Supplementary Material of

Robust Open-Loop Control for Attractor Avoidance of Complex Biological Networks

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I. DETAILED PROCEDURES OF EXAMPLE

We revisit Examples 1 and 2 by including all the detailed procedures that are omitted in the paper. The state equation of F^0 is restated here for ease of understanding.

A. Example 1

Consider a simple BN F^0 with ten nodes (n = 10) whose state equations are given by

F^0:

x01_ = x07_ | x06_ | x01_

x02_ = ~x07_ & ~x10_ | x01_

x03_ = x04_ & x05_ & x06_ | x04_ & x08_

x04_ = ~x03_ & ~x08_ | ~x02_ & ~x03_

x05_ = ~x04_ & ~x09_ | x06_

x06_ = x05_ & x07_ | x03_ & x05_

x07_ = x02_ & x06_ & x07_ | x01_ & x02_ & x07_

x08_ = x05_ & x08_ | x04_ & x08_ | x04_ & x05_

x09_ = ~x06_ | ~x03_ | x02_

x10_ = x10_ | ~x07_ | ~x02_

where x+ denotes x(t+1) and '(t)' is omitted for brevity. The desired attractor set is identified as

$D^0 = \{c_1, \ldots, c_8\}$

c_1 = (0,0,0,1,0,0,0,0,1,1)

c_2 = (1,1,*,*,*,*,1,*,1,1)

c_3 = (1,1,0,1,0,0,0,0,1,1)

c_4 = (1,1,0,1,0,0,1,0,1,1)

c_5 = (1,1,0,0,1,1,1,1,1,1)

c_6 = (1,1,*,*,*,*,1,*,1,0)

c_7 = (1,1,0,0,1,1,1,1,1,0)

c_8 = (1,1,0,1,0,0,1,0,1,0)

By permanently fixing certain nodes to BN F^0, we make a population of BNs (F^1 and F^2)with node mutations whose state equations are given by

F^1: (x03_ = 0):

x01_ = x01_ | x06_ | x07_

x02_ = x01_ | (~x07_ & ~x10_)

x04_ = ~x02_ | ~x08_

x05_ = x06_

x06_ = x05_ & x07_

x07_ = (x01_ & x02_ & x07_) | (x02_ & x06_ & x07_)

x08_ = (x04_ & x05_) | (x04_ & x08_) | (x05_ & x08_)

x10_ = x10_ | ~x02_ | ~x07_

We identify the desired attractor set as the collection of attractors that match the target attractor specified by D^0.

$D^1 = \{c_1, \ldots, c_6\}$

c_1 = (0,0,0,1,0,0,0,0,1,1)

c_2 = (1,1,0,1,0,0,0,0,1,1)

c_3 = (1,1,0,1,0,0,1,0,1,1)

c_4 = (1,1,0,0,1,1,1,1,1,1)

c_5 = (1,1,0,0,1,1,1,1,1,0)

c_6 = (1,1,0,1,0,0,1,0,1,0)

F^2: (x04_ = 1):

x01_ = x01_ | x06_ | x07_

x02_ = x01_ | (~x07_ & ~x10_)

x03_ = x08_ | (x05_ & x06_)

x05_ = x06_

x06_ = (x03_ & x05_) | (x05_ & x07_)

x07_ = (x01_ & x02_ & x07_) | (x02_ & x06_ & x07_)

x08_ = x05_ | x08_

x09_ = x02_ | ~x03_ | ~x06_

x10_ = x10_ | ~x02_ | ~x07_

The desired attractor set is identified as

$D^2 = \{c_1, \ldots, c_4\}$

c_1 = (0,0,0,1,0,0,0,0,1,1)

c_2 = (1,1,0,1,0,0,0,0,1,1)

c_3 = (1,1,0,1,0,0,1,0,1,1)

c_4 = (1,1,0,1,0,0,1,0,1,0)

B. Example 2

We run Algorithm 1 to solve the attractor avoidance control problem of F^1 and F^2 in Example 1. By applying Algorithm 1, we derive [i,b] = [8,0] which maximizes the size of the avoidable undesired attractor set. After applying constant control x08_ := 0, we show the state equation of reduced H^j := $H^j_{|[8,0]}$(j=1,2), respectively.

H^1:

x01_ = x01_ | x06_ | x07_

x02_ = x01_ | (~x07_ & ~x10_)

x05_ = x06_

x06_ = x05_ & x07_

x07_ = (x01_ & x02_ & x07_) | (x02_ & x06_ & x07_)

x10_ = x10_ | ~x02_ | ~x07_

H^2:

x01_ = x01_ | x06_ | x07_

x02_ = x01_ | (~x07_ & ~x10_)

x03_ = x05_ & x06_

x05_ = x06_

x06_ = (x03_ & x05_) | (x05_ & x07_)

x07_ = (x01_ & x02_ & x07_) | (x02_ & x06_ & x07_)

x09_ = x02_ | ~x03_ | ~x06_

x10_ = x10_ | ~x02_ | ~x07_

We find FVS of each H to investigate whether additional control input is necessary or not. By fixing all possible state values of the nodes in FVS set to reduced BN, we can infer the possible number of point attractors. In this case, both of FVS has four nodes, we use 2^4 = 16 states to compute the number of point attractors in reduced BNs.

FVS node set of H^1= [x01_, x05_, x07_, x10_], [x01_, x06_, x07_, x10_]

FVS node set of H^2= [x01_, x06_, x07_, x10_]

The outcome of FVS control shows that $\Psi(H^1)$ = 6 and $\Psi(H^2)$ = 7, both of which are greater than |D1| = |D2| = 4. Thus, we have to find additional control inputs. By applying Algorithm 1 again, Φ' = {[5,0]} or {[6,0]}, and the final solution is Φ = {[8,0], [5,0]}, {[8,0], [6,0]}. In our numerical experiments, cases where additional control inputs were required occurred in about 16% of random BNs.

II. DETAILED METHODS ON ATTRACTOR AVOIDANCE CONTROL IN EXPERIMENTS OF COMPLEX BNS

For the experiments on attractor avoidance control of random complex BNs, optimization methods were applied to reduce time complexity. The core optimization focuses on the process of calculating the FVS. Specifically, the FVS is computed only if applying the current control solution reduces the network size below the predetermined threshold, 10 (corresponding to Algorithm 1). Furthermore, the size of the FVS is restricted to a maximum of 8 to remain within a practical scope. Even if multiple sets of the FVS are calculated, only one is selected to find the number of point attractors. Finally, the algorithm is designed to terminate immediately once at least one control candidate solution is determined. This approach guarantees the existence of a solution, but the final set of solutions may vary due to the specific order and selection.

TABLE S1
Detailed experimental results of the attractor avoidance control problem on random complex BNs

Number of elements in control inputs												
Network size	Average						95% confidence interval					
	Proposed algorithm	Brute force search	LDOI control	Stable motif control	Biobalm control	FVS control	Proposed algorithm	Brute force search	LDOI control	Stable motif control	Biobalm control	FVS control
node_10	1.26	2.14	2.24	2.72	2.43	3.91	0.48	0.85	1.16	1.07	0.98	1.16
node_20	1.85	2.62	4.40	3.30	2.94	5.30	0.82	0.95	4.61	1.23	1.30	1.51
node_30	1.95	2.72	6.65	3.66	3.2	6.53	0.86	0.98	6.45	1.24	1.27	2.31
node_40	2.02	3.22	10.43	-	3.86	8.57	1.05	1.14	11.19	-	1.60	2.26
Computational time (secs)												
Network size	Average						95% confidence interval					
	Proposed algorithm	Brute force search	LDOI control	Stable motif control	Biobalm control	FVS control	Proposed algorithm	Brute force search	LDOI control	Stable motif control	Biobalm control	FVS control
node_10	1.71	1.01	1.44	1.41	1.13	1.05	0.15	0.005	0.08	0.16	0.03	0.01
node_20	6.72	1.39	2.19	3.47	1.37	1.40	0.43	0.28	0.14	0.55	0.14	0.11
node_30	22.64	2.59	3.13	32.53	2.78	9.72	0.58	0.48	0.16	1.24	0.56	0.72
node_40	50.34	12.15	4.72	-	10.80	565.85	0.53	0.95	0.25	-	1.07	1.13

To generate random complex BNs, we utilized 'BNGenerator', a software tool that constructs random networks based on biological Boolean logics extracted from the Cell Collective (https://cellcollective.org/). 'BNGenerator' is adapted from https://github.com/choonlog/OutputStabilization.

Note that all the compared methods are not tailored specifically for the attractor avoidance control problem, as they were originally proposed control inputs for the state and/or output stabilization. Thus, for each BN population, we first apply each method to solving the global stabilization problem for every desired attractor within each abnormal BN. From the resulting set of solutions, we select the one with the minimum cardinality (it would be natural that recursive applications to all desired attractors increase the computational time). Consequently, the final solution is derived from the intersection of solutions from abnormal BNs in each population; if no common solution exists, the method fails to identify a final control input. All the compare methods are executed until the lapse of the maximum runtime of 24 hours. Networks for which no solution is found within 24 hours are excluded from the final analysis. In summary, for the stable motif control, only 98, 94, and 0 BN populations are analyzed for networks with 20, 30, and 40 nodes, respectively. For the biobalm control, 99 BN populations are examined for the network with 40 nodes. Other than these cases, 100 BN populations are analyzed for the attractor avoidance control problems. The following Python packages are utilized to implement the comparison procedure:

pystablemotifs.drivers.minimal_drivers for brute force search *pystablemotifs.drivers.GRASP* for greedy randomized adaptive search procedure (GRASP) search (GRASP_iterations = 2,000) for LDOI control *ar=pystablemotifs.AttractorRepertorie.from_primes* and *ar.succession_diagram.reprogram_to_trap_spaces* for stable motif control (adopted from https:// github.com/jcrozum/pystablemotifs); specifically, '*AttractorRepertoire.from_primes'* is employed to identify the complete set of attractors, while '*reprogram_to_trap_spaces*' is used to determine the control inputs based on the succession diagram; the computational time includes all steps, including attractor identification and control input search. *cana.control.feedback_vertex_set_driver_nodes*(graph='structural', method='bruteforce', max_search=10, keep_self_loops=True) for FVS control (adopted from https://github.com/CASCI-lab/CANA) *biobalm.SuccessionDiagram.from_rules* and *biobalm.control.succession_control* for biobalm control (adopted from https://github.com/jcrozum/biobalm)

TABLE S2
Specification of complex biological BNs

Network	Node number	Link number	Avg/ max in-degree	Mutated nodes	Size of undesired/desired	Undesired attractor set	Desired attractor set
EMT network	19	34	1.81 / 6	{Ex_TGFB=ON}	4 / 1	'**0**1*****11*****00*', '1001111001011010110 01', '0100010110111101 00000', '10**11100*0**0*011**1'	'1010111000000000011111'
				{miR_200=ON}	2 / 2	'10**11100*0*00*0111*1', '1001111001010010111 01'	'1010101000000000011111', '1010111000000000011111'
MAPK signaling network	53	108	2.04 / 5	{FGFR3=ON}	40 / 8	'***00**0*00**11***1**0**1******1111****1*0**0000*0***', '***00**0*01**10***1**0**1******1111****1*0**0000*0***'	'0110101010010100101111111100111111111011011010111111 0', '0110101010010110101111111100111111111011011010111111 0'
				{EGFR=ON}	40 / 16	'1**00**0*10**01*001010**1****0*1111*0*01*0**0000*0*0*', '***00**0*10**000*01*10**1******1111****1*0**0000*0***', …	'01111011111100001011111111001111111110110010010011110', '01101010111100101011111111001111111110110010000011110', …

For the EMT network, the undesired attractor set is defined by EMT hybrid phenotypes, which represent mixed epithelial and mesenchymal characteristics associated with high metastatic and invasive potential. Conversely, the desired attractor set is characterized by high expression of ‘CDH1_HIGH_’ and low expression of ‘VIM_HIGH_’. For the MAPK signaling network, the undesired attractor set comprises non-apoptotic phenotypes representing proliferative characteristics. The desired attractor set is defined by high expression levels of ‘Apoptosis_’ and ‘Growth_Arrest_’, and low expression level of ‘Proliferation_’.

Desired attractor set for the abnormal MAPK signaling network with {FGFR3_=ON}:

'0110101010010100101111111100111111111011011010101111110',
'0110101010010110101111111100111111111011011010101111110',
'0111101110010110101111111100111111111011011011111111110',
'0110101010110110101111111100111111111011011010101111110',
'0111101110010100101111111100111111111011011011111111110',
'0111101110110110101111111100111111111011011011111111110',
'0110101010110100101111111100111111111011011010101111110',
'0111101110110100101111111100111111111011011011111111110'

Undesired attractor set for the abnormal MAPK signaling network with {FGFR3_=ON}:
'***00**0*00**11***1**0**1******1111****1*0**0000*0***',
'***00**0*01**10***1**0**1******1111****1*0**0000*0***',
'***0***0*01**100**1**0**1******1111****1*0**0000*0***',
'***0***0*00*0100**1**0**1**0***1111**0*100*00000*0**0',
'***00**0*00**100**1**0**1******1111**0*1*0**0000*0***',
'***00**0*00*0100**1**0**1**0***1111**0*100*00000*0**0',
'***00**0*01**100**1**0**1******1111**0*1*0**0000*0***',

'***0***0*00**110**1**0**1******1111****1*0**0000*0***',
'1**00**0*00**1100*10*0**1****0*1111*0001*0**0000*0*0*',
'***00**0*01*0110**1**0**1**0***1111**0*100*00000*0**0',
'***00**0*00*0110**1*****1**0***1111**0*100*00000****0',
'***00**0*00*0100**1*****1**0***1111**0*100*00000****0',
'***0***0*00*0110**1*****1**0***1111**0*100*00000****0',
'***0***0*01*0100**1*****1**0***1111**0*100*00000****0',
'***00**0*01*0100**1**0**1**0***1111**0*100*00000*0**0',
'1**00**0*01**1100*10*0**1****0*1111*0001*0**0000*0*0*',
'1**00**0*00**1000*10*0**1****0*1111*0001*0**0000*0*0*',
'***0***0*01**110**1**0**1******1111****1*0**0000*0***',
'***00**0*00**110**1**0**1******1111**0*1*0**0000*0***',
'1**00**0*01**1000*10*0**1****0*1111*0001*0**0000*0*0*',
'***0***0*01*0100**1**0**1**0***1111**0*100*00000*0**0',
'1**00**0*01**11*0*10*0**1****0*1111*0*01*0**0000*0*0*',
'***00**0*01**11***1**0**1******1111****1*0**0000*0***',
'***00**0*01**100**1**0**1******1111****1*0**0000*0***',
'***00**0*01**110**1**0**1******1111**0*1*0**0000*0***',
'***0***0*00*0100**1*****1**0***1111**0*100*00000****0',
'***00**0*01*0100**1*****1**0***1111**0*100*00000****0',
'***00**0*00*0110**1**0**1**0***1111**0*100*00000*0**0',
'***0***0*00*0110**1**0**1**0***1111**0*100*00000*0**0',
'1**00**0*00**11*0*10*0**1****0*1111*0*01*0**0000*0*0*',
'***00**0*01*0110**1*****1**0***1111**0*100*00000****0',
'1**00**0*01**10*0*10*0**1****0*1111*0*01*0**0000*0*0*',
'***0***0*00**100**1**0**1******1111****1*0**0000*0***',
'***0***0*01*0110**1**0**1**0***1111**0*100*00000*0**0',
'***00**0*01**110**1**0**1******1111****1*0**0000*0***',
'***00**0*00**10***1**0**1******1111****1*0**0000*0***',
'1**00**0*00**10*0*10*0**1****0*1111*0*01*0**0000*0*0*',
'***00**0*00**100**1**0**1******1111****1*0**0000*0***',
'***0***0*01*0110**1*****1**0***1111**0*100*00000****0',
'***00**0*00**110**1**0**1******1111****1*0**0000*0***'

Desired attractor set for the abnormal MAPK signaling network with {EGFR_=ON}:

'0111101111110000101111111100111111111011001001001110',
'0110101011110010101111111100111111111011001000001110',
'0111101111010010101111111100111111111011011011111110',
'0110101011010010101111111100111111111011011010111110',
'0111101111010000101111111100111111111011011011111110',
'0111101111010000101111111100111111111011001001001110',
'0110101011010000101111111100111111111011001000001110',
'0110101011010010101111111100111111111011001000001110',
'0110101011110010101111111100111111111011011010111110',
'0111101111010010101111111100111111111011001001001110',
'0111101111110000101111111100111111111011011011111110',
'0111101111110010101111111100111111111011011011111110',
'0111101111110010101111111100111111111011001001001110',
'0110101011110000101111111100111111111011001000001110',
'0110101011010000101111111100111111111011011010111110',
'0110101011110000101111111100111111111011011010111110'

Undesired attractor set for the abnormal MAPK signaling network with {EGFR_=ON}:

'1**00**0*10**01*001010**1****0*1111*0*01*0**0000*0*0*',
'***00**0*10**000*01*10**1******1111****1*0**0000*0***',
'***0***0*10**000*01*10**1******1111****1*0**0000*0***',
'1**00**0*10**010001010**1****0*1111*0001*0**0000*0*0*',

'***0***0*10*0000*01*10**1**0***1111**0*100100000*0**0',
'***00**0*10*0000*01*10**1**0***1111**0*100100000*0**0',
'***00**0*10**01**01*10**1******1111****1*0**0000*0***',
'1**00**0*10**00*001010**1****0*1111*0*01*0**0000*0*0*',
'***00**0*11**01**01*10**1******1111****1*0**0000*0***',
'***0***0*11*0000*01*10**1**0***1111**0*100100000*0**0',
'***00**0*11**000*01*10**1******1111**0*1*0**0000*0***',
'1**00**0*11**010001010**1****0*1111*0001*0**0000*0*0*',
'***00**0*11*0010*01*10**1**0***1111**0*100100000*0**0',
'1**00**0*11**000001010**1****0*1111*0001*0**0000*0*0*',
'***00**0*10**000*01*10**1******1111**0*1*0**0000*0***',
'***00**0*11*0010*01*1***1**0***1111**0*100100000****0',
'***00**0*11*0000*01*10**1**0***1111**0*100100000*0**0',
'***00**0*10**010*01*10**1******1111**0*1*0**0000*0***',
'***00**0*10*0010*01*10**1**0***1111**0*100100000*0**0',
'***0***0*11*0010*01*10**1**0***1111**0*100100000*0**0',
'***00**0*10**010*01*10**1******1111****1*0**0000*0***',
'***00**0*11**010*01*10**1******1111****1*0**0000*0***',
'***0***0*11**000*01*10**1******1111****1*0**0000*0***',
'***0***0*11*0010*01*1***1**0***1111**0*100100000****0',
'***0***0*10*0010*01*1***1**0***1111**0*100100000****0',
'***00**0*10*0010*01*1***1**0***1111**0*100100000****0',
'***00**0*11*0000*01*1***1**0***1111**0*100100000****0',
'1**00**0*10**000001010**1****0*1111*0001*0**0000*0*0*',
'***00**0*11**00**01*10**1******1111****1*0**0000*0***',
'***0***0*10*0000*01*1***1**0***1111**0*100100000****0',
'***00**0*10*0000*01*1***1**0***1111**0*100100000****0',
'1**00**0*11**00*001010**1****0*1111*0*01*0**0000*0*0*',
'***00**0*11**010*01*10**1******1111**0*1*0**0000*0***',
'***00**0*10**00**01*10**1******1111****1*0**0000*0***',
'***0***0*10**010*01*10**1******1111****1*0**0000*0***',
'***00**0*11**000*01*10**1******1111****1*0**0000*0***',
'***0***0*10*0010*01*10**1**0***1111**0*100100000*0**0',
'***0***0*11*0000*01*1***1**0***1111**0*100100000****0',
'***0***0*11**010*01*10**1******1111****1*0**0000*0***',
'1**00**0*11**01*001010**1****0*1111*0*01*0**0000*0*0*'

TABLE S3
Detailed experimental results of the attractor avoidance control problem on complex biological BNs

Network	Algorithms	Computation times (sec.)	No. min. control inputs	No. solution sets	Control input sets
EMT network	Proposed algorithm	7.27	2	1	'~HA_&~SNAIL1_'
	Brute force search	0.05	0	0	-
	LDOI control	1.45	0	0	-
	Stable motif control	0.78	0	0	-
	Biobalm control	0.20	0	0	-
	FVS control	0.12	0	0	-
MAPK signaling network	Proposed algorithm	19.47	1	1	'TGFBR_stimulus_'
	Brute force search	3752.41	4	8	'DNA_damage_&EGFR_stimulus_&FGFR3_stimulus_&TGFBR_stimulus_','DNA_damage_&FGFR3_stimulus_&TGFBR_stimulus_&~EGFR_stimulus_','TGFBR_stimulus_&~DNA_damage_&~EGFR_stimulus_&~FGFR3_stimulus_','EGFR_stimulus_&FGFR3_stimulus_&TGFBR_stimulus_&~DNA_damage_','FGFR3_stimulus_&TGFBR_stimulus_&~DNA_damage_&~EGFR_stimulus_','EGFR_stimulus_&TGFBR_stimulus_&~DNA_damage_&~FGFR3_stimulus_','DNA_damage_&TGFBR_stimulus_&~EGFR_stimulus_&~FGFR3_stimulus_','DNA_damage_&EGFR_stimulus_&TGFBR_stimulus_&~FGFR3_stimulus_'
	LDOI control	23.69	0	0	-
	Stable motif control	54.14	4	8	'DNA_damage_&EGFR_stimulus_&FGFR3_stimulus_&TGFBR_stimulus_','DNA_damage_&FGFR3_stimulus_&TGFBR_stimulus_&~EGFR_stimulus_','TGFBR_stimulus_&~DNA_damage_&~EGFR_stimulus_&~FGFR3_stimulus_','EGFR_stimulus_&FGFR3_stimulus_&TGFBR_stimulus_&~DNA_damage_','FGFR3_stimulus_&TGFBR_stimulus_&~DNA_damage_&~EGFR_stimulus_','EGFR_stimulus_&TGFBR_stimulus_&~DNA_damage_&~FGFR3_stimulus_','DNA_damage_&TGFBR_stimulus_&~EGFR_stimulus_&~FGFR3_stimulus_','DNA_damage_&EGFR_stimulus_&TGFBR_stimulus_&~FGFR3_stimulus_'
	Biobalm control	5.33	4	8	'TGFBR_stimulus_&~DNA_damage_&~EGFR_stimulus_&~FGFR3_stimulus_','DNA_damage_&EGFR_stimulus_&TGFBR_stimulus_&~FGFR3_stimulus_','EGFR_stimulus_&TGFBR_stimulus_&~DNA_damage_&~FGFR3_stimulus_','DNA_damage_&FGFR3_stimulus_&TGFBR_stimulus_&~EGFR_stimulus_','FGFR3_stimulus_&TGFBR_stimulus_&~DNA_damage_&~EGFR_stimulus_','EGFR_stimulus_&FGFR3_stimulus_&TGFBR_stimulus_&~DNA_damage_','DNA_damage_&TGFBR_stimulus_&~EGFR_stimulus_&~FGFR3_stimulus_','DNA_damage_&EGFR_stimulus_&FGFR3_stimulus_&TGFBR_stimulus_'
	FVS control	1184.46	0	0	-

Note that '~HA_&~SNAIL1_' implies that control inputs are set to HA_:=0, SNAIL1_:=0; '-' indicates 'not applicable'.