The WT, N12D, and I71N time-course data were fit via regularized nonlinear least-squares to the first-order kinetic model

$$C \xrightarrow{k_1} N \xrightarrow{k_2} L$$

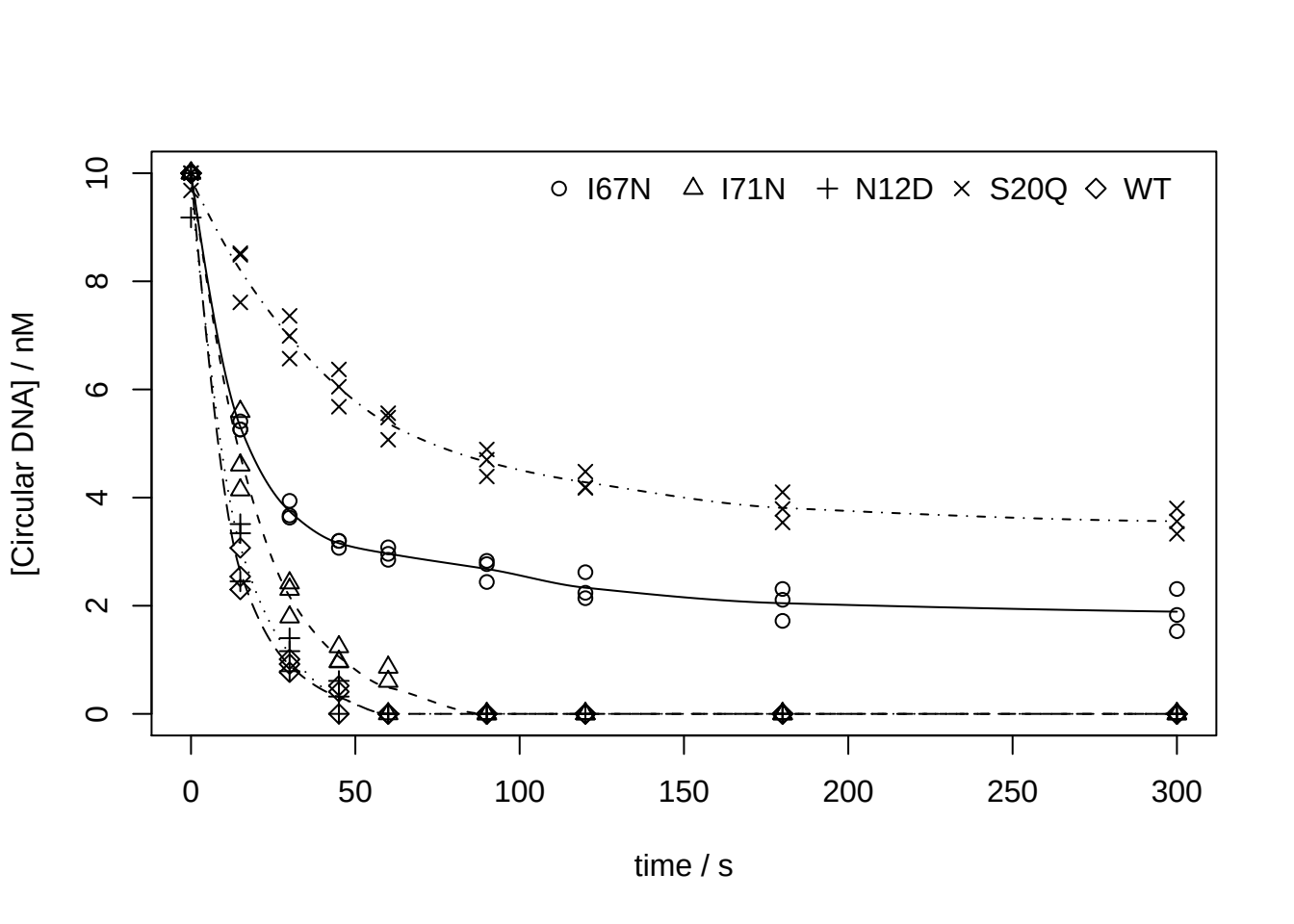
which describes the stepwise conversion of DNA from circular to nicked to linear form. Regression curves (Figure S4) are shown overlaid with data and empirical monotonic Hermite smoothing-spline interpolants. These figures show no inconsistency between the first order kinetic model and the observed data for WT, N12D and I71N proteins.

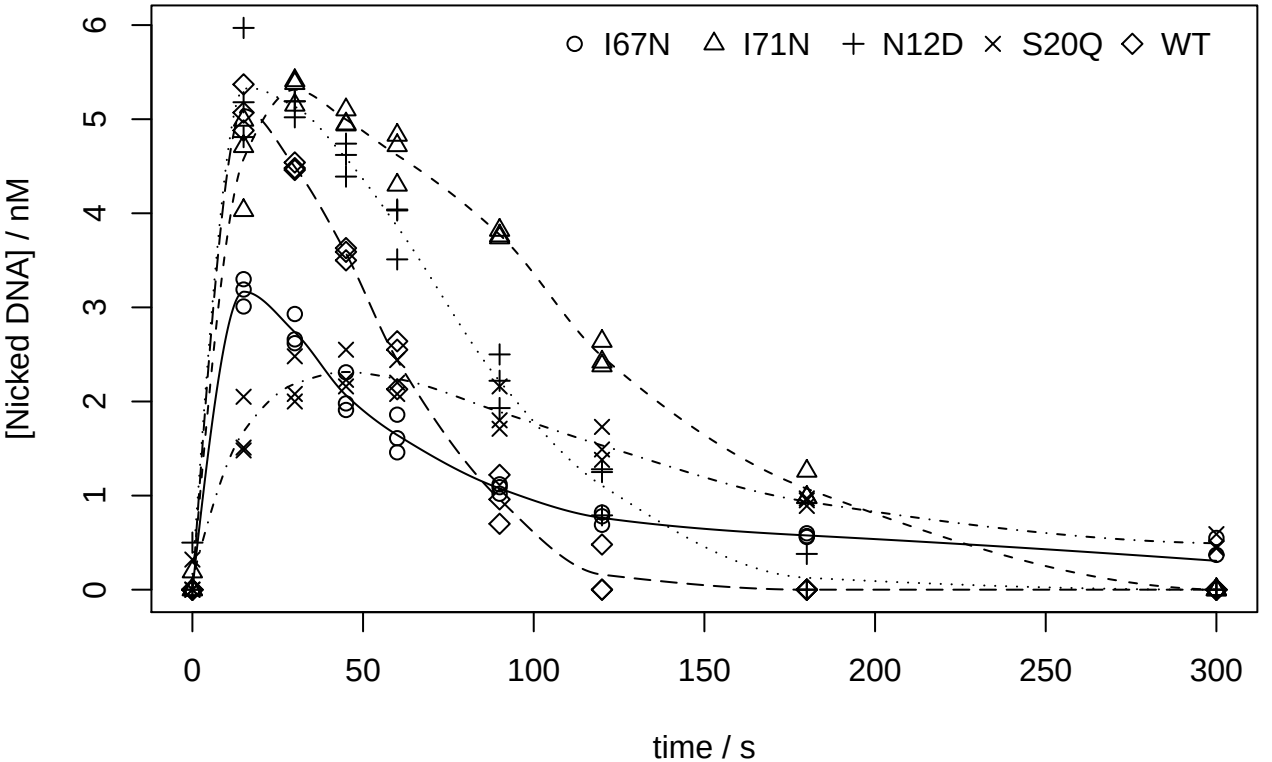
In contrast, the first-order model above cannot account for the behavior of the I67N or S20Q mutants. Instead, values of k_1 were estimated in the absence of nicked or linear forms. For S20Q, circular DNA concentrations were fit to the empirical formula

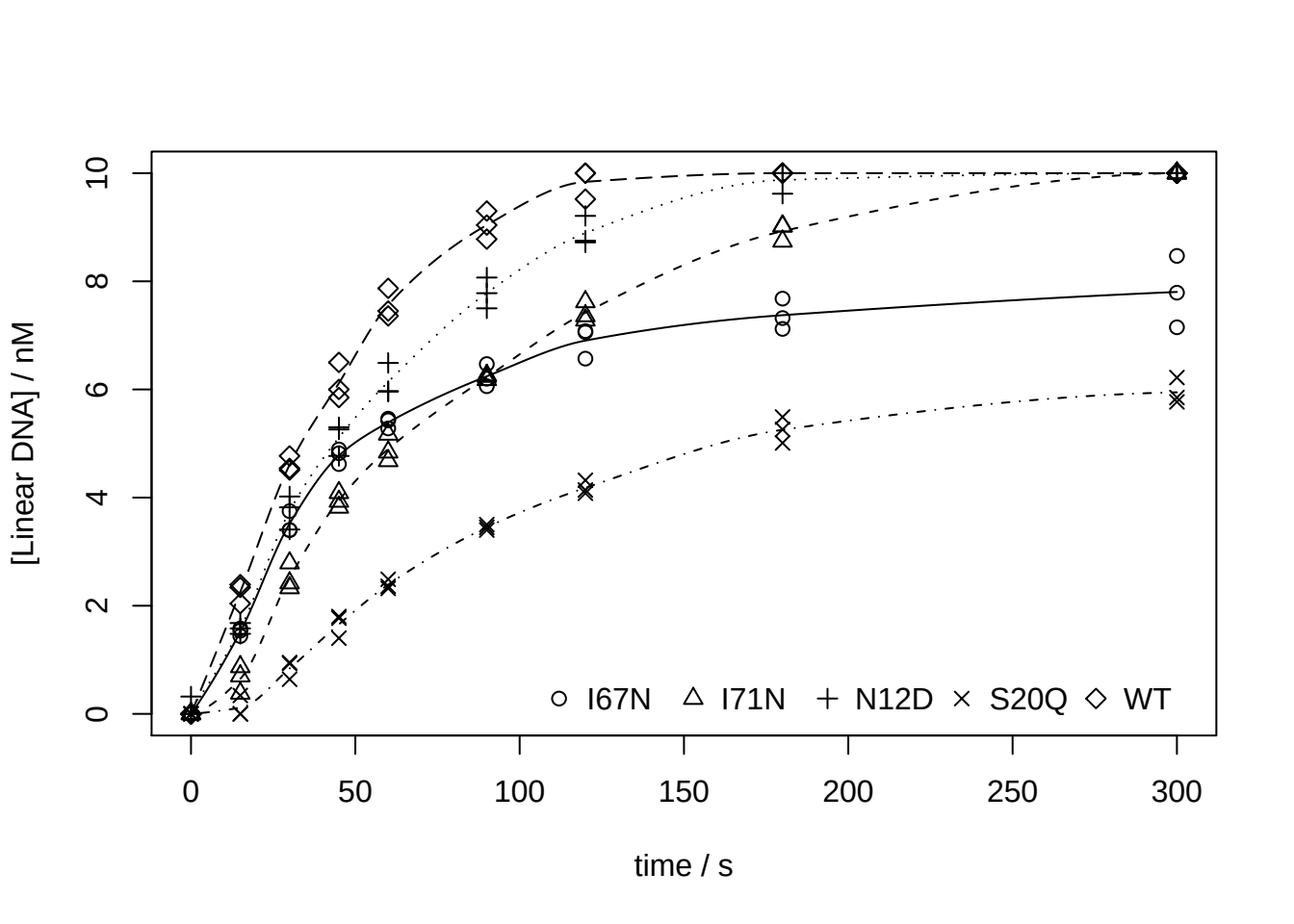
$$C = C_0 + \exp(-\kappa_1 t + \kappa_2) + \kappa_3$$

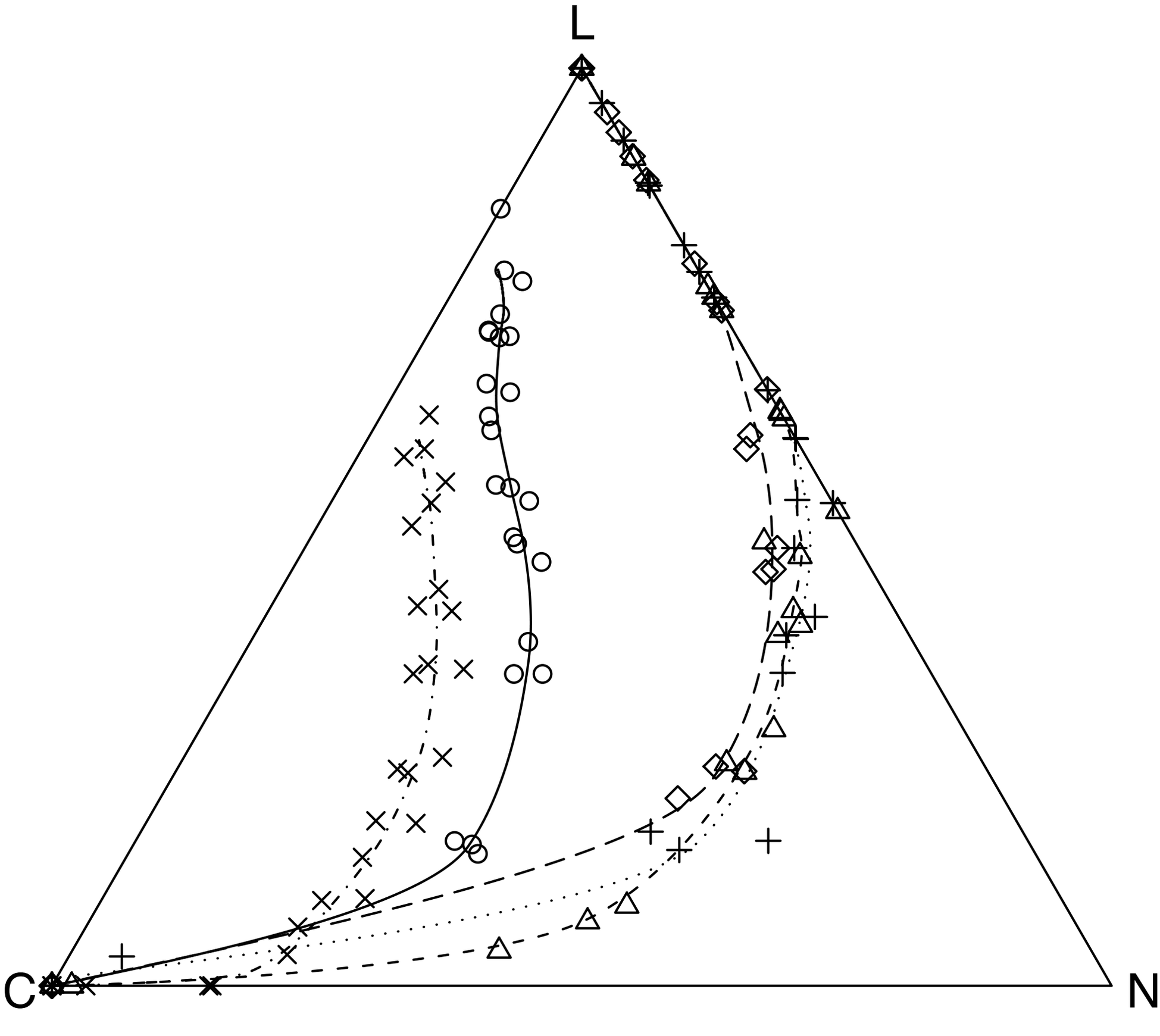
where the comparable initial reaction velocity is given as $k_1 = \kappa_1 e^{\kappa_2}$. This empirical model could not reproduce the dynamic behavior of the I67N mutant due to the rapid initial conversion of C to N. Instead, the initial reaction velocity was estimated by the slope of the smoothing spline at time zero. The empirical differences in reaction kinetics between the I67N and S20Q mutants suggest that each has distinct mechanistic changes compared to each other, not just WT.

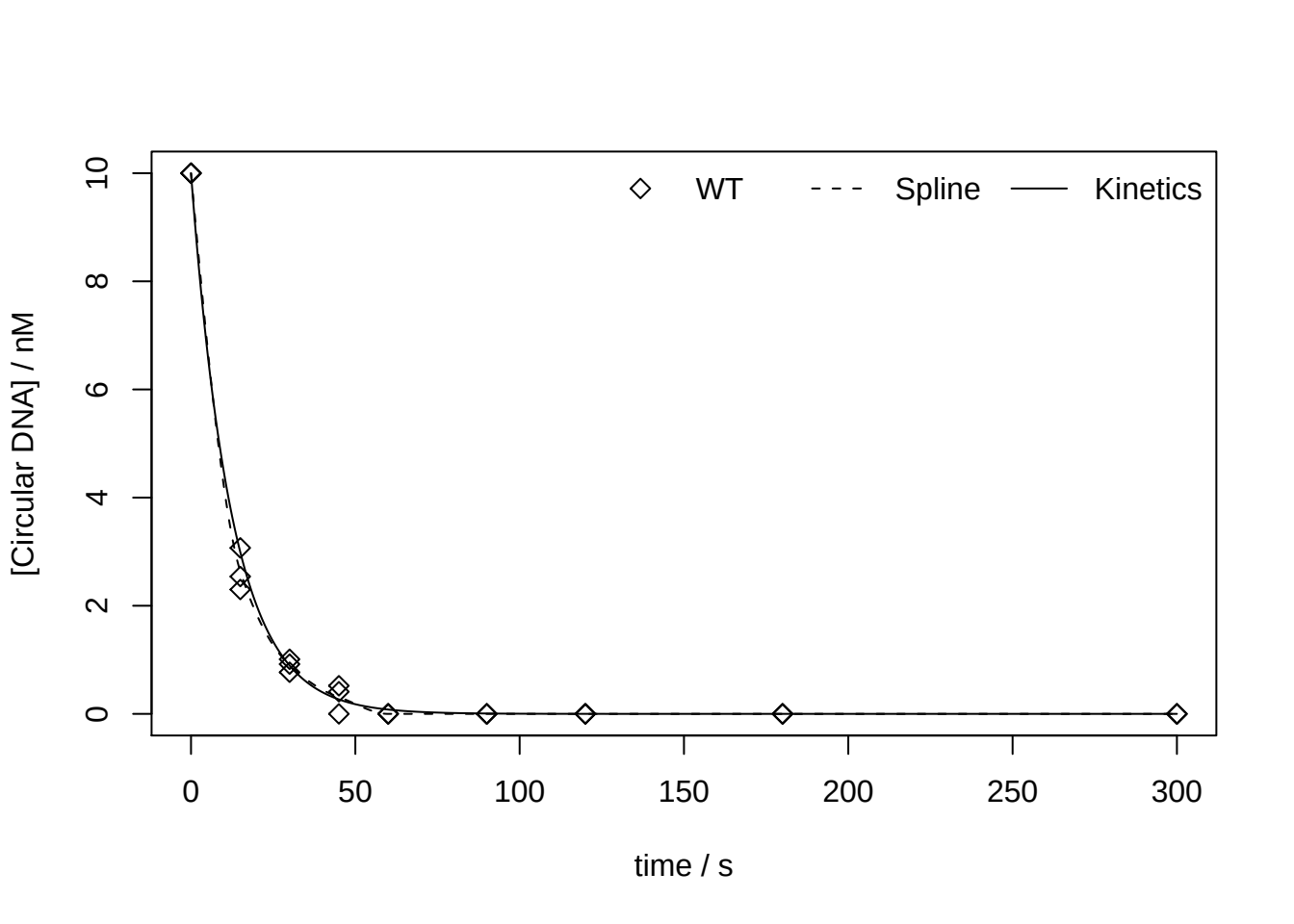
Since the total concentration of circular, nicked, and linear DNA is presumed constant, a ternary plot showing the relative proportion of each component is presented in Figure S4. Ternary-plot trajectories are displayed without regard to time, and the overall shapes of the reaction regression curves suggest mechanistic differences without regard to differences in reaction rate. Specifically, the plot suggests that the (WT, N12D, I71N), (I67N), and (S20Q) reaction curves are all mechanistically distinct since they group into three observably different classes.

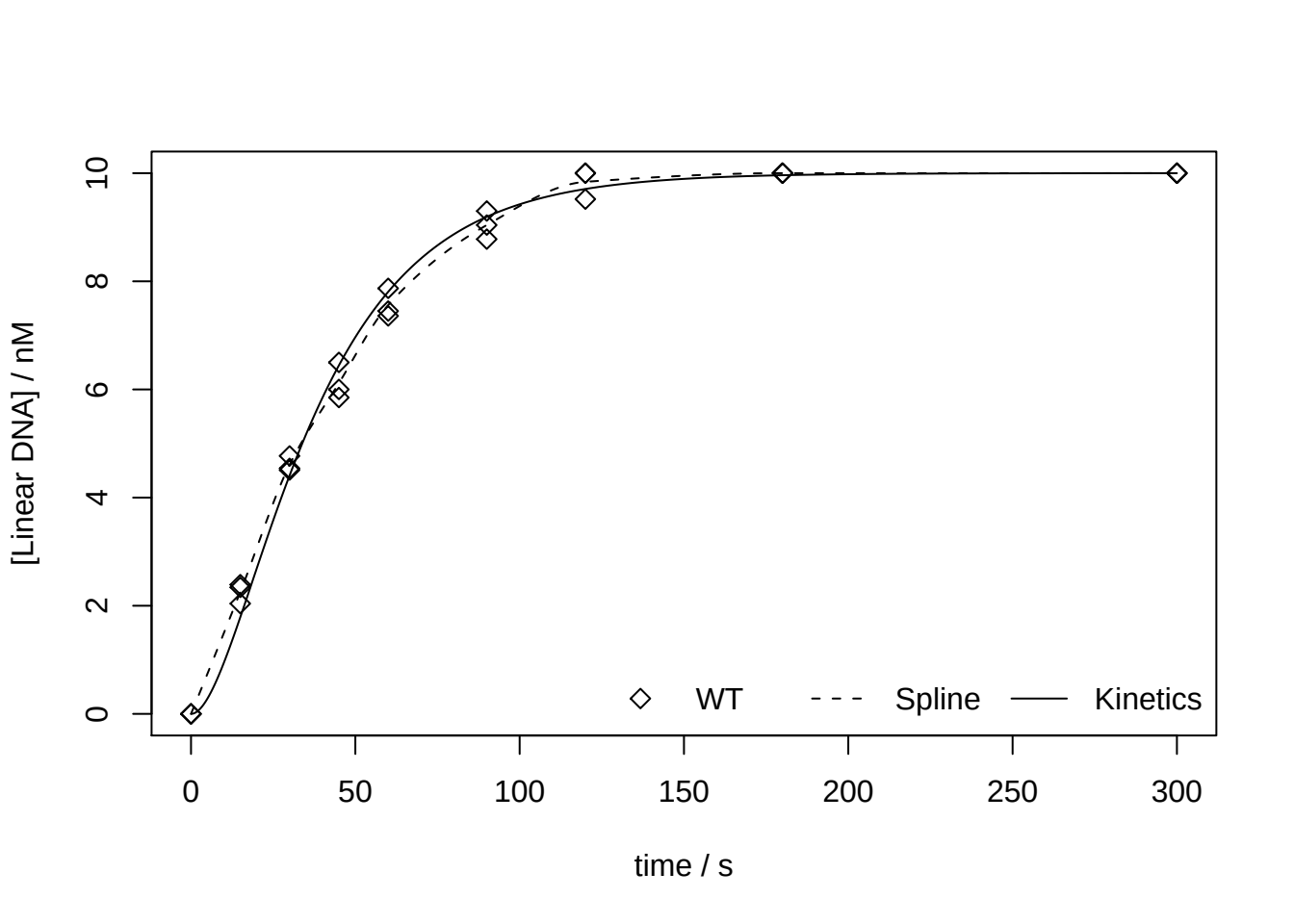

I67N
I71N
N12D
S20Q
WT
[Circular DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s

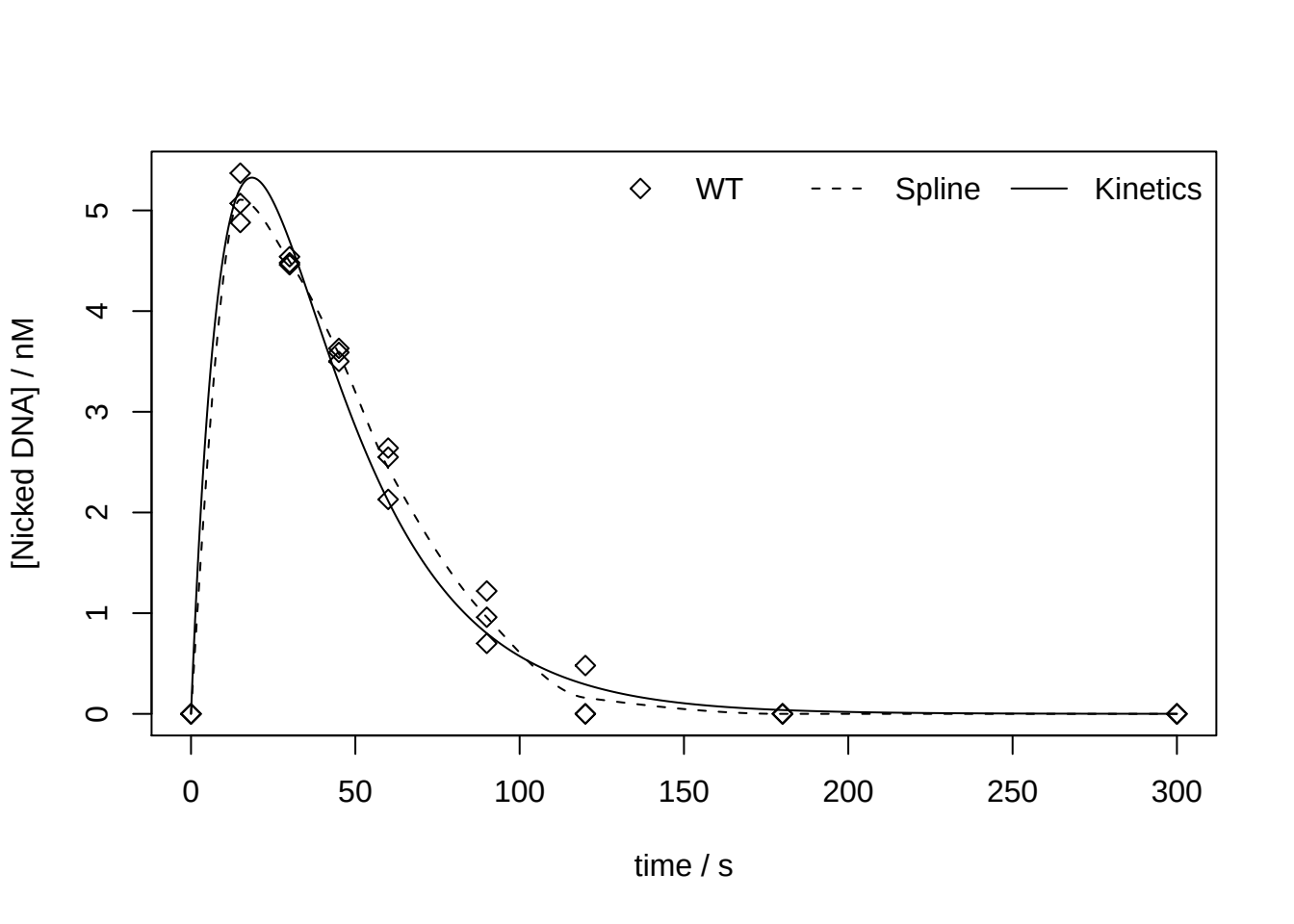

I67N
I71N
N12D
S20Q
WT
[Nicked DNA] / nM
0
1
2
3
4
5
6
0
50
100
150
200
250
300
time / s

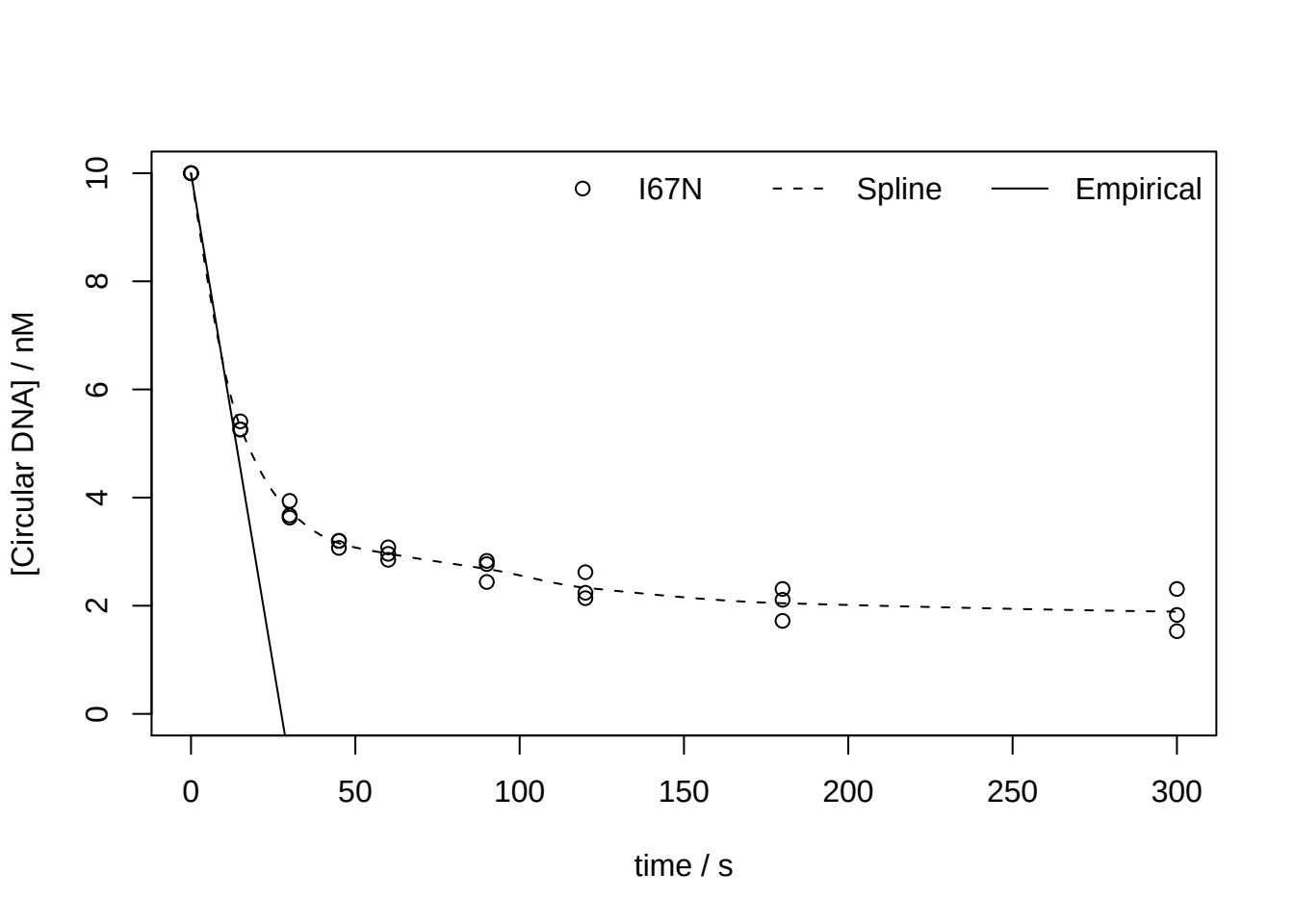

[Linear DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s
I67N
I71N
N12D
S20Q
WT

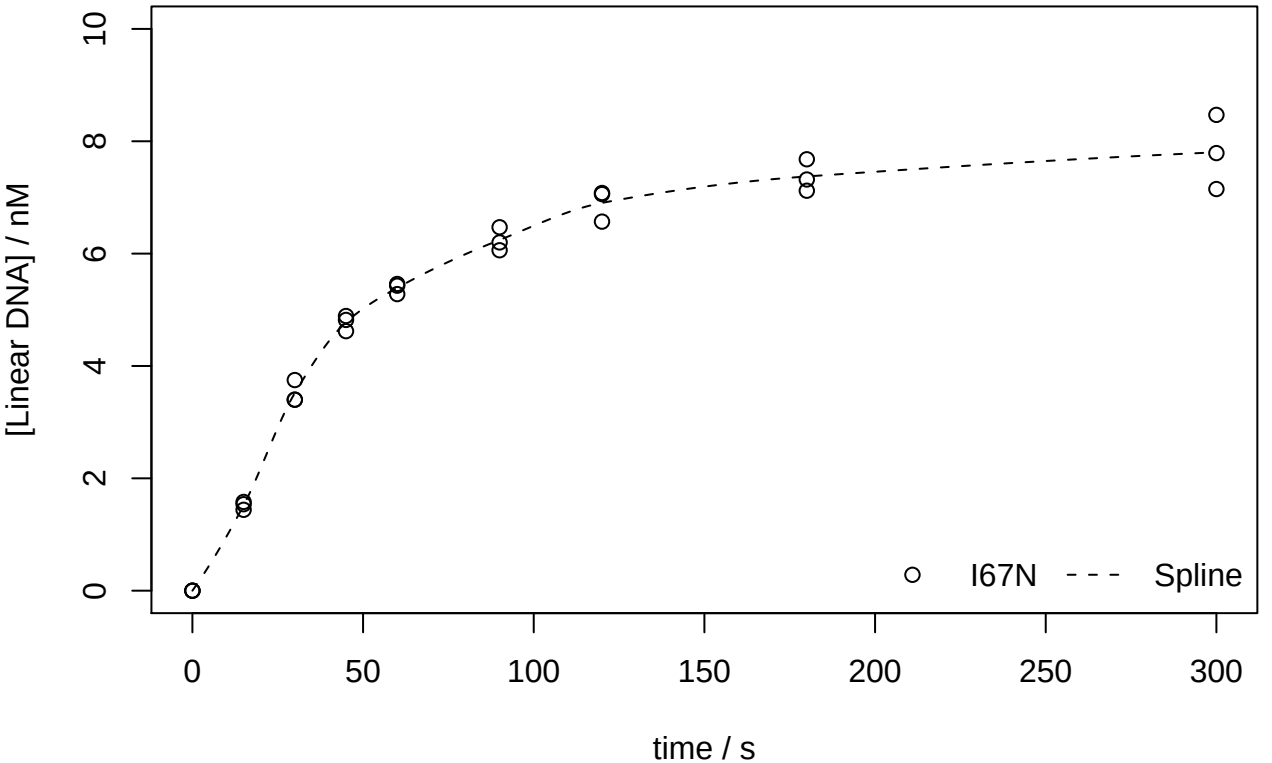

L
C
N
○ I67N △ I71N + N12D × S20Q ◇ WT

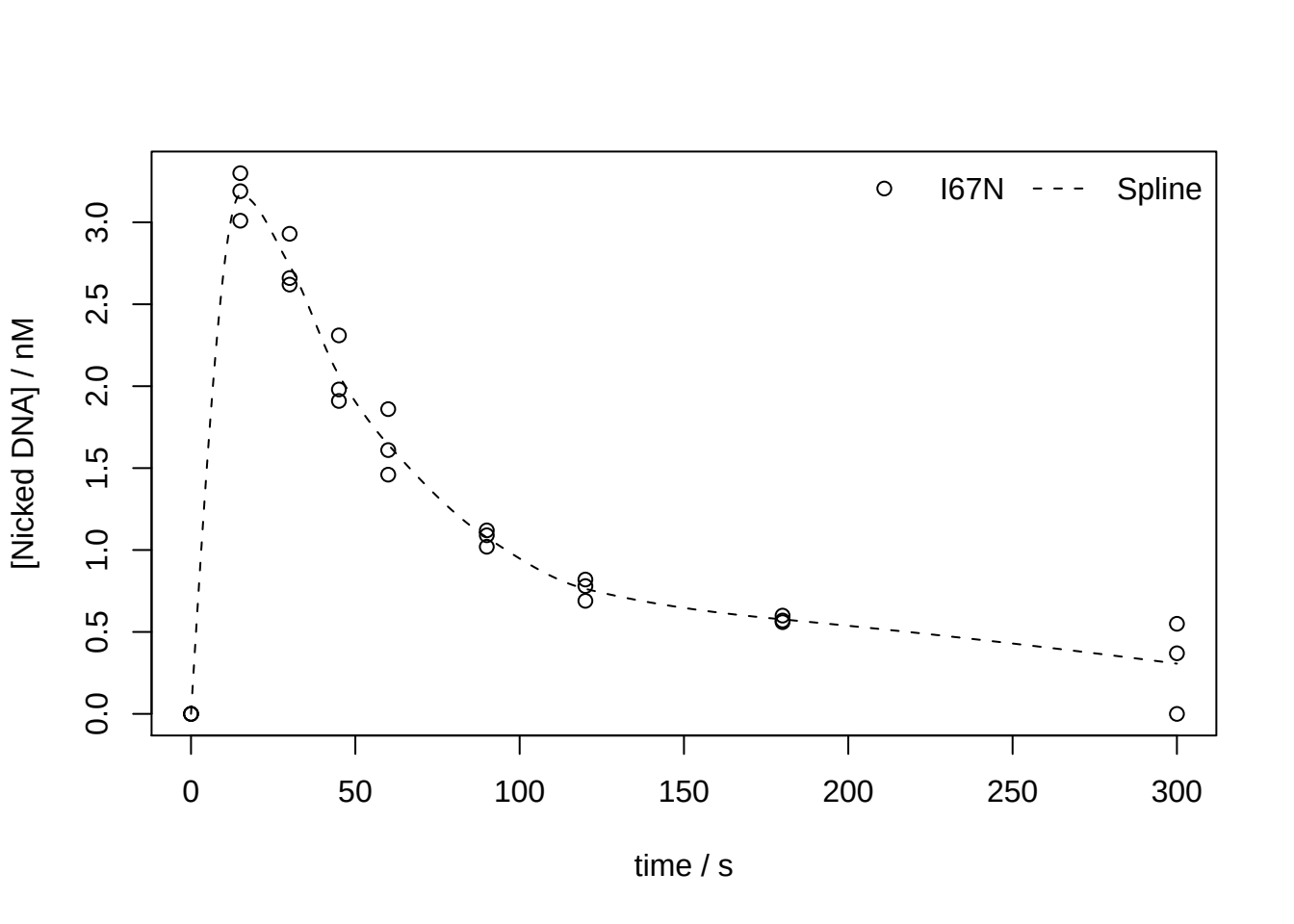

WT
Spline
Kinetics
[Circular DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s

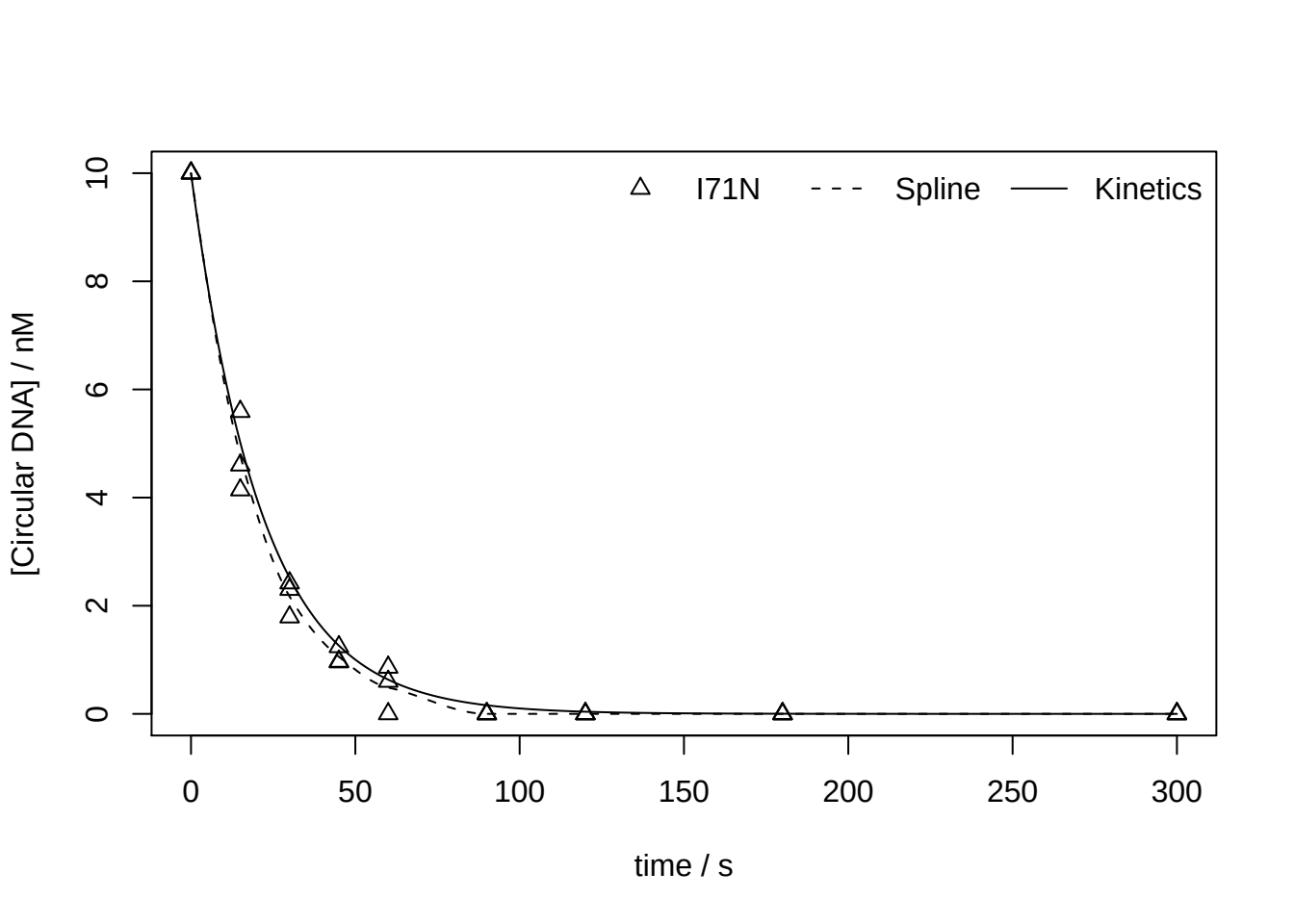

[Linear DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s
WT
Spline
Kinetics

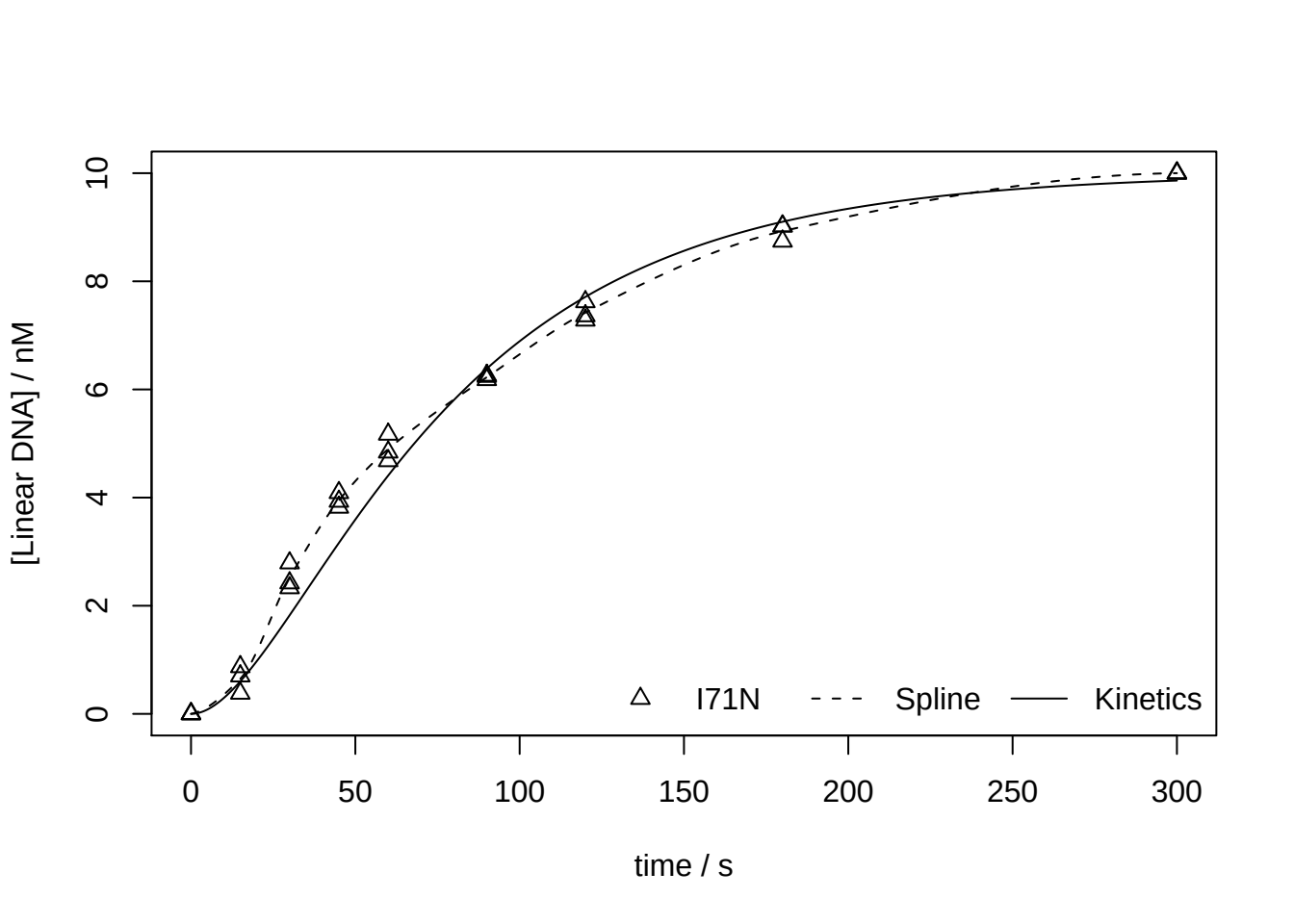

WT
Spline
Kinetics
[Nicked DNA] / nM
0
1
2
3
4
5
0
50
100
150
200
250
300
time / s

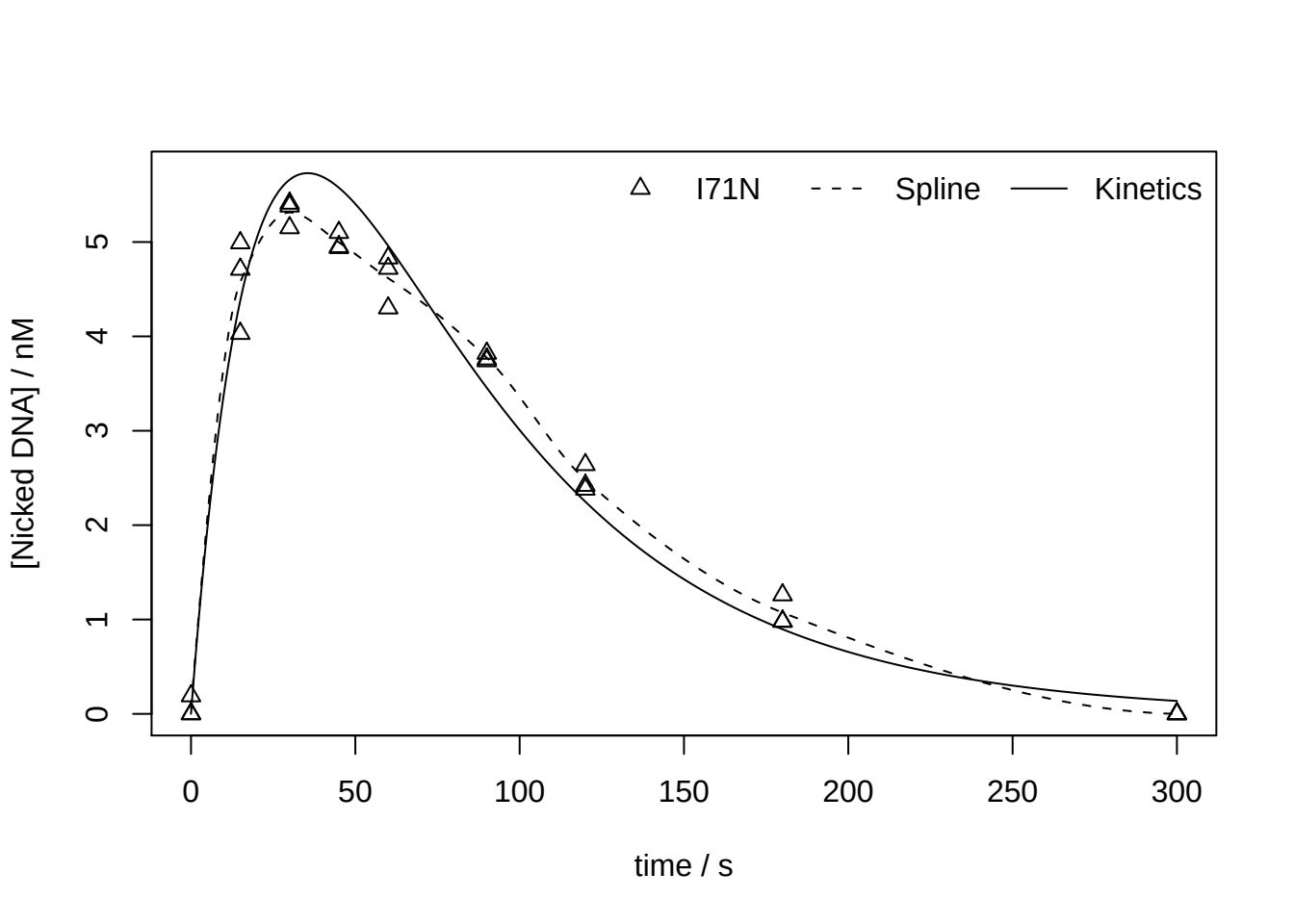

I67N
Spline
Empirical
[Circular DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s

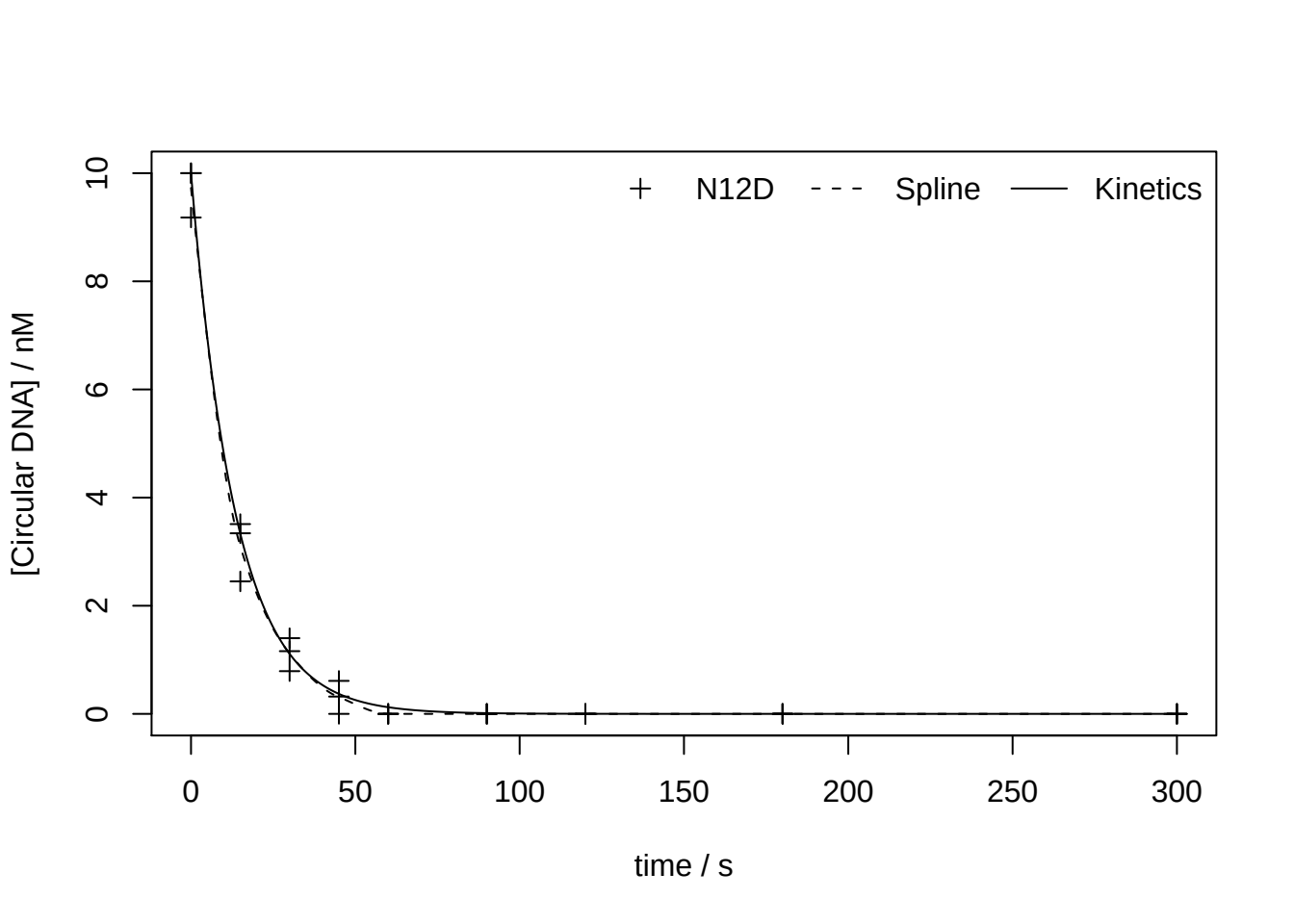

[Linear DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s
I67N
Spline

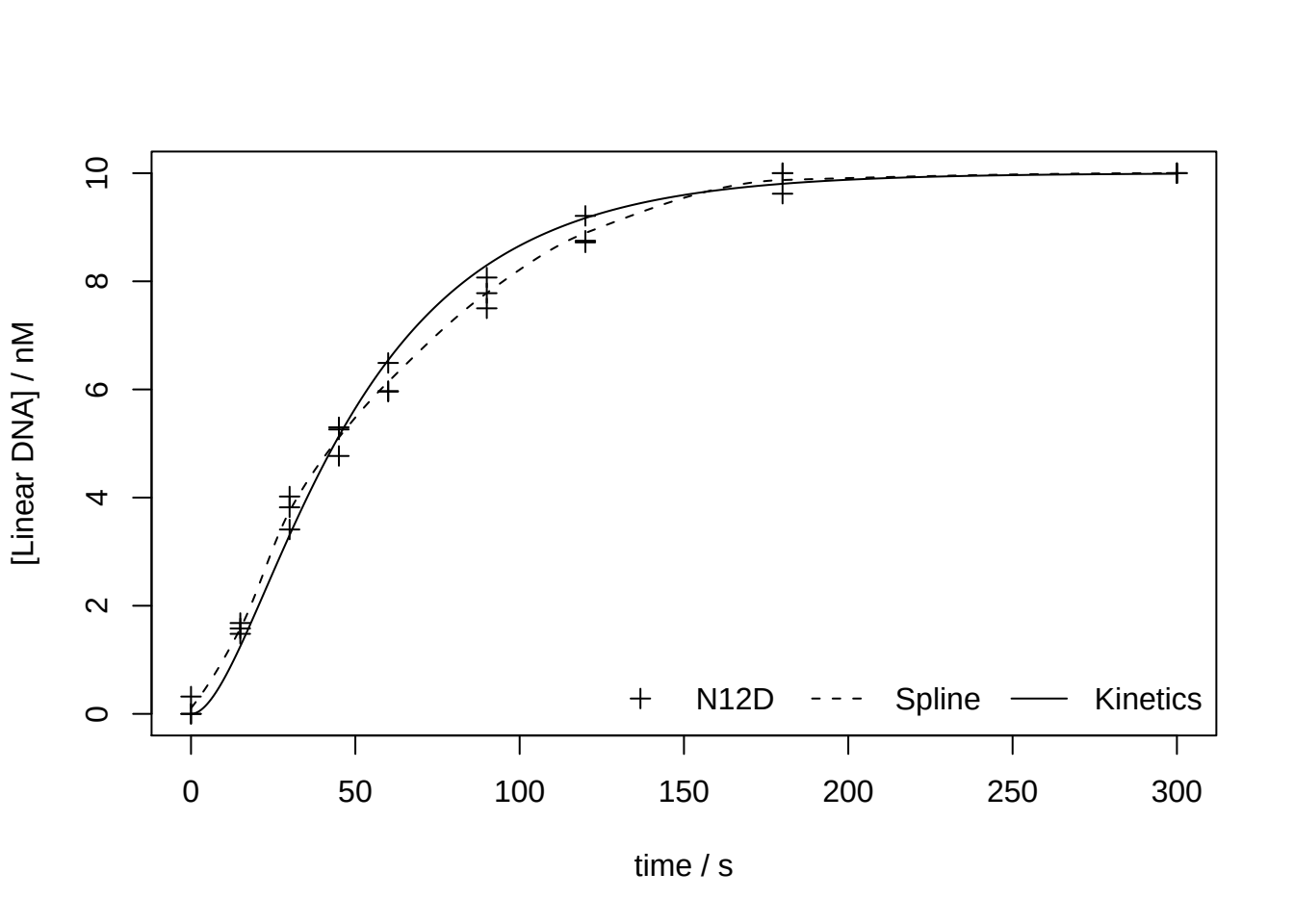

I67N
Spline
[Nicked DNA] / nM
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
50
100
150
200
250
300
time / s


I71N
Spline
Kinetics
[Circular DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s


[Linear DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s
I71N
Spline
Kinetics


I71N
Spline
Kinetics
[Nicked DNA] / nM
0
1
2
3
4
5
0
50
100
150
200
250
300
time / s


N12D
Spline
Kinetics
[Circular DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s


[Linear DNA] / nM
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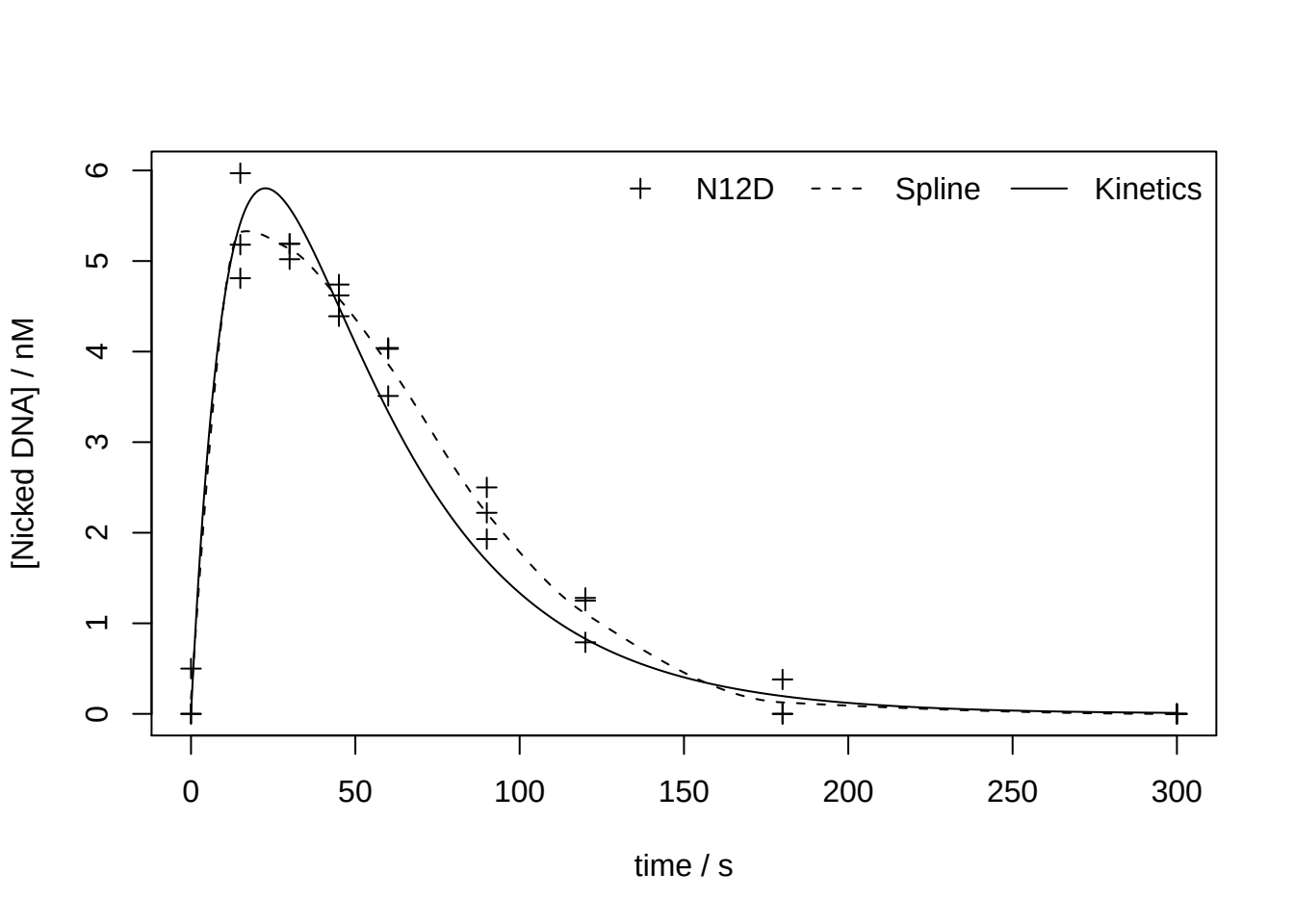
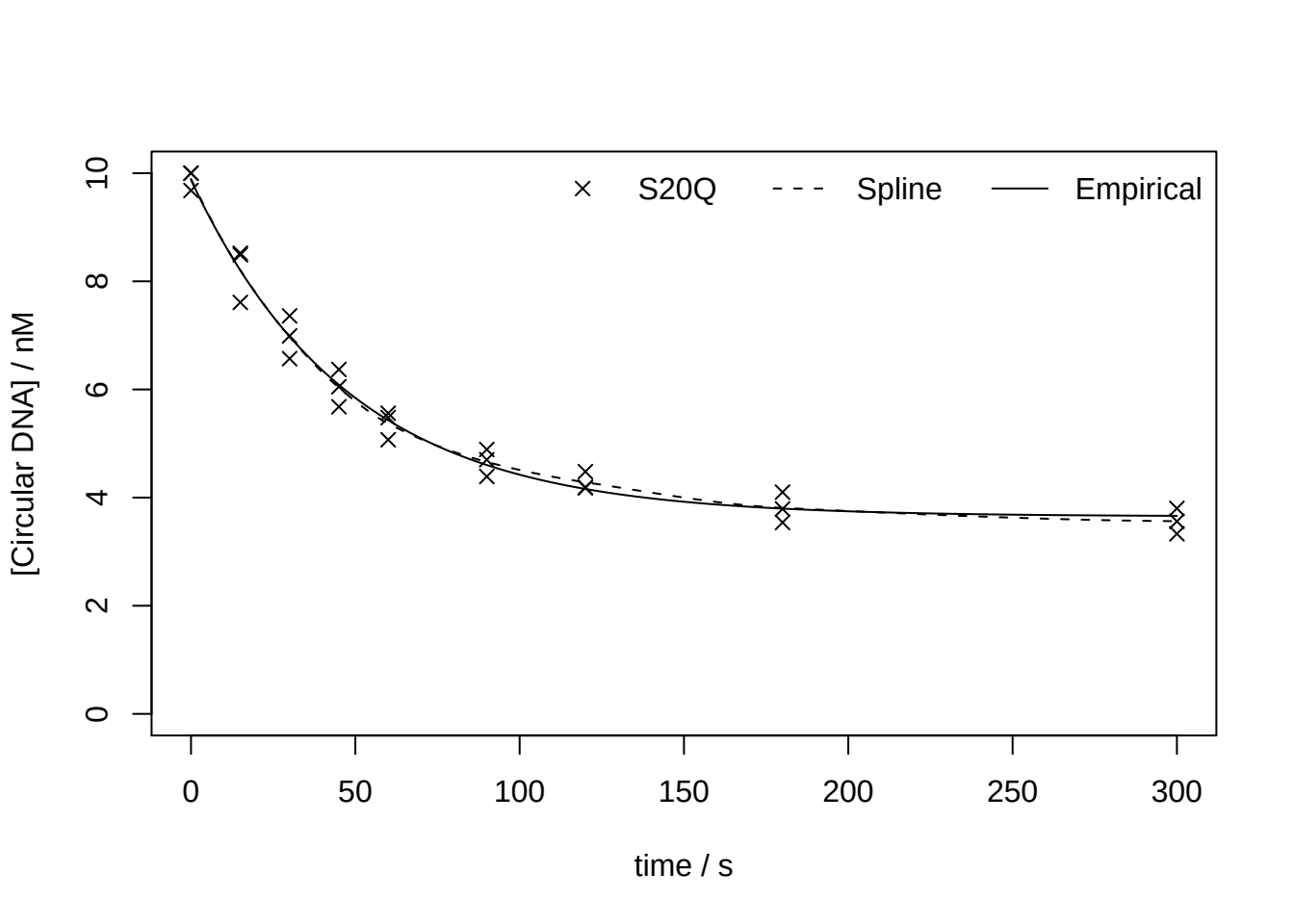
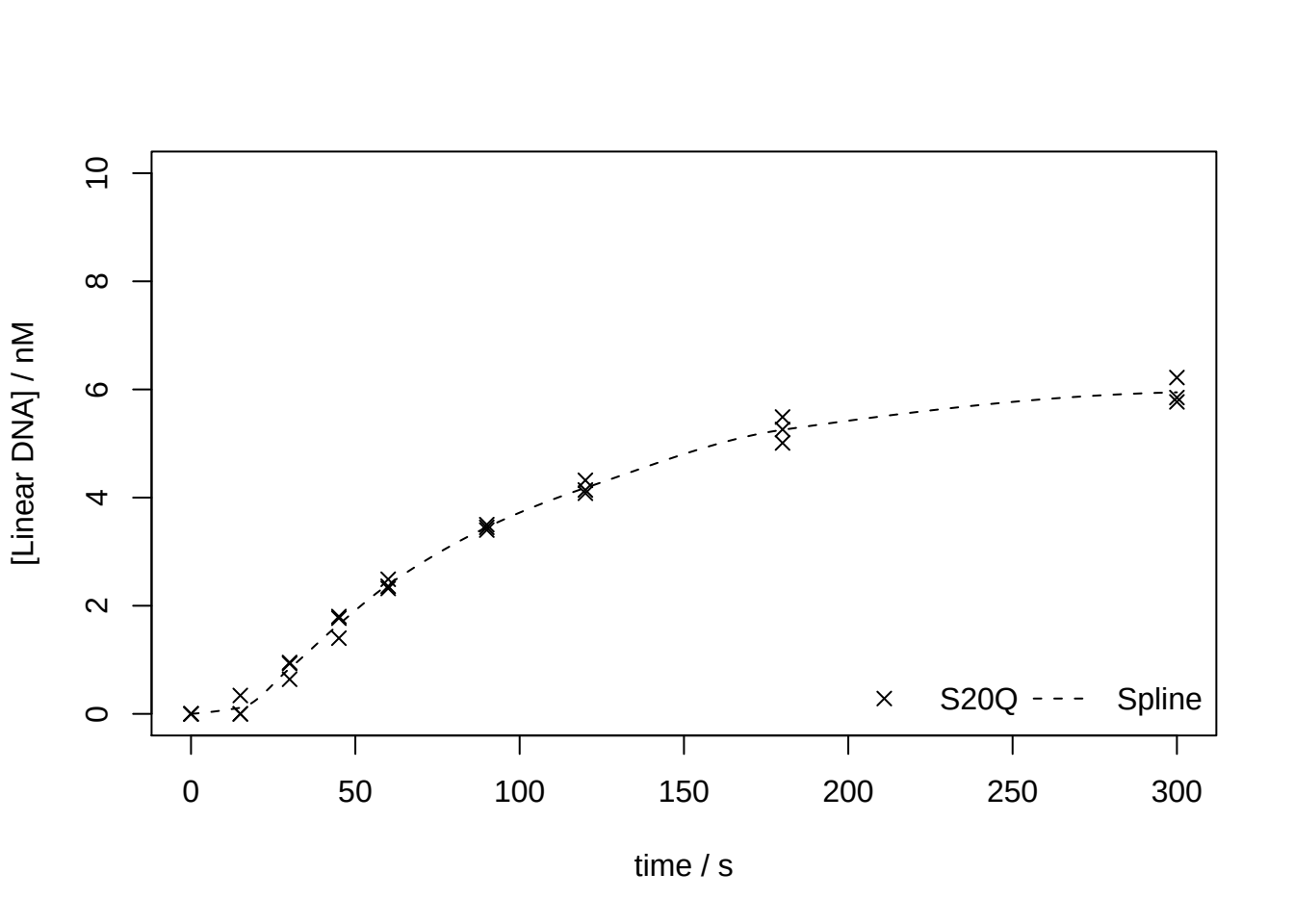
4
6
8
10
0
50
100
150
200
250
300
time / s
N12D
Spline
Kinetics


N12D
Spline
Kinetics
[Nicked DNA] / nM
time / s
0
1
2
3
4
5
6
0
50
100
150
200
250
300


S20Q
Spline
Empirical
[Circular DNA] / nM
0
2
4
6
8
10
0
50
100
150
200
250
300
time / s


[Linear DNA] / nM
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2
4
6
8
10
0
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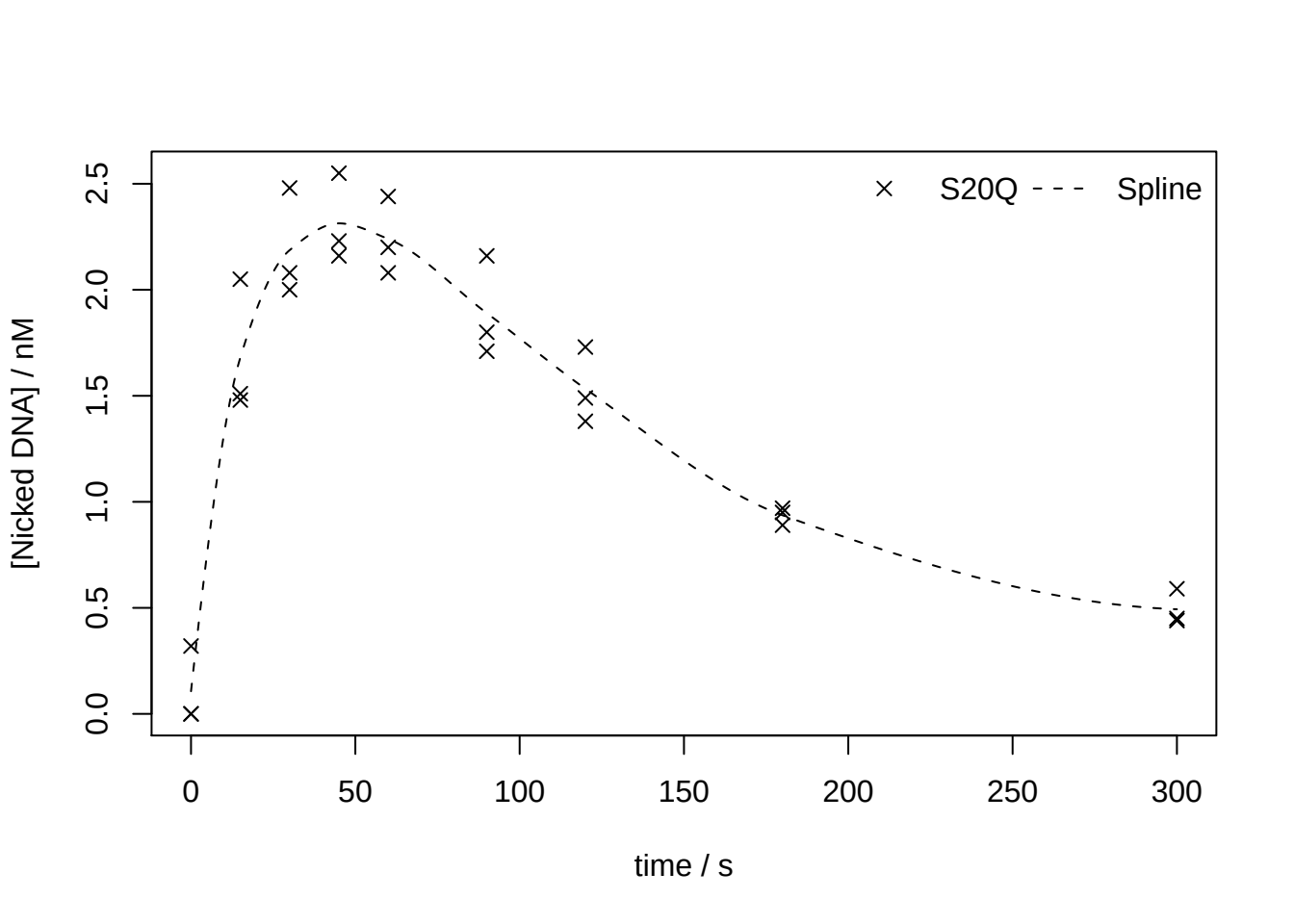
100
150
200
250
300
time / s
S20Q
Spline


S20Q
Spline
[Nicked DNA] / nM
time / s
0.0
0.5
1.0
1.5
2.0
2.5
0
50
100
150
200
250
300