Crystal Structures of MEK1 Binary and Ternary Complexes with Nucleotides and Inhibitors – Supporting information

Table 1: primers used for the crystallization constructs

fNKF	5'GGAGGCCCCTTAACAAATTTGGAATGGACAGCCGACCTCCC
rNKF	5'GGGAGGTCGGCTGTCCATTCCAAATTTGTTAAGGGGCCTCC
fN36	5'GCTCTAGCTCCTCCAGCTTCTTGCCCTGAAAATA CAGG3'
rN36	5'CCTGTATTTTCAGGGCAAGAAGCTGGAGGAGCTAGAGC
fCdel384	5'GCAGGTTGGCTCTGCTCCACCATCGGCCTTAACCAGTAAAAGGGTGGGCGCGCCGACCCAGC
rCdel384	5'GCTGGGTCGGCGCGCCCACCCTTTTACTGGTTAAGGCCGATGGTGGAGCAGAGCCAACCTGC

Table 2: MEK1 (SwissPROT accession code Q02750) and MEK2 (P36507) sequence alignment

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MEK1  -MPKKKPTP--IQLNP-APDGSAVNGTSSAETNLEALQKKLEELELDEQQRKRLEAFLTQ  56
MEK2  MLARRKPVLPALTINPTIAEGPSPTSEGASEANLVDLQKKLEELELDEQQKKRLEAFLTQ  60
       :.::**.   : :**  .:*.: .. .::*:**  ***************:*********
MEK1  KQKVGELKDDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEIKPAIRNQIIRELQ  116
MEK2  KAKVGELKDDDFERISELGAGNGGVVTKVQHRPSGLIMARKLIHLEIKPAIRNQIIRELQ  120
      * ************:************ **.*:****:************************
MEK1  VLHECNSPYIVGFYGAFYSDGEISICMEHMDGGSLDQVLKKAGRIPEQILGKVSIAVIKG  176
MEK2  VLHECNSPYIVGFYGAFYSDGEISICMEHMDGGSLDQVLKEAKRIPEEILGKVSIAVLRG  180
      ****************************************:* ****:**********::*
MEK1  LTYLREKHKIMHRDVKPSNILVNSRGEIKLCDFGVSGQLIDSMANSFVGTRSYMSPERLQ  236
MEK2  LAYLREKHQIMHRDVKPSNILVNSRGEIKLCDFGVSGQLIDSMANSFVGTRSYMAPERLQ  240
      *:******:**************************************************:*****
MEK1  GTHYSVQSDIWSMGLSLVEMAVGRYPIPPPDAKELELMFG-CQVEGDAAETP---PRPRT  292
MEK2  GTHYSVQSDIWSMGLSLVELAVGRYPIPPPDAKELEAIFGRPVVDGEEGEPHSISPRPRP  300
      ********************:**************** :**   *:*: .*.    ****.
MEK1  PGRPLSSYGMDSRPPMAIFELLDYIVNEPPPKLPSGVFSLEFQDFVNKCLIKNPAERADL  352
MEK2  PGRPVSGHGMDSRPAMAIFELLDYIVNEPPPKLPNGVFTPDFQEFVNKCLIKNPAERADL  360
      ****:*.:******.*******************.***: :**:****************
MEK1  KQLMVHAFIKRSDAEEVDFAGWLCSTIGLNQPSTPTHAAGV  393
MEK2  KMLTNHTFIKRSEVEEVDFAGWLCKTLRLNQPGTPTRTAV-  401

      * *  *:*****:.**********.*: ****.***::*
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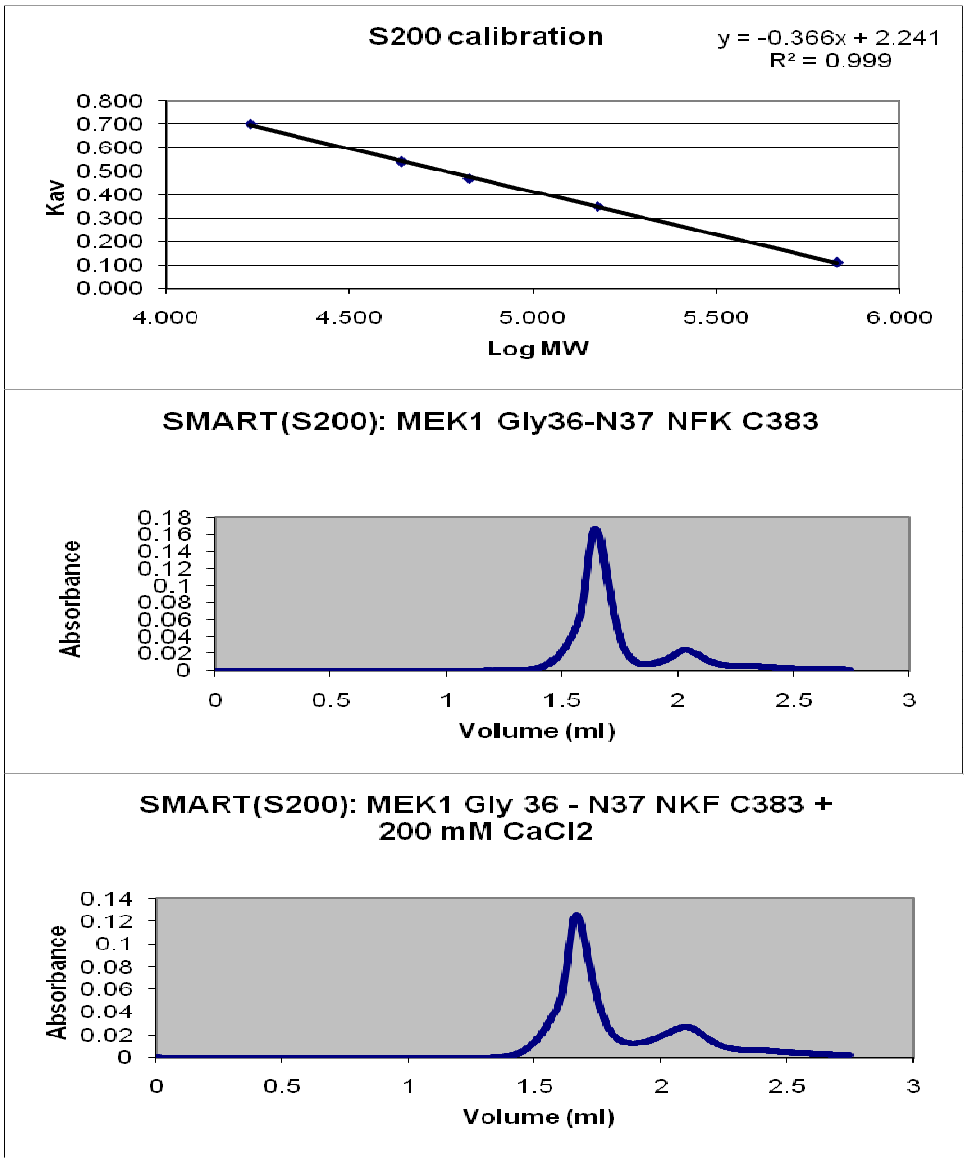
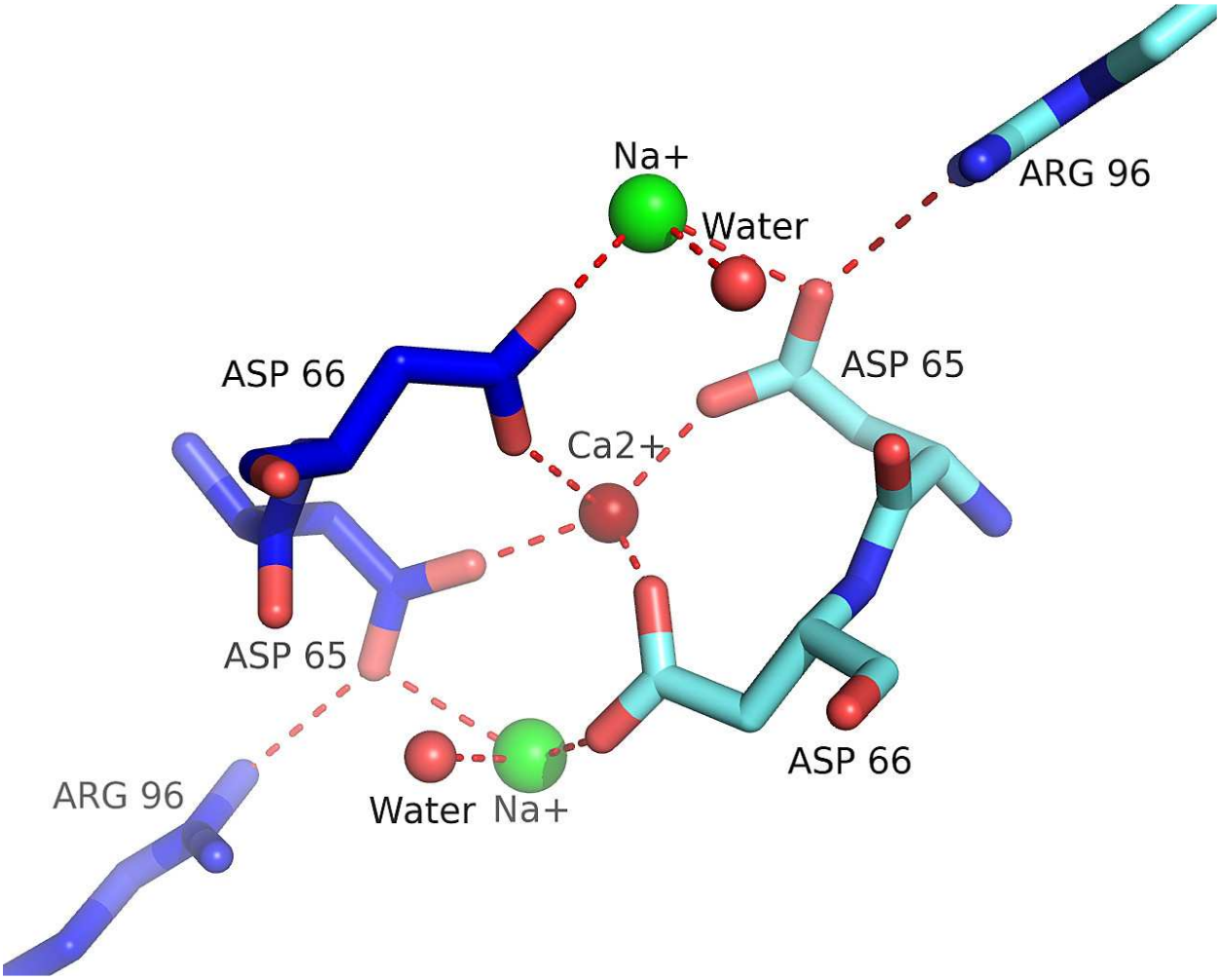
The crystallized construct is indicated in red (an N-terminal Gly at position 36, which is a left-over from the TEV protease cleavage, is not shown). The overall homology is 81% over the entire MEK1 sequence and 88% over the crystallized sequence.

Figure 1: Size-exclusion chromatography experiments.



The top graphics represents the column calibration using standards with a molecular weight of 670 kD, 150 kD, 67 kD, 44 kD, and 17 kD, respectively. The column volume is 2.0 ml. The middle graphics is the chromatogram run with the same buffer as the crystallization cocktail except for the absence of calcium and precipitant (PEG 4K). The protein elutes at a volume of 1.65 ml (main peak), which corresponds to a size of 26 kD according to the calibration above. The smaller peak around 2 ml corresponds to a smaller molecular weight (oxidized DTT). The last chromatogram is run as the previous experiment but with the addition of 200 mM Calcium Chloride: the same concentration as in the crystallization conditions. The protein elutes at 1.67 ml, or a size of 24 kD: the results with and without calcium match within the experimental errors and indicate a monomer in solution.

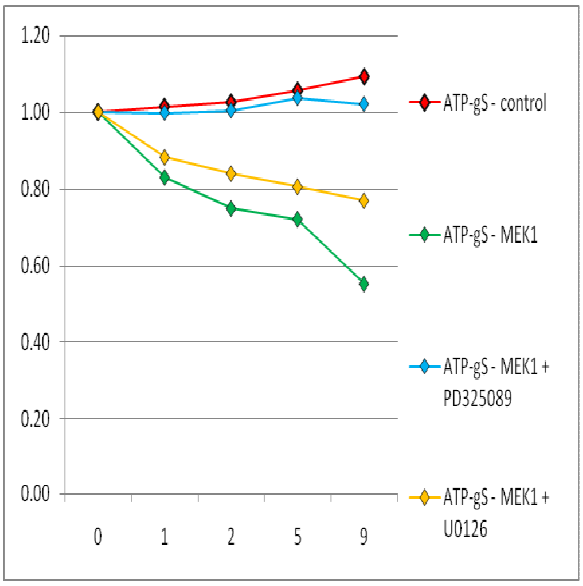
Figure 2: Calcium-binding site at the crystallographic dimer interface.

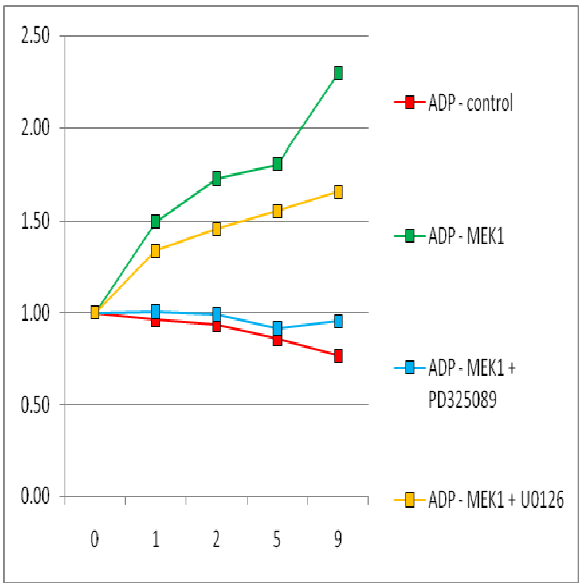


The residues near the calcium (carbons colored blue for one monomer, cyan for the other), the water molecules (red), sodium (green) and calcium (dark red) cations are represented as ball-and-sticks. The structure is oriented with the crystallographic 2-fold perpendicular to the figure. Hydrogen-bonds are shown as red dotted lines. The calcium is at the center of a distorted tetrahedron made by the aspartates of two consecutive amino acids, Asp 65 and Asp 66.

Table 3: Time-dependent ATP-γS hydrolysis (continued on next page).

	ATP-γS (control)		ATP-γS + MEK1		ATP-γS + MEK1 + PD325089		ATP-γS + MEK1 + U0126	
Day	ADP	ATP-γS	ADP	ATP-γS	ADP	ATP-γS	ADP	ATP-γS
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	0.96	1.01	1.49	0.83	1.00	1.00	1.33	0.88
2	0.94	1.03	1.73	0.75	0.99	1.00	1.46	0.84
5	0.86	1.06	1.81	0.72	0.91	1.04	1.55	0.81
9	0.77	1.09	2.29	0.55	0.95	1.02	1.66	0.77





ATP-γS is mixed with either buffer (control, red lines), MEK1 (orange lines), MEK1 with an excess of the PD325089 allosteric inhibitor (light blue lines), or MEK1 with an excess of the U0126 allosteric inhibitor (orange lines). Concentration of each nucleotide is measured right after mixing (axis label 0), or after 1, 2 5 or 9 days, respectively (note that the abscissa is not to scale to save space). The vertical axis on the left and the right graphs represent the ratio of the ATPγ-S or ADP concentrations, respectively, over their initial concentrations at day 0. Note that commercially available ATP-γS contains some amount of ADP and therefore the ADP concentration at day 0 is not zero.