



# Computational Prediction and Analysis of the DR6-NAPP interaction

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### Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that involves a devastating clinical course and that lacks an effective treatment. A biochemical model for neuronal development, recently proposed by Nikolaev and co-workers, that may also have implications for AD, hinges on a novel protein-protein interaction between the death cell receptor six (DR6) ectodomain and an N-terminal fragment of amyloid precursor protein (NAPP), specifically, the growth factor like domain of NAPP (GFD NAPP). Given all of this, we used a pure computational work-flow to dock a binding competent homology model of the DR6 ectodomain to a binding competent crystal structure of GFD NAPP. The DR6 homology model was built according to a template supplied by the neurotrophin p75 receptor. The best docked model was selected according to an empirical estimate of the binding affinity and represents a high quality model of probable structural accuracy, especially with respect to the residue-level contribution of GFD NAPP. The final model was tested and verified against a variety of biophysical and theoretical data sets. Particularly worth noting is the excellent observed agreement between the theoretically calculated DR6-GFD NAPP binding free energy and the experimental quantity. The model is used to provide a satisfying structural and energetic interpretation of DR6-GFD NAPP binding and to suggest the possibility of a mechanism for spontaneous apoptosis. The evidence suggests that the DR6-NAPP model proposed here is of probable accuracy and that it will prove useful in future studies, modeling work and structure-based AD drug design.

### Key experimental data

Work performed and cited by Nikolaev and co-workers resulted in a body of experimental data that was used to test and validate the final DR6-GFD NAPP Model. Key data includes:

- NAPP binds to DR6 (EC50=4.6 nM,  $dG_{bind,exp} = -11.5$  kcal/mol)
- Anti-GFD NAPP (22C11) antibody prevents NAPP from binding to DR6
- Anti-GFD NAPP (22C11) data strongly implicates the GFD NAPP alpha helix, as a key DR6 recognition motif
- N-terminal APLP2 also binds to DR6 with similar affinity
- p75 binds to NAPP, but with lower affinity than DR6 (EC50=300 nM,  $dG_{bind,exp} = -9.0$  kcal/mol)
- Crystal structure of GFD NAPP is known (1MWP)
- No crystal structure of the DR6 is available but
  - 1) Sequence of DR6 is known
  - 2) Structure of p75, in complex with NGF, is known (1SG1)

### Building and testing the DR6-GFD NAPP model using a validated work-flow

- Retrieve GFD NAPP crystal structure (1MWP) from the PDB
- Build a homology model of DR6 using a p75 template structure (I-TASSER)
- Minimize the DR6 model structure using AMBER99 and GB/SA
- Validate the DR6 homology model
- Dock the minimized DR6 model to GFD NAPP (ClusPro)
- Minimize the top10 docked DR6-GFD NAPP models
- Assuming rigid body association, calculate  $dG_{bind,MM-GB/SA}$  for each model
- Using an empirical energy function, calculate  $dG_{bind,empirical}$  for each model

$$dG_{bind,empirical} = -0.79 \Delta X_{c1} - 0.075 \Delta X_{c11} - 0.65 X_{ab} - 0.86 X_{bb} - 0.00089 X_{gap} - 0.089 \Delta X_{bar} - 0.33$$

- Compare  $dG_{bind,empirical}$  with  $dG_{bind,exp}$
- Use PPI-Pred to predict DR6-GFD NAPP interface residues; compare results with model interface residues
- Compare model GFD NAPP interface residues with 22C11 residues data
- Other tests (can account for APLP2 data; can account for p75-NAPP data)
- **Entire modelling and testing work-flow previously validated on p75-NGF**

### The DR6 ectodomain homology model is a high quality model of probable accuracy

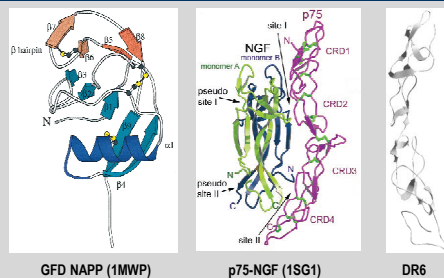
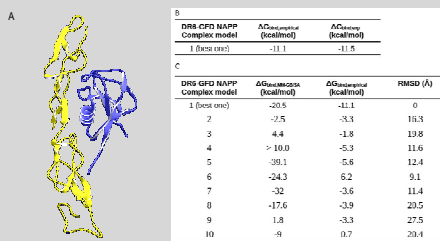


Table 1 We built a DR6 Ectodomain (67=211) Homology Model Using the I-TASSER server. The model was optimized using implicit solvent energy minimization. The accuracy and physical quality of our I-TASSER model was evaluated using a variety of internal I-TASSER and external QIAGEN, QMCA, and DIALS tools.

Model quality	DR6 I-TASSER model	Minimized DR6 I-TASSER model	p75 template reference (1sg1)
I-TASSER C-score*	1.31	N/A	N/A
I-TASSER IIC-score*	0.9	N/A	N/A
I-TASSER RMSD*	2.2	N/A	N/A
ProSA p-score	-3.87	-4.35	-4.35
EMSAV score	0.008	0.04	0.04
DR6E wire ray	100.80	124.85	126.03
Minimization energy*	N/A	-1081.22	N/A

### The best DR6-GFD NAPP model is physically plausible and in nice with experiment

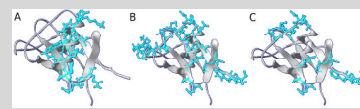
We used the ClusPro docking server to dock the GFD NAPP crystal structure to the minimized and validated DR6 homology model. The top 10 results from the ClusPro server were retained for further analysis. The top 10 complexes were then minimized and scored using and MM-GB/SA methodology and an empirical methodology.



The best ClusPro generated DR6-GFD NAPP docked model (model 1) is depicted in (A). An empirical estimate of the binding affinity for model 1 is provided in (B), along with the experimental binding affinity. Free energy scores for the top (10) ClusPro models are provided in (C), along with RMSD values with respect to model 1. The nice agreement between the theoretical and experimental binding affinity strongly suggests that the proposed DR6-GFD NAPP model is a good one of probable accuracy.

### The interface residues implied by the DR6-GFD NAPP model agree nicely with experiment and theoretical calculation

We next tested the DR6-GFD NAPP model by comparing the interface residues implied by the model with independently predicted interface residues (PPI-Pred) and the GFD NAPP interface residues suggested by the 22C11 data.



(A) 22C11 ; (B) Model 1 ; (C) PPI-Pred

ClusPro predicted interface residues for GFD NAPP:  
 CR6:CR6:756,760,761,762,763,764,765,766,767,768,769,770,771,772,773,774,775,776,777,778,779,780,781,782,783,784,785,786,787,788,789,790,791,792,793,794,795,796,797,798,799,800,801,802,803,804,805,806,807,808,809,810,811,812,813,814,815,816,817,818,819,820,821,822,823,824,825,826,827,828,829,830,831,832,833,834,835,836,837,838,839,840,841,842,843,844,845,846,847,848,849,850,851,852,853,854,855,856,857,858,859,860,861,862,863,864,865,866,867,868,869,870,871,872,873,874,875,876,877,878,879,880,881,882,883,884,885,886,887,888,889,890,891,892,893,894,895,896,897,898,899,900,901,902,903,904,905,906,907,908,909,910,911,912,913,914,915,916,917,918,919,920,921,922,923,924,925,926,927,928,929,930,931,932,933,934,935,936,937,938,939,940,941,942,943,944,945,946,947,948,949,950,951,952,953,954,955,956,957,958,959,960,961,962,963,964,965,966,967,968,969,970,971,972,973,974,975,976,977,978,979,980,981,982,983,984,985,986,987,988,989,990,991,992,993,994,995,996,997,998,999,1000,1001,1002,1003,1004,1005,1006,1007,1008,1009,1010,1011,1012,1013,1014,1015,1016,1017,1018,1019,1020,1021,1022,1023,1024,1025,1026,1027,1028,1029,1030,1031,1032,1033,1034,1035,1036,1037,1038,1039,1040,1041,1042,1043,1044,1045,1046,1047,1048,1049,1050,1051,1052,1053,1054,1055,1056,1057,1058,1059,1060,1061,1062,1063,1064,1065,1066,1067,1068,1069,1070,1071,1072,1073,1074,1075,1076,1077,1078,1079,1080,1081,1082,1083,1084,1085,1086,1087,1088,1089,1090,1091,1092,1093,1094,1095,1096,1097,1098,1099,1100,1101,1102,1103,1104,1105,1106,1107,1108,1109,1110,1111,1112,1113,1114,1115,1116,1117,1118,1119,1120,1121,1122,1123,1124,1125,1126,1127,1128,1129,1130,1131,1132,1133,1134,1135,1136,1137,1138,1139,1140,1141,1142,1143,1144,1145,1146,1147,1148,1149,1150,1151,1152,1153,1154,1155,1156,1157,1158,1159,1160,1161,1162,1163,1164,1165,1166,1167,1168,1169,1170,1171,1172,1173,1174,1175,1176,1177,1178,1179,1180,1181,1182,1183,1184,1185,1186,1187,1188,1189,1190,1191,1192,1193,1194,1195,1196,1197,1198,1199,1200,1201,1202,1203,1204,1205,1206,1207,1208,1209,1210,1211,1212,1213,1214,1215,1216,1217,1218,1219,1220,1221,1222,1223,1224,1225,1226,1227,1228,1229,1230,1231,1232,1233,1234,1235,1236,1237,1238,1239,1240,1241,1242,1243,1244,1245,1246,1247,1248,1249,1250,1251,1252,1253,1254,1255,1256,1257,1258,1259,1260,1261,1262,1263,1264,1265,1266,1267,1268,1269,1270,1271,1272,1273,1274,1275,1276,1277,1278,1279,1280,1281,1282,1283,1284,1285,1286,1287,1288,1289,1290,1291,1292,1293,1294,1295,1296,1297,1298,1299,1300,1301,1302,1303,1304,1305,1306,1307,1308,1309,1310,1311,1312,1313,1314,1315,1316,1317,1318,1319,1320,1321,1322,1323,1324,1325,1326,1327,1328,1329,1330,1331,1332,1333,1334,1335,1336,1337,1338,1339,1340,1341,1342,1343,1344,1345,1346,1347,1348,1349,1350,1351,1352,1353,1354,1355,1356,1357,1358,1359,1360,1361,1362,1363,1364,1365,1366,1367,1368,1369,1370,1371,1372,1373,1374,1375,1376,1377,1378,1379,1380,1381,1382,1383,1384,1385,1386,1387,1388,1389,1390,1391,1392,1393,1394,1395,1396,1397,1398,1399,1400,1401,1402,1403,1404,1405,1406,1407,1408,1409,1410,1411,1412,1413,1414,1415,1416,1417,1418,1419,1420,1421,1422,1423,1424,1425,1426,1427,1428,1429,1430,1431,1432,1433,1434,1435,1436,1437,1438,1439,1440,1441,1442,1443,1444,1445,1446,1447,1448,1449,1450,1451,1452,1453,1454,1455,1456,1457,1458,1459,1460,1461,1462,1463,1464,1465,1466,1467,1468,1469,1470,1471,1472,1473,1474,1475,1476,1477,1478,1479,1480,1481,1482,1483,1484,1485,1486,1487,1488,1489,1490,1491,1492,1493,1494,1495,1496,1497,1498,1499,1500,1501,1502,1503,1504,1505,1506,1507,1508,1509,1510,1511,1512,1513,1514,1515,1516,1517,1518,1519,1520,1521,1522,1523,1524,1525,1526,1527,1528,1529,1530,1531,1532,1533,1534,1535,1536,1537,1538,1539,1540,1541,1542,1543,1544,1545,1546,1547,1548,1549,1550,1551,1552,1553,1554,1555,1556,1557,1558,1559,1560,1561,1562,1563,1564,1565,1566,1567,1568,1569,1570,1571,1572,1573,1574,1575,1576,1577,1578,1579,1580,1581,1582,1583,1584,1585,1586,1587,1588,1589,1590,1591,1592,1593,1594,1595,1596,1597,1598,1599,1600,1601,1602,1603,1604,1605,1606,1607,1608,1609,1610,1611,1612,1613,1614,1615,1616,1617,1618,1619,1620,1621,1622,1623,1624,1625,1626,1627,1628,1629,1630,1631,1632,1633,1634,1635,1636,1637,1638,1639,1640,1641,1642,1643,1644,1645,1646,1647,1648,1649,1650,1651,1652,1653,1654,1655,1656,1657,1658,1659,1660,1661,1662,1663,1664,1665,1666,1667,1668,1669,1670,1671,1672,1673,1674,1675,1676,1677,1678,1679,1680,1681,1682,1683,1684,1685,1686,1687,1688,1689,1690,1691,1692,1693,1694,1695,1696,1697,1698,1699,1700,1701,1702,1703,1704,1705,1706,1707,1708,1709,1710,1711,1712,1713,1714,1715,1716,1717,1718,1719,1720,1721,1722,1723,1724,1725,1726,1727,1728,1729,1730,1731,1732,1733,1734,1735,1736,1737,1738,1739,1740,1741,1742,1743,1744,1745,1746,1747,1748,1749,1750,1751,1752,1753,1754,1755,1756,1757,1758,1759,1760,1761,1762,1763,1764,1765,1766,1767,1768,1769,1770,1771,1772,1773,1774,1775,1776,1777,1778,1779,1780,1781,1782,1783,1784,1785,1786,1787,1788,1789,1790,1791,1792,1793,1794,1795,1796,1797,1798,1799,1800,1801,1802,1803,1804,18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