A new validated mathematical model of the Wnt signaling pathway predicts effective combinational therapy by sFRP and Dkk

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Short title: Validated model of Wnt pathway

Keywords: Wnt pathway, signal transduction, computational modeling, sFRP, Dkk, ODE
Synopsis
The Wnt signaling pathway controls cell proliferation and differentiation, and its deregulation is implicated in different diseases, including cancer. Learning how to manipulate this pathway could substantially contribute to therapy. We developed a mathematical model describing the initial sequence of events in the Wnt pathway, from ligand binding to β-catenin accumulation, and the effects of inhibitors, such as secreted Frizzled-related proteins (sFRP) and Dickkopf (Dkk). Model parameters were retrieved from experimental data reported in the literature. The model was retrospectively validated by accurately predicting the effects of Wnt3a and sFRP1 on β-catenin level in two independent published experiments ($R^2$ between 0.63 and 0.91). Prospective validation was obtained by testing the model’s accuracy in predicting the effect of Dkk1 on Wnt-induced β-catenin accumulation ($R^2$~0.94). Model simulations under different combinations of sFRP1 and Dkk1 predicted a clear synergistic effect of these two inhibitors on β-catenin accumulation, which may point towards a new treatment avenue. Our model allows precise calculation of the effect of inhibitors applied alone or in combination, and provides a flexible framework for identifying potential targets for intervention in the Wnt signaling pathway.

Introduction
The Wnt signaling pathway controls mechanisms that direct cell proliferation, polarity, and fate determination during embryonic development and in tissue homeostasis. Numerous studies have linked mutations in the Wnt pathway to various diseases, including cancer [1-2]. Many genes in the Wnt pathway, initially discovered as transiently functioning in development, have turned out to be oncogenes and tumor suppressors [3-4]. The special significance of Wnt signaling in stem and progenitor cells implies its role in regulating cancer stem cells (CSCs), which are thought to be associated with relapse, metastasis and drug resistance [5-6].

Significant effort is currently being invested worldwide in the development of therapeutic agents that function by manipulating the Wnt pathway, with particular focus on agents that may provide novel approaches to limiting tumor growth [3, 6-7]. For example, researchers are currently developing anti-cancer therapeutic applications for regulatory proteins, such as sFRP [8-9]. Since the Wnt pathway is involved primarily in embryogenesis and in tissue repair in adults, compounds that affect this pathway are not expected to cause significant toxicity [6].

There are at least 19 Wnt ligands and 10 Frizzled receptors, activating at least three intracellular signaling pathways [4, 10-11]. This vast network is influenced by a wide range of regulators, including two main classes of extracellular inhibitors: those directly interacting with Wnt proteins (e.g. sFRP, WIF) and those binding to Wnt receptors or co-receptors (e.g. Dkk binding to LRP) [12-13]. The complexity of interactions among ligands, antagonists and receptors makes the Wnt signaling pathway amenable to therapeutic intervention at many target points. It is not intuitively clear which component of the system is the best target for therapeutic intervention, or how such interventions should be designed in order to achieve the best clinical outcomes.

Mathematical models based on biological information are effective in improving the understanding of complex signaling pathways and their roles in disease control [14]. Such models are simplified to include only the main components of the signaling pathways, yet ensuring that the fundamental properties of the system are retrieved [15]. Theoretical and numerical analysis of such models may be used to predict
system behavior under various scenarios, and these predictions can then be compared to experimental data ([15]; see [16] for an example of an application of this approach to the MAPK pathway). However, as quantitative information on signal transduction pathways is rarely available, most mathematical models of signal transduction pathways are yet to be experimentally substantiated.

Among the Wnt-activated pathways, the canonical Wnt pathway is the best characterized. It regulates the transcriptional co-activator β-catenin, which controls the expression of specific target genes [4]. To study the dynamical behavior of the intracellular components of this pathway, Lee and colleagues [17] formulated and parameterized a mathematical model using data from experiments in Xenopus extracts. Other researchers further analyzed this model and extended it by adding pathway-related reactions (e.g., [18-21]; see review in [22]). These models focus on the intracellular steps of the canonical Wnt pathway. However, modeling the initial steps of this pathway, i.e., the binding of extracellular ligands and inhibitors to membrane receptors, is essential for elucidating the roles of different Wnt signal inhibitors. Such knowledge could pave the way to the development of innovative therapeutic interventions. For example, a relatively simple model for the Wnt and Notch signaling pathways suggested that exogenous Dkk1 is a potential modulator of stem cell fate decision, a prediction that was verified experimentally in mammary CSCs [23]. However, this model's simplicity, while rendering it analytically tractable, was an impediment to quantitatively evaluating the chemical reactions in these pathways [24].

In this work, we developed a detailed mechanistic model for the extracellular and intracellular parts of the canonical Wnt pathway. The predictive ability of this general model was retrospectively validated using quantitative data from independent experiments, testing the effects of Wnt3a and sFRP on β-catenin accumulation. We also conducted experiments measuring inhibition by Dkk1, which prospectively validated model predictions of this effect. Simulations of the combined effects of sFRP1 and Dkk1 predicted synergism between these inhibitors.

**Materials and methods**

1. **Mathematical model of the Wnt pathway**

1a. **Basic assumptions and kinetic model**

In our model, schematically described in Figure 1, the interactions among the Wnt ligand, its inhibitors, and cell surface receptors are represented in detail, whereas the intracellular part is represented more generally. The model captures the following processes: The pathway is activated by binding of Wnt ligand to Frizzled receptor [25-26]. The resulting receptor-ligand complex may recruit an unoccupied LRP receptor and create a ternary complex, consisting of Wnt, Frizzled and LRP [27-29]. The latter complex transduces the signal inside the cell and interferes with the destruction cycle of β-catenin [30-31].

The intracellular level of β-catenin is regulated by a specific destruction complex comprising Axin, APC and GSK3β. This complex binds β-catenin and causes its phosphorylation. Phosphorylated β-catenin dissociates from the destruction complex and is rapidly degraded [4].

Three assumptions of our model are consistent with those of Lee et al. [17]: (i) Upon dissociation from phosphorylated β-catenin, the destruction complex may bind another β-catenin molecule. (ii) β-catenin is produced at a constant rate. (iii) There exists an additional slow degradation path of β-catenin, independent of the destruction
complex. Lee et al. also assumed that the reactions assembling the destruction complex are at rapid equilibrium, with the exception of GSK3β binding [17]. To further reduce the modeling complexity of the intracellular part, we assume that the destruction complex is at rapid equilibrium with all its components. This implies that its total concentration is constant. Further, we represent β-catenin phosphorylation, dissociation from the complex and degradation as a one-step process.

The regulation of β-catenin destruction by the Wnt signal is carried out via the intracellular domain of the LRP receptor. When bound in ternary complex with Wnt and Frizzled, the LRP receptor binds intracellular Axin [32-33]. For simplicity, our model assumes that the whole destruction complex is bound to the intracellular part of the activated LRP, in line with the above assumption of equilibrium between the complex and its components. We also assume that the binding of the destruction complex to the intracellular domain of LRP is reversible, and upon dissociation, the receptor complex decomposes into its components [34]. The bound destruction complex cannot participate in the β-catenin destruction cycle. Hence, high Wnt concentration leads to the formation of active ternary Wnt-Frizzled-LRP complexes, which reduce β-catenin destruction, allowing its accumulation.

The model includes down-regulation of the pathway by sFRP, which competes with Frizzled for Wnt binding [35-36], and Dkk, which binds to LRP and abolishes formation of the ternary complex [13, 37]. The total concentrations of all system components, except for β-catenin, are assumed to be constant over time of interest.

1b. Model equations
We translated the above description into ordinary differential equations (ODEs), assuming well-mixing and the law of mass action for all the protein interactions. The model describes the dynamics of 13 state variables, representing concentrations of the modeled pathway components by a system of seven ODEs. We close the system by six conservation equations for the total concentrations of Wnt ($W_T$), destruction complex ($C_T$), sFRP ($S_T$) and Dkk ($D_T$) and for the numbers of Frizzled ($F_T$) and LRP ($L_T$) receptors:

$$
\dot{S}_T = k_{1SW} S - k_{1S} S_w ,
$$

$$
\dot{L}_D = k_4 DL - k_4 L_D ,
$$

$$
\dot{F}_T = k_2 FW - k_{2F} F_T - K_{sw-in} k_5 LF_T + k_3 L_F ,
$$

$$
\dot{L}_F = K_{sw-in} k_5 LF_T - k_3 L_F - k_3 L_F C ,
$$

$$
\dot{C}_L = k_3 L_F C - k_3 C_L ,
$$

$$
\dot{C} = -K_{sw-in} k_5 L_F C + K_{sw-in} k_3 C_L - k_3 CB + k_{-6} C_B ,
$$

$$
\dot{B} = k_7 - k_8 B - k_6 CB ,
$$

$$
\dot{C}_B = C_T - C - K_{sw-in} C_L ,
$$

$$
L = L_T - L_F - C_L - L_D ,
$$

$$
F = F_T - F_W - L_F - C_L ,
$$

$$
W = W_T - (F_T + L_F + C_L) \cdot K_{sw-ex} - S_W ,
$$

$$
S = S_T - S_w ,
$$

$$
D = D_T - D_F \cdot K_{sw-ex} .
$$

The modeled variables represent the following: extracellular free Wnt ($W$); Frizzled receptors—free ($F$) and bound to Wnt ($F_W$); free LRP receptors ($L$); ternary receptor...
complexes Frizzled/Wnt/LRP ($L_F$); intracellular destruction complex—free (C), bound by the ternary receptor complex ($C_1$) and bound to β-catenin ($C_b$); intracellular free β-catenin ($B$); extracellular sFRP—free ($S$) and bound to Wnt ($S_W$); extracellular free Dkk ($D$); and LRP receptors bound to Dkk ($L_D$).

Reaction rates are given by the coefficients $k_{5i}$, where $i$ is the reaction step index as shown in Figure 1, and the sign corresponds to the reaction direction. Association reaction rates, $k_i$, have units of $M^{-1}s^1$, while dissociation rates have units of $s^1$. Note that variables in different cell compartments have different units: intracellular and extracellular proteins are measured in molars ($M$), while free or bound receptors in cell membrane are measured in number per cell, receptor/cell. To compute reactions between components measured in different units, we introduced partition coefficients, $K_{su-ex}$ and $K_{su-in}$, translating numbers in cell membrane to extracellular and intracellular concentrations, respectively. These coefficients were determined as follows: $K_{su-ex} = \frac{N_{cells}}{A_v \cdot V_{exp}}$, $K_{su-in} = \frac{1}{A_v \cdot V_{cell}}$, where $N_{cells}$ is the number of cells, $V_{exp}$ is the experimental volume (both depending on experimental conditions), $V_{cell}$ is the intracellular compartment volume and $A_v$ is Avogadro’s number. For the estimation of $K_{su-ex}$ we assumed that experimental volume is a well-mixed external compartment. The estimation of $K_{su-in}$ is based on a similar assumption for intracellular cytoplasmatic volume.

1c. Computer implementation

The model was solved numerically by an ODE solver in MATLAB. Whenever some of the parameters were adjusted, it was performed by repetitive applications of a local search algorithm (trust region) in MATLAB, each time beginning with a random initial guess for the values of adjusted parameters; other parameters were set at literature-based values (see below).

2. Data acquisition from the literature

To fine-tune and subsequently validate the model we used published experimental data on the time course of β-catenin accumulation induced by different Wnt3a concentrations (Figure 4CD in [38]), as well as accumulation of β-catenin after a fixed period of stimulation by Wnt3a and inhibition of this effect by sFRP at different concentrations (Figures 1C and 2 in [39] and our previous work [40], respectively). All the experiments were conducted in L cells, which do not express cadherins; hence, the cytoplasmatic β-catenin is free and not bound to the membrane. This makes these data suitable for comparison with our model, which does not include the binding of β-catenin to cadherins.

For all the published measurements we extracted average values. We also report s.e.m. estimations, whenever available in the relevant publication source.

3. Parameter evaluation

The initial estimation of parameter values and ranges was based on various literature sources (Supplementary data). Then the data extracted from [38] were used to fine-tune the model for Wnt effect. This was done in two steps: (1) adjustment of alternative subsets of model parameters to fit part of the experimental data (denoted "partial training set") and (2) selection of the best-predictive parameter set, using the whole experimental data set.
We formed a partial training set by selecting three of the ten Wnt3a doses experimentally tested in [38] (0, 12.5 and 400 ng/ml). All the time-course measurements of β-catenin accumulation for these doses were included in the partial training set.

At the first step, we considered several alternative choices of a subset of adjusted parameters, focusing on those parameters for which our initial estimation based on literature was likely to be less reliable. Each subset of model parameters was adjusted by fitting model predictions to the partial training set, while other parameters were set at their initially estimated values (reported in Supplementary data). See Appendix A for the details of parameter adjustment.

Subsequently, in order to select the best-predictive parameter set among the sets obtained in the first step, we simulated the application of all the ten Wnt3a doses experimentally tested in [38], using each of these parameter sets, and compared the resulting model predictions to the data. To select the best parameter set, we used several statistical tests for correlation between observed and predicted values (see Appendix B). For example, we calculated the coefficient of determination, $R^2$, defined as $R^2 = 1 - \frac{SS_{err}}{SS_{tot}}$. Here, $SS_{err}$ is the sum of squares of differences between model-predicted and observed measurements, $SS_{err} = \sum_{j=1}^{10} \sum_{i=1}^{10} M_{i,j} (E_{i,j} - P_{i,j})^2$, where $E_{i,j}$ are the experimental data points, $P_{i,j}$ are the corresponding model predictions, and $M$ is a matrix of weights (see Appendix A). $SS_{tot}$ is the total sum of squares of the data, proportional to the sample variance, $SS_{tot} = \sum_{j=1}^{10} \sum_{i=1}^{10} M_{i,j} (E_{i,j} - \bar{E})^2$, where, $\bar{E} = \frac{\sum_{j=1}^{10} \sum_{i=1}^{10} M_{i,j} E_{i,j}}{\sum_{j=1}^{10} \sum_{i=1}^{10} M_{i,j}}$ is the weighted mean of the observed data. This is the most general definition of $R^2$; it implies that $R^2 \leq 1$, and results that are closer to 1 indicate a better fit. Its value is not bounded from below, and when the prediction error is larger than data variance, it becomes negative, indicating a significant disagreement between the model and the data. This index was used also for comparing model predictions to experimental data during model validation.

The best-predictive parameter set (Table 1) was used for further model simulations.

4. Experiments testing Dkk1 inhibition of Wnt-induced β-catenin accumulation

Proteins. Recombinant mouse Wnt3a and Dkk1 were purchased from R&D Systems (Minneapolis, MN).

Assaying Wnt3a signaling by measuring β-catenin accumulation. The experiments were conducted under the same conditions reported in [40]. Mouse fibroblasts (L cells) were plated on 24-well plates, 250,000 cells per well, and grown overnight. They were incubated for 30 minutes in a serum-free medium, and, then, for another 30 minutes, with Dkk1 at 0 to 10 nM. Wnt3a (0.5 nM final concentration) was then added. Two hours later, the cells were lysed and β-catenin levels were detected by immunoblotting with anti-β-catenin mouse monoclonal antibody (BD Transduction Laboratories), and measured in BioRad ChemiDoc XRS. The
membranes were re-blotted with mouse anti-β-actin antibody (Sigma). Numerical data of band intensity were obtained using Quantity One X software. β-catenin band intensities were standardized with respect to β-actin band intensities. The densitometric intensity of experimental β-catenin bands was corrected by subtracting the negative control. These data were normalized to a scale where the relative densitometric value of β-catenin accumulation, induced by 0.5 nM of Wnt3a, 2 hours after treatment was set at 100%. The results were expressed as the mean outcomes of three experiments ± s.e.m., analyzed by ANOVA followed by a post-hoc multiple comparison test (using SPSS 16.0 software).

5. Model validation
5a. Predicting the effect of Wnt3a and sFRP

From two independent experimental works [39-40], we extracted data on the inhibitory effect of sFRP (see section 2). Both works report accumulation of β-catenin under several different concentrations of Wnt3a after 3 [39] or 2 hours [40]. In order to compare the results of the two experiments with model predictions, we scaled each one of them, separately, to the results of [38], using linear units scaling. The proportion coefficient between the measurement units of the experiment in [40] and those of [38] was found by minimizing the sum of square of differences between β-catenin measurements in the two experiments at t=2 hours, for the same concentrations of Wnt3a. This coefficient is equal to the proportion between the corresponding values of parameter λ, which translates the simulated β-catenin level to the experimental measurement units (see Table 1 and Appendix A). Similar scaling was performed for the data from [39], using the average between measurements at t=2 and t=4 hours in [38] for each Wnt3a concentration, since no measurements at t=3 hours were available there. The obtained proportion coefficients were used to compute the values of λ for simulating each of these experiments.

To simulate the experiments testing the effect of Wnt3a, we used the same initial conditions as in the previously described simulation, and the experiment volume and cell number reported for each experiment. The concentrations of Wnt3a were set to 0.5 nM and 2.5 nM, as reported for the experiments [40] and [39], respectively. The model was simulated over 2 or 3 hours, corresponding to the duration of the respective experiment, and total computed β-catenin accumulation was compared to the experimental results.

Further, the model was used to predict the inhibition effects of sFRP1 and sFRP2. To this end, we simulated the sFRP inhibition experiments, with S_T values ranging from 0 to 16 and from 0 to 500 nM, covering the range of experimental sFRP concentrations in [40] and [39], respectively. The inhibition of β-catenin accumulation was computed as the ratio between Wnt-induced β-catenin accumulation with and without sFRP. For this computation, the base level of β-catenin, i.e., that obtained for W_T=S_T=0, was subtracted from both values in the ratio, as done with the experimental results.

An additional adjustment of the rate parameters for sFRP2-Wnt binding was performed using the results of the sFRP2 inhibition experiment, which had been conducted with chicken sFRP2 [39] (while the binding coefficients in the model were initially estimated for mouse sFRP2 [40]). We used the search algorithm to determine the best-fit values for k_l and k_r, while all the other model parameters remained fixed.

5b. Model validation using experimental results of inhibition by Dkk1
We used the model to predict Dkk1 inhibition of Wnt-induced β-catenin accumulation. The initial values were defined as above, and experimental volume and cell number were set in accordance with section 4. The values of $D_r$ ranged from 0 to 10 nM. The inhibition was computed as the ratio between β-catenin levels with and without Dkk1, after 2.5 hours. Baseline β-catenin level was subtracted from both values.

6. Predicting the combined effect of sFRP and Dkk

We simulated the combined effect of sFRP1 and Dkk1 on Wnt-induced β-catenin accumulation, using the experimental volume and cell numbers as in section 4. We checked different Wnt3a concentrations, in the range of 0.05 to 5 nM. For each Wnt3a dose, we simulated the effects of adding different combinations of sFRP1 and Dkk1 concentrations in the experimentally relevant ranges of concentrations (0–40 nM for Dkk1 and 0–300 nM for sFRP1).

Results
1. Model calibration

The mathematical model for the canonical Wnt pathway was constructed on the basis of published biological knowledge (see Fig. 1 and section 1 in Materials and Methods), and calibrated by experimental data [38]. For details of model parameterization see Materials and Methods section 3.

We found that adjusting as few as 4 parameters to a subset of the data, comprising measurements for 3 Wnt3a concentrations (0, 12.5 and 400 ng/ml), was sufficient to obtain a good fit to data for all other Wnt3a concentrations (Fig. 2) with unbiased residual distribution (Fig. 3). The adjustment of these 4 parameters resulted in the final set of parameters of our model (Table 1). Predictions generated using this parameter set, when compared to the complete data set [38], had a mean absolute error of 0.025 and coefficient of determination $R^2\approx0.965$ (for definition see Materials and Methods section 3). These results demonstrate that our model accurately predicts the time course of Wnt-induced β-catenin accumulation.

2. Model validation by independent experiments

2a. Validation by predicting the effect of Wnt3a

The model predicted the effect of Wnt3a in independent experiments (Fig.4), with $R^2$ values of 0.626 for the experiments that had employed Western blot [40], and 0.908 for those that used ELISA as an assay [39]. Hannoush et al. [38], whose data we used to fine-tune the model, also used ELISA, which may explain the better accuracy of model prediction for the experimental results of Galli et al. [39].

2b. Validation by predicting sFRP inhibitory effect

Our model accurately predicts the sFRP1 effect on Wnt-induced β-catenin accumulation found in [40] and in [39] (Fig. 5A and Fig. 5B, respectively). $R^2$ values for predicted vs. observed results are 0.893 and 0.911, respectively.

Prediction accuracy for the effect of sFRP2 was lower: $R^2\approx0.507$ (Fig. 6A) and $R^2\approx–0.65$ (Fig. 6B, solid line), respectively. In the latter case, the model clearly underestimated the experimental effect, as reflected by the negative coefficient of determination. However, the binding coefficients used in these model simulations were estimated for mouse sFRP2 [40], whereas the experiment shown in Fig. 6B was conducted with chicken sFRP2 [39]. In this experimental work, it was also reported...
that commercially purchased mouse sFRP2 shows less potency than purified chicken sFRP2, and inhibited Wnt3a at doses comparable to those of sFRP1 [39]. Indeed, we found that increasing the binding rate to \( k_b \approx 8.89 \times 10^5 M^{-1}s^{-1} \) and decreasing the dissociation rate to \( k_d \approx 7.8 \times 10^5 s^{-1} \) (both contributing to a decrease in the dissociation constant) render the model predictions of the experimental observations accurate \( (R^2 \approx 0.914, \text{see Fig. } 6B, \text{ dashed line}) \).

2c. Prospective validation by experiments evaluating Dkk1 inhibitory effect

The model predicted that low Dkk1 concentrations would inhibit Wnt3a-induced β-catenin accumulation \( (IC_{50} \approx 2.4 \text{ nM}, \text{see Fig. 7A, solid line}) \). Subsequently, our experimental results in L cells validated the model predictions with \( R^2 \approx 0.944 \) (Figure 7); Dkk1 inhibited β-catenin accumulation in a dose-dependent manner with \( IC_{50} \approx 3.2 \text{ nM} \).

3. Model predictions of the combined effect of sFRP and Dkk

Figure 8A,B show simulation results of combined application of sFRP and Dkk in different concentrations, under 0.5 and 5 nM Wnt3a, respectively. The effect of each inhibitor in the combination increased with its concentration; Dkk1 inhibited β-catenin to a specific level with lower concentrations than sFRP1 (also due to lower \( K_d \)). At larger Wnt3a concentration higher levels of the inhibitors are required to achieve the same relative inhibition.

These results were used to examine whether the combined effect of sFRP1 and Dkk1 is additive or synergistic. For this purpose, we created isoboles [41] – curves that represent the set of sFRP1 and Dkk1 dose pairs that yield a specified effect. Each isobole in Figure 8C,D represents different combinations of sFRP1 and Dkk1 that inhibit β-catenin accumulation to a fixed level, in the presence of a given Wnt3a concentration. The convex form of the curves suggests a synergistic effect of the two inhibitors in all the Dkk1 and sFRP1 concentrations tested, i.e., the effect of the combination is greater than additive, since an additive effect would have resulted in linear curves. Hence, lower concentrations of Dkk1 and sFRP1 can be combined to achieve significant inhibition of β-catenin. We also simulated application of the same concentration combinations, varying each of the model parameter values up to ±50%, and observed similar synergistic behavior under all of the Dkk1, sFRP1 and Wnt3a concentrations tested (data not shown). This result implies that the observed synergistic effect is robust, probably imposed by the pathway structure.

Table 2 shows different combinations of sFRP1 and Dkk1 that are expected to inhibit Wnt-induced β-catenin accumulation to the same level, in the presence of a fixed Wnt3a concentration. The synergistic effect of Dkk1 and sFRP1 is clearly seen in Table 2 by comparing predicted concentrations of combined sFRP1 and Dkk1 (columns \( b, c \) and \( d \)) to the concentrations needed to achieve the same effect by each inhibitor alone (columns \( a \) and \( e \)). The ratio between sFRP1 and Dkk1 doses that separately achieve the same effect is termed \textit{potency ratio} (second column from right). Column \( c \) shows the maximally synergistic combination of sFRP1 and Dkk1, i.e., the point where the sum of sFRP1 and Dkk1 doses, scaled by the potency ratio, is minimal. The ratio between Dkk1 and sFRP1 in the maximally synergistic combination increases when higher doses of Wnt3a are applied (see rightmost column). Interestingly, this ratio is approximately the same as the potency ratio (compare last two columns in Table 2).


**Discussion**

We employed mathematical modeling to investigate the Wnt canonical pathway and to provide a new mechanistic framework for this pathway, which can serve as a platform for discovering new anti-cancer treatments. Whereas previous mathematical models of the Wnt pathway have focused on its intracellular components (see review in [22]), our model details the initial sequence of events occurring along the pathway, from the ligands and inhibitors binding the membranal and extracellular components to β-catenin regulation. This allows exploration of quantitative effects of different extracellular pathway inhibitors, including therapeutic agents.

Our model accurately predicted experimental results of the effects of Wnt and sFRP, obtained in different laboratories, by different assays, and across a wide range of concentrations. In addition, model predictions concerning Dkk1 effect were prospectively validated experimentally. Note that binding rates for these inhibitors, which are most influential on model predictions, were already fixed from literature in the initial stage of parameter estimation. This demonstrates that our model is able to quantitatively predict the effects of inhibitors of the Wnt pathway.

For the effect of sFRP2 on Wnt-induced β-catenin accumulation, the model was less accurate (Fig. 6). This can be explained by imprecise estimation of the rates of sFRP2 binding to Wnt3a. In one of the experiments [39] chicken sFRP2 was used, while the reaction rates assumed in our model were taken from mouse sFRP2 experiments [40]. Evaluating the chicken sFRP2-Wnt3a reaction rates by adjusting the model to the experimental data of Galli et al. [39], resulted in lower estimation for the dissociation constant. This concurs with the observation that mouse sFRP2 is less potent than chicken sFRP2 [39]. This demonstrates the applicability of our model for determining unknown specific parameters of Wnt pathway inhibitors (including potential drugs), when experimental data of their effect are available.

The Dkk1 dose-response relationship in our experiment varied from that recently observed by Bourhis et al. [42], with a difference of two orders of magnitude in IC_{50}. The reason underlying this discrepancy may be that in the latter study, the constant of dissociation (K_D) between Dkk1 and LRP was significantly higher than previously reported K_D values [13, 37]. This difference may result from different protein expression systems and purification procedures.

Despite the abundance of published research on the canonical Wnt pathway, there are still parts of this pathway whose underlying mechanism is not clear. For example, our modeling hypothesis that Wnt-Frizzled complex is created and subsequently binds to LRP is consistent with previous experimental results, but to the best of our knowledge had not been explicitly stated before. Experimental validation of our model lends support to the basic biological assumptions laid at its foundation, including this hypothesis.

Our general mechanistic model characterizes the canonical Wnt signaling pathway for different biological systems, and it is not tailored to a specific experimental setup. Hence, the model can be used to forecast results of various experiments, by input of a few parameters that characterize the specific experiment (e.g. cell number). New biological information, such as the binding affinities between specific Wnt ligands, their receptors and different inhibitors may also be easily incorporated into the model. In addition, new assumptions about the Wnt canonical pathway can be implemented in the mathematical model. For example, some studies suggest that Dishevelled polymerization induces large aggregates (signalosomes) of Wnt receptors and destruction complex (reviewed in [43]). This aggregation was best demonstrated when Wnt signalling proteins were over-expressed [44-45], and it still remains to be
Appendix A: Parameter Adjustment

Parameter adjustment to data from [38] was initially performed for several choices of parameter subsets. The considered subsets included four to seven parameters, e.g., \{F_0, k_3, k_6, \lambda\}, \{k_3, k_5, k_6, \lambda\}, \{F_0, k_3, k_5, k_6, \lambda\}, \{F_0, F_C, k_3, k_5, k_6, \lambda\}, \{F_0, F_C, k_3, k_5, k_6, \lambda\}.

Given the vector \(v\) of the values of adjusted parameters, the model was simulated as follows. We set \(S_i = 0\) and \(D_i = 0\), since in [38] no inhibitors were added, and used the reported experiment volume and cell number. For each Wnt3a concentration, \(W_j\), the model was initiated with free Wnt concentration (and \(W_T\) equal to \(W_j\), and other variables at their unique steady-state values assumed in the absence of Wnt. The model was numerically integrated from time \(t=0\) to \(t=t_{10}=35\) hours. To compare the simulations to the experimental results, we interpreted the latter in terms of the model variables. We assumed that the fluorescence measurements reported in the experiments were in direct proportion to the total concentration of \(\beta\)-catenin, with proportion coefficient \(\lambda\). Model predictions for \(\beta\)-catenin accumulation at time \(t_i\) were computed as \(P_{ij}(v)=\lambda B(t_i)+C_B(t_i))\), for a given Wnt3a concentration \(W_j\), and for parameter values \(v\). Here, \(B\) and \(C_B\) are the model variables representing, respectively, the concentration of free \(\beta\)-catenin and the concentration of \(\beta\)-catenin bound by the destruction complex. The value of \(\lambda\) was included in all subsets of adjusted parameters.

For each choice of a subset of adjusted parameters, we determined the parameter values by minimizing the Goal Function (GF), computed by comparing simulation results to the data in the partial training set. The data sampled from [38] were represented as a matrix \(E\), where \(E_{ij}\) is the \(\beta\)-catenin fluorescence intensity at time \(t_i\) under concentration \(W_j\) of Wnt3a, \(1 \leq i, j \leq 10\). We also constructed a corresponding matrix \(M\) of weights, \(M_{ij} \in \{0,1,2\}\) being the number of values available from graphical sampling of measurement \(E_{ij}\). The partial training set was defined as
\{E_{i,j}|i=1,\ldots,10; j\in\{1,5,10\}\}, corresponding to the doses \(W_0=0\), \(W_5=12.5\) and \(W_{10}=400\ ng/ml\).

We defined \(GF\) as the sum of squares of differences between model-predicted and observed measurements, with weights taken from matrix \(M\).

\[
GF(v) = \sum_{j=1}^{10} \sum_{i=1}^{10} M_{i,j}(E_{i,j} - P_{i,j})^2,
\]

where \(J=\{1,5,10\}\), and \(P_{i,j}\) are the corresponding model predictions under the parameter set \(v\). The values of the adjusted parameters that minimize \(GF\) were found using the search algorithm with initial guesses randomly chosen within the relevant parameter ranges (reported in Supplementary Data).

**Appendix B: Tests Used for the Model calibration**

The statistical tests used for evaluating the correlation between model-predicted and observed experimental values were:

(i) Mean residual between the simulation results and the experimental data points;
(ii) Average of the square of absolute and relative error per data point;
(iii) The absolute value of absolute and relative error per data point;
(iv) Linear regression for predicted vs. observed data points;
(v) Linear regression as in (iv), forcing intersection with y-axis to 0;
(vi) Coefficient of determination, \(R^2\) (defined in Materials and Methods section 3).

For tests (i)-(iii) smaller scores indicate better prediction; in test (iv) better prediction should give the intersection closer to zero and the slope and correlation coefficient closer to 1; in test (v) both slope and correlation coefficient should be closer to 1; in test (vi) \(R^2\) should be closer to 1. When calculating average relative error in (ii) and (iii), the experimental points with values less than 0.005 (which is <1% of the maximal experimental result) were excluded from the data set, to prevent bias.

**Funding**

This work was partly supported by grant #012930 from the NEST FP6 program and by the Chai Foundation.

**References**


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signaling complex reveals multiple wnt and dkk1 binding sites on lrp6. J Biol Chem. 285, 9172-9179

Tables

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<tr>
<th>Parameter name</th>
<th>Value (Reference)</th>
<th>Units</th>
<th>Biological Meaning</th>
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<td>for sFRP1: $4.33 \cdot 10^4$ [40]</td>
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<td>sFRP-Wnt binding rate</td>
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Accepted Manuscript

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Table 1. The model parameters. Parameter values were estimated directly from literature (see Supplementary Data) or by fitting to experimental data. The parameter interpretations are given in the right column. Experiment-specific parameters are shown separately for each of the simulated setups.

*The value of \( \lambda \) scales the measurement units for \( \beta \)-catenin level in the specific experimental system; its value was fitted for [38]. For other experimental systems it was determined by scaling the \( \beta \)-catenin measurement units to those used in [38]. This parameter is relevant when \( \beta \)-catenin accumulation is reported in non-relative units (Figs 2-4).

Table 2. Simulated combinations of sFRP1 and Dkk1 yielding inhibition of \( \beta \)-catenin accumulation to specific levels. For each Wnt3a concentration and \( \beta \)-catenin accumulation level, five different combinations of sFRP1 and Dkk1 are suggested. Each row contains five points on one of the isoboles shown in Figures 8C,D, e.g. the values in the grey (second) row correspond to the points marked \( a \) to \( e \) in Fig. 8D. These points are chosen as follows: in column \( a \) – Dkk1 alone; \( e \) – sFRP1 alone; \( b \) – half of the maximal Dkk1 concentration (specified in \( a \) and corresponding sFRP1 concentration; \( d \) – half of the maximal sFRP1 concentration (specified in \( e \)) and corresponding Dkk1 concentration; \( c \) – maximally synergistic combination of sFRP1 and Dkk1 doses (see Results). The last column presents the ratio between sFRP1 and Dkk1 concentrations in column \( c \), and the column next leftward to it presents the potency ratio (between sFRP1 in column \( e \) and Dkk1 in column \( a \)).
Figure 1. A schematic description of the mathematical model for the Wnt signaling pathway. The scheme shows the modeled reactions, indexed 1 to 8, as follows: (1) interaction between sFRP and Wnt, (2) interaction between Wnt and Frizzled, (3) interaction between Wnt-Frizzled complex and LRP, (4) interaction between Dkk and LRP, (5) interaction between ternary complex (Frizzled/Wnt/LRP) and destruction complex (GSK/Axin/APC), (6) interaction between β-catenin and destruction complex, (7) production of β-catenin, and (8) degradation of β-catenin, independent of destruction complex; forward and backward rate constants are indexed accordingly. The central part depicts the reactions transducing the signal from Wnt to the destruction complex, the grey parts show reactions between inhibitors and relevant pathway components, and the boxed part shows β-catenin regulation by the destruction complex.
Figure 2. Model simulation of Wnt effect on $\beta$-catenin accumulation, compared to experimental data. Four of the model parameters were adjusted using partial training data set, containing measurements of $\beta$-catenin accumulation under three Wnt3a concentrations (0, 12.5 and 400ng/ml). The model was then simulated, using the resulted parameter set, under all experimental Wnt3a concentrations, and simulation results were compared to the complete data. Markers represent averages (with s.e.m. bars, whenever available) from two independent experiments, each carried out in quadruplicate [38], and lines are results of model simulations. Markers and lines of different types correspond to different Wnt3a doses, reported in labels to the right of the corresponding lines. The grayed labels (solid lines) mark the doses included in the partial training set.
Figure 3. Statistical testing for the best-predictive parameter set: observed vs. predicted. (A) Simulation results vs. experimental results (B) Residues vs. experimental results. All axes units are normalized β-catenin units, as reported for the experiment [38].
Figure 4. Model predictions of Wnt3a effect on β-catenin accumulation, compared to different experimental sources. (A) Data from [40] (circles) showing means and s.e.m. of 3 or 4 experiments, are presented along with model predictions (line), for \( t = 2 \) hours. (B) Data from [39] (circles) showing means and s.e.m. of 3 to 6 independent replicates, are presented along with model predictions (line), for \( t = 3 \) hours.
Figure 5. Model predictions of sFRP1 effect on β-catenin accumulation, compared to experimental results. (A) β-catenin inhibition as a function of sFRP1 concentration (means of 3 or 4 experiments with s.e.m. bars), as reported in [40] (circles), along with model predictions for the same experiment (line). (B) A similar comparison between means of 3 independent replicates reported by [39] (circles), and model predictions (line). The s.e.m. bars are shown whenever available. In accordance with the experiments’ setup, Wnt3a concentration is 0.5 and 2.5 nM, and β-catenin is sampled at t=2 and t=3 hours in (A) and (B), respectively. β-catenin accumulation is relative to maximal level (with Wnt3a only) and to base level (no Wnt3a).
Figure 6. Model predictions of sFRP2 effect on β-catenin accumulation, compared to experimental results. (A) β-catenin inhibition as a function of sFRP2 concentration (means of 3 or 4 experiments with s.e.m. bars), as reported in [40] (circles), along with model predictions for the same experiment (solid line). (B) A similar comparison between means of 3 independent replicates from [39] (circles) and predictions of the model before (solid line) and after (dashed line) adjusting of Wnt3a-sFRP2 binding reaction rates. The s.e.m. bars are shown whenever available. In accordance with the experiments setup, Wnt3a concentration is 0.5 and 2.5 nM, and β-catenin is sampled at t=2 and t=3 hours in (A) and (B), respectively. Accumulation of β-catenin is relative to maximal level (with Wnt3a only) and to base level (no Wnt3a).
Figure 7. Inhibition of β-catenin accumulation by Dkk1 – prospectively validated model predictions. (A) The effect of Dkk1 on Wnt3a-induced β-catenin accumulation, as predicted by the model (line) and experimentally tested thereafter (circles). L cells were treated for two hours with 0.5 nM Wnt3a in combination with increasing concentrations of Dkk1. β-catenin band intensities were standardized with respect to β-actin. β-catenin accumulation is reported relative to maximal level (with Wnt3a only) and to base level (no Wnt3a). Averages and s.e.m. of 3 independent experiments are shown. (B) The effect of DKK1 on β-catenin accumulation in a representative experiment. β-catenin levels are shown in a Western blot, as compared to the β-actin loading control.
Figure 8. Combined effect of sFRP1 and Dkk1, predicted by the model. (A-B) β-catenin accumulation as a function of sFRP1 and Dkk1 concentrations. Wnt3a concentration was set to 0.5 and 5 nM in A and B, respectively. β-catenin accumulation is relative to maximal level (with Wnt only) and to base level (no Wnt). The surface color is changing with the height, representing β-catenin accumulation level. The black solid curves on the surface are contours at several fixed β-catenin accumulation levels (isoboles). (C-D) Isobolograms (graphs of isoboles) for the combined effect of Dkk1 and sFRP1. Each curve represents all combinations of sFRP1 and Dkk1 that inhibit β-catenin accumulation to a fixed level, in the presence of a given Wnt3a concentration. Panel C shows isoboles for β-catenin inhibition to 10% of the maximal level. Different curves correspond to different concentrations of Wnt3a. In panel D, isoboles are shown for different fixed β-catenin accumulation levels, between 5% and 50% of the maximal level, obtained with 0.5 nM Wnt3a. Points a to e correspond to the values in the second line of Table 2. In all panels, β-catenin is sampled at t=3 hours.