Transcription factor target identification with limited data using Gaussian process models

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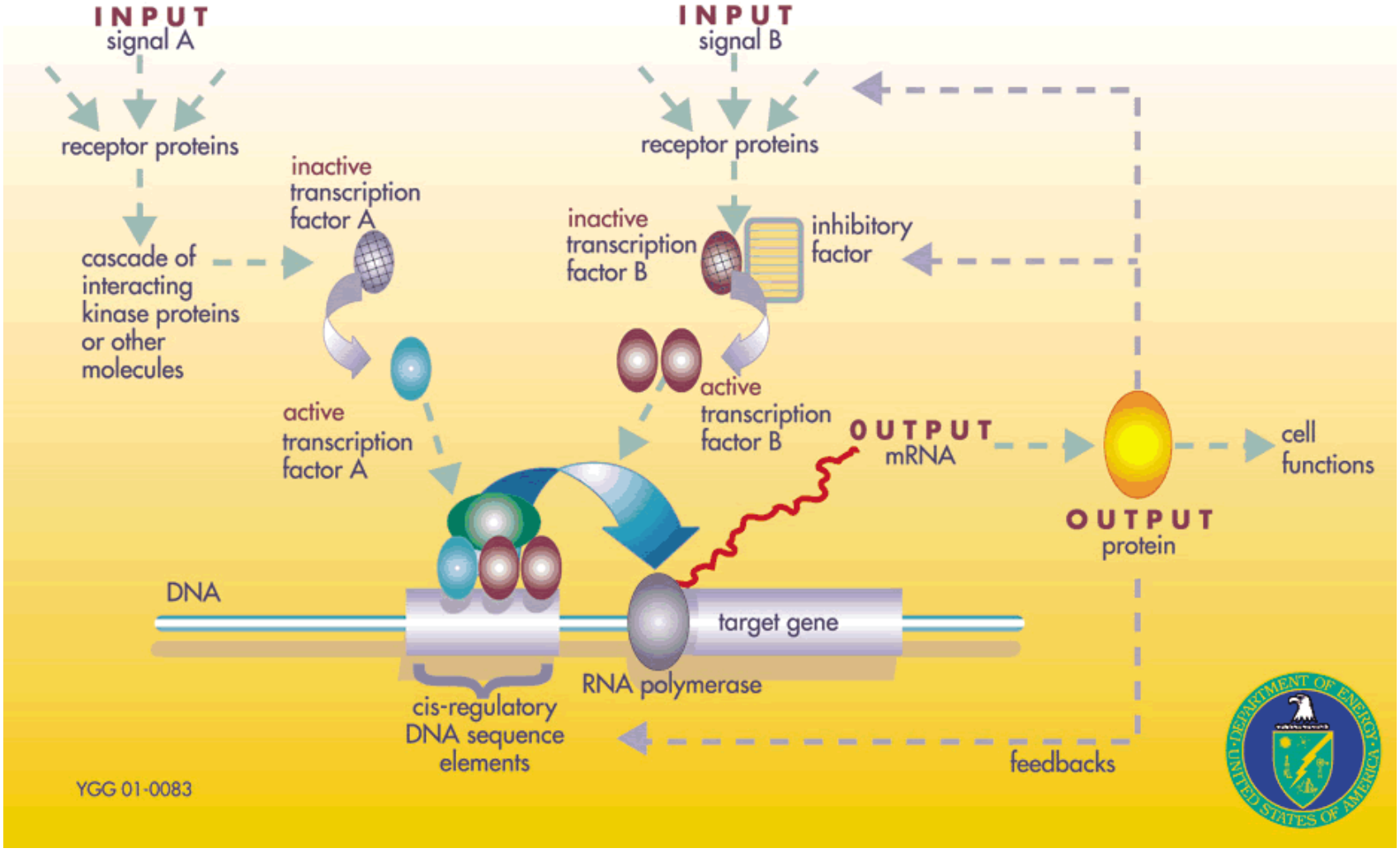
A GENE REGULATORY NETWORK



The data

- High-throughput measurements of nucleic acids (esp. mRNA)
- Detecting proteins require more targeted techniques

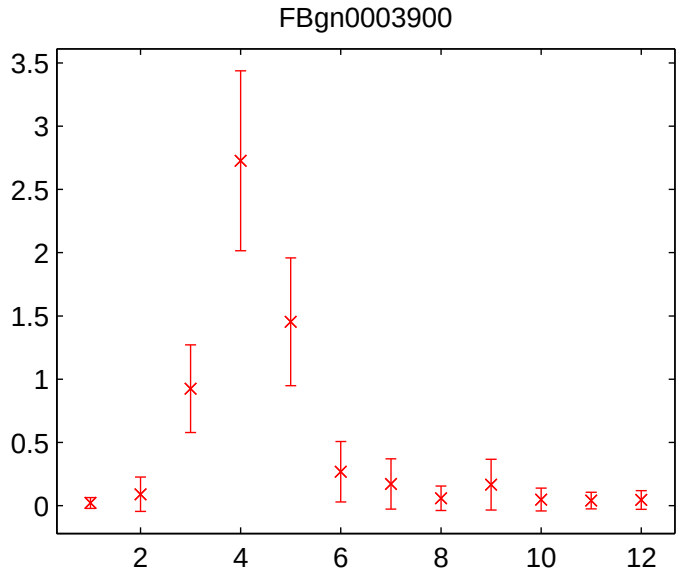


Figure: Sample expression time series

Our goal

- To infer activities of unobserved chemical species through the effects they have on others
- Application: Infer local regulatory relationships, i.e. direct targets of transcription factors (TFs)
- Data: time series mRNA expression data
  (DNA (genes) $\xrightarrow{\text{transcription}}$ mRNA $\xrightarrow{\text{translation}}$ Protein)

Outline

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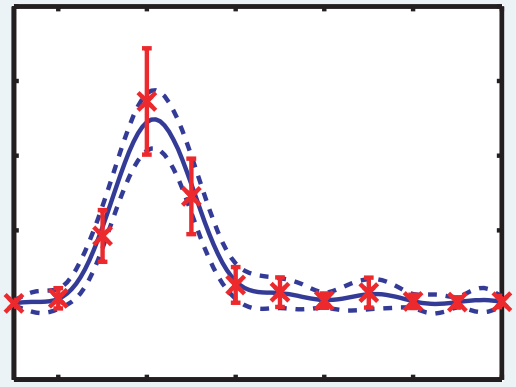
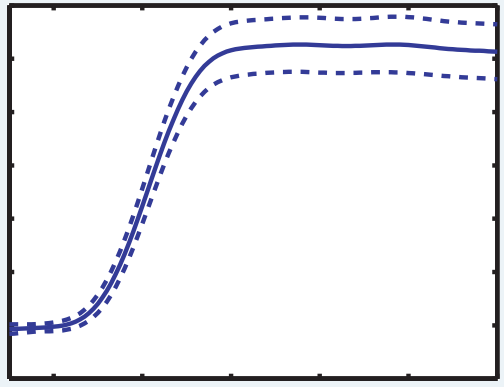
The ODE model

y(t) = TF mRNA profile



f(t) = protein activity profile

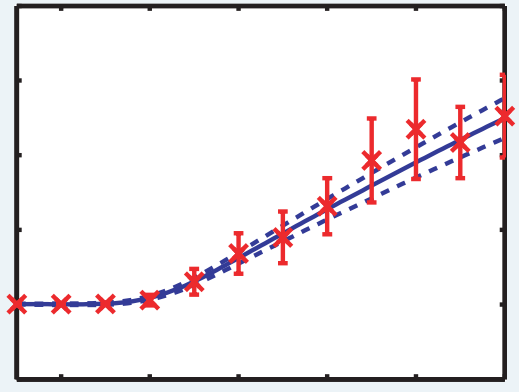


$$y(t) \sim \mathcal{GP}(m(t), k(t,t'))$$

$$\frac{\mathrm{d}f(t)}{\mathrm{d}t} = \sigma y(t) - \delta f(t)$$

$$\frac{\mathrm{d}x_j(t)}{\mathrm{d}t} = B_j + S_j f(t) - D_j x_j(t)$$

x(t) = target expression profile



(Observations are denoted in red, inferred functions in blue.)

Gaussian process ODE modelling

- Use Gaussian process priors on activity time courses
  - Functional prior, specified by mean and covariance functions
  - No need for time discretisation
- Two alternatives for "bootstrapping" the TF protein activity
  - Training set of known targets (cf. **?**, Genome Biology)
  - Hierarchical model with TF translation from measured mRNA

Gaussian Processes

- Gaussian Process

$$f(t) \backsim \mathcal{GP}\left(m(t), k\left(t, t'\right)\right)$$

where

$$\begin{aligned} m(t) &= \mathbb{E}[f(t)] = \langle f(t) \rangle \\ k\left(t, t'\right) &= \mathbb{E}\left[\left(f(t) - m(t)\right)\left(f\left(t'\right) - m\left(t'\right)\right)\right] \end{aligned}$$

The joint covariance

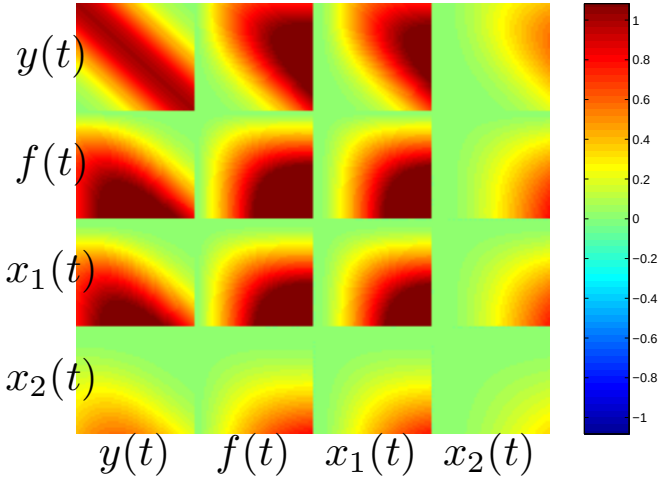
RBF covariance function for $y(t)$

$$f(t) = \sigma \exp(-\delta t) \int_0^t y(u) \exp(\delta u) \, \mathrm{d}u$$

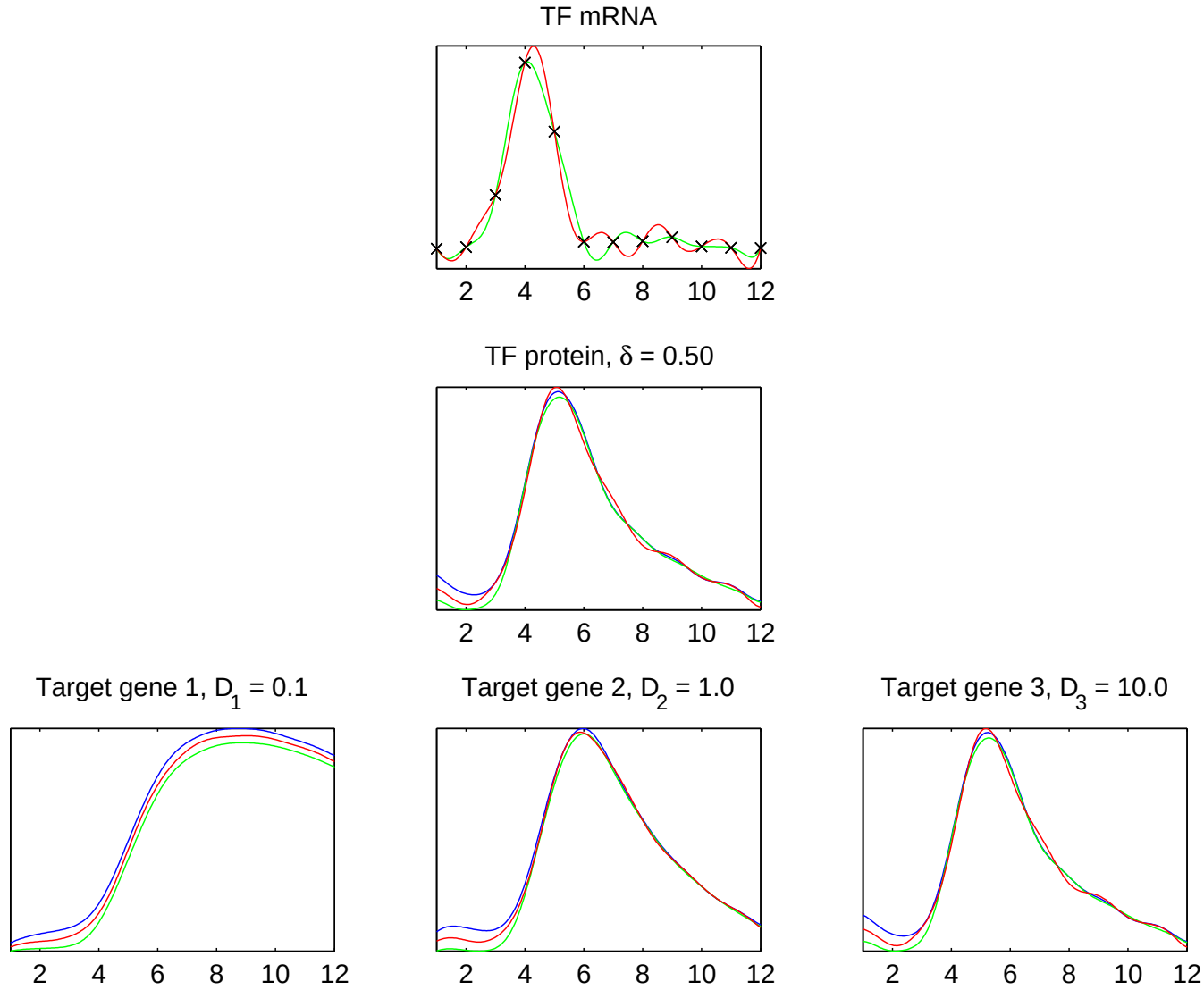
$$x_i(t) = \frac{B_i}{D_i} + S_i \exp(-D_i t) \int_0^t f(u) \exp(D_i u) \, \mathrm{d}u.$$

- Joint distribution for $x_1(t)$, $x_2(t)$, $f(t)$ and $y(t)$.
- Here:

δ	D_1	S_1	D_2	S_2
0.1	5	5	0.5	0.5



Covariance samples



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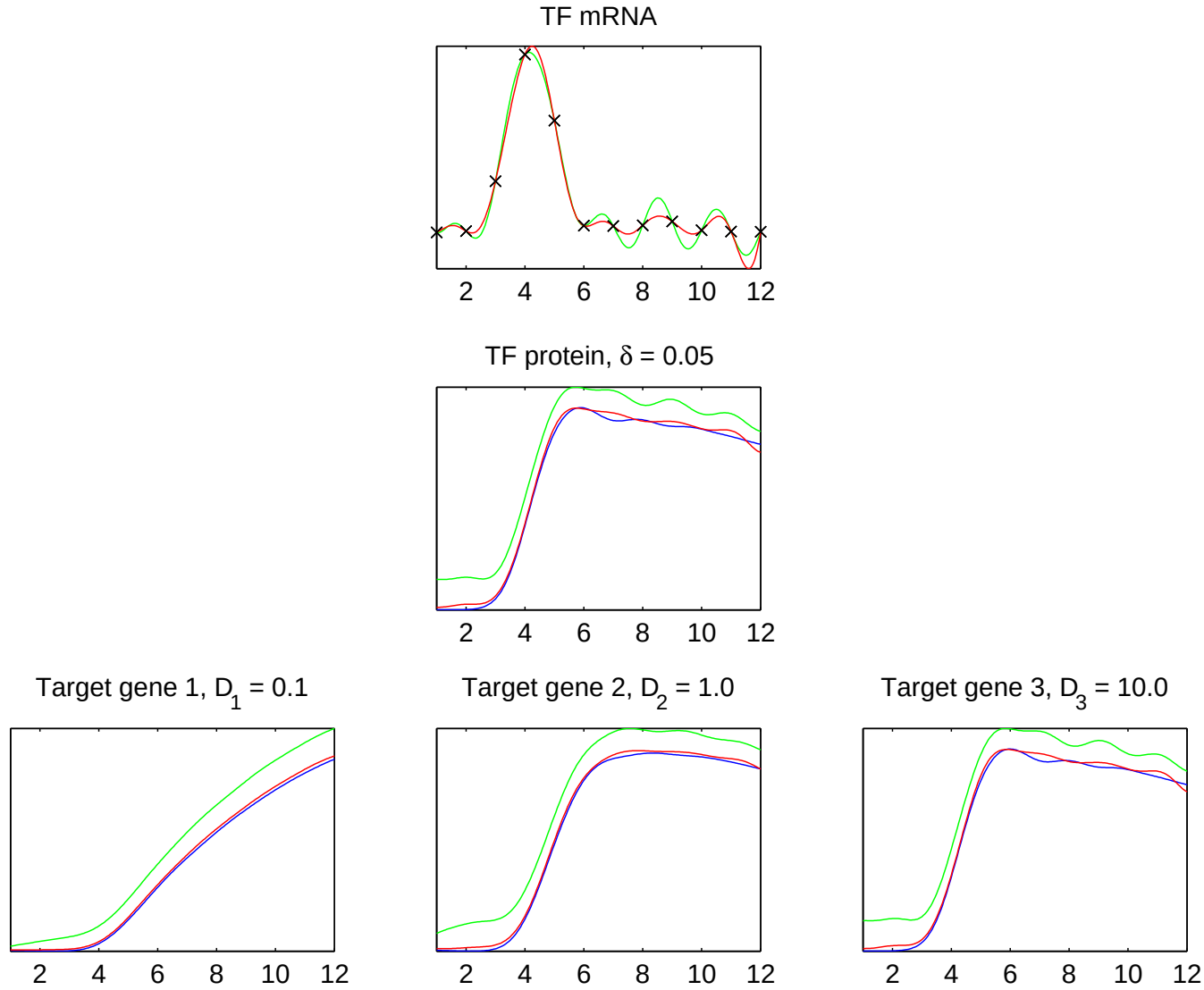

Outline

Target ranking: motivation

- Finding target genes of TFs is an obvious first step in reverse engineering gene regulatory networks
- Typical techniques
  - Measure TF binding locations in the genome using ChIP-chip/-seq
  - Observe gene expression in mutants with the TF disabled (knockouts) or overexpressed
- Endless potential applications in understanding disease and other biological phenomena

Case study: Mesoderm and muscle development in Drosophila

- Focus on two TFs regulating mesoderm and muscle development in fruit fly Drosophila: Twist and Mef2
- Assume no post-translational regulation of these TFs
- Expression data: 12 time points at 1 h intervals, 3 repeats
- Data averaged over the whole embryo

Application of the models to target ranking

- First apply the two-layer model for each target gene independently
- Ranking by model likelihood

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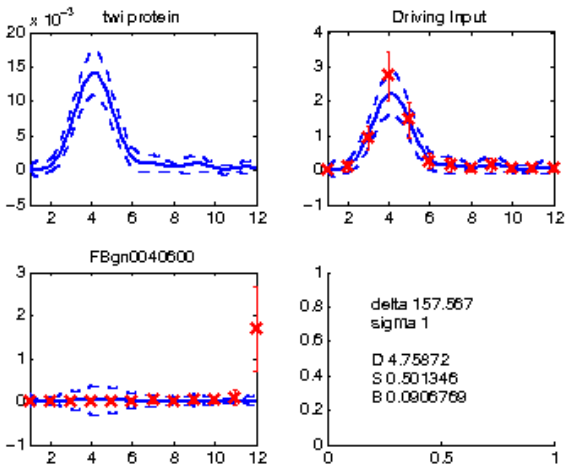

Figure: Fitted two-layer (GPDISIM) models

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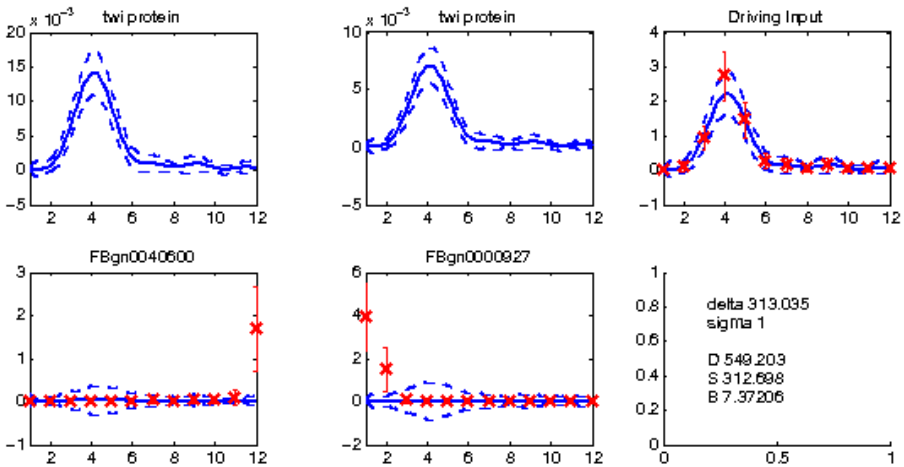

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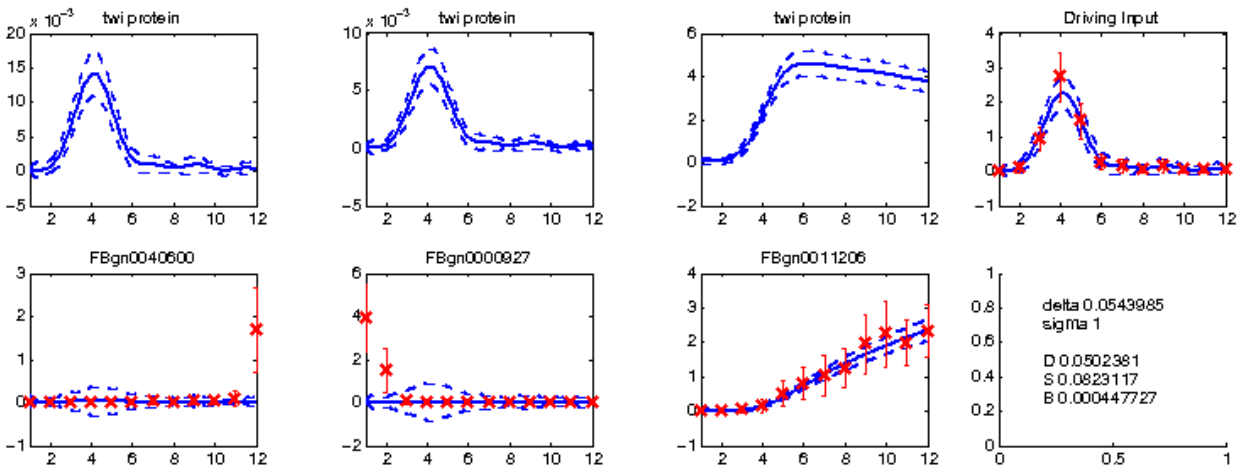

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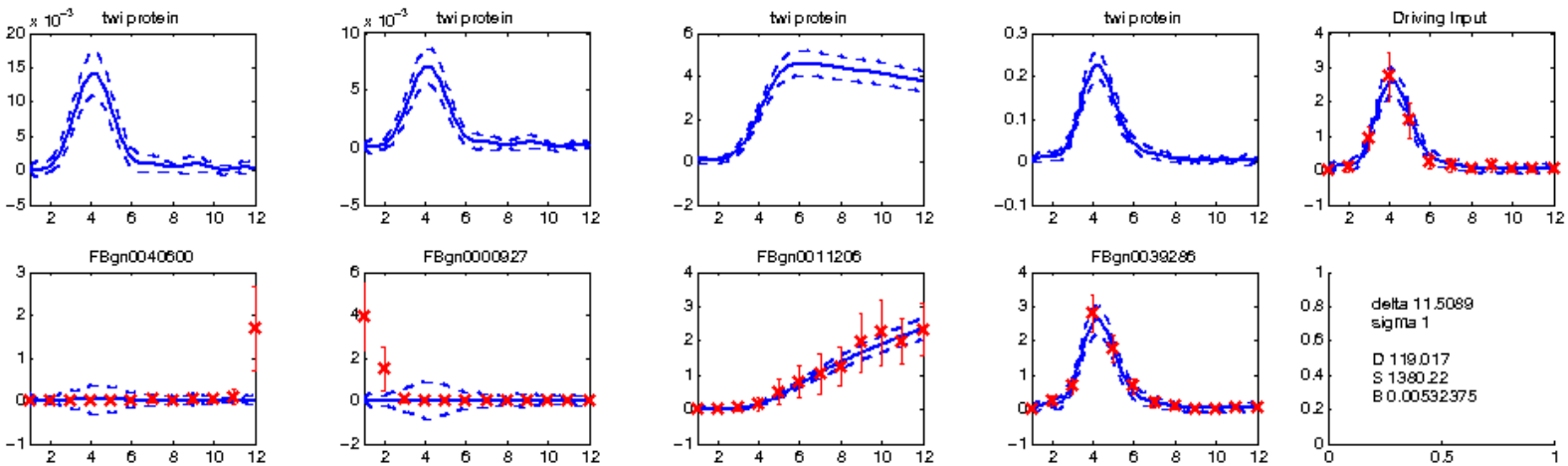


Figure: Fitted two-layer (GPDISIM) models

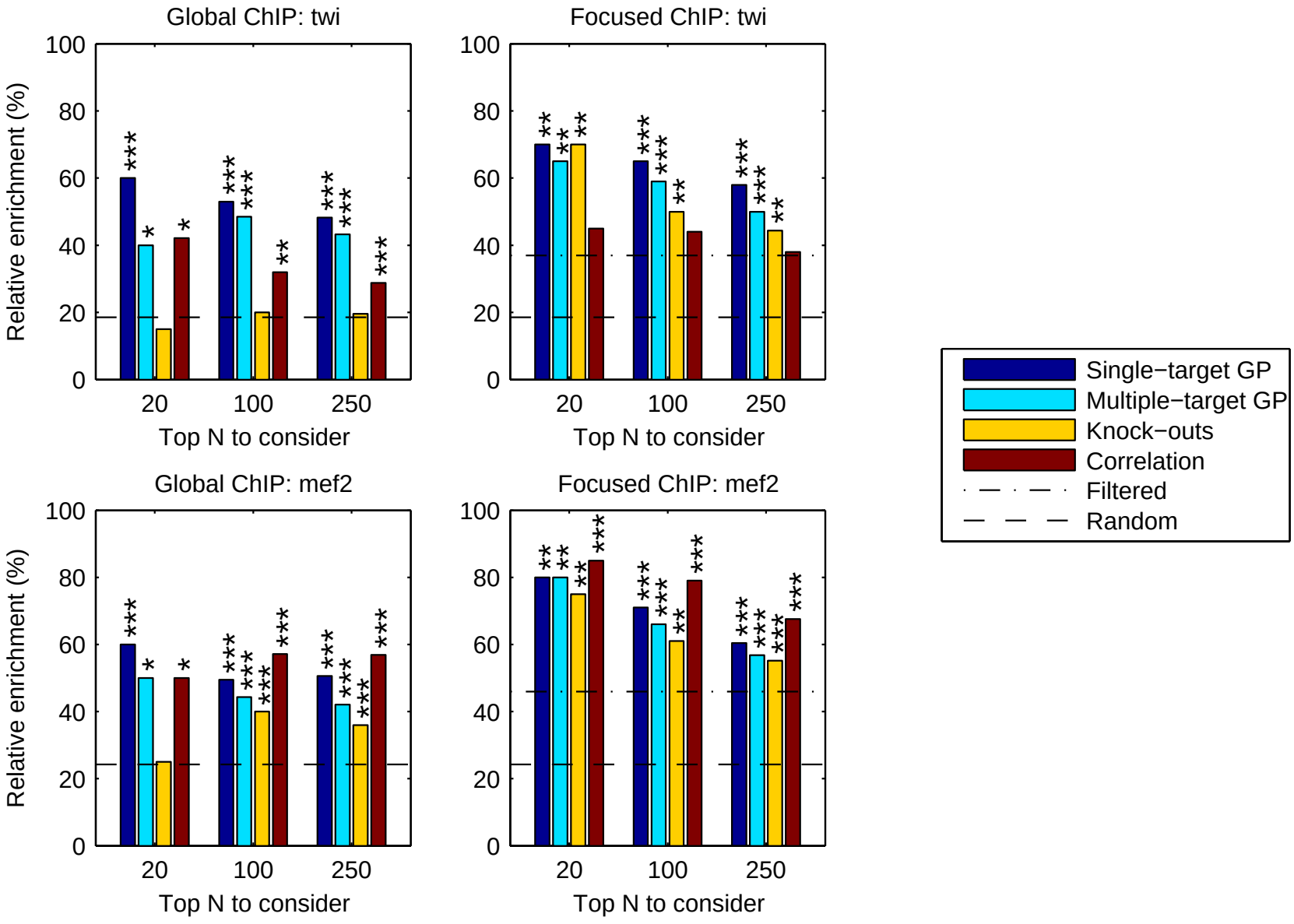
Application of the models to target ranking

- Need to exclude inactive genes
  - Threshold the average z-score of gene activity
  - Threshold the likelihood ratio against a model with $S = 0$
- Using a set of identified likely targets as a training set, learn multiple-target models for training set + each target individually

Evaluation methods

- Evaluate the ranking methods by taking a number of top-ranked targets and record the number of "positives" (**?**):
  - targets with ChIP-chip binding sites within 2 kb of gene
  - (targets differentially expressed in TF knock-outs)
- Compare against
  - Ranking by correlation of expression profiles
  - Ranking by q-value of differential expression in knock-outs
- Optionally focus on genes with annotated expression in tissues of interest

Results



'***': $p < 0.001$, '**': $p < 0.01$, '*': $p < 0.05$

Single-TF Target Ranking: Summary

- The two-layer translation/transcription model provides impressive target ranking results
- Works with very short time series, even 6-7 time points
- More details in the paper:

A. Honkela, C. Girardot, E. H. Gustafson, Y.-H. Liu, E. E. M. Furlong, N. D. Lawrence, and M. Rattray.
Model-based method for transcription factor target identification with limited data.
Proc Natl Acad Sci U S A, 107(17):7793–7798, Apr 2010.
doi:10.1073/pnas.0914285107

Outline

Extending the model

- The linear single-TF model is about as far as exact inference takes us
- More complicated models require approximate techniques (e.g. MCMC)
- With MCMC there are no restrictions on the functional forms of models used

The full model

- Consider the ODE transcription regulation model for multiple TFs

$$\frac{dx_j(t)}{dt} = B_j + S_j g(f_1(t), \ldots, f_l(t); \mathbf{w}_j) - D_j x_j(t)$$

- $g(\cdot)$ a positive sigmoidal activation function
- $\mathbf{w}_j$ interaction weights between the jth gene and the set of l TFs

Gene regulation with multiple TFs

$$\frac{dx_j(t)}{dt} = B_j + S_j g(f_1(t), \ldots, f_I(t); \mathbf{w}_j) - D_j x_j(t),$$

- $g(\cdot)$ is assumed to be a multiple-TF hill function:

$$g(f_1(t), \ldots, f_I(t); \mathbf{w}_j) = \frac{\prod_{i=1}^{I} f_i(t)^{w_{ji}}}{\gamma_j^{\sum_{i=1}^{I} w_{ji}} + \prod_{i=1}^{I} f_i(t)^{w_{ji}}}$$

  where w_{ji} can be both positive and negative

- The above can also be written as the sigmoid function:

$$g(f_1(t), \ldots, f_I(t); \mathbf{w}_j) = \frac{1}{1 + e^{-w_{j0} - \sum_{i=1}^{I} w_{ji} \log f_i(t)}}$$

  where we defined $w_{j0} = -\sum_{i=1}^{I} w_{ji} \log \gamma_j$ as new parameter

Bayesian inference from mRNA data: priors

$$x_j(t) = \frac{B_j}{D_j} + \left(A_j - \frac{B_j}{D_j} \right) e^{-D_j t} + S_j \int_0^t g(f_1(u), \ldots, f_l(u); \mathbf{w}_j) e^{-D_j(t-u)} du$$

$$f_i(t) = \int_0^t y_i(t) e^{-d_i(t-u)} du$$

- We place priors on:
  - Kinetics: $\Theta = \{A_j, B_j, D_j, S_j\}_{j=1}^N$ (uniform or log normal)
  - Decays of TF mRNA: $\{d_i\}$, (uniform or log normal)
  - Interaction weights: $\{\mathbf{w}_j\}$, (Gaussian priors with optionally positivity constraints and/or spike and slab sparse priors)
  - mRNA functions $y_i(t)$: Gaussian processes (through a transformation that ensures positivity of $y_i(t)$)
  - Lengthscales of Gaussian processes (uniform or gamma) and noise variances in the likelihoods (gamma)

Bayesian inference from mRNA data: MCMC (Michalis)

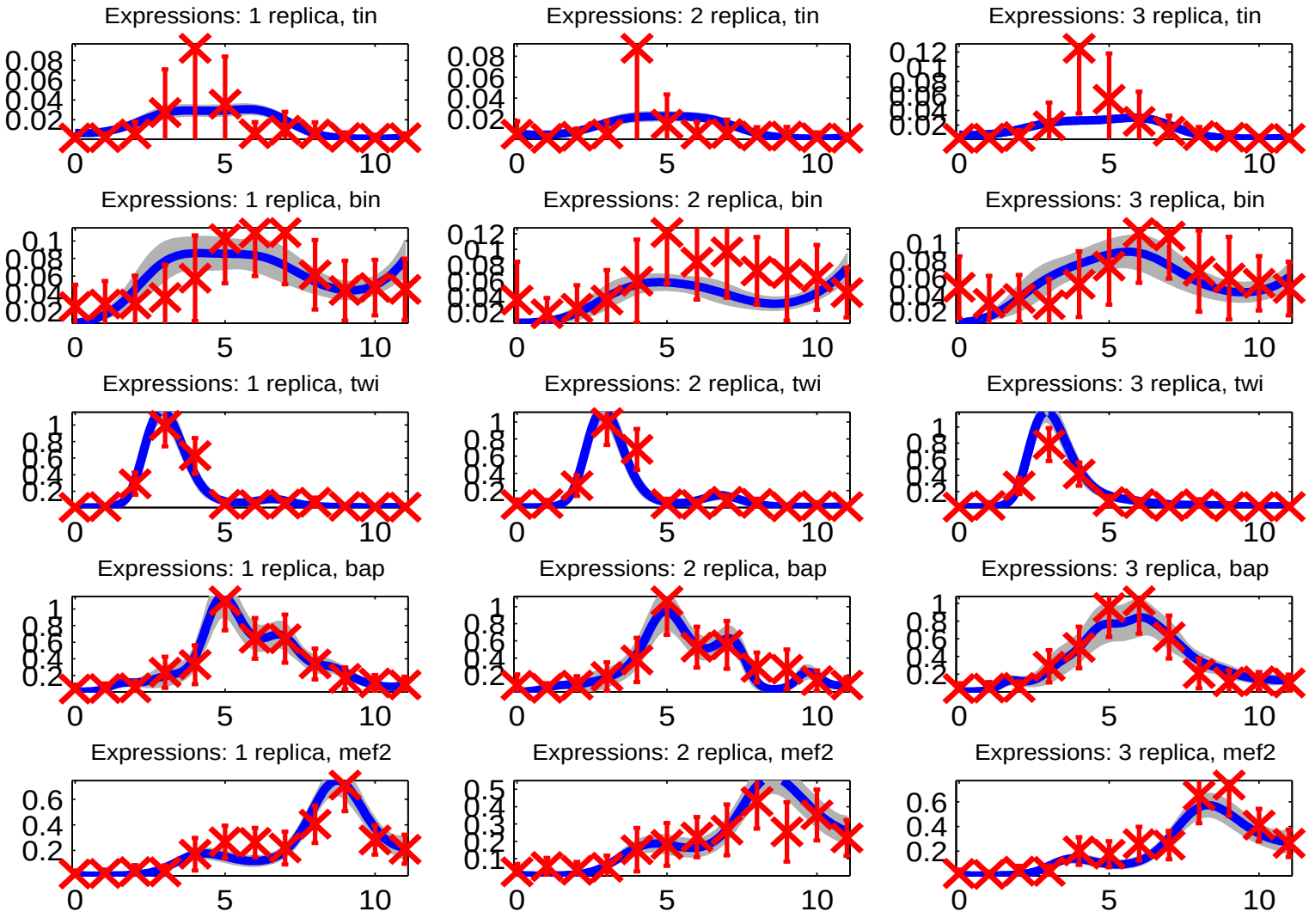
$$\text{joint} = p(\widetilde{X}|X)p(\widetilde{Y}|Y)p(\bar{Y}_i)p(\Theta)p(W)p(\{d_i\}_{i=1}^{I})p(\{\sigma_j^2\})p(\{\ell^2\}_{i=1}^{I})$$

- Many Metropolis-Hastings steps involving sampling Gaussian process functions, kinetic parameters, interaction weights, etc.
- We can afford training the model using MCMC in moderate-sized networks, e.g. with 100 genes and 5 TFs and 3 replicas, but not genome-wide (too slow for that)
- But once the model in trained, we can do genome-wide prediction (e.g. gene target identification) and this is fast

Experiments in Drosophila data

- Microarray dataset containing three replicas of 12 time points collected hourly throughout Drosophila embryogenesis in wild-type embryos
- 92 target genes
- 5 TFs (including Twist and Mef2 which regulate mesoderm and muscle development)
- ChIP information is used to define the (deterministic) sparse prior one interaction between TFs and target genes (**?**)

Experiments in Drosophila data



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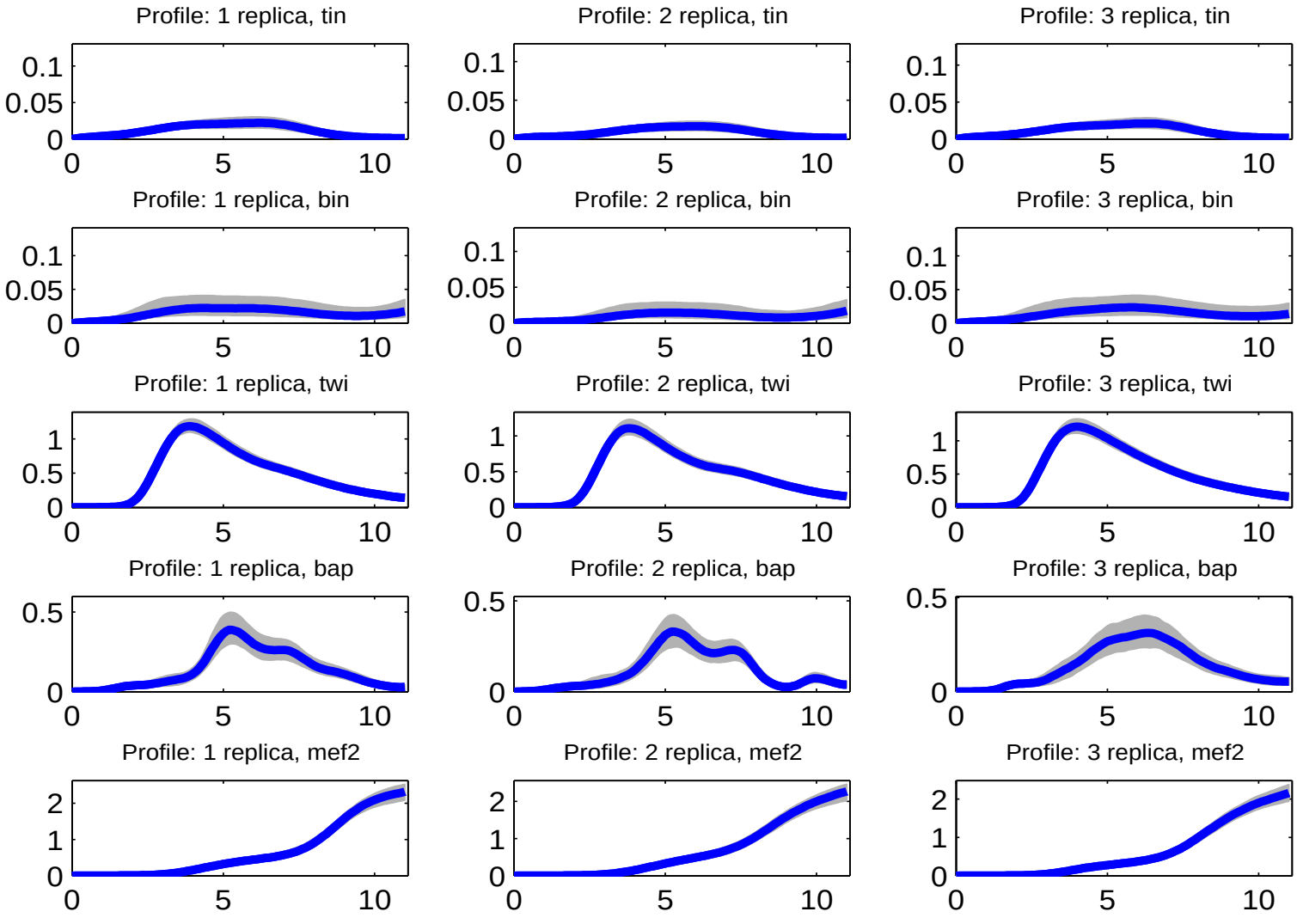

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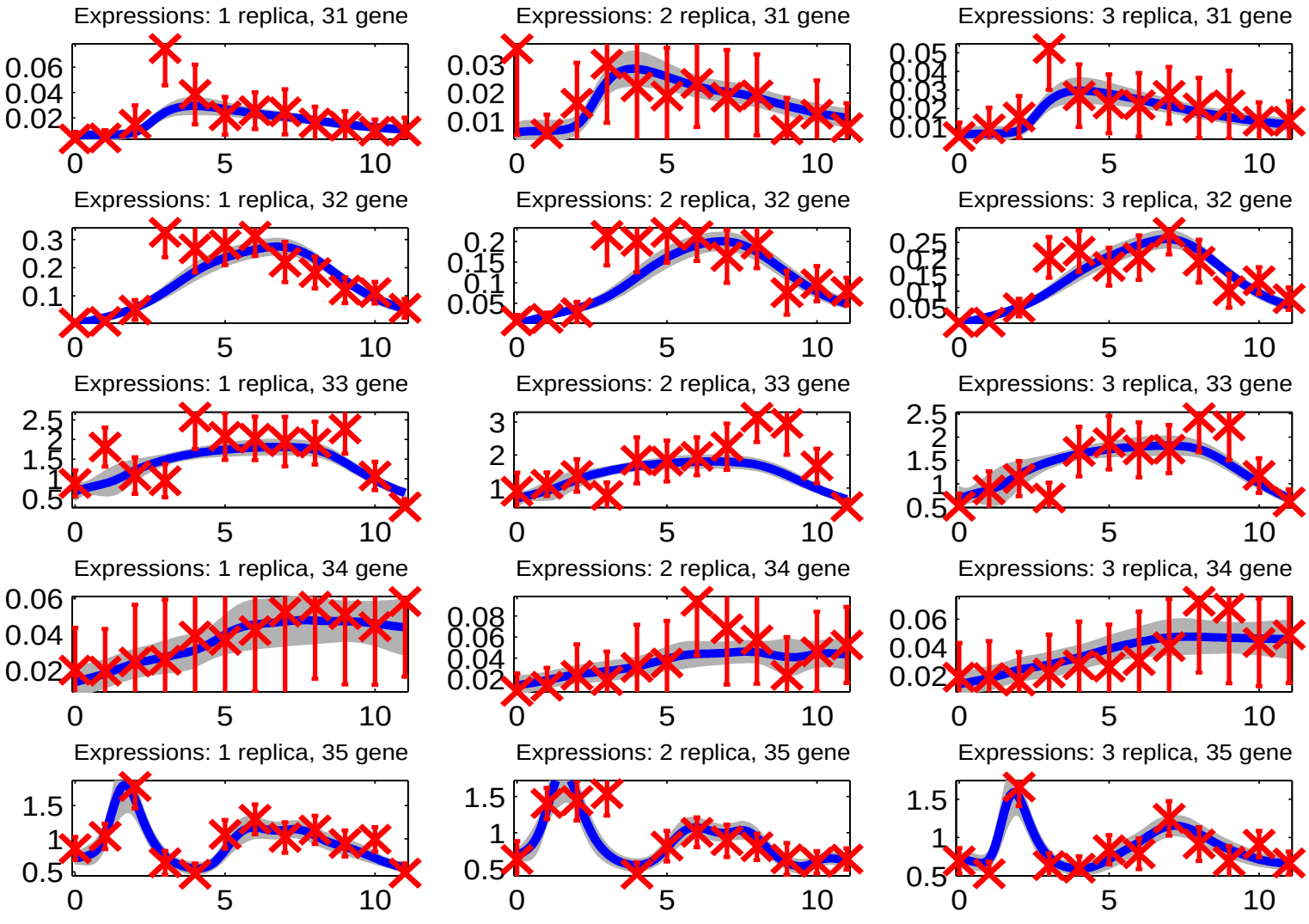

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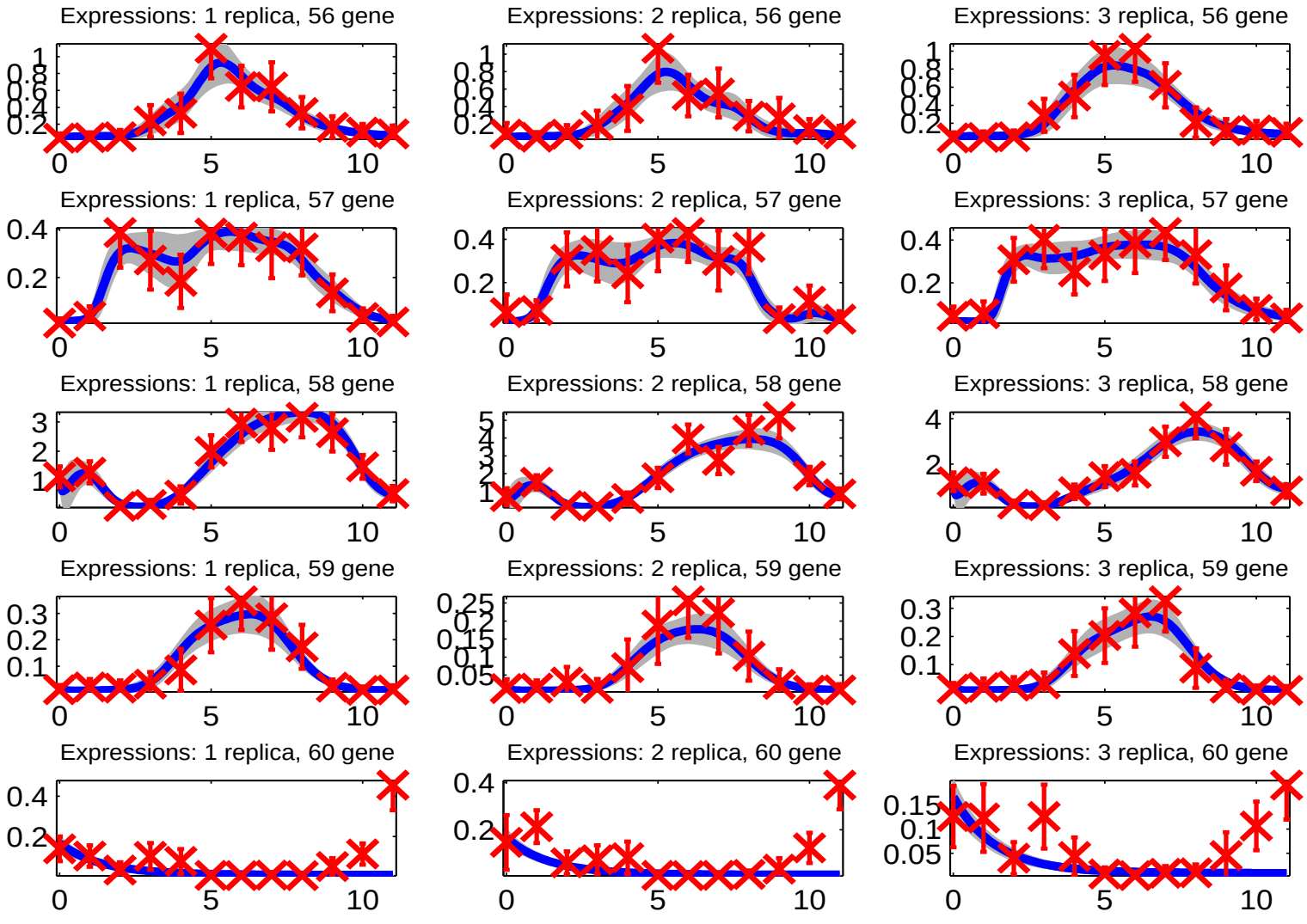

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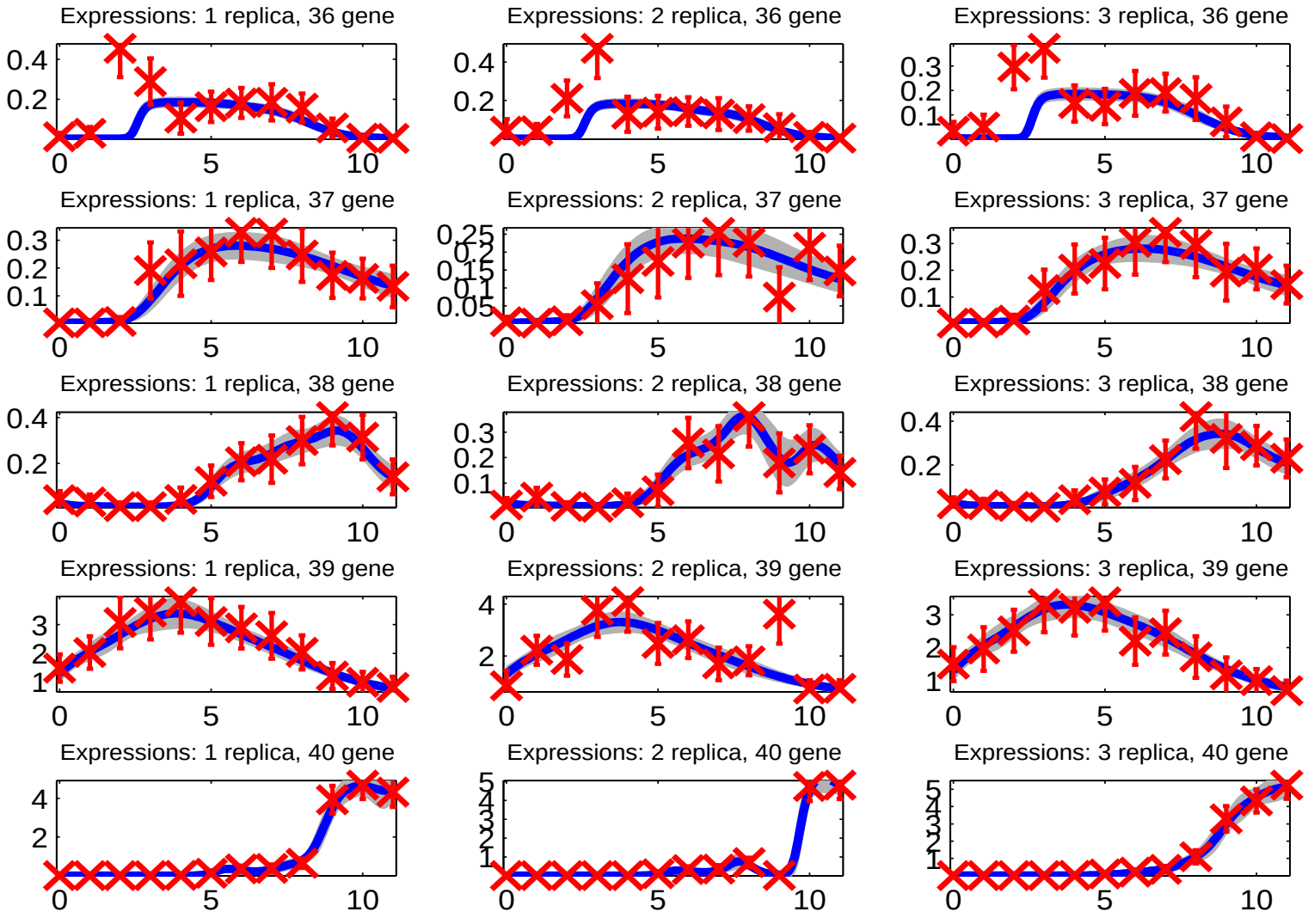


Genome-wide gene ranking/identification

- The trained model gives the posterior distribution of TF profiles
- It can be used to make (probabilistic) statements about if a certain TF combination regulates a test gene $*$?
  - Infer its interaction weights with a suitable prior
  - Compare models restricting a set of interaction weights to zero

Genome-wide gene ranking/identification

- Model comparison is based on **?** estimate of marginal likelihood
- Fast sampling, < 1 min per gene per model
  - All functions are drawn from posterior samples
  - Relatively few parameters to sample

Multiple-TF Models: Summary

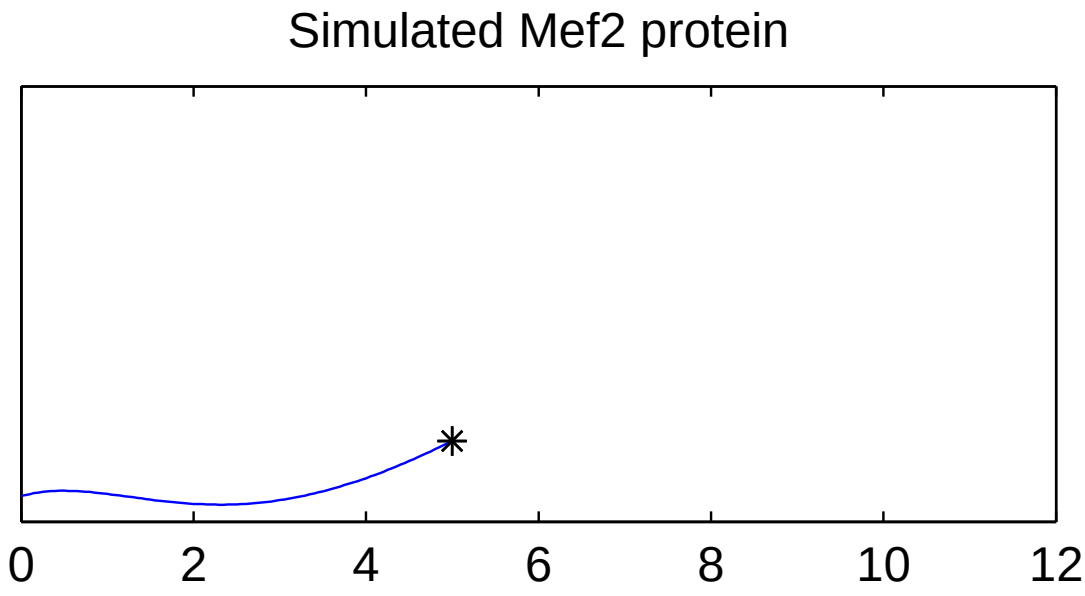
- Realistic models of combinatorial regulation
- MCMC techniques applicable to genome-wide screenings
- Amount of available data clearly a limiting factor in identifying the models

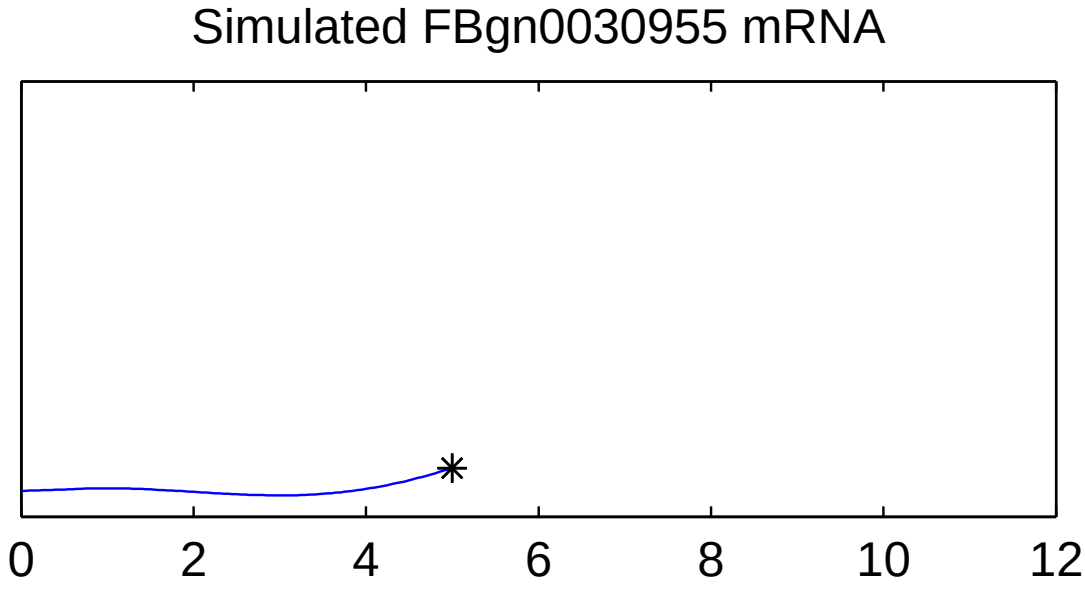
Outline

Molecular biology time series

- Biological systems are dynamic, observing their time evolution very helpful
- Time series measurements of gene expression, protein activity, protein binding, ...
- Problem: most of these assays are highly disruptive to the sample
- Therefore: time series = series of independent experiments run for different lengths of time
- This has implications for modelling...

Simulated molecular biology time series





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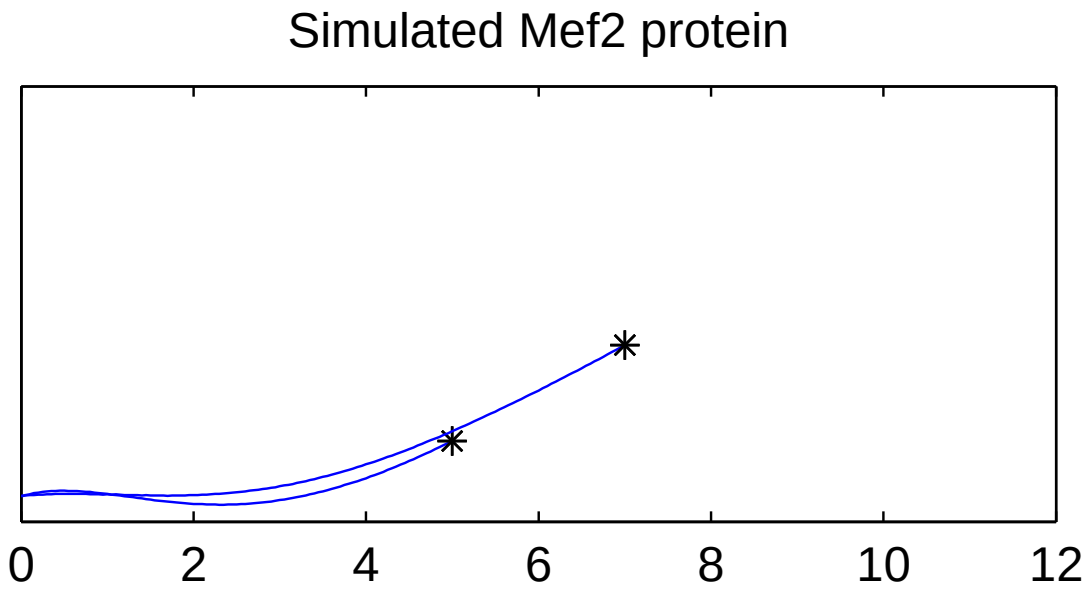

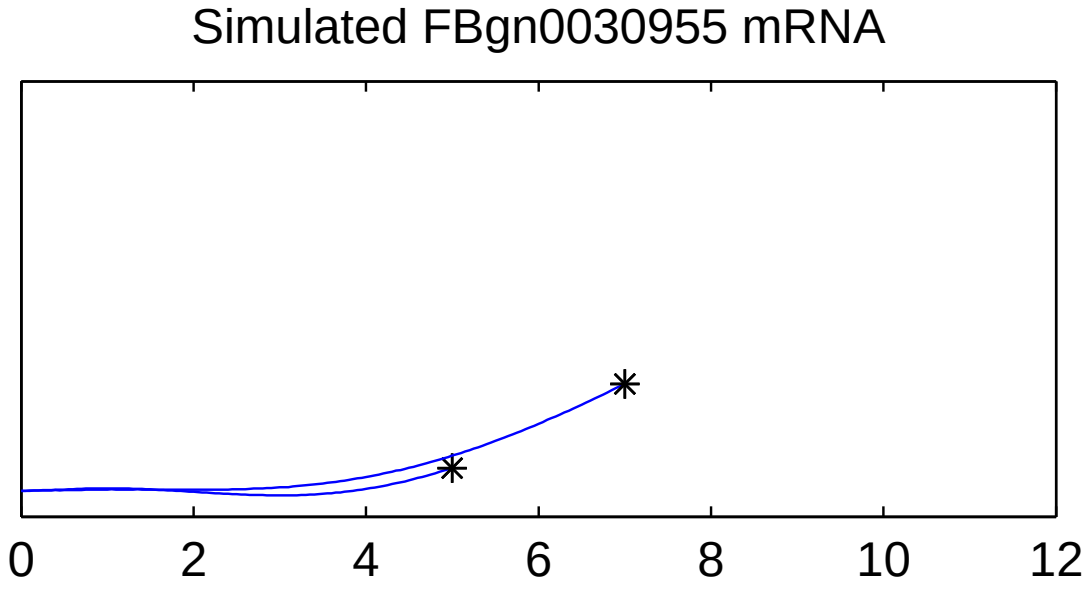

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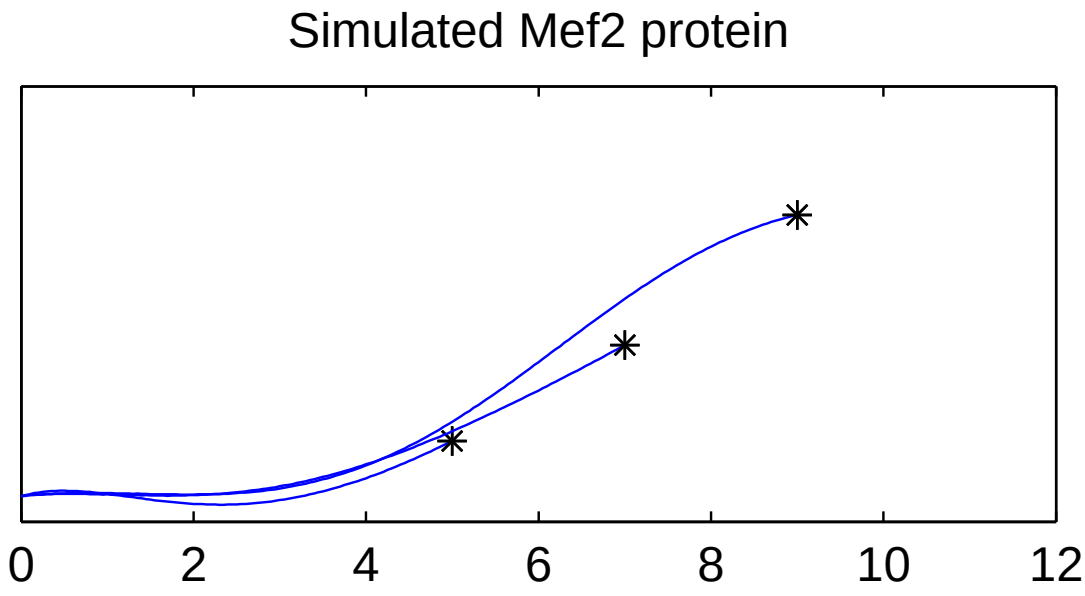

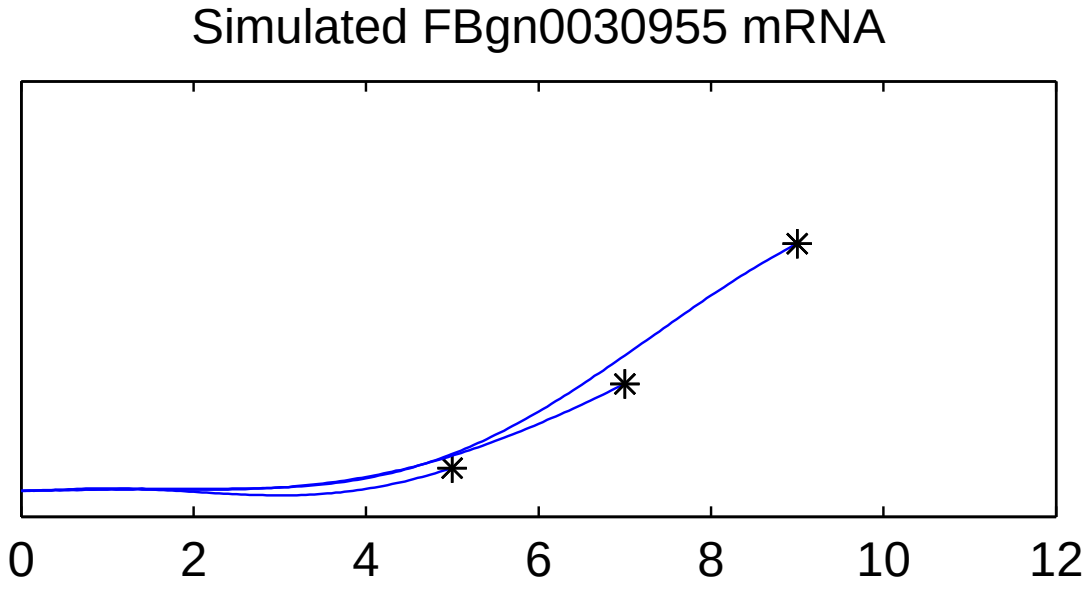

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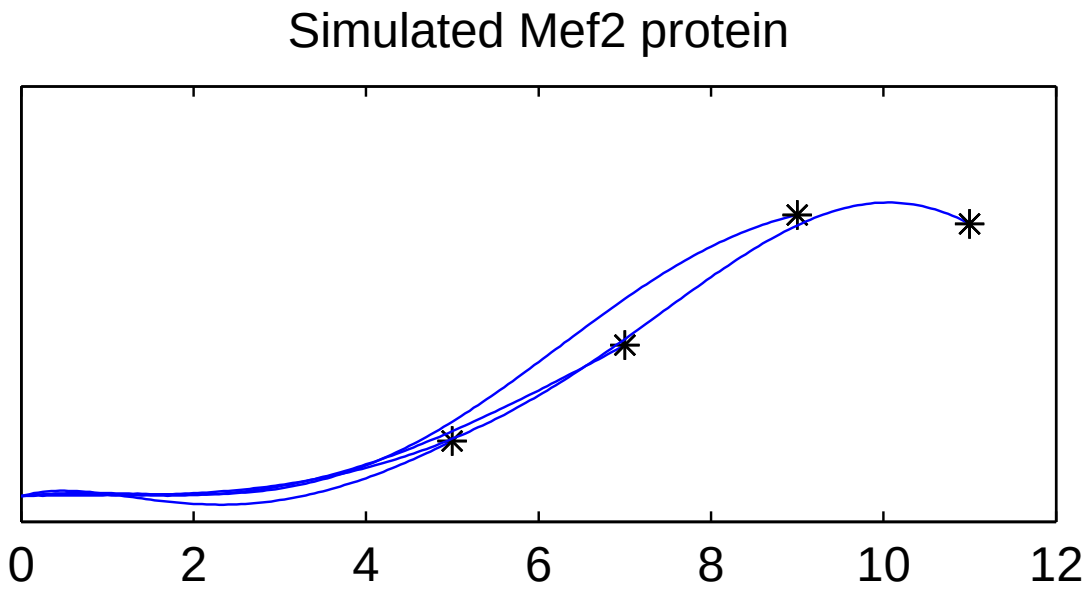

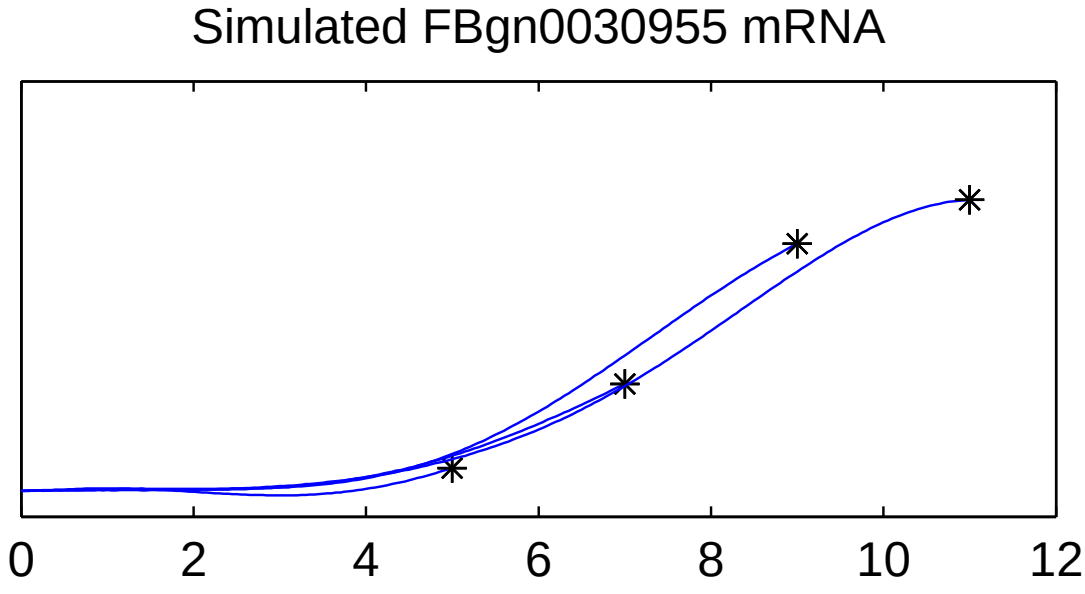

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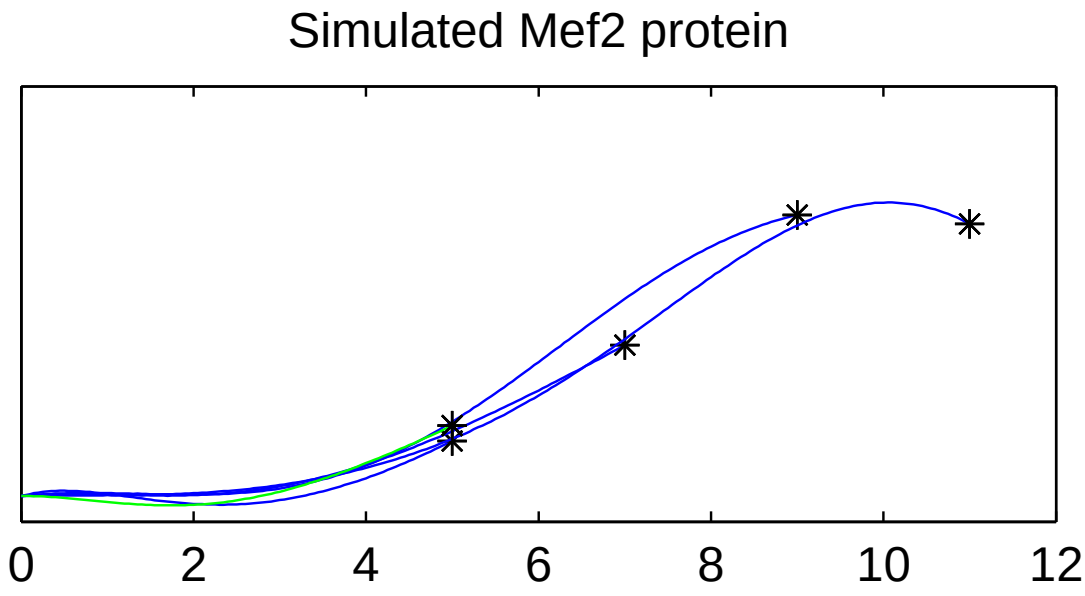

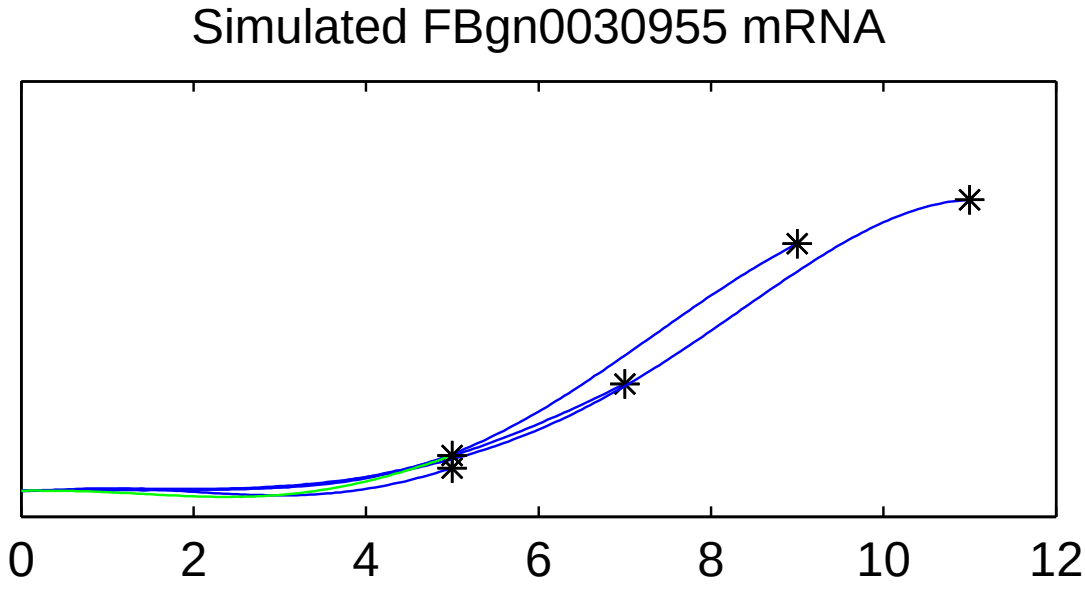

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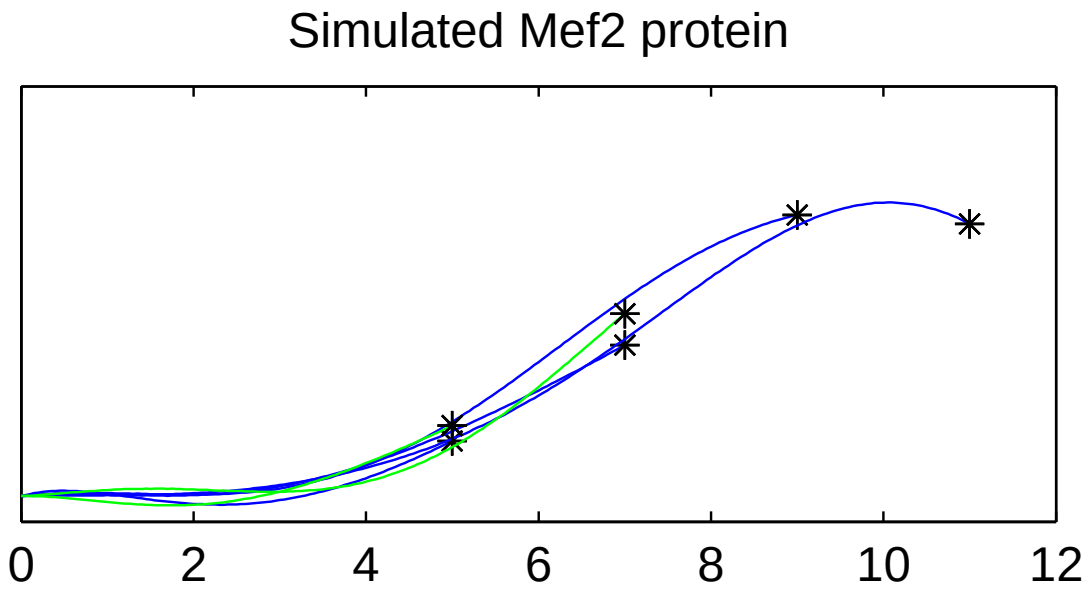

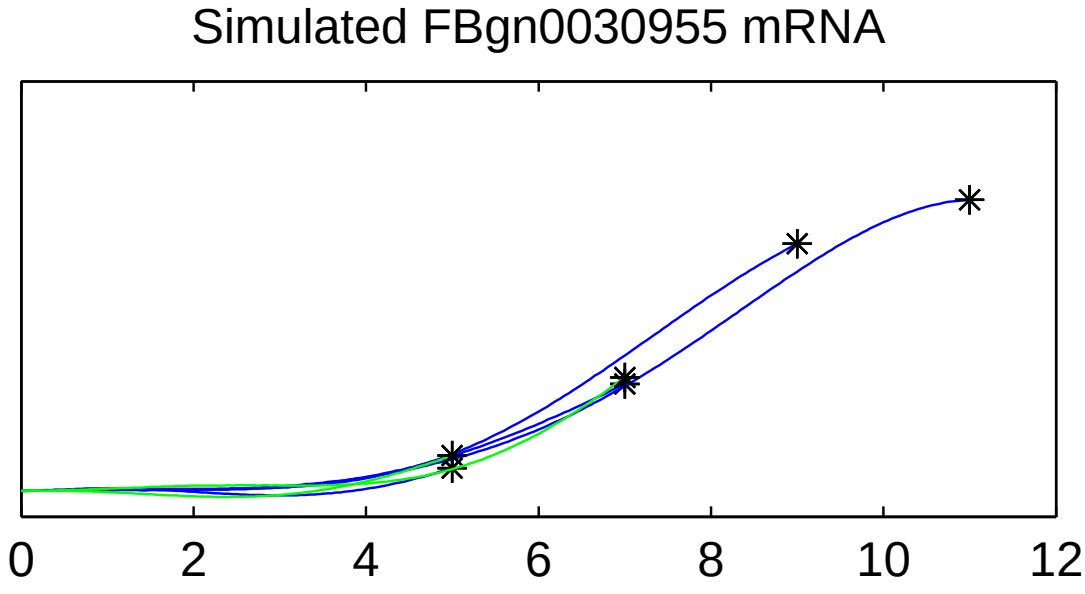

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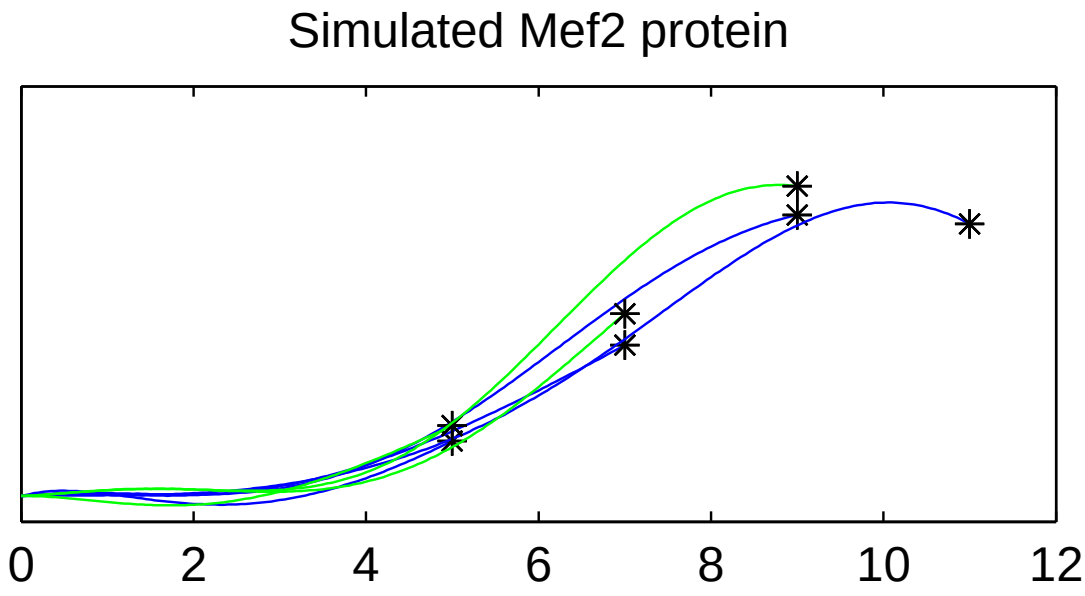

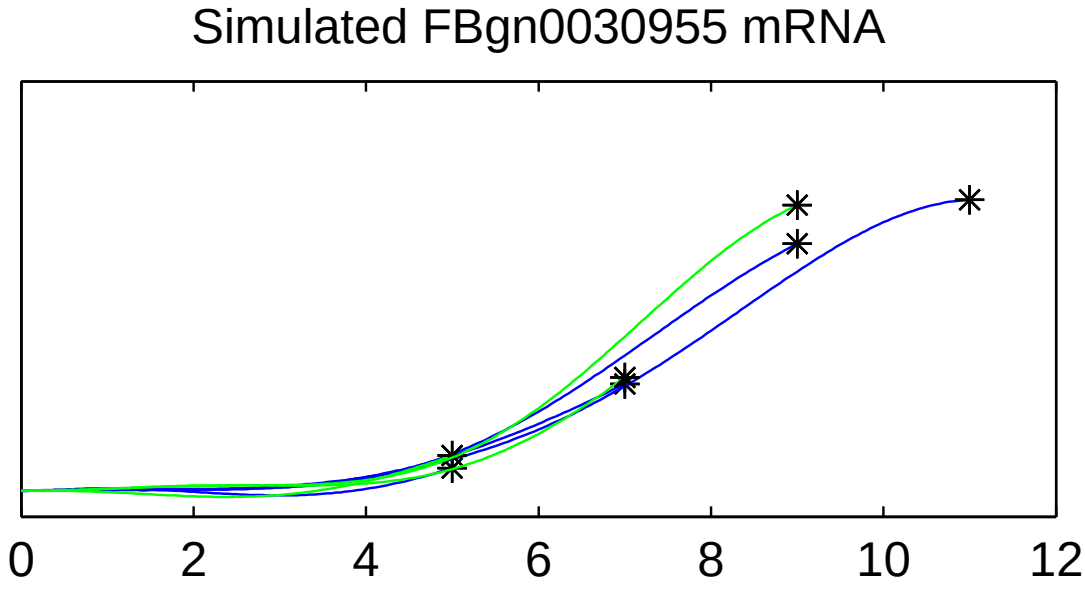

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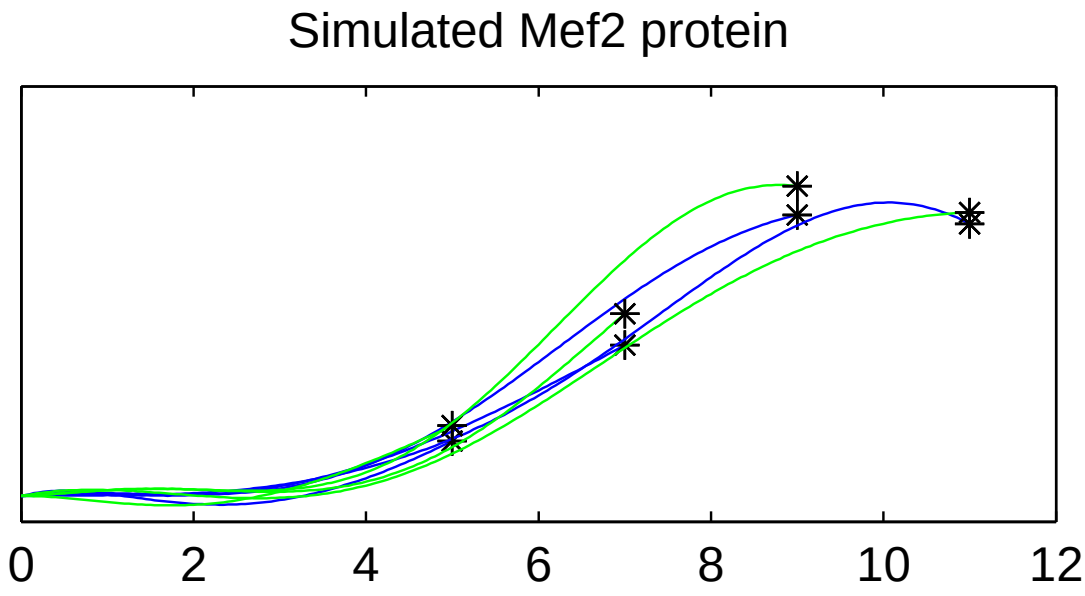

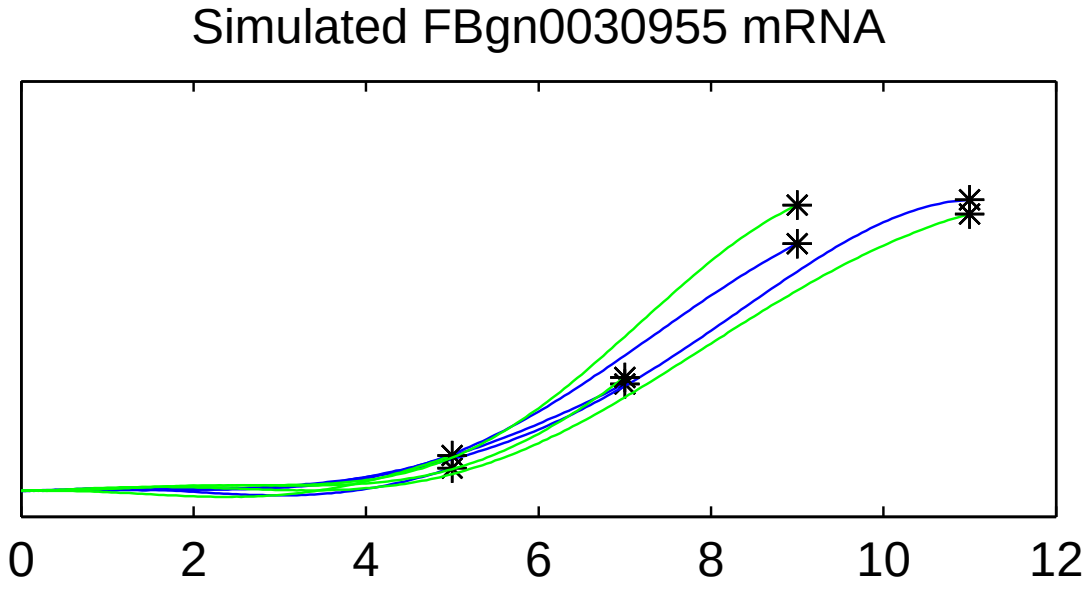

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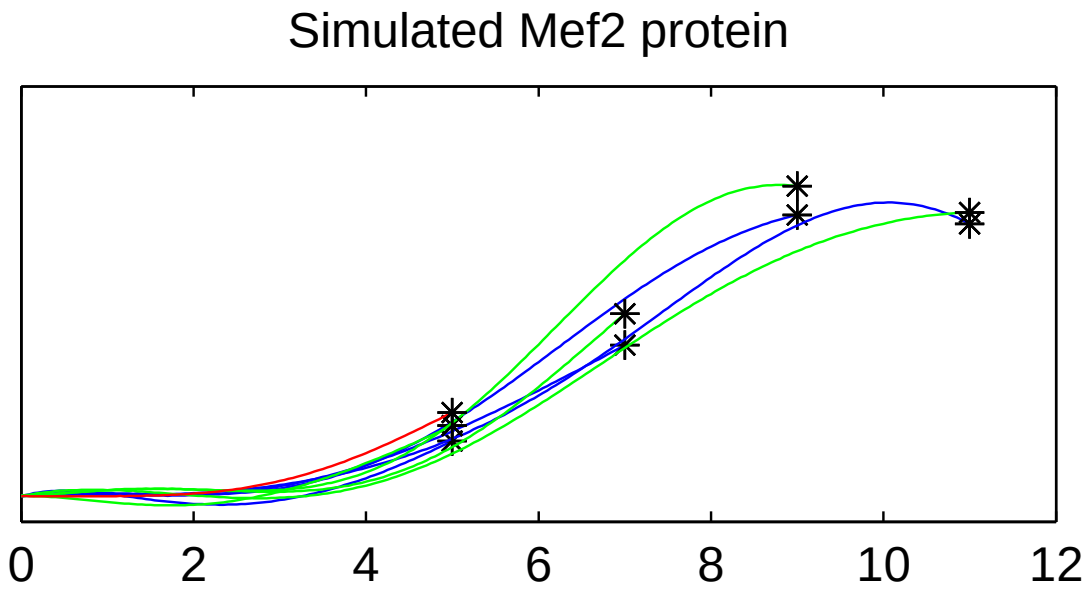
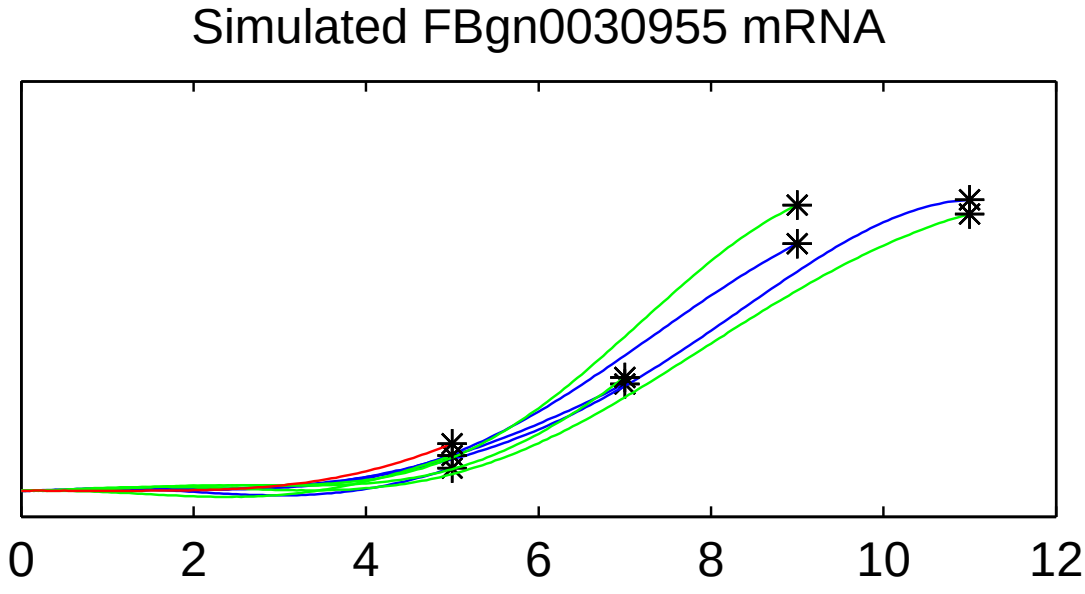
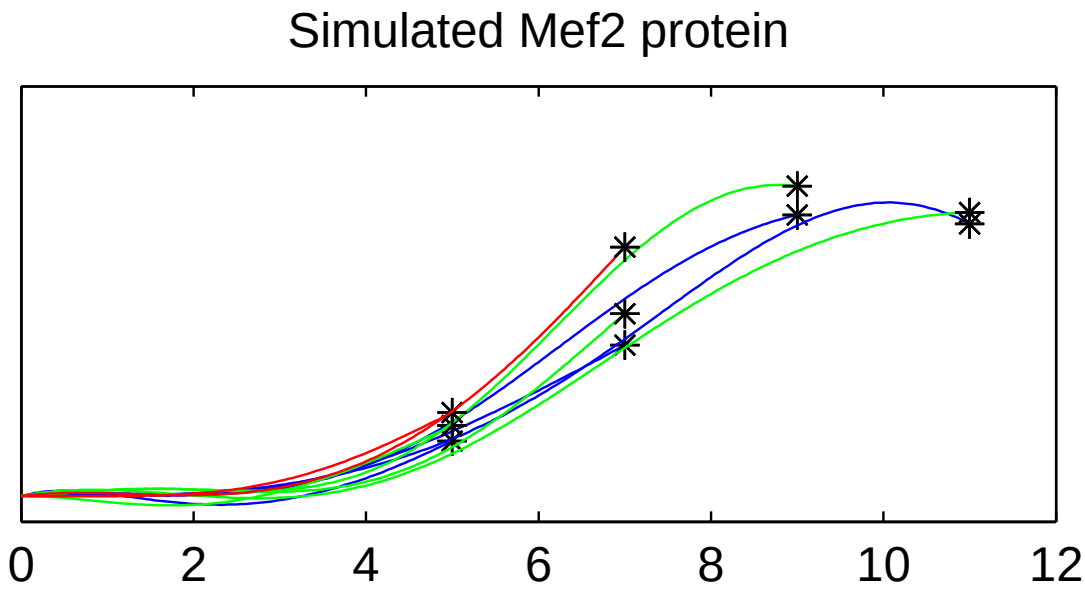
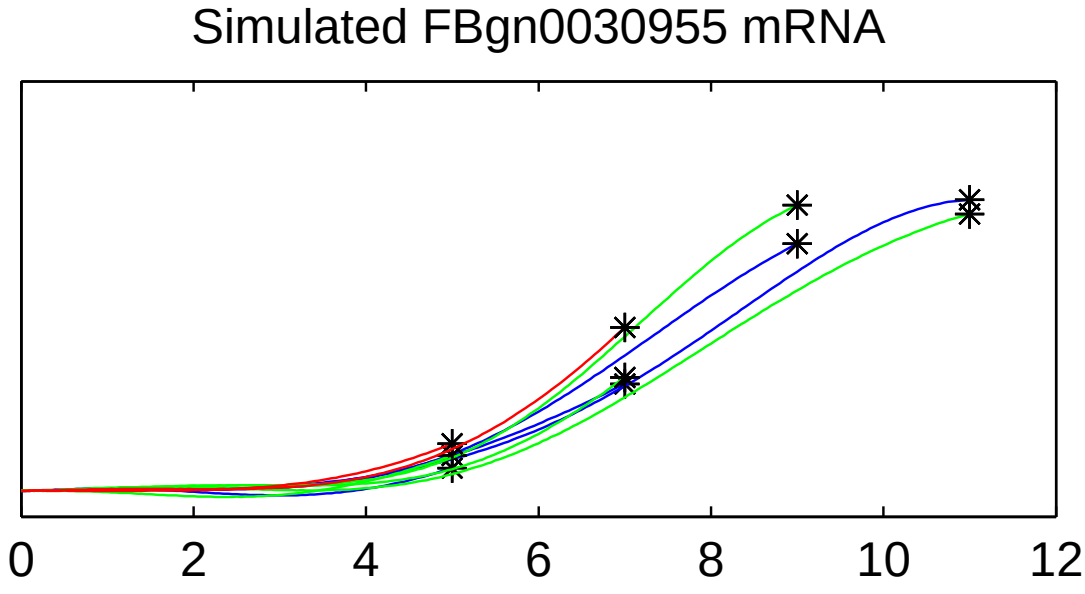




Simulated molecular biology time series





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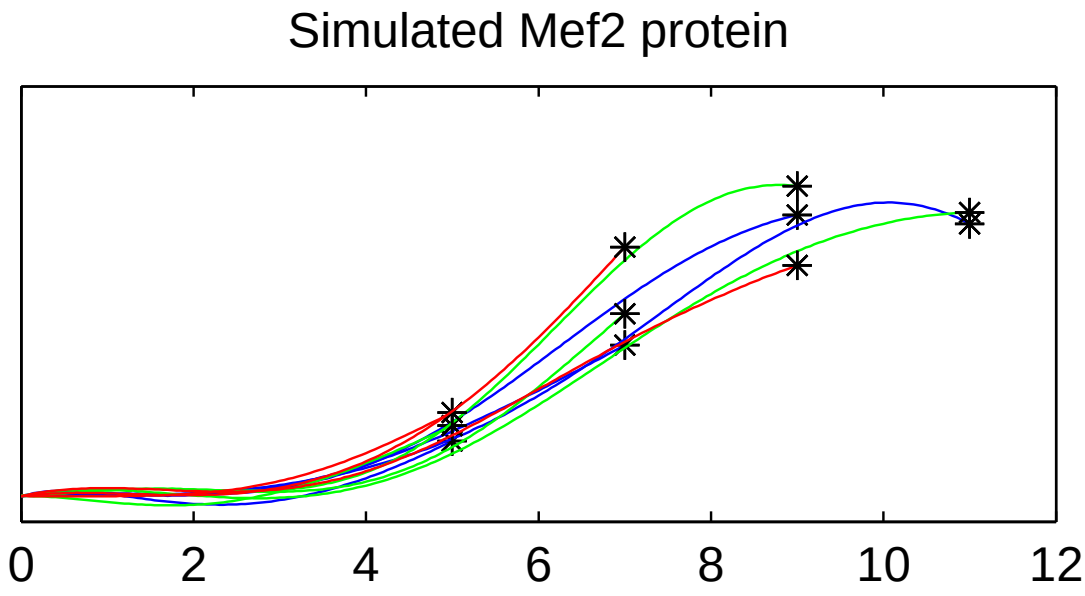
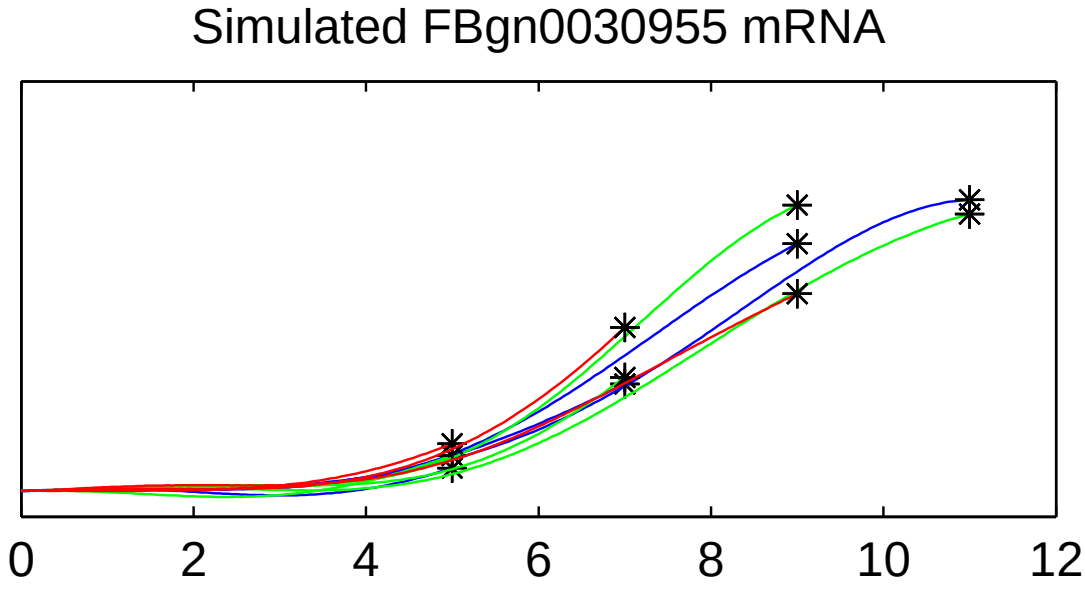
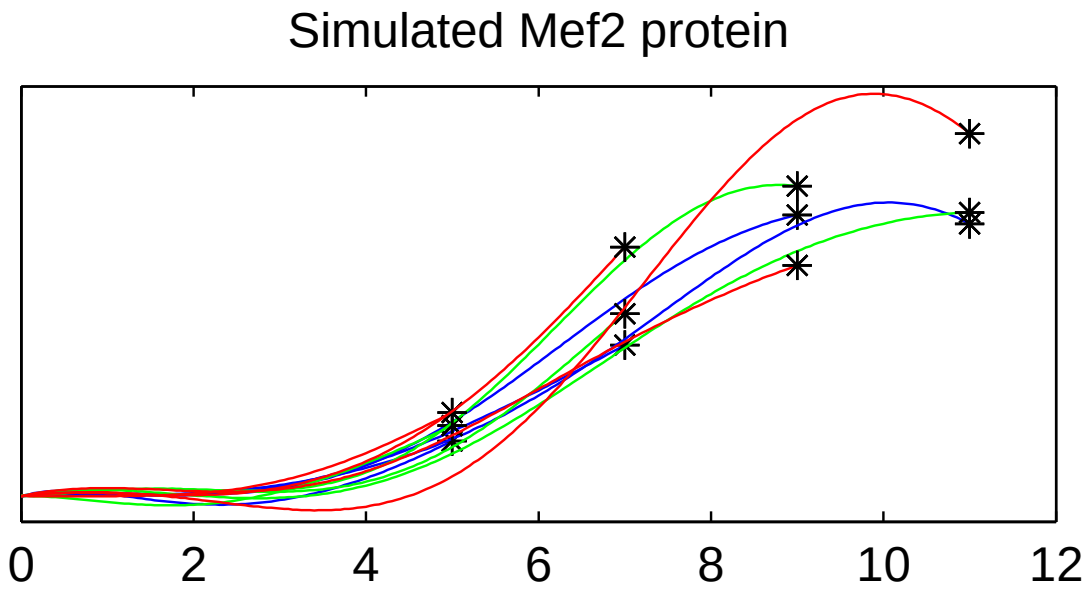
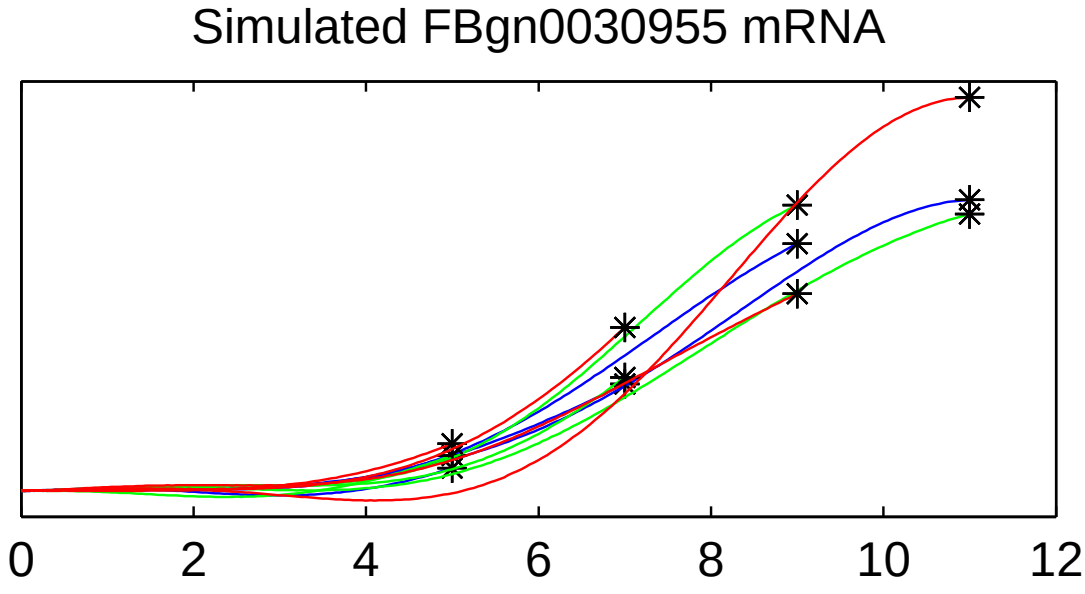


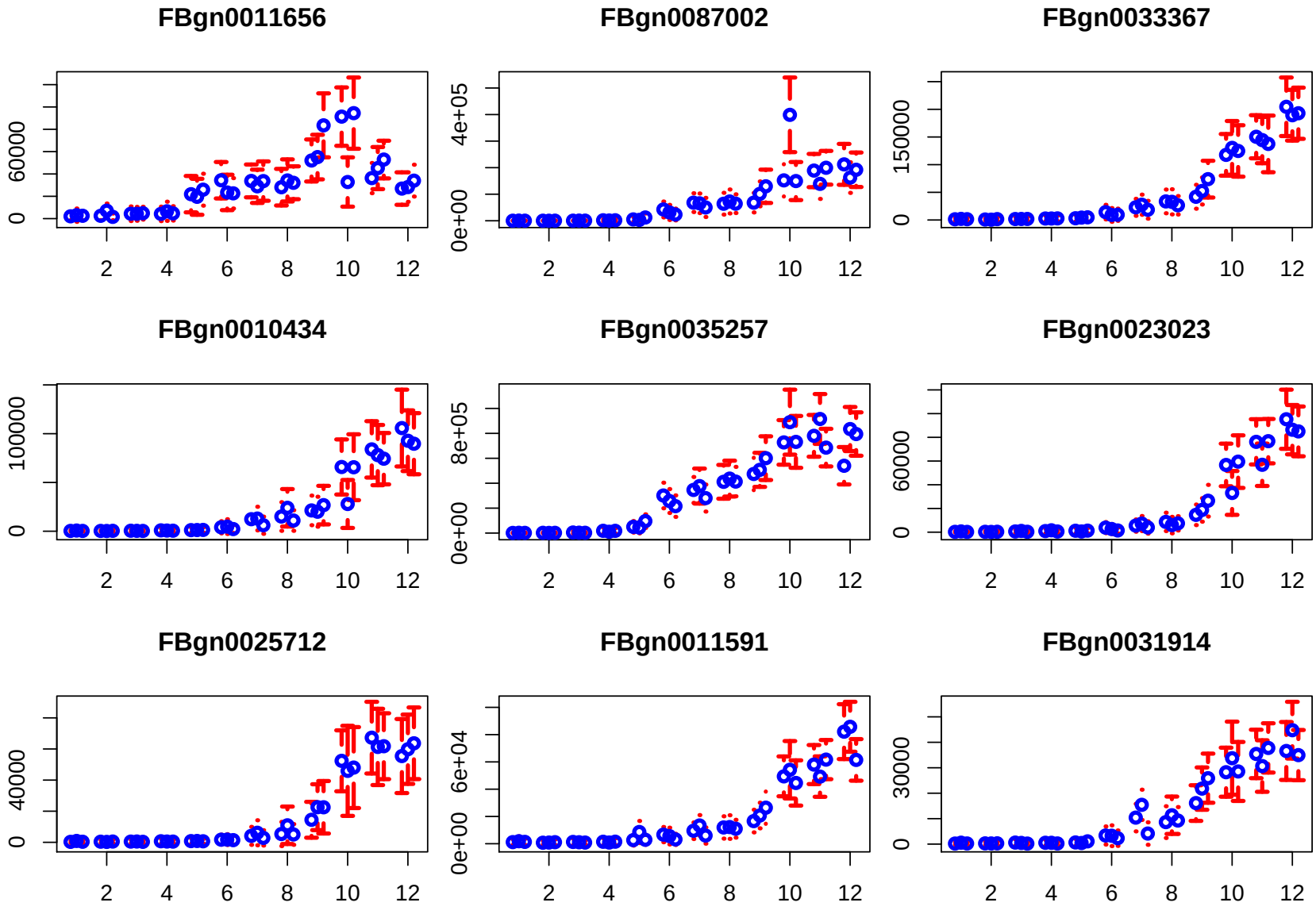


Simulated molecular biology time series





Real gene expression time series



Example model: Linear ODE model of transcription

- Linear Activation Model (**?**, Genome Biology)

$$\frac{\mathrm{d}x_j(t)}{\mathrm{d}t} = B_j + S_j f(t) - D_j x_j(t)$$

- $x_j(t)$ – concentration of gene j's mRNA
- $f(t)$ – concentration of active transcription factor
- Model parameters: baseline B_j, sensitivity S_j and decay D_j
- Placing a Gaussian process (GP) prior on $f(t)$ leads to a joint GP over all concentration profiles (**?**, Bioinformatics)

How to connect the model to data?

1. Assume independent profiles for each complete (biological) repeat
   - Loses statistical power for extra independence assumptions
   - Is it meaningful to order the repeats?
2. Assume one shared underlying profile with independent observations
   - Potentially sensitive to outliers

Exchangeability analysis

Assume $x_j^k(t_i)$ observation of kth repeat of jth gene at ith time

	$x_\cdot^k(t_i) \leftrightarrow x_\cdot^{k'}(t_i)$ "swap arrays"	$x_j^k(t_i) \leftrightarrow x_j^{k'}(t_i)$ "swap single gene"
"Reality"	Yes	No
1. Independent profiles	No	No
2. Shared profile	Yes	Yes

Solution: hierarchical GP model

- Assume the underlying $f(t)$ is composed of a shared and an experiment-specific part $f_{ik}(t)$

$$\frac{\mathrm{d}x_j(t)}{\mathrm{d}t} = B_j + S_j[f_{\text{shared}}(t) + f_{ik}(t)] - D_j x_j(t)$$

- Covariance is of the same form as usual
- Introduces additional covariance terms for measurements from the same experiment
- Alternative parametrisations of variance of $f_{ik}(t)$
  - Shared across all experiments
  - Sampled independently for each experiment

Exchangeability analysis revisited

Assume $x_j^k(t_i)$ observation of kth repeat of jth gene at ith time

	$x_\cdot^k(t_i) \leftrightarrow x_\cdot^{k'}(t_i)$ "swap arrays"	$x_j^k(t_i) \leftrightarrow x_j^{k'}(t_i)$ "swap single gene"
"Reality"	Yes	No
1. Independent profiles	No	No
2. Shared profile	Yes	Yes
3. Hierarchical model	Yes	No

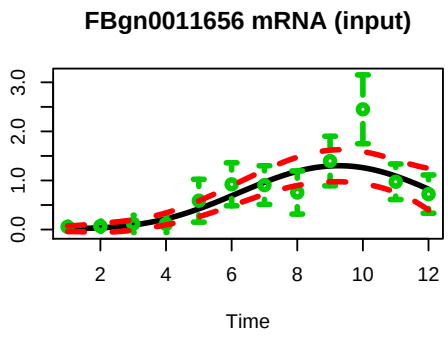
ODE model of translation and transcription

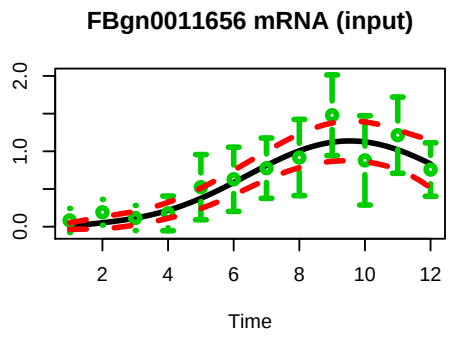
- Assume TF is transcriptionally regulated with related mRNA $y(t)$
- This yields a system of ODEs (**?**)

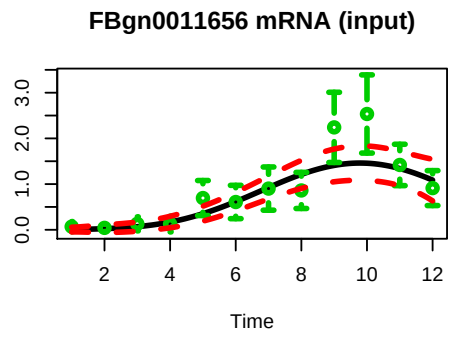
$$\frac{\mathrm{d}f(t)}{\mathrm{d}t} = \sigma y(t) - \delta f(t)$$
$$\frac{\mathrm{d}x_j(t)}{\mathrm{d}t} = B_j + S_j f(t) - D_j x_j(t)$$

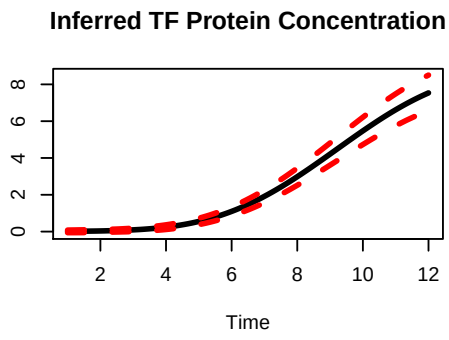
- The corresponding GP model can be derived analogously to the previous case

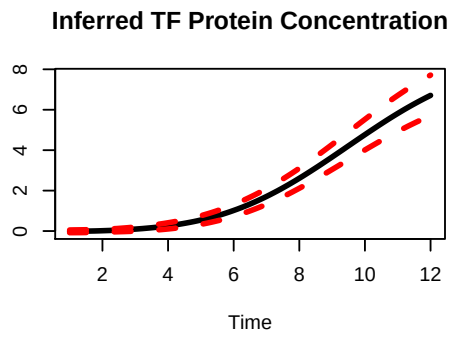
Independent profiles

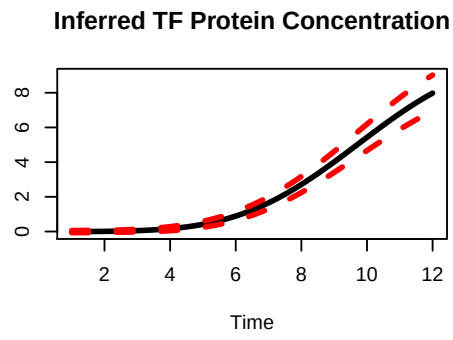


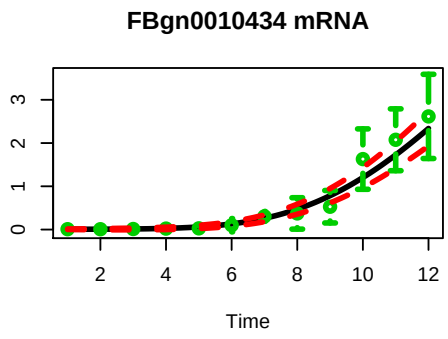


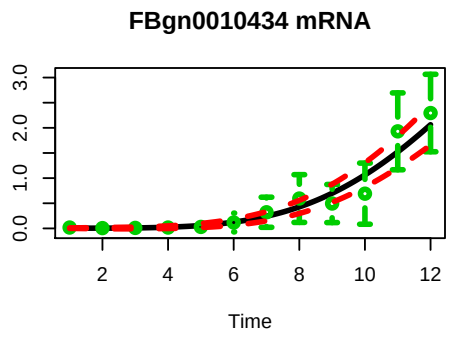


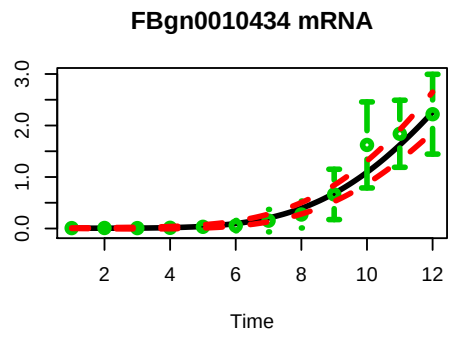




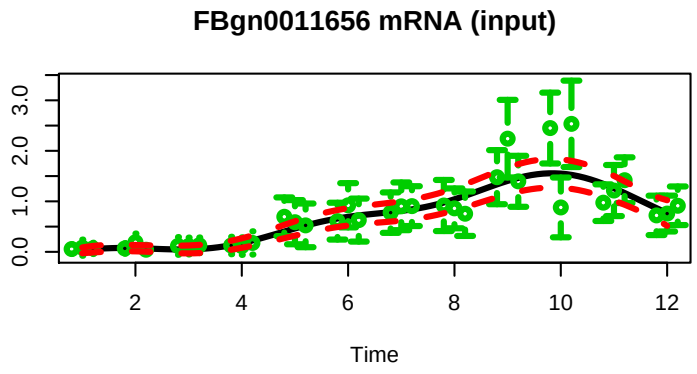


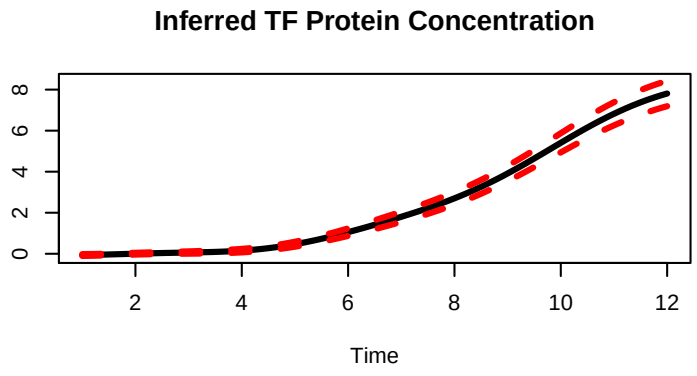


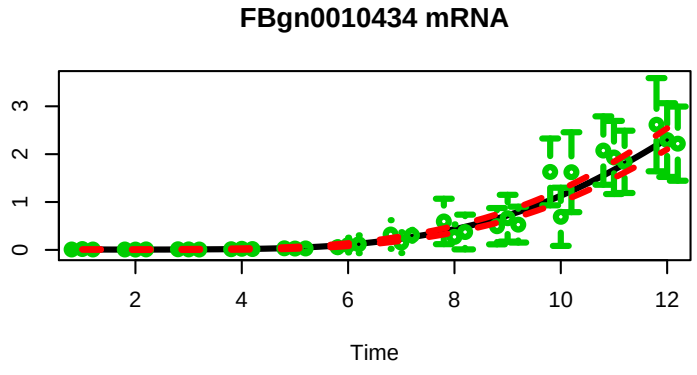




Hierarchical model







Conclusion

- Transcription factor target identification with ODE models
  - Very good performance with linear single-TF models
- Non-linear multiple-TF models also feasible
- Linear model can be extended to account for the experimental structure of time series assays
  - Previous approaches have invalid exchangeability assumptions
- Future work
  - Stochastic differential equation models
  - Incorporation of new data modalities

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References

Now available in Bioconductor:

tigre — Transcription factor Inference through Gaussian process Reconstruction of Expression