



A Parameter Estimation Framework for Kinetic Models of Biological Systems

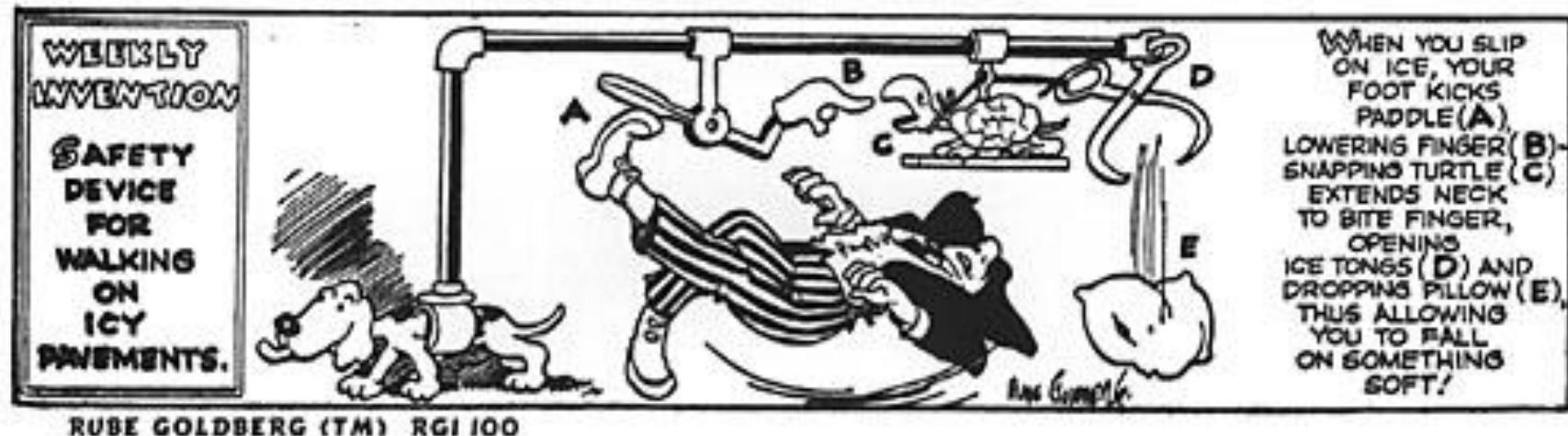
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Outline

- Introduction and Background
- Proposed Framework
- Example case model
- Conclusion and Outlook

Humans think linear



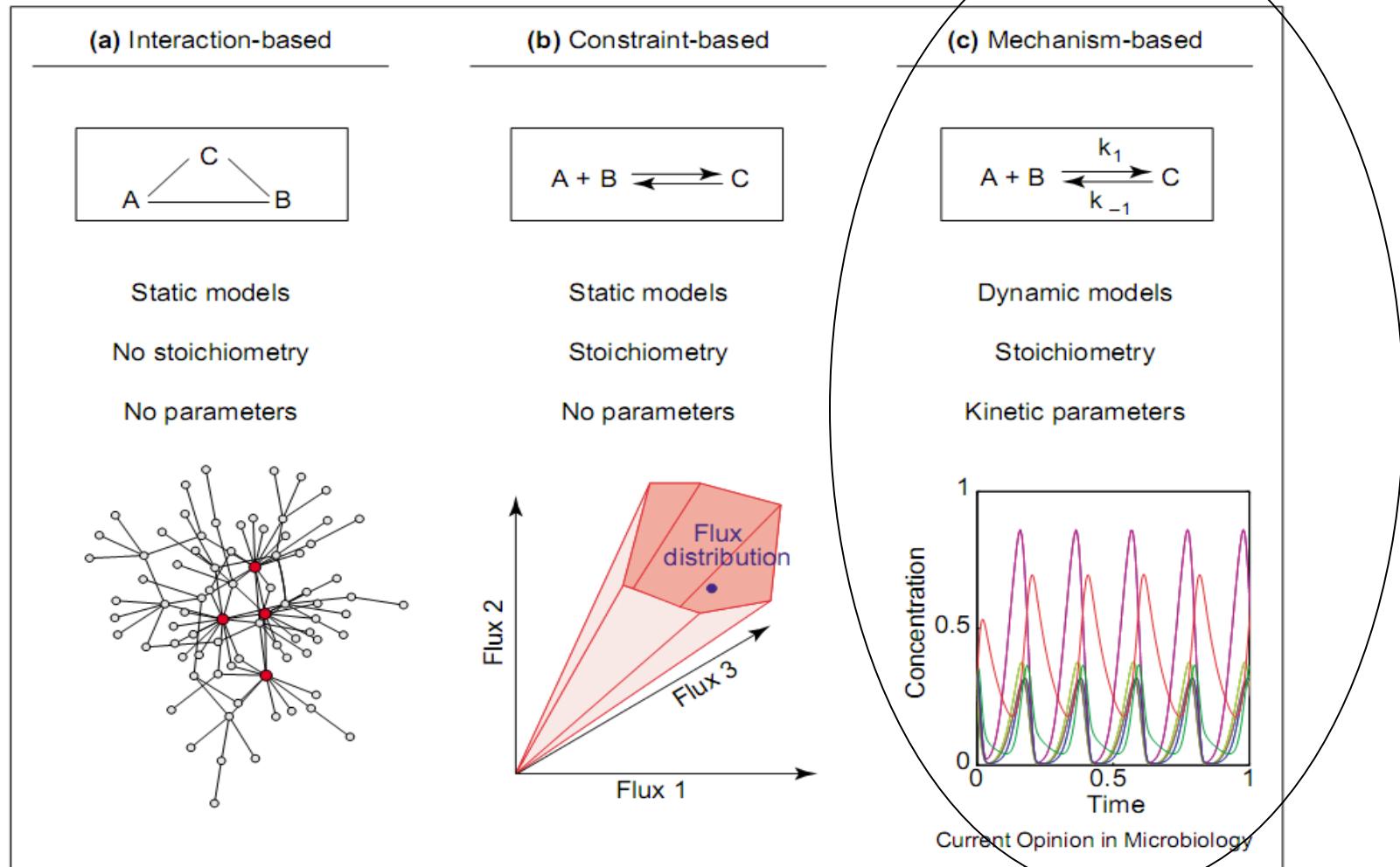
...but biological systems contain:

- non-linear dynamic interaction between components
- positive and negative feedback loops

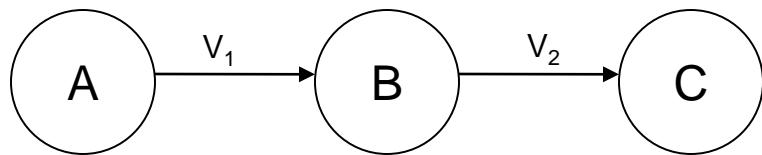
Therefore we need modelling to understand such complex systems

Systems biology is a melding of mathematical modeling, computational approaches and biological experimentation

Different models in systems biology

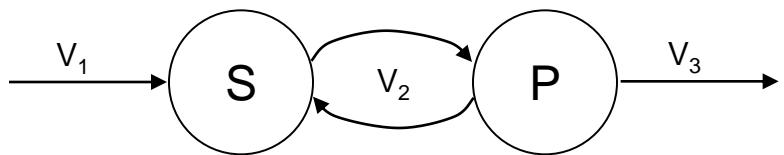


Ref: Jorg Stelling (2004) Mathematical modeling in microbial systems biology.
Current Opinion in Systems Biology, 7, 513-518.



- Model: A system of equations for describing the rate of change of the concentration of each of the metabolites.
- Described with a system of ODEs,
 - e.g. $\frac{d[B]}{dt} = v_1(t) - v_2(t)$
 - v_1 and v_2 are the fluxes.
 - » *Flux*: the flow of material between two metabolite pools.
 - Each flux is represented by a corresponding rate law.

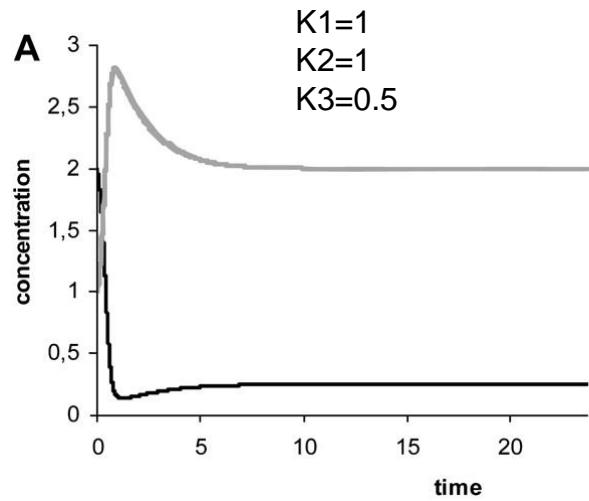
The Selkov Oscillator:



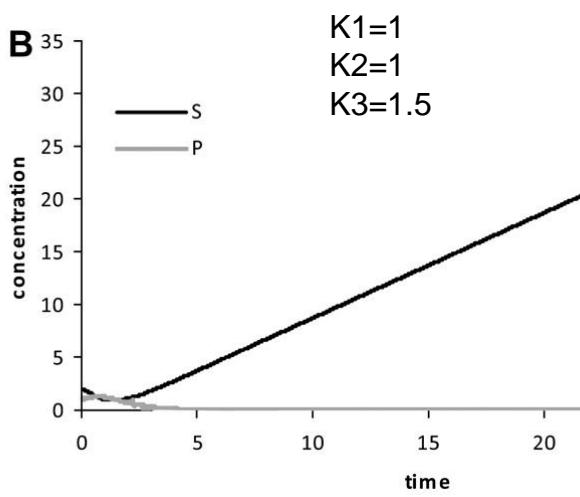
ODE:

$$\frac{dS(t)}{dt} = v_1 - v_2 = k_1 - k_2 SP^2$$

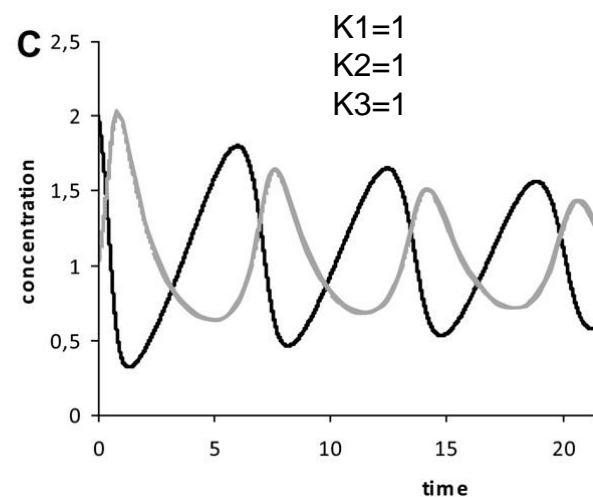
$$\frac{dP(t)}{dt} = v_2 - v_3 = k_2 SP^2 - k_3 P$$



(A) Converging



(B) Diverging



(C) Oscillatory

Ref: Schallau K., Junker B. H., Simulating Plant Metabolic Pathways with Enzyme-Kinetic Models Plant Physiol. 2010;152:1763-1771

The typical approach to Kinetic modeling consists of five phases:

1. The collection of information on network structure and regulation,
2. Selection of the mathematical model framework,
3. Estimation of the parameter values,
4. Model diagnostics, and
5. Model application.

Ref: PhD Dissertation "Parameter estimation and network identification in metabolic pathway systems", I-Chun Chou

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Parameter Estimation

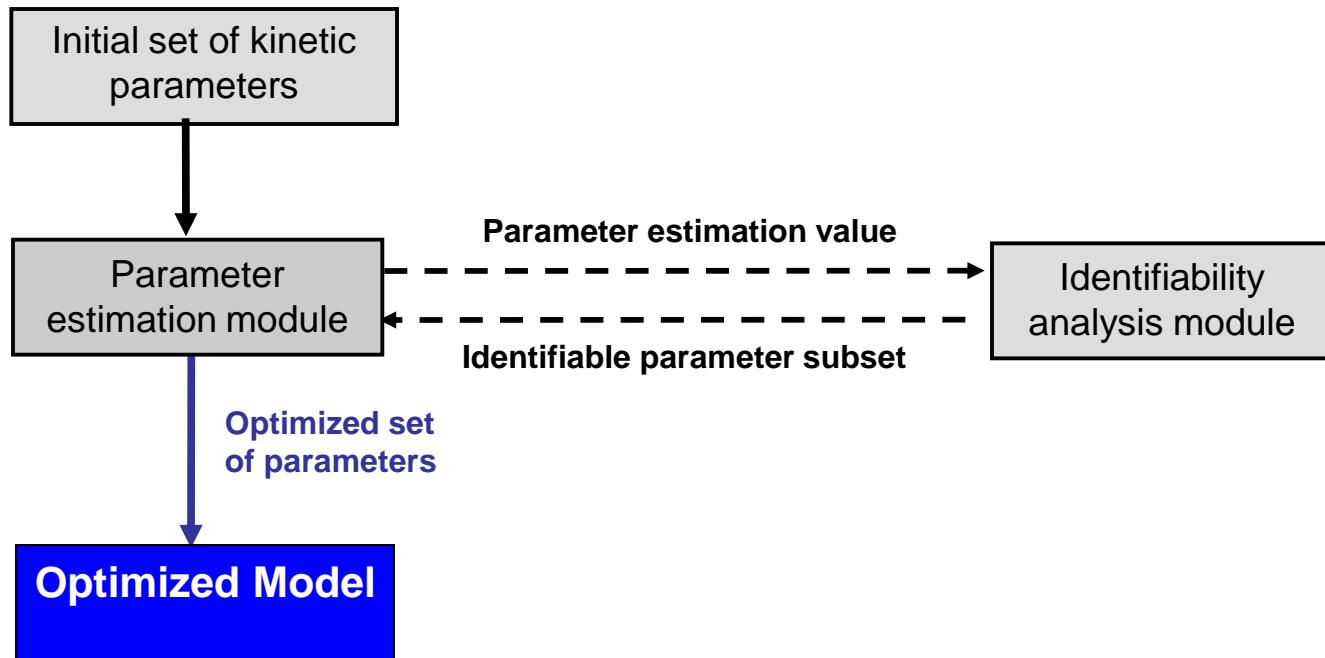
Parameter estimation is a method to determine unknown kinetic parameters in a model by mathematically fitting simulated data to measured data.

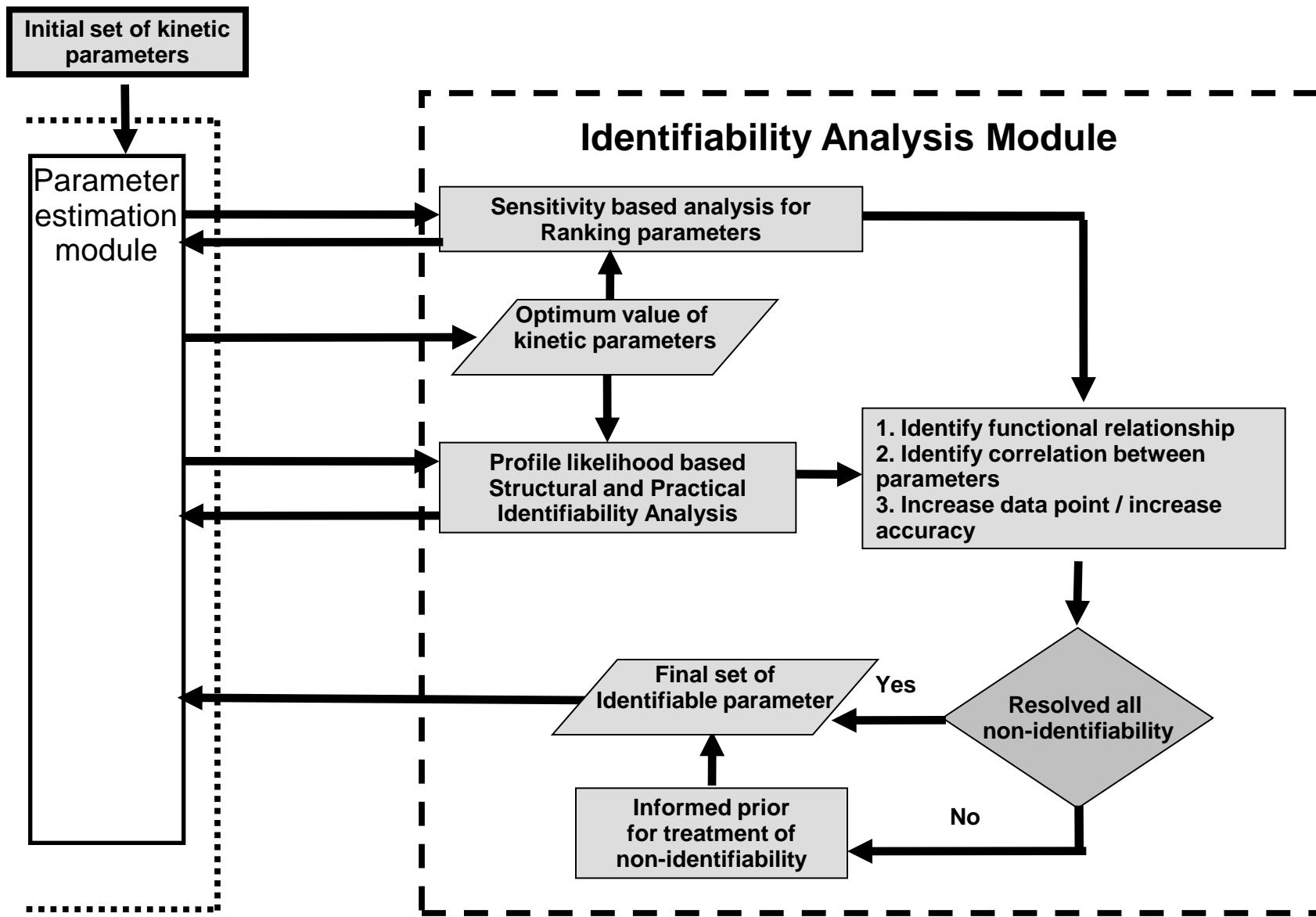
$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

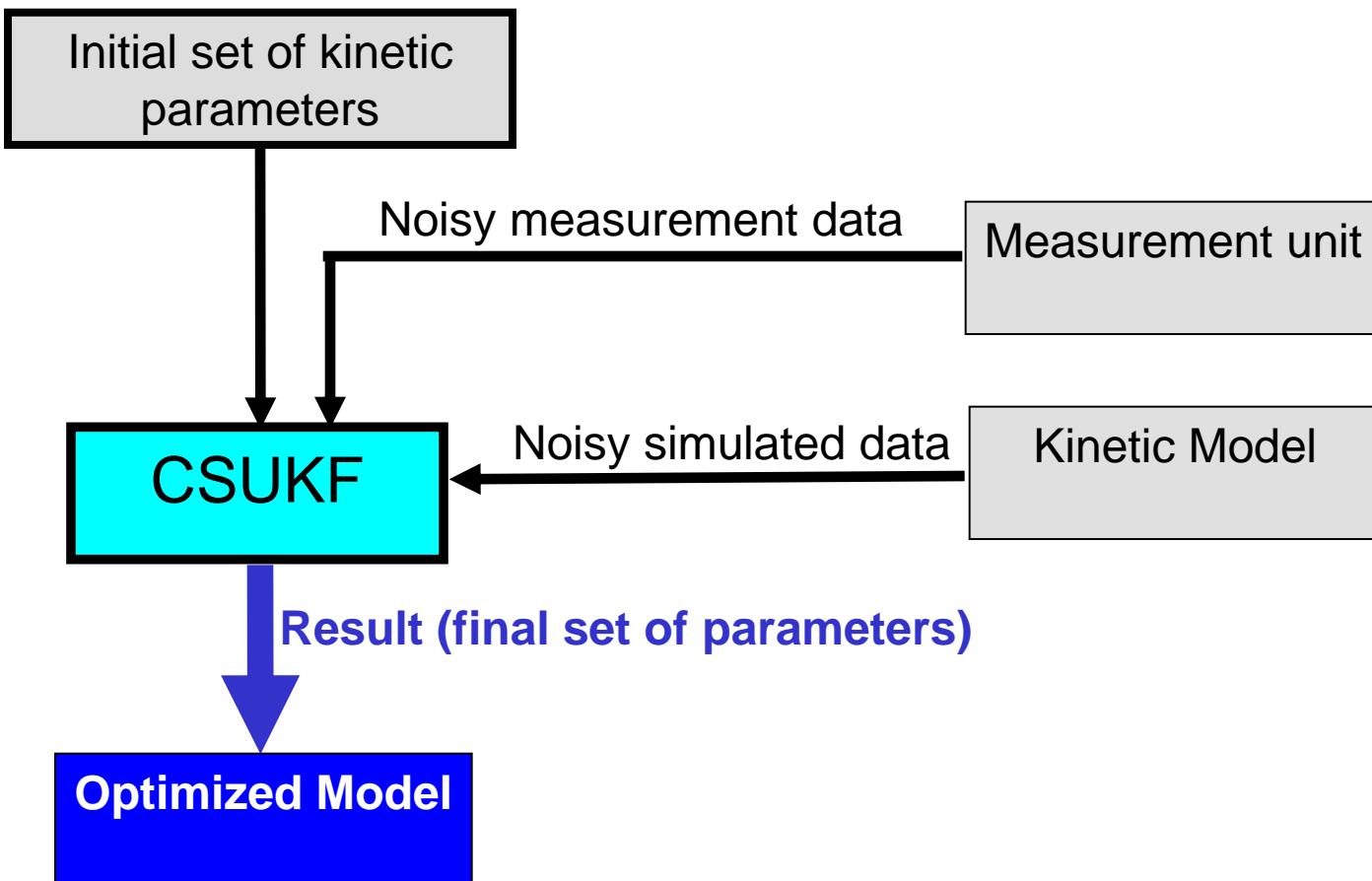
Parameter estimation can become very complex with a large Kinetic Model.

Michaelis–Menten equation

Complete framework







The algorithm ...

- ... has an inherent property of describing dynamic systems.
- ... supports recursive estimation based on past data.
- ... can predict the parameter even when some of the states of the model are hidden or unobserved.
- ... considers both the state and measurement error.
- ... provides a convenient measure of the estimation accuracy.
- Most widely used algorithm in control theory is the Kalman Filter

- In control theory biological models are represented with **state-space equations**.
- State-space is a mathematical representation of a physical system related by first order ODE.
 - Internal state is represented with **state equation**
 - System output is given through **observation equation**

$$\dot{x} = f(x, \theta, t) + w$$

$$y = h(x) + e$$

- Parameter estimation is converted into state estimation by extending the state space definition

$$\dot{x} = f(x, \theta, t) + w, \quad x(t_0) = x_0$$

$$\dot{\theta} = 0 \quad \theta(t) = \theta_0$$

$$y = h(x) + e$$

Why the Unscented Version?

- The application of the KF is only applicable to linear systems while biological models are mostly non-linear.
- Two extensions of the KF for non-linear systems have been proposed:
 - the Extended Kalman Filter (EKF), and
 - the Unscented Kalman Filter (UKF).
- The EKF has the following drawbacks:
 - linearization produces an unstable filter if the system is too non-linear, and
 - the calculation complexity is very high, which often leads to significant implementation difficulties.
- In consideration of these drawbacks, the UKF was selected for my project.

Unscented Kalman Filter

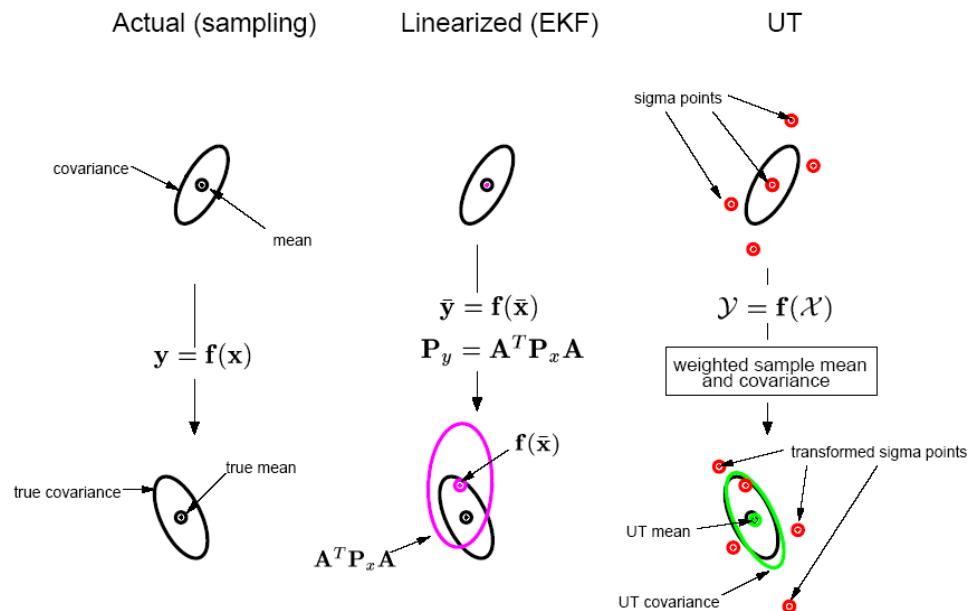
- Recursive estimation method

- Propagates the probability distribution function (PDF) through the unscented transformation (UT).

- Considers both the state and the measurement errors.

- In the UT a set of sample points or sigma points are initially chosen.

- These sigma points are then propagated through the nonlinear function.

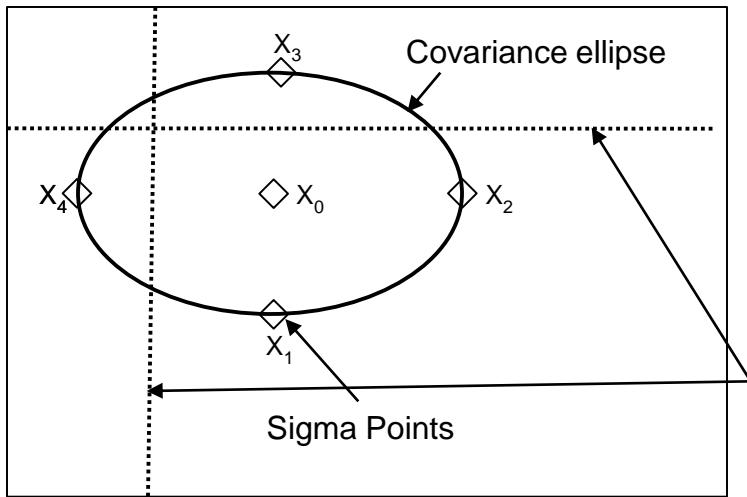


[Source: Book Chapter on “The Unscented Kalman Filter” Eric A. Wan And Rudolph van der Merwe]

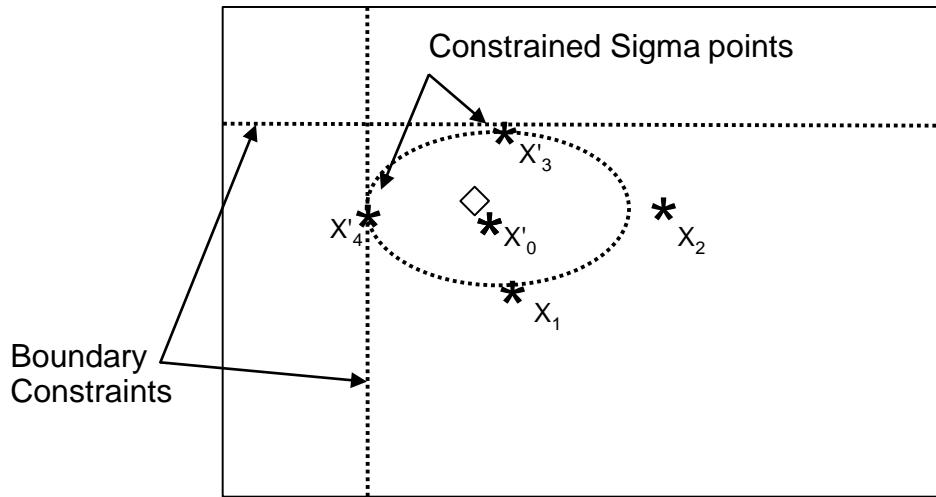
Reasons for CSUKF

- In Biological system constraints are important
 - To include prior information about the parameters.
 - Make parameter values biologically relevant.
- There is no general mechanism for incorporating constraints into the state space.
- The UKF suffers from numerical stability should the covariance not be positive definite.
 - The Square-Root variant of the UKF was designed to maintain stability, and
 - is a natural choice as the basis for further extensions.

Constrained SUKF



(a) Unconstrained sigma points



(b) Constrained sigma points

- Sigma points are chosen in such a way that accommodates boundaries or inequality constraints such as: $X \geq 0$
- Weights are also adjusted accordingly.

- We select the sigma points within the constraint boundary

$$L(k) \leq x(k) \leq U(k)$$

- Define the direction of the sigma points

$$s = [\sqrt{P} \quad -\sqrt{P}]$$

- The sigma points are initialised as

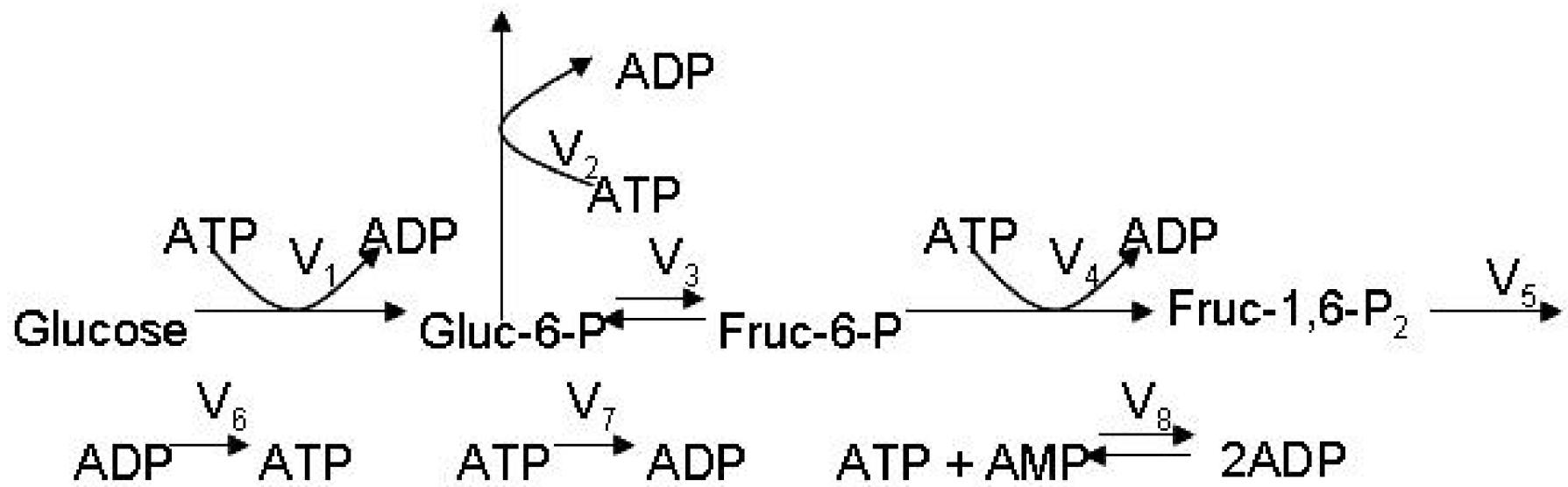
$$\chi(k) = \begin{cases} \hat{x}(k-1) & , j=0 \\ \hat{x}(k-1) + \zeta_j \text{col}_j(S) & , 1 \leq j \leq 2n \end{cases}$$

- Step sizes are defined as

$$\zeta_j = \min(\text{col}_j(\Theta)) \quad , 1 \leq j \leq 2n$$

$$\Theta_{i,j} \equiv \begin{cases} \sqrt{n+\lambda} & , S_{i,j} = 0 \\ \min(\sqrt{n+\lambda}, \frac{U_i(k) - \hat{x}_i(k-1)}{S_{i,j}}) & , S_{i,j} > 0 \\ \min(\sqrt{n+\lambda}, \frac{L_i(k) - \hat{x}_i(k-1)}{S_{i,j}}) & , S_{i,j} < 0 \end{cases}$$

Schematic diagram of the simplified Glycolysis model



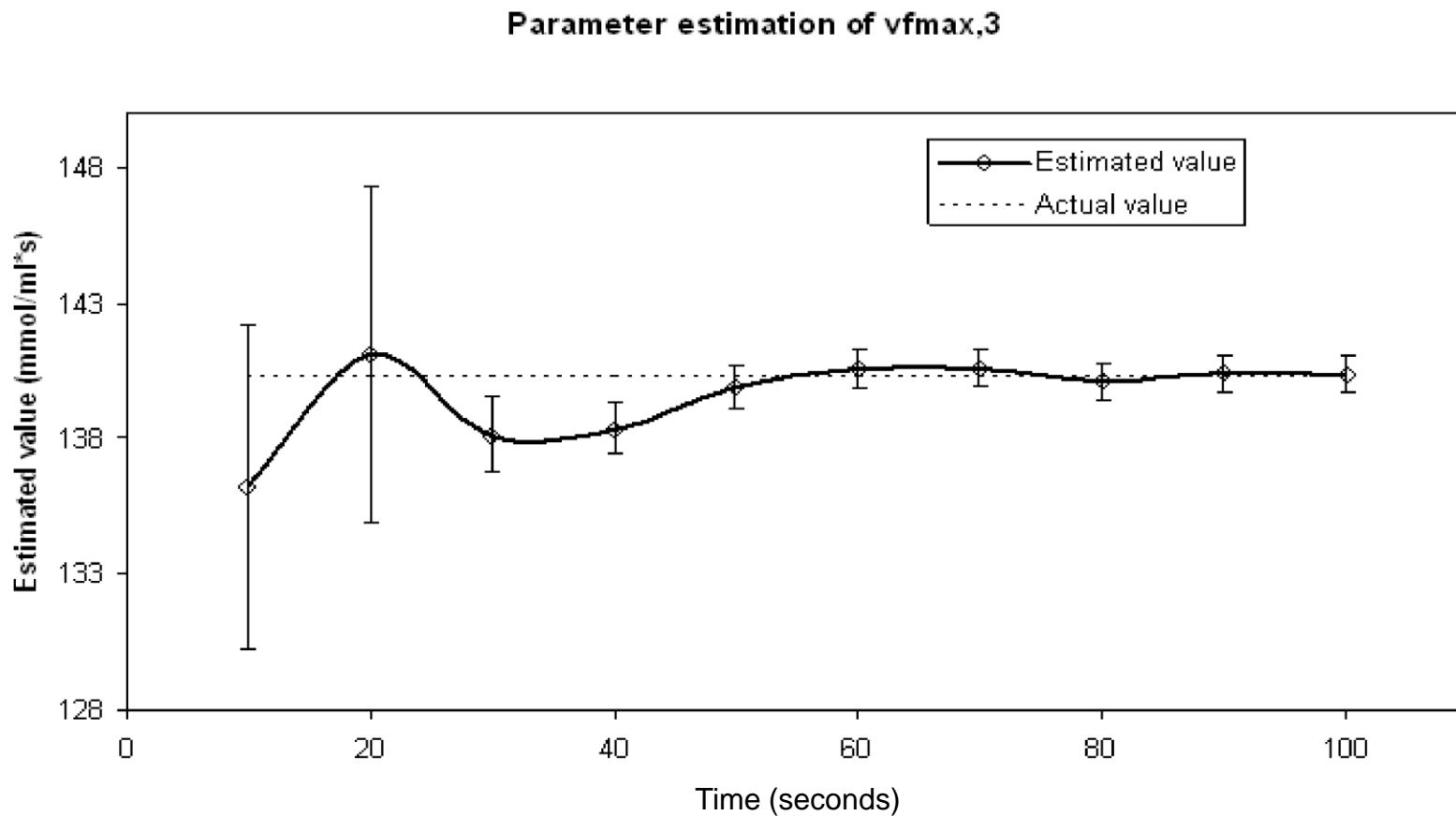
Ref: Hynne *et al.*. Biophysical Chemistry (2001), 94, 121-163

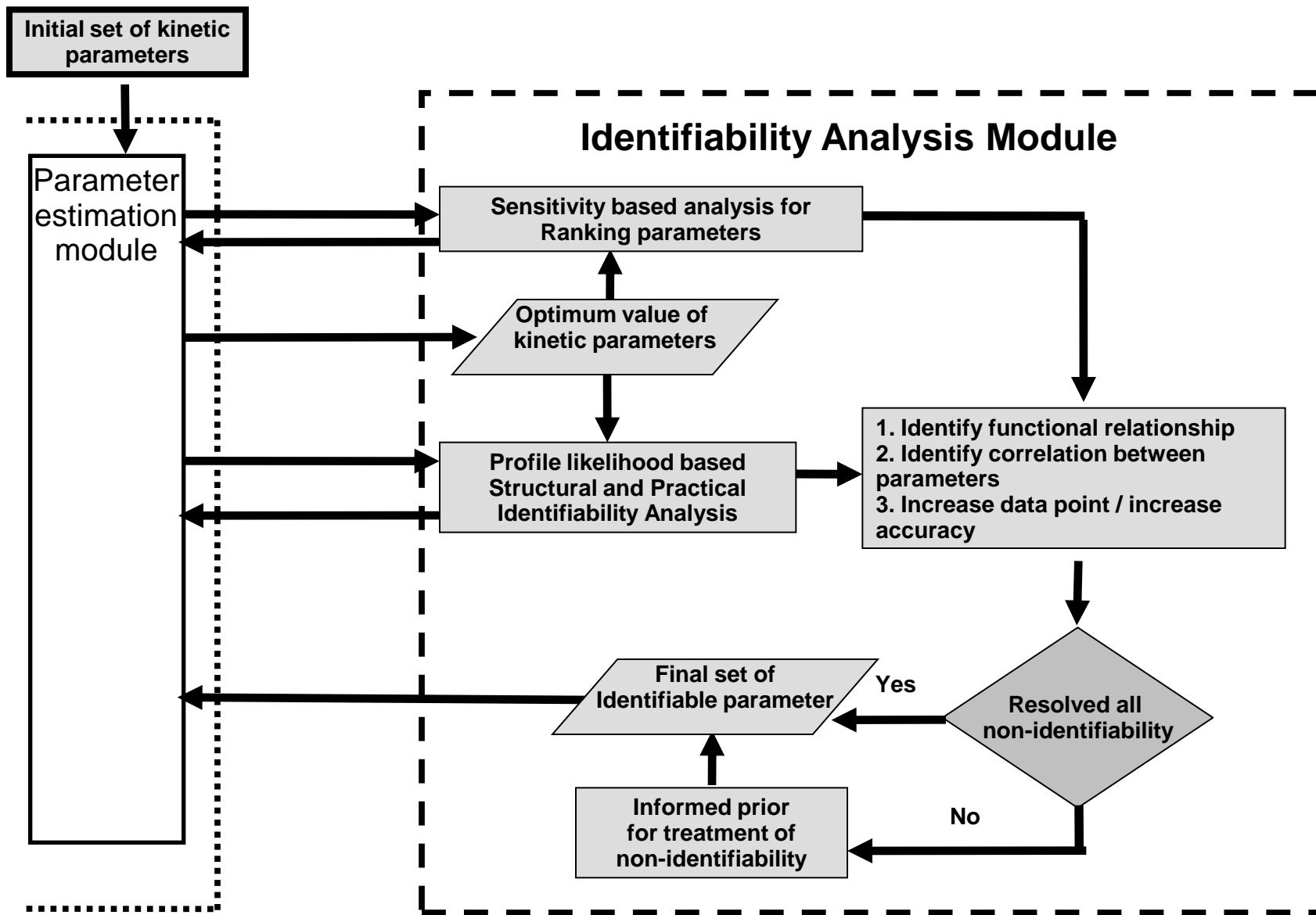
Parameter Name	Actual Value	Estimation	
		Average	Std. Dev.
k2	2.26	2.26	0.076
Vfmax,3	140.28	140.23	0.592
Vmax,4	44.72	44.74	0.266
k8r	133.33	133.33	0.387

Table: Summary statistics of the parameter estimation values obtained from CSUKF. For each estimated parameter, the mean and standard deviation are calculated from 100 runs.

Trajectory of the parameter estimation of $V_{\max,3}^f$.

- Showing standard deviations at ten second intervals.



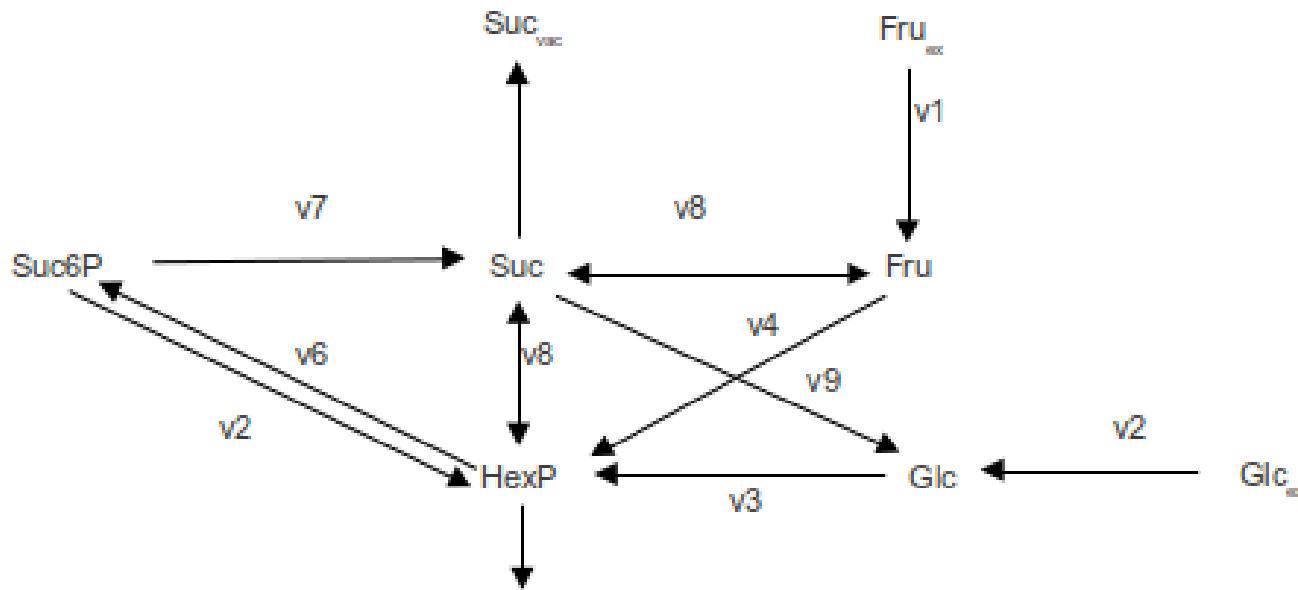


Sensitivity Coefficient Matrix

- Calculate the rate of change of each metabolite with respect to the rate of change of each parameter (a partial differential equation).
- Form a matrix with the values from the partial differential equations.
 - Each row corresponds to a metabolite.
 - Each column corresponds to a parameter.
- Parameter having the highest influence on the metabolite will have the highest value in its column.

$$Z = \frac{\partial \mathbf{X}}{\partial \theta} = \begin{bmatrix} z_{11} & z_{12} & \cdots & z_{1n} \\ z_{21} & z_{22} & \cdots & z_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ z_{n1} & z_{n2} & \cdots & z_{nn} \end{bmatrix}$$

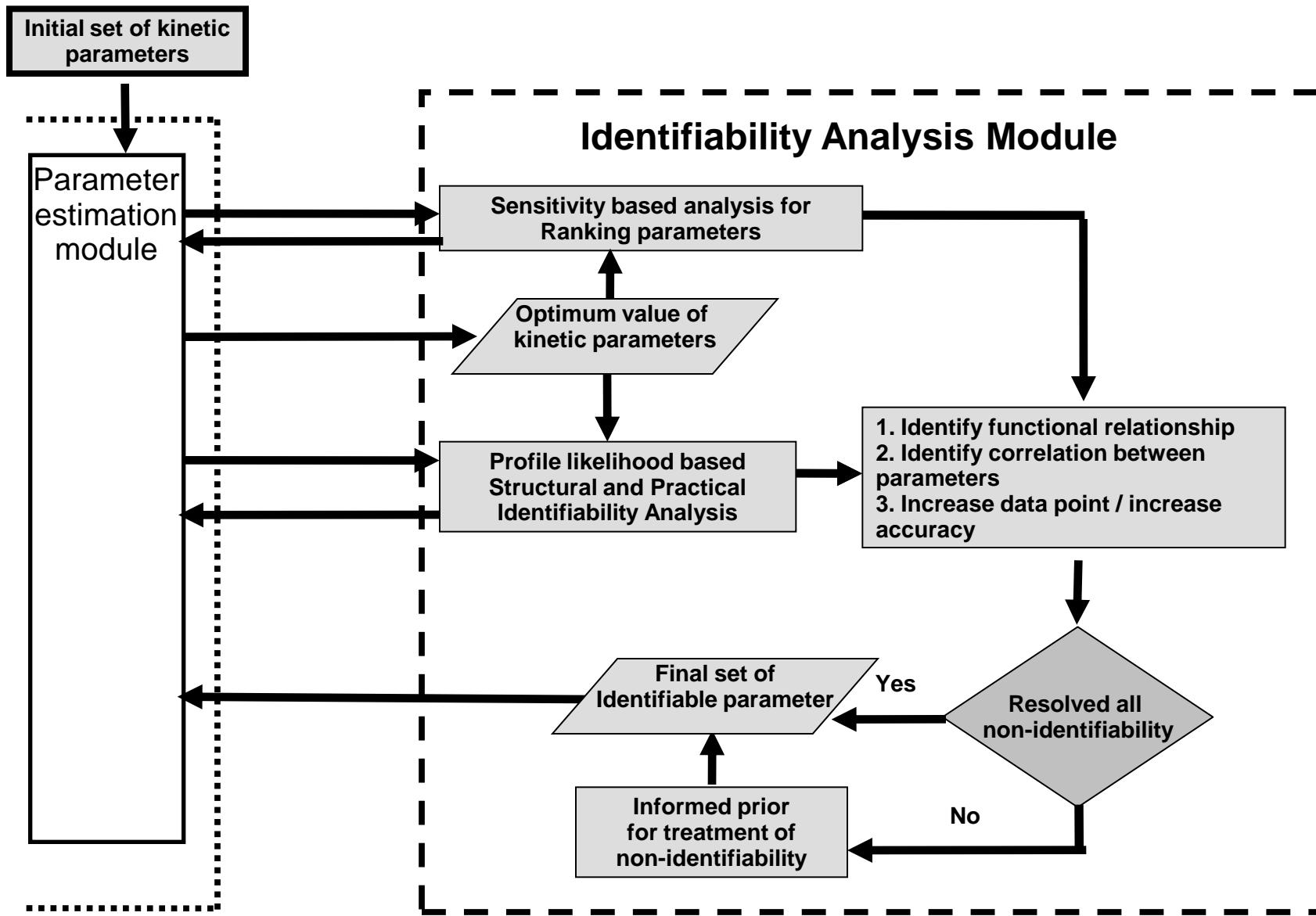
Schematic diagram of sucrose accumulation of sugarcane model



Ref: Rohwer et al. Biochemical Journal (2001), 358(2), 437–445

Parameter Name	CSUKF		Ranking
	Mean	S.D.	
Ki1Fru	1.00	0.01	4
Ki2Glc	1.00	0.01	9
Ki3G6P	0.67	1.46	5
Ki4F6P	0.63	0.85	NI
Ki6Suc6P	0.45	0.77	8
Ki6UDPGlc	0.32	0.40	3
Vmax6r	0.34	0.67	1
Km6UDP	4.73	3.45	6
Km6Suc6P	5.97	4.58	2
Ki6F6P	0.65	1.06	NI
Vmax11	0.28	0.19	7
Km11Suc	21.43	21.82	NI

Table: The mean and standard deviation of the estimated parameters is calculated from 50 repetitions. The ranking is chosen to be the most commonly occurring rankings from the 50 runs. The NI stands for Non-identifiable.



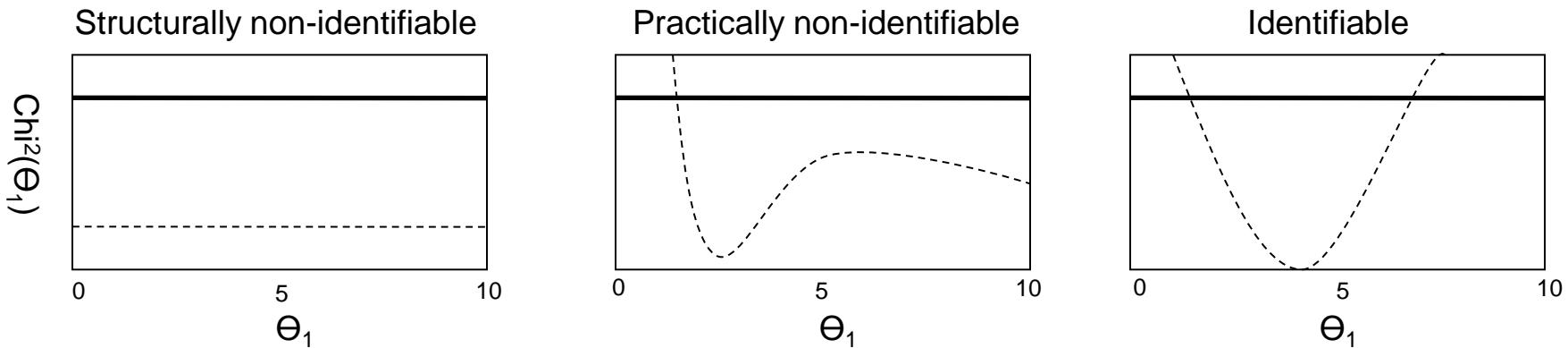
Question: Is it possible to estimate parameters?

Applied identifiability analysis as observability analysis becomes too complicated with large biological models

Based on:

- The model structure and parameterization of the model.
 - *Structural identifiability.*
- The experimental data used for estimation.
 - *Practical identifiability.*
 - Considers both the time step and measurement error.

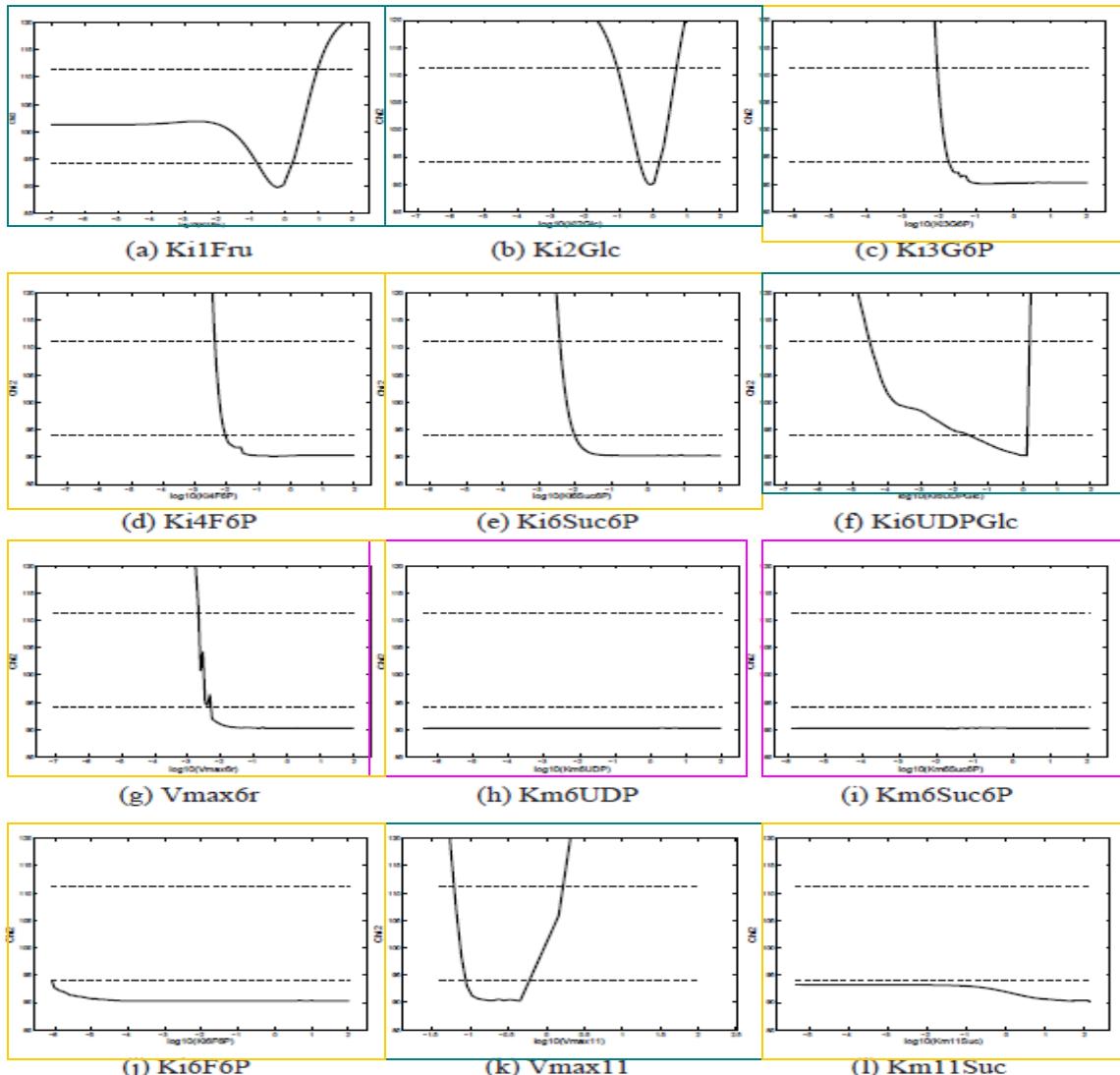
The ***profile likelihood*** of a set of parameter values is the ***probability*** that those values (with one fixed) could give rise to the observed measurements.



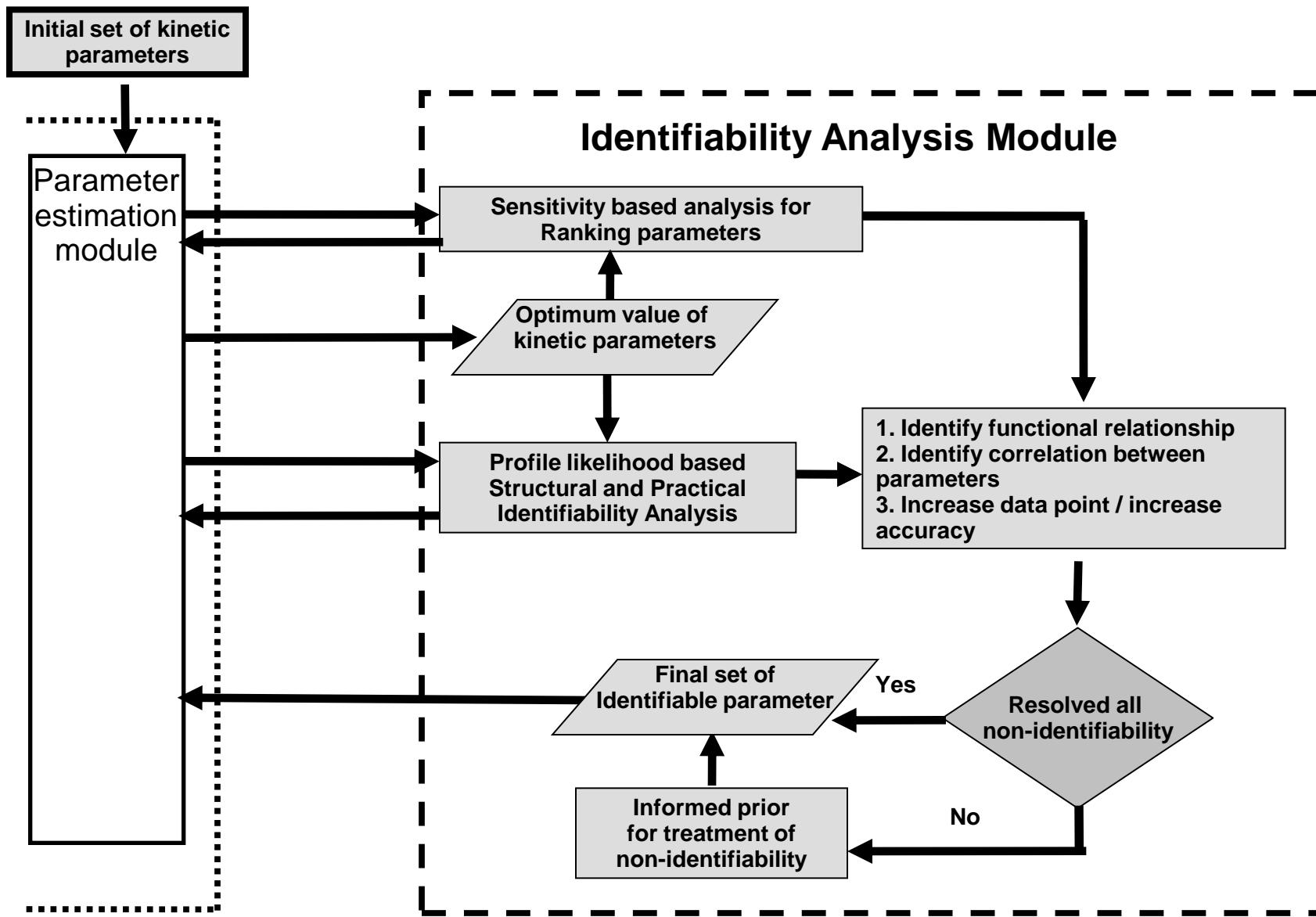
- Structural non-identifiability manifests a flat profile likelihood, entirely below the χ^2 threshold.
- With practical non-identifiability the likelihood crosses the χ^2 threshold exactly once and flattens out as $\Theta_1 \rightarrow \infty$.
- An identifiable parameter manifests in a likelihood with a parabolic shape crossing the χ^2 threshold exactly twice.

Identifiability analysis result

Profile likelihood based identifiability analysis.



- Structural non-identifiability
 - Obtain measurement data to change the mapping function
 - Determine the functional relationship between the parameters
- Practical non-identifiability
 - Increase number of data points in the measurement data
 - Increase the accuracy of the measurement data



Determining the relationship

- Identify correlated parameters

- Obtain the covariance matrix from CSUKF as

$$P_{\theta} = VV^T$$

- Calculate the correlation coefficient between θ_i and θ_j as

$$\text{corr}(i, j) = \frac{P_{(i, j)}}{\sqrt{P_{(i, i)}P_{(j, j)}}}$$

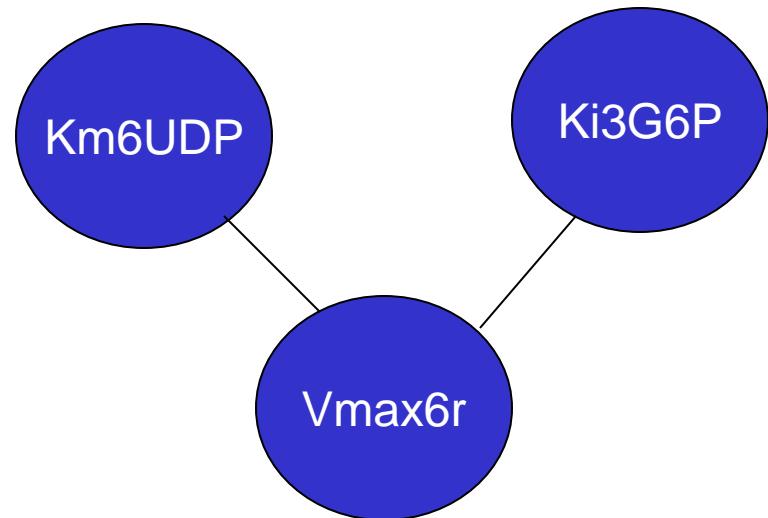
- Identify non-linear functionally related parameters

- Use the test function of the Mean Optimal Transformation Approach (MOTA) that uses the Alternating Conditional Expectation as

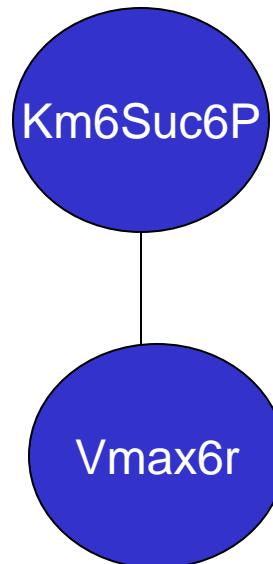
$$\xi(Y^{ace}) = \alpha + \sum_{i=1}^n \phi(X_i^{ace}) + \varepsilon$$

- MOTA interprets this transformation as a functional relationship.

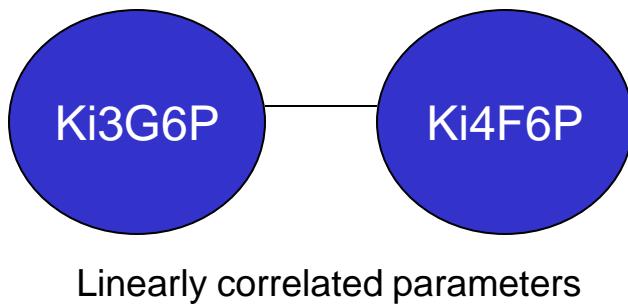
Determining Relationship



Related parameters when applied with K_m6UDP



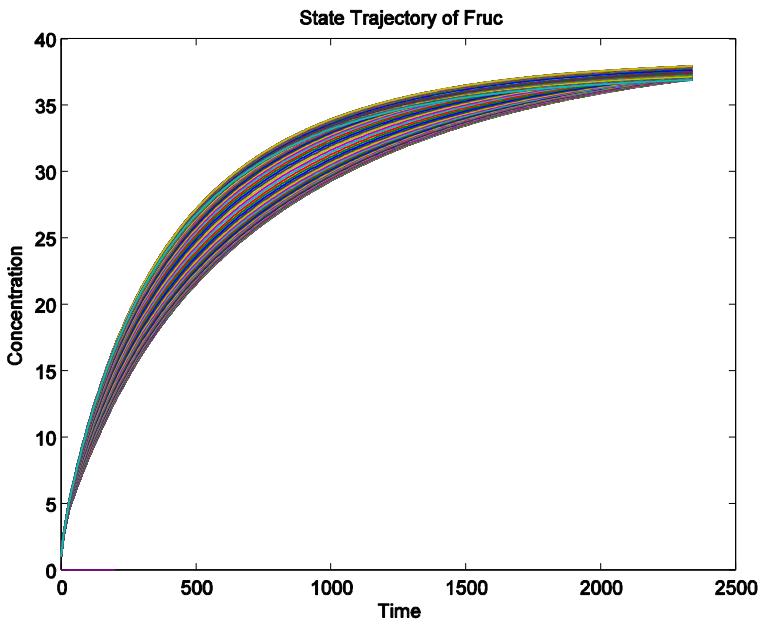
Related parameters when applied with $K_m6Suc6P$



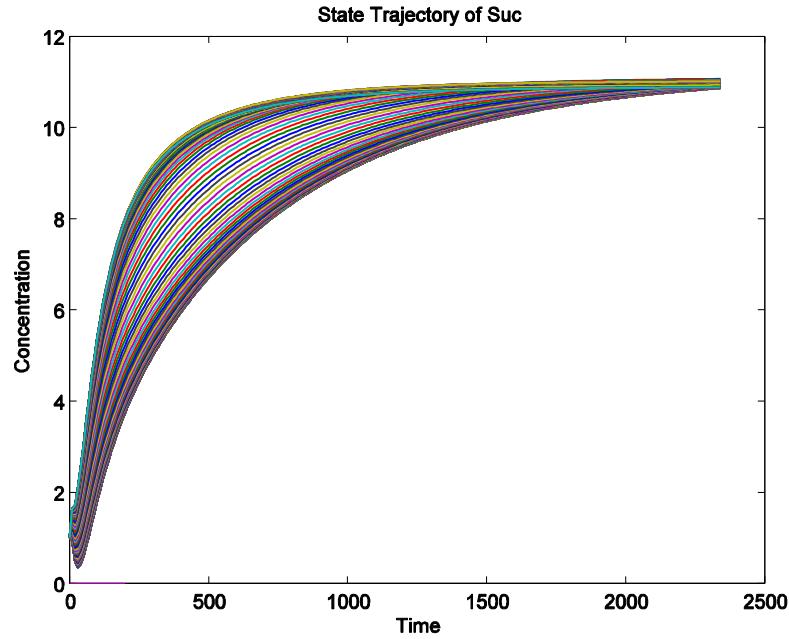
Linearly correlated parameters

Determining points of measurement data

Figure: Trajectory along the values of Km11Suc used during the calculation of the profile likelihood. Places of larger variability denotes points where measurement of a species would efficiently estimate the parameter



a) Trajectory of Fruc

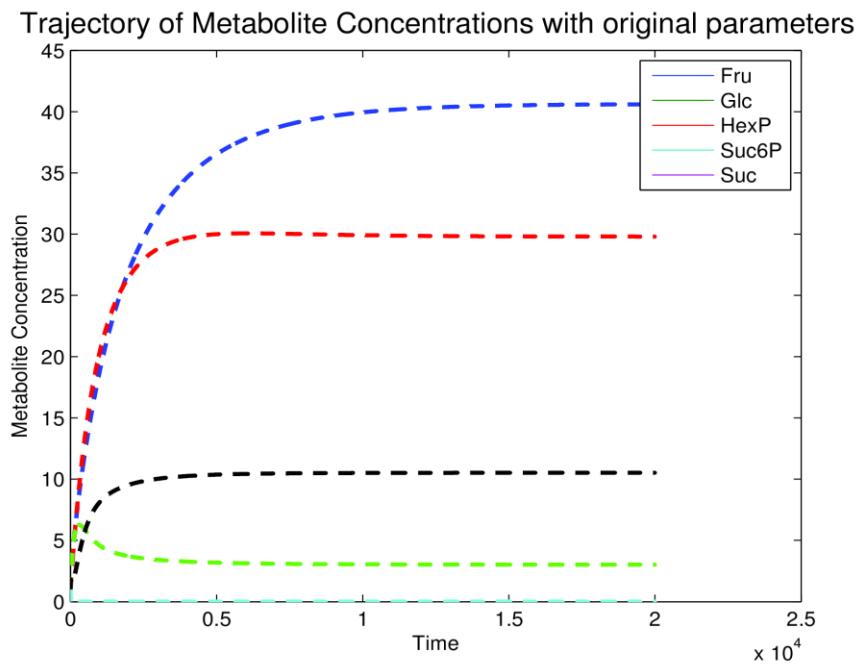
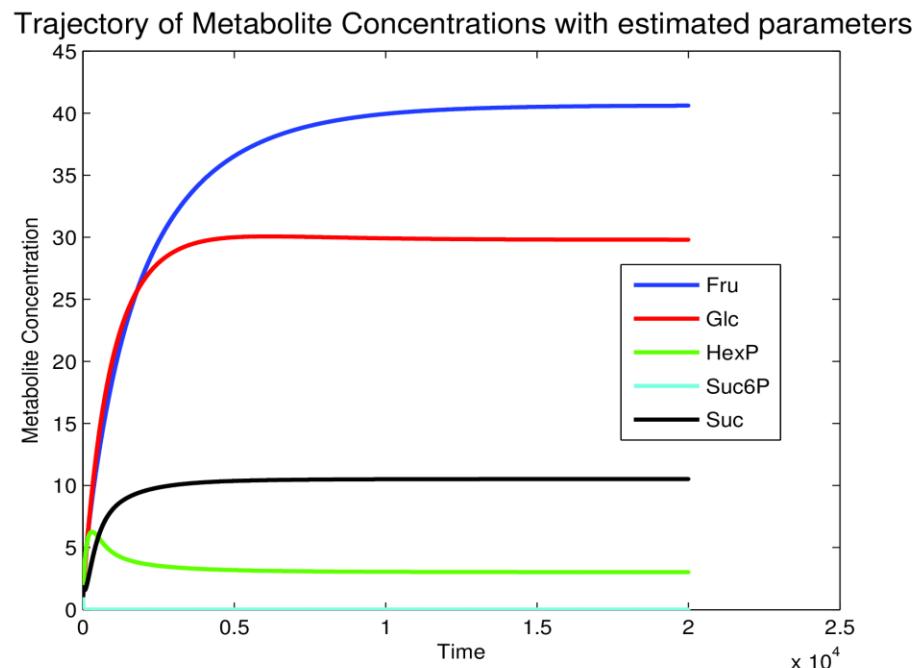


b) Trajectory of Suc

Parameter Name	Obtained	Estimated Value	Original Value
Ki1Fru	Estimated	0.99	1.0
Ki2Glc	Estimated	1.0	1.0
Ki3G6P	Estimated	0.10	0.10
Ki4F6P	Measured	10.00	10.00
Ki6Suc6P	Estimated	0.05	0.07
Ki6UDPGlc	Estimated	1.16	1.4
Vmax6r	Measured	0.20	0.20
Km6UDP	Estimated	0.40	0.30
Km6Suc6P	Estimated	0.16	0.10
Ki6F6P	Measured	0.40	0.40
Vmax11	Estimated	0.99	1.0
Km11Suc	Estimated	99.59	100.0

Table: Final parameter values after solving all non-identifiability problems. To achieve this, three non-identifiable parameters (Ki4F6P, Vmax6r and Ki6F6P) were explicitly measured and the rest were estimated.

System Dynamics



Using the informed prior

- A parameter vector θ with two elements $\beta^{(1)}, \beta^{(2)}$

- With two sets of θ

$$\theta_1 = \{\beta_1^{(1)}, \beta_1^{(2)}\} \quad \text{and} \quad \theta_2 = \{\beta_2^{(1)}, \beta_2^{(2)}\}$$

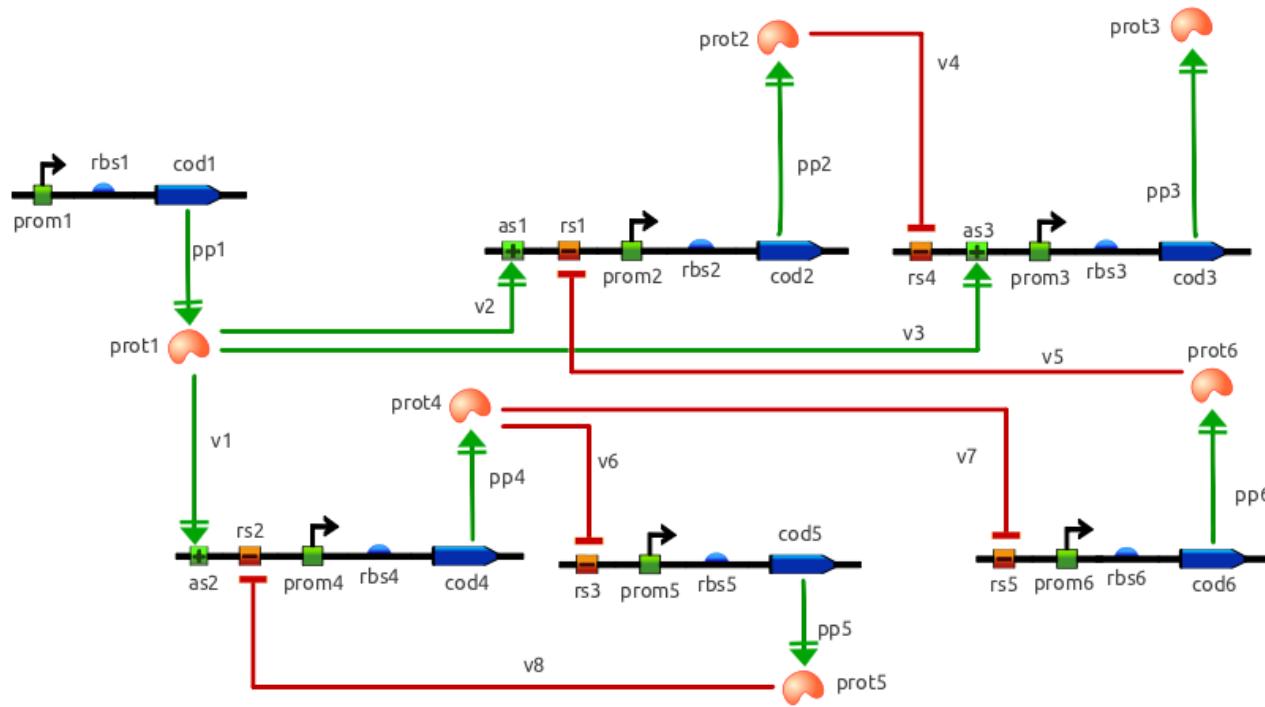
- If the likelihood function is of $\beta^{(1)} + \beta^{(2)}$ then it is non-identifiable.
- If an informed prior assigns $\beta^{(1)} = y$ with probability one then $\theta_1 = \theta_2$ is possible if and only if $\beta_1^{(2)} = \beta_2^{(2)}$
- This makes the model identifiable.
- Both the P and Q matrices along with the mean value of CSUKF is used to introduce this informed prior.

Parameter Name	Original Value	Estimated value	Std. Dev.
Ki1Fru	1.00	1.00	0.010
Ki2Glc	1.00	1.00	0.010
Ki3G6P	0.10	0.16	0.008
Ki4F6P	10.00	6.26	1.160
Ki6Suc6P	0.07	0.25	0.001
Ki6UDPGlc	1.40	0.14	0.001
Vmax6r	0.20	0.07	0.000
Km6UDP	0.30	4.69	0.550
Km6Suc6P	0.10	3.49	0.010
Ki6F6P	0.40	0.93	0.005
Vmax11	1.00	1.03	0.002
Km11Suc	100.00	104.64	2.120

Table: Result of all the 12 parameter estimation using informed prior. 100 runs of the estimation was made to calculate the statistics.

Gene regulatory network

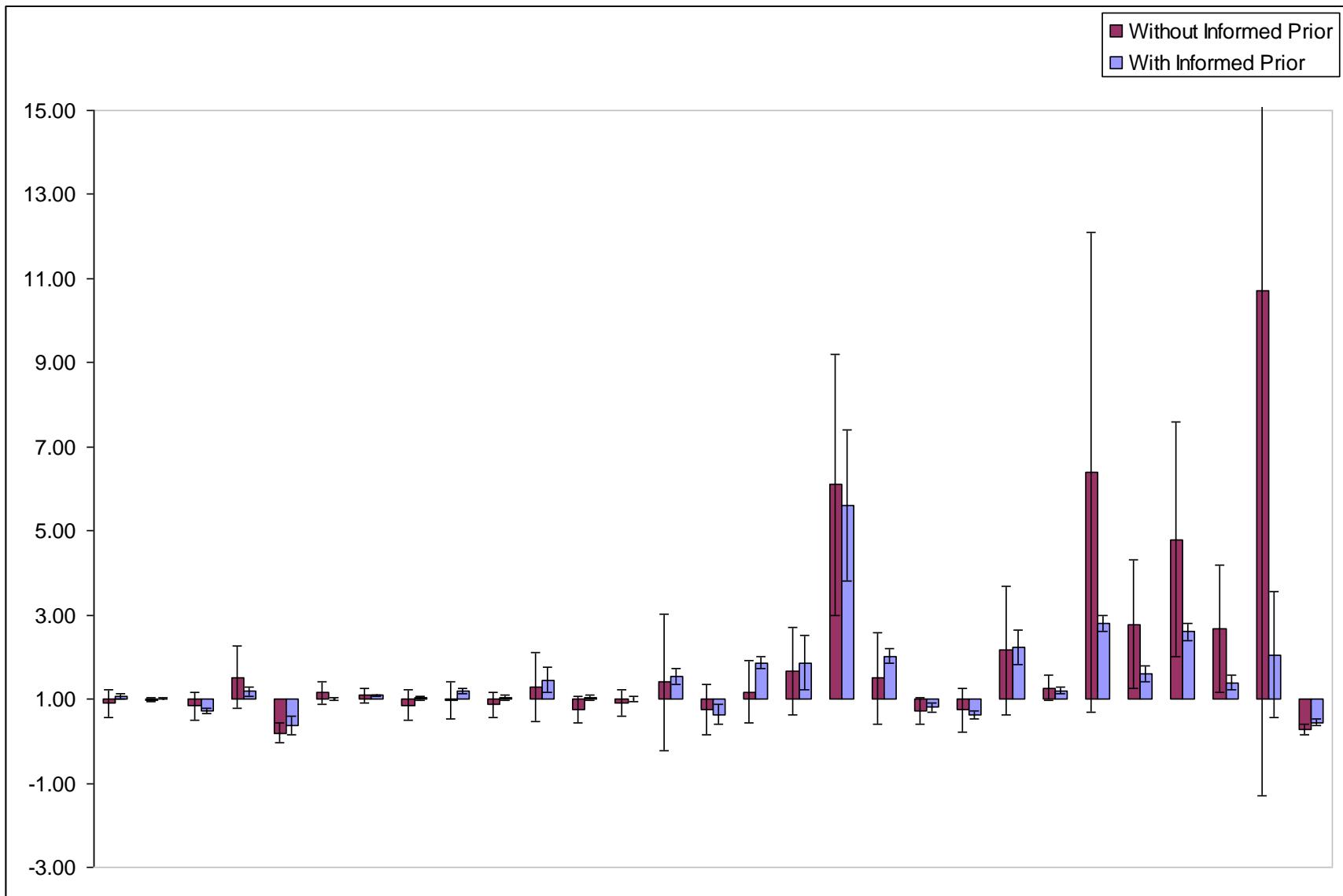
Schematic diagram of gene regulatory network



Ref: <http://www.the-dream-project.org/challenges/dream6-estimation-model-parameters-challenge>

- A two phase experiment was designed with mutant and wildtype data.
- First phase experiment
 - Mutant data with high RBS4 activity is used.
 - Divided into two stages:
 - In the first stage the P and Q matrices are initialized with small random number.
 - In the second stage the P and Q matrices are initialized based on the ranking of the parameters calculated in the first stage.
- Second phase experiment
 - Wild type data is used.
 - The mean and covariance calculated at the first phase is used to form the informed prior for the second phase.

Sugarcane Model



- Apply the framework to calculating fluxes with data from dynamic ^{13}C labeling experiments,
- Enhance accuracy when used with informed prior, and
- Find new targets for metabolic engineering.



Acknowledgement

- Thanks to:
 - Dr. Björn Junker
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 - Prof. Falk Schreiber
 - All the members of Systems Biology Group
 - BMBF for funding



Bundesministerium
für Bildung
und Forschung



To you for your kind attention and patience



- We select the sigma points within the constraint boundary

$$L(k) \leq x(k) \leq U(k)$$

- Define the direction of the sigma points

$$S = [\sqrt{P} \quad -\sqrt{P}]$$

- The sigma points are initialised as

$$\chi(k) = \begin{cases} \hat{x}(k-1) & , j=0 \\ \hat{x}(k-1) + \zeta_j \text{col}_j(S) & , 1 \leq j \leq 2n \end{cases}$$

- Step sizes are defined as

$$\zeta_j = \min(\text{col}_j(\Theta)) \quad , 1 \leq j \leq 2n$$

$$\Theta_{i,j} \equiv \begin{cases} \sqrt{n+\lambda} & , S_{i,j} = 0 \\ \min(\sqrt{n+\lambda}, \frac{U_i(k) - \hat{x}_i(k-1)}{S_{i,j}}) & , S_{i,j} > 0 \\ \min(\sqrt{n+\lambda}, \frac{L_i(k) - \hat{x}_i(k-1)}{S_{i,j}}) & , S_{i,j} < 0 \end{cases}$$

- The weight varies linearly with the step size

$$W_0 = b$$

$$W_j = a\zeta_j + b \quad , 1 \leq j \leq 2n$$

The values of a and b are

$$a = \frac{n}{(n+\lambda)} \frac{(1-2\lambda)}{\sum \zeta_j}$$

$$b = \frac{\lambda}{(n+\lambda)}$$

- The covariance matrix of regular UKF can be written as

$$P(k) = \left(\sum_{j \in l^+} \sqrt{W_j^C} (\chi_j(k-1) - \hat{x}(k)) \sqrt{W_j^C} (\chi_j(k-1) - \hat{x}(k))^T + \sqrt{Q} \sqrt{Q}^T \right) - \\ \left(\sum_{j \in l^-} \sqrt{|W_j^C|} (\chi_j(k-1) - \hat{x}(k)) \sqrt{|W_j^C|} (\chi_j(k-1) - \hat{x}(k))^T \right)$$

$$P(k) = V^{pos}(k)(V^{pos}(k))^T - V^{neg}(k)(V^{neg}(k))^T$$

- The weights vary in magnitude and sign, due to asymmetric nature of sigma points, we decompose square-root factor into two parts

Initialization

The state estimation is initialized with the expected value of the state vector, and an initial square-root factor of the estimation covariance matrix is calculated.

$$\hat{x}(0) = E[x(0)]$$

$$V(0) = \text{chol}\left\{E[(x(0) - \hat{x}(0))(x(0) - \hat{x}(0))^T]\right\}$$

For $k \in \{1 \dots T\}$:

The sigma points, χ , are calculated so as to satisfy the constraints $L(k) \leq x(k) \leq U(k)$

$$\chi(k-1) = \begin{cases} \hat{x}(k-1) & , j=0 \\ \hat{x}(k-1) + \zeta_j \cdot \text{col}_j(S) & , 1 \leq j \leq 2n \end{cases}$$

where χ is based on the direction, $S = [V(k-1) \quad -V(k-1)]$ and step size, ζ

$$\zeta_j = \min(\text{col}_j(\Theta)) \quad , 1 \leq j \leq 2n$$

$$\sqrt{n + \lambda} \quad , S_{i,j} = 0$$

$$\text{and, } \Theta_{i,j} \equiv \begin{cases} \min(\sqrt{n + \lambda}, \frac{U_i(k) - \hat{x}_i(k-1)}{S_{i,j}}) & , S_{i,j} > 0 \\ \min(\sqrt{n + \lambda}, \frac{L_i(k) - \hat{x}_i(k-1)}{S_{i,j}}) & , S_{i,j} < 0 \end{cases}$$

The constrained mean and covariance weights are calculated, also based on the step size

$$\begin{aligned} W_0^M &= b \\ W_0^C &= b + (1 - \alpha^2 + \beta) \\ W_j^M &= W_j^C = a\zeta_j + b \quad , 1 \leq j \leq 2n \end{aligned}$$

where,

$$a = \frac{n}{(n + \lambda)} \frac{(1 - 2\lambda)}{\sum \zeta_j}$$

$$b = \frac{\lambda}{(n + \lambda)}$$

Time Update

$$\chi^x(k) = f(\chi^x(k-1))$$

$$\hat{x}^-(k) = W^M (\chi^x(k))^T$$

$$G^x(k) = qr \left\{ \begin{bmatrix} \sqrt{W_j^C} (\chi_j^x(k) - \hat{x}^-(k)) & \sqrt{Q} \end{bmatrix}_{j \in l^+} \right\} \quad , l^+ = \{ j \mid W_j^C \geq 0 \}$$

$$V_x^{neg}(k) = \left[\begin{bmatrix} \sqrt{|W_j^C|} (\chi_j^x(k) - \hat{x}^-(k)) \end{bmatrix}_{j \in l^-} \right] \quad , l^- = \{ j \mid W_j^C < 0 \}$$

The prior Cholesky factor is found by performing a downdate of the positive and negative square roots

$$V_x^-(k) = cholupdate(G^x(k), V_x^{neg}(k), '-')$$

Measurement Update

To incorporate the additive process noise, R , in the measurement update stage, sigma points are redrawn and unconstrained weights are calculated

$$\chi(k) = \left[\hat{x}^-(k) \quad \hat{x}^-(k) \pm \sqrt{(n + \lambda)}(V_x^-(k)) \right]$$

$$W_0^M = b$$

$$W_0^C = b + (1 - \alpha^2 + \beta)$$

$$W_j^M = W_j^C = b \quad , 1 \leq j \leq 2n$$

Now the measurement update can be performed

$$\chi^y(k) = h(\chi(k))$$

$$\hat{y}^-(k) = W^M(\chi^y(k))^T$$

$$G^y(k) = qr \left\{ \begin{bmatrix} \sqrt{W_j^C}(\chi_j^y(k) - \hat{y}^-(k)) & \sqrt{R} \end{bmatrix}_{j \in l^+} \right\} \quad , l^+ = \{ j \mid W_j^C \geq 0 \}$$

$$V_y^{neg}(k) = \left[\begin{bmatrix} \sqrt{|W_j^C|}(\chi_j^y(k) - \hat{y}^-(k)) \end{bmatrix}_{j \in l^-} \right] \quad , l^- = \{ j \mid W_j^C < 0 \}$$

The prior Cholesky factor is found by performing a downdate of the positive and negative square roots

$$V_y^-(k) = cholupdate(G^y(k), V_y^{neg}(k), '-')$$

The cross correlation covariance estimation, P^{xy} , may now be calculated

$$P^{xy}(k) = \sum_{j=0}^{2n} W_j^C (\chi_j^x(k) - \hat{x}^-(k)) (\chi_j^y(k) - \hat{y}^-(k))^T$$

From which the posterior state estimation may be calculated

$$\hat{x}(k) = \hat{x}^-(k) + K(k) (y_{meas}(k) - \hat{y}^-(k))$$

where

$$K(k) = \frac{P^{xy}(k)}{(\hat{V}_y^-(k))^T \hat{V}_y^-(k)}$$

y_{meas} are the actual measurement values. Finally the square root factor of the estimation covariance is updated

$$V(k) = cholupdate(V_x^-(k), K(k) V_y^-(k), '-')$$

Sugarcane Model

Parameter Name	Actual value	Without Informed Prior				Parameter Name	Actual value	Without Informed Prior					
		With Informed Prior		Without Informed Prior				With Informed Prior		Without Informed Prior			
		Estimate	Std. Dev.	Estimate	Std. Dev.			Estimate	Std. Dev.	Estimate	Std. Dev.		
p_degradation_rate	0.80	0.85	0.05	0.72	0.27	v1_Kd	1.00	1.54	0.18	1.40	1.62		
pro1_strength	3.00	3.04	0.05	2.94	0.15	v1_h	4.00	2.54	0.92	2.98	2.36		
pro2_strength	8.00	5.85	0.47	6.66	2.58	v2_Kd	1.00	1.87	0.15	1.17	0.74		
pro3_strength	6.00	7.12	0.68	9.14	4.43	v2_h	2.00	3.74	1.28	3.32	2.09		
pro4_strength	8.00	2.93	1.78	1.50	1.87	v3_Kd	0.10	0.56	0.18	0.61	0.31		
pro5_strength	3.00	3.03	0.07	3.46	0.80	v3_h	2.00	4.05	0.34	2.99	2.20		
pro6_strength	3.00	3.27	0.03	3.27	0.54	v4_Kd	10.00	8.04	1.12	7.17	3.10		
rbs1_strength	3.90	3.98	0.23	3.33	1.41	v4_h	4.00	2.49	0.42	2.97	2.06		
rbs2_strength	5.00	5.94	0.33	4.82	2.18	v5_Kd	1.00	2.22	0.41	2.16	1.52		
rbs3_strength	5.00	5.13	0.32	4.31	1.56	v5_h	1.00	1.20	0.08	1.27	0.29		
rbs4_strength	1.00	1.46	0.29	1.29	0.81	v6_Kd	0.10	0.28	0.02	0.64	0.57		
rbs5_strength	5.00	5.23	0.31	3.77	1.56	v6_h	2.00	3.20	0.39	5.55	3.07		
rbs6_strength	5.00	5.03	0.28	4.55	1.64	v7_Kd	0.10	0.26	0.02	0.48	0.28		
						v7_h	2.00	2.78	0.35	5.34	3.03		
						v8_Kd	0.20	0.41	0.30	2.14	2.40		
						v8_h	4.00	1.77	0.33	1.12	0.50		

Orthogonal based Algorithm

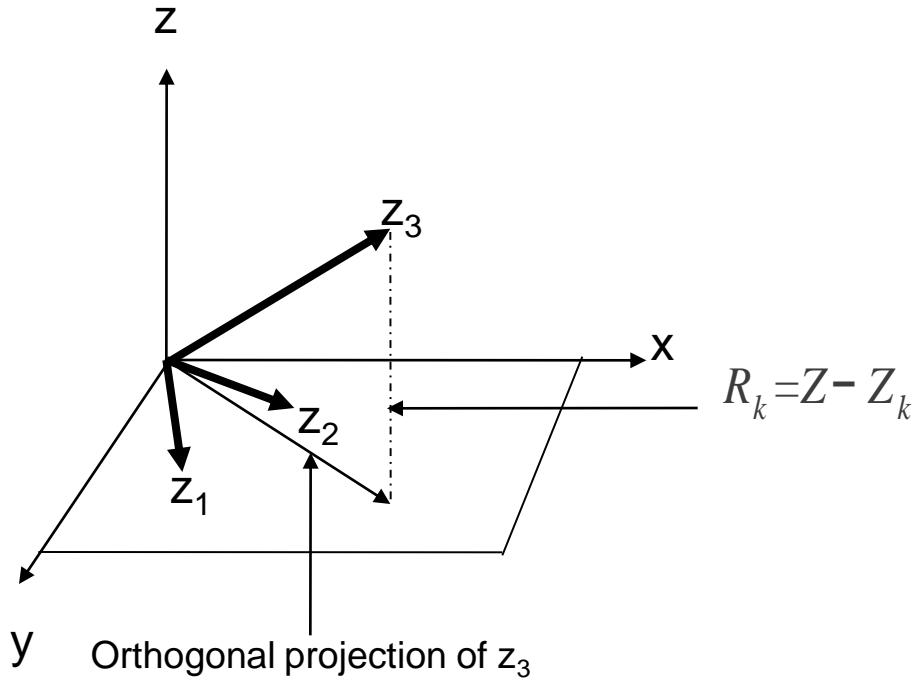


Fig: Orthogonal Projection

Ref: McAuley et. al. Modeling ethylene/butene copolymerization with multi-site catalysts: parameter estimability and experimental design

- Sigma points and corresponding weights are calculated as:

$$X_0 = \bar{x}$$

$$W_0 = \frac{\kappa}{(n+\kappa)}$$

$$X_i = \bar{x} + (\sqrt{(n+\kappa)P_x})_i$$

$$W_i = \frac{1}{2(n+\kappa)}$$

$$X_{i+n} = \bar{x} - (\sqrt{(n+\kappa)P_x})_i$$

$$W_{i+n} = \frac{1}{2(n+\kappa)}$$

- These sigma vectors are propagated through the nonlinear function,

$$Y_i = f [X_i]$$

- The mean and covariance are then derived from the weighted average of the transformed points as:

$$\bar{y} = \sum_{i=0}^{2n} W_i Y_i$$

$$P_{yy} = \sum_{i=0}^{2n} W_i \{Y_i - \bar{y}\} \{Y_i - \bar{y}\}^T$$

- The transformed mean and covariance are then fed into the normal Kalman filter.

CLE approach

Metabolic state	stationary			instationary	
Isotopic state	no labeling	stationary	instationary	no labeling	instationary
Concentrations (intracellular)	c	c	c	c	c
Fluxes	v	v	v	v	v
Isotopomers					
Results	Net-fluxes	Net- and exchange-fluxes	Net- and exchange-fluxes	Enzyme kinetics	Enzyme kinetics