

THE GLYCOPHORIN A TRANSMEMBRANE DOMAIN DIMER:
A MODELLING STUDY BY MD-SIMULATION.

By

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Abstract:

Understanding of the conformation and energetics of helix-helix interactions is essential as recent studies suggest specific roles for transmembrane helix association in a range of functions. I have studied the dimerisation of the transmembrane helix of glycophorin A, (GpA), and searched for its stable low-energy configurations.

Energyminimisation (EM) and molecular dynamics (MD) has been used on a large number of relative orientations of the two helices using the CHARMM-program, and for the resulting conformations I have calculated the accessible surfacearea, contactarea and distances between certain vital residues of this dimer. The results of these calculations was then compared with the corresponding values for a model of glycophorin A determined in an earlier study by another group.

This work in theoretical biophysics was made at Stockholm University (SU), department of biochemistry.

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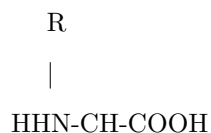
1. INTRODUCTION

1.1 Proteins

Proteins fall into the general class of polymers, which are simply linear molecules built up from simple repeating units called monomers. In the case of proteins the monomers are the so called amino acids.

The proteins have the property of acquiring very specific folded three-dimensional conformations and an elusive goal, for more than 40 years now, has been to predict this structure given only the sequence of amino acids.

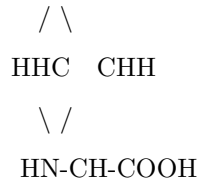
The general structure for 19 of the 20 amino acids normally used to build proteins is:



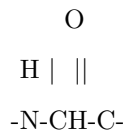
where R is the so called sidechain. The difference between two amino acids in this group lies solely in the chemical structure of R.

The 20'th natural amino acid, Proline, is similar but has the sidechain bonded to the nitrogen atom:



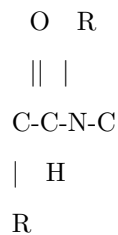


The 20 amino acids are assembled into proteins by linking them together via so called peptide bonds. In the process of linking of two amino acids one watermolecule is formed from the nitrogenhydrogen of one of the amino acids and one hydrogen and one oxygen from the carboxylgroup from the other amino acid, i.e it is a condensationprocess. In proteins many such amino acids are linked together to form a linear polypeptidechain. The so called polypeptidebackbone is simply a repetition of the basic amino acid unit:



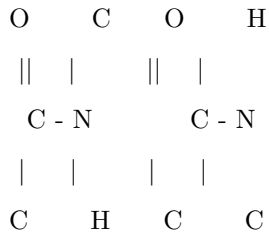
where the carbon in the middle is called the alpha-carbon. Such a unit plus its sidechain, R, is described as an amino acid residue when incorporated into a polypeptide chain.

In principle rotation may occur about any of the three bonds of the polypeptide backbone:



However, the peptide bond appears to have partial double-bonded character so that the six atoms depicted below have a strong tendency to be coplanar. Rotation of this bond is markedly restricted, but two configurations of the planar peptide bond are possible (C-alpha's in trans and cis configurations

respectively):



The remaining two bonds of the polypeptide backbone have single bond character, as do many of the bonds in the side chains.

This means that each residue may exist in a number of conformations, perhaps 10 on average, implying that even a relatively small polypeptide chain of 100 residues may exist in up to 10^{100} conformations. A fraction of these conformations are not possible in practice, (atoms occupying the same space etc.), yet the number of conformations even with a more conservative estimation of possible conformations per residue, leaves us with an astronomical number of conformations. Because of a factor known as conformational entropy is lost on adopting a single conformation it is highly unlikely that the polypeptide exists in a single stable conformation. This factor has the value $R \ln N$, where N is the number of possible conformations.

The contribution to the free energy of the disordered polymer is given by $-RT \ln N$. At 25 C this has the value of -136kcal/mole for a chain of 100 residues. In order to have half the population of molecules in one distinct conformation this is the minimum energy required by stabilising interactions.

As a consequence of this large conformational entropy, polypeptides will tend to exist in many different conformational states unless many stabilizing interactions are present simultaneously. Yet we know that proteins are able to do this.

1.2 The problem

One central question now arises: Is it possible to predict the conformation of a polypeptide into which it folds naturally, given only the amino acid sequence ?

Generally this problem is very complex and, although the general principles of protein association seem to be fairly well understood, one has rendered very limited progress in the applications of these principles to specific associations. The main problems in modelling these processes is the enormous conformational space that has to be scanned in the simulations, uncertainty in the exact form of the energy function to be used and how to incorporate entropy effects, plus the fact that most folded conformations are usually studied in an environment of liquid water, and the most important characteristic of all intermolecular forces is that they are generally more dependent upon the properties of this solvent than on the nature and strength of the intermolecular forces themselves. The most important noncovalent effect in water, the hydrophobic effect, is almost certainly a consequence of the strong interaction of water molecules with each other, rather than of a direct interaction between the solute molecules, and the constantly changing interactions between ensembles of many molecules in the liquid state makes modelling of proteins in water a very difficult task.

In our case however, the dimerisation of the transmembrane helix in GpA, the problem is simplified. Firstly we only study the transmembrane region of the dimer so it seems reasonable to model the dimer in vacuo, i.e because there is no water in this region, which discards hydrophobicity effects, and the dielectric of the inner lipid bilayer environment is low, (Treutlein et al., 1992), and secondly, we know from experiments that the secondary structure of GpA in this region consists of two alpha helices, a fact that greatly reduces the number of degrees of freedom and consequently the span of the conformational space.

2. METHODOLOGY

2.1 Molecular Dynamics (MD)

The fundamental idea of MD is to use Newton's equations of motion, $F = m \cdot a$, for every atom in the molecule. If F is a conservative force we can write $F = -\text{grad } E$, where E is the energyfunction of the system.

Several computer programs have been developed for the purpose of MD-simulations, one of them, the one I have used, is the CHARMM-program (Chemistry at Harvard Macromolecular Mechanics).

2.2 The CHARMM-program

CHARMM uses an empirical energyfunction to model the system, (L. Nilsson & M. Karplus, 1986). This energyfunction reads:

$$E = E_b + E_{th} + E_{ph} + E_{om} + E_{vdw} + E_{el} + E_{hb} + E_{cr} + E_{cph}$$

Where:

E_b = bond potential

E_{th} = bond angle potential

E_{ph} = dihedral (torsion) angle potential

E_{om} = improper torsions

E_{vdw} = van der Waals interactions

E_{el} = electrostatic potential

E_{hb} = hydrogen bonding potential

E_{cr} = constraints on atom distances (harmonic potential)

E_{cph} = constraints on dihedral angles (harmonic potential)

Of these terms two are especially important in what follows and it seems appropriate to write them out explicitly. They are:

$$E_{vdw} = \sum_{i,j} \text{exc}(i,j) (A(ij)/r^{12} - B(ij)/r^6) \text{sw}((r(ij)^2, r(\text{on})^2, r(\text{off})^2))$$

where $r(ij)$ = distance between atom i and atom j

$A(ij)$ and $B(ij)$ defines the shape of the van der Waals wells

sw is a switching function defined by:

$$\text{sw}(x, x(\text{on}), x(\text{off})) = 1 \text{ when } x \leq x(\text{on})$$

$$\text{sw}(x, x(\text{on}), x(\text{off})) = (x(\text{off}) - x)^2 (x(\text{off}) + 2x - 3x(\text{on})) / (x(\text{off}) - x(\text{on}))^3$$

when $x(\text{on}) < x \leq x(\text{off})$

$$\text{sw}(x, x(\text{on}), x(\text{off})) = 0 \text{ when } x > x(\text{off})$$

and

$$E_{el} = \sum_{i,j} \text{exc}(i,j) (Q(i)Q(j) / (4\pi \epsilon_0)) * (1 - 2r(ij)^2 / r(\text{cut})^2 + r(ij)^4 / r(\text{cut})^4)$$

excl is a exclusion function defined as :

$\text{excl}(ij) = 0$ if atoms are connected by angles or bonds or $i = j$.

$\text{excl}(ij) = 1$ otherwise.

2.3 The simulation protocols

After elaborate testing and guided by the methodology used in

the work done by another group, (Treutlein et al., 1992), I have decided to use four different schemes for the simulations.

They are:

- A.1 Separate the two superimposed helices by 7 Angstrom.
- 2 Tilt the first helix by 30 respectively 35 degrees relative to the lengthaxis of the second helix.
- 3 Rotate the first helix by 180 degrees with respect to its lengthaxis in order to get the residues of one helix face to face with the corresponding residue in the other helix.
- 4 Rotate the second helix by an angle ϕ_5 around its lengthaxis.
- 5 Rotate the first helix by an angle ϕ_6 around its lengthaxis.
- 6 Randomize the orientations of the sidechains.
- 7 Execute an energyminimisation (300 steps) with harmonically constrained backbone.
- 8 Execute a MD-simulation (5ps long) at the temperature of 400K.
- 9 Execute an energyminimisation (800 steps) without harmonic constraints.
- 10 Do a loop over the hole range 0-330 degrees for ϕ_5 and ϕ_6 .

The choice of 7 Angstroms in step 1 is somewhat arbitrary but tests indicated that this distance lies in the range of 5 to 9 Angstroms. Steps 2 and 3 are motivated by experiments. Specifically step 3 is motivated by mutational analyses which indicates that 9 residues are especially important in the folding process. It is then natural to assume that the distances between each of these and its counterpart in the other helix is short. However since we don't know if the direction of the separation in step 7 is optimal in order to get short distances between these especially important residues, we have to scan over the hole range

0-330 degrees for the angles ϕ_5 and ϕ_6 . This motivates step 10 above. This scanning procedure is done in steps of 30 degrees for each angle.

The orientations of the sidechains is unknown, therefore, in order to have something to start from, I have chosen to randomize these orientations, i.e step 6. Different so called ISEED numbers was used in order to compare if, and in what way, the final results of the simulations was depending upon the initial sidechain conformation.

Step 7 to 9 is the so called simulated annealing procedure. Step 7 is needed in order to stabilize the molecule so that the MD in step 8 doesn't crash.

Steps 1 to 6 is identical in the second scheme but after that it differs from the one above according to:

B.7 Execute an energyminimisation (300 steps) with harmonically constrained backbone, shorter van der Waals radiuses for the sidechain atoms and no electrostatic interactions at all.

8 Execute an energyminimisation (300 steps) with harmonically constrained backbone but now with electrostatics and "standard" vdw-radiuses for all atoms.

9 Execute a MD-simulation (5ps long) at the temperature of 400K.

10 Execute an energyminimization (800 steps) without harmonic constraints.

11 Do a loop over the hole range 0-330 degrees for ϕ_5 and ϕ_6 .

The third and fourth simulation protocol is quite different from the two above. In the third protocol, (protocol C), steps 7 and the following are altered to :

- C.7 Execute an energyminimisation (300 steps) with fix backbone atoms, shorter van der Waals radiuses for the sidechain atoms and no electrostatic interactions at all.
- 8 Do a loop where all atoms are fixed except the sidechain atoms of one residue, the van der Waal's radiuses are restored to their original values and do the following during each loop-turn:
- a. Execute an energyminimisation (300 steps)
 - b. Execute a MD-simulation (5ps long at 400K)
 - c. Execute an energyminimisation (200 steps)
 - d. Change residue to the next in line as long as the residue number is less than 23 and go to a.
9. Execute an energyminimisation (800 steps) with all atoms loose for the entire molecule.

The fourth protocol, (protocol D), is identical to protocol C except for step 7 where the backbone atoms are harmonically constrained instead of being fixed.

2.4 Tests of the protocols

In order to get some kind of measure of which protocol that gives results that best fit the results of Treutlein's group, (Treutlein et al., 1992), I did the following:

- 1a. For each protocol, instead of steps 1 to 5, I started from the so called wildtype conformation of the molecule, the one used by Treutlein et. al.

- 1b. For each protocol I created five versions, which differs from each other only in that they use different ISEED-numbers in the process of randomising the sidechains, step 6 above. Let's call these A.x, A.y, A.z, A.u, A.v, B.x, B.y, B.z, B.u, B.v, C.x, C.y, C.z, C.u, C.v, D.x, D.y, D.z, D.u and D.v, where the capital letters stands for the different protocols and the lowercase letters distinguishes between which ISEED number that is used.
- 1c. I created conformations from the wild type using only step 1 to 6 above, i.e no simulated annealing. Let's call these x.rand, y.rand, z.rand, u.rand and v.rand respectively.
- 1d. I created conformations from the wild type using only the process of simulated annealing in protocol A to D , i.e I didn't randomise the sidechains this time. Let's call these A.norand, B.norand etc.

2.4.1 RMSD measurements

After this I created programs that measures the root mean squared distance,(RMSD), between the atoms in the two helices.

I divided these measurements into 9 different measures. They are:

- 2a. All atoms
- 2b. All sidechain atoms
- 2c. All backbone atoms
- 2d. All atoms in residues 3,4,7,8,11,14,15,19 and 22.
- 2e. All C-beta
- 2f. All C-alpha
- 2g. All atoms in helix-A belonging to residue 3,4,7,8,11,14,15,19 or 22.
- 2h. All atoms in helix-B belonging to residue 3,4,7,8,11,14,15,19 or 22.
- 2i. All sidechain atoms belonging to residue 3,4,7,8,11,14,15,19

or 22.

(Residues 3,4,7,8,11,14,15,19 and 22 are the nine residues mentioned above under section 2.3).

I used these measurements for the RMSD between:

3a. The wildtype and A.x to A.v , the wildtype and B.x to B.v and so on. The results, only averages, are shown in table 1a to 1d below.

3b. The wildtype and x.rand to v.rand. The result, only averages, is shown in table 2 below.

3c. x.rand and A.x, y.rand and A.y, z.rand and A.z, u.rand and A.u, v.rand and A.v, and then between x.rand and B.x and so on. The results are shown in table 3a to 3d below.

3d. All pairwise combinations of A.x to A.v.
All pairwise combinations of B.x to B.v
All pairwise combinations of C.x to C.v
All pairwise combinations of D.x to D.v

The results are shown in table 4a to 4d below.

3e. All pairwise combinations of x.rand to v.rand. The result is shown in table 5 below.

3e. The wild type and A.norand to D.norand.

The results are shown in table 6 below.

3f. All pairwise combinations of A.x to D.x
All pairwise combinations of A.y to D.y
All pairwise combinations of A.z to D.z
All pairwise combinations of A.u to D.u

All pairwise combinations of A.v to D.v

The results are shown in table 7 below.

The complete versions of these tables, i.e not only average values, can be found in the appendix.

Table 1a A.x-A.v/w.t

Table 1b B.x-B.v/w.t

| measure | Average | measure | Average |
|---------------------|---------|---------------------|---------|
| All atoms | 1.596 | All atoms | 1.616 |
| All backbone | 0.629 | All backbone | 0.639 |
| All sidechain | 2.249 | All sidechain | 2.282 |
| All atoms res.3-22 | 1.245 | All atoms res.3-22 | 1.200 |
| C-beta | 1.914 | C-beta | 1.859 |
| C-alpha | 1.626 | C-alpha | 1.573 |
| helix-A | 1.228 | helix-A | 1.215 |
| helix-B | 1.144 | helix-B | 1.073 |
| All sidech.res.3-22 | 1.688 | All sidech.res.3-22 | 1.619 |

Table 1c C.x-C.v/w.t

Table 1d D.x-D.v/w.t

| measure | Average | measure | Average |
|---------------------|---------|---------------------|---------|
| All atoms | 1.739 | All atoms | 1.718 |
| All backbone | 0.695 | All backbone | 0.097 |
| All sidechain | 2.452 | All sidechain | 2.530 |
| All atoms res.3-22 | 1.241 | All atoms res.3-22 | 1.176 |
| C-beta | 1.875 | C-beta | 1.957 |
| C-alpha | 1.601 | C-alpha | 1.626 |
| helix-A | 1.162 | helix-A | 1.149 |
| helix-B | 1.161 | helix-B | 1.122 |
| All sidech.res.3-22 | 1.705 | All sidech.res.3-22 | 1.772 |

Table 2 x.rand-v.rand/w.t

| measure | Average |
|--------------|---------|
| All atoms | 1.812 |
| All backbone | 4.85E-4 |

| | |
|---------------------|-------|
| All sidechain | 2.677 |
| All atoms res.3-22 | 1.193 |
| C-beta | 1.987 |
| C-alpha | 1.653 |
| helix-A | 1.141 |
| helix-B | 1.154 |
| All sidech.res.3-22 | 1.811 |

Table 3a A.x/x.rand-A.v/v.rand

Table 3b B.x/x.rand-B.v/v.rand

| measure | Average | measure | Average |
|--------------------|---------|--------------------|---------|
| All atoms | 1.486 | All atoms | 1.514 |
| All backbone | 0.629 | All backbone | 0.639 |
| All sidechain | 2.091 | All sidechain | 2.128 |
| All atoms res.3-22 | 0.995 | All atoms res.3-22 | 0.966 |
| C-beta | 1.436 | C-beta | 1.422 |
| C-alpha | 1.249 | C-alpha | 1.225 |
| helix-A | 0.888 | helix-A | 0.853 |
| helix-B | 0.882 | helix-B | 0.935 |

| measure | Average | measure | Average |
|---------------------|---------|---------------------|---------|
| All sidech.res.3-22 | 1.290 | All sidech.res.3-22 | 1.241 |

Table 3c C.x/x.rand-C.v/v.rand

Table 3d D.x/x.rand-D.v/v.rand

| measure | Average | measure | Average |
|---------------------|---------|---------------------|---------|
| All atoms | 1.287 | All atoms | 0.650 |
| All backbone | 0.695 | All backbone | 0.097 |
| All sidechain | 1.749 | All sidechain | 0.956 |
| All atoms res.3-22 | 0.918 | All atoms res.3-22 | 0.437 |
| C-beta | 1.271 | C-beta | 0.735 |
| C-alpha | 1.116 | C-alpha | 0.606 |
| helix-A | 0.707 | helix-A | 0.441 |
| helix-B | 0.923 | helix-B | 0.411 |
| All sidech.res.3-22 | 1.134 | All sidech.res.3-22 | 0.711 |

Table 4a Pairwise all A.x to A.v

Table 4b Pairwise all B.x to B.v

| measure | Average | measure | Average |
|---------|---------|---------|---------|
|---------|---------|---------|---------|

| | | | | |
|---------------------|-------|---------------------|-------|--|
| All atoms | 1.687 | All atoms | 1.706 | |
| ----- | ----- | ----- | ----- | |
| All backbone | 0.685 | All backbone | 0.679 | |
| ----- | ----- | ----- | ----- | |
| All sidechain | 2.381 | All sidechain | 2.409 | |
| ----- | ----- | ----- | ----- | |
| All atoms res.3-22 | 1.238 | All atoms res.3-22 | 1.174 | |
| ----- | ----- | ----- | ----- | |
| C-beta | 1.882 | C-beta | 1.764 | |
| ----- | ----- | ----- | ----- | |
| C-alpha | 1.601 | C-alpha | 1.506 | |
| ----- | ----- | ----- | ----- | |
| Helix-A | 1.168 | Helix-A | 1.111 | |
| ----- | ----- | ----- | ----- | |
| Helix-B | 1.099 | Helix-B | 1.132 | |
| ----- | ----- | ----- | ----- | |
| All sidech.res.3-22 | 1.775 | All sidech.res.3-22 | 1.759 | |
| ----- | ----- | ----- | ----- | |

Table 4c Pairwise all C.x to C.v

Table 4d Pairwise all D.x to D.v

| | | | | |
|--------------------|---------|--------------------|---------|--|
| ----- | ----- | ----- | ----- | |
| measure | Average | measure | Average | |
| ----- | ----- | ----- | ----- | |
| All atoms | 1.834 | All atoms | 1.795 | |
| ----- | ----- | ----- | ----- | |
| All backbone | 0.763 | All backbone | 0.049 | |
| ----- | ----- | ----- | ----- | |
| All sidechain | 2.577 | All sidechain | 2.643 | |
| ----- | ----- | ----- | ----- | |
| All atoms res.3-22 | 1.291 | All atoms res.3-22 | 1.183 | |
| ----- | ----- | ----- | ----- | |
| C-beta | 1.92 | C-beta | 2.002 | |
| ----- | ----- | ----- | ----- | |
| C-alpha | 1.646 | C-alpha | 1.653 | |
| ----- | ----- | ----- | ----- | |

| | | | |
|---------------------|-------|---------------------|-------|
| Helix-A | 1.247 | Helix-A | 1.231 |
| Helix-B | 1.183 | Helix-B | 1.052 |
| All sidech.res.3-22 | 1.792 | All sidech.res.3-22 | 1.783 |

Table 5 Pairwise all x.r to v.r

Table 6 w.t/A.norand-w.t/D.norand

| measure | Average | measure | Average |
|---------------------|---------|---------------------|---------|
| All atoms | 1.878 | All atoms | 0.7702 |
| All backbone | 1E-14 | All backbone | 0.5448 |
| All sidechain | 2.767 | All sidechain | 0.9392 |
| All atoms res.3-22 | 1.21 | All atoms res.3-22 | 0.6614 |
| C-beta | 2.04 | C-beta | 0.7813 |
| C-alpha | 1.687 | C-alpha | 0.7345 |
| Helix-A | 1.249 | helix-A | 0.5548 |
| Helix-B | 1.086 | helix-B | 0.6225 |
| All sidech.res.3-22 | 1.828 | All sidech.res.3-22 | 0.6642 |

Table 7 Pairwise all A.x to D.v for fixed ISEED.

| | | | | | | Average | |
|---------|-----------|-----------|-----------|-----------|-----------|----------|--|
| measure | x-Average | y-Average | z-Average | u-Average | v-Average | of | |
| | | | | | | Averages | |
| 2a | 1.08 | 0.81 | 1.19 | 1.25 | 1.29 | 1.12 | |
| 2b | 0.59 | 0.53 | 0.74 | 0.63 | 0.6 | 0.62 | |
| 2c | 1.45 | 1.04 | 1.61 | 1.71 | 1.79 | 1.52 | |
| 2d | 0.73 | 0.76 | 0.85 | 0.98 | 0.88 | 0.84 | |
| 2e | 0.97 | 1.07 | 1.25 | 1.45 | 1.19 | 1.19 | |
| 2f | 0.87 | 0.94 | 1.08 | 1.25 | 1.06 | 1.04 | |
| 2g | 0.57 | 1.19 | 0.90 | 0.91 | 0.53 | 0.71 | |
| 2h | 0.78 | 0.69 | 0.56 | 0.91 | 1.01 | 0.79 | |
| 2i | 0.97 | 0.81 | 0.97 | 1.34 | 1.22 | 1.06 | |

Now, what does these tables tell us ?

According to tables 1a to 1d we can say the following:

Of all the four protocols A to D

Protocol A gives us the lowest RMSD's to the wildtype if we consider all atoms or all sidechain atoms, but it gives the highest RMSD's if we

consider only atoms in the especially important residues or only the alpha carbons or only atoms in helix-A.

Protocol B gives us the lowest RMSD's to the wildtype if we consider only the alpha carbons or only the beta carbons or only atoms in helix-B or only sidechain atoms in the important residues.

Protocol C gives us the highest RMSD's to the wildtype if we consider all atoms or only the backbone atoms or only atoms in helix-B.

Protocol D gives us the lowest RMSD's if we consider only the backbone atoms or only atoms in the especially important residues or only atoms in helix-A, but it gives us the highest RMSD's if we consider only all the sidechain atoms or only the beta carbons or only the alpha carbons or only sidechain atoms in the important residues.

What Table 2 tells us is somewhat dissapointing, because it tells us that the process of simulated annealing, in all our protocols, only reduces the RMSD's to the wild type marginally.

Tables 3a to 3d tells us that, of all the protocols, protocol D gives us the conformations that have the lowest RMSD's to the randomized conformations. This means that this protocol does not take us significantly closer to the wildtype conformation, e.g the average RMSD between the randomized conformations and the resulting conformations from protocol D, for only atoms in the especially important residues, is 0.711 Angstroms which is significantly lower than the corresponding value in Table 2, which is 1.811 Angstroms. For this reason I discard protocol D as a possible candidate for the title "best protocol of A to D". Of the remaining three protocols protocol A and protocol B seems to be the two that can bring us decently close to the wildtype conformation, in regard to RMSD's.

Tables 4a to 4d tells us, for each protocol, how much the spread is in the RMSD's when we consider the resulting conformations from different starting conformations, i.e different randomisations of the sidechains. The results are once again quite depressing. The dependence upon starting

conformation of the sidechains is quite severe, a fact that of course wasn't desirable at all. However, the lowest values are found for protocol A and B, a fact that further emphasizes that these two protocols are the ones that may be interesting.

An interesting question that now arises is the following: 'is these spreads in the RMSD's low compared to the values we get when we measure the corresponding RMSD's between the randomized conformations ?'

This question is answered by the results in table 5, and tragically enough, the answer is that the difference is small.

Table 6 tells us, for each protocol, how far we get from the wild type conformation when we don't randomize the sidechains. From this table we can see that in this sense protocol D is the "weakest" and protocol B is the most powerful. Sadly enough, as we saw in table 1b, protocol B does not take us particularly close to the wildtype conformation when we start from a conformation with randomised sidechain conformations.

Table 7, see appendix for the full table, measures pairwise RMSD's between the resulting conformations from the protocols. If we for each initial sidechain conformation, i.e first for x then for y and so on, mark which pair that gets the lowest RMSD in each measurement, i.e 2a to 2i, and then counts the number of times this happens for each protocol pair, and if we then do the same thing for the highest RMSD's, we get the following result, where L stands for number of lowest marks and H for number of highest marks.

| | | | | | | |
|--|-------|--|---|----|---|---|
| | — | | — | | — | |
| | | | | L | | |
| | | | | H. | | |
| | — | | — | | — | |
| | A - B | | | 15 | | |
| | | | | 2 | | . |
| | — | | — | | — | |
| | A - C | | | 6 | | |
| | | | | 0 | | |
| | — | | — | | — | |
| | A - D | | | 1 | | |
| | | | | 24 | | |
| | — | | — | | — | |
| | B - C | | | 16 | | |
| | | | | 2 | | |

| | | | |
|-------|---|----|--|
| | | | |
| B - D | 0 | 12 | |
| | | | |
| C - D | 7 | 5 | |
| | | | |

This result indicates that protocol B has low pairwise RMSD's with both protocol A and with protocol C, but definitely not with protocol D.

Protocol A has the highest number of H's in regard to protocol D, whereas protocol C has nearly equally many H's and L's in regard to protocol D.

As we have seen, protocol D is certainly the worst of the four protocols, so the fact that protocol A and B gives conformations that have quite high RMSD's to the resulting conformations from D is only strengthens our belief in that these two are the only interesting protocol's.

However, the results in table 2 and 5 wasn't very uplifting, since they told us that none of these protocols decreased the RMSD values more than marginally from the ones we got with exclusively randomized sidechain conformations. How about another kind of measure of the ability to take us close to the wildtype conformation ? Well, I've decided to look at the dihedral angles. Hopefully these measurements gives more positive results than the RMSD measurements did.

2.4.2 Dihedral angle measurements

What is meant by a dihedral angle between two atoms ? Well, we need four atoms to specify the dihedral angle between two atoms, in this case the dihedral between atoms b and c. Atoms a and b defines one plane, and atoms c and d another. The dihedral angle between b and c is the angle between these two planes, see fig. 2.1 below..

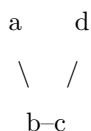


fig.2.1

I have measured the dihedral angle between the alpha carbon and beta carbon in five of the especially important residues, residues 3, 4, 8, 15 and 19. (In residues 7, 11,14 and 22 we have the amino acid glycine which as its sidechain only has a hydrogen atom.) The differences between the dihedrals of the wildtype conformation and the resulting conformations from the protocols respectively the difference in the dihedrals between the wildtype conformation and the exclusively randomized conformations are shown in table 8 below.

In table 9 I've summarized the average values of the results in table 8.

Table 10 shows the correlation coefficients between the resulting conformations from the protocols repectively between the resulting conformations from the protocols and the conformation with exclusively randomized sidechains.

Table 8

| | HELIX | RESIDUE | ISEED | PROT.A | PROT.B | PROT.C | PROT.D | RANDOM. |
|---|-------|---------|---------|---------|---------|---------|---------|---------|
| A | 3 | x | 108.039 | 105.07 | 107.405 | 106.088 | 119.876 | |
| A | 4 | x | 120.949 | 118.563 | 120.102 | 127.647 | 70.642 | |
| A | 8 | x | 104.1 | 104.343 | 105.06 | 117.074 | 144.067 | |
| A | 15 | x | 87.797 | 93.194 | 116.595 | 126.331 | 136.876 | |
| A | 19 | x | 126.457 | 124.348 | 121.81 | 133.947 | 147.688 | |
| B | 3 | x | 102.834 | 4.5989 | 72.9612 | 105.653 | 96.3772 | |
| B | 4 | x | 109.77 | 123.757 | 111.161 | 110.082 | 141.598 | |
| B | 8 | x | 6.942 | 109.341 | 111.308 | 119.616 | 125.737 | |
| B | 15 | x | 121.822 | 119.998 | 121.918 | 110.773 | 84.2284 | |
| B | 19 | x | 6.829 | 102.093 | 96.9982 | 119.825 | 117.699 | |
| A | 3 | y | 145.173 | 106.308 | 149.217 | 136.502 | 111.446 | |
| A | 4 | y | 118.208 | 119.984 | 123.654 | 122.091 | 107.604 | |
| A | 8 | y | 121.889 | 120.248 | 119.379 | 114.658 | 156.307 | |
| A | 15 | y | 3.568 | 2.694 | 3.899 | 3.183 | 13.999 | |
| A | 19 | y | 6.4048 | 8.6108 | 8.1725 | 3.538 | 32.9712 | |
| B | 3 | y | 0.4794 | 8.8533 | 6.793 | 16.903 | 51.1937 | |
| B | 4 | y | 112.851 | 105.289 | 149.926 | 102.074 | 119.659 | |
| B | 8 | y | 112.201 | 113.414 | 116.413 | 110.262 | 79.064 | |

| |
|--|
| B 15 y 128.026 4.722 3.737 6.474 56.773 |
| B 19 y 85.8222 84.428 84.2819 116.828 101.814 |
| A 3 z 105.318 103.52 17.9503 4.770 0.7799 |
| A 4 z 5.055 4.396 10.493 3.841 0.543 |
| A 8 z 8.88 8.704 3.121 6.918 24.691 |
| A 15 z 113.671 114.448 115.267 126.779 88.593 |
| A 19 z 4.0977 122.82 122.301 129.998 88.0779 |
| B 3 z 143.299 144.877 139.94 127.938 118.293 |
| B 4 z 3.066 2.646 2.397 2.038 13.886 |
| B 8 z 128.909 119.259 136.895 112.28 125.347 |
| B 15 z 4.517 0.155 5.943 7.996 1.891 |
| B 19 z 100.786 100.175 98.9973 118.663 122.799 |
| A 3 u 109.71 117.717 176.051 156.585 153.403 |
| A 4 u 2.814 2.437 4.309 15.337 54.444 |
| A 8 u 10.092 9.774 5.401 4.523 51.378 |
| A 15 u 117.545 117.008 118.437 110.939 102.876 |
| A 19 u 123.943 106.738 93.1057 103.484 145.76 |
| B 3 u 139.9 139.571 126.101 137.255 137.911 |
| B 4 u 120.326 135.118 126.756 124.287 169.521 |
| B 8 u 114.583 130.102 115.986 112.373 101.517 |
| B 15 u 120.246 124.808 4.72 111.247 70.694 |
| B 19 u 101.691 7.17 98.1871 119.362 124.642 |
| A 3 v 105.011 105.028 104.35 103.671 95.4111 |
| A 4 v 2.614 4.192 4.986 3.844 3.971 |
| A 8 v 7.502 7.815 9.275 5.449 10.19 |
| A 15 v 2.243 0.293 1.453 4.034 33.866 |
| A 19 v 100.252 17.6634 97.5977 113.988 84.0617 |
| B 3 v 107.843 100.744 94.4412 107.671 94.9632 |
| B 4 v 5.864 1.993 2.435 12.809 42.278 |
| B 8 v 105.736 2.663 109.115 120.819 126.627 |
| B 15 v 2.041 107.562 2.607 120.788 66.247 |
| B 19 v 85.4155 9.177 98.2129 129.925 159.273 |
| —— —— —— —— —— —— —— —— |

Table 9

| AVERAGE OF THE DIHEDRAL ANGLE DIFFERENCES | |
|--|-------|
| PROTOCOL A | 76.66 |
| PROTOCOL B | 72.97 |
| PROTOCOL C | 77.95 |
| PROTOCOL D | 85.38 |
| RANDOMIZED | 88.59 |

Table 10

| | A | B | C | D | RAND. |
|-------|-----------|-----------|-----------|-----------|-----------|
| A | ///////// | 0.63 | 0.73 | 0.69 | 0.66 |
| B | 0.63 | ///////// | 0.73 | 0.75 | 0.62 |
| C | 0.73 | 0.73 | ///////// | 0.88 | 0.84 |
| D | 0.69 | 0.75 | 0.88 | ///////// | 0.87 |
| RAND. | 0.66 | 0.62 | 0.84 | 0.87 | ///////// |

From table 9 we can see that all four protocols gives conformations with lower average differences of the dihedrals to the wildtype conformation than the conformation with exclusively randomized sidechains does. This is, of course, a positive result, and, as we can see, protocol B gives the lowest average value. However, we can not take these numbers too seriously since the uncertainties, i.e the standard deviations, for these are of the same order as the numbers themselves.

The results in table 10 is quite difficult to interpret. The fact that protocol B has higher correlations with protocol C and D than with A is somewhat puzzling, since A and B are very similar to each other but not to C or D. The explanation, I think, must be that protocols B, C and D uses reduced van der Waals radiuses in the first step of the annealing procedure, i.e the first energyminimisation, while protocol A uses the "standard" radiuses. Further, the correlation between protocols C and D are high, which isn't too unexpected, and these two protocols also gives high correlation coefficients with the conformation resulting from taking the wildtype conformation and randomising the sidechains. As we can see, once again protocol B is the most powerful of the four protocols in regard to ability to take us far away from the initial conformation.

Let us now concentrate our attention to protocol B.

3. CALCULATIONS OF ACCESSIBLE SURFACEAREA, CONTACTAREA AND DISTANCES BETWEEN THE ESPECIALLY IMPORTANT RESIDUES.

After deciding to use protocol B, I went on by investigating if this protocol could give any interesting information regarding the relationship between energy, accessible surfacearea, contactarea and distances between the vital residues, in this case the ones that Treutlein et.al. found to be most important, Treutlein et.al.,1992, which is residues 3, 4, 7, 11 and 15.

I've only considered two of the five randomisations that was used in the investigations above, namely protocols B.x and B.z. This time I've used the original B-protocol, i.e the one in section 2.3 which doesn't start from the wildtype conformation. The loop in step 11 is done in

30 degree steps for phi5 and phi6.

For the resulting conformations from these two B-protocols I have calculated a number of correlation coefficients, these are shown in table 11 respectively table 12a and table 12b.

Table 11

| | final | final | Energy | Energy | surface | surface |
|----------|---------|---------|--------|--------|---------|---------|
| measure | access. | access. | B.x | B.z | area | area |
| | B.x | B.z | | | B.x | B.z |
| final | | | | | | |
| access. | | | | | | |
| surf.ar. | | 0.93 | 0.71 | 0.69 | 0.23 | 0.38 |
| B.x | | | | | | |
| final | | | | | | |
| access | | | | | | |
| surf.ar. | 0.93 | | 0.66 | 0.74 | 0.32 | 0.28 |
| B.z | | | | | | |
| Energy | | | | | | |
| B.x | 0.71 | 0.66 | | 0.71 | 0.29 | 0.34 |
| Energy | | | | | | |
| B.z | 0.69 | 0.74 | 0.71 | | 0.19 | 0.13 |
| contact | | | | | | |
| surface | | | | | | |
| area | 0.23 | 0.32 | 0.29 | 0.19 | | 0.74 |
| B.x | | | | | | |
| contact | | | | | | |
| surface | | | | | | |

| | | | | | | |
|------|------|------|------|------|------|------------|
| area | 0.38 | 0.28 | 0.34 | 0.13 | 0.74 | ////////// |
| B.z | | | | | | ////////// |

Table 12a Correlationtable for protocol B.x

| | | | | | | | | | | |
|------------|------------|-------|------------|------------|------------|------------|------------|-------|--------|--------|
| initial | final | area | area | Ener. | surf. | res.3 | res.4 | res.7 | res.11 | res.15 |
| ////////// | | | | | | | | | | |
| acc.surf. | 0.90 | 0.65 | 0.11 | -0.10 | -0.14 | -0.15 | -0.21 | -0.21 | | |
| area | ////////// | | | | | | | | | |
| final | ////////// | | | | | | | | | |
| acc.surf. | 0.90 | 0.71 | 0.23 | 0.05 | 0.12 | 0.03 | 0.02 | 0.06 | | |
| area | ////////// | | | | | | | | | |
| Energy | 0.65 | 0.71 | ////////// | 0.29 | 0.37 | -0.11 | 0.32 | 0.21 | -0.04 | |
| contact | | | ////////// | | | | | | | |
| surface | 0.11 | 0.23 | 0.29 | ////////// | 0.49 | 0.44 | 0.57 | 0.64 | 0.56 | |
| area | | | ////////// | | | | | | | |
| dist. | | | | ////////// | | | | | | |
| resid.3 | -0.10 | -0.05 | 0.37 | 0.49 | ////////// | 0.18 | 0.96 | 0.85 | 0.39 | |
| dist. | | | | | ////////// | | | | | |
| resid.4 | -0.14 | 0.12 | -0.11 | 0.44 | 0.18 | ////////// | 0.29 | 0.49 | 0.89 | |
| dist. | | | | | | ////////// | | | | |
| resid.7 | -0.15 | -0.03 | 0.32 | 0.57 | 0.96 | 0.29 | ////////// | 0.94 | 0.51 | |
| dist. | | | | | | | ////////// | | | |

| | | | | | | | | | | | | | | | | | | |
|----------|-------|--|------|--|-------|--|------|--|------|--|------|--|------|--|------|------|---|--|
| resid.11 | -0.21 | | 0.06 | | 0.21 | | 0.64 | | 0.85 | | 0.49 | | 0.94 | | | 0.71 | | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| dist. | | | | | | | | | | | | | | | | | | |
| resid.15 | -0.21 | | 0.06 | | -0.04 | | 0.56 | | 0.39 | | 0.89 | | 0.51 | | 0.71 | | | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |

Table 12b Correlationtable for protocol B.z

| | | | | | | | | | | | | | | | | | | |
|-----------|-----------|--|-----------|--|-------|--|-------|--|-------|--|-------|--|-------|--|--------|--|--------|--|
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| | initial | | final | | cont. | | dist. | | dist. | | dist. | | dist. | | dist. | | dist. | |
| | acc.surf. | | acc.surf. | | Ener. | | surf. | | res.3 | | res.4 | | res.7 | | res.11 | | res.15 | |
| | area | | area | | area | | | | | | | | | | | | | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| initial | | | | | | | | | | | | | | | | | | |
| acc.surf. | | | 0.90 | | 0.72 | | 0.21 | | -0.09 | | -0.10 | | -0.13 | | -0.23 | | -0.22 | |
| area | | | | | | | | | | | | | | | | | | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| final | | | | | | | | | | | | | | | | | | |
| acc.surf. | 0.90 | | | | 0.74 | | 0.28 | | 0.02 | | 0.17 | | 0.03 | | 0.00 | | 0.07 | |
| area | | | | | | | | | | | | | | | | | | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| Energy | 0.72 | | 0.74 | | | | 0.13 | | 0.23 | | -0.21 | | 0.18 | | 0.04 | | -0.15 | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| contact | | | | | | | | | | | | | | | | | | |
| surface | 0.21 | | 0.28 | | 0.13 | | | | 0.38 | | 0.35 | | 0.44 | | 0.53 | | 0.48 | |
| area | | | | | | | | | | | | | | | | | | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| dist. | | | | | | | | | | | | | | | | | | |
| resid.3 | -0.09 | | 0.02 | | 0.23 | | 0.38 | | | | 0.03 | | 0.95 | | 0.83 | | 0.40 | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| dist. | | | | | | | | | | | | | | | | | | |
| resid.4 | -0.10 | | 0.17 | | -0.21 | | 0.35 | | 0.03 | | | | 0.17 | | 0.42 | | 0.82 | |
| | — | | — | | — | | — | | — | | — | | — | | — | | — | |
| dist. | | | | | | | | | | | | | | | | | | |
| resid.7 | -0.13 | | 0.03 | | 0.18 | | 0.44 | | 0.95 | | 0.17 | | | | 0.92 | | 0.52 | |

| | | | | | | | | | | |
|----------|-------|------|-------|------|------|------|------|------|------|--|
| | | | | | | | | | | |
| dist. | | | | | | | | | | |
| resid.11 | -0.23 | 0.00 | 0.04 | 0.53 | 0.83 | 0.42 | 0.92 | | 0.76 | |
| | | | | | | | | | | |
| dist. | | | | | | | | | | |
| resid.15 | -0.22 | 0.07 | -0.15 | 0.48 | 0.40 | 0.82 | 0.52 | 0.76 | | |
| | | | | | | | | | | |

As we can see, high absolute values of the correlation coefficients are very rare in table 11. The only significant results in this table is the correlation between respectively:

1. the final accessible surface areas
2. the contact areas
3. the energies
4. the final accessible surface area and the energy

The fact that the correlation coefficients between energy and the final accessible surface area has a positive sign is not desirable, since we expect increasing energy values with decreasing contact area, i.e increasing accessible surface area, but as we can see, table 11 tells us that increasing contact area does not mean decreasing accessible surface area, even if this correlation is weak, 0.2-0.4.

In table 12a and 12b the measurement "dist.resid.3" stands for the interatomic distance between the beta carbon of residue 3 in helix-A and the corresponding beta carbon in helix-B, and so on for "dist.resid.4" to "dist.resid.15" except that in the case of residues 7 and 11 I have measured the distance between the alpha carbons since the amino acid in these residues is glycine.

The strongest correlations in table 12a and 12b are the same in both tables, they are the correlations between:

1. initial and final accessible surface areas

2. energy and final accessible surface area
3. "dist.resid.3" and "dist.resid.7"
4. "dist.resid.3" and "dist.resid.11"
5. "dist.resid.4" and "dist.resid.15"
6. "dist.resid.7" and "dist.resid.11"
7. "dist.resid.11" and "dist.resid.15"

The first of these only shows that the annealing procedure does not alter the accessible surface area, more than marginally.

The last five are easily explained by the fact that an ideal alpha helix has 3.6 residues per turn.

The second correlation is plausible in the light of the following argument: Intuitively one imagines the energy to decrease with increasing contact area, and an increase in contact area should mean a reduction of the accessible surface area, consequently the sign of the correlation between energy and accessible surface area is positive, (so far so good), but as we can see, table 12a and 12b tells us that the energy increases with increasing contact area. Although the latter fact is a bit bothering, I do not think that these correlations are significant, their values lies in the range 0.2-0.3, which means that this is quite a weak correlation.

Further, we can also see that the correlation coefficients between the initial accessible surface areas and the residue distances, are negative, but that the corresponding correlation coefficients for the final accessible surface areas are positive, although this can not be taken to seriously since the correlations are weak. The latter fact is also true for the contact areas, but in this case the correlations are stronger.

How can we explain this ?

A possible explanation is as follows:

The amino acids in residue 3,4,7,11 and 15 are small. When the two helices are rotated, with respect to each other, the distances between the residues changes, and if this distance increases for one of our especially important residues, it could happen that another, bigger amino acid, gets rotated into the interface of the two helices. This, of course, reduces the accessible surface area, but increases the contact surface area of

the interface. This explains the negative sign in the correlations between initial accessible surface area and the residue distances, respectively the positive sign in the correlations between the residue distances and the contact area. A possible explanation for the positive sign, in the correlations between final accessible surface area and the residue distances, is that the annealing procedure, for each rotamer, increases the distances between large-amino-acid residues, at least if they overlap initially. This increases the accessible surface area.

Table 13 answers the important question:

” Does short residue distances, between the especially important residues, implicate a small contact surface area ? ”

To answer this question I picked all final conformations from protocol B.z, that has a contact area less than 900 Angstroems squared, which is significantly lower than the average value, and then I compared the average residue distances for these conformations with the overall average value for these distances. As we can see the answer to the question above is yes. I have also included the initial residue distances and contact areas, i.e before the annealing, in table 13, and this information tells us two more things about the effect of the annealing procedure:

1. It increases the distances between the especially important residues.
2. It reduces the contact surface area.

This is consistent with, and strengthens our belief in, the explanation above for the positive sign in the correlations between the residue distances and the final accessible surface area.

Table 13

| | all initial | all final | init. low contact | final low contact |
|----------|---------------|---------------|--------------------|--------------------|
| | conformations | conformations | area conformations | area conformations |
| distance | | | | |

| | | | | | | | | |
|----------|---------|--|---------|--|--------|--|--------|--|
| residue | 11.41 | | 11.54 | | 8.69 | | 8.09 | |
| 3 to 3 | | | | | | | | |
| ----- | | | | | | | | |
| distance | | | | | | | | |
| residue | 10.66 | | 11.04 | | 8.84 | | 9.99 | |
| 4 to 4 | | | | | | | | |
| ----- | | | | | | | | |
| distance | | | | | | | | |
| residue | 8.57 | | 9.37 | | 6.16 | | 6.64 | |
| 7 to 7 | | | | | | | | |
| ----- | | | | | | | | |
| distance | | | | | | | | |
| residue | 7.38 | | 8.88 | | 4.68 | | 5.51 | |
| 11 to 11 | | | | | | | | |
| ----- | | | | | | | | |
| distance | | | | | | | | |
| residue | 8.09 | | 9.66 | | 5.37 | | 6.75 | |
| 15 to 15 | | | | | | | | |
| ----- | | | | | | | | |
| contact | | | | | | | | |
| surface | 1154.84 | | 1021.72 | | 976.82 | | 861.64 | |
| area | | | | | | | | |
| ----- | | | | | | | | |

To further investigate the effect of our annealing procedure in protocol B, I did the following:

I measured the RMSD's between the backbone atoms of the wildtype conformation and the final conformations from protocol B.z. Then I plotted these RMSD's against energy and computed the correlation coefficient.

All this is depicted in diagram 1, where I've also included a line to show the energy of the wildtype after the same annealing procedure as in protocol B, (no randomisations of the sidechains).

The correlation is negative, -0.27, which is not so good since it indicates that the energy decreases with increasing RMSD between the backbone atoms

of the wildtype conformation and the final conformations from protocol B.z. We can also see that only one of the final conformations from protocol B.z has lower energy than the wildtype conformation, and that the RMSD for this conformation is quite great, approximately 3.5 Angstroms.

The last table, table 14 below, shows final accessible surface area, contact area and energy for the model of the GpA wildtype used by Brungers group, Treutlein et. al.,1992, after application of the annealing procedure of protocol B. In the first case I've used the same randomisation of the sidechain orientations as in protocol B.z and in the second case no randomisation was used.

Table 14

| | Wildtype | Wildtype |
|-------------------------------|------------|------------------|
| | randomized | no randomisation |
| final accessible surface area | 3527.8 | 3657.8 |
| final contact area | 1055.8 | 955.6 |
| Energy | 221.5 | 169.2 |

This table tells us a great deal about the problems we have with our protocol. We see that if we randomize the orientations of the sidechains then protocol B.z is not capable to take us to a conformation with especially low energy, although the contact area for this final conformation is bigger than the contact area of the non randomized

final conformation. This indicates that our annealing procedure is not powerful enough to move on from an energetically unfavourable conformation, e.g. with atoms stucked in certain positions, to more favourable ones. This was in fact confirmed by visual inspection of certain high energy conformations, where the initial randomized conformations were compared with the final conformations. It turned out that if some atoms were stucked inside a ring, e.g. the ones in Phenylalanine or Tyrosine, then they remained there even after the annealing procedure.

4. DISCUSSION

As we have seen, of our four protocols, protocol B is the one that, given a conformation with randomized sidechain orientations, gets us closest to the model of the wildtype conformation used by Brunger's group, (Treutlein et. al., 1992). However, the results of this study has not been very successful, and the central question of course, is why ?

One major reason seems to be that none of our protocols manage to pack the sidechains properly after the randomisations of their orientations.

This fact seems clear in the light of the last test I did. That this was the case stood clear early on in my investigations, when I used protocol A. In fact this was the reason for using reduced van der Waals radiuses in the other protocols, but as we have seen the improvements have been very limited.

APPENDIX

Table 1a

| measure | A.x | A.y | A.z | A.u | A.v | Average |
|---------|-----|-----|-----|-----|-----|---------|
|---------|-----|-----|-----|-----|-----|---------|

| | | | | | | |
|---------------------|---------|---------|---------|---------|---------|-------|
| All atoms | 1.65681 | 1.49021 | 1.62094 | 1.67585 | 1.53447 | 1.596 |
| All backbone | 0.56589 | 0.68237 | 0.59847 | 0.66726 | 0.63069 | 0.629 |
| All sidechain | 2.38356 | 2.06976 | 2.26689 | 2.35616 | 2.16849 | 2.249 |
| All atoms res.3-22 | 1.29741 | 1.35389 | 1.11982 | 1.2477 | 1.20603 | 1.245 |
| C-beta | 2.06686 | 2.06201 | 1.68756 | 1.96253 | 1.79276 | 1.914 |
| C-alpha | 1.73684 | 1.75614 | 1.44095 | 1.65242 | 1.54431 | 1.626 |
| helix-A | 1.42826 | 1.35577 | 1.08849 | 1.19973 | 1.06783 | 1.228 |
| helix-B | 1.09834 | 1.21732 | 1.03552 | 1.17701 | 1.19037 | 1.144 |
| All sidech.res.3-22 | 1.82939 | 1.94831 | 1.38619 | 1.74305 | 1.53152 | 1.688 |

Table 1b

| measure | B.x | B.y | B.z | B.u | B.v | Average |
|--------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 1.76229 | 1.4618 | 1.54076 | 1.72144 | 1.59143 | 1.616 |
| All backbone | 0.67103 | 0.64257 | 0.59641 | 0.68436 | 0.60281 | 0.639 |
| All sidechain | 2.48614 | 2.0438 | 2.18926 | 2.42323 | 2.26842 | 2.282 |
| All atoms res.3-22 | 1.36575 | 1.3065 | 1.12965 | 1.19708 | 0.9996 | 1.200 |
| C-beta | 2.12182 | 2.02743 | 1.76295 | 1.8746 | 1.50821 | 1.859 |

| | | | | | | |
|---------------------|---------|---------|---------|---------|---------|-------|
| C-alpha | 1.80142 | 1.71443 | 1.4849 | 1.57777 | 1.28499 | 1.573 |
| helix-A | 1.46683 | 1.37216 | 1.10821 | 1.19729 | 0.93146 | 1.215 |
| helix-B | 1.15568 | 1.10684 | 1.02551 | 1.10367 | 0.97377 | 1.073 |
| All sidech.res.3-22 | 1.19678 | 1.853 | 1.49451 | 1.63655 | 1.14269 | 1.619 |

Table 1c

| measure | C.x | C.y | C.z | C.u | C.v | Average |
|---------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 1.88208 | 1.49093 | 1.78598 | 1.73462 | 1.80218 | 1.739 |
| All backbone | 0.73551 | 0.72931 | 0.73585 | 0.64728 | 0.62876 | 0.695 |
| All sidechain | 2.65156 | 2.05702 | 2.51272 | 2.46428 | 2.57349 | 2.452 |
| All atoms res.3-22 | 1.37851 | 1.31633 | 1.15458 | 1.2529 | 1.10434 | 1.241 |
| C-beta | 2.12987 | 1.92946 | 1.71804 | 1.93059 | 1.66484 | 1.875 |
| C-alpha | 1.81373 | 1.66819 | 1.47182 | 1.63725 | 1.41587 | 1.601 |
| helix-A | 1.41611 | 1.28955 | 0.90739 | 1.20259 | 0.99578 | 1.162 |
| helix-B | 1.2389 | 1.15692 | 1.18547 | 1.12825 | 1.09416 | 1.161 |
| All sidech.res.3-22 | 1.9711 | 1.90428 | 1.52195 | 1.65407 | 1.47281 | 1.705 |

Table 1d

| measure | D.x | D.y | D.z | D.u | D.v | Average |
|---------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 1.88944 | 1.34642 | 1.76093 | 1.88291 | 1.71015 | 1.718 |
| All backbone | 0.09946 | 0.10075 | 0.09632 | 0.09612 | 0.09379 | 0.097 |
| All sidechain | 2.78573 | 1.99123 | 2.57201 | 2.77799 | 2.52454 | 2.530 |
| All atoms res.3-22 | 1.30792 | 1.15034 | 0.97531 | 1.29622 | 1.15 | 1.176 |
| C-beta | 2.1681 | 1.92009 | 1.6687 | 2.14604 | 1.88198 | 1.957 |
| C-alpha | 1.80414 | 1.59444 | 1.36826 | 1.78753 | 1.57629 | 1.626 |
| helix-A | 1.3639 | 1.19829 | 0.89372 | 1.30279 | 0.98746 | 1.149 |
| helix-B | 1.17906 | 1.07906 | 0.98214 | 1.21234 | 1.15831 | 1.122 |
| All sidech.res.3-22 | 2.00766 | 1.84462 | 1.49759 | 1.8321 | 1.67642 | 1.772 |

Table 2

| measure | x.rand | y.rand | z.rand | u.rand | v.rand | Average |
|--------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 1.95646 | 1.48678 | 1.8147 | 2.01965 | 1.78465 | 1.812 |
| All backbone | 4.85E-4 | 4.85E-4 | 4.85E-4 | 4.85E-4 | 4.85E-4 | 4.85E-4 |
| All sidechain | 2.89374 | 2.20053 | 2.65755 | 3.00257 | 2.63283 | 2.677 |
| All atoms res.3-22 | 1.38563 | 1.21981 | 0.90440 | 1.32414 | 1.13016 | 1.193 |

| | | | | | | |
|---------------------|---------|---------|---------|---------|---------|-------|
| C-beta | 2.29343 | 2.04511 | 1.55072 | 2.20107 | 1.84317 | 1.987 |
| C-alpha | 1.9134 | 1.69695 | 1.27187 | 1.83167 | 1.54985 | 1.653 |
| helix-A | 1.44105 | 1.22649 | 0.72256 | 1.32232 | 0.99354 | 1.141 |
| helix-B | 1.21807 | 1.19368 | 0.99804 | 1.24513 | 1.11395 | 1.154 |
| All sidech.res.3-22 | 2.10662 | 1.94915 | 1.41084 | 1.92591 | 1.662 | 1.811 |

Table 3a

| measure | A.x/x.r | A.y/y.r | A.z/z.r | A.u/u.r | A.v/v.r | Average |
|---------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 1.56019 | 1.27165 | 1.34735 | 1.60152 | 1.65125 | 1.486 |
| All backbone | 0.56586 | 0.68238 | 0.59845 | 0.66727 | 0.63068 | 0.629 |
| All sidechain | 2.22924 | 1.73115 | 1.88432 | 2.2605 | 2.34976 | 2.091 |
| All atoms res.3-22 | 0.99568 | 0.93633 | 0.93978 | 1.1504 | 0.95203 | 0.995 |
| C-beta | 1.51817 | 1.27791 | 1.39755 | 1.76684 | 1.21886 | 1.436 |
| C-alpha | 1.29195 | 1.12582 | 1.19157 | 1.50449 | 1.12997 | 1.249 |
| helix-A | 0.78047 | 0.78422 | 1.0352 | 1.19522 | 0.64250 | 0.888 |
| helix-B | 1.10533 | 0.87576 | 0.65481 | 0.84720 | 0.92662 | 0.882 |
| All sidech.res.3-22 | 1.53229 | 1.03228 | 1.04315 | 1.52555 | 1.31801 | 1.290 |

Table 3b

| measure | B.x/x.r | B.y/y.r | B.z/z.r | B.u/u.r | B.v/v.r | Average |
|---------------------|---------|---------|----------|---------|---------|---------|
| All atoms | 1.3555 | 1.3062 | 1.55867 | 1.77864 | 1.57316 | 1.514 |
| All backbone | 0.671 | 0.64256 | 0.59641 | 0.68434 | 0.60277 | 0.639 |
| All sidechain | 1.88056 | 1.80616 | 2.19219 | 2.52095 | 2.24007 | 2.128 |
| All atoms res.3-22 | 0.80326 | 1.01097 | 0.88294 | 1.06865 | 1.06518 | 0.966 |
| C-beta | 1.05806 | 1.4958 | 1.31273 | 1.62634 | 1.61955 | 1.422 |
| C-alpha | 0.95355 | 1.28427 | 1.11975 | 1.38356 | 1.38403 | 1.225 |
| helix-A | 0.76498 | 0.96634 | 0.769325 | 1.037 | 0.7263 | 0.853 |
| helix-B | 0.75835 | 0.91825 | 0.73823 | 0.95192 | 1.3093 | 0.935 |
| All sidech.res.3-22 | 1.08914 | 1.10347 | 0.92149 | 1.44821 | 1.64504 | 1.241 |

Table 3c

| measure | C.x/x.r | C.y/y.r | C.z/z.r | C.u/u.r | C.v/v.r | Average |
|--------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 1.28408 | 1.30855 | 1.36238 | 1.29811 | 1.18283 | 1.287 |
| All backbone | 0.73549 | 0.72931 | 0.73584 | 0.64728 | 0.62873 | 0.695 |
| All sidechain | 1.73018 | 1.76805 | 1.84265 | 1.78831 | 1.61475 | 1.749 |
| All atoms res.3-22 | 0.89182 | 0.99516 | 0.86373 | 1.00002 | 0.84024 | 0.918 |

| | | | | | | |
|---------------------|---------|---------|---------|---------|---------|-------|
| C-beta | 1.25703 | 1.34634 | 1.14681 | 1.45559 | 1.14793 | 1.271 |
| C-alpha | 1.10233 | 1.19292 | 1.02148 | 1.25987 | 1.00573 | 1.116 |
| helix-A | 0.82809 | 0.75043 | 0.60695 | 0.8176 | 0.53208 | 0.707 |
| helix-B | 0.83443 | 0.96439 | 0.77179 | 1.0164 | 1.03014 | 0.923 |
| All sidech.res.3-22 | 1.23497 | 1.09826 | 0.98986 | 1.25411 | 1.09299 | 1.134 |

Table 3d

| measure | D.x/x.r | D.y/y.r | D.z/z.r | D.u/u.r | D.v/v.r | Average |
|---------------------|---------|---------|---------|---------|---------|---------|
| All atoms | 0.74097 | 0.63852 | 0.62352 | 0.65777 | 0.58794 | 0.650 |
| All backbone | 0.09943 | 0.10072 | 0.09631 | 0.09610 | 0.09376 | 0.097 |
| All sidechain | 1.09566 | 0.93968 | 0.91895 | 0.95598 | 0.86911 | 0.956 |
| All atoms res.3-22 | 0.44361 | 0.45554 | 0.38652 | 0.46653 | 0.43502 | 0.437 |
| C-beta | 0.74218 | 0.77344 | 0.64443 | 0.77521 | 0.73776 | 0.735 |
| C-alpha | 0.61452 | 0.63512 | 0.53192 | 0.64371 | 0.60531 | 0.606 |
| helix-A | 0.45098 | 0.4194 | 0.46095 | 0.47196 | 0.40110 | 0.441 |
| helix-B | 0.42070 | 0.47366 | 0.27911 | 0.42413 | 0.45615 | 0.411 |
| All sidech.res.3-22 | 0.75 | 0.69431 | 0.60764 | 0.75019 | 0.75474 | 0.711 |

Table 4a

| | meas | A.x-y | A.x-z | A.x-u | A.x-v | A.y-z | A.y-u | A.y-v | A.z-u | A.z-v | A.u-v | Aver. |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|
| 2a | 1.5101 | 1.7564 | 1.8161 | 1.5430 | 1.8217 | 1.8843 | 1.5342 | 1.5099 | 1.7394 | 1.7588 | 1.687 | |
| 2b | 0.5580 | 0.6601 | 0.6717 | 0.6259 | 0.7350 | 0.8394 | 0.4969 | 0.6705 | 0.7801 | 0.8098 | 0.685 | |
| 2c | 2.1615 | 2.5006 | 2.6003 | 2.1894 | 2.5620 | 2.6407 | 2.1986 | 2.0949 | 2.4040 | 2.4573 | 2.381 | |
| 2d | 1.1571 | 1.3180 | 1.2649 | 1.2114 | 1.2325 | 1.3109 | 1.2199 | 1.0235 | 1.2129 | 1.4251 | 1.238 | |
| 2e | 1.8216 | 2.0611 | 1.9650 | 1.8993 | 1.8443 | 1.9072 | 1.964 | 1.5018 | 1.7198 | 2.1320 | 1.882 | |
| 2f | 1.5347 | 1.7360 | 1.6605 | 1.5979 | 1.5707 | 1.6471 | 1.6451 | 1.2872 | 1.4954 | 1.8319 | 1.601 | |
| 2g | 1.1954 | 1.1272 | 1.2366 | 1.1841 | 1.2152 | 1.4236 | 1.2360 | 1.1568 | 0.7645 | 1.1406 | 1.168 | |
| 2h | 1.0112 | 1.3581 | 1.0760 | 1.1183 | 1.0395 | 0.8365 | 1.1823 | 0.7234 | 1.2718 | 1.3705 | 1.099 | |
| 2i | 1.7316 | 1.9436 | 1.7544 | 1.9171 | 1.7086 | 1.8451 | 1.8430 | 1.4805 | 1.5756 | 1.9461 | 1.775 | |

Table 4b

| | meas | B.x-y | B.x-z | B.x-u | B.x-v | B.y-z | B.y-u | B.y-v | B.z-u | B.z-v | B.u-v | Aver. |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|
| 2a | 1.6040 | 1.7843 | 1.7933 | 1.7539 | 1.6628 | 1.8857 | 1.5820 | 1.7697 | 1.4174 | 1.8069 | 1.706 | |
| 2b | 0.6670 | 0.6923 | 0.7757 | 0.6827 | 0.7128 | 0.6546 | 0.5526 | 0.7707 | 0.6339 | 0.6469 | 0.679 | |
| 2c | 2.2549 | 2.5093 | 2.5328 | 2.4864 | 2.3417 | 2.6992 | 2.2596 | 2.4572 | 1.9685 | 2.5825 | 2.409 | |

| | | | | | | | | | | | |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
| 2d | 1.0835 | 1.1457 | 1.3769 | 1.2835 | 1.2145 | 1.3326 | 1.0959 | 1.1014 | 0.9512 | 1.1562 | 1.174 |
| 2e | 1.6248 | 1.6976 | 1.9712 | 1.9354 | 1.7971 | 2.0384 | 1.7018 | 1.6272 | 1.4769 | 1.7676 | 1.764 |
| 2f | 1.3869 | 1.4588 | 1.7256 | 1.6477 | 1.5372 | 1.7352 | 1.4337 | 1.3836 | 1.2469 | 1.4996 | 1.506 |
| 2g | 1.0815 | 0.8636 | 1.4216 | 1.1157 | 1.2781 | 1.3762 | 0.9959 | 1.0873 | 0.8494 | 1.0446 | 1.111 |
| 2h | 1.0476 | 1.2598 | 1.2238 | 1.3601 | 0.9709 | 1.1885 | 1.1560 | 0.9844 | 0.9370 | 1.1968 | 1.132 |
| 2i | 1.7745 | 1.6161 | 1.9254 | 1.9506 | 1.8126 | 1.9680 | 1.7521 | 1.6170 | 1.4845 | 1.6907 | 1.759 |

Table 4c

| | meas | C.x-y | C.x-z | C.x-u | C.x-v | C.y-z | C.y-u | C.y-v | C.z-u | C.z-v | C.u-v | Aver. |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|
| 2a | 1.8167 | 2.0035 | 1.6938 | 1.7597 | 2.1293 | 1.7440 | 1.8235 | 1.7227 | 1.7947 | 1.8505 | 1.834 | |
| 2b | 0.9088 | 0.7354 | 0.7737 | 0.6750 | 1.0214 | 0.7102 | 0.6916 | 0.7091 | 0.7415 | 0.6602 | 0.763 | |
| 2c | 2.4926 | 2.8449 | 2.3722 | 2.5024 | 2.9420 | 2.4750 | 2.5791 | 2.4249 | 2.4977 | 2.6390 | 2.577 | |
| 2d | 1.3245 | 1.3728 | 1.3382 | 1.2606 | 1.6184 | 1.2269 | 1.2127 | 1.2687 | 1.2952 | 0.9951 | 1.291 | |
| 2e | 1.8662 | 2.0456 | 2.0147 | 1.9289 | 2.2829 | 1.8458 | 1.8230 | 1.9277 | 1.9870 | 1.4764 | 1.92 | |
| 2f | 1.6369 | 1.7567 | 1.7229 | 1.6326 | 1.9970 | 1.5738 | 1.5502 | 1.6445 | 1.6865 | 1.2599 | 1.646 | |
| 2g | 1.2224 | 1.2323 | 1.3610 | 1.2036 | 1.5830 | 1.3553 | 1.1774 | 1.2929 | 1.2402 | 0.8019 | 1.247 | |
| 2h | 1.1801 | 1.4073 | 1.1871 | 1.2143 | 1.3390 | 0.9034 | 1.1849 | 1.1419 | 1.1724 | 1.0977 | 1.183 | |
| 2i | 1.9076 | 1.7083 | 1.9853 | 1.8843 | 2.0634 | 1.7945 | 1.7992 | 1.5984 | 1.6837 | 1.4949 | 1.792 | |

Table 4d

| | meas | D.x-y | D.x-z | D.x-u | D.x-v | D.y-z | D.y-u | D.y-v | D.z-u | D.z-v | D.u-v | Aver. |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|
| 2a | 1.8488 | 1.9347 | 1.7871 | 1.5762 | 1.8689 | 1.7965 | 1.8119 | 1.7184 | 1.8481 | 1.76 | 1.795 | |
| 2b | 0.0605 | 0.0462 | 0.0442 | 0.0488 | 0.0578 | 0.0560 | 0.0532 | 0.0332 | 0.0427 | 0.0473 | 0.049 | |
| 2c | 2.7203 | 2.845 | 2.6566 | 2.3431 | 2.7452 | 2.6579 | 2.6499 | 2.5134 | 2.7068 | 2.5960 | 2.643 | |
| 2d | 1.2127 | 1.1102 | 1.2207 | 1.1707 | 1.1810 | 1.2551 | 1.2803 | 1.0957 | 1.2561 | 1.0464 | 1.183 | |
| 2e | 2.0534 | 1.8864 | 2.0680 | 1.9793 | 1.9896 | 2.1119 | 2.1741 | 1.8494 | 2.1134 | 1.7931 | 2.002 | |
| 2f | 1.6953 | 1.5542 | 1.7073 | 1.6370 | 1.6476 | 1.7478 | 1.7911 | 1.5290 | 1.7508 | 1.4717 | 1.653 | |
| 2g | 1.3024 | 1.0959 | 1.3867 | 1.2397 | 1.4214 | 1.3366 | 1.2257 | 1.2348 | 1.1688 | 0.9012 | 1.231 | |
| 2h | 1.0756 | 1.0862 | 1.0039 | 1.0228 | 0.8171 | 1.1142 | 1.1998 | 0.8742 | 1.1916 | 1.1366 | 1.052 | |
| 2i | 1.8405 | 1.5849 | 1.8958 | 1.9579 | 1.8115 | 1.9369 | 1.9014 | 1.5544 | 1.7583 | 1.5924 | 1.783 | |

Table 5

| | meas | xr-yr | xr-zr | xr-ur | xr-vr | yr-zr | yr-ur | yr-vr | zr-ur | zr-vr | ur-vr | Aver. |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|
| 2a | 1.9196 | 2.0414 | 1.8109 | 1.5901 | 2.0178 | 1.9606 | 1.9557 | 1.7656 | 1.9347 | 1.7864 | 1.878 | |
| 2b | 10E-15 | 11E-15 | 79E-16 | 10E-15 | 43E-16 | 12E-15 | 82E-16 | 43E-16 | 19E-15 | 14E-15 | 1E-14 | |
| 2c | 2.8431 | 3.0018 | 2.690 | 2.3583 | 2.9603 | 2.9219 | 2.8563 | 2.5782 | 2.8238 | 2.6341 | 2.767 | |

| | | | | | | | | | | | |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
| 2d | 1.2126 | 1.1954 | 1.1285 | 1.2418 | 1.1902 | 1.2154 | 1.3736 | 1.1170 | 1.1715 | 1.2497 | 1.21 |
| 2e | 2.0742 | 2.0071 | 1.9124 | 2.0870 | 1.9912 | 2.0407 | 2.3258 | 1.8845 | 1.9561 | 2.1204 | 2.04 |
| 2f | 1.7036 | 1.6632 | 1.5779 | 1.7312 | 1.6538 | 1.6906 | 1.9190 | 1.5575 | 1.6271 | 1.7487 | 1.687 |
| 2g | 1.3003 | 1.1545 | 1.2523 | 1.2602 | 1.3671 | 1.4125 | 1.1883 | 1.3153 | 1.0719 | 1.1702 | 1.249 |
| 2h | 1.0449 | 1.1974 | 0.9614 | 1.1568 | 0.9552 | 0.9184 | 1.4144 | 0.8351 | 1.1076 | 1.2686 | 1.086 |
| 2i | 1.7926 | 1.7114 | 1.6708 | 2.0178 | 1.9026 | 1.9111 | 2.0456 | 1.7106 | 1.6365 | 1.8797 | 1.828 |

Table 6

| measure | w.t./A.nor. | w.t./B.nor. | w.t./C.nor. | w.t./D.nor. | Average |
|---------------------|-------------|-------------|-------------|-------------|---------|
| All atoms | 0.884438 | 0.944574 | 0.835259 | 0.416625 | 0.7702 |
| All backbone | 0.711549 | 0.718862 | 0.657982 | 0.090636 | 0.5448 |
| All sidechain | 1.02933 | 1.13113 | 0.984286 | 0.611905 | 0.9392 |
| All atoms res.3-22 | 0.760298 | 0.814314 | 0.713111 | 0.357747 | 0.6614 |
| C-beta | 0.799614 | 0.957865 | 0.769897 | 0.597898 | 0.7813 |
| C-alpha | 0.792996 | 0.89779 | 0.754201 | 0.493058 | 0.7345 |
| helix-A | 0.614756 | 0.712324 | 0.523427 | 0.368652 | 0.5548 |
| helix-B | 0.72706 | 0.737143 | 0.715538 | 0.310064 | 0.6225 |
| All sidech.res.3-22 | 0.727468 | 0.867643 | 0.678276 | 0.383282 | 0.6642 |

| | | | | | | | | | |
|--|--|--|--|--|--|--|--|--|--|
| | | | | | | | | | |
|--|--|--|--|--|--|--|--|--|--|

Table 7

| | 2a | 2b | 2c | 2d | 2e | 2f | 2g | 2h | 2i |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 34x-41x | 1.0824 | 0.4457 | 1.5136 | 0.7374 | 1.0869 | 0.9397 | 0.4216 | 0.9146 | 1.1227 |
| 34x-46x | 1.1906 | 0.6167 | 1.6121 | 0.7919 | 1.0253 | 0.9408 | 0.5796 | 0.8538 | 1.1580 |
| 34x-47x | 1.3330 | 0.5567 | 1.8676 | 0.8675 | 1.2707 | 1.0963 | 0.6518 | 0.978 | 1.2830 |
| 41x-46x | 0.8116 | 0.5152 | 1.0667 | 0.5412 | 0.6485 | 0.6104 | 0.4813 | 0.5137 | 0.6358 |
| 41x-47x | 1.0749 | 0.6603 | 1.4283 | 0.7233 | 0.8764 | 0.8214 | 0.6342 | 0.7248 | 0.7825 |
| 46x-47x | 0.9663 | 0.7242 | 1.1956 | 0.7204 | 0.8988 | 0.8307 | 0.6456 | 0.6666 | 0.8634 |
| Average | 1.0765 | 0.5865 | 1.4473 | 0.7303 | 0.9678 | 0.8732 | 0.5690 | 0.7752 | 0.9743 |
| | | | | | | | | | |
| 34y-41y | 0.5817 | 0.3383 | 0.7821 | 0.6778 | 1.0684 | 0.8984 | 0.6419 | 0.6095 | 0.7031 |
| 34y-46y | 0.6081 | 0.3535 | 0.8182 | 0.6234 | 0.9670 | 0.8151 | 0.4436 | 0.7108 | 0.7425 |
| 34y-47y | 1.0480 | 0.6783 | 1.3578 | 0.9093 | 1.2242 | 1.0875 | 0.7274 | 0.8315 | 0.9621 |
| 41y-46y | 0.5649 | 0.4416 | 0.6840 | 0.5880 | 0.8086 | 0.7138 | 0.6142 | 0.3892 | 0.6323 |
| 41y-47y | 1.0181 | 0.6383 | 1.3310 | 0.8866 | 1.2350 | 1.0876 | 0.841 | 0.7564 | 0.8620 |
| 46y-47y | 1.0279 | 0.7265 | 1.2932 | 0.8959 | 1.1227 | 1.0296 | 0.6266 | 0.8423 | 0.9400 |
| Average | 0.8081 | 0.5294 | 1.0444 | 0.7635 | 1.0710 | 0.9386 | 0.6491 | 0.69 | 0.8070 |

| | | | | | | | | | |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 34v-46v | 1.4095 | 0.6595 | 1.9600 | 0.7808 | 0.8276 | 0.8067 | 0.3723 | 0.8800 | 0.8019 |
| | | | | | | | | | |
| 34v-47v | 1.5235 | 0.6255 | 2.1547 | 0.8694 | 1.0532 | 1.004 | 0.5685 | 0.8416 | 1.1800 |
| | | | | | | | | | |
| 41v-46v | 1.020 | 0.3688 | 1.4542 | 0.7906 | 1.2632 | 1.0569 | 0.5628 | 0.9355 | 1.3678 |
| | | | | | | | | | |
| 41v-47v | 1.4261 | 0.5978 | 2.0140 | 1.0261 | 1.5579 | 1.3306 | 0.7593 | 1.2209 | 1.5776 |
| | | | | | | | | | |
| 46v-47v | 0.9505 | 0.6209 | 1.2327 | 0.7597 | 0.9957 | 0.8819 | 0.4199 | 0.9592 | 0.9256 |
| | | | | | | | | | |
| Average | 1.2857 | 0.5956 | 1.7870 | 0.8779 | 1.1897 | 1.0569 | 0.5330 | 1.0061 | 1.2155 |
| | | | | | | | | | |
| | | | | | | | | | |
| Average | | | | | | | | | |
| of | 1.120 | 0.617 | 1.521 | 0.842 | 1.186 | 1.039 | 0.712 | 0.788 | 1.062 |
| Average | | | | | | | | | |
| | | | | | | | | | |