Probabilistic model checking of complex biological pathways

J. Heath, M. Kwiatkowska, G. Norman, D.Parker, O. Tymschyshyn

University of Birmingham

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Motivation and objectives

- to analyze a complex biological pathway of the Fibroblast Growth Factor (FGF)
- to adopt a stochastic modelling approach
- to illustrate the applicability of the probabilistic model checker PRISM
- to create a model of FGF
- to define interesting properties of the model
- to calculate the **exact** quantitative measure of these properties

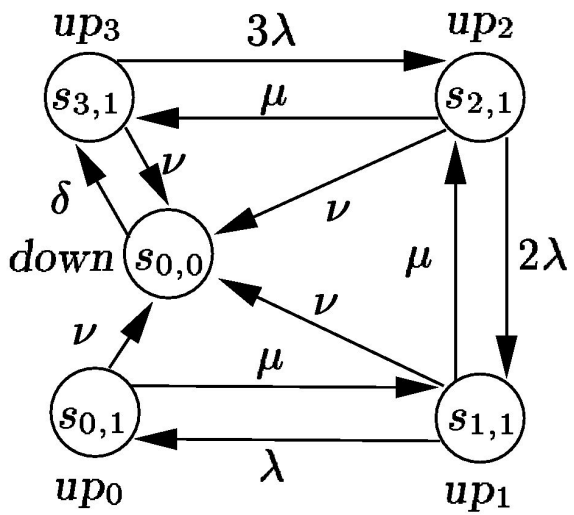
Reaction rules for the pathway of FGF

1. FGF binds to FGFR
FGF+FGFR $\leftrightarrow$ FGFR:FGF (k_{on} = 5e+8M^{-1}s^{-1}, k_{off}=1e−1s^{-1})
2. Whilst FGFR:FGF exists
FGFR Y653 $\rightarrow$ FGFR Y653P (k_{cat}=0.1s^{-1})
FGFR Y654 $\rightarrow$ FGFR Y654P (k_{cat}=0.1s^{-1})
3. When FGFR Y653P and FGFR Y654P
FGFR Y463 $\rightarrow$ FGFR Y463P (k_{cat}=70s^{-1})
FGFR Y583 $\rightarrow$ FGFR Y583P (k_{cat}=70s^{-1})
FGFR Y585 $\rightarrow$ FGFR Y585P (k_{cat}=70s^{-1})
FGFR Y766 $\rightarrow$ FGFR Y766P (k_{cat}=70s^{-1})
4. FGFR binds FRS2
FGFR+ FRS2 $\leftrightarrow$ FGFR:FRS2 (k_{on} = 1e+6M^{-1}s^{-1}, k_{off}=2e−2s^{-1})
5. When FGFR Y653P, FGFR Y654P and FGFR:FRS2
FRS2 Y196 $\rightarrow$ FRS2 Y196P (k_{cat}=0.2s^{-1})
FRS2 Y290 $\rightarrow$ FRS2 Y290P (k_{cat}=0.2s^{-1})
FRS2 Y306 $\rightarrow$ FRS2 Y306P (k_{cat}=0.2s^{-1})
FRS2 Y382 $\rightarrow$ FRS2 Y382P (k_{cat}=0.2s^{-1})
FRS2 Y392 $\rightarrow$ FRS2 Y392P (k_{cat}=0.2s^{-1})
FRS2 Y436 $\rightarrow$ FRS2 Y436P (k_{cat}=0.2s^{-1})
FRS2 Y471 $\rightarrow$ FRS2 Y471P (k_{cat}=0.2s^{-1})
6. Reverse when Shp2 bound to FRS2:
FRS2 Y196P $\rightarrow$ FRS2 Y196 (k_{cat}=12s^{-1})
FRS2 Y290P $\rightarrow$ FRS2 Y290 (k_{cat}=12s^{-1})
FRS2 Y306P $\rightarrow$ FRS2 Y306 (k_{cat}=12s^{-1})
FRS2 Y382P $\rightarrow$ FRS2 Y382 (k_{cat}=12s^{-1})
FRS2 Y436P $\rightarrow$ FRS2 Y436 (k_{cat}=12s^{-1})
FRS2 Y471P $\rightarrow$ FRS2 Y471 (k_{cat}=12s^{-1})
FRS2 Y392P $\rightarrow$ FRS2 Y392 (k_{cat}=12s^{-1})
7. FRS2 effectors bind phosphoFRS2:
Src+FRS2 Y196P $\leftrightarrow$ Src:FRS2 Y2196P (k_{on} = 1e+6M^{-1}s^{-1}, k_{off}=2e−2s^{-1})
Grb2+FRS2 Y306P $\leftrightarrow$ Grb2:FRS2 Y306P(k_{on} = 1e+6M^{-1}s^{-1}, k_{off}=2e−2s^{-1})
Shp2+FRS2 Y471P $\leftrightarrow$ Shp2:FRS2 Y471P(k_{on} = 1e+6M^{-1}s^{-1}, k_{off}=2e−2s^{-1})
8. When Src:FRS2 we relocate/remove
Src:FRS2 $\rightarrow$ relocate out ($t_{1/2}$=15min)
9. When Plc:FGFR it degrades FGFR
PLC+FGFRY 766 $\leftrightarrow$ PLC:FGFR 766(k_{on} = 1e+6M^{-1}s^{-1}, k_{off}=2e−2s^{-1})
PLC:FGFR 766 $\rightarrow$ degFGFR ($t_{1/2}$=60min)
10. Spry appears in time-dependent manner:
$\rightarrow$ Spry ($t_{1/2}$=15min)
11. Spry binds Src and is phosphorylated:
Spry+Src $\leftrightarrow$ Spry Y55:Src (k_{on} = 1e+5M^{-1}s^{-1}, k_{off}=1e−4s^{-1})
Spry Y55:Src $\rightarrow$ Spry Y55P:Src (k_{cat}=10s^{-1})
Spry Y55P+Src $\leftrightarrow$ Spry Y55P:Src (k_{on} = 1e+5M^{-1}s^{-1}, k_{off}=1e−4s^{-1})
Spry Y55P+Cbl $\leftrightarrow$ Spry Y55P:Cbl (k_{on} = 1e+5M^{-1}s^{-1}, k_{off}=1e−4s^{-1})
Spry Y55P+Grb2 $\leftrightarrow$ Spry Y55P:Grb2(k_{on} = 1e+5M^{-1}s^{-1}, k_{off}=1e−4s^{-1})
12. phosphoSpry binds Cbl which degrades/removes FRS2
Spry Y55P:Cbl+FRS2 $\leftrightarrow$ FRS-Ubi (k_{cat}=8.5e−4s^{-1})
FRS2-Ubi $\rightarrow$ degFrs2 ($t_{1/2}$=5min)
13. Spry is dephosphorylated by Shp2: (when Shp2 bound to FRS2)
Spry Y55P $\rightarrow$ Spry Y55 (k_{cat}=12s^{-1})
14. Grb2 binds Sos
Grb2+Sos $\leftrightarrow$ Grb2:Sos (k_{on} = 1e+5M^{-1}s^{-1}, k_{off}=1e−4s^{-1})

Modelling of a biological system in PRISM I

- based on simulation-based techniques for discrete stochastic models
- PRISM provides:
  - modelling language – continuous-time Markov chains (CTMCs)
  - extended by rewards associated with states and transitions
  - specification language
  - symbolic approache to probabilistic model checking

Example of CTMCs



$$\mathbf{R} = \begin{pmatrix} 0 & 3\lambda & 0 & 0 & \nu \\ \mu & 0 & 2\lambda & 0 & \nu \\ 0 & \mu & 0 & \lambda & \nu \\ 0 & 0 & \mu & 0 & \nu \\ \delta & 0 & 0 & 0 & 0 \end{pmatrix} \quad \text{and} \quad \mathbf{E} = \begin{pmatrix} 3\lambda+\nu \\ 2\lambda+\mu+\nu \\ \lambda+\mu+\nu \\ \mu+\nu \\ \delta \end{pmatrix}$$

- R - rate matrix
- E - total rate matrix - $E(s) = \sum_{s' \in S} R(s, s')$
- Probability of leaving s within t time units: $1 - e^{-E(s) \cdot t}$
- Probability of transition from s to s' within t time units: $P(s, s', t) = \frac{R(s,s')}{E(s)} \cdot (1 - e^{-E(s) \cdot t})$

Modelling of a biological system in PRISM II

- protein to protein reactions - complexation, decomplexation and degradation
- model consist of a set of reactive modules
- two alternative approaches to modelling

Reactions:
1. $A+B \longleftrightarrow A{:}B$ (complexation)
2. $A \longrightarrow$ (degradation)

Reaction rates:
- complexation : r_1
- decomplexation : r_2
- degradation : r_3

(a) System of reactions

```
module M
  ab : [0..2] init 1;

  // 0: a degraded, b free 1: a,b free 2: a,b bound
  [] ab=1 → r1 : (ab'=2); // bind
  [] ab=2 → r2 : (ab'=1); // unbind
  [] ab=1 → r3 : (ab'=0); // degrade
endmodule
```

(b) PRISM encoding 1

```
module A
  a : [0..1] init 1;

  [bind] a=1 → r1 : (a'=0);
  [rel]  a=0 → r2 : (a'=1);
  []     a=1 → r3 : (a'=0);
endmodule
```

```
module B
  b : [0..1] init 1;

  [bind] b=1 → (b'=0);
  [rel]  b=0 → (b'=1);
endmodule
```

```
module AB
  ab : [0..1] init 0;

  [bind] ab=0 → (ab'=1);
  [rel]  ab=1 → (ab'=0);
endmodule
```

(c) PRISM encoding 2

```
rewards a=1 : 1; endrewards
```

(d) Reward structure 1

```
rewards [bind] true : 1; endrewards
```

(e) Reward structure 2

Fragment of the PRISM model

```
formula Frs = relocFrs2=0 ∧ degFrs2=0; // FRS2 not relocated or degraded
module FRS2
  FrsUbi     : [0..1] init 0; // ubiquitin modification of FRS2
  relocFrs2  : [0..1] init 0; // FRS2 relocated
  degFrs2    : [0..1] init 0; // FRS2 degraded
  Y196P      : [0..1] init 0;... Y471P : [0..1] init 0; // phosphorylation of receptors
  // compounds bound to FRS2
  FrsFgfr    : [0..1] init 0; // 0: FGFR not bound, 1: FGFR bound
  FrsGrb     : [0..2] init 0; // 0: Grb2 not bound, 1: Grb2 bound, 2: Grb2:Sos bound
  FrsShp     : [0..1] init 0; // 0: Shp2 not bound, 1: Shp2 bound
  FrsSrc     : [0..8] init 0;
  // 0: Src not bound        1: Src bound,          2: Src:Spry
  // 3: Src:SpryP,           4: Src:SpryP:Cbl,      5: Src:SpryP:Grb
  // 6: Src:SpryP:Grb:Cbl,   7: Src:SpryP:Grb:Sos,  8: Src:SpryP:Grb:Sos:Cbl
                                  ...
  // phosphorylation of receptors (5)
  [] Frs∧Y653P=1∧Y654P=1∧FrsFgfr=1∧Y196P=0 → 0.2 : (Y196P′=1); // Y196
                                  ...
  [] Frs∧Y653P=1∧Y654P=1∧FrsFgfr=1∧Y471P=0 → 0.2 : (Y471P′=1); // Y471
  // dephosphorylation of Y196 (6) - remove Src if bound
  []          Frs∧FrsShp=1∧Y196P=1∧FrsSrc=0 → 12 : (Y196P′=0);
  [src_rel]   Frs∧FrsShp=1∧Y196P=1∧FrsSrc>0 → 12 : (Y196P′=0)∧(FrsSrc′=0);
                                  ...
  // dephosphorylation of Y471 (6) - remove Shp2 since bound
  [shp_rel] Frs∧FrsShp=1∧Y471P=1 → 12 : (Y471P′=0)∧(FrsShp′=0);
                                  ...
  // Src:FRS2→degFRS2 [8]
  [] Frs∧FrsSrc>0 → 1/(15*60) : (relocFrs2′=1);
                                  ...
  // Spry55p:Cbl+FRS2→Frs-Ubi [12]
  [] Frs∧FrsSrc=4,6,8 ∧ FrsUbi=0 → 0.00085 : (FrsUbi′=1);
  // FRS2-Ubi→degFRS2 [12]
  [] Frs∧FrsUbi=1 → 1/(5*60) : (degFrs2′=1);
                                  ...
  // Grb2+Sos↔Grb2:Sos [14]
  [sos_bind_frs]  Frs∧FrsGrb=1  → 1      : (FrsGrb′=2);          // Grb:FRS2
  [sos_bind_frs]  Frs∧FrsSrc=5,6→ 1      : (FrsSrc′=FrsSrc+2);// Grb:SpryP:Src:FRS2
  [sos_rel_frs]   Frs∧FrsGrb=2  → 0.0001 : (FrsGrb′=1);          // Grb:FRS2
  [sos_rel_frs]   Frs∧FrsSrc=7,8→ 0.0001 : (FrsSrc′=FrsSrc−2);// Grb:SpryP:Src:FRS2
                                  ...
endmodule
```

Specification of properties I

- extension of Continuous stochastic logic (CSL)

Basic syntax

- $\varphi := tt \mid A \mid \neg\varphi \mid \varphi \wedge \varphi \mid \mathcal{P}_{\bowtie p}(\psi)$
- $\psi := \mathcal{X}(\varphi) \mid \varphi \mathcal{U} \varphi \mid \varphi \mathcal{U}^{I} \varphi$

Steady-state probabilities extension

- $\mathcal{S}_{\bowtie p}(\varphi)$

Reward extension

- $\mathcal{R}_{\bowtie p}(\mathcal{F}\varphi) \mid \mathcal{R}_{\bowtie p}(\mathcal{C}^{\leq T})$
- definition of rewards

Specification of properties II

Examples

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7. $\mathcal{R}_{=?}(\mathcal{F}\ (a = 0 \wedge ab = 0))$ + definition of rewards
8. What is the expected number of times that the proteins A and B bind before A degrades

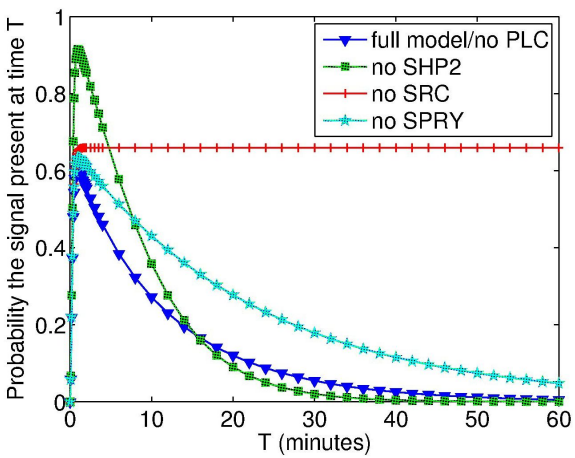
Results I

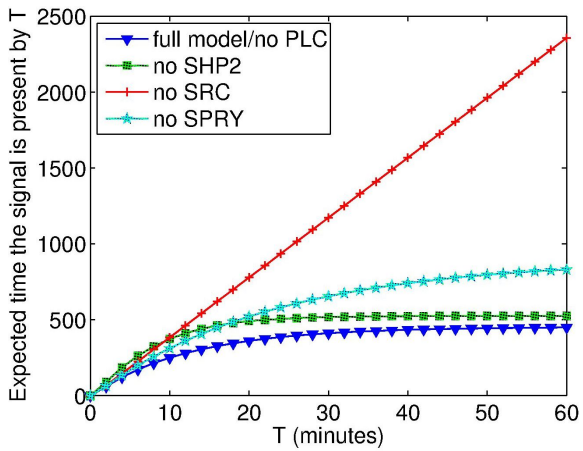
Size of the model

- 80 616 states
- 560 000 transitions

Some inspected properties

1. What is the probability that Grb2 is bound to FRS2 at the time instant T? – $\mathcal{P}_{=?}(\mathit{true}\ \mathcal{U}^{[T,T]}\ a_{grb2})$





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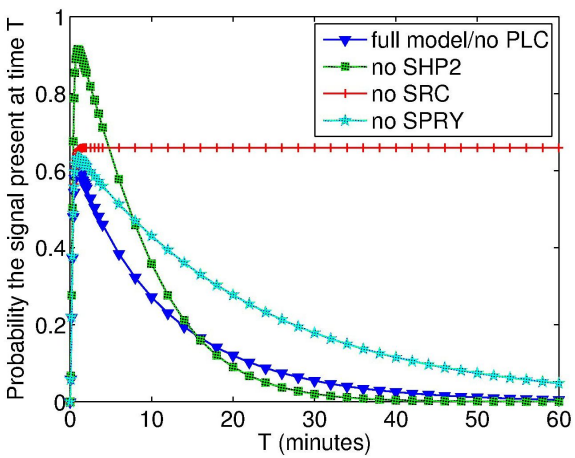

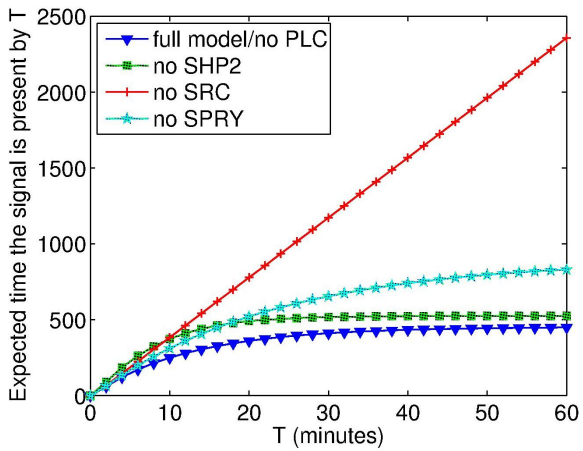

Results II

Long run and expected reachability properties

1. What is the long-run probability that Grb2 is bound to FRS2? – $\mathcal{S}_{=?}(a_{grb2})$

	probability bound	expected no. of bindings	expected time bound (min)
full model	7.54e-7	43.1027	6.27042
no Shp2	3.29e-9	10.0510	7.78927
no Src	0.659460	283.233	39.6102
no Spry	4.6e-6	78.3314	10.8791
no Plc	0.0	51.5475	7.56241

Table 1. Long run and expected reachability properties for the signal

Results II

Long run and expected reachability properties

1. What is the long-run probability that Grb2 is bound to FRS2? – $\mathcal{S}_{=?}\, a_{grb2}$
2. What is the expected number of times Grb2 binds to FRS2 before degradation or relocation occurs? – $\mathcal{R}_{=?}(\mathcal{F}\ (a_{src} \wedge a_{plc} \wedge a_{spry}))$

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Conclusion

- created a model of a complex biological pathway of FGF
- calculated the exact quantitative measure of interesting properties
- illustrated the applicability of the probabilistic model checker PRISM

Thank you for your attention.