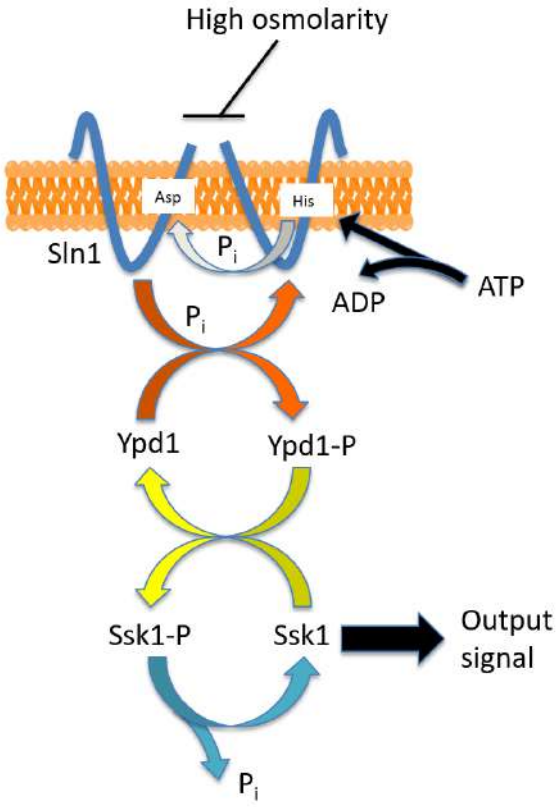
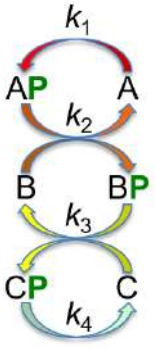
Tutorial 4: Exploring Coupled ODEs Modelling a Biological System

Problem Statement

This Tutorial will demonstrate the use of ROptimus to address a problem from yet another problem class. We will employ ROptimus to recover the rate constants for a system of coupled ordinary differential equations (ODEs) modelling a biological pathway. Specifically, we will study a phosphorelay system from the high osmolarity glycerol (HOG) pathway in yeast. A phosphorelay system is a network involving multiple proteins in which, after an initial phosphorylation event using ATP (or an alternate phosphate donor), the phosphorylation and dephosphorylation events of proteins in the network proceed without further consumption of ATP (Klipp et al. 2009). The below diagram illustrates the phosphorelay system that will be studied in detail (Klipp et al. 2009):



Under normal circumstances, the transmembrane protein Sln1, which is present as a dimer, autophosphorylates at a histidine residue (consuming ATP). The phosphate group is then transferred to an aspartate residue of Sln1. Thereafter, the phosphate is transferred to the protein Ypd1 and finally to the protein Ssk1. Ssk1 is continuously dephosphortylated to give an output signal. The signalling pathway is inhibited by an increase is osmolarity outside of the cell (Klipp et al. 2009). If we let A represent Sln1, B represent Ypd1, C represent Ssk1 and XP represent the phophorylated form of protein X, then the above network can be represented by the below schematic (Klipp et al. 2009):



where each k_i represents the rate constant for the relevant phosphorylation/dephosphorylation reaction.

The above graphic allows us to arrive at the following equations to describe the temporal behavior of the phosphorelay system:

$$\frac{d}{dt}[A] = -k_1[A] + k_2[AP][B]$$
$$\frac{d}{dt}[B] = -k_2[AP][B] + k_3[BP][C]$$
$$\frac{d}{dt}[C] = -k_3[BP][C] + k_4[CP]$$

Moreover, under the generally accepted assumption that the degradation and production of proteins occurs on a time scale that far exceeds that of phosphorylation events, we have the following conservation relationships (Klipp et al. 2009):

$$[A]_{total} = [A] + [AP]$$
$$[B]_{total} = [B] + [BP]$$
$$[C]_{total} = [C] + [CP]$$

where $[A]_{total}$, $[B]_{total}$ and $[C]_{total}$ are constants. Differentiating, we have:

$$\frac{d}{dt}[AP] = -\frac{d}{dt}[A]$$
$$\frac{d}{dt}[BP] = -\frac{d}{dt}[B]$$
$$\frac{d}{dt}[CP] = -\frac{d}{dt}[C]$$

Given this model of the phosphorelay system, the question we desire to answer is as follows: given initial concentrations of the three proteins $\{[A]_i, [B]_i, [C]_i\}$ and target concentrations of the three proteins $\{[A]_t, [B]_t, [C]_t\}$, what are the values $\{k_1, k_2, k_3, k_4\}$ that result in the proteins having the target concentrations at steady state when the system is allowed to equilibrate from the initial concentrations? This formulation assumes that no information is known about the rate constants and that initial and target concentrations can be determined experimentally, which is often the case in practice (Raue et al. 2013). The problem formulation could be altered depending on the information that is known or that can be determined experimentally.

Defining ROptimus Inputs

Having outlined how the behaviour of the phosphorelay system can be modelled using a system of differential equations, we can now proceed with defining input parameters for `Optimus()`. We will create a variable state that will be a numeric vector holding the names and initial concentrations of all species in the network. For this tutorial, we will choose $[A]_i = [B]_i = [C]_i = 100$ and $[AP]_i = [BP]_i = [CP]_i = 0$. Note that the units are arbitrary and that the total sum of units across this vector will remain constant throughout the simulation of the dynamics of the phosphorelay system.

```
state  <- c(cA=100, cB=100, cC=100, cAP=0, cBP=0, cCP=0)
```

Next, we will create a variable target which will be a numeric vector holding the names and target concentration of all species in the network. We will arbitrarily choose target values of $[A]_t = 90$, $[B]_t = 20$, $[C]_t = 70$, $[AP]_t = 10$, $[BP]_t = 80$ and $[CP]_t = 30$. Note that the chosen target values must be consistent with the above defined conservation equations, meaning we must have $[X]_i + [XP]_i = [X]_t + [XP]_t, \forall\, X \in \{A, B, C\}$.

```
target <- c(cA=90, cB=20, cC=70, cAP=10, cBP=80, cCP=30)
```

In order to determine the steady state behavior of the ODE system, we will employ the function `ode()` from the R library `deSolve` (this function interfaces with the Fortran library typically used to solve systems of differential equations). This function requires as input a function that describes the dynamics of the ODE system. We will call this function `model()`. At a high level, `model()` will simply define the equations derived in the previous section that describe the network. It should contain equations that use the objects with the names specified within state above, and should have equations that assign the outcomes to new objects that have the same order and names as specified in state, but with "d" at the beginning (a more detailed description of the requirements of `model()` can be found in the R documentation of `ode()`).

```
model <- function(t, state, K){

  with( as.list(c(state, K)), {
    # rate of change
    dcA  <- -k1*cA+k2*cAP*cB
    dcB  <- -k2*cAP*cB+k3*cBP*cC
    dcC  <- -k3*cBP*cC+k4*cCP
    dcAP <- -dcA
    dcBP <- -dcB
    dcCP <- -dcC
    # return the rate of change
    list(c(dcA, dcB, dcC, dcAP, dcBP, dcCP))
  })
}
```

The variables `state` and `target`, and the function `model()` should be stored as entries in a list `DATA` which will be given to the functions `m()` and `u()` as inputs.

```
DATA <- NULL
DATA$state  <- state
DATA$target <- target
DATA$model  <- model
```

We will make `K` be a numeric vector holding the set of rate constants $\{k_1, k_2, k_3, k_4\}$. We will (arbitrarily) initialize each rate constant to have value 1.0.

```
K <- c(k1=1.0, k2=1.0, k3=1.0, k4=1.0)
```

The function `m()` will take as input the vector `K` of rate constants and the list `DATA`. It will return an object `O` that contains the concentrations of the six species in the network when the system is simulated from the initial state specified in `DATA` using the `K` rate constants for 10 time steps. Note that it is not necessarily

guaranteed that the system will reach a steady state after 10 time steps; the number of time steps was chosen such that the optimisation procedure would terminate within 1-2 hours in this example. `m()` will call the function `ode()` from the library `deSolve`, so we must first ensure that `deSolve` is installed.

```
install.packages("deSolve")
```

```
library(deSolve)
m <- function(K, DATA){
  state <- DATA$state
  model <- DATA$model

  span = 10.0

  times <- c(0, span)
  O   <- ode(y=state, times=times, func=model, parms=K)[2,2:(length(state)+1)]
  return(O)
}
```

Recall that the function `u()` must return an energy `E` and a quality `Q` of the candidate solution. Here, `u()` will set both `E` and `Q` to be the RMSD between the steady state concentrations of the network corresponding to the current set of rate constants `K`, as determined by `m()`, and the target concentrations.

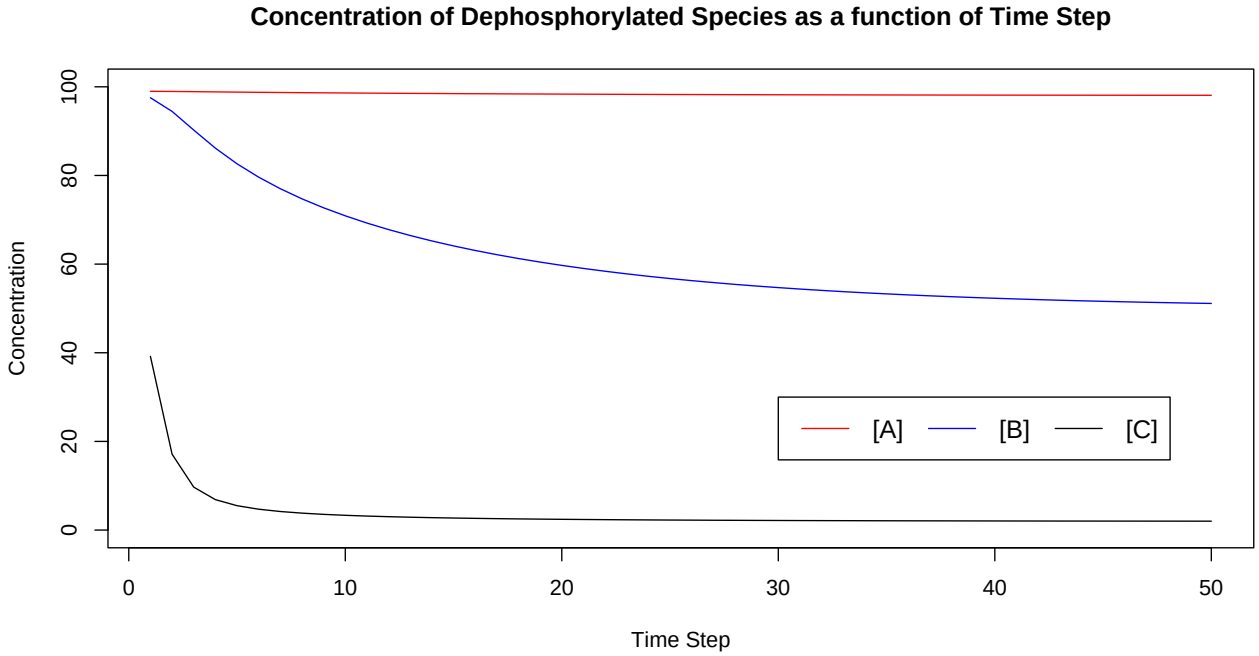
```
u <- function(O, DATA){
  target   <- DATA$target
  RESULT   <- NULL
  RESULT$Q <- sqrt(mean((O-target)^2)) # measure of the fit quality
  RESULT$E <- RESULT$Q # the pseudo energy derived from the above measure

  return(RESULT)
}
```

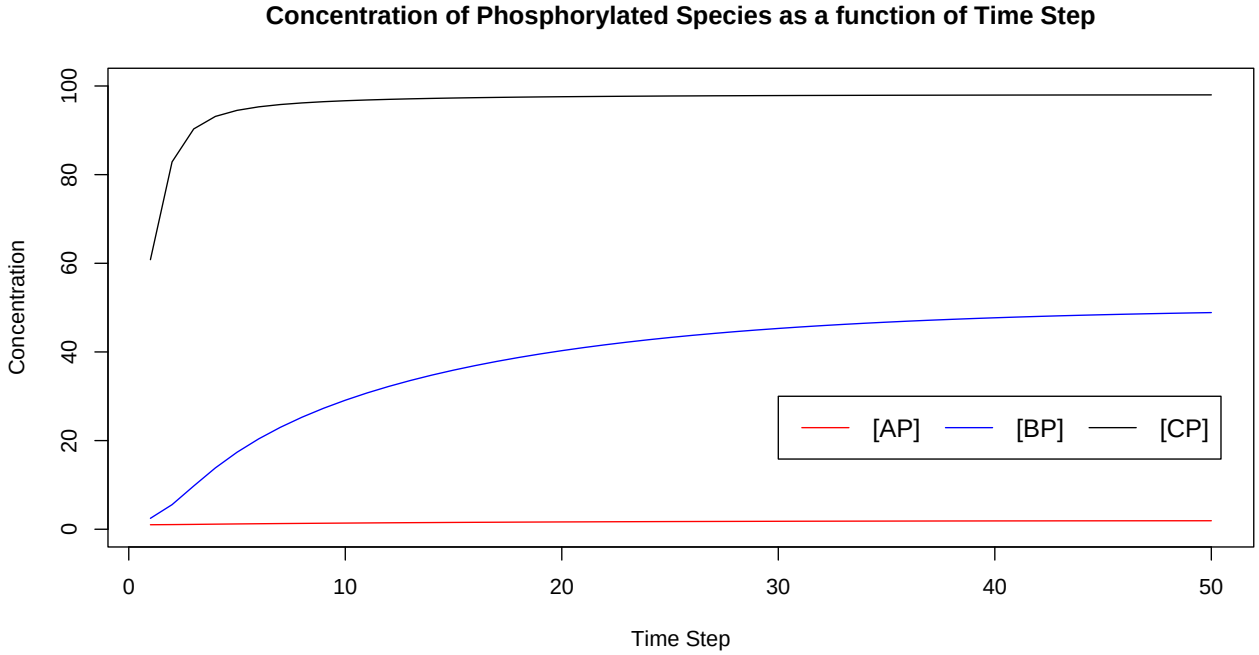
The final mandatory input to `Optimus()` that must be defined is the alteration function `r()`. Just as in **Tutorial 1**, for each snapshot of `K`, we shall randomly select one of its four coefficients, then either increment or decrement (chosen randomly) it by 0.0002, returning the altered set of coefficients. Since we are dealing with rate constants in this case, if ever `r()` were to make an entry in K negative, that entry will automatically be set to 0.

```
r <- function(K){
  K.new <- K
  # Randomly selecting a coefficient to alter:
  K.ind.toalter <- sample(size=1, x=1:length(K.new))
  # Creating a potentially new set of coefficients where one entry is altered
  # by either +move.step or -move.step, also randomly selected:
  move.step <- 0.0002
  K.new[K.ind.toalter] <- K.new[K.ind.toalter] + sample(size=1, x=c(-move.step, move.step))

  ## Setting the negative coefficients to 0
  neg.ind <- which(K.new < 0)
  if(length(neg.ind)>0){ K.new[neg.ind] <- 0 }

  return(K.new)
}
```

Exploring the System Dynamics

Before calling `Optimus()` to solve this problem, let us first simulate the system of ODEs from the chosen initial state using a few sets of arbitrary rate constants to become familiar with how the system evolves. The below graphs illustrate the evolution of the system for 50 time steps for the rate constants $\{k_1 = 1.0, k_2 = 1.0, k_3 = 1.0, k_4 = 1.0\}$:





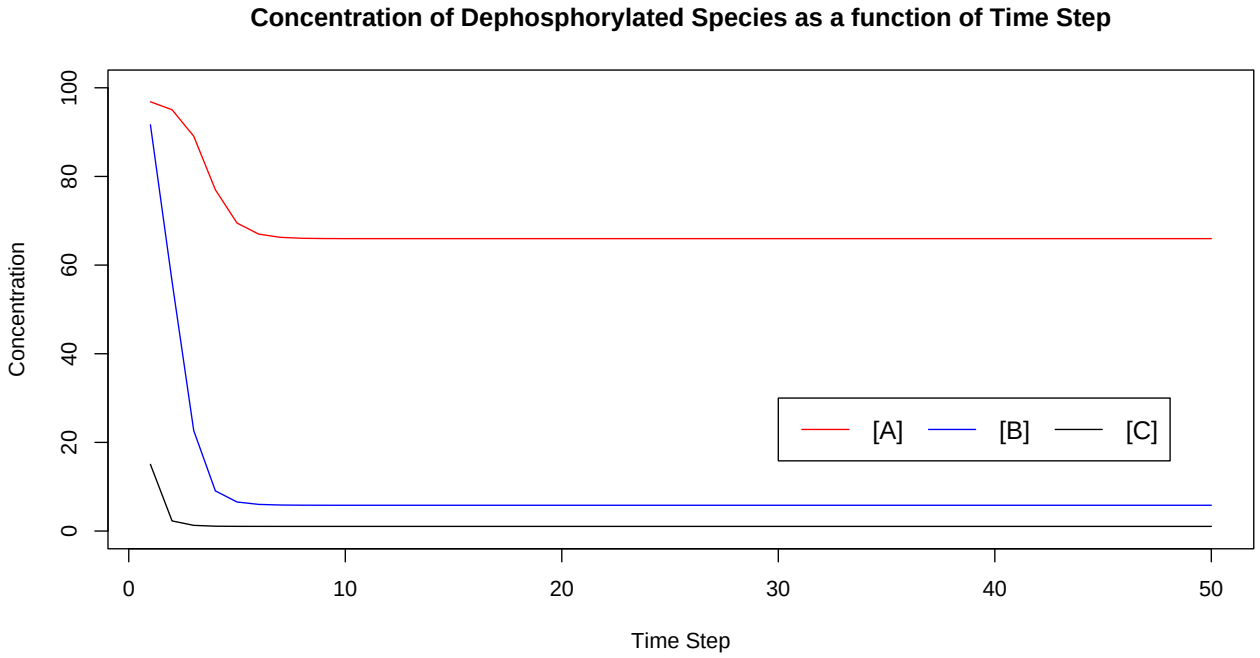
The table below summarises the initial and final concentrations of the various species when the system is simulated for 50 time steps using the rate constants $\{k_1 = 1.0, k_2 = 1.0, k_3 = 1.0, k_4 = 1.0\}$:

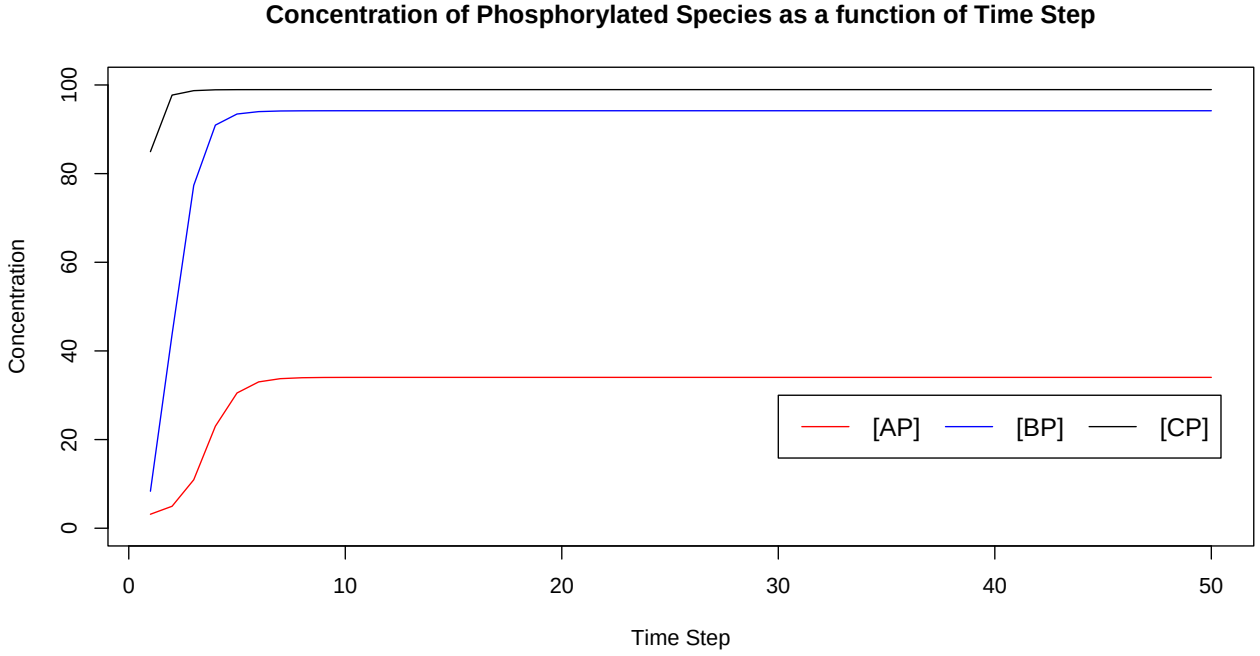
	[A]	[B]	[C]	[AP]	[BP]	[CP]

Table 11: System summary for k1 = k2 = k3 = k4 = 1.0.

	[A]	[B]	[C]	[AP]	[BP]	[CP]
Initial	100.00000	100.00000	100.000000	0.000000	0.00000	0.00000
Final (after 50 time steps)	98.08145	51.12118	2.004924	1.918549	48.87882	97.99508

If instead we use the set of rate constants $\{k_1 = 1.5, k_2 = 0.5, k_3 = 1.0, k_4 = 1.0\}$, the system evolves as follows:





The table below summarizes the initial and final concentrations of the various species when the system is

simulated for 50 time steps using the rate constants $\{k_1 = 1.5, k_2 = 0.5, k_3 = 1.0, k_4 = 1.0\}$:

Table 12: System summary for k1 = 1.5, k2 = 0.5, k3 = k4 = 1.0.

	[A]	[B]	[C]	[AP]	[BP]	[CP]
Initial	100.00000	100.000000	100.000000	0.00000	0.00000	0.00000
Final (after 50 time steps)	65.96628	5.814787	1.050583	34.03372	94.18521	98.94942

Acceptance Ratio Simulated Annealing ROptimus Run

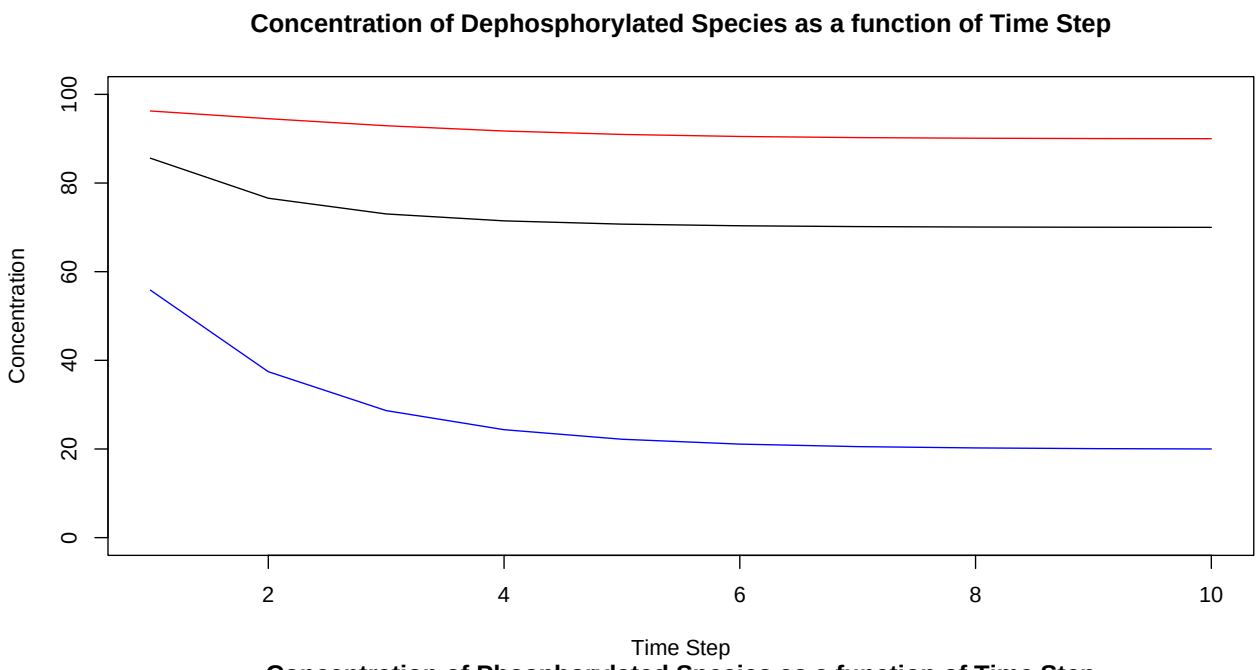
We will now call Acceptance Ratio Simulated Annealing ROptimus to solve our problem. Similarly to **Tutorial 2**, we will execute 200 000 optimisation iterations and perform 2 annealing cycles. We will set `DUMP.FREQ` to have a value of 100 000.

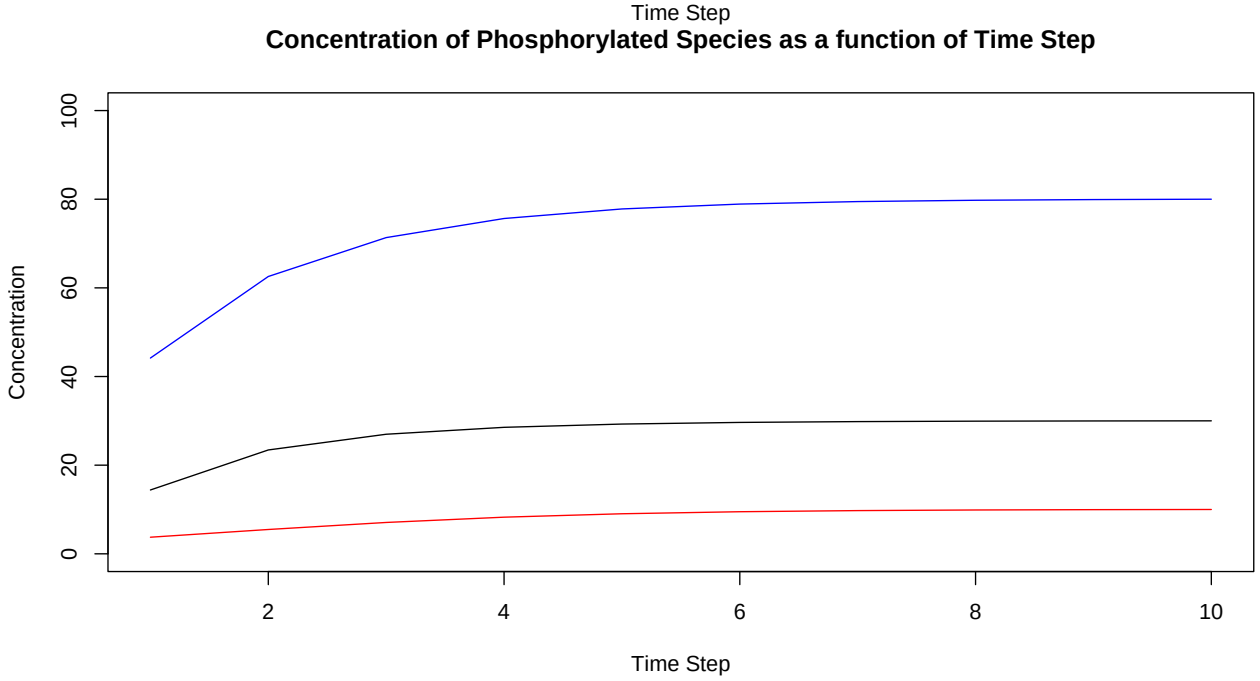
```
Optimus(NCPU=4, OPTNAME="DE_4_SA",
        NUMITER=200000, CYCLES=2, DUMP.FREQ=100000, LONG=FALSE,
        OPT.TYPE="SA",
        K.INITIAL=K, rDEF=r, mDEF=m, uDEF=u, DATA=DATA)
```

Table 13: 4-core Acceptance Ratio Simulated Annealing results from ROptimus run.

	E (RMSD)	K1	K2	K3	K4
CPU 1	0.0012516	0.7974	0.3586	0.0128	2.3886
CPU 2	0.0017556	0.7850	0.3532	0.0126	2.3512
CPU 3	0.0013625	0.8098	0.3642	0.0130	2.4262
CPU 4	0.0012516	0.7974	0.3586	0.0128	2.3886

Of the 4 optimisation replicas, CPU 1 and 4 find the best set of rate constants, $\{k_1 = 0.7974, k_2 = 0.3586, k_3 = 0.0128, k_4 = 2.3886\}$. This set of rate constants results in an RMSD (after 10 iterations) of 0.0012516. Let us simulate how the system evolves according to these rate constants for 10 time steps:





The table below summarizes the initial and final concentrations of the various species when the system is simulated for 10 time steps using the rate constants $\{k_1 = 0.7974, k_2 = 0.3586, k_3 = 0.0128, k_4 = 2.3886\}$:

Table 14: System summary for k1 = 0.7974, k2 = 0.3586, k3 = 0.0128, k4 = 2.3886.

	[A]	[B]	[C]	[AP]	[BP]	[CP]
Initial	100.00000	100.00000	100.00000	0.00000	0.00000	0.00000
Final (after 10 time steps)	89.99814	20.00081	69.99924	10.00186	79.99919	30.00076

As can be seen in the above table, the concentration of the species in the system after 10 time steps are very

close to the target values $[A]_t = 90$, $[B]_t = 20$, $[C]_t = 70$, $[AP]_t = 10$, $[BP]_t = 80$ and $[CP]_t = 30$. As alluded to earlier, it is possible that the system has not reached a steady state after 10 time steps, however the above graphs suggest that the concentrations after 10 steps are already extremely close to, if not equal to, steady state values. The optimisation process could be re-executed after increasing the value of the parameter `span` in the function `m()` to simulate the system for a larger number of time steps.

Acceptance Ratio Replica Exchange ROptimus Run

Let us now examine how replica exchange ROptimus using 12 cores performs on this task. We will use 200 000 optimisation iterations and set `STATWINDOW` to 50, similarly to **Tutorials 2** and **3**.

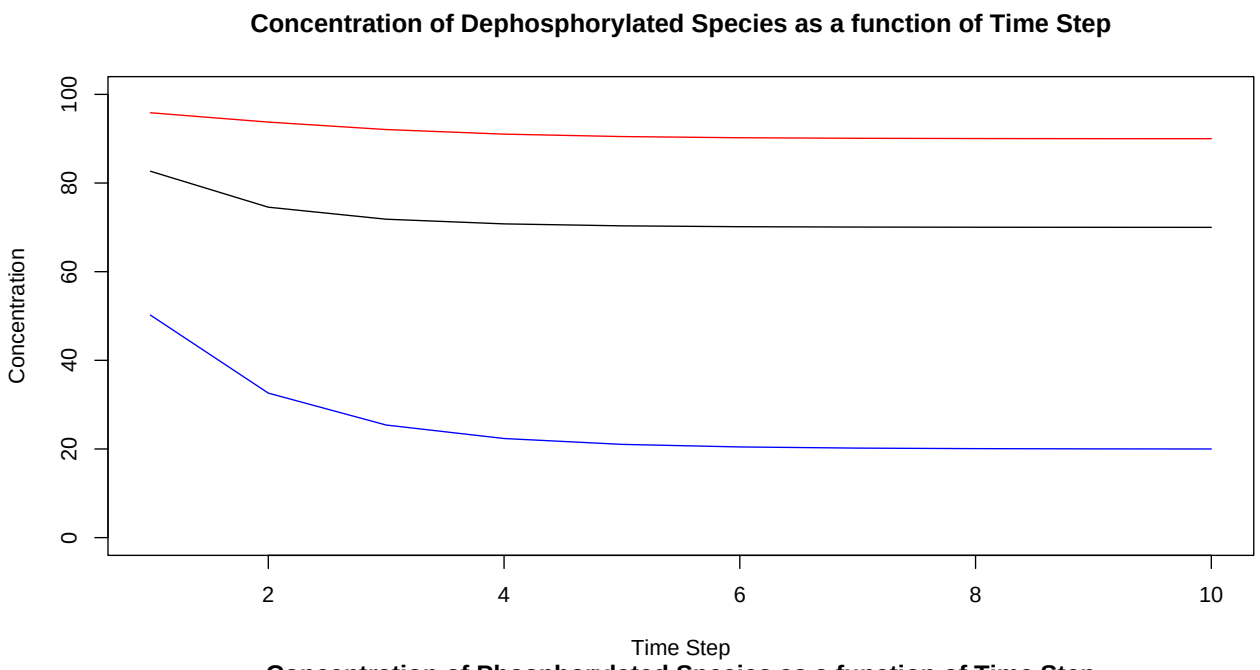
```
ACCRATIO <- c(90, 82, 74, 66, 58, 50, 42, 34, 26, 18, 10, 2)
```

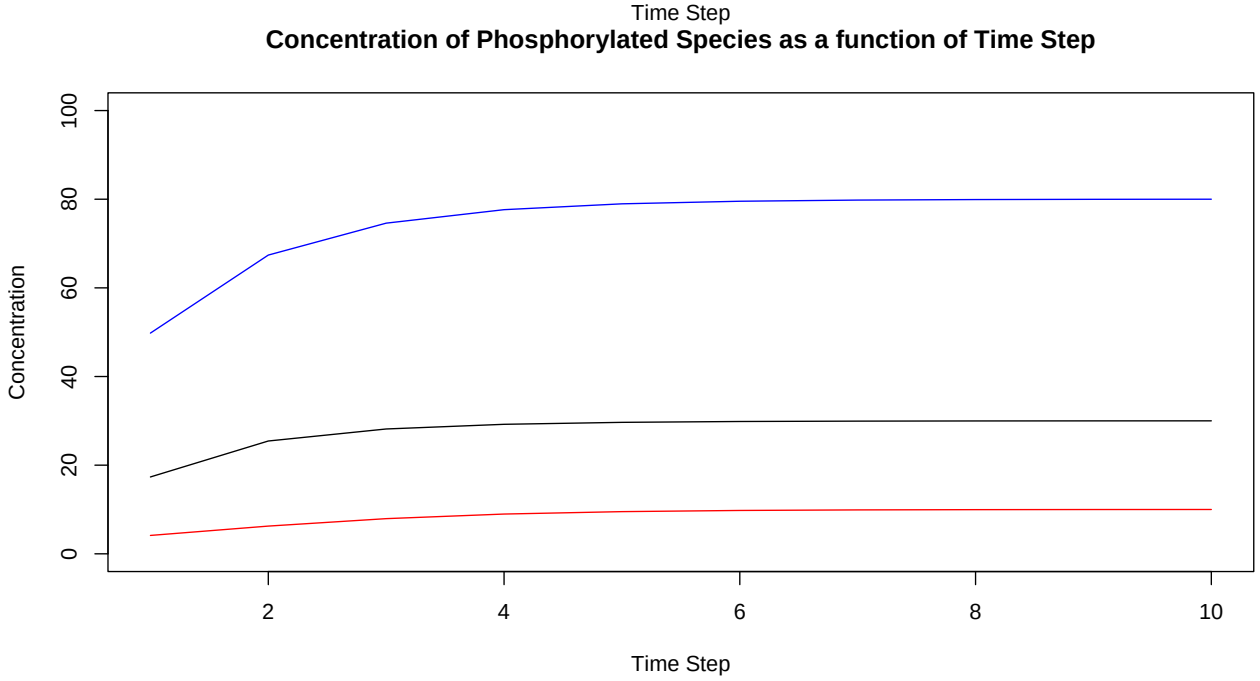
```
Optimus(NCPU=12, OPTNAME="DE_12_RE",
        NUMITER=200000, STATWINDOW=50, DUMP.FREQ=100000, LONG=FALSE,
        OPT.TYPE="RE", ACCRATIO=ACCRATIO,
        K.INITIAL=K, rDEF=r, mDEF=m, uDEF=u, DATA=DATA)
```

Table 15: 12-core Acceptance Ratio Replica Exchange results from ROptimus run.

	Replica Acceptance Ratio	E (RMSD)	K1	K2	K3	K4
CPU 1	90	0.0026225	0.9088	0.4090	0.0146	2.7246
CPU 2	82	0.0026343	0.8346	0.3754	0.0134	2.5012
CPU 3	74	0.0019724	0.7234	0.3252	0.0116	2.1644
CPU 4	66	0.0020945	0.9586	0.4312	0.0154	2.8748
CPU 5	58	0.0029243	0.9338	0.4200	0.0150	2.8002
CPU 6	50	0.0025811	0.8964	0.4034	0.0144	2.6872
CPU 7	42	0.0028685	0.8592	0.3866	0.0138	2.5752
CPU 8	34	0.0025121	0.8840	0.3978	0.0142	2.6500
CPU 9	26	0.0011265	0.9834	0.4424	0.0158	2.9492
CPU 10	18	0.0025811	0.8964	0.4034	0.0144	2.6872
CPU 11	10	0.0012516	0.7974	0.3586	0.0128	2.3886
CPU 12	2	0.0017556	0.7850	0.3532	0.0126	2.3512

Of the 12 optimisation replicas, CPU 9 (26% acceptance ratio) finds the best set of rate constants, $\{k_1 = 0.9834, k_2 = 0.4424, k_3 = 0.0158, k_4 = 2.9492\}$. This set of rate constants results in an RMSD (after crude 10 iteration limit) of 0.0011265, which is lower than the RMSD of the solution found by Acceptance Ratio Simulated Annealing ROptimus (0.0012516) done with the use of more modest computational resources. Let us simulate how the system evolves according to these rate constants for 10 time steps:





The table below summarises the initial and final concentrations of the various protein species when the system is simulated for 10 time steps using the rate constants $\{k_1 = 0.9834, k_2 = 0.4424, k_3 = 0.0158, k_4 = 2.9492\}$:

Table 16: System summary for k1 = 0.9834, k2 = 0.4424, k3 = 0.0158, k4 = 2.9492.

	[A]	[B]	[C]	[AP]	[BP]	[CP]
Initial	100.00000	100.00000	100.00000	0.00000	0.00000	0.00000
Final (after 10 time steps)	89.99807	19.99983	70.00022	10.00193	80.00017	29.99978

Here again, we see that the protein concentrations after 10 time steps are remarkably close to the target

values $[A]_t = 90$, $[B]_t = 20$, $[C]_t = 70$, $[AP]_t = 10$, $[BP]_t = 80$ and $[CP]_t = 30$ and the graphs suggests that these concentrations have either converged or are very close to converging to the steady state.

Summary

We have seen how ROptimus can be employed to recover rate constants for a system of coupled ODEs that describes a biological pathway. Given an initial state of the system and a target state, both Acceptance Ratio Simulated Annealing (SA) ROptimus and Replica Exchange (RE) ROptimus found a set of rate constants that resulted in the desired system behaviour upon simulation of the system. In the current setup in this tutorial, RE slightly outperformed SA, both however are not directly comparable unless their allocated resources and times are equalised.

Table 17: Summary of protein concentrations after 10 time steps.

	[A]	[B]	[C]	[AP]	[BP]	[CP]
Target	90.00000	20.00000	70.00000	10.00000	80.00000	30.00000
ROptimus (AR Simulated Annealing)	89.99814	20.00081	69.99924	10.00186	79.99919	30.00076
ROptimus (AR Replica Exchange)	89.99807	19.99983	70.00022	10.00193	80.00017	29.99978

Table 18: Summary of recovered rate constants.

	E (RMSD)	K1	K2	K3	K4
ROptimus (AR Simulated Annealing)	0.0012516	0.7974	0.3586	0.0128	2.3886
ROptimus (AR Replica Exchange)	0.0011265	0.9834	0.4424	0.0158	2.9492