

Supplementary Information

Protein Flexibility and Conformational Entropy in Ligand Design Targeting the Carbohydrate Recognition Domain of Galectin-3

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Table S1. Summary of quality statistics for the crystal structure of the L2-Gal3 complex.

Space group	P2 ₁ 2 ₁ 2 ₁
Resolution (Å)	25.00 – 1.20 (1.23 – 1.20)
Wavelength (Å)	0.90778
Unit cell (Å)	a =35.80, b = 57.69, c = 62.41
Completeness (%)	99.6 (96.9)
No. of observed reflections	274,413
No. of unique reflections	41,094
Mean <I/σ>	11.5 (2.2)
R _{merge} (I) (%)	8.1 (65.6)
R _{cryst} (F) (%)	13.8 (32.2)
R _{free} (F) (%)	19.3
No. of amino acids	138
No. of water molecules	232
No. of chloride ions	1
rms deviations from ideal bond lengths (Å)	0.011
Average B-factors protein (Å ²)	17.4
Average B-factors 5-78 ligand (Å ²)	32.9

Table S2a: Mean values, standard deviations and standard error of mean for ^{15}N backbone amide relaxation rates.

500 MHz	R_1 (s^{-1})	Std	SEM	R_2 (s^{-1})	Std	SEM	$\{^1\text{H}\}$ - ^{15}N -NOE	Std	SEM
Apo	2.01	0.11	0.01	9.75	1.08	0.10	0.74	0.06	0.01
Lac	1.82	0.15	0.01	9.53	2.07	0.19	0.69	0.38	0.04
L2	1.90	0.09	0.01	9.92	0.87	0.08	0.76	0.05	0.00
L3	1.92	0.09	0.01	9.75	0.80	0.08	0.77	0.04	0.00
600 MHz	R_1 (s^{-1})	Std	SEM	R_2 (s^{-1})	Std	SEM	$\{^1\text{H}\}$ - ^{15}N -NOE	Std	SEM
Apo	1.63	0.07	0.01	10.40	1.25	0.12	0.79	0.06	0.01
Lac	1.45	0.09	0.01	10.44	1.67	0.16	0.72	0.35	0.03
L2	1.51	0.07	0.01	10.63	1.02	0.09	0.78	0.06	0.01
L3	1.53	0.07	0.01	10.54	0.86	0.08	0.79	0.04	0.00

Table S2b: Mean values, standard deviations and standard error of mean for ^{15}N side-chain relaxation rates.

500 MHz	R_1 (s^{-1})	Std	SEM	R_2 (s^{-1})	Std	SEM	$\{^1\text{H}\}$ - ^{15}N -NOE	Std	SEM
Apo	1.15	0.77	0.31	7.89	4.39	1.79	-0.66	1.84	0.75
Lac	1.52	0.25	0.10	7.84	1.92	0.79	0.56	0.24	0.10
L2	1.62	0.21	0.08	8.54	1.46	0.55	0.63	0.24	0.09
L3	1.62	0.19	0.08	8.52	1.86	0.76	0.61	0.27	0.11
600 MHz	R_1 (s^{-1})	Std	SEM	R_2 (s^{-1})	Std	SEM	$\{^1\text{H}\}$ - ^{15}N -NOE	Std	SEM
Apo	0.96	0.56	0.23	8.38	4.19	1.71	-0.50	1.66	0.68
Lac	1.23	0.08	0.03	8.48	1.82	0.74	0.58	0.22	0.09
L2	1.27	0.11	0.04	9.49	1.75	0.66	0.64	0.28	0.10
L3	1.27	0.12	0.05	9.18	1.88	0.77	0.62	0.23	0.09

Table S2c: Mean values, standard deviations and standard error of mean for ^2H side-chain methyl relaxation rates. Units in s^{-1} .

	R_z^a	Std	SEM	R_+^b	Std	SEM	R_{z2}^c	Std	SEM	R_{+z}^d	Std	SEM
Apo	21.40	9.34	1.13	90.38	26.20	3.18	18.07	8.03	0.97	75.50	24.99	3.03
Lac	21.58	10.77	1.31	90.38	25.52	3.09	18.03	7.74	0.94	75.80	27.06	3.28
L2	21.45	10.62	1.29	88.39	24.25	2.92	18.05	7.33	0.89	73.51	26.65	3.21
L3	21.70	11.74	1.45	90.42	28.04	3.45	16.77	7.23	0.89	72.37	27.68	3.41

^a $R(D_z)$

^b $R(D_+)$

^c $R(3D_z^2-2)$

^d $R(D_+D_z + D_zD_+)$

Table S2d: Mean values, standard deviations and standard error of mean for ^{15}N R_{2dd} backbone amide relaxation rates.

500 MHz	R_{2dd} (s^{-1})	Std	SEM
Apo	6.18	0.53	0.05

Table S3a: Backbone model-free order parameters for apo-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O_s^2	std	O_f^2	std	τ_s (ps)	std
115	0.72	0.01	41	5								
116	0.78	0.01	36	9								
118	0.91	0.01										
119	0.90	0.01			0.5	0.2						
120	0.87	0.01										
122	0.85	0.01	33	13								
125	0.87	0.01					0.95	0.02	0.91	0.02	1290	1257
126	0.79	0.01	26	9	0.4	0.2						
127	0.84	0.01	35	12	0.7	0.2						
129	0.87	0.01	43	18	0.3	0.2						
130	0.87	0.01	32	11	0.3	0.1						
131	0.94	0.01										
132	0.90	0.01										
134	0.90	0.01										
135	0.89	0.01			0.5	0.2						
136	0.89	0.01	46	15								
137	0.89	0.01										
138	0.89	0.01			0.4	0.1						
139	0.87	0.01	34	16	0.7	0.2						
142	0.77	0.01					0.92	0.01	0.84	0.01	842	189
143	0.84	0.01			0.4	0.2						
144	0.91	0.01										
145	0.87	0.01										
146	0.90	0.01										
147	0.87	0.01			0.4	0.2						
148	0.85	0.01										
150	0.88	0.01	36	16								

151	0.86	0.01	36	14	0.7	0.2						
154	0.87	0.01	22	13	0.7	0.1						
155	0.87	0.01			0.5	0.2						
156	0.91	0.01										
157	0.94	0.01										
158	0.96	0.01										
159	0.88	0.01	28	17								
160	0.87	0.02			0.8	0.3						
163	0.90	0.01			0.4	0.2						
164	0.88	0.01	77	22	0.5	0.2						
165	0.82	0.01					0.92	0.01	0.89	0.01	888	292
167	0.91	0.01	73	28								
168	0.82	0.01	49	9								
169	0.79	0.01					0.95	0.02	0.84	0.03	940	636
171	0.89	0.01	54	22								
172	0.91	0.01										
173	0.86	0.01	66	22	6.7	0.3						
174	0.91	0.01			0.5	0.2						
175	0.83	0.01	45	14	0.7	0.2						
176	0.91	0.01										
177	0.86	0.01	29	14	0.5	0.2						
178	0.89	0.01	64	19								
179	0.90	0.01			1.5	0.2						
180	0.82	0.01			0.6	0.1						
181	0.90	0.01			0.8	0.3						
182	0.88	0.01										
183	0.92	0.01			1.3	0.3						
184	0.85	0.01	57	15	0.8	0.2						
185	0.86	0.01			0.8	0.2						

186	0.86	0.03			2.7	0.6
187	0.85	0.01	50	16	0.6	0.2
189	0.84	0.01	40	14	4.5	0.2
190	0.88	0.02	38	23	0.9	0.3
192	0.71	0.01	17	5		
193	0.82	0.01			0.8	0.2
194	0.84	0.02	51	27		
195	0.86	0.01	38	15		
196	0.88	0.01				
198	0.89	0.01				
199	0.88	0.01			0.5	0.2
200	0.89	0.01				
202	0.86	0.01			0.7	0.2
203	0.92	0.01				
204	0.90	0.01			0.5	0.2
205	0.90	0.01	49	16	0.6	0.1
207	0.95	0.02			2.0	0.3
208	0.93	0.01			1.0	0.2
209	0.90	0.01				
210	0.87	0.01			3.4	0.2
212	0.89	0.01			0.6	0.2
213	0.88	0.01			1.0	0.2
214	0.88	0.01	51	17	0.7	0.2
215	0.89	0.01	41	20	1.9	0.2
216	0.85	0.00				
218	0.87	0.01			1.3	0.2
219	0.89	0.01			8.4	0.3
220	0.90	0.01			0.4	0.2
221	0.87	0.01	37	14	0.8	0.2

222	0.89	0.01			0.7	0.2						
223	0.89	0.01										
224	0.89	0.01			0.7	0.3						
225	0.76	0.01	27	8	0.5	0.2						
227	0.87	0.02										
228	0.84	0.01					0.95	0.02	0.89	0.02	1377	1257
229	0.95	0.01	66	44								
230	0.91	0.01										
231	0.91	0.01			0.3	0.2						
232	0.77	0.01					0.93	0.03	0.83	0.04	627	257
233	0.95	0.01										
234	0.89	0.01	52	19								
235	0.91	0.01										
236	0.87	0.01			1.1	0.2						
237	0.93	0.01										
238	0.91	0.01	57	23								
240	0.85	0.01	65	13	0.8	0.2						
241	0.87	0.01										
243	0.92	0.01			0.5	0.3						
244	0.87	0.01			0.2	0.1						
245	0.88	0.01					0.96	0.02	0.92	0.02	1138	1169
246	0.89	0.01										
247	0.89	0.01	23	14								
248	0.89	0.01										
249	0.84	0.01	32	13								
250	0.82	0.01					0.94	0.02	0.87	0.03	903	555

Table S3b: Backbone modelfree order parameters for lac-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O_s^2	std	O_f^2	std	τ_s (ps)	std
114	0.06	0.01					0.08	0.01	0.73	0.02	587	22
115	0.39	0.01					0.66	0.01	0.60	0.01	2408	489
116	0.78	0.01	38	9								
118	0.89	0.01	48	22								
119	0.80	0.01	45	10								
120	0.85	0.01										
122	0.84	0.01			0.4	0.2						
124	0.04	0.01					0.06	0.01	0.70	0.01	668	18
125	0.86	0.01	32	13	0.3	0.2						
126	0.78	0.01	15	9								
127	0.81	0.01	33	10	1.0	0.2						
129	0.86	0.01										
130	0.83	0.01	43	9	0.4	0.2						
131	0.87	0.02	51	20	0.7	0.3						
132	0.86	0.01			0.5	0.3						
134	0.87	0.01										
135	0.91	0.01										
136	0.88	0.01										
137	0.85	0.01										
138	0.89	0.01										
139	0.85	0.01	61	14	1.9	0.3						
141	0.08	0.02					0.12	0.03	0.69	0.05	483	72
142	0.77	0.01	41	6								
143	0.84	0.01										
144	0.87	0.01	27	17								
145	0.80	0.01	76	11	0.4	0.2						
146	0.89	0.01										

147	0.84	0.01	15	9								
148	0.82	0.01			0.5	0.2						
149	0.88	0.01										
150	0.87	0.01			0.5	0.2						
151	0.85	0.01			1.1	0.2						
154	0.85	0.01	31	12								
155	0.85	0.01			0.5	0.2						
156	0.88	0.01										
157	0.89	0.01										
158	0.91	0.01										
159	0.86	0.01			0.6	0.2						
160	0.86	0.01			0.4	0.2						
163	0.86	0.01	53	24								
164	0.89	0.01	63	24								
167	0.86	0.01	30	17								
168	0.81	0.01	24	10								
169	0.77	0.01					0.93	0.02	0.83	0.02	977	554
171	0.88	0.01										
172	0.87	0.01										
173	0.85	0.01			0.4	0.2						
174	0.87	0.01			0.5	0.2						
175	0.81	0.01			1.0	0.2						
176	0.88	0.01										
177	0.65	0.01					0.81	0.01	0.80	0.01	603	75
178	0.86	0.01	21	11								
179	0.86	0.01			1.4	0.2						
180	0.80	0.01	13	7	0.5	0.1						
181	0.86	0.01										
182	0.83	0.01			0.7	0.2						

183	0.92	0.01										
184	0.83	0.01	17	10	0.5	0.2						
185	0.86	0.01										
186	0.80	0.02	31	18	1.3	0.5						
187	0.82	0.02					0.95	0.02	0.86	0.02	2871	2331
189	0.74	0.01	67	7	3.7	0.2						
190	0.89	0.01										
192	0.66	0.01	12	4	0.3	0.2						
193	0.83	0.01										
194	0.82	0.01										
195	0.85	0.01										
196	0.83	0.01	21	10	0.8	0.2						
198	0.87	0.01										
199	0.90	0.01										
200	0.87	0.01										
201	0.86	0.01	28	12								
202	0.84	0.01			0.4	0.2						
203	0.90	0.01										
204	0.89	0.01										
205	0.87	0.01	39	14	0.4	0.2						
207	0.86	0.01	55	17								
208	0.88	0.01			0.6	0.2						
209	0.87	0.01										
210	0.85	0.01										
212	0.91	0.01										
213	0.85	0.01	20	12	0.5	0.2						
214	0.90	0.01	35	22								
215	0.88	0.01			0.8	0.2						
216	0.84	0.01										

218	0.89	0.01			0.6	0.3						
219	0.88	0.02	32	21	6.8	0.4						
220	0.85	0.01			0.6	0.2						
222	0.88	0.01										
223	0.86	0.01										
224	0.87	0.01	40	26								
225	0.60	0.01					0.79	0.01	0.76	0.01	603	70
227	0.75	0.02	57	12	0.7	0.4						
228	0.86	0.01										
229	0.91	0.01										
230	0.90	0.01										
231	0.86	0.01	29	15	1.1	0.2						
232	0.77	0.01	53	8								
233	0.90	0.01			0.6	0.2						
234	0.85	0.01			0.5	0.2						
235	0.88	0.01										
236	0.86	0.01	43	14								
237	0.92	0.01										
238	0.89	0.01										
240	0.87	0.01										
241	0.88	0.01										
243	0.93	0.01										
244	0.85	0.01										
245	0.80	0.01	58	9								
246	0.86	0.01										
247	0.86	0.01										
248	0.88	0.01			0.4	0.2						
249	0.73	0.01	58	7								

Table S3c: Backbone modelfree order parameters for L2-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O^2_s	std	O^2_f	std	τ_s (ps)	std
114	0.64	0.02					0.76	0.03	0.84	0.02	527	128
115	0.68	0.01					0.89	0.01	0.77	0.01	980	137
116	0.76	0.00	34	6								
118	0.87	0.01					0.96	0.02	0.91	0.02	929	424
119	0.87	0.00										
120	0.86	0.00										
122	0.82	0.01	28	6	0.3	0.1						
125	0.87	0.00			0.7	0.1						
126	0.81	0.00	22	6								
127	0.81	0.01	21	7	1.2	0.1						
129	0.85	0.01					0.97	0.04	0.88	0.04	1000	559
130	0.86	0.01			0.9	0.1						
131	0.91	0.01			0.3	0.1						
132	0.87	0.01										
133	0.89	0.00			0.2	0.1						
134	0.87	0.01	21	9								
135	0.85	0.01					0.95	0.01	0.89	0.01	2252	1452
136	0.85	0.01					0.96	0.01	0.89	0.01	2858	2591
137	0.87	0.01										
139	0.84	0.01	33	9	1.6	0.1						
142	0.76	0.00	37	4	0.6	0.1						
143	0.85	0.01	18	8								
144	0.88	0.00			0.6	0.1						
145	0.88	0.01			0.2	0.1						
146	0.89	0.00			0.2	0.1						
147	0.89	0.01										

148	0.85	0.00											
149	0.87	0.01	28	8									
150	0.87	0.00											
151	0.87	0.01	20	10	0.3	0.1							
154	0.87	0.00	19	8	0.5	0.1							
155	0.86	0.01	21	10	0.5	0.1							
156	0.90	0.00											
157	0.89	0.01	33	13	0.3	0.1							
158	0.89	0.01			0.8	0.1							
159	0.89	0.01											
160	0.85	0.00			0.5	0.1							
163	0.88	0.01	39	13	0.5	0.2							
164	0.88	0.01	23	11	0.7	0.1							
167	0.85	0.01					0.98	0.01	0.87	0.01	2705	1759	
168	0.80	0.00	14	4	0.6	0.1							
169	0.81	0.00	28	5									
170	0.88	0.00			0.3	0.1							
171	0.87	0.01			0.3	0.1							
172	0.86	0.01	34	10	0.6	0.1							
173	0.86	0.01	23	12	0.2	0.1							
174	0.88	0.01			0.3	0.1							
175	0.84	0.00			0.5	0.1							
176	0.91	0.00											
177	0.86	0.01	19	12									
178	0.87	0.00	37	7									
179	0.84	0.01	20	6	1.6	0.1							
180	0.83	0.00			0.4	0.1							
181	0.86	0.00											
182	0.85	0.01			0.4	0.1							

183	0.88	0.00			0.9	0.1
184	0.83	0.00	19	7	0.7	0.1
185	0.87	0.00				
186	0.81	0.01	18	11	1.2	0.1
187	0.86	0.01			0.8	0.1
189	0.79	0.01	13	8	4.9	0.2
190	0.85	0.01	37	8	0.9	0.1
192	0.65	0.01	16	2	0.7	0.1
193	0.82	0.01	20	7		
194	0.85	0.01	39	11		
195	0.83	0.00	31	7	0.6	0.1
196	0.83	0.01	25	6	0.9	0.1
198	0.86	0.00	27	7		
199	0.88	0.01	17	9		
200	0.87	0.00				
201	0.87	0.01	21	8		
202	0.85	0.01	19	7	0.3	0.1
203	0.90	0.01	32	15		
204	0.88	0.01	17	9	0.7	0.1
207	0.89	0.01			2.1	0.1
208	0.89	0.01			1.2	0.1
209	0.88	0.00			0.3	0.1
210	0.87	0.01	20	12	1.6	0.2
211	0.88	0.01			5.0	0.1
212	0.89	0.00	18	10		
213	0.87	0.00			0.8	0.1
214	0.89	0.00	22	11		
215	0.88	0.01			1.5	0.2
216	0.83	0.00			0.5	0.1

218	0.89	0.00			1.0	0.1						
219	0.91	0.00			5.6	0.1						
220	0.88	0.01	30	12	0.4	0.1						
222	0.90	0.00										
223	0.85	0.01			0.7	0.1						
224	0.83	0.01	26	11	1.5	0.1						
225	0.72	0.01	20	4	1.5	0.1						
227	0.79	0.01	47	6	0.9	0.2						
228	0.82	0.01	15	7	0.5	0.1						
229	0.88	0.00			1.0	0.1						
230	0.88	0.01					0.98	0.00	0.90	0.01	1478	1533
231	0.88	0.01	15	8	0.7	0.1						
232	0.77	0.01	35	5	0.2	0.1						
233	0.91	0.01	52	10	0.5	0.1						
234	0.88	0.00										
235	0.87	0.01	22	12	0.5	0.1						
236	0.87	0.00										
237	0.91	0.00										
238	0.88	0.00										
239	0.86	0.00										
240	0.88	0.00	16	7								
241	0.85	0.01					0.97	0.01	0.87	0.00	4027	2907
243	0.88	0.02					0.95	0.02	0.93	0.01	1387	2372
244	0.78	0.02					0.91	0.02	0.86	0.01	5345	2265
245	0.85	0.01					0.94	0.01	0.90	0.01	1298	315
246	0.87	0.00										
247	0.82	0.00					0.95	0.01	0.87	0.01	1765	990
248	0.88	0.00	25	7								
249	0.79	0.01					0.96	0.07	0.83	0.07	433	263

Table S3d: Backbone modelfree order parameters for L3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O^2_s	std	O^2_f	std	τ_s (ps)	std
115	0.72	0.00	36	4								
116	0.72	0.01					0.90	0.01	0.80	0.01	1168	181
118	0.85	0.01					0.96	0.01	0.89	0.01	2470	1453
119	0.87	0.01										
120	0.84	0.01	29	6	0.4	0.1						
122	0.81	0.00			1.1	0.1						
125	0.89	0.00										
126	0.76	0.01	9	4	0.9	0.1						
127	0.81	0.01	23	6	1.6	0.1						
129	0.85	0.00			0.6	0.1						
130	0.83	0.01	12	6	1.6	0.1						
131	0.88	0.01	43	14	1.0	0.2						
132	0.87	0.01			0.4	0.1						
134	0.88	0.00	23	10								
135	0.85	0.01					0.97	0.01	0.88	0.01	2007	777
136	0.86	0.01					0.97	0.01	0.89	0.01	1652	995
137	0.87	0.01			0.3	0.1						
139	0.86	0.01			1.5	0.2						
142	0.75	0.00	25	4	1.3	0.1						
143	0.85	0.00										
144	0.89	0.00			0.4	0.1						
145	0.88	0.00										
146	0.88	0.01			0.5	0.1						
147	0.87	0.01			0.5	0.1						
148	0.83	0.00	10	6	0.3	0.1						
149	0.86	0.01	16	6	0.3	0.1						

150	0.84	0.01	14	9	0.8	0.1						
151	0.84	0.00			1.1	0.1						
154	0.87	0.00	15	7	0.4	0.1						
155	0.83	0.01			1.1	0.1						
157	0.91	0.00	24	15								
158	0.90	0.01			0.9	0.2						
159	0.88	0.00										
160	0.87	0.01			0.7	0.2						
163	0.88	0.01	27	13	0.4	0.2						
164	0.87	0.01	17	10	0.9	0.1						
167	0.84	0.01					0.96	0.01	0.87	0.01	1725	625
168	0.79	0.00	18	4	1.0	0.1						
169	0.81	0.00	23	6								
171	0.87	0.00	14	9								
172	0.88	0.00										
173	0.88	0.01	28	12								
174	0.87	0.01			0.9	0.1						
175	0.81	0.00			1.2	0.1						
176	0.86	0.01			1.0	0.1						
177	0.84	0.01			0.9	0.1						
179	0.82	0.01			2.2	0.1						
180	0.82	0.00	12	4	0.4	0.1						
181	0.85	0.01	21	8	1.0	0.1						
182	0.82	0.01	14	6	1.4	0.1						
183	0.89	0.01	27	10	0.4	0.1						
184	0.82	0.01	10	6	1.1	0.1						
185	0.86	0.01	30	9								
186	0.81	0.01	33	8	1.3	0.2						
187	0.87	0.00	17	8								

189	0.82	0.01	14	5	3.8	0.1
190	0.83	0.01	22	7	1.4	0.1
192	0.64	0.01	11	3	1.0	0.1
193	0.81	0.00			0.9	0.1
194	0.81	0.01	38	8	1.0	0.2
195	0.83	0.01			1.1	0.1
196	0.81	0.01	13	5	1.3	0.1
198	0.87	0.00				
199	0.88	0.01	22	10	0.3	0.1
200	0.88	0.00				
201	0.86	0.01	13	6	0.3	0.1
202	0.85	0.01			0.4	0.1
203	0.89	0.01			0.4	0.1
204	0.88	0.01			0.9	0.2
207	0.86	0.01			2.5	0.2
208	0.87	0.01			1.9	0.1
209	0.82	0.01			1.4	0.1
210	0.84	0.01	19	6	2.6	0.2
212	0.87	0.01	42	9	0.3	0.1
213	0.87	0.00			0.5	0.1
214	0.90	0.00	28	11		
215	0.86	0.01	16	9	1.9	0.1
216	0.83	0.00	19	5		
219	0.87	0.01			6.0	0.2
220	0.87	0.01			0.8	0.1
222	0.87	0.00			0.6	0.1
223	0.86	0.01			0.7	0.1
224	0.86	0.01			0.9	0.2
225	0.74	0.01	13	4	1.0	0.1

227	0.77	0.01	28	8	1.9	0.2						
228	0.83	0.00	47	7								
229	0.90	0.01	43	9	0.5	0.1						
230	0.89	0.00										
231	0.88	0.00			0.5	0.1						
232	0.75	0.00	28	5	0.8	0.1						
233	0.90	0.00	34	12	0.8	0.1						
234	0.85	0.01	17	5	0.6	0.1						
235	0.87	0.00			0.8	0.1						
236	0.87	0.00										
237	0.91	0.01	32	15	0.2	0.1						
238	0.88	0.01	28	12								
240	0.88	0.00										
241	0.86	0.01					0.98	0.02	0.88	0.02	3957	1950
243	0.88	0.01					0.94	0.01	0.94	0.01	1350	912
244	0.87	0.00	18	10								
245	0.88	0.00										
246	0.86	0.00	30	6	0.4	0.1						
247	0.88	0.00										
248	0.86	0.01	21	11	0.6	0.1						
249	0.78	0.01	25	5	0.5	0.1						
250	0.80	0.01	21	5	0.8	0.1						

Table S3e: Side-chain modelfree order parameters for apo-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O_s^2	std	O_f^2	std	τ_s (ps)	std
129	0.59	0.03	71	13								
162	0.91	0.03			7.2	0.7						
168	0.73	0.01	73	8	0.8	0.2						
181	0.03	0.02	48	15	1.7	0.5						
183	0.03	0.00	23	1	9.2	0.1						
224	0.90	0.02			1.9	0.3						

Table S3f: Side-chain modelfree order parameters for lac-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O_s^2	std	O_f^2	std	τ_s (ps)	std
129	0.41	0.04					0.60	0.05	0.69	0.03	901	148
162	0.87	0.01			1.5	0.2						
168	0.68	0.01					0.82	0.02	0.83	0.01	814	112
181	0.91	0.02	88	56								
186	0.79	0.02	34	19								
224	0.88	0.01	44	16	0.7	0.2						

Table S3g: Side-chain modelfree order parameters for L2-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O_s^2	std	O_f^2	std	τ_s (ps)	std
129	0.57	0.01	75	5								
144	0.83	0.03			1.9	0.5						
162	0.88	0.01	39	10	1.3	0.2						
168	0.74	0.01	61	4	1.0	0.1						
181	0.93	0.01			0.7	0.1						
186	0.85	0.01			1.8	0.2						
224	0.89	0.01	42	10	1.6	0.1						

Table S3h: Side-chain model-free order parameters for L3-Gal3 from ^{15}N relaxation rates.

Residue	O^2	std	τ_e (ps)	std	R_{ex} (s^{-1})	std	O_s^2	std	O_f^2	std	τ_s (ps)	std
129	0.53	0.02					0.72	0.02	0.74	0.01	571	86
162	0.88	0.01			2.0	0.1						
168	0.75	0.01	74	4	0.3	0.1						
181	0.93	0.01			0.9	0.1						
186	0.83	0.01			1.9	0.1						
224	0.88	0.01			2.1	0.1						

Table S3i: Side-chain model-free order parameters for apo-Gal3 from ^2H relaxation rates.

Residue	Atom	O^2	std	τ_e (ps)	std	τ_c (ns)	std
114	1	0.51	0.02	44	2		
114	2	0.48	0.01	30	1		
115	1	0.22	0.00	27	0		
115	2	0.44	0.01	50	1		
116	1	0.46	0.01	77	1		
120	1	0.63	0.03	33	2		
126	2	0.77	0.05	81	5		
126	1	0.78	0.03	70	3		
127	1	0.25	0.01	87	1		
130	1	0.48	0.01	6	0		
131	1	0.51	0.01	32	1		
132	1	0.38	0.01	14	1		
132	2	0.67	0.02	31	1		
134	1	0.82	0.02	27	2		
135	2	0.38	0.01	38	1		
137	2	0.55	0.01	66	1		

138	1	1.00	0.10	139	12		
138	2	0.90	0.03	27	2		
142	1	0.92	0.05	68	4		
145	1	0.77	0.02	16	2		
145	2	0.78	0.02	33	2		
146	1	0.83	0.01	43	1		
147	1	0.52	0.08	61	5	4.2	0.8
147	2	0.28	0.01	70	2		
170	1	0.41	0.02	72	2		
170	2	0.49	0.01	70	1		
171	2	0.85	0.02	27	2		
172	2	0.87	0.03	23	2		
172	1	0.82	0.05	61	4		
175	2	0.96	0.04	29	3		
189	2	0.79	0.05	32	3	5.9	0.4
200	1	0.78	0.02	14	1		
200	2	0.80	0.02	24	2		
202	2	0.69	0.06	44	5		
202	1	0.52	0.01	29	1		
203	2	0.62	0.02	38	2		
204	1	0.88	0.03	35	2		
204	2	0.91	0.03	19	2		
211	1	0.85	0.02	37	2		
212	1	0.97	0.03	39	2		
213	1	0.94	0.05	67	4		
216	1	0.91	0.02	36	2		
218	1	0.74	0.06	55	6		
225	1	0.76	0.02	51	2		
225	2	0.76	0.02	45	1		

228	2	0.62	0.03	56	3		
228	1	0.61	0.03	65	3		
231	1	0.71	0.02	24	1		
231	2	0.89	0.03	17	2		
234	1	0.71	0.04	21	3		
234	2	0.68	0.06	40	5		
236	2	0.82	0.02	23	2		
240	1	0.57	0.01	17	1		
242	1	0.42	0.04	32	4		
243	2	0.68	0.04	46	2	5.3	0.4
245	1	0.68	0.03	84	4		
248	2	0.62	0.02	56	2		
249	1	0.45	0.01	13	0		
250	1	0.38	0.01	17	0		
250	2	0.55	0.01	39	1		

Table S3j: Side-chain model-free order parameters for lac-Gal3 from ^2H relaxation rates.

Residue	Atom	O^2	std	τ_e (ps)	std	τ_c (ns)	std
113	2	0.02	0.00	9	0		
114	1	0.45	0.02	46	2		
114	2	0.42	0.01	35	1		
115	1	0.20	0.00	27	1		
115	2	0.40	0.01	51	1		
116	2	0.44	0.01	80	2		
120	1	0.63	0.03	38	3		
126	2	0.66	0.04	95	6		
126	1	0.66	0.03	74	4		
127	2	0.24	0.01	87	2		
130	1	0.44	0.01	5	1		

131	1	0.48	0.01	33	1		
132	1	0.34	0.01	15	1		
132	2	0.65	0.01	33	2		
134	1	0.77	0.08	27	4	7.7	0.9
135	2	0.35	0.01	40	1		
137	2	0.51	0.01	67	2		
138	1	0.75	0.10	179	19		
138	2	0.83	0.03	34	3		
142	1	0.87	0.05	74	6		
145	1	0.73	0.02	17	2		
145	2	0.72	0.02	32	2		
146	1	0.77	0.01	46	1		
147	1	0.27	0.01	75	2		
147	2	0.28	0.01	71	2		
156	1	0.87	0.06	97	9		
170	1	0.36	0.01	74	2		
170	2	0.45	0.01	78	2		
171	2	0.76	0.02	28	2		
172	1	0.80	0.02	24	3		
172	2	0.74	0.03	62	4		
175	2	0.90	0.04	28	4		
189	2	0.58	0.01	41	1		
200	1	0.71	0.02	15	2		
200	2	0.77	0.02	25	2		
202	1	0.54	0.05	54	7		
202	2	0.51	0.01	30	2		
203	2	0.65	0.11	36	6	6.9	1.5
204	1	0.83	0.03	38	3		
204	2	0.83	0.02	23	2		

211	1	0.82	0.02	39	2		
212	1	0.86	0.03	40	3		
213	1	0.82	0.04	70	5		
216	1	0.87	0.02	37	2		
218	1	0.69	0.05	54	7		
219	2	0.71	0.09	100	14		
225	1	0.70	0.02	53	3		
225	2	0.71	0.02	44	2		
228	2	0.63	0.03	54	4		
228	1	0.56	0.02	65	4		
231	1	0.87	0.07	13	5	5.9	0.5
231	2	0.84	0.03	21	2		
234	1	0.65	0.03	18	4		
234	2	0.63	0.06	45	7		
236	1	0.52	0.01	15	2		
236	2	0.78	0.02	21	2		
240	1	0.55	0.01	16	1		
242	2	0.65	0.03	33	4		
242	1	0.45	0.05	35	6		
243	2	0.45	0.01	57	1		
245	1	0.72	0.03	82	5		
248	2	0.55	0.02	58	2		
249	1	0.43	0.01	13	1		
250	1	0.35	0.00	18	1		
250	2	0.51	0.01	40	1		

Table S3k: Side-chain model-free order parameters for L2-Gal3 from ^2H relaxation rates.

Residue	Atom	O^2	std	τ_e (ps)	std	τ_c (ns)	std
113	2	0.01	0.00	8	0		
114	1	0.47	0.01	46	1		
114	2	0.42	0.01	32	1		
115	1	0.21	0.00	26	0		
115	2	0.40	0.01	52	1		
116	1	0.44	0.01	85	2		
120	1	0.57	0.02	36	2		
126	2	0.67	0.03	87	4		
126	1	0.69	0.02	79	2		
127	2	0.26	0.01	85	1		
130	1	0.50	0.02	4	1	6.8	0.3
131	1	0.49	0.01	31	1		
132	1	0.39	0.01	13	1		
132	2	0.65	0.02	33	1		
134	1	0.83	0.07	24	3	7.1	0.6
135	2	0.32	0.00	40	1		
137	2	0.49	0.01	64	1		
138	2	0.82	0.02	30	2		
142	1	0.83	0.03	66	3		
145	1	0.73	0.02	15	2		
145	2	0.70	0.02	33	2		
146	1	0.81	0.02	30	2		
147	1	0.29	0.01	72	2		
147	2	0.55	0.08	56	5	4.0	0.6
155	2	0.82	0.04	79	4		
156	1	0.82	0.05	90	6		
170	1	0.41	0.02	68	2		

171	2	0.77	0.02	22	2		
172	1	0.82	0.03	23	2		
172	2	0.72	0.03	58	3		
189	2	0.73	0.04	34	2	6.0	0.4
200	1	0.67	0.01	15	1		
200	2	0.77	0.01	24	1		
202	2	0.55	0.01	26	1		
203	2	0.56	0.02	41	2		
204	1	0.82	0.02	38	1		
204	2	0.82	0.02	20	2		
211	1	0.78	0.02	40	1		
212	1	0.88	0.02	41	2		
213	1	0.84	0.03	69	3		
216	1	0.88	0.02	36	1		
218	1	0.70	0.04	61	5		
219	2	0.75	0.06	77	7		
225	2	0.71	0.01	47	1		
228	2	0.62	0.02	55	3		
228	1	0.53	0.02	75	3		
231	1	0.63	0.01	26	1		
231	2	0.80	0.02	20	2		
234	1	0.68	0.03	12	3		
236	1	0.53	0.01	15	1		
236	2	0.80	0.02	24	1		
240	1	0.55	0.01	16	1		
240	2	0.63	0.01	43	1		
242	2	0.61	0.03	35	3		
242	1	0.69	0.07	56	7		
243	2	0.46	0.01	55	1		

245	1	0.73	0.03	97	4		
248	2	0.55	0.01	57	1		
249	1	0.43	0.00	11	0		
250	1	0.48	0.02	10	1	5.5	0.3
250	2	0.50	0.01	40	1		

Table S31: Side-chain model-free order parameters for L3-Gal3 from ^2H relaxation rates.

Residue	Atom	O^2	std	τ_e (ps)	std	τ_c (ns)	std
113	2	0.01	0.00	9	0		
114	1	0.51	0.01	45	1		
114	2	0.47	0.01	30	1		
115	1	0.21	0.00	26	0		
115	2	0.44	0.01	49	1		
116	1	0.53	0.02	96	2		
120	1	0.56	0.02	36	2		
126	2	0.75	0.03	86	4		
126	1	0.63	0.02	73	3		
127	2	0.25	0.01	87	1		
130	1	0.47	0.00	4	0		
131	1	0.54	0.01	30	1		
132	1	0.43	0.03	9	2	6.4	0.5
132	2	0.73	0.02	27	2		
134	1	0.78	0.02	26	1		
135	2	0.35	0.01	40	1		
137	2	0.55	0.01	59	1		
138	2	0.87	0.03	28	2		
142	1	0.84	0.04	73	4		
145	1	0.75	0.02	17	2		
145	2	0.74	0.02	32	2		

146	1	0.91	0.02	40	2		
147	1	0.26	0.01	72	2		
147	2	0.27	0.01	70	2		
155	2	0.79	0.04	93	4		
156	1	0.98	0.06	78	5		
170	1	0.57	0.08	63	4	5.3	0.8
170	2	0.54	0.01	73	1		
171	2	0.82	0.02	27	2		
172	2	0.65	0.04	67	5		
189	2	0.64	0.01	36	1		
200	1	0.73	0.01	12	1		
200	2	0.77	0.02	24	1		
202	1	0.75	0.05	41	6		
202	2	0.58	0.01	27	1		
203	2	0.57	0.02	42	2		
204	1	0.89	0.02	32	2		
204	2	0.83	0.02	23	2		
211	1	0.83	0.02	33	2		
212	1	0.90	0.02	43	2		
213	1	0.84	0.03	72	3		
216	1	0.88	0.02	39	2		
218	1	0.83	0.06	46	5		
219	2	0.81	0.08	71	9		
228	2	0.62	0.03	60	3		
228	1	0.65	0.02	63	3		
231	1	0.65	0.01	21	1		
231	2	0.84	0.02	18	2		
234	1	0.72	0.03	23	3		
234	2	0.69	0.05	31	5		

236	1	0.64	0.05	8	3	6.5	0.5
236	2	0.83	0.02	20	2		
240	1	0.54	0.01	17	1		
242	1	0.88	0.08	38	7		
245	1	0.98	0.03	73	3		
248	2	0.59	0.01	54	1		
249	1	0.45	0.00	11	0		
250	1	0.38	0.00	16	1		
250	2	0.56	0.01	38	1		

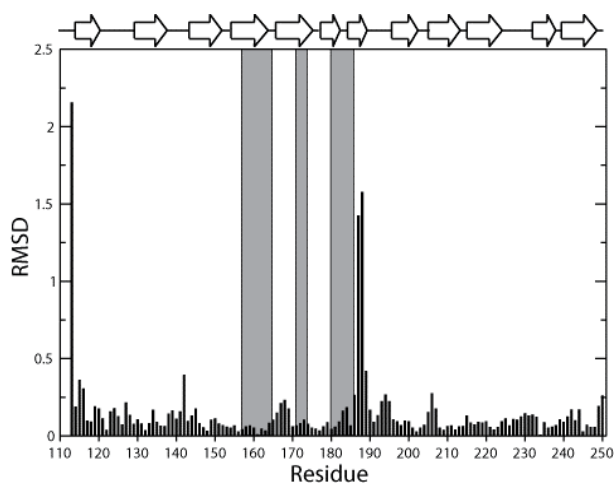


Figure S1a: Atom coordinate RMSD between each lactose-bound state and the apo state, plotted versus residue number. Secondary structure elements are indicated at the top of the graph. Residues within 5 Å of the ligand are highlighted by grey bars.

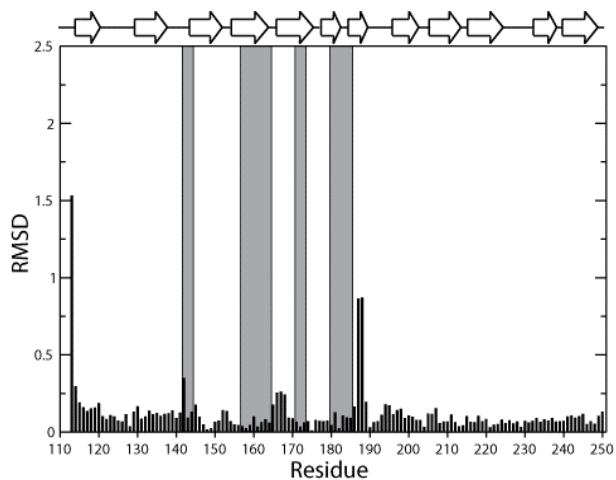


Figure S1b: Atom coordinate RMSD between each L2-bound state and the apo state, plotted versus residue number. Secondary structure elements are indicated at the top of the graph. Residues within 5 Å of the ligand are highlighted by grey bars.

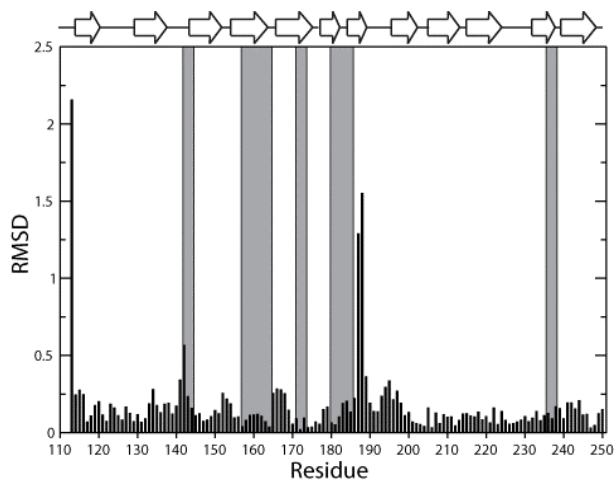


Figure S1c: Atom coordinate RMSD between each L2-bound state and the apo state, plotted versus residue number. Secondary structure elements are indicated at the top of the graph. Residues within 5 Å of the ligand are highlighted by grey bars.

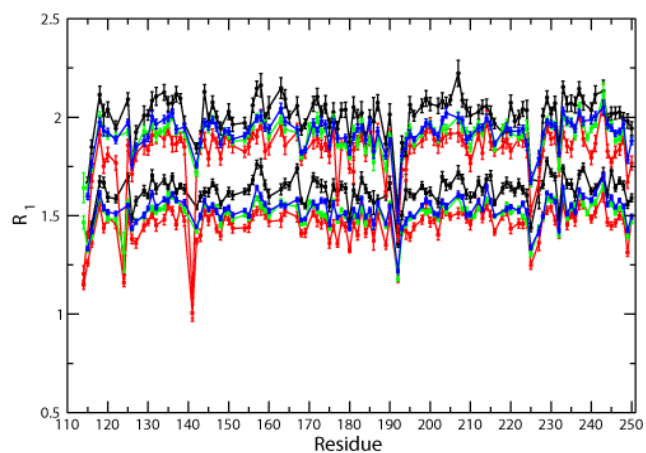


Figure S2a: ^{15}N R_1 relaxation rates for all backbone amides at 500 (circles) and 600 MHz (squares). (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

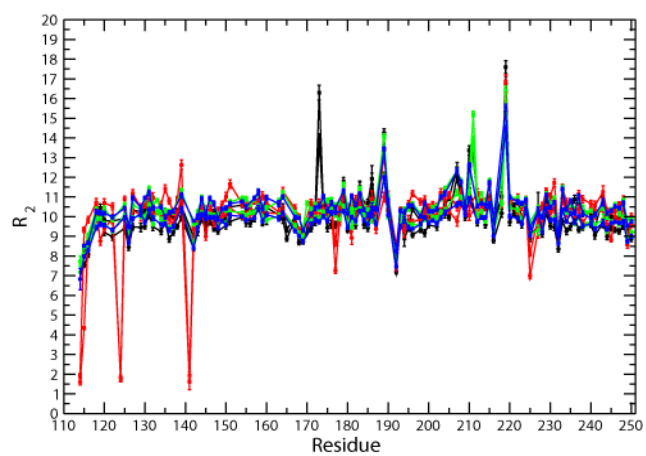


Figure S2b: ^{15}N R_2 relaxation rates for all backbone amides at 500 (circles) and 600 MHz (squares). (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

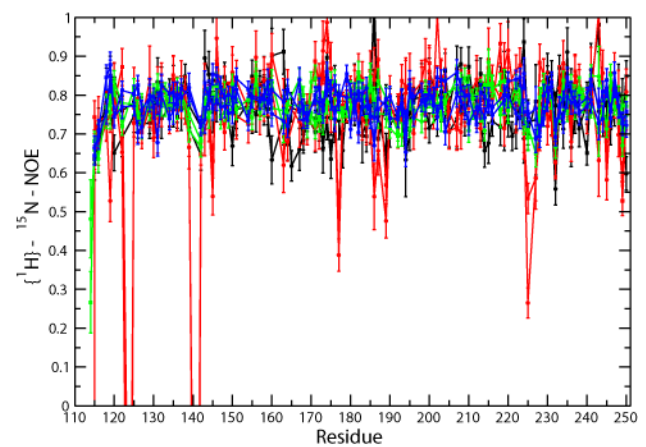


Figure S2c: $\{^1\text{H}\}-^{15}\text{N}$ NOE for all backbone amides at 500 (circles) and 600 MHz (squares). (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

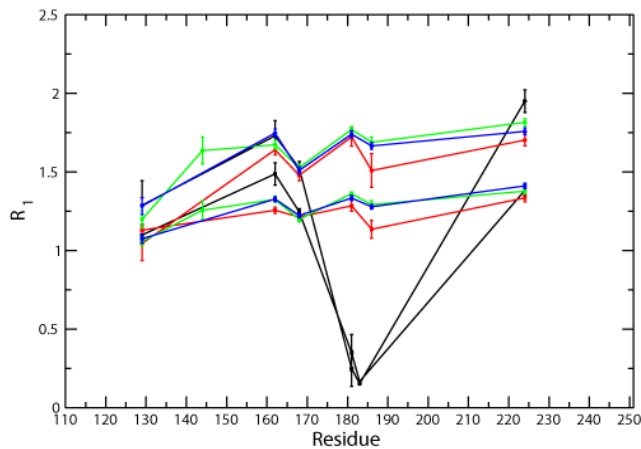


Figure S2d: ^{15}N R_1 relaxation rates for all sidechain arginines and tryptophanes at 500 (circles) and 600 MHz (squares). (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

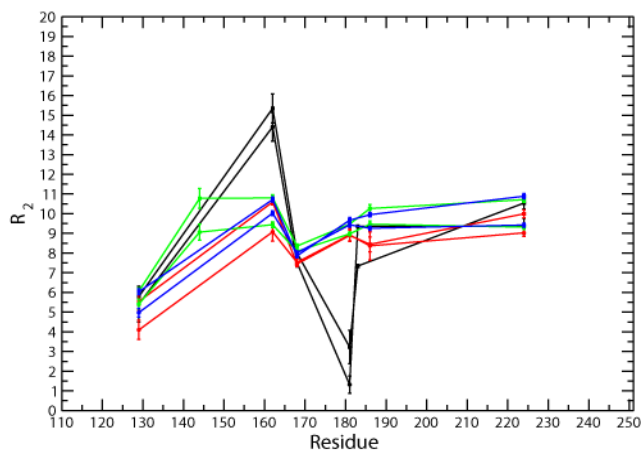


Figure S2d: ^{15}N R_2 relaxation rates for all sidechain arginines and tryptophanes at 500 (circles) and 600 MHz (squares). (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

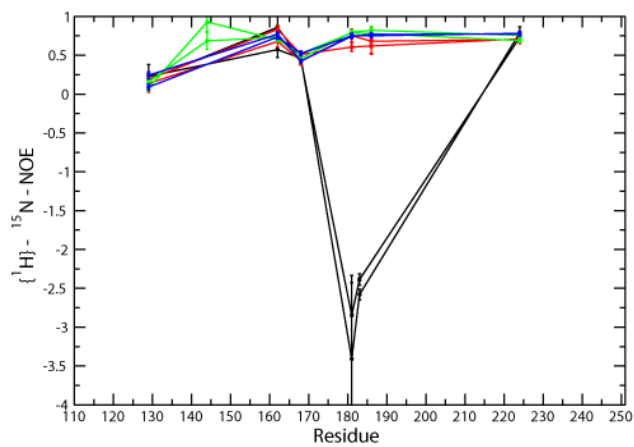


Figure S2e: $\{^1\text{H}\}-^{15}\text{N}$ NOE for all sidechain arginines and tryptophanes at 500 (circles) and 600 MHz (squares). (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

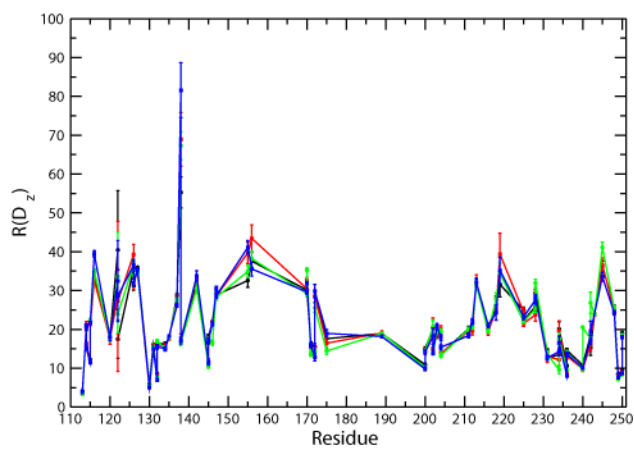


Figure S2f: $R(D_z)$ relaxation rates for methyl groups at 600 MHz. (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

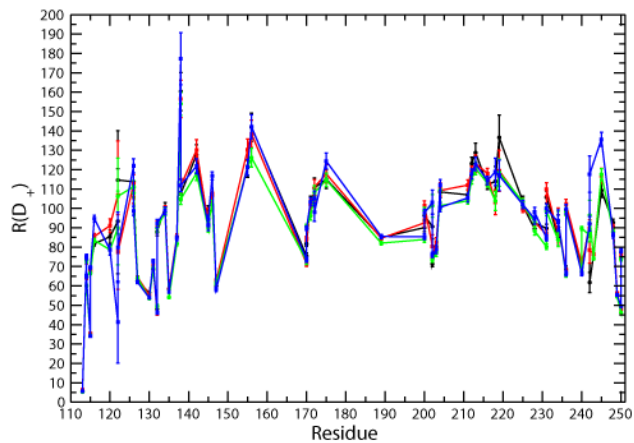


Figure S2g: $R(D_+)$ relaxation rates for methyl groups at 600 MHz. (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

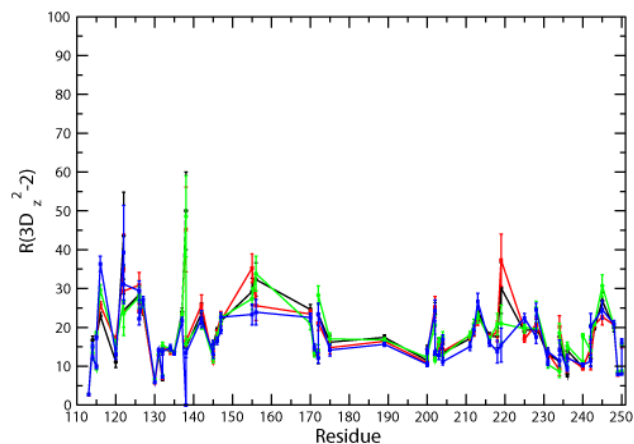


Figure S2h: $R(3D_z - 2)$ relaxation rates for methyl groups at 600 MHz. (black) apo Gal3, (red) lac-

Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

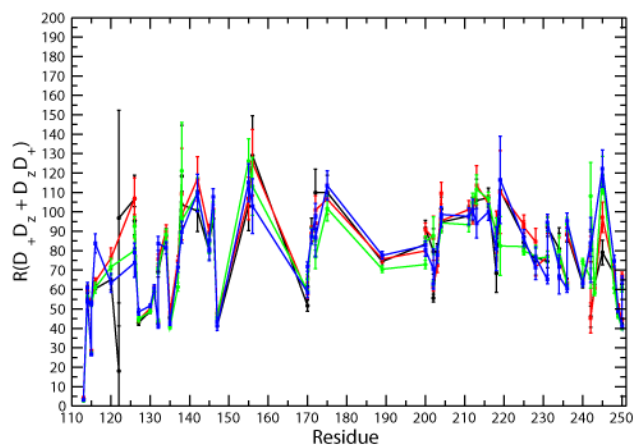


Figure S2i: $R(D_+D_z + D_zD_+)$ relaxation rates for methyl groups at 600 MHz. (black) apo Gal3, (red) lac-Gal3, (green) L2-Gal3, and (blue) L3-Gal3.

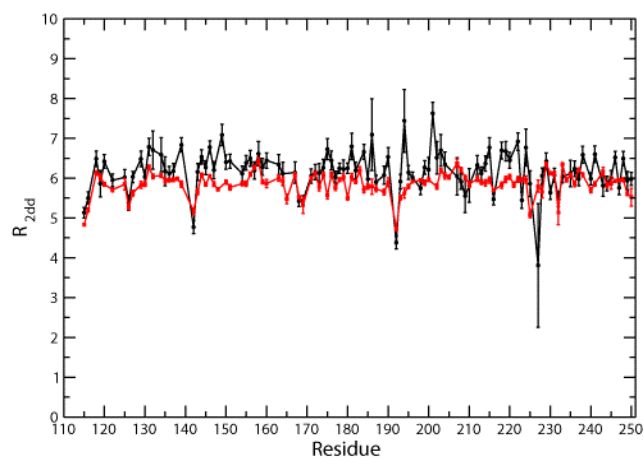


Figure S3: ^{15}N $R_{2\text{dd}}$ relaxation rates for backbone amides in apo-Gal3 at 500 MHz. (black) experimental rates, and (red) theoretical rates calculated from model-free parameters.