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

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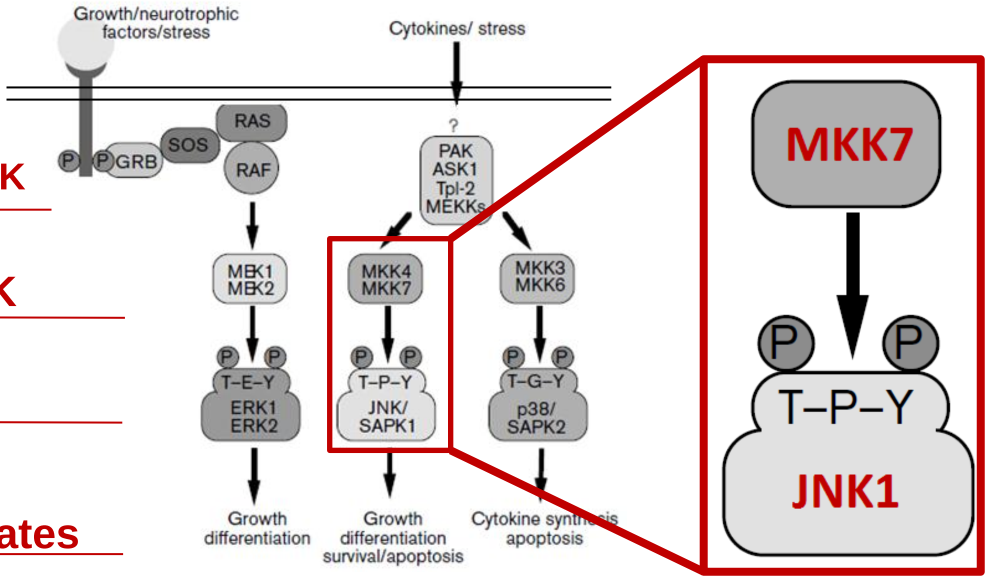
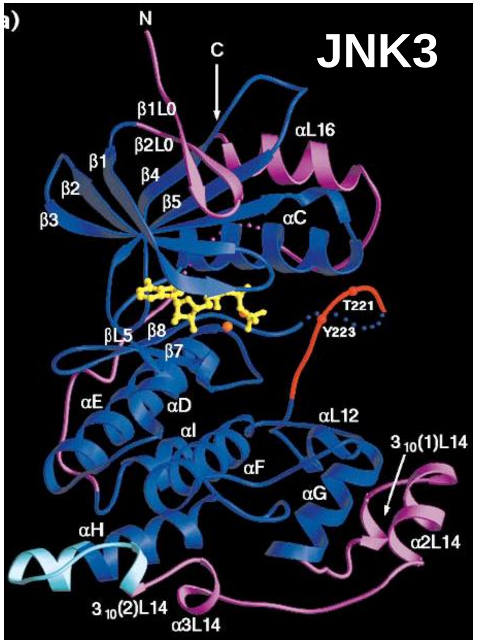
# Mitogen-Activated Protein Kinase networks

- MAP**KKK**
- MAP**KK**
- MAP**K**
- MAPK **substrates**

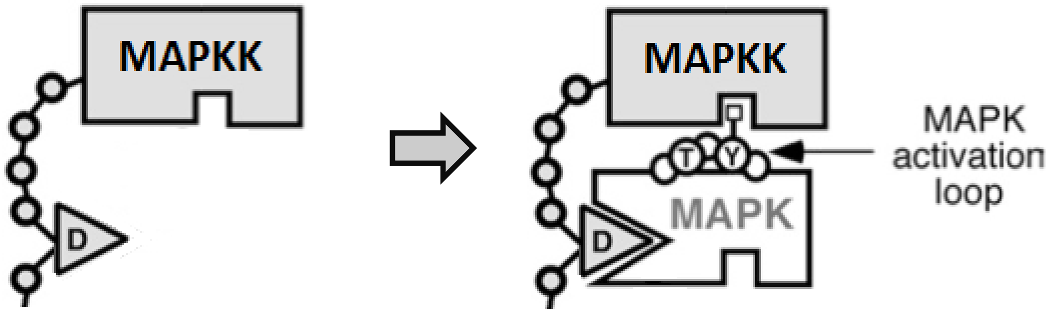


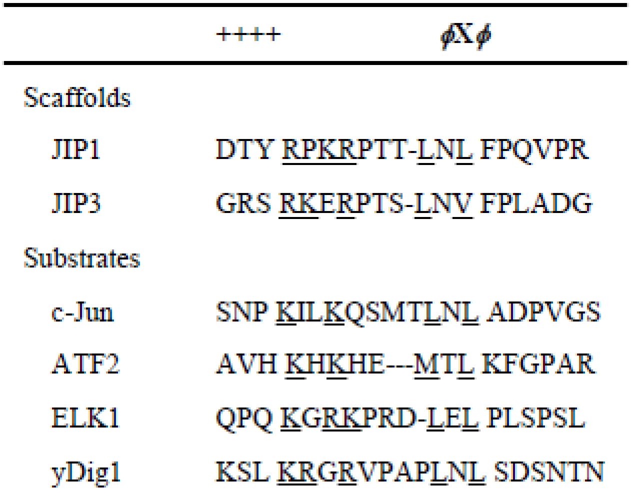
Keyse S. M., Curr Opin In Cell Biol (2000)



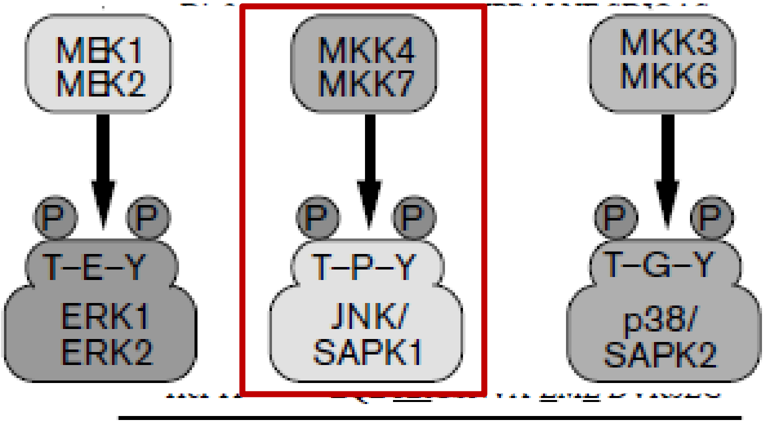
Xie X., Structure 1998

# MAPKK-MAPK, MAPK-substrate, MAPK phosphatase





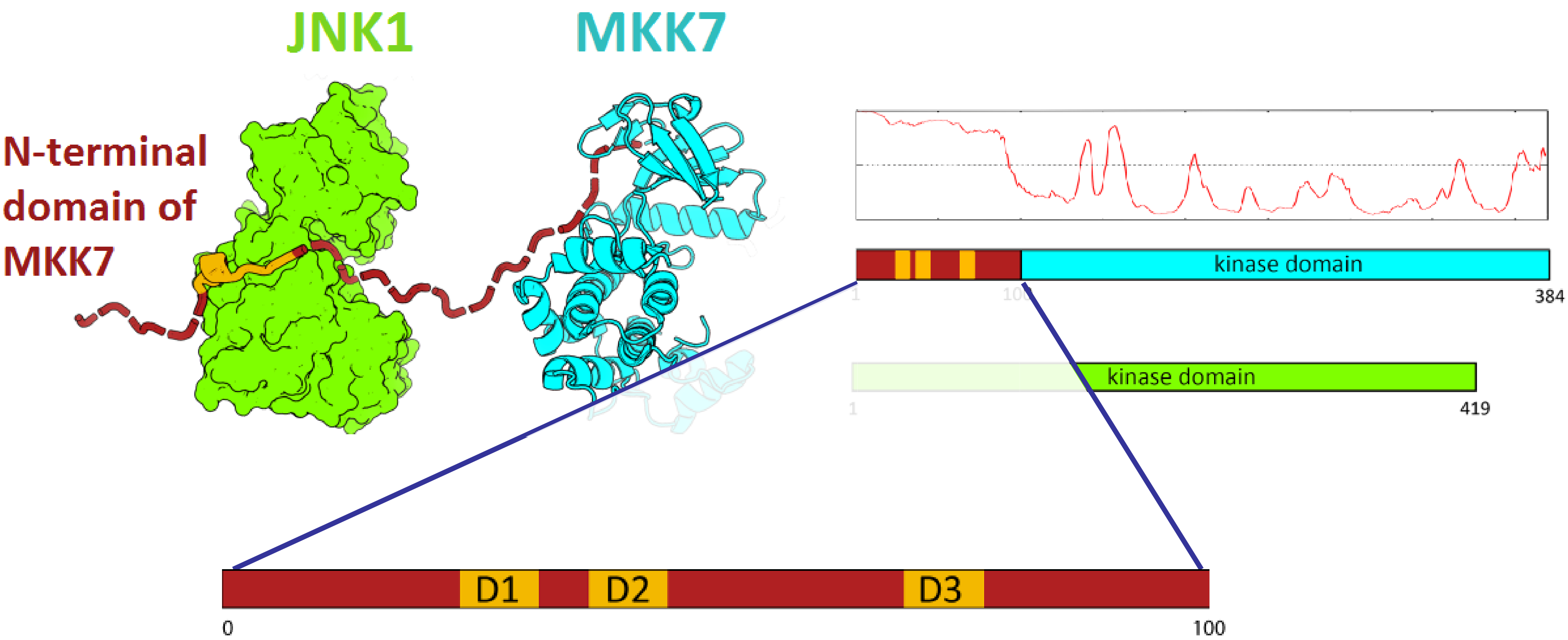
| | ++++ | ϕXϕ |
|---|---|---|
| Scaffolds | | |
| JIP1 | DTY RPKRPTT-LNL FPQVPR | |
| JIP3 | GRS RKERPTS-LNV FPLADG | |
| Substrates | | |
| c-Jun | SNP KILKQSMTLNL ADPVGS | |
| ATF2 | AVH KHKHE---MTL KFGPAR | |
| ELK1 | QPQ KGRKPRD-LEL PLSPSL | |
| yDig1 | KSL KRGRVPAPLNL SDSNTN | |



| Peptide | Sequence |
|---|---|
| | +++ ϕxϕ |
| **MEK1** 🟦 | MP KKKPTP--IQL NPAPDG |
| **MEK2** ◆ | MLA RRKPVLPALTI NPTIAE |
| **MKK3** ▲ | GKS KRKKD---LRI SCMSKP |
| **MKK6** 🟩 | SQSKG KKRNPG--LKI PKEAFE |
| **MKK4** ● | MQG KRKA----LKL NFANPP |
| **MKK7-D2** | Q RPRPT---LQL PLANDG |

Bardwell L., Biochem Soc Trans (2006)
Bardwell A. J. et al., JBiol Chem (2009)

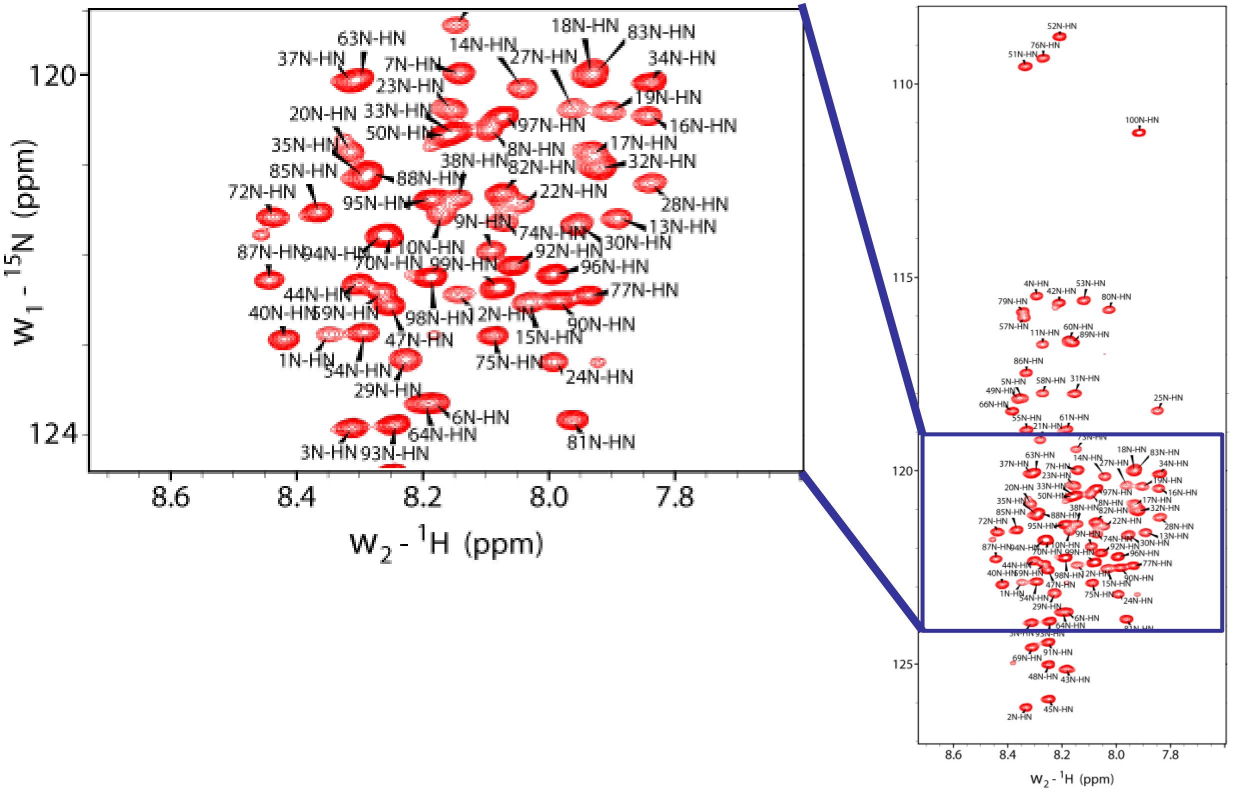
# MKK7 - JNK interaction



$^{1}$MAASSLEQKL $^{11}$SRLEAKLKQE $^{21}$NREA**RRRIDL** $^{31}$**NL**DISPQ**RPR** $^{41}$**PTLQL**PLAND
$^{51}$GGSRSPSSES $^{61}$SPQHPTPPA**R** $^{71}$**PRHMLGL**PST $^{81}$LFTPRSMESI $^{91}$EIDQKLQEIM

# Assignment

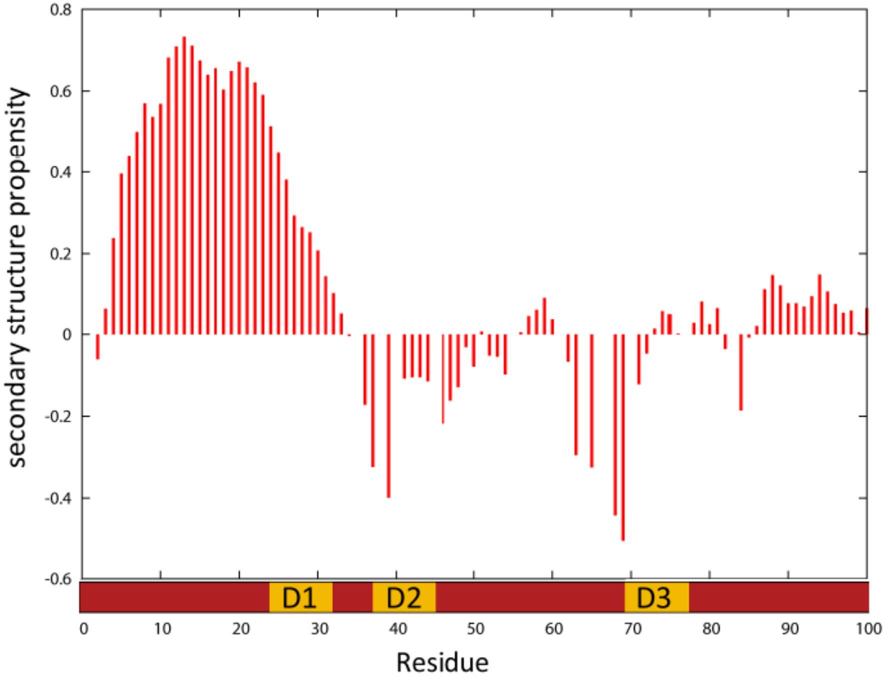
- 100 residues
- 12 % prolines
- 87 out of 88 non-proline residues are assigned
- $^{1}$H spectral dispersion shows that the domain is disordered
- two interchangeable P-X-X-P motifs need to be confirmed

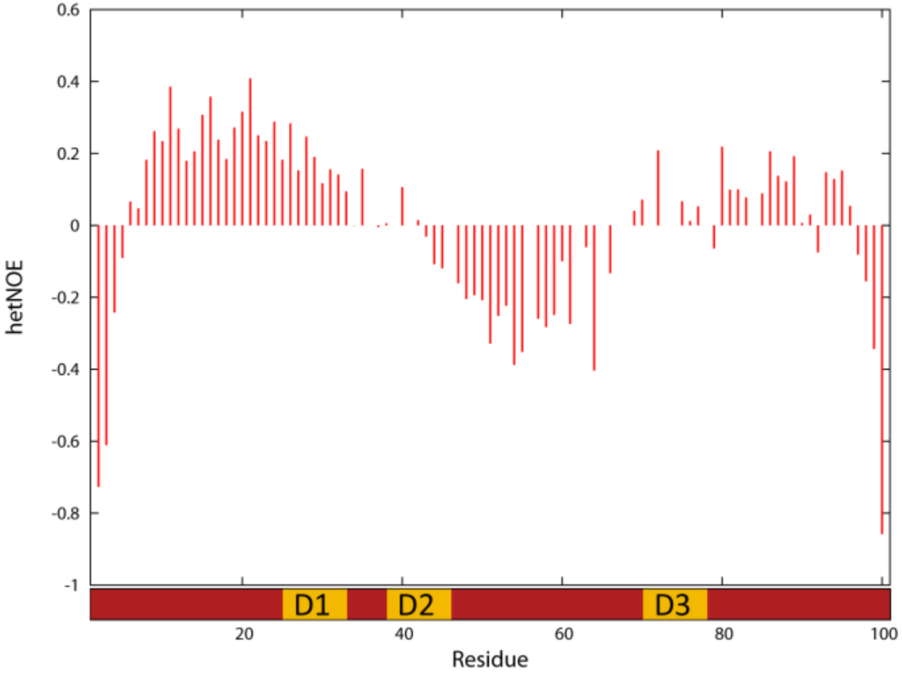


$^{1}$MAASSLEQKL $^{11}$SRLEAKLKQE $^{21}$NREARRRIDL $^{31}$NLDIS**P**QR**P**R $^{41}$**P**TLQL**P**LAND

$^{51}$GGSRS**P**SSES $^{61}$S**P**QH**P**T**PP**AR $^{71}$**P**RHMLGL**P**ST $^{81}$LFT**P**RSMESI $^{91}$EIDQKLQEIM

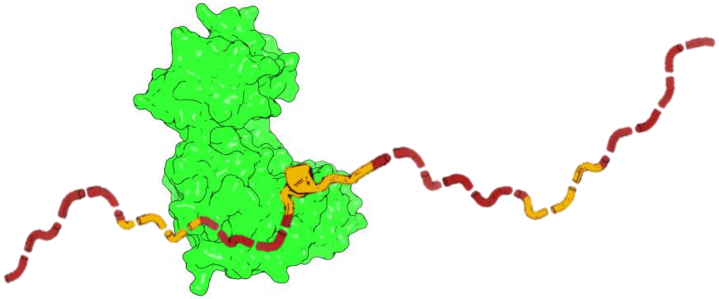
# Characterization of N-terminal domain of MKK7





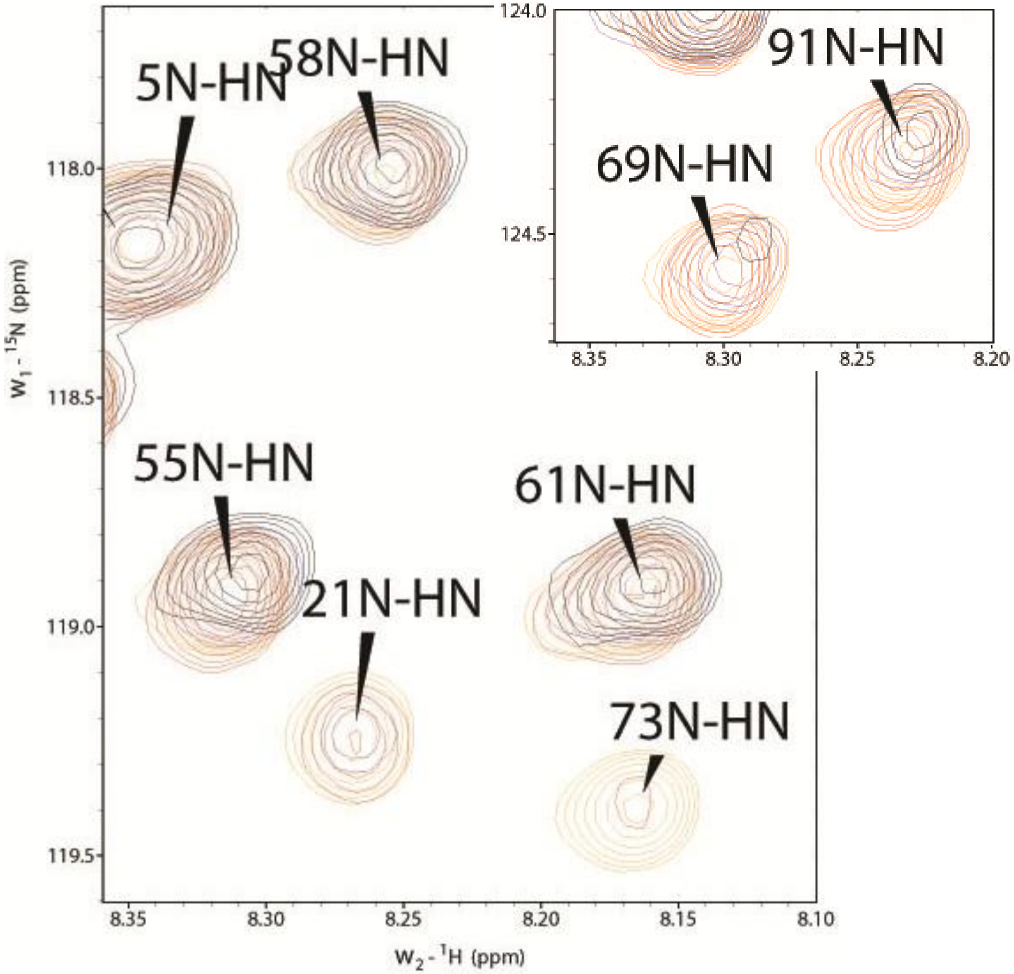
- a region with α-helical propensity at the N-terminus
- the rest of the N-terminal domain of MKK7 is extended and flexible
- C-terminus not as extended and flexible as in the central region

# Titration with JNK1

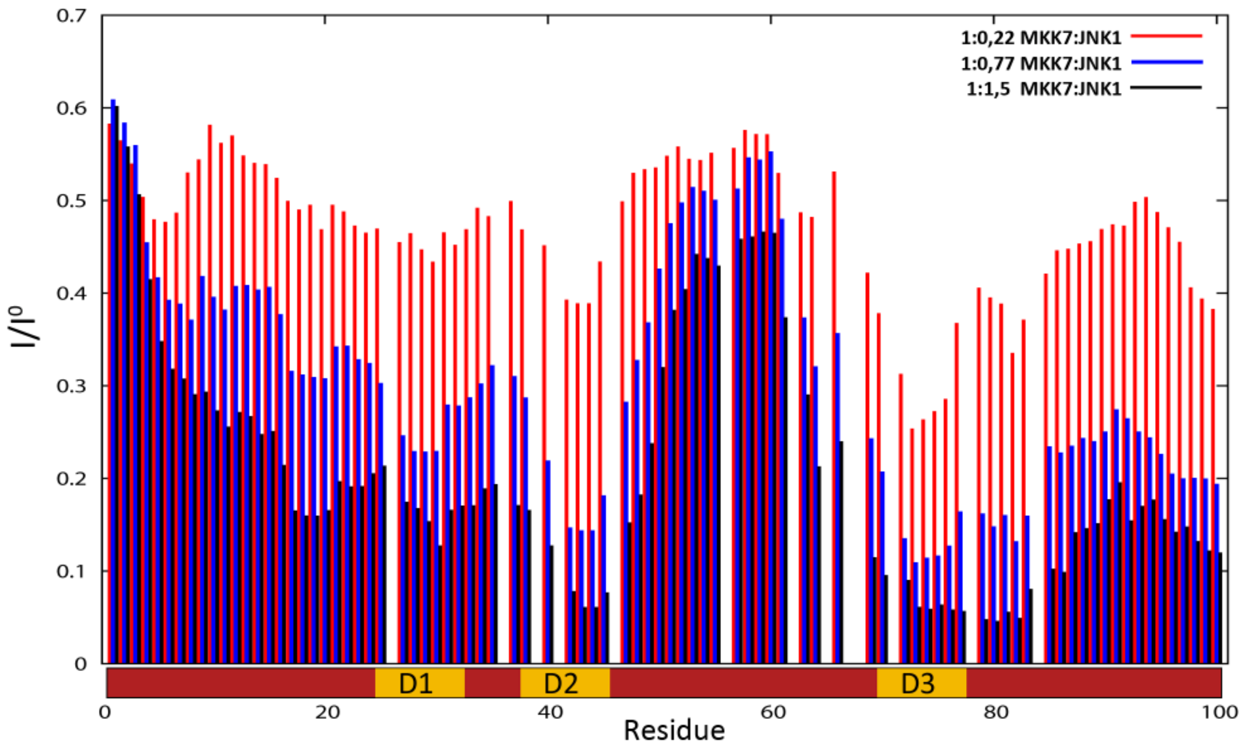


Expected:

- high $M_W$ complex
  (43 kDa + 11 kDa)
- Three possibly interchanging binding sites
- residues outside the D-site motif interact too
- N- and C-termini far away from the D-sites are less likely to bind

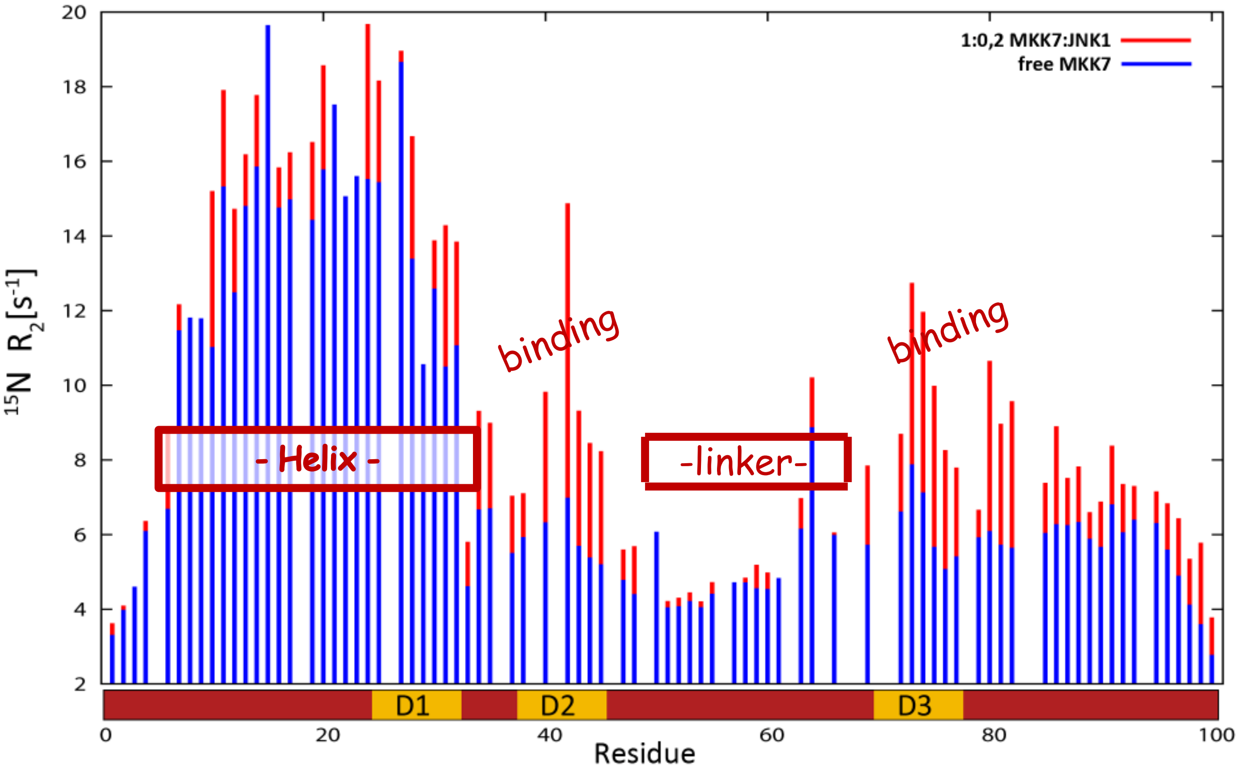


# Peak intensities drop in restricted regions



- $^{1}H$-$^{15}N$ HSQC of [1H,15N] MKK7 – JNK1 complex at three different ratios
  - 1 : 0,22
  - 1 : 0,77
  - 1 :~1,5
- normalized peak intensities
- averaged with a window function over 5 residues
- D1 < D2 < D3

# Measurement of $T_2$ relaxation rates confirms mapped binding regions



- Comparison between the unbound form (blue) and complex at a ratio 1:0,22 [1H,15N] MKK7 – JNK1 (red)
- $T_2$ relaxation rates do not increase in the linker region between the D2 and D3 sites

$^{32}$DISPQRPR $^{41}$PTLQLPLAND $^{51}$GGSRSPSSES $^{61}$SPQHPTPPAR $^{71}$PRHMLGLPST $^{81}$LFT

# Perspectives

- behavior of the N-terminal domain in full length MKK7

- More information on D-site affinities
- Better description of local conformational sampling along the N-terminal domain of MKK7
- Comparison with other interacting MKK – MAPK pairs
- ➢ Understanding of features governing specificity