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► **To cite this version:**

Athanassios Stavrakoudis. Computational modeling and molecular dynamics simulations of a cyclic peptide mimotope of the CD52 antigen complexed with CAMPATH-1H antibody. *Molecular Simulation*, 2010, 36 (02), pp.127-137. 10.1080/08927020903124593 . hal-00564475

HAL Id: hal-00564475

<https://hal.science/hal-00564475>

Submitted on 9 Feb 2011

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**Computational modeling and molecular dynamics
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antigen complexed with CAMPATH-1H antibody**

Journal:	<i>Molecular Simulation/Journal of Experimental Nanoscience</i>
Manuscript ID:	GMOS-2009-0056.R1
Journal:	Molecular Simulation
Date Submitted by the Author:	10-Jun-2009
Complete List of Authors:	Stavrakoudis, Athanassios; Ioannina University, Economics
Keywords:	antigen/antibody interactions, computer simulation, disulphide cyclization, molecular dynamics, QSAR
Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.	
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11 **Computational modeling and molecular dynamics**
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13 **simulations of a cyclic peptide mimotope of the CD52 antigen**
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15 **complexed with CAMPATH-1H antibody**
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Summary

A peptide mimotope of the CD52 antigen with the sequence T₁SSPSAD₇ has been co-crystallized with the CAMPATH-1H antibody. Molecular dynamics simulations in explicit water of the T₁SSPSAD₇ peptide in both antibody free and bound states showed that the peptide's β -turn was remained stable in the bound state but it was eliminated in the free state. Based on the observation that Thr₁ and Ala₆ residues made close contacts through their side chain, a new peptide mimotope is proposed: (S,S)-C₁SSPSCD₇. Thr₁ and Ala₆ residues have been mutated in Cys residues and a disulfide bond has been imposed. The new analogue has been simulated in both antibody bound and free states with molecular dynamics in explicit water. It was found that the peptide remained in stable β -turn conformation, both in complexed and free states. The difference in configurational entropy was estimated to be 0.15 kJK⁻¹mol⁻¹. However, despite the structural similarity, the cyclic analogue lost more than 25% of its buried surface area contact with the antibody and a couple of critical hydrogen bond interactions were broken. It is concluded that design of cyclic analogues that mimic the bound conformation of peptides should be carefully performed and conformational "freezing" does not necessarily guarantee better binding.

Keywords: antigen/antibody interactions; CAMPATH-1H; CD52 antigen; computer simulation; disulphide cyclization; molecular dynamics; QSAR; β -turn

Introduction

Antibodies are well known multi-domain protein molecules for their capability of specifically bind other protein or non-protein molecules, the so called antigens [1]. Functionality of antigen binding is localized close to N-terminal part of their protein chains which have significant variability in residue composition from antibody to antibody, V_H of the heavy chain and V_L of the light chain respectively. Antigen binding topography [2] has been attributed to six fragments that constitute the Complementary Determining Regions (CDRs). Today there is a vast number of clinical [3, 4] or therapeutic [5, 6] applications of antibodies.

CAMPATH-1H is a humanized [7] monoclonal antibody against the CD52 antigen [8]. It has been used successfully for the treatment of leukemia, autoimmune disease and transplant rejection [9, 10]. The CAMPATH-1H antibody has a highly basic binding site. It has been shown that the antibody binds the peptide mimotope $T_1SSPSAD_7$, and the complex structure has been determined by X-Ray [11]. The peptide's conformation in bound state was found to be type I β -turn around Pro_5Ser_6 residues. In contrast to other cases, where the CDR-H3 is essential to antigen binding [2], the CDR-L3 of CAMPATH-1H dominates antigen binding [11]. Molecular dynamics simulations of the $T_1SSPSAD_7$ peptide in complex (with CAMPATH-1H) and free states revealed that β -turn conformation of the peptide is retained only in the complexed state [12]. This fact indicates that the antibody induces the β -turn conformation upon complexation process.

Design of therapeutic compounds based on β -turn conformation is a well known process [13]. It is possible that stabilization of the β -turn in the Pro_5Ser_6 fragment could enhance binding properties of the peptide mimotope. Among of many ways to introduce structure stabilization in a peptide fragment, one of the most frequent used is disulfide

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4 cyclization [14-17]. Other notable examples of biological activity enhancement through
5 peptide cyclization can be found in the literature [18, 19]. The T₁SSPSAD₇ peptide does
6 offer directly an opportunity. Here, a molecular dynamics (MD) study of the peptide
7 mimotope (S,S)C₁SSPSCD₇ of the CD52 antigen is presented, in both antibody free and
8 bound states. This side chain proximity of Thr₁ and Ala₆ of the original mimotope offers an
9 excellent opportunity for disulfide engineering: Thr₁ and Ala₆ can be substituted by
10 cysteine residues and a cyclic structure can be imposed through disulfide bond formation.
11 Since there are no major conformational changes to be expected, the resulted cyclic
12 peptide can be used instead of its original linear precursor, under the assumption that the
13 antibody can bind the designed peptide.
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26 Molecular dynamics simulations offer a solid background for studying biomolecular
27 structure and interactions. This computational based approach has been shown to be very
28 valuable and its importance is well described [20-23]. Similar computational approaches
29 have been showed to add valuable information to existed experimental data of
30 antibody/antigen complexes [24-26], in searching for active conformational templates [27,
31 28], to establish a model of protein/peptide dimerization based on a disulfide bridge [29], or
32 to explore pathways of protein folding [30]. This study presents the engineering of a
33 peptide mimotope through disulfide cyclization and exploration of its structural properties in
34 bound and free states through molecular dynamics simulations. It is proposed that the
35 cyclic peptide has stable β -turn conformation in solution and its structure at the bound
36 state it is very close to the crystal structure of its linear precursor bound at the CAMPATH-
37 1H antibody's binding site. Reduction of the entropy required for binding of the
38 T₁SSPSAD₇ peptide could possibly result in better binding properties. On the other hand,
39 restriction on mobility can hinder small or even subtle conformational changes needed for
40 optimal binding and biological activity. Molecular dynamics simulations offer an excellent
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4 framework to study both aspects of the proposed engineering of the linear peptide: a) if
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6 the cyclic peptide retains in free and bound states the bound conformation of the linear
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8 analogue and b) if the cyclic peptide retains good binding affinity as the linear analogue.
9

10 11 12 **Methodology**

13 14 15 ***Computational modeling of the system***

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17 Initial coordinates were extracted from CAMPATH-1H complexed structure as
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19 deposited at PDB, access code 1ce1 [11]. Molecular modeling of the disulfide cyclic
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21 (S,S)₂C₁SSPSCD₇ peptide and starting conformation for the subsequent MM/MD studies
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23 was built with the VMD program [31]. Residues Thr₁ and Ala₆ of chain P (peptide) were
24
25 mutated to Cys and a disulfide bond was imposed. Topology and force field parameters for
26
27 all atoms were assigned from the CHARMM27 parameter set [32]. The antibody/antigen
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29 complex was solvated with TIP3P [33] water molecules using a rectangular box with
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31 dimensions 9.96x8.49x11.41 nm³. This allowed a distance of at least 1.8 nm between any
32
33 protein atom and the edges of the box in order to avoid simulation artifacts [34]. Water
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35 molecules found in the X-Ray structure were also included and treated with the TIP3P
36
37 model. The system was neutralized by placing 62 Na⁺ and 70 Cl⁻ ions, using VMD's
38
39 autoionize plugin, in order to approximate ionic strength of 0.25 M. From this point on, all
40
41 subsequently MM and MD runs were performed with NAMD (v2.6) [35] using 8 CPUs of a
42
43 Linux cluster. Non-bonded van der Waals interactions were gradually turned off at a
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45 distance between 1.0 and 1.2 nm. The non-bonded pair list was updated every 10 steps at
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47 a distance 1.4 nm. Long range electrostatics were computed every second step with the
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49 PME method [36], with a grid spacing of less than 0.1 nm. Bonds to hydrogen atoms were
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51 constrained with the SHAKE [37] with a relative tolerance of 10⁻⁸, allowing a 2 fs step
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4 during MD runs. The whole system, consisted by 91076 atoms, was energy minimized with
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6 5000 steps of conjugate gradients. After minimization the temperature of the system was
7
8 gradually increased with Langevin dynamics, using the NVT ensemble, to 310 K, during a
9
10 period 3000 steps, by stepwise reassignment of velocities every 500 steps. The simulation
11
12 continued until 100000 steps (0.2 ns). At minimization and equilibration stages, heavy
13
14 atoms of the antibody/antigen complex (included the crystallographic water molecules)
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16 were restrained to their initial positions with a force constant of $50 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$. The force
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18 constant of positional restraints was then decreased to $5 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$ for another 100000
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20 steps and finally positional restraints were totally eliminated for subsequent 200000 steps
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22 of NVT equilibration period. The simulation was continued under constant pressure, with
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24 Langevin piston method [38], thus NPT ensemble, for 20 ns. Pressure was maintained at 1
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26 atm and temperature was kept at 310 K. The results presented here are from this,
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28 isothermal–isobaric ensemble, MD run. Snapshots were saved to disk at 1 ps interval for
29
30 further analysis. A similar trajectory was produced with only the peptide antigen present.
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32 The same computational procedure was applied. The length of this trajectory (free state)
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34 was 50 ns.
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40 ***Conformational analysis***

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42 Conformational analysis and visual inspection of structures were performed with
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44 VMD [31] , Carma [39] and Eucb [40] software packages. Appropriate circular statistics
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46 were taken into consideration for calculation of descriptive values of dihedral angles [41].
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48 Trajectory analysis of hydrogen bonds were performed with a single geometrical criterion:
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50 a hydrogen bond was accepted if the Acceptor-Donor distance was less than 0.33 nm and
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52 the Donor-Hydrogen-Acceptor angle greater than 120° . Hydrophobic interactions were
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54 assigned when two non-polar heavy atoms were in less than 0.4 nm distance proximity.
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Secondary structure assignment was performed with STRIDE [42]. Structural figures were prepared with PYMOL [43].

The Root Mean Square Distance (RMSD) between the backbone atoms of the trajectory frames of polypeptide chains and the corresponding atoms of the X-Ray structure, calculated for frame t , is given by Equation 1, where x^m, y^m, z^m are the Cartesian coordinates found at the X-Ray structure and x^t, y^t, z^t are the Cartesian coordinates of trajectory frame t . N is the number of atoms.

$$RMSD_t = \frac{1}{N} \sqrt{\sum_{i=1}^N (x_i^m - x_i^t)^2 + (y_i^m - y_i^t)^2 + (z_i^m - z_i^t)^2} \quad (1)$$

The Root Mean Square Fluctuation (RMSF) of an atom is a measure of the deviation between the position of the atom and some reference position:

$$RMSF = \sqrt{\frac{1}{T} \sum_{i=1}^T (x_i - \bar{x})^2} \quad (2)$$

Where T is the number of trajectory frames and \bar{x} is the time-averaged position. Practically, RMSF calculates the mobility of an atom during the MD trajectory, thus higher RMSF values indicate higher mobility and lower RMSF values indicate restricted mobility. RMSD and RMSF calculations were performed after removing the global translation/rotation of the protein system by atom-positional least-squared fitting the protein atoms of the trajectory onto the coordinates of the first frame.

Buried Surface Calculation

Calculation of buried surface area (BSA) was performed with the NACCESS program [44], based on the formula:

$$BSA = Sp + Sa - Sc \quad (3)$$

thus as the difference of the surface accessible area of the complex (S_c) from the sum of the surface accessible areas of the peptide (S_p) and antibody (S_a) respectively. Calculations were performed to all frames of the compP (peptide/antibody complex) trajectory in order to get and characterize the times series of BSA.

Entropy calculations

Schlitter's formulation [45] was used for the calculation of the configurational entropy (S):

$$S_{true} < S = \frac{1}{2} k_B \ln \det \left[1 + \frac{k_B T e^2}{\hbar^2} \mathbf{M} \boldsymbol{\sigma} \right] \quad (4)$$

where S is an upper estimation of the true entropy (S_{true}), k_B is Boltzmann's constant, T is the absolute temperature (in which the system was simulated), e is Euler's number, \hbar is Plank's constant divided by 2π , \mathbf{M} is the mass matrix that holds on the diagonal the masses belonging to the atomic Cartesian degrees of freedom, and $\boldsymbol{\sigma}$ is the covariance matrix of atom positional fluctuations:

$$\sigma_{ij} = (x_i - \langle x_i \rangle)(y_i - \langle y_i \rangle), \quad (5)$$

Entropy calculations were performed with the backbone atoms (N, C $^\alpha$ and C') of the peptide from the bound and free trajectories respectively, at 0.1 ns interval (100 frames).

Two separate trajectories (for example free and complex trajectories of a peptide) can be combined, thus one trajectory can be appended at the end of the other trajectory, and the plot of configurational entropy S against time can be used as assessment of the overlap between configurational spaces sampled in two simulations [46]. Such trajectories

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4 have been derived for the backbone (bb) atoms (N, C^α, C') of the peptide from the last 10
5 ns of the free (f) and bound (b) trajectories. Both appending sequences were applied
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7 resulting in S_{bb}^{f+b} and S_{bb}^{b+f} calculations, where the bound trajectory was appended to the
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9 free (f+b) or the free trajectory was appended to the bound one (b+f). Plotting the
10
11 calculated values of S from both the combined trajectories over time demonstrates the
12
13 relative size and overlap of sampled trajectories. Plotting of S over time after the
14
15 combination of two trajectories results in three cases [46], briefly described as:
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21 1. S increases after appending one trajectory after to the other, with a jump observed
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23 at this point, thus the two trajectories do not overlap, or there is only a small
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25 overlap between them.
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- 28 2. S evolves smoothly after the appending of the trajectories, without an observable
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30 perturbation of the line of S over time, thus the two trajectories show significant
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32 overlap.
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- 35 3. S curve increases during the time of the first trajectory but decreases a little after
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37 the appending of the second trajectory, thus the second trajectory samples a
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39 smaller configurational space than the first one, which also contains the
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41 configurational space visited by the second one.
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44 Such an analysis of the combined trajectories is more advantageous than comparing
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46 directly the configurational entropies of two independent trajectories and can provide
47
48 information about the extent of sampling overlap (if any) between two trajectories. The
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50 coverage of the free trajectory with respect to the complex trajectory is:
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$$53 \Delta S_{bb}^{b+f} \equiv S_{bb}(\text{complex, peptide}) - S_{bb}(\text{complex}) \quad (6)$$

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and the coverage of the bound (complex) trajectory with respect to the free trajectory is:

$$\Delta S_{bb}^{f+c} \equiv S_{bb}(peptide, complex) - S_{bb}(peptide) \quad (7)$$

Results and discussion

Crystal structure of the antigen-antibody complex

The central part of the T₁SSPSAD₇ peptide was found in β -turn conformation in the crystal structure complexed with the CAMPATH-1H antibody. Secondary structure analysis with the STRIDE revealed that the peptide was in CCTTTTC conformational state (where C stands for Coil and T for Turn conformational states). Two consecutive β -turns were found. The fragment Ser₃-Pro-Ser-Ala₆ constituted the first type I β -turn which stabilized by the Ser₃:O-Ala₆:N hydrogen bond. The corresponding donor-acceptor distance was found 0.32 nm. Backbone dihedral angles (φ , ψ) were (-73.1°, -15.0°) and (-93.0°, -1.5°) for residues Pro₄ and Ser₅ respectively. These values are very close to those corresponding to an idealized type I β -turn, (-60°, -30°) and (-90°, 0°) for residues $i+1$ and $i+2$ respectively. The fragment Pro₄-Ser-Ala-Asp₇ constituted the second type IV β -turn, which was not accompanied by hydrogen bond stabilization. The distance between Pro₄:C α and Asp₇:C α atoms was found 0.653 nm and the torsion angle Pro₄:C α -Ser₅:C α -Ala₆:C α -Asp₇:C α was found 81.3°. These data indicate that this turn is relatively unstable.

Another important intermolecular contact that stabilized the turn structure was the close contact between the side chain atoms Thr₁:C γ and Ala₆:C β . The corresponding distance was 0.33 nm, thus a relatively strong hydrophobic interaction was in presence.

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4 Most of the antigen-antibody contacts involved antibody's light chain, especially the
5 L3 region of CDRs. This was an unusual finding, since most of these contacts have been
6 observed to involve the CDR-H3 [2]. Numerous hydrogen bond interactions were also
7 observed between the peptide antigen and antibody's CDRs, as it is shown at Fig. 5 of
8 [11]. This is well expected since the peptide contains numerous polar side chains. Table I
9 lists the hydrogen bond interactions between the peptide antigen and antibody's light and
10 heavy chains.
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23 ***RMSF and RMSD analysis***

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25 Fig. 1 shows the RMSF of C^α atoms as calculated from the free and bound
26 trajectories for the T₁SSPSAD₇ peptide. The peptide showed only limited mobility in the
27 bound state and remarkable small RMSF values (<0.1 nm) confirmed this fact. This also
28 illustrates the goodness of fit of the peptide into antibody's binding site.
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34 Most of antibody's heavy and light chains C^α atoms showed RMSF values close to
35 0.1 nm. It is interesting to note that some exposed loop regions of the heavy chain showed
36 increased RMSF values between 0.3 and 0.5 nm. For example, the RMSF peak at 0.4 nm
37 corresponds to residue 165 of the heavy chain. A similar situation has been observed with
38 the simulation of the linear peptide complexed with the CAMPATH-1H antibody [12].
39 Residues of the CDRs that they were found to have contacts with the peptide antigen
40 showed minimal RMS fluctuations between 0.05 and 0.1 nm (Fig. 1). This fact indicates
41 that the conformation of the CDRs was not influenced from the cyclization of the peptide
42 and that interactions between antibody and peptide were remained almost intact. Small
43 RMSF fluctuations are indicative of minimal conformational mobility and the minimal effect
44 that the cyclization of the peptide had on antibody's conformation is corroborated by the
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4 small RMSF values of the CDR regions, as well as by the similarity of the RMSF profiles
5 during the current and previous (with the linear peptide [12]) simulations.
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9 RMSF values of peptide's C^α atoms of the MD trajectory of peptide in bound state
10 (compP) were found between 0.06 and 0.1 nm (Fig. 1). These low RMSF values underline
11 the conformational rigidity of the peptide in the bound state. In the free state (freeP
12 trajectory), RMSF values of peptide's C^α atoms were found between 0.05 and 0.15 nm.
13 Although a raise in RMSF values is expected when the peptide is simulated in the
14 unbound state (freeP trajectory), the difference in RMSF values is relatively small. For
15 example, the corresponding RMSF values ranged between 0.15 and 0.28 nm during the
16 simulation of the linear peptide [12]. The decreased RMSF values illustrate the effect of
17 cyclization on peptide's structure. The Asp₇ residue, located at the C-terminal part of the
18 peptide, showed the highest RMSF values in both compP and freeP trajectories (0.1 and
19 0.15 nm respectively). On the other hand, the Ser₃ residue showed the smaller RMSF
20 value in both compP and freeP trajectories, with values close to 0.05 nm in both cases. In
21 general, RMSF values of peptide's C^α atoms were found with remarkable covariance in
22 comp and freeP trajectories (Fig. 1).
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39 Antibody's heavy and light chains showed moderate mobility during the MD
40 trajectory, as revealed from backbone RMSD time series plot (Fig. 1). RMSD (values in
41 parentheses indicate standard deviation) of the heavy chain exhibited values between 0.13
42 and 0.34 nm which averaged at 0.23 nm (0.04 nm). Light chain showed less mobility than
43 the heavy one with RMSD values between 0.10 and 0.26 nm which averaged at 0.17 nm
44 (0.03 nm). These time series were relative stable through the simulation time which
45 indicates the stability of the trajectory. However, during the last 10 ns of the compP
46 trajectory there is a small gradual increase in RMSD values of antibody's heavy chain. The
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4 observed values are within expected range in a MD simulation, but there is some evidence
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6 of conformational change in the heavy chain.
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9 Peptide's backbone atoms RMSD time evolution (in the bound state, Fig. 1c)
10 showed limited fluctuation and recorded values were found to be between 0.1 and 0.26
11 nm. Time series average value was found 0.21 nm (0.016 nm). These relatively low
12 values, along with the previously analyzed RMS fluctuations, indicate the very low
13 flexibility of the peptide when bound to antibody's binding site. A trajectory movie
14 (supplement material) also provides visual evidence about this fact.
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22 Much like the previous observations concerning the RMSD of the peptide bound to
23 the antibody, when the peptide was simulated in the absence of the antibody (free state) it
24 showed significantly increased mobility (Fig. 1d). Backbone RMSD values of the peptide
25 ranged between approximately 0.05 and 0.25 nm during the 50 ns trajectory. These values
26 averaged at 0.16 nm (0.05 nm). RMSD fluctuation remained relative stable during MD
27 trajectory. In line with the RMSF analysis, RMSD analysis indicates that the peptide
28 remained closed in its initial conformation, which was actually that found in the crystal
29 structure. If the results of MD of the linear peptide in the free state are taken into
30 consideration, where RMSD values reached 0.4 nm, it assumed here that disulphide
31 cyclization, not only reduced the conformational space available to the peptide, but also
32 resulted in "fixing" the conformation close to those found in the bound state.
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46 From this point of view, analysis of the freeP trajectory corroborates the initial
47 design hypothesis of this work that substitution of Thr₁ and Ala₆ by Cys residues and
48 disulphide cyclization will "freeze" the conformation of the peptide. This can be seen in
49 Fig. 2 where representative structures of the cyclic peptide from trajectories compP and
50 freeP are depicted.
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Backbone conformation and secondary structure of the peptide antigen

The initial (crystal structure) conformational state of the T₁SSPSAD₇ peptide was found CCTTTTC according to STRIDE assignment. A type I β -turn around the Pro₄Ser₅ existed in the central part of the peptide. Previous MD studies showed that this β -turn remains stable when the peptide was simulated in the complex state, but disappeared when simulated in the free (unbound) state. Disulfide cyclization, after substitution of Thr₁ and Ala₆ with cysteine residues, is expected to stabilize this β -turn. Trajectory analysis of the peptide's secondary structure revealed that this conformation was retained for 82% of the frames, during the compP trajectory. Analysis of the freeP trajectory revealed two main conformational clusters (Table 2) with secondary structure assignments CCTTTTC and TTTTTTC (percentage of occurrence was 45% and 43% respectively). Although the turn conformation of the central part of the peptide was retained in both conformational clusters, a second turn appeared, at the N-terminal part of the peptide. Overall, both trajectories showed remarkable similarity in secondary structure assignments, which corroborates the idea that disulfide cyclization can stabilize the solution conformation of the peptide close to the bound structure of the linear precursor peptide. Of course, even after cyclization, the peptide can access bigger conformational space in free state than in complex state. But the fact that both trajectories showed similar secondary structures illustrates the possible success of this molecular design.

The β -turn structure observed at the Ser₃-Pro₄-Ser₅-Ala₆ peptide's fragment was very well conserved during both trajectories. However, the initial type I particular conformation was only retained during the compP trajectory for 57%. Another 25% of the trajectory frames were found with type IV β -turn. Thus, type I was the dominant conformation, but significant part of the trajectory escaped to slightly different backbone

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4 conformation. During the freeP trajectory the type I β -turn of the Ser₃-Pro₄-Ser₅-Ala₆
5 peptide's fragment was purely conserved, it appeared for only 20% of the frames.
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7 Ramachadran plots of backbone dihedrals (Fig. 3) demonstrate that it is basically due to
8 Ser₅'s ψ dihedral angle. However, the fluctuation of ψ_5 did not resulted in total
9 disappearance of the β -turn. The Ser₃-Pro₄-Ser₅-Ala₆ fragment retained in β -turn
10 conformation (for total 91% of the simulation time) but type IV (42%) and type VIII (29%)
11 also appeared. The freeP trajectory revealed another β -turn formation at the Cys₁-Ser₂-
12 Ser₃-Pro₄ fragment, not existed at the initial conformation. For 47% of the simulation time
13 (Table 3) this fragment was found in either type IV of type VIII β -turn conformation. As it
14 shown in Table 3, both turns co-existed for approximately 43% of the time, nearly half of
15 the trajectory.
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28 Analysis of intra-peptide hydrogen bonds during the compP trajectory showed that
29 the Ser₃:O-Ala₆:N distance and the Ser₃:O-Ala₆:H^N-Ala₆:N angle satisfied the hydrogen
30 bond criteria for only 23% of the trajectory time. Thus, the hydrogen bond that stabilized
31 the β -turn of the central part of the peptide was only poorly conserved. The corresponding
32 percentage of frames was 45% when the linear peptide simulated in the bound state [12].
33 Although, the existence of a hydrogen bond is not an absolute requirement for the
34 acceptance of a β -turn structure, it is clear that the stabilization of the β -turn due to the
35 existence of the intramolecular hydrogen bond was scaled down after the imposed
36 disulphide bond. On the other hand, a new hydrogen bond appeared between Ser₃:O
37 and Ser₅:N atoms which corresponds to a γ inverse turn. This interaction appeared for
38 approximately 65% of the time. Thus, the bulge in the central part of the peptide fragment
39 was not completely lost but it was somewhat altered in comparison with the initial crystal
40 structure. Despite the disruption of the canonical hydrogen bond interaction, the fragment
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4 Ser₃-Pro-Ser-Cys₆ remained in β -turn structure, as measured by Ser₃:C ^{α} -Cys₆:C ^{α} distance
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6 the Ser₃:C ^{α} -Pro₄:C ^{α} -Ser₅:C ^{α} -Cys₆:C ^{α} dihedral angle, for approximately 80% of the time.
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9 Simulation of the peptide in the free state (freeP trajectory) showed that the initial
10 turn conformation was retained for 85% of the time (Table 3). The initial CCTTTTC
11 conformational state was found in 82% of the frames in the compP trajectory and 45% in
12 the freeP trajectory. Compared with the 5% of the CCTTTTC conformation of the linear
13 peptide, found in a previous research, the 45% of the frames demonstrates the effect of
14 the cyclization in the conservation of the turn structure. From this point of view, disulfide
15 cyclization succeeded in its purpose: it offered a structural framework of β -turn
16 stabilization.
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27 ***Antibody-antigen interactions***

28 The binding site of the CAMPATH-1H antibody is highly basic. A number of polar hydrogen
29 bonds between the peptide and the antibody has been reported to contribute to the
30 stability of the complex. However, several of these hydrogen bonds were poorly conserved
31 in the compP trajectory (Table 1).
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38 One of the consequences of the disulphide cyclization of the peptide antigen was
39 the loss of a hydrogen bond interaction between Glu_{101H}:O ^{ϵ 2} and Cys_{1P}:S^Y side chain
40 atoms. The original distance of 0.24 nm between these two atoms fluctuated between 0.29
41 and 0.86 nm during compP trajectory. The substitution had also some effect to the nearby
42 residue. Hydrogen bond between Ser_{2P}:N and Ser_{2P}:O^Y was conserved for only 30% of the
43 compP trajectory.
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51 Ser_{3P} and His_{81L} were found in hydrogen bond state via their side chains. The
52 Ser_{3P}:O^Y-His_{91L}:N ^{δ 1} distance ranged between 0.252 and 0.60 nm during com-MD trajectory.
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54 The corresponding distance in the X-Ray structure was 0.264 nm. Trajectory analysis
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4 showed that these two side chains remained in hydrogen bond state for 56% of the
5 simulation time. It should be noted here that hydroxyl group of Ser_{3P} acted as acceptor and
6 imidazolium group of His_{91L} acted as donor of this hydrogen bond. Arg_{96L}'s side chain also
7 contributed a hydrogen bond to Ser_{3P}'s side chain for 17% of the simulation time. The
8 Arg_{96L}:Nⁿ²-Ser_{3P}:O^y distance ranged between 0.287 and 0.886 nm during com-MD
9 trajectory, while the corresponding distance in the X-Ray structure was 0.30 nm. Thus,
10 side chain of Ser_{3P} remained in hydrogen bond state with CDR-L3 residues for
11 approximately 73% of the simulation time.
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13
14 Pro_{4P}, lying in the center of the peptide's sequence, dominated the hydrophobic
15 interactions with the antibody. Pro_{4P}'s side chain aliphatic atoms were found to make
16 several contacts (distance less than 0.4 nm) with Tyr_{32L}'s (CDR-L1) side chain.
17 Considering the X-Ray structure of the complex, there were found 9 pairs of interatomic
18 distances of less than 0.4 nm between Pro_{4P}'s and Tyr_{32L}'s side chain carbon atoms. Of
19 course, not all of them were conserved during com-MD trajectory. However, seven of them
20 (not at the same necessarily) were found to exist (for at least 20% of the trajectory time) in
21 distance less than 0.4 nm. The escape of the 0.4 nm cutoff distance can be attributed to
22 minor fluctuations of the χ^1 angle of Tyr_{32L} which was averaged at -59° (7.7°) during com-
23 MD trajectory. While this side chain dihedral did not changed substantially during the
24 simulation time, it must be noted that a small fluctuation of 15° for example, can result in
25 more than 0.05 nm increase in distance between side chain atoms of Pro_{4P} and Tyr_{32L}.
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29 During the second half of the compP trajectory, Pro_{4P}:O and Arg_{L94}:Nⁿ² atoms were
30 found in hydrogen bond state (for 47% of the total simulation time). The original distance
31 (X-ray) of these two atoms was 0.68 nm. This fact indicates that a medium sized
32 conformational change occurred at the CDR-L3 region that probably stabilized the
33 complex. Thus, some new bonds can be formed in place of the broken ones.
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5 Ser_{5P} dominated hydrogen bond interactions between peptide and antibody. In the
6 original, X-Ray, structure, there were found three hydrogen bonds between peptide and
7 antibody. Namely, Ser_{5P}:O-Arg_{94L}:Nⁿ¹ with distance 0.3 nm, Ser_{5P}:O^y-His_{91L}:O with distance
8 0.263 nm and Ser_{5P}:O^y-Arg_{96L}:N^e with distance 0.284 nm. This means that Ser_{5P}:O^y atom
9 acted both as hydrogen bond donor, (with His_{91L}:O as acceptor) and acceptor (with
10 Arg_{96L}:N^e as donor). The initial Ser_{5P}:O-Arg_{94L}:Nⁿ¹ hydrogen bond was retained for only
11 29% of the com-MD trajectory. However, another hydrogen bond between Ser_{5P}:O and
12 Arg_{96L}:Nⁿ² atoms, not present in the initial structure, acted in a complementary mode, and
13 existed for 41% of the simulation time. The hydrogen bond between Ser_{5P}:O^y and
14 Arg_{96L}:N^e atoms was retained for 87% of the simulation time during com-MD trajectory,
15 while hydrogen bond between Ser_{5P}:O^y and His_{91L}:O was retained for 61% of the
16 simulation time.
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30 Finally, a severe loss of the hydrogen bonding occurred at the C-terminal of the
31 peptide. Due to the basic nature of antibody's binding site, numerous hydrogen bonds and
32 salt bridges existed between the Asp_{7P} residue and antibody's basic residues. With the
33 exception of Asp_{7P}-Arg_{94L}, all other interactions were significantly suppressed or
34 extinguished. For example, a very strong hydrogen bond between Arg_{H52}:N^e and Asp_{P7}:O^{δ1}
35 atoms was completely lost during compP trajectory.
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45 ***Configurational entropy***

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47 One of the main objectives of the current work was to examine to what extent the
48 cyclic peptide access the same conformational space in complex and free state. As it is
49 expected, conformational fluctuation of peptide in the bound state can be rather prohibited.
50 On the other hand, the imposed cyclization through disulfide bond formation prohibits the
51 opening of the turn conformation to an extended one. Thus, it is assumed that the cyclic
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4 peptide should share (at least partly) the same conformational space in both free and
5 bound states facilitating the antibody binding of the peptide due to reduction of the entropic
6 cost. The similarity of the peptide's structure in the bound state with the crystal structure of
7 its linear precursor has been already discussed.
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13 Similarity of the peptide structures obtained from freeP and compP MD trajectories
14 can be seen in Fig. 2. However, configurational entropy offers a more quantitative way to
15 estimate the difference or similarity or the overlap of the trajectories. As it can be seen
16 from Fig. 4 the peptide sampled similar conformational spaces in both the compP and
17 freeP trajectories. However, sampling during compP was only a subset of the
18 conformational space sampled during freeP trajectory. This is something to be expected
19 because of the restriction to flexibility that the antibody imposes to the peptide. On the
20 other hand, the difference in configurational entropy was estimated to be $0.15 \text{ kJ K}^{-1} \text{ mol}^{-1}$, a
21 relatively moderate value, indicating that the peptide's conformation in free and bound
22 states do not differ dramatically. From this point of view the design of the cyclic peptide
23 was successful: "active" conformation was retained in the solution for 50 ns, which is not a
24 negligible time. Thus it can be expected that the antibody could bind the peptide from its
25 solution conformation with less entropic cost. But despite the structural similarity, is this
26 really "active" conformation?
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44 ***Buried surface area calculations***

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47 The connection between thermodynamics of protein/ligand association and
48 antibody/antigen complex formation and BSA is well described in the literature [47-49]. Fig.
49 5 shows the time evolution of buried surface area at the interface between antibody and
50 peptide over simulation time as calculated according to Equation 3 from the compP
51 trajectory. Time series of BSA ranged between 2.13 and 5.50 nm^2 and averaged at 3.67
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4 nm² (std 0.50 nm²). The BSA of the starting conformation was estimated to 4.87 nm².
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6 From these values it can be roughly estimated that the BSA between peptide and antibody
7 significantly decreased through the molecular dynamics trajectory. The majority of the
8 trajectory frames (69%) had BSA less than 3.87 nm², a value that it is 1 nm² below those
9 found in the crystal structure. It must be noted that variations of the BSA during MD
10 trajectories are to be expected because of the dynamic nature of the protein structure.
11 Moreover, a recent study [50] questioned the precision of the calculated solvent-accessible
12 surface area of proteins, and an error of 0.5-1 nm² in the calculation of BSA of
13 protein/protein complexes has been suggested. But even with findings taken into
14 consideration, the difference of experimental value (X-ray) and the average value of the
15 MD trajectory (4.87-3.67=1.2 nm²) is far away from to be considered a measurement error.
16 The loss of the ~25% (1.2/4.87) of the BSA between peptide and antibody can not be
17 neglected and it is concluded that the affinity of the antibody for the peptide has been
18 significantly reduced after the disulphide cyclization.
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35 As it can be seen from Fig. 5, a sharp fall in the value of BSA can be observed at
36 approximately 2.5 ns of the simulation time. Mean value of BSA calculated during the last
37 17 ns (omitting the first 3 ns) was found only 3.53 nm². These observations underline the
38 importance of sufficient sampling during MD simulations. Short runs (1-2 ns) can produce
39 misleading results and should be avoided.
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45 It has been proposed recently [47] that the free energy of protein/ligand binding can
46 be estimated by the empirical formula:
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$$\Delta G_{\max}^{\circ} \approx -30 - \frac{\text{CSA}_{\text{apolar,bindingsite}}}{10} \text{ kJmol}^{-1} \quad (8)$$

49
50 where CSA (Connolly surface area) is the non-polar accessible surface area. The
51 parametrization of the equation (8) has been performed with Connolly method of surface
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4 area calculation, but if we take into consideration that 1 kJmol⁻¹ equivalents with 20 Å² in
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6 Connolly's methods and 37 ± 7 Å² in Lee-Richards method (NACCESS) then we can use
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8 the above formula with appropriate scaling. For the initial complex (X-ray) ΔG_{max}^o was
9
10 calculated -56.3 kJmol⁻¹. Using the average trajectory value of 367 Å² of the ASA of
11
12 antibody/peptide complex, which scales to 198.4 Å² of CSA, the value of -49.8 was
13
14 estimated for the ΔG_{max}^o of the antibody/cyclic peptide. Thus, it is estimated that the
15
16 difference in the Gibbs free energy of binding between the linear and cyclic peptides is 6.5
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18 kJmol⁻¹. Although the results should be interpreted with care due to intrinsic limitations of
19
20 such calculations, it is clear that reduce in BSA between antibody and peptide resulted in
21
22 lower binding affinity.
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28 **Concluding remarks**

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31 Molecular modeling techniques coupled with extensive molecular dynamics
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33 simulations in explicit solvent have been utilized in order to design and computationally
34
35 validate the effect of disulfide bond formation on a peptide that binds to the CAMPATH-1H
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37 antibody. As cyclization is routinely used in order to enhance binding properties and/or
38
39 biological activity of bioactive peptides, the initial proposed molecular model seemed quite
40
41 reasonable guess: the T₁SSPAP₇ peptide could be replaced by the (S,S)C₁SSPCP₇ one,
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43 given the proximity of Thr₁ and Ala₆ side chains. After the initial building and molecular
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45 mechanics calculations, the stability of the antibody/peptide complex as well as
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47 conformation of the peptide in both bound and free states were explored with extensive
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49 molecular dynamics simulations in explicit water.
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53 Analysis of the results showed that the proposed model is quite promising in order
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55 to replace the linear one as peptide mimotope. The peptide remain stable into the binding
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4 groove of the CAMPATH-1H antibody and experienced moderate only fluctuation around
5 the initial structure. Moreover, the initial conformation of β -turn around the Pro₄Ser₅
6 fragment was perfectly conserved during the 20ns MD study in complexed state and 50 ns
7 in free state.
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12 The study of the cyclic peptide in the free state revealed also interesting
13 characteristics of its conformation. The results corroborated the hypothesis that the
14 engineering of the peptide and disulfide bond formation can substantially enhance
15 peptide's conformational stability in water. The β -turn around the Pro₄Ser₅ fragment was
16 retained for 91% of the time (50 ns). On the contrary, a previous study showed the
17 complete abolishment of turn conformation of the linear peptide in solution. The adoption
18 of a well-structured conformation of the peptide in solution is thus attributed to the
19 cyclization.
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30 Estimation of configurational entropy profiles of the peptide in free and bound
31 states was used in order to extract information about the similarity of the conformational
32 space accessed. A heuristic formula that uses combined trajectories of the two separate
33 simulations was used. Results indicated that the two MD trajectories overlap and they
34 sampled largely common areas of the conformational space. More specifically, the compP
35 trajectory sampled a subspace of the conformational space of sampled by the freeP
36 trajectory.
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45 The peptide retained in the free state most of its conformational characteristics
46 found in the complexed state. But a significant reduced tendency for hydrogen bonding
47 interactions between the peptide and antibody was observed. It seemed that the antibody
48 lost (at least partly) its affinity to bind the peptide. Analysis of the buried surface area
49 between the peptide and antibody showed that 25% of the contact surface was lost. This
50 has probably severe effects in antibody's affinity for the peptide. So, while the cyclization
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4 of peptide results in solution conformation that retains most of peptide's conformational
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6 characteristics, it did not help the antibody bonding.
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9 While cyclic peptides are still a very promising replacement of their linear
10 analogues, their usage in computer-aided design should be carefully implemented and
11 their conformational rigidity should not be over-emphasized. The current study presents
12 results that has two sides, a) disulphide cyclization effectively reduced the flexibility of the
13 linear peptide and transformed a complete unordered structure to a quite stable β -turn
14 conformation and b) minor changes in antigen/antibody contacts resulted in gradual loss of
15 several interactions that eliminated the buried surface area by approximately 25% and
16 thus reducing the binding affinity.
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32 **Acknowledgments**

33
34 NAMD parallel execution has been performed at the Research Center of Scientific
35 Simulations (RCSS) of University of Ioannina. The open source community is also
36 gratefully acknowledged for releasing to the public all the computational tools (Linux,
37 NAMD, GNU tools, etc) needed for this research.
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List of figures

Fig. 1 (a) RMSF of C^α atoms of antibody's heavy (H) and light (L) chains, (b) RMSF of peptide's C^α atoms in bound (compP) and free (freeP) states, (c) time evolution of backbone atoms (N, C^α, C', O) RMSD during the compP trajectory of antibody's heavy (H) and light (L) chains and peptide (P) and (d) time evolution of backbone atoms (N, C^α, C', O) RMSD during the freeP trajectory of the peptide. For interpretation of colors in this figure the reader is referred to the online version of this article.

Fig. 2 (a) Five snapshots of peptide's structure from the compP trajectory (every 4 ns) and (b) Five snapshots of peptide's structure from the freeP trajectory (every 10 ns). Peptide frames are superimposed on the backbone atoms of the corresponding atoms from the crystal structure (in magenta). Hydrogen atoms are omitted for the clarity of representation. For interpretation of colours in this figure the reader is referred to the online version of this article.

Fig. 3 Ramachadran map (backbone ϕ/ψ dihedrals) for peptide's residues in the bound (compP) and free (freeP) trajectories. Horizontal axis is for ϕ angle and vertical axis is for ψ angle. Two dimension probability density plots obtained are shown, 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. For interpretation of colours in this figure the reader is referred to the online version of this article.

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4 **Fig. 4** Plot of estimated entropies of backbone atoms of the (S,S) C_1 SSPSCD₇ peptide.
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6 Frames from the freeP were appended to the compP trajectory and *vice-versa*. Last 10 ns
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8 from the two trajectories were used, assuming equilibrium was reach. See methods for
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10 details of calculation.
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15 **Fig. 5** Time series of non-polar buried surface area (BSA) between peptide and antibody
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17 from the compP trajectory. See methods for details of calculation. The dashed horizontal
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19 line at 4.87 nm² indicates the experimental (X-ray) value of BSA of the crystallized linear
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21 analogue [11] while the dashed line at 3.87 nm² indicates the BSA value reduced by 1
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23 nm². This is to emphasize the fact that the great majority of the frames were found with
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25 significantly reduced BSA values.
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Table 1. Hydrogen bonds found between the peptide mimotope (P) and antibody's heavy (H) and light (L) chains. Percentage of frames (out of total number of trajectory frames) is given.

Donor	Acceptor	Distance ^a (nm)	% Occurance
Ser _{P2} :O ^Y	His _{H103} :O	0.300	-
Ser _{P2} :N	His _{H103} :O	0.300	32
His _{L91} :N ^{δ1}	Ser _{P3} :O ^Y	0.263	28
Ser _{P3} :O ^Y	His _{L91} :O	0.263	54
Arg _{L94} :N ^{η2}	Pro _{P4} :O	0.680	57
Arg _{L94} :N ^{η1} - Arg _{L96} :N ^{η6}	Ser _{P5} :O	0.300	86
Arg _{L96} :N ^{ε,η2}	Ser _{P5} :O ^Y	0.284	87
Arg _{H52} :N ^{η2}	Cys _{P6} :O	0.330	65
Ser _{P5} :O ^Y	His _{L91} :O	0.263	63
Ser _{P5} :N	His _{L91} :O	0.263	-
Arg _{H52} :N ^ε	Asp _{P7} :O ^{δ1}	0.244	-
Tyr _{H33} :O ^η	Asp _{P7} :O ^{δ1}	0.272	3
Lys _{H56} :N ^ζ	Asp _{P7} :O ^{δ1}	0.410	44
Arg _{L94} :N ^{η1,2}	Asp _{P7} :O ^{η1}	0.307, 0.412	93
Tyr _{H33} :O ^η	Asp _{P7} :O ^{δ2}	0.238	2
Lys _{H56} :N ^ζ	Asp _{P7} :O ^{δ2}	0.295	55

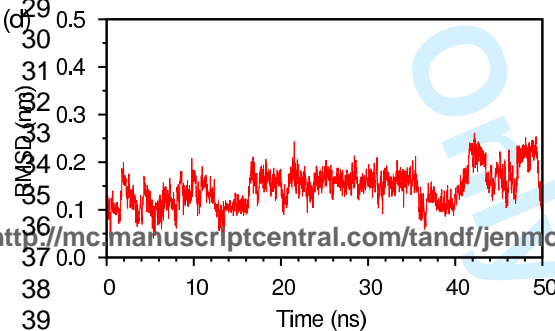
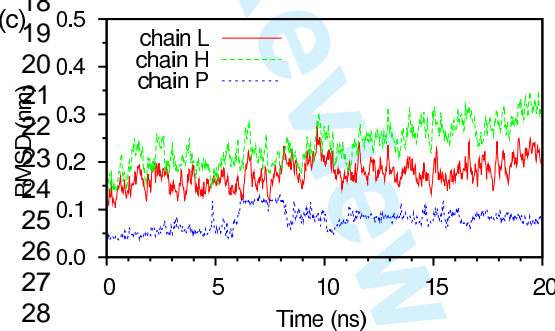
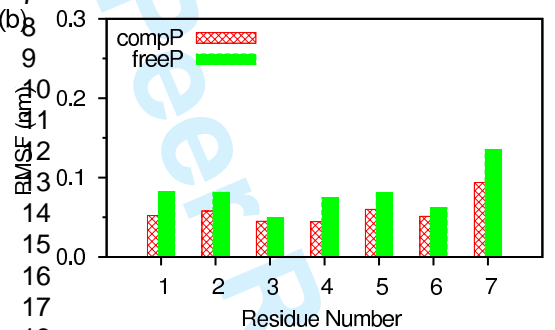
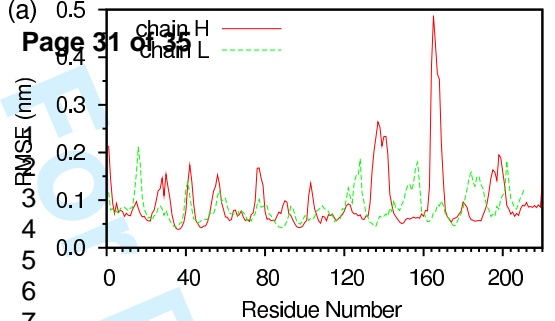
^a Donor-Acceptor distance found in the crystal structure of antibody with the linear peptide

Table 2. Main conformational clusters after secondary structure assignment of the compP and freeP MD trajectories.

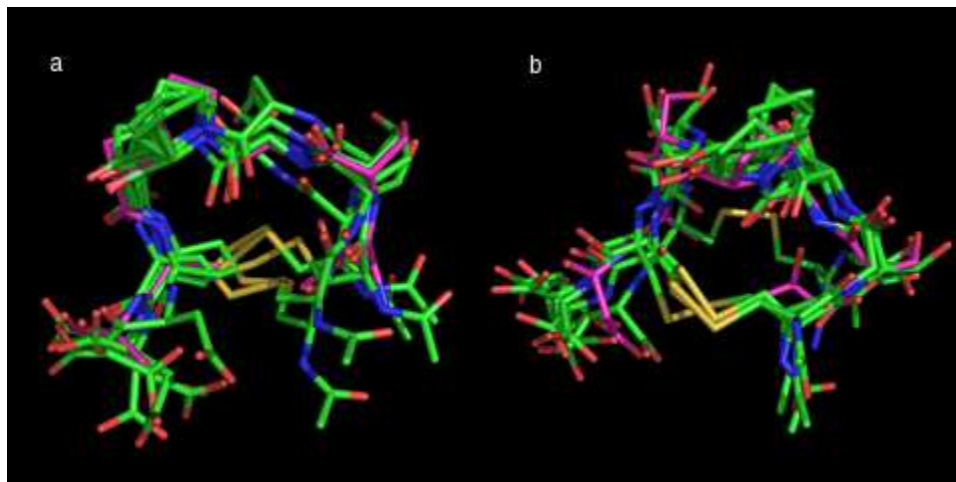
Secondary Structure	Trajectory	
	compP	freeP
CCTTTTC	82	45
TTTTTTC	2	43
CCCCCCC	14	7

Table 3. β -turn occurrence during the two MD trajectories. Percentage of frames are given.

Fragment	Type	Trajectory	
		compP	freeP
Cys1-Pro4	VIII		21
	IV		28
Ser3-Cys6	I	51	31
	VIII		25
	IV	18	30
Pro4-Asp7	I	4	
	IV	78	



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40x20mm (300 x 300 DPI)

Pre Review Only

