X-ray Crystal Structure of ERK5 (MAPK7) in Complex with a Specific Inhibitor

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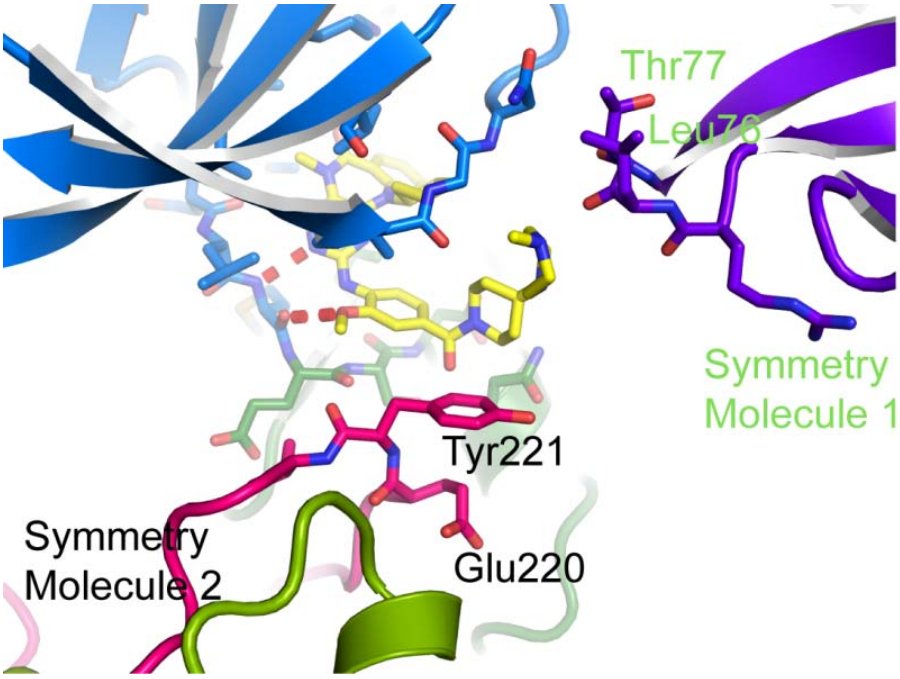
Supplementary Information

Contents

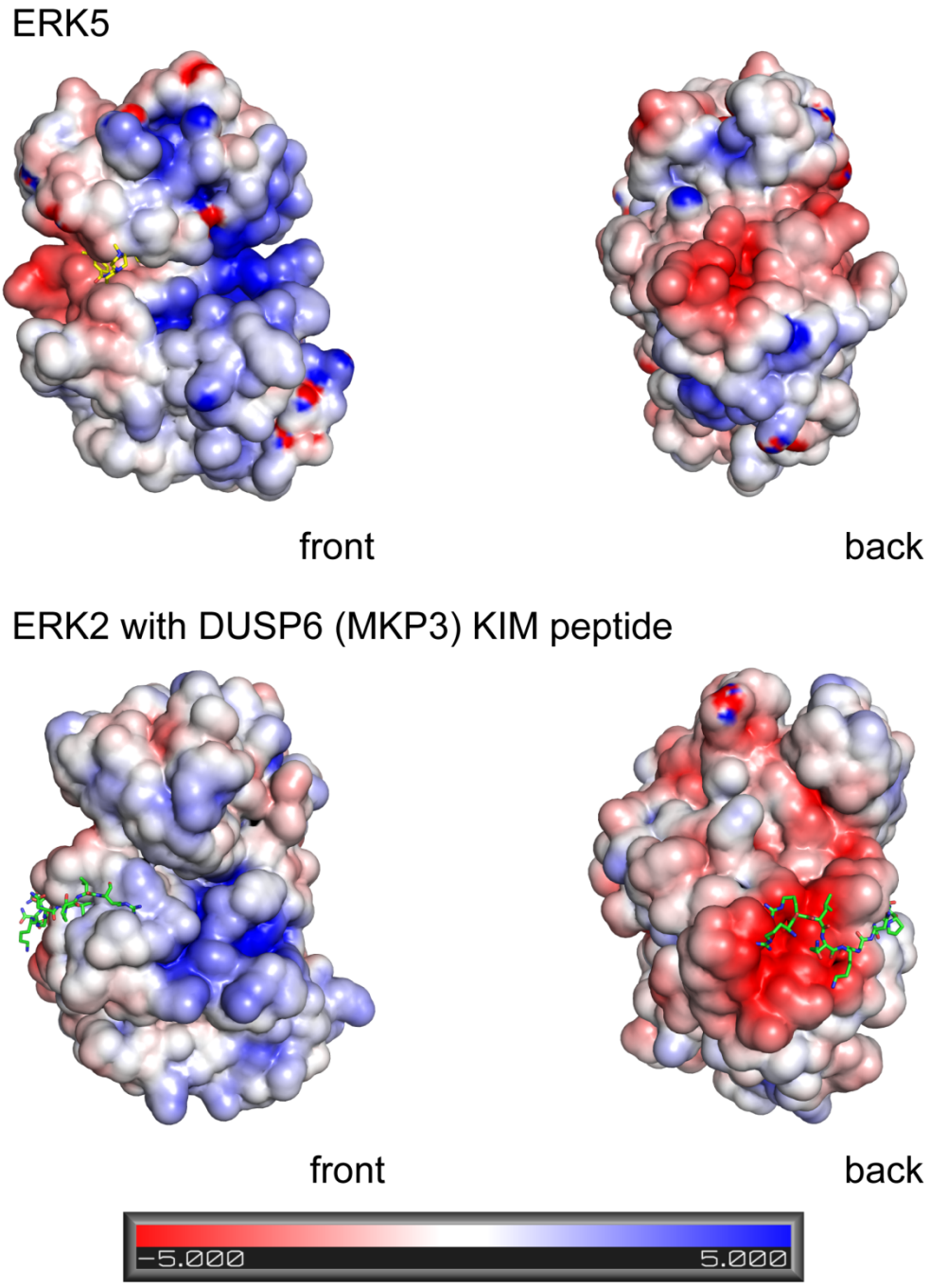
Supplementary Figure 1

Interaction of the inhibitor **25** (in yellow) with two other ERK5 molecules in the crystal lattice. The symmetry-related ERK5 molecules are shown in purple (1st molecule), and green and pink (2nd molecule, activation loop in pink).



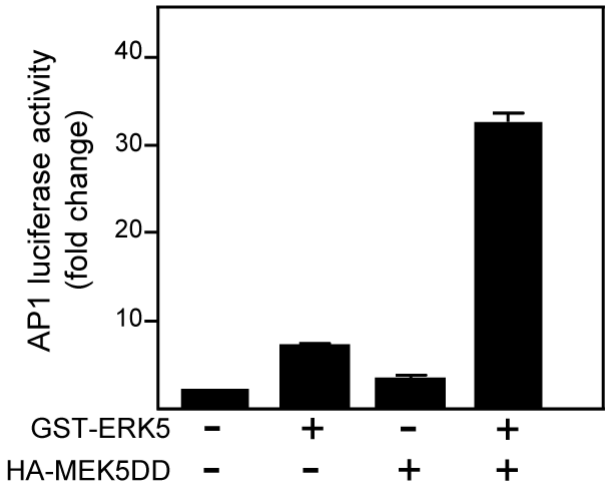
Supplementary Figure 2

Charged surface comparison of ERK5 with the complex between ERK2 and a DUSP6 (MKP3) Kinase Interaction Motif (KIM) docking peptide (PDB ID 2FYS) (Liu S. et al., PNAS, 2006, 103, 5326-5331). The KIM peptide in the ERK2 structure is shown in green, and the inhibitor in the ERK5 structure is shown in yellow. Scale in electronvolts.



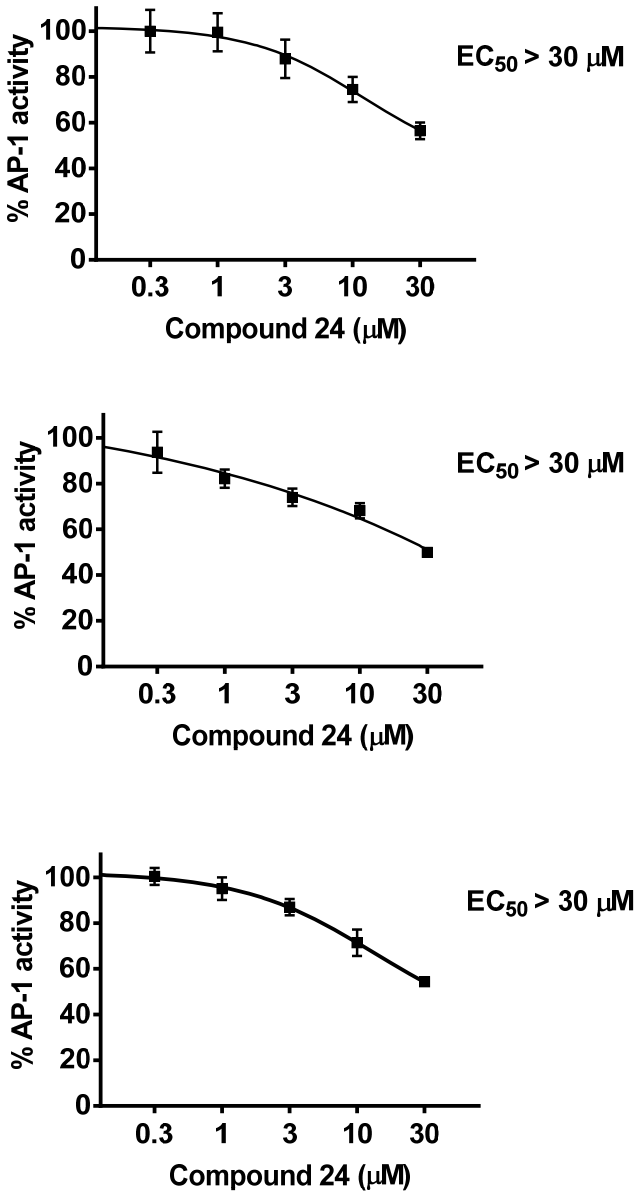
Supplementary Figure 3

Activation of ERK5 by active MEK5 (MEK5DD) induces AP1 transcriptional activity. The pAP1-luciferase reporter and pRL-CMV-Renilla plasmids were co-transfected with plasmids encoding for the indicated proteins and 24 h later lysates were subjected to the dual-luciferase assay. Each value is the mean ± s.d. of four different determinations, each performed in triplicate, and normalized using the Renilla values.



Supplementary Figure 4

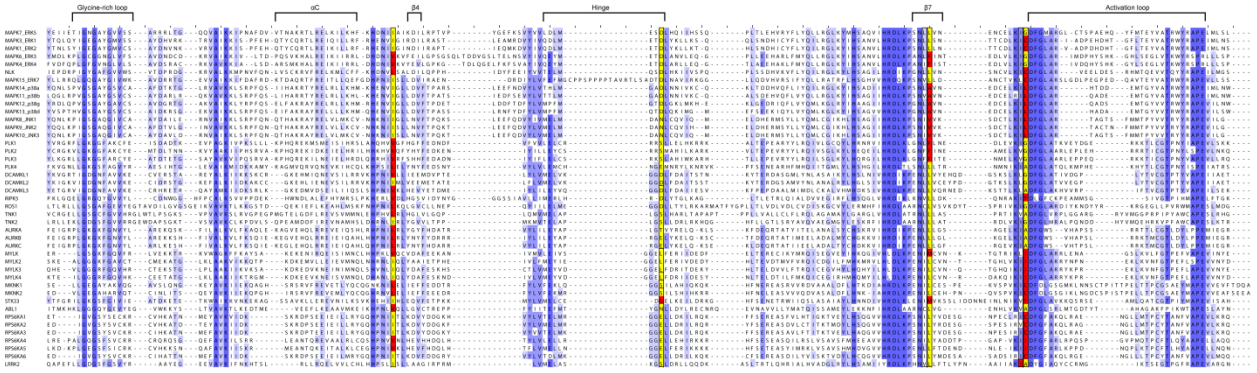
EC_{50} for **24** (ERK5-mediated AP-1 transcriptional activity assay).



Compound 24 EC_{50} >30 μM

Supplementary Figure 5

Sequence alignment of the N-terminal regions of various kinases relevant for the discussion of inhibitor selectivity for the ERK5-IN-1 inhibitor series.



ERK5 Protein Sequences used for crystallisation

Expressed protein sequence (including His6 tag):

MGHHHHHHSSGVDLGTENLYFQSMAEPLKEEDGEDGSAEPPGPVKAEPAHTAASVAAKNLALLKARSFDV
TFDVGDEYEIIETIGNGAYGVVSSARRRLTGQQVAIKKIPNAFDVVTNAKRTLRELKILKHFKHDNIIAI
KDILRPTVPYGEFKSVYVVLDLMESDLHQIIHSSQPLTLEHVRYFLYQLLRGLKYMHSAQVIHRDLKPSN
LLVNENCELKIGDFGMARGLCTSPAEHQYFMTEYVATRWYRAPELMLSLHEYTQAIDLWSVGCIFGEMLA
RRQLFPGKNYVHQLQLIMMVLGTPSPAVIQAVGAERVRAYIQSLPPRQPVPWETVYPGADRQALSLLGRM
LRFEPSARISAAAALRHPFLAKYHDPDDEPDCAPPFDFAFDREALTRERIKEAIVAEIEDFHARREGIRQ

Crystallised protein sequence (after TEV protease digestion):

SMAEPLKEEDGEDGSAEPPGPVKAEPAHTAASVAAKNLALLKARSFDVTFDVGDEYEIIETIGNGAYGVV
SSARRRLTGQQVAIKKIPNAFDVVTNAKRTLRELKILKHFKHDNIIAIKDILRPTVPYGEFKSVYVVLDL
MESDLHQIIHSSQPLTLEHVRYFLYQLLRGLKYMHSAQVIHRDLKPSNLLVNENCELKIGDFGMARGLCT
SPAEHQYFMTEYVATRWYRAPELMLSLHEYTQAIDLWSVGCIFGEMLARRQLFPGKNYVHQLQLIMMVLG
TPSPAVIQAVGAERVRAYIQSLPPRQPVPWETVYPGADRQALSLLGRMLRFEPSARISAAAALRHPFLAK
YHDPDDEPDCAPPFDFAFDREALTRERIKEAIVAEIEDFHARREGIRQ