

Structural Characterization of a Protein A Mimetic Peptide Dendrimer Bound to Human IgG

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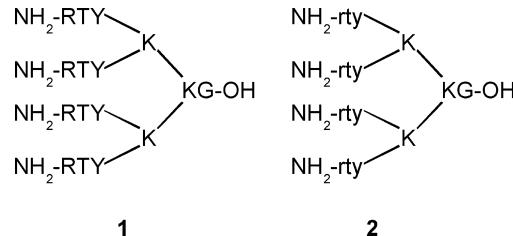
Understanding the chemical physical properties of protein binding sites is at the basis of the rational design of protein ligands. The hinge region of the Fc fragment of immunoglobulin G is an important and well characterized protein binding site, known to interact with several natural proteins and synthetic ligands. Here, we report structural evidence that a *Staphylococcus aureus* Protein A mimetic peptide dendrimer, deduced by a combinatorial approach, binds close to the C γ 2/C γ 3 interface of the constant fragment of a human IgG1 molecule, partially hindering the Protein A binding site. The X-ray analysis evidenced a primary binding site located between a terminal Arg residue of the ligand peptidic arm and a hydrophobic protein site consisting of Val308, Leu309, and His310. A molecular dynamic analysis of the model derived from the X-ray structure showed that in water at room temperature the complex is further stabilized by the formation of at least one more contact between a terminal Arg residue of the second arm of the peptide and the carboxylic group of a protein amino acid, such as Glu318, Asp312, or Asp280. It appears thus that stability of the Fc–dendrimer complex is determined by the synergistic formation of multiple bonds of different nature between the dendrimer arms and the protein accessible sites. The electrostatic and van der Waals energies of the complex were monitored during the MD simulations and confirmed the energetic stability of the two interactions.

1. Introduction

The development of elegant yet simple molecular constructs amenable to interact with biomolecules in a predictable way or to mimic the complex machinery of naturally selected biomolecular systems represents an ambitious objective for supramolecular chemists.^{1–6} A common motif in this research field is the need to set the rules of a minimalist approach to molecular recognition with synthetically accessible model systems endowed with functional activity. Some years ago, exploiting the molecular diversity generated in combinatorial libraries, one of us reported a peptide dendrimer that strongly binds to the constant fragment (Fc) of immunoglobulins of the G class (IgG).^{7,8} The peptide libraries were screened by competitive ELISA assays seeking for a peptide able to disrupt the interaction of IgG with protein A, an IgG binding protein of bacterial origin.^{9,10} Peptide dendrimer **1** (Scheme 1), comprising four copies of the tripeptide Arg-Thr-Tyr linked to an asymmetric polylysine core, emerged as the best inhibitor (pseudo-linear formula (ArgThrTyr)₄Lys(α,ϵ)₂Lys(α,ϵ)Gly).

As expected from the experimental design, the dendrimeric peptide **1** acted as a protein A mimetic molecule. Although the combinatorial libraries were prepared using proteinogenic L-amino acids, the diastereomeric peptide **2**, a partial inverse^{11–13} analogue of **1**, with the terminal residues replaced by D-amino

SCHEME 1: Schematic Representation of Dendrimeric Peptides (Lowercase Letters Indicate D Amino Acids)



1

2

acids, was still able to bind IgG with high affinity.¹⁴ Once immobilized on solid supports such as agarose or methacrylate based resins,^{15,16} peptide **2** proved to be an effective affinity ligand for the purification of antibodies from complex mixtures such as sera and cell culture supernatants. Affinity matrixes based on peptide **2** compete, in terms of capacity, purity, and yield of purified antibodies,¹⁷ with standard materials routinely used for downstream processing. Besides contrasting the interaction between Protein A and IgG, peptide **2** hinders the recognition of IgG by human Fc γ receptors *in vitro* as well as *in vivo* on experimental animal models showing high potential for the therapeutic treatment of autoimmune diseases, including *Lupus erythematosus*.^{18–20} Protein A and Fc γ receptors, as well as other endogenous or exogenous receptors,^{21–25} bind to immunoglobulins exploiting different loci at the boundary between the C γ 2 and C γ 3 domains of the IgG constant portion. This region is usually referred to in the literature as the “consensus binding site”²⁶ and represents an important target for putative therapeutics. The biochemical properties of peptide **2** infer that its binding site on the IgG surface should be very close to the C γ 2/C γ 3 interface.

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The aim of this study is twofold. On the one side, we intend to increase our understanding of the nature of the interaction between peptide dendrimer **2** and IgG. Such knowledge can in fact be exploited to modify the chemical composition of the peptide in order to increase its affinity and selectivity for immunoglobulins. Then, from a more fundamental standpoint, we wanted to study the capability of synthetic peptides to interact with the hinge region of the Fc fragment of immunoglobulin G, which is an important and well characterized protein binding site, known to interact with several natural proteins and synthetic ligands.

The adopted approach, described in section 2 of this paper, comprises X-ray molecular structure determination and molecular dynamic investigation of the bimolecular complex formed, in the solid state, by the peptide dendrimer **2** and the Fc fragment of a human IgG1 molecule (IgG isotype 1). [The PDB accession code for the crystal structure of the Fc-peptide dendrimer **2** complex is 3D6G (www.rcsb.org/pdb/).] The ligand is located in the cavity comprised between two or three Fc dimers, and thus experiences intermolecular interactions different from those it would establish in a pure water environment. Moreover, since the dendrimer has nine charged groups, eight of which are due to the four Arg residues and one to the terminal Gly of the Lys scaffold, its binding geometry may depart from the conformation determined by crystallography once the system is studied in water because of the enhanced mobility of these groups when immersed in a polar environment. To analyze this possibility, molecular dynamics (MD) simulations were performed starting from the X-ray crystallographic structure considering explicitly the presence of a periodic shell of water molecules. The X-ray structure and the results of the simulations are discussed in section 3.

2. Method and Theoretical Background

2.1. Peptide Synthesis. The peptide dendrimer was synthesized on solid phase, according to reported methods,¹⁴ by using amino acids in the D or L configuration and following the Fmoc strategy; TBTU/HOBT were used throughout the synthesis. On a Gly functionalized Wang resin, two subsequent couplings of Fmoc-Lys(Fmoc)-OH were performed in order to introduce the tetradentate lysine core. Then, the following amino acids were coupled to the polylysine core: Fmoc-D-Tyr(OtBu)-OH, Fmoc-D-Thr(OtBu)-OH, Fmoc-D-Arg(Pbf)-OH. After completion of all synthesis steps, the resin was washed with DMF, methanol, and DCM and dried overnight in a vacuum. The peptide was cleaved from the solid support by treating the dry resin with a mixture of TFA/thioanisole/phenol/TIS (88:3:3:4.5:1.5 w/w) for 3 h. The crude peptide was precipitated with cold ethyl ether. The resulting precipitate was centrifuged at 3500 rpm (1534 g) for 15 min and the solvents discarded. After washing with fresh diethyl ether, the crude peptide was dissolved in water and lyophilized. The crude peptide was purified by reverse phase HPLC on a semipreparative Grace-Vydac peptide and protein C18 column.

2.2. X-ray Data. IgG from the anticancer drug Herceptin^{27,28} (gift from Genentech Inc., CA), which possesses a fully human sequence in the Fc fragment, was used in the crystallographic study. Protein was cleaved by papain²⁹ and purified on an affinity and gel filtration chromatography column. The dendrimer ligand was dissolved in ethanol to get 100 mM solution and added to 20 μ L of solution containing the Fc dimer at a 26 mg/mL concentration in a 20 mM Hepes buffer, pH 7.5, at the ligand to protein molar ratio 5:1. Crystals of the Fc dimer in complex with the dendrimer were grown using the hanging drop method at 18 °C under the following crystallization conditions: 10–20%

PEG 20K, 0.1 M MES pH 6.5. The crystals were in the shape of rods with an elongated hexagonal base with dimensions of 0.25 mm × 0.1 mm × 0.05 mm. Diffraction data were collected in EMBL/DESY Hamburg, beamline X-11; MAR CCD 165 mm. During diffraction measurements, the crystal was frozen in a stream of nitrogen at 100 K and 20% v/v R,R-2,3-butanediol in water was used as a cryoprotectant. Images were indexed, integrated, and scaled using the HKL2000 suite of programs.³⁰ The crystal structure of the Fc-dendrimer complex was solved in the $P2_12_12_1$ space group by molecular replacement using the Molrep program from the CCP4 package^{31,32} using the Fc fragment of the IgG PDB: 1HZH structure as a model.³³ The refinement of the crystal structure was then performed with Refmac5 with included TLS parameters.³⁴ Visualization of rebuilding was performed in the Coot 3-D graphic program according to the experimental electron density map.³⁵ The libraries of ligand and saccharide chains were prepared in Monomer Library Sketcher using the CCP4i and ProDrg programs.^{31,32,36} The position of the ligand was determined on the basis of differential electron density maps and occupancy by analysis of temperature factors. The crystal structure of the Fc-dendrimer complex is deposited in the PDB with accession code 3D6G. This crystal structure has one of the highest resolutions of the Fc fragment of IgG1 deposited in the PDB to date.

2.3. MD Simulations. The molecular dynamics (MD) simulation approach followed in order to model the dendrimer–protein complex consisted of a three-step procedure that can be summarized as follows:

(Step 1) MD simulation protocol with restraints imposed on the Fc receptor and on the dendrimer branch that binds in the BS1 site for a time span of 2 ns. This step is devoted to the relaxation of the globular conformation of the dendrimer determined from the X-ray crystal structure in the presence of water molecules.

(Step 2) Simulated annealing with 15 ps temperature spikes of 400 K followed by 100 ps of MD simulations at 300 K for a total simulation time of 2 ns performed with restraints imposed on the Fc receptor and on the dendrimer branch that binds in the BS1 site. This step is devoted to the search of the most favorable secondary binding sites performed without disrupting the primary binding site. No protein denaturation was observed.

(Step 3) Unrestrained MD simulation protocol in explicit water for a time span of 10 ns.

The MD simulation protocol employed in steps 1 and 3 consists of the following:

- (i) 2000 minimization cycles to remove any possible unfavorable contact between solvent and solute; in this step, the complex is restrained with a harmonic potential of the form $k(\Delta x)^2$, where Δx is the displacement and k the force constant ($k = 500 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$).
- (ii) 1500 cycles minimization without restraints.
- (iii) Simulated annealing of 20 ps at constant volume to raise the temperature of the system from 0 to 300 K; in this phase, a weak restraint is imposed on the complex ($k = 10 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$) to avoid wild structural fluctuations.
- (iv) 100 ps run at constant pressure (equilibration phase) to allow the water density to relax.
- (v) MD run for a standard period of 10 ns at constant pressure and temperature (300 K).

In order to determine interaction energies between dendrimer **2** and IgG, an additional simulation of dendrimer **2** solvated in water was performed adopting the same computational protocol described above, using as a starting point for the simulations the structure assumed by dendrimer **2** in the X-ray crystal. The total simulation time was 30 ns.

All of the simulations were performed in explicit water in a cubic box using a nonbonded cutoff of 12 Å adopting a simulation protocol tested in our previous studies of complexes between IgG and ligands.^{37,38} Periodic boundary conditions (PBCs) were applied, and long-range electrostatic interactions were evaluated using the particle mesh Ewald (PME) method. In all of the simulation steps, the temperature has been controlled with a Langevin dynamics algorithm, with a collision frequency of $g = 1 \text{ ps}^{-1}$. The pressure in the equilibration phase is controlled by means of a weak coupling Berendsen scheme. The SHAKE algorithm was used for all of the covalent bonds involving hydrogen, which allowed using a time step of 2.0 fs. The force field employed in all of the simulations is the AMBER ff03; the nonstandard LYX and CYX residue libraries have been built following the standard procedure described in the AMBER suite documentation, with atomic charges computed at the B3LYP/6-31 g(d,p) level and fitted using the RESP formalism and bond angle and dihedral parameters directly taken from the AMBER ff03 force field.³⁹ Structures to be used for successive analysis were extracted every 500 fs. The analysis of the trajectory has been carried out using the ANAL and MMPBSA programs provided in the AMBER 8 computational suite.⁴⁰

3. Results and Discussion

3.1. X-ray Structure. The crystal structure of the Fc–dendrimer **2** complex, reported in Figure 1A, clearly shows that one of the dendrimer arms binds close to the C γ 2/C γ 3 interface, though only in the vicinity of the consensus binding site. The dendrimer core is mostly positioned in proximity of the water channel between the C γ 2 and C γ 3 domains. Three arms of the dendrimer assume a branched conformation, with the charged groups creating single interactions with polar groups from symmetry related molecules. The fourth arm protrudes toward the protein surface and establishes strong interactions with the protein, among which are several hydrogen bonds. The crystal structure of the complex was determined in the P₂12₁ space group, with the Fc dimer and one ligand molecule in the independent unit of the crystal lattice. Different environments surround the two chains of the dimer, which explains why only a single interaction with a monomeric chain is observed. In Figure 1, the binding site of dendrimer **2** on the IgG1(Fc) surface is compared to the Fc-domain B of Protein A complex, PDB: 1L6X.⁴¹ This complex was determined in the C222₁ space group and contained one chain of the Fc dimer and one ligand molecule in the independent unit. For the purpose of comparison, it is shown as a dimer located on the crystallographic 2-fold axis in Figure 1B. Due to different space groups and crystal packing of both structures, the interactions between ligand and protein are not the same. As it is clearly displayed, the dendrimer partially hinders the binding site of Protein A, thus providing a first explanation of its affinity as well as of its inhibitory activity.²⁰ In closer details, the X-ray data evidence the presence of a specific interaction between protein amino acids Val308, Leu309, and His310 of the C γ 2 domain and the terminal Arg of the dendrimer arm. The intermolecular interactions are due to the formation of hydrogen bonds between the guanidinium group of Arg, the main chain backbone of Leu309, and the imidazole ring of His310. As shown below, this is the main

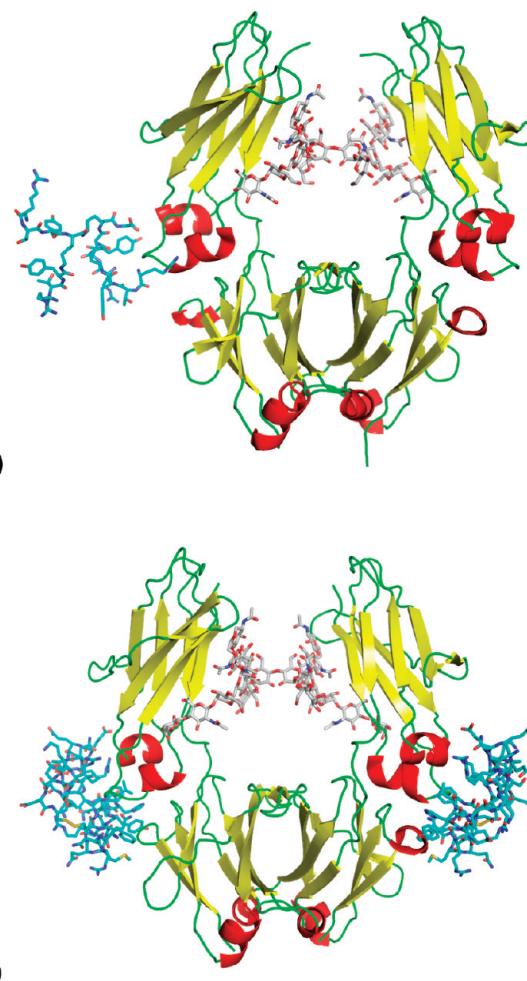
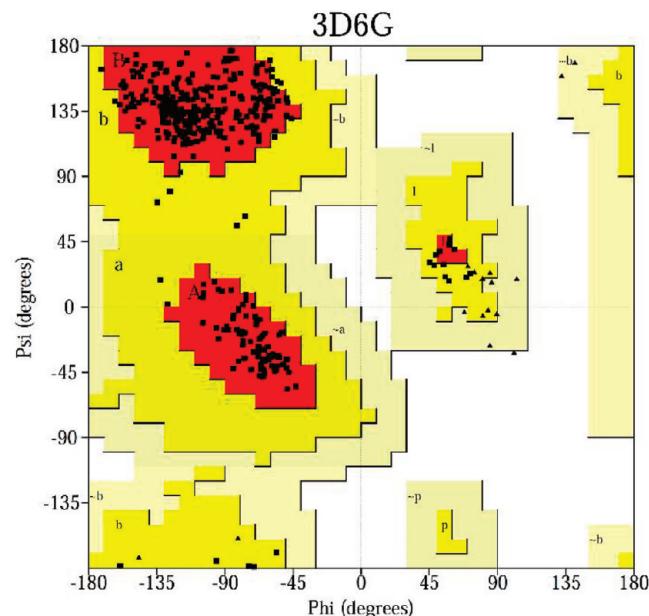


Figure 1. Crystal structures of the Fc fragment of human IgG in the form of the complex with dendrimer ligand (A) (PDB: 3D6G) and (B) with domain B of protein A (PDB: 1L6X)—the dimer is located on the crystallographic 2-fold axis.⁴¹

binding site of the dendrimer with IgG1(Fc) and was thus dubbed BS1. In addition, Ile253, which forms a hydrophobic knob with Ser254 and is known to be one of the key residues of the consensus binding site, is also in proximity of BS1. Interestingly enough, it has recently been observed, in a study of the physical–chemical properties of the consensus binding site, that the region of the human IgG (Fc) surface positioned in the C γ 2 domain near the 309-helix and the 280s loop is similar, in terms of hydrophobicity and solvent accessibility, to the consensus binding site, thus suggesting it has comparable ligand binding properties.²⁶ Both protein chains are glycosylated at Asn297, and the whole saccharide chains are very well visible in the electron density map. The saccharide chains from both monomers create hydrogen bonds between each other, which is responsible for the shape of the Fc dimer. The Ramachandran plot of the X-ray structure (Figure 2) proofs the proper conformation of all residues, since all amino acids are positioned in the favored and allowed regions. Moreover, all of the sugar moieties appear in the proper conformation. Additional statistic parameters, such as R , R_{free} , and rmsd, reported in Table 1, confirm the good refinement of the crystal structure.

3.2. Molecular Modeling. Several MD simulations were performed exploiting different simulated annealing protocols in which selected parts of the ligand were restrained to the X-ray structure. The simulation protocol was designed to help the system relax from the crystallographic pose and to establish



Plot statistics	
Residues in most favoured regions	342
Residues in additional allowed regions	23
Residues in generously allowed regions	0
Residues in disallowed regions	0
Number of non-glycine and non-proline residues	365
Number of end residues (excl. Gly and Pro)	1
Number of glycine residues (shown as triangles)	18
Number of proline residues	35
Total number of residues	419

Figure 2. Ramachandran plot of the crystal structure of the Fc-dendrimer ligand complex.

TABLE 1: Data Collection and Structure Refinement Statistics

data collection	
wavelength (Å)	0.8162
space group	$P_{2_1}2_1$
cell dimensions: a, b, c (Å), α, β, γ (deg)	49.78, 79.16, 139.22, 90, 90, 90
resolution (Å)	40.0–2.30 (2.38–2.30) ^a
unique reflections	25480 (2442)
completeness %	99.3 (97.5)
R_{merge}	0.064 (0.599)
redundancy	10.8 (5.3)
refinement statistics	
no. of reflections working/test set	24234/806
$R_{\text{work}}/R_{\text{free}}$ (%)	18.8/25.7
mean isotropic B value (Å ²)	72.42
no. of atoms	4002
- protein	3384
- dendrimer	152
- saccharides	198
- water	268
rms deviations bond lengths (Å)	0.021
rms deviations bond angles (deg)	1.860
Ramachandran plot	
most favored (%)	93.7
additionally allowed	6.3
generously allowed	0.0
PDB code	3D6G

^a Values in parentheses correspond to the highest resolution shell.

new interactions with the protein within a reasonable computational time, without losing the binding site conformation. Molecular dynamics simulations revealed that in the presence of water at 300 K the dendrimer abandons its globular conformation and one or, in some cases, even two of its arms

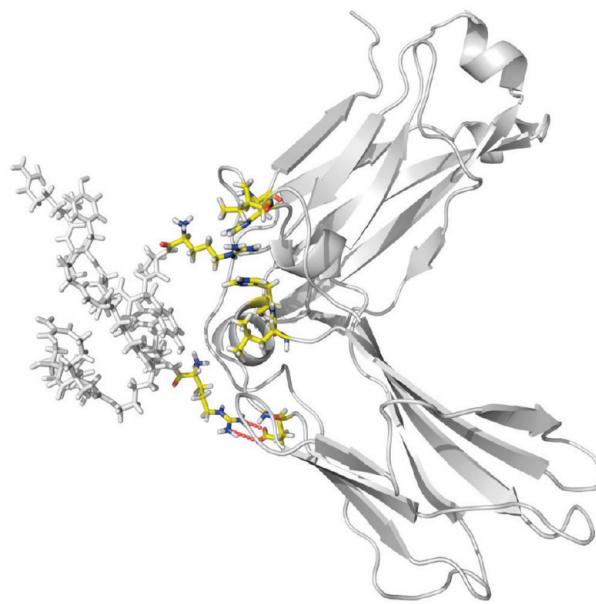


Figure 3. Structure of the dendrimer ligand in complex with the Fc fragment of human IgG (A) determined through MD simulations. The key protein and dendrimer amino acids are shown in yellow.

establish ionic interactions with negatively charged protein amino acids. In Figure 3, an ionic bond between an Arg guanidinium group and Glu318 is evidenced, which can be considered as the second interaction point of the dendrimer (BI2). Though once formed the BI2 ionic bond is maintained during the MD simulation time span, different simulation protocols lead to the formation of various ionic interactions, involving negatively charged protein residues and positively charged Arg dendrimer residues. Despite the variability of BI2, BS1 was always maintained in all simulations. The BS1 structure experiences a certain mobility, as during the simulations the Arg terminal residue slightly drifts away from the X-ray binding site toward a binding pocket defined by His310, Ile253, and His435. The change in the binding site structure, compared to the X-ray structure in Figure 4, can reasonably be attributed to the establishment of secondary interactions by part of the other arms of the dendrimer with the protein. The fact that the site is maintained and not lost is indirect evidence of its energetic stability. The identification of a secondary interaction with respect to BS1 opens up the question about why this interaction was not identified in the X-ray crystal structure. A first consideration is that the X-ray structure refers to a solid crystal in which a very small amount of water is present, located mainly in the channels between the C γ 2 and C γ 3 domains, while the system studied through MD simulations is uniformly solvated in water. A possible explanation is thus that, during the crystallization process, the secondary interactions, involving charged Arg groups and not as strong as the primary interaction, are disrupted with the progressive decrease of the water content in the hanging drop. In this situation, it is likely that the dendrimer arms involved in the BI2 bonds retract and coalesce in a globular structure, similar to that presented in Figure 1A, located in the position in which most of the remaining water is concentrated, i.e., in the intermolecular water channel. In the MD calculations, water is reinserted in the simulation domain, so that what we observe in our simulations is probably the inverse of the process that took place during crystallization. The possibility that this dendrimer arm movement actually takes place is in part confirmed a posteriori by the MD simulation results, which clearly show that the ionic bonds formed in the

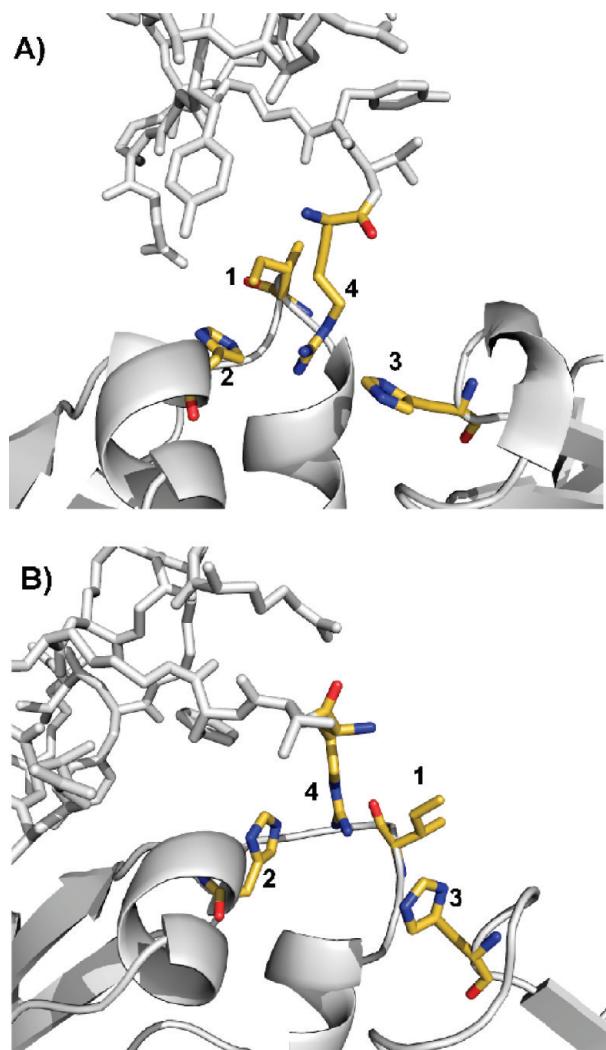


Figure 4. Comparison between the X-ray structure of the dendrimer binding site BS1 (A) and the one determined through MD simulations (B). The BS1 structural change involves a modification of the relative position of the dendrimer Arg (4) with respect to the hydrophobic pocket defined by His310 (2), His435 (3), and Ile253 (1).

BI2 interactions have a high mobility, as discussed more in detail below when commenting on different MD snapshots.

Three snapshots of the dendrimer–protein complex relaxed in solution are sketched in Figures 5–7. The structures reported have been obtained through different MD production runs, starting from slightly different guess structures derived with the employment of the simulated annealing protocol. These structures have been reported in order to point out that, while the BS1 site maintains a stable configuration in all of the MD simulations performed, the secondary arms of the dendrimer show a high mobility. This mobility results in the ability to form a variety of polar interactions with negatively charged Fc residues that produces a stabilization effect on the dendrimer–Fc complex. In these structures, both amino–carboxyl and guanidinium–carboxyl interactions have been found to contribute to the dendrimer–protein complex stabilization. In the figure description, the surface residues have been labeled with their residue number according to the absolute protein numeration, while the ligand residues have been labeled with progressive numbers as a function of the structure description.

In the complex shown in Figure 5, two guanidinium–carboxyl contacts are visible between Arg1 and Glu318 and between Arg2 and Asp280. It can be observed that the main binding site (BS1)

is maintained: the main dendrimer binding arm protrudes in the pocket between Ile253 and His310 interacting with the aliphatic portion of the Arg3 side chain.

In the complex reported in Figure 6, it is possible to observe the interaction between the amino group of the Arg2 amino acid backbone with the carboxyl group belonging to the Asp312 side chain and the guanidinium–carboxyl interaction between Arg1 and Glu283.

In the third structure, represented in Figure 7, a configuration characterized by the presence of one secondary binding arm has been reported, that exhibits a main carboxyl–guanidinium interaction involving the terminal Arg1 residue of dendrimer 2 and the Glu318 residue of IgG. This structure also shows the simultaneous capability of the same terminal Arg to form an interaction between its backbone amino group and the carboxyl group of the Asp312 residue. In this picture, it is also possible to observe the peculiar configuration of the main binding site (BS1), where the knob constituted by Gln311, adjacent to His310 and Ile253, interacts with both the Arg2 and the Tyr3 residues.

MD simulations allow one to determine the affinity of the ligand for the protein by measuring the change of electrostatic and van der Waals interaction energy between environment and dendrimer when it is in complex with IgG and that calculated through a different simulation of the single dendrimer 2 ligand performed in a pure water environment. The calculated energy changes for a fully equilibrated MD simulation are reported in Figure 8. The energetic analysis reveals that the complex binding energy is dominated by the increase of van der Waals interaction energies that takes place upon the formation of the complex, though also electrostatic interaction energies are globally negative and thus contribute to the complex stability. In interpreting the preponderance of van der Waals over electrostatic energies, it should be remarked that the energies reported in Figure 8 are energy changes and not absolute interaction energies, so that the small contribution of electrostatic energies to the complex stability must be taken to mean that the electrostatic energies of interaction of dendrimer 2 with IgG are similar to those that the ligand has with water when the protein is not present. From this standpoint, the change of structure of the peptide from the globular structure it assumes when dissolved in water to the extended structure it assumes when in complex with IgG can be attributed to the van der Waals interactions it establishes with the protein. Finally, in the analysis of Figure 8, it can be pointed out that the two minima have a precise physical meaning, as they are related to the formation and successive disruption of ionic bonds involving Arg residues of the dendrimer and charged groups of the protein.

4. Conclusions

The intelligent design of ligands able to bind selectively human IgG requires both a deep understanding of the molecular structure and accessibility of the antibody hot spots as well as viable synthetic routes of molecules with functional activity. In this framework, this study contributes to our understanding of the interaction between ligands and IgG in two ways.

First, it was shown that the high affinity of the dendrimer under investigation for IgG is determined by the establishment of multiple interactions by which a first dendrimer arm binds through hydrogen bonding and hydrophobic interactions to a specific protein site, while a second one forms ionic bonds with negatively charged protein amino acids. The binding of dendrimer 2 to IgG appears thus not to be just an effect of the high effective molarity of functional groups

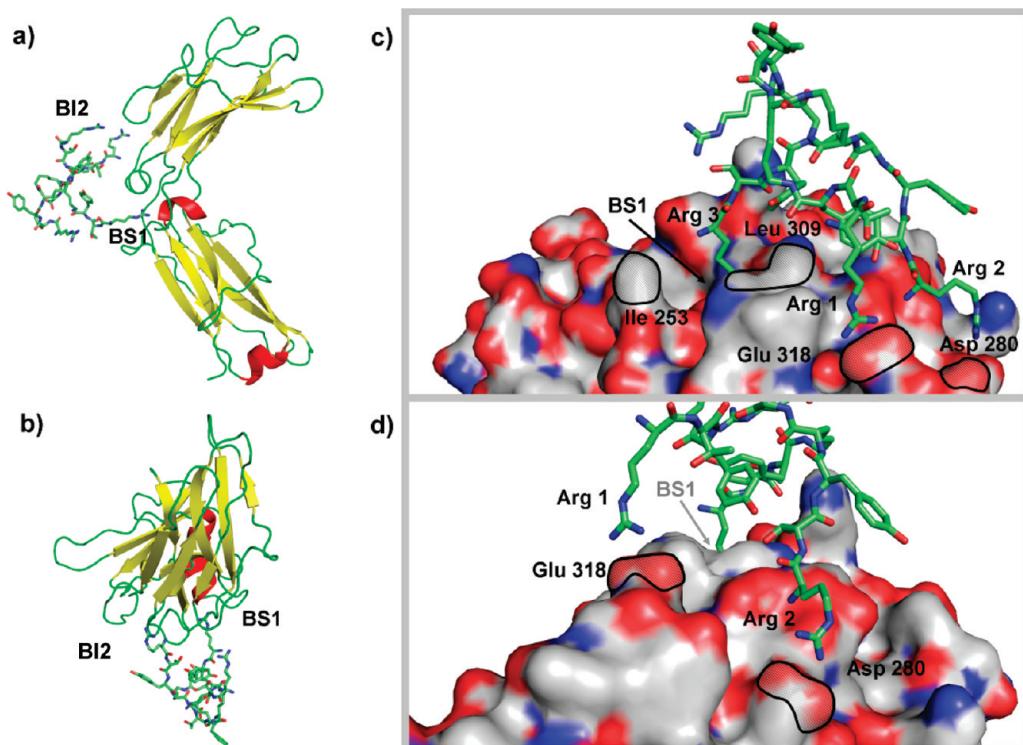


Figure 5. Complex 1: In this particular configuration, the dendrimer–Fc complex shows two secondary interactions due to two guanidinium–carboxyl hydrogen bonds. In this snapshot, the guanidinium groups of two dendrimer terminal Arg interact with Glu318 and Asp280. The Fc domain is represented as a surface, while the ligand has been depicted through a stick representation. The oxygen atoms are depicted in red, the nitrogen atoms in blue. For the sake of clarity, the carbon atoms have been depicted in gray in the receptor and in green in the ligand. The portions of the molecular surface that belong to the interacting residues have been marked with a black line.

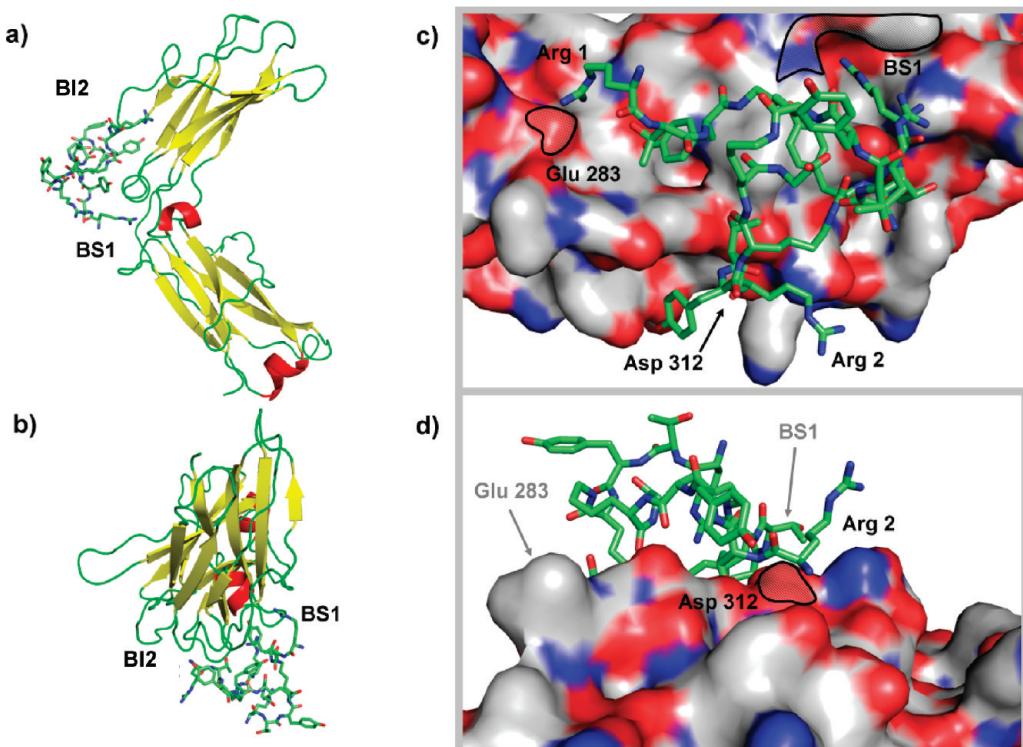


Figure 6. In this configuration, the dendrimer–Fc complex shows a double secondary interaction due to the formation of a guanidinium–carboxyl hydrogen bond between a dendrimer terminal Arg and Glu283. The second interaction is an amino–carboxyl bond formed by another terminal Arg with Asp312. In this picture, the Fc domain is represented as a surface, while the ligand has been depicted through a stick representation. The oxygen atoms are depicted in red, the nitrogen atoms in blue. For the sake of clarity, the carbon atoms have been depicted in gray in the receptor and in green in the ligand. The portions of the molecular surface that belong to the interacting residues have been marked with a black line.

competing for a single binding site but rather the result of synergistic binding events occurring in two different sites

of the protein surface. This remarkable property, despite the structural simplicity of **2**, is certainly the result of the built-

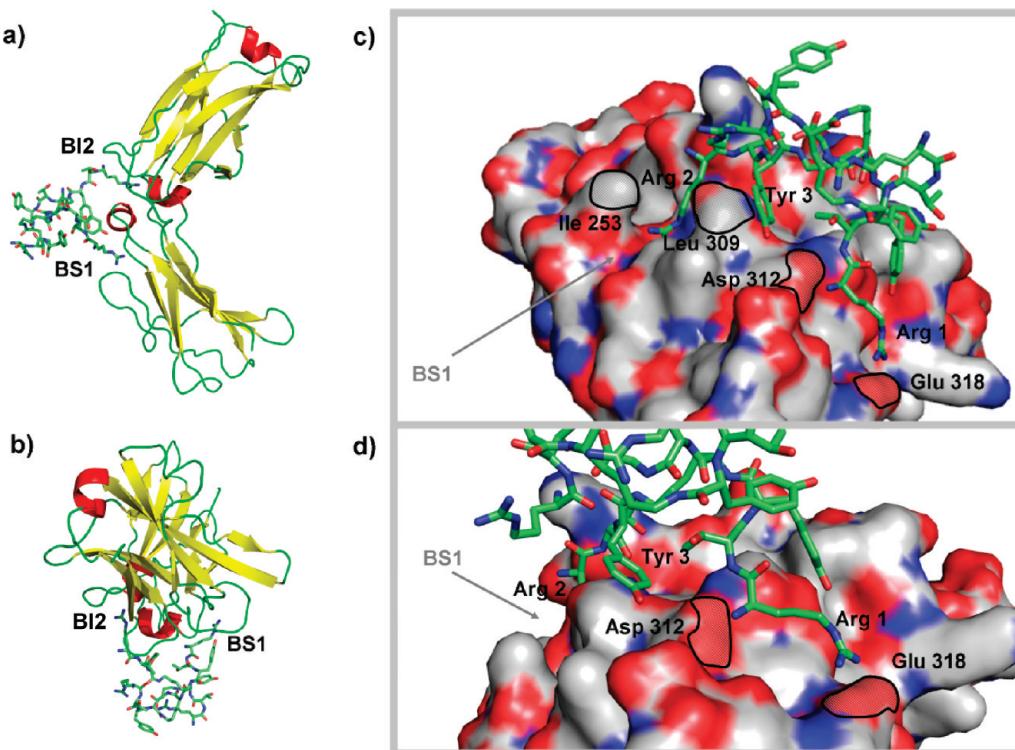


Figure 7. A single secondary interaction is shown in this picture: the Arg1 residue shows a major interaction between its guanidinium group and Glu318 and also the propensity to interact through its amino group with Asp312. In this picture, the Fc domain is represented as a surface, while the ligand has been depicted through a stick representation. The oxygen atoms are depicted in red, the nitrogen atoms in blue. For the sake of clarity, the carbon atoms have been depicted in gray in the receptor and in green in the ligand. The portions of the molecular surface that belong to the interacting residues have been marked with a black line.

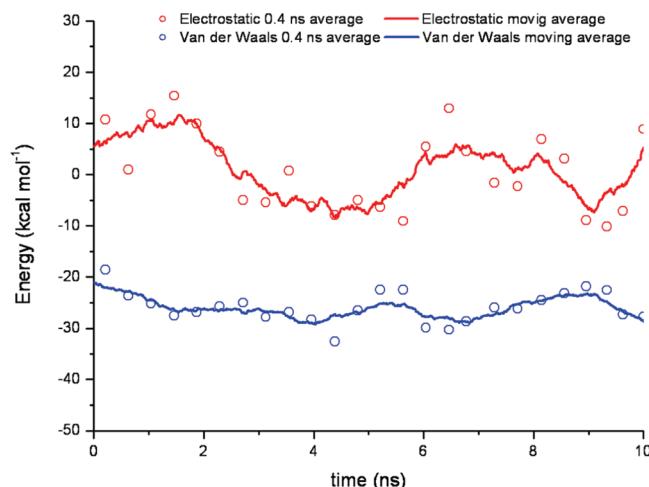


Figure 8. Time evolution of electrostatic and van der Waals relative energy changes between environment and ligand ($\Delta\Delta E$, kcal/mol) of the dendrimer when it is in complex with IgG and when it is in a pure water solution.

in selection criteria of combinatorial approach followed in the development of the molecule.

A second important result of this study is the identification of a new IgG binding site, located in the proximity but not overlapped with the consensus binding site, which provides important information that can be exploited to design synthetic ligands, with improved affinity and selectivity for IgG and

greater potential to treat diseases where IgG/Fc γ interaction plays a major role.⁴²

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