# Supplemental Materials

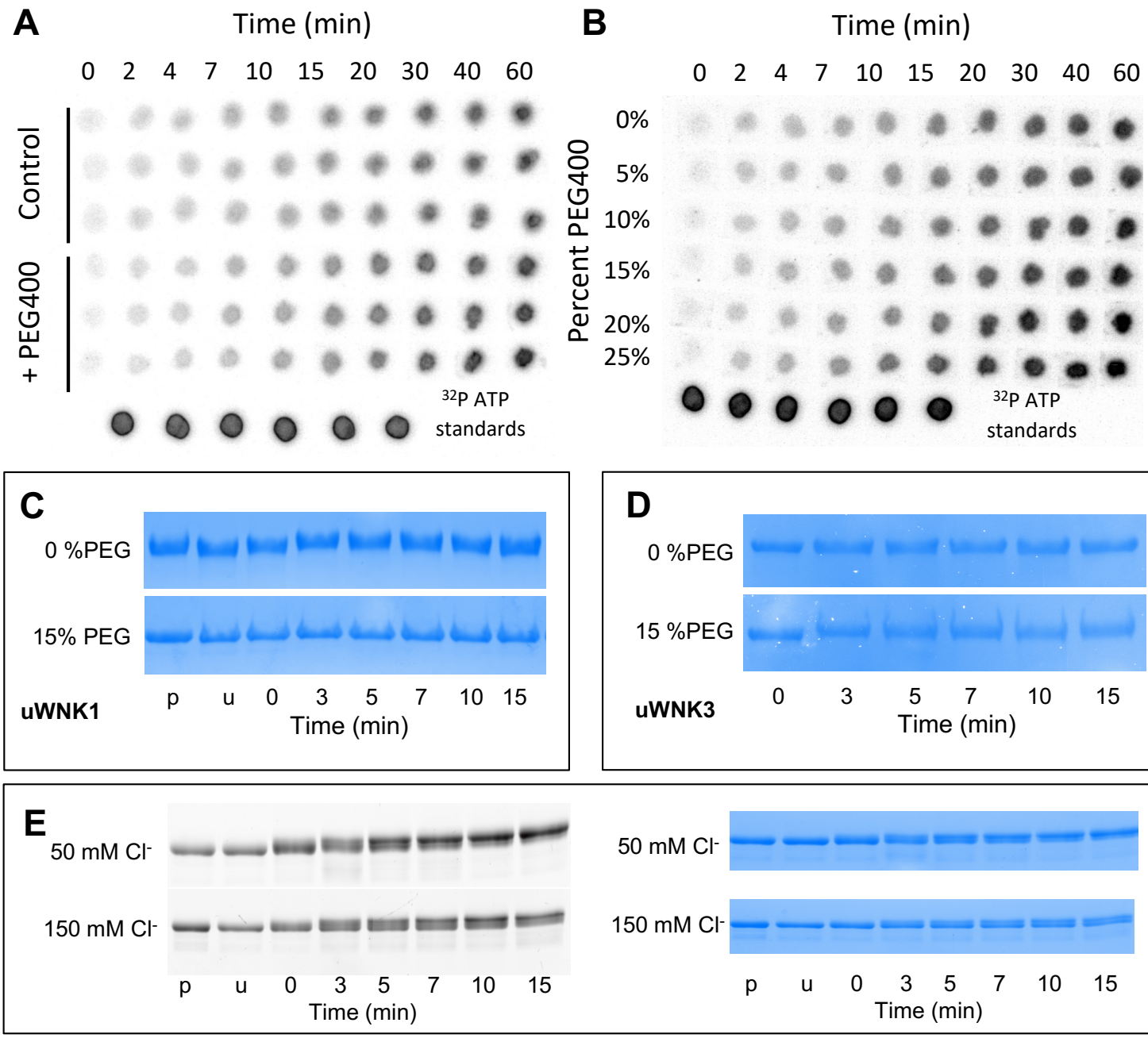
***Molecular Biology of the Cell***

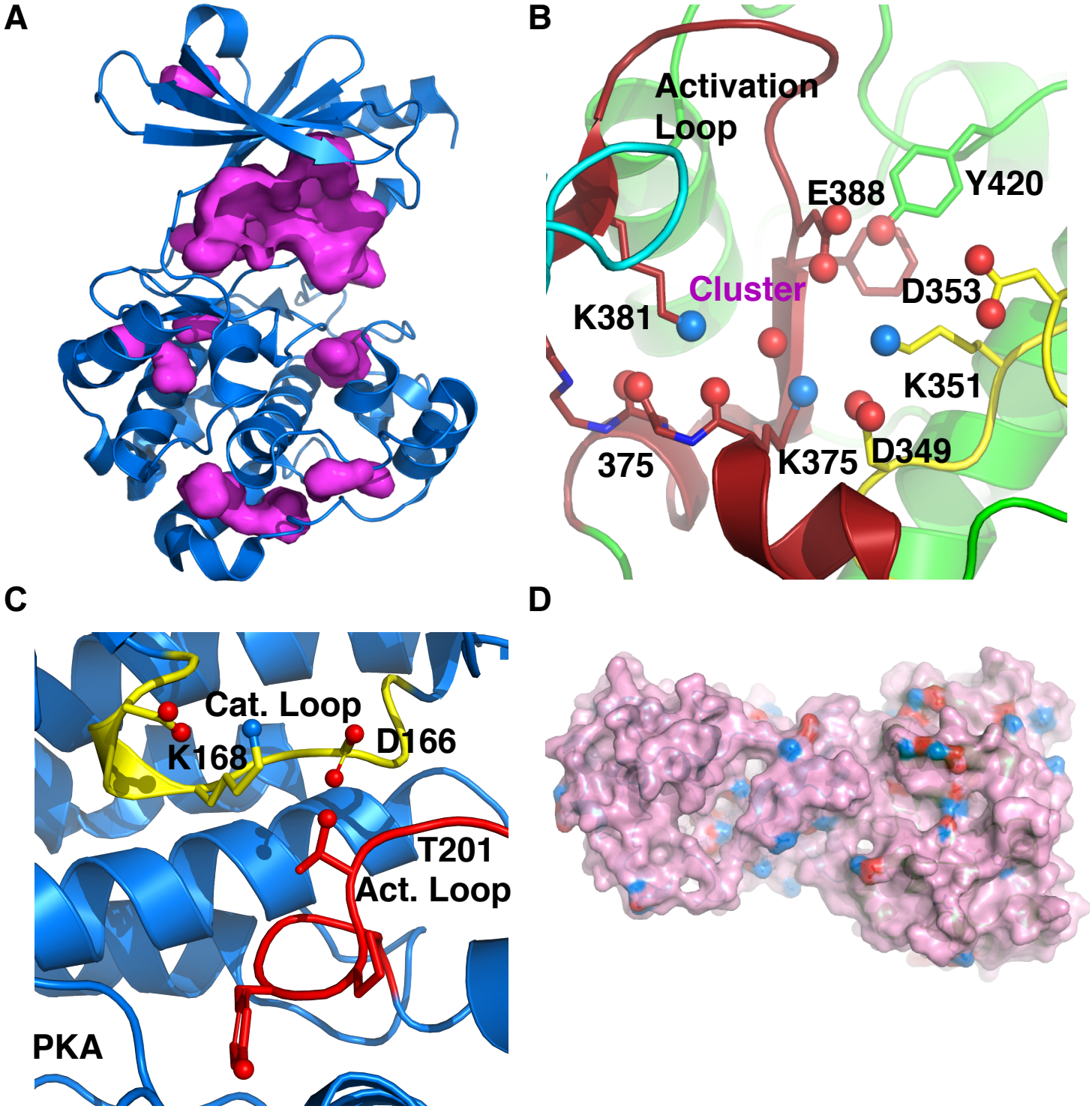
Akella et al.

**SUPPORTING INFORMATION**

**Supporting Information**. The following files are available free of charge.

**Figure S1.** Blots corresponding to data in Figure 1 and Coomassie stain of Pro-Q Diamond gels from Figure 2.
**Figure S2.** Structural insights
**Table S1.** Autophosphorylation sites in WNK3
**Table S2.** SAXS data collection and analysis without and with 15% PEG400
**Table S3.** CRYSOL3 analysis of SAXS data for uWNK3 without PEG400
**Table S4.** Statistics of WNK1SA crystallographic data and refinement statistics (PDB file 6CN9)


A
Time (min)
0 2 4 7 10 15 20 30 40 60
Control
+ PEG400
32P ATP
standards
B
Time (min)
0 2 4 7 10 15 20 30 40 60
Percent PEG400
0%
5%
10%
15%
20%
25%
32P ATP
standards
C
0 %PEG
15% PEG
uWNK1
p u 0 3 5 7 10 15
Time (min)
D
0 %PEG
15 %PEG
uWNK3
0 3 5 7 10 15
Time (min)
E
50 mM Cl-
150 mM Cl-
p u 0 3 5 7 10 15
Time (min)
50 mM Cl-
150 mM Cl-
p u 0 3 5 7 10 15
Time (min)


A
B
Activation
Loop
E388
Y420
Cluster
D353
K381
K351
375
K375
D349
C
Cat. Loop
K168
D166
T201
Act. Loop
PKA
D

SUPPORTING INFORMATION

Article title: Osmosensing by WNK Kinases

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**Table S1. Autophosphorylation sites in WNK3**

| WNK3 phosphopeptide | Location | 1) From *E. coli* | 2) Dephos | 3) Rephos |
|---|---|---|---|---|
| $^{1'}$MKE**T***AAAKF$^{9'}$ | S-Tag | **50%** | **0%** | **30%** |
| $^{10'}$ERQHMDSPDLGTLVPRG**S***M$^{28'}$ | Thrombin site | **90%** | **0%** | **40%** |
| $^{118}$KAVATSP**S***GRFL$^{129}$ | Beta sheet 1 | 20% | 20% | 20% |
| $^{283}$ITGPTG**S***VKIGDLGLATL$^{300}$ | Beta sheet 7 | 5% | 5% | 10% |
| $^{301}$MRT**S***F$^{305}$ | Minor act. loop | **95%** | **30%** | **50%** |
| $^{306}$AK**S***VIGTPEFMAPEMY$^{321}$ | Main act. loop | **95%** | **0%** | **80%** |
| $^{359}$RKVT**S***GIKPASF$^{370}$ | Helix G | 7% | 0% | 1% |
| $^{404}$FAEDTKLP**T***TENLY$^{418'}$ | TEV site | 20% | 0% | 2% |

* in red denotes phosphorylation site
AA numbering with ‘ denotes nonphysiological tag sequence (underlined)

**Table S2. SAXS data collection and analysis without and with 15% PEG400**

**Data Collection at Beamline BioCAT (18-ID)**

| Parameter | uWNK3 | uWNK3 + 15% PEG400 |
|---|---|---|
| Wavelength (Å) | 1.0 | 1.0 |
| s-range ($Å^{-1}$) | 0.0-0.25 | 0.0-0.25 |
| Exposure time (sec) | 0.5 | 0.5 |
| Concentration (mg/mL) | 5 | 5 |

**Results**

| | | |
|---|---|---|
| $I_0$ | 0.00008 | 0.005 |
| Rg from P(r) (Å) | 28.2 | 27.1 |
| Rg from Guinier plot (Å) | 27.3 | 26.8 |
| Dmax (Å) | 95 | 108 |
| Porod Volume ($Å^3$) | 68000 | 67000 |
| Molecular weight (kD) | | |
| From Porod volume | 47 | 13 |
| From Vc | 44 | 15 |
| From sequence | 79.6 | 40 |
| OLIGOMER | | |
| Dimer/Monomer (%) | 70/30 | 40/60 |

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$q = 4\pi\sin\theta/\lambda$
Rg radius of gyration
P(r) pairwise distribution function
Porod volume ($I(q) \alpha q^{-4}$) (Putnam *et al.*, 2007)
Vc (Rambo and Tainer, 2013)
OLIGOMER (Konarev *et al.*, 2003)

**Table S3. CRYSOL3 analysis of SAXS data for uWNK3 without PEG400**

| | Dimer | Monomer |
|---|---|---|
| Total water beads | 2285 | 1248 |
| Beads-convex surface | 1961 | 1070 |
| Beads-concave surface | 61 | 39 |
| Beads- internal cavities | 263 | 138 |
| $\chi^2$ | 2.6 | 22 |
| Radius of Gyration | 30 | 21 |

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CRYSOL3 **(Franke *et al.*, 2017)**

**Table S4. Statistics of WNK1SA crystallographic data and refinement (PDB file 6CN9)**

| | |
|---|---|
| Space group | P1 |
| Unit cell dimensions (Å) | *a*=38.30Å, *b*=57.8Å, *c*=65.7Å<br>$\alpha$=91.3°, $\beta$=89.99°, $\gamma$=90.89° |
| Wavelength (Å) | 0.97905 |
| Resolution (Å) | 50-1.8 |
| No. of unique reflections (last) | 50,614 (5012) |
| Average multiplicity (last shell) | 4.3 (4.3) |
| Completeness (%) (last shell) | 97.6 (96.9) |
| Intensity I/σI (%) | 27.9 / 10.7 |
| Rsym (last shell)[a] | 0.077 (0.144) |
| Rwork / Rfree[b] | 0.16 / 0.22 |
| Non-H protein atoms | 5317 |
| Waters | 905 |
| RMSD in bond length (Å)[c] | 0.03 |
| RMSD in bond angles (°)[c] | 1.24 |
| Average B-values (Å$^2$) | 26.9 |
| Ramachandran plot statistics (%) | |
| Most favored | 96.1 |
| Additionally allowed | 2.6 |
| Disallowed | 1.3 |

[a] $R_{sym} = \sum | I_{avg} - I_j | / \sum I_j$.

[b] $R_{factor} = \sum | F_o - F_c | / \sum F_o$, where $F_o$ and $F_c$ are observed and calculated structure factors, respectively, $R_{free}$ was calculated from a randomly chosen 5% of reflections excluded from the refinement, and $R_{factor}$ was calculated from the remaining 95% of reflections.

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

## References

Franke, D., Petoukhov, M.V., Konarev, P.V., Panjkovich, A., Tuukkanen, A., Mertens, H.D.T., Kikhney, A.G., Hajizadeh, N.R., Franklin, J.M., Jeffries, C.M., and Svergun, D.I. (2017). ATSAS 2.8: a comprehensive data analysis suite for small-angle scattering from macromolecular solutions. J Appl Crystallogr *50*, 1212-1225.

Konarev, P.V., Volkov, V.V., Sokolova, A.V., Koch, M.H., and Svergun, D.I. (2003). PRIMUS: a Windows PC-based system for small-angle scattering analysis. J. Appl. Crystallogr. *36*, 1277-1282.

Putnam, C.D., Hammel, M., Hura, G.L., and Tainer, J.A. (2007). X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution. Q Rev Biophys *40*, 191-285.

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