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A MOLECULAR DYNAMICS STUDY OF THE CORRELATIONS BETWEEN SOLVENT-ACCESSIBLE SURFACE, MOLECULAR VOLUME AND FOLDING STATE

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Abstract

We analyzed the correlations between molecular volume, solvent-accessible surface and folding state (secondary structure content) for unfolded conformers of alpha (holo and apo Myoglobin) and beta (Retinol Binding Protein) proteins, and a small water-soluble alanine-rich alpha helical peptide. Conformers with different degrees of folding were obtained using Molecular Dynamics at constant Temperature and Pressure with implicit solvent (dielectric constant adjustment) for all four systems, and with explicit solvent for the single helix peptide. Our results support the view that unfolded conformations are not necessary extended, that volume variation is not a good indication of folding state and that the simple model of water penetrating the interior of the protein does not explain the increase in volume upon unfolding.

Key-words. Protein folding, unfolding, pressure, volume, solvent accessibility, myoglobin, retinol binding protein, alanine helix, molecular dynamics.

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1. Introduction

The characterization of unfolded states has been an important topic in protein folding research[1-5]. The early view of unfolded states as extended polypeptide chains has changed to a more complex picture. The current view of an unfolded state is an ensemble of partially folded conformers of the protein, where the extent of unfolding depends on the denaturing conditions[3-11].

An important physico-chemical characteristic of an unfolded state is its volume. The changes in protein volume upon unfolding are normally small. It has been found experimentally that the volume changes on denaturation of proteins are usually less than 1% of the native volume[12-13]. Consequently, experimental determinations of volume changes are very sensitive to errors in volume measurements/estimates. For this reason, any attempts at studying the volume changes during unfolding must be done in such way that the volumes of the conformers to be compared are consistently calculated. Also, to minimize errors, it is better to have a series of intermediate volumes between the initial (native) and final (unfolded) states. Experimentally this is a though task, especially because only the native and the unfolded volumes can normally be measured. However, this is easily achieved using Molecular Dynamics (MD) calculations.

The effects of hydrostatic pressure on protein structure are important because of applications in food sterilization and control of enzymatic reactions. Pressure generally induces a decrease in the volume of the protein[13-17]. The volume change upon pressure-induced unfolding is usually positive at low pressure but negative at pressures above 100-200MPa. This behavior is the opposite of what is expected if the model of stabilization of

structure by hydrophobic interactions is accepted[18]. According to this model, the denaturation process, which is characterized by the exposure of many non-polar groups to the solvent, should resemble the simple model of a non-polar molecule being transferred from a non-polar environment (the protein core) into a polar one (water). This is true for some thermal unfolding data, but this simple model does not explain the behavior of the protein volume during pressure-induced unfolding. Compression in pressure-induced unfolding, and expansion in native to molten globule transition induced by different denaturing agents[19] have been explained as resulting from water being forced into the interior of the protein. However, all the proposed explanations for the volume behavior during unfolding are still controversial[6,13,20].

In this work, we used MD at Constant Pressure and Temperature (NPT) to generate conformers with various degrees of folding (ranging from native to partially folded and unfolded) for the holo and the apo form of Myoglobin (an alpha protein), the Retinol Binding Protein (a beta protein), and a short alanine-rich water-soluble peptide. The folding state of the conformers was characterized in terms of remaining secondary structures relative to the native conformation. The correlation between folding state molecular volume and solvent-accessible surfaces was then investigated. For the alanine-rich alpha-helical peptide, simulations were performed using both implicit and explicit solvent approaches.

2. Method

We performed a series of NPT MD simulations at 300K for the apo and the holo forms of Myoglobin (ApoMb and HoloMb, respectively), the Retinol Binding Protein (RBP) and a small alpha helical alaninine-rich peptide (AK helix). We used the available three-dimensional structures (3D) for Myoglobin (PDB code 4mbn; crystallographic resolution of 2 Å) and RBP (PDB code 1rbp; crystallographic resolution of 2.0Å) as starting conformation. The 3D structure of apomyoglobin has *not* been experimentally determined. Therefore, we started with the set of coordinates from the X-ray crystal structure of the holo form with the heme group deleted. The initial coordinates were minimized using periodic boundary conditions (PBC). The cell parameters were also minimized. Simulations were performed using the AMBER[21] force field (FF) modified to include terms explicitly describing hydrogen bonds (HB).

The simulation box was allowed to change its shape but the cell angles were kept fixed at 90°. We used an implicit solvent description with the dielectric constant based on the corresponding values for water at half of the pressure and at the temperature of interest (300 K). The dielectric constant was *not* taken as distance-dependent. This approach was validated by our previous results of NPT MD simulations for holomyoglobin[22]. The pressure dependence of the dielectric constant of water at constant temperature was estimated[23] using a modification to the Bradley and Pitzer equation[24] adjusted to fit experimental values[25].

We used a dynamics time step of 0.001 ps, a temperature relaxation time of 0.01 ps, and the Rahman-Parrinello mass term equal 0.1. The system was weakly coupled to a thermal bath[26].

We considered values of external hydrostatic pressures between 1 atm and 1.0 GPa. For each simulation, the pressure was increased from the previous value in steps of 25 MPa/10 ps, until the desired pressure was reached. At the desired pressure, 100 ps of dynamics was performed. The final conformation obtained at each pressure was used as the input to the simulation at the next higher pressure.

The non-bonded interactions were calculated using spline cutoffs (R_{cut} = 9 Å). The degree of protonation was based on pH 7. Thus, glutamic and aspartic acids were negatively charged, lysine and arginine were positively charged, and histidines were neutral. To neutralize charges we included a Na^+ counter ion for each Glu and Asp and a Cl^- counter ion for each Lys and Arg, using a program developed by Nagarajan[27].

To evaluate the differences between implicit and explicit solvent NPT-MD calculations, we simulated a small soluble system, at 300 K, using the same pressure interval and the same simulation parameters used for the proteins. We chose the single alpha helical peptide Ac-AAAAKAAAAKAAAAKA-NH2 which is soluble in water and has 80% of helical content[28]. The helix was solvated in a pre-equilibrated box of 447 water molecules.

3. Results and Discussion

The effect of the pressure on the conformation and stability of the systems studied was analyzed in terms of variation of volume, percentage of remaining secondary structures and solvent-accessible surfaced area.

Tables 1 and 2 show dipole moments (DP), hydrophilic residues solvent-accessible surface (HISA), hydrophobic residues solvent-accessible surface (HOSA), total solvent-accessible surface (TSAS), estimated volumes (TEV) and percentage of remaining secondary structures (%RSS) calculated for the MD conformers of apo and holo Myoglobin, Retinol Binding protein (Table 1), and for AK helix (Table 2). The percentage of molecular volume variation as a function of pressure relative to the 0.1 MPa (native) conformation is shown in Figure 1 for ApoMb, HoloMb, RBP and for AK helix using implicit and explicit solvent approaches. Figure 2 shows the correlation between volume and percentage of remaining secondary structure, while Figure 3 shows the correlation between volume and solvent-accessible surfaces for the pressure-induced conformers. Figure 4 shows the correlation between solvent-accessible surface, secondary structure content and molecular volume.

Volume variation.

We used the last MD conformer at each pressure to represent the folding state of the system at that pressure, assuming that the MD was long enough for the properties of a conformer to be close to the overall properties of the ensemble. The volumes of the

conformers were calculated using Connolly's procedure[29-30]. The calculated volumes are not precise estimates of the absolute value of the volumes of the protein under pressure. Nonetheless, they can be compared to each other to generate an overall picture of the volume variation among conformers with different degrees of folding.

The estimated volumes for the conformers of the holo form of myoglobin (an alpha helical protein) under pressure were obtained from previous calculations[22]. The total estimated volumes (TEV) calculated for the MD conformers of apo and holo Myoglobin, Retinol Binding protein are shown in Table 1, and in Table 2 for the AK helix. The percentage of molecular volume variation as a function of pressure relative to the 0.1 MPa (native) conformation is shown in Figure 1 for ApoMb, HoloMb, RBP and for AK helix using implicit and explicit solvent approaches. The Apomyoglobin volume decreases with pressure until 300 MPa. Between 400 MPa and 600 MPa, the volume increases as the unfolding progresses, until the protein reaches a volume 4% above its initial volume, at 600 MPa. Above 600 MPa, the volume starts to decrease again. From 800 MPa to 1.0 GPa, the protein is compressed to its smaller volumes, around 4% below the volume at 1atm. The volume of the holomyoglobin exhibits a different behavior. It remains below its volume at 1atm during the entire range of pressures (from 1atm to 1.2 GPa). The holomyoglobin is initially compressed to 94 % of its volume at 0.1 MPa (1 atm) until 400 MPa, while still in a native-like state. As the pressure is further increased, the volume of the protein starts to expand until it reaches a molten globule state at 700 MPa[22]. The holomyoglobin molten globule has a volume less than 1 % smaller than the volume of the unfolded state at 1.0 GPa.

The volume variation for RBP (a beta barrel protein) is similar to that of apo myoglobin. This protein has its volume gradually reduced between 10 MPa and 300 MPa (4 % smaller than at 0.1MPa). Rbp expands again between 400 MPa and 600 MPa, and the volumes at 500 MPa and 600 MPa are higher than at 0.1 MPa. From the percentages of remaining secondary structures (%RSS) shown in Table 1, for both systems, the expansion starts when approximately 80 % of the initial secondary structure content is already lost. The expanded conformers with 17 % or less of remaining secondary structures present a volume higher than the native structure.

The general behavior that can be drawn from these three systems simulations is that the native and native-like conformations are compressible while retaining most of their secondary structures, but the systems expand as they lose organization. After losing 20%-30% of their initial secondary structure content, the three systems have a volume that is 2%-4% smaller than the native (0.1 MPa) state. The solvent accessible surfaces (total (TSAS), hydrophilic residues (HISA), and hydrophobic residues (HOSA)), also shown in table 1, follow a similar decrease-increase trend, with their values being always smaller than those of the native ones after 20%-30% of the secondary structure content is lost. Because there is no explicit water filling the voids during the dynamics, the compressibility of the systems and/or the onset pressures are not expected to correspond to experimental values. However, the simulated structural behavior at high pressures should correlate to experiments.

The main difference between the AK helix MD simulations with and without explicit solvent is the degree of unfolding. The helix unravels much easier with implicit solvent which may be an artifact of the implicit solvent approximation. However, in both

cases we reach very similar values for solvent-accessible surfaces and volume after 25% (71% and 78% RSS column in Table 2) of the secondary structure content is lost. The estimated volume at 0.1 MPa is higher for the explicit water case, which highlights that the helix is less compressible in explicit water. The total solvent-accessible surface (TSAS) and the solvent-accessible surface of the hydrophilic residues (HISA), also shown in Table 2, are larger for the explicit water at 0.1 MPa, reflecting the interactions with the explicit water molecules.

The results for the AK helix indicate, at least for small peptides, the influence of the explicit water molecules on the degree of folding of the peptide. A similar conclusion has been reached[31] in MD studies of small peptides at 1 atm of pressure. However, one must keep in mind is that neither implicit nor explicit solvent current models may actually reproduce correctly the role of the solvent in pressure-induced unfolding. Water models regularly used in MD simulations were developed to reproduce water properties at regular ranges of temperature and pressure. It has already been suggested that some popular water models fail in describing water properties at extreme conditions[32-33] (high temperatures and pressures). Since water properties such as viscosity and compressibility are very likely to play a role in the protein unfolding, inclusion of water in the simulations without a model that correctly predicts its properties at extreme conditions may lead to results that are as approximated as implicit solvent models.

Overall, the volume variations for all four systems were less than 6 %, and all the systems (including the explicit water AK helix) showed a decrease in volume at high pressure, consistent with experimental results[19].

Volume and folding state.

From the results shown in Table 2 and Figure 2, the simulations using implicit and explicit solvent for the AK helix seem to indicate that variations of the protein volume with pressure alone cannot be taken as an indication of the folding state of the protein. The single helix exhibits very compact but yet folded states (as indicated by secondary structure content) in explicit water, while some unfolded conformers from the implicit solvent calculations are also very compact.

A major problem in estimating volume variations upon unfolding has been the description of the unfolded state[34-35]. Most of the studies on this topic had considered a complete extended model for the unfolded state. As a result of this approximation, the estimated partial volumes are always higher than the experimental values[34]. It can be argued whether implicit solvent calculations lead to realistic folding states. Nonetheless the unfolded (low content of secondary structures) conformers obtained by implicit solvent MD simulations are certainly better representations of the unfolded state of the protein than the simple extended model normally used. In spite of the fact that approximated force fields are used in the MD simulations, the resulting conformers arise naturally from the forces being applied to the system. Also, because the conformer corresponding to the previous pressure is used as initial conformation for the next point, consecutive NPT-MD conformers are correlated and so are their properties. Thus, volume variations reveal that even in the absence of explicit water molecules the volume can still increase from one pressure-induced conformer to the next. This result seems to support the idea that the increase in volume upon unfolding is not caused by water penetrating the interior of the protein[1,5], a process that cannot be described in implicit solvent

calculations. On the other hand, if water penetrating the interior of the protein is not the process responsible for the unfolding, the question of what drives unfolding remains unanswered.

Solvent –accessible surface and folding state.

Figure 4 shows the correlation between solvent-accessible surface area (SAS), percentage of remaining secondary structure (%RSS) and volume variation (%ΔV) for the systems simulated in this work. The isolated single helix exhibits the smallest change in SAS in both implicit and explicit solvent simulations. Assuming that less than 50% of remaining secondary structure characterizes an unfolded state, we see that the SAS values decrease with pressure for folded conformers and tend to increase as the percentage of remaining secondary structure decreases. After the system is unfolded, an increase in pressure will cause a decrease in the SAS. Excluding the native state, the lower SAS value corresponds to the more compressed conformer in all the cases studied. These highly compressed conformers retain around 30 % of the initial secondary structure content.

Our results confirm that a high SAS value is a good indication of an unfolded state as these states do present higher SAS values when compared to the native one. However, it is possible to have configurations of the polypeptide chain with almost no secondary structure left that present SAS values comparable to the native state (as indicated by the SAS values for some of the unfolded conformers of ApoMb).

Role of solvent on estimated volumes from MD.

Recently, we published a set of molecular dynamics simulations (MD) at constant temperature and pressure (TPN) for the unfolding of metmyoglobin[22]. These simulations described the pressure-induced unfolding pathway through a series of MD conformers, one of then being identified as a molten globule intermediate. A good agreement between experimental and theoretical data was reached in that work. Those results suggest that the driving force for the pressure unfolding is the hydrogen bond migration (a main-chain effect), while the hydrophobic effects regulate the side chain interactions that are responsible for the solvent exposure, volume and tertiary packing. The interactions between water and non-polar groups were not explicitly represented in that work (which used implicit solvent). Thus, the volume changes observed in the TPN MD unfolding pathway under pressure should reflect the lack of explicit hydrophobic interactions between protein and solvent. By comparing the volume changes in MD simulation with implicit solvent to experimental data, and to MD simulation data with explicit solvent, it is possible to determine the role of water in the protein's volume behavior under pressure. Since MD calculations with explicit solvent are time-consuming, we chose to perform that comparison using a small water-soluble alanine-rich helical peptide. Both implicit and explicit solvent calculations for AK helix show an increase in volume at low pressure (10 MPa) and a decrease in volume at intermediate pressure (500 MPa to 700 MPa). The volume at 0.1 MPa is higher for the explicit solvent case reflecting the influence of water molecules in the molecular volume of the protein. The differences between the two curves may reflect water/protein interaction and degree of unfolding. The implicit solvent

case induces higher unfolding than the explicit case (which keeps at least 70 % of secondary structure during the simulations).

The effect of pressure on pancreatic trypsin inhibitor (BPTI) was studied experimentally between 1 and 2,000 bar using amide N-15 chemical shifts[36]. This study suggests that the pressure-induced structural changes are larger in the helical and loop regions than in the beta-sheet. The authors correlate pressure-induced structural changes to the local compressibility and, because the compressibility can be related with volume fluctuation, their result is taken as an indication that the volume fluctuation is larger in helical and loop regions than in beta-sheet. Comparing the volume variations for the helical proteins and the beta protein, one can see that in both cases the maximum compression is about 4 % of the initial volume, and there is no significant difference between their behavior.

4. Conclusions

Whether MD with implicit solvent can realistically describe the pressure-induced unfolding pathway, or a single set of calculation is appropriate to represent an ensemble of conformers (which is the current view of an unfolded state) is admittedly arguable. However, there is no doubt that the conformers obtained using MD represent different folding states that a particular system can assume, regardless of what conditions would led to them experimentally. The analysis of partially folded and unfolded conformers presented here shows that these conformers do not need to be extended and they can have different degrees of organized structures. Conformers with almost no secondary

structures can have very compact volume and present solvent-accessible surfaces comparable to a native conformation. Conformers with low secondary structure content consistently present higher solvent accessibility compared to the native state. This leads to the conclusion that a high value of solvent-accessible surface is a good indication of unfolding, but values comparable to the native protein do not imply a folded state. Volume variation cannot be directly correlated to degree of folding as the protein can assume very compact forms that do not contain organized (secondary) structures.

One proposed model for pressure unfolding[19,37] supposes that changes in the water properties induced by pressure facilitate the incorporation of water molecules into the hydrophobic core of the protein. As a result of water penetration, the protein swells and consequently unfolds. There is no doubt that interactions with water will determine the evolution of the unfolding with pressure. However if the unfolding was solely driven by water penetration in the protein's core, implicit solvent NPT simulations would not necessary lead to different degrees of unfolding at different pressures for different systems. All systems were structurally stable at 1atm NPT simulation and a dielectric constant adjusted according to pressure was the only water property included in the calculation. Nonetheless that was enough to induce unfolding at high pressure in the simulation.

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Captions

Table 1. Dipole moments (DP), hydrophilic (HISA) and hydrophobic (HOSA) residues solvent-accessible surface, total solvent-accessible surface (TSAS), estimated volumes (TEV) and percentage of remaining secondary structures (%RSS) for apo and holo Myoglobin (ApoMb and HoloMb, respectively) and Retinol Binding Protein (RBP). Properties estimated for conformers obtained by NPT MD at 300 K.

Table 2. Dipole moments (DP), hydrophilic residues solvent-accessible surface (HISA), hydrophobic residues solvent-accessible surface (HOSA), total solvent-accessible surface (TSAS), estimated volumes (TEV) and percentage of remaining secondary structures (%RSS) for AK helix. Properties estimated for conformers obtained by NPT-MD with implicit and explicit solvent (water) at 300K and pressures (P) from 0.1.MPa to 1.2 GPa.

Figure 1. Predicted Percentage of volume variation (ΔVolume %) as a function of pressure for apo and holo Myoglobin (ApoMb and HoloMb, respectively), Retinol Binding Protein (RBP), and AK helix.

Figure 2. Percentage of remaining secondary structure (%RSS) as a function of volume for apo and holo Myoglobin (ApoMb and HoloMb, respectively), Retinol Binding

Protein (RBP), and AK helix implicit and explicit solvent calculation (AK helix implicit and AK helix explicit).

Figure 3. Correlation between volume and solvent-accessible surfaces for the pressure-induced conformers of apo and holo Myoglobin (ApoMb and HoloMb, respectively), Retinol Binding Protein (RBP), and AK helix.

Figure 4. Correlation between solvent-accessible surface area (SAS), percentage of remaining secondary structures (%RSS) and volume variation (%ΔV) for a) apo and holomyoglobin (apoMB and holoMb), b) Retinol Binding Protein (RBP) and c) AK helix implicit and explicit solvent calculation (AK helix imp and AK helix exp).

Table 1. Dipole moments (DP), hydrophilic (HISA) and hydrophobic (HOSA) residues solvent-accessible surface, total solvent-accessible surface (TSAS), estimated volumes (TEV) and percentage of remaining secondary structures (%RSS) for apo and holo Myoglobin (ApoMb and HoloMb, respectively) and Retinol Binding Protein (RBP). Properties estimated for conformers obtained by NPT MD at 300 K.

P(MPa)	DP (Debye)	HISA (Å^2)	HOSA (Å^2)	TSAS (Å^2)	TEV (Å^3)	%RSS
ApoMb						
0.1	325.8	2497	4164	6662	25669	100
10	276.8	2566	4149	6714	25708	94
100	223.6	2427	4024	6450	25365	89
200	312.2	2489	3969	6458	25144	73
300	321.8	2460	3817	6277	25120	39
400	271.8	2601	4059	6661	25257	32
500	330.2	3185	4516	7701	26462	17
600	485.6	3290	4949	8239	26777	0
700	420.1	3005	4590	7595	25899	0
800	321.6	2538	3997	6535	24712	3
900	226.2	2525	4049	6574	24766	3
1000	176.1	2529	4178	6707	24775	3
HoloMb						
0.1	181.6	2856	4152	7009	26806	100
10	178.2	2667	3876	6543	26302	91
100	179.7	2612	3939	6551	25950	94
200	126.1	2339	3835	6174	25588	95
300						
400	154.9	2498	3729	6227	25263	79

500						
600	157.4	2695	3882	6577	25611	70
700	167.2	2749	4123	6872	26010	58
800	213.6	2886	4173	7060	25937	51
900						
1000	156.7	2900	4181	7082	26136	37
1200	229.2	3431	4366	7796	26702	32
RBP						
0.1	419.7	3441	4073	7514	29885	100
10	400.0	3543	4064	7608	29819	100
100	484.0	3474	3983	7457	29481	87
200	478.9	3146	3824	6970	28854	84
300	523.1	3071	3875	6946	28658	78
400	435.9	3372	4148	7520	29148	19
500	304.4	3444	5036	8480	30079	17
600	423.7	4380	4998	9378	31026	10

Table 2. Dipole moments (DP), hydrophilic residues solvent-accessible surface (HISA), hydrophobic residues solvent-accessible surface (HOSA), total solvent-accessible surface (TSAS), estimated volumes (TEV) and percentage of remaining secondary structures (%RSS) for AK helix. Properties estimated for conformers obtained by NPT-MD with implicit and explicit solvent (water) at 300K and pressures (P) from 0.1.MPa to 1.2 GPa.

P	a) AK helix implicit solvent calculation						b) AK helix explicit solvent calculation					
(MPa)	DP (Debye)	HISA (Å^2)	HOSA (Å^2)	TSAS (Å^2)	TEV (Å^3)	%RSS	DP (Debye)	HISA (Å^2)	HOSA (Å^2)	TSAS (Å^2)	TEV (Å^3)	%RSS
0.1	48.45	387	948	1335	2654	100	36.10	518	902	1420	2745	100
10	52.46	448	899	1346	2668	78	19.88	493	958	1451	2760	93
100	56.90	440	902	1342	2686	28	28.00	493	936	1429	2728	100
200	30.64	438	901	1339	2681	57	34.21	491	923	1414	2747	100
300	35.13	526	925	1452	2780	57	33.54	475	917	1392	2706	100
400	37.42	506	853	1359	2729	0	22.31	467	906	1373	2685	100
500	36.26	438	869	1307	2672	21[a)]	30.25	463	930	1392	2715	93

600	19.50	467	798	1265	2631	50	19.86	469	902	1371	2700	71
700	51.22	457	796	1253	2600	57[a)]	18.08	474	884	1358	2682	71
800	29.91	469	863	1332	2674	36[b)]	11.62	481	839	1320	2645	71
900	25.92	464	818	1282	2621	28	20.08	467	847	1314	2635	100
1000	22.65	419	891	1310	2658	28	36.13	412	898	1310	2625	100

[a)]helix 3_{10} [b)]helix π

Figure 1. Predicted Percentage of volume variation (ΔVolume %) as a function of pressure for apo and holo Myoglobin (ApoMb and HoloMb, respectively), Retinol Binding Protein (RBP), and AK helix implicit and explicit solvent calculation (AK helix implicit and AK helix explicit).

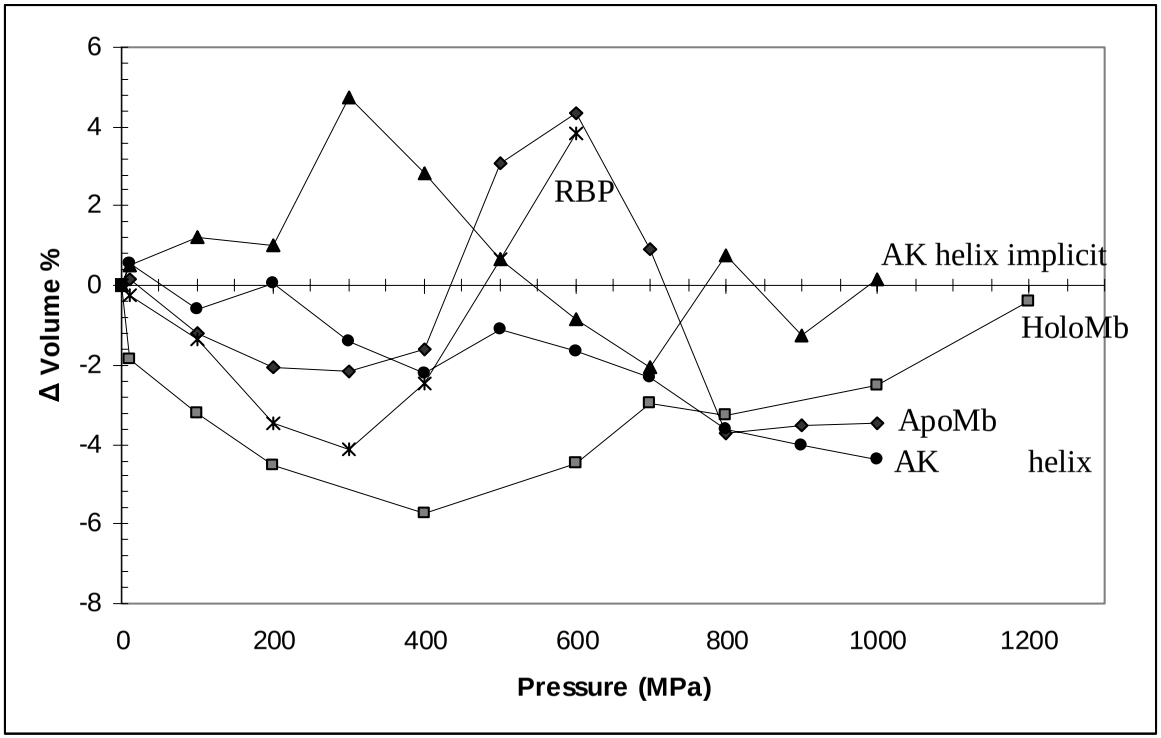


Figure 2. Percentage of remaining secondary structure (%RSS) as a function of volume for apo and holo Myoglobin (ApoMb and HoloMb, respectively), Retinol Binding Protein (RBP), and AK helix implicit and explicit solvent calculation (AK helix implicit and AK helix explici

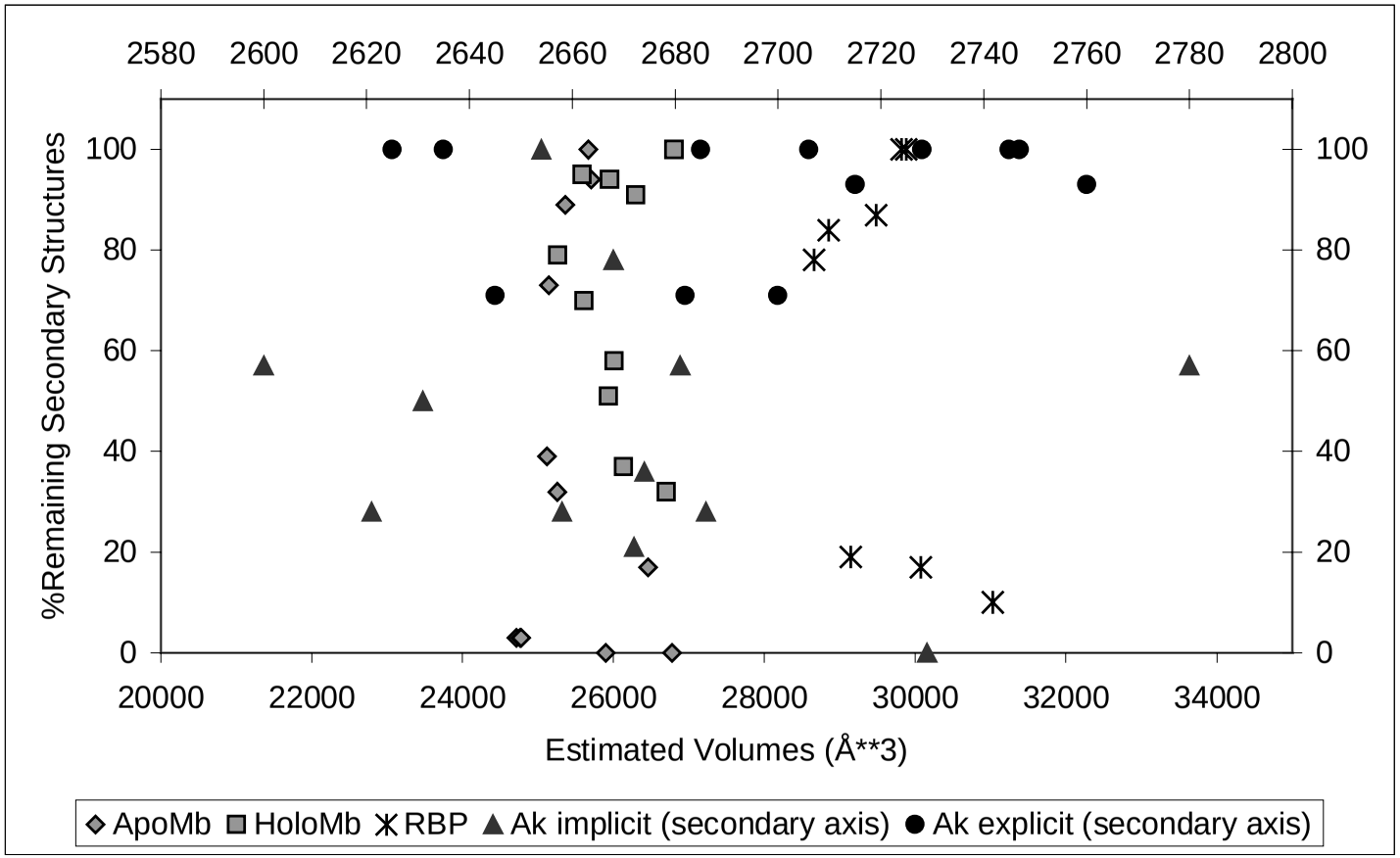


Figure 3. Correlation between volume and solvent-accessible surfaces for the pressure-induced conformers of apo and holo Myoglobin (ApoMb and HoloMb, respectively), Retinol Binding Protein (RBP), and AK helix.

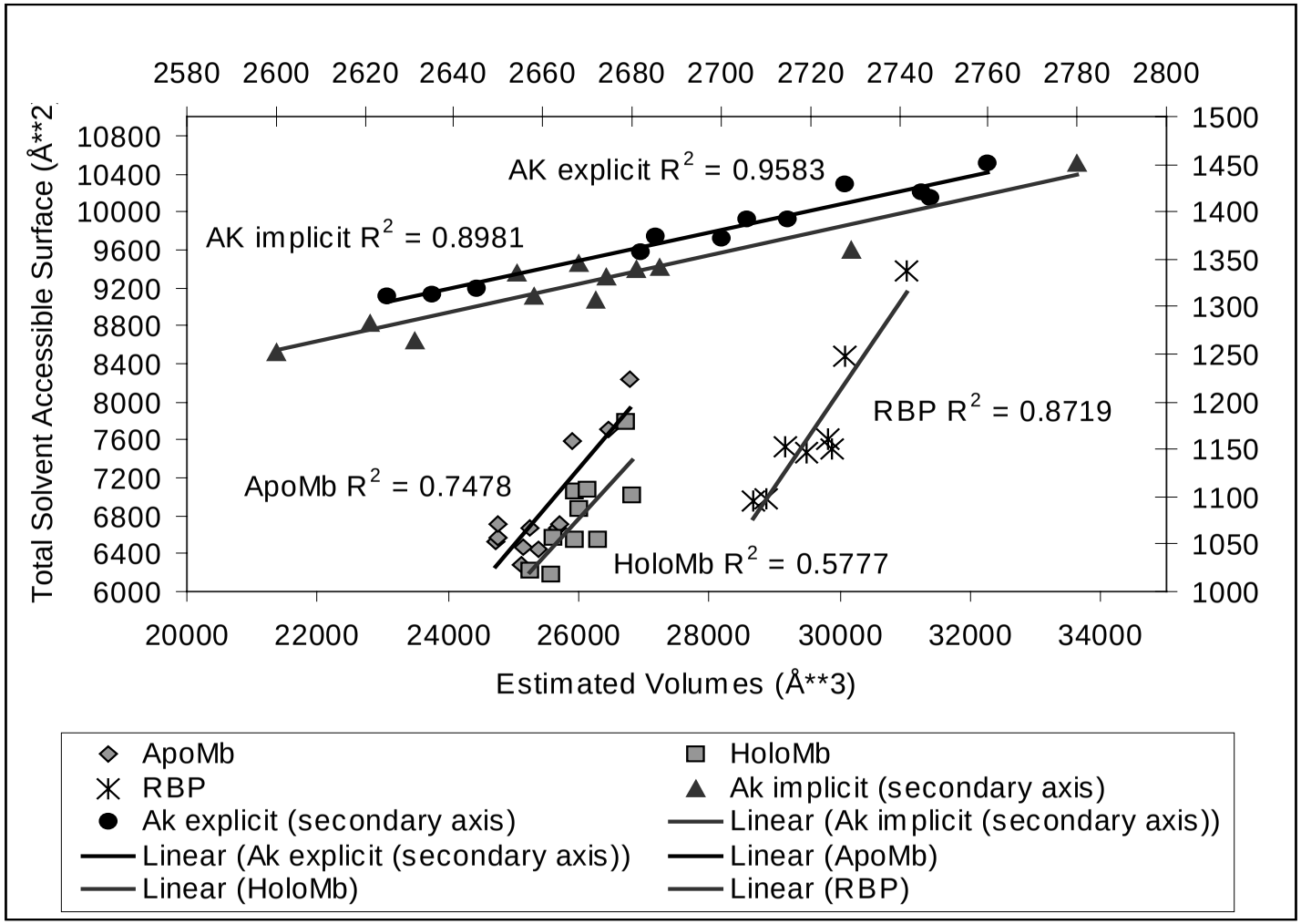
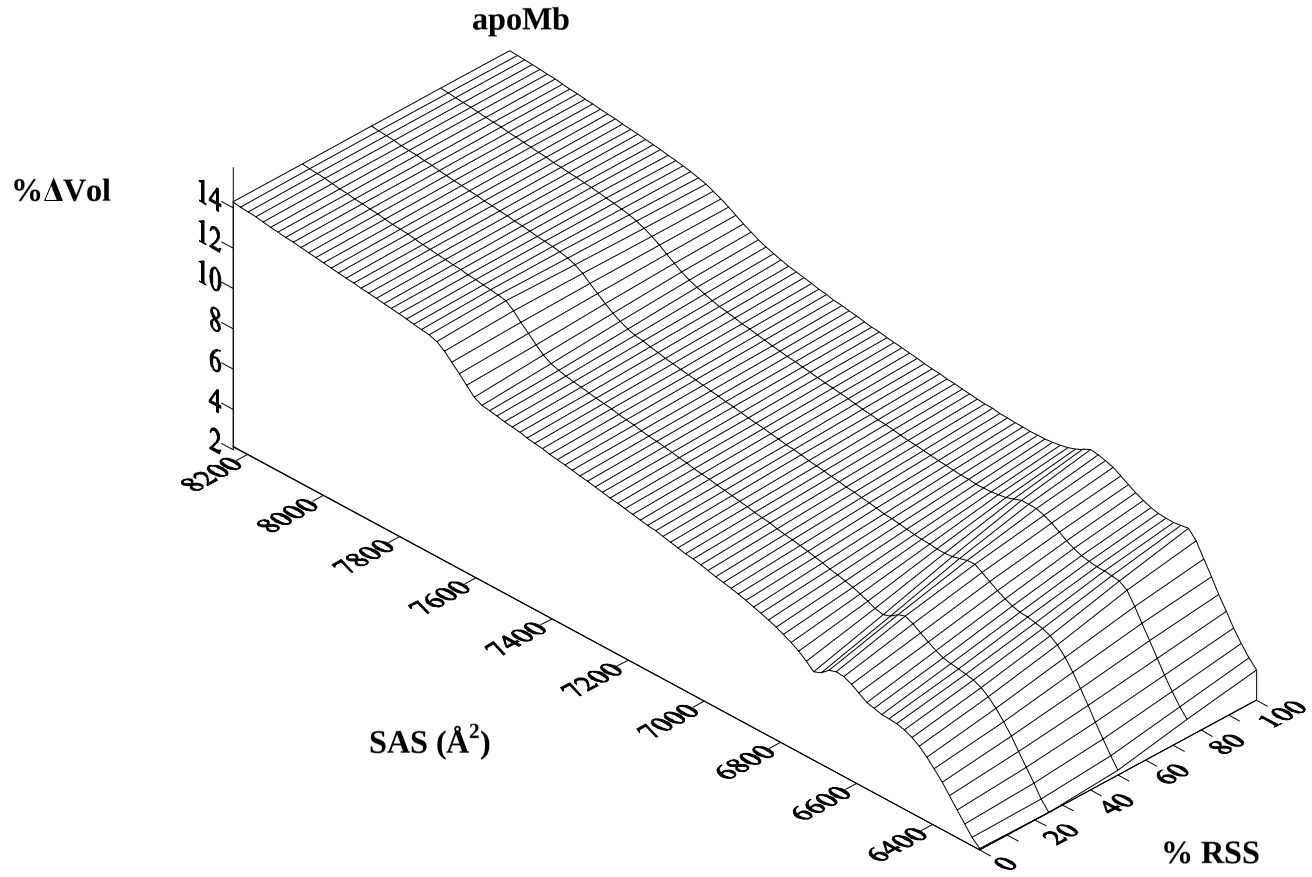
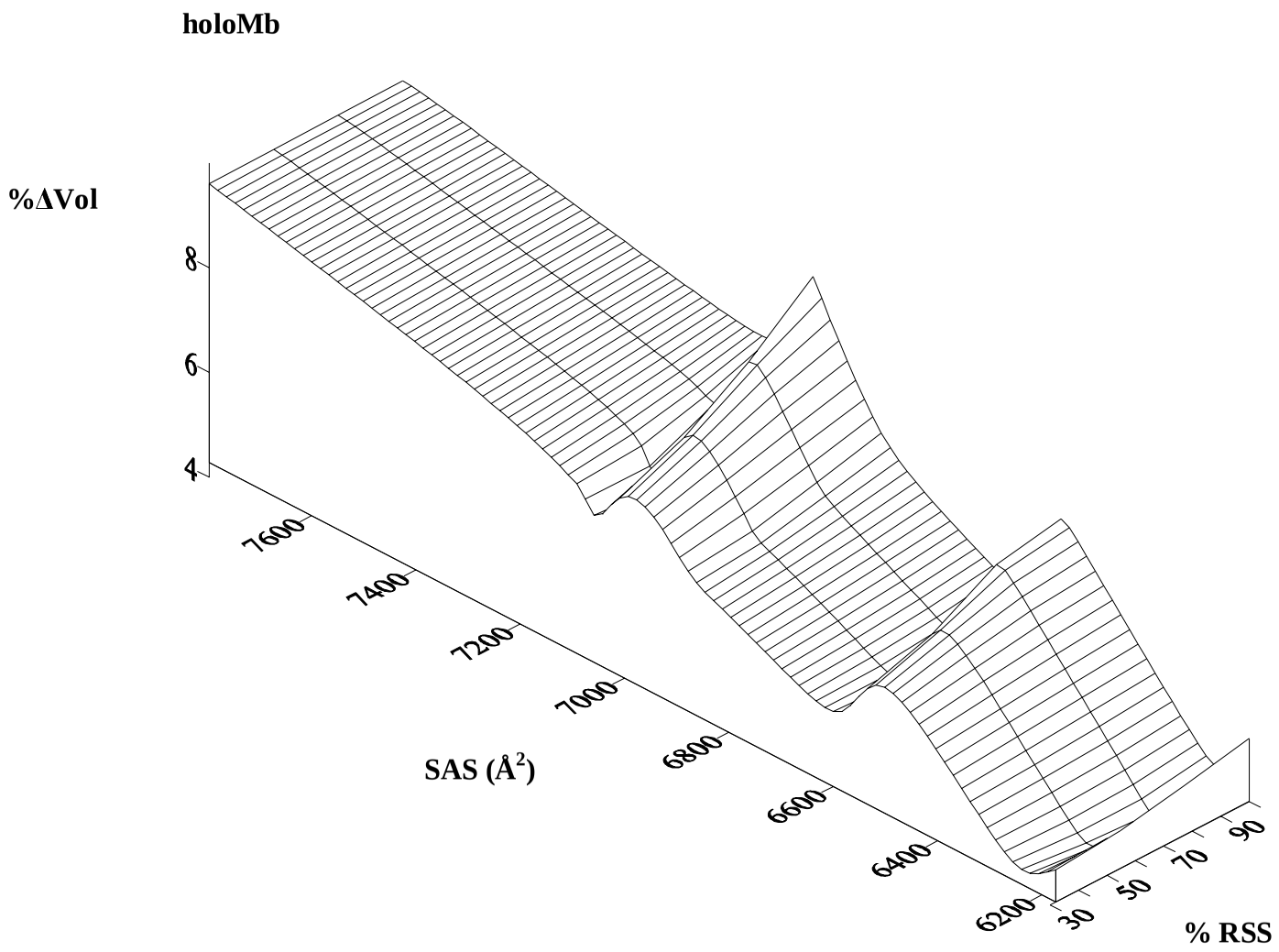


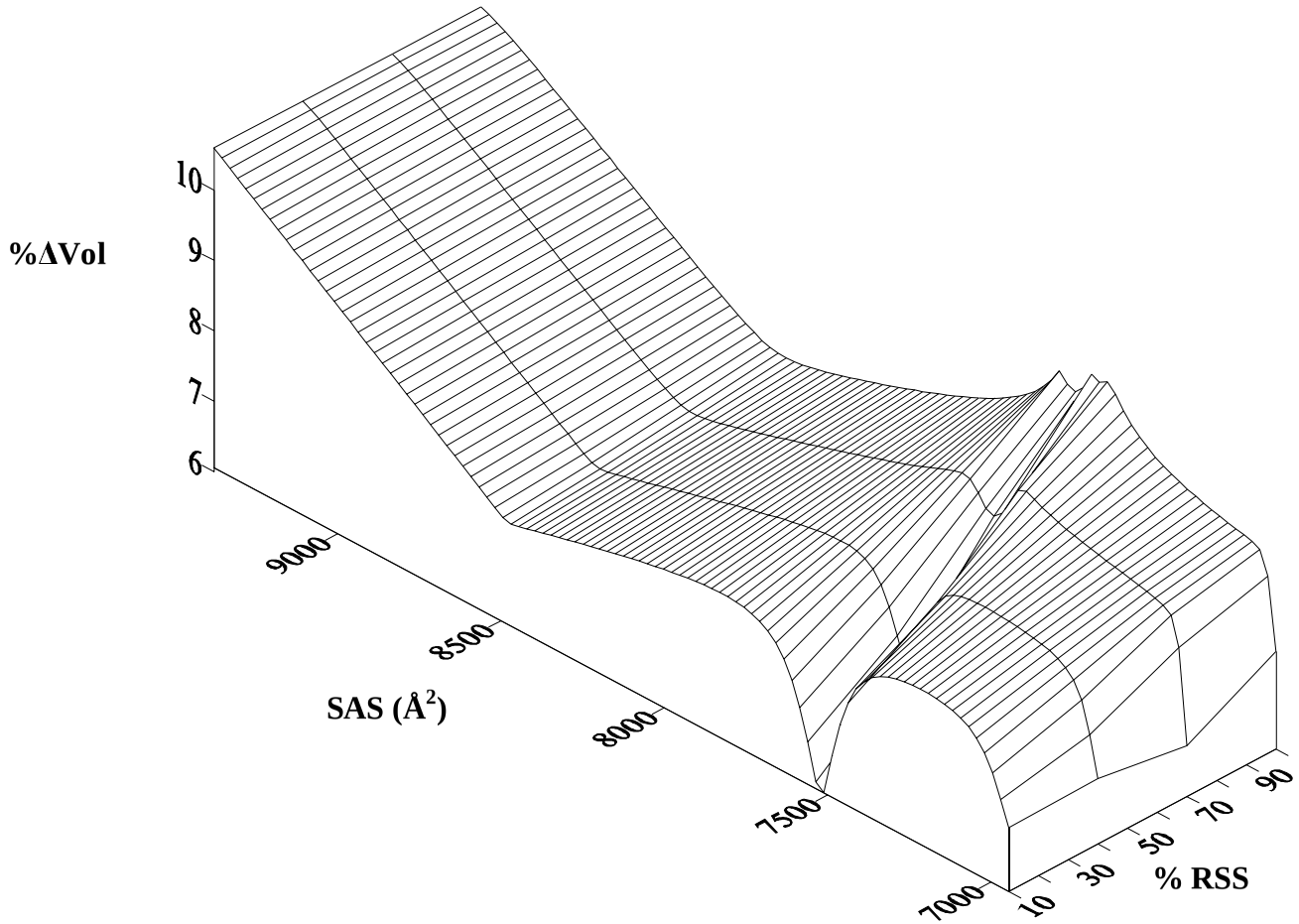
Figure 4. Correlation between solvent-accessible surface area (SAS), percentage of remaining secondary structures (%RSS) and volume variation (%ΔV) for a) apo and holomyoglobin (apoMB and holoMb), b) Retinol Binding Protein (RBP) and c) AK helix implicit and explicit solvent calculation (AK helix imp and AK helix exp) .

4.a)



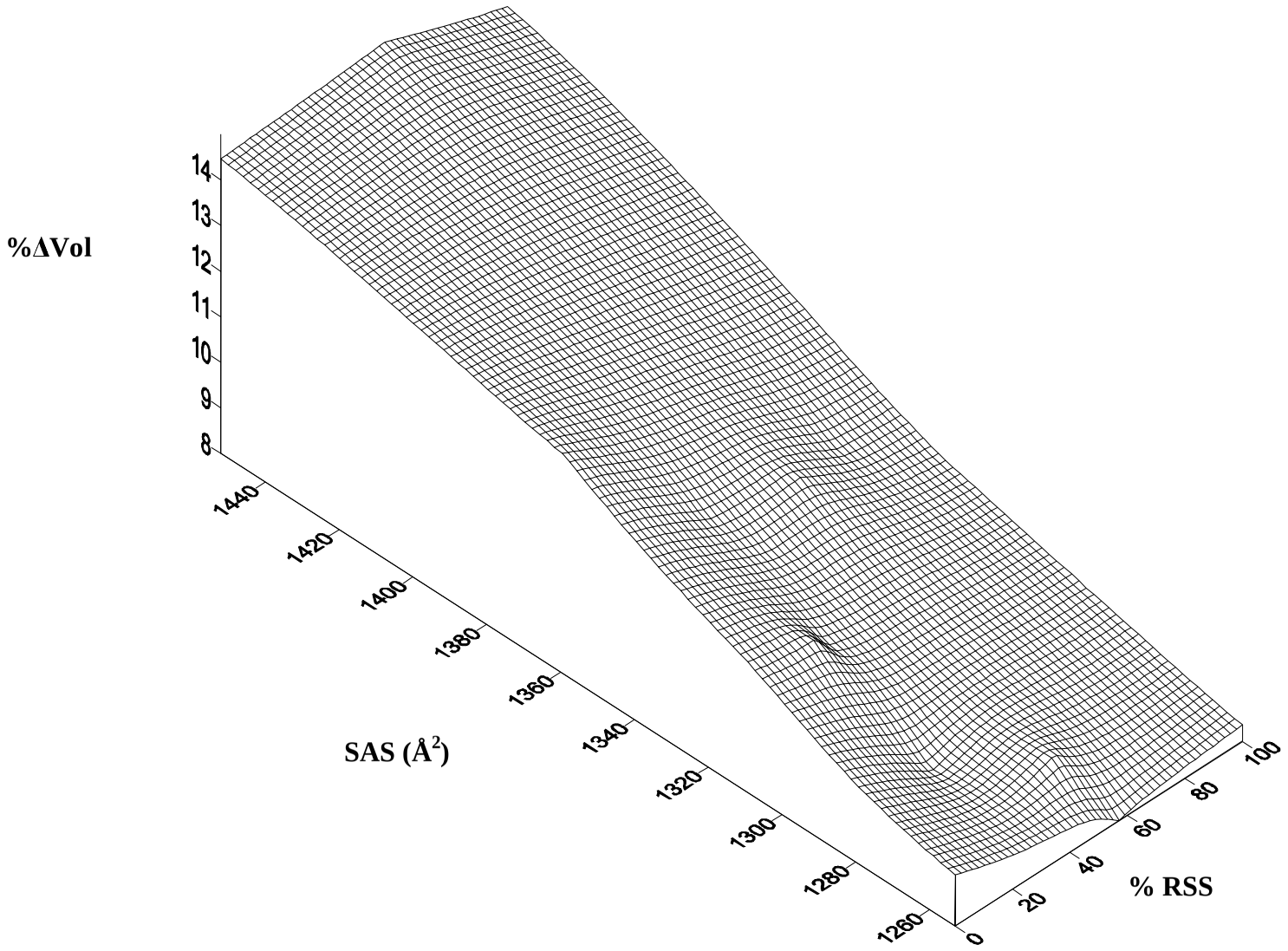

holoMb
%ΔVol
8
6
4
7600
7400
7200
7000
6800
6600
6400
6200
SAS (Å²)
30
50
70
90
% RSS

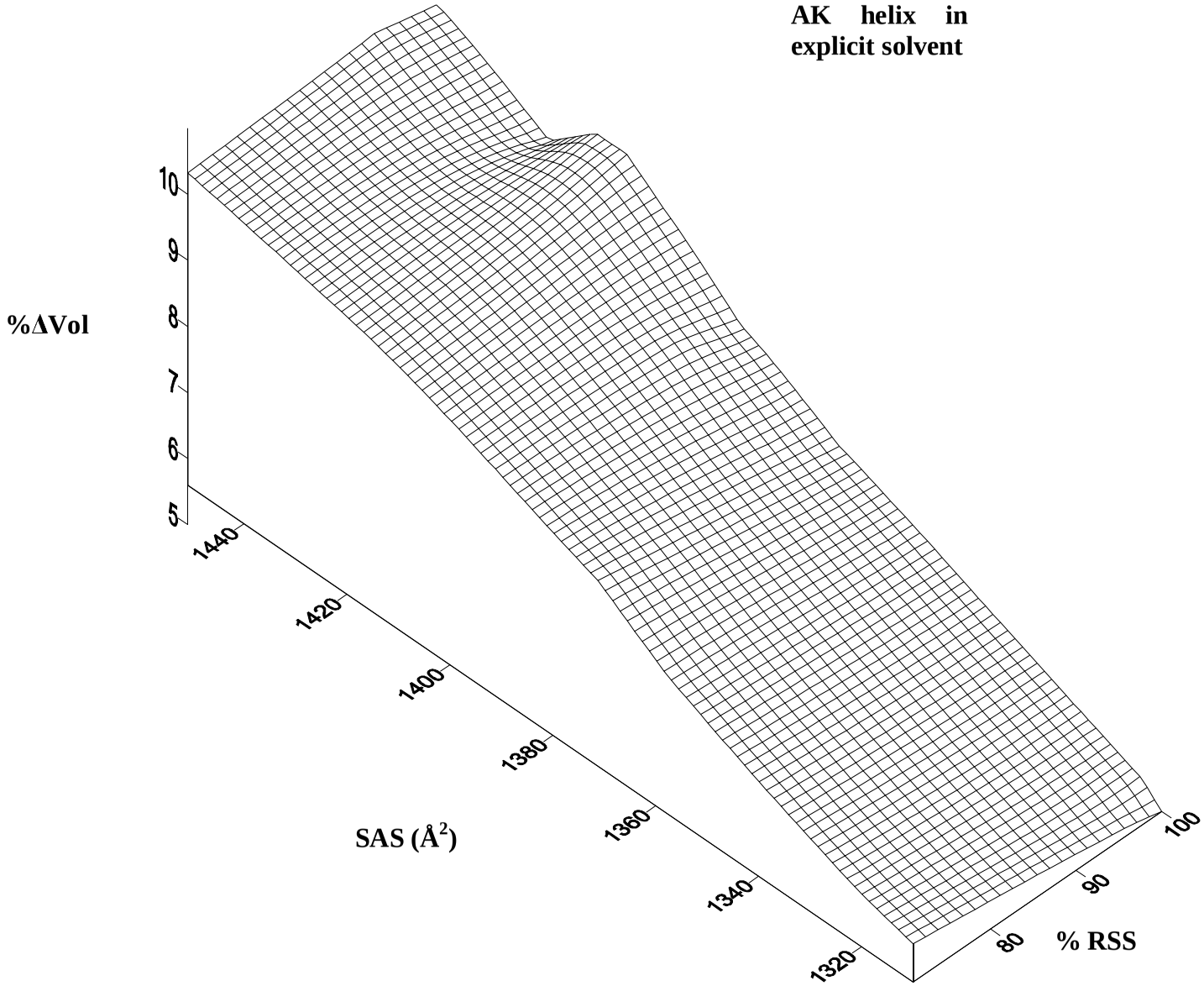
4.b) **RBP**



4.c)

AK helix in implicit solvent




AK helix in explicit solvent
%ΔVol
10
9
8
7
6
5
1440
1420
1400
1380
1360
1340
1320
SAS (Å2)
100
90
80
% RSS