

# The homology modelling results

## 1. PSI-BLAST

3 PSI-BLAST iterations were performed to extract the position specific scoring matrix (PSSM) from UniRef90 database. 109 hits were found in overall. 5 templates were selected with a highest total scores for both proteins. Templates for homology modelling for both NKX2-5 normal and variant are shown in Tab.S1.

**Table S1. The selected templates for homology modelling (both normal and variant)**

Template	Total score	BLAST E-value	Align score	Cover	ID	Resolution	Header
1	90.95	2,00E-21	240.0	53%	5FLV-I	3.00 Å	XRAY Homeobox protein Nkx-2.5 [Mus musculus] <NKX25_MOUSE(134-197)> (226 residues with quality score 0.717), released 2016-02-10
2	43.08	2,00E-26	255.0	21%	1FTT-A	Unknown or NMR	NMR Homeobox protein Nkx-2.1 [Rattus norvegicus] <NKX21_RAT(161-227)> (68 residues with quality score 0.805), released 1996-01-29
3	35.92	2,00E-18	113.0	32%	6M3D-C	1.60 Å	XRAY Segmentation polarity homeobox protein engrailed [Drosophila melanogaster] <HMEN_DROME(453-512)> (108 residues with quality score 1.000), released 2020-09-16
4	30.41	4,00E-20	175.0	21%	2L9R-A	Unknown or NMR	NMR Homeobox protein Nkx-3.1 [Homo sapiens] <NKX31_HUMAN(132-189)> (69 residues with quality score 0.816), released 2011-04-20
5	30.41	2,00E-25	164.0	19%	4RDU-D	1.85 Å	XRAY Homeobox protein DLX-5 [Homo sapiens] <DLX5_HUMAN(135-198)> (62 residues with quality score 0.969), released 2014-11-19

## 2. FASTA sequences

Fasta sequence for NKX2-5 (normal)

>NKX2-5

```
MFPSPALTPTPFSVKDILNLEQQQRLAAAGELSARLEATLAPSSCMLAAFKPEAYAGPE
AAAPGLPELRAELGRAPSPAKCASAFPAAPAFYPRAYSDPDPKDPRAEKKELCALQKAV
ELEKTEADNAERPRARRRRKPRVLFSQAQVYELERRFKQQRYSAPERDQLASVLKLTST
QVKIWFQNRRYKCKRQRQDQTLELVGLPPPPPPARRIAVPVLVRDGGKCLGDSAPYAPA
YGVGLNPYGYNAYPAYPGYGGAAACSPGYSCTAAYPAGPSPAQPATAAANNFVNFVGVGDL
NAVQSPGIPQNSGVSTLHGIRAW
```

Fasta sequence for NKX2-5 (mutation)

>NKX2-5 P.PRO280LEU.

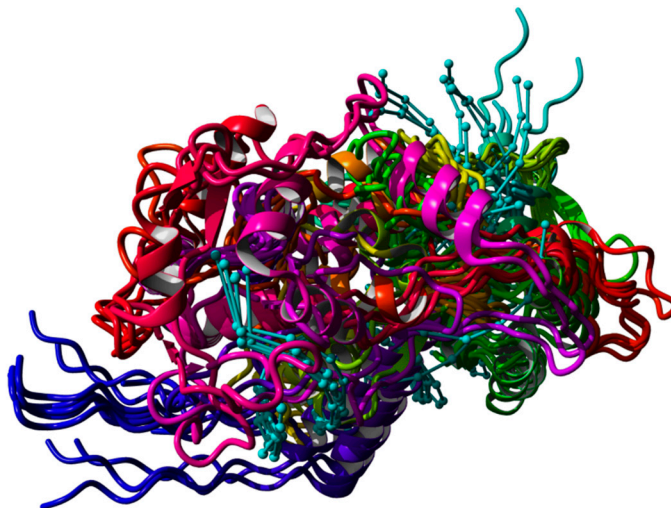
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MFPSPALTPTPFSVKDILNLEQQQRLAAAGELSARLEATLAPSSCMLAAFKPEAYAGPE
AAAPGLPELRAELGRAPSPAKCASAFPAAPAFYPRAYSDPDPKDPRAEKKELCALQKAV
```

ELEKTEADNAERPRARRRRRKPRVLFSQAQVYELERRFKQQRYSAPERDQLASVLKLTST  
 QVKIWFQNRRYKCKRQRQDQTLELVGLPPPPPPPARRIAVPVLVRDGGKPCLGDSAPYAPA  
 YGVGLNPYGYNAYPAYPGYGGAACSPGYSCTAAYPAGPSLAQPATAAANNFVNFGVGD  
 NAVQSPGIPQSNSGVSTLHGIRAW

### 3. Models used during homology modelling for NKX2-5 normal

**Table S2. The list of 13 models sorted by their Z-scores for NKX2-5 normal**

Rank	Z-score	Structure	State	Model ID	Filename	Original number	Residues	Comment
1	1.443		monomer	1FTT-A	<a href="#">nkx2-5_1ftt-a.yob</a>	6	143-197	Optimal
2	1.165		monomer	4RDU-D	<a href="#">nkx2-5_4rdu-d.yob</a>	13	138-198	Optimal
3	0.799		monomer	2L9R-A	<a href="#">nkx2-5_2l9r-a.yob</a>	12	145-196	Optimal
4	-0.805		monomer	6M3D-C01	<a href="#">nkx2-5_6m3d-c01.yob</a>	7	9-197	Good
5	-0.894		monomer	6M3D-C03	<a href="#">nkx2-5_6m3d-c03.yob</a>	9	9-197	Good
6	-0.978		monomer	6M3D-C05	<a href="#">nkx2-5_6m3d-c05.yob</a>	11	9-197	Good
7	-1.177		monomer	6M3D-C04	<a href="#">nkx2-5_6m3d-c04.yob</a>	10	9-197	Satisfactory
8	-1.296		monomer	6M3D-C02	<a href="#">nkx2-5_6m3d-c02.yob</a>	8	9-197	Satisfactory
9	-2.466		monomer	5FLV-I02	<a href="#">nkx2-5_5flv-i02.yob</a>	2	141-324	Poor
10	-2.606		monomer	5FLV-I01	<a href="#">nkx2-5_5flv-i01.yob</a>	1	141-324	Poor
11	-2.684		monomer	5FLV-I03	<a href="#">nkx2-5_5flv-i03.yob</a>	3	141-324	Poor
12	-2.859		monomer	5FLV-I04	<a href="#">nkx2-5_5flv-i04.yob</a>	4	141-324	Poor
13	-2.936		monomer	5FLV-I05	<a href="#">nkx2-5_5flv-i05.yob</a>	5	141-324	Poor



**Figure S8.** Aligned models from Tab. S2 (file nkx2-5\_normal\_13models.pdb)

**Table S3.** List of hybrid models obtained from the best parts of models listed in Tab.S2

Transfer	First residue	Last residue	Length	From model	Score
1	126	210	85	4RDU-D	<b>-6.419</b>
2	1	145	145	6M3D-C05	<b>-4.417</b> accepted
3	190	324	135	5FLV-I03	<b>-2.657</b> accepted
6	144	157	14	6M3D-C02	<b>-2.655</b> accepted
7	119	127	9	6M3D-C03	<b>-2.638</b> accepted
8	141	157	17	6M3D-C04	<b>-2.604</b> accepted
9	140	159	20	6M3D-C04	<b>-2.597</b> accepted
10	146	159	14	6M3D-C04	<b>-2.578</b> accepted
11	149	157	9	6M3D-C02	<b>-2.573</b> accepted
12	146	157	12	6M3D-C02	<b>-2.540</b> accepted
13	148	159	12	6M3D-C04	<b>-2.535</b> accepted
16	210	215	6	5FLV-I02	<b>-2.530</b> accepted

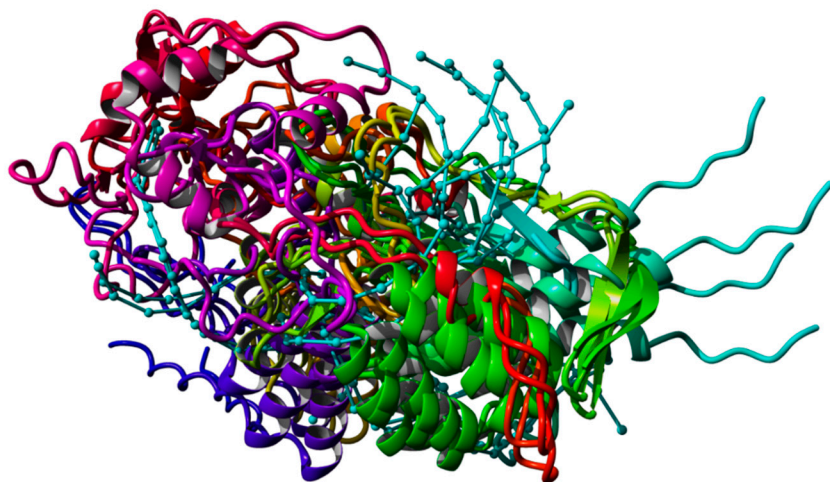


Figure S9 Superimposed hybrid models from Tab.S3 (file nkx2-5\_normal\_7models.pdb)

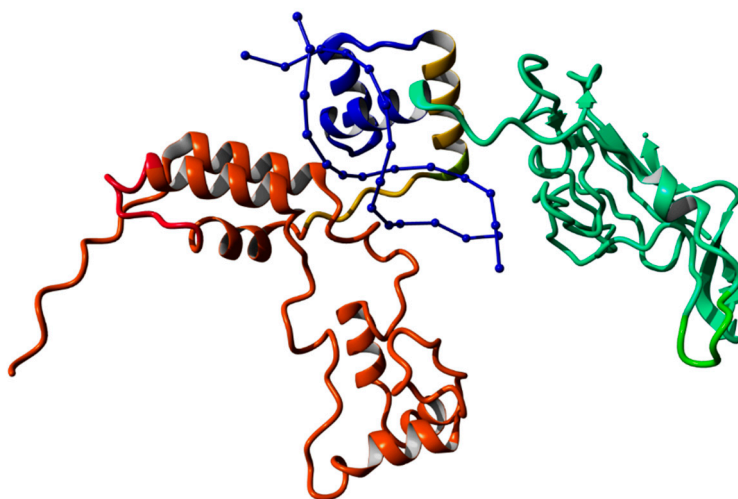













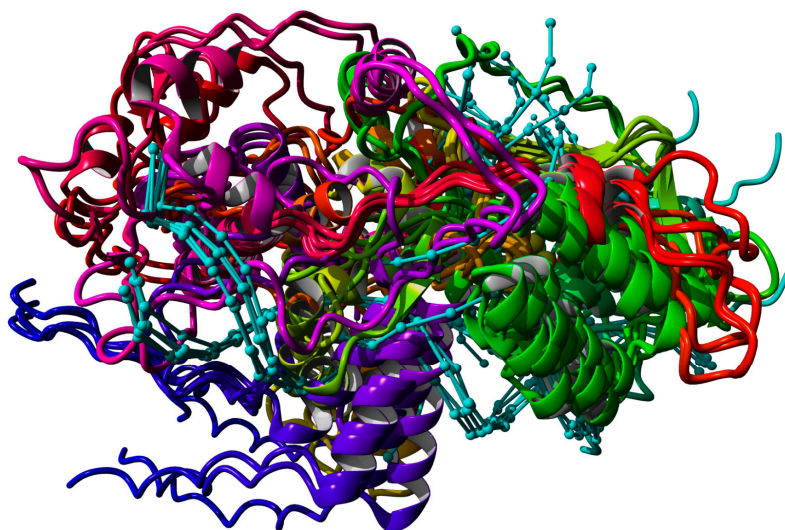


Figure S10 Final model for NKX2-5 normal with an overall Z-score -2.250 (Dihedrals -3.003 bad, Packing 1D - 1.805 satisfactory, Packing 3D -2.389 poor). (file nkx2-5\_normal.pdb)

#### 4. Models used during homology modelling for NKX2-5 variant

**Table S4. The list of 13 models sorted by their Z-scores for NKX2-5 variant**

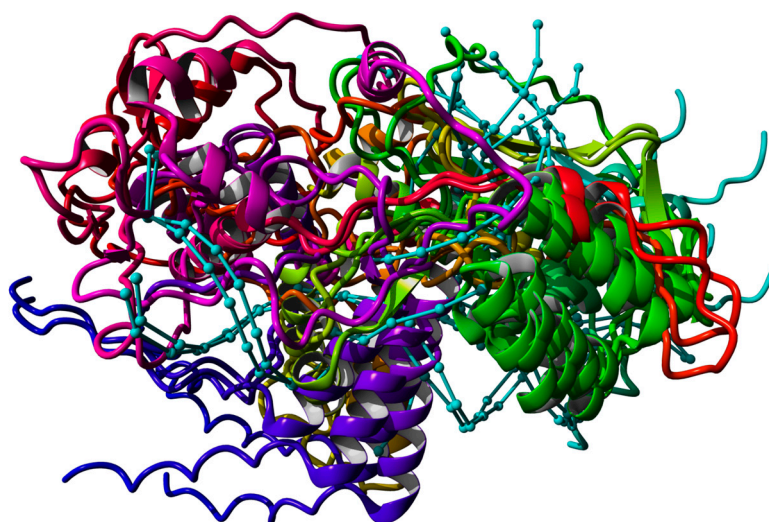
Rank	Z-score	Structure	State	Model ID	Filename	Original number	Residues	Comment
1	1.443		monomer	1FTT-A	<a href="#">nkx2-5 p.Pro280Leu. 1ftt-a.yob</a>	6	143-197	Optimal
2	1.165		monomer	4RDU-D	<a href="#">nkx2-5 p.Pro280Leu. 4rd-d.yob</a>	13	138-198	Optimal
3	0.799		monomer	2L9R-A	<a href="#">nkx2-5 p.Pro280Leu. 2l9r-a.yob</a>	12	145-196	Optimal
4	-0.805		monomer	6M3D-C01	<a href="#">nkx2-5 p.Pro280Leu. 6m3d-c01.yob</a>	7	9-197	Good
5	-0.894		monomer	6M3D-C03	<a href="#">nkx2-5 p.Pro280Leu. 6m3d-c03.yob</a>	9	9-197	Good
6	-0.978		monomer	6M3D-C05	<a href="#">nkx2-5 p.Pro280Leu. 6m3d-c05.yob</a>	11	9-197	Good
7	-1.177		monomer	6M3D-C04	<a href="#">nkx2-5 p.Pro280Leu. 6m3d-c04.yob</a>	10	9-197	Satisfactory
8	-1.296		monomer	6M3D-C02	<a href="#">nkx2-5 p.Pro280Leu. 6m3d-c02.yob</a>	8	9-197	Satisfactory
9	-2.359		monomer	5FLV-I02	<a href="#">nkx2-5 p.Pro280Leu. 5flv-i02.yob</a>	2	141-324	Poor
10	-2.492		monomer	5FLV-I04	<a href="#">nkx2-5 p.Pro280Leu. 5flv-i04.yob</a>	4	141-324	Poor
11	-2.774		monomer	5FLV-I05	<a href="#">nkx2-5 p.Pro280Leu. 5flv-i05.yob</a>	5	141-324	Poor
12	-2.828		monomer	5FLV-I01	<a href="#">nkx2-5 p.Pro280Leu. 5flv-i01.yob</a>	1	141-324	Poor
13	-2.973		monomer	5FLV-I03	<a href="#">nkx2-5 p.Pro280Leu. 5flv-i03.yob</a>	3	141-324	Poor



**Figure S11. Aligned models from Tab. S2 (file nkx2-5\_variant\_13models.pdb)**

**Table S5. List of hybrid models obtained from the best parts of models listed in Tab.S4**

Transfer	First residue	Last residue	Length	From model	Score
1	126	210	85	4RDU-D	<b>-6.419</b>
2	1	145	145	6M3D-C05	<b>-4.419</b> accepted
3	190	324	135	5FLV-I05	<b>-2.704</b> accepted
4	141	157	17	6M3D-C04	<b>-2.688</b> accepted
5	146	159	14	6M3D-C04	<b>-2.678</b> accepted
6	119	127	9	6M3D-C03	<b>-2.649</b> accepted
7	144	157	14	6M3D-C02	<b>-2.629</b> accepted
8	152	157	6	6M3D-C04	<b>-2.612</b> accepted
10	181	198	18	5FLV-I02	<b>-2.608</b> accepted
11	170	187	18	5FLV-I02	<b>-2.574</b> accepted
12	119	122	4	6M3D-C03	<b>-2.572</b> accepted
13	184	189	6	6M3D-C02	<b>-2.549</b> accepted
15	210	215	6	5FLV-I02	<b>-2.545</b> accepted
16	119	122	4	6M3D-C03	<b>-2.522</b> accepted
18	152	155	4	6M3D-C04	<b>-2.514</b> accepted
20	209	214	6	5FLV-I05	<b>-2.511</b> accepted



**Figure S12. Aligned models from Tab. S5 (file nkx2-5\_variant\_7models.pdb)**

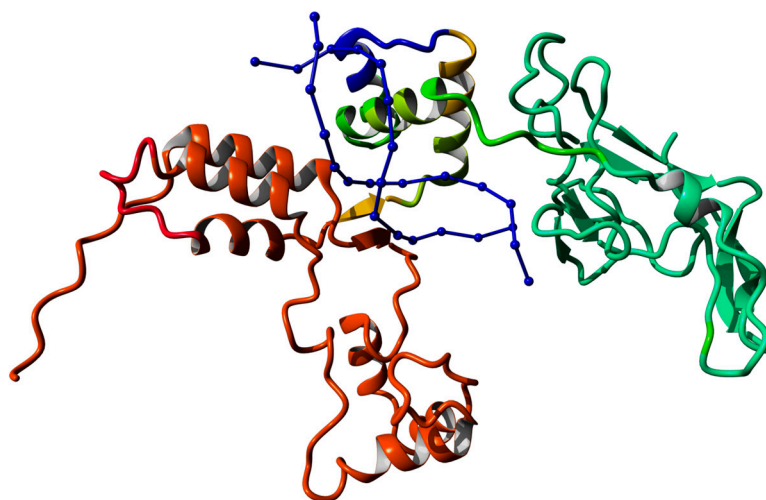


Figure S13. Final model for NKX2-5 variant with an overall Z-score -2.310 (Dihedrals -3.261 bad, Packing 1D - 1.845 satisfactory, Packing 3D -2.404 poor). (file nkx2-5\_variant.pdb)