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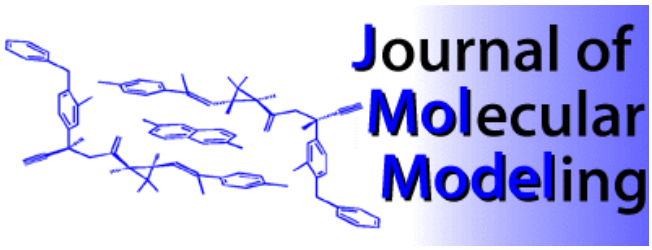

Substrate Access to Cytochrome P450cam: A Comparison of a Thermal Motion Pathway Analysis with Molecular Dynamics Simulation Data[$]

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Abstract

Cytochrome P450 enzymes are hemoprotein monooxygenases that catalyse the oxidation of a variety of compounds. The mechanism by which camphor, the natural substrate of Cytochrome P450cam (P450cam), accesses the active site is a long-standing puzzle, although putative access channels have been proposed. A thermal motion pathway (TMP) analysis was performed on the crystal structure of P450cam with camphor bound. Hereby, three distinct thermal motion pathway families (TMPFs) were found. Possible substrate access channels obtained by this analysis based on B-factors are compared with exit channels explored by molecular dynamics simulations (MDS) by imposing an artificial expulsion force on the substrate in addition to the standard MD force field. Two out of three TMPFs are supported by results obtained with the random expulsion MDS method. However, the pathway found by the TMP method to have the highest average B-factor could not be observed by MDS. The pathway proposed from crystallographic data, which is a small opening above the active site located near residues 185, 87 and 395 corresponds to the TMPF with the second highest average B-factor.

Keywords: Protein-ligand binding, Temperature factors, Protein channel, Protein dynamics.

Introduction

Cytochrome P450 enzymes (P450s) are hemoprotein monooxygenases that catalyse the oxidation of a variety of compounds [1]. P450s play a critical role in the synthesis and degradation of physiologically important compounds, e.g. steroids, fatty acids, prostaglandins, and of many xenobiotics, e.g. drugs, alcohols, pesticides, procarcinogens. The mechanism by which camphor, the natural substrate of Cytochrome P450cam (P450cam), accesses the active site is a long-standing puzzle, although putative access channels have been proposed [2] . While there is recent crystallographic and molecular dynamics simulation (MDS) evidence for opening of a substrate access channel in P450BM-3 [3–5] , for P450cam such conformational changes upon substrate access have not been observed in different crystal structures or by standard molecular dynamics simulations.

Here, a thermal motion pathway (TMP) [6] analysis was performed on the crystal structure of P450cam with cam-

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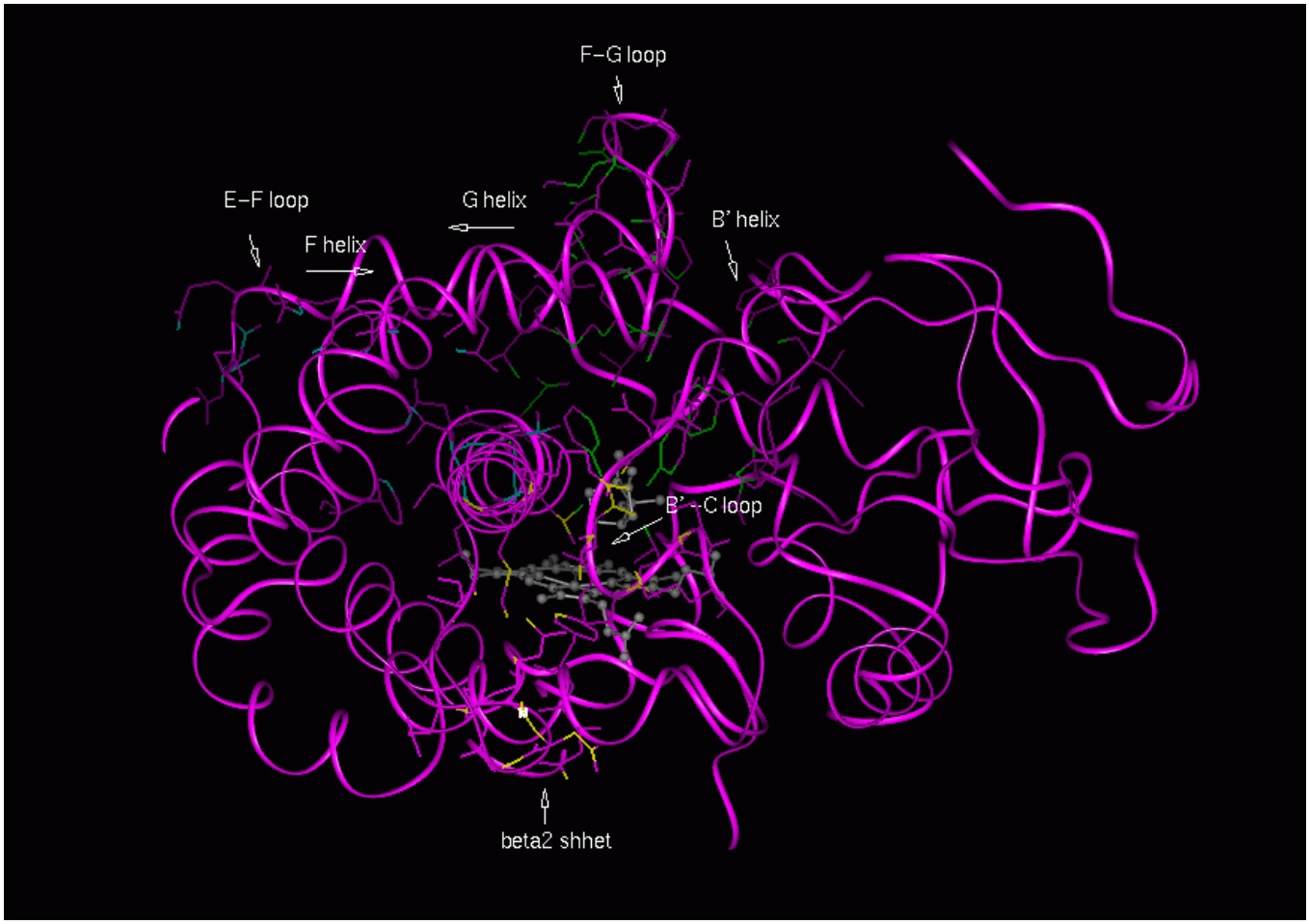

Figure 1. *Overview of the location of all 3 TMPFs in P450cam. Atoms belonging to TMPF1 are displayed in yellow, atoms of TMPF2 in green and atoms of TMPF3 in light blue. The main chain of P450cam is displayed as a ribbon. Heme and camphor are colored in white. Secondary structure elements involved in possible camphor access pathways are indicated.*

phor bound. As crystallographically determined temperature factors (B-factors) indicate the flexibility of protein atoms, protein regions with high thermal factors connecting the active site to the surface may indicate ligand exit/access channels. Possible substrate access channels obtained by the TMP analysis of atomic B-factors are compared with exit channels explored by MDS by imposing an artificial expulsion force on the substrate in addition to the standard MD force field.

Methods

TMP-analysis

Atoms bordering the active site of the crystal structure of P450cam with camphor bound (Brookhaven PDB file: 2cpp) are grouped into triplets. The maximum distance allowed between two atoms in a triplet is 4.5 Å. Starting from these primary triplets, secondary triplets are defined in close contact to the primary ones and sorted by average thermal factors. The secondary triplet with the highest average thermal factor is retained and used as a new starting point. The above steps are repeated until the protein surface is reached via pathways with elevated thermal factors. All TMPs with an average thermal factor <B> larger than 20Å^2 (for comparison the mean thermal factor of the protein is 18.75 Å^2) were found to belong to three distinct pathway families.

Each pathway family (TMPF) consists of **n** individual pathways (Table 2). Within one family, atoms belonging to individual pathways overlap by at least 30%. A TMPF consists in total of **m** atoms (Table 2). Each pathway is characterized by an average B factor <B>, a pathway family by a $<B>_{fam}$, which is an average over all individual <B>. $<B>_{min}$ and $<B>_{max}$ are those pathways within a pathway family that have the lowest and highest average B factors.

Molecular Dynamics Simulations

Simulations were performed with the ARGOS program [7] and the CHARMm 22 all atom force field. A shell of 1226 TIP3P water molecules was put around the crystal structure of P450cam with camphor bound in the active site (2cpp.pdb).

Table 1. *Comparison of protein atoms in TMPFs and MDS trajectories*

	TRAJ1/TMPF1	TRAJ2a/TMPF2	TRAJ2b/TMPF2	TRAJ3/TMPF3
atom overlap between TMPFi and TRAJi	3/32 (9%)	24/64 (38%)	10/64 (16%)	17/26 (65%)
atom overlap between pathway of $<B>_{max}$ of TMPFi and TRAJi	3/18 (17%)	4/21 (19%)	1/21 (5%)	11/15 (73%)
residue overlap between TMPFi and TRAJi	3/20 (15%)	16/30 (53%)	7/30 (23%)	11/14 (79%)

The simulations were performed with a twin range cutoff of 8/10Å and Coulomb interactions were damped by force-shifting [8] .

Possible substrate access channels to the active site were explored by imposing an artificial random expulsion force on the ligand in addition to the standard MD force field. Force constants of 800 to 1000kJ/mol·nm were used. The direction of the expulsion force was kept as long as the substrate covers a certain distance within a specified time. As a camphor molecule in the gaseous state at room temperature covers an average distance of 0.0022 Å/fs, distance thresholds between 0.005 and 0.02 Å/10 MD steps (20fs) were cho-

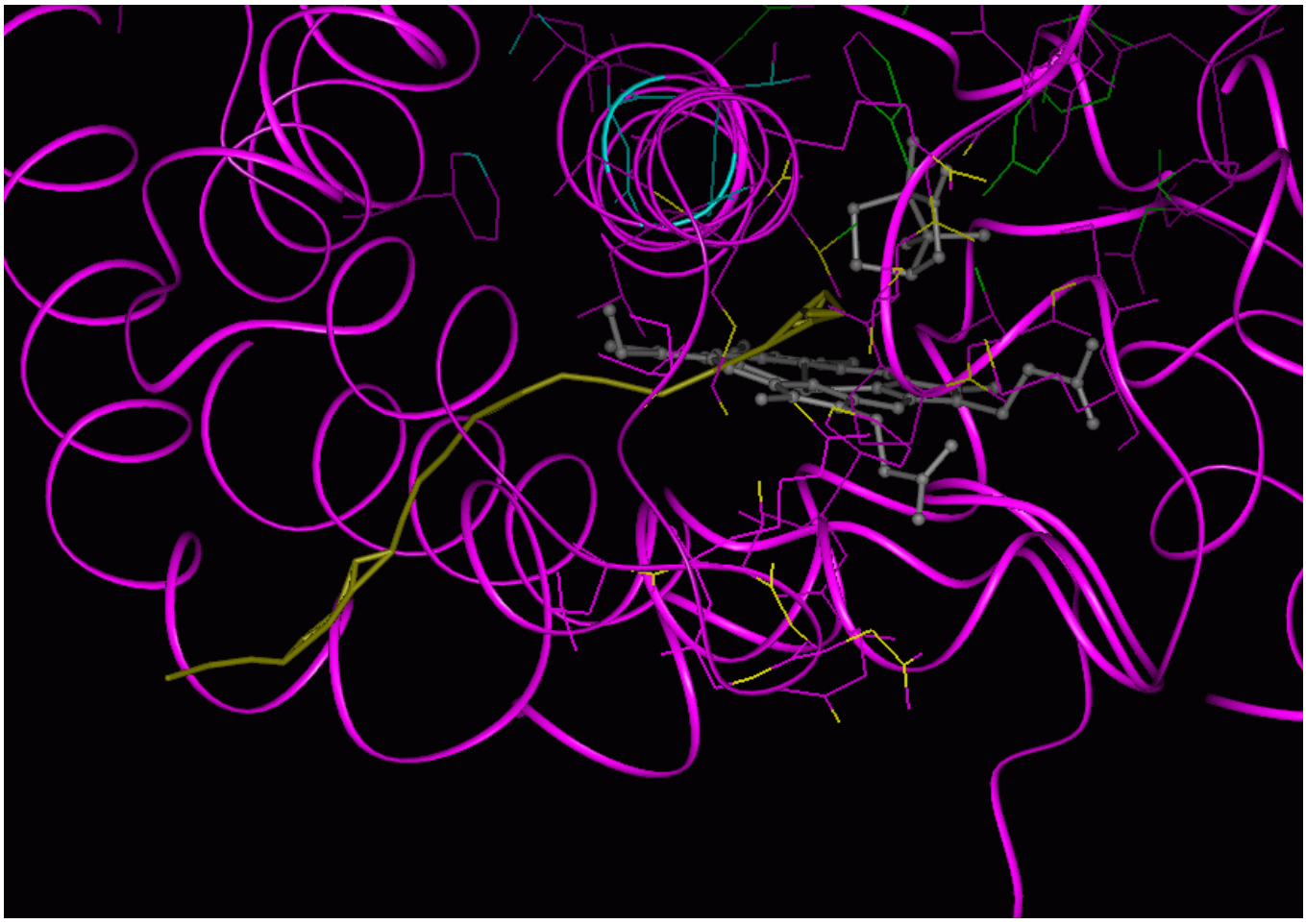

Figure 2. *Comparison between TMPF1 and TRAJ1. Atoms belonging to TMPF1 are coloured in yellow. The trace drawn by the center of mass of camphor along its expulsion trajectory is also shown in yellow.*

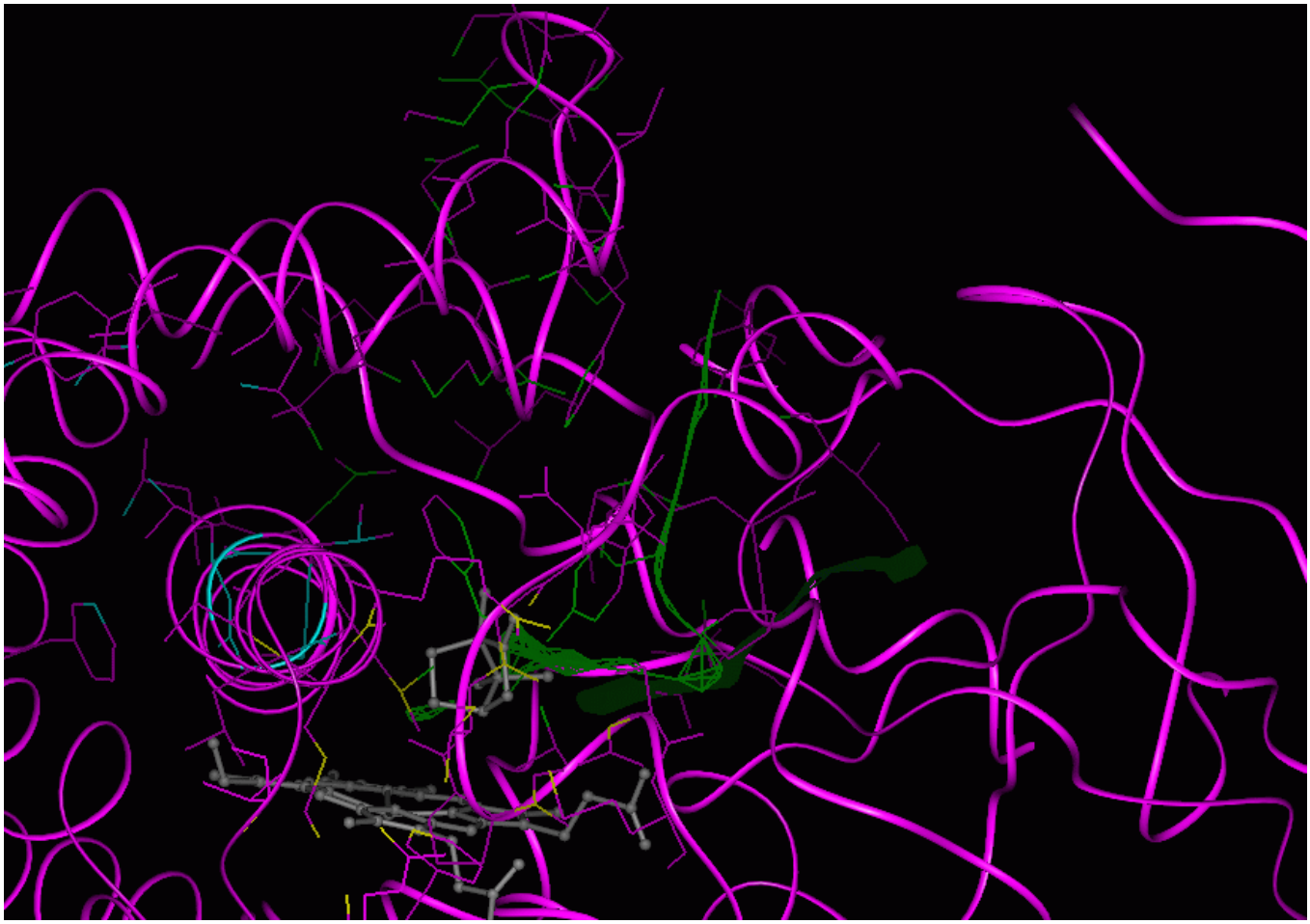

Figure 3. *Comparison between TMPF2 and TRAJ2a and TRAJ2b. Atoms belonging to TMPF2 are displayed in green. The trace of center of mass of camphor along TRAJ2a is shown in light gree and that of TRAJ2b in dark green.*

sen. If the threshold distance was not covered, a new vector was randomly chosen, so that a new direction for camphor expulsion was probed. Six simulations were run and four exit channels found, all four being close to the TMPs. Two of them are near to each other: TRAJ2a and TRAJ2b, where the abbreviation "TRAJ" stands for trajectory. The nomenclature for the simulation results is such that TRAJ1 is close to TMPF1, and so on.

Results and Discussion

The location of pathways found by TMP analysis within P450cam secondary structure elements (Figure 1) are as follows:

1. Atoms of TMPF1 are located within the I-helix, the B'-C loop, and the β^2-sheet.
2. Atoms of TMPF2 are within the I-helix, the B-B' loop. the B'helix, the β^3-sheet, the F&G helices, and the F-G loop,
3. Atoms of TMPF3 are located within the I-helix, the F&G helices, the E-F loop, and the E helix.

Comparison of pathways found by TMP and MDS

Table 1 shows the overlap between the set of atoms delineating the exit channel in MDS (protein non-hydrogen atoms within 3.5 Å of any camphor atom upon expulsion were considered) and the whole set of atoms belonging to a TMPF and the pathway with the highest individual $<B>_{max}$, respectively. Comparison was performed on an atom as well as a residue basis.

The best atom overlap is obtained for TMPF3: 65% of the atoms and 79% of the residues of TRAJ3 and TMPF3 overlap. A MDS pathway is necessarily a subset of TMPF, as a TMPF is a result of clustering of several individual TMPs. Therefore a 100% overlap between TRAJi and TMPFi is not to be expected. This can also be seen from Figure 4, where TRAJ3 and TMPF3 are shown.

Satisfactory overlap is also observed for TRAJ2a (Figure 3). TRAJ1 and TRAJ2b initially reside within TMPFs 1 and 2, resp., but show deviations during the later stage of expulsion (Figures. 2 and 3).

In order to investigate whether atoms displaced during camphor expulsion in MDS are similar to the atoms belong-

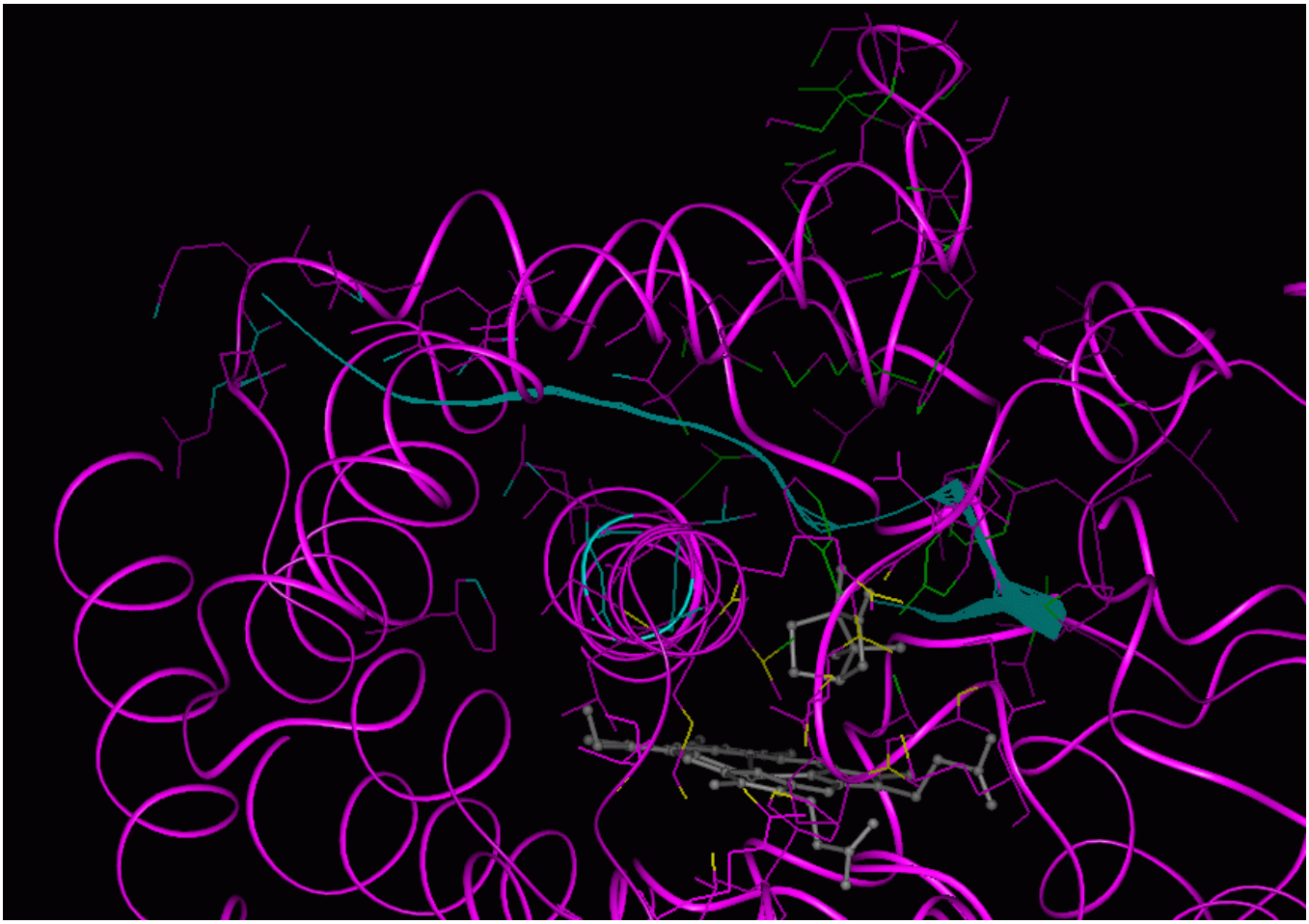

Figure 4. *Comparison between TMPF3 and TRAJ3. Atoms belonging to TMPF3 and the trace of center of mass of camphor along TRAJ3 are shown in blue.*

ing to a TMPF, temperature factors of atoms *k*, B_k^{TRAJi}, observed during trajectory i were averaged over the atoms of TMPFj, yielding

$$\left\langle B^{TRAJi} \right\rangle_{TMPFj} = \frac{1}{N_j} \sum_{k \in TMPFj} B_k^{TRAJi}$$

N_j is the total number of atoms of TMPFj. If the atoms in a TMPF are the ones that are displaced during camphor exit in MDS, then the following inequalities are observed:

$$\langle B \rangle_{TMPF1}^{TRAJ1} > \langle B \rangle_{TMPF2}^{TRAJ1} \quad \text{and} \quad \langle B \rangle_{TMPF1}^{TRAJ1} > \langle B \rangle_{TMPF3}^{TRAJ1}.$$

With the exception of TRAJ2b these inequalities can be observed, as the data in Table 2 show.

Conclusions

From crystallographic data, the only putative pathway proposed, has been a small opening above the active site located near the residues 185, 87 and 395 [2] . This pathway corresponds to the TMPF2 and is supported by results obtained with the random expulsion MDS method (TRAJ2a). Good correspondence between both methods was also found for a second pathway (TMPF3) located along the F-G helices, E-helix and E-F loop. The pathway with the highest average B-factor, TMPF1, however could not be observed with the random expulsion MDS method. Further details of the MDS will be reported elsewhere.

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Table 2. *Comparison of protein mobility along TMPFs and MDS trajectories*

	$<B>_{min}/Å^2$	$<B>_{max}/Å^2$	$<B>_{fam}/Å^2$	n [a]	m [b]	TRAJ1 $<B>/Å^2$	TRAJ2a $<B>/Å^2$	TRAJ2b $<B>/Å^2$	TRAJ3 $<B>/Å^2$
TMPF1	27.38	31.80	30.85	7	32	14.2	18.1	8.9	17.8
TMPF2	21.50	28.63	25.67	13	64	7.2	24.0	7.4	22.3
TMPF3	20.40	25.23	23.51	18	26	9.6	10.0	6.4	24.4

[a] number of pathways
[b] number of atoms

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