Supporting Information

for

Dynamic modelling of phosphorolytic cleavage catalyzed by pyrimidine-nucleoside phosphorylase

Robert T. Giessmann[#,1,*], Niels Krausch[#,1], Felix Kaspar [1], Mariano Nicolas Cruz Bournazou [2,3], Anke Wagner [1,4], Peter Neubauer [1] and Matthias Gimpel [1]

[1] Laboratory of Bioprocess Engineering, Department of Biotechnology, Technische Universität Berlin, Ackerstr. 76, ACK24, D-13355 Berlin, Germany
[2] Institute of Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland
[3] DataHow AG, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland
[4] BioNukleo GmbH, Ackerst. 76, D-13355 Berlin, Germany
* Correspondence: r.giessmann@tu-berlin.de

[#] The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

Further material can be found at:

https://doi.org/10.5281/zenodo.3243519

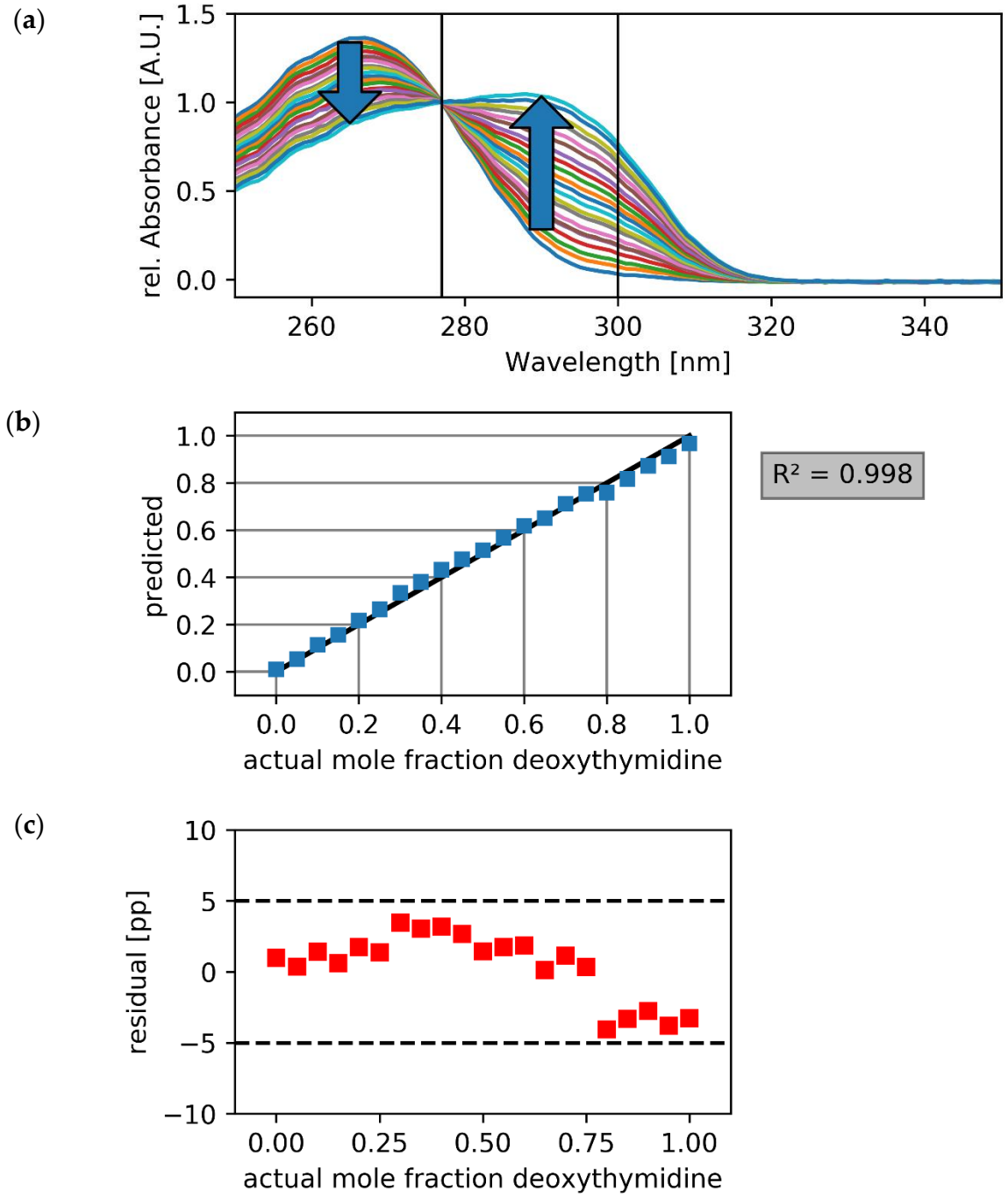


Figure S1. : "Predicted vs actual" plot. (**a**) Overlay of normalized absorptions of deoxythymidine / thymine mixtures. The acquired spectra were normalized to 277 nm (the isosbestic point estimated from enzymatic conversion reactions). In direction of increasing thymine (product) concentration, one can see an increase in extinction at ~300 nm. This ratio ($Abs_{300/277}$) allows to estimate the mole fraction of each substance in the mixture. (**b**) Predicted mole fraction of deoxythymidine in a mixture of thymine and deoxythymidine assayed as described in Materials and Methods, versus actual mole fraction. The coefficient of determination ($R^2 > 0.99$) indicates excellent agreement between actual and predicted mole fractions. (**c**) Residuals between predicted and actual mole fractions for the same data set. The absolute errors are approximately constant and limited by the 5 percentage points (pp) bounds indicated by dashed lines.

Table S1. Experimental conditions in this study. List of all experimental conditions used in this study.

Exp. Condition #	Nucleoside / mM	Phosphate / mM	Enzyme / mM
1	0.8	2	0.0125
2	0.8	2	0.0500
3	0.8	80	0.0125
4	0.8	80	0.0500
5	5	2	0.0125
6	5	2	0.0500
7	2	75	0.0250
8	0.8	2	0.0125
9	0.8	2	0.0500
10	0.8	80	0.0125
11	0.8	80	0.0500
12	5	2	0.0125
13	5	2	0.0500
14	2	75	0.0250
15	5	80	0.0125
16	0.8	28	0.0250
17	5	28	0.0250
18	0.8	54	0.0375
19	5	54	0.0375
20	5	80	0.0125
21	5	80	0.0500
22	0.8	28	0.0250
23	5	28	0.0250
24	0.8	54	0.0375
25	5	54	0.0375
26	2	75	0.0250
27	2.2	2	0.0250
28	2.2	80	0.0250
29	2.2	28	0.0375
30	2.2	54	0.0125
31	2	75	0.0250
32	2.2	2	0.0250
33	2.2	80	0.0250
34	2.2	28	0.0375
35	2.2	54	0.0125
36	2.2	54	0.0500
37	3.6	2	0.0375
38	2	75	0.0250
39	3.6	80	0.0375
40	3.6	28	0.0125
41	3.6	28	0.0500
42	3.6	54	0.0250
43	2	75	0.0250
44	3.6	80	0.0375
45	3.6	28	0.0125
46	3.6	28	0.0500
47	3.6	54	0.0250
48	2	75	0.0250

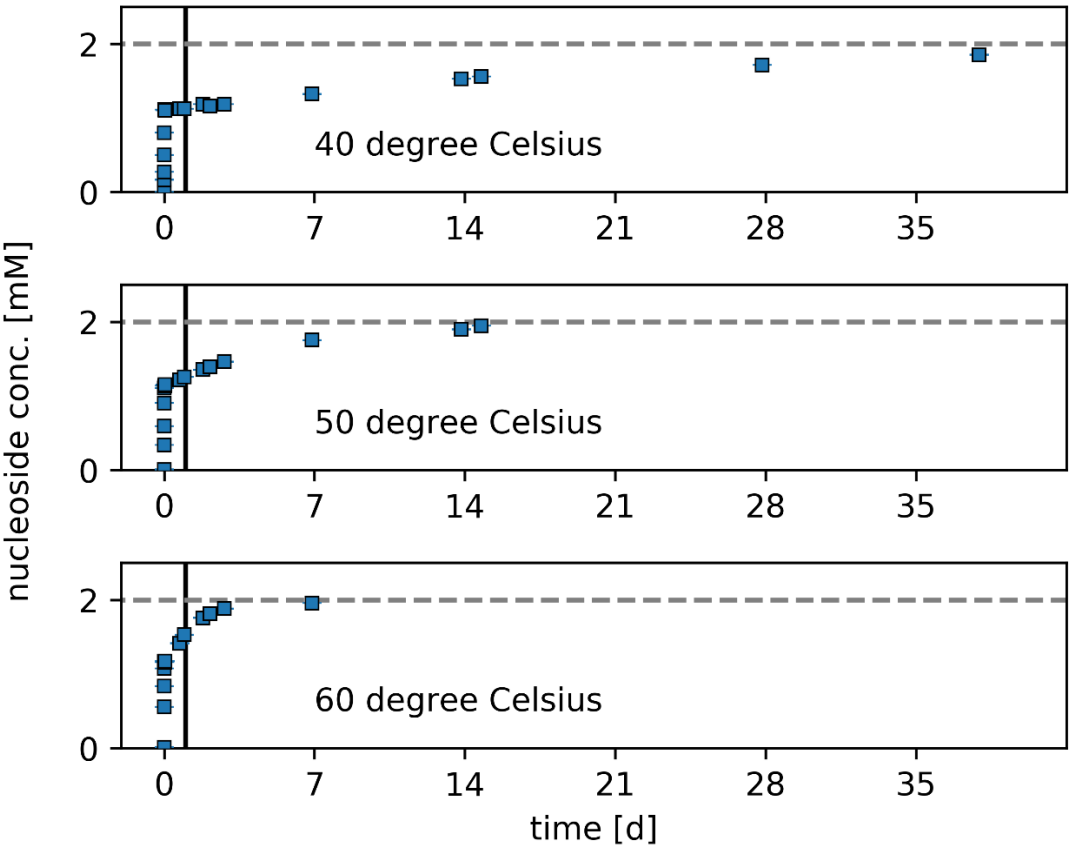
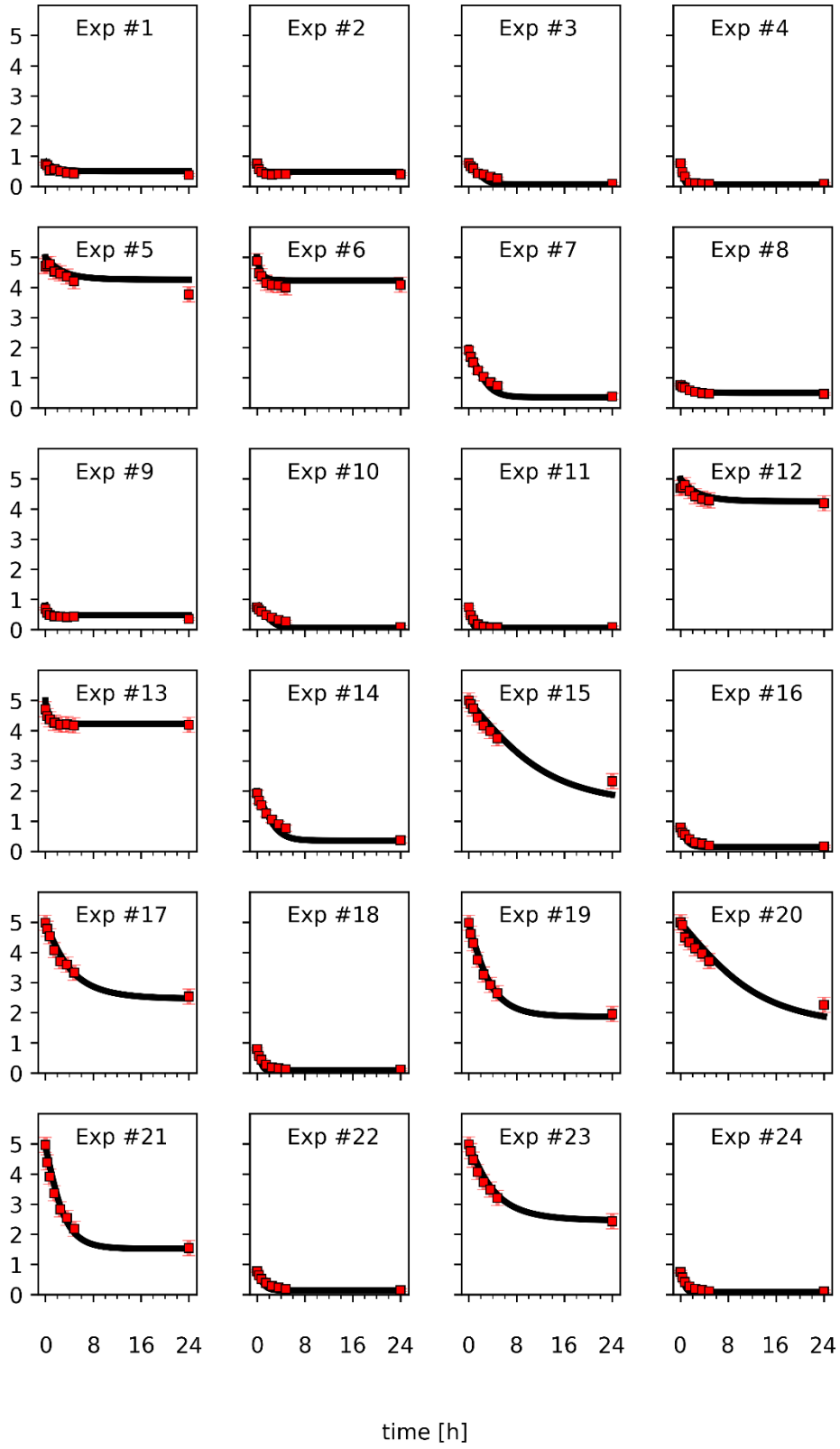


Figure S2. Degradation progress of deoxyribose-1-phosphate at elevated temperatures. To ensure enzyme activity and stability of deoxyribose-1-phosphate under the given assay conditions, we investigated the progress of decomposition of deoxyribose-1-phosphate over prolonged times. The vertical black line indicates the assay duration investigated in the main text (1 day = 24 hours), whereas the horizontal grey line indicates full conversion (2 mM). 1 mL aliquots of one master mix were heated at indicated temperatures (40, 50, and 60°C, respectively). The experimental conditions were: 2 mM deoxythymidine, 10 mM phosphate, 50 mM buffer (pH 9.0 at 25°C), and excessive amounts of enzyme (approximately 25-fold higher than in normal experiments). The equilibrium was reached after a maximum of 1 hour of reaction. To prevent evaporation of reaction mixture at 50 and 60°C, the reaction solutions were overlayed with mineral oil of BioReagent grade. The reaction at 40°C did not show significant differences in its level of equilibrium during the first day, whereas the reactions at 50 and 60°C respectively showed progress towards full conversion, which can be explained by decomposition of deoxyribose-1-phosphate. This also indicates that the enzyme is stable over the duration of assay.

Figure S3.



(Figure S3 continued on the next page)

Figure S3. (continued)

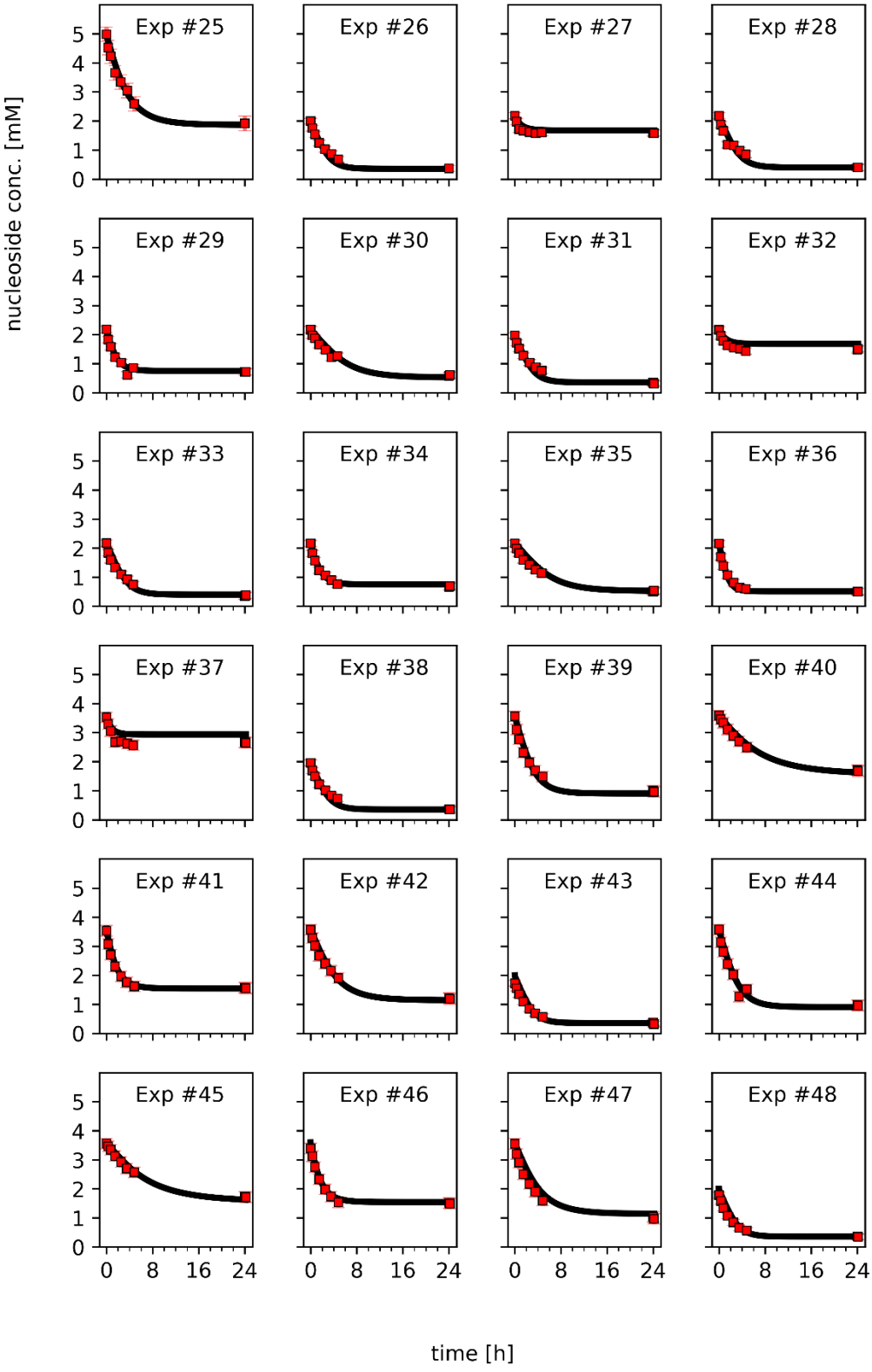


Figure S3. Fits of all experiments. The panels show all experimental data sets labelled as in Table S1, and the model fit with best parameter set k. Concentrations (red squares) are given with the 95% confidence interval as described in Materials and Methods, and the model fit (black line) is calculated with the parameter set k (see Results).

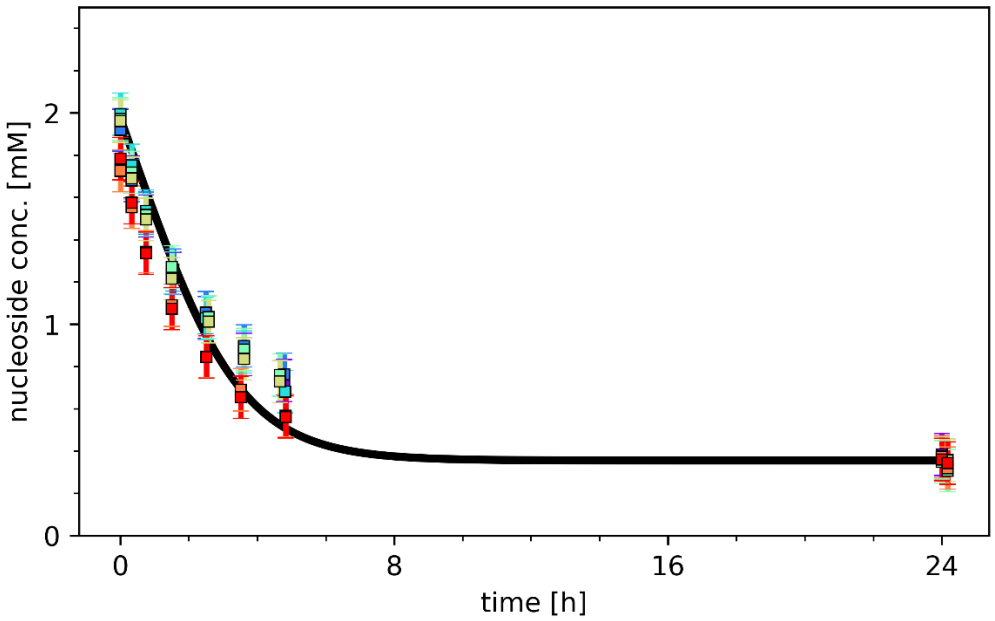


Figure S4: Comparison of inter-day controls. Shown is an overlay of concentrations (colored squares) with 95% confidence intervals, and the model fit for the parameter set k (black line). Shown are experiments with numbers Exp #7, #14, #26, #31, #38, #43, #48 (see Table S1). All of these experiments had an initial concentration of 2 mM nucleoside, 75 mM phosphate, and 0.025 mM enzyme. This condition corresponds to the definition of enzymatic activity, and was conducted at least once per day during generation of the data set.