Supplementary Figures

Figure S1. Dependence of input parameters within *trj_cavity* upon cavity characterization. **(A)** Mean internal cavity surface (purple spheres) calculated for Der p 2, using different values of the *-dim* option, as indicated inset, and a value of 1.4 Å for the *–spacing* option. The estimated volumes are 1.31 nm^3 (dim = 2, 3); 1.25 nm^3 (dim = 4), 0.87 nm^3 (dim = 5), and 0.75 nm^3 (dim = 6). The protein is shown perpendicular to the cavity entrance axis (top panel), and rotated by 90° (bottom panel), with both cartoons representation and all-atoms overlapping in CPK format to highlight the source of cavity exit/entry tunnels. **(B)** Mean internal cavity surface (purple spheres) calculated for Der p 2, using different values of the *–spacing* option, as indicated inset, and a value of 6 for the *–dim* option. The crystal structure of Der p 2 (left) with two modelled bound lysophosphatidylcholine molecules is compared to cavities using grid sizes of 0.5 Å (volume = 1.58 nm^3); 0.8 Å (volume = 0.95 nm^3**)**; and 1.4 Å (volume = 0.75 nm^3**)**. Protein is shown in cartoons representation. **(C)** Mean internal cavity surface calculated for Der p 2, using alternative input index groups (specified using the *–n* option), and values of 6 for the *-dim* option and 1.4 Å for the *–spacing* option. Two different index groups were specified, corresponding to either the protein region coloured in red alone, or the protein regions including both red and cyan (i.e. the entire protein); the corresponding internal cavities are displayed in opaque blue spheres or transparent blue surface, respectively. Protein is displayed in cartoons representation.

Figure S2. Predicted sugar binding mode in $PulA_{Kox.}$ In **(A)** the internal cavity for $PulA_{Kox}$ detected by *trj_cavity* (transparent blue) is shown, with possible sugar-binding (purple/orange sticks format) and catalytic (spacefill format) residues shown in violet, and in **(B)** the approximate predicted site for recognition of sugar substrate (CPK sticks format) is highlighted, following structural alignment with $PulA_{KPn}$. Binding would be sterically blocked by overlap of cavities in N1 and A domains. The protein is shown in cartoons format, coloured according to the five domains: N1 (dark blue), N2 (green), N3 (cyan), A (yellow) and C (purple). Membrane lipids are shown in CPK sticks format.

Figure S3. Quantitative measures of dynamic protein and cavity descriptors. **(A)** Comparison of the cavity volume estimated using *trj_cavity* (red line) with that from MDpocket (black line), calculated for the Der p 2 hydrophobic cavity during 100 ns of simulation. **(B)** Protein

backbone root-mean-squared deviation (RMSD) calculated for Der p 2. **(C)** Comparison of the bottleneck radius estimated using *trj_cavity* (red line) with that from Caver (black line), calculated for the NavMs channel during 1000 ns of simulation in the presence of sodium.

Figure 1

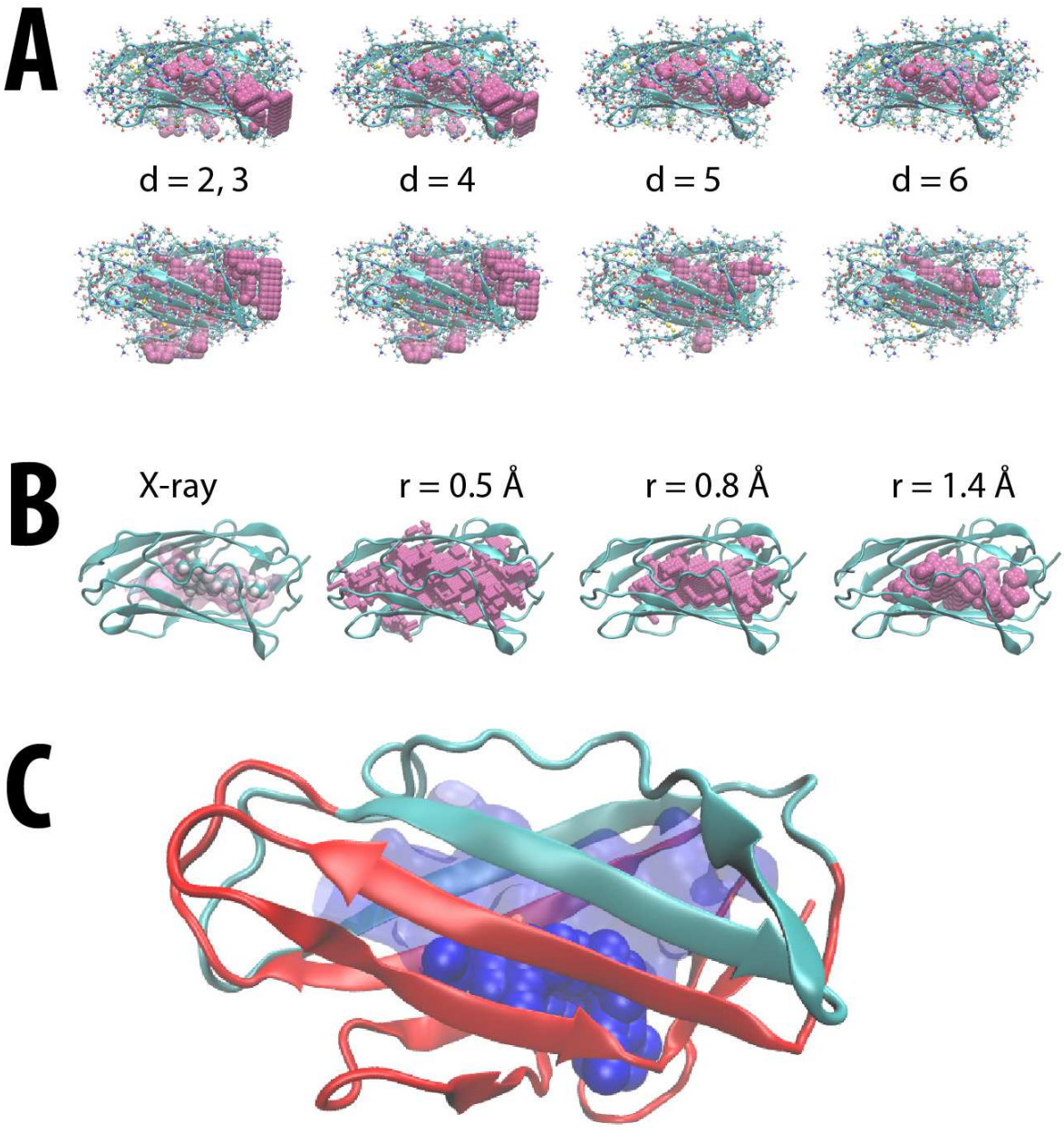


Figure 2

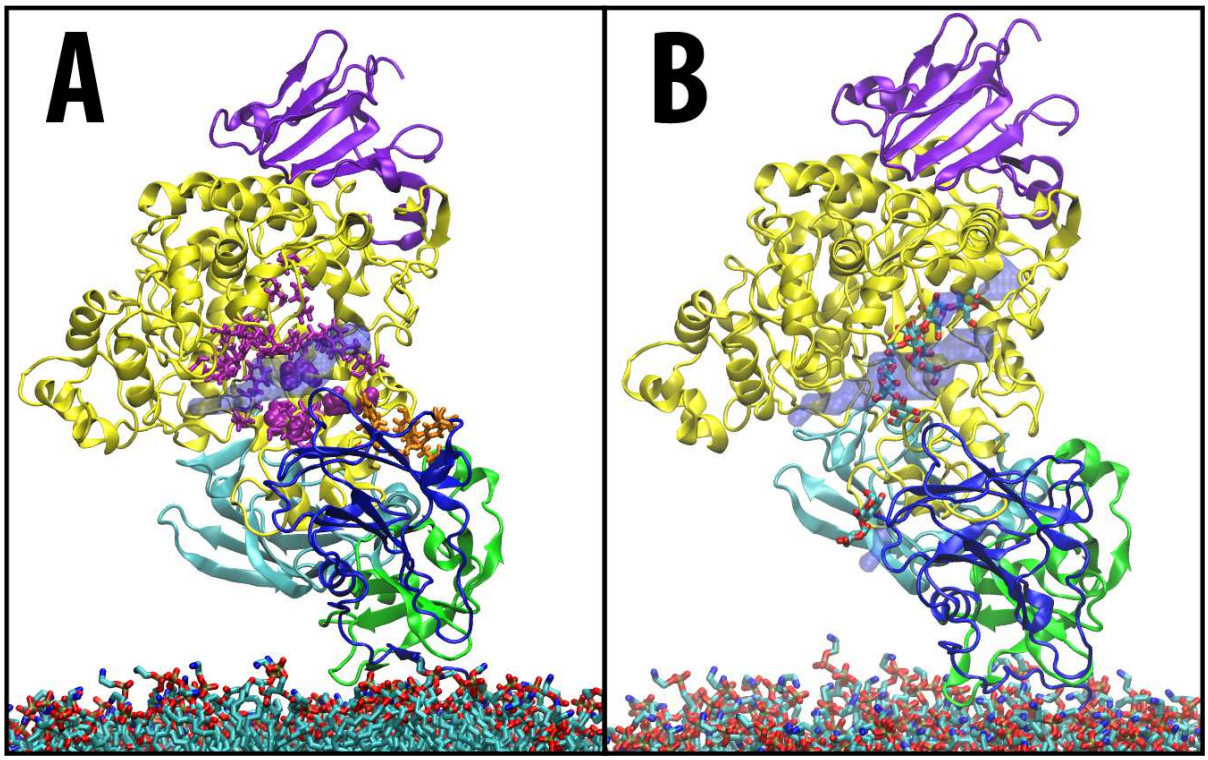
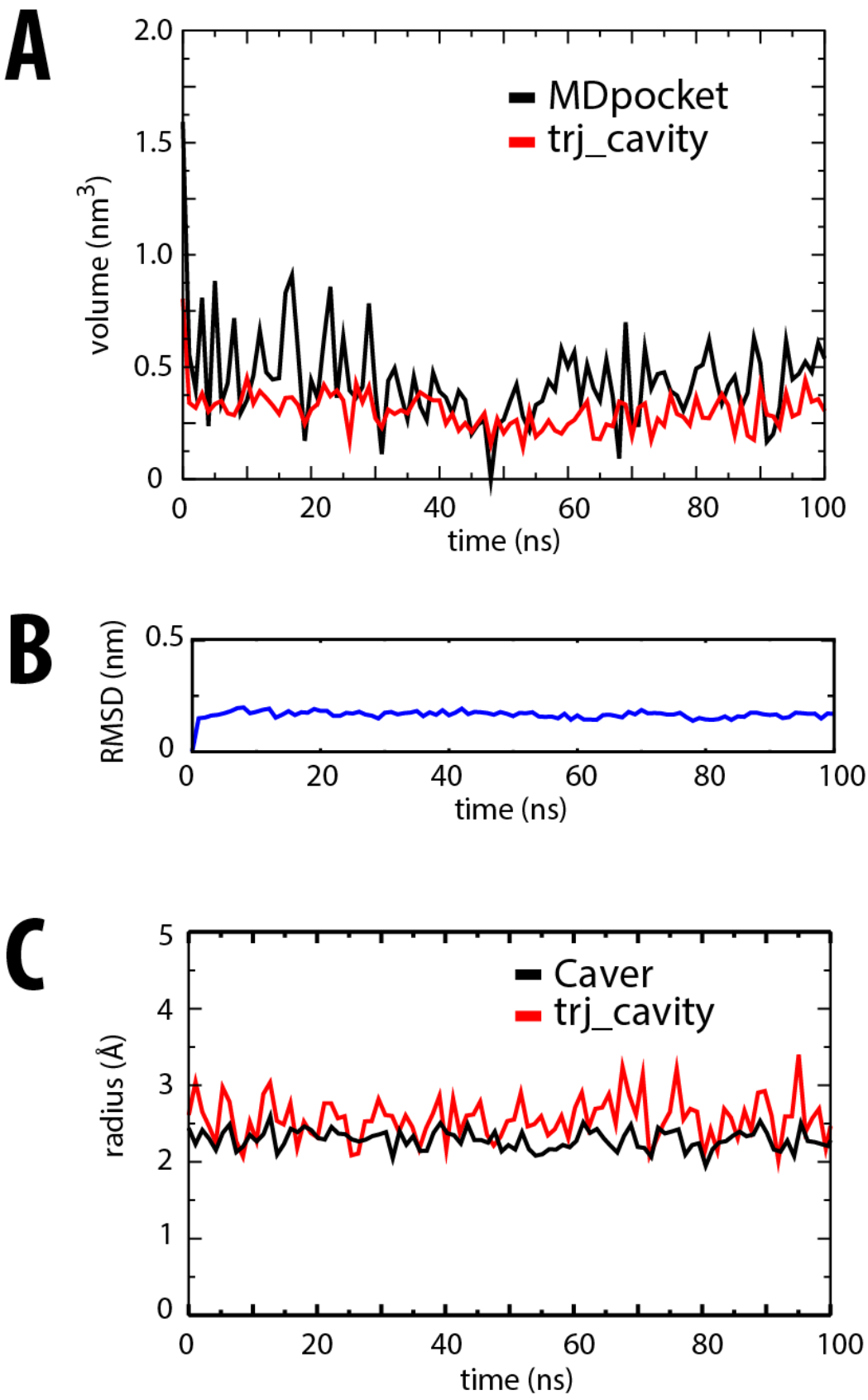


Figure 3


A
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1.5
1.0
0.5
0
volume (nm3)
MDpocket
trj_cavity
0
20
40
60
80
100
time (ns)
B
0.5
0
RMSD (nm)
0
20
40
60
80
100
time (ns)
C
5
4
3
2
1
0
radius (Å)
Caver
trj_cavity
0
20
40
60
80
100
time (ns)