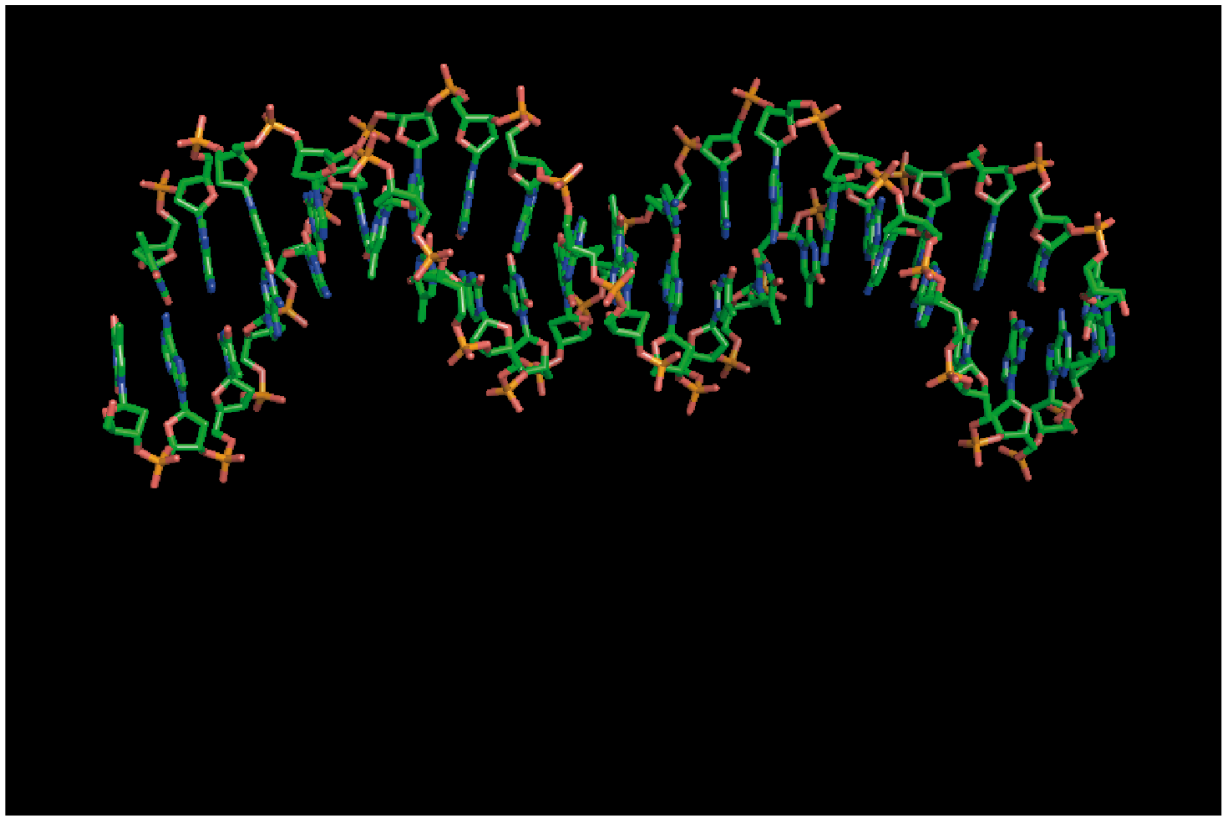
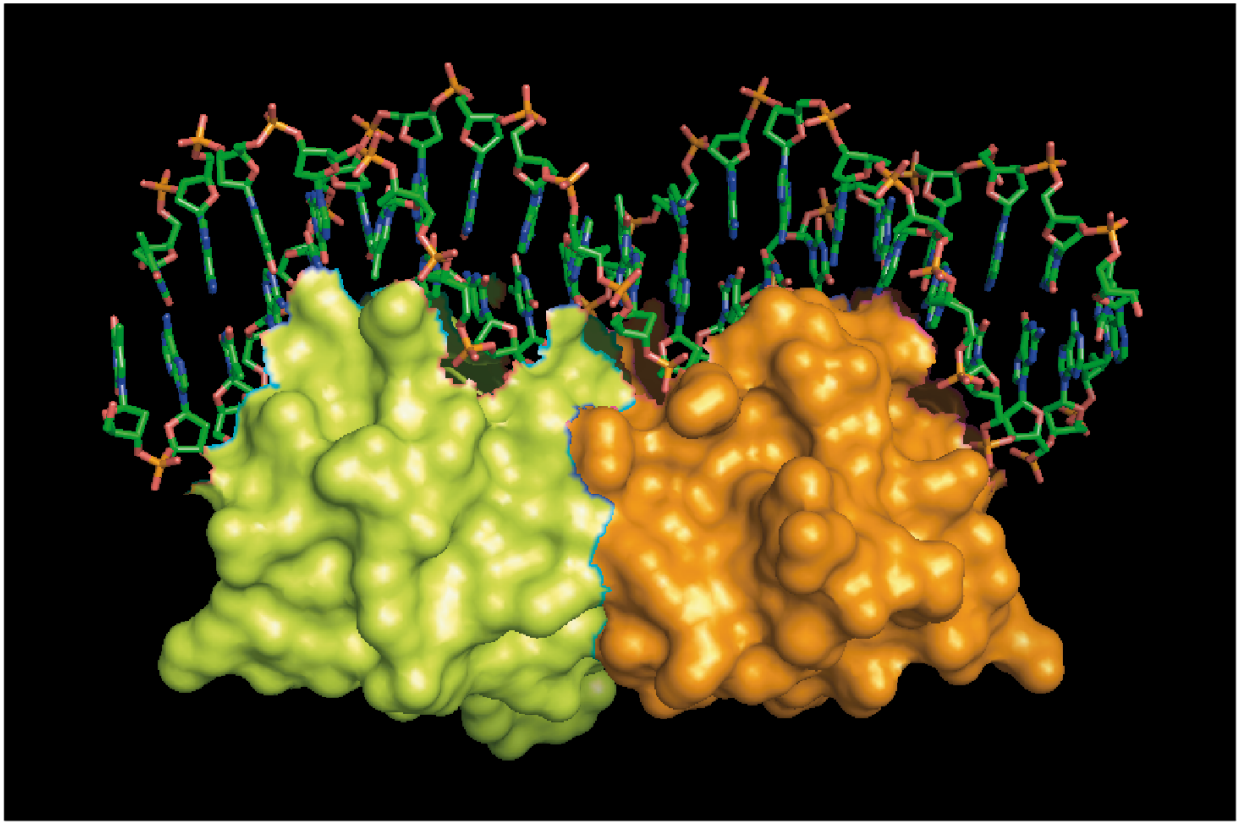
Susan Weininger
Molecular Lock Corporation

Construction of de novo biological process control circuits: parts and engineering principles

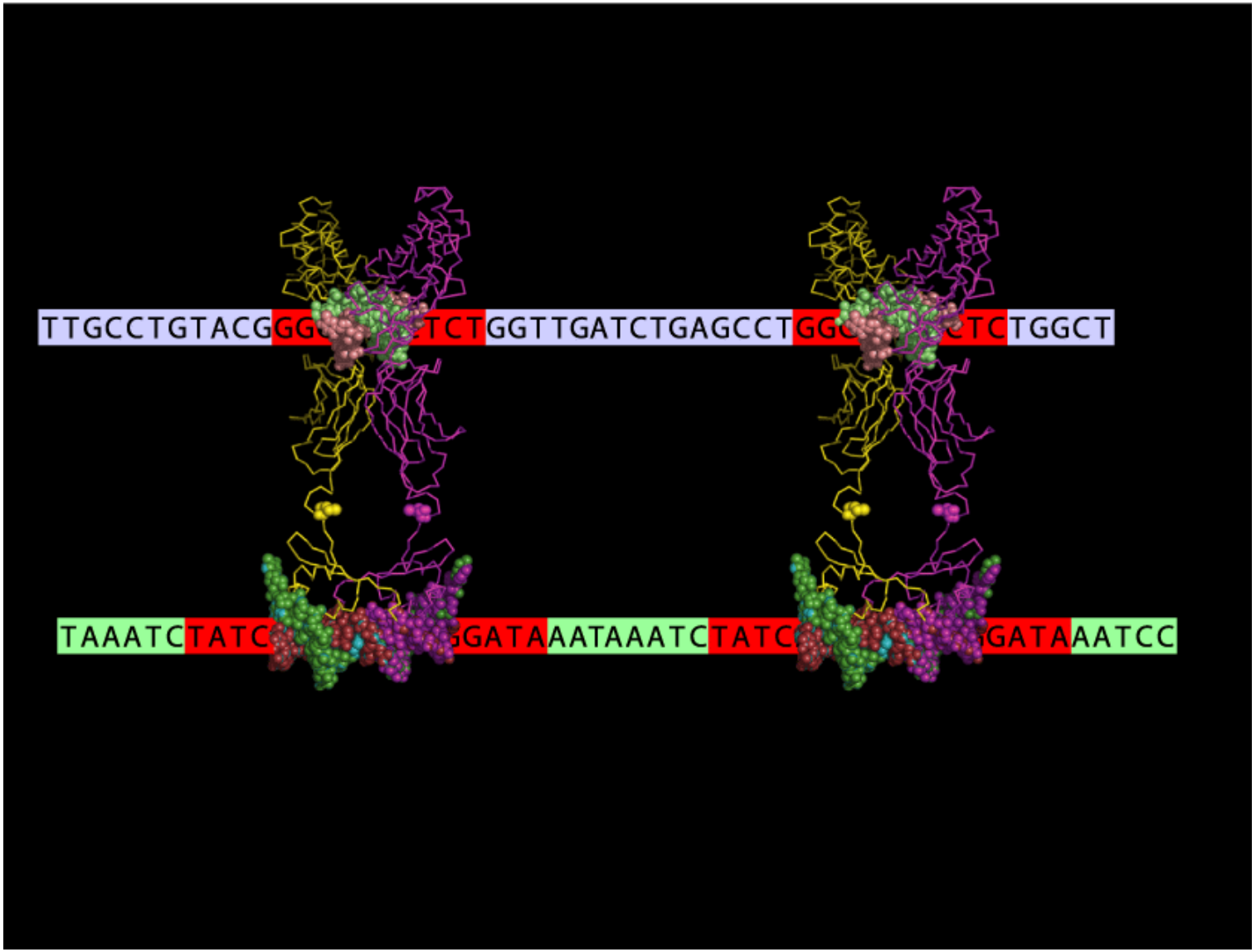
Stanford EE380: Computer Systems Colloquium
October 14, 2009



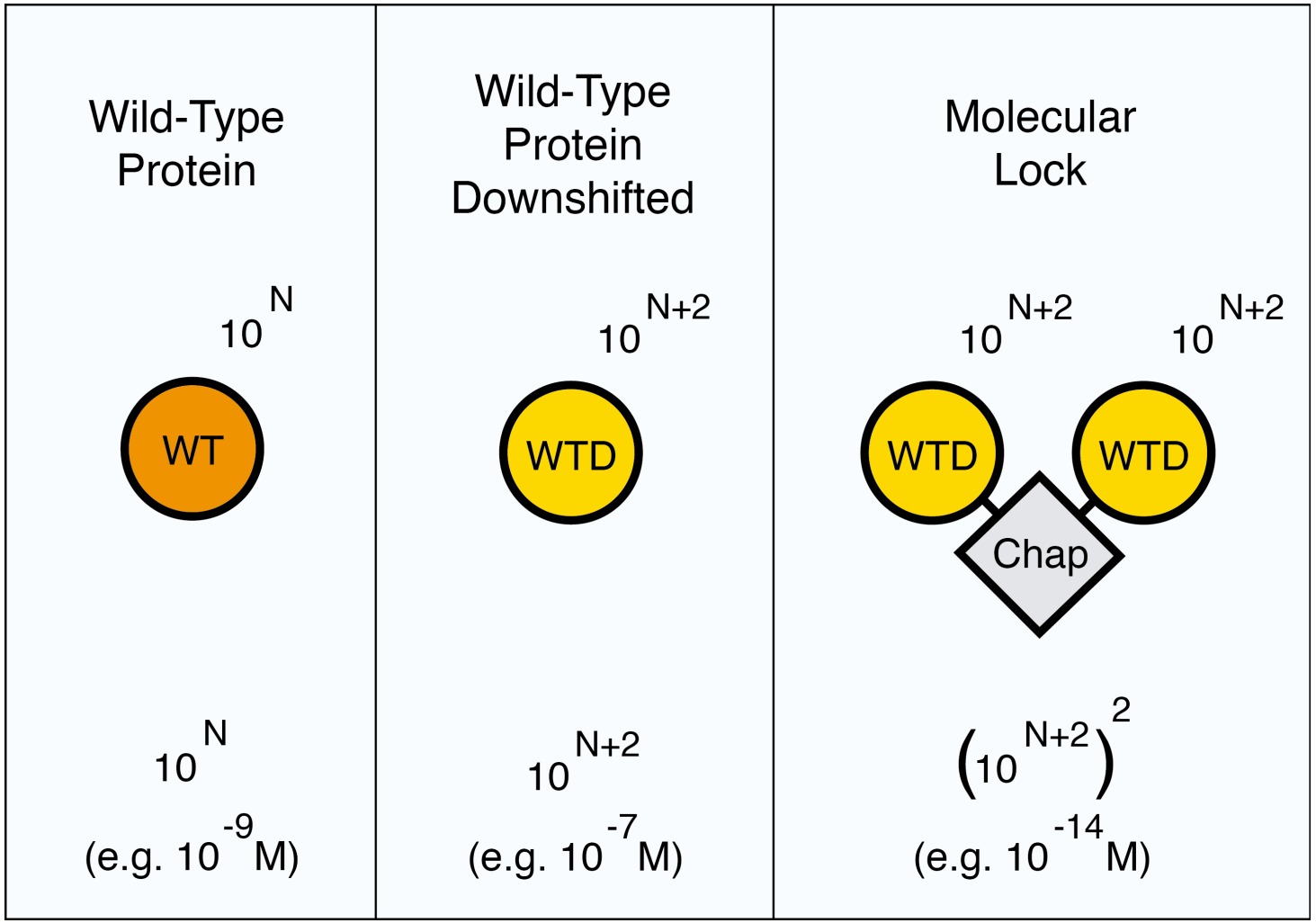
Structure → Sequence → Cloning → PCR → sRNA



Molecular Locks are molecular assemblies that are cooperatively assembled to bind to specific nucleic acid targets.

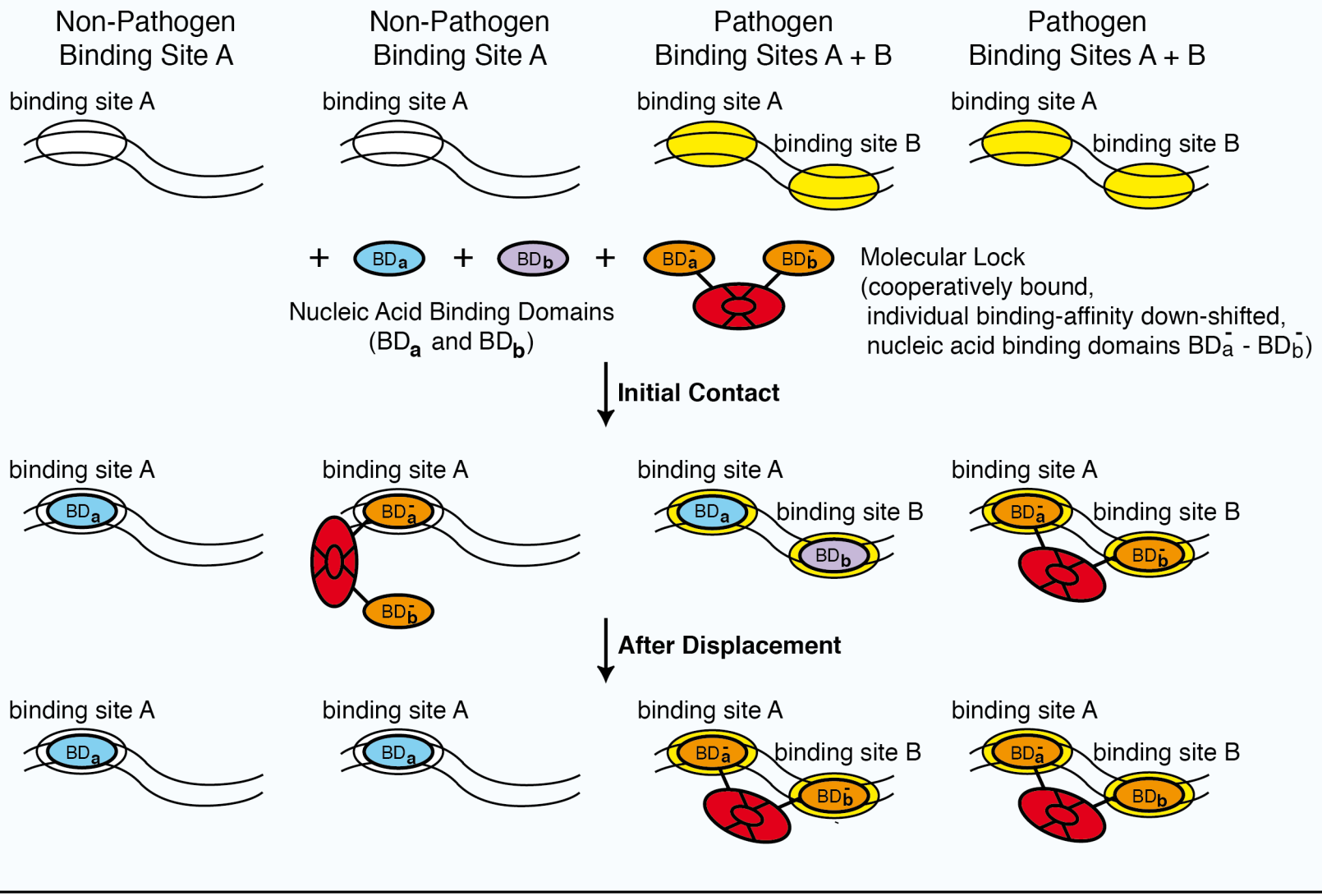


Cooperative assembly allows the Molecular Lock to bind tightly to its target without individual components interfering with sites that have some but not all of the features of the target. This minimizes the impact of the Molecular Lock on normal cellular control and trafficking (side effects).



Downshifting the binding affinity of the binding domains of a multivalent Molecular Lock provides selectivity for composite targets containing sequences found elsewhere in the genome.

Molecular Locks and Binding Competition

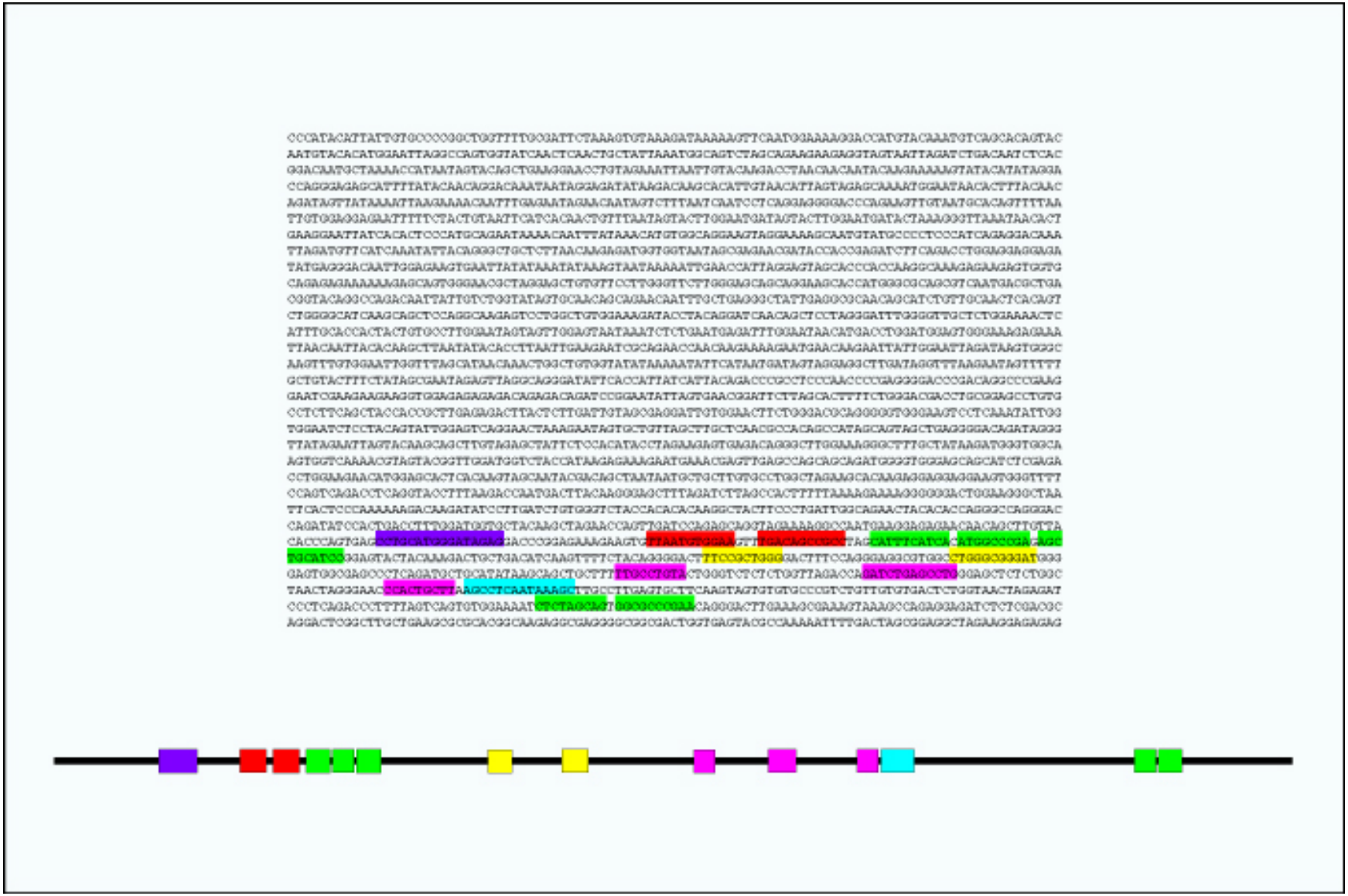


Molecular Lock Development Steps

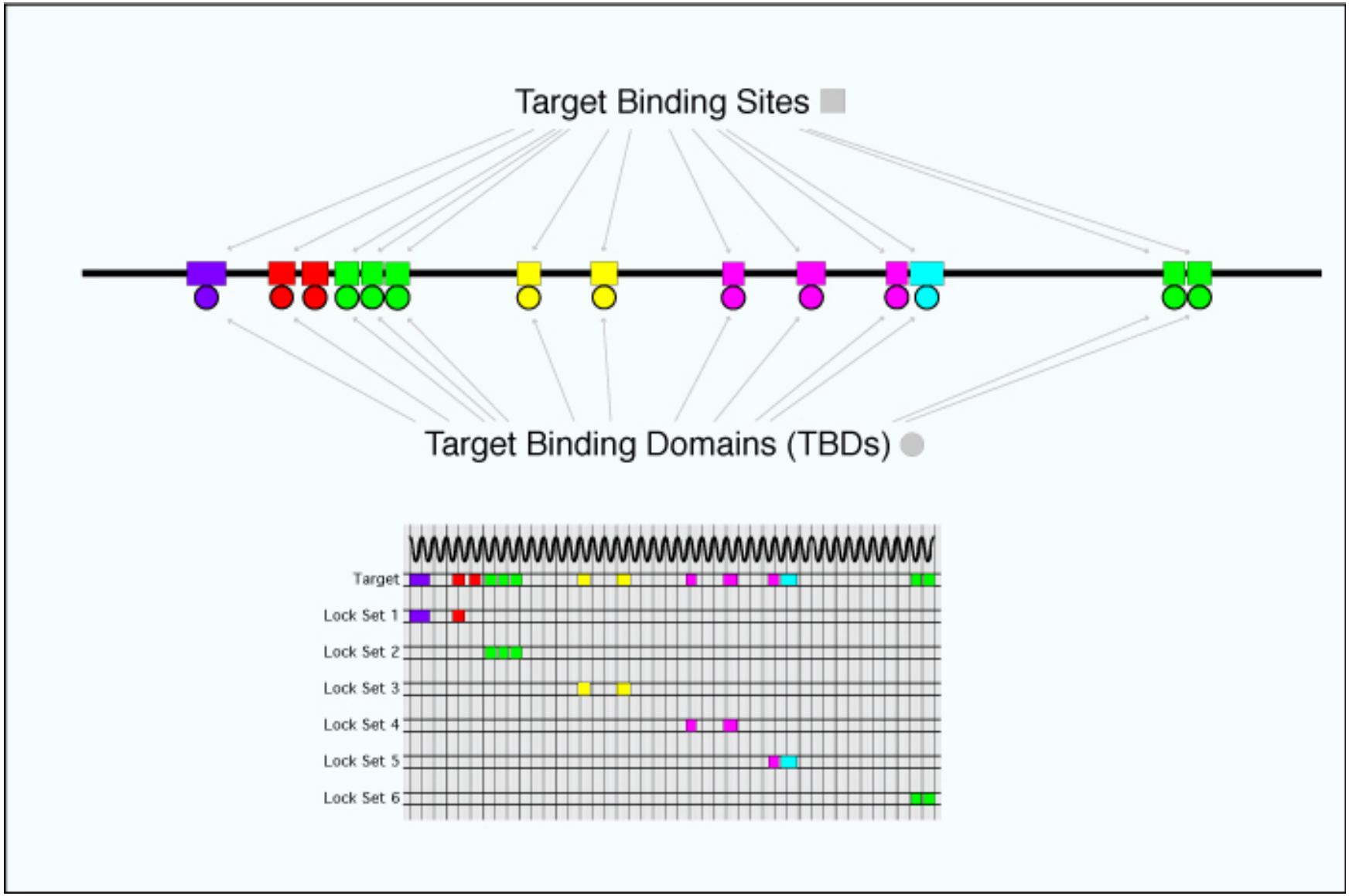
1) **Select target and identify protein binding sites within target.**
2) **Evaluate spacing of target binding sites and the radial presentation of proteins bound to the target.**
3) **Select oligomerization domains so that the nucleic acid binding domains cooperatively bind to the target in the right geometry.**
4) **Express nucleic acid binding – oligomerization domain hybrids and characterize the binding of these hybrid proteins in gel shifts.**
5) **Check the specificity of assembled Molecular Lock using Lock and Drop™.**

Select target and identify protein binding sites within target (Target shown: HIV LTR)

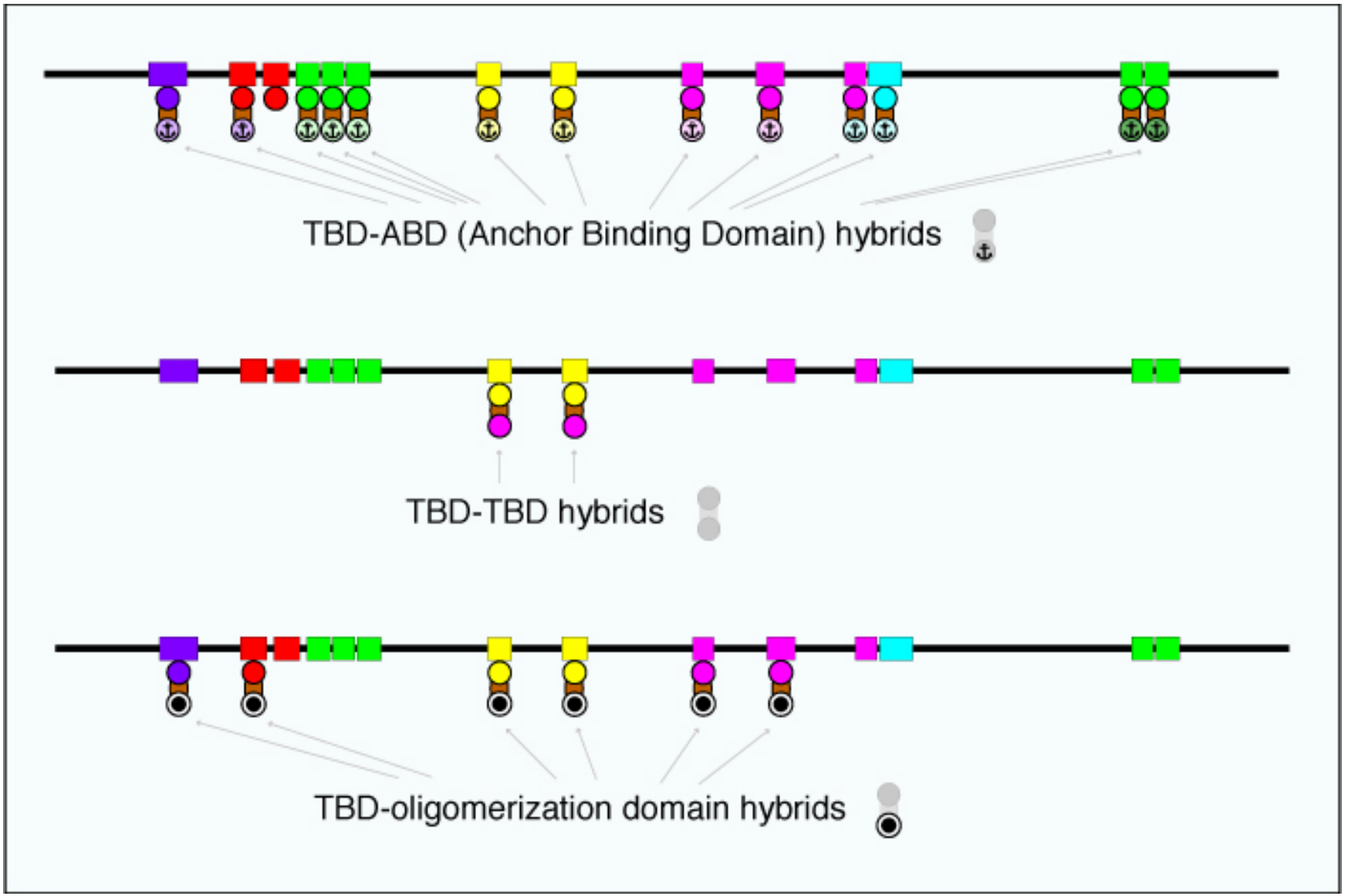
Evaluate spacing of target binding sites.



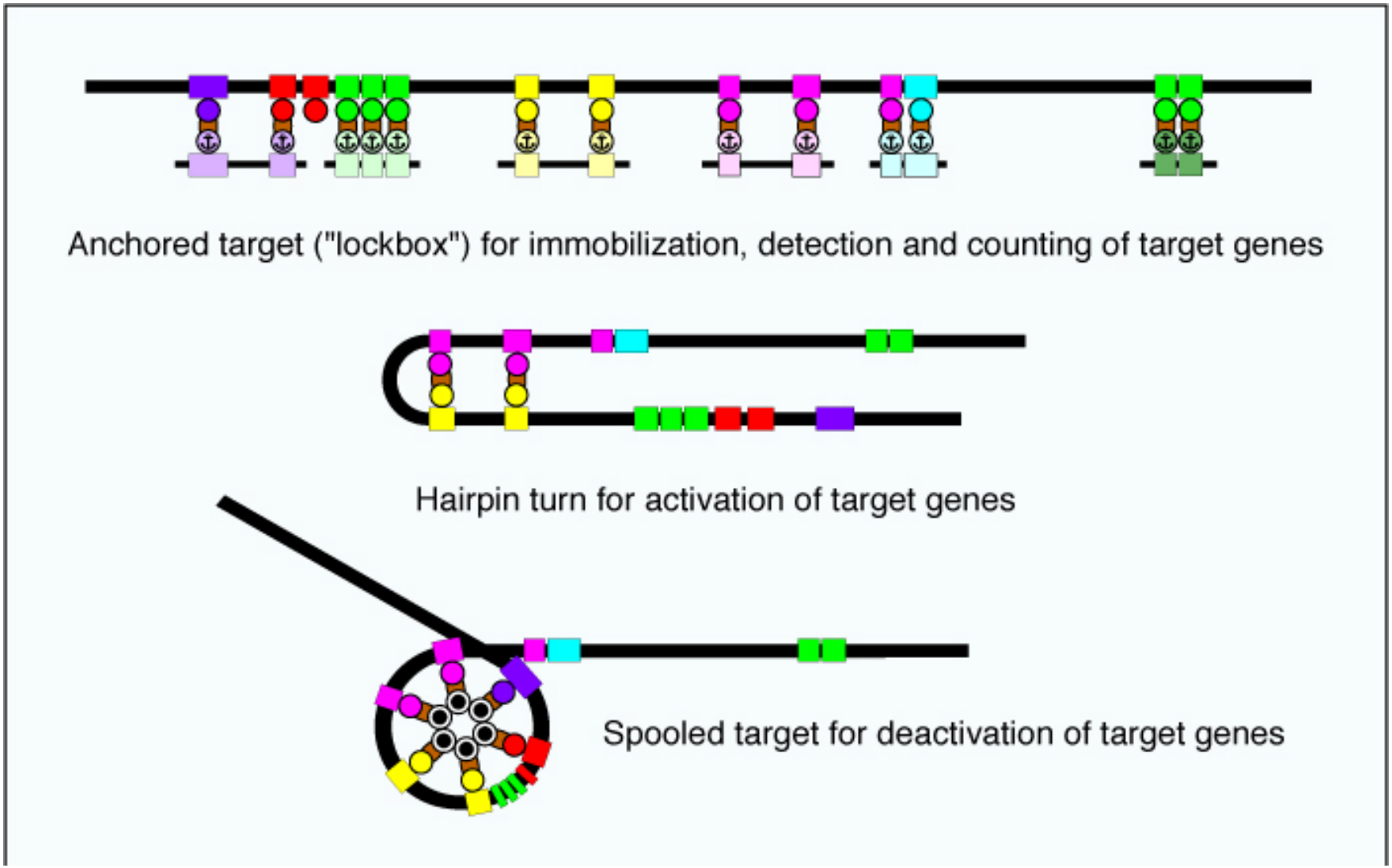
Evaluate radial presentation of proteins bound to the target.

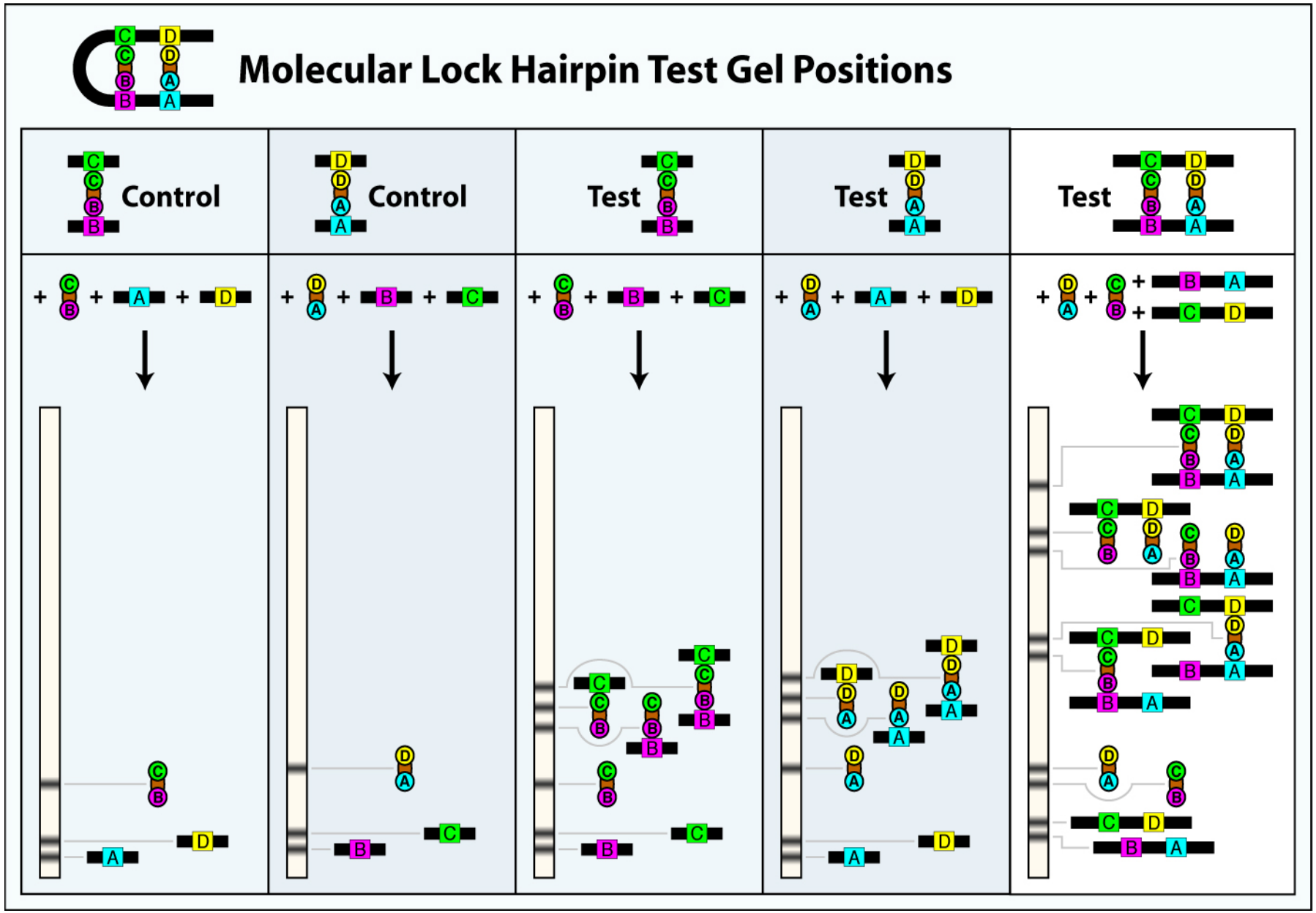


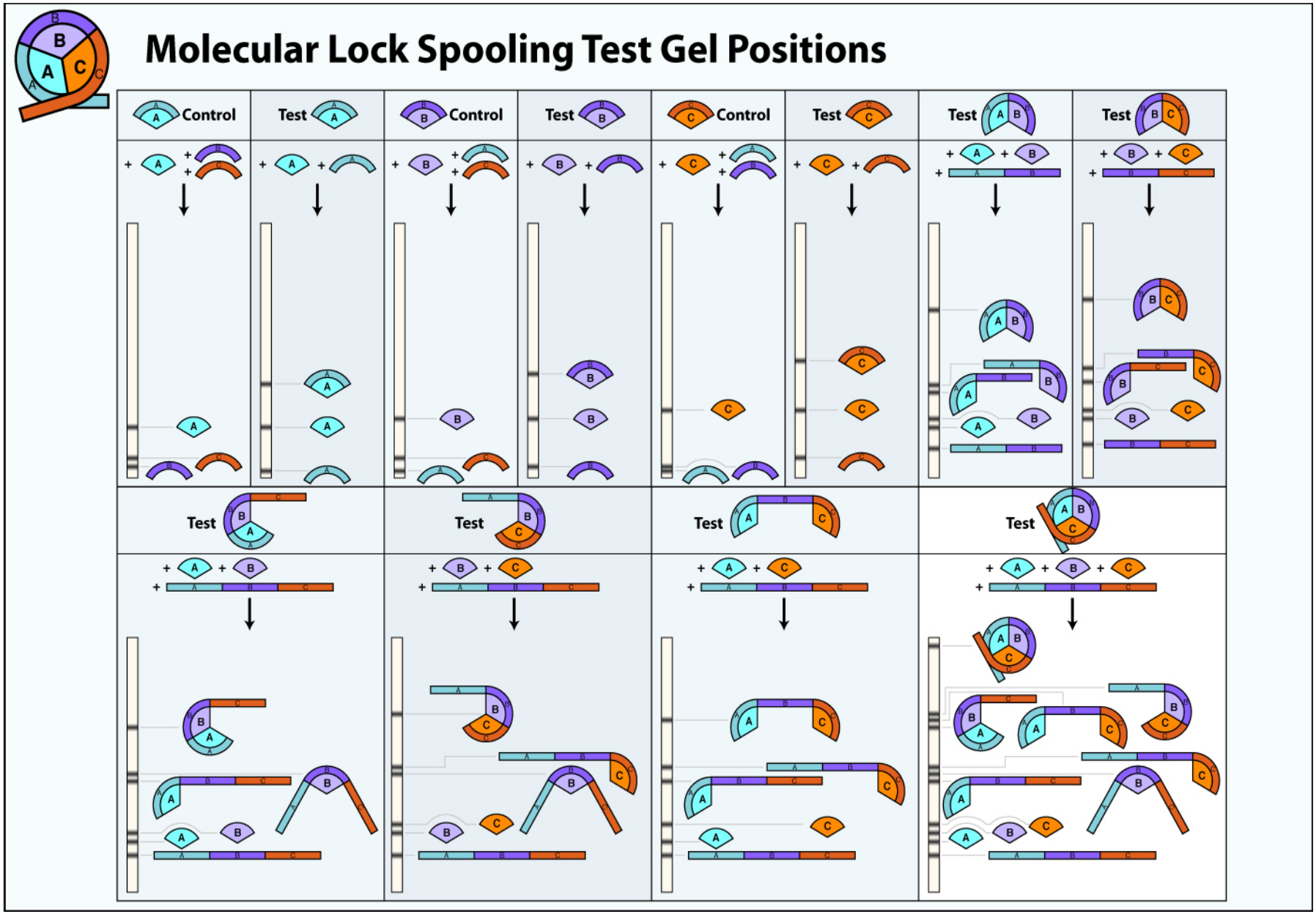
Express nucleic acid binding-oligomerization domain hybrids

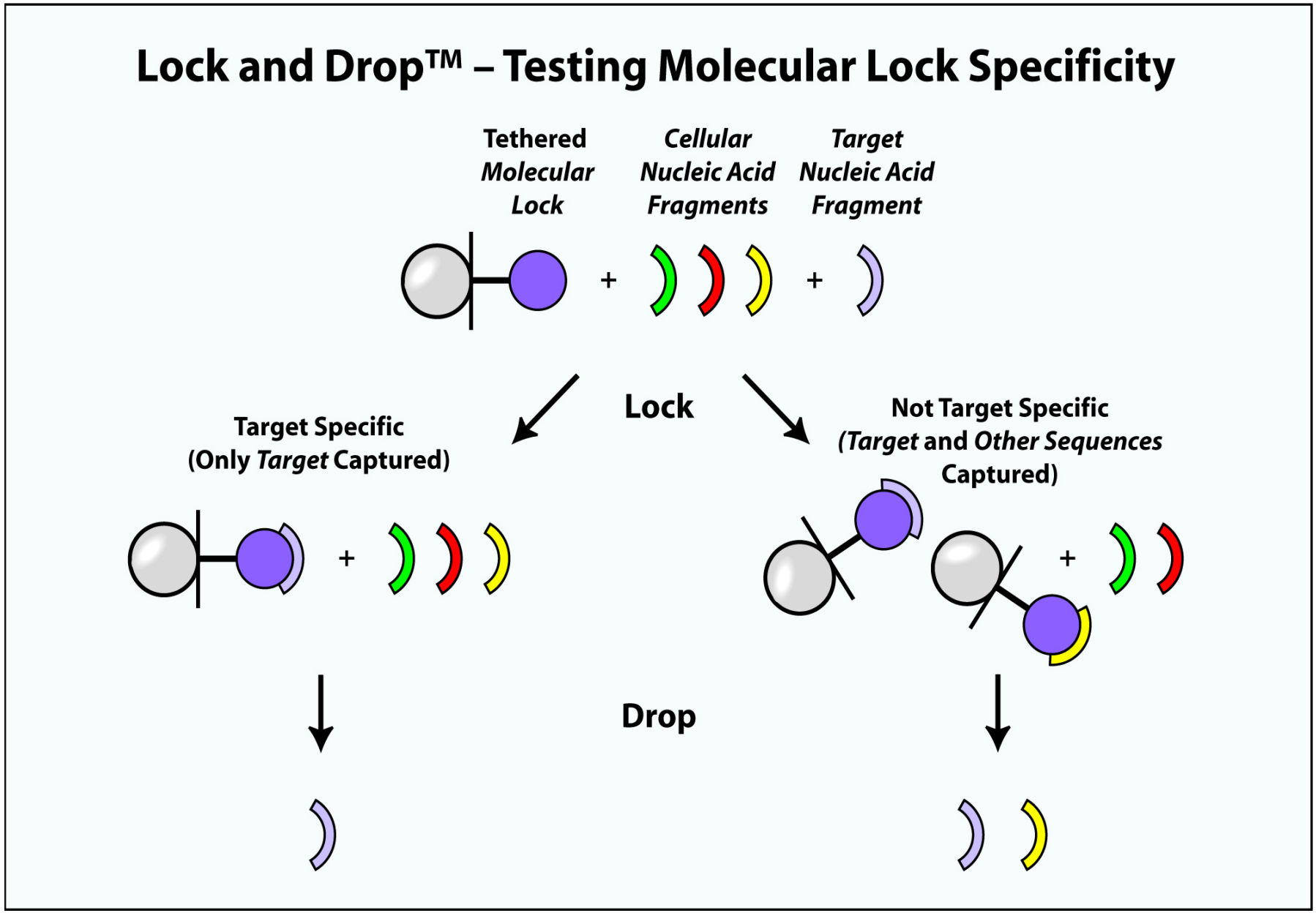


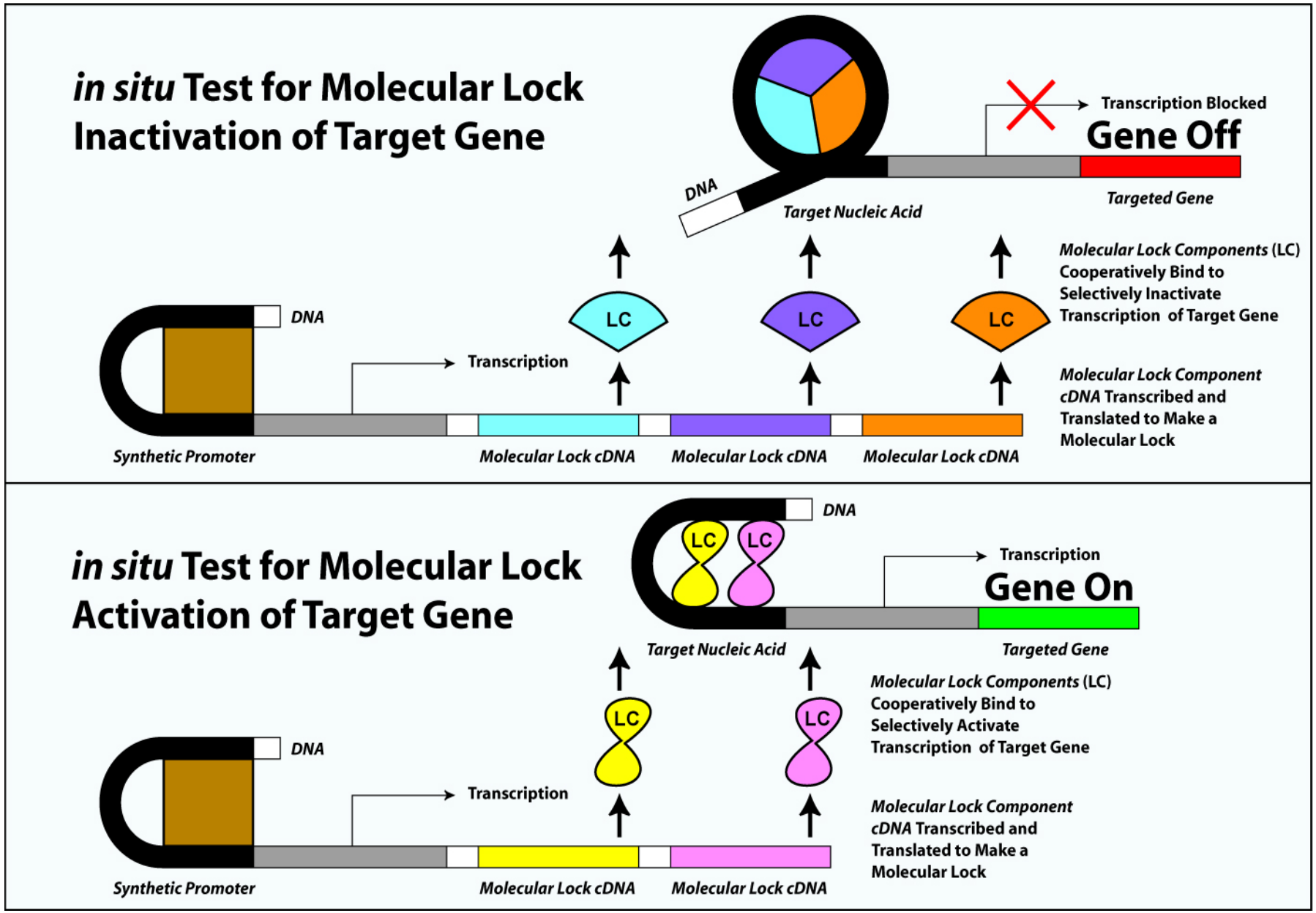
Select oligomerization domains so that the nucleic acid binding domains cooperatively bind to the target in the right geometry.

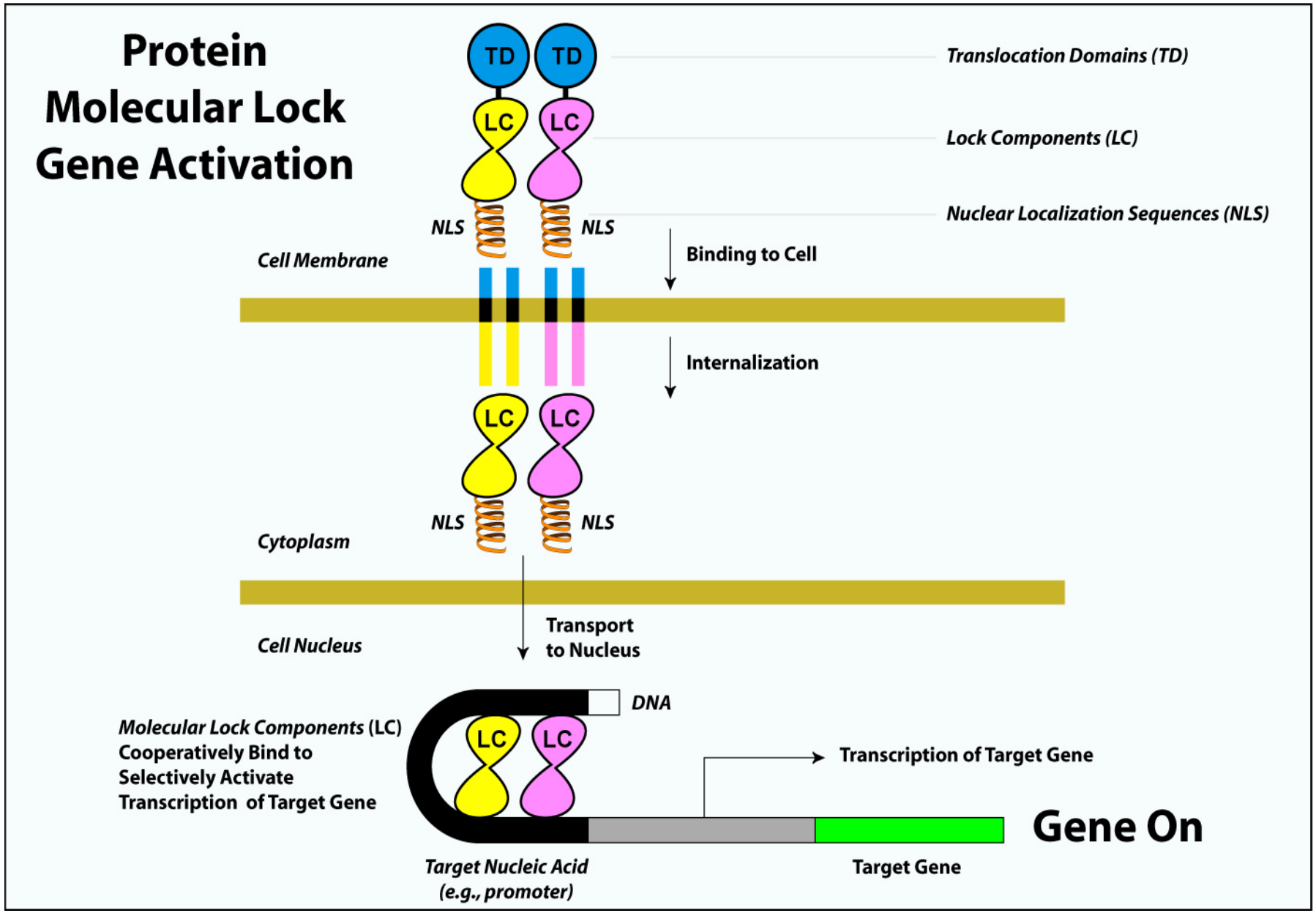


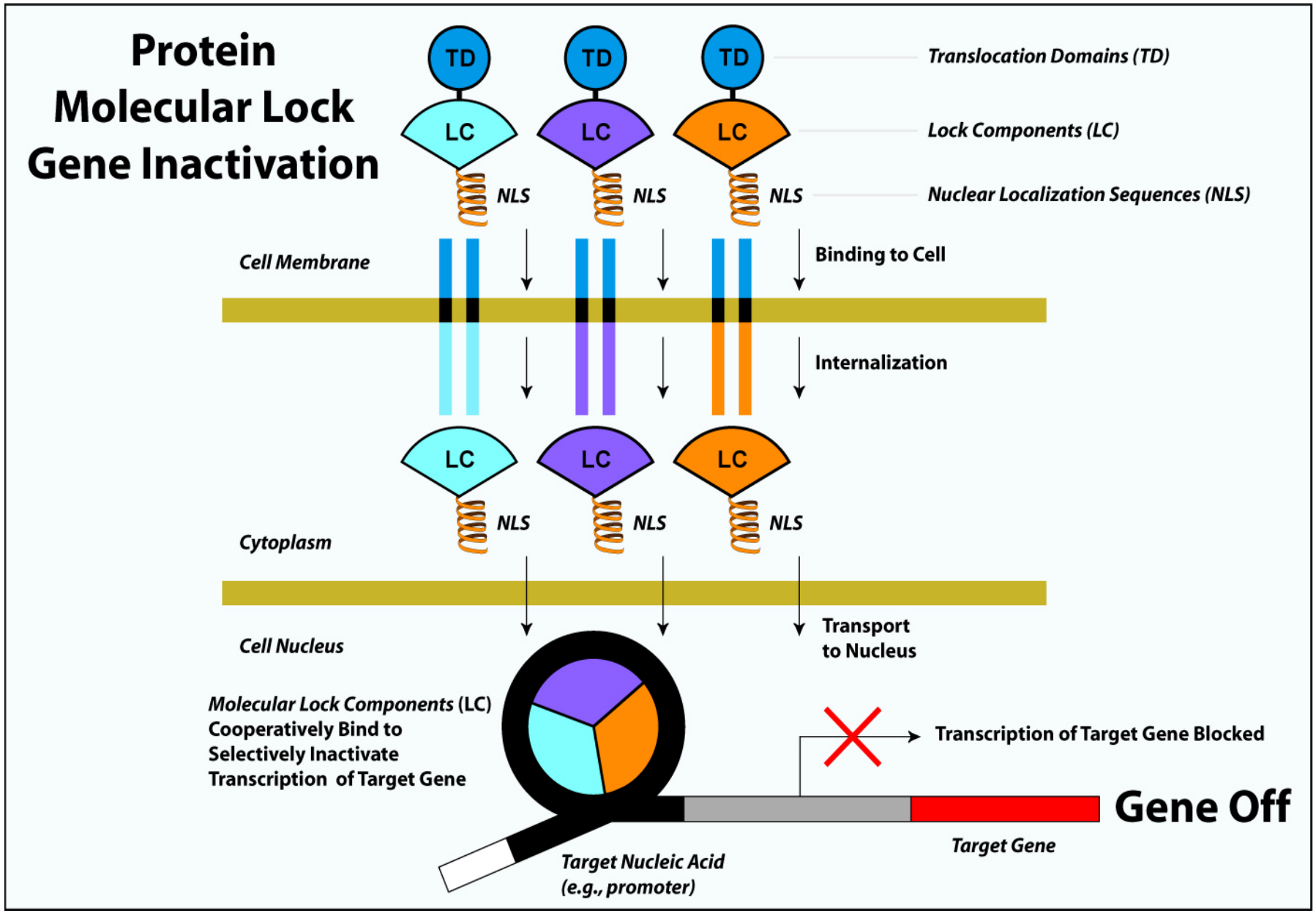




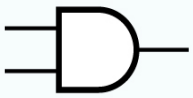








AND

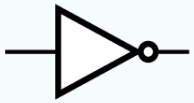


Input		Output
A	B	A AND B
0	0	0
0	1	0
1	0	0
1	1	1

OR

Input		Output
A	B	A OR B
0	0	0
0	1	1
1	0	1
1	1	1

NOT

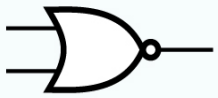


Input	Output
A	NOT A
0	1
1	0

NAND

Input		Output
A	B	A NAND B
0	0	1
0	1	1
1	0	1
1	1	0

NOR

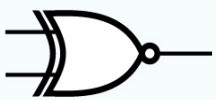


Input		Output
A	B	A NOR B
0	0	1
0	1	0
1	0	0
1	1	0

XOR

Input		Output
A	B	A XOR B
0	0	0
0	1	1
1	0	1
1	1	0

XNOR

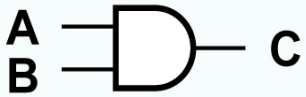


Input		Output
A	B	A XNOR B
0	0	1
0	1	0
1	0	0
1	1	1

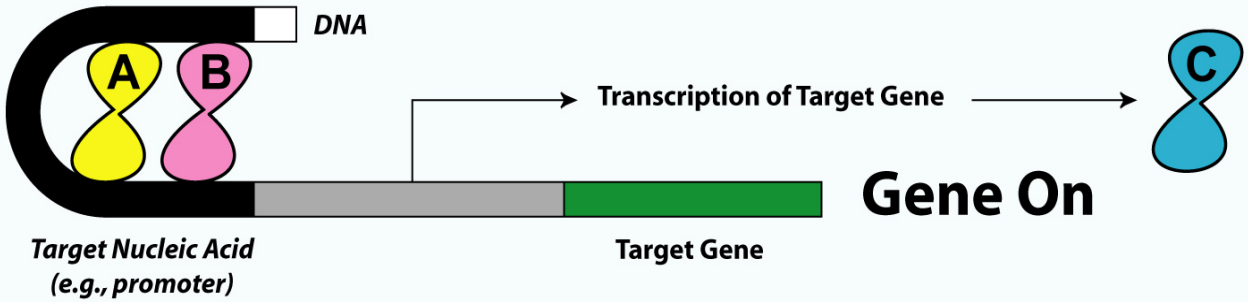
The structure and action of Molecular Locks makes them uniquely suited to engineering new biological circuits that can run parallel to existing naturally occurring biological circuits.

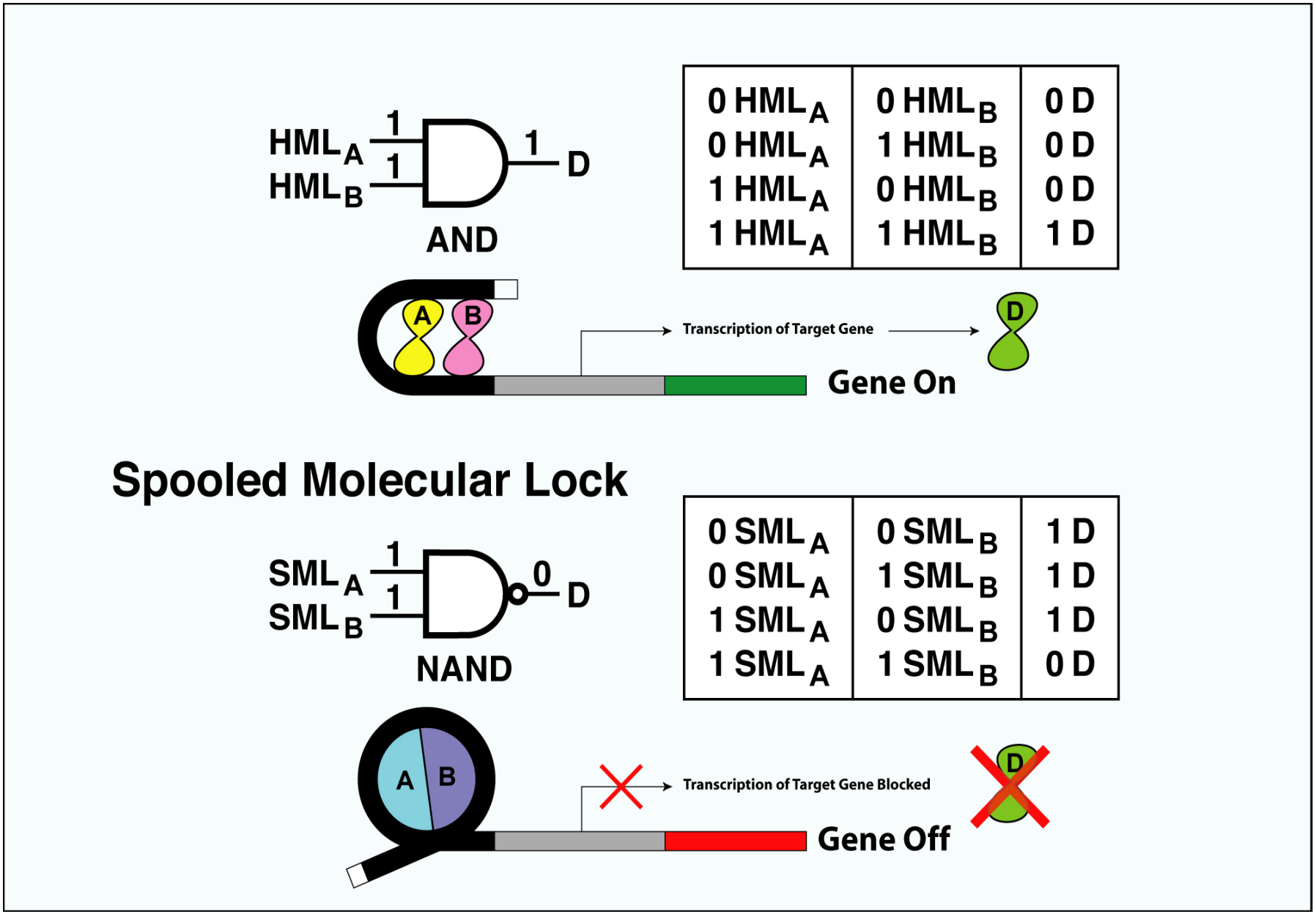
The following examples use free protein as the signal.

AND

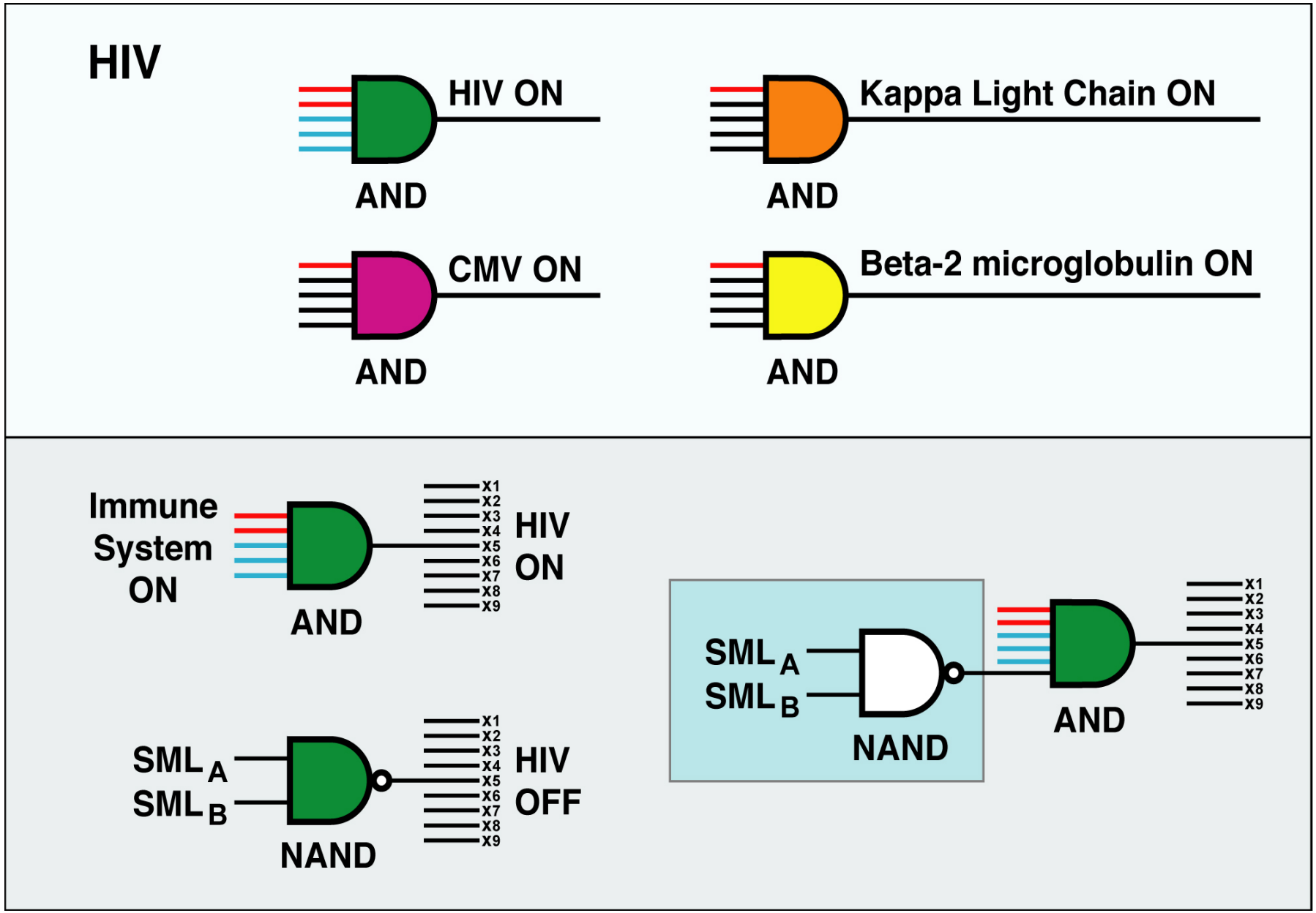


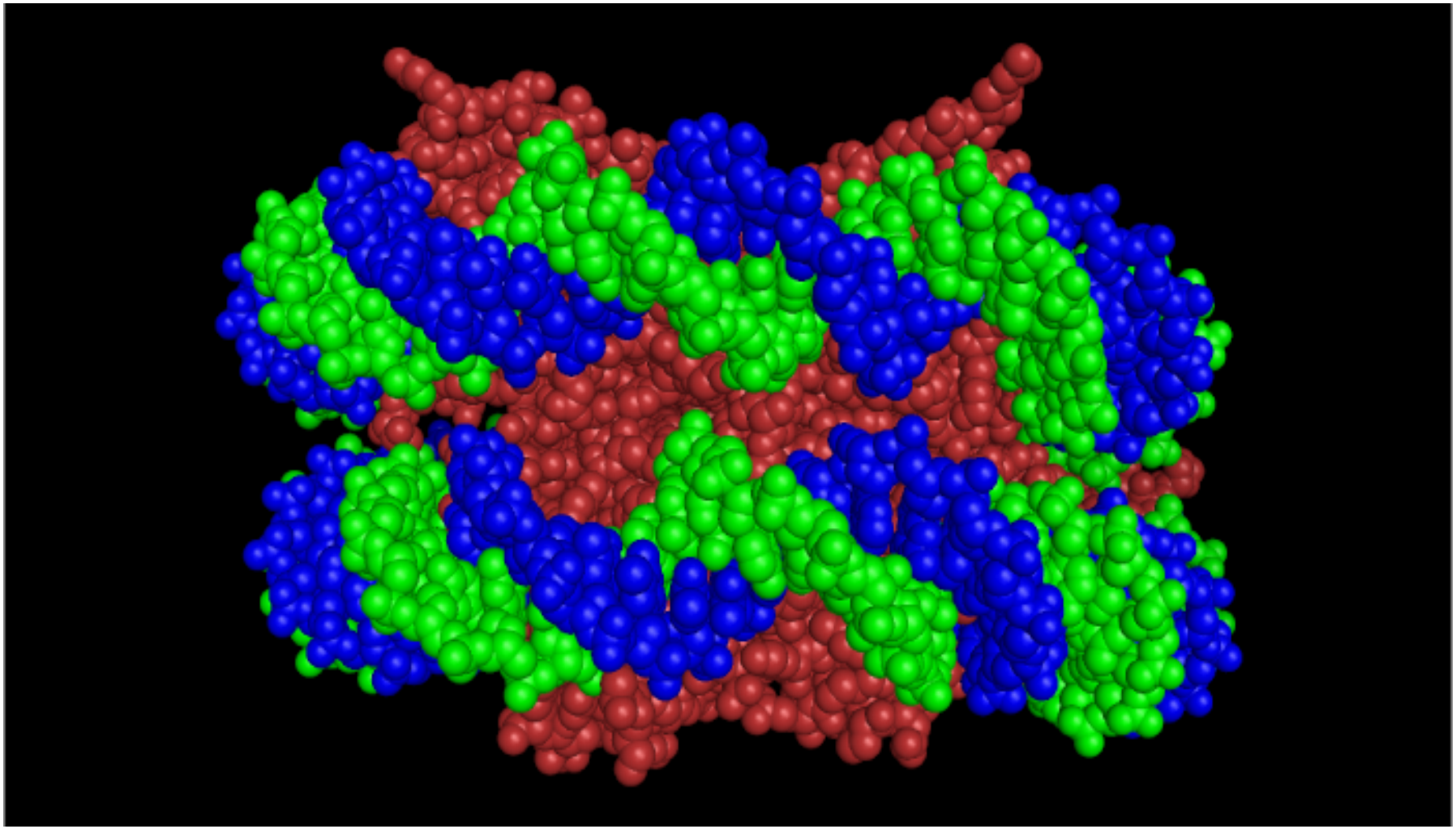
The binding components of the multivalent Molecular Lock must all be present for the Molecular Lock to cooperatively bind to the nucleic acid target. A Molecular Lock in the hairpin configuration can be used to activate a gene, expressing a new protein.



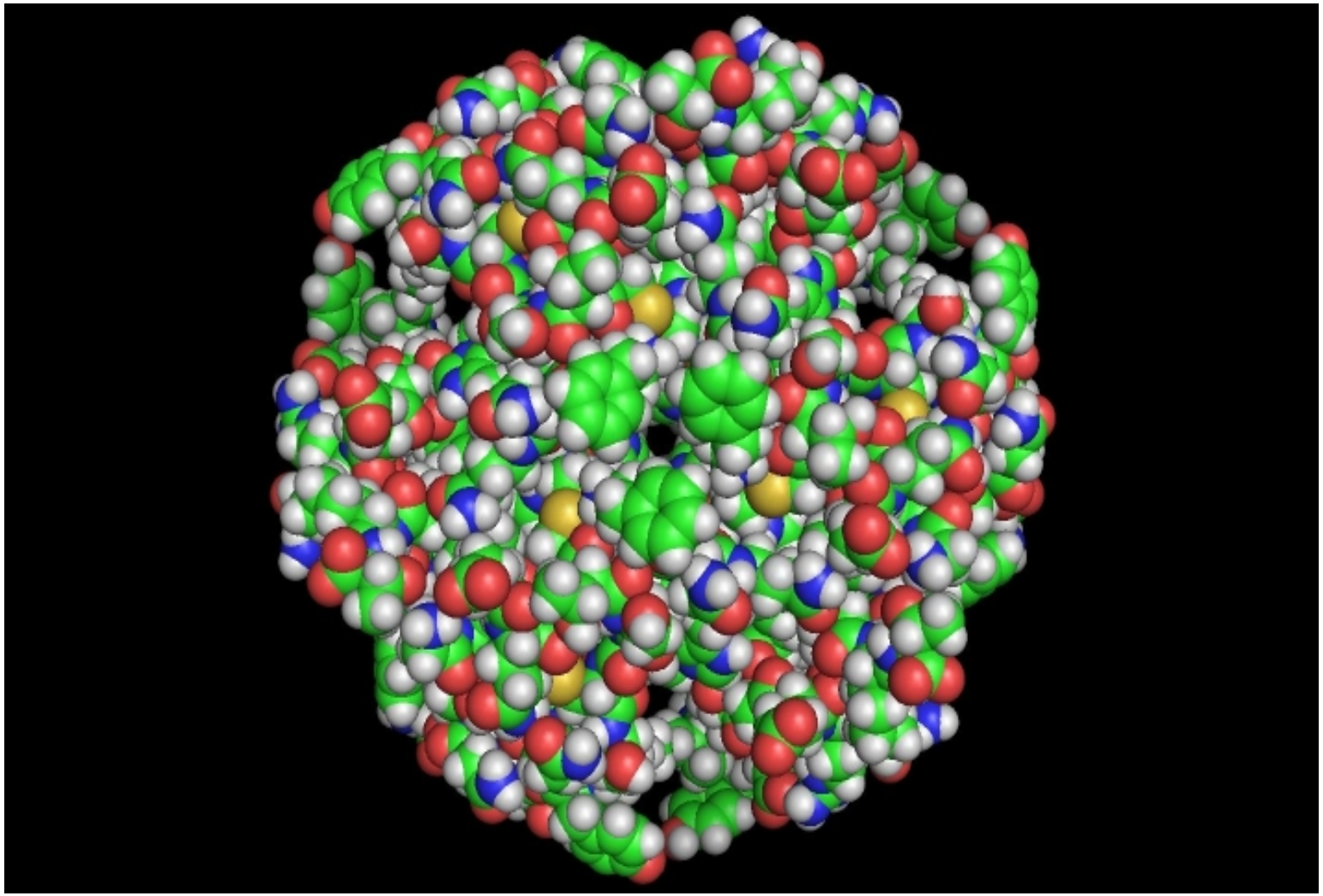


Cooperative assembly allows the Molecular Lock to bind tightly and selectively to its target, discriminating sites that have some but not all of the features of the target. For example, identical NF-κB binding sites are present in the HIV-LTR (the control region of HIV) and in the human genome (e.g., the Beta-2-microglobulin promoter, the Kappa light chain promoter); a Molecular Lock can discriminate for an HIV-LTR target containing such a site in a human genome sample.



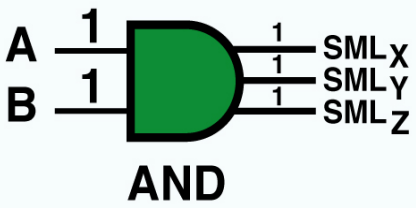


Histones are naturally occurring structures in which protein spools the DNA, shutting off its transcription.

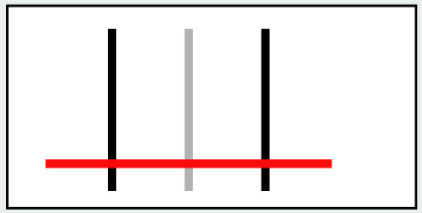


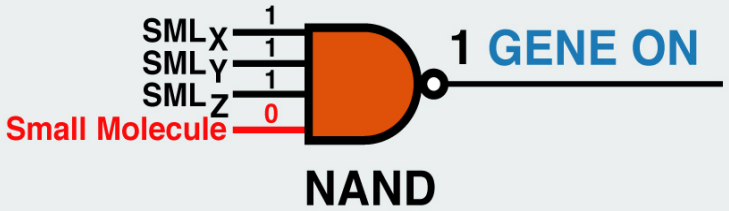
Molecular Lock scaffolds are similar in size to Histone protein assemblies.

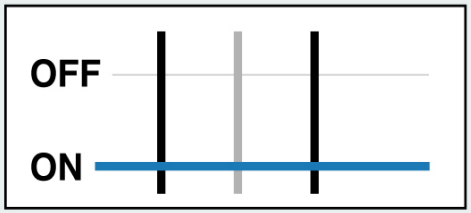
Constitutive

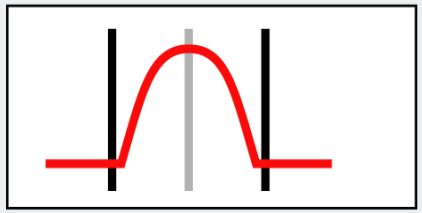


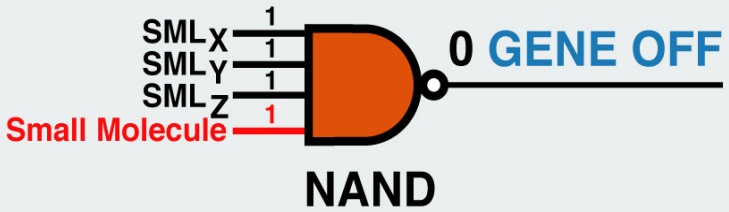
Small Molecular Control

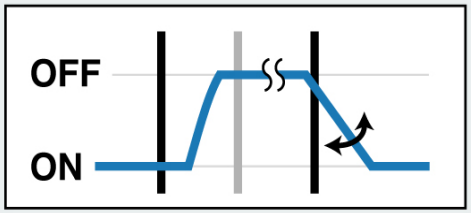




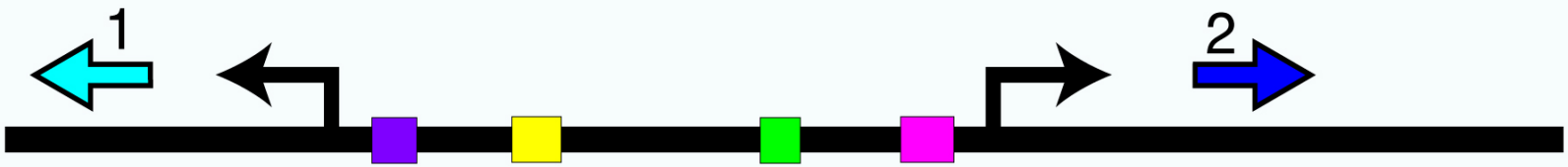


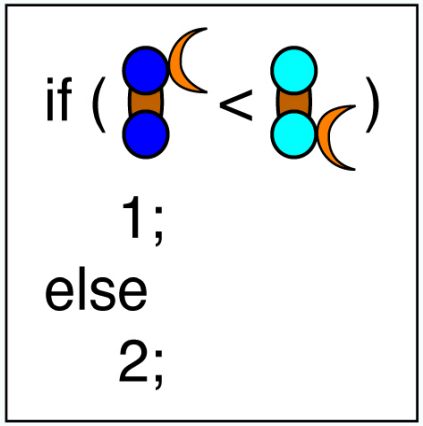


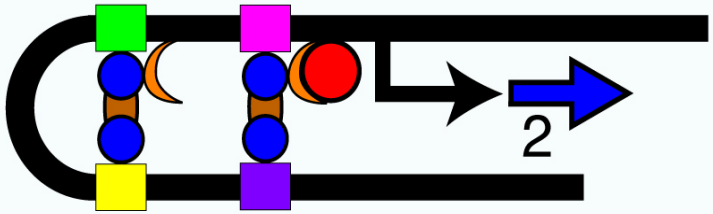


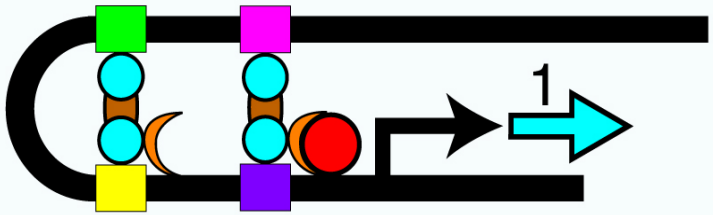


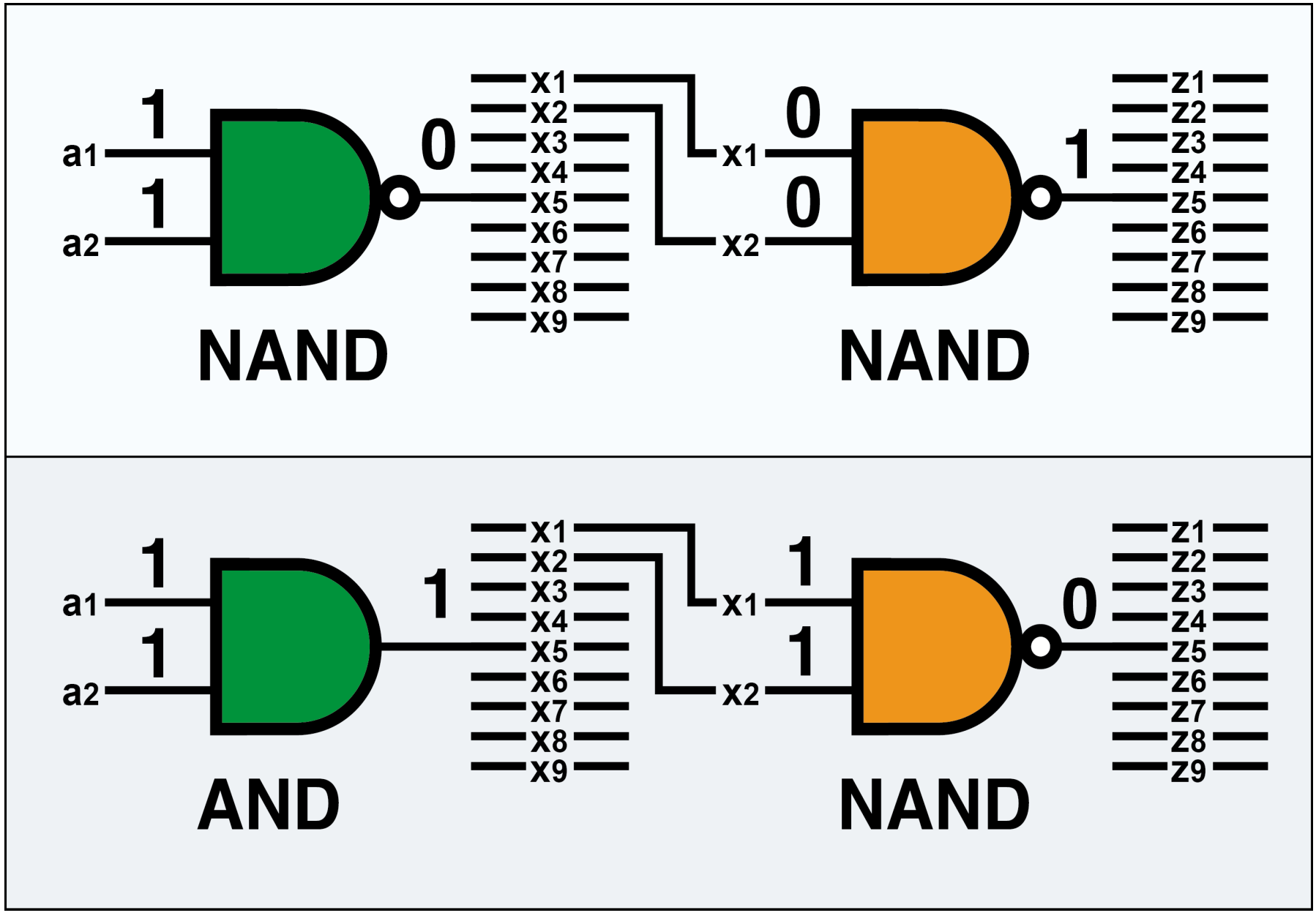
Control Flow

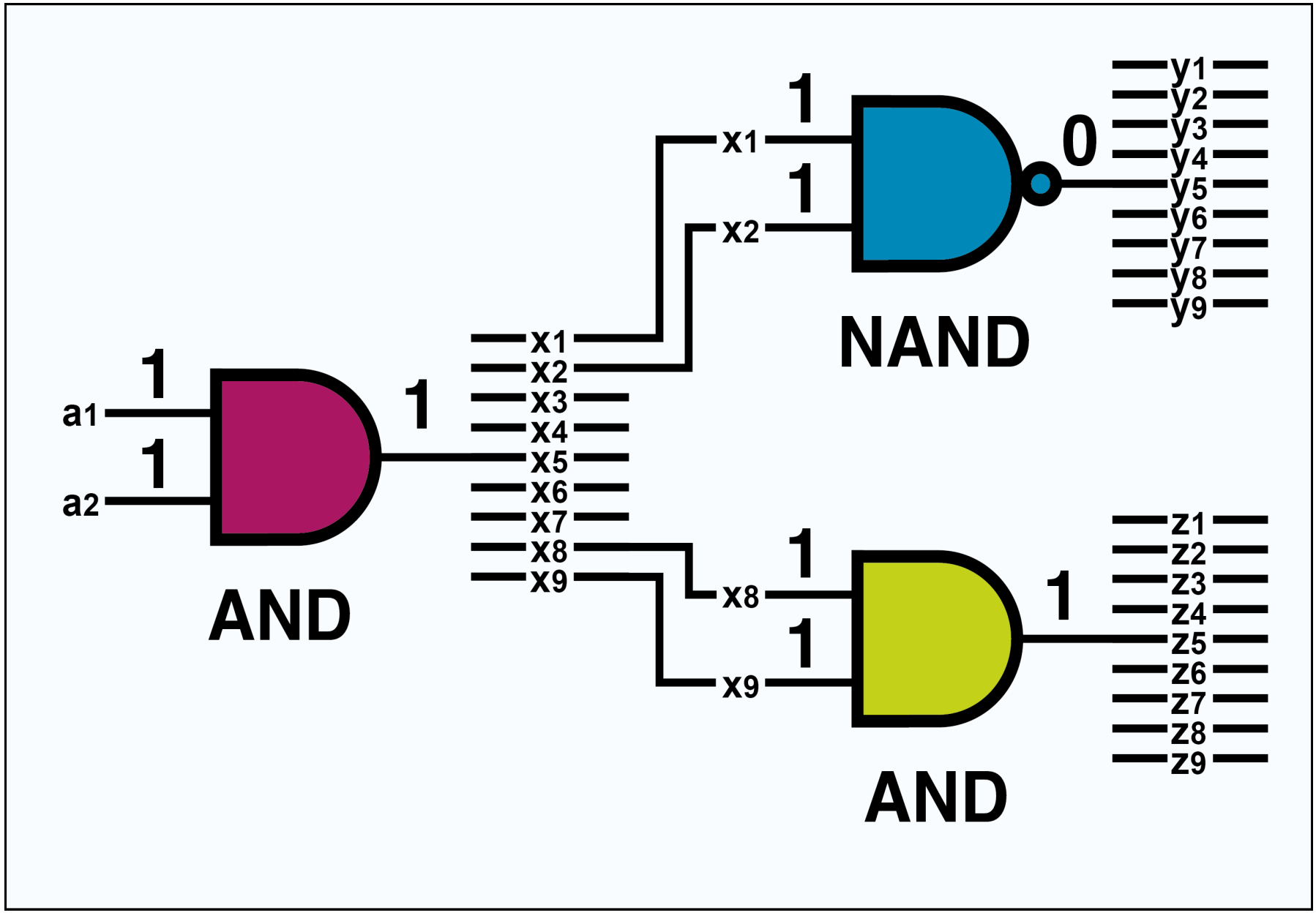






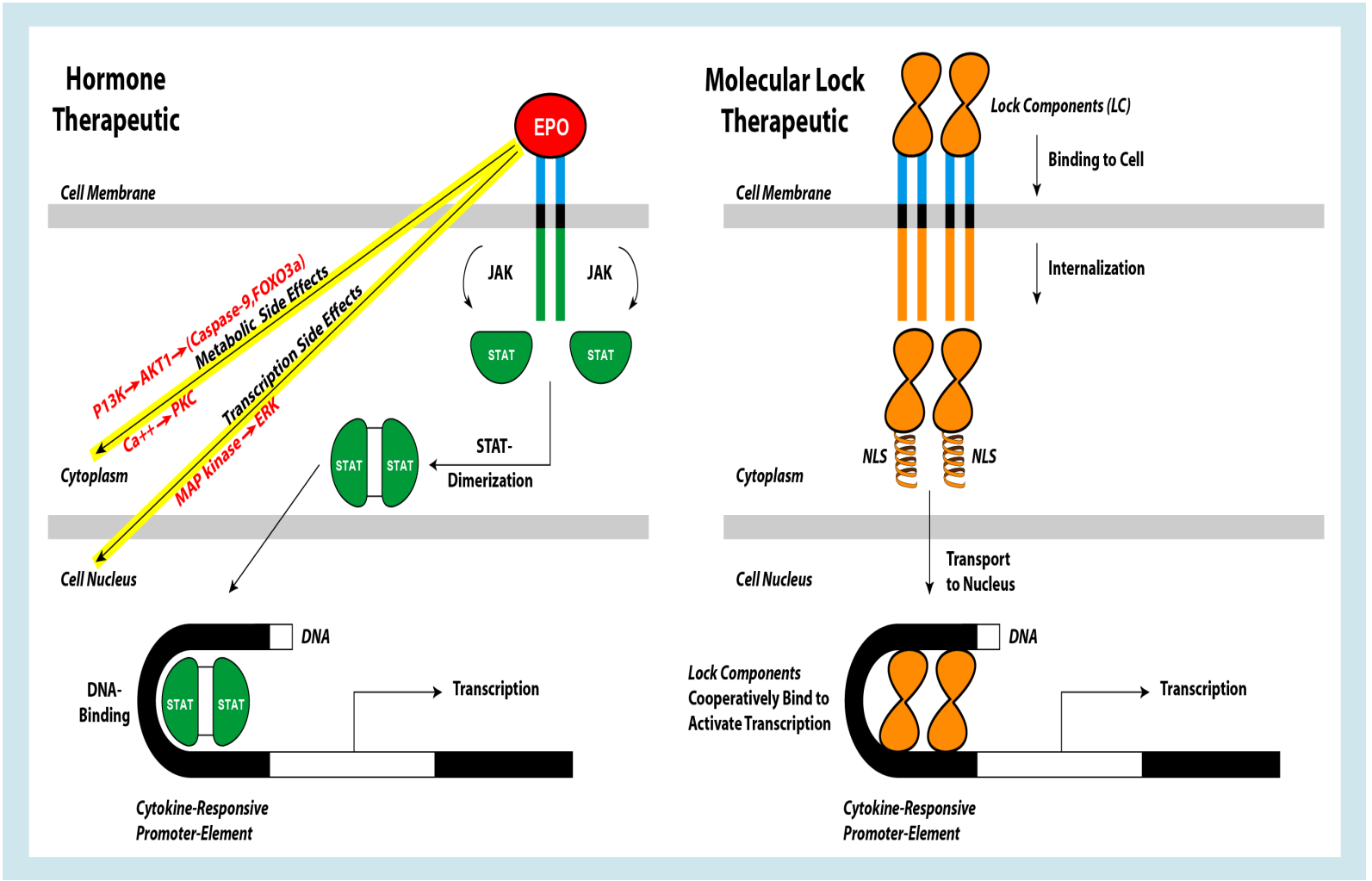




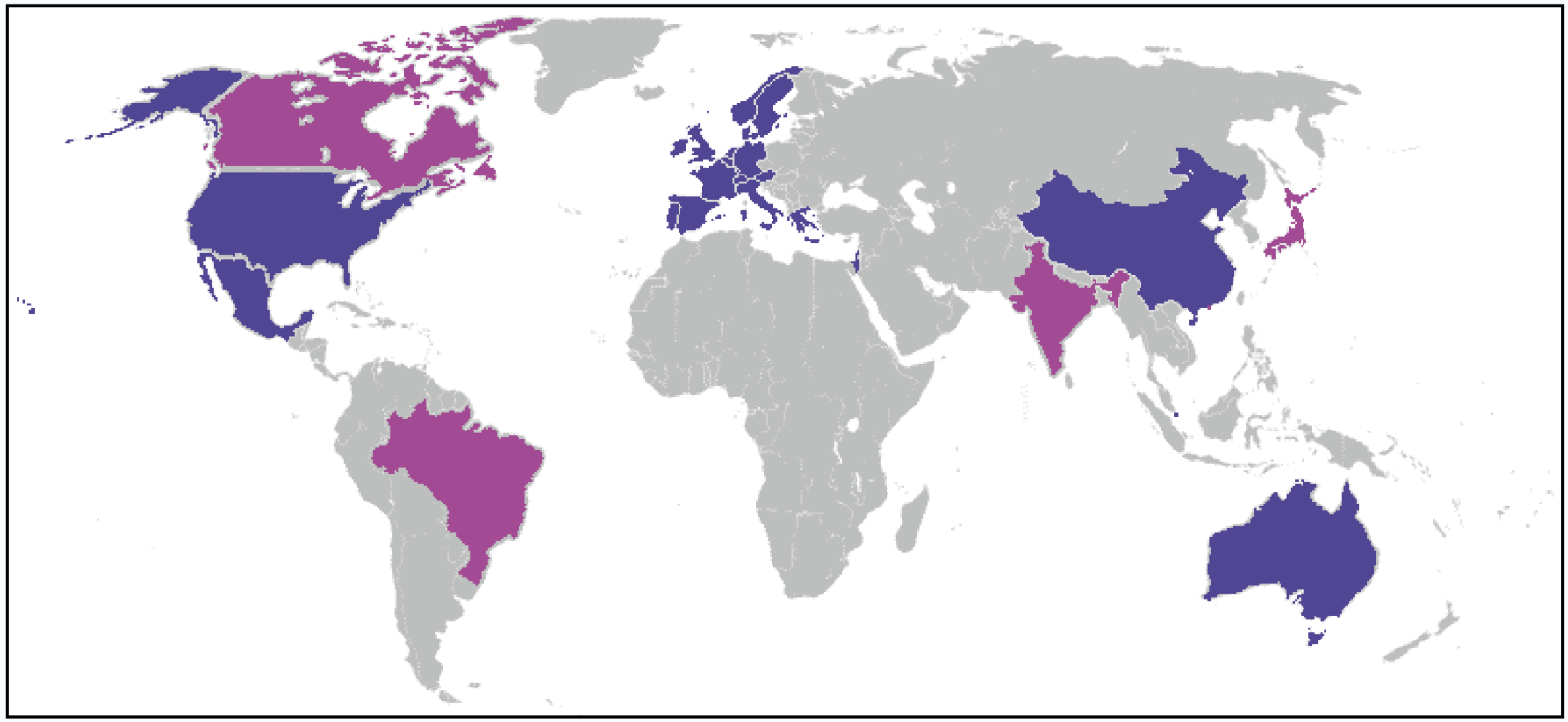


The challenge of engineering new biological circuits should not be limited to creating pharmaceutically active responses to pathogenic and genetic diseases, but rather to creating an entirely new immune system.

The Gene Pool, Inc. is developing Molecular Locks as treatments for infection, cancer, heart disease, diabetes, and obesity. Molecular Locks may replace hormones, monoclonal antibodies, antibiotics, and small molecule drugs. Molecular Locks are designed to target specific cells and modulate the transcription and translation of specific genes and pathways that are often the ultimate target of existing drugs. Existing drugs often have collateral, unwanted impacts on pathways and cell types; these impacts may be eliminated by using Molecular Locks.



Molecular Locks can activate or deactivate specific pathways that are the desired targets of existing drugs without serious, unintended collateral effects.



Arthur and Susan Weininger have developed and patented the Molecular Lock technology. Over two dozen patents have issued worldwide.