PacDOCK: a web server for the comparison and the clustering of molecular docking results

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SUPPLEMENTARY DATA

S1 ADDITIONAL MATERIALS AND METHODS

All the re-dockings were computed with OpenEye programs: MakeReceptor, OMEGA, and HYBRID [1].

The receptor files were downloaded from RCSB PDB [2] and set up using MakeReceptor. The docking box enclosing the active site was determined by creating an initial box around the bound ligand and then extending each side of the box by 5 Å around the ligand.

The ligand of the structures 5CGC (51D) and 1DNE (netropsin) were downloaded in SDF format from RSCB PDB, while the ligand of the structure 6DDF (DAMGO) and the other ligands used for comparison with other web servers were extracted from the complex structure using MakeReceptor.

For 51D, netropsin, and DAMGO, a file containing 10000 ligand conformers was generated using OMEGA with the mode "pose". The minimum Root Mean Square Cartesian distance below which two conformers are duplicates (RMS) was set to 0.1 Å. For the ligand used for comparison with other web servers, the same software was used to generate 1000 conformers with an RMS of 0.5 Å.

The three docking calculations in the case of 51D, netropsin, and DAMGO were run using HYBRID, generating 10000 docked poses for each ligand with a docking resolution set to "High" (translational step-size of 1.0 Å and rotational step-size of 1.0 Å). For the other ligands, 100 poses per ligand were generated and the resolution was set to "Standard" (translational step-size of 1.0 Å and rotational step-size of 1.5 Å).

Regarding the clustering cut-off selection in the following examples, the commonly accepted cut-off of 2.0 Å may be useful in the case of flexible ligands. On the other hand, in the case of more rigid ligands the use of such a cut-off can produce only one cluster. Therefore, it may be useful to choose a smaller cut-off (1.0 or 0.5 Å in our examples) to better distinguish between the various poses.

S2   USAGE EXAMPLE: 5CGC

ProRMSD performs the RMSD calculation after matching the atoms of the ligand in the experimental pose and the re-docked pose on the basis of the atom type, the atoms to which they are bound, and the bond order.

Table S1. Atom indexes for the experimental and re-docked poses of the ligand.

Table S2. Atom matching between ligand atoms in the experimental pose and the re-docked pose.

Experimental pose		Re-docked pose	
Atom index	Coordinates (x, y, z)	Atom index	Coordinates (x, y, z)
1 Cl	-24.1790 20.8910 44.1790	1 C	-21.0621 18.4565 47.2079
2 F	-25.0390 18.2510 43.3230	2 C	-25.9419 13.3724 39.3465
3 C	-24.0300 18.1900 44.2130	3 C	-22.4757 17.1703 45.6821
4 C	-23.5200 19.3770 44.7100	4 C	-22.6665 19.5780 45.7416
5 C	-22.4900 19.3650 45.6240	5 C	-24.3337 15.7216 42.8659
6 C	-21.9580 18.1510 46.0550	6 C	-25.7355 14.7591 39.2197
7 C	-22.4660 16.9530 45.5590	7 C	-25.5596 13.0846 40.6340
8 C	-23.5090 16.9640 44.6400	8 C	-24.1563 13.6085 44.5132
9 C	-24.0150 15.6600 44.1290	9 C	-22.0765 18.4012 46.2028
10 C	-24.4050 15.5000 42.7970	10 C	-23.4648 17.1162 44.7000
11 C	-24.8580 14.2550 42.3780	11 C	-24.0548 18.2930 44.2389
12 N	-24.8980 13.2540 43.2510	12 C	-23.6557 19.5239 44.7597
13 C	-24.5130 13.4360 44.4990	13 C	-23.8754 15.8368 44.1625
14 N	-24.0830 14.6030 44.9380	14 C	-24.6948 14.4496 42.4712
15 N	-25.2490 14.0660 41.0520	15 N	-20.2383 18.5014 48.0241
16 C	-25.5550 12.8780 40.4720	16 N	-25.2592 15.3139 40.3375
17 C	-25.8690 13.1270 39.1830	17 N	-23.7733 14.7974 45.0166
18 C	-25.7500 14.5050 38.9610	18 N	-24.6185 13.3662 43.2717
19 N	-25.3770 15.0660 40.0800	19 N	-25.1633 14.2704 41.1789
20 C	-20.8890 18.1419 47.0030	20 F	-25.0062 18.2416 43.2930
21 N	-20.0410 18.1570 47.7560	21 Cl	-24.3787 20.9806 44.1940

Experimental pose		Re-docked pose	
Atom index	Coordinates (x, y, z)	Atom index	Coordinates (x, y, z)
1 Cl	-24.1790 20.8910 44.1790	21 Cl	-24.3787 20.9806 44.1940
2 F	-25.0390 18.2510 43.3230	20 F	-25.0062 18.2416 43.2930
3 C	-24.0300 18.1900 44.2130	11 C	-24.0548 18.2930 44.2389
4 C	-23.5200 19.3770 44.7100	12 C	-23.6557 19.5239 44.7597
5 C	-22.4900 19.3650 45.6240	4 C	-22.6665 19.5780 45.7416
6 C	-21.9580 18.1510 46.0550	9 C	-22.0765 18.4012 46.2028
7 C	-22.4660 16.9530 45.5590	3 C	-22.4757 17.1703 45.6821
8 C	-23.5090 16.9640 44.6400	10 C	-23.4648 17.1162 44.7000
9 C	-24.0150 15.6600 44.1290	13 C	-23.8754 15.8368 44.1625
10 C	-24.4050 15.5000 42.7970	5 C	-24.3337 15.7216 42.8659
11 C	-24.8580 14.2550 42.3780	14 C	-24.6948 14.4496 42.4712
12 N	-24.8980 13.2540 43.2510	18 N	-24.6185 13.3662 43.2717
13 C	-24.5130 13.4360 44.4990	8 C	-24.1563 13.6085 44.5132
14 N	-24.0830 14.6030 44.9380	17 N	-23.7733 14.7974 45.0166
15 N	-25.2490 14.0660 41.0520	19 N	-25.1633 14.2704 41.1789
16 C	-25.5550 12.8780 40.4720	7 C	-25.5596 13.0846 40.6340
17 C	-25.8690 13.1270 39.1830	2 C	-25.9419 13.3724 39.3465
18 C	-25.7500 14.5050 38.9610	6 C	-25.7355 14.7591 39.2197
19 N	-25.3770 15.0660 40.0800	16 N	-25.2592 15.3139 40.3375
20 C	-20.8890 18.1419 47.0030	1 C	-21.0621 18.4565 47.2079
21 N	-20.0410 18.1570 47.7560	15 N	-20.2383 18.5014 48.0241

Table S3. RMSD values for the re-docked poses of the ligand with respect to the experimental pose calculated by using ProRMSD and two similar web servers: DockRMSD and LigRMSD [3, 4].

	ProRMSD	DockRMSD	LigRMSD
RMSD	0.297	0.297	0.30

Note: RMSD value for the web server LigRMSD was reported with two decimal digits, as this is how the value is reported by the web server.

ClusDOCK performs the cluster analysis with three different algorithms. In this example, the gromos algorithm was employed, with a cut-off of 0.5 Å and a minimum cluster size of 1 structure. The steps that lead to cluster formations are illustrated below, in Table S4-S5.

Table S4. Pairwise RMSD matrix (10×10) in which each element contains the RMSD of the ith binding pose calculated with respect to the jth binding pose of the re-docking. Considering a cut-off of 0.5 Å, the number of neighbours is calculated (RMSD values below the cut-off are highlighted in gold). Structures 1 and 3 have the highest number of neighbours, i.e., six. Among these, the first pose (highlighted in green) has the best scoring function, thus it represents the centre of the most populated cluster, and forms together with all its neighbours (poses no. 2, 3, 4, 6, 8, and 9; highlighted in yellow) a cluster. Therefore, the first cluster is composed of structures 1, 2, 3, 4, 6, 8, and 9, and presents the scoring function of structure 1, the centroid of the cluster. These structures are thereafter eliminated from the pool of structures.

Pose no.	1	2	3	4	5	6	7	8	9	10
1	0	0.478	0.393	0.350	0.750	0.485	2.682	0.361	0.403	1.048
2	0.478	0	0.290	0.472	0.665	0.302	2.662	0.531	0.606	0.907
3	0.393	0.290	0	0.286	0.614	0.427	2.650	0.362	0.414	0.859
4	0.350	0.472	0.286	0	0.566	0.505	2.669	0.352	0.307	0.835
5	0.750	0.665	0.614	0.566	0	0.734	2.711	0.689	0.664	0.512
6	0.485	0.302	0.427	0.505	0.734	0	2.640	0.523	0.581	0.893
7	2.682	2.662	2.650	2.669	2.711	2.640	0	2.653	2.661	2.743
8	0.361	0.531	0.362	0.352	0.689	0.523	2.653	0	0.143	0.869
9	0.403	0.606	0.414	0.307	0.664	0.581	2.661	0.143	0	0.844
10	1.048	0.907	0.859	0.835	0.512	0.893	2.743	0.869	0.844	0
No. of neighbours	6	4	6	5	0	3	0	4	4	0

Table S5. Pairwise RMSD matrix (3×3) recalculated after the elimination of the first cluster structures. The values of RMSD calculated between the three remaining structures are higher than the cut-off, thus they remain as singleton clusters, each one with its relative scoring function.

Pose no.	5	7	10
5	0	2.711	0.512
7	2.711	0	2.743
10	0.512	2.743	0
No. of neighbours	0	0	0

Figure S1. Result of cluster analysis with the gromos algorithm on 51D re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	7	1, 2, 3, 4, 6, 8, 9	−17.532913
2	1	5	−16.862759
3	1	7	−16.757294
4	1	10	−16.584923

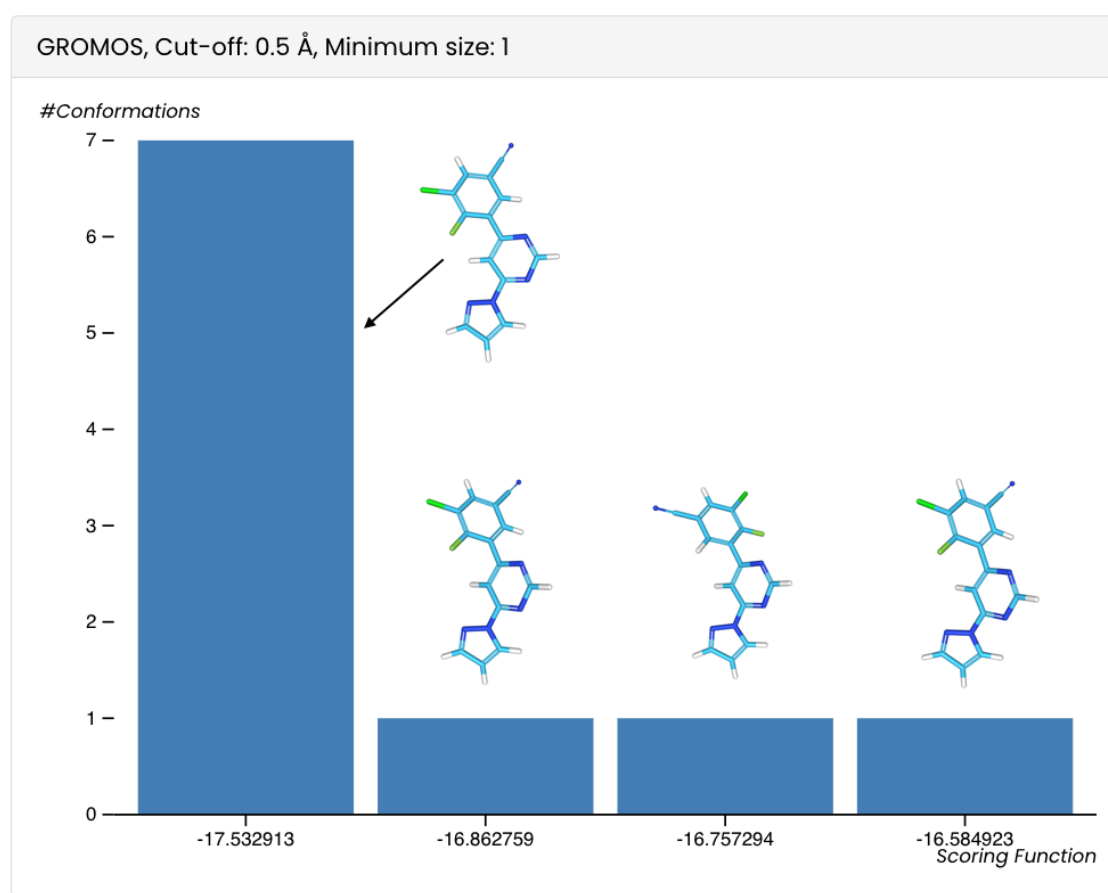GROMOS, Cut−off: 0.5 Å, Minimum size: 1

Figure S2. Result of cluster analysis with the single-linkage algorithm on 51D re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	7	1, 2, 3, 4, 6, 8, 9	−17.532913
2	1	5	−16.862759
3	1	7	−16.757294
4	1	10	−16.584923

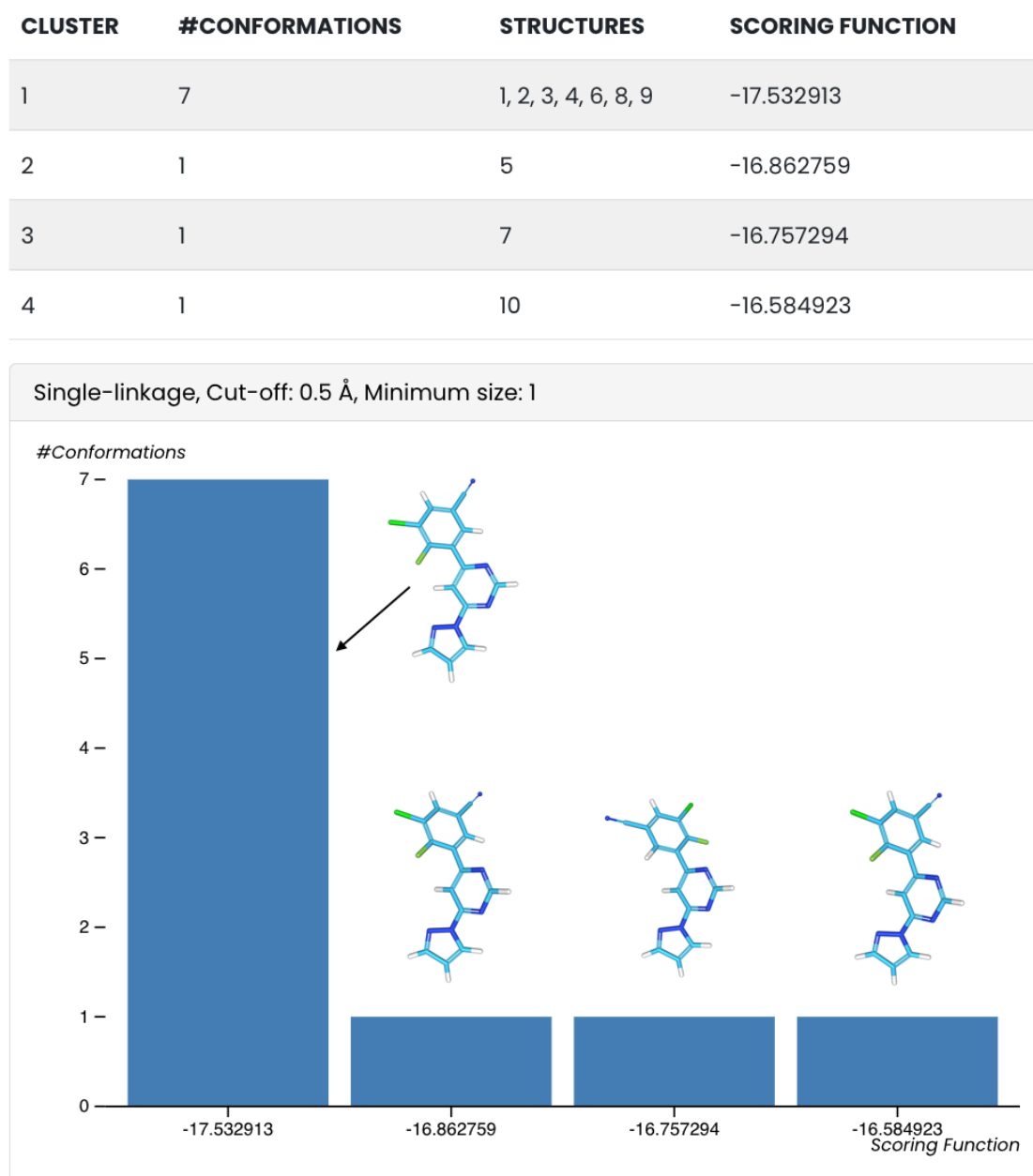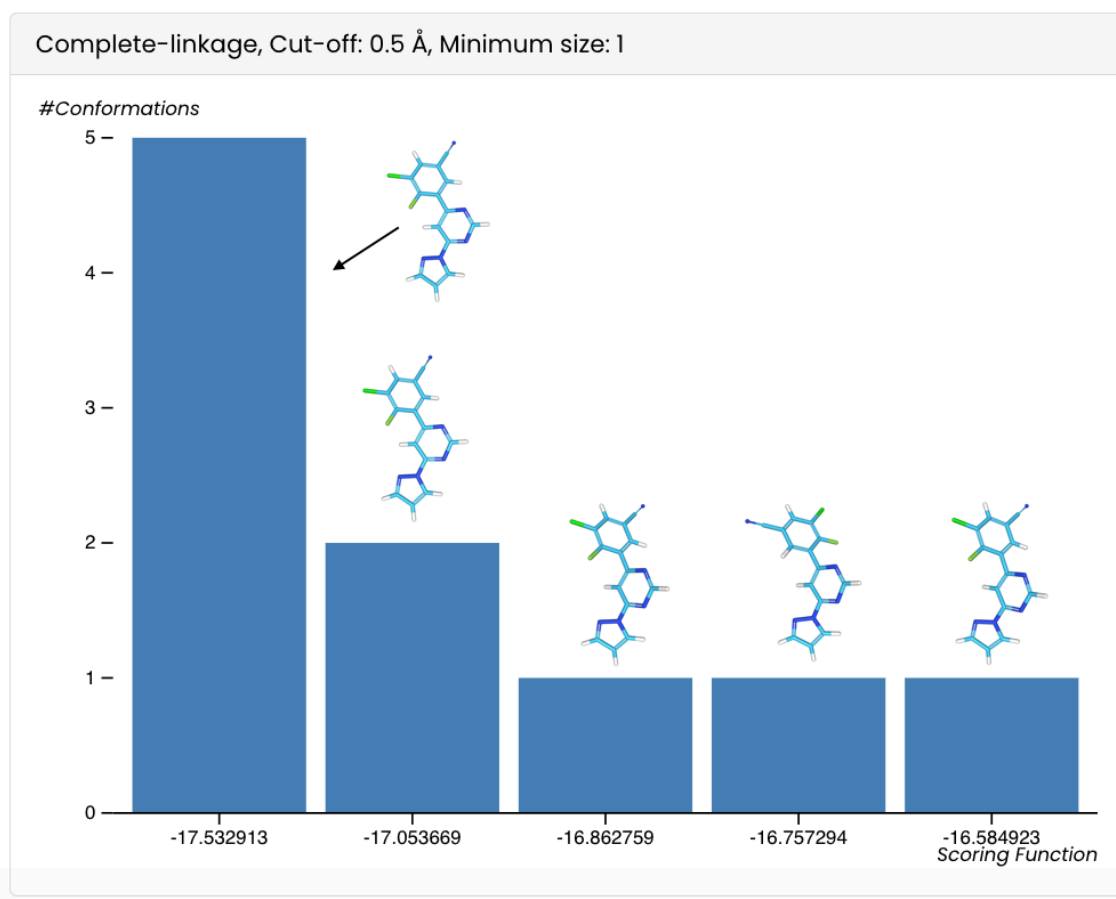Single-linkage, Cut-off: 0.5 Å, Minimum size: 1

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Figure S3. Result of cluster analysis with the complete-linkage algorithm on 51D re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	5	1, 3, 4, 8, 9	-17.532913
2	2	2, 6	-17.053669
3	1	5	-16.862759
4	1	7	-16.757294
5	1	10	-16.584923

In this example, the use of the gromos and single-linkage algorithms returns the same clusters, while the complete-linkage algorithm allows one more cluster to be identified by splitting the most populated cluster obtained in the other cases into two smaller assemblies.

S3   USAGE EXAMPLE: 1DNE

ProRMSD performs the RMSD calculation after matching the atoms of the ligand in the experimental pose and the re-docked pose on the basis of the atom type, the atoms to which they are bound, and the bond order.

Table S6. Atom indexes for the experimental and re-docked poses of netropsin.

Experimental pose		Re-docked pose	
Atom index	Coordinates (x, y, z)	Atom index	Coordinates (x, y, z)
1 C	12.778 25.090 69.997	1 C	9.900 21.094 78.437
2 N	12.564 23.865 69.582	2 C	10.534 23.415 73.774
3 N	13.724 25.870 69.495	3 C	7.776 21.699 77.997
4 N	11.932 25.495 70.926	4 C	9.901 25.319 72.753
5 C	11.942 26.559 71.802	5 C	9.072 21.819 77.545
6 C	10.694 26.076 72.652	6 C	10.859 24.329 72.741
7 O	9.764 26.699 72.217	7 C	9.086 20.552 79.406
8 N	10.763 24.979 73.300	8 C	9.388 23.873 74.384
9 C	9.797 24.401 74.177	9 C	8.664 23.261 75.511
10 C	10.169 23.638 75.260	10 C	9.489 19.709 80.561
11 C	9.058 23.320 75.956	11 C	12.597 17.172 82.844
12 N	7.991 23.902 75.327	12 C	12.279 25.161 70.834
13 C	6.546 23.869 75.661	13 C	14.986 25.514 68.234
14 C	8.464 24.620 74.250	14 C	6.627 20.569 79.901
15 C	8.757 22.437 77.093	15 C	7.857 25.838 74.085
16 O	7.555 22.157 77.313	16 C	12.054 17.946 81.702
17 N	9.698 21.901 77.868	17 C	13.538 24.717 70.115
18 C	9.308 20.948 78.918	18 C	11.304 19.187 82.170
19 C	10.189 20.461 79.908	19 N	7.797 20.926 79.129
20 C	9.491 19.561 80.697	20 N	9.012 25.031 73.755
21 N	8.199 19.491 80.122	21 N	13.837 17.431 83.233
22 C	7.071 18.674 80.537	22 N	15.260 26.451 67.244
23 C	8.118 20.316 79.072	23 N	9.461 22.523 76.429
24 C	9.843 18.641 81.727	24 N	11.925 24.266 71.875
25 O	9.053 17.837 82.249	25 N	10.761 19.938 81.071
26 N	11.191 18.352 81.915	26 N	13.867 25.658 69.075
27 C	11.390 17.251 82.965	27 N	11.808 16.267 83.405
28 C	12.707 17.156 83.587	28 N	15.833 24.422 68.389
29 C	12.822 16.987 85.085	29 O	7.444 23.368 75.612
30 N	11.790 17.016 85.907	30 O	8.710 18.859 80.984
31 N	14.011 16.822 85.653	31 O	11.676 26.184 70.527

Table S7. Atom matching between netropsin atoms in the experimental pose and the re-docked pose.

Experimental pose		Re-docked pose	
Atom index	Coordinates (x, y, z)	Atom index	Coordinates (x, y, z)
1 C	12.778 25.090 69.997	13 C	14.986 25.514 68.234
2 N	12.564 23.865 69.582	28 N	15.833 24.422 68.389
3 N	13.724 25.870 69.495	22 N	15.260 26.451 67.244
4 N	11.932 25.495 70.926	26 N	13.867 25.658 69.075
5 C	11.942 26.559 71.802	17 C	13.538 24.717 70.115
6 C	10.694 26.076 72.652	12 C	12.279 25.161 70.834
7 O	9.764 26.699 72.217	31 O	11.676 26.184 70.527
8 N	10.763 24.979 73.300	24 N	11.925 24.266 71.875
9 C	9.797 24.401 74.177	6 C	10.859 24.329 72.741
10 C	10.169 23.638 75.260	2 C	10.534 23.415 73.774
11 C	9.058 23.320 75.956	8 C	9.388 23.873 74.384
12 N	7.991 23.902 75.327	20 N	9.012 25.031 73.755
13 C	6.546 23.869 75.661	15 C	7.857 25.838 74.085
14 C	8.464 24.620 74.250	4 C	9.901 25.319 72.753
15 C	8.757 22.437 77.093	9 C	8.664 23.261 75.511
16 O	7.555 22.157 77.313	29 O	7.444 23.368 75.612
17 N	9.698 21.901 77.868	23 N	9.461 22.523 76.429
18 C	9.308 20.948 78.918	5 C	9.072 21.819 77.545
19 C	10.189 20.461 79.908	1 C	9.900 21.094 78.437
20 C	9.491 19.561 80.697	7 C	9.086 20.552 79.406
21 N	8.199 19.491 80.122	19 N	7.797 20.926 79.129
22 C	7.071 18.674 80.537	14 C	6.627 20.569 79.901
23 C	8.118 20.316 79.072	3 C	7.776 21.699 77.997
24 C	9.843 18.641 81.727	10 C	9.489 19.709 80.561
25 O	9.053 17.837 82.249	30 O	8.710 18.859 80.984
26 N	11.191 18.352 81.915	25 N	10.761 19.938 81.071
27 C	11.390 17.251 82.965	18 C	11.304 19.187 82.170
28 C	12.707 17.156 83.587	16 C	12.054 17.946 81.702
29 C	12.822 16.987 85.085	11 C	12.597 17.172 82.844
30 N	11.790 17.016 85.907	27 N	11.808 16.267 83.405
31 N	14.011 16.822 85.653	21 N	13.837 17.431 83.233

Table S8. RMSD values for the re-docked poses of netropsin with respect to the experimental pose calculated by using ProRMSD, DockRMSD, and LigRMSD.

	ProRMSD	DockRMSD	LigRMSD
RMSD	2.219	2.219	2.22

Note: RMSD value for the web server LigRMSD was reported with two decimal digits, as this is how the value is reported by the web server.

ClusDOCK performs the cluster analysis with three different algorithms. In this example, the gromos algorithm was employed, with a cut-off of 1.0 Å and a minimum cluster size of 1 structure. The steps that lead to cluster formations are illustrated below, in Table S9-S14.

Table S9. Pairwise RMSD matrix (20×20) in which each element contains the RMSD of the ith binding pose calculated with respect to the jth binding pose of netropsin re-docking. Considering a cut-off of 1.0 Å, the number of neighbours is calculated (RMSD values below the cut-off are highlighted in gold). Structures 3, 6, and 14 have the highest number of neighbours, i.e., five. Among these, the third pose (highlighted in green) has the best scoring function, thus it represents the centre of the most populated cluster, and forms together with all its neighbours (poses no. 5, 6, 8, 16, and 17; highlighted in yellow) a cluster. Therefore, the first cluster is composed of structures 3, 5, 6, 8, 16, and 17, and presents the scoring function of structure 3, the centroid of the cluster. These structures are thereafter eliminated from the pool of structures.

Pose no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0.166	11.600	3.411	11.609	11.607	4.079	11.602	3.623	0.661	1.147	4.136	1.022	0.933	3.724	11.639	11.612	1.101	11.426	11.449
2	0.166	0	11.602	3.435	11.606	11.612	4.096	11.604	3.650	0.675	1.128	4.162	1.032	0.923	3.749	11.650	11.612	1.039	11.422	11.446
3	11.600	11.602	0	11.732	0.629	0.400	11.413	0.479	11.787	11.614	11.807	11.432	11.797	11.802	11.789	0.910	0.804	11.819	1.275	1.424
4	3.411	3.435	11.732	0	11.794	11.723	1.696	11.775	0.789	3.303	3.110	1.731	3.139	3.373	2.155	11.711	11.696	3.583	11.589	11.550
5	11.609	11.606	0.629	11.794	0	0.854	11.502	0.350	11.873	11.628	11.810	11.531	11.808	11.798	11.803	1.353	0.764	11.796	1.065	1.304
6	11.607	11.612	0.400	11.723	0.854	0	11.383	0.588	11.763	11.605	11.809	11.406	11.799	11.808	11.763	0.559	0.775	11.833	1.382	1.463
7	4.079	4.096	11.413	1.696	11.502	11.383	0	11.473	1.476	3.814	3.713	0.393	3.802	4.079	3.041	11.337	11.340	4.294	11.268	11.178
8	11.602	11.604	0.479	11.775	0.350	0.588	11.473	0	11.841	11.614	11.807	11.498	11.799	11.795	11.782	1.043	0.705	11.803	1.165	1.345
9	3.623	3.650	11.787	0.789	11.873	11.763	1.476	11.841	0	3.432	3.287	1.509	3.314	3.578	2.362	11.728	11.758	3.805	11.679	11.626
10	0.661	0.675	11.614	3.303	11.628	11.605	3.814	11.614	3.432	0	1.035	3.897	1.016	1.057	3.594	11.617	11.600	1.223	11.431	11.431
11	1.147	1.128	11.807	3.110	11.810	11.809	3.713	11.807	3.287	1.035	0	3.796	0.319	0.565	3.355	11.834	11.790	0.817	11.582	11.582
12	4.136	4.162	11.432	1.731	11.531	11.406	0.393	11.498	1.509	3.897	3.796	0	3.868	4.151	3.163	11.353	11.372	4.383	11.305	11.211
13	1.022	1.032	11.797	3.139	11.808	11.799	3.802	11.799	3.314	1.016	0.319	3.868	0	0.444	3.387	11.821	11.796	0.813	11.591	11.599
14	0.933	0.923	11.802	3.373	11.798	11.808	4.079	11.795	3.578	1.057	0.565	4.151	0.444	0	3.455	11.842	11.799	0.474	11.594	11.612
15	3.724	3.749	11.789	2.155	11.803	11.763	3.041	11.782	2.362	3.594	3.355	3.163	3.387	3.455	0	11.755	11.697	3.563	11.594	11.569
16	11.639	11.650	0.910	11.711	1.353	0.559	11.337	1.043	11.728	11.617	11.834	11.353	11.821	11.842	11.755	0	1.073	11.882	1.731	1.688
17	11.612	11.612	0.804	11.696	0.764	0.775	11.340	0.705	11.758	11.600	11.790	11.372	11.796	11.799	11.697	1.073	0	11.812	1.055	1.026
18	1.101	1.039	11.819	3.583	11.796	11.833	4.294	11.803	3.805	1.223	0.817	4.383	0.813	0.474	3.563	11.882	11.812	0	11.593	11.618
19	11.426	11.422	1.275	11.589	1.065	1.382	11.268	1.165	11.679	11.431	11.582	11.305	11.591	11.594	11.594	1.731	1.055	11.593	0	0.512
20	11.449	11.446	1.424	11.550	1.304	1.463	11.178	1.345	11.626	11.431	11.582	11.211	11.599	11.612	11.569	1.688	1.026	11.618	0.512	0
No. of neighbours	3	3	5	1	4	5	1	4	1	2	3	1	3	5	0	2	4	3	1	1

Table S10. Pairwise RMSD matrix (14×14) recalculated after the elimination of the first cluster structures. The structure 14 has the highest number of neighbours, thus it represents the centre of the new cluster, which includes its neighbours (poses no. 1, 2, 11, 13, and 18), and presents the scoring function of structure 14. These structures are thereafter eliminated from the pool of structures.

Pose no.	1	2	4	7	9	10	11	12	13	14	15	18	19	20
1	0	0.166	3.411	4.079	3.623	0.661	1.147	4.136	1.022	0.933	3.724	1.101	11.426	11.449
2	0.166	0	3.435	4.096	3.650	0.675	1.128	4.162	1.032	0.923	3.749	1.039	11.422	11.446
4	3.411	3.435	0	1.696	0.789	3.303	3.110	1.731	3.139	3.373	2.155	3.583	11.589	11.550
7	4.079	4.096	1.696	0	1.476	3.814	3.713	0.393	3.802	4.079	3.041	4.294	11.268	11.178
9	3.623	3.650	0.789	1.476	0	3.432	3.287	1.509	3.314	3.578	2.362	3.805	11.679	11.626
10	0.661	0.675	3.303	3.814	3.432	0	1.035	3.897	1.016	1.057	3.594	1.223	11.431	11.431
11	1.147	1.128	3.110	3.713	3.287	1.035	0	3.796	0.319	0.565	3.355	0.817	11.582	11.582
12	4.136	4.162	1.731	0.393	1.509	3.897	3.796	0	3.868	4.151	3.163	4.383	11.305	11.211
13	1.022	1.032	3.139	3.802	3.314	1.016	0.319	3.868	0	0.444	3.387	0.813	11.591	11.599
14	0.933	0.923	3.373	4.079	3.578	1.057	0.565	4.151	0.444	0	3.455	0.474	11.594	11.612
15	3.724	3.749	2.155	3.041	2.362	3.594	3.355	3.163	3.387	3.455	0	3.563	11.594	11.569
18	1.101	1.039	3.583	4.294	3.805	1.223	0.817	4.383	0.813	0.474	3.563	0	11.593	11.618
19	11.426	11.422	11.589	11.268	11.679	11.431	11.582	11.305	11.591	11.594	11.594	11.593	0	0.512
20	11.449	11.446	11.550	11.178	11.626	11.431	11.582	11.211	11.599	11.612	11.569	11.618	0.512	0
No. of neighbours	3	3	1	1	1	2	3	1	3	5	0	3	1	1

Table S11. Pairwise RMSD matrix (8×8) recalculated. Among the structures that have the highest number of neighbours, the number 4 has the best scoring function, thus it represents the centre of the new cluster, which includes its neighbour (pose no. 9), and presents the scoring function of structure 4. These structures are thereafter eliminated from the pool of structures.

Pose no.	4	7	9	10	12	15	19	20
4	0	1.696	0.789	3.303	1.731	2.155	11.589	11.550
7	1.696	0	1.476	3.814	0.393	3.041	11.268	11.178
9	0.789	1.476	0	3.432	1.509	2.362	11.679	11.626
10	3.303	3.814	3.432	0	3.897	3.594	11.431	11.431
12	1.731	0.393	1.509	3.897	0	3.163	11.305	11.211
15	2.155	3.041	2.362	3.594	3.163	0	11.594	11.569
19	11.589	11.268	11.679	11.431	11.305	11.594	0	0.512
20	11.550	11.178	11.626	11.431	11.211	11.569	0.512	0
No. of neighbours	1	1	1	0	1	0	1	1

Table S12. Pairwise RMSD matrix (6×6) recalculated. Among the structures that have the highest number of neighbours, the number 7 has the best scoring function, thus it represents the centre of the new cluster, which includes its neighbour (pose no. 12), and presents the scoring function of structure 7. These structures are thereafter eliminated from the pool of structures.

Pose no.	7	10	12	15	19	20
7	0	3.814	0.393	3.041	11.268	11.178
10	3.814	0	3.897	3.594	11.431	11.431
12	0.393	3.897	0	3.163	11.305	11.211
15	3.041	3.594	3.163	0	11.594	11.569
19	11.268	11.431	11.305	11.594	0	0.512
20	11.178	11.431	11.211	11.569	0.512	0
No. of neighbours	1	0	1	0	1	1

Table S13. Pairwise RMSD matrix (4×4) recalculated. Among the structures that have the highest number of neighbours, the number 19 has the best scoring function, thus it represents the centre of the new cluster, which includes its neighbour (pose no. 20), and presents the scoring function of structure 19. These structures are thereafter eliminated from the pool of structures.

Pose no.	10	15	19	20
10	0	3.594	11.431	11.431
15	3.594	0	11.594	11.569
19	11.431	11.594	0	0.512
20	11.431	11.569	0.512	0
No. of neighbours	0	0	1	1

Table S14. Pairwise RMSD matrix (2×2) recalculated. The value of RMSD calculated between the two remaining structures is higher than the cut-off, thus they remain as singleton clusters, each one with its relative scoring function.

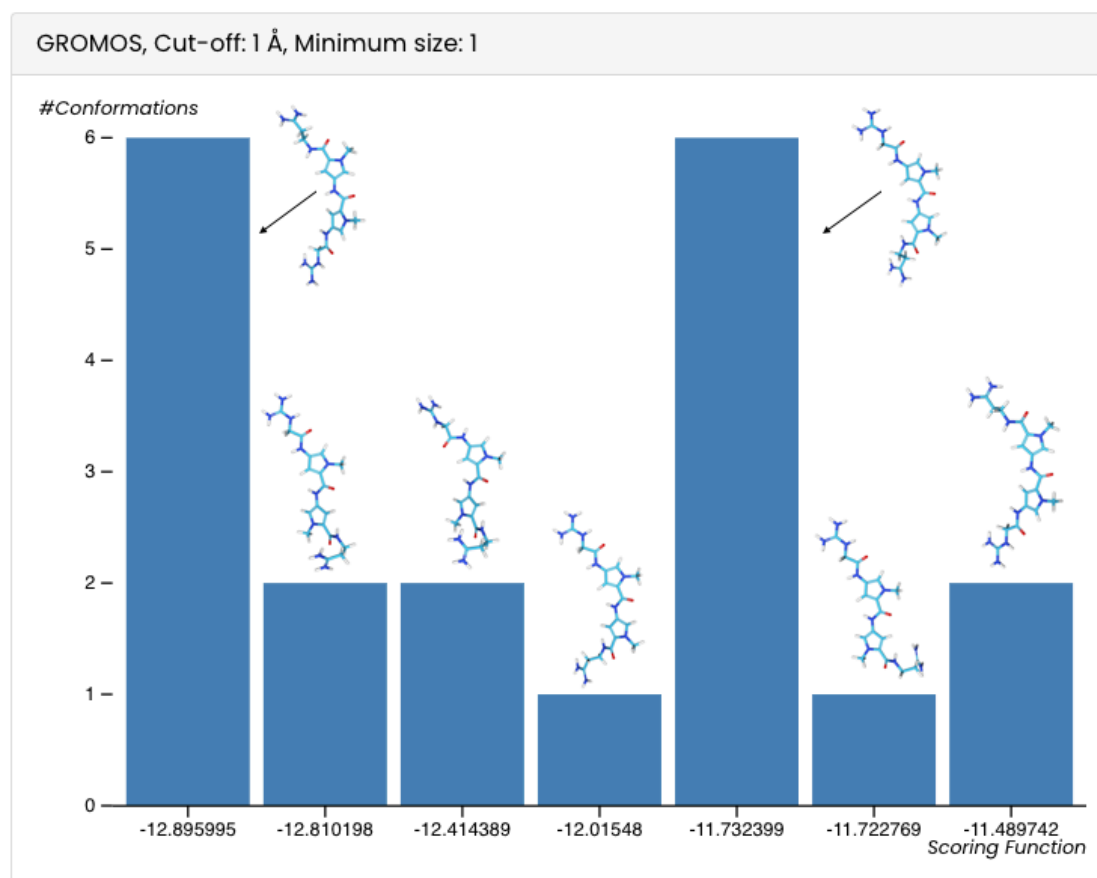| Pose no.	10	15
10	0	3.594
15	3.594	0
No. of neighbours	0	0

Figure S4. Result of cluster analysis with the gromos algorithm on netropsin re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

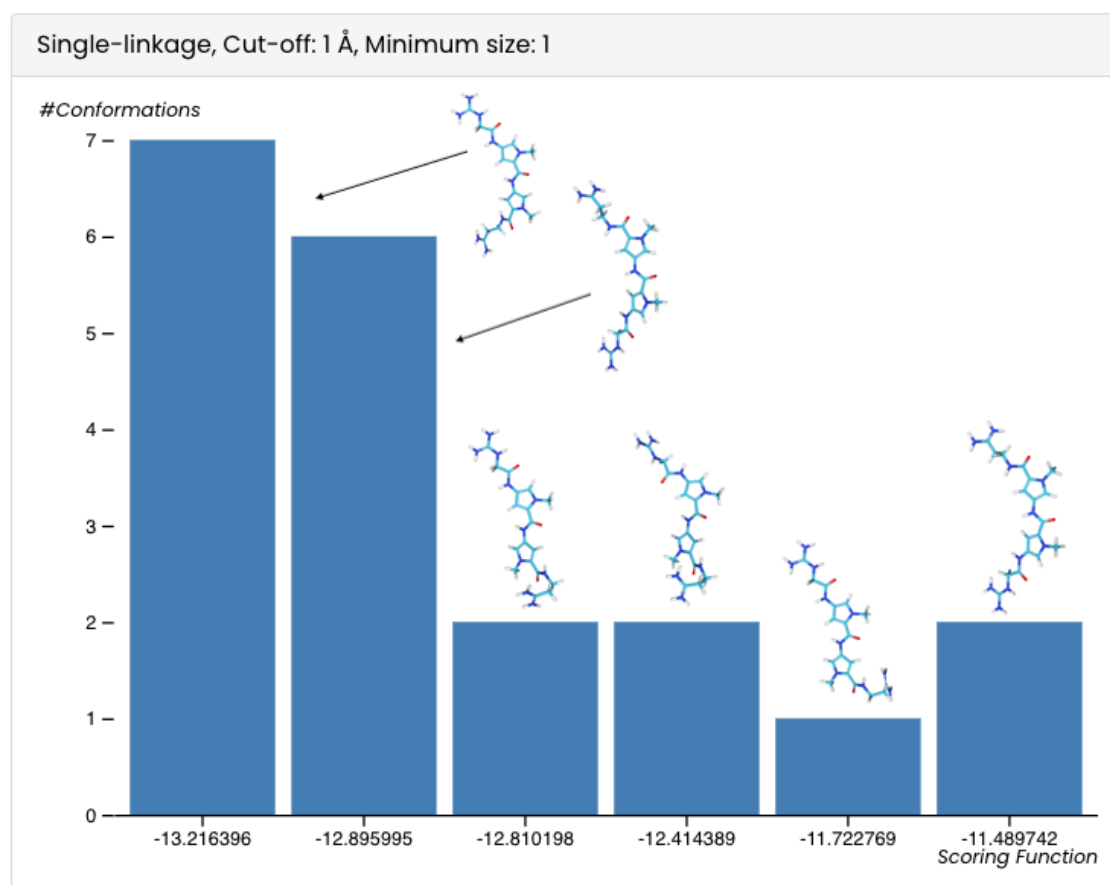| CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	6	3, 5, 6, 8, 16, 17	-12.895995
2	2	4, 9	-12.810198
3	2	7, 12	-12.414389
4	1	10	-12.01548
5	6	14, 1, 2, 11, 13, 18	-11.732399
6	1	15	-11.722769
7	2	19, 20	-11.489742

Figure S5. Result of cluster analysis with the single-linkage algorithm on netropsin re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

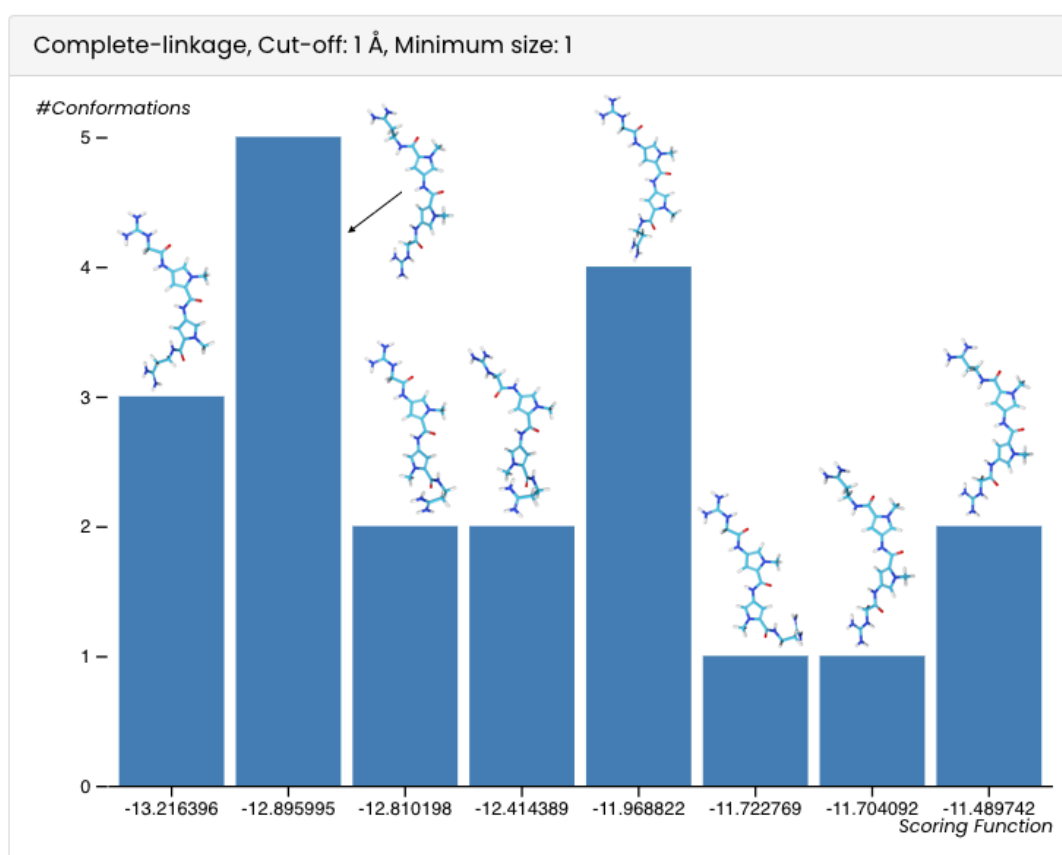| CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	7	1, 2, 10, 11, 13, 14, 18	−13.216396
2	6	3, 5, 6, 8, 16, 17	−12.895995
3	2	4, 9	−12.810198
4	2	7, 12	−12.414389
5	1	15	−11.722769
6	2	19, 20	−11.489742

Figure S6. Result of cluster analysis with the complete-linkage algorithm on netropsin re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	3	1, 2, 10	–13.216396
2	5	3, 5, 6, 8, 17	–12.895995
3	2	4, 9	–12.810198
4	2	7, 12	–12.414389
5	4	11, 13, 14, 18	–11.968822
6	1	15	–11.722769
7	1	16	–11.704092
8	2	19, 20	–11.489742

In this example, the three different algorithms produce a different number of clusters and a different population for each cluster. By using the gromos algorithm, the two largely populated clusters have the same number of binding poses, with the representative poses that are in one case very similar to the experimental one (pose #14, RMSD = 2.089 Å, slightly better than pose #1) and in the other case is completely upside-down with respect to the reference structure. The single-linkage and the complete-linkage algorithms appear to allow for a different distribution of the binding poses, with the single-linkage algorithm providing the most populated cluster (7 structures) able to include all the binding poses that are more similar to the experimental reference.

S4   USAGE EXAMPLE: 6DDF

This section presents an example of PacDOCK usage for the complete analysis of docking results with RMSD calculation, visualisation of the docked pose with respect to the reference pose and receptor-ligand interactions, and clustering. This example regards the structure of the μ-opioid receptor (μOR)-G_i protein complex bound to the agonist peptide DAMGO and nucleotide-free G_i with a resolution of 3.5 Å (PDB ID: 6DDF). The μ-opioid receptor is a G-protein-coupled receptor (GPCR) and the target of most clinically and recreationally used opioids. The induced positive effects of analgesia and euphoria are mediated by μOR signalling through the adenylyl cyclase-inhibiting heterotrimeric G protein G_i [5].

ProRMSD. Starting from the PDB file, DAMGO was separated from the receptor μOR, and then re-docked into the target using the docking program OpenEye HYBRID (*see Section S1*). From the docking program output, the first pose of the ligand was used to create the input file to calculate the RMSD of this pose with respect to the experimental one by using ProRMSD with the default RMSD calculation mode. The atom matching procedure is required for the RMSD calculation since the atoms in the experimental and the docked pose are labelled differently (Figure S3). Through the use of ProRMSD, it is possible to calculate RMSD also in this case, where atoms are randomly labelled. The atomic order in the structure files before and after the matching is shown in Tables S15 and S16. The value of RMSD calculated between the reference and the docked pose is 1.973 Å. The RMSD calculation comparison with DockRMSD and LigRMSD is shown in Table S17.

PacVIEW. The same two structures used for the RMSD calculation with ProRMSD were visualised using PacVIEW in the Docking results mode. As shown in Figure S7, it is possible to visualise the spatial differences between the two poses of the ligand. Moreover, the ligand was displayed within the allosteric site of the receptor and the key interactions were investigated by using PacVIEW in the Receptor-Ligand interactions mode (Figure S3).

ClusDOCK. The first twenty poses from the re-docking were clustered with ClusDOCK by using the gromos algorithm, a cut-off of 1.0 Å, and a minimum cluster size of 1 structure (the pairwise RMSD matrix is reported in Table S18). The cluster analysis shows the grouping of the twenty poses in three different clusters. Interestingly, the first cluster, thus the most populated, is also the one with the most favourable scoring function. Additional information on the clustering can be found in Table S19 and the results are shown in Figures S8-S10.

ProRMSD performs the RMSD calculation after matching the atoms of the ligand in the experimental pose and the re-docked pose on the basis of the atom type, the atoms to which they are bound, and the bond order.

Table S15. Atom indexes for the experimental and re-docked poses of DAMGO.

Experimental pose		Re-docked pose	
Atom index	Coordinates (x, y, z)	Atom index	Coordinates (x, y, z)
1 N	119.032 149.353 141.168	1 C	120.519 147.766 147.642
2 C	119.627 150.658 140.911	2 C	120.834 148.364 148.862
3 C	118.634 151.725 141.330	3 C	120.199 148.558 146.539
4 O	117.440 151.459 141.405	4 C	120.830 149.754 148.979
5 C	119.990 150.808 139.438	5 C	120.195 149.948 146.656
6 C	120.865 151.993 139.143	6 C	119.967 152.942 138.112
7 C	122.213 151.968 139.453	7 C	121.630 151.197 138.273
8 C	120.349 153.136 138.558	8 C	120.923 153.888 138.480
9 C	123.026 153.050 139.191	9 C	122.586 152.143 138.642
10 C	121.157 154.227 138.289	10 C	120.511 150.546 147.876
11 C	122.494 154.175 138.606	11 C	120.320 151.597 138.008
12 O	123.302 155.254 138.342	12 C	122.233 153.489 138.745
13 C	118.125 154.001 143.534	13 C	118.152 152.613 146.121
14 N	119.116 152.928 141.617	14 C	117.832 151.766 142.968
15 O	118.538 154.955 144.185	15 C	119.189 154.127 148.502
16 C	118.226 154.002 142.029	16 C	117.713 150.944 139.593
17 C	118.737 155.338 141.536	17 C	118.696 153.686 141.606
18 N	117.595 152.911 144.082	18 C	116.966 151.510 148.034
19 C	117.551 152.699 145.517	19 C	120.506 152.050 148.002
20 C	118.944 152.630 146.119	20 C	119.288 150.576 137.611
21 O	119.893 152.361 145.381	21 C	116.977 152.172 145.266
22 C	120.881 154.394 147.485	22 C	120.243 155.963 149.819
23 N	119.047 152.847 147.437	23 C	121.378 156.524 148.982
24 O	121.664 154.429 146.538	24 C	117.878 152.404 141.586
25 C	117.939 152.991 148.347	25 C	119.140 152.605 148.421
26 C	120.305 153.144 148.128	26 C	118.510 149.942 138.773
27 C	121.394 152.051 148.125	27 N	117.186 152.573 143.895
28 C	120.910 150.669 147.924	28 N	120.048 154.567 149.499
29 C	120.042 148.064 147.544	29 N	118.435 151.440 140.668
30 C	120.038 150.085 148.843	30 N	118.089 152.257 147.473
31 C	121.353 149.941 146.821	31 N	117.617 148.862 138.262
32 C	119.598 148.786 148.646	32 O	119.091 153.243 145.627
33 C	120.918 148.637 146.627	33 O	118.286 150.649 143.207
34 N	120.253 155.512 148.116	34 O	118.505 154.857 147.788
35 O	119.037 158.187 147.081	35 O	116.559 151.264 139.316
36 C	120.891 156.702 147.563	36 O	123.167 154.412 139.103
37 C	120.060 157.367 146.442	37 O	122.579 155.827 149.287

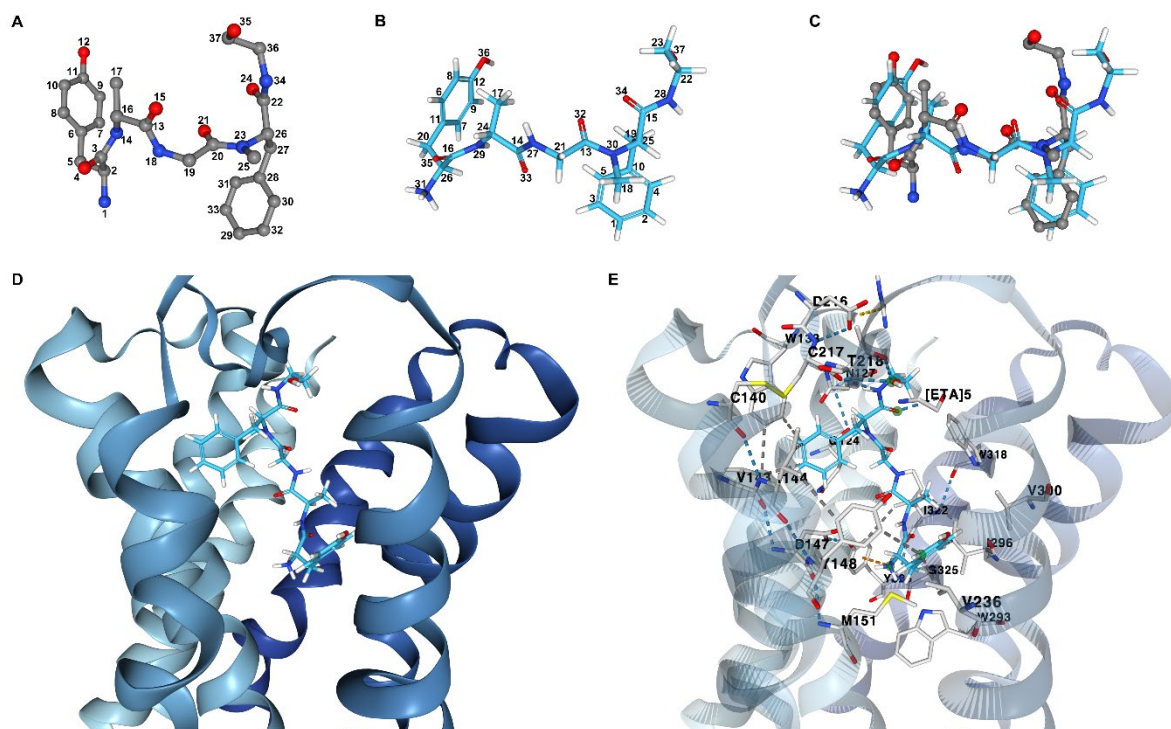Table S16. Atom matching between DAMGO atoms in the experimental pose and the re-docked pose.

Experimental pose		Re-docked pose	
Atom index	Coordinates (x, y, z)	Atom index	Coordinates (x, y, z)
1 N	119.032 149.353 141.168	31 N	117.617 148.862 138.262
2 C	119.627 150.658 140.911	26 C	118.510 149.942 138.773
3 C	118.634 151.725 141.330	16 C	117.713 150.944 139.593
4 O	117.440 151.459 141.405	35 O	116.559 151.264 139.316
5 C	119.990 150.808 139.438	20 C	119.288 150.576 137.611
6 C	120.865 151.993 139.143	11 C	120.320 151.597 138.008
7 C	122.213 151.968 139.453	7 C	121.630 151.197 138.273
8 C	120.349 153.136 138.558	6 C	119.967 152.942 138.112
9 C	123.026 153.050 139.191	9 C	122.586 152.143 138.642
10 C	121.157 154.227 138.289	8 C	120.923 153.888 138.480
11 C	122.494 154.175 138.606	12 C	122.233 153.489 138.745
12 O	123.302 155.254 138.342	36 O	123.167 154.412 139.103
13 C	118.125 154.001 143.534	14 C	117.832 151.766 142.968
14 N	119.116 152.928 141.617	29 N	118.435 151.440 140.668
15 O	118.538 154.955 144.185	33 O	118.286 150.649 143.207
16 C	118.226 154.002 142.029	24 C	117.878 152.404 141.586
17 C	118.737 155.338 141.536	17 C	118.696 153.686 141.606
18 N	117.595 152.911 144.082	27 N	117.186 152.573 143.895
19 C	117.551 152.699 145.517	21 C	116.977 152.172 145.266
20 C	118.944 152.630 146.119	13 C	118.152 152.613 146.121
21 O	119.893 152.361 145.381	32 O	119.091 153.243 145.627
22 C	120.881 154.394 147.485	15 C	119.189 154.127 148.502
23 N	119.047 152.847 147.437	30 N	118.089 152.257 147.473
24 O	121.664 154.429 146.538	34 O	118.505 154.857 147.788
25 C	117.939 152.991 148.347	18 C	116.966 151.510 148.034
26 C	120.305 153.144 148.128	25 C	119.140 152.605 148.421
27 C	121.394 152.051 148.125	19 C	120.506 152.050 148.002
28 C	120.910 150.669 147.924	10 C	120.511 150.546 147.876
29 C	120.042 148.064 147.544	1 C	120.519 147.766 147.642
30 C	120.038 150.085 148.843	4 C	120.830 149.754 148.979
31 C	121.353 149.941 146.821	5 C	120.195 149.948 146.656
32 C	119.598 148.786 148.646	2 C	120.834 148.364 148.862
33 C	120.918 148.637 146.627	3 C	120.199 148.558 146.539
34 N	120.253 155.512 148.116	28 N	120.048 154.567 149.499
35 O	119.037 158.187 147.081	37 O	122.579 155.827 149.287
36 C	120.891 156.702 147.563	22 C	120.243 155.963 149.819
37 C	120.060 157.367 146.442	23 C	121.378 156.524 148.982

Table S17. RMSD values for the re-docked poses of DAMGO with respect to the experimental pose calculated by using ProRMSD, DockRMSD, and LigRMSD.

	ProRMSD	DockRMSD	LigRMSD
RMSD	1.973	1.973	1.97

Note: RMSD value for the web server LigRMSD was reported with two decimal digits, as this is how the value is reported by the web server.

Figure S7. Analysis of re-docking results of the structure of the μ-opioid receptor (μOR)-G$_i$ protein complex bound to the agonist peptide DAMGO. Experimental pose of the ligand in ball and stick representation and with carbon, nitrogen, and oxygen atoms coloured by element: grey, blue, and red, respectively. Docked pose of the ligand in licorice representation and with carbon, nitrogen, oxygen, and hydrogen atoms coloured in light blue, blue, red, and white, respectively. Receptor in cartoon representation, with atom index colour style. (A) Atom indexes of the experimental pose of the ligand. (B) Atom indexes of the docked pose of the ligand assigned by OpenEye HYBRID docking program. (C) Ligand docked pose compared with ligand experimental pose. (D) Ligand docked pose within the receptor binding pocket. (E) Interactions between the receptor and the ligand in the docked pose within the binding pocket. Selected residues from the receptor are in licorice representation with carbon, oxygen, nitrogen, and sulphur atoms coloured light grey, red, blue, and yellow, respectively. These residues are labelled in black and selected interactions are shown as dashed lines. The ligand atoms involved in the main interactions are surrounded by a green sphere. The receptor cartoon representation has been opacified for clarity.

ClusDOCK performs the cluster analysis with three different algorithms. In this example, the gromos algorithm was employed, with a cut-off of 1.0 Å and a minimum cluster size of 1 structure. The steps that lead to cluster formations are illustrated below, in Table S18-S19.

Table S18. Pairwise RMSD matrix (20×20) in which each element contains the RMSD of the ith binding pose calculated with respect to the jth binding pose of DAMGO re-docking. Considering a cut-off of 1.0 Å, the number of neighbours is calculated (RMSD values below the cut-off are highlighted in gold). There are multiple structures that have the highest number of neighbours, i.e., ten. Among these, the first pose (highlighted in green) has the best scoring function, thus it represents the centre of the most populated cluster, and forms together with all its neighbours (poses no. 2, 3, 4, 5, 7, 8, 9, 10, 13, 17, and 18; highlighted in yellow) a cluster. Therefore, the first cluster is composed of structures 1, 2, 3, 4, 5, 7, 8, 9, 10, 13, 17, and 18; and presents the scoring function of structure 1, the centroid of the cluster. These structures are thereafter eliminated from the pool of structures.

Pose no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0	0.196	0.588	0.521	0.613	9.166	0.413	0.564	0.466	0.270	9.126	9.180	0.769	9.117	9.180	9.186	0.668	0.641	9.104	9.106
2	0.196	0	0.465	0.455	0.536	9.152	0.375	0.488	0.408	0.174	9.115	9.166	0.700	9.108	9.156	9.172	0.557	0.536	9.095	9.096
3	0.588	0.465	0	0.432	0.297	9.083	0.327	0.380	0.519	0.531	9.055	9.098	0.409	9.055	9.089	9.105	0.397	0.480	9.042	9.034
4	0.521	0.455	0.432	0	0.607	9.148	0.497	0.593	0.174	0.558	9.117	9.165	0.707	9.117	9.155	9.169	0.431	0.421	9.099	9.090
5	0.613	0.536	0.297	0.607	0	9.075	0.295	0.244	0.707	0.601	9.044	9.091	0.174	9.041	9.061	9.099	0.515	0.630	9.030	9.024
6	9.166	9.152	9.083	9.148	9.075	0	9.092	9.090	9.166	9.143	0.309	0.157	9.066	0.500	1.478	0.318	9.224	9.255	0.379	0.425
7	0.413	0.375	0.327	0.497	0.295	9.092	0	0.237	0.552	0.399	9.058	9.105	0.438	9.050	9.093	9.112	0.496	0.595	9.042	9.043
8	0.564	0.488	0.380	0.593	0.244	9.090	0.237	0	0.677	0.512	9.057	9.104	0.338	9.050	9.058	9.111	0.476	0.616	9.041	9.042
9	0.466	0.408	0.519	0.174	0.707	9.166	0.552	0.677	0	0.492	9.133	9.182	0.832	9.131	9.183	9.185	0.527	0.481	9.114	9.108
10	0.270	0.174	0.531	0.558	0.601	9.143	0.399	0.512	0.492	0	9.105	9.154	0.769	9.094	9.148	9.160	0.627	0.638	9.086	9.093
11	9.126	9.115	9.055	9.117	9.044	0.309	9.058	9.057	9.133	9.105	0	0.326	9.037	0.243	1.483	0.448	9.195	9.226	0.308	0.478
12	9.180	9.166	9.098	9.165	9.091	0.157	9.105	9.104	9.182	9.154	0.326	0	9.083	0.453	1.489	0.287	9.240	9.272	0.433	0.567
13	0.769	0.700	0.409	0.707	0.174	9.066	0.438	0.338	0.832	0.769	9.037	9.083	0	9.036	9.041	9.092	0.579	0.710	9.023	9.014
14	9.117	9.108	9.055	9.117	9.041	0.500	9.050	9.050	9.131	9.094	0.243	0.453	9.036	0	1.547	0.563	9.195	9.228	0.412	0.679
15	9.180	9.156	9.089	9.155	9.061	1.478	9.093	9.058	9.183	9.148	1.483	1.489	9.041	1.547	0	1.514	9.203	9.246	1.414	1.495
16	9.186	9.172	9.105	9.169	9.099	0.318	9.112	9.111	9.185	9.160	0.448	0.287	9.092	0.563	1.514	0	9.247	9.280	0.486	0.608
17	0.668	0.557	0.397	0.431	0.515	9.224	0.496	0.476	0.527	0.627	9.195	9.240	0.579	9.195	9.203	9.247	0	0.230	9.179	9.172
18	0.641	0.536	0.480	0.421	0.630	9.255	0.595	0.616	0.481	0.638	9.226	9.272	0.710	9.228	9.246	9.280	0.230	0	9.207	9.196
19	9.104	9.095	9.042	9.099	9.030	0.379	9.042	9.041	9.114	9.086	0.308	0.433	9.023	0.412	1.414	0.486	9.179	9.207	0	0.357
20	9.106	9.096	9.034	9.090	9.024	0.425	9.043	9.042	9.108	9.093	0.478	0.567	9.014	0.679	1.495	0.608	9.172	9.196	0.357	0
No. of neighbours	11	11	11	11	11	6	11	11	11	11	6	6	11	6	0	6	11	11	6	6

Table S19. Pairwise RMSD matrix (8×8) recalculated. Among the structures that have the highest number of neighbours, the number 6 has the best scoring function, thus it represents the centre of the second cluster, which includes its neighbour (poses no. 11, 12, 14, 16, 19, and 20), and presents the scoring function of structure 6. These structures are thereafter eliminated from the pool of structures.

Pose no.	6	11	12	14	15	16	19	20
6	0	0.309	0.157	0.500	1.478	0.318	0.379	0.425
11	0.309	0	0.326	0.243	1.483	0.448	0.308	0.478
12	0.157	0.326	0	0.453	1.489	0.287	0.433	0.567
14	0.500	0.243	0.453	0	1.547	0.563	0.412	0.679
15	1.478	1.483	1.489	1.547	0	1.514	1.414	1.495
16	0.318	0.448	0.287	0.563	1.514	0	0.486	0.608
19	0.379	0.308	0.433	0.412	1.414	0.486	0	0.357
20	0.425	0.478	0.567	0.679	1.495	0.608	0.357	0
No. of neighbours	6	6	6	6	0	6	6	6

Structure number 15 remains as a singleton cluster with its scoring function.

Figure S8. Result of cluster analysis with the gromos algorithm on DAMGO re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

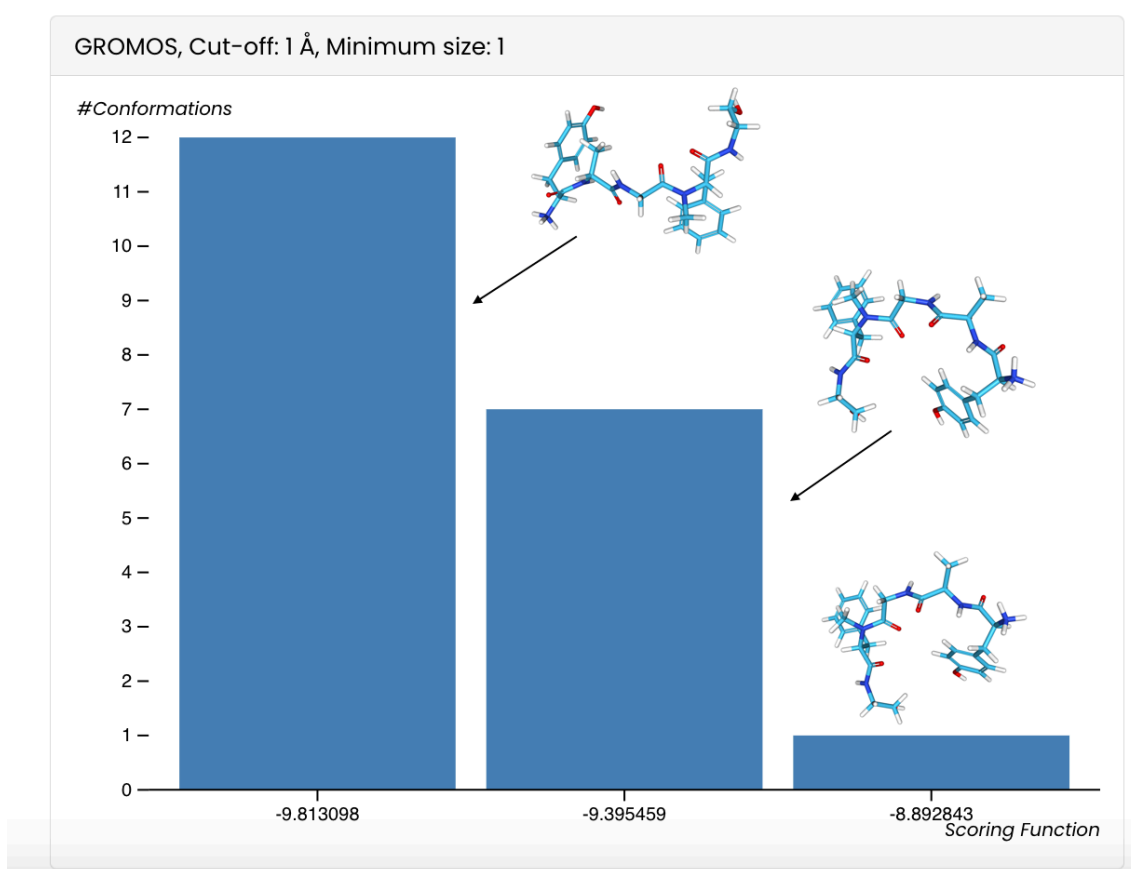| CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	12	1, 2, 3, 4, 5, 7, 8, 9, 10, 13, 17, 18	−9.813098
2	7	6, 11, 12, 14, 16, 19, 20	−9.395459
3	1	15	−8.892843

Figure S9. Result of cluster analysis with the single-linkage algorithm on DAMGO re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	12	1, 2, 3, 4, 5, 7, 8, 9, 10, 13, 17, 18	-9.813098
2	7	6, 11, 12, 14, 16, 19, 20	-9.395459
3	1	15	-8.892843

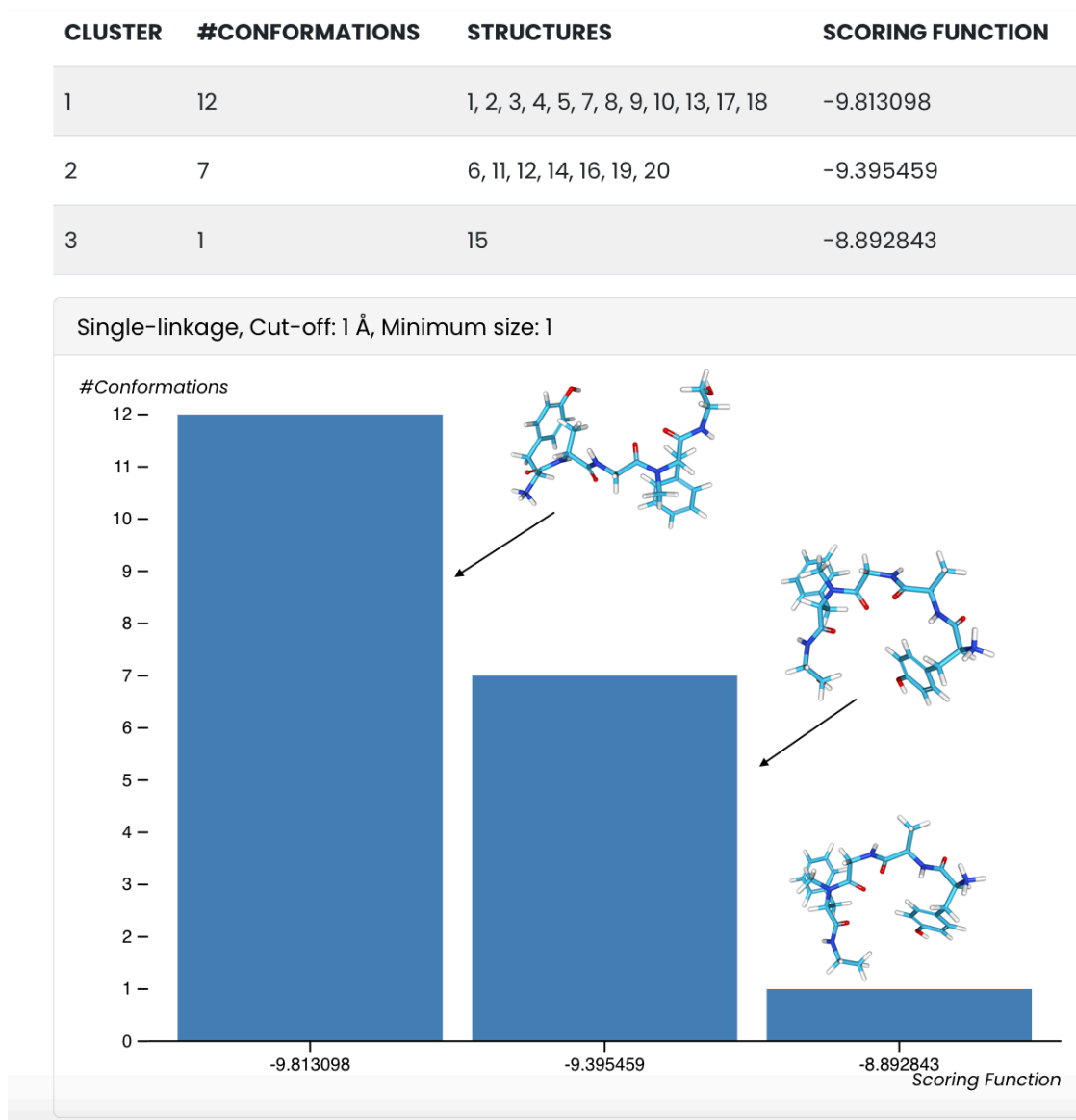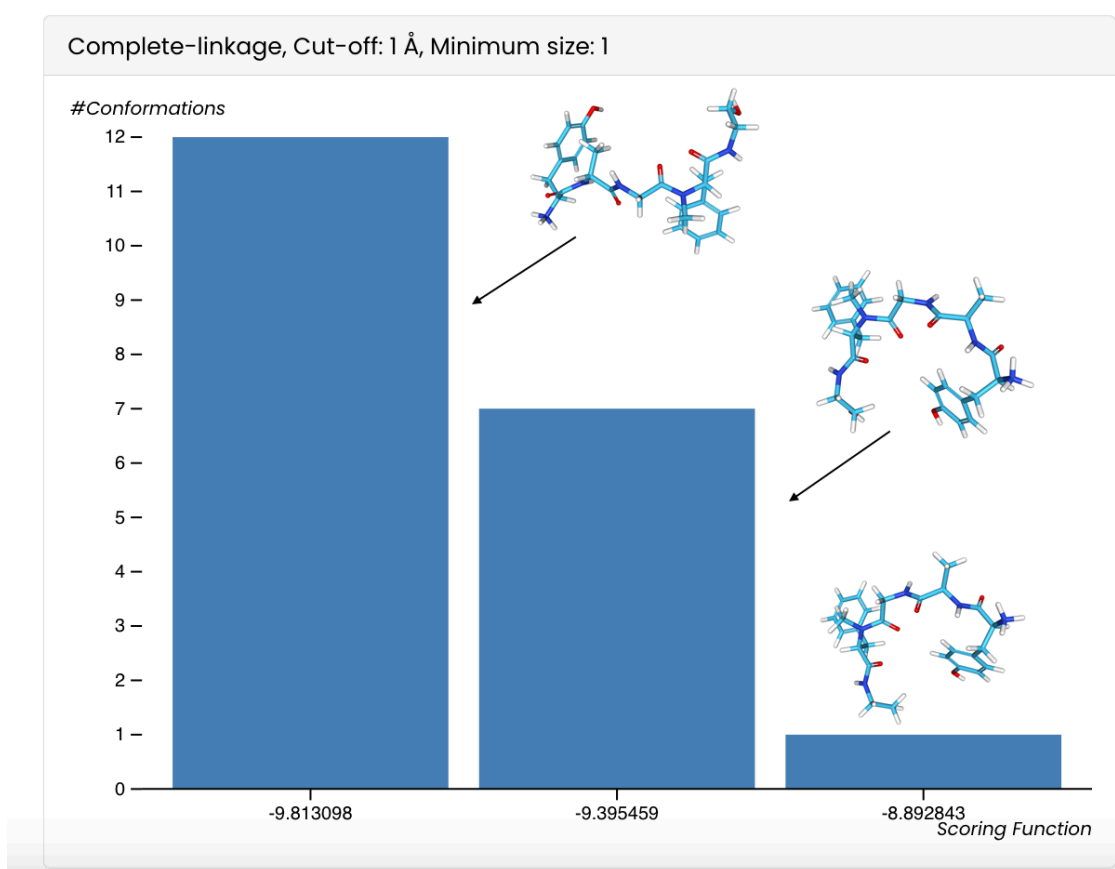Single-linkage, Cut-off: 1 Å, Minimum size: 1

Figure S10. Result of cluster analysis with the complete-linkage algorithm on DAMGO re-docking results. (A) For each cluster is reported the cluster population, the structure indexes, and the scoring function of the centroid of the cluster that was selected as the most representative structure. The order of the clusters depends on their scoring function (the first one has the lowest scoring function). (B) Bar plot that shows on the y-axis the population for each cluster and on the x-axis the scoring function of the centroid of each cluster. The representative structure of each cluster is also reported.

CLUSTER	#CONFORMATIONS	STRUCTURES	SCORING FUNCTION
1	12	1, 2, 3, 4, 5, 7, 8, 9, 10, 13, 17, 18	−9.813098
2	7	6, 11, 12, 14, 16, 19, 20	−9.395459
3	1	15	−8.892843

In this example, the three clustering algorithms are able to distinguish between different binding poses and give the same results.

S5   COMPARISON WITH SIMILAR WEB SERVERS

Table S20. Comparison of functionalities between different web servers for RMSD calculations.

Web Server	Input file formats	Reference pose vs docked pose	Reference pose vs multiple docked poses	RMSD among similar compounds	Superimposed RMSD	Backbone RMSD
PacDOCK (**ProRMSD**)	SDF, PDB, MOL2, MOL	YES	YES	NO	YES	YES
DockRMSD	MOL2	YES	NO	NO	NO	NO
LigRMSD	MOL	YES	YES*	YES	NO	NO

***Each docked pose must be contained in its own file and a zip file of all MOL files must be uploaded.

To test ProRMSD ability and to compare its performance with DockRMSD and LigRMSD, the ligands of ten different target-ligand complex structures downloaded from RCSB PDB were separated from the target, and then re-docked into the target using the docking program OpenEye HYBRID (*see Section S1).*

The RMSD values of the first tenth docked poses with respect to the reference experimental pose were calculated with the three different web servers.

Note: RMSD values for the web server LigRMSD were reported with two decimal digits, as this is how the value is reported by the web server.

Table S21. List of PDB identification codes of the selected complex structures for re-docking.

PDB ID	CLASSIFICATION	LIGAND	METHOD	RESOLUTION (Å)
1DNE	DNA	NT	X-ray	2.40
2PIV	Hormone receptor	T3	X-ray	1.95
3LFA	Transferase	1N1	X-ray	2.10
4EIY	Membrane protein	ZMA	X-ray	1.80
4LRM	Transferase/Transferase inhibitor	YUN	X-ray	3.53
5CGC	Signaling protein	51D	X-ray	3.10
6DDF	Membrane Protein	DAMGO	cryo-EM	3.50
6DUK	Transferase/Transferase inhibitor	JBJ	X-ray	2.20
6ZKK	Electron transport	970	cryo-EM	3.70
7LGS	Hydrolase/Hydrolase inhibitor	Q6K	X-ray	3.10

The complex structures used for this test were chosen on the basis of their ligands, in an attempt to represent different classes of molecules of pharmaceutical interest, with criteria outlined in Table S22.

Table S22. Re-docking structures selection criteria.

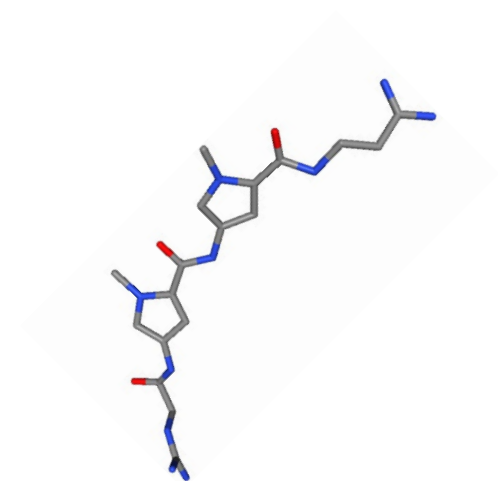| CRITERIA	LIGAND
PARTIAL SYMMETRY	NT
HIGHLY FLEXIBILITY	1N1; DAMGO
HETEROATOMS	T3; JBJ; 51D
MULTIPLE RINGS	Q6K; ZMA
CONDENSED RINGS	970; YUN

Table S23. RMSD values of NT re-docking.

1DNE - NT	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	2.219	2.219			2.22		
POSE 2	2.100	2.100			2.10		
POSE 3	11.796	11.796			11.78	0.02	0.14
POSE 4	4.053	4.053			4.05		
POSE 5	11.759	11.759			11.74	0.02	0.16
POSE 6	11.819	11.818			11.81	0.01	0.08
POSE 7	4.382	4.382			4.35	0.03	0.74
POSE 8	11.789	11.789			11.77	0.02	0.16
POSE 9	4.167	4.167			4.17		
POSE 10	2.068	2.068			2.07		

Figure S11. NT ligand.

Table S24. RMSD values of T3 re-docking.

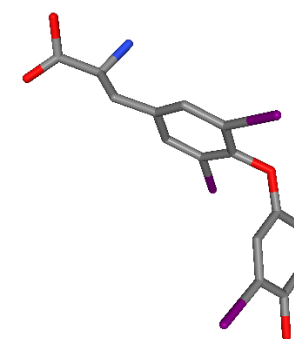| 2PIV - T3	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	2.999	2.999			2.63	0.37	14.03
POSE 2	2.985	2.985			2.62	0.37	13.93
POSE 3	2.852	2.852			2.43	0.42	17.37
POSE 4	2.874	2.874			2.45	0.42	17.31
POSE 5	2.934	2.934			2.56	0.37	14.61
POSE 6	2.931	2.931			2.48	0.45	18.19
POSE 7	2.868	2.868			2.37	0.50	21.01
POSE 8	3.090	3.09			2.77	0.32	11.55
POSE 9	2.946	2.946			2.58	0.37	14.19
POSE 10	3.166	3.166			2.65	0.52	19.47

Figure S12. T3 ligand.

Table S25. RMSD values of 1N1 re-docking.

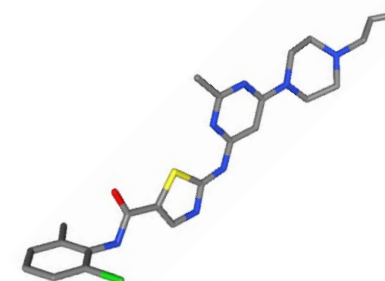| 3LFA – 1N1	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	11.857	11.847	0.010	0.08	11.85	0.01	0.06
POSE 2	11.857	11.850	0.007	0.06	11.85	0.01	0.06
POSE 3	11.853	11.845	0.008	0.07	11.85		
POSE 4	11.854	11.844	0.010	0.08	11.84	0.01	0.12
POSE 5	11.854	11.844	0.010	0.08	11.84	0.01	0.12
POSE 6	11.860	11.853	0.007	0.06	11.85	0.01	0.08
POSE 7	11.845	11.837	0.008	0.07	11.84	0.01	0.04
POSE 8	11.768	11.760	0.008	0.07	11.76	0.01	0.07
POSE 9	2.281	2.253	0.028	1.24	2.25	0.03	1.38
POSE 10	11.848	11.840	0.008	0.07	11.84	0.01	0.07

Figure S13. 1N1 ligand.

Table S26. RMSD values of ZMA re-docking.

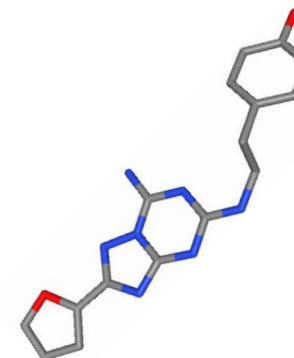| 4EIY - ZMA	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	1.431	1.431			1.07	0.36	33.74
POSE 2	1.570	1.570			1.25	0.32	25.60
POSE 3	1.449	1.449			1.09	0.36	32.94
POSE 4	1.532	1.532			1.20	0.33	27.67
POSE 5	1.487	1.487			1.14	0.35	30.44
POSE 6	1.644	1.644			1.34	0.30	22.69
POSE 7	1.661	1.661			1.36	0.30	22.13
POSE 8	2.205	2.205			2.21		
POSE 9	2.219	2.219			2.22		
POSE 10	2.317	2.317			2.32		

Figure S14. ZMA ligand.

Table S27. RMSD values of YUN re-docking.

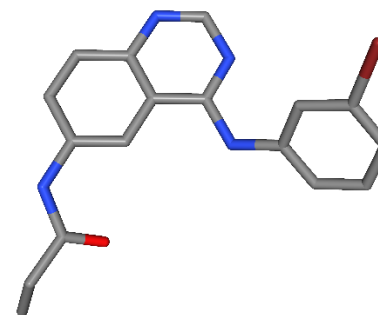| 4LRM - YUN	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	6.410	6.410			6.41		
POSE 2	6.413	6.413			6.41		
POSE 3	6.715	6.715			6.71		
POSE 4	6.589	6.589			6.59		
POSE 5	6.597	6.597			6.60		
POSE 6	6.514	6.514			6.51		
POSE 7	6.559	6.559			6.56		
POSE 8	6.570	6.570			6.57		
POSE 9	6.530	6.530			6.53		
POSE 10	6.520	6.520			6.52		

Figure S15. YUN ligand.

Table S28. RMSD values of 51D re-docking.

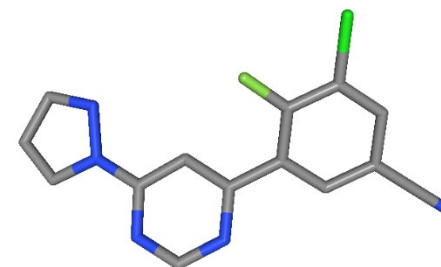| 5CGC – 51D	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	0.297	0.297			0.30		
POSE 2	0.427	0.427			0.43		
POSE 3	0.293	0.293			0.29		
POSE 4	0.264	0.264			0.26		
POSE 5	0.535	0.535			0.53		
POSE 6	0.538	0.538			0.54		
POSE 7	2.681	2.681			2.68		
POSE 8	0.395	0.395			0.39		
POSE 9	0.411	0.411			0.41		
POSE 10	0.906	0.906			0.91		

Figure S16. 51D ligand.

Table S29. RMSD values of DAMGO re-docking.

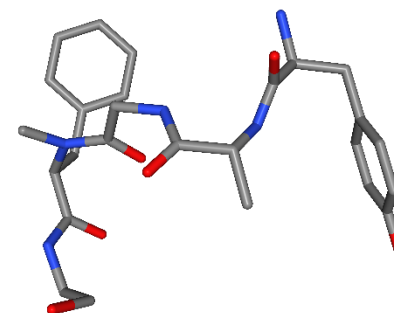| 6DDF - DAMGO	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	1.973	1.973			1.97		
POSE 2	1.974	1.974			1.97		
POSE 3	1.910	1.910			1.91		
POSE 4	1.999	1.999			2.00		
POSE 5	1.864	1.864			1.86		
POSE 6	8.400	8.400			8.38	0.02	0.24
POSE 7	1.843	1.843			1.84		
POSE 8	1.848	1.848			1.85		
POSE 9	2.034	2.034			2.03		
POSE 10	1.956	1.956			1.96		

Figure S17. DAMGO ligand.

Table S30. RMSD values of JBJ re-docking.

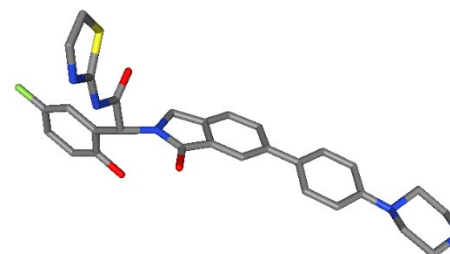| 6DUK - JBJ	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	1.188	1.188			1.01	0.18	17.62
POSE 2	1.228	1.228			1.02	0.21	20.39
POSE 3	1.217	1.217			1.01	0.21	20.50
POSE 4	1.337	1.337			1.15	0.19	16.26
POSE 5	1.363	1.363			1.18	0.18	15.51
POSE 6	1.207	1.207			1.00	0.21	20.70
POSE 7	1.348	1.348			1.19	0.16	13.28
POSE 8	1.205	1.205			0.93	0.28	29.57
POSE 9	1.283	1.283			1.05	0.23	22.19
POSE 10	1.330	1.330			1.15	0.18	15.65

Figure S18. JBJ ligand.

Table S31. RMSD values of 970 re-docking.

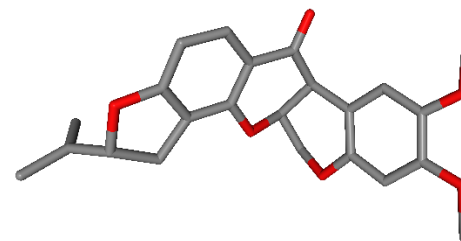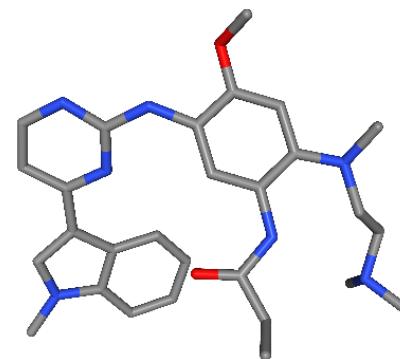| 6ZKK – 970	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	0.668	0.668			0.67		
POSE 2	8.110	8.110			8.11		
POSE 3	1.217	1.217			1.22		
POSE 4	7.722	7.722			7.72		
POSE 5	7.009	7.009			7.01		
POSE 6	4.536	4.536			4.54		
POSE 7	8.863	8.863			8.86		
POSE 8	7.365	7.365			7.36		
POSE 9	7.850	7.850			7.85		
POSE 10	7.497	7.497			7.50		

Figure S19. 970 ligand.

Table S32. RMSD values of Q6K re-docking.

7LGS – Q6K	ProRMSD	DockRMSD	Absolute difference (Å)	Relative difference (%)	LigRMSD	Absolute difference (Å)	Relative difference (%)
POSE 1	1.180	1.162	0.018	1.55	1.16	0.02	1.72
POSE 2	1.267	1.251	0.016	1.28	1.25	0.02	1.36
POSE 3	2.437	2.423	0.014	0.58	2.42	0.02	0.70
POSE 4	1.199	1.182	0.017	1.44	1.18	0.02	1.61
POSE 5	2.601	2.601			2.60		
POSE 6	2.302	2.285	0.017	0.74	2.28	0.02	0.96
POSE 7	1.321	1.306	0.015	1.15	1.31	0.01	0.84
POSE 8	2.612	2.612			2.61		
POSE 9	2.371	2.364	0.007	0.30	2.36	0.01	0.47
POSE 10	2.357	2.349	0.008	0.34	2.35	0.01	0.30

Figure S20. Q6K ligand .

REFERENCES

1. McGann,M. (2012) FRED and HYBRID docking performance on standardized datasets. *J. Comput. Aided Mol. Des.*, **26**, 897–906.

2. Berman,H.M., Westbrook,J., Feng,Z., Gilliland,G., Bhat,T.N., Weissig,H., Shindyalov,I.N. and Bourne,P.E.. (2000) The Protein Data Bank. *Nucleic Acids Research*, **28**, 235–242.

3. Bell,E.W. and Zhang,Y. (2019) DockRMSD: an open-source tool for atom mapping and RMSD calculation of symmetric molecules through graph isomorphism.

4. Velázquez-Libera,J.L., Durán-Verdugo,F., Valdés-Jiménez,A., Núñez-Vivanco,G. and Caballero,J. (2020) LigRMSD: a web server for automatic structure matching and RMSD calculations among identical and similar compounds in protein-ligand docking. *Bioinformatics*, **36**, 2912–2914.

5. Koehl,A., Hu,H., Maeda,S., Zhang,Y., Qu,Q., Paggi,J.M., Latorraca,N.R., Hilger,D., Dawson,R., Matile,H., *et al.* (2018) Structure of the μ-opioid receptor–Gi protein complex. *Nature*, **558**, 547–552.