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On the personalised modelling of cancer signalling

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Abstract: Dynamic modelling has long been used to understand fundamental principles of cell signalling and its dysregulation in cancer. More recently, these models have also been used to understand the individual risks of cancer patients, and predict their survival probabilities. However, the current methodologies for integrating tumour data and generating patient-specific simulations suffer from the lack of general applicability; they only work for cell signalling models in which only posttranslational protein modifications are considered, so that the total protein concentrations are conserved. Here, we present novel, generally applicable method. The method is based on a simple theoretical framework for modelling gene-regulation, and the indirect estimation of patient-specific parameters from tumour data. Because our method does not require time-invariance of the total-protein concentrations, it can be applied to models of any nature, including the many cancer signalling models involving gene-regulation.

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1. INTRODUCTION

The dynamic states of cancer cell signalling are intimately linked to cancer cell fate decisions and clinical outcomes (Kolch et al., 2015; Fey et al., 2012). Recently, multiple studies have shown that patient-specific differences in the dynamic behaviour of these signalling networks underlie individual pathogenetic changes and disease manifestation (Fey et al., 2015; Flanagan et al., 2015; Lindner et al., 2013; Murphy et al., 2013). For example, a dynamic model of the JNK pathway could predict the survival probabilities of cancer patients in neuroblastoma, a common childhood cancer (Fey et al., 2015). These predictions were based on personalised simulations for over 700 patients, and revealed that a high amplitude, switch-like JNK activation was associated with neuroblastoma cell death, and better patient survival.

To generate the patient-specific simulations, the signalling model was personalised by adjusting the total protein concentration of each model component to the measured values from the patient’s tumour sample. This approach works for purely posttranslational signalling models in which the total protein concentration of each gene is conserved (constant), and the only dynamic changes arise from the regulation of protein-protein interactions and enzyme activities, for example by phosphorylation.

In fact, all the personalised models mentioned are based on this simple principle of directly using the measured mRNA or protein concentrations as static parameters in the model (Fey et al., 2015; Flanagan et al., 2015; Lindner et al., 2013; Murphy et al., 2013). Thus, all these personalised models are based on the assumption that the total protein concentrations of the modelled genes are constant for all genes. Because only then do these measured concentrations constitute static parameters in the model that can readily be adjusted using the measured tumour data. Unfortunately, this assumption is often violated and prevents the application of this approach to systems in which the protein levels themselves are regulated.

Regulation of gene expression on the level of mRNA and total protein is quite common (Kolch et al., 2015; Nakakuki et al., 2010). Examples that are particularly relevant for cancer are regulation of the cell-cycle or the DNA-damage response. In these systems, both mRNA and total protein concentrations are regulated, and changing over time. Moreover, these dynamic changes are critical aspects of the systems behaviour. So far, the assumption of time-invariant total protein levels has hampered personalised modelling of these important processes. Thus, there is an urgent need for novel model-personalisation methodologies that are generally applicable.

Here we propose such a methodology.

In contrast to the current approach, the here proposed methodology does not require that the total-protein concentrations are constant. Instead, we propose a theoretical framework in which the patient-specific mRNA and total protein concentrations arise naturally from patient-specific mRNA synthesis parameters. Rather than time-invariant parameters, the mRNA and total protein concentrations are now dynamic states described by ordinary differential equations. This framework allows us to personalise models of any nature, involving signalling, gene-regulation or both, by indirectly estimating the patient-specific synthesis parameters.

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The current manuscript is organised as follows. First, we will present a simple, yet generally applicable theoretical framework for the personalised modelling of gene expression. Second, we show how the patient-specific parameters can be estimated from expression data measured in the patients’ tumour samples. Third, we illustrate the methodology by testing it on simulated data from a p53 DNA-damage response model.

2. A MODELLING FRAMEWORK FOR PERSONALISED GENE EXPRESSION

Although often considered separately, the posttranslational and gene-regulatory levels of cell signalling are intrinsically linked (Fig. 1). On the one hand, signalling on the protein level affects the gene-regulatory level, for instance by triggering mRNA synthesis through the activation of transcription factors. On the other hand, the gene-regulatory level affects the signalling level by controlling the expression of the proteins that mediate and process the signals.

2.1 Combined modelling of gene-regulation and signalling

Mathematically, we can think of signalling as a system consisting of two layers, linked by feedback (Fig. 1). Each layer consists of a set of ordinary differential equations. The signalling layer has the concentrations of all posttranslational protein forms as states, and takes the vector of protein concentrations as input. Vice versa, the gene-regulatory layer has the mRNA concentrations as states and takes the vector of protein concentrations as input.

Formally, we have the following system

\[ \frac{d}{dt} y = f(y, p) + B \text{diag}(k_{\text{transl}}) x \]

\[ \frac{d}{dt} x = \rho + g(y, k) - \text{diag}(k_{\text{rdeg}}) x, \]

where \( x \in \mathbb{R}^n \) and \( y \in \mathbb{R}^m \) are vectors of mRNA and protein concentrations, respectively. \( f(y, p) \) and \( g(y, k) \) are functions describing posttranslational modifications and gene-regulatory effects, respectively. Note that \( f(y, p) \) should contain a degradation term, i.e. \( f(y, p) = f(y, p) - \text{diag}(k_{\text{rdeg}}) y \). \( B \in \mathbb{R}^{n \times m} \) is a matrix containing \( m \) different unit vectors \( e_i \in \mathbb{R}^n \), indicating the subset of unmodified proteins \( y_i \) that are translated (synthesised) from their corresponding mRNA templates. \( \text{diag} k_{\text{transl}} \in \mathbb{R}^{m \times n} \) and \( \text{diag} k_{\text{rdeg}} \in \mathbb{R}^{m \times n} \) are diagonal matrices containing the protein-translation and mRNA-degradation rate constants. \( \rho \in \mathbb{R}^n \) is a vector of patient-specific parameters.

Remark on the notation. In the following, and for simplicity of presentation, we often do not explicitly denote the dependency of \( f \) and \( g \) on the kinetic parameters \( p \) and \( k \). That is, when referring to \( f \) and \( g \) we mean the functions \( f : y \mapsto f(y, p) \) and \( g : y \mapsto g(y, p) \), respectively.

2.2 Rationale and assumptions behind the approach

The method is based on linking the measured patient-specific gene-expression differences on the systems level to parameter changes in the model on individual gene level. Because we want the patient-specific parameters to be identifiable, we assume that the basal mRNA-synthesis rate for each gene varies between patients, but all other parameters do not.

Assumption 1. The observed gene-expression differences in tumour samples are caused by patient-specific differences of the basal mRNA-synthesis rates.

Although some other parameters, most notably mRNA-degradation and protein-translation rate constants, might also vary between patients, we only estimate the patient-specific mRNA-synthesis rates. All other kinetic parameters not directly relating to gene expression – such as catalytic rate constants and binding affinities – are presumed not to vary between patients.

Remark on violating Assumption 1. Although it is a theoretical requirement for formulating the methodology, violating Assumption 1 has limited practical consequences. As we will see later in Sec. 4.3, accurate, albeit less precise simulation results can be obtained even when the rates of mRNA-degradation and protein-translation for each gene differ between patients.

3. ESTIMATING THE PATIENT-SPECIFIC PARAMETERS

Our aim is to estimate the patient specific parameter \( \rho \) from gene expression measurements of the patients’ tumours. Such tumour data are usually obtained from tissue samples collected in biopsies or surgery, and would usually reflect the homeostatic, unperturbed state of the patient’s tumour. Thus, these tumour data would correspond to the unstimulated steady-state of our model (1).

Assumption 2. Measured gene expression values from tumour samples reflect basal (unstimulated) steady-state values.

This is a fair assumption considering that most tumour data are collected prior to pharmacological treatment (de Gramont et al., 2015; Juty et al., 2015).

Put more formally, the goal is to estimate \( \rho \) from steady state measurements of either \( x \) or \( y \), based on the model in (1) with given functions \( f, g \) and given kinetic parameters \( p, k, k_{\text{transl}} \) and \( k_{\text{rdeg}} \).

For the steady-state we have

\[ 0 = f(y, p) + B \text{diag}(k_{\text{transl}}) x, \]

\[ 0 = \rho + g(y, k) - \text{diag}(k_{\text{rdeg}}) x. \]

The next step is to solve (2) for the patient-specific parameters \( \rho \). Two scenarios can be distinguished.
3.1 Estimation based on mRNA measurements (x is measured)

In the first scenario, all mRNA concentrations are measured. The following theorem provides the main result.

Theorem. Assume that the function \( f : y \mapsto f(y,p) \) is injective. Then we can estimate the patient specific parameters \( \rho \) from steady state measurements of \( x \).

Proof (by construction). Because \( f \) is injective, a left-inverse of \( f \) with \( f_{\text{inv}} \circ f = Id_y \) exists. Thus, in steady-state \( \frac{d}{dt} y = 0 \), it hold that

\[
\dot{y} = f_{\text{inv}}(B \text{diag}(k_{\text{transl}})x, p),
\]

which means given steady-state measurements of \( y \), we know the steady-state of \( x \). Further, it follows from the steady-state condition \( \frac{d}{dt} x = 0 \) of \( x \), that

\[
\rho = k_{\text{deg}}^T x - g \circ f_{\text{inv}}(B \text{diag}(k_{\text{transl}})x).
\]

\[\square\]

Equation (4) is the analytical solution to the problem in (2), and provides an explicit formula for estimating the patient-specific parameters from steady-state mRNA measurements.

3.2 Estimation based on protein measurements (y is measured)

In the second scenario, the protein concentrations are measured. The following theorem provides the main result.

Theorem. Assume non-zero translation rates for all genes, i.e. all elements of \( k_{\text{transl}} \) are greater than zero. Then we can estimate the patient-specific parameters from steady-state measurements of \( y \).

Proof. By definition \( B \) is composed of a subset of a unit vectors, e.g. \( B = [e_i, e_j, \ldots] \). It holds that \( B^T B = I \), which means we can solve (2a) for \( x \)

\[
x = -\text{diag}^{-1}(k_{\text{transl}})B^T f(y, p).
\]

(5)

To see this, note that right-multiplying (2a) with \( B^T \), gives \( 0 = B^T f(y) + B^T B \text{diag}(k_{\text{transl}})x \). After substitution into (2b) we can solve for the patient-specific parameter

\[
\rho = -k_{\text{deg}}^T \text{diag}^{-1}(k_{\text{transl}})Bf(y, p) - g(y, k).
\]

\[\square\]

Equation (6) is the analytical solution to the problem in (2), and provides an explicit formula for estimating the patient-specific parameters from steady-state protein measurements.

4. EXAMPLE

To illustrate the proposed methodology, we applied it to a simple toy model inspired by the p53 DNA damage response system, using simulated data for testing. On the gene-regulatory level, p53 induces the expression of MDM2, which in turn promotes the degradation of p53 (Fig. 2). On the posttranslational level, the phosphorylation of p53, for example induced by DNA damage, inhibits p53 degradation, thus stabilising p53 protein, resulting in increased p53 protein expression. Thus, this system contains an interesting crosstalk between the posttranslational level describing p53 phosphorylation and the gene-regulatory level describing p53 expression. Indeed, this was our rationale behind choosing this model. We wanted to look at a system that contains a feedback loop involving both transcriptional and post-translational events, as we thought such a system would constitute the most difficult challenge for model personalisation.

4.1 Equations of the example system

Our example system, based on a core model of the p53 DNA-damage response, is a prototypical example for a signalling system that involves gene-regulation (Fig. 2). Note that there are many other gene-regulatory systems that follow similar regulatory patterns (Avraham and Yarden, 2011), for example NF-\( \kappa \)B (Perkins, 2012).

The model equations are

\[
\begin{align*}
\dot{y}_1 &= -v_1(y_1) + v_2(y_3) - p_{-1} y_2 y_1 + k_{t,1} x_1, \\
\dot{y}_2 &= -p_{-2} y_2 + k_{t,2} x_2, \\
\dot{y}_3 &= +v_1(y_1) - v_2(y_3) - p_{-3} y_3, \\
\dot{x}_1 &= \rho_1 - k_{\text{deg},1} x_1, \\
\dot{x}_2 &= \rho_2 + k_1 H(y_1 + y_3) - k_{\text{deg},2} x_2,
\end{align*}
\]

where \( y_1, y_2, y_3 \) and \( x_1, x_2 \) denote the protein and mRNA concentrations of \( Y_1, Y_2, pY_1 \) and \( X_1, X_2 \), respectively, and with parameters described below, and functions

\[
\begin{align*}
v_1(y_1) &= p_1 u(t) \frac{y_1}{y_1 + K_{m,1}}, \\
v_2(y_3) &= p_2 \frac{y_3}{y_3 + K_{m,2}},
\end{align*}
\]

(7f)

(7g)

describing the rates of \( Y_1 \) phosphorylation and dephosphorylation, respectively, and the Hill function

\[
H(y) = \frac{y^n}{K_h^n + y^n},
\]

(7h)

describing the \( Y_1 \) mediated induction of \( X_2 \) synthesis. \( u(t) \) denotes a possibly time-dependent input signal, which we can interpret as the activity of the upstream kinase catalysing the phosphorylation of \( Y_1 \). Constants \( \rho_1 \) and \( \rho_2 \) are patient specific parameters that, for the purposes of our simulation tests, are randomly chosen from a lognormal distribution (denoted \( \ln \mathcal{N} \)) with mean \( \mu = 1 \) and standard deviation \( \sigma = 0.33 \). All other, nonrandom parameters are

\[
\begin{align*}
k_{t,1} &= 10, & k_{t,2} &= 10, \\
p_{-1} &= 5, & p_{-2} &= 5, & p_{-3} &= 0.5, \\
k_1 &= 5, & k_{\text{deg},1} &= 1, & k_{\text{deg},2} &= 1, \\
p_1 &= 100, & K_{m,1} &= 1, & K_h &= 2, \\
p_2 &= 10, & K_{m,2} &= 1, & n &= 2.
\end{align*}
\]

(8a)

(8b)

(8c)

(8d)

(8e)

What makes this system particularly interesting is the link between the gene-expression and posttranslational levels, in which the phosphorylation of \( Y_1 \) regulates the expression of the total \( Y_1 \) protein concentration \( [Y_1]_{\text{total}} = y_1 + y_3 \). This link is established by a condition on the parameter values \( p_{-1} \gg p_{-3} \) which makes the degradation of \( Y_1 \) in the unphosphorylated form \( y_1 \) much faster than the degradation of the phosphorylated form \( y_3 \).

Figure 3 shows the behaviour of the model with the nominal patient-specific parameters \( \rho_1 = 1, \rho_2 = 1 \) in response to a unit step input at \( t = 10 \), that is \( u = 0 \) for \( t < 10 \), and \( u = 1 \) for \( t \geq 10 \).
We can easily verify that the example system is of the form 
\[ y \in (1), \]
where we presume that the mRNA levels are measured in the basal, unstimulated steady state, for which it holds that \( u = 0, \) and \( \dot{x}_1 = \ldots = \dot{x}_3 = 0. \)

We can easily verify that the example system is of the form in (1), where \( y = [y_1, y_2, y_3]^T, \) and
\[
 f(y) = \begin{bmatrix}
    -v_1(y_1) + v_2(y_3) - p_{\text{deg}} y_1 \\
    v_1(y_1) - v_2(y_3) - 3y_3 \\
    v_1(y_1) - v_2(y_3) - p_{\text{deg}} y_1 \\
\end{bmatrix},
B = \begin{bmatrix}
    1 & 0 & 0 \\
    0 & 1 & 0 \\
    0 & 0 & 1 \\
\end{bmatrix}
\]

and \( k_{\text{transl}} = [k_{t1,1}, k_{t1,2}], x = [x_1, x_2]^T. \)

Further, we can verify that \( f \) is injective for \( u = 0, \) and the inverse \( f^{-1} \) exists. To see this, we solve the steady state equations for 7a-c as follows. From (7b) we obtain
\[
y_2 = \frac{k_{t1,2} x_2}{p - 2}.
\]

From (7f) follows with \( u = 0 \) that \( v_1 = 0, \) and therewith from (7c) that the only possible steady-state for \( pY1 \) is
\[
y_3 = 0.
\]
Fig. 5. Correlation between the real and estimated patient-specific parameter values. Test 2: Correct model, noisy measurements.

Correct model, noisy measurements  A more realistic scenario is when the patient data are corrupted with measurement noise. In this case, we observed a good correlation between the real and estimated patient-specific parameters with about 90% and 53% of the variability explained for \( \rho_1 \) and \( \rho_2 \), respectively (Fig. 5). Accordingly, the simulated protein levels in response to a unit step input also correlated well with the real response (Fig. 6).

Incomplete model, perfect measurements  Next we assessed what happens when Assumption 1 is violated, and the real system contains additional patient-specific parameters, but no measurement noise. Specifically, we used variations in the mRNA-degradation constants \( k_{r \text{deg},1}, k_{r \text{deg},1} \) and the protein-translation parameters \( k_{t,1}, k_{t,1} \) in addition to \( \rho_1, \rho_2 \) for generating the patient-specific data. Basically, this means that the model used for re-engineering the patient-specific differences (1) is incomplete: Because our methodology keeps the degradation and translation parameters constant, all patient specific differences will be mapped onto \( \rho_1 \) and \( \rho_2 \). Naturally, this impacted on the patient-specific parameter estimates (Fig. 7), which are now less correlated with the real values (20 – 50% of variation explained). In contrast, the simulated dynamic responses correlated well with the real responses. About 38% of the variability in the steady state response was explained by the simulations (Fig. 8).

The fact that the response predictions of the output were more precise than some parameter estimates, is explained as follows. The algorithm maps all patient-specific differences onto \( \rho_1 \) and \( \rho_2 \), even the differences arising from \( k_{\text{rdeg}} \) and \( k_t \). Because they include these differences, the patient-specific parameter estimates are more variable. In contrast, the estimated mRNA concentrations are precise, because (4) guaranties that the modelled mRNA values match the measured values \( x_{\text{model}} = x_{\text{measured}} \). This means that for predicting the dynamic responses, the precision of the state-estimates is more important than the precision of the parameter-estimates.

Incomplete model, noisy measurements  Finally, we also analysed the worst case scenario, in which both the model and the measurements contain errors. The results were not markedly different from the ones obtained for noise-free measurements (Fig. 9). Despite the fact that the patient-specific estimates were not very precise (especially for \( \rho_2 \) with a correlation coefficient of only \( R = 0.42 \)), the output predictions were precise, with the simulated pY1 responses explaining 48% of the variability present in the real responses (Fig. 9).

5. CONCLUSIONS

This paper proposes a generally applicable methodology for personalising models of cancer signalling. The advantage of using such personalised dynamic models over static biomarkers, has already been demonstrated in several case studies (Fey et al., 2015; Flanagan et al., 2015; Murphy et al., 2013; Lindner et al., 2013). In contrast to previous approaches, the here proposed methodology is not restricted to models in which the total protein concentrations are time-invariant parameters.
The mathematical framework underlying the methodology is based on two central biological principles: 1) mRNA serves as template for protein synthesis; 2) individual, cell-type specific differences arise from changes in gene expression.

Elaborating on the latter, a basic assumption in our approach is that the individual gene expression patterns arise from patient-specific differences in the basal gene expression rate parameters. Importantly, our approach is robust with respect to violating this assumption: The methodology can still be applied when other, unmodelled patient-specific differences are present.

Another, relatively weak assumption in framework is that all non-patient-specific parameters in the model are known. These parameters would usually be provided by the model, ideally as a result from constructing and calibrating the model using data from cell-culture experiments and parameter estimation. Literature reported values are also often used in these models, although care has to be taken for adopting parameter values out of context. In any case, measurements of mRNA and protein half-lives do exist for many genes (Schwanhüser et al., 2011).

The validity of i) the approach and ii) the personalised model could be tested experimentally by measuring i) the synthesis and degradation parameters for example in pulse-chase experiments (Larance and Lamond, 2015), and ii) the dynamic responses in time-course experiments. This would i) test the assumption of patient-specific synthesis parameters, and ii) (in-)validate the predicted dynamic responses. Both experiments would require live cancer-cells from patients. Isolating live cancer-cells from tumour-samples can be technically challenging but the resulting cells are easy to assay. Tumour explants (little pieces of live tumour tissue) are easier to keep alive in the lab, but also contain non-cancerous cells and not all biochemical assays can be applied.

In conclusion, our methodology makes the personalised modelling of dynamic cell-signalling possible. In conjunction with tumour data (TCGA database, Tomczak et al., 2015), it should be used to test the predictive and prognostic utility of the many signalling models constructed to date (BioModels database, Juty et al., 2015).

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