Supporting Information for

Biophysical Characterization of a Disabled Double Mutant of Soybean Lipoxygenase: The "Undoing" of Precise Substrate Positioning Relative to Metal Cofactor and an Identified Dynamical Network

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Table S1. Kinetic parameters of SLO and mutants in 0.1 M borate (pH 9) at 30°C

Enzyme	k_{cat} (s^{-1})	$E_{a(H)}$[f] (kcal/mol)	$^{D}k_{cat}$	ΔE_a[g] (kcal/mol)
WT[a]	297 (12)	2.1 (0.2)	81 (5)	0.9 (0.2)
I553V[b]	91 (5)	2.4 (0.5)	77 (6)	2.6 (0.5)
I553L[b]	273 (10)	0.4 (0.7)	81 (3)	3.4 (0.6)
I553A[a]	280 (10)	1.9 (0.2)	93 (4)	4.0 (0.3)
I553G[b]	58 (4)	0.03 (0.04)	178 (16)	5.3 (0.7)
L546A[a]	4.8 (0.6)	4.1 (0.4)	93 (9)	1.9 (0.6)
L754A[a]	0.31 (0.02)	4.1 (0.3)	112 (11)	2.0 (0.5)
L546A/L754A (DM)[c]	0.025 (0.01)	9.9 (0.2)	692 (43)	0.3 (0.7)
L546A/I553A[d]	2.21 (0.09)	3.8 (0.4)	128 (3)	2.8 (0.4)
L754A/I553A[d]	0.56 (0.03)	6.9 (0.2)	85 (7)	3.9 (0.5)

[a]From Ref [1].
[b]From Ref [2].
[c]The values of k_{cat}, $^{D}k_{cat}$ and ΔE_a are From Ref [3], while the $E_{a(H)}$ value is from Ref [4].
[d]From Ref [5].
[e]From Ref [6].
[f]Determined from the temperature dependence of k_{cat}.
[g].$\Delta Ea = E_{a(D)} - E_{a(H)}$.
[h]These are computed according to the vibronically non-adiabatic multi-dimensional model, Ref [3].

Table S2. Weighted average rates of HDX (in min^{-1})

144-160					
Temp	WT	I553G	L546A	L754A	DM
10	0.027 (0.004)	0.03 (0.004)	0.019 (0.004)	0.018 (0.004)	0.020 (0.004)
20	0.051 (0.008)	0.054 (0.013)	0.047 (0.01)	0.038 (0.008)	0.055 (0.012)
25	0.071 (0.014)	0.079 (0.015)	0.043 (0.01)	0.079 (0.025)	0.076 (0.016)
30	0.066 (0.016)	0.10 (0.03)	0.072 (0.014)	0.048 (0.009)	0.055 (0.018)
40	0.20 (0.05)	0.26 (0.09)	0.25 (0.06)	0.076 (0.03)	0.25 (0.16)
167-185					
Temp	WT	I553G	L546A	L754A	DM
10	0.076 (0.03)	0.046 (0.02)	0.039 (0.01)	0.064 (0.03)	0.017 (0.007)
20	0.058 (0.03)	0.016 (0.01)	0.025 (0.01)	0.021 (0.007)	0.058 (0.02)
25	0.016 (0.005)	0.022 (0.006)	0.008 (0.007)	0.004 (0.006)	0.029 (0.01)
30	0.018 (0.008)	0.020 (0.007)	0.014 (0.005)	0.015 (0.004)	0.010 (0.005)
40	0.027 (0.007)	0.024 (0.003)	0.025 (0.004)	0.019 (0.004)	0.016 (0.003)
186-206					
Temp	WT	I553G	L546A	L754A	DM
10	0.076 (0.02)	0.065 (0.02)	0.047 (0.01)	0.040 (0.009)	0.044 (0.01)
20	0.077 (0.02)	0.080 (0.03)	0.050 (0.01)	0.052 (0.01)	0.077 (0.02)
25	0.054 (0.01)	0.073 (0.02)	0.23 (0.1)	0.068 (0.03)	0.12 (0.04)
30	0.09 (0.1)	0.091 (0.03)	0.050 (0.01)	0.040 (0.009)	0.30 (0.15)
40	0.13 (0.05)	0.040 (0.01)	0.12 (0.04)	0.030 (0.01)	0.37 (0.15)
212-238					
Temp	WT	I553G	L546A	L754A	DM
10	0.14 (0.02)	N.C.	0.098 (0.03)	0.091 (0.02)	N.C.
20	0.32 (0.1)	N.C.	0.36 (0.07)	0.29 (0.08)	N.C.
25	0.37 (0.1)	N.C.	0.44 (0.1)	0.67 (0.2)	N.C.
30	0.96 (0.3)	N.C.	0.58 (0.2)	0.64 (0.3)	N.C.
40	0.060 (0.03)	N.C.	0.025 (0.01)	0.015 (0.01)	N.C.
239-256					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
257-273					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
274-283					

Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
284-296					
Temp	WT	I553G	L546A	L754A	DM
10	0.026 (0.005)	0.025 (0.006)	N.C.	N.C.	0.018 (0.005)
20	0.065 (0.01)	0.21 (0.06)	N.C.	N.C.	0.056 (0.009)
25	0.11 (0.03)	0.11 (0.04)	N.C.	N.C.	0.19 (0.1)
30	0.20 (0.1)	0.18 (0.14)	N.C.	N.C.	0.34 (0.2)
40	0.38 (0.1)	0.38 (0.18)	N.C.	N.C.	0.44 (0.25)
284-299					
Temp	WT	I553G	L546A	L754A	DM
10	0.041 (0.006)	0.30 (0.11)	0.09 (0.02)	0.027 (0.005)	0.063 (0.001)
20	0.048 (0.006)	0.41 (0.08)	0.25 (0.06)	0.036 (0.005)	0.091 (0.01)
25	0.08 (0.04)	0.33 (0.08)	0.39 (0.08)	0.10 (0.03)	0.18 (0.05)
30	0.11 (0.03)	0.48 (0.22)	0.84 (0.22)	0.13 (0.02)	0.18 (0.04)
40	0.29 (0.08)	0.60 (0.22)	0.88 (0.11)	0.30 (0.09)	0.57 (0.14)
297-305					
Temp	WT	I553G	L546A	L754A	DM
10	0.10 (0.02)	0.50 (0.12)	0.39 (0.11)	0.047 (0.02)	0.15 (0.04)
20	0.10 (0.02)	0.92 (0.16)	0.53 (0.08)	0.088 (0.01)	0.38 (0.09)
25	0.15 (0.06)	1.6 (0.3)	0.92 (0.18)	0.33 (0.08)	0.59 (0.2)
30	0.58 (0.16)	1.7 (0.5)	1.8 (0.3)	0.27 (0.06)	1.07 (0.3)
40	0.81 (0.19)	4.4 (3.0)	2.6 (0.4)	0.45 (0.1)	1.9 (0.4)
306-316					
Temp	WT	I553G	L546A	L754A	DM
10	0.14 (0.04)	0.49 (0.12)	0.46 (0.11)	0.052 (0.02)	0.18 (0.05)
20	0.09 (0.01)	0.90 (0.10)	0.61 (0.09)	0.10 (0.02)	0.26 (0.04)
25	0.15 (0.06)	0.98 (0.16)	0.69 (0.08)	0.33 (0.08)	0.45 (0.1)
30	0.48 (0.2)	1.2 (0.3)	1.7 (0.22)	0.22 (0.05)	0.92 (0.3)
40	0.44 (0.11)	1.7 (0.9)	1.8 (0.21)	0.34 (0.06)	1.3 (0.3)
316-334					
Temp	WT	I553G	L546A	L754A	DM
10	0.024 (0.004)	0.052 (0.007)	0.032 (0.006)	0.015 (0.004)	0.024 (0.004)
20	0.016 (0.003)	0.058 (0.013)	0.051 (0.008)	0.019 (0.003)	0.040 (0.008)
25	0.012 (0.003)	0.056 (0.008)	0.056 (0.01)	0.019 (0.006)	0.041 (0.01)
30	0.019 (0.002)	0.087 (0.02)	0.21 (0.06)	0.021 (0.004)	0.14 (0.06)
40	0.030 (0.006)	0.060 (0.018)	0.30 (0.09)	0.026 (0.004)	0.067 (0.02)
316-338					
Temp	WT	I553G	L546A	L754A	DM
10	0.013 (0.004)	0.033 (0.005)	0.021 (0.004)	0.011 (0.003)	0.013 (0.003)

20	0.017 (0.003)	0.030 (0.006)	0.035 (0.005)	0.017 (0.003)	0.030 (0.006)
25	0.019 (0.003)	0.042 (0.005)	0.033 (0.005)	0.017 (0.004)	0.037 (0.01)
30	0.026 (0.003)	0.073 (0.02)	0.077 (0.02)	0.025 (0.0 5)	0.029 (0.006)
40	0.049 (0.009)	0.092 (0.02)	0.20 (0.05)	0.033 (0.006)	0.081 (0.02)
317-334					
Temp	WT	I553G	L546A	L754A	DM
10	0.009 (0.004)	0.046 (0.005)	0.029 (0.005)	0.010 (0.002)	0.024 (0.002)
20	0.013 (0.007)	0.067 (0.013)	0.047 (0.006)	0.016 (0.004)	0.038 (0.006)
25	0.015 (0.006)	0.058 (0.010)	0.061 (0.013)	0.018 (0.005)	0.041 (0.008)
30	0.014 (0.003)	0.053 (0.008)	0.18 (0.05)	0.019 (0.004)	0.092 (0.06)
40	0.022 (0.006)	0.056 (0.017)	0.26 (0.08)	0.025 (0.005)	0.21 (0.08)
347-355					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.C.
20	N.D.	N.D.	N.D.	N.D.	N.C.
25	N.D.	N.D.	N.D.	N.D.	N.C.
30	N.D.	N.D.	N.D.	N.D.	N.C.
40	N.D.	N.D.	N.D.	N.D.	N.C.
362-388					
Temp	WT	I553G	L546A	L754A	DM
10	0.068 (0.01)	N.C.	0.034 (0.01)	0.033 (0.006)	0.032 (0.006)
20	0.12 (0.03)	N.C.	0.085 (0.02)	0.073 (0.01)	0.079 (0.02)
25	0.14 (0.03)	N.C.	0.25 (0.07)	0.28 (0.1)	0.21 (0.08)
30	0.55 (0.2)	N.C.	0.27 (0.09)	0.19 (0.04)	0.42 (0.09)
40	0.96 (0.3)	N.C.	0.76 (0.1)	0.63 (0.1)	0.92 (0.3)
407-413					
Temp	WT	I553G	L546A	L754A	DM
10	0.047 (0.003)	0.052 (0.003)	0.042 (0.003)	0.045 (0.003)	0.044 (0.003)
20	0.12 (0.02)	0.18 (0.03)	0.11 (0.02)	0.11 (0.02)	0.12 (0.01)
25	0.14 (0.02)	0.25 (0.05)	0.32 (0.08)	0.34 (0.007)	0.30 (0.05)
30	0.42 (0.2)	0.41 (0.1)	0.41 (0.08)	0.43 (0.1)	0.49 (0.15)
40	0.61 (0.3)	0.48 (0.2)	0.57 (0.2)	0.68 (0.3)	0.73 (0.3)
414-423					
Temp	WT	I553G	L546A	L754A	DM
10	0.25 (0.06)	0.16 (0.03)	0.28 (0.06)	0.27 (0.07)	0.23 (0.06)
20	0.19 (0.03)	0.22 (0.04)	0.26 (0.05)	0.65 (0.2)	0.30 (0.06)
25	0.18 (0.03)	0.33 (0.11)	0.24 (0.04)	0.22 (0.03)	0.24 (0.05)
30	0.35 (0.06)	0.26 (0.11)	0.30 (0.04)	0.36 (0.08)	0.30 (0.05)
40	0.34 (0.2)	0.62 (0.15)	0.19 (0.05)	0.23 (0.07)	0.33 (0.07)

424-435					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.

30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
436-449					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
450-462					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
466-477					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
493-503					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
504-521					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
522-540					
Temp	WT	I553G	L546A	L754A	DM
10	0.005 (0.002)	0.007 (0.004)	0.011 (0.005)	0.005 (0.006)	0.012 (0.004)
20	0.009 (0.002)	0.014 (0.002)	0.011 (0.002)	0.008 (0.004)	0.018 (0.004)
25	0.020 (0.003)	0.016 (0.001)	0.020 (0.002)	0.017 (0.003)	0.018 (0.004)
30	0.028 (0.003)	0.024 (0.004)	0.023 (0.003)	0.016 (0.004)	0.026 (0.002)
40	0.041 (0.005)	0.041 (0.007)	0.043 (0.004)	0.047 (0.006)	0.039 (0.005)
541-554					
Temp	WT	I553G	L546A	L754A	DM
10	0.001 (0.004)	0.025 (0.003)	0.003 (0.005)	0.003 (0.005)	0.003 (0.006)

20	0.004 (0.001)	0.042 (0.004)	0.008 (0.001)	0.005 (0.002)	0.011 (0.004)
25	0.012 (0.001)	0.073 (0.009)	0.017 (0.001)	0.012 (0.002)	0.011 (0.001)
30	0.019 (0.002)	0.14 (0.03)	0.030 (0.002)	0.018 (0.003)	0.022 (0.002)
40	0.047 (0.004)	0.07 (0.02)	0.060 (0.008)	0.053 (0.003)	0.045 (0.006)
555-565					
Temp	WT	I553G	L546A	L754A	DM
10	0.001 (0.002)	0.007 (0.002)	0.003 (0.004)	0.002 (0.004)	0.001 (0.001)
20	0.003 (0.001)	0.011 (0.001)	0.004 (0.001)	0.004 (0.002)	0.011 (0.003)
25	0.009 (0.001)	0.020 (0.001)	0.008 (0.001)	0.009 (0.001)	0.009 (0.001)
30	0.015 (0.001)	0.031 (0.003)	0.011 (0.001)	0.012 (0.002)	0.015 (0.002)
40	0.019 (0.003)	0.093 (0.008)	0.016 (0.002)	0.017 (0.002)	0.015 (0.002)
566-576					
Temp	WT	I553G	L546A	L754A	DM
10	0.004 (0.001)	0.012 (0.002)	0.008 (0.002)	0.007 (0.001)	0.009 (0.002)
20	0.016 (0.001)	0.019 (0.002)	0.016 (0.001)	0.015 (0.002)	0.019 (0.002)
25	0.024 (0.002)	0.027 (0.003)	0.022 (0.002)	0.024 (0.002)	0.025 (0.002)
30	0.043 (0.004)	0.044 (0.006)	0.036 (0.004)	0.034 (0.005)	0.033 (0.004)
40	0.086 (0.01)	0.062 (0.02)	0.09 (0.02)	0.067 (0.01)	0.080 (0.017)
586-603					
Temp	WT	I553G	L546A	L754A	DM
10	0.089 (0.01)	0.09 (0.01)	0.056 (0.01)	0.064 (0.01)	0.055 (0.01)
20	0.16 (0.03)	0.30 (0.04)	0.17 (0.03)	0.13 (0.02)	0.14 (0.03)
25	0.24 (0.04)	0.34 (0.07)	0.29 (0.07)	0.37 (0.08)	0.30 (0.06)
30	0.41 (0.2)	0.33 (0.1)	0.41 (0.09)	0.37 (0.1)	0.41 (0.16)
40	0.64 (0.2)	0.48 (0.2)	0.33 (0.1)	0.031 (0.02)	0.74 (0.3)
604-617					
Temp	WT	I553G	L546A	L754A	DM
10	0.021 (0.009)	0.015 (0.006)	0.014 (0.006)	0.013 (0.006)	N.C.
20	0.022 (0.003)	0.036 (0.01)	0.025 (0.005)	0.022 (0.007)	N.C.
25	0.051 (0.01)	0.037 (0.005)	0.047 (0.009)	0.049 (0.01)	N.C.
30	0.045 (0.02)	0.099 (0.05)	0.22 (0.1)	0.069 (0.02)	N.C.
40	0.068 (0.02)	0.23 (0.1)	0.096 (0.04)	0.043 (0.01)	N.C.
619-632					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.C.	N.C.	N.C.	N.D.
20	N.D.	N.C.	N.C.	N.C.	N.D.
25	N.D.	N.C.	N.C.	N.C.	N.D.
30	N.D.	N.C.	N.C.	N.C.	N.D.
40	N.D.	N.C.	N.C.	N.C.	N.D.
633-648					
Temp	WT	I553G	L546A	L754A	DM
10	0.012 (0.003)	0.011 (0.003)	0.009 (0.003)	0.007 (0.003)	0.010 (0.003)
20	0.013 (0.003)	0.011 (0.002)	0.013 (0.001)	0.009 (0.003)	0.022 (0.003)
25	0.015 (0.002)	0.021 (0.004)	0.016 (0.003)	0.016 (0.003)	0.019 (0.003)
30	0.023 (0.003)	0.026 (0.01)	0.021 (0.003)	0.020 (0.003)	0.020 (0.003)

40	0.035 (0.006)	0.036 (0.004)	0.037 (0.007)	0.026 (0.004)	0.032 (0.005)
649-669					
Temp	WT	I553G	L546A	L754A	DM
10	0.046 (0.004)	0.047 (0.005)	0.033 (0.004)	0.033 (0.004)	N.C.
20	0.093 (0.01)	0.15 (0.03)	0.077 (0.01)	0.080 (0.009)	N.C.
25	0.15 (0.02)	0.17 (0.04)	0.19 (0.05)	0.21 (0.04)	N.C.
30	0.24 (0.06)	0.25 (0.05)	0.29 (0.05)	0.021 (0.04)	N.C.
40	0.55 (0.1)	0.45 (0.1)	0.59 (0.06)	0.48 (0.1)	N.C.
690-703					
Temp	WT	I553G	L546A	L754A	DM
10	N.D.	N.D.	N.D.	N.D.	N.D.
20	N.D.	N.D.	N.D.	N.D.	N.D.
25	N.D.	N.D.	N.D.	N.D.	N.D.
30	N.D.	N.D.	N.D.	N.D.	N.D.
40	N.D.	N.D.	N.D.	N.D.	N.D.
704-726					
Temp	WT	I553G	L546A	L754A	DM
10	0.053 (0.006)	0.043 (0.006)	0.037 (0.06)	N.C.	0.032 (0.005)
20	0.061 (0.02)	0.078 (0.03)	0.044 (0.009)	N.C.	0.063 (0.01)
25	0.069 (0.02)	0.056 (0.01)	0.032 (0.01)	N.C.	0.073 (0.02)
30	0.043 (0.01)	0.049 (0.02)	0.030 (0.01)	N.C.	0.037 (0.01)
40	0.069 (0.02)	0.053 (0.01)	0.10 (0.03)	N.C.	0.055 (0.01)
727-737					
Temp	WT	I553G	L546A	L754A	DM
10	0.020 (0.002)	0.022 (0.002)	0.015 (0.002)	0.013 (0.001)	0.017 (0.001)
20	0.049 (0.005)	0.059 (0.007)	0.039 (0.003)	0.039 (0.003)	0.050 (0.003)
25	0.091 (0.01)	0.087 (0.01)	0.063 (0.01)	0.069 (0.01)	0.088 (0.01)
30	0.15 (0.04)	0.13 (0.04)	0.10 (0.02)	0.097 (0.02)	0.11 (0.02)
40	0.25 (0.01)	0.21 (0.2)	0.097 (0.04)	0.23 (0.09)	0.045 (0.009)
738-745					
Temp	WT	I553G	L546A	L754A	DM
10	0.030 (0.004)	0.051 (0.004)	0.034 (0.005)	0.028 (0.003)	0.030 (0.003)
20	0.073 (0.008)	0.20 (0.03)	0.13 (0.01)	0.088 (0.01)	0.11 (0.01)
25	0.15 (0.02)	0.25 (0.04)	0.27 (0.04)	0.23 (0.04)	0.28 (0.04)
30	0.30 (0.09)	0.29 (0.09)	0.51 (0.1)	0.29 (0.06)	0.39 (0.11)
40	0.85 (0.2)	0.017 (0.006)	0.80 (0.4)	0.67 (0.2)	0.96 (0.4)
751-761					
Temp	WT	I553G	L546A	L754A	DM
10	0.055 (0.02)	0.37 (0.1)	0.10 (0.03)	0.023 (0.005)	0.040 (0.009)
20	0.073 (0.01)	0.70 (0.2)	0.26 (0.07)	0.041 (0.01)	0.14 (0.03)
25	0.11 (0.02)	0.48 (0.2)	0.85 (0.5)	0.23 (0.09)	0.46 (0.16)
30	0.65 (0.74)	0.25 (0.3)	0.007 (0.007)	0.12 (0.03)	0.63 (0.4)
40	0.099 (0.05)	0.007 (0.007)	0.012 (0.006)	0.036 (0.02)	0.018 (0.09)
762-781					
Temp	WT	I553G	L546A	L754A	DM

10	0.025 (0.002)	0.027 (0.002)	0.019 (0.002)	0.018 (0.002)	0.019 (0.002)
20	0.056 (0.006)	0.076 (0.01)	0.046 (0.005)	0.045 (0.004)	0.053 (0.006)
25	0.11 (0.02)	0.097 (0.01)	0.064 (0.01)	0.10 (0.02)	0.10 (0.02)
30	0.12 (0.02)	0.15 (0.03)	0.15 (0.03)	0.10 (0.02)	0.12 (0.02)
40	0.34 (0.06)	0.40 (0.09)	0.37 (0.05)	0.38 (0.1)	0.25 (0.1)
782-796					
Temp	WT	I553G	L546A	L754A	DM
10	0.012 (0.001)	0.017 (0.003)	0.009 (0.002)	0.010 (0.002)	0.017 (0.004)
20	0.034 (0.002)	0.047 (0.004)	0.031 (0.004)	0.030 (0.004)	0.033 (0.003)
25	0.044 (0.008)	0.046 (0.007)	0.046 (0.003)	0.044 (0.004)	0.048 (0.006)
30	0.077 (0.02)	0.059 (0.009)	0.049 (0.01)	0.071 (0.02)	0.049 (0.07)
40	0.046 (0.01)	0.030 (0.008)	0.043 (0.01)	0.039 (0.01)	0.038 (0.01)
797-820					
Temp	WT	I553G	L546A	L754A	DM
10	0.24 (0.08)	0.28 (0.1)	0.29 (0.1)	0.13 (0.05)	0.27 (0.1)
20	0.027 (0.009)	0.021 (0.008)	0.024 (0.004)	0.029 (0.01)	0.38 (0.19)
25	0.021 (0.006)	0.026 (0.008)	0.022 (0.02)	0.020 (0.007)	0.032 (0.011)
30	0.024 (0.005)	0.020 (0.008)	0.022 (0.005)	0.018 (0.004)	0.018 (0.004)
40	0.030 (0.007)	0.027 (0.003)	0.029 (0.005)	0.018 (0.004)	0.025 (0.005)
821-836					
Temp	WT	I553G	L546A	L754A	DM
10	0.024 (0.004)	0.029 (0.005)	0.031 (0.007)	0.046 (0.01)	0.037 (0.009)
20	0.020 (0.004)	0.013 (0.004)	0.025 (0.005)	0.026 (0.008)	0.034 (0.008)
25	0.014 (0.004)	0.019 (0.004)	0.016 (0.004)	0.019 (0.006)	0.026 (0.006)
30	0.020 (0.003)	0.020 (0.006)	0.018 (0.003)	0.017 (0.003)	0.017 (0.003)
40	0.036 (0.005)	0.042 (0.004)	0.037 (0.008)	0.026 (0.013)	0.024 (0.003)

N.D. = not determined

N.C. = no coverage

Table S3. Apparent activation energies for HDX (kcal/mol)

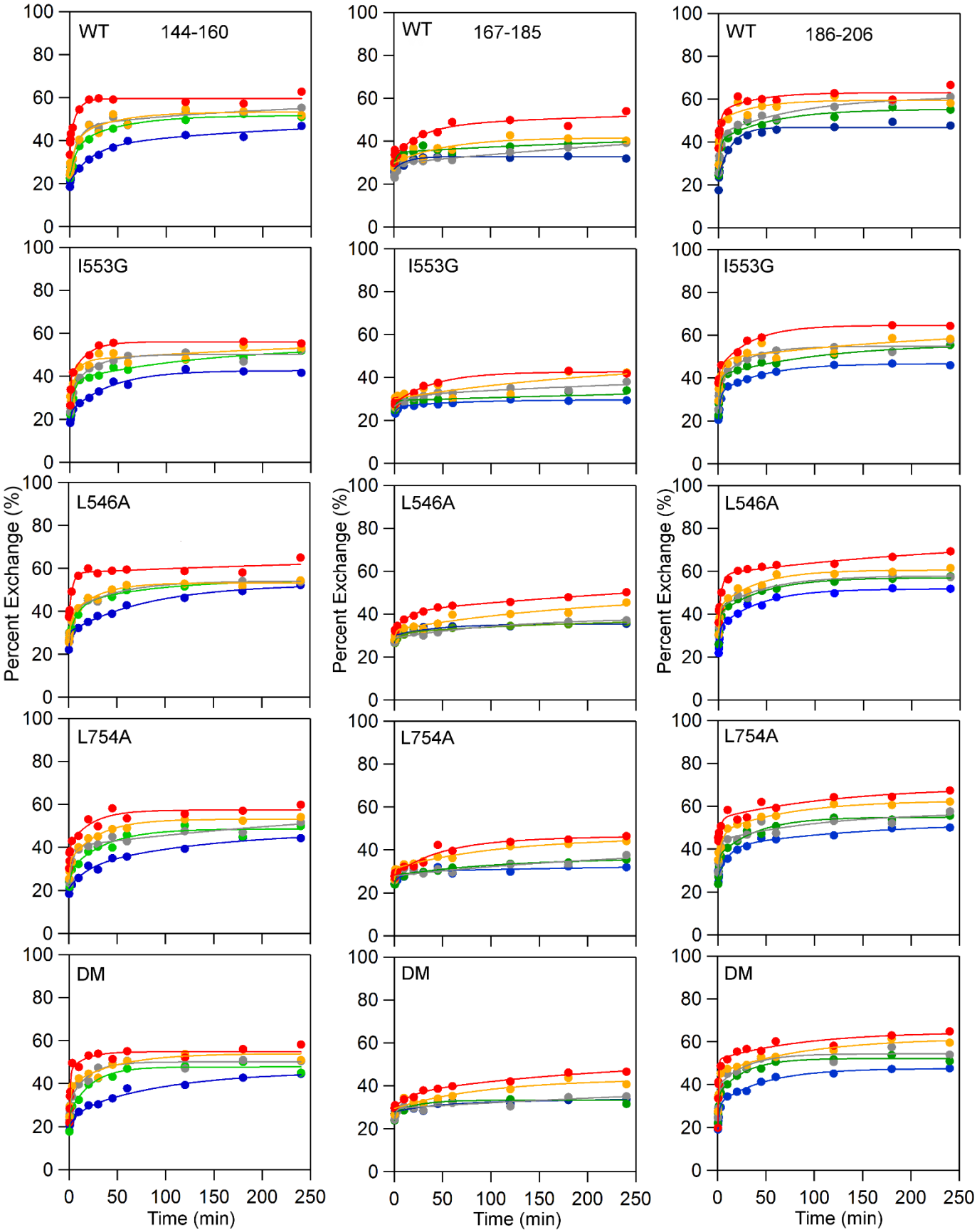
	WT	I553G	L546A	L754A	DM
144-160	10.9 (1.8)	12.4 (1.0)	14.2 (2.3)	8.1 (2.9)	13.2 (3)
167-185	-7.6 (4.5)	-3.5 (3.2)	-3.6 (5.2)	-7.4 (7.9)	-3.2 (5.8)
186-206	3.1 (2.3)	8.8 (3.5)	5.0 (5.7)	6.4 (3.3)	13.7 (2.2)
212-238*	15.1 (3.2)	N.C.	15.3 (2.5)	18.0 (3.2)	N.C.
239-256	N.D.	N.D.	N.D.	N.D.	N.D.
257-273	N.D.	N.D.	N.D.	N.D.	N.D.
274-283	N.D.	N.D.	N.D.	N.D.	N.D.
284-296	16.2 (0.8)	15.8 (7.9)	N.C.	N.C.	20.2 (3.4)
284-299	11.7 (2.2)	3.9 (1.2)	14.3 (2.4)	14.9 (2.3)	12.8 (2.1)
297-305	14.0 (4.2)	12.5 (1.2)	12.1 (1.9)	14.0 (3.3)	17.5 (1.5)
306-316	8.9 (4.4)	7.1 (0.6)	9.0 (2.3)	11.4 (3.4)	12.7 (2.1)
316-334	1.2 (3.1) 6.7 (3.1)*	1.5 (1.6)	14.2 (3.3)	3.1 (0.3)	7.7 (4.1) 12.9 (5.3)*
316-338	7.6 (1.3)	6.9 (2.0)	13.1 (2.6)	6.7 (0.6)	9.6 (2.3)
317-334	4.8 (0.8)	0.7 (1.2)	13.9 (2.5)	5.2 (0.6)	13.0 (2.3)
347-355	N.D.	N.D.	N.D.	N.D.	N.C.
362-388	16.6 (3.3)	N.C.	18.5 (1.9)	17.3 (3.2)	20.7 (1.8)
407-413	15.8 (2.2)	13.3 (2.3)	16.2 (2.8)	16.8 (2.6)	18.7 (1.6)
414-423	2.7 (2.3)	7.3 (1.7)	-1.8 (1.3)	-1.7 (3.9)	2.0 (0.9)
424-435	N.D.	N.D.	N.D.	N.D.	N.D.
436-449	N.D.	N.D.	N.D.	N.D.	N.D.
450-462	N.D.	N.D.	N.D.	N.D.	N.D.
466-477	N.D.	N.D.	N.D.	N.D.	N.D.
493-503	N.D.	N.D.	N.D.	N.D.	N.D.
504-521	N.D.	N.D.	N.D.	N.D.	N.D.
522-540	13.2 (1.7)	10.3 (0.5)	8.4 (1.8)	13 (1.9)	6.8 (0.8)
541-554	23.1 (1.9)	14.2 (2.6)	18.2 (1.2)	17.4 (1.9)	15.6 (1.6)
555-565	18.5 (3.2)	15.4 (1.9)	10.6 (1.6)	13.3 (1.8)	15.0 (5.6)
566-576	18.0 (1.3)	10.2 (0.9)	14.1 (1.0)	13.4 (0.3)	12.5 (0.8)
586-603	12.1 (0.9)	9.1 (2.4)	5.6 (4.1)	10.3 (3.8)	N.C.
604-617	7.5 (2.6)	16.1 (2.1)	14.1 (5.6)	8.5 (3.6)	N.C.
619-632	N.D.	N.C.	N.C.	N.C.	N.D.
633-648	6.6 (1.5)	7.7 (1.7)	8.3 (0.7)	8.3 (1.3)	6.0 (1.6)
649-669	14.8 (0.7)	12.9 (1.3)	17.6 (1.5)	15.9 (1.9)	N.C.
690-703	N.D.	N.D.	N.D.	N.D.	N.D.
704-726	0.8 (1.8)	0.4 (2.0)	4.4 (3.6)	N.C.	1.9 (3.0)
727-737*	17.2 (1.0)	15.1 (0.5)	16.1 (0.2)	17.5 (0.6)	16.7 (1.3)
738-745	20.1 (1.2)	15.2 (1.6)	19.3 (1.1)	18.9 (1.7)	20.6 (1.5)
751-761**	7.3 (2.3)	4.1 (5.7)	22.7 (6.1)	23.2 (12.2)	26.1 (5.4)
762-781	15.1 (1.2)	15.5 (0.4)	17.7 (1.4)	17.5 (1.8)	15.0 (1.2)
782-796*	15.4 (1.1)	10.4 (2.5)	15.2 (2.8)	16.6 (0.6)	9.5 (1.4)
797-820*	1.6 (2.0)	1.2 (3.8)	-5.1 (16.2)	3.3 (2.5)	-1.6 (6.8)
821-836	2.0 (2.9)	2.5 (3.8)	0.2 (3.2)	-3.9 (2.7)	-3.7 (1.9)

*Only fit 4 of 5 data points. ** Only fit 3 of 5 data points.

Table S4: Data collection and refinement statistics

	L546A	L754A	DM
PDB ID:	5TQN	5TR0	5TQO
Wavelength (Å)	0.826556	0.826545	0.826561
Temperature (K)	293	293	300
Space group	P1 2_1 1	P1 2_1 1	P1 2_1 1
Cell parameters			
a b c (Å)	91.52 92.66	91.45 92.65 100.72	91.61 92.81 101.27
α β γ (°)	100.75 90.00 93.47 90.00	90.00 93.39 90.00	90 94.11 90
Copies per a.s.u	2	2	2
Resolution (Å)	45.68-1.80 (1.83-1.80)	45.65-1.85 (1.88-1.85)	68.34-1.70 (1.79-1.70)
Rmerge	0.068 (0.832)	0.099 (0.812)	0.096 (0.687)
I/σI	11.1 (1.5)	13.3 (1.7)	7.3 (1.6)
Completeness (%)	99.8 (99.9)	99.7 (99.7)	99.13 (98.66)
Redundancy	3.5 (3.6)	2.9 (2.9)	3.7 (3.7)
Refinement			
Resolution (Å)	45.68-1.80 (1.82-1.80)	45.65-1.85 (1.87-1.85)	68.341-1.7 (1.742-1.7)
Number of reflections			
Total	154938 (5099)	142544 (4605)	183987 (13034)
Test	7863 (253)	7248 (249)	2020 (129)
R_{work}/R_{free} (%)	13.69/16.21 (29.44/35.19)	14.30/17.20 (26.79/33.46)	14.20/16.49 (25.71/29.03)
Number of non H atoms	21360	21587	21492
Protein	20634	20886	20591
Ligand	2	2	2
Solvent	724	699	899
Wilson B-factor	22.70	22.22	17.92
Average B-factor	27.07	27.26	22.89
Protein	26.90	27.11	22.50
Ligand	27.12	30.66	14.7
Solvent	31.99	31.57	31.83
Rmsd			
Rmsd bond lengths	0.011	0.011	0.006
Rmsd bond angles	1.021	1.045	0.769
Ramachandran plot			
Favored (%)	95.69	95.84	96.01
Allowed (%)	4.27	4.16	3.75
Outliers (%)	0.04	0	0.24

Statistics for the highest-resolution shell are shown in parentheses.


WT 144-160
WT 167-185
WT 186-206
I553G
L546A
L754A
DM
Percent Exchange (%)
Time (min)
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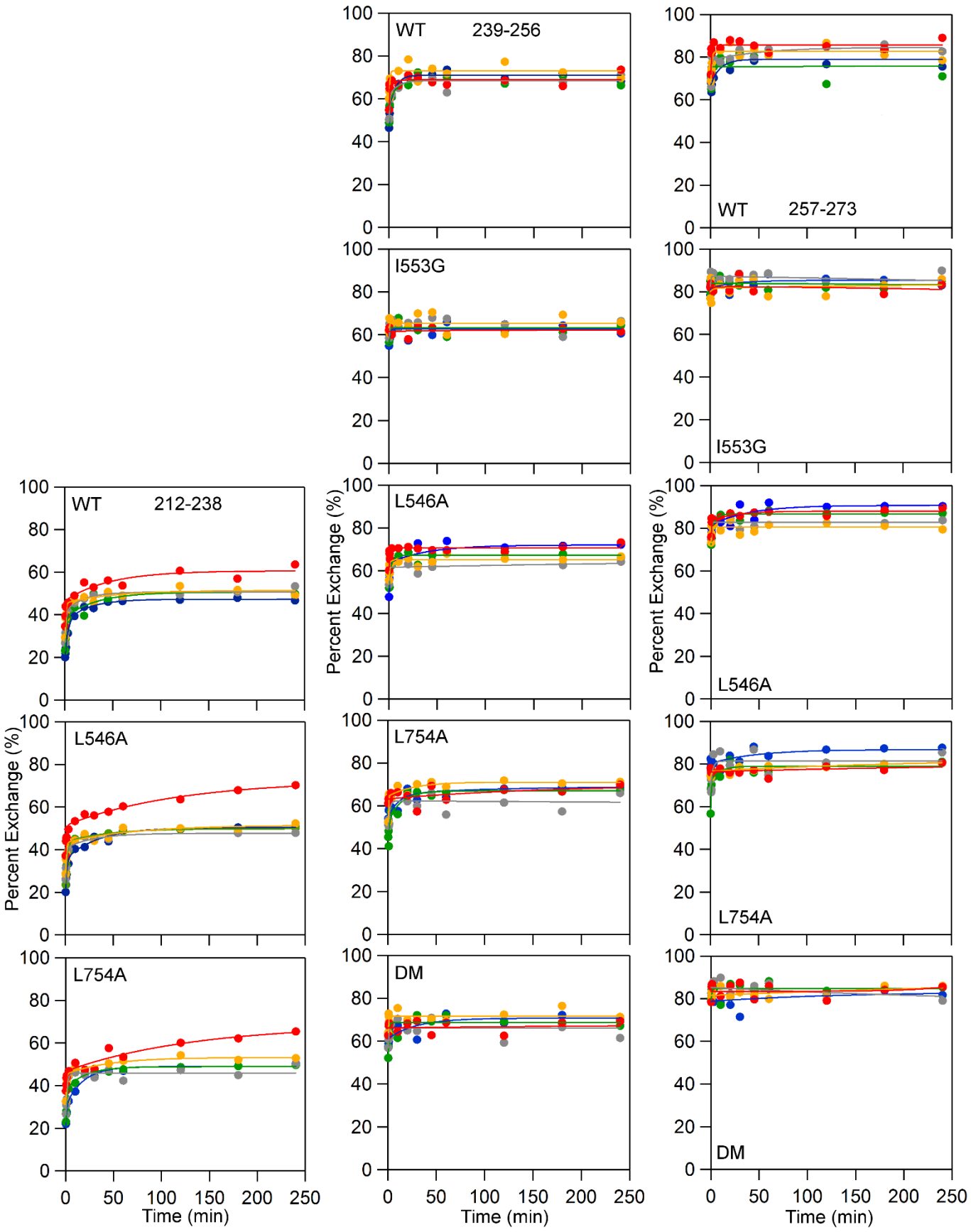
20
40
60
80
100
0
50
100
150
200
250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WT 239-256
WT 257-273
I553G
L546A
L754A
DM
Percent Exchange (%)
Time (min)
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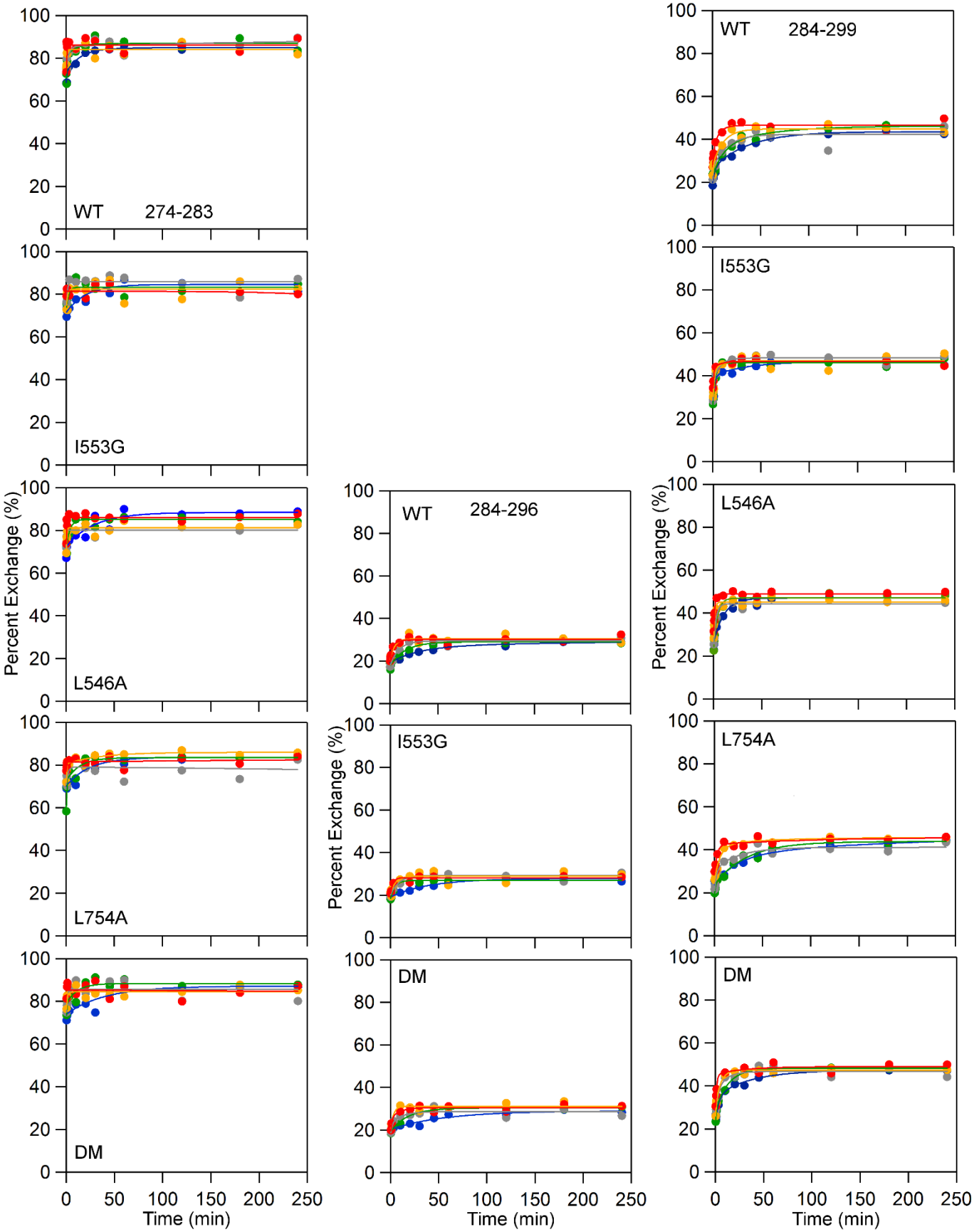
40
60
80
100
0
50
100
150
200
250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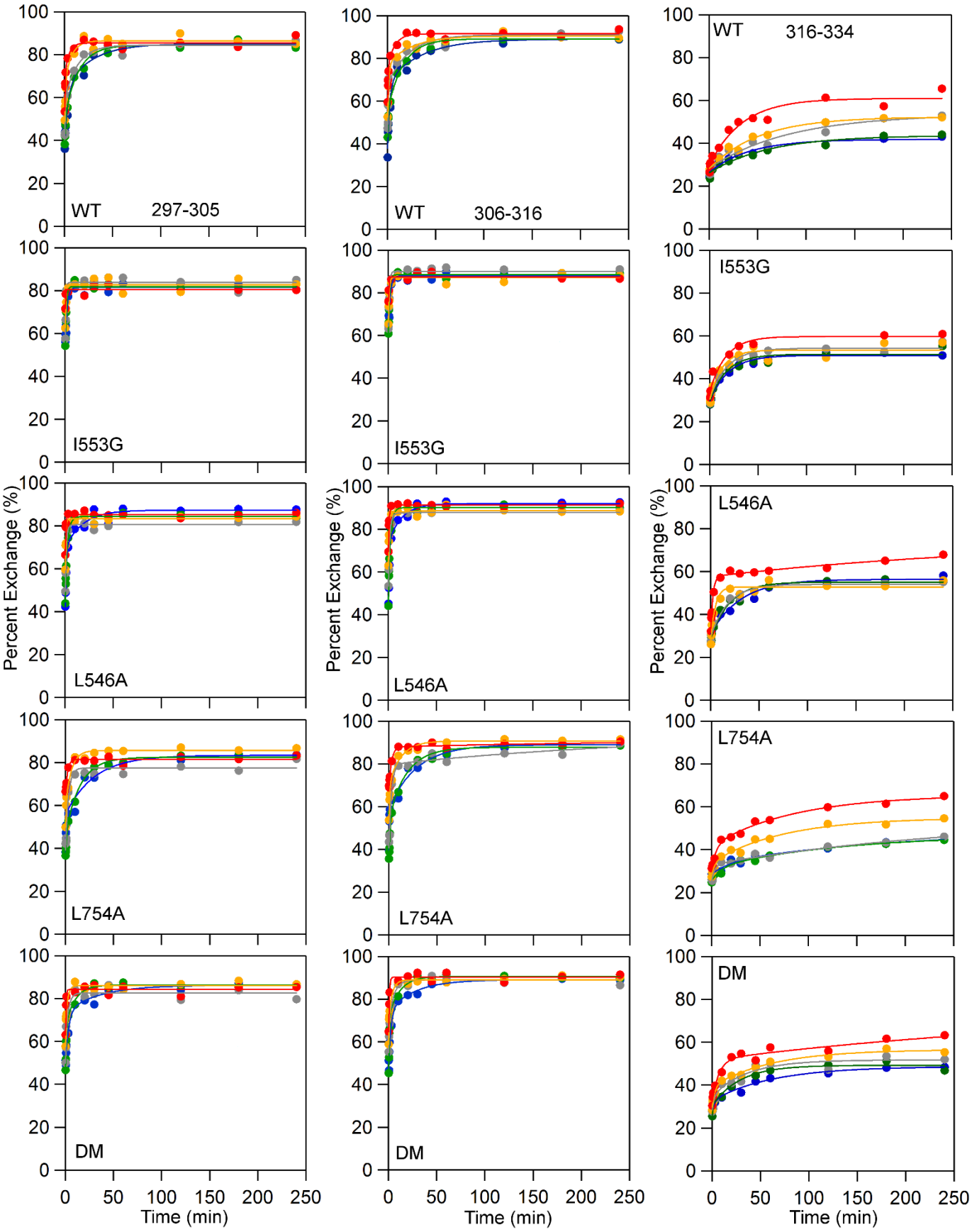
I553G
L546A
L754A
DM
WT 284-299
I553G
L546A
L754A
DM
WT 284-296
I553G
DM
Percent Exchange (%)
Time (min)
0
20
40
60
80
100
0
50
100
150
200
250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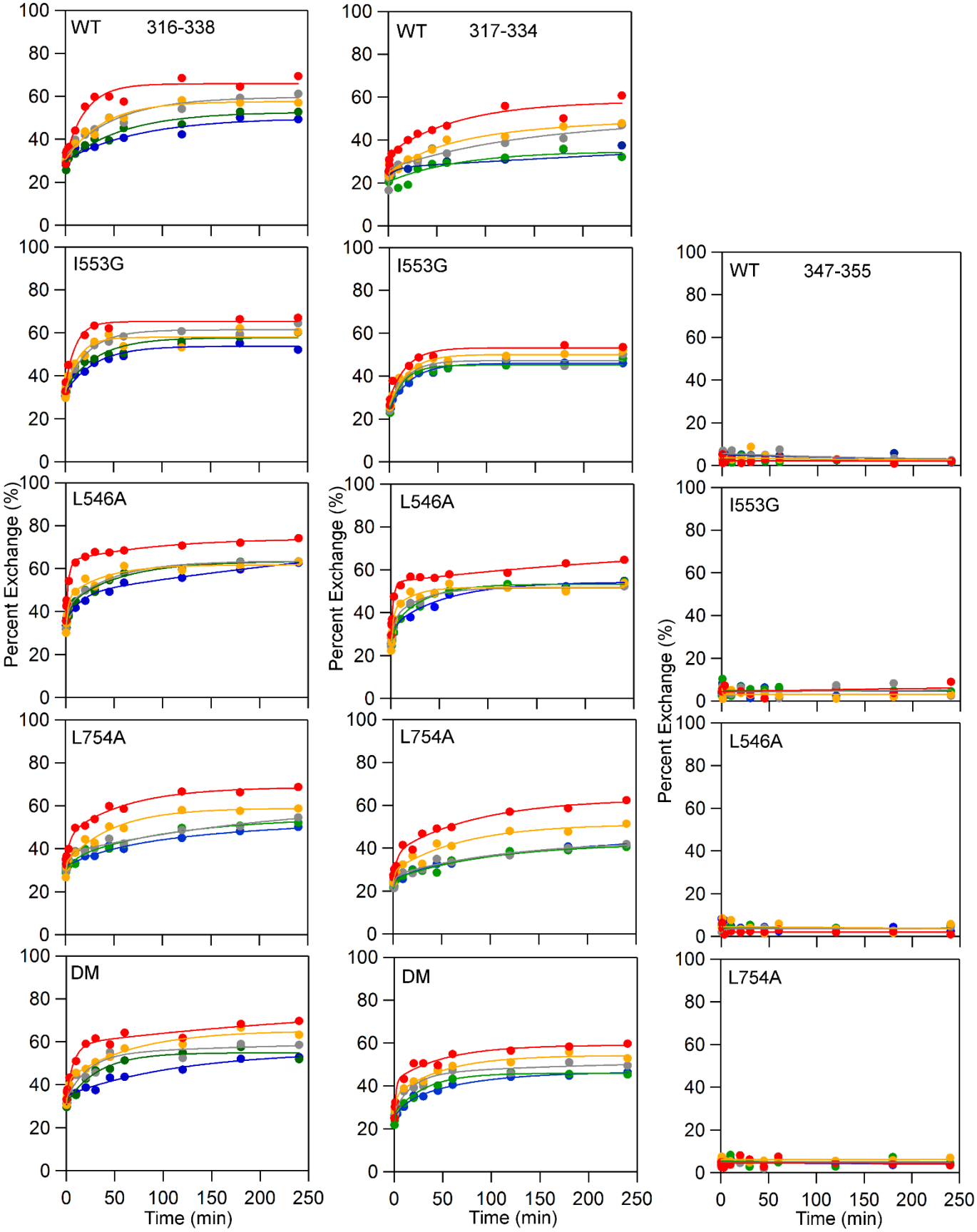
WT 306-316
WT 316-334
I553G
I553G
I553G
L546A
L546A
L546A
L754A
L754A
L754A
DM
DM
DM
Percent Exchange (%)
Time (min)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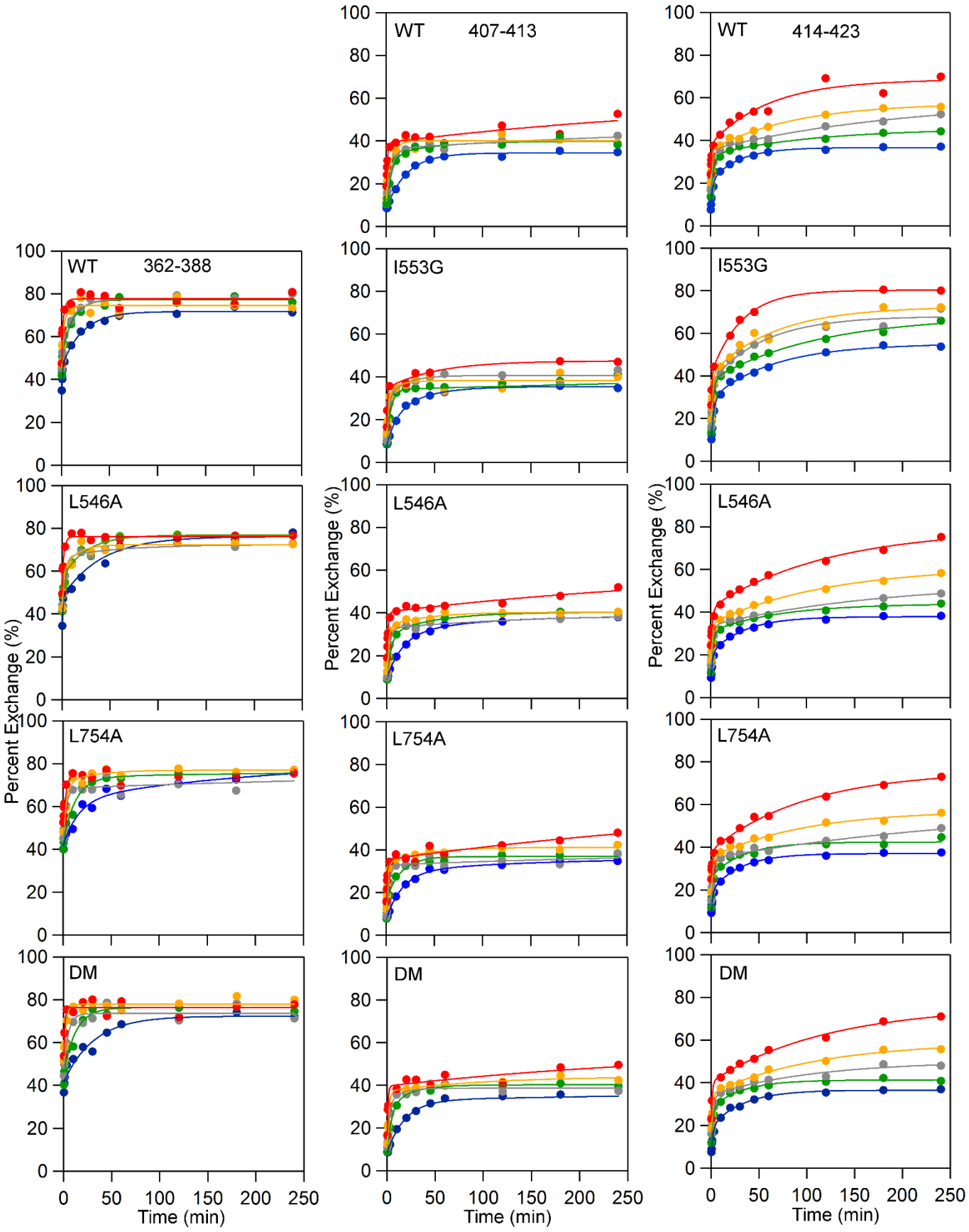
WT 317-334
WT 347-355
I553G
I553G
I553G
L546A
L546A
L546A
L754A
L754A
L754A
DM
DM
Percent Exchange (%)
Percent Exchange (%)
Percent Exchange (%)
Time (min)
Time (min)
Time (min)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I553G
I553G
L546A
L546A
L546A
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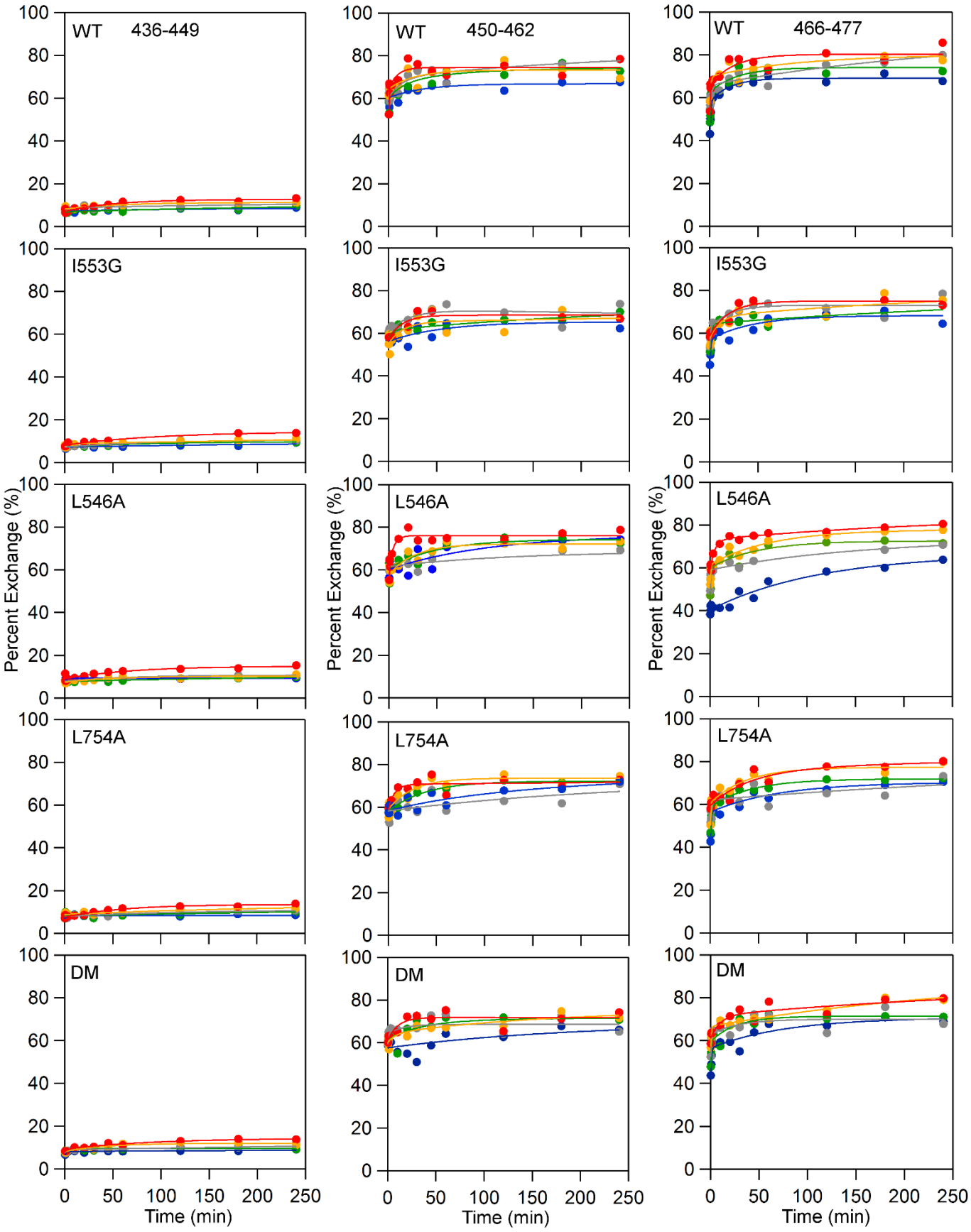
L754A
L754A
DM
DM
DM
Percent Exchange (%)
Percent Exchange (%)
Time (min)
Time (min)
Time (min)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WT 450-462
WT 466-477
I553G
L546A
L754A
DM
Percent Exchange (%)
Time (min)
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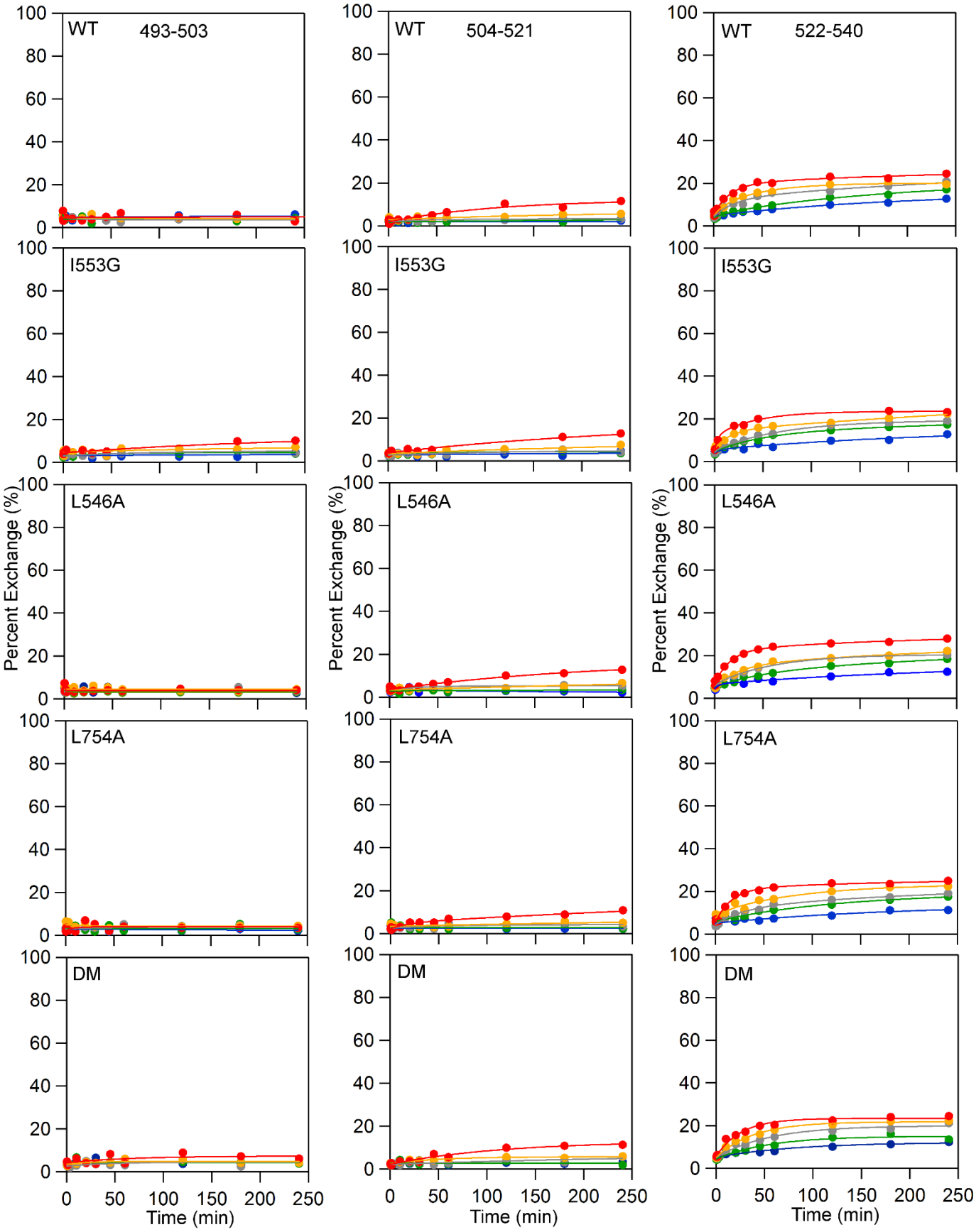
60
80
100
0
50
100
150
200
250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WT 504-521
WT 522-540
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L546A
L754A
DM
Percent Exchange (%)
Time (min)
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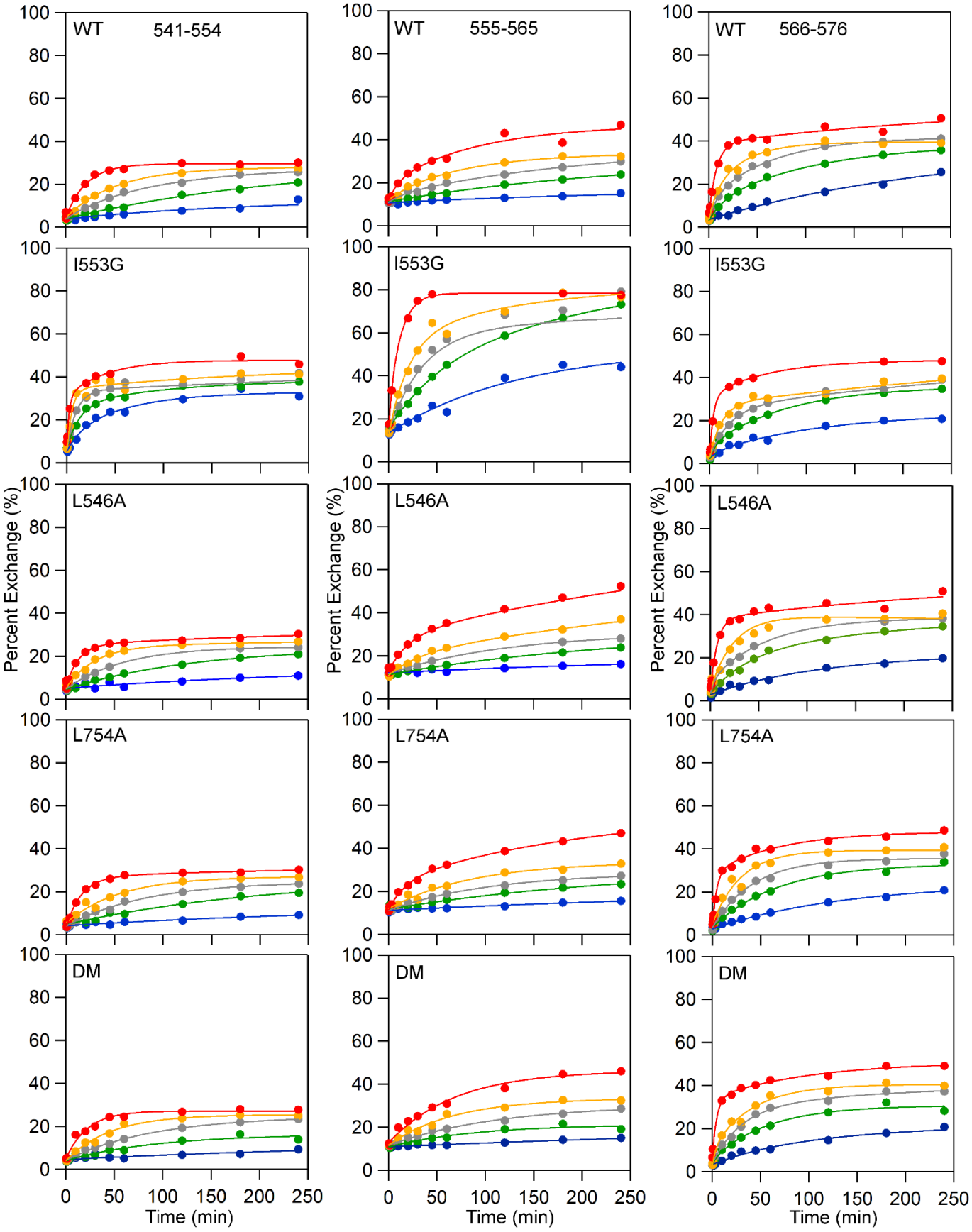
100
150
200
250
0
20
40
60
80
100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WT 555-565
WT 566-576
I553G
L546A
L754A
DM
Percent Exchange (%)
Time (min)
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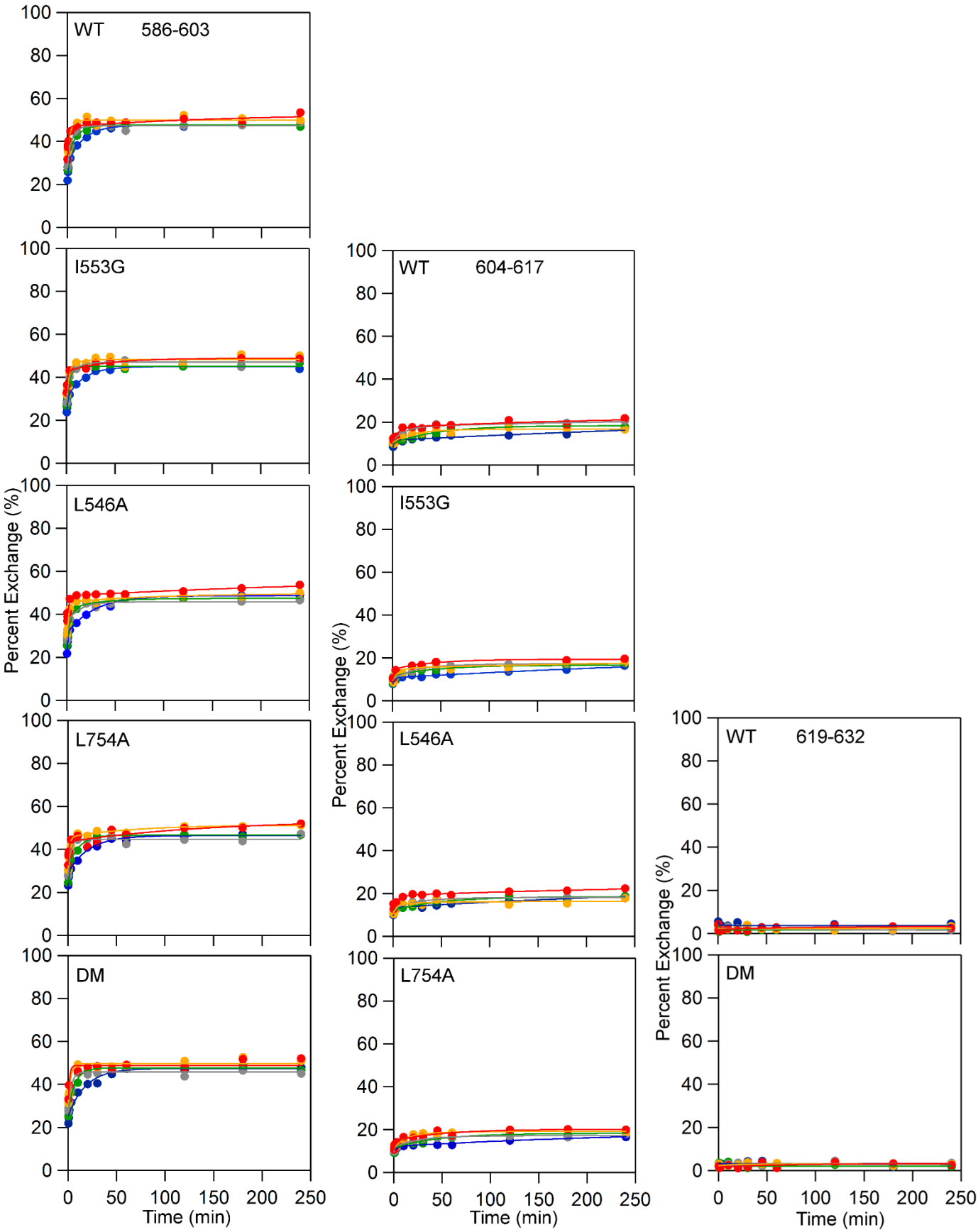
60
80
100
0
50
100
150
200
250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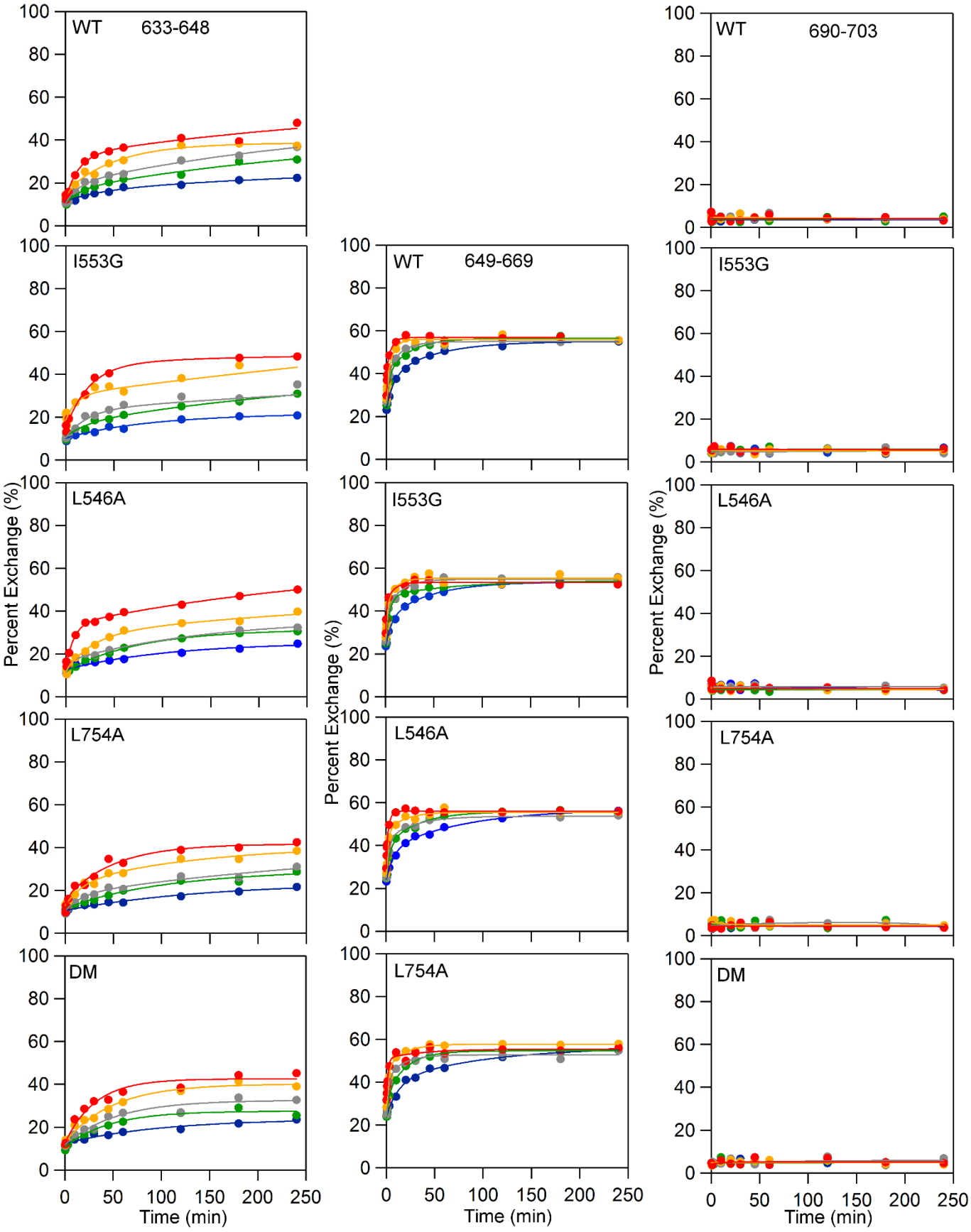
I553G
L546A
L754A
DM
WT 604-617
I553G
L546A
L754A
WT 619-632
DM
Percent Exchange (%)
Time (min)
0
20
40
60
80
100
0
50
100
150
200
250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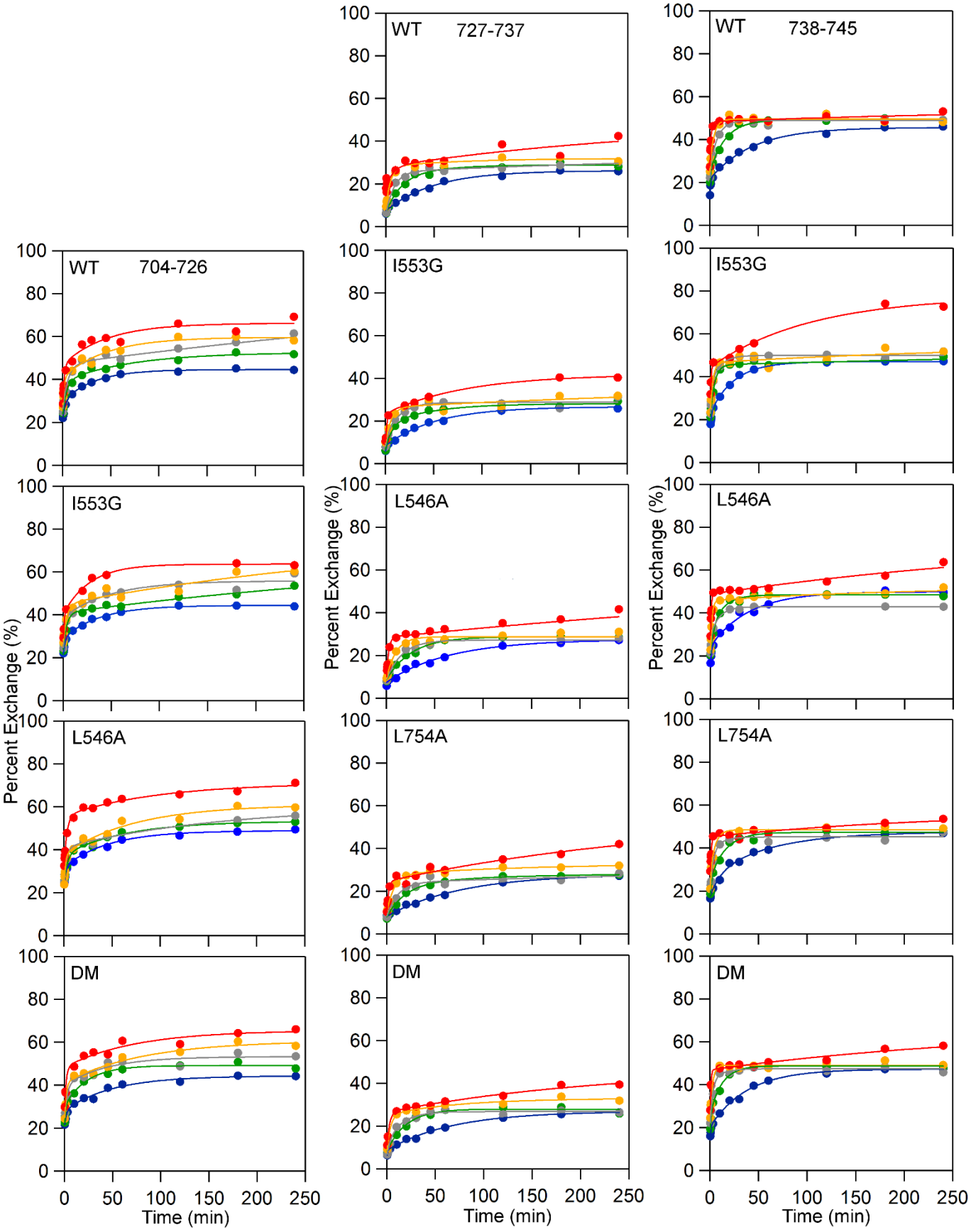
I553G
L546A
L754A
DM
WT 649-669
I553G
L546A
L754A
WT 690-703
I553G
L546A
L754A
DM
Percent Exchange (%)
Time (min)
0
20
40
60
80
100
0
50
100
150
200
250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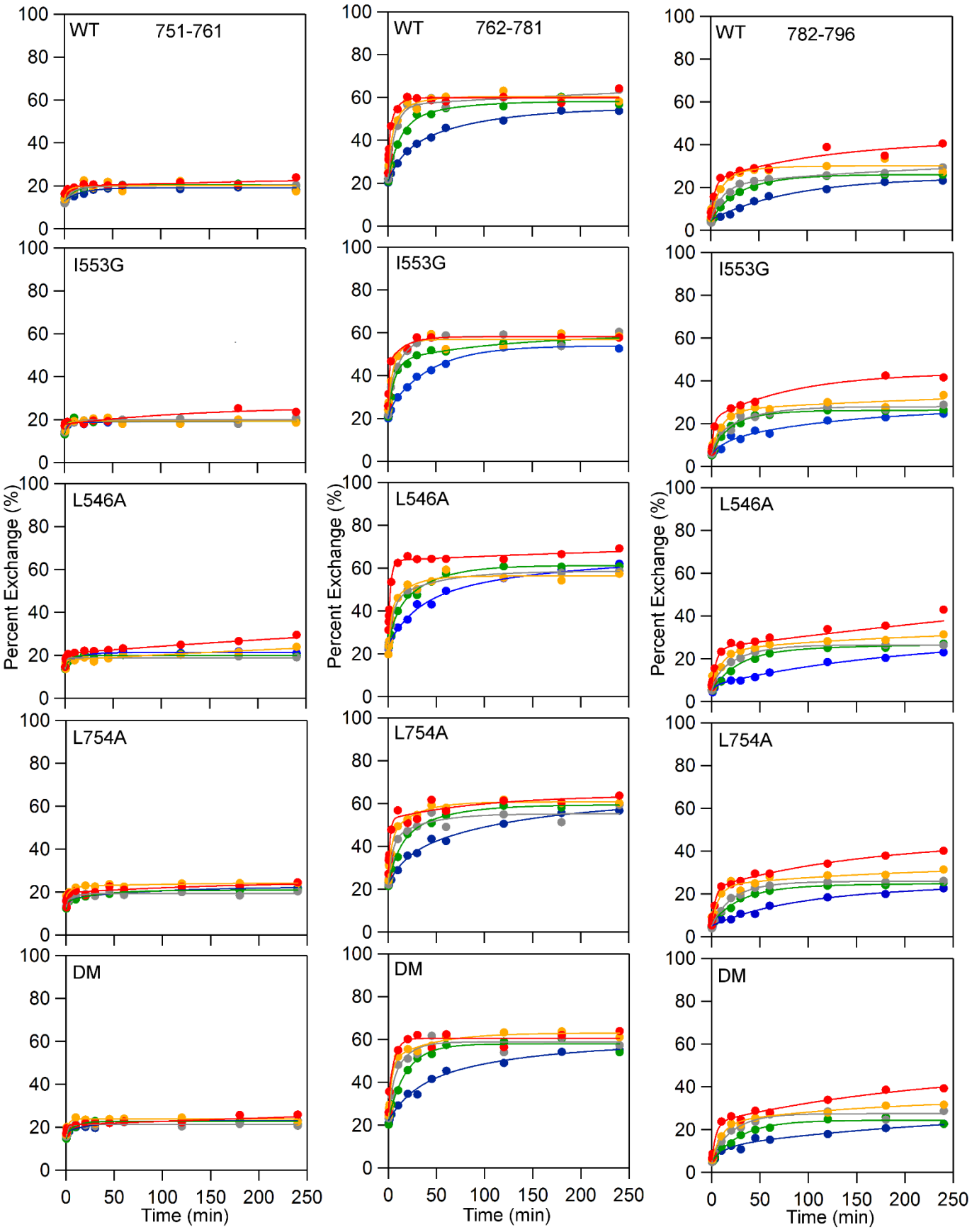
WT 727-737
WT 738-745
I553G
I553G
I553G
L546A
L546A
L546A
L754A
L754A
L754A
DM
DM
DM
Percent Exchange (%)
Percent Exchange (%)
Percent Exchange (%)
Time (min)
Time (min)
Time (min)
0
20
40
60
80
100
0
50
100
150
200
250


WT 751-761
WT 762-781
WT 782-796
I553G
L546A
L754A
DM
Percent Exchange (%)
Time (min)
0
20
40
60
80
100
0
50
100
150
200
250

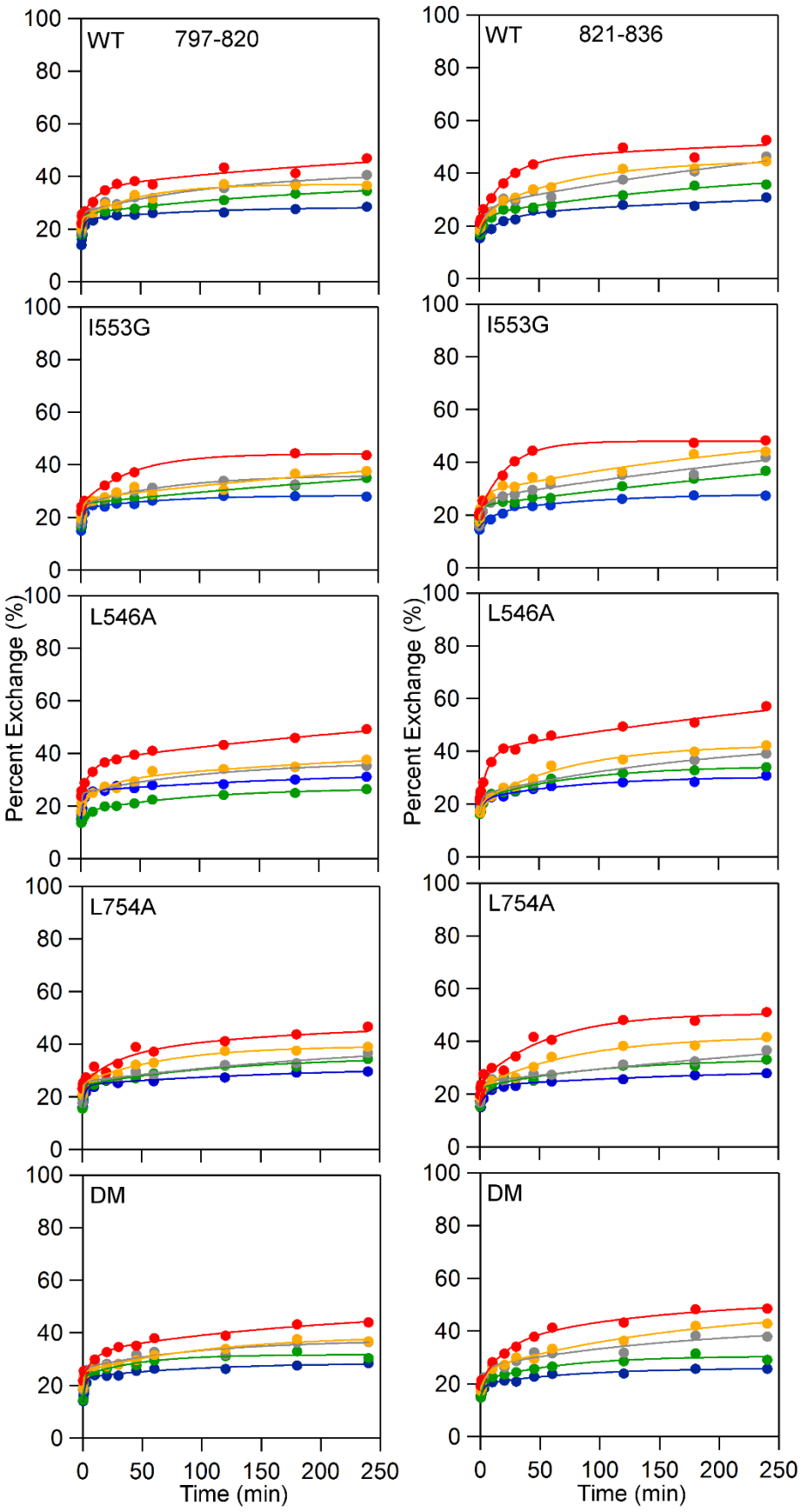


Figure S1: Compilation of the complete sets of experimental HDXMS traces for WT, I553G, L546A, L754A and DM.

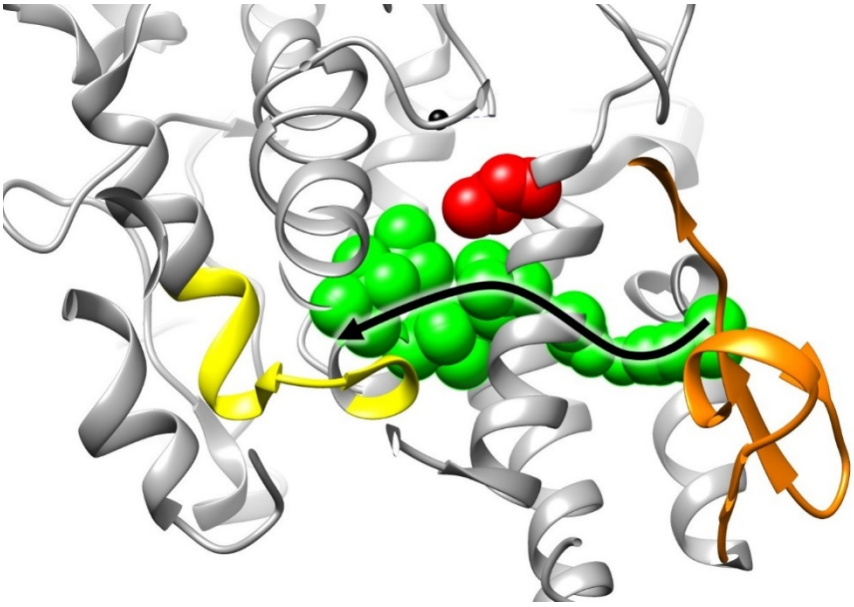


Figure S2: Previously identified network connecting the solvent-exposed loop and active site.[6] The active site and surface loop are color coded as yellow (peptide 555-565) and orange (peptide 284-299, 317-334), respectively. The residues Y317, S749, V750, L546 and I553, colored in green and presented in the space-filling mode, have been assigned to the connectivity for the thermal activation from solvent interface to active site. The L754 (colored in red) is outside the network.[6]

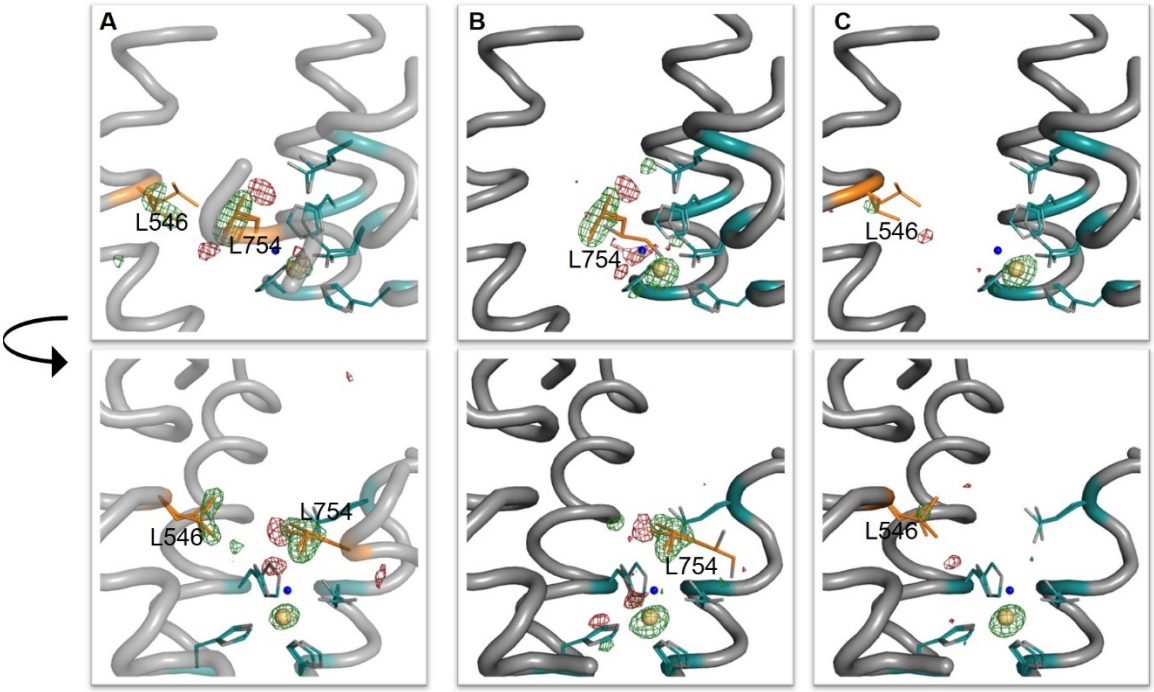


Figure S3: Isomorphous difference map for room temperature structures (A) WT – DM; (B) WT – L754A; (C) WT – L546A Shown in red (negative) and green (positive) is the WT – mutant isomorphous difference map contoured at 0.33 $e^-/Å^3$. Ligands to the iron (gold sphere) are in teal, and residues 546 and 754 are in orange. The map shows slightly higher iron occupancy in WT. Two views for each mutant are shown separately in the upper and lower panels.

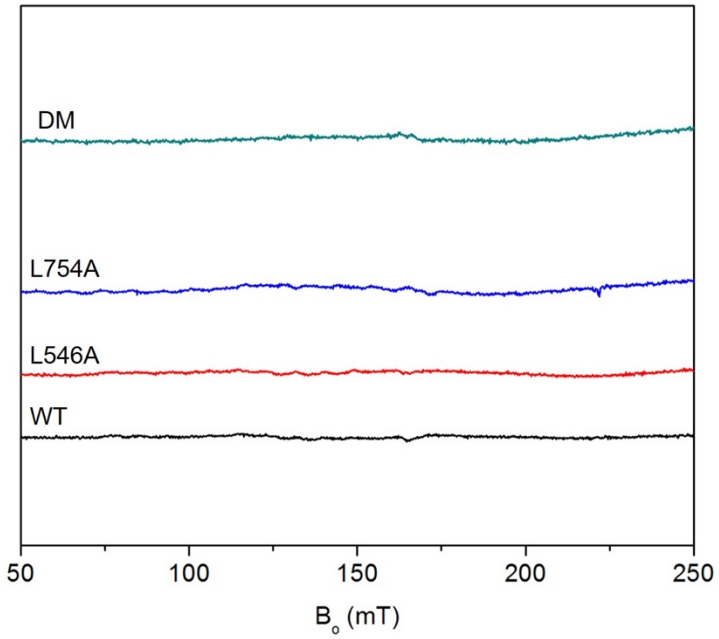


Figure S4: EPR spectra of resting (reduced) WT, L546A, L754A and DM SLO-1 (140 μM) in 0.1 M potassium phosphate pH = 7.0 buffer. The peak intensities here and in other spectra of L546A, L754A and DM have been corrected for the iron content of each variant: WT (0.9), L546A (0.50), L754 (0.70), DM (0.70).

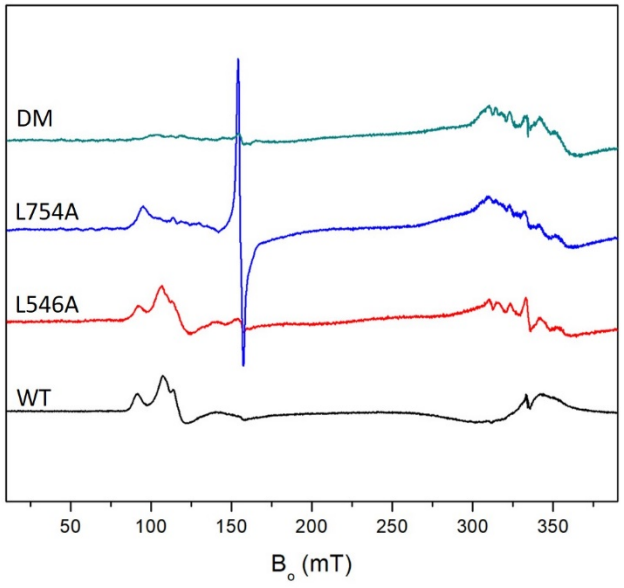


Figure S5: Expanded EPR spectra of oxidized WT, L546A, L754A and DM SLO-1, showing the g=2 region. The latter is generally attributed to varying levels of contaminating manganese ion in the protein preparations.

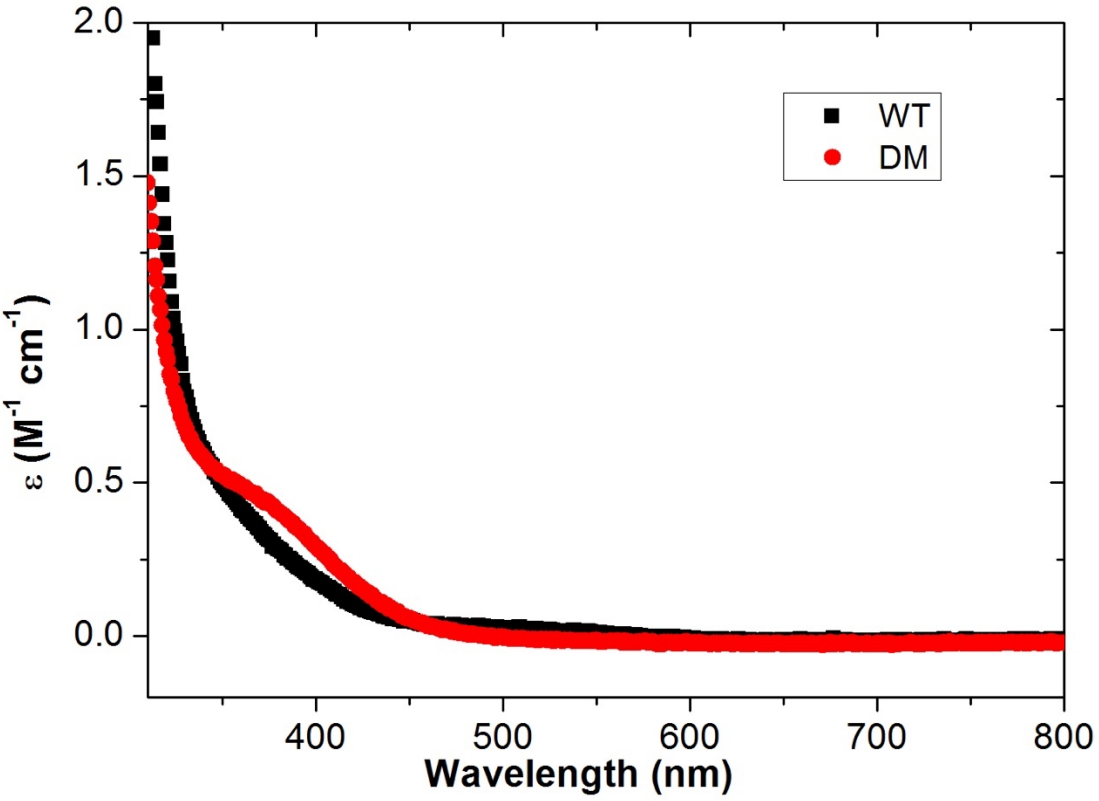


Figure S6: Room temperature absorption spectra of the WT and DM Fe(III) lipoxygenase. These traces represent the absorbance following reaction of Fe(II) SLO and linoleic acid, corrected for each enzyme sample Fe(II) SLO prior to addition of linoleic acid. The absorption spectrum for lineoleic acid is below 240nm. The absorption of DM is after concentration correction due to the reduced iron content (70%) relative to WT (90%).

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