

Supporting Information for: Dual Role of Strigolactone Receptor Signaling Partner in Inhibiting Substrate Hydrolysis

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Details of adaptive sampling protocol

Table S1: Adaptive sampling metrics and selection criteria for *apo* system.

Round	Sampling Metrics	Selection Criteria
1	-	Initial structure
2	A223-D3 N-terminus distance A223-D3 C-terminus distance	4 random clusters, 25 points per cluster
3	A223-D3 N-terminus distance A223-C-terminus distance	4 random clusters, 25 points per cluster
4-9	A223-D3 N-terminus distance A223-D3 C-terminus distance D3 orientation relative to D14	100 clusters, 5 points each from 20 least populated
10-12	A223-D3 N-terminus distance A223-D3 C-terminus distance D3 orientation relative to D14 Distance between CTH terminals	100 clusters, 5 points each from 20 least populated
13-14	TICA coordinate 1 TICA coordinate 2	25 points each from 4 undersampled regions
15	TICA coordinate 3 TICA coordinate 5	25 points each from 4 undersampled regions

Table S2: Summary of adaptive rounds for *apo* system

Round	Parallel Trajectories	Trajectory length (ns)	Aggregate (μ s)
1	1	130	0.130
2	100	130	12.9
3	100	130	13.0
4	100	130	13.0
5	100	130	13.0
6	100	130	13.0
7	100	130	13.0
8	100	130	13.0
9	100	130	12.6
10	100	130	13.0
11	100	130	13.0
12	100	130	13.0
13	100	130	12.2
14	100	130	13.0
15	100	130	12.7
Total (μ s)			180.5

Table S3: Adaptive sampling metrics and selection criteria for *holo* system.

Round	Sampling Metrics	Selection Criteria
1	-	Initial structure
2	I120-D3 N-terminus distance D-ring-S97 distance	4 random clusters, 25 points per cluster
3	A223-D3 N-terminus distance D-ring-S97 distance A-ring-S97 distance	100 clusters, 5 points each from 20 least populated and D-ring-S97 distance < 3nm
4	A223-D3 N-terminus distance D-ring-S97 distance B-ring-L283 distance	100 clusters, 5 points each from 20 least populated and D-ring-S97 distance < 3nm
5-6	A223-D3 N-terminus distance D-ring-S97 distance B-ring-L283 distance K230-A279	100 clusters, 5 points each from 20 least populated and D-ring-S97 distance < 3nm
7-8	A223-D3 N-terminus distance D-ring-S97 distance B-ring-L283 distance K230-A279	100 clusters, 5 points each from 20 least populated and D-ring-S97 distance < 3nm
9	A223-D3 N-terminus distance D-ring-S97 distance B-ring-L283 distance K230-A279 CTH helical content	200 clusters, 5 points each from 40 least populated
10	TICA coordinate 1 TICA coordinate 3	50 points each from 4 undersampled regions
11	S220-H247 distance TICA coordinate 4	200 points from undersampled region
12	TICA coordinate 1 TICA coordinate 3	200 points from undersampled region

Table S4: Summary of adaptive rounds for *holo* system

Round	Parallel Trajectories	Trajectory length (ns)	Aggregate (μs)
1	1	120	0.120
2	100	120	12.0
3	100	120	12.0
4	100	120	12.0
5	100	120	12.0
6	100	120	12.0
7	100	120	12.0
8	200	120	12.0
9	200	120	24.0
10	200	120	23.6
11	200	120	24.0
12	200	120	23.3
13	200	120	23.3
Total (μs)			202.3

Markov state model construction and validation

MSM Features

To construct our Markov state models, we first calculated a set of inter-residue distance and helical content features from our simulation data. The full set of features used for MSM construction is shown in Table S5.

Table S5: Featurizations used for MSM construction. C- α distances were used for all inter-residue distances.

	<i>Apo</i>	<i>Holo</i>
CTH Dissociation	A223-D3 N-terminus A223-D3 C-terminus K230-A279 A223-E276 L283-A223 R284-K230	A223-D3 N-terminus A223-D3 C-terminus K230-A279
CTH-T1 distance	G149-R284	-
CTH-T4 distance	F195-D3 N Terminus	-
CTH-D14 Contacts	R267-D3 N-terminus T238-D3 C-terminus Y132-T275 E240-L283	D131-D3 C-terminus
CTH Structure	Helical Content N-C Terminal Distance	Helical Content N-C Terminal Distances
D-loop contacts	T216-H247 R217-H247 D218-H247 V219-H247 S220-H247 V221-H247 P222-H247	T216-H247 R216-H247 D217-H247 I218-H247 M219-H247 V220-H247 P221-H247
Ligand - catalytic triad	- -	A-ring-S97 D-ring-S97
Ligand - D14	- - -	A-ring-A223 D-ring-A223 D-ring-F195
Ligand - CTH	- -	D-ring-G285 B-ring-L283

MSM hyperparameter selection

To select lag times for our MSMs, we plotted implied timescales for models estimated at a series of lag times and chose a lag time at which the implied timescale stopped changing with increasing lag time. Implied timescale plots are shown in Fig. S1.

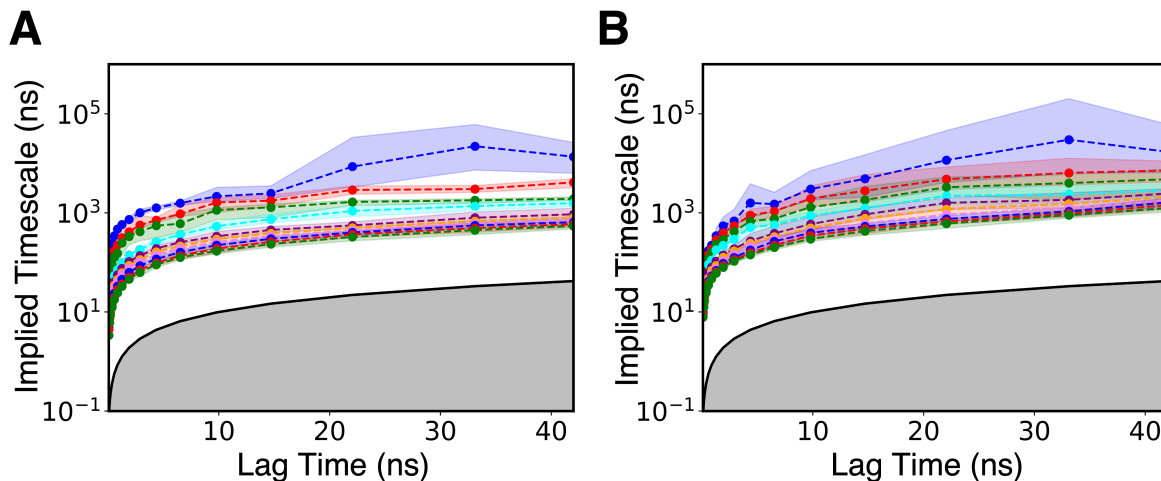


Figure S1: Implied timescale plots for the *apo* (A) and *holo* (B) systems. Lag time was chosen as 15 ns for both systems.

To select number of TICA components and number of clusters to discretize our simulation data for MSM construction, we performed a grid search in which we calculated a cross-validation score for MSMs calculated with different parameter sets. Cross-validation scores for different parameter sets are shown in Fig. S2. Final parameters used for MSM construction are shown in Table S6.

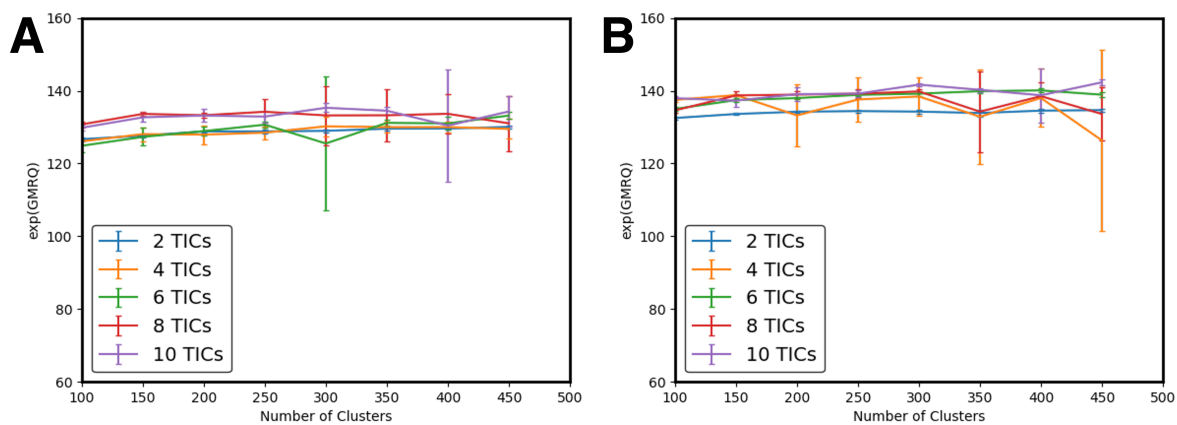


Figure S2: Cross-validation scores calculated with different hyperparameter sets for the *apo* (A) and *holo* (B) systems.

Table S6: Final parameters used for MSM construction

	<i>Apo</i>	<i>Holo</i>
Lag time (ns)	15	15
Number of TICA components	10	6
Number of clusters	350	400

Chapman-Kolmogorov validation

To validate our MSMs, we employed the Chapman-Kolmogorov test. Briefly, if a system exhibits Markovian behavior, the n th power of a transition matrix estimated at lag time τ should equal the transition matrix estimated at lag time $n\tau$. Results of the Chapman-Kolmogorov test are shown in Fig. S3.

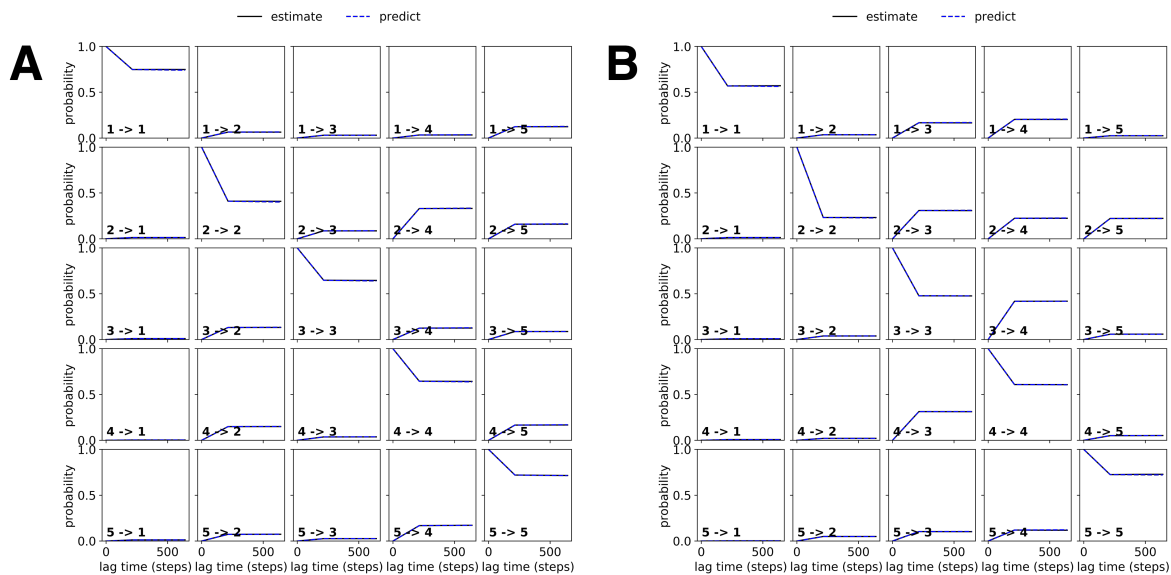


Figure S3: Chapman-Kolmogorov tests for the *apo* (A) and *holo* (B) systems. The test shows good agreement between estimated and predicted MSMs, indicating that the models follow the Markov property.

RMSD Calculations

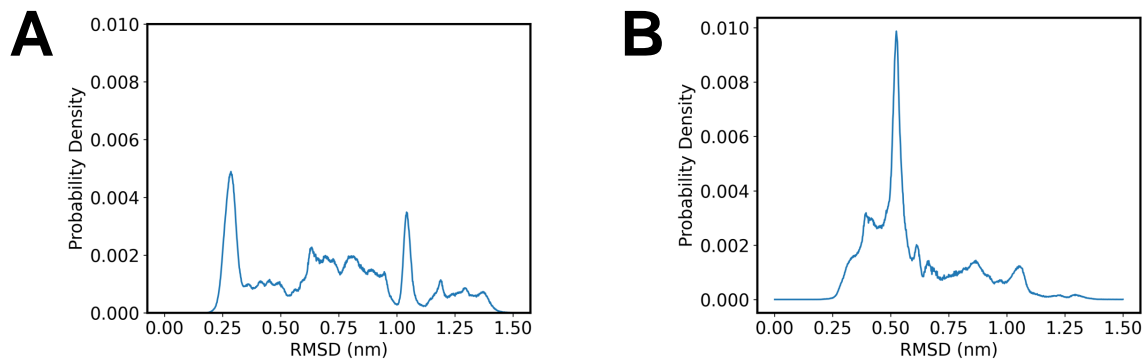


Figure S4: Probability density as a function of RMSD *apo* (A) and *holo* (B) systems. The peak at 0.25 nm for the *apo* system reflects the narrow free energy well and limited number of stable positions for the associated state. The peak at 0.50 nm for the *holo* system also corresponds to the associated state, demonstrating the larger flexibility in position compared to the *apo* state.

Transition path theory

During transition path theory calculation for the *holo*, we coarse-grained our data by assigning MSM clusters to a set of eleven macrostates defined by positions of the GR24 ligand and CTH. Definitions for different ligand and CTH positions are shown in Table S7 and macrostates are shown in Table S8. All calculated fluxes between the eleven macrostates are shown in Table S9.

Table S7: Definitions of ligand binding and CTH association used for TPT calculation

State	Parameters
GR24 Bound	Minimum D-ring-S97 distance < 0.6 nm AND Mean A-ring-S97 distance > Mean D-ring-S97 distance AND Not included in T1/T4 associated state
GR24 Inversely Bound	Mean A-ring-S97 distance < 1.5 nm AND Mean D-ring-S97 distance < 1.5 nm AND Not included in T1/T4 associated state
GR24-T1/T4 Association	Minimum A-ring-E138 distance < 1 nm AND D-ring-F196 distance < 1.3 nm
GR24-CTH Association	Minimum B-ring-A279 distance < 0.9 nm AND B-ring-L283 distance < 0.9 nm
GR24 Unbound	Not included in any other GR24 state
CTH-D14 Association	Minimum N-term-A223 distance < 2.0 nm AND Minimum C-Term-K230 distance < 1.4 nm AND Minimum C-term-V239 distance < 1.9 nm AND Minimum A279-A223 distance < 1.2 nm
CTH-D14 Dissociation	Not included in CTH-D14 associated state

Table S8: Definitions of the eleven macrostates used for TPT calculation

State Number	Definition
1	GR24 Bound CTH Associated
2	GR24 Inverse CTH Associated
3	GR24+T1/T4 CTH Associated
4	GR24+CTH CTH Associated
5	GR24 Unbound CTH Associated
6	GR24 Bound CTH Dissociated
7	GR24 Inverse CTH Dissociated
8	GR24+T1/T4+CTH CTH Dissociated
9	GR24+T1/T4 CTH Dissociated
10	GR24+CTH CTH Dissociated
11	GR24 Unbound CTH Dissociated

Table S9: Rate constants of flux between TPT states (μs^{-1})

	1	2	3	4	5	6	7	8	9	10	11
1	-	0.27	1.25	0.82	5.61	0.18	0.26	0.36	0.80	1.30	2.30
2	0.32	-	1.02	0.83	1.45	0.18	0.26	0.42	0.63	0.90	1.65
3	0.43	0.31	-	1.58	6.89	0.22	0.35	0.57	1.49	2.68	5.52
4	0.41	0.32	3.02	-	5.61	0.20	0.33	0.56	1.48	2.42	6.32
5	1.06	0.33	2.94	1.62	-	0.22	0.35	0.64	1.49	2.34	4.98
6	0.42	0.28	4.42	1.45	7.52	-	0.32	0.51	1.28	2.96	10.24
7	0.42	0.30	4.56	1.72	8.42	0.21	-	0.54	1.26	3.12	6.58
8	0.39	0.32	2.99	1.61	6.85	0.21	0.33	-	1.23	2.32	6.02
9	0.43	0.28	2.84	1.45	6.14	0.19	0.29	0.48	-	2.43	5.16
10	0.45	0.29	2.74	1.50	6.39	0.21	0.33	0.52	1.30	-	4.94
11	0.42	0.36	2.93	1.73	5.20	0.22	0.33	0.59	1.42	2.38	-