Dynamic Deterministic Effects Propagation Networks (DDEPN) - exemplary workflow

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Abstract

Network modelling in systems biology has become an important tool to study molecular interactions, especially in the medical field like cancer research. The understanding of the interplay of proteins in cellular signalling is the basis for the development of novel drugs and therapies. Here, we set up a new method for the reconstruction of signalling networks from time course protein data after external perturbation. We show how to use protein expression and phosphorylation data measured on Reverse Phase Protein Arrays to infer a signalling network among proteins of the ERBB signalling cascade in a human breast cancer cell line.

Our method models the signalling dynamics by a boolean signal propagation mechanism that defines a sequence of state transitions for a given network structure. A likelihood score is proposed that describes the probability of our measurements given a particular state transition matrix. We identify the optimal sequence of state transitions via a Hidden Markov Model. Network structure search is performed by a genetic algorithm that optimises the overall likelihood of a population of candidate networks. We test our method on simulated networks and data and show its increased performance in comparison to another Dynamical Bayesian Network approach. The reconstruction of a network in our real data results in several known signalling chains from the ERBB network, showing the validity and usefulness of our approach.

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1 QuickStart: using DDEPN for network inference on simulated data sets

This section shows an exemplary workflow to reconstruct a signalling network from simulated data. An analysis on real data can be performed analogously. Details on formatting the input data matrix as well as arguments for the function calls can be found in subsequent sections.

1.1 Simulating data

In this section we show how to generate artificial networks and data. A reference signalling network is simulated and used to sample measurements that incorporate the network structure.

First, simulate a network with 6 nodes and 2 distinct input stimuli.

```
> set.seed(12345)
> n <- 6
> signet <- signalnetwork(n = n, nstim = 2, cstim = 0, prop.inh = 0.2)
> net <- signet$phi
> stimuli <- signet$stimuli
> weights <- signet$weights
```

Second get intensities for each protein that are based on the network structure generated above.

```
> dataset <- makedata(net, stimuli, mu.bg = 1200, sd.bg = 400,
+     mu.signal.a = 2000, sd.signal.a = 1000)
```

1.2 Running the Genetic Algorithm (GA)

Now run the genetic algorithm to reconstruct the network from the data generated above and compare it to the originally sampled network *net*.

```
> ret <- ddepn(dataset$datx, phiorig = net, inference = "netga",
+     maxiterations = 15, p = 30, q = 0.3, m = 0.8, usebics = TRUE)
```

After the reconstruction, the generated network can be viewed as follows:

```
> plotrepresult(ret)
```

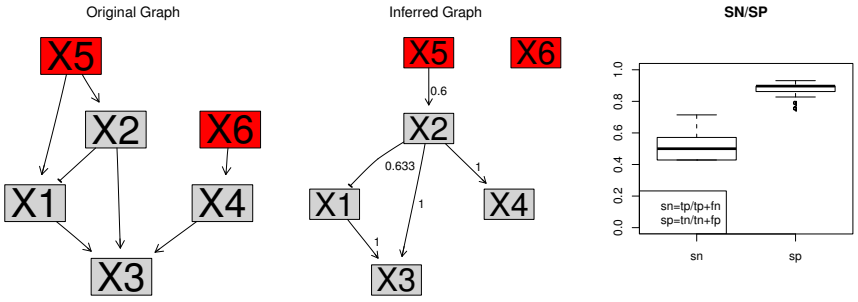


Figure 1: Result plot of the genetic algorithm. Upper left: the given graph; upper right: the inferred graph; lower left: transitive reduction of the inferred graph; lower right: sensitivity/specificity plot for comparing the original and inferred graphs

1.3 Running Markov Chain Monte Carlo Sampling (MCMC)

An example run for MCMC sampling follows. Here, the package *multicore* is needed, since two parallel and independent MCMC runs are performed. If *multicore* is not available on the machine, just set *multicores=FALSE* to perform sampling for a single chain.

```
> B <- matrix(0.5, nrow = n, ncol = n, dimnames = dimnames(net))
> maxiterations <- 5000
> burnin <- 1000
> library(multicore)
> ret <- ddepn(dataset$datx, phiorig = net, inference = "mcmc",
+     maxiterations = maxiterations, burnin = burnin, usebics = FALSE,
+     lambda = 5, B = B, multicores = TRUE, cores = 2)
```

After the sampling one can examine the sampling run:

```
> plotrepresult(ret$samplings[[1]])
```

The returned list *ret* contains two elements, another list with name samplings (*ret$samplings*), which holds the different runs for the MCMC. In case of *multicores=FALSE*, only one run is performed and *ret$samplings* holds only one element. Otherwise *cores* runs are performed independently in parallel, and *ret$samplings* holds *cores* elements. The second element in *ret* with name *ltraces* is a matrix and holds the score traces of all runs, where each column corresponds to one trace. Output diagnostics can be produced using the R-package *coda*. See figure 3 for some example plots.

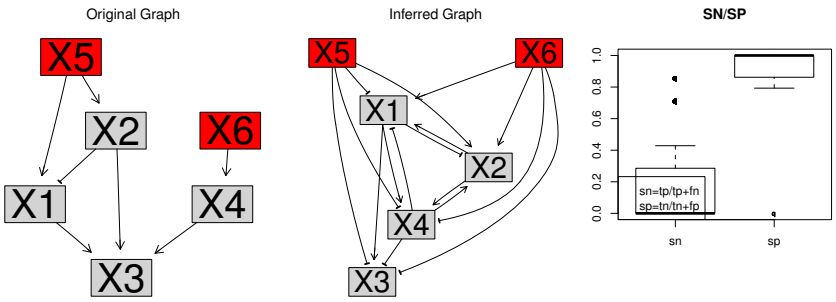


Figure 2: Result plot of MCMC sampling, analogous to figure 1.

```
> library(coda)
> mcmc1 <- mcmc(data = ret$ltraces[-c(1:burnin, maxiterations),
+     1])
> mcmc2 <- mcmc(data = ret$ltraces[-c(1:burnin, maxiterations),
+     2])
> mcmcl <- mcmc.list(mcmc1, mcmc2)
> pdf("mcmcoutput1.pdf", width = 12, height = 6)
> plot(mcmcl)
> dev.off()
> pdf("mcmcoutput2.pdf", width = 12, height = 6)
> gelman.plot(mcmcl)
> dev.off()
```

2 Notes on formatting constraints for the arguments of *ddepn*

There is only one neccessary argument to the function call of *ddepn*: The data matrix *dat*. Optionally, the stimuli list *stimuli*, a reference network *phiorig* and seed networks *phi* can be passed to *ddepn*. Each of these arguments is described briefly below.

Input data matrix *dat*

The data matrix contains all measurements for the nodes in the rows (e.g. proteins or genes), and the experiments and time points in the columns. There are some special needs on how to name the columns. We allow several treatments to be included in the data matrix. Examples for these treatments are stimulation by growth factors or inhibition by application of a drug. We refer generally to each of these as 'treatment'.

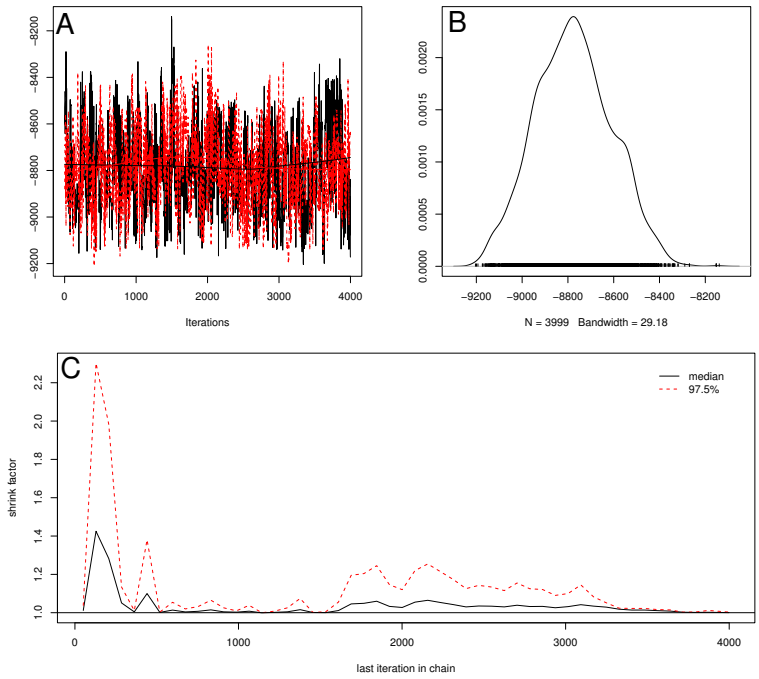


Figure 3: Using package coda for some MCMC output analysis. A: Traces for 2 MCMC runs; B: Distributionplot of the sampling; C: gelman.plot of two MCMC samplings

Each of the treatments will be included in the final network as a node, e.g. stimulation by the growth hormone *EGF* is added to the data matrix as row with name *EGF* (and thus appears as node *EGF* in the final network). The expression values for the stimuli nodes are set to 0 in each column of the data matrix, but are never used in the algorithm and regarded as dummy values. Effects originating in these nodes are estimated in the reconstruction process.

To distinguish the different experimental conditions in the matrix, the columns of the data matrix have to be named in the format *treatment_time*, where treatment also can be a combination of several treatments, e.g. stimulation by *EGF* and simultaneous inhibition by a drug *X*. In this case, each stimulus has to be separated by an ampersand (&). The time point is separated from the stimuli via an underscore character (_), and can be on whatever scale (minutes, hours etc.). An example data matrix is shown below. In this table, the dummy rows for the treatments are already included (rows EGF and X). However, they are not mandatory as input to *ddepn* and, if missing, will be generated automatically, only requiring the correct labeling of the

columns.

	EGF_1	EGF_1	EGF_2	EGF_2	EGF&X_1	EGF&X_1	EGF&X_2	EGF&X_2
EGF	0	0	0	0	0	0	0	0
X	0	0	0	0	0	0	0	0
AKT	1.45	1.8	0.99	1.6	1.78	1.8	1.56	1.58
ERK	1.33	1.7	1.57	1.3	0.68	0.34	0.62	0.47
MEK	0.45	0.8	0.99	0.6	0.78	0.8	0.56	0.58

stimuli

The user might pass an argument stimuli directly, describing the structure of the treatments, i.e. which treatments were done simultaneously. This is usually generated automatically using the properly labeled column names of the data matrix and will be removed soon. However a short example exemplifies the use of *stimuli*:
Consider two experimental conditions, first stimulation by *EGF*, second simultaneous stimulation by *EGF* and inhibition by *X*. The *stimuli* list holds two elements:

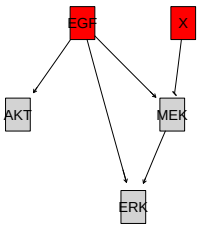
```
 > stimuli
[[1]]
EGF
 1

[[2]]
EGF  X
 1   2
```

Each element is a named numeric vector, holding the row numbers of the treatments in the data table labeled by the names of the treatments.

Reference network *phiorig*

If desired, a reference network *phiorig* can be given, used to compare the edges of the inferred network to it. The user **must** ensure that all treatments are included as nodes, since the inference will estimate effects from these.
The format of the network must be an adjacency matrix, where each entry corresponds to an edge from the node specified by the rowname to the node specified by the column name. Two types of edges are allowed: 1 for activation, 2 for inhibition. 0 means no edge between the pair of nodes. An example network and corresponding adjacency matrix are shown in figure 4.



Φ	EGF	X	AKT	MEK	ERK
EGF	0	0	1	1	1
X	0	0	0	2	0
AKT	0	0	0	0	0
MEK	0	0	0	0	1
ERK	0	0	0	0	0

Figure 4: Example network. Left: graph representation, Right: adjacency matrix

Seed networks *phi*

Both the GA and MCMC sampler require single or multiple seed networks. If not given, an unconnected network is used as seed for each individual in the GA population or the start networks for each MCMC run, respectively. However, the user can provide own seed networks using the argument *phi*. This can either be an adjacency matrix or a list of adjacency matrices. Again, the treatments **must** be included as nodes. If given a single adjacency matrix, it is used as seed network for each of the individuals in the population of networks during the genetic algorithm, or as independent seeds for parallel MCMC samplings. If given as list, its length must equal the number of individuals in the population in the GA (specified by the function argument *p*), or the number of independent runs in the MCMC sampler (specified by the argument *cores*). The format of the adjacency matrices is the same as for *phiorig*.

3 Prior knowledge inclusion

Currently, two methods for using biological prior knowledge are implemented. We refer to the first as *Laplace* prior ([1, 4]), and to the second as *ScaleFree* prior ([2]).

Laplace prior

The laplace prior penalises deviations of edges in the inferred network from prior edge probabilities, aquired from network databases (e.g. KEGG [3]). The package includes a snapshot of the KEGG database, downloaded in August 2009.

```
> data(kegggraphs)
> length(kegggraphs)
```

The list *kegggraphs* includes 195 elements, each of which has 3 members, a string *name* specifying the name of the pathway, a graphNEL object *g* and an igraph object *ig*, which for the time being is not used:

```
> kegggraphs[[1]]

$name
[1] "Glycolysis / Gluconeogenesis"

$g
A graphNEL graph with directed edges
Number of Nodes = 64
Number of Edges = 256

$ig
NULL
```

Similar to the set of reference networks from KEGG, one can download other reference networks from different sources (e.g. Reactome, Transpath) and compile the prior distribution from them. Each graphNEL object can be converted to a detailed adjacency list (including inhibitions as entries with value 2):

```
> kegggraph.to.detailed.adjacency(gR = kegggraphs[[1]]$g)
```

To obtain prior probabilites for each edge between all pairs of nodes present in *kegggraphs*, we follow the approach suggested by [4]. We count the total number of node pairs M occurring in all reference networks as well as the number of node pairs m that are connected via an edge in all reference networks. The prior probability matrix $\mathcal{B}$ is defined as:

$$\mathcal{B} = \frac{m}{M}$$

We compute a normalising factor Z as described in [4] for a given hyperparameter λ. The prior probability density for each edge between node i and j is calculated as laplace type distribution:

$$P(\Phi_{ij}|\lambda) = \frac{1}{2\lambda} exp(\frac{-|\Phi_{ij} - \mathcal{B}_{ij}|}{\lambda})$$

In the function call to *ddepn*, just pass arguments B and *lambda* and set *usebics=FALSE* to use the laplace prior for inference.

```
> ddepn(data, lambda = 2, B = B, usebics = FALSE)
```

ScaleFree prior

According to [2] we set up a prior distribution that penalises high node degrees in the inferred network. The assumption is that for biological networks the degree of a node follows a power law distribution, i.e. the probability of seeing k nodes follows

$$P(k) \propto k^{-\gamma}.$$

We set up the prior distribution as described in [2]. To use the ScaleFree prior, just pass the arguments *gam* (the exponent γ), *it* (the number of permutations) and factor K to the function call of *ddepn*, and again set argument *usebics=FALSE*.

```
> ddepn(data, gam = 2.2, it = 500, K = 0.8, usebics = FALSE)
```

4 Use cases for GA and MCMC inference

This section shows the various types of calls to *ddepn* with all of the different settings (inference type, prior type).

Data generation:

```
> library(ddepn)
> set.seed(12345)
> n <- 6
> signet <- signalnetwork(n = n, nstim = 2, cstim = 0, prop.inh = 0.2)
> net <- signet$phi
> stimuli <- signet$stimuli
> weights <- signet$weights
> dataset <- makedata(net, stimuli, mu.bg = 1200, sd.bg = 400,
+     mu.signal.a = 2000, sd.signal.a = 1000)
```

GA, use BICs optimisation and no prior

```
> maxiterations = 15
> p = 30
```

```
> q = 0.3
> m = 0.8
> ret <- ddepn(dataset$datx, phiorig = net, inference = "netga",
+     maxiterations = maxiterations, p = p, q = q, m = m, usebics = TRUE)
```

GA, use laplace prior

```
> maxiterations = 15
> p = 30
> q = 0.3
> m = 0.8
> B <- matrix(0.5, nrow = n, ncol = n, dimnames = dimnames(net))
> lambda <- 2
> ret <- ddepn(dataset$datx, phiorig = net, inference = "netga",
+     maxiterations = maxiterations, p = p, q = q, m = m, usebics = FALSE,
+     lambda = lambda, B = B)
```

GA, use scalefree prior

```
> maxiterations = 15
> p = 30
> q = 0.3
> m = 0.8
> B <- matrix(0.5, nrow = n, ncol = n, dimnames = dimnames(net))
> gam <- 2.2
> it <- 500
> K <- 0.8
> ret <- ddepn(dataset$datx, phiorig = net, inference = "netga",
+     maxiterations = maxiterations, p = p, q = q, m = m, usebics = FALSE,
+     gam = gam, it = it, K = K)
```

MCMC, use laplace prior

```
> maxiterations <- 1000
> burnin <- 100
> B <- matrix(0.5, nrow = n, ncol = n, dimnames = dimnames(net))
> lambda <- 2
> ret <- ddepn(dataset$datx, phiorig = net, inference = "mcmc",
+     maxiterations = maxiterations, burnin = burnin, usebics = FALSE,
+     lambda = lambda, B = B)
```

MCMC, use scalefree prior

```
> B <- matrix(0.5, nrow = n, ncol = n, dimnames = dimnames(net))
> gam <- 2.2
> it <- 500
> K <- 0.8
> ret <- ddepn(dataset$datx, phiorig = net, inference = "mcmc",
+     maxiterations = maxiterations, burnin = burnin, usebics = FALSE,
+     gam = gam, it = it, K = K)
```

Session Information

The version number of R and packages loaded for generating the vignette were:

- R version 2.11.1 (2010-05-31), `i486-pc-linux-gnu`
- Locale: `LC_CTYPE=de_DE@euro`, `LC_NUMERIC=C`, `LC_TIME=de_DE@euro`, `LC_COLLATE=de_DE@euro`, `LC_MONETARY=C`, `LC_MESSAGES=de_DE@euro`, `LC_PAPER=de_DE@euro`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=de_DE@euro`, `LC_IDENTIFICATION=C`
- Base packages: base, datasets, graphics, grDevices, methods, stats, tools, utils

References

[1] Holger Froehlich, Mark Fellmann, Holger Sueltmann, Annemarie Poustka, and Tim Beissbarth. Large scale statistical inference of signaling pathways from rnai and microarray data. *BMC Bioinformatics*, 8(11):386, 2007.

[2] Takeshi Kamimura and Hidetoshi Shimodaira. A scale-free prior over graph structures for bayesian inference of gene networks. Online.

[3] M.˜Kanehisa, M.˜Araki, S.˜Goto, M.˜Hattori, M.˜Hirakawa, M.˜Itoh, T.˜Katayama, S.˜Kawashima, S.˜Okuda, T.˜Tokimatsu, and Y.˜Yamanishi. Kegg for linking genomes to life and the environment. *Nucleic Acids Res.*, 36:D480 – D484, 2008.

[4] Adriano˜V Werhli and Dirk Husmeier. Reconstructing gene regulatory networks with bayesian networks by combining expression data with multiple sources of prior knowledge. *Stat Appl Genet Mol Biol*, 6:Article15, 2007.