# The calcium binding properties and structure prediction of the Hax-1 protein

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Hax-1 is a protein involved in regulation of different cellular processes, but its properties and exact mechanisms of action remain unknown. In this work, using purified, recombinant Hax-1 and by applying an *in vitro* autoradiography assay we have shown that this protein binds Ca²⁺. Additionally, we performed structure prediction analysis which shows that Hax-1 displays definitive structural features, such as two α-helices, short β-strands and four disordered segments.
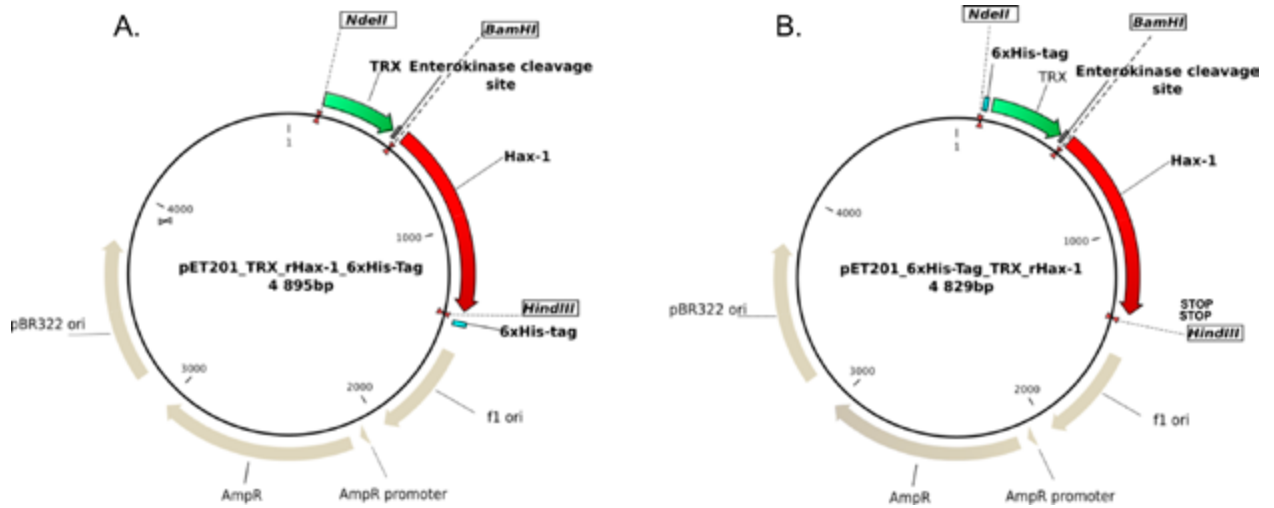
**Supplementary Figure 1. Generation of the pET201_6xHis_TRX_Hax-1 expression vector. Restriction sites used in cloning and the relevant features of the two vectors are marked.**
(**A**) A map of the initial pET201_TRX_Hax-1_6xHis vector used in cloning. (**B**) A map of the modified pET201_6xHis_TRX_Hax-1 expression vector. The vector was re-designed to encode the His-tag at the N-terminus, in frame with thioredoxin, and to destroy the C-terminal His-tag by the addition of two stop codons.
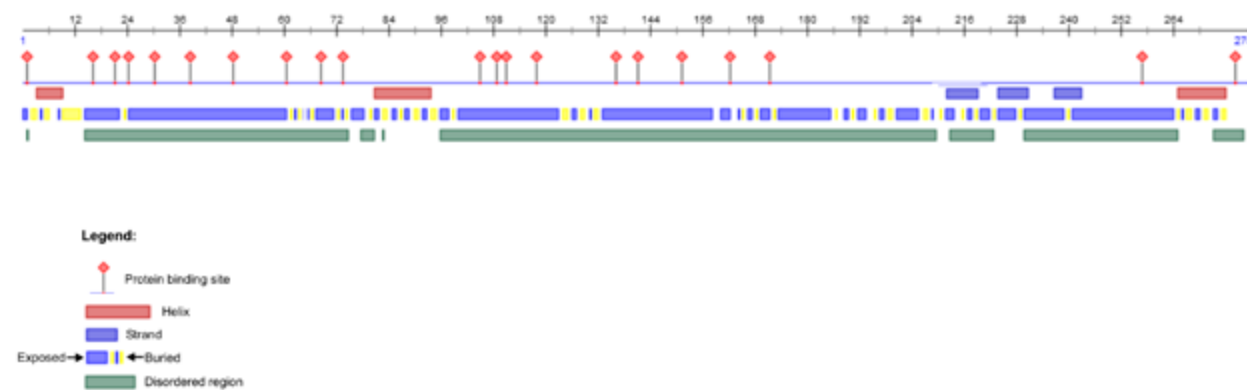
Supplementary Figure 2. Rat Hax-1 secondary structure prediction (PredictProtein 2013).
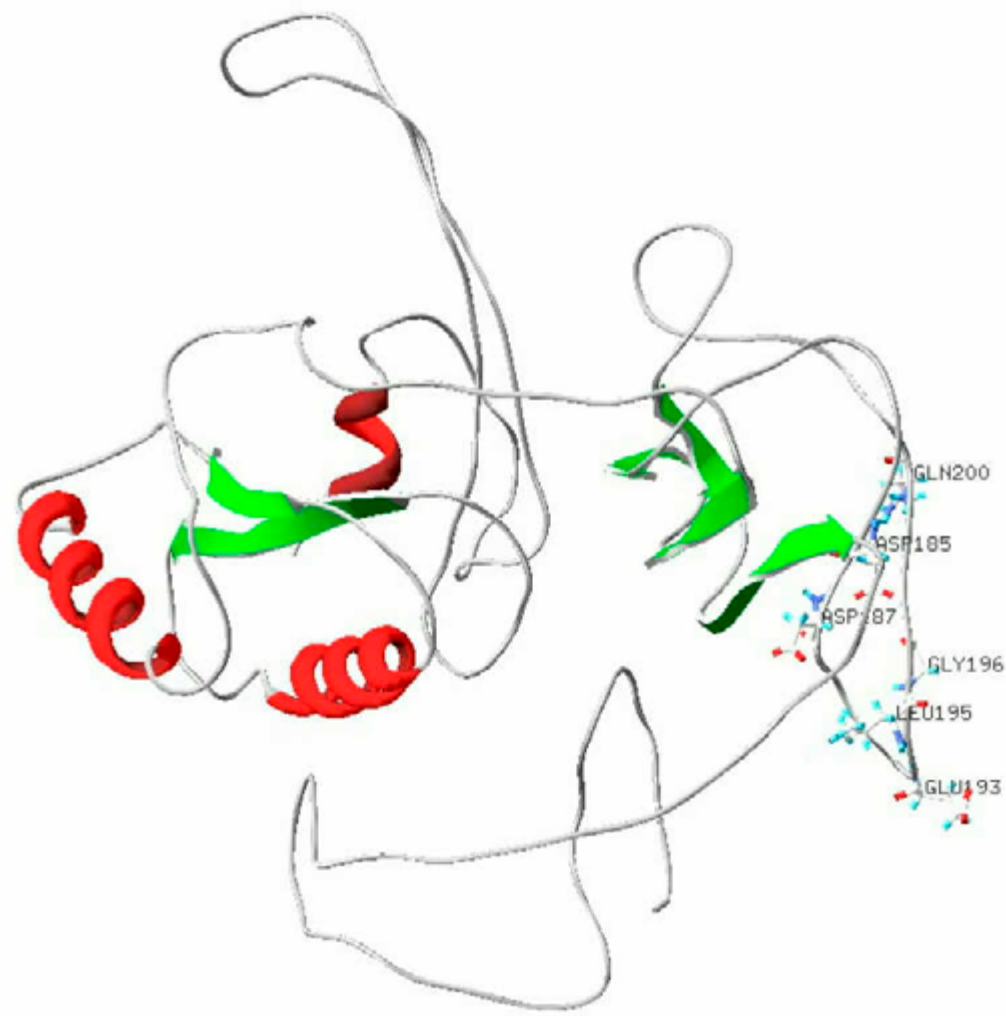


Video 1. An animation of the rat Hax-1 protein model, depicted in Fig. 4.
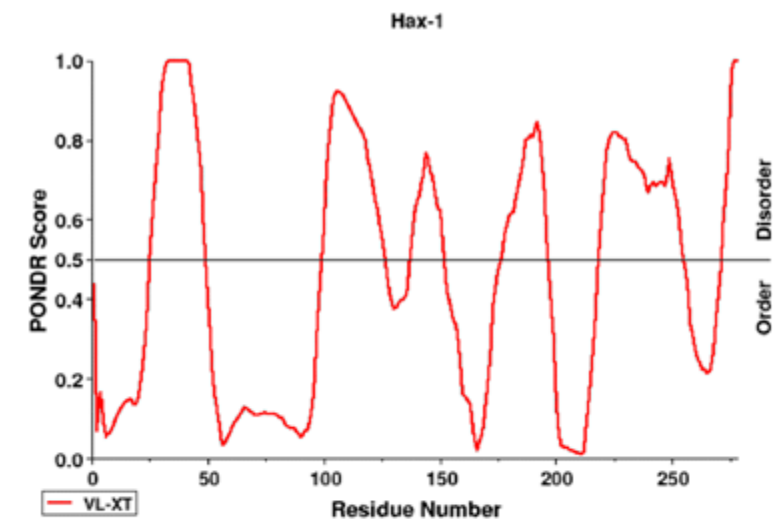


PONDR Protein Disorder Predictor
Developed by P. Romero, X. Li, A.K. Dunker,Z. Obradovic, E. Garner.
VL3 Predictor
Developed by P. Radivojac and A.K. Dunker
DEPP Predictor
Developed by P. Radivojac
VSL2 Predictor
Developped by K. Peng and Z. Obradovic
============================VLXT NNP STATISTICS=================================
Predicted residues: 278Number Disordered Regions: 6
Number residues disordered: 128Longest Disordered Region: 36
Overall percent disordered: 46.04Average Prediction Score: 0.4567
Predicted disorder segment [26]-[49]Average Strength= 0.8749
Predicted disorder segment [100]-[126]Average Strength= 0.7811
Predicted disorder segment [138]-[151]Average Strength= 0.6638
Predicted disorder segment [177]-[196]Average Strength= 0.7004
Predicted disorder segment [219]-[254]Average Strength= 0.7189
Predicted disorder segment [272]-[278]Average Strength= 0.8322
============================PREDICTOR OUTPUT=================================
          "D" = Disordered          " " = Ordered
=============================================================================
1 MSVFDLFRGF FGFPGPRSHR DPFFGGMTRD DDDDEDDEEE EDSGAWGRES
VLXT                DDDDD DDDDDDDDDD DDDDDDDDD

51 YAFDGFHPTE EFGFSFSPRG GMRFHGNFGF DDLVRDFNSI FSEMGAWTLP
VLXT                                    D

101 SHSPELPGPE SETPGVRLRE GQTLRDSMLK YPDSHQPRIF EGVLESHAKP
VLXT DDDDDDDDDD DDDDDDDDDD DDDDDD          DDD DDDDDDDDDD

151 ESSKPAPDWG SQGPFHRLDD TWPVSPHSRA REDKDLDSQV SQEGLGPLLQ
VLXT D                DDDD DDDDDDDDDD DDDDDD

201 PQPKSYFKSI SVTKITKPDG TVEEHRTVVD SEGRRETTVT HQEAHDSSRS
VLXT                DD DDDDDDDDDD DDDDDDDDDD DDDDDDDDDD

251 DPDPPRSSAL DDPFSILDLL LGRWFRSR
VLXT DDDD          DDDDDDD

Supplementary Figure 3. Rat Hax-1 analyzed by PONDR.