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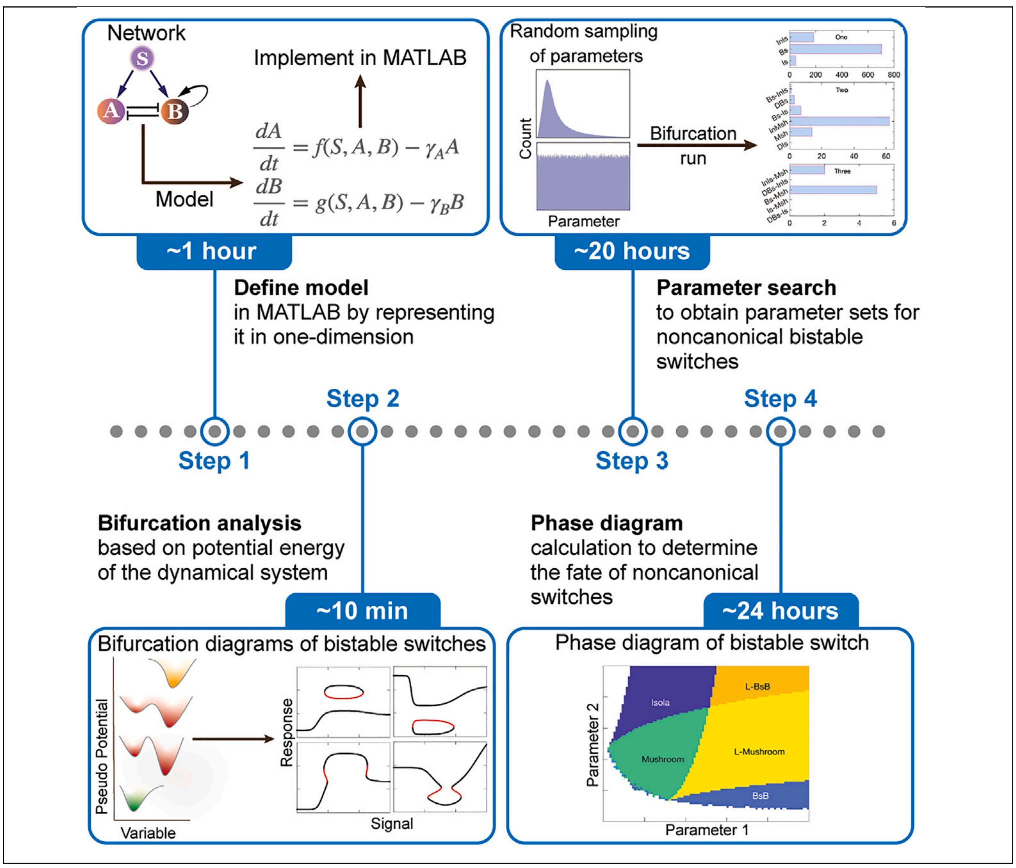
Protocol for potential energy-based bifurcation analysis, parameter searching, and phase diagram analysis of noncanonical bistable switches



We have explored the design principles of noncanonical bistable switches using high-throughput bifurcation analysis of positive feedback loops under dual signaling. Here, we present a protocol to carry out bifurcation analysis using pseudo-potential energy of the dynamical system. We also describe steps to perform automated parameter searching for canonical and noncanonical switches and multi-parameter phase diagram analysis of these switches.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

A protocol for bifurcation analysis based on the potential energy of the dynamical system

Steps for high-throughput parameter screening for noncanonical switches

Approach to delineate the robustness of a network in generating noncanonical switches

Detailed steps to calculate the phase diagram of noncanonical bistable switches

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Protocol for potential energy-based bifurcation analysis, parameter searching, and phase diagram analysis of noncanonical bistable switches

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SUMMARY

We have explored the design principles of noncanonical bistable switches using high-throughput bifurcation analysis of positive feedback loops under dual signaling. Here, we present a protocol to carry out bifurcation analysis using pseudo-potential energy of the dynamical system. We also describe steps to perform automated parameter searching for canonical and noncanonical switches and multi-parameter phase diagram analysis of these switches.

For complete details on the use and execution of this protocol, please refer to Das et al.[1]

BEFORE YOU BEGIN

Bistability is a crucial mechanism to generate all-or-none response that dictates various decision-making processes in a living cell. A canonical bistable switch consists of a single bistable region and with variation of stimulus concentration the expression level of the regulator changes either from high-to-low or low-to-high with distinct signaling thresholds. Typically, a positive feedback loop, either due to mutual activation or mutual inhibition between two regulators, with embedded ultrasensitivity leads to a bistable signal response curve. We developed a new tool for investigating bistability and multistability in networks with positive feedback loop by exploiting the pseudo-potential energy of the corresponding dynamical system.[1–3] The method relies on the determination of stable and unstable steady states from the minima and maxima, respectively, in the pseudo-potential energy landscape. We determined the bifurcation diagrams of multistable switches utilizing the pseudo-potential energy of the system. The method has been implemented in MATLAB and applied to determine the topological requirements of generating variety of canonical and non-canonical bistable switches from mutual activation and mutual inhibition loops under dual signaling.[1]

The protocol described here consists of three parts. The first part of the protocol generates one-parameter (1-p) bifurcation diagrams of bistable switches from ppMISA network (Figure 1A). The second part details the automated parameter searching for canonical and noncanonical bistable switches from the network under random sampling of parameters. The final part describes the fate of a bistable switch under simultaneous variation of two parameters to construct a phase diagram of the bistable switches.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
MATLAB R2016b with Signal Processing Toolbox	MathWorks (Natick, MA)	https://www.mathworks.com/products/matlab.html
MATLAB codes for simulation	This work	https://github.com/dbarikUoH/Protocol_NonCanonicalSwitch (10.5281/zenodo.8375309)
Other		
Table with parameter ranges used in the parameter search calculation; Table detailing the jump pattern-based identification of bistable switches	Das et al.[1]	https://doi.org/10.1016/j.isci.2023.106379

STEP-BY-STEP METHOD DETAILS

Download codes

Timing: 10 min

Download the zip file that contains the MATLAB codes and relevant model parameters from the GitHub link (https://github.com/dbarikUoH/Protocol_NonCanonicalSwitch) mentioned in the 'key resources table'. Extract the folders from the zip file by unzipping the file. Note that the directories 'Bifurcation1p', 'ParameterSearch' and 'PhaseDia' contain MATLAB codes to perform 1-p bifurcation analysis, parameter searching for bistability and phase diagram calculation, respectively.

Pseudo-potential energy based 1-p bifurcation analysis

Timing: 10–15 min

This section lists the steps to carry out 1-p bifurcation analysis using the pseudo-potential energy of the dynamical system corresponding to the positive feedback regulated network motifs. We describe here the details of pseudo-potential energy based 1-p bifurcation analysis of ppMISA network under OR logic gate (Figure 1A).[1] The MATLAB code generates 1-p bifurcation diagram by monitoring the extrema in the effective pseudo-potential energy of the dynamical system. Further, the code identifies the bistable switch provided it falls under the group of 189 bistable switches listed in the Table S1.

Note: The group of 189 switches contains both canonical and noncanonical switches. It includes 84 reversible and 105 consequential irreversible switches. The irreversible switches include irreversible at the lowest (42), highest (42) and both at the lowest and highest (21) values of the bifurcation parameter. These switches originate from the fusion of canonical bistable and noncanonical isola, mushroom, inverted isola and inverted mushroom switches (Figure 1) in different numbers and orientations.

The steps of the bifurcation analysis are listed below.

1. Browse to the 'Bifurcation1p' directory.
   a. Run the code by typing 'STAR_1p_bifurcation_ppMISA_OR' in the MATLAB command window. The steps bifurcation calculation are mentioned next.

   Note: The code generates the bifurcation diagram − a plot of the steady state value of the dynamical variable, B, as a function of the bifurcation parameter, S.

2. At every S, calculate the effective pseudo-potential energy (V(S,B)) of the dynamical system by integrating the right hand side (pseudo force term) of the one dimensional representation of the dynamical system (Figure 1B).

Note: The statements below calculate the force and pseudo potential energy of the dynamical system, respectively.

```
[f]=BS_model_bifurcation(S0,b1,param); pot=dx2*cumtrapz(-f);
```

⚠ CRITICAL: The pseudo force term in the one-dimensional representation of the dynamical system is defined in the function file BS_model_bifurcation.m. The theoretical method of pseudo-potential energy based bifurcation can be found in Das et al.[1] and Dey et al.[2,3]

3. Determine the locations of extrema in the potential energy using the findpeaks function in MATLAB.
   a. Store the locations of the minima (stable steady states) and maxima (unstable steady states) in separate arrays.
   b. Repeat the above steps for a range of S ($0 \le S \le 1000$) to construct the 1-p bifurcation plot of the bistable switch.
4. Using the statement below, determine the number of jumps in the stable branch at the bifurcation points and their locations required for the identification of switches.

```
[sum_diffss2,bfrSN,jmpN,jmpLocSN]=BS_jumpSS_SN(peaks_ss,vss,vuss);
```

Note: Identification of the bistable switch, from the list of 189 switches is based the number of bifurcation points, number of jumps in the stable branch at the bifurcation points and the location of these jumps (see Figure 1F and Table S1). See Das et al.[1] for the details of the method.

5. Use the function BS_switchType_189BS.m to identify the type of the bistable switch making use of jump pattern calculated in the previous step.

```
BS_switchType_189BS(peaks_ss,sum_diffss2,jmpN,jmpLocSN)
```

Note: In the MATLAB command window, the code prints the abbreviated name of the identified switch. Identification of a bistable switch is an add-on feature and is not mandatory for bifurcation analysis. Therefore, this part of the calculation can be omitted by commenting the relevant line (line # 124).

6. Use the statement below to plot the 1-p bifurcation diagram (Figure 1C).

```
plot_bifurcation(bfrSN,vss,vuss)
```

Note: In the bifurcation plot, the arrays corresponding to the stable and unstable steady states are plotted as a function of S. The maximum value (sig_max) and the step length (dsig) of the bifurcation parameter are chosen as 1000 and 0.1, respectively. The step length can be modified to alter the resolution of the bifurcation diagram and if required the sig_max can be increased to avoid truncation of the bifurcation diagram at the largest value of S. The upper limit (xE) in the numerical integration of force is chosen as 10000 and it can be increased to avoid the truncation of the bifurcation diagram in the steady state axis (B-axis in the bifurcation plot).

7. Uncomment the line # 140–148 to overlay the bifurcation diagram on top of the contours of the pseudo-potential energy (Figure 1D).

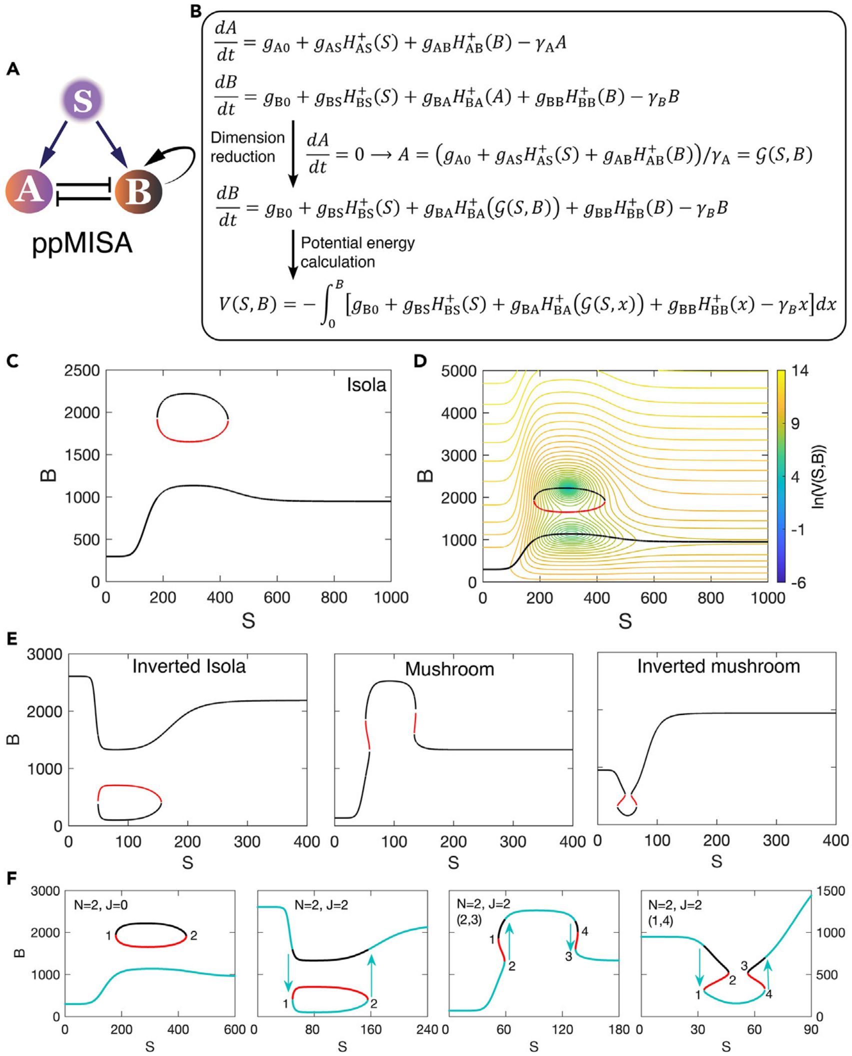

Figure 1. Outcome of pseudo-potential energy based 1-p bifurcation analysis

(A–F) The network diagram of the ppMISA network (A). The two dimensional dynamical system is reduced to a one dimensional system in gene B by applying quasi steady state approximation on gene A. The effective pseudo-potential energy (V(S,B)) is calculated by integrating the effective force term in the right hand side of the one dimensional representation (B). The default parameter set in the code generates an isola bistability where the stable and unstable steady states are represented by black and red lines, respectively (C). The isola bifurcation is overlaid on top of the contours of ln(V(S,B)) (D). The vertical color-bar represents the values of ln(V(S,B)). Inverted isola, mushroom and inverted mushroom can be generated by choosing the appropriate parameter set provided in the code (E). Switches are identified based on the number of bifurcation points (N), number of jumps (J) and their locations (F). Isola and inverted isola switches both possess two saddle-node bifurcation points (N = 2). Based on the array structure used in our calculations,[1] the stable branch in the isola (cyan line) does not exhibit any jump (J = 0) as opposed to the inverted isola with two jumps (J = 2). Both the mushroom and inverted mushroom possess four bifurcation points (N = 4), however locations of jumps are different for the mushroom (2,3) and inverted mushroom (1,4). See Table S1 for the values of N, J and the location of jumps for all 189 switches.

Note: The contour plot takes a longer time to plot in MATLAB. By default, the code loads the parameter for isola bistability and generates an isola bistable switch (Figure 1C). The parameter sets for inverted isola, mushroom and inverted mushroom are also provided and can be loaded to obtain these switches by uncommenting the line # 36, 37 and 38, respectively, for inverted isola, mushroom and inverted mushroom switches (Figure 1E).

Parameter searching for canonical and noncanonical bistable switches using high-throughput bifurcation analysis

Timing: 18–20 h

This section details the steps to run potential energy based high-throughput bifurcation analysis to determine the ability of a network to generate reversible canonical and noncanonical bistable switches and to search for parameter sets for these switches with a maximum of three bistable regions (84 in total). By carrying out 1-p bifurcation analysis for a randomly sampled parameter set, we determine the occurrence probability (percentage chance) and total counts of these switches for a network. We segregate the parameter sets based on the type of switches leading to switch specific parameter sets. In addition, we created a list of 14 switches by grouping together topologically similar switches from the 84 switches (see Table S2). We determine the counts and occurrence probabilities of these 14 switches as done in Das et al.[1]

Note: To improve computational efficiency, we perform parameter search calculation in two steps. With a randomly chosen parameter set, first we carry out a low-resolution bifurcation analysis and if the set generates bistability then we perform a high-resolution bifurcation calculation for accurate identification of the bistable switch.

The steps of the parameter search calculations are following:

8. Browse to the 'ParameterSearch' directory to locate the necessary MATLAB files relevant to parameter search calculation.
9. The first step in the searching is sampling of parameters randomly. Run the 'Thre_inwards_ppMISA_OR.m' in MATLAB to generate the values of threshold parameters of S, A and B genes in the ppMISA network. The steps for generating threshold values are the following:
   a. Determine the threshold values of S, in regulating A and B genes, from the unregulated steady state expression of S by choosing the synthesis and degradation parameters of S from independent uniform distributions of specific ranges.[1]
   b. Calculate the threshold values of A from the network segment considering only the inward regulatory interactions of A (S –> A |– B).
   c. Use similar method to obtain the threshold values of B (S –> B |– A).

Note: The threshold parameters in the Hill functions of the model equations are generated using half-function rule to ensure that the sampled threshold parameters are not biased favoring either activation or inhibition.[4] The threshold values of these three regulators are stored in three separate '.dat' files and are used in the main code of the parameter search calculation. All other parameters are sampled from independent uniform distributions within the main code of the parameter searching.

10. Run the 'STAR_parSearch_189BS_ppMISA_OR.m' (main code) from the current folder to perform the parameter searching of canonical and noncanonical bistable switches. The steps in the main code of the parameter searching are mentioned next.
11. Define the sample size of random parameter sampling (nr), initial values of the counts of all bistable switches and arrays required to store the segregated parameters.
12. Load the values of the threshold parameters obtained from half-functional rule calculations (Step 9).
13. Sample all other parameters from independent uniform distributions with defined ranges.[1]
14. Specify the numerical parameters for the low- and high-resolution 1-p bifurcation calculations next.
15. With the randomly chosen parameter set, run the low-resolution bifurcation calculation for the entire range of the bifurcation parameter ($0 \leq S \leq 1000$) and the dynamical variable ($0 \leq B \leq 10000$) with larger step lengths of S and B.

Note: The step length of S (dsig1) and B (dx1), respectively, are 0.5 and 8.

16. Run the high-resolution bifurcation calculation, with much smaller step lengths of S and B, provided the low-resolution run results in reversible bistability with the sampled parameter set.

Note: Parameter sets leading to monostability, irreversible bistability and higher order multistability (e.g., tristability) are discarded. In the high-resolution calculation, the step length of S is calculated based on the locations of first and last bifurcation points obtained from the low-resolution run.

⚠ CRITICAL: During the high-resolution bifurcation calculation, various measures are taken to reduce computational cost.

a. High-resolution bifurcation calculation is not carried out for the entire range of the bifurcation parameter ($0 \le S \le 1000$). Instead, the range is determined from the locations of first (S_F) and last (S_L) bifurcation points obtained from the low-resolution run. The high-resolution bifurcation calculation is run between $S_0(= S_F - \Delta S)$ and $S_N(= S_F + \Delta S)$. We choose a small value of ΔS satisfying the condition that $S_0 \ge 0$ and $S_N \le 1000$.
b. The step length of S is chosen based on the magnitude of ($S_F - S_L$). We chose a bigger step length for a larger value of this difference. The following statement determines the range and step length of S to be used in the high-resolution calculation.

```
[sigE3,sigE3F1,dsig2nw,dsig2]=ParRegion_highres(nmbr_peaks_ss,sum_diffss,dSc,Sct,dSr,dSn,sigE1,sigE2,dsig2);
```

c. In the high-resolution run, the upper limit (xE1) in the numerical integration of the force is adoptively chosen based on the value of largest steady state obtained from the low-resolution run.

17. Determine the number of jumps in the stable branch at the bifurcation points and their locations required for the identification of switches (See Step 4 and Table S1).

⚠ CRITICAL: A number of measures are implemented to avoid erroneous identification of the switches.

a. If the high-resolution bifurcation run generates a higher order multistability (for example tristability), which was not detected in the low-resolution run due to the larger step size of S, the corresponding parameter set is discarded.
b. If the bifurcation diagram is truncated in the steady state axis (B-axis) either at the lowest (S = 0) or largest (S = 1000) values of S, then the jump-based identification of bistability may lead to an erroneous result. If such a rare situation arises, then the corresponding parameter set is also discarded.

Note: The default value of the maximum value of the dynamical variable (xE) is 10000 and it can be increased if truncation occurs frequently.

18. Use the statement below to identify the type of bistable switches, count their numbers and segregate their parameters in separate arrays.

```
[Cnt,Indx,Par]=BS_switchSegregation_189BS(kk,ic1,data,peaks_ss,sum_diffss2,jmpN,jmpLocSN,Cnt,Indx,Par);
```

19. Repeat the steps 15–18 for the sample size (nr) number of times to determine the count of various switches.
20. Use the statement below to store the counts of the 84 reversible bistable switches and their parameters in structured arrays.

```
[ppMISA_OR_189BS]=BS_saveCntParIndx_189BS(Cnt,Indx,Par);
```

Note: Figure 2 presents the counts and the occurrence probabilities obtained from the MATLAB run with a parameter sample size (nr) of 10000. For the ease of visualization, the counts and occurrence probabilities of bistable switches with one, two and three bistable regions are plotted separately. The counts of 84 switches and their segregated parameters are saved in the 'parSearch_ppMISA_OR_189BS.mat' file.

21. The statement below stores the counts of the group of 14 bistable switches and their parameters in structured arrays.

```
[ppMISA_OR_14BS]=BS_saveCntParIndx_14BS(Cnt,Par);
```

Note: Figure 3 reports the counts and occurrence probabilities of these 14 switches (See Table S2). The counts and parameters of the group of 14 bistable switches, reported in Das et al.,[1] are stored in the 'parSearch_ppMISA_OR_14BS.mat' file.

Optional: One-parameter bifurcation diagrams can be plotted during the parameter searching by uncommenting the lines # 972–974. However, for parameter searching with a larger sample size (nr > 1000), these lines must be left commented to improve computational efficiency.

Note: The parameter searching calculation takes about 18–20 hours for a sample size of 10000 in a workstation with 2×Intel(R) Xeon(R) Gold 6226R CPU @ 2.90GHz processors. To get a sense of the calculation, a trial run can be performed with a smaller sample size, say 1000, while plotting the 1-p bifurcation diagrams by uncommenting the line # 972–974. The code is provided for the ppMISA model however the method can be applied to other regulatory motifs reported in Das et al.[1]

⚠ CRITICAL: The maximum value of the bifurcation parameter (sig_max) and the dynamical variable (xE) were chosen to be 1000 and 10000, respectively, in the low-resolution run. With the selected ranges of various parameters, most of the bistable switches fall within those two limits. However, the maximum values of those two parameters may need to be modified as required due to altered ranges of various parameters. For example, with an increased range of expression rate (or decreased range of degradation rate) of B, the maximum value of the dynamical variable may need to be increased accordingly.

Calculation of phase diagrams of noncanonical bistable switches

Timing: 20–24 h

This section describes the steps to run phase diagram analysis of a bistable switch. In the phase diagram calculation, we determine the fate of a switch under simultaneous variation of two other parameters. In the phase diagram calculation, for each combination of two secondary parameters, we run 1-p bifurcation analysis and determine the type of resulting switch. By repeating this process for a range of combination of these two parameters, we create a phase diagram where we plot the 'identity index' of the resulting switches as a function of these two parameters.

Note: Here we considered a total of 189 bistable switches that include both reversible and irreversible switches. The basic structure of the phase diagram calculation involves running a low-resolution bifurcation run with selected values of the two relevant parameters followed by the high-resolution bifurcation calculation to identify the type of bistability.

The steps of the phase diagram calculation are listed below.

22. Browse to the 'PhaseDia' directory where all the necessary MATLAB codes are available.
23. Run the MATLAB code 'STAR_phaseDia_189BS_ppMISA_OR.m' to generate the phase diagram under the variation of the mutual regulatory strengths of the gene A and gene B (g_{AB} and g_{BA}) in the ppMISA network.
24. Define the initial counts of 189 switches and then load the parameter values for the starting bistable switch (a mushroom) from the data file parameter_phaseDia.mat.

```
load parameter_phaseDia.mat
```

Note: The parameter set of the starting bistable switch provided in the code corresponds to mushroom bistability. Modify the parameter set accordingly to start with a different starting switch.

25. Choose the phase diagram related parameters associated with the g_{AB} and g_{BA}. These parameters include their initial values, step lengths and the number of values.
26. Specify the numerical parameters for the low- and high-resolution bifurcation calculations.
27. Initiate the nested loops to choose the values of g_{AB} and g_{BA} independently for the phase diagram calculation.
28. Perform the low-resolution bifurcation calculation with the particular combination of g_{AB} and g_{BA}.
29. Subsequently carry out the high-resolution bifurcation calculation with various speed enhancing features as detailed in the previous section (see Step 16).
30. Determine the number of jumps in the stable branch at the bifurcation points and their locations (see Step 17).
31. Determine the type of bistability next and assign the 'identity index' of the switch obtained.

Note: Each type of switch is assigned an identity index (phaseIndx) between the number 1 to 189 (See Table S1). The phaseIndx values for monostability and tristability are 0 and 200, respectively. The abbreviated names of the resulting switches are printed in the MATLAB window during the calculation.

32. Repeat the steps 27–31 to construct the phase diagram.
33. Store the counts of various types of bistable switches in the structured array using the statement below

```
[ppMISA_OR_189BS]=BS_saveCnt_189BS(Cnt);
```

Note: These counts are not used for any other analysis in the phase diagram calculation and thus can be commented if needed.

34. Use the statement below to plot a two-dimensional surface plot of the color-coded phaseIndx as a function of g_{BA} and g_{AB} (Figure 4A).

```
plot_phaseDia(Val_gba,Val_gab,phaseDia)
```

Note: Phase diagram plot is color coded based on the phaseIndx values of the switches. In a particular diagram, if the range of phaseIndx values becomes wide then switches with close phaseIndx values can be difficult to identify due to poor color contrast in the surface plot. To improve the color contrast, we have provided an additional function plot_phaseDia_Rscl(Val_gba,Val_gab,phaseDia) that modifies the phaseIndx values based on the total number of phases present in the diagram and thereby reducing the range of the phaseIndx values. This function also plots the phase diagram with modified phaseIndx values creating a greater contrast of colors among the phases (Figure 4B). Note that phaseIndx values in this modified phase diagram cannot be used for manual identification of the phases in the surface plot and the unaltered phaseIndx values (Figure 4A) must be used for the identification of phases from the surface plot.

35. Save the counts of various types of switches, phaseIndx and the values of g_{BA} and g_{AB} in the data file phaseDia_189BS_ppMISA_gab_gba.mat using the command below.

```
save phaseDia_189BS_ppMISA_gab_gba.mat phaseDia Val_gab Val_gba ppMISA_OR_189BS
```

Optional: During the phase diagram calculation 1-p bifurcation diagrams can be plotted during a trial run for visual inspection of the 1-p bifurcation diagrams by uncommenting line # 511–515.

Note: The phase diagram is calculated with 101 × 101 values of g_{BA} and g_{AB} with a step length of 1 for both of the parameters and it takes about 20 hours in a workstation with 2×Intel(R) Xeon(R) Gold 6226R CPU @ 2.90GHz. A trial calculation can be done with 20 × 20 grid with a step length of 5 to get a sense of the phase diagram and during the trial calculation 1-p bifurcation diagrams can also be plotted for visual inspection.

EXPECTED OUTCOMES

The entire protocol consists of three different types of calculations. In the first part 1-p bifurcation calculation is performed using the pseudo-potential energy of the one-dimensional representation of the dynamical system. Four different parameter sets are provided in the code corresponding to isola, inverted isola, mushroom, and inverted mushroom bistable switches. The bifurcation diagrams obtained from these calculations are reported in the Figures 1C–1E. In the next part of the protocol, parameter searching is performed to determine the capacity of the ppMISA network in producing diverse types of reversible bistable switches. Figure 2 reports the counts and occurrence probabilities of 84 different reversible bistable switches and Figure 3 plots these quantities for a group of 14 switches obtained by combining counts of topologically similar switches. The parameter search calculation serves two purposes, first it determines the robustness of a network in generating a particular type of bistability and second it finds out the parameter set required to obtain a particular switch. Finally, the protocol generates phase diagram of bistable switches by varying two parameters simultaneously (Figure 4). The phase diagram determines the transition of a particular type of switch to another type while other parameters are varied.

The codes perform these calculations for the ppMISA network. The model equation files can be modified for other networks reported in the Das et al.[1] or any other networks that can be reduced to an effective one-dimensional dynamical system.

LIMITATIONS

Dimension reduction by applying quasi-steady state approximation on the dynamical variables is key to the pseudo-potential energy based bifurcation analysis. The multidimensional dynamical system representing the network must be reduced to a one-dimensional dynamical system such that an effective potential energy of the system can be defined and calculated.[1–3] The method is currently limited to networks where such reduction is possible. We have limited the search for non-canonical bistable switches up to three bistable regions. However, theoretically, there are many

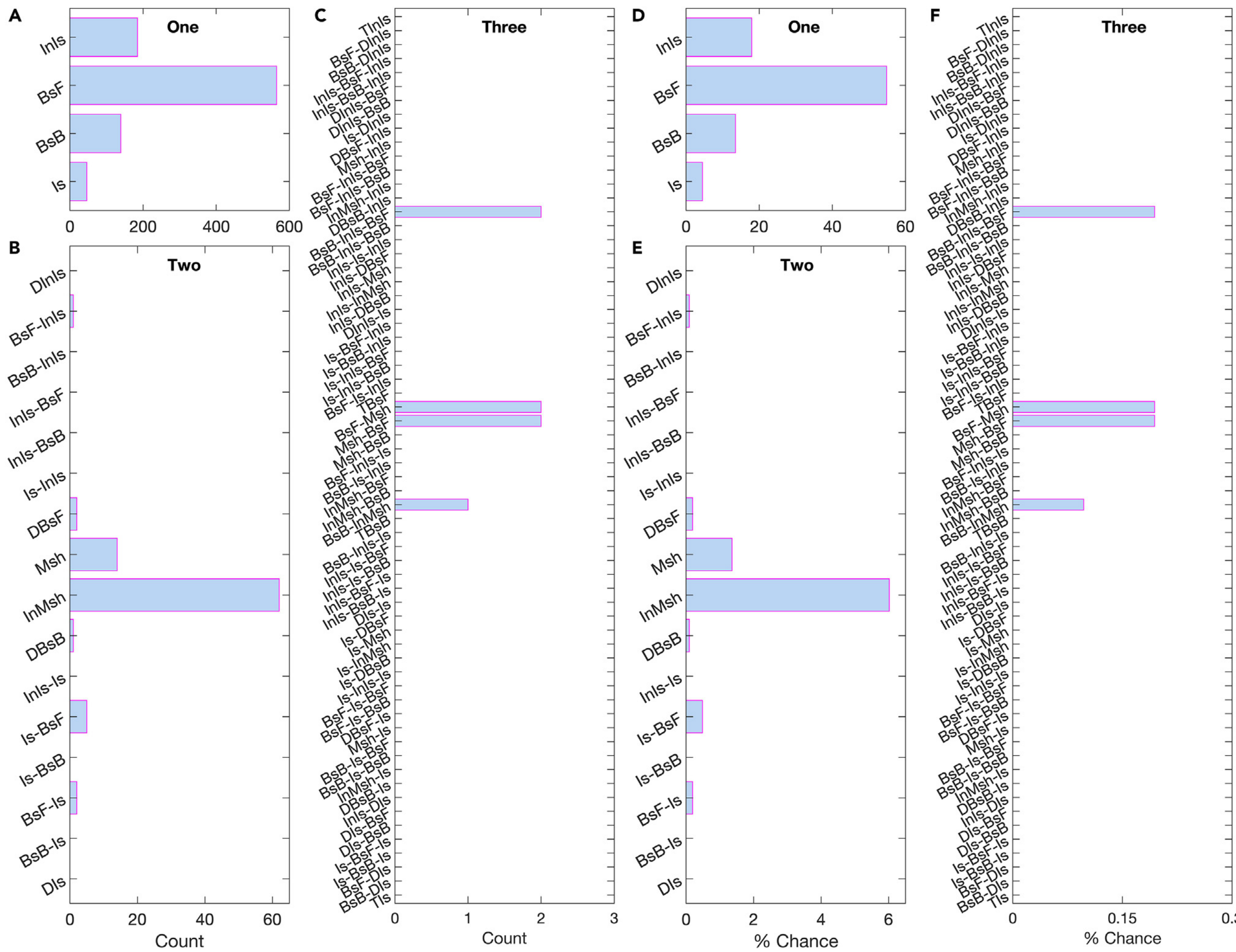

Figure 2. Outcome of the parameter searching for noncanonical bistable switches

(A–F) Bar plot of the counts (A–C) and occurrence probabilities (percentage chance) (D–F) of 84 reversible bistable switches obtained from parameter search calculation with a sample size of 10000. The plots are segregated based on the number of bistable regions as indicated inside each plot. See the Table S1 for the schematic representations of all the switches.

other noncanonical bistable switches with more than three bistable regions are possible. The possibility of such switches can be included in the parameter searching protocol. Finally, the protocol only deals with bistable switches originating from the saddle-node bifurcations. However bistable switches can originate from pitchfork bifurcation as well and such bistable switches are left out from the parameter searching protocol.

TROUBLESHOOTING

Problem 1

The bifurcation diagram may get truncated in the S and B axis depending on the choice of the numerical parameters in the model. It is particularly important for the B axis as truncation would lead to erroneous identification of the switch (Step 5).

Potential solution

To avoid the truncation in the steady state axis (B axis) the upper limit (xE) in the integration of the force term must be increased. The maximum value of the S (sig_max) can be increased to avoid the truncation of the switch at the largest value of S.

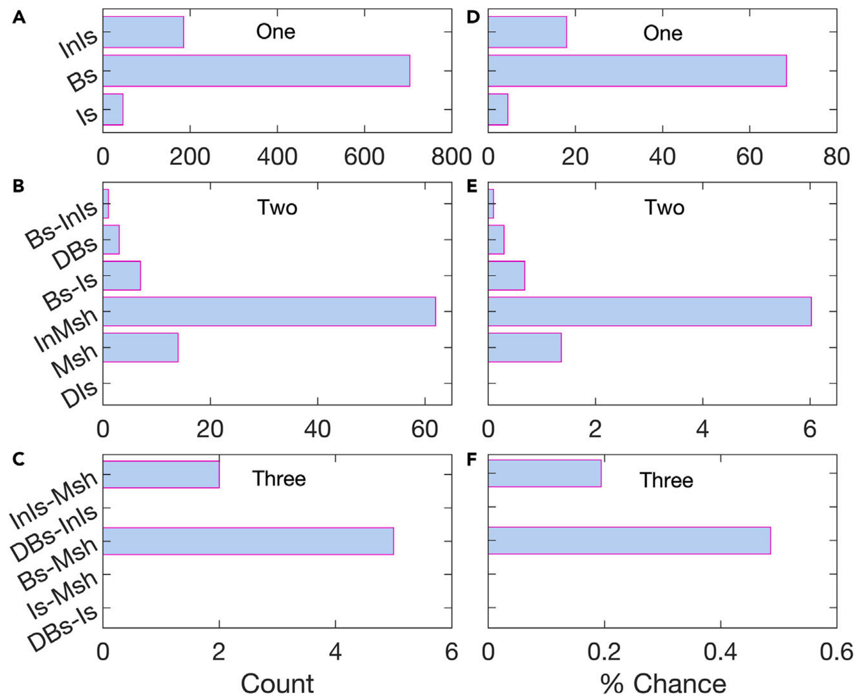

Figure 3. Outcome of parameter searching for the group of 14 bistable switches

(A–F) The counts (A–C) and occurrence probabilities (percentage chance) (D–F) of 14 types of bistable switches are segregated based on the number of bistable regions. See Das et al.[1] for the counts and occurrence probabilities of these switches with a much bigger sample size of 900,000. Table S2 lists these 14 switches along with their 'parent' switches.

Problem 2

In the parameter search calculation, MATLAB may return error message if the relevant data files for the threshold parameters are not present in the directory (Step 10).

Potential solution

The code for generating the threshold parameters must be run first (Step 9) and then the code for the parameter search can be run.

Problem 3

In the high-resolution bifurcation calculation in parameter searching (Step 16), the step length of S is determined based on the magnitude of the difference between the first and last bifurcation points obtained from low-resolution run. To reduce computational cost, a bigger step length is used for bigger magnitude of this difference. Due to this a mushroom switch with a very narrow bistable region in one side can be erroneously identified as canonical bistable switch.

Potential solution

To avoid such misidentification, in the high-resolution calculation the step length of S can be reduced from the default values by decreasing the value of 'fac' that factorizes the step length by the chosen value. The default value of 'fac' is 1 (line # 686 in the main code for parameter searching).

Problem 4

The phase boundaries in the phase diagram plot (Figure 4) can be rough depending on the step length of the two parameters against which phase diagram is calculated. In addition, the phase boundary of a mushroom (or inverted mushroom) switch can be noisy due to stochastic appearance of canonical bistable switch at the boundary.

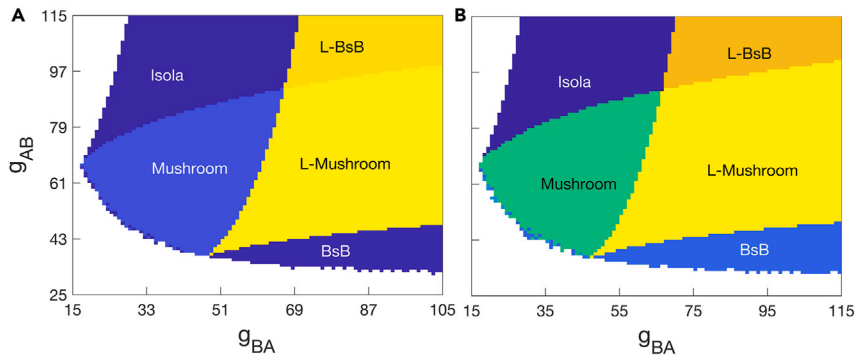

Figure 4. Phase diagram of bistable switches

(A and B) The phase diagram plots 'phaseIndx' as a function of mutual regulatory strengths of A (g_{AB}) and B (g_{BA}) (A). In the modified phase diagram, the 'phaseIndx' are changed according to the total number of phases and thereby result in a greater color contrast among phases (B).

Potential solution

- To avoid the roughness in the phase boundary the step length of phase parameters must be decreased (Step 25). Note that with decreased step length the computational cost would also increase. In such a case the phase diagram calculation can be performed in several smaller grids simultaneously and finally the phase diagram data of these smaller grids can be joined together to construct the entire diagram.
- The noisy edge of the mushroom switch can be removed by reducing the step length of the bifurcation parameter, S (Step 26).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Debashis Barik (dbariksc@uohyd.ac.in).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The codes and datasets generated during this study are available at GitHub [https://github.com/dbarikUoH/Protocol_NonCanonicalSwitch].

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2023.102665.

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AUTHOR CONTRIBUTIONS

D.B. and S.D. designed and tested the protocol and wrote the manuscript; D.B. supervised the study.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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