Modeling and validating chronic pharmacological manipulation of circadian rhythms

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Supplementary Methods

Gene Expression Studies

Animals for qPCR experiments. Male mice, 8 weeks old, strain C57Bl/6J-0664 were ordered from Jackson Labs and entrained to 12 hour light/dark cycle for at least 1 week prior to use. Mice were housed in solid bottom cages and fed standard chow with water available ad libitum. All procedures used in this study were approved by, and in accordance with, the guidelines of the Pfizer Animal Care and Use Committee. Animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. Mice were dosed at ZT11, one hour before lights

off, by subcutaneous injection, with 8 mice per treatment group. PF-670462 (4-[3-cyclohexyl-5-(4-fluoro-phenyl)-3*H*-imidazol-4-yl]-pyrimidin-2-ylamine) was dissolved in 20% beta-cyclodextran sulfobutyl ether in water at a 10 ml/kg dose volume. Mice were sacrificed at time noted via CO2 euthanasia. Hypothalami and cortex were micro dissected and immediately frozen on dry ice, then stored at -80C until processed.

RNA extraction and qPCR. RNA isolation was carried out using Macherey-Nagel NucleoSpin kit (catalog number 740 790.4) using the NucleoVac96 Vacuum Manifold (catalog number 740 681) according to the manufacturer's instructions. Samples were homogenized in lysis buffer using a 3mm steel bead (Qiagen) in a Retsch MM300 shaker at 20 Hz for five minutes. Synthesis of cDNA was carried out with the Applied Biosystems High Capacity RNA to cDNA kit (#4387406) following manufacturers protocol using 9ul of RNA as eluted from NucleoSpin kit, with cDNA diluted to a final volume of 170ul with water. Quantitative PCR was performed in a 10ul reaction containing 4.5 ul of cDNA, 0.5ul 20X Taqman assay mix, and 5ul Taqman Fast Universal PCR Master Mix (Applied Biosystems #4367848) in 384 well plates (Applied Biosystems) using the cycling conditions specified by the manufacturer on the Applied Biosystems (AB) 7900 instrument. Taqman gene expression assays purchased from Applied Biosystems are detailed in Table S8. Relative quantity for each gene was calculated in Excel using raw Ct data exported from AB SDS 2.3 software. The DDCt method was employed as described by Livac and Schmittgen (1), utilizing actin as the reference gene, the lowest abundance sample acting as reference sample. The average relative quantity for each timepoint is graphed with errors bars representing SEM.

Behavior Studies

Animals. Adult male C57BL/6J mice were used for all experiments. After acclimating for 1 week, the mice were anesthetized with 2.5% isoflurane and prepped for surgical implantation of an indwelling telemeter unit. A midline incision through skin and muscle was made in the ventral abdominal region, and a sterile telemeter (TA10TA-F20; Data Sciences International, St. Paul, MN) was inserted into the peritoneal cavity. The muscle was closed with absorbable suture and the skin was closed with wound clips. The animals were given 5 mg/kg s.c. carprofen and were allowed to recover in a clean cage beneath a heating lamp. Once ambulatory, the animals were placed in their home cages, the cages were placed on matrixed receivers, and the telemeters were activated and tested on the data collection system (Dataquest Acquisition Software, Data Sciences International). Telemeters were programmed to record activity and temperature and the collected data were uploaded into Matlab-based (The Mathworks Inc., Natick, MA) analysis software (ClockLab; ActiMetrics, WilmetteWilmette, IL) containing algorithms to determine and predict activity onsets and associated circadian parameters. Each cage contained a running wheel to encourage activity and was housed within a light-tight isolation chamber (four cages per box; Plastic Design, Inc., North Chelmsford, MA) with constant ventilation. Lighting for each box was set at 250 to 300 lux, and the light cycle was controlled with a timer (ChronTrol, San Diego, CA). The timing of lights on and off in each box was also recorded and checked daily. The mice were kept in a 12:12 LD (light/dark cycle of 12 h each) for at least 2 weeks after recovery from transmitter implantation surgery before initiation of the study to ensure their sleep/wake cycles were entrained to the LD cycle.

Before dosing, baseline data were reviewed over a 7d period to ensure that animals met exclusion criteria. Animals were excluded if their baseline (daily period) was less than 23.9h or greater than 24.1h. Animals were also excluded if the characteristics of their data were insufficient to allow for reliable determination of activity onsets. Treatment groups were randomized across isolation chambers such that each chamber contained animals from different treatment groups. In each experiment, animals were either shifted into DD (DD dosing) or maintained in LD (LD dosing) and all were treated at circadian time 11 (CT11), which was 1h before the lights would normally have been turned off with the indicated dose of PF-670462 for the indicated number of days. After the last day of dosing, mice in LD were shifted into DD and circadian measures followed for an additional 7 days. Data were then downloaded and for the 1d or 3d dosing, phase shifts were calculated as the time difference in behavior onset between the predosing week and after dosing as analyzed by the Clocklab software. For chronic dosing, phase shifts were calculated as the daily additive shift in onset. One-way ANOVA with Tukey's or Dunnett's post-test were used to analyze phase shifts.

Pharmacokinetic Characterization

Distribution Profiles. A single dose (32mg/kg) of PF-00670462 was administered to male C57/Bl6 mice (n=2-3 per dose/time point) as a subcutaneous injection based on pretreatment body weight and a dosing volume of 10 mL/kg. Drug was prepared on the same day of study in a dosing vehicle composed of 5/5/90 DMSO:cremophor:0.9% saline. For sample collection, mice were placed under isofluorane anesthesia at 0.5, 1, 2,

4, and 8h post dose, blood samples were obtained by cardiac puncture and collected into EDTA-containing tubes, which were stored on wet ice until plasma isolation. Subsequently, CSF was acquired via cistern magna puncture, transferred to a polypropylene tube, and immediately frozen on dry ice. The whole brain was extracted, rinsed of excess blood with ice-cold saline, placed into a tared vial, weighed, and frozen on dry ice. For bioanalytical sample preparation, plasma and CSF samples were used as is, whereas brain tissue samples were first homogenized in a 4-fold volume (w:v) of 60% isopropanol solution. Individual standard curves were prepared in respective control matrices. Samples (50uL) were processed using an acetonitrile-mediated matrix precipitation methodology followed by a liquid chromatography-tandem mass spectrometry assay.

Target Occupancy. All procedures were carried out in accordance with Institutional Animal Care and Use Committee guidelines under Animal Usage Protocol No. 15933. Male C57BL/6J mice from Jackson Laboratories, 9-12 weeks of age, were housed on a 12-hour light/dark cycle and given food and water ad libitum. Animals were allowed to acclimate for 5 days after arrival and were always used within 10 days of arrival. [^{3}H]PF-05236216 was prepared by the Pfizer Radiochemical Synthesis Group in Groton, CT. Studies were performed using lot 00703442-029-rvc001, 70.7 Ci/mmol, 0.770 mCi/ml in ethanol. The [^{3}H]PF-05236216 dosing solution was prepared by diluting the appropriate amount of stock solution in 0.9% saline to achieve a 100 mCi/ml solution. The appropriate amount of PF-670462, lot PF-00670462-00-0009, was weighed to achieve the dosing concentration specified (mg/kg) and dissolved in 20% sulfobutylether b-cyclodextrin (SBE BCD). Mice received a 10 ml/kg subcutaneous (SC)

injection of PF-670462 at 30 minutes prior to euthanasia. They then received a 3 ml/kg intraorbital injection of [^{3}H]PF-05236216 at 1 minute prior to euthanasia. PF-05175333 (100 mg/kg, SC) was used to determine the level of non-specific binding. Note that all doses were corrected for salt content of the drug. Mice were euthanized by live decapitation 30 minutes after dosing and brains removed. Hypothalami were rapidly dissected and homogenized for approximately 3 seconds each using a polytron at its highest setting (5-6) in 10 volumes (100 mg/ml) of ice-cold assay buffer (50 mM Tris, 2 mM $MgCl_2$, pH 7.4). Two replicates (200 ml each) of the resulting homogenates were filtered using a vacuum manifold apparatus with 25 mm Whatman GF/B filters presoaked in 0.3% polyethyleneimine (PEI). The filters were soaked overnight in 10 ml of Biosafe scintillation fluid. The samples were then counted on a liquid scintillation counter. The unused portions of the brains were frozen on dry ice and reserved for drug exposure analysis.

Modeling Studies

Incorporation of PF-670462 into the model. We incorporated PF-670462 into a recently developed mathematical model of intracellular mammalian circadian clocks (2). PF-670462 binds CK1δ/ε and inhibits the phosphorylation of CK1δ/ε (Figure S2). The inhibition of phosphorylation reduced the degradation rate of PER1/2, nucleus translocation of PER1/2 and binding rate of PER1/2 and CRY1/2 in the model. We considered the concentration of PF-670462 in four compartments: in plasma, brain tissue, cytoplasm and nucleus (Figure S2 and Table S6). To describe the import into and export out of these four compartments, 6 parameters were added. Because PF-670462 is

absorbed to plasma very fast (<0.5h) (Figure 4a), we assumed that dosing is directly applied to the plasma compartment for simplicity. The addition of a new compartment (e.g. skin) describing drug administration did not change the behavior of model (data not shown). We also considered a clearance rate for free CK1δ/ε in plasma. The binding and unbinding rates of PF-670462 to CK1δ/ε are also considered. Since CK1δ/ε can freely exit the nucleus of the cell in the original model (2), we allowed for the rate of nuclear export of CK1δ/ε to be different when it is bound to PF-670462. Finally, we needed to choose an initial concentration of free PF-670462 in plasma when a 32 mg/kg dose is administrated (higher or lower doses can be determined by appropriately scaling this value). In total, this adds 11 parameters to the model while other parameters of the original model were left unchanged (Table S5).

Definition of concentration in the model. We followed a convention from the circadian modeling field whereby all concentrations are defined as number of molecules/Vr, where Vr is a reference volume (2-5). Here, we chose Vr to be the volume of distribution in the cell cytosol (Figure S2a) as has been done previously. To convert to concentrations in the standard form used in multi-compartment models would require knowledge of the actual volumes of distribution, which remain unknown in some compartments. It would also require knowledge of the fraction of the flux of free PF-670462 from brain to a single cell, the fraction of the flux from plasma that goes specifically to brain rather than other tissues, and measures of absolute concentrations of clock proteins in single cells, which are still lacking (2, 6, 7). For these reasons, concentrations are viewed as relative and not absolute. We scaled variables to match data presented in Figure 4a.

Ordinary differential equations of the mathematical model. Equations that describe the dynamics of CK1 inhibitor (PF-670462) are added to the original model (2), which are highlighted in red. The parameters and variables are described in Table S5-7. The equations of the original model are adopted from the supplementary information in Kim and Forger (2012) (2).

1) Promoter Activity

E-box

GR'=bin*(Sum[x[0][kk][0][1][1],(8)])*(1-G-GR)-unbin*GR

G'=bin*x[0][0][0][1][1]*(1-G-GR)-unbin*G

GrR'=binr*(Sum[x[0][kk][0][1][1],{kk,1,2}])*(1-Gr-GrR)-unbinr*GrR

Gr'=binr*x[0][0][0][1][1]*(1-Gr-GrR)-unbinr*Gr

GcR'=binc*(Sum[x[0][kk][0][1][1],{kk,1,2}])*(1-Gc-GcR)-unbinc*GcR

Gc'=binc*x[0][0][0][1][1]*(1-Gc-GcR)-unbinc*Gc

RORE

GBR'=binrev*(revn+revng+revngp+revnp)*GB-unbinrev*GBR

GB'=-binrev*(revn+revng+revngp+revnp)*GB+unbinrev*GBR

GBRb'=binrevb*(revn+revng+revngp+revnp)*GBb-unbinrevb*GBRb

GBb'=-binrevb*(revn+revng+revngp+revnp)*GBb+unbinrevb*GBRb

2) Transcription

MnPo'=trPo*G-tmc*MnPo-umPo*MnPo

McPo'=tmc*MnPo-umPo*McPo

MnPt'=trPt*G-tmc*MnPt-umPt*MnPt

McPt'=tmc*MnPt-umPt*McPt

MnRt'=trRt*Gc-tmc*MnRt-umRt*MnRt

McRt'=tmc*MnRt-umRt*McRt

MnRev'=trRev*x[0][0][0][1][1]*Gr-tmcrev*MnRev-umRev*MnRev

McRev'=tmcrev*MnRev-umRev*McRev

MnRo'=trRo*G*GB-tmc*MnRo-umRo*MnRo

McRo'=tmc*MnRo-umRo*McRo

MnB'=trB*GBb-tmc*MnB-umB*MnB

McB'=tmc*MnB-umB*McB

MnNp'=trNp*GB- tmc*MnNp - umNp*MnNp

McNp'=tmc*MnNp-umNp*McNp

3) Secondary Feedback Loop

B'=tlb*McB-cbin*B*Cl+uncbin*BC-ub*B

Cl'=tlnp*McNp+tlc-cbin*B*Cl+uncbin*BC-uc*Cl

BC'=cbin*B*Cl-uncbin*BC-phos*BC-ubc*BC

cyrev'=tlrev*McRev-(nlrev+urev)*cyrev-ag*cyrev*(x[0][0][2][0][0])+nerev*revn+dg*cyrevg

revn'=-(nerev+urev)*revn-ag*Nf*revn*(x[0][0][2][1][0])+nlrev*cyrev+dg*(revng)

cyrevg'=ag*cyrev*x[0][0][2][0][0]-(dg+gto+urev+nlrev)*cyrevg+nerev*revng

revng'=ag*Nf*revn*x[0][0][2][1][0]-(dg+gto+urev+nerev)*revng+nlrev*cyrevg

cyrevgp'=gto*cyrevg-(dg+uprev+nlrev)*cyrevgp+nerev*revngp

revngp'=gto*revng-(dg+uprev+nerev)*revngp+nlrev*cyrevgp

cyrevp'=dg*(cyrevgp)-(uprev+nlrev)*cyrevp+nerev*revnp

revnp'=dg*(revngp)-(uprev+nerev)*revnp+nlrev*cyrevp

4) Translation

x[j][k][l][m][n]'=

If[(j=1)&&(k=0)&&(l=0)&&(m=0)&&(n=0),tlp*McPo,0]

+If[(j=3)&&(k=0)&&(l=0)&&(m=0)&&(n=0),tlp*McPt,0]

+If[(j=0)&&(k=1)&&(l=0)&&(m=0)&&(n=0),tlr*McRo,0]

+If[(j=0)&&(k=2)&&(l=0)&&(m=0)&&(n=0),tlr*McRt,0]

5) Dynamics of Free PF-670462

pInh'==-(uinp+nlpin)*pInh+nepin*bInh

bInh'=(nlbin+nepin)*bInh+nlpin*pInh+nebin*Inh

Inh'= nlbin*bInh-(nebin+nlin)*Inh+ nein*nInh+(-inbin*Inh

*Sum[x[jj][kk][ll][0][0],{jj,0,6},{kk,0,2},{ll,1,3,2}]) +inubin*Sum[x[jj][kk][ll][0][0],{jj,0,6},{kk,0,2},{ll,4,5}]

nInh'== Inh*nlin-(nein)* nInh-

(inbin*Nf*nInh* Sum[x[jj][kk][ll][1][nn],{jj,0,6},{kk,0,2},{ll,1,3,2},{nn, 0,1}])+ inubin*Sum[x[jj][kk][ll][1][

nn],{jj,0,6},{kk,0,2},{ll,4,5},{nn,0,1}]

6) Binding/Unbinding

CK1-CK1 inhibitor (PF-670462)

[x[j][k][l][m][n]'=

If[(l==1)&&(m==0)&&(n==0),-inbin*Inh*x[j][k][l][m][n]+ inubin*x[j][k][4][m][n],0]+

If[(l==3)&&(m==0)&&(n==0),-inbin*Inh*x[j][k][l][m][n]+ inubin*x[j][k][5][m][n],0]+

If[(l==4)&&(m==0)&&(n==0), inbin*Inh*x[j][k][1][m][n]-inubin*x[j][k][l][m][n],0]+

If[(l==5)&&(m==0)&&(n==0), inbin*Inh*x[j][k][3][m][n]-inubin*x[j][k][l][m][n],0]+

If[(l==1)&&(m==1),-inbin*Nf*nInh*x[j][k][l][m][n]+

inubin*x[j][k][4][m][n],0]+

If[(l==3)&&(m==1),-inbin*Nf*nInh*x[j][k][l][m][n]+ inubin*x[j][k][5][m][n],0]+

If[(l==4)&&(m==1), inbin*Nf*nInh*x[j][k][1][m][n]-inubin*x[j][k][l][m][n],0]+

If[(l==5)&&(m==1), inbin*Nf*nInh*x[j][k][3][m][n]-inubin*x[j][k][l][m][n],0]

PER-CRY

x[j][k][l][m][n]'=

If[(k==0)&&(n== 0)&&((j==2)||(j==4)||(j==5)||(j==6)),-

ar* If[m==1,Nf,1]*x[j][k][l][m][n]* Sum[x[0][kk][0][m][0],{kk,1,2}]+ dr*Sum[x[j][kk][l][m][n],{kk,1,2}],0]+

If[(j==0)&&((k==1)||(k==2))&&(l==0)&&(n==0),-

ar* If[m==1,Nf,1]*x[j][k][l][m][n]* Sum[x[jj][0][ll][m][0],{jj,{2,4,5,6}},{ll,0,5}]+ dr*Sum[x[jj][k][ll][m][n],{jj,

{2,4,5,6}},{ll,0,5}],0]+

If[((j==2)||(j==4)||(j==5)||(j==6))&&((k==1)||(k== 2))&&(n==0), ar*If[m==1,Nf,1]*x[0][k][0][m][n]*x[j][0][l][m][0]- dr*x[j][k][l][m][n],0]+

If[(k==0)&&(n== 1)&&((j==2)||(j==4)||(j==5)||(j==6))&&(m==1),-

ar*Nf* x[j][k][l][m][n]*Sum[x[0][kk][0][m][0],{kk,1,2}]+ dr*Sum[x[j][kk][l][m][n],{kk,1,2}],0]+

If[(j==0)&&((k==1)||(k==2))&&(l==0)&&(m==1)&&(n== 0),-

ar*Nf*x[j][k][l][m][n]* Sum[x[jj][0][ll][m][1],{jj,{2,4,5,6}},{ll,0,5}]+ dr*Sum[x[jj][k][ll][m][1],{jj,{2,4,5,6}},{ll,0,5}],0]+

If[((j==2)||(j==4)||(j==5)||(j==6))&&((k==1)||(k== 2))&&(m==1)&&(n==1), ar*Nf*x[j][0][l][m][n]*x[0][k][0][m][0]-dr*x[j][k][l][m][n],0]+

If[(k==0)&&(n== 0)&&((j==2)||(j==4)||(j==5)||(j==6))&&(m==1),-

ar*Nf* x[j][k][l][m][n]*Sum[x[0][kk][0][m][1],{kk,1,2}]+ dr*Sum[x[j][kk][l][m][1],{kk,1,2}],0]+

If[(j==0)&&((k==1)||(k==2))&&(l==0)&&(m==1)&&(n== 1),-

ar*Nf*x[j][k][l][m][n]* Sum[x[jj][0][ll][m][0],{jj,{2,4,5,6}},{ll,0,5}]+ dr*Sum[x[jj][k][ll][m][n],{jj,{2,4,5,6}},{ll,0,5}],0]+

If[((j==2)||(j==4)||(j==5)||(j==6))&&((k==1)||(k== 2))&&(m==1)&&(n==1), ar*Nf*x[j][0][l][m][0]*x[0][k][0][m][1]-dr*x[j][k][l][m][n], 0]

PER-CKI

x[j][k][l][m][n]'=

If[(l==0)&&(j>0)&&(n==0),-

ac*If[m==1,Nf,1]*x[j][k][l][m][n]* Sum[x[0][0][ll][m][0],{ll,1,4,3}]+ dc*Sum[x[j][k][ll][m][n],{ll,1,4,3}],0]+

If[(j==0)&&(k==0)&&((l==1)||(l==4))&&(n==0),-

ac* If[m==1,Nf,1]*x[j][k][l][m][n]* Sum[x[jj][kk][0][m][0],{jj,1,6},{kk,0,2}]+ dc*Sum[x[jj][kk][l][m][0],{jj,1,6},{kk,0,2}],0]+

If[(j>0)&&((l==1)||(l==4))&&(n==0), ac*If[m==1,Nf,1]*x[0][0][l][m][0]*x[j][k][0][m][n]-

dc*x[j][k][l][m][n],0]+

If[(l==0)&&(j>0)&&(m==1)&&(n==1),-

ac*Nf*x[j][k][l][m][n]* Sum[x[0][0][ll][m][0],{ll,1,4,3}]+ dc*Sum[x[j][k][ll][m][n],{ll,1,4,3}],0]+

If[(j==0)&&(k==0)&&((l==1)||(l==4))&&(m==1)&&(n== 0),-

ac*Nf*x[j][k][l][m][n]* Sum[x[jj][kk][0][m][1],{jj,1,6},{kk,0,2}]+ dc*Sum[x[jj][kk][l][m][1],{jj,1,6},{kk,0,2}],0]+

If[(j>0)&&((l==1)||(l==4))&&(m==1)&&(n==1), ac*Nf*x[0][0][l][m][0]*x[j][k][0][m][n]-
dc*x[j][k][l][m][n], 0]+
If[(j>2)&&(l==2)&&(n==0),-ac*If[m==1,Nf,1]* x[j][k][l][m][n]*x[0][0][1][m][0]+dc*x[j][k][3][m][n],0]+
If[(j==0)&&(k==0)&&(l==1)&&(n==0),-
ac*If[m==1,Nf,1]* x[j][k][l][m][n]*Sum[x[jj][kk][2][m][0],{jj,3,6},{kk,0,2}]+ dc*Sum[x[jj][kk][3][m][0],{jj,3
,6},{kk,0,2}],0]+ If[(j>2)&&(l==3)&&(n==0), ac*If[m==1,Nf,1]*x[j][k][2][m][n]*x[0][0][1][m][0]-
dc*x[j][k][l][m][n],0]+
If[(j>2)&&(l==2)&&(n==0),-ac*If[m==1,Nf,1]* x[j][k][l][m][n]*x[0][0][4][m][0]+dc*x[j][k][5][m][n],0]+
If[(j==0)&&(k==0)&&(l==4)&&(n==0),-
ac*If[m==1,Nf,1]* x[j][k][l][m][n]*Sum[x[jj][kk][2][m][0],{jj,3,6},{kk,0,2}]+ dc*Sum[x[jj][kk][5][m][0],{jj,3
,6},{kk,0,2}],0]+
If[(j>2)&&(l==5)&&(n==0), ac*If[m==1,Nf,1]*x[j][k][2][m][n]*x[0][0][4][m][0]- dc*x[j][k][l][m][n],0]+
If[(l==2)&&(j>2)&&(m==1)&&(n==1),-ac*Nf*x[j][k][l][m][n]* x[0][0][1][m][0]+dc*x[j][k][3][m][n],0]+
If[(j==0)&&(k==0)&&(l==1)&&(m==1)&&(n==0),-
ac*Nf* x[j][k][l][m][n]*Sum[x[jj][kk][2][m][1],{jj,3,6},{kk,0,2}]+ dc*Sum[x[jj][kk][3][m][1],{jj,3,6},{kk,0,2}
],0]+
If[(j>2)&&(l==3)&&(m==1)&&(n==1), ac*Nf*x[0][0][1][m][0]*x[j][k][2][m][n]-dc*x[j][k][l][m][n],0]+
If[(l==2)&&(j>2)&&(m==1)&&(n==1),-ac*Nf*x[j][k][l][m][n]* x[0][0][4][m][0]+dc*x[j][k][5][m][n],0]+
If[(j==0)&&(k==0)&&(l==4)&&(m==1)&&(n==0),-
ac*Nf* x[j][j][l][m][n]*Sum[x[jj][kk][2][m][1],{jj,3,6},{kk,0,2}]+ dc*Sum[x[jj][kk][5][m][1],{jj,3,6},{kk,0,2}]
,0]+
If[(j>2)&&(l==5)&&(m==1)&&(n==1), ac*Nf*x[0][0][4][m][0]*x[j][k][2][m][n]-dc*x[j][k][l][m][n], 0]

PER-GSK3β

x[j][k][l][m][n]'=
If[(j>2)&&((l==0)||(l==1)),-If[m==1,Nf,1]*agp* x[j][k][l][m][n]*x[0][0][2][m][0]+dg*x[j][k][l+2][m][n],0]+
If[(j>2)&&(l==4),-If[m==1,Nf,1]*agp*x[j][k][l][m][n]* x[0][0][2][m][0]+dg*x[j][k][5][m][n],0]+
If[(j>2)&&((l==2)||(l==3)), If[m==1,Nf,1]*agp*x[j][k][l-2][m][n]*x[0][0][2][m][0]- dg*x[j][k][l][m][n],0]+
If[(j>2)&&(l==5), If[m==1,Nf,1]*agp*x[j][k][4][m][n]*x[0][0][2][m][0]- dg*x[j][k][l][m][n],0]+

If[(j==0)&&(k==0)&&(l==2)&&(n==0),-

If[m==1,Nf,1]*agp* Sum[x[jj][kk][ll][m][nn],{jj,3,6},{kk,0,2},{ll,{0,1,4}},{nn, 0,1}]*x[j][k][l][m][n]+ dg*Sum[x[jj][kk][ll][m][nn],{jj,3,6},{kk,0,2},{ll,{2,3,5}},{nn,0, 1}],0]

PER-BMALs-CLOCK/NPAS2

x[j][k][l][m][n]'=

If[(j>0)&&(m==1)&&(n==0),-bbin*Nf*x[j][k][l][m][n]* x[0][0][0][m][1]+unbbin*x[j][k][l][m][1],0]+

If[(j==0)&&(k==0)&&(n>0)&&(l==0)&&(m==1),-

bbin*Nf* x[0][0][0][m][n]* Sum[x[jj][kk][ll][m][0],{jj,1,6},{kk,0,2},{ll,0,5}]+ unbbin*Sum[x[jj][kk][ll][m][n],{jj,1,6},{kk,0,2},{ll,0,5}], 0]+

If[(j>0)&&(m==1)&&(n>0), bbin*Nf*x[j][k][l][m][0]*x[0][0][0][m][n]- unbbin*x[j][k][l][m][n],0]

CRY-BMALs-CLOCK/NPAS2

x[j][k][l][m][n]'=

If[(j=0)&&(k>0)&&(l=0)&&(m=1)&&(n=0),-

cbbin*Nf*x[j][k][l][m][n]*x[0][0][0][m][1]+uncbbin*x[j][k][l][m][1],0]+

If[(j=0)&&(k=0)&&(l=0)&&(m=1)&&(n=1),-

cbbin*Nf*Sum[x[0][kk][0][m][0],{kk,1,2}]*x[j][k][l][m][n]+uncbbin*Sum[x[0][kk][0][m][n],{kk,1,2}],0]+

If[(j=0)&&(k>0)&&(l=0)&&(m=1)&&(n=1),cbbin*Nf*x[j][k][l][m][0]*x[0][0][0][m][n]-

uncbbin*x[j][k][l][m][n],0]

REV-ERBs-GSK3β

x[j][k][l][m][n]'=

If[(j=0)&&(k=0)&&(l=2)&&(m=0)&&(n=0),-ag*cyrev*x[j][k][l][m][n]+(dg)*cyrevg+(dg)*cyrevgp,0]+

If[(j=0)&&(k=0)&&(l=2)&&(m=1)&&(n=0),-ag*Nf*revn*x[j][k][l][m][n]+(dg)*revng+(dg)*revngp,0]

7) Translocation

PER binding proteins

x[j][k][l][m][n]'=

If[((j=2)||(j=4)||(j=5)||(j=6))&&(m=1),-ne*If[(n=0),1,0]*x[j][k][l][m][n]+If[(n=0),1,0]*nl*x[j][k][l][0][n],0]+

If[((j=2)||(j=4)||(j=5)||(j=6))&&(m=0),ne*If[(n=0),1,0]*x[j][k][l][1][n]-If[(n=0),1,0]*nl*x[j][k][l][m][n],0]+

BMALs-CLOCK/NPAS2

x[j][k][l][m][n]'=

If[(j=0)&&(k=0)&&(l=0)&&(m=1)&&(n=1, nlbc*x[j][k][l][0][n],0]+

If[(j=0)&&(k=0)&&(l=0)&&(m=0)&&(n=1),-nlbc*x[j][k][l][m][n],0]+

CK1 and GSK3b Kinase

x[j][k][l][m][n]'=

If[(j=0)&&(k=0)&&((l=1)||(l=2))&&(m=1)&&(n=0),-lne*x[j][k][l][m][n],0]+

If[(j=0)&&(k=0)&&((l=1)||(l=2))&&(m=0)&&(n=0),lne*x[j][k][l][1][n],0]

CK1 inhibitor (PF-670462)

x[j][k][l][m][n]'=

If[(j==0)&&(k==0)&&((l==4))&&(m==1)&&(n==0),-lnei* x[j][k][l][m][n],0]+

If[(j==0)&&(k==0)&&((l==4))&&(m==0)&&(n==0), lnei*x[j][k][l][1][n],0]

8) Phosphorylation

x[j][k][l][m][n]'=

If[((j=1))&&(l=1)&&(k=0)&&(m=0)&&(n=0),-hoo*x[j][k][l][m][n],0]+

If[((j=2))&&(l=1)&&(k=0)&&(m=0)&&(n=0),+hoo*x[1][k][l][m][n],0]+

If[((j=3)||(j=5))&&((l=1)||(l=3))&&(k=0),-hto*x[j][k][l][m][n],0]+

If[((j=4)||(j=6))&&((l=1)||(l=3))&&(k=0),hto*x[j-1][k][l][m][n],0]+

If[((j=3)||(j=4))&&((l=2)||(l=3)),-gto*x[j][k][l][m][n],0]+If[((j=5)||(j=6))&&((l=2)||(l=3)),gto*x[j-2][k][l][m][n],0]+

If[(j=0)&&(k=0)&&(l=0)&&(m=0)&&(n=1),phos*BC,0]+

9) Degradation

CRY

x[j][k][l][m][n]'=

If[(j=0)&&(k=1)&&(l=0)&&(n=0),-uro*x[j][k][l][m][n],0]+

If[(j=0)&&(k=2)&&(l=0)&&(n=0),-urt*x[j][k][l][m][n],0]+

If[(j=0)&&(k=1)&&(l=0)&&(m=1)&&(n=1),-uro*x[j][k][l][m][n],0]+

If[(j=0)&&(k=2)&&(l=0)&&(m=1)&&(n=1),-urt*x[j][k][l][m][n],0]+

PER

x[j][k][l][m][n]'=

If[((j==1)||(j==3)||(j==5))&&(k==0),-If[(m==0)&&(n==1), 0,1]*upu*x[j][k][l][m][n],0]+

If[((j==2))&&(k==0),-If[(m==0)&&(n==1),0,1]*up* x[j][k][l][m][n],0]+

If[((j==4)||(j==6))&&(k==0),-If[(m==0)&&(n==1),0,1]*up* x[j][k][l][m][n],0]+

If[(j==0)&&(k==0)&&(l==1)&&(n==0), up*Sum[x[jj][0][ll][m][nn],{jj,4,6,2},{nn,0,1},{ll,1,3,2}]+ up*Sum[x[jj][0][ll][m][nn],{jj,2,2},{nn,0,1},{ll,1,3,2}]+ upu*Sum[x[jj][0][ll][m][nn],{jj,1,5,2},{nn,0,1},{ll,1,3,2}], 0]

+

If[(j==0)&&(k==0)&&(l==4)&&(n==0), up*Sum[x[jj][0][ll][m][nn],{jj,4,6,2},{nn,0,1},{ll,4,5}]+ up*Sum[x[jj][0][ll][m][nn],{jj,2,2},{nn,0,1},{ll,4,5}]+ upu*Sum[x[jj][0][ll][m][nn],{jj,1,5,2},{nn,0,1},{ll,4,5}], 0]+

If[(j==0)&&(k==0)&&(l==2)&&(n==0), up*Sum[x[jj][0][ll][m][nn],{jj,4,6,2},{ll,{2,3,5}},{nn,0, 1}]+ up*Sum[x[jj][0][ll][m][nn],{jj,2,2},{ll,{2,3,5}},{nn,0,1}]+ upu*Sum[x[jj][0][ll][m][nn],{jj,1,5,2},{ll,{2,3,5}},{nn,0,1}],0]+

If[(j==0)&&(k==0)&&(l==0)&&(n==1)&&(m==1), up*Sum[x[jj][0][ll][m][n],{jj,4,6,2},{ll,0,5}]+ up*Sum[x[jj][0][ll][m][n],{jj,2,2},{ll,0,5}]+ upu*Sum[x[jj][0][ll][m][n],{jj,1,5,2},{ll,0,5}],0]

BMALs-CLOCK/NPAS2

x[j][k][l][m][n]'=

If[(j>0)&&(k=0)&&(m=1)&&(n=1),-ubc*x[j][k][l][m][n],0]+

If[(j=0)&&(k=0)&&(l=0)&&(n=1),-ubc*x[j][k][l][m][n],0]+

If[(j>0)&&(k=0)&&(m=1)&&(n=0),ubc*x[j][k][l][m][1],0]

REV-ERBs

x[j][k][l][m][n]'=

If[(j=0)&&(k=0)&&(l=2)&&(m=0)&&(n=0),urev*cyrevg+uprev*cyrevgp,0]+

If[(j=0)&&(k=0)&&(l=2)&&(m=1)&&(n=0),urev*revng+uprev*revngp,0]

11) Transcriptional Activity of GSK3β

gto'=trgto*G*GB-ugto*gto

12) Light Activity

ltn'=60*lta*(1-ltn)-ltb*ltn

MnPo'=trPo*G-tmc*MnPo-umPo*MnPo+lono*19.9*lta*(1-ltn)*trPo

MnPt'=trPt*G-tmc*MnPt-umPt*MnPt+lont*19.9*lta*(1-ltn)*trPt

Supplementary References

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