

Time: 2024.09.10-2024.09.12

1. **Experiment:** Using HDock to evaluating the binding energy
2. **Time:** 2024.09.10-2024.09.12
3. **Member:** Xudong Tang, Yang Jin, Binxuan Zhang, Kaiqing Zhang, Xuanton Liu
4. **Method:**

HDock is an efficient protein-protein interaction docking tool that combines template matching and free docking algorithms to accurately predict intermolecular interactions. During the HDock precision screening phase, binding score calculations consider structural alignment between bait proteins and target proteins, enabling more effective identification of protein interactions with enhanced stability and higher binding affinity. This serves as a crucial screening metric that improves result accuracy.

(1) The procedure of HDock:

- ① Data input: The HDock web server is available at <http://hdock.phys.hust.edu.cn/>. Both sequence and structure information of ligand and receptor should be uploaded through the website separately. The docking process will be carried out in the server. Click submit to wait for the results. Generally, the waiting time is approximately 30 minutes. The submitted sequences should be in FASTA format and contain only standard amino acids. The structure should be in PDB format. In addition, residue information of the binding site is also possible to be submitted as a filter in the docking process.
- ② Docking preparation: First, the homologous sequences of molecules will be searched by sequence similarity search. The template with the highest sequence coverage, highest sequence similarity and highest resolution will be selected to build the template-based model. ClustalW is used for sequence alignment, and MODELLER is used for model construction. This step will be executed on the server.
- ③ Global docking: HDockLite, a layered docking program based on FFT, is used to globally sample the presumed binding directions. We subjected the screened proteins to HDock refined screening, where adjusting the Spacing and Angle parameters to 1.2 and 15 provides highly accurate docking results, offering data references for protein-protein interaction strength. Docking scores are generally negative values, with more negative scores indicating a higher probability of valid binding models. The ranked binding modes are clustered with an RMSD cutoff of 5 Å. If two binding modes have a ligand RMSD of ≤ 5 Å, the one with the better score is kept. This step will be executed on the server. The docking model of HDockLite and the template-based docking model of MODELLER will be provided for download through web interaction.
- ④ Results analysis: The server will send the top 10 docking models to the website. The results page contains an interactive visualization window and a summary table. The summary table usually contains docking energy score, confidence score and ligand RMSD. If the SAXS data file is provided, the SAXS chi-square of the model can also be displayed. Results can also be downloaded as a PDB file, using the molecular visualization software PyMOL to open and view the predicted model. The docking results of HDock are generally referenced by the docking score and input into the formula: $\text{Confidence_score} = 1.0/[1.0 + e^{(0.02 \cdot (\text{Docking_Score} + 150))}]$. Taking a docking score of -200 as a reference, the confidence score at this point is approximately 0.7. In the official documentation, a confidence score > 0.7 (closer to 1) indicates a high likelihood that the two protein molecules will bind; a confidence score between 0.5 and 0.7 indicates that the two proteins may bind; a confidence score < 0.5 indicates that it is unlikely that the two molecules will bind. Therefore, a docking score lower than -200 corresponds to a very high probability of binding.

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5. Result:

Table.1 HDock Scores for IL-2 mimics targeting IL-2R α and IL-2R β

IL-2R α and IL-2R β Complex	HDock	IL-2R β and IL-2R β Complex	HDock
B73	-266.18	B41	-283.09
B30	-262.99	B78	-274.87
B19	-259.94	B66	-271.51
B12	-256.36	B73	-268.54
B46	-256.1	B19	-266.05
B85	-254.93	B42	-264.04
B50	-254.4	B53	-262.29
B28	-253.48	B70	-249.3
B42	-252.07	B14	-248.62
B66	-247.32	B69	-248.6
B92	-244.83	B63	-245.8
B56	-241.86	B2	-244.07
B78	-240.6	B45	-243.82
B53	-240.58	B75	-243.7
B54	-240.03	B90	-243.13
B67	-239.77	B30	-242.64
B60	-239.71	B99	-241.69
B14	-238.68	B71	-240.88
B75	-238.26	B18	-240.85
B9	-236.76	B58	-240.04
B77	-235.83	B86	-237.62
B33	-234.25	B98	-237.13
B80	-232.22	B34	-233.13
B64	-232.13	B36	-233.12
B36	-232.1	B76	-233.09
B58	-231.73	B25	-232.64

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B16	-230.57	B50	-232.58
B4	-230.56	B26	-232.26
B11	-229.48	B16	-231.97
B2	-228.91	B12	-229.92
B86	-228.15	B24	-228.96
B93	-228.03	B56	-228.32
B10	-227.84	B87	-227.6
B71	-227.61	B92	-225.01
B25	-226.9	B51	-223.91
B96	-226.73	B88	-222.72
B37	-226.47	B77	-221.42
B79	-226.42	B82	-220.4
B87	-226.02	B67	-220.16
B43	-225.68	B46	-219.73
B82	-225.21	B4	-218.47
B68	-224.88	B0	-218.14
B76	-224.12	B28	-216.02
B18	-223.68	B96	-215.17
B89	-223.59	B10	-214.62
B35	-222.33	B20	-213.94
B38	-222.26	B80	-212.86
B98	-221.76	B32	-212.4
B40	-221.54	B83	-211.39
B70	-221.54	B44	-210.94
B26	-221.29	B68	-210.78
B13	-220.32	B74	-209.05
B39	-219.83	B35	-209
B45	-219.81	B84	-208.86
B41	-219.33	B31	-207.51
B49	-217.12	B3	-207.22
B97	-217.1	B38	-206.82

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B72	-216.79	B49	-205.16
B99	-216.48	B11	-204.37
B15	-215.88	B37	-204.01
B22	-215.35	B97	-203.77
B29	-215.22	B61	-203.35
B52	-215.13	B39	-203.2
B90	-214.74	B54	-201.1
B51	-214.42	B27	-200.1
B20	-214.39	B93	-199.98
B74	-213.96	B43	-199.63
B27	-213.23	B17	-199.02
B88	-212.92	B52	-198.86
B63	-211.11	B33	-198.72
B17	-210.81	B85	-198.55
B31	-210.55	B48	-198.42
B55	-209.91	B5	-198.4
B32	-209.14	B72	-196.92
B5	-206.15	B40	-196.5
B47	-206.07	B94	-195.94
B34	-205.46	B1	-193.57
B3	-205.42	B15	-193.53
B57	-205.28	B55	-190.82
B62	-204.36	B7	-189.85
B84	-203.91	B57	-189.79
B83	-201.54	B79	-189.54
B24	-200.87	B64	-189.35
B1	-200.44	B91	-189.31
B61	-200.07	B9	-189.02
B7	-199.55	B22	-188.94
B48	-197.47	B60	-186.98
B44	-196.46	B89	-185.69

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B0	-195.97	B29	-184
B69	-195.65	B62	-182.49
B65	-195.24	B8	-181.75
B21	-195.03	B13	-180.98
B8	-192.53	B47	-179.07
B91	-188.54	B21	-177.55
B94	-185.84	B81	-176.32
B6	-183.34	B6	-172.16
B59	-180.23	B59	-167.59
B95	-174.85	B23	-167.13
B23	-167.66	B95	-161.25
B81	-163.65	B65	

Table.2 HDock Scores for IL-2 mimics targeting IL-2R α and IL-2R γ

IL-2R α and IL-2R γ Complex	HDock	IL-2R γ and IL-2R γ Complex	HDock
G14	-289.8	G14	-290.86
G50	-281.82	G35	-289.62
G97	-273.2	G71	-276.1
G2	-261.96	G9	-274.13
G33	-254.27	G83	-266.59
G70	-254.27	G2	-263.08
G37	-251.79	G63	-258.95
G66	-248.84	G61	-257.87
G83	-248.61	G97	-255.6
G71	-244.66	G50	-255.49
G51	-244.46	G26	-252.8
G18	-242.97	G70	-252.07
G28	-242.86	G30	-249.95
G61	-241.7	G90	-249.32
G35	-241.13	G57	-248.37

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G23	-240.98	G36	-247.29
G65	-240.87	G37	-247.22
G44	-240.73	G42	-246.37
G69	-240.24	G44	-244.12
G30	-238.8	G12	-243.49
G56	-238.58	G16	-243.02
G43	-238.15	G18	-242.61
G92	-238.03	G28	-241.43
G11	-236.9	G32	-241.03
G98	-236.19	G88	-240.63
G67	-235.11	G23	-239.76
G36	-234.53	G43	-239.59
G15	-233.55	G68	-239.25
G85	-232.64	G11	-236.5
G90	-232.34	G1	-236.28
G74	-231.44	G66	-235.87
G95	-230.47	G19	-235.43
G1	-229.89	G76	-231.9
G9	-229.06	G15	-231.52
G73	-228.44	G39	-231.4
G63	-228.37	G69	-229.72
G19	-227.89	G77	-229.33
G8	-227.69	G7	-228.79
G39	-227.37	G67	-228.73
G76	-225.45	G87	-228.67
G58	-225.28	G85	-227.87
G57	-225.23	G98	-227.86
G49	-224.89	G58	-227.72
G84	-224.87	G73	-227.27
G46	-224.37	G0	-226.34
G13	-223.61	G53	-226.24

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G0	-223.35	G75	-225.41
G31	-221.81	G60	-224.77
G68	-221.7	G92	-223.79
G47	-221.26	G49	-223.55
G78	-221.21	G45	-221.54
G88	-219.56	G10	-221.17
G55	-219.21	G31	-221.15
G29	-218.42	G38	-220.99
G75	-218.07	G95	-220.49
G41	-216.91	G33	-220.16
G77	-212.81	G51	-220.02
G87	-212.36	G41	-219.89
G24	-212.1	G84	-217.6
G45	-211.87	G4	-215
G42	-211.6	G82	-212.68
G5	-211.34	G29	-211.89
G81	-210.87	G47	-211.24
G32	-209.65	G6	-210.7
G94	-209.51	G13	-210.55
G54	-209.4	G74	-209.54
G52	-209.21	G86	-209.33
G7	-208.07	G55	-208.45
G53	-207.78	G54	-207.32
G25	-205.98	G20	-204.86
G10	-205.45	G34	-203.87
G59	-205.08	G99	-203.16
G60	-204.46	G59	-202.84
G89	-204.37	G25	-202.44
G17	-202.19	G24	-201.79
G79	-201.97	G81	-201.56
G27	-201.81	G3	-200.46

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G64	-201.52	G72	-199.78
G22	-201.46	G27	-198.77
G20	-200.93	G52	-196.96
G40	-200.92	G5	-196.58
G34	-198.18	G78	-196.1
G72	-197.62	G40	-195.96
G99	-197.26	G21	-195.94
G38	-195.1	G46	-195.23
G91	-194.48	G22	-192.55
G16	-194.05	G17	-192.42
G4	-190.55	G64	-191.91
G86	-189.76	G79	-190.15
G26	-189.49	G91	-189.24
G48	-189.13	G48	-186.86
G21	-188.47	G89	-186.59
G82	-186.88	G65	-185.23
G93	-185.74	G80	-183.47
G80	-181.05	G94	-181.76
G96	-177.09	G56	-181.69
G6	-174.79	G8	-174.57
G62	-173.53	G96	-168.89
G3	-169.35	G93	-166.7
G12		G62	-154.13