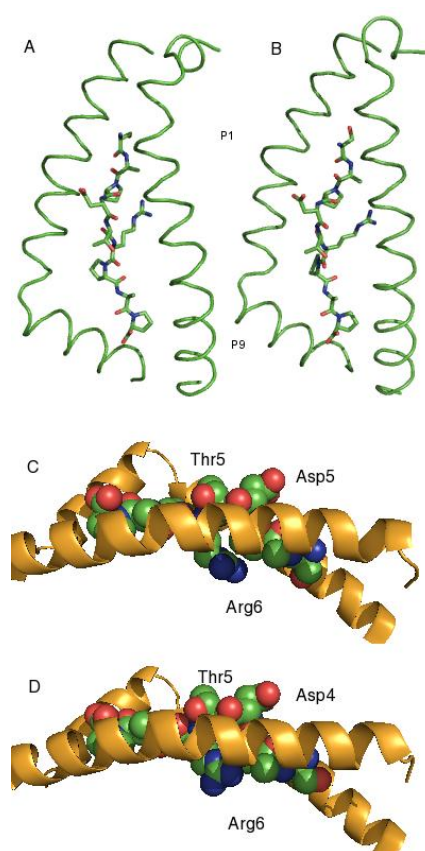


Figure 4: Peptide's (sticks) orientation in MHC (ribbons) binding groove in trA (A) and trB (B) trajectories. Exposure to the solvent of the region Asp4-Thr5, while Arg6 side chain orientates towards the beta-sheet floor, in the interior of the binding groove of pocket C, in trA (C) and trB (D) trajectories.

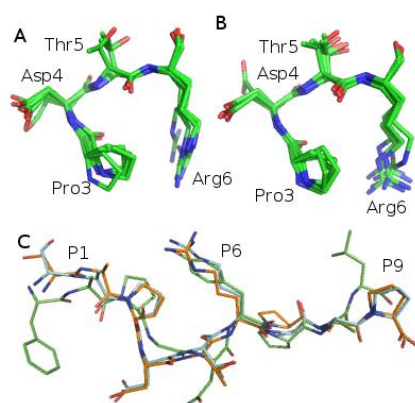


Figure 5: A) and B) Backbone overlay of the Pro3-Arg6 region of the peptide from the trA and trB trajectories respectively. This fragment has been found in β -turn conformation for considerable amount of time. C) Backbone superimposition of SEV9 peptide (green) from the X-ray structure with representative structures from trA (cyan) and trB (orange) trajectories. The differentiation of backbone conformation at fragment Asp4-Thr5 is well seen. Side chains of residues 2, 3, 6 and 7 share common orientation towards the MHC binding groove. Interestingly, conformations of residues at positions 1 and 9 deviate from the original structure.

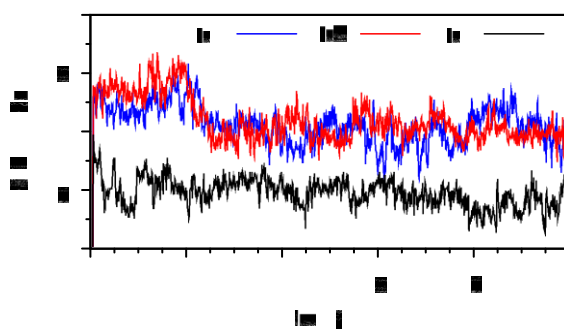


Figure 6: A) Total number of hydrogen bonds between the peptide and the MHC molecule, as evolved over simulation time. Data were averaged every 10 ps. B) Total number of water mediated hydrogen bonds between the peptide and MHC molecule, as evolved over simulation time. Data were taken every 10 ps.

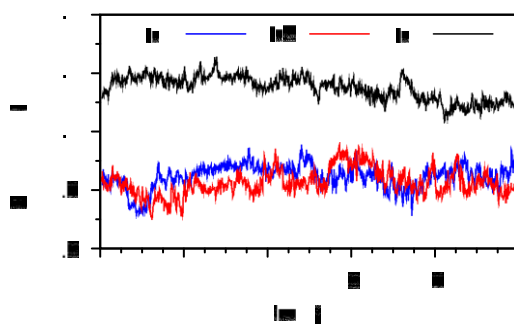
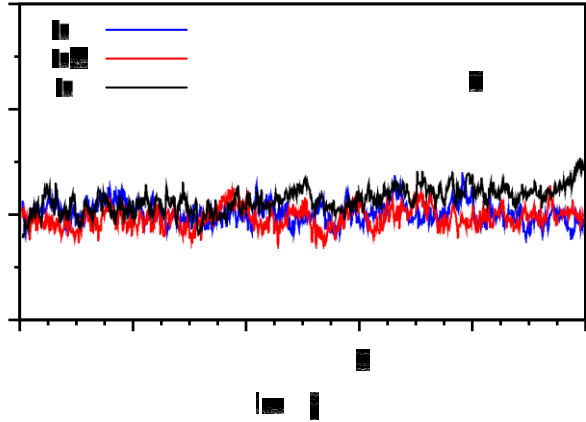
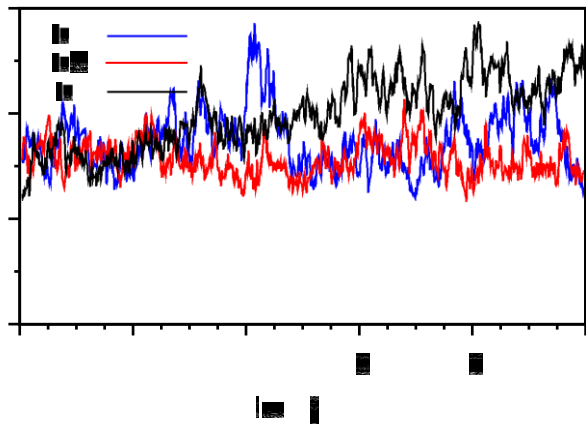
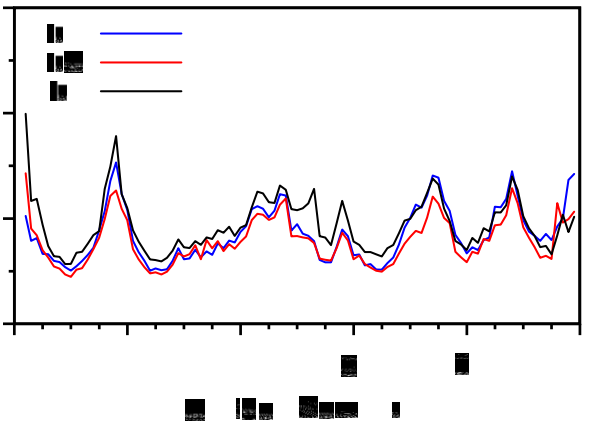
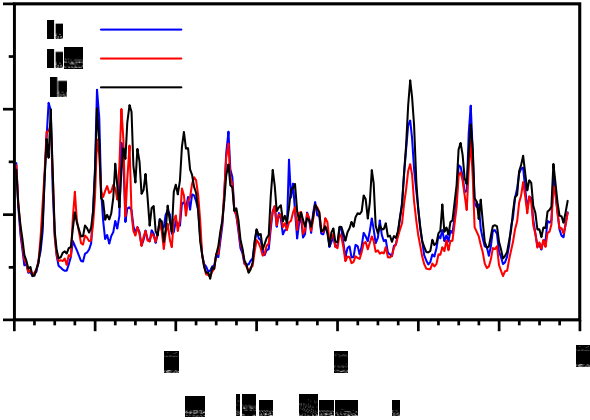


Figure 7: Time series of apolar buried surface area (BSA) between the peptide and the MHC molecule in tr0, trA and trB trajectories.

Figure 1

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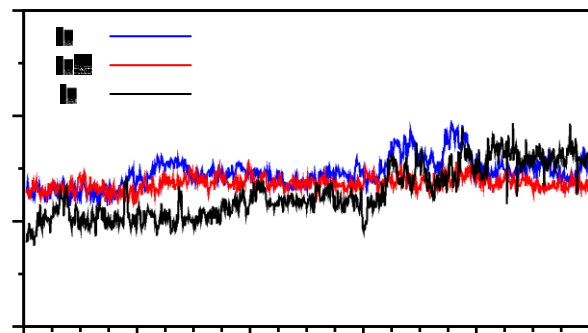
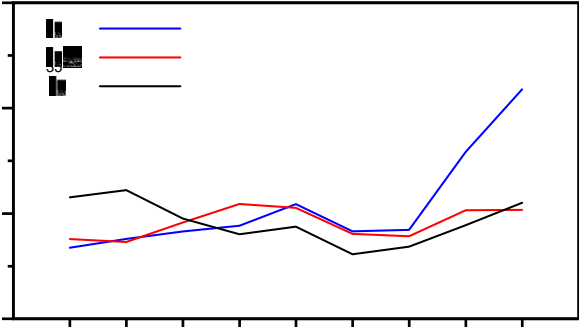




Figure 6

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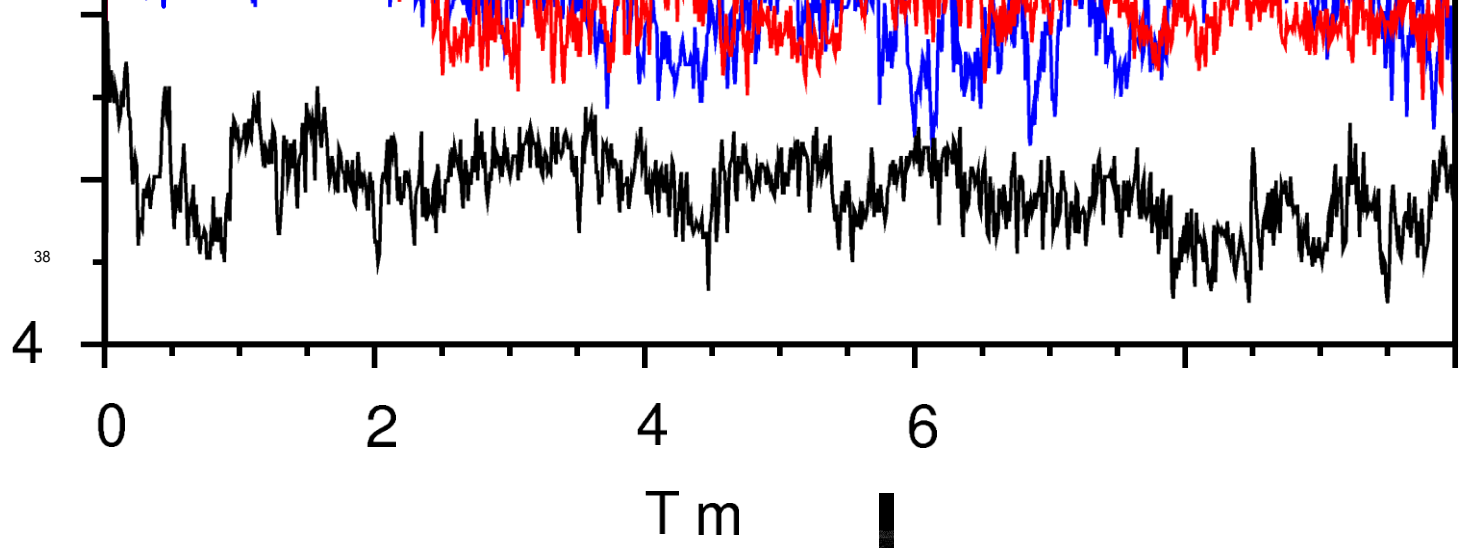
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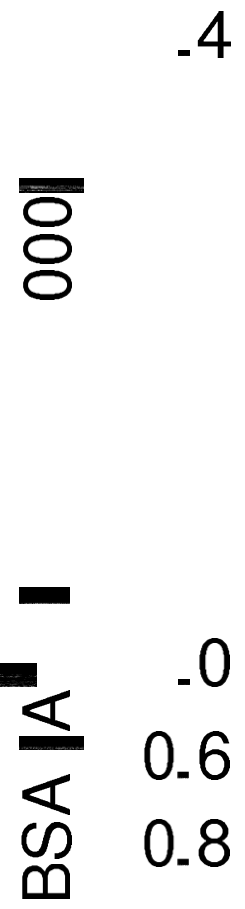
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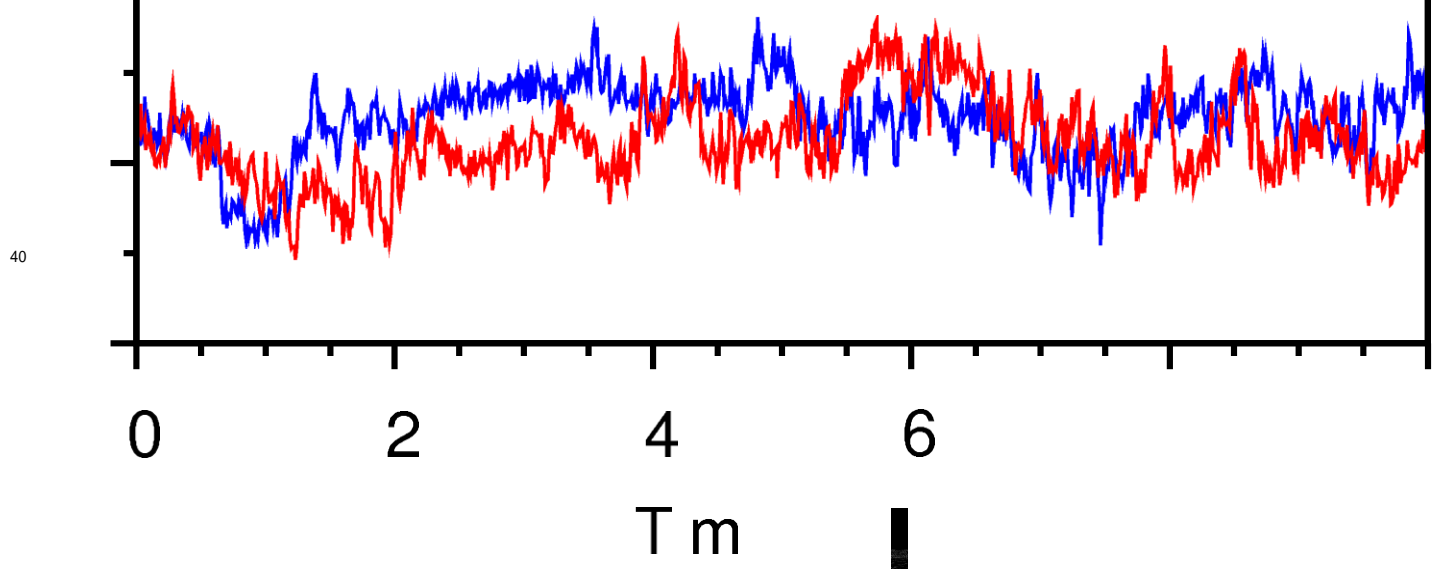
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Figure 7

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