The small GTPases K-Ras, N-Ras and H-Ras have distinct biochemical properties determined by allosteric effects

SUPPLEMENTAL DATA

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Abstract

This document contains the detailed description of (a) the raw experimental data; (b) the mathematical models; (c) the nonlinear regression procedures. This information relates to the analysis of the single-turnover kinetic data on hydrolysis of GTP catalyzed by various isoforms of Ras. This document also contains input files for the software package DynaFit (Kuzmic, 1996, 2009) which was used to perform all kinetic analyses.

Key words: enzyme kinetics; mathematics; Ras; hydrolysis

Contents

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1. Raw Experimental Data

The data tables below contain the nominal concentration of radioactive inorganic phosphate, in nanomoles per liter, vs. the reaction time, in minutes. The starting concentration of the Ras–GTP complex is 10 nM in every experiment. Thus, the expected maximum concentration of inorganic phosphate formed upon nearly complete conversion is also 10 nM. The right-most column in each table contains the *coefficient of variation*, CV, defined as

$$CV = 100 \times \frac{\text{standard deviation}}{\text{average}} \quad .$$

1.1. Wild-type Ras

1.1.1. H-Ras

t, min	replicate 1	replicate 2	[Pi], nM replicate 3	average	std.dev.	CV,%
0	0.89	0.53	0.27	0.56	0.31	55
5	0.89	0.65	0.19	0.57	0.35	62
10	1.39	1.28	0.42	1.03	0.53	51
15	1.29	1.36	1.06	1.24	0.16	13
20	1.00	1.61	0.28	0.96	0.67	69
25	1.91	2.35	0.71	1.66	0.85	51
30	2.20	1.95	0.93	1.69	0.67	40
35	2.39	2.63	1.05	2.03	0.85	42
40	2.44	3.03	0.45	1.97	1.35	69
60	3.07	3.83	3.80	3.57	0.43	12
75	3.59	3.50	0.95	2.68	1.50	56
90	2.14	4.18	2.67	3.00	1.06	35
120	3.94	4.03	2.76	3.58	0.71	20
150	4.46	4.43	3.51	4.14	0.54	13
180	4.57	4.08	2.20	3.61	1.25	35
240	4.41	5.16	3.03	4.20	1.08	26
300	6.26	1.86	1.95	3.36	2.51	75

1.1.2. K-Ras

t, min	[Pi], nM replicate 1	replicate 2	replicate 3	average	std.dev.	CV,%
0	0.23	0.16	0.16	0.18	0.04	22
5	0.37	0.20	0.28	0.28	0.09	30
10	0.58	0.47	0.36	0.47	0.11	23
15	0.76	0.73	0.15	0.55	0.34	62
20	0.98	0.99	0.17	0.71	0.47	65
25	1.30	0.82	0.17	0.76	0.57	75
30	1.61	1.05	0.18	0.95	0.72	76
35	1.55	1.63	0.24	1.14	0.78	69
40	1.81	1.70	0.56	1.36	0.69	51
60	2.81	1.88	0.39	1.69	1.22	72
75	2.31	2.49	0.31	1.71	1.21	71
90	3.24	1.80	1.44	2.16	0.95	44
120	4.10	2.50	1.63	2.74	1.25	46
150	3.84	2.57	1.60	2.67	1.12	42
180	4.04	3.53	1.56	3.04	1.31	43
240	5.95	4.25	1.64	3.95	2.17	55
300	6.63	0.81	2.97	3.47	2.94	85

1.1.3. N-Ras

t, min	[Pi], nM replicate 1	replicate 2	replicate 3	average	std.dev.	CV,%
0	0.19	0.17	0.07	0.14	0.06	45
5	0.39	0.25	0.17	0.27	0.11	39
10	0.53	0.35	0.23	0.37	0.15	42
15	0.87	0.39	0.09	0.45	0.39	88
20	1.30	0.63	0.27	0.73	0.53	72
25	0.71	0.78	0.14	0.54	0.35	65
30	1.47	0.75	0.24	0.82	0.62	76
35	1.43	1.14	0.18	0.92	0.65	71
40	1.92	1.23	0.38	1.18	0.77	66
60	2.02	1.16	0.21	1.13	0.91	80
75	2.77	1.85	0.39	1.67	1.20	72
90	3.22	1.81	0.58	1.87	1.32	71
120	2.87	1.64	0.74	1.75	1.07	61
150	2.28	2.56	0.80	1.88	0.95	50
180	4.12	3.42	0.88	2.81	1.71	61
240	4.56	3.31	1.44	3.10	1.57	51
300	6.52	1.10	2.56	3.39	2.81	83

1.2. Wild-type Ras in the presence of Raf

1.2.1. H-Ras

t, min	replicate 1	replicate 2	[Pi], nM replicate 3	average	std.dev.	CV,%
0	0.20	0.17	0.37	0.25	0.11	43
5	0.34	0.30	0.63	0.42	0.18	42
10	0.46	0.55	0.86	0.62	0.21	34
15	0.77	0.86	1.76	1.13	0.55	49
20	0.81	0.63	1.37	0.94	0.39	41
25	1.34	1.20	1.53	1.36	0.16	12
30	0.88	1.24	2.39	1.50	0.79	52
35	1.49	1.17	1.85	1.50	0.34	23
40	1.57	1.67	2.52	1.92	0.53	27
60	2.01	1.39	3.39	2.27	1.03	45
75	1.91	1.79	2.96	2.22	0.64	29
90	2.18	2.02	3.54	2.58	0.84	32
120	2.39	2.02	4.19	2.87	1.16	40
150	2.72	1.80	4.36	2.96	1.30	44
180	2.29	2.11	5.43	3.28	1.86	57
210	2.67	2.64	5.32	3.54	1.54	43
240	3.05	3.07	4.45	3.52	0.80	23

1.2.2. K-Ras

t, min	replicate 1	replicate 2	[Pi], nM replicate 3	average	std.dev.	CV,%
0	0.15	0.17	0.28	0.20	0.07	34
5	0.22	0.20	0.46	0.29	0.15	50
10	0.44	0.31	0.76	0.50	0.23	46
15	0.46	0.46	1.05	0.66	0.34	52
20	0.52	0.46	1.18	0.72	0.40	56
25	0.79	0.77	1.29	0.95	0.29	31
30	0.71	0.74	1.27	0.90	0.31	35
35	1.16	0.92	1.64	1.24	0.36	29
40	0.94	0.82	1.36	1.04	0.28	27
60	1.47	1.27	2.77	1.84	0.82	44
75	2.05	1.33	2.73	2.04	0.70	34
90	1.67	1.36	3.19	2.08	0.98	47
120	1.74	1.60	3.29	2.21	0.93	42
150	2.15	1.89	3.80	2.61	1.04	40
180	2.47	2.01	3.74	2.74	0.90	33
210	2.28	1.82	3.61	2.57	0.93	36
240	2.99	2.34	4.57	3.30	1.15	35

1.2.3. N-Ras

t, min	[Pi], nM replicate 1	replicate 2	replicate 3	average	std.dev.	CV,%
0	0.10	0.07	0.22	0.13	0.08	62
5	0.13	0.12	0.29	0.18	0.10	54
10	0.12	0.17	0.43	0.24	0.17	70
15	0.25	0.20	0.55	0.33	0.19	57
20	0.33	0.28	0.62	0.41	0.18	44
25	0.37	0.29	0.61	0.42	0.17	39
30	0.48	0.43	0.95	0.62	0.29	46
35	0.62	0.40	1.16	0.73	0.39	54
40	0.64	0.46	1.28	0.80	0.43	54
60	0.85	0.63	1.93	1.13	0.70	62
75	0.90	1.01	1.99	1.30	0.60	46
90	1.20	1.10	2.67	1.65	0.88	53
120	1.55	1.19	2.63	1.79	0.75	42
150	1.85	1.32	2.64	1.94	0.67	34
180	1.74	1.07	3.71	2.17	1.37	63
210	2.00	1.49	3.79	2.43	1.21	50
240	2.16	2.05	4.20	2.81	1.21	43

1.3. H-Ras mutants

1.3.1. H-Ras H166Y

t, min	[Pi], nM replicate 1	replicate 2	replicate 3	average	std.dev.	CV,%
0	0.73	0.90	1.54	1.06	0.43	41
5	1.03	1.34	2.21	1.52	0.61	40
10	1.74	1.64	3.62	2.33	1.11	48
15	2.26	2.04	4.60	2.97	1.42	48
20	2.72	2.59	5.39	3.57	1.58	44
25	2.52	2.92	7.13	4.19	2.55	61
30	2.91	3.29	7.39	4.53	2.48	55
35	3.35	3.71	7.85	4.97	2.50	50
40	2.77	4.14	9.56	5.49	3.59	65
60	5.04	4.42	10.47	6.64	3.33	50
75	5.42	5.40	10.37	7.06	2.86	40
90	6.10	6.31	13.32	8.58	4.11	48
120	6.27	6.22	14.10	8.86	4.54	51
150	6.31	7.15	14.88	9.45	4.72	50
180	6.89	6.55	13.86	9.10	4.12	45
210	6.26	6.89	14.32	9.16	4.48	49
240	5.99	6.64	14.50	9.04	4.74	52
300	7.86	7.38	13.68	9.64	3.50	36

1.3.2. H-Ras Q95L

t, min	[Pi], nM replicate 1	replicate 2	replicate 3	average	std.dev.	CV,%
0	0.77	0.78	0.95	0.84	0.10	12
5	1.51	1.24	1.40	1.38	0.14	10
10	2.28	1.59	1.51	1.79	0.43	24
15	2.66	1.77	2.04	2.16	0.46	21
20	3.29	2.84	2.60	2.91	0.35	12
25	3.43	2.70	2.92	3.02	0.37	12
30	4.93	3.49	3.40	3.94	0.86	22
35	6.38	3.36	3.76	4.50	1.64	36
40	5.79	4.13	3.80	4.57	1.07	23
60	7.53	5.30	5.43	6.09	1.25	21
75	7.91		5.72	4.56	4.05	89
90	7.16	4.89	6.64	6.23	1.19	19
120	8.12	7.14	6.01	7.09	1.05	15
150	9.47	6.08	10.48	8.67	2.31	27
180	7.95	8.56	6.54	7.69	1.04	13
210	8.92	6.21	7.30	7.48	1.36	18
240	10.26	6.85	4.63	7.25	2.84	39
300	11.01	8.31	5.95	8.42	2.53	30

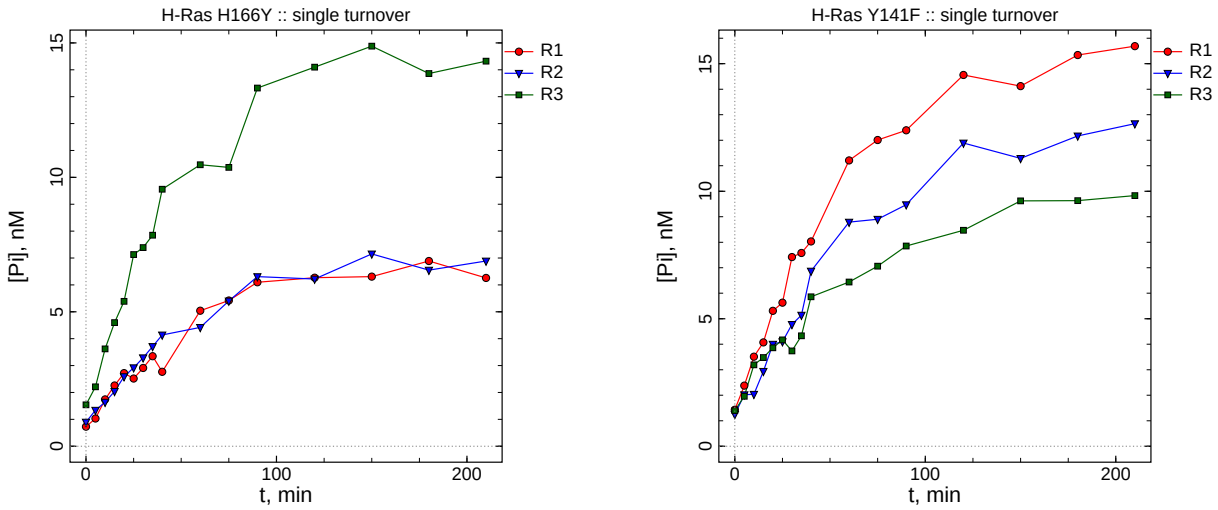
1.3.3. H-Ras Y141F

t, min	[Pi], nM replicate 1	replicate 2	replicate 3	average	std.dev.	CV,%
0	1.43	1.25	1.40	1.36	0.10	7
5	2.38	2.02	1.96	2.12	0.23	11
10	3.51	2.04	3.19	2.91	0.77	27
15	4.07	2.94	3.48	3.50	0.56	16
20	5.31	4.00	3.86	4.39	0.80	18
25	5.63	4.10	4.17	4.63	0.86	19
30	7.42	4.78	3.74	5.31	1.90	36
35	7.58	5.14	4.33	5.68	1.70	30
40	8.03	6.87	5.86	6.92	1.09	16
60	11.21	8.79	6.44	8.81	2.38	27
75	12.01	8.90	7.06	9.32	2.50	27
90	12.39	9.47	7.85	9.90	2.30	23
120	14.56	11.89	8.47	11.64	3.05	26
150	14.12	11.29	9.62	11.67	2.28	19
180	15.34	12.17	9.63	12.38	2.86	23
210	15.69	12.65	9.83	12.72	2.93	23
240	15.76	11.41	9.20	12.12	3.33	28
300	14.76	12.56	9.62	12.31	2.58	21

1.4. Reproducibility of signal amplitude vs. half-time to conversion

Under normal circumstances, in a well-reproduced replicated experiment, the *CV* values are expected to fall below approximately 10–15%. Note that in the tables above the *CV* values are

significantly higher, due to the special nature of multi-step analytical procedure. Importantly, the overall *shape* (i.e., half-time to complete conversion) is well reproduced. This is illustrated in *Figure S1*.



(a) **(b)**

Figure S1: Raw data. Signal amplitude (theoretically expected value 10 nM) is not well reproduced in replicated experiments, whereas half-time to full GTP conversion is well reproduced. **(a)** H-Ras mutant H166Y. **(b)** H-Ras mutant Y141F.

2. Mathematical and statistical methods

2.1. Mathematical representation of the molecular mechanism

Under the conditions of the single-turnover assay, the hydrolysis of GTP pre-incubated with Ras is irrreversible and follows the simplest possible first-order rate law, according to the reaction scheme displayed in *Figure S2*.

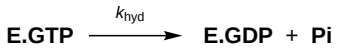


Figure S2: Postulated molecular mechanism for single-turnover hydrolysis of GTP pre-incubated with Ras (enzyme "E").

In the symbolic notation of the software package DynaFit [1, 2] the molecular mechanism is encoded as

```
[mechanism]
   E.GTP ---> E.GDP + Pi      :     k.hyd
```

When presented by the above input code, the software automatically generates the system of first-order ordinary differential Eqns (S1)–(S3).

$$\frac{\mathrm{d[E.GTP]}}{\mathrm{d}t} = -k_{.\mathrm{hyd}}[\mathrm{E.GTP}] \tag{S1}$$

$$\frac{\mathrm{d[E.GDP]}}{\mathrm{d}t} = +k_{.\mathrm{hyd}}[\mathrm{E.GTP}] \tag{S2}$$

$$\frac{\mathrm{d[Pi]}}{\mathrm{d}t} = +k_{.\mathrm{hyd}}[\mathrm{E.GTP}] \tag{S3}$$

The fitting function (model equation) for each individual data set is

$$F(t) = F_0 + \sum_{i=1}^{n_S} r_i c_i(t) \quad , \tag{S4}$$

where

$F(t)$	the experimental signal observed at time t
F_0	offset on the signal axis (a property of the instrument and/or of the initial conditions)
n_S	number of unique molecular species participating in the reaction mechanism
$c_i(t)$	the concentration of the ith species at time t
r_i	the molar response coefficient of the ith species

In this case, $n_S = 3$. The molecular species participating in the mechanism are E.GTP, E.GDP, Pi. The concentrations of these molecular species at time t are computed from their initial concentrations (at time zero, $t = 0$) by solving an initial-value problem defined by a system of simultaneous first-order Ordinary Differential Equations (ODEs) listed immediately above.

2.2. *Global regression model*

The amplitude of the experimental signal (i.e., the nominal maximum [Pi] concentration expected at total conversion of initially present GTP) is *not* reproducible from one replicate to the next, as illustrated in *Figure S1*. Thus, the regression model for the *global* fit [3] of combined kinetic traces was constructed as follows:

- The molar response coefficient of radioactive inorganic phosphate (r_{Pi} in Eqn (S4)) was set to the initial value "1.00" but treated as a locally adjustable model parameter, i.e., specific to each individual kinetic trace.
- Similarly, the offset on the signal axis (F_0 in Eqn (S4)) was also treated as a locally adjustable model parameter, i.e., specific to each individual kinetic trace. This value corresponds to the small amount of radioactive inorganic phosphate present already at the start of the reaction ($t = 0$).
- In contrast, the intrinsic hydrolysis rate constant, k_{hyd} in Eqns (S1)–(S3), was treated as the only *globally* optimized model parameter. In other words, by definition the same value of k_{hyd} was assumed to apply identically to all three replicated kinetic traces.

These particular model construction principles are embodied by the input script listed in section 2.3.

2.3. *Example script: DynaFit software*

The following input script for the software package DynaFit [1, 2] was utilized to perform global fit [3] of a typical data set, in this case, involving the H-Ras mutant Y141F.

```
[task]
   task = fit
   data = progress
[mechanism]
   E.GTP ---> E.GDP + Pi :    k.hyd
[constants]
   k.hyd = 0.01 ??
[concentrations]
   E.GTP = 10
[data]
   directory ./project/Ras/data
graph H-Ras Y141F
   sheet H-Y141F.csv
   column 2 | offset = auto ? | response Pi = 1 ? | label R1
   column 3 | offset = auto ? | response Pi = 1 ? | label R2
   column 4 | offset = auto ? | response Pi = 1 ? | label R3
[output]
   directory ./project/Ras/output/H-Y141F
[settings]
{Output}
   XAxisLabel = t, min
   YAxisLabel = [Pi], nM
   WriteTeX = y
{Filter}
   XMax = 210
```

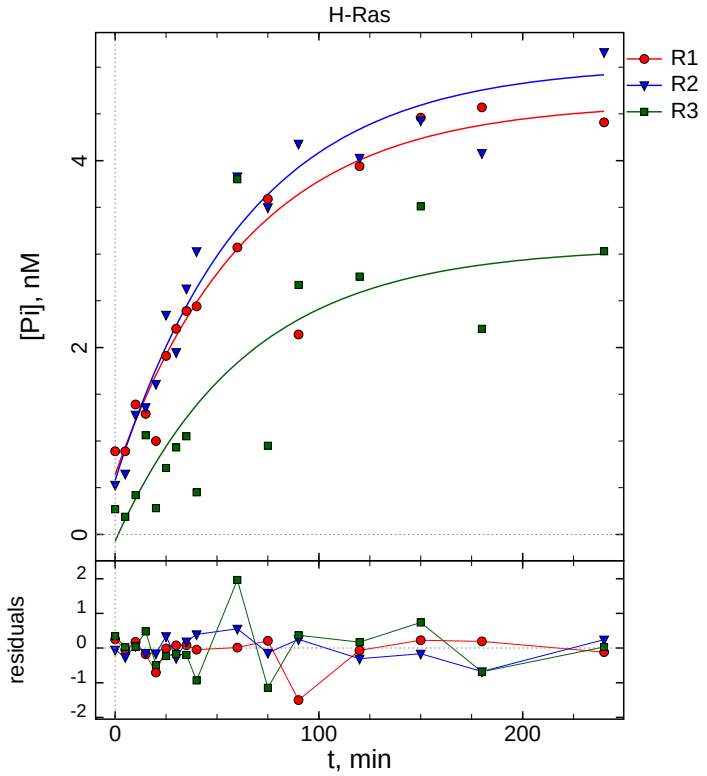
```
{TrustRegion}
   RobustFit = y
{ConfidenceIntervals}
   LevelPercent = 68
[end]
```

3. Results

3.1. Wild-type Ras

3.1.1. H-Ras

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES

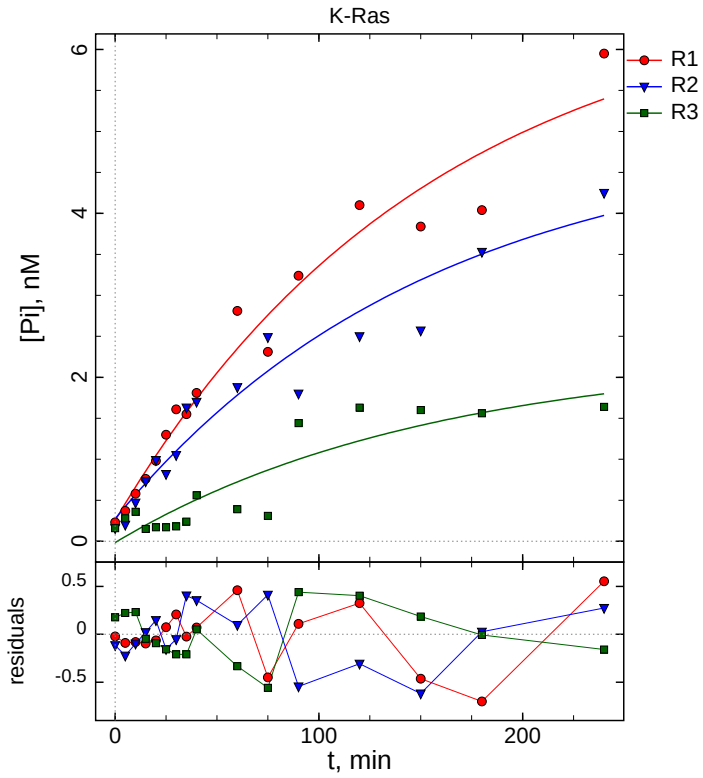


BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.0155394	0.0157 ± 0.0032	20.4	0.0126	0.0191	
2	$r_{(\mathrm{Pi})}$ / 1	0.398392	0.384 ± 0.045	11.7			
3	offset / 1	0.638595	0.61 ± 0.29	47.5			
4	$r_{(\mathrm{Pi})}$ / 2	0.444858	0.437 ± 0.045	10.3			
5	offset / 2	0.576968	0.6 ± 0.3	50.0			
6	$r_{(\mathrm{Pi})}$ / 3	0.314924	0.318 ± 0.045	14.2			
7	offset / 3	-0.0730555	-0.074 ± 0.28	> 100			

3.1.2. K-Ras

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES

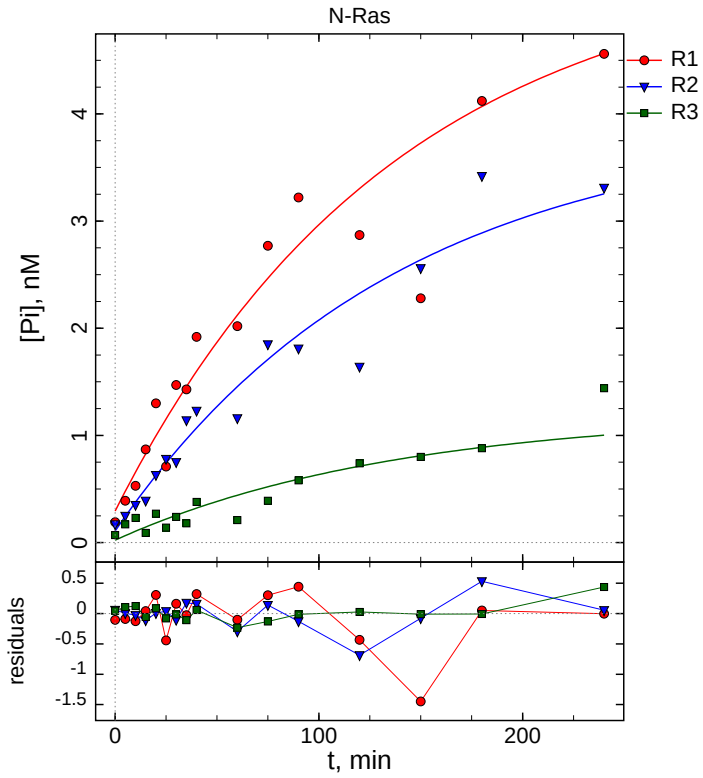


BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.00645581	0.0057 ± 0.0014	24.6	0.0042	0.0072	
2	$r_{(Pi)}$ / 1	0.653113	0.692 ± 0.095	13.7			
3	offset / 1	0.252638	0.3 ± 0.15	50.0			
4	$r_{(Pi)}$ / 2	0.469944	0.488 ± 0.071	14.5			
5	offset / 2	0.273632	0.32 ± 0.14	43.8			
6	$r_{(Pi)}$ / 3	0.230496	0.247 ± 0.047	19.0			
7	offset / 3	-0.0164548	-0.0064 ± 0.13	>> 100			

3.1.3. N-Ras

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES



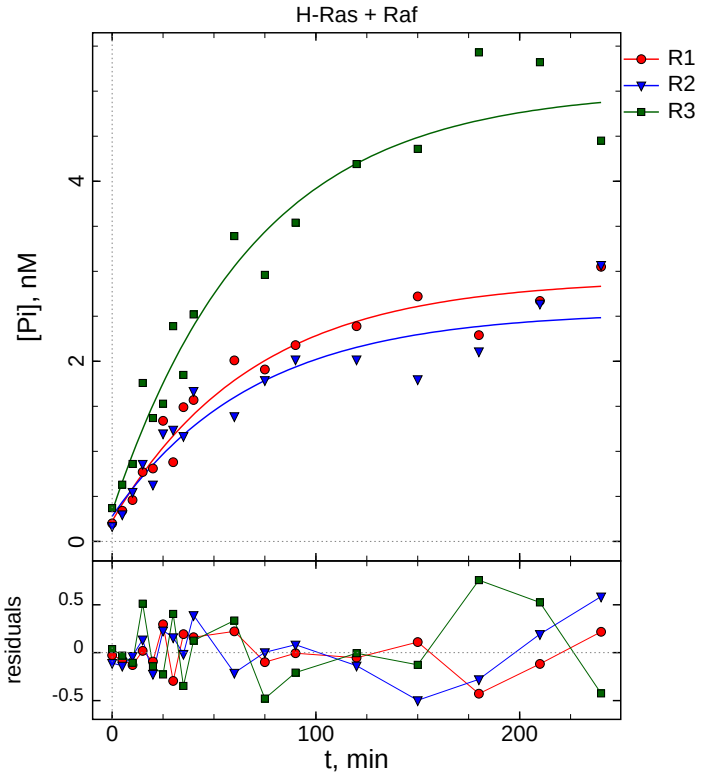
BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.00725892	0.0062 ± 0.0017	27.4	0.0043	0.0082	
2	$r_{(\mathrm{Pi})}$ / 1	0.517497	0.512 ± 0.079	15.4			
3	offset / 1	0.294471	0.41 ± 0.15	36.6			
4	$r_{(\mathrm{Pi})}$ / 2	0.381264	0.414 ± 0.066	15.9			
5	offset / 2	0.108655	0.13 ± 0.14	> 100			
6	$r_{(\mathrm{Pi})}$ / 3	0.118724	0.144 ± 0.039	27.1			
7	offset / 3	0.0224758	0.0037 ± 0.13	>> 100			

3.2. *Wild-type Ras in the presence of Raf*

3.2.1. *H-Ras*

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES

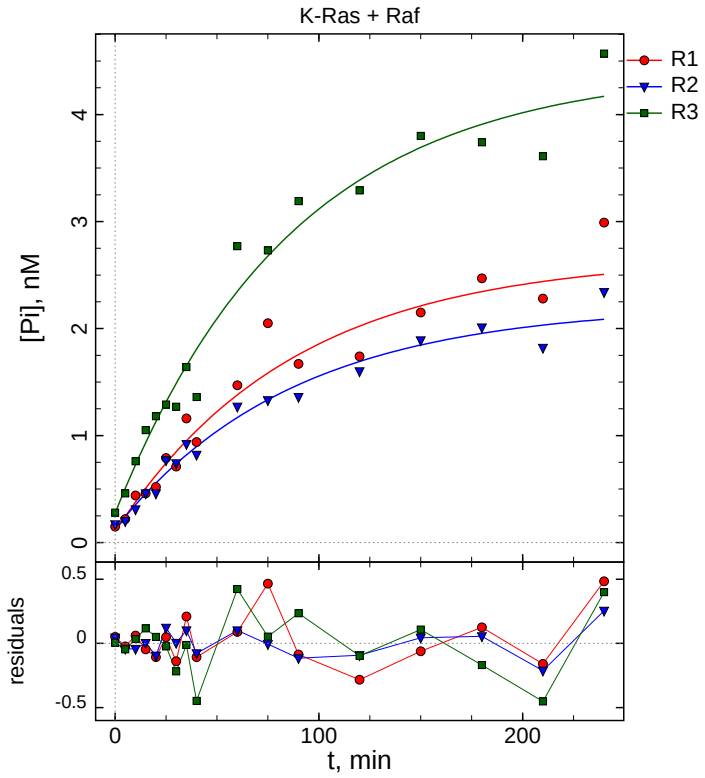


BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.0144875	0.0136 ± 0.0017	12.5	0.0119	0.0154	
2	$r_{(\mathrm{Pi})}$ / 1	0.268946	0.268 ± 0.023	8.6			
3	offset / 1	0.22624	0.26 ± 0.14	53.8			
4	$r_{(\mathrm{Pi})}$ / 2	0.227706	0.233 ± 0.023	9.9			
5	offset / 2	0.278186	0.29 ± 0.14	48.3			
6	$r_{(\mathrm{Pi})}$ / 3	0.468692	0.48 ± 0.024	5.0			
7	offset / 3	0.332573	0.38 ± 0.16	42.1			

3.2.2. *K-Ras*

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES

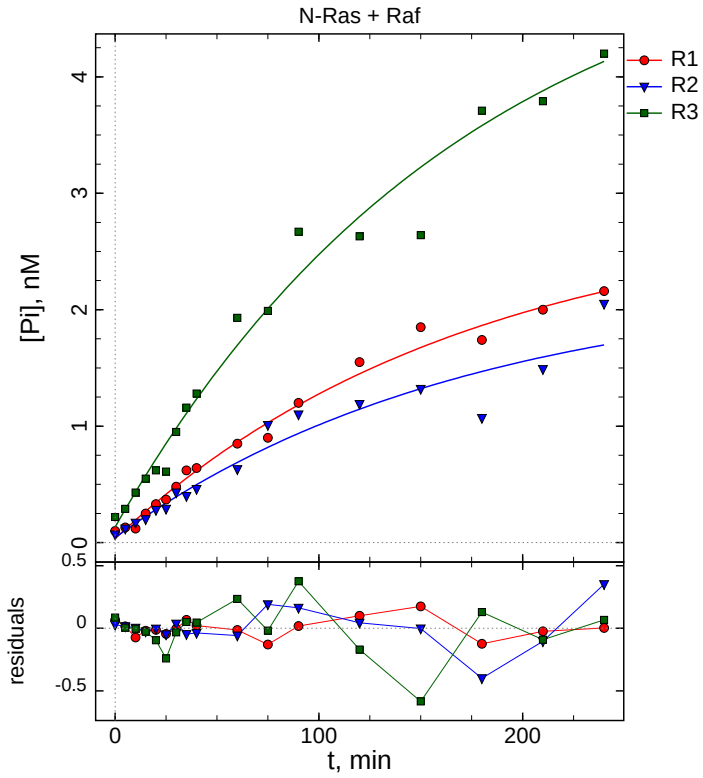


BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.0114947	0.0113 ± 0.0012	10.6	0.01	0.0126	
2	$r_{(Pi)}$ / 1	0.256986	0.268 ± 0.017	6.3			
3	offset / 1	0.0999755	0.094 ± 0.095	> 100			
4	$r_{(Pi)}$ / 2	0.209391	0.212 ± 0.017	8.0			
5	offset / 2	0.125458	0.128 ± 0.092	71.9			
6	$r_{(Pi)}$ / 3	0.415915	0.42 ± 0.019	4.5			
7	offset / 3	0.274938	0.27 ± 0.11	40.7			

3.2.3. *N-Ras*

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES



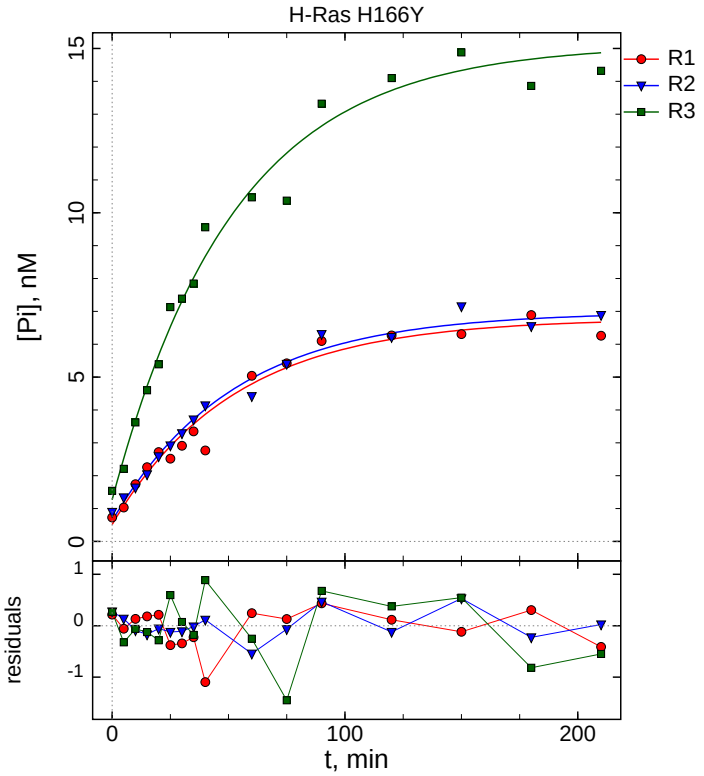
BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.00577513	0.00587 ± 0.00092	15.7	0.00493	0.00684	
2	$r_{(\mathrm{Pi})}$ / 1	0.283162	0.282 ± 0.027	9.6			
3	offset / 1	0.0341227	0.029 ± 0.067	> 100			
4	$r_{(\mathrm{Pi})}$ / 2	0.220826	0.221 ± 0.023	10.4			
5	offset / 2	0.0418638	0.041 ± 0.066	> 100			
6	$r_{(\mathrm{Pi})}$ / 3	0.533286	0.52 ± 0.043	8.3			
7	offset / 3	0.133561	0.14 ± 0.078	55.7			

3.3. Site-directed mutants of H-Ras

3.3.1. H-Ras H166Y

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES

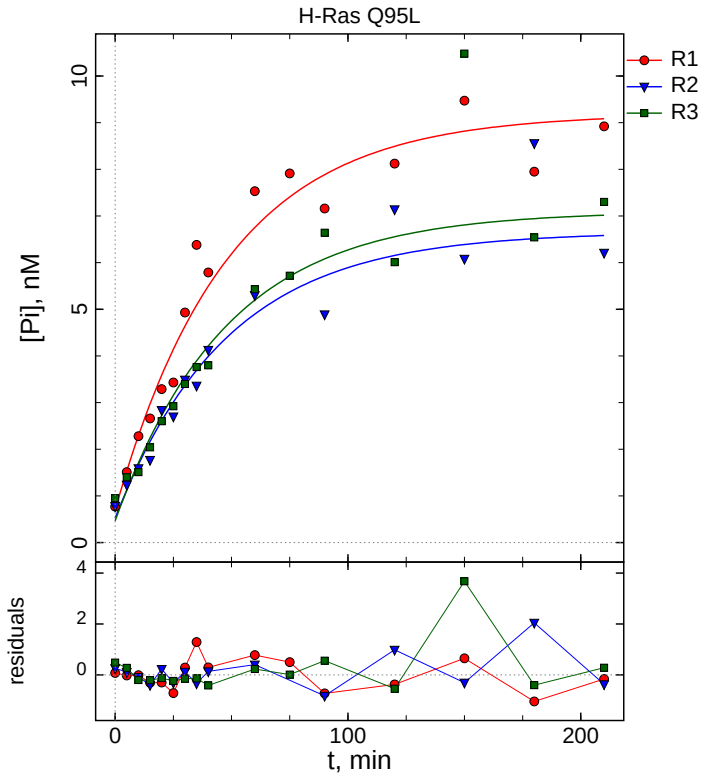


BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.0191252	0.0192 ± 0.0012	6.2	0.018	0.0204	
2	$r_{(Pi)}$ / 1	0.626939	0.627 ± 0.036	5.7			
3	offset / 1	0.515065	0.47 ± 0.24	51.1			
4	$r_{(Pi)}$ / 2	0.635628	0.638 ± 0.036	5.6			
5	offset / 2	0.623862	0.61 ± 0.24	39.3			
6	$r_{(Pi)}$ / 3	1.38483	1.361 ± 0.036	2.6			
7	offset / 3	1.27014	1.35 ± 0.28	20.7			

3.3.2. *H-Ras Q95L*

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES

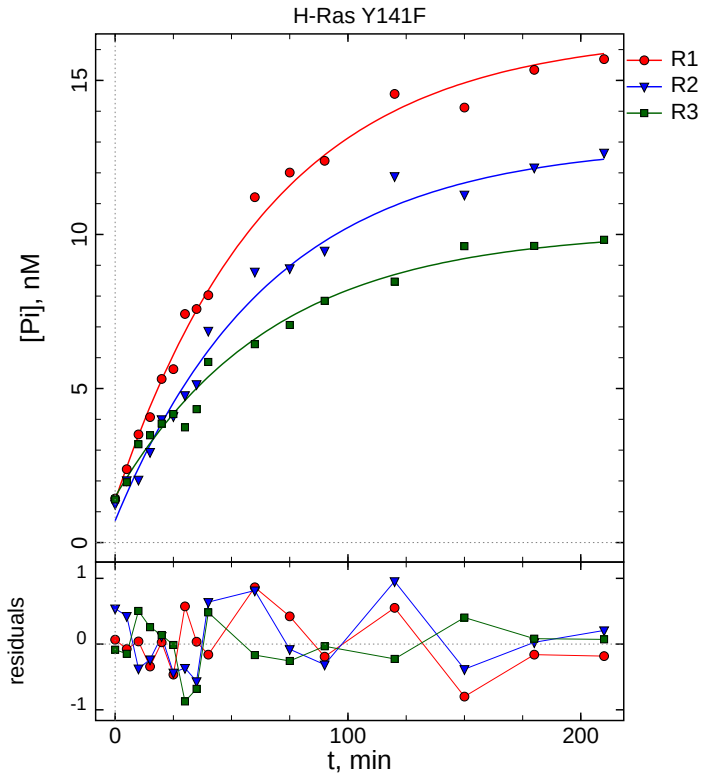


BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.0208156	0.0194 ± 0.0027	13.9	0.0168	0.0221	
2	$r_{(\mathrm{Pi})}$ / 1	0.849533	0.839 ± 0.063	7.5			
3	offset / 1	0.694502	0.91 ± 0.45	49.5			
4	$r_{(\mathrm{Pi})}$ / 2	0.613619	0.662 ± 0.063	9.5			
5	offset / 2	0.521035	0.47 ± 0.43	91.5			
6	$r_{(\mathrm{Pi})}$ / 3	0.663194	0.742 ± 0.063	8.5			
7	offset / 3	0.466624	0.34 ± 0.44	> 100			

3.3.3. *H-Ras Y141F*

EXPERIMENTAL DATA AND BEST-FIT MODEL CURVES



BEST-FIT MODEL PARAMETERS

#	par/set	initial	final ± std.err.	cv,%	low	high	note
1	k_{hyd}	0.0149559	0.01521 ± 0.00084	5.5	0.01438	0.01605	
2	$r_{(\mathrm{Pi})}$ / 1	1.51644	1.508 ± 0.038	2.5			
3	offset / 1	1.36308	1.36 ± 0.25	18.4			
4	$r_{(\mathrm{Pi})}$ / 2	1.22633	1.233 ± 0.038	3.1			
5	offset / 2	0.709562	0.69 ± 0.24	34.8			
6	$r_{(\mathrm{Pi})}$ / 3	0.86486	0.866 ± 0.037	4.3			
7	offset / 3	1.48457	1.41 ± 0.22	15.6			

References

[1] P. Kuzmic, Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV proteinase, Anal. Biochem. 237 (1996) 260–273.

[2] P. Kuzmic, DynaFit–a software package for enzymology, Meth. Enzymol. 467 (2009) 247–280.

[3] J. M. Beechem, Global analysis of biochemical and biophysical data, Meth. Enzymol. 210 (1992) 37–54.