## Protocol S1: Comparison of CAVER 3.0, MOLE 1.2 and MolAxis 1.4

The crystal structures were prepared for the comparison by removing all ligands with the exception of HEM. Atoms with alternate conformations were retained in the conformation with higher occupancy. Analyses by CAVER 3.0 were performed using the default settings except for the frame clustering threshold of 2 and the probe radius of $0.6 \AA$. The starting points, shell radius, the number of approximating balls and the application of the average surface were adjusted for each protein in the test set as follows:

| PDB-ID | Starting point $(\mathbf{x}, \mathbf{y}, \mathbf{z}$ coordinates) |  | Shell radius $[\boldsymbol{\AA}]$ | No. of approximating <br> spheres | Average surface |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 2OAR | 62.150 | 160.98 | -0.050 | 15 | 12 |
| 2BG9 | 61.170 | 63.440 | 23.860 | 15 | 4 |
| 1BL8 | 82.530 | 26.552 | 46.581 | 5 | 12 |
| 1AKD | 17.012 | 24.139 | 7.790 | 3 | 12 |
| 2ACE | 4.397 | 68.330 | 63.294 | 5 | 12 |
| 1MXT | 17.083 | -0.533 | 12.495 | 3 | 12 |
| 1MO | no | yes |  |  |  |
| 1THG | 56.786 | 17.650 | 16.101 | 5 | 12 |
| 2JBV | 84.060 | 35.959 | 74.901 | 3 | 12 |

MOLE 1.2 [1] was set to calculate 50 tunnels for each protein in the test set. The calculation was performed without clustering in order to retrieve all tunnels, including those of high similarity. The starting points were set as follows:

| PDB | Starting point ( $\mathbf{x}, \mathbf{y}, \mathbf{z}$ coordinates) |  |  |
| :---: | :---: | :---: | ---: |
| 2OAR | 62.150 | 160.980 | -0.050 |
| 2BG9 | 61.170 | 63.440 | 23.860 |
| 1BL8 | 82.530 | 26.552 | $46 . .581$ |
| 1AKD | 17.012 | 24.139 | 7.790 |
| 2ACE | 4.397 | 68.330 | 63.294 |
| 1MXT | 17.083 | -0.533 | 12.495 |
| 1MQF | 56.786 | 17.650 | 16.101 |
| 1THG | 51.745 | 4.591 | 7.890 |
| 2JBV | 84.060 | 35.959 | 74.901 |

Analyses by MolAxis $1.4[2,3]$ were performed using approximation resolution of $0.1 \AA$. For chamber proteins, the calculations were started from a point within $3 \AA$ of the user-specified starting point. For transmembrane proteins (2BG9, 1BL8 and 2OAR), the search for the channel was confined to a "via sphere" of $3 \AA$ radius centered at the user-specified point along the channel vector. The radius of the outer sphere and the display cutoff were adjusted in individual cases.

| PDB | Starting point $(\mathbf{x}, \mathbf{y}, \mathbf{z}$ coordinates) |  | Outer sphere <br> radius $[\AA]$ | Display radius <br> cutoff $[\AA]$ | Channel <br> vector |  |
| :---: | :---: | ---: | ---: | :---: | :---: | :---: |
| 2OAR | $27.654^{a}$ | $129.514^{a}$ | $1.14^{a}$ | 50 | 11 | $1,1,1$ |
| 2BG9 | $61.170^{a}$ | $63.440^{a}$ | $23.860^{a}$ | 140 | 12 | $0,0,1$ |
| 1BL8 | $82.530^{a}$ | $26.552^{a}$ | $46.581^{a}$ | 70 | 5 | $0,0,1$ |
| 1AKD | 17.012 | 24.139 | 7.790 | 30 | 3.6 | - |
| 2ACE | 4.397 | 68.330 | 63.294 | 30 | 3.5 | - |
| 1MXT | 17.083 | -0.533 | 12.495 | 30 | 4 | - |
| 1MQF | 56.786 | 17.650 | 16.101 | 30 | 4 | - |
| 1THG | 51.745 | 4.591 | 7.890 | 30 | 4 | - |
| 2JBV | 84.060 | 35.959 | 74.901 | 30 | 3 | - |

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## References

1. Petrek M, Kosinova P, Koca J, Otyepka M (2007) MOLE: a Voronoi diagram-based explorer of molecular channels, pores, and tunnels. Structure 15: 1357-1363.
2. Yaffe E, Fishelovitch D, Wolfson HJ, Halperin D, Nussinov R (2008) MolAxis: efficient and accurate identification of channels in macromolecules. Proteins 73: 72-86.
3. Yaffe E, Fishelovitch D, Wolfson HJ, Halperin D, Nussinov R (2008) MolAxis: a server for identification of channels in macromolecules. Nucleic Acids Res 36: W210-215.

[^0]:    ${ }^{a}$ center of the "via sphere"

