Role of intrinsically disordered regions in kinase signalling pathways: Substrate recognition in the c-Jun N-terminal kinase (JNK) pathway

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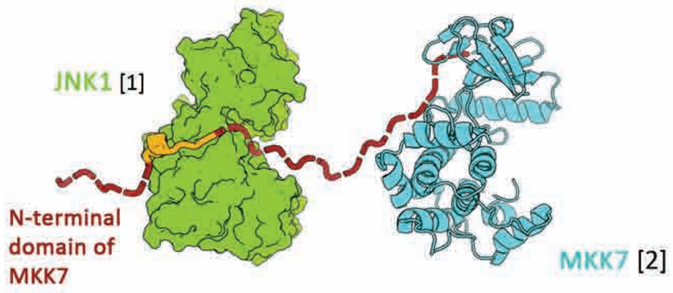
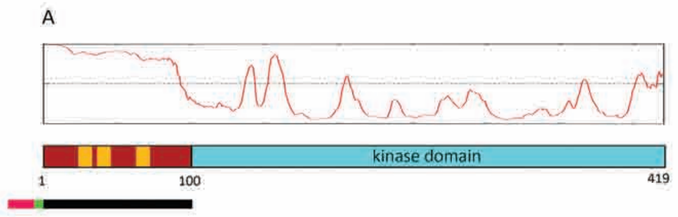
Introduction

Mitogen-activated protein kinase (MAPK) cell signalling pathways feature three sequentially acting protein kinases making up a signalling module: an MKKK (MAPK kinase kinase) that phosphorylates and thereby activates an MKK (MAPK kinase), which then activates the MAPK by phosphorylation. Our work focuses on the c-Jun N-terminal kinase (JNK) signalling pathway with special emphasis on the interaction between the two kinases MKK7 and JNK1.





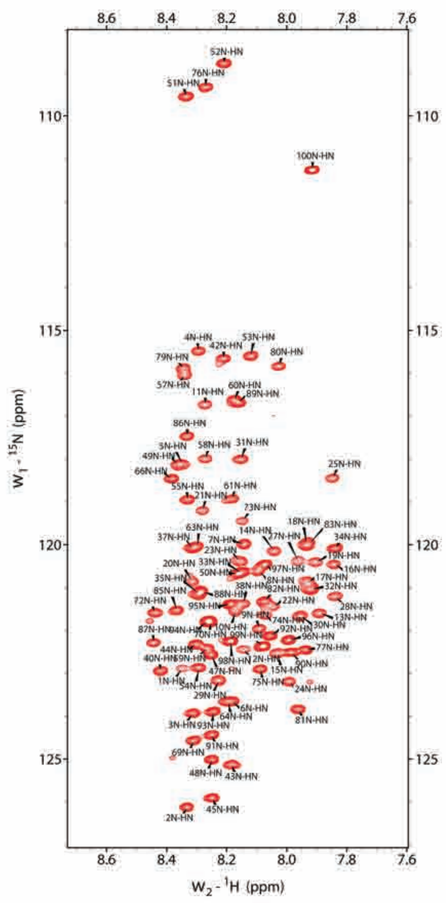
A: Pondr-fit disorder prediction for MKK7 [3]. Rectangle representation of domains is diplayed below with D-sites in yellow color.

B: Rectangle representation of JNK1-α1 and the corresponding construct used in this study.

Below: Sequence of MKK7 N-terminal domain construct. The three D-sites are colored in yellow. They all share the common motif R-X-R-X-X-L-X-L.

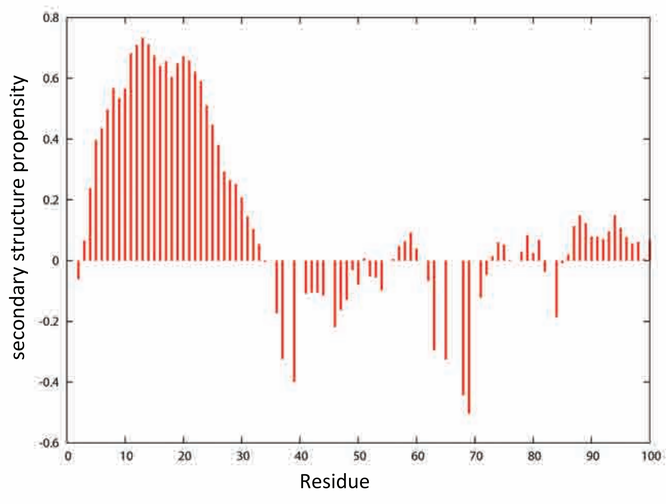
$^{-2}$GH ^{1}MAASSLEQKL ^{11}SRLEAKLKQE ^{21}NREARRRIDL ^{31}NLDISPQRPR ^{41}PTLQLPLAND
^{51}GGSRSPSSES ^{61}SPQHPTPPAR ^{71}PRHMLGLPST ^{81}LFTPRSMESI ^{91}EIDQKLQEIM

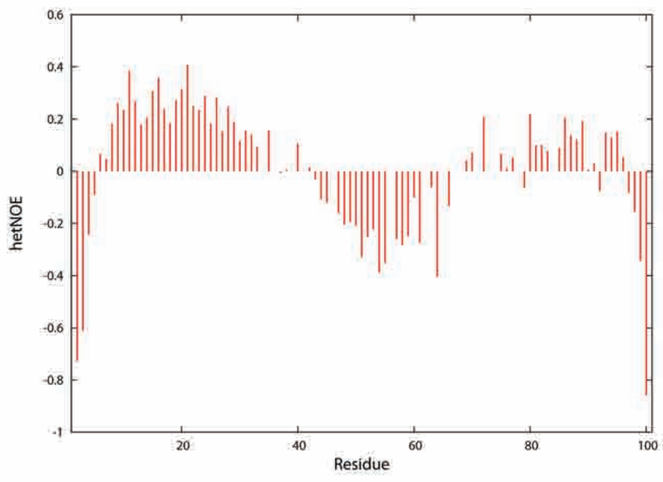
Assignment and structural characterisation of N-terminal domain of MKK7



left: ^{1}H-^{15}N HSQC of MKK7 N-terminal domain. The limited ^{1}H spectral dispersion shows that the protein is disordered. 87 residues out of 88 non-proline residues are assigned, prolines comprise 12 % of the sequence, no connectivity through prolines causes 4 ambiguities.

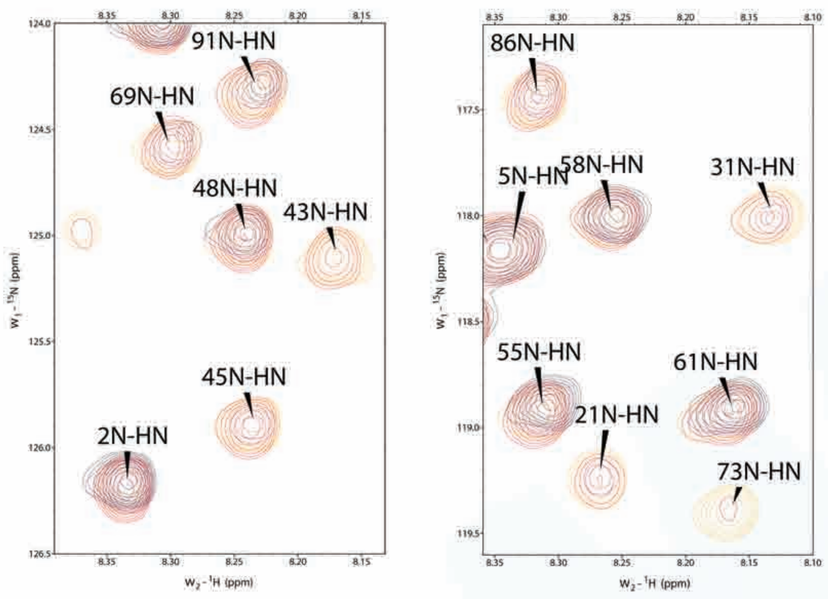
below: Experimental SSP values [4] for intrinsically disordered N-terminal domain of MKK7. First 30 residues are partialy populating an alpha helical state. Regions between 35 - 55 and 65 - 75 have a tendency for a beta strand or an extended conformation.





{^{1}H}-^{15}N heteronuclear NOEs of the intrinsically disordered N-terminal domain of MKK7, measured on 600 MHz spectrometer. Negative and small positive values demonstrate disorder in this MKK7 domain. Slightly higher values for the first 40 amino acids are in agrement with observed increased alpha helical propensity in the secondary chemical shifts.

Chemical shifts do not change during MKK7 N-terminal domain titration with JNK1

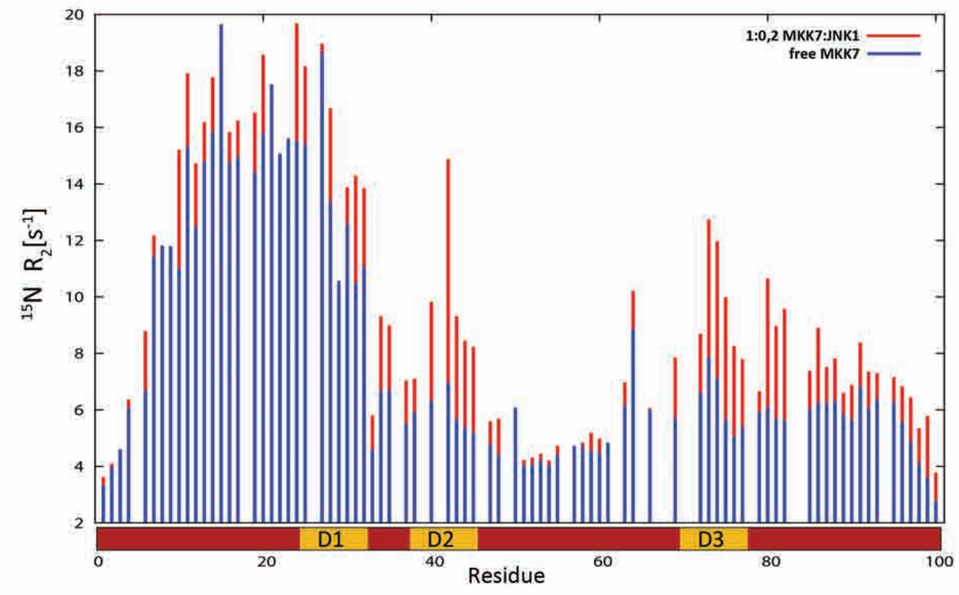


Regions of overlayed titration ^{1}H-^{15}N HSQC spectra of the MKK7 N-terminal domain.

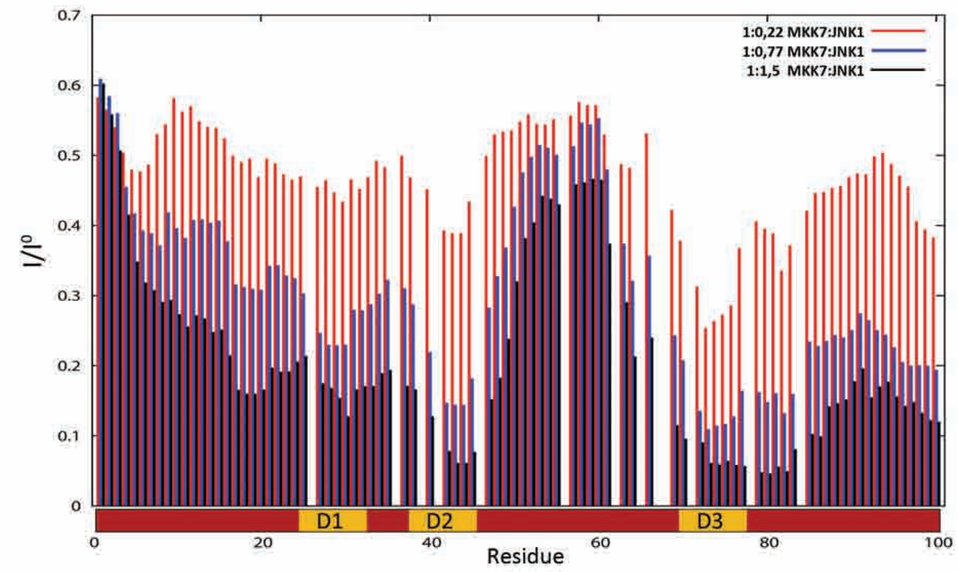
Increasing the JNK1/MKK7 N-terminal domain ratio makes intensities of some peaks drop while other peaks' intensities do not decrease much. Only a few peaks display minor chemical shift changes during titration.

Free form in orange. JNK1:MKK7 ratio 1:0,22 in red. JNK1:MKK7 ratio 1:0,77 in purple. JNK1:MKK7 ratio 1:1,5 in black.

Identification of interacting regions in MKK7



Transverse ^{15}N relaxation for the intrinsically disordered N-terminal domain of MKK7 in free form (blue) and after JNK1 is added (red). Concentration of MKK7 N-terminal domain was the same in both experiments. JNK1 is added at a ratio 1:0,22 (MKK7:JNK1). Relaxation rate increases the most for the residues of the D2 and D3 sites. Relaxation rates do not change in the central region.



Titration of MKK7 N-terminal domain with JNK1. Peak intensities were extracted from 1H-15N HSQC spectra. They were normalized by dividing with intensities of the free MKK7 N-terminal domain. Residues of D2 and D3 sites display largest decrease in intensities. Binding events do not perturb the intensities of the peaks in the far N-terminal and in the central region. The results are in conformity with ^{15}N transverse relaxation rates.

References:

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