## Supplementary information

A novel mechanism by which small molecule inhibitors induce the DFG flip in Aurora A.
Mathew P. Martin, Jin-Yi Zhu, Harshani R. Lawrence, Roberta Pireddu, Yunting Luo, Riazul Alam, Sevil Ozcan, Said M. Sebti, Nicholas J. Lawrence, and Ernst Schönbrunn ${ }^{*}$

Supplementary Table 1. Summary of data collection and structure refinement ${ }^{a}$.

| Structure (PDB ID) | $\begin{gathered} 1 \\ (3 \cup O 5) \end{gathered}$ | $\begin{gathered} 3 \\ (3 \cup O 4) \end{gathered}$ | $\begin{gathered} \hline 4 \\ (3 U O D) \end{gathered}$ | $\begin{gathered} 5 \\ \text { (3UP2) } \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: |
| Data Collection |  |  |  |  |
| Space group | P6122 | P6122 | P6, 22 | P6, 22 |
| Unit cell dimensions (A) | $\begin{array}{r} a=81.79 \\ b=81.79 \\ c=173.77 \\ \hline \end{array}$ | $\begin{gathered} a=83.08 \\ b=83.08 \\ c=172.76 \end{gathered}$ | $\begin{aligned} & \mathrm{a}=81.98 \\ & \mathrm{~b}=81.98 \\ & \mathrm{c}=173.81 \end{aligned}$ | $\begin{gathered} a=82.44 \\ b=82.44 \\ c=173.14 \end{gathered}$ |
| Resolution range | $\begin{gathered} 20-2.70 \\ (2.75-2.70) \end{gathered}$ | $\begin{gathered} 20-2.45 \\ (2.50-2.45) \end{gathered}$ | $\begin{gathered} 20-2.50 \\ (2.60-2.50) \end{gathered}$ | $\begin{gathered} 20-2.30 \\ (2.40-2.30) \\ \hline \end{gathered}$ |
| Unique reflections | $\begin{aligned} & 9540 \\ & (473) \end{aligned}$ | $\begin{gathered} 13594 \\ (773) \end{gathered}$ | $\begin{aligned} & 12584 \\ & (1353) \\ & \hline \end{aligned}$ | $\begin{aligned} & 16126 \\ & (1860) \end{aligned}$ |
| Completeness (\%) | 94.9 (96.7) | 99.6 (99.9) | 99.7 (99.9) | 99.7 (99.9) |
| I/OI | 36.9 (7.3) | 60.9 (9.1) | 51.8 (7.8) | 54.4 (10.7) |
| $\mathbf{R}_{\text {merge }}{ }^{\text {b }}$ (\%) | 14.1 (31.6) | 3.4 (20.7) | 4.4 (22.9) | 3.1 (15.8) |
| Structure refinement |  |  |  |  |
| Protein atoms Average B-factor ( $\AA^{2}$ ) | $\begin{gathered} 2195 \\ 50 \end{gathered}$ | $\begin{gathered} 2188 \\ 58 \end{gathered}$ | $\begin{gathered} 2188 \\ 52 \end{gathered}$ | $\begin{gathered} 2188 \\ 50 \\ \hline \end{gathered}$ |
| Ligand atoms | 23 | 29 | 27 | 28 |
| Average B-factor ( $\AA^{2}$ ) | 35 | 43 | 39 | 38 |
| Solvent molecules | 39 | 49 | 45 | 98 |
| Average B-factor ( $\AA^{2}$ ) | 44 | 46 | 44 | 46 |
| r.m.s.d. ${ }^{\text {c }}$ bonds ( $(\mathrm{A})$ | 0.013 | 0.004 | 0.009 | 0.010 |
| r.m.s.d.angles (9) | 1.2 | 1.0 | 1.2 | 1.2 |
| $\mathbf{R}_{\text {cryst }}{ }^{\text {d }}$ (\%) | 21.7 | 22.0 | 20.6 | 20.8 |
| $\mathrm{R}_{\text {free }}{ }^{e}$ (\%) | 27.3 | 25.7 | 26.2 | 24.9 |
| $\mathrm{R}_{\text {free }}$ reflection set size | 416 (4.5 \%) | 612 (4.5 \%) | 567 (4.5 \%) | 775 (4.5 \%) |
| Coordinate error (Å) <br> (ML method from PHENIX) | 0.37 | 0.27 | 0.22 | 0.17 |
| ${ }^{a}$ The structure with compound 2 has been deposited as PDB code 3UP7 (Lawrence et al, submitted). <br> ${ }^{b} R_{\text {merge }}=$ quality of amplitudes $(F)$ in the scaled data set, Diederichs \& Karplus (1997), <br> Nature Struct. Biol. 4, 269-275. <br> ${ }^{c}$ r.m.s.d. $=$ root mean square deviation from ideal values. <br> ${ }^{d} R_{\text {cryst }}=100 \times \Sigma\left\|F_{\text {obs }}-F_{\text {model }}\right\| / F_{\text {obs }}$ where $F_{\text {obs }}$ and $F_{\text {model }}$ are observed and calculated structure factor amplitudes, respectively. <br> ${ }^{e} R_{\text {free }}$ is $R_{\text {cryst }}$ calculated for randomly chosen unique reflections, which were excluded from the refinement. |  |  |  |  |

Continuation of Table 1.

| Structure (PDB ID) | $\begin{gathered} \hline 6 \\ (3 U N Z) \\ \hline \end{gathered}$ | $\begin{gathered} 7 \\ (3 \cup O 6) \\ \hline \end{gathered}$ | $\begin{gathered} \hline 8 \\ (3 \mathrm{UOH}) \\ \hline \end{gathered}$ | $\begin{gathered} 9 \\ (3 \cup O J) \\ \hline \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: |
| Data Collection |  |  |  |  |
| Space group | P 32 | $\mathrm{P} 3_{2}$ | P32 | P 32 |
| Unit cell dimensions ( A ) | $\begin{aligned} & a=85.78 \\ & b=85.78 \\ & c=76.56 \end{aligned}$ | $\begin{aligned} & a=85.71 \\ & b=85.71 \\ & c=76.66 \end{aligned}$ | $\begin{aligned} & a=85.75 \\ & b=85.75 \\ & c=76.94 \\ & \hline \end{aligned}$ | $\begin{aligned} & a=85.71 \\ & b=85.71 \\ & c=77.08 \end{aligned}$ |
| Resolution range | $\begin{gathered} 20-2.80 \\ (2.90-2.80) \end{gathered}$ | $\begin{gathered} 20-2.80 \\ (2.90-2.80) \end{gathered}$ | $\begin{gathered} 20-2.80 \\ (2.90-2.80) \end{gathered}$ | $\begin{gathered} 20-2.90 \\ (3.00-2.90) \end{gathered}$ |
| Unique reflections | $\begin{aligned} & 15425 \\ & (1514) \\ & \hline \end{aligned}$ | $\begin{aligned} & 15518 \\ & (1509) \end{aligned}$ | $\begin{aligned} & 15512 \\ & (1541) \\ & \hline \end{aligned}$ | $\begin{aligned} & 13920 \\ & (1330) \end{aligned}$ |
| Completeness (\%) | 99.4 (99.4) | 99.0 (99.7) | 99.5 (99.9) | 99.1 (99.0) |
| I/OI | 27.6 (4.6) | 31.8 (4.7) | 30.9 (5.3) | 25.5 (4.5) |
| $\mathbf{R}_{\text {merge }}{ }^{\text {b }}$ (\%) | 7.8 (39.7) | 6.8 (37.4) | 6.2 (32.2) | 6.5 (41.1) |
| Structure refinement |  |  |  |  |
| Protein atoms Average B-factor ( $\AA^{2}$ ) | $\begin{gathered} 4318 \\ 58 \\ \hline \end{gathered}$ | $\begin{gathered} 4340 \\ 69 \\ \hline \end{gathered}$ | $\begin{gathered} 4340 \\ 64 \\ \hline \end{gathered}$ | $\begin{gathered} 4318 \\ 70 \\ \hline \end{gathered}$ |
| Ligand atoms Average $B$-factor $\left(\AA^{2}\right)$ | 48 39 | 48 | 48 | 50 |
| Solvent molecules Average B-factor ( $\dot{A}^{2}$ ) | $\begin{aligned} & 38 \\ & 44 \end{aligned}$ | 21 46 | 34 44 | 28 49 |
| r.m.s.d. ${ }^{\text {c }}$ bonds ( $(\mathrm{A})$ | 0.007 | 0.013 | 0.009 | 0.013 |
| r.m.s.d.angles (9) | 1.3 | 1.5 | 1.2 | 1.9 |
| $\mathbf{R}_{\text {cryst }}{ }^{\text {d }}$ (\%) | 23.1 | 22.9 | 23.0 | 22.7 |
| $\mathrm{R}_{\text {free }}{ }^{e}$ (\%) | 27.7 | 27.6 | 28.8 | 27.4 |
| $\mathrm{R}_{\text {free }}$ reflection set size | 695 (4.5 \%) | 614 (4.0 \%) | 699 (4.5 \%) | 557 (4.0 \%) |
| Coordinate error (Å) <br> (ML method from PHENIX) | 0.42 | 0.41 | 0.42 | 0.48 |

Continuation of Table 1.

| Structure (PDB ID) | $\begin{gathered} 10 \\ \text { (3UOK) } \end{gathered}$ | $\begin{gathered} 11 \\ \text { (3UOL) } \\ \hline \end{gathered}$ | CDK2 -1 (3UNJ) | CDK2 -7 (3UNK) |
| :---: | :---: | :---: | :---: | :---: |
| Data Collection |  |  |  |  |
| Space group | P3 ${ }_{2}$ | P3 ${ }_{2}$ | $\mathrm{P} 2{ }_{1} 2_{1} 2_{1}$ | $\mathrm{P} 2{ }_{12} 2_{2}$ |
| Unit cell dimensions (Å) | $\mathrm{a}=85.74$ | $\mathrm{a}=85.75$ | $\mathrm{a}=53.22$ | $\mathrm{a}=52.95$ |
|  | $\mathrm{b}=85.74$ | $\mathrm{b}=85.75$ | $\mathrm{b}=71.96$ | $\mathrm{b}=72.02$ |
|  | c=76.69 | c=76.72 | $\mathrm{c}=72.65$ | c=72.37 |
| Resolution range | $\begin{gathered} 20-2.95 \\ (3.00-2.95) \end{gathered}$ | $\begin{gathered} 20-2.40 \\ (2.50-2.40) \end{gathered}$ | $\begin{gathered} 20-1.90 \\ (2.00-1.90) \end{gathered}$ | $\begin{gathered} 20-2.10 \\ (2.20-2.10) \end{gathered}$ |
|  | 13174 | 24248 | 22465 | 16682 |
| Unique reflections | (598) | (2768) | (3133) | (2127) |
| Completeness (\%) | 99.5 (99.8) | 98.4 (98.0) | 99.4 (99.2) | 100 (99.8) |
| I/ $/ \mathrm{I}$ | 22.4 (3.8) | 30.2 (7.5) | 18.5 (7.5) | 20.7 (6.9) |
| $\mathbf{R}_{\text {merge }}{ }^{\text {b }}$ (\%) | 7.7 (47.4) | 4.4 (23.9) | 5.9 (23.2) | 7.5 (23.8) |
| Structure refinement |  |  |  |  |
| Protein atoms | 4358 | 4320 | 2366 | 2371 |
| Average B-factor ( $\AA^{2}$ ) | 57 | 58 | 27 | 28 |
| Ligand atoms | 50 | 52 | 23 | 24 |
| Average B-factor ( $\AA^{2}$ ) | 36 | 43 | 21 | 25 |
| Solvent molecules | 17 | 124 | 214 | 153 |
| Average B-factor ( $\hat{A}^{2}$ ) | 37 | 50 | 31 | 27 |
| r.m.s.d. ${ }^{\text {c bonds ( }}$ ( ${ }^{\text {( }}$ ) | 0.010 | 0.012 | 0.010 | 0.010 |
| r.m.s.d. angles (9) | 1.5 | 1.5 | 1.4 | 1.3 |
| $\mathbf{R}_{\text {cryst }}{ }^{\text {d }}$ (\%) | 22.3 | 23.6 | 19.9 | 18.0 |
| $\mathbf{R}_{\text {free }}{ }^{e}$ (\%) | 27.6 | 27.9 | 26.1 | 23.4 |
| $\mathrm{R}_{\text {free }}$ reflection set size | $\begin{gathered} 525 \\ (4.0 \%) \\ \hline \end{gathered}$ | $\begin{gathered} 1067 \\ (4.4 \%) \\ \hline \end{gathered}$ | $\begin{gathered} 1124 \\ (5.0 \%) \end{gathered}$ | $\begin{gathered} 835 \\ (5.0 \%) \\ \hline \end{gathered}$ |
| Coordinate error (Å) (ML method from PHENX) | 0.44 | 0.40 | 0.25 | 0.21 |



Supplementary Figure $1 \mathrm{IC}_{50}$ determination of bisanilinopyrimidine inhibitors with Aurora A.


Supplementary Figure 2 Binding studies of bisanilinopyrimidine inhibitors with Aurora A by isothermal titration calorimetry (ITC).


Supplementary Figure 3 Comparison of the DFG-in dead-end complexes (stereo presentations). The structures of 2-5 (magenta) were aligned with the structure of $\mathbf{1}$ (cyan). Shown are the inhibitors, the ADFG segment, Lys162 and distances in Å. a) Aurora A-2. b) Aurora A-3. c) Aurora A-4. d) Aurora A-5.


Supplementary Figure 4 Comparison of the DFG-out dead-end complexes (stereo presentations). The structures of 6-9 (magenta) were aligned with the structure of $\mathbf{1}$ (cyan). Shown are the inhibitors, the ADFG segment, Lys162 and distances in Å. a) Aurora A-6. b) Aurora A-7. c) Aurora A-8. d) Aurora A-9.


Supplementary Figure 5 Binding modes of bisanilinopyrimidine inhibitors with Aurora A. Crystal structures were determined for Aurora A liganded with different ortho-substituted bisanilinopyrimidine inhibitors. The hinge region (residues 211 - 213 ) is indicated in orange, the DFG (residues $274-276$ ) in cyan, the activation loop (residues 277 - 293) in magenta, other residues in grey and the inhibitor in yellow. The orange dotted lines indicate the close distance of electronegative groups to the methyl group of Ala273. The $\mathrm{F}_{\mathrm{o}}-\mathrm{F}_{\mathrm{c}}$ electron density resulting from refinement omitting the inhibitor is shown as red mesh, contoured at $2.5 \sigma$. The insets are surface representations of the overall structures. Compounds $\mathbf{1 - 5}$ are DFG-in inhibitors, compounds 6-9 are DFG-out inhibitors.


Supplementary Figure 6 Crystal structures of 10 and 11 bound to Aurora A (Stereo presentations of the binding interactions between the DFG-out inhibitors $\mathbf{1 0}$ a) and $\mathbf{1 1}$ b) to Aurora A. The Fo-Fc electron density map of the omitted inhibitor is contoured at $2.5 \sigma$ and shown in red mesh. Potential hydrogen bonding and van der Waals interactions are indicated as black and green dotted lines, respectively.


Supplementary Figure 7 Co-crystal structures of Aurora A inhibitors with CDK2. a) Structure of $\mathbf{1}$ bound to CDK2. b) Structure of 7 bound to CDK2. Shown as red mesh is the Fo-Fc electron density map from refinements omitting the inhibitors, contoured at $2.5 \sigma$.
a



Supplementary Figure 8 Interactions of Trp277 in the DFG-in and DFG-out states (stereo presentations). a) In the DFG-in state with compound 1, the main chain carbonyl oxygen of $\operatorname{Trp} 277$ forms hydrogen bonding interactions with the guanidinium group of Arg255 (black dotted lines). The indole moiety is surrounded by mostly polar residues with weak potential for VDW interactions (green dotted lines). b) In the DFG-out state with compound 7, the indole moiety is positioned in a strictly hydrophobic pocket with high VDW interaction potential; polar interactions between $\operatorname{Trp} 277$ and neighboring residues no longer exist.

