**Water and Chloride as allosteric inhibitors in WNK kinase osmosensing**

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**This file includes:**

Supplementary files 1a-1h

**Supplementary file 1a**. Crystallographic Data and Refinement of WNK1/S382A and WNK1/SA/PEG400

**WNK1/SA\* WNK1/SA/PEG400**

Space group P1 P1211

Unit cell dimensions *a, b, c* (Å) 38.25, 57.72, 65.60 38.32, 56.81, 65.28

Angles  (°) 89.0, 89.6, 89.2 90, 95.4, 90

Wavelength (Å) 0.9795 0.9795

Resolution (Å) 50-2.0 (2.0-2.04) 30-2.0 (2.0-2.05)

Unique reflections (last shell) 38697 17297

Completeness (%) (last shell) 89 (83) 96 (93)

I/last shell) 13.3(2.6) 29.8(8.8)

Rsym, Rpim (last shell)a 0.04, 0.045 (0.20, 0.24) 0.14, 0.06 (0.76, 0.29)

Redundancy (last shell) 1.7 (1.1) 7.6 (7.7)

CC1/2 (last shell) 0.99 (0.90) 0.56(0.55)

Wilson B factor 28.9 18.5

**Structure**

Rwork/Rfreeb  (last shell) 0.16/0.22 (0.21/0.25) 0.199/0.233 (0.20/0.21)

Non-H protein atoms 4900 2281

Waters 453 250

Cryoprotectant (glycerol/PEG400) 9 0

RMSD in bond length (Å)c 0.006 0.016

RMSD in bond angles (°)c 1.38 1.65

Average B-values (Å2) 31.9 41.0

Ramachandran plot stats. (%)

Most favored region 94.6 90.8

Disallowed region 1.1 3.4

Molprobity Score 1.9 1.7

Residues missing from the model None 379-385

a Rsym = ∑ | Iavg - Ij | / ∑ Ij.

b Rfactor = ∑ | Fo - Fc | / ∑ Fo , where Fo and Fc are observed and calculated structure factors,

respectively, Rfree was calculated from a randomly chosen 5% of reflections excluded form

the refinement, and Rfactor was calculated from the remaining 95% of reflections.

c r.m.s.d is the root-mean-square deviation from ideal geometry.

\* Newly crystallized form independent from 6CN9.

**Supplementary file 1b.** Cell constant and C-C Superposition Comparisons

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| A. Coordinate  sets | WNK1/SA  +PEG400 | WNK1/SA  +PEG400 | WNK1/SA  (6CN9) | pWNK1  (5W7T) |
| Crystal system | Monoclinic  indexing | Triclinic  indexing | Triclinic | Monoclinic |
| Space Group | P21 | P1 | P1 | P21 |
| *a (*Å)  *b (*Å)  c *(*Å)  *α* (˚)  ** (˚)  ** (˚) | 38.32  56.81  65.28  90.0  95.4  90.0 | 38.32  56.82  65.89  90.1  95.4  90.0 | 38.31  57.77  65.66  91.3  90.0  90.9 | 45.18  62.23  120.90  90.0  92.3  90.0 |
| C-C overlay | PEG-6CN9A | PEG-6CN9B | PEG-6CN9 | PEG-pWNK |
| *Å* | 0.79 | 1.3 | 3.2 | 0.82 |

**Supplementary file 1c.** WNK3/1 expression level, and activation loop S308/S382 phosphorylation and activity

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Mutants** | **Expression** | **Purification** | **Phos (%) from**  ***E. coli*** | **Auto-phos** | **Cl- Sensitivity** |
| wt WNK3 | Very good | 26 mg/L | 98% | N/A | Very Sensitive |
| WNK3/E314A | Ok | 7 mg/L | 100% | Much faster | Insensitive |
| WNK3/E314Q | Ok | 6 mg/L | 99% | Faster | Insensitive |
| WNK3/K236A | Very good | 20 mg/L | 97% | Similar WT | Similar WT |
| WNK3/K307A | Good | 16 mg/L | 90% | Similar WT | Similar WT |
| WNK3/M301A | Very good | 23 mg/L | 96% | Slower | Very sensitive |
| WNK3/Y346F | Very good | 25 mg/L | 97% | Slower | Very sensitive |
| WNK3/D279N | Ok | 9 mg/L | 92% | Slower | Very sensitive |
| wt WNK1 | Good | 15 mg/L | 95% | Slower | Very Sensitive |
| WNK1/E388A | Ok | 10 mg/L | 94% | Faster WNK1 | < wt WNK1 |

**Supplementary file 1d.** WNK1 and WNK3 peptides monitored by LC-MS

**Monitored peptide (M+2H)2+ % acetonitrile**

Wild type WNK1 AKSVIGTPEFMAPEMY 885.5 22.5%

AKS\*VIGTPEFMAPEMY 925.5 23.5%

WNK1/E388A AKSVIGTPAFMAPEMY 885.5 18.0%

AKS\*VIGTPAFMAPEMY 925.5 18.8%

Wild type WNK3 MRTSF 641.1 10.1%

MRTS\*F 721.1 11.5%

AKSVIGTPEFMAPEMY 885.5 22.5%

AKS\*VIGTPEFMAPEMY 925.5 23.5%

E314A MRTSF 641.1 10.1%

MRTS\*F 721.1 11.5%

AKSVIGTPAFM 561.5 18.0%

AKS\*VIGTPAFM 601.5 18.8%

K236A MRTSF 641.1 10.1%

MRTS\*F 721.1 11.5%

AKSVIGTPEFMAPEMY 885.5 22.5%

AKS\*VIGTPEFMAPEMY 925.5 23.5%

K307A MRTSF 641.1 10.1%

MRTS\*F 721.1 11.5%

AASVIGTPEFMAPEMY 856.9 30.3%

AAS\*VIGTPEFMAPEMY 897.0 33.2%

M301A ARTSF 581.1 7.3%

ARTS\*F 661.1 8.6%

AKSVIGTPEFMAPEMY 885.5 22.5%

AKS\*VIGTPEFMAPEMY 925.5 23.5%

Y346F MRTSF 641.1 10.1%

MRTS\*F 721.1 11.5%

AKSVIGTPEFMAPEMY 885.5 22.5%

AKS\*VIGTPEFMAPEMY 925.5 23.5%

D297N MRTSF 641.1 10.1%

MRTS\*F 721.1 11.5%

AKSVIGTPEFMAPEMY 885.5 22.5%

AKS\*VIGTPEFMAPEMY 925.5 23.5%

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Wild type and mutant WNK1- and WNK3-specific chymotrypsin-derived activation loop peptides monitored

to generate autophosphorlation progress curves. M*/z* for the (M+2H)2+ of peptide and percent

acetonitrile where peptide elutes from a C18 HPLC column (0.1% formic acid in acetonitrile/water

HPLC mobile phase).

**Supplementary file 1e.** uWNK1 and uWNK3 autophosphorylation assay conditions

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component |  |  |  |  |
| HEPES pH7.4 | 20 mM | 20 mM | 20 mM |  |
| Mg Gluconate | 20 mM | 20 mM | 20 mM |  |
| Final [Cl-] | 50 mM | 150 mM | 250 mM |  |
| ATP | 5 mM | 5 mM | 5 mM |  |
| uWNK1/uWNK3 | 4 M | 4 M | 4 M |  |
| Final Volume | 50 L | 50 L | 50 L |  |
| Guanidine-HCl to final conc. of 1M to stop the reaction.. | | |  |  |

**Supplementary file 1f.** Wildtype and E388A uWNK1 autophosphorylation were fit to a basic autocatalytic mechanism using DynaFit software. Modeled progress curves superimposed on mass spectrometry data are shown in Figure 5E and 5F.

**Mechanism**

uWNK + pWNK → 2 pWNK :kP

uWNK + ion ⇄ uWNK\_ion :ion\_on ion\_off

**ODE System**

d[uWNK]/dt = - kP[uWNK][pWNK] - ion\_on[uWNK][ion] + ion\_off[uWNK\_ion]

d[pWNK]/dt = + kP[uWNK][pWNK]

d[ion]/dt = - ion\_on[uWNK][ion] + ion\_off[uWNK\_ion]

d[uWNK\_ion]/dt = + ion\_on[uWNK][ion] - ion\_off[uWNK\_ion]

**Optimized Parameters**

**uWNK1 wt uWNK1 E388A**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Initial Value | Final Value | Std. Error | CV(%) |
| KP | 0.18 | 0.183 | 0.008 | 4.4 |
| Ion\_on | 2e6 | 3.54e-6 | 5.4e-7 | 15.2 |
| Ion\_off | 0.2 | 0.119 | 0.013 | 11.1 |
| Kionbound |  | 2.98e-5 |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Initial Value | Final Value | Std. Error | CV(%) |
| KP | 0.18 | 0.150 | 0.007 | 4.7 |
| Ion\_on | 2e6 | 4.15e-6 | 6e-7 | 14.4 |
| Ion\_off | 0.2 | 0.103 | 0.011 | 10.5 |
| Kionbound |  | 4.03e-5 |  |  |

**Supplementary file 1g.** Molecular weight vs [WNK] by static light scattering

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **WT/ Mutants** | **MW at 0.8 mg/mL (KDa)** | **St. Dev.** | **MW at 1.8 mg/mL** | **St. Dev.** |
| wt WNK3 | 65 | 0.6 | 78 | 0.5 |
| WNK3/E314Q | 51 | 0.2 | 52 | 0.1 |
| WNK3/E314A | 56 | 0.1 | 77 | 1.1 |
| WNK3/K236A | 61 | 0.3 | 85 | 0.2 |
| WNK3/K307A | 59 | 0.3 | 65 | 0.3 |
| WNK3/M301A | 64 | 0.4 | 69 | 0.2 |
| WNK3/Y346F | 68 | 0.8 | 76 | 0.1 |
| WNK3/D279N | 39 | 0.1 | 41 | 0.1 |
| wt WNK1 | 38 | 0.5 | 35 | 0.1 |
| WNK1/E388A | 39 | 0.1 | 35 | 0.6 |

**Supplementary file 1h**. Crystallographic Data and Refinement of WNK3/SA/E314A

**WNK3/SA/E314A**

Space group P1211

Unit cell dimensions *a, b, c* (Å) 50.17, 113.60, 67.52

Angles  (°) 90, 101.4, 90

Wavelength (Å) 0.9795

Resolution (Å) 43-3.3 (3.36-3.3)

Unique reflections (last shell) 7500

Completeness (%) (last shell) 70(55)

I/last shell) 9.8(1.0)

Rsym, Rpim (last shell)a 0.17, 0.08 (0.76, 0.54)

Redundancy (last shell) 5.2 (3.7)

CC1/2 (last shell) 0.96(0.60)

Wilson B factor 81.9

**Structure**

Rwork/Rfreeb  (last shell) 0.188/.270 (0.30/0.42)

Non-H protein atoms 4255

Waters 117

RMSD in bond length (Å)c 0.004

RMSD in bond angles (°)c 1.62

Average B-values (Å2) 87.1

Ramachandran plot stats. (%)

Most favored region 88.4

Disallowed region 1.6

Molprobity Score 1.9

Residues missing from the model A:/303-314;B:/303-315

a Rsym = ∑ | Iavg - Ij | / ∑ Ij.

b Rfactor = ∑ | Fo - Fc | / ∑ Fo , where Fo and Fc are observed and calculated structure factors,

respectively, Rfree was calculated from a randomly chosen 5% of reflections excluded form

the refinement, and Rfactor was calculated from the remaining 95% of reflections.

c r.m.s.d is the root-mean-square deviation from ideal geometry.