Supporting Information

GPU-Accelerated Implementation of Continuous Constant pH Molecular Dynamics in Amber: pK_a Predictions with Single-pH Titration Simulations

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Table S1: Calculated pK_a's for SNase, RNase H, and BACE1 from pH replica-exchange simulations with different exchange attempts in comparison to single-pH simulations and experiment

Protein	Residue	Expt	2 ps^{-1}	1 ps^{-1}	0.5 ps^{-1}	Single	Protein	Residue	Expt	2 ps^{-1}	Single	Single (10 ns)
SNase	His8	6.5	6.5	6.3	6.3	6.4(0.06)	**BACE1**	Glu1		4.0	4.0(0.03)	4.2(0.01)
	Glu10	2.8	3.7	3.9	4.1	4.0(0.02)		Asp4		5.0	5.3(0.03)	5.1(0.03)
	Asp19*	2.2	1.8	1.2	1.4	1.5(0.17)		Glu17		3.2	4.2(0.04)	4.5(0.10)
	Asp21*	6.5	4.1	6.2	6.0	6.1(0.17)		Asp32*	5.2	4.2	6.6(0.25)	6.8(0.05)
	Asp40	3.9	2.8	3.2	3.3	3.2(0.09)		His45		5.5	5.6(0.06)	5.5(0.08)
	Glu43	4.3	3.7	3.4	3.4	3.7(0.10)		His49		6.4	6.1(0.07)	6.1(0.08)
	Glu52	3.9	3.9	3.8	3.8	3.8(0.04)		Asp62		1.7	2.6(0.09)	2.3(0.06)
	Glu57	3.5	3.4	3.5	3.5	3.5(0.02)		Glu77		4.3	4.5(0.04)	4.4(0.02)
	Glu67	3.8	4.5	4.3	4.2	4.2(0.01)		Glu79		4.7	4.2(0.16)	4.4(0.08)
	Glu73	3.3	3.9	3.7	3.7	3.8(0.04)		Asp83		5.3	2.2(0.07)	3.4(0.16)
	Glu75	3.3	2.6	3.3	3.6	3.4(0.22)		His89		7.4	7.3(0.07)	7.1(0.04)
	Asp77	$<$2.2	1.9	2.3	2.2	2.0(0.07)		Glu104		3.9	3.9(0.04)	3.7(0.03)
	Asp83	$<$2.2	2.1	2.4	2.6	2.6(0.31)		Asp106		2.8	2.8(0.03)	2.8(0.02)
	Asp95	2.2	4.3	4.9	4.5	4.3(0.11)		Glu116		7.0	6.2(0.07)	5.7(0.08)
	Glu101	3.8	3.5	3.7	3.6	3.9(0.08)		Glu125		3.8	4.2(0.04)	4.1(0.02)
	His121	5.2	6.8	6.9	6.7	6.7(0.05)		Asp130		1.8	1.8(0.06)	2.4(0.23)
	Glu122	3.9	3.0	3.3	4.0	3.3(0.11)		Asp131		4.3	3.8(0.05)	3.8(0.04)
	Glu129	3.8	4.5	4.2	4.2	4.2(0.05)		Glu134		4.4	4.7(0.05)	4.8(0.06)
	Glu135	3.8	4.2	4.0	4.1	3.9(0.05)		Asp138		3.2	3.5(0.07)	4.1(0.09)
	rmse†		1.03	0.93	0.84	0.79		His145		6.5	6.3(0.08)	6.0(0.04)
	rmsd‡		0.31	0.2	0.24	-		Glu165		4.1	4.0(0.03)	3.7(0.07)
RNase H	Glu6	4.5	4.3	4.9		4.9(0.29)		Asp180		2.4	2.8(0.05)	2.7(0.09)
	Asp10*	6.1	4.8	6.7		7.1(0.18)		His181		6.4	6.7(0.03)	6.3(0.03)
	Glu32	3.6	3.2	3.1		3.3(0.09)		Glu196		3.3	3.2(0.07)	2.9(0.10)
	Glu48	4.4	2.5	3.0		3.8(0.12)		Glu200		3.5	3.0(0.03)	3.4(0.08)
	Glu57	3.2	4.1	4.6		4.7(0.06)		Glu207		2.4	3.6(0.10)	3.4(0.05)
	Glu61	3.9	2.8	2.4		2.4(0.05)		Asp212		1.2	0.9(0.02)	1.4(0.10)
	His62	7.0	6.9	6.9		6.6(0.07)		Asp216		1.2	2.0(0.04)	2.2(0.04)
	Glu64	4.4	3.1	3.3		3.4(0.04)		Glu219		3.5	3.9(0.04)	3.8(0.05)
	Asp70*	2.6	2.6	1.5		1.7(0.17)		Asp223		4.2	4.5(0.05)	4.8(0.02)
	His83	5.5	6.2	6.0		6.1(0.05)		Asp228*	3.5	2.4	< 1	< 1
	Asp94	3.2	3.2	2.9		3.0(0.07)		Glu242		4.2	4.2(0.02)	4.3(0.01)
	Asp102	$<$2.0	3.4	3.9		3.5(0.15)		Glu255		3.8	4.1(0.04)	4.0(0.04)
	Asp108	3.2	3.1	2.8		3.0(0.04)		Asp259		3.1	2.7(0.03)	2.8(0.02)
	His114	$<$5.0	7.0	6.3		6.8(0.05)		Glu265		4.0	3.9(0.03)	3.9(0.02)
	Glu119	4.1	3.9	3.8		3.8(0.04)		Glu290		3.0	3.6(0.04)	3.5(0.02)
	His124	7.1	6.2	5.5		5.5(0.06)		Glu310		3.8	3.5(0.10)	3.9(0.07)
	His127	7.9	6.6	6.6		6.4(0.02)		Asp311		2.6	3.4(0.19)	3.9(0.16)
	Glu129	3.6	4.6	3.3		3.3(0.05)		Asp317		4.4	3.6(0.04)	3.7(0.06)
	Glu131	4.3	4.3	4.5		4.5(0.05)		Asp318		5.1	4.4(0.13)	4.2(0.07)
	Asp134	4.1	4.3	4.1		4.3(0.12)		Glu339		5.5	5.2(0.19)	4.7(0.13)
	Glu135	4.3	4.2	4.1		4.2(0.04)		Asp346		1.9	1.6(0.01)	2.3(0.13)
	Glu147	4.2	3.9	3.6		3.7(0.09)		His360		4.8	4.7(0.09)	6.2(0.22)
	Asp148	$<$2.0	2.4	2.6		2.8(0.05)		His362		7.0	7.7(0.07)	6.8(0.14)
	Glu154	4.4	3.8	3.2		3.6(0.05)		Asp363		2.6	3.0(0.13)	2.8(0.07)
	rmse†		0.81	0.88		0.83		Glu364		4.2	4.0(0.03)	4.0(0.03)
	rmsd‡		0.56	0.24		-		Glu371		3.5	3.2(0.06)	3.2(0.05)
								Asp378		5.2	4.7(0.07)	4.1(0.19)
								Glu380		3.8	4.1(0.06)	4.0(0.03)
								Asp381		4.6	4.0(0.04)	4.0(0.04)

*Residues undergoing linked titration. These pK_a values were obtained from best fits to the two-proton titration model. † Root-mean-square error with respect to the experimental pK_a's. ‡ Root-mean-square deviation with respect to the pK_a's from 2-ns single-pH simulations. For BACE1, results from the 10-ns single pH simulations are also listed.

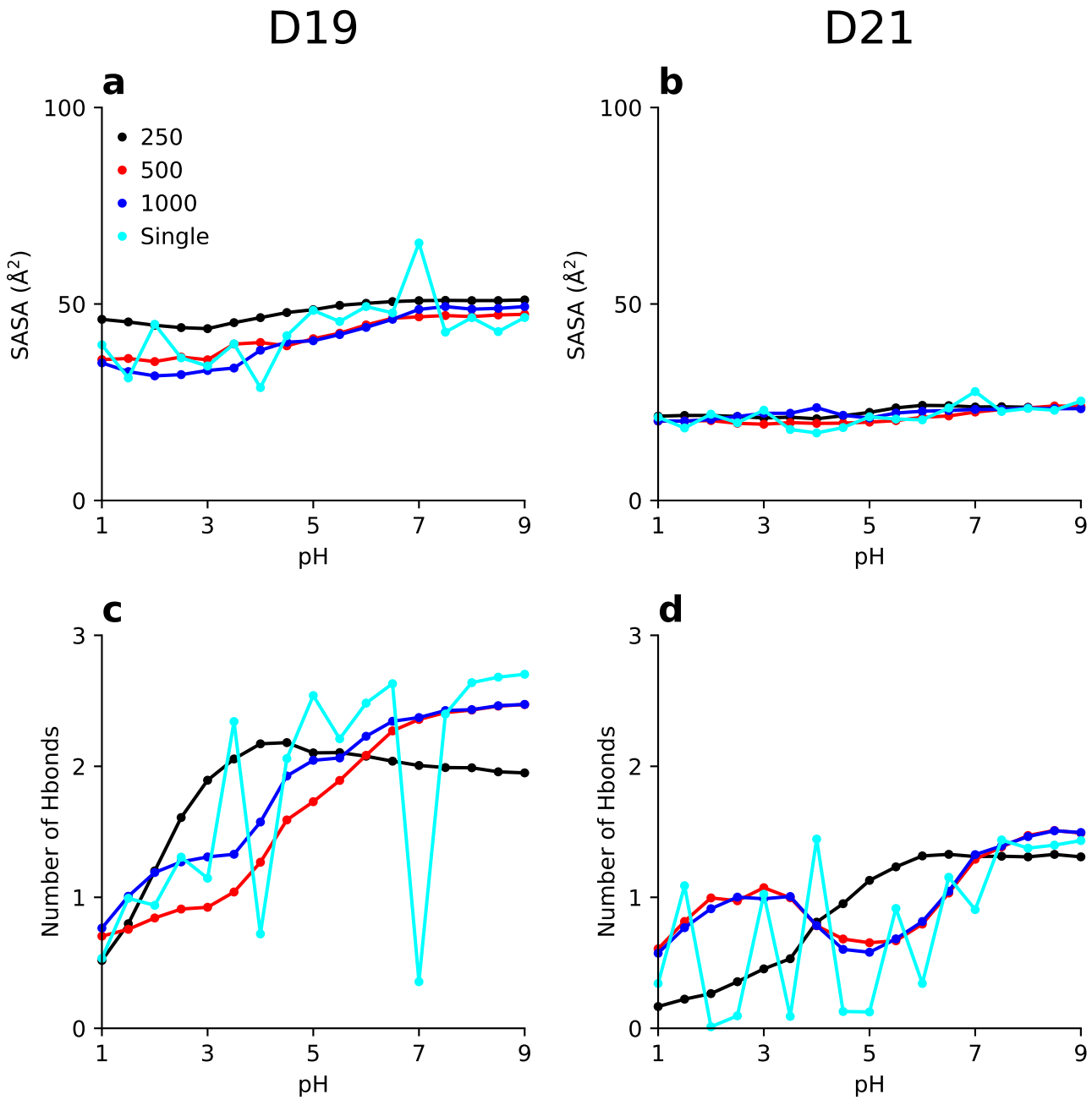


Figure S1: The solvent-accessible surface area (SASA) and number of hydrogen bonds as functions of pH for Asp 19 and Asp 21 in SNase from the replica-exchange simulations with 250 steps between replica exchange attempts, 500 steps between attempts, 1000 steps between attempts, and from the single-pH simulations.

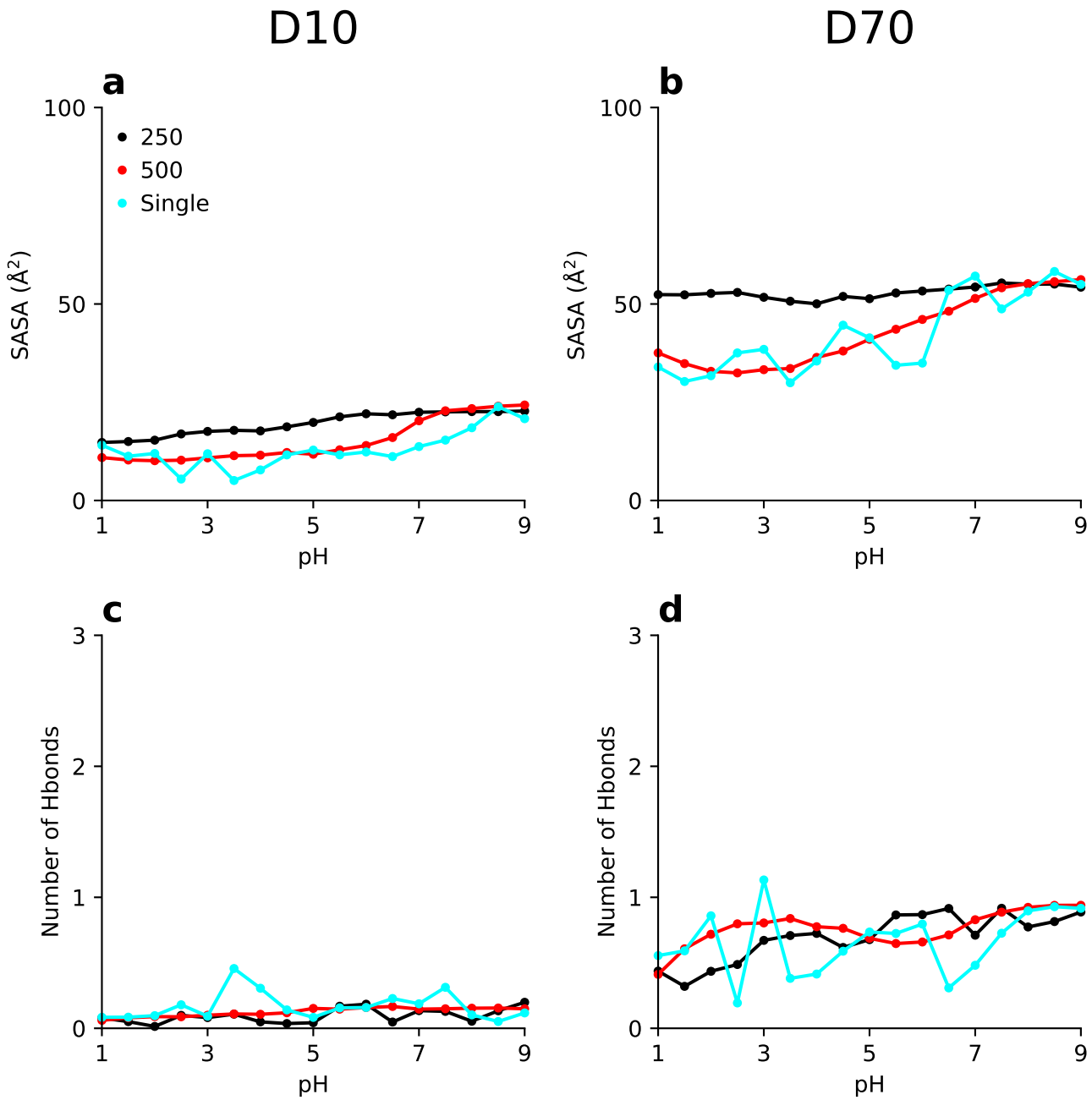


Figure S2: The solvent-accessible surface area (SASA) and number of hydrogen bonds as functions of pH for Asp 10 and Asp 70 in RNase H from the replica-exchange simulations with 250 steps between replica exchange attempts, 500 steps between attempts, 1000 steps between attempts, and from the single-pH simulations.

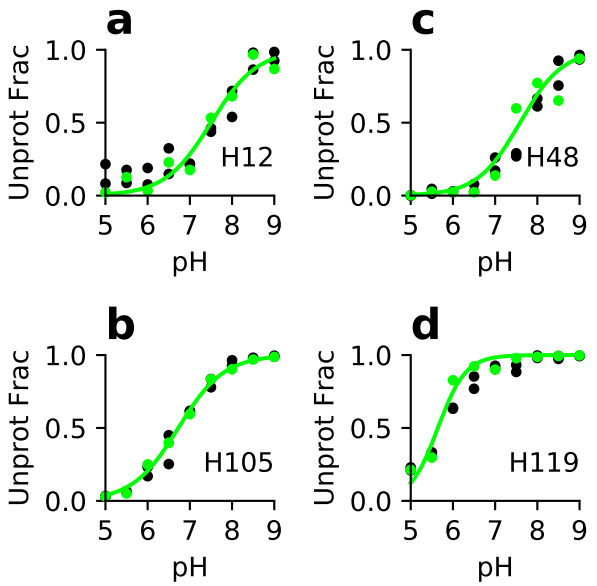


Figure S3: **The data underlying the bootstrap error analysis for the His ((a) H12 (b) H105 (c) H48 (d) H119) residues in RNase A.** Three single-pH simulations were run at pH = 5, 6, 7, 8, and 9. The first data set and fits to the Henderson-Hasselbalch equation are in green.

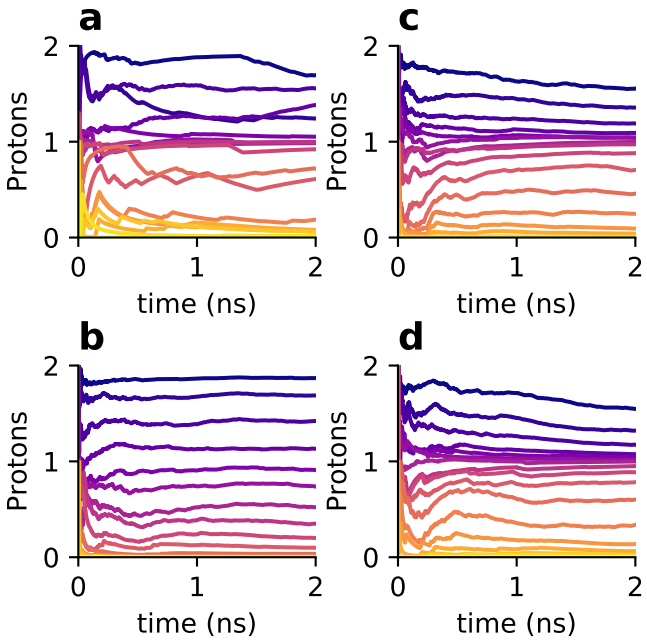


Figure S4: **Total number of protons on Asp19 and Asp21 in SNase as a function of time.** (a) Single-pH simulations. Replica-exchange simulations with swap attempt rates of (b) 2 ps^{1} and (c) 1 ps^{1}.

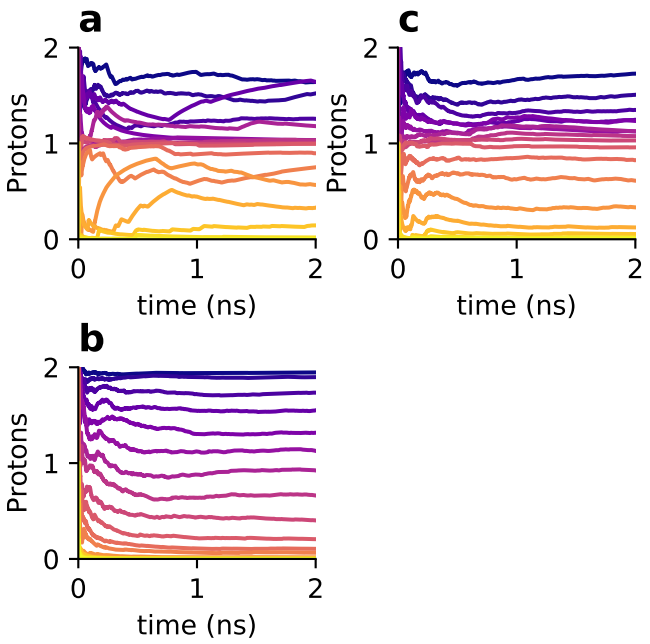
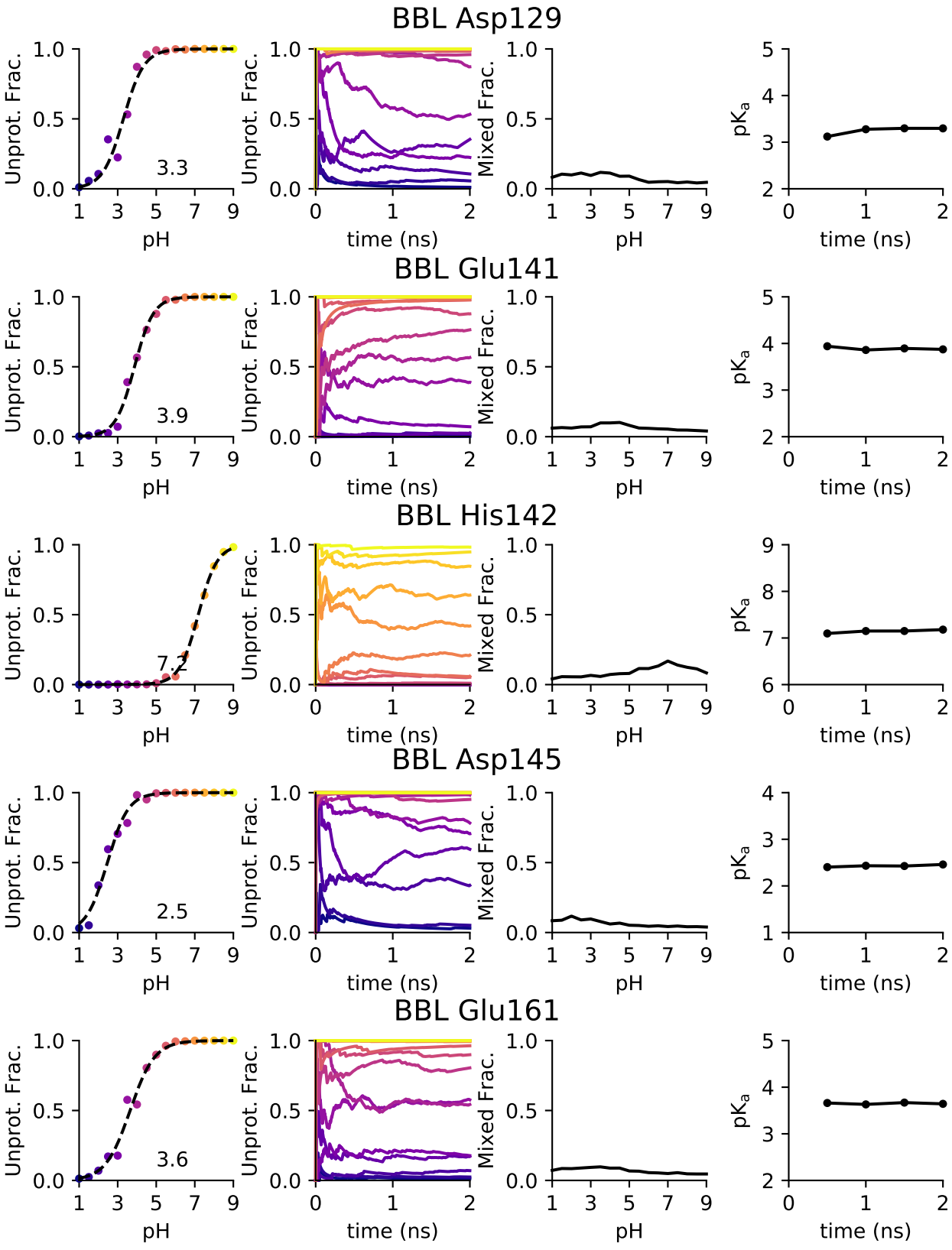
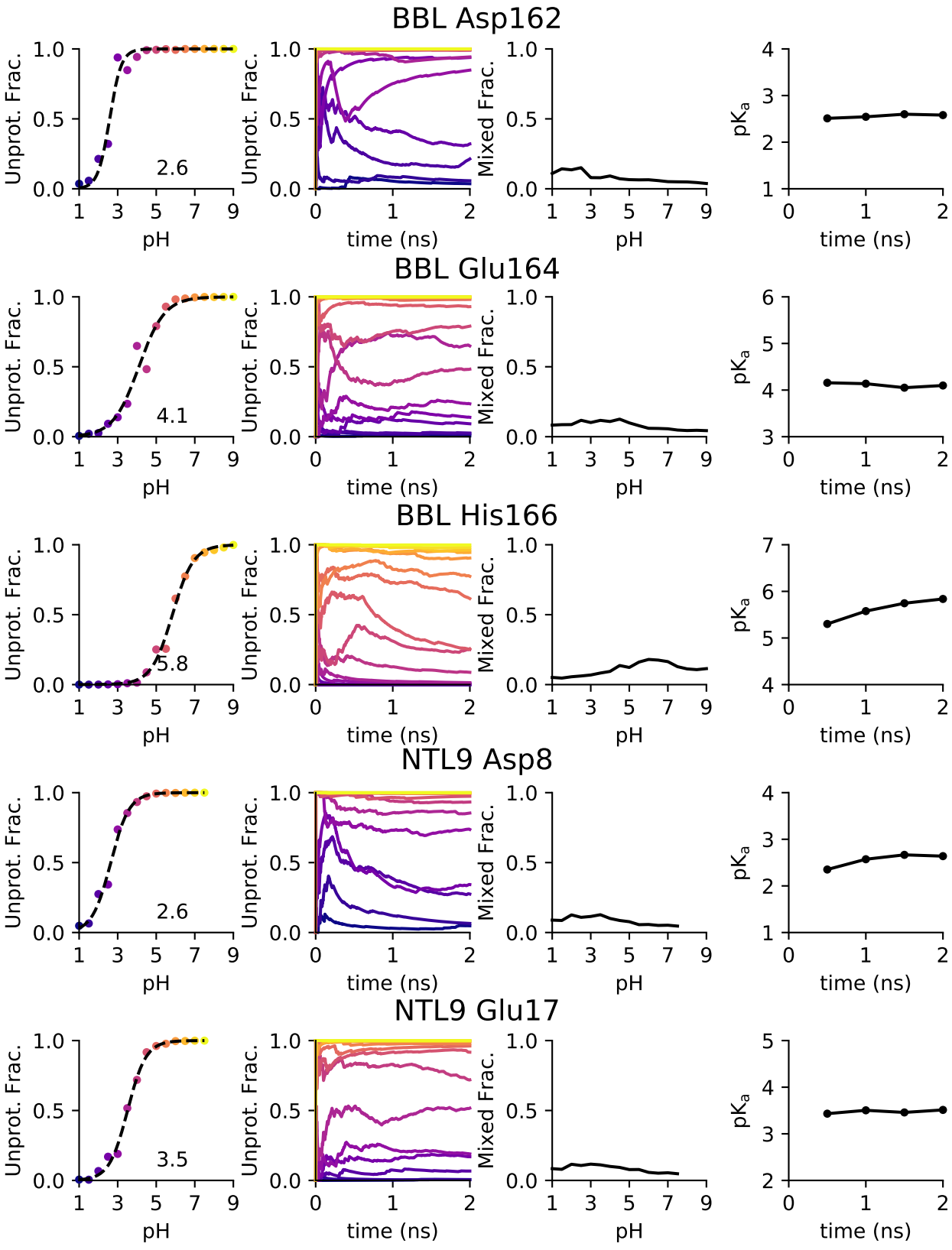
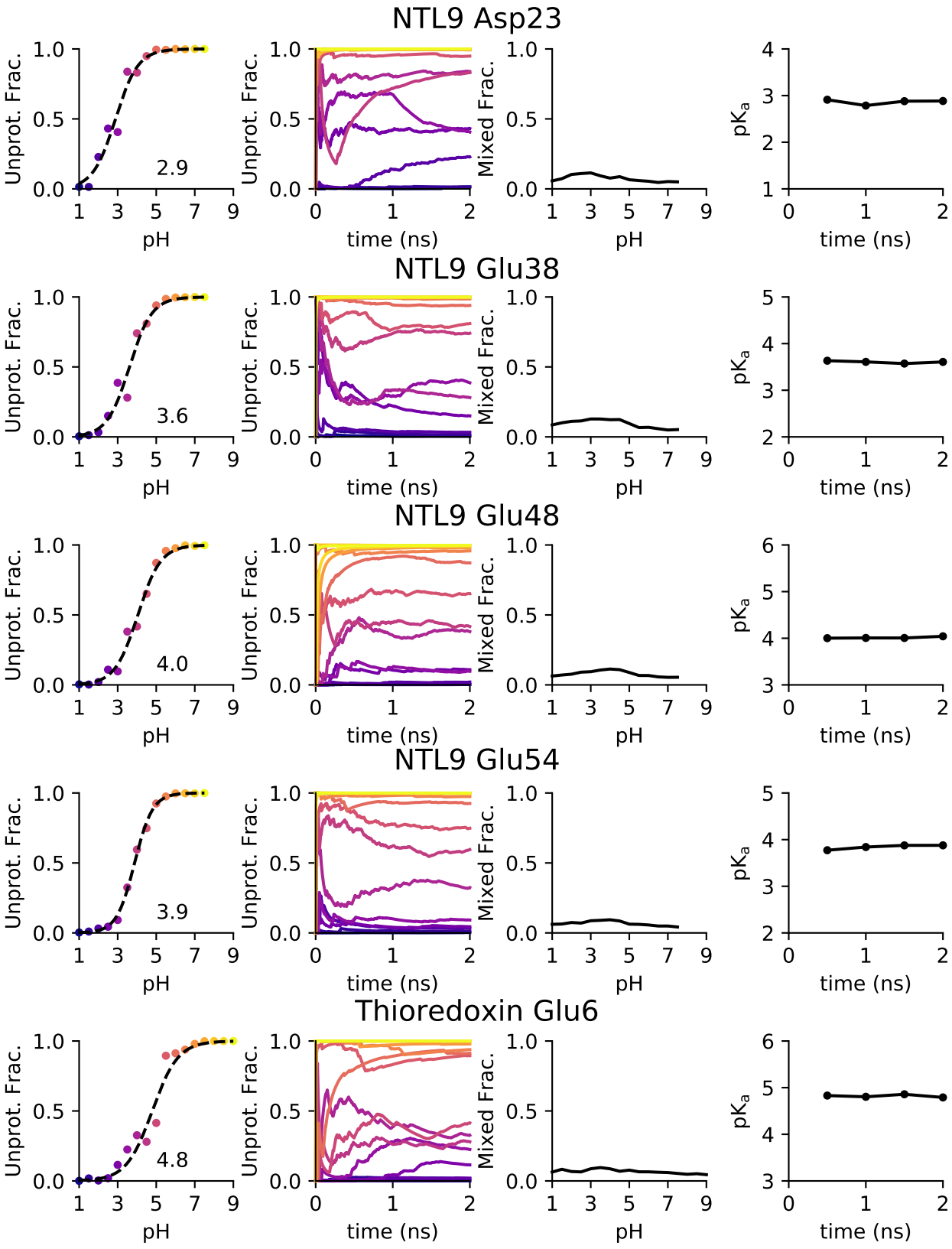
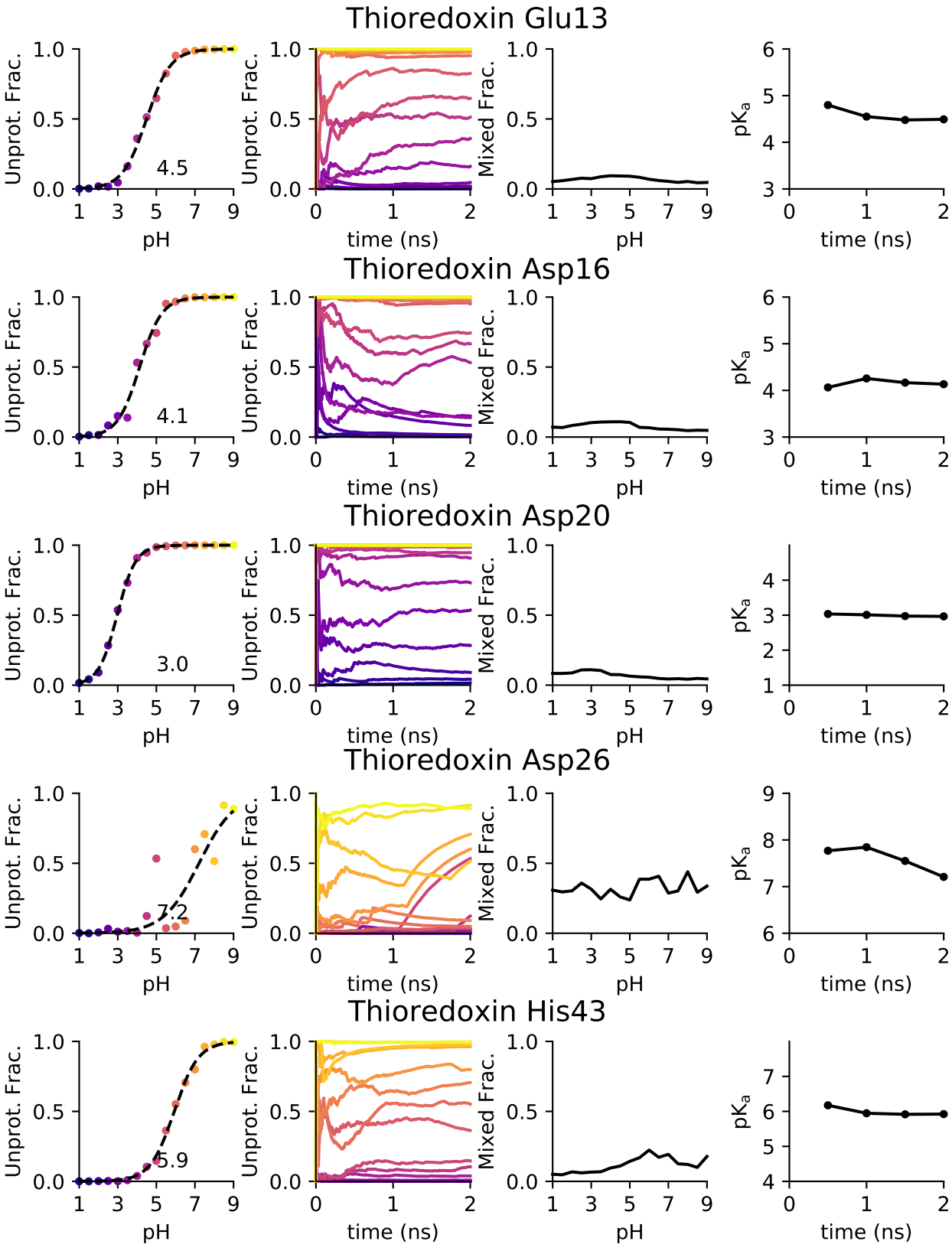


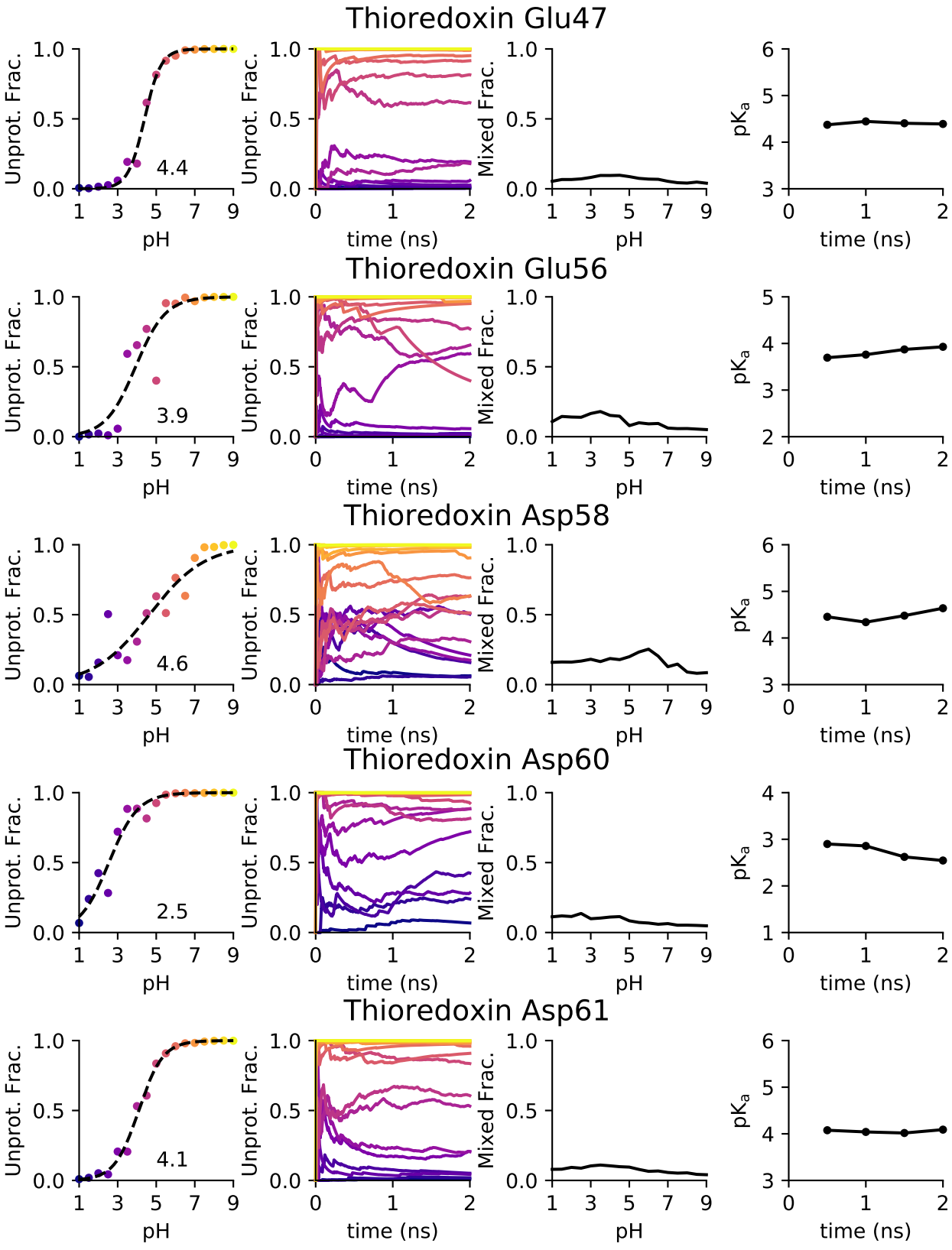
Figure S5: **Total number of protons on Asp10 and Asp70 in RNaseH as a function of time.** (a) Single-pH simulations. Replica-exchange simulations with swap attempt rates of (b) 2 ps^{1} and (c) 1 ps^{1}.


BBL Asp129
Unprot. Frac.
3.3
pH
time (ns)
Mixed Frac.
pKa
BBL Glu141
3.9
BBL His142
7.2
BBL Asp145
2.5
BBL Glu161
3.6


BBL Asp162
Unprot. Frac.
pH
2.6
time (ns)
Mixed Frac.
pK$_a$
BBL Glu164
4.1
BBL His166
5.8
NTL9 Asp8
2.6
NTL9 Glu17
3.5


NTL9 Asp23
NTL9 Glu38
NTL9 Glu48
NTL9 Glu54
Thioredoxin Glu6
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
2.9
3.6
4.0
3.9
4.8


Thioredoxin Glu13
Unprot. Frac.
pH
4.5
time (ns)
Mixed Frac.
pK$_a$
Thioredoxin Asp16
4.1
Thioredoxin Asp20
3.0
Thioredoxin Asp26
7.2
Thioredoxin His43
5.9


Thioredoxin Glu47
Unprot. Frac.
pH
4.4
time (ns)
Mixed Frac.
pK$_a$
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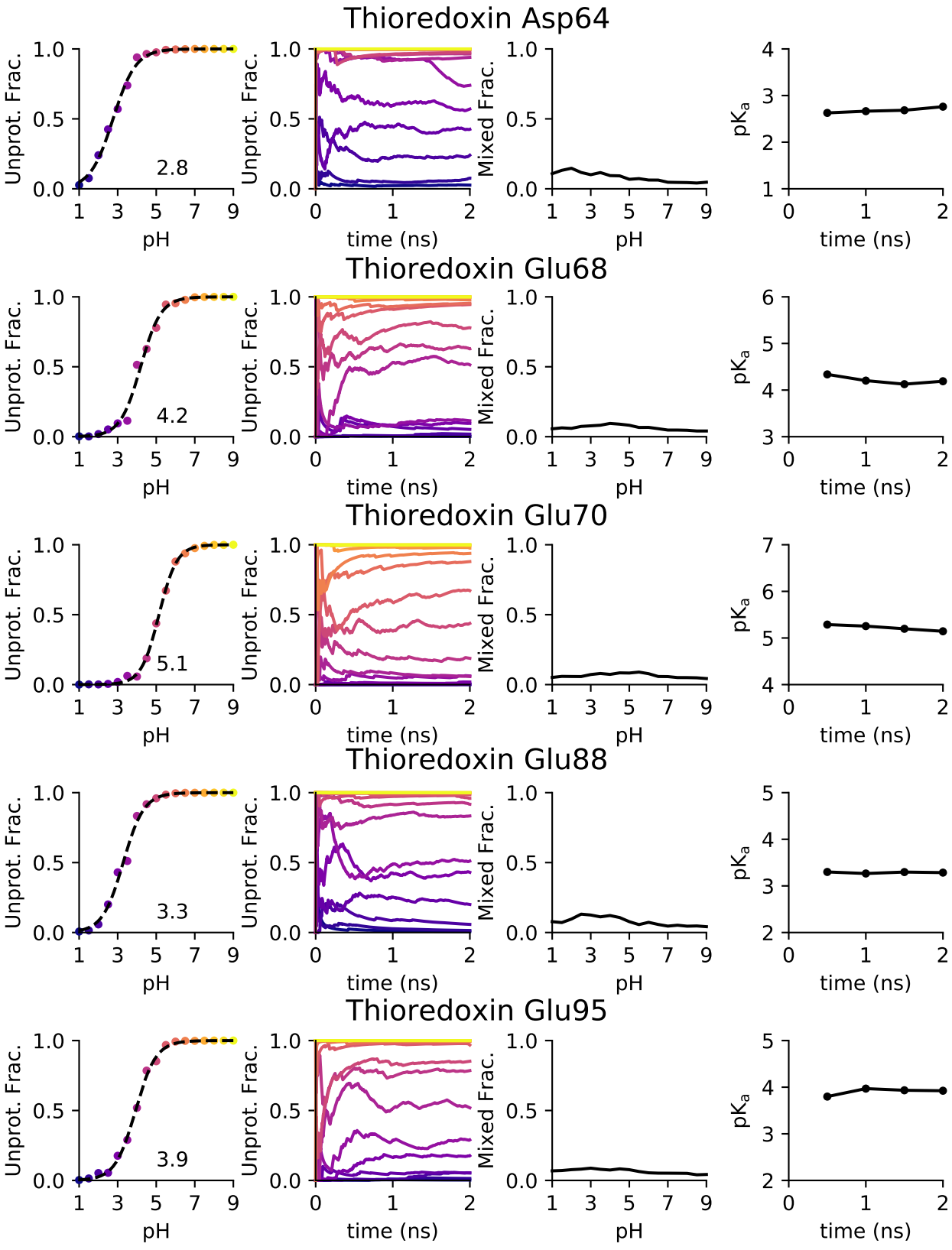
3.9
Thioredoxin Asp58
4.6
Thioredoxin Asp60
2.5
Thioredoxin Asp61
4.1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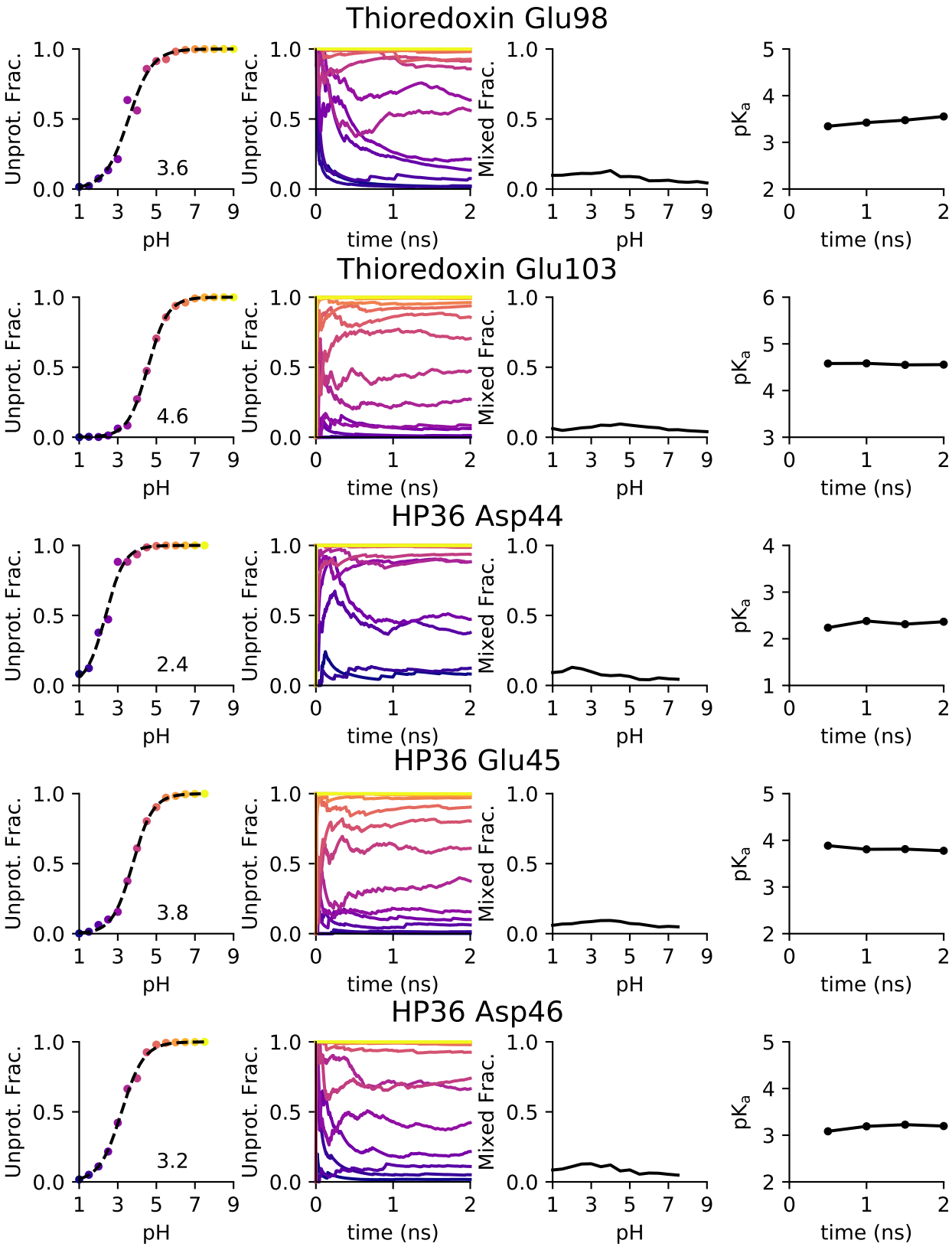
Unprot. Frac.
pH
2.8
time (ns)
Mixed Frac.
pK$_a$
Thioredoxin Glu68
4.2
Thioredoxin Glu70
5.1
Thioredoxin Glu88
3.3
Thioredoxin Glu95
3.9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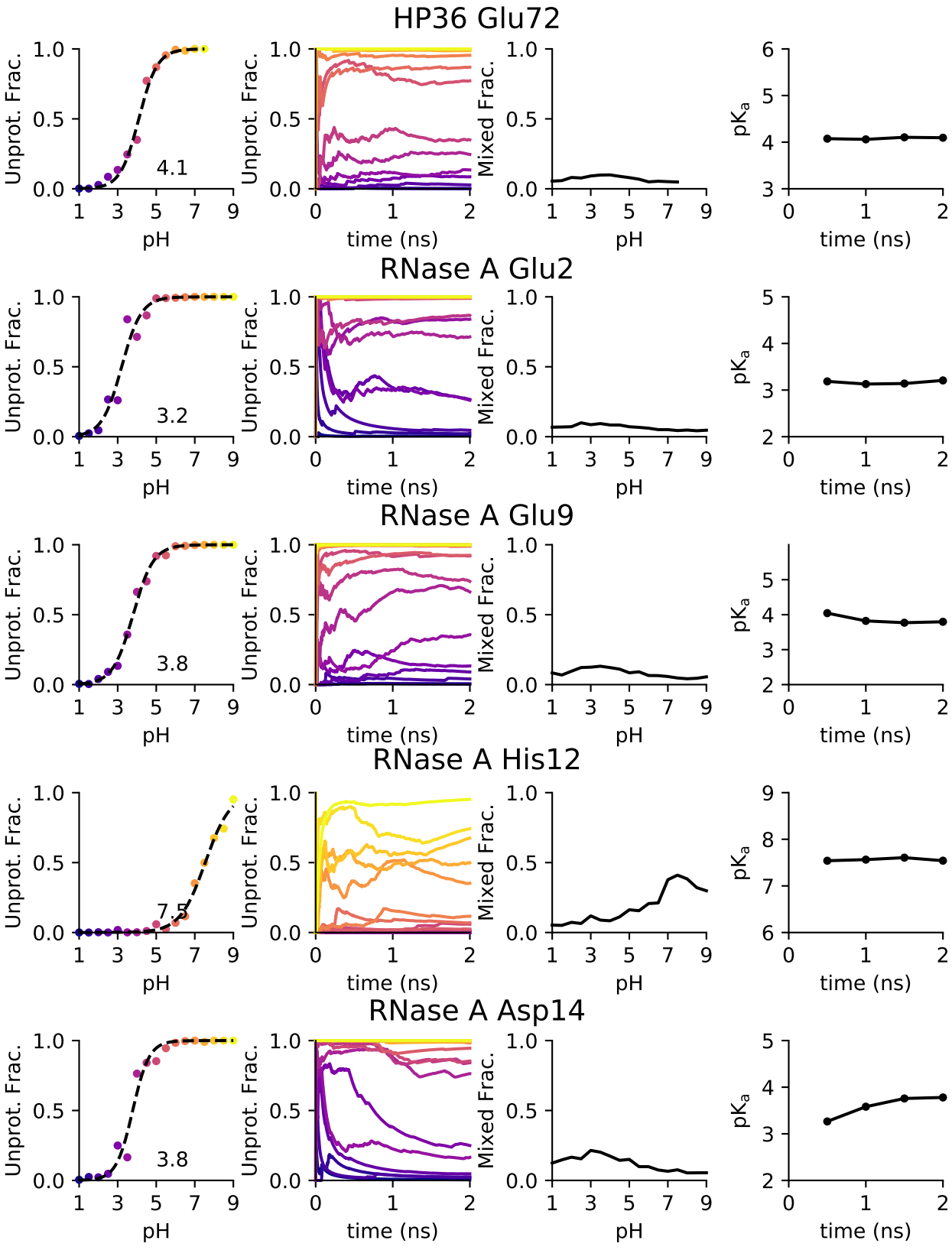
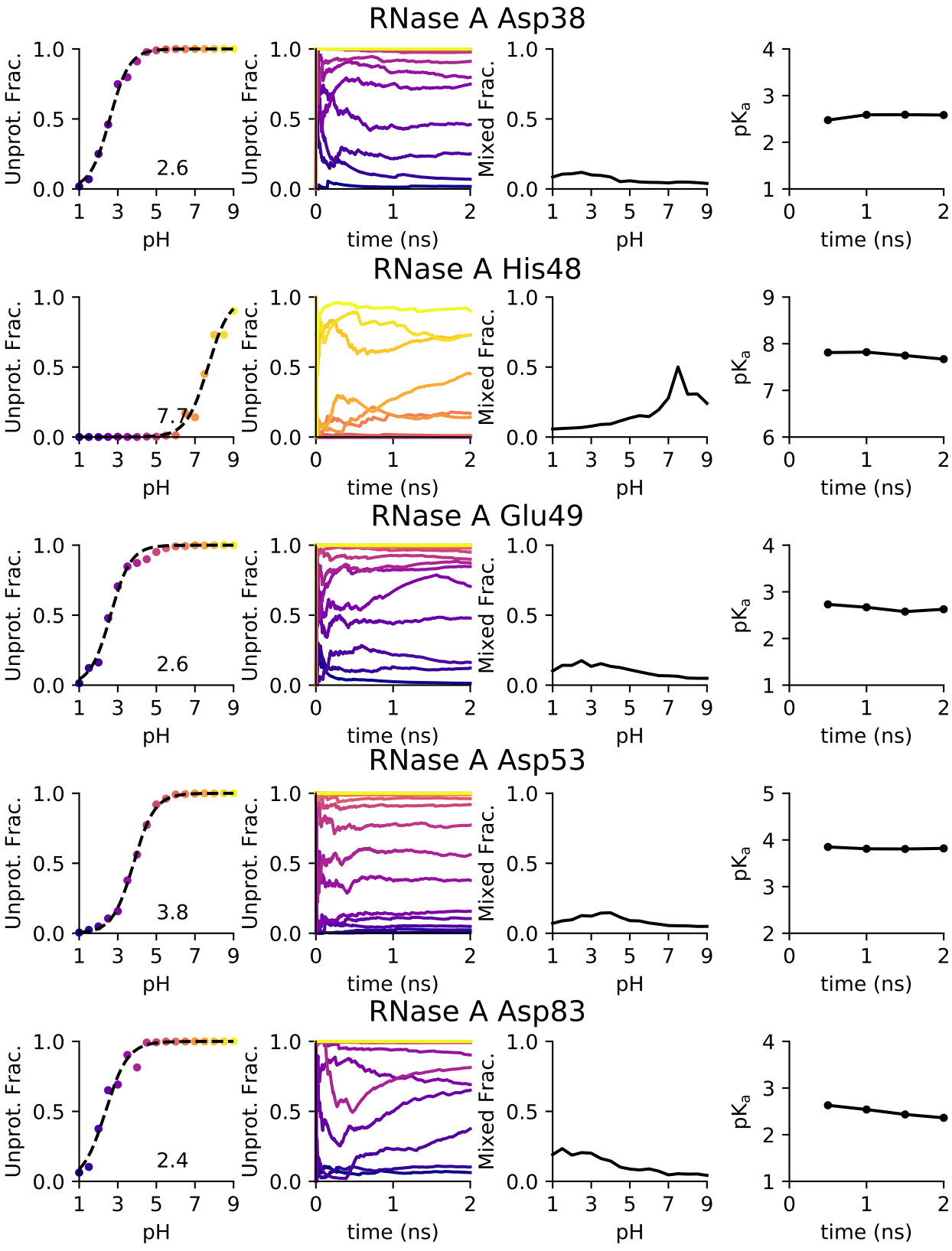
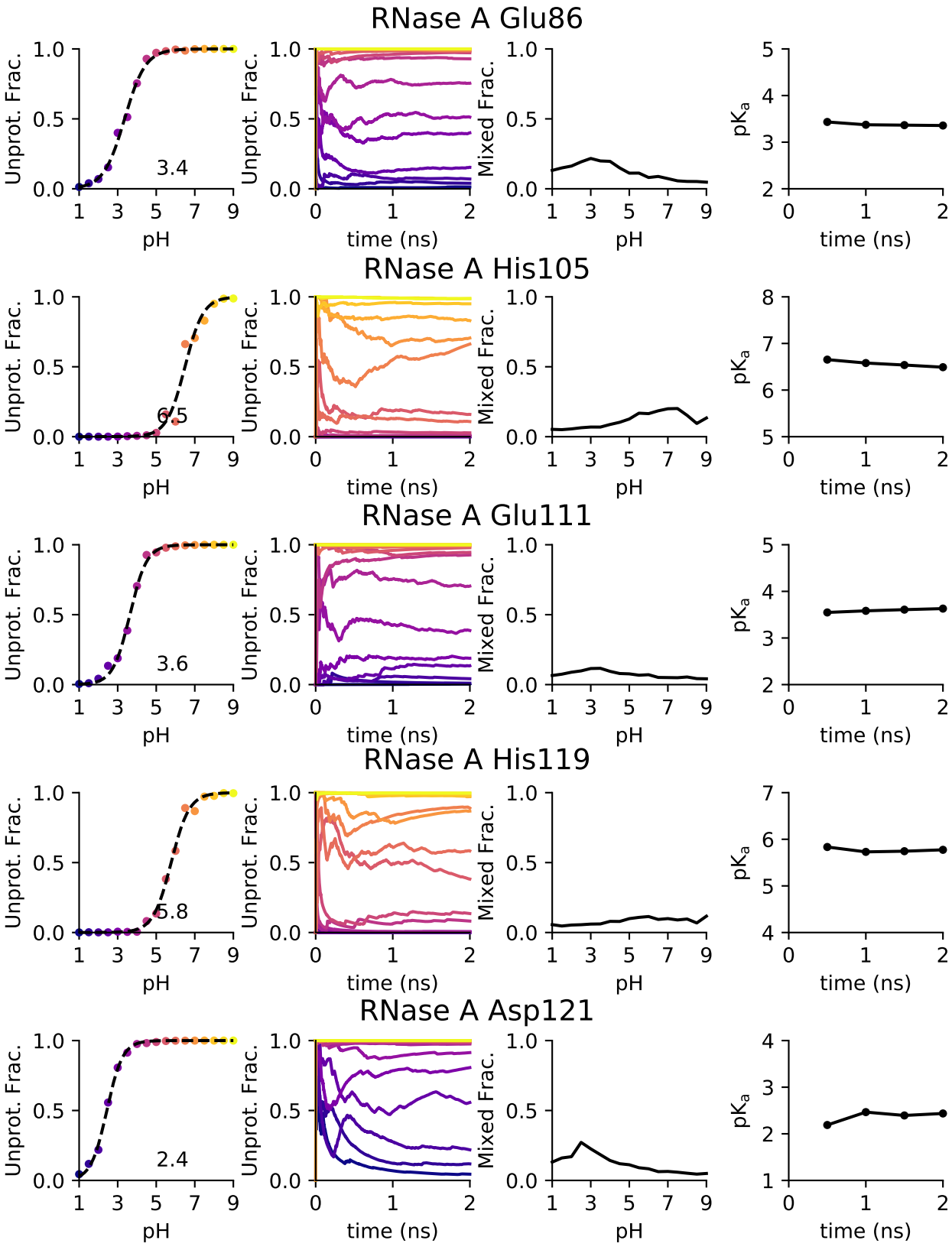
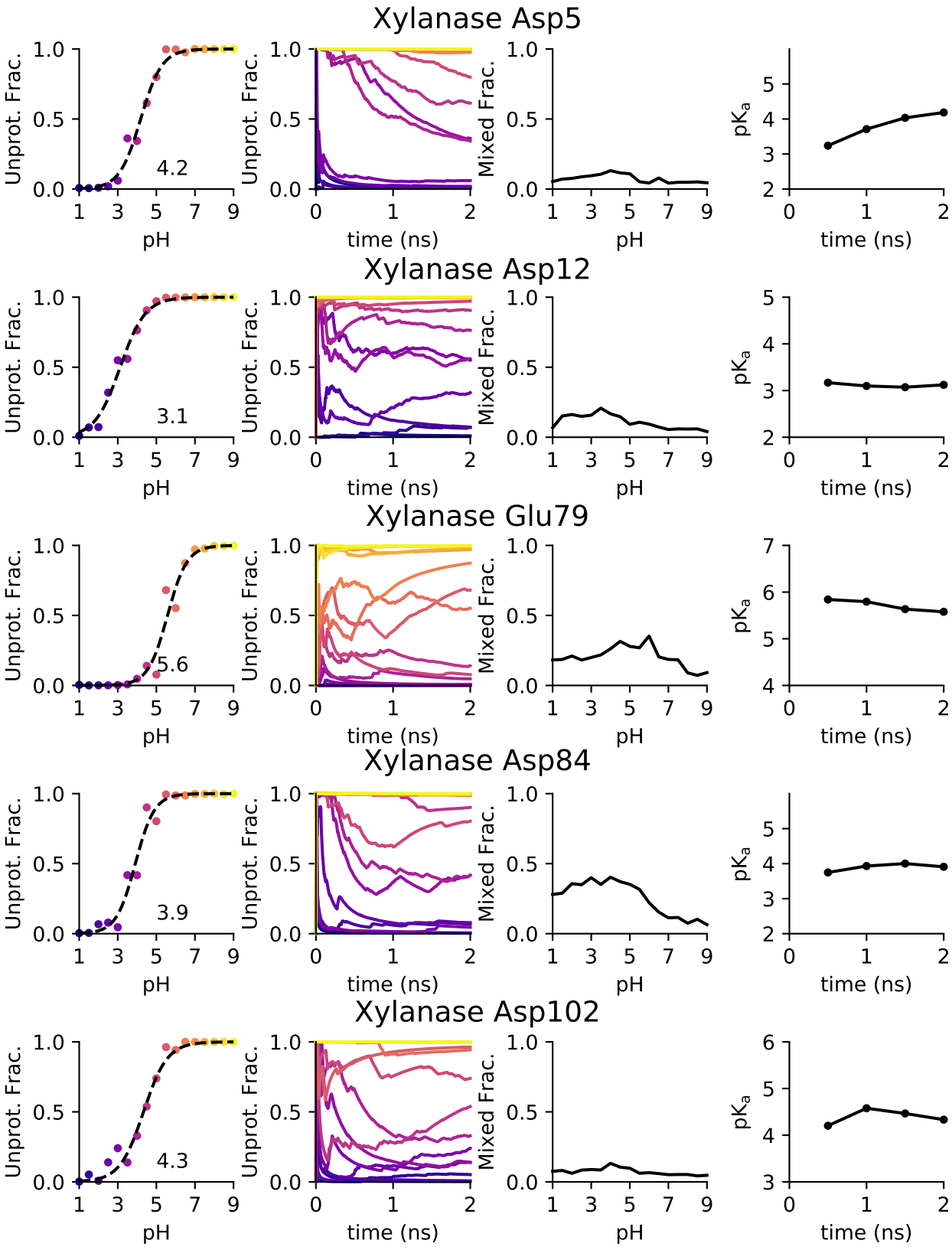
Unprot. Frac.
pH
3.6
time (ns)
Mixed Frac.
pK$_a$
Thioredoxin Glu103
4.6
HP36 Asp44
2.4
HP36 Glu45
3.8
HP36 Asp46
3.2


HP36 Glu72
RNase A Glu2
RNase A Glu9
RNase A His12
RNase A Asp14
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
4.1
3.2
3.8
7.5
3.8


RNase A Asp38
RNase A His48
RNase A Glu49
RNase A Asp53
RNase A Asp83
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
2.6
7.7
2.6
3.8
2.4


RNase A Glu86
Unprot. Frac.
pH
3.4
time (ns)
Mixed Frac.
pKa
RNase A His105
6.5
RNase A Glu111
3.6
RNase A His119
5.8
RNase A Asp121
2.4


Xylanase Asp5
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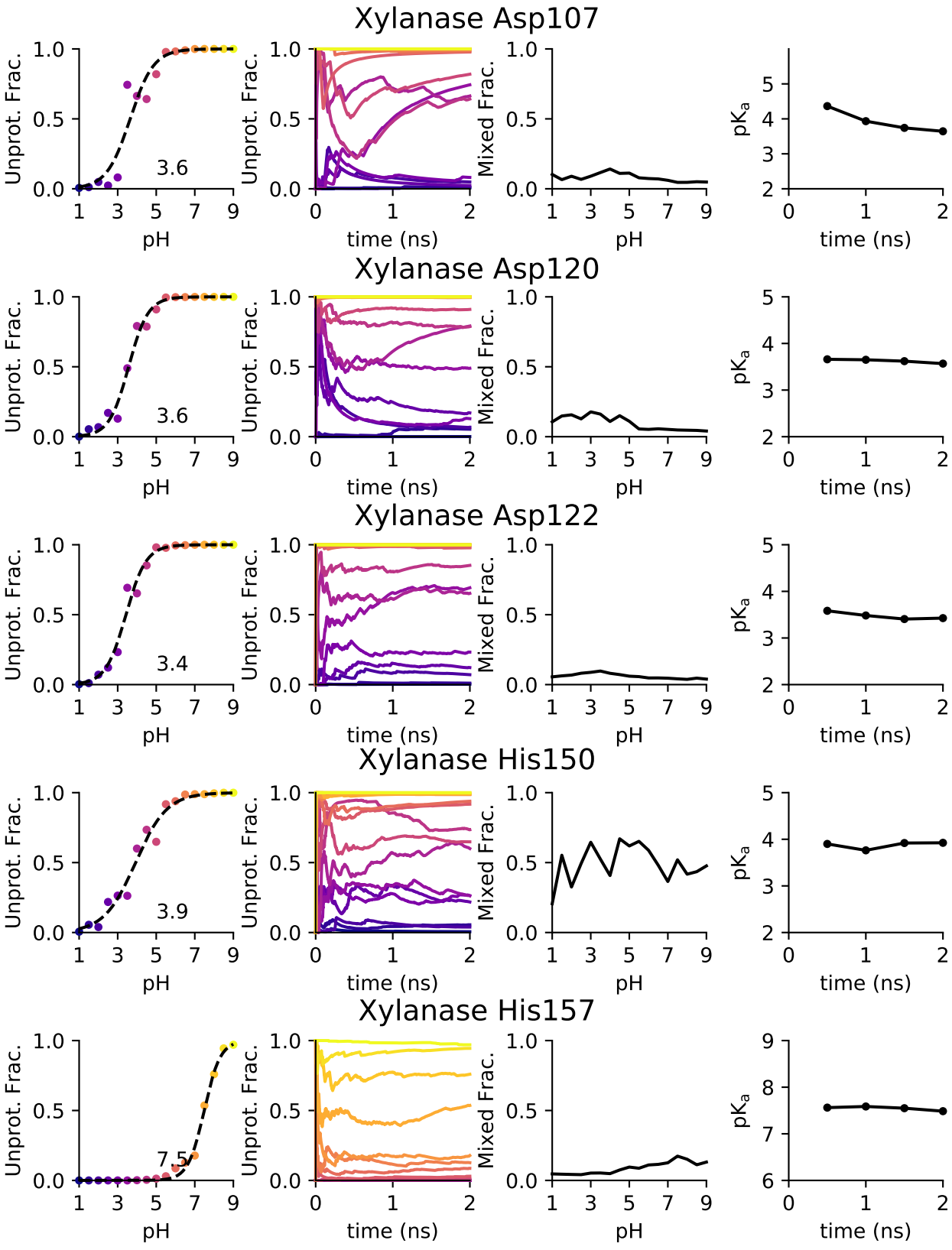
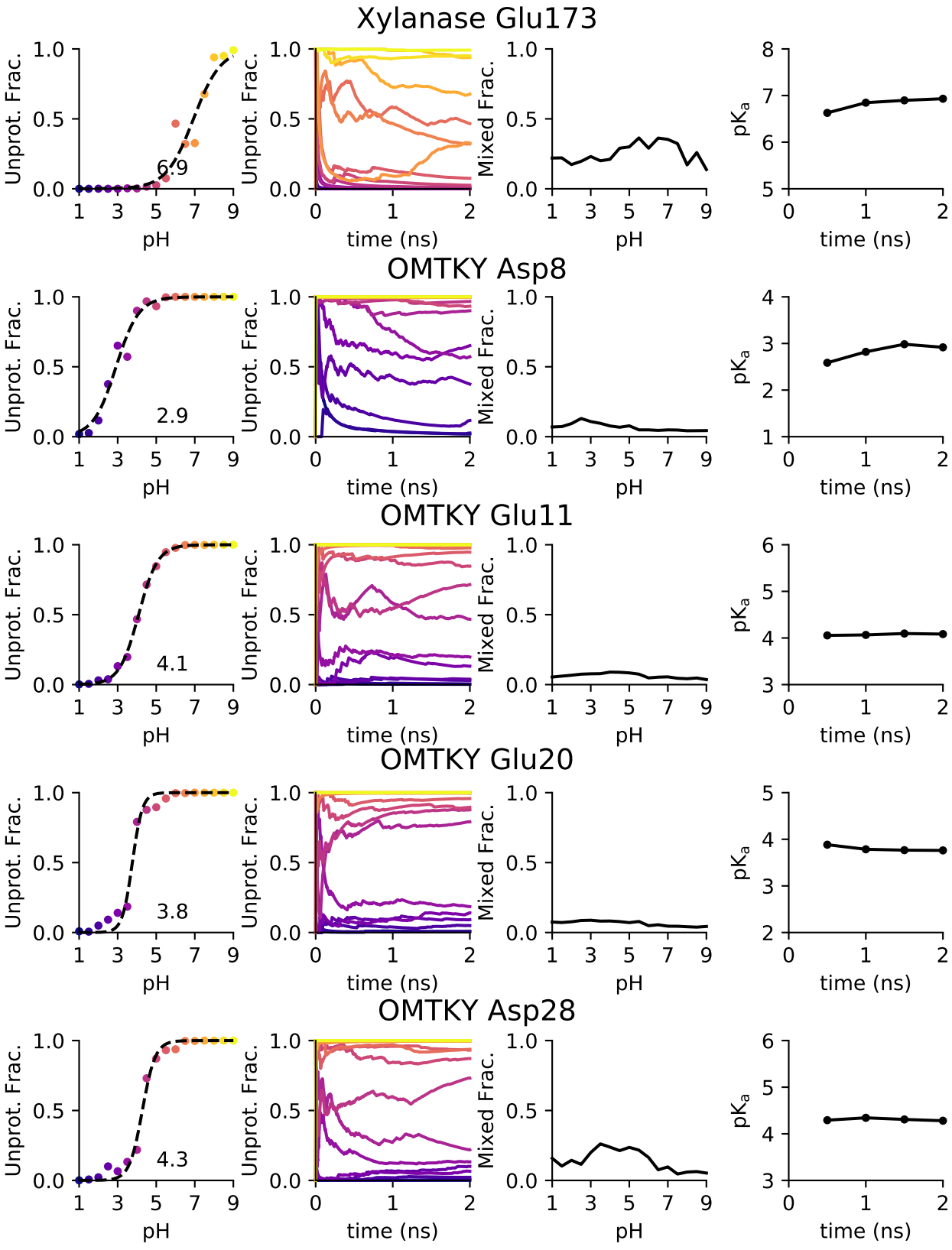
Xylanase Glu79
Xylanase Asp84
Xylanase Asp102
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
4.2
3.1
5.6
3.9
4.3


Xylanase Asp107
Xylanase Asp120
Xylanase Asp122
Xylanase His150
Xylanase His157
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
3.6
3.6
3.4
3.9
7.5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Unprot. Frac.
pH
6.9
time (ns)
Mixed Frac.
pK$_a$
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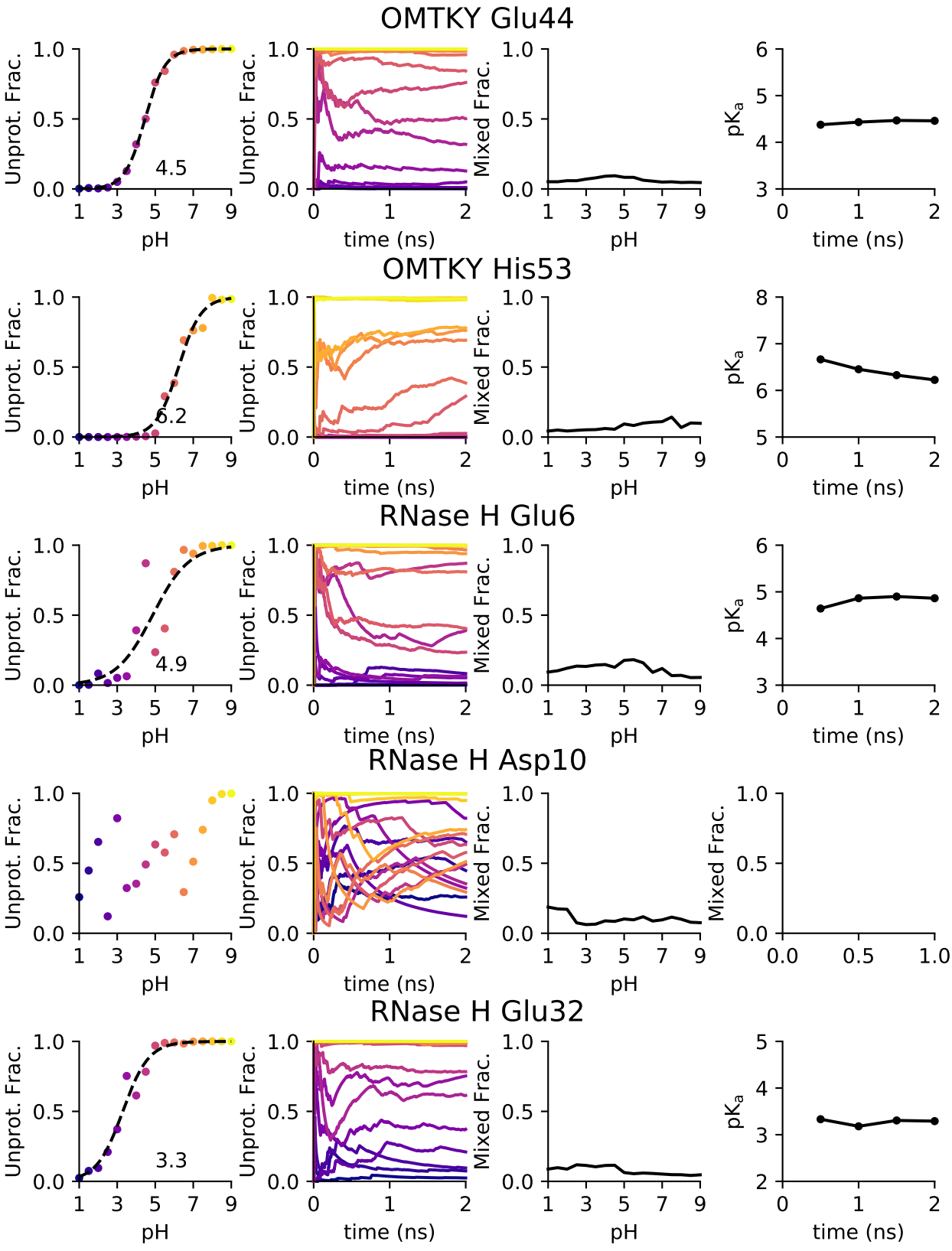
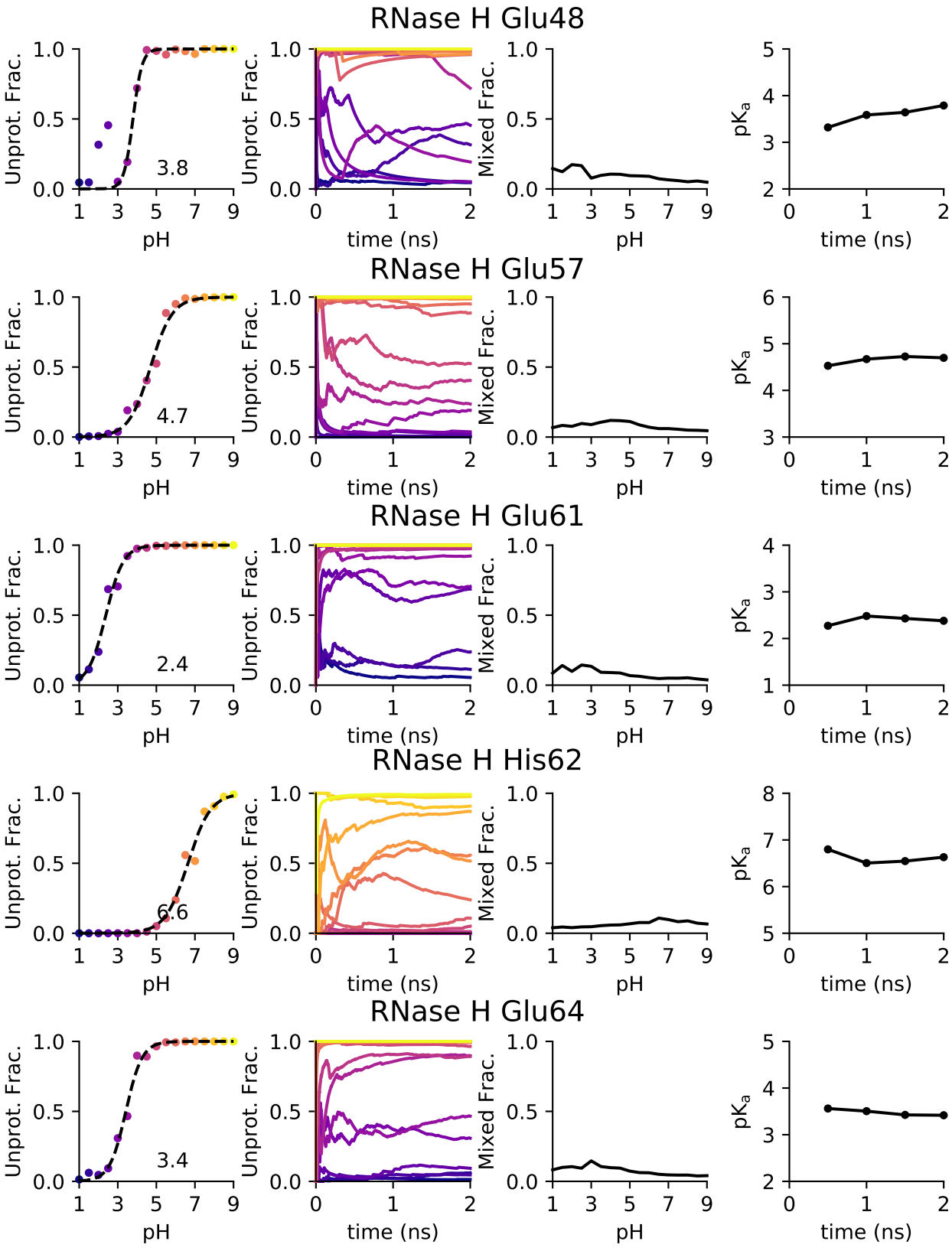
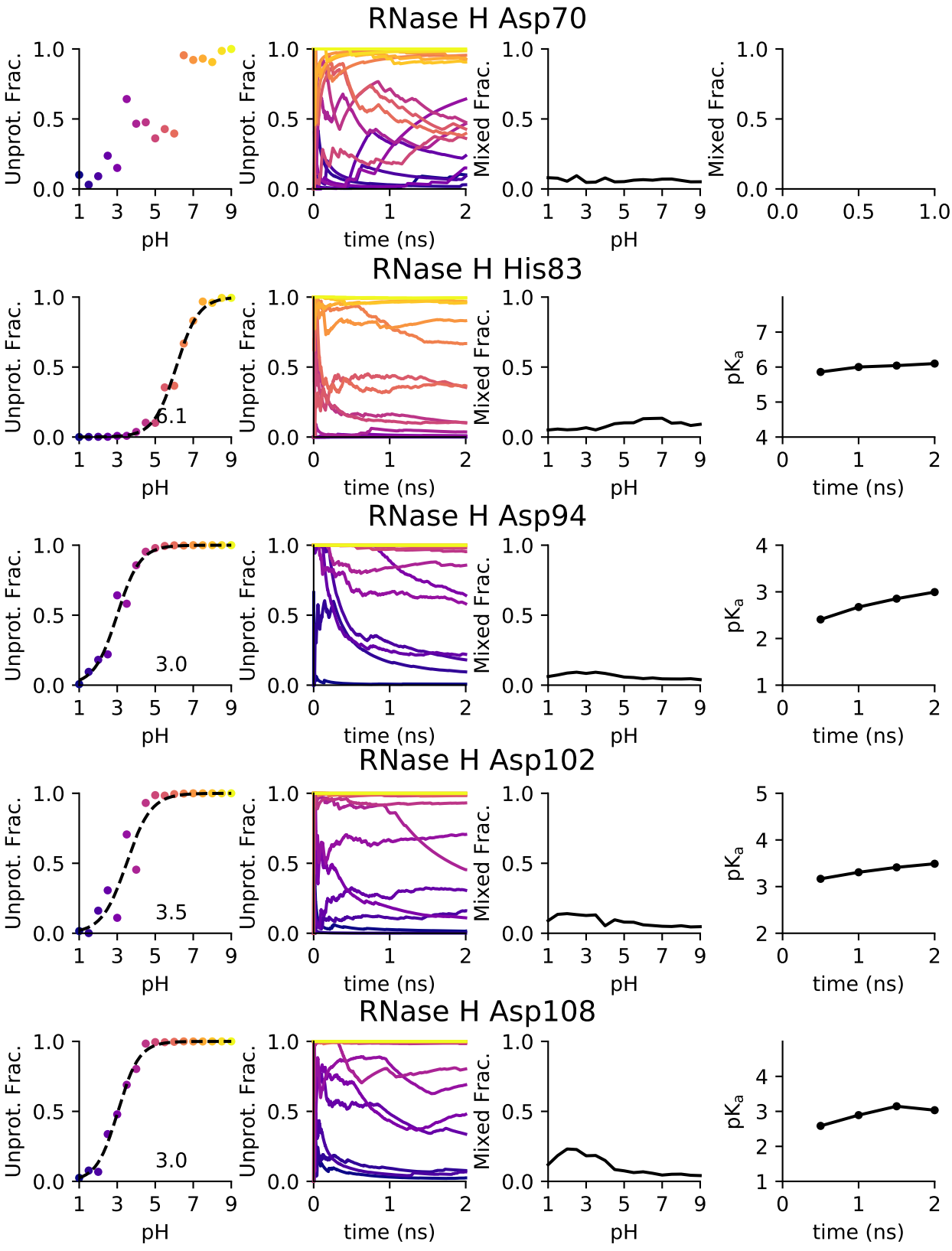
4.1
OMTKY Glu20
3.8
OMTKY Asp28
4.3


OMTKY Glu44
Unprot. Frac.
4.5
pH
time (ns)
Mixed Frac.
pK$_a$
OMTKY His53
6.2
RNase H Glu6
4.9
RNase H Asp10
RNase H Glu32
3.3


RNase H Glu48
RNase H Glu57
RNase H Glu61
RNase H His62
RNase H Glu64
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
3.8
4.7
2.4
6.6
3.4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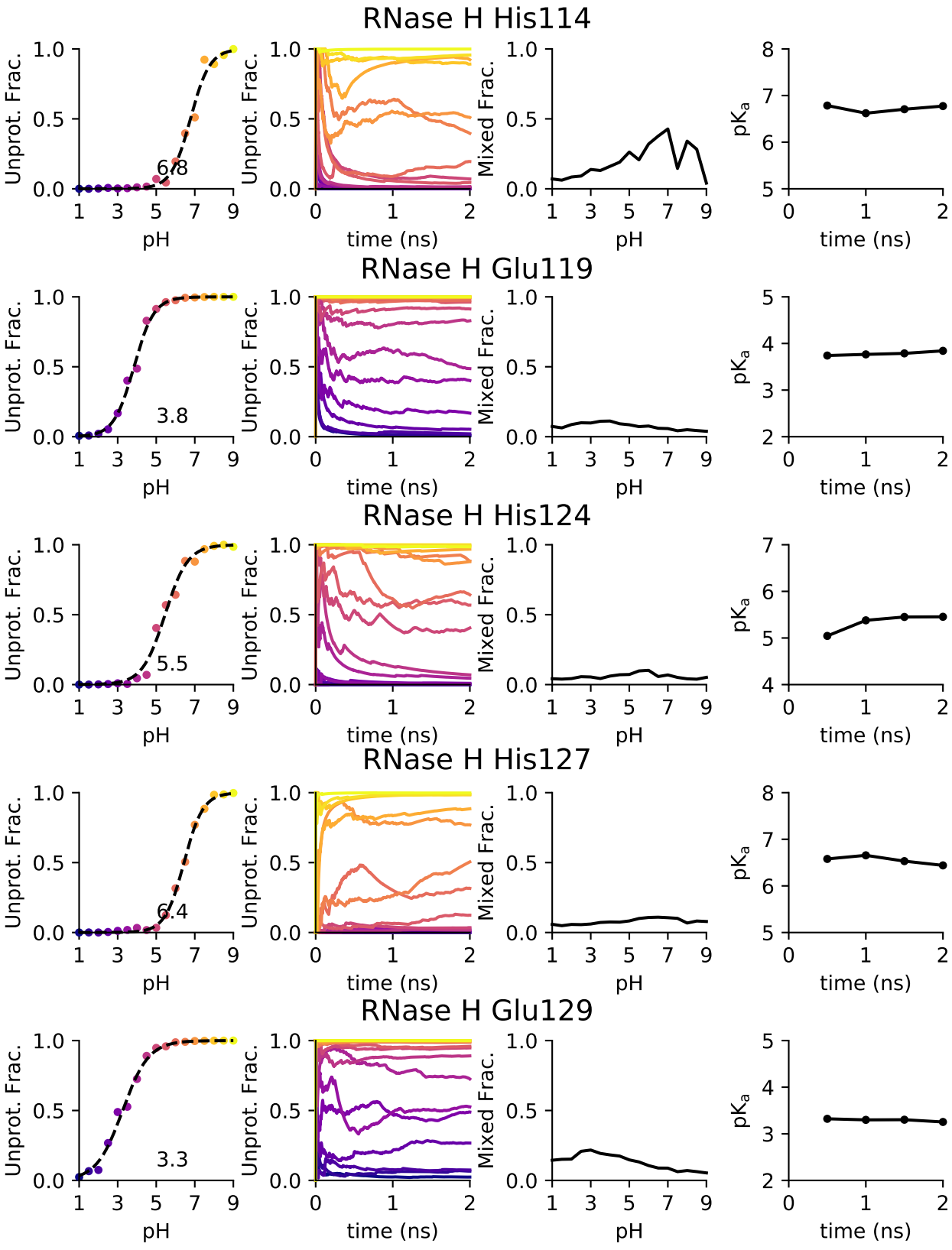
6.1
RNase H Asp94
3.0
RNase H Asp102
3.5
RNase H Asp108
3.0
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)


RNase H His114
Unprot. Frac.
pH
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time (ns)
Mixed Frac.
pK$_a$
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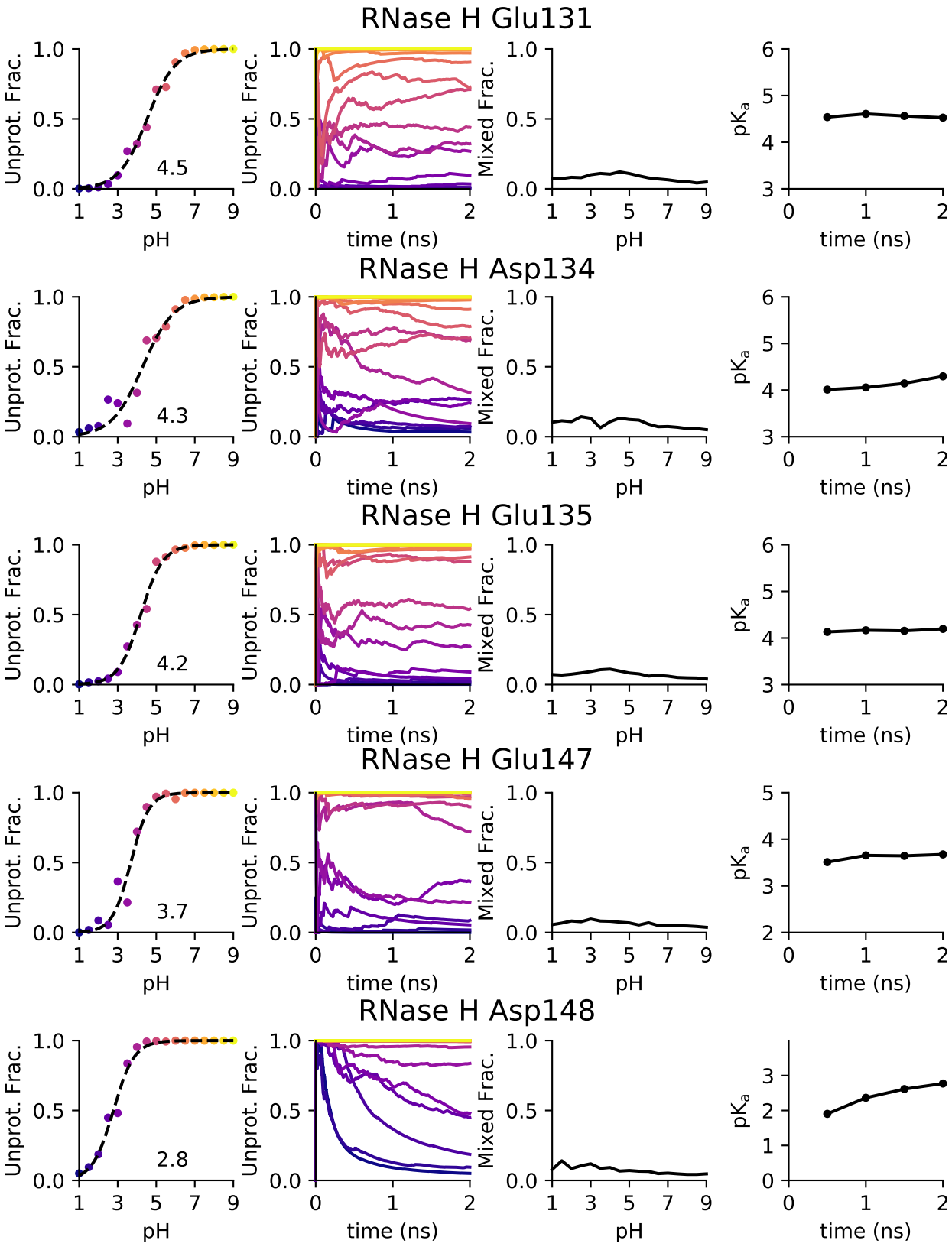
RNase H His124
5.5
RNase H His127
6.4
RNase H Glu129
3.3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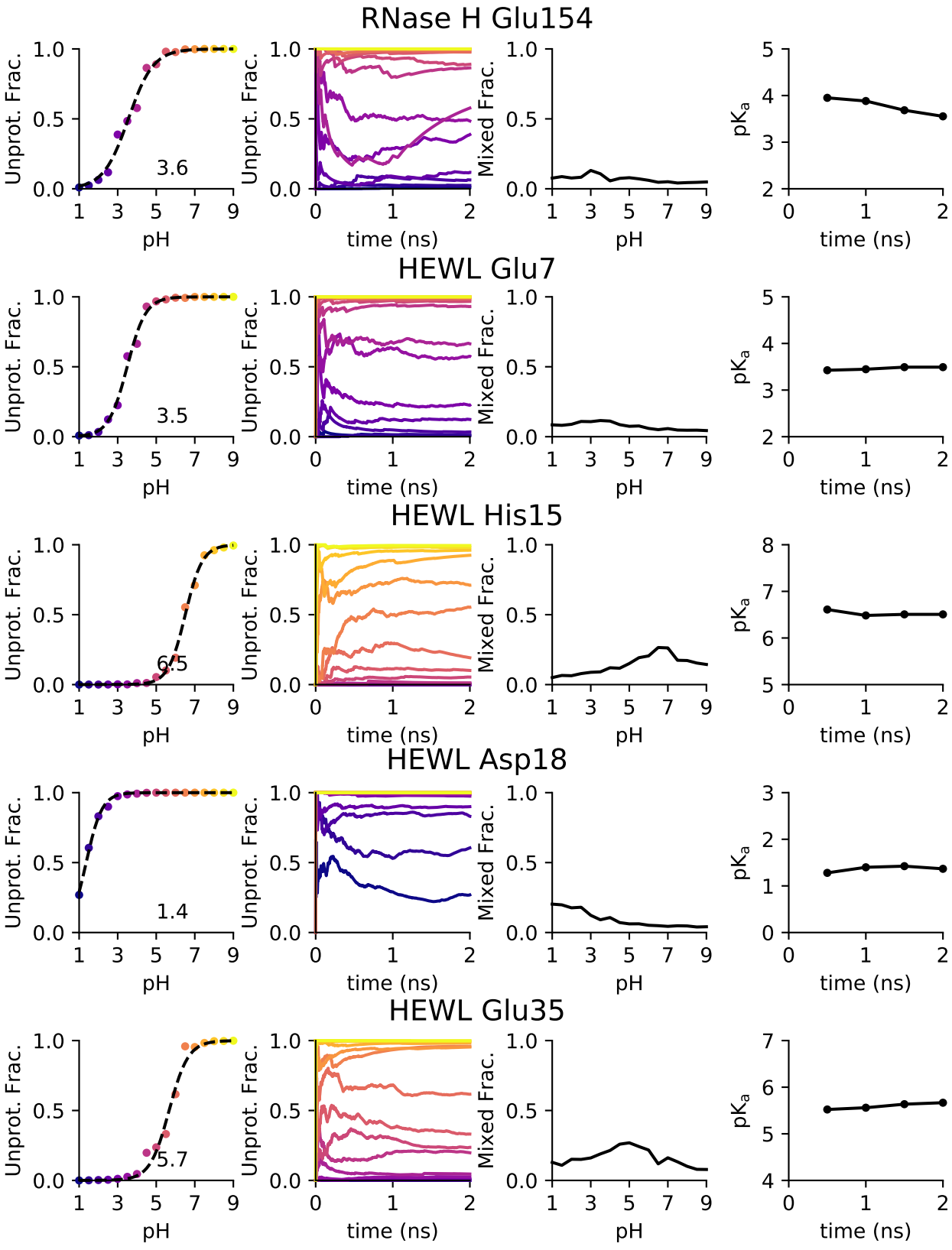
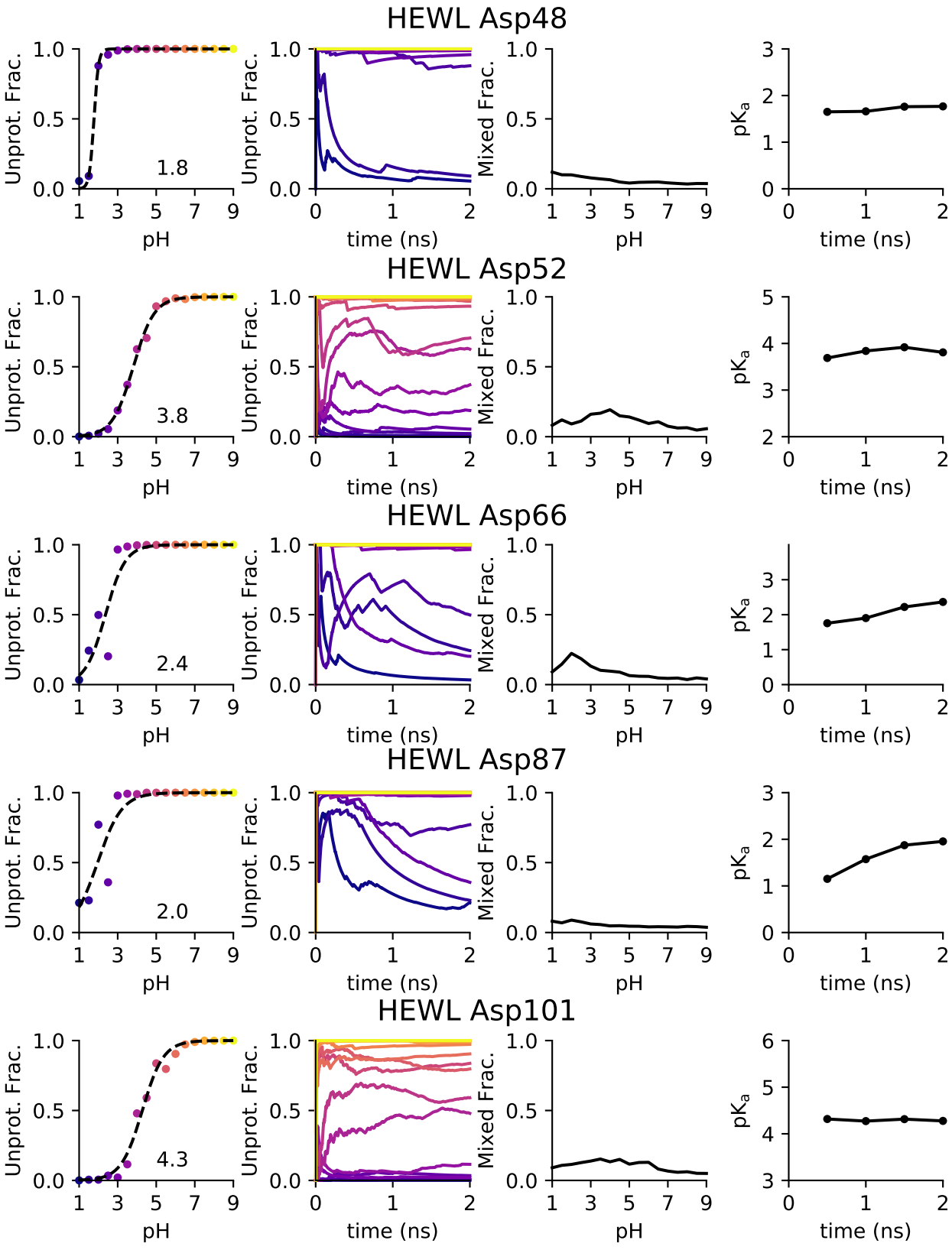
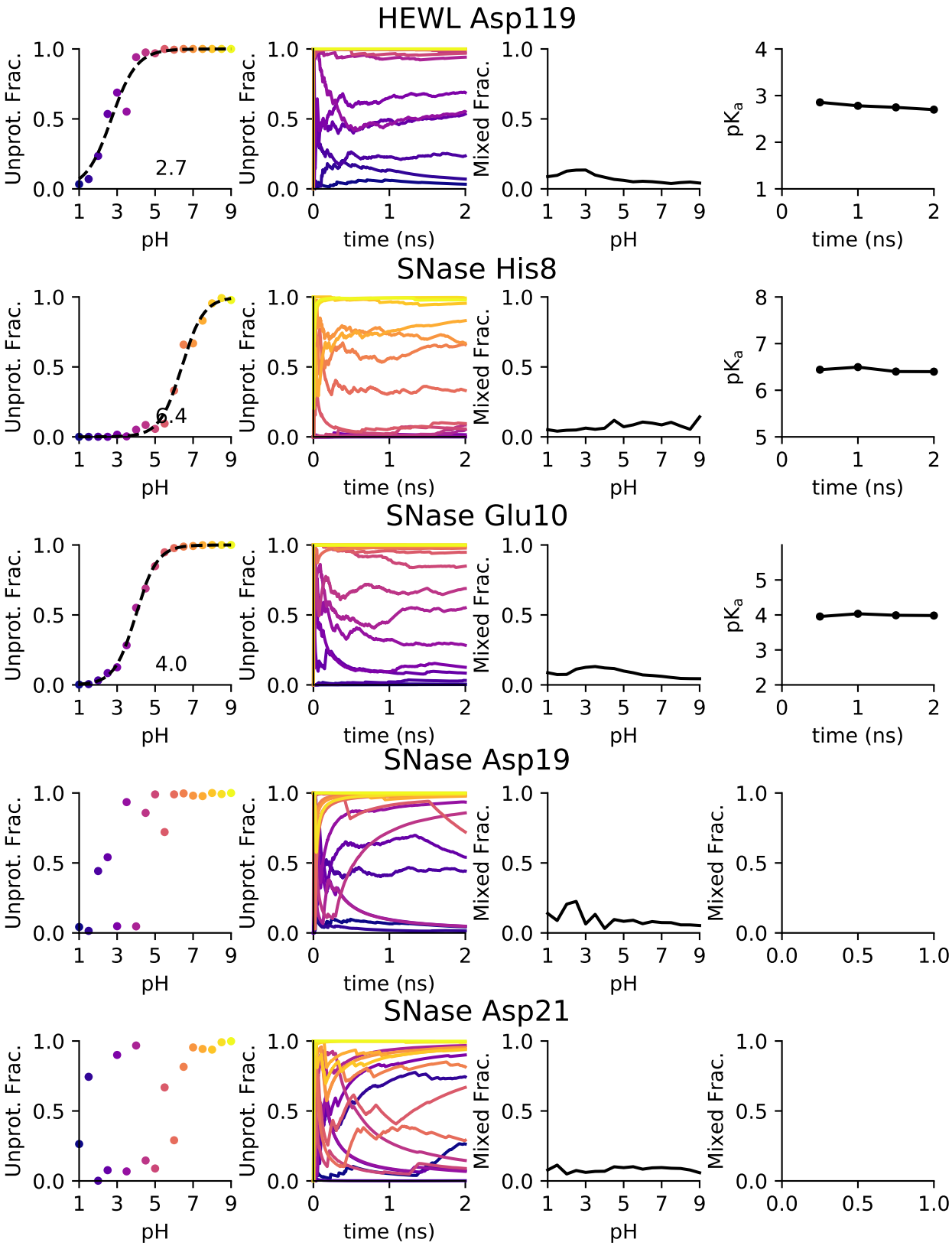
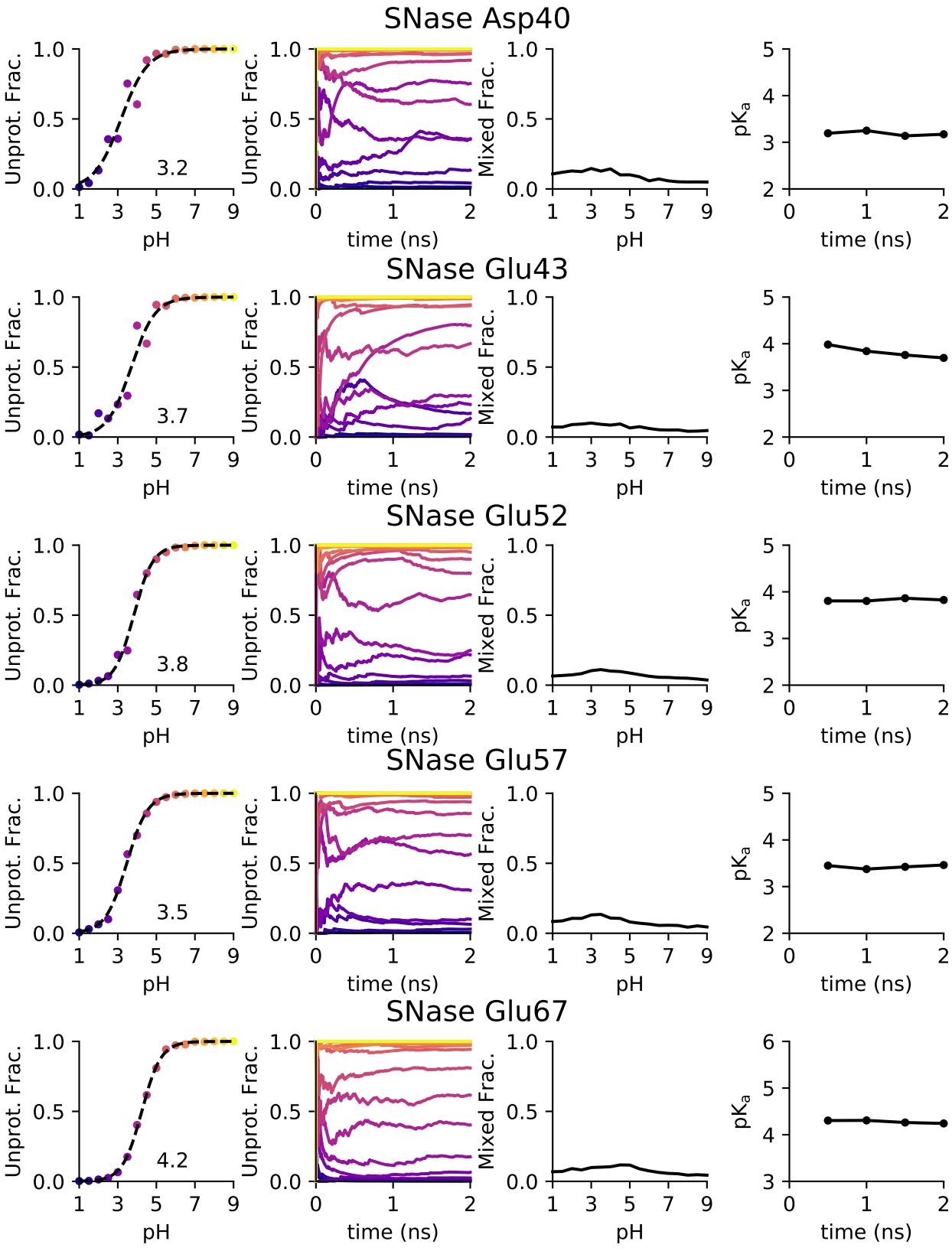
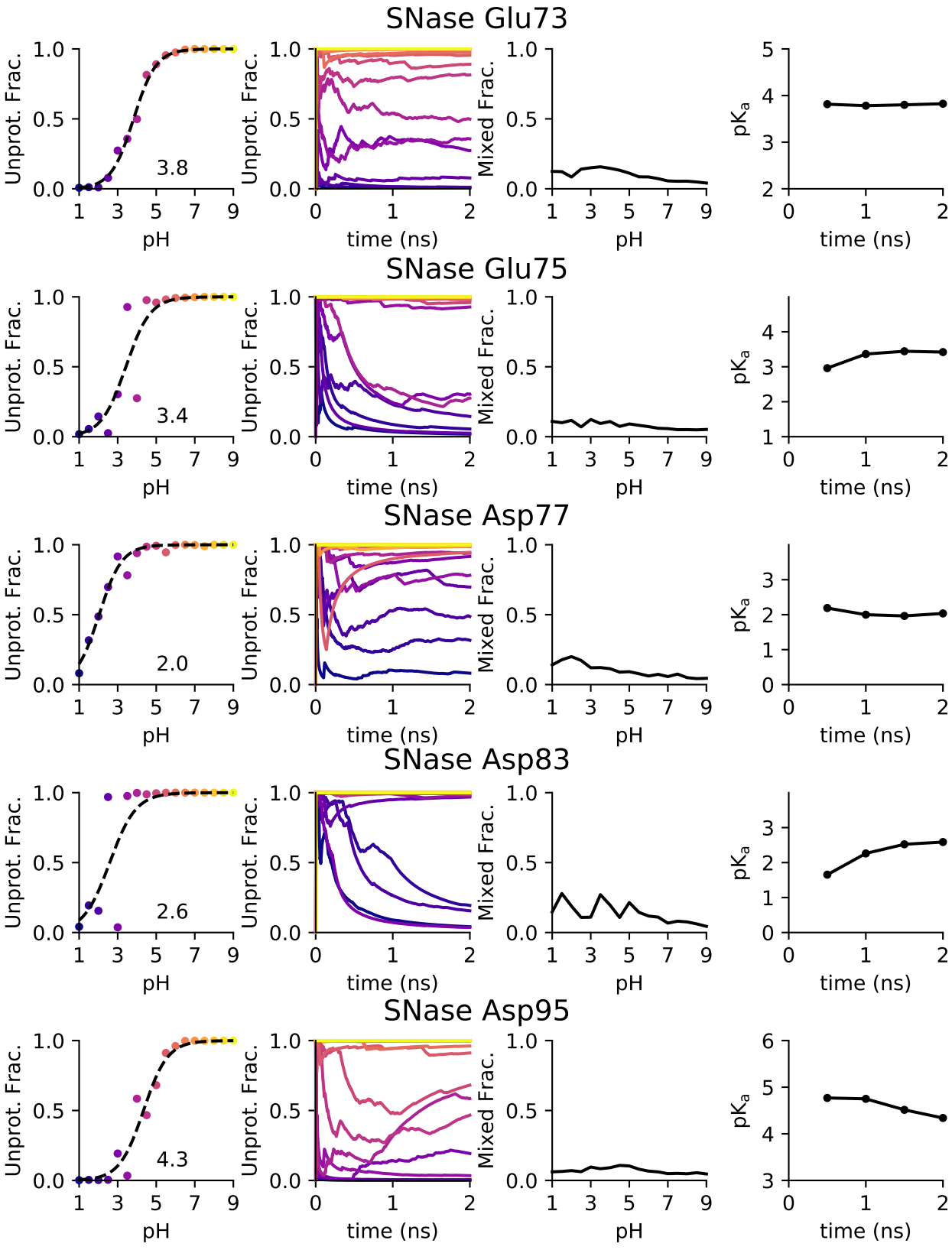
RNase H Asp134
RNase H Glu135
RNase H Glu147
RNase H Asp148
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
4.5
4.3
4.2
3.7
2.8

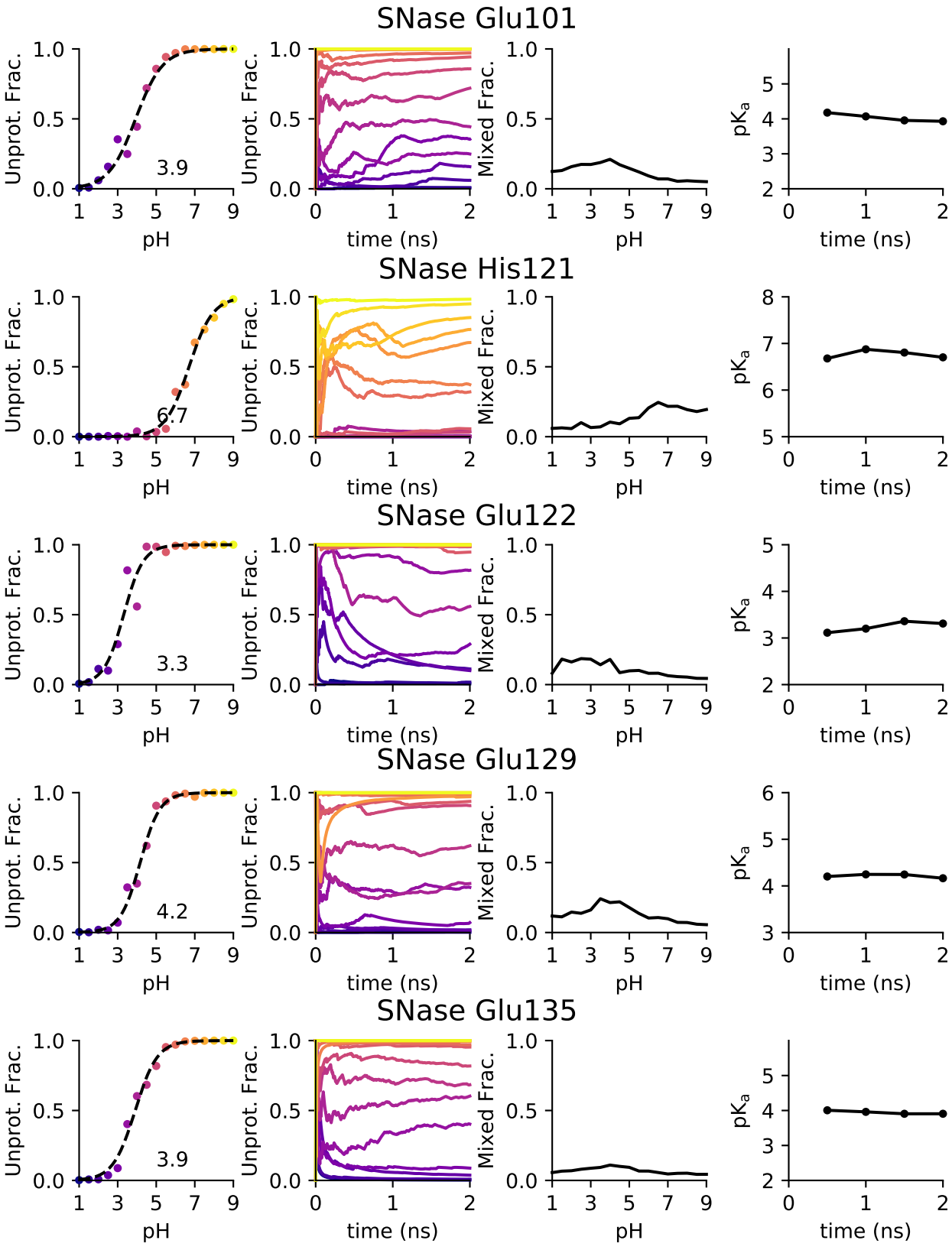

RNase H Glu154
HEWL Glu7
HEWL His15
HEWL Asp18
HEWL Glu35
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
3.6
3.5
6.5
1.4
5.7

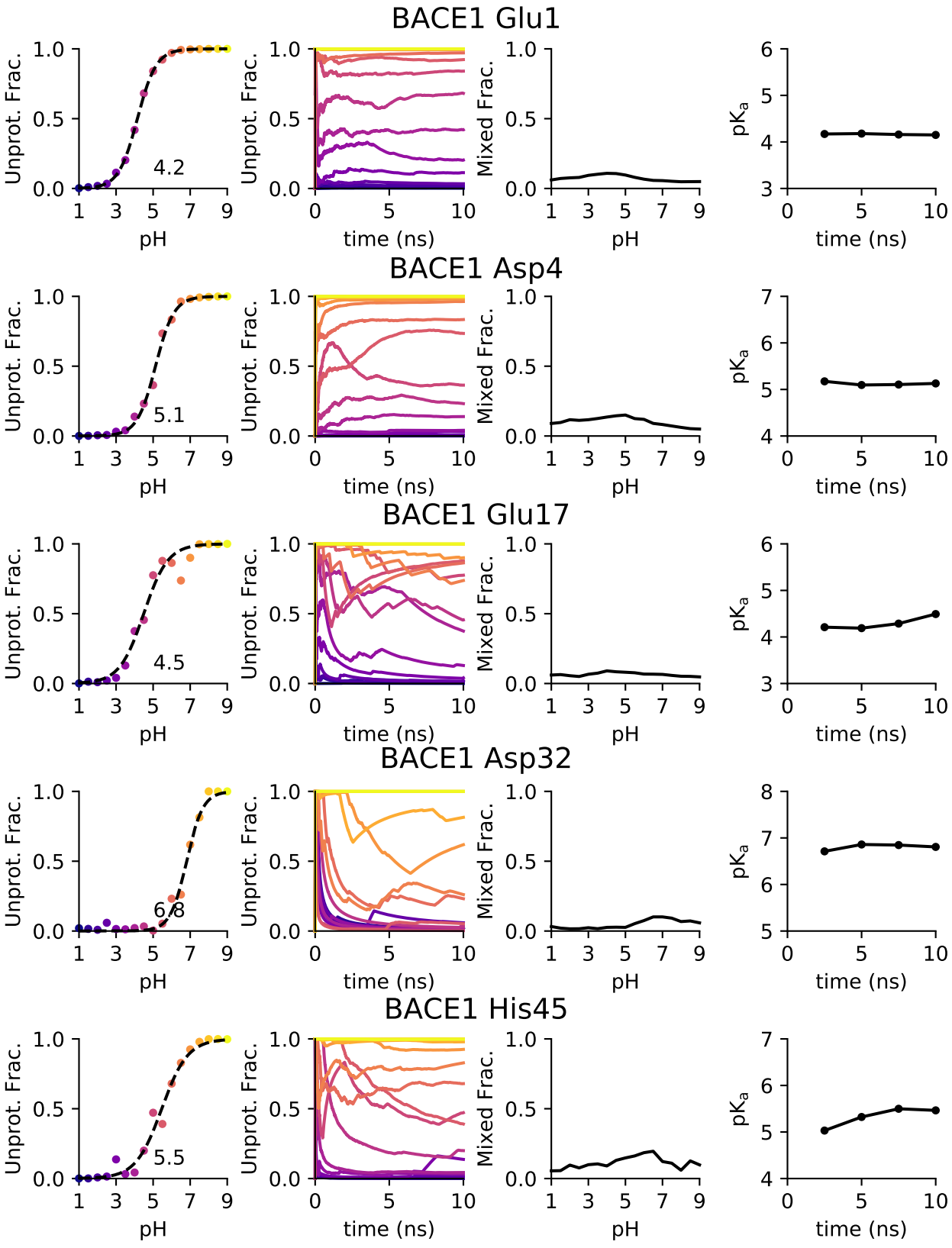

HEWL Asp48
Unprot. Frac.
1.8
pH
Unprot. Frac.
time (ns)
Mixed Frac.
pH
pKa
time (ns)
HEWL Asp52
Unprot. Frac.
3.8
pH
Unprot. Frac.
time (ns)
Mixed Frac.
pH
pKa
time (ns)
HEWL Asp66
Unprot. Frac.
2.4
pH
Unprot. Frac.
time (ns)
Mixed Frac.
pH
pKa
time (ns)
HEWL Asp87
Unprot. Frac.
2.0
pH
Unprot. Frac.
time (ns)
Mixed Frac.
pH
pKa
time (ns)
HEWL Asp101
Unprot. Frac.
4.3
pH
Unprot. Frac.
time (ns)
Mixed Frac.
pH
pKa
time (ns)

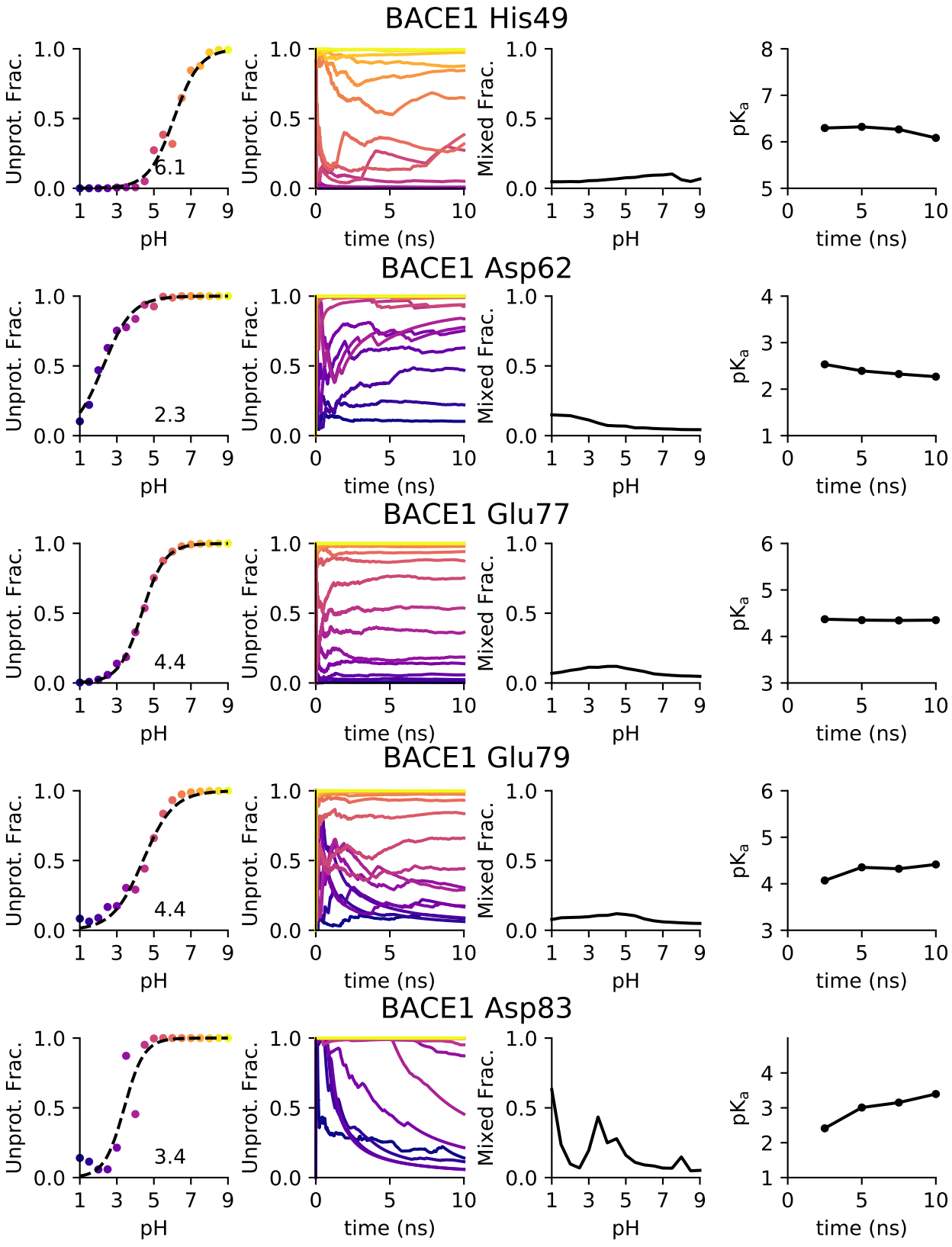

HEWL Asp119
SNase His8
SNase Glu10
SNase Asp19
SNase Asp21
Unprot. Frac.
Mixed Frac.
pH
time (ns)
pK$_a$
2.7
6.4
4.0

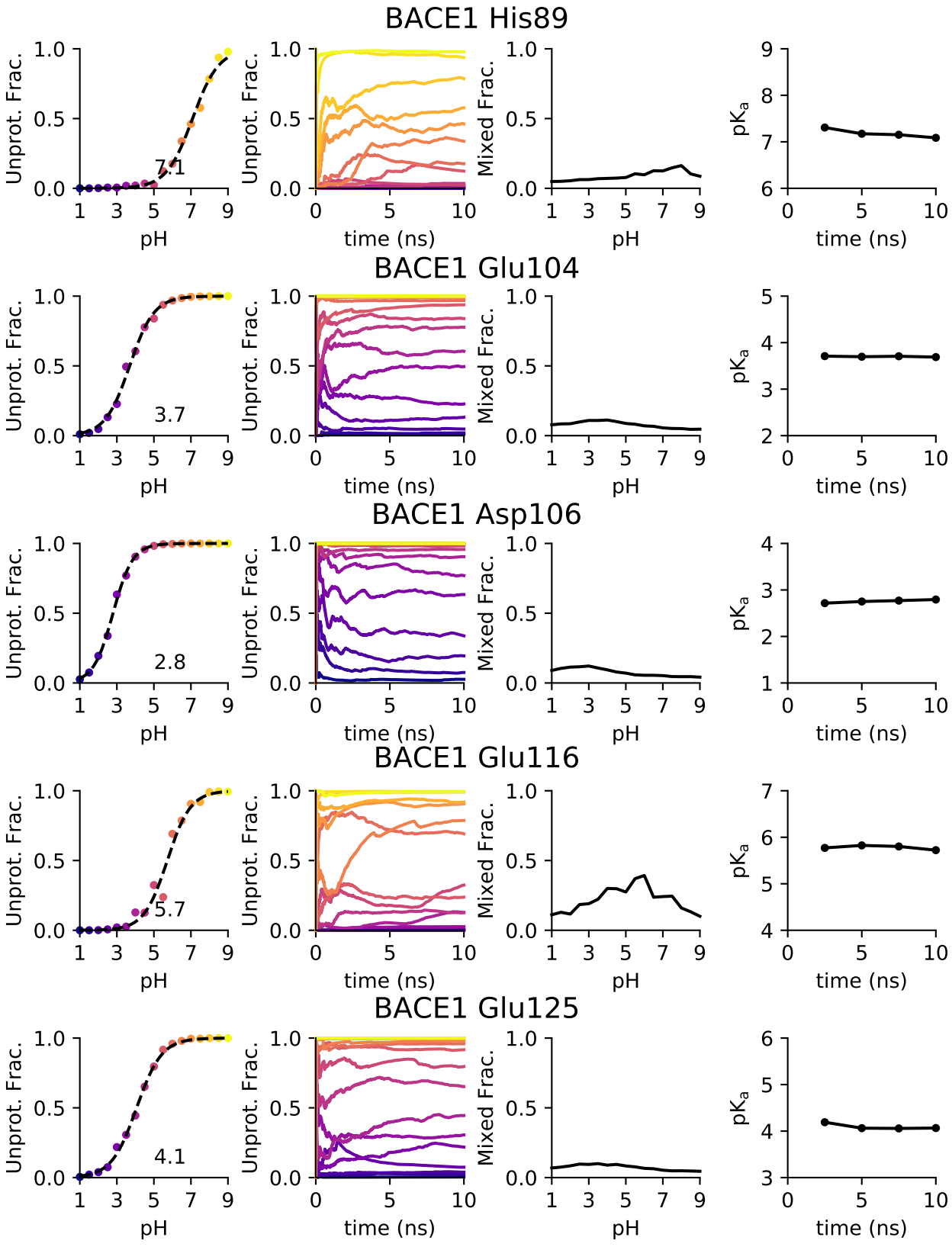

SNase Asp40
SNase Glu43
SNase Glu52
SNase Glu57
SNase Glu67
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
3.2
3.7
3.8
3.5
4.2

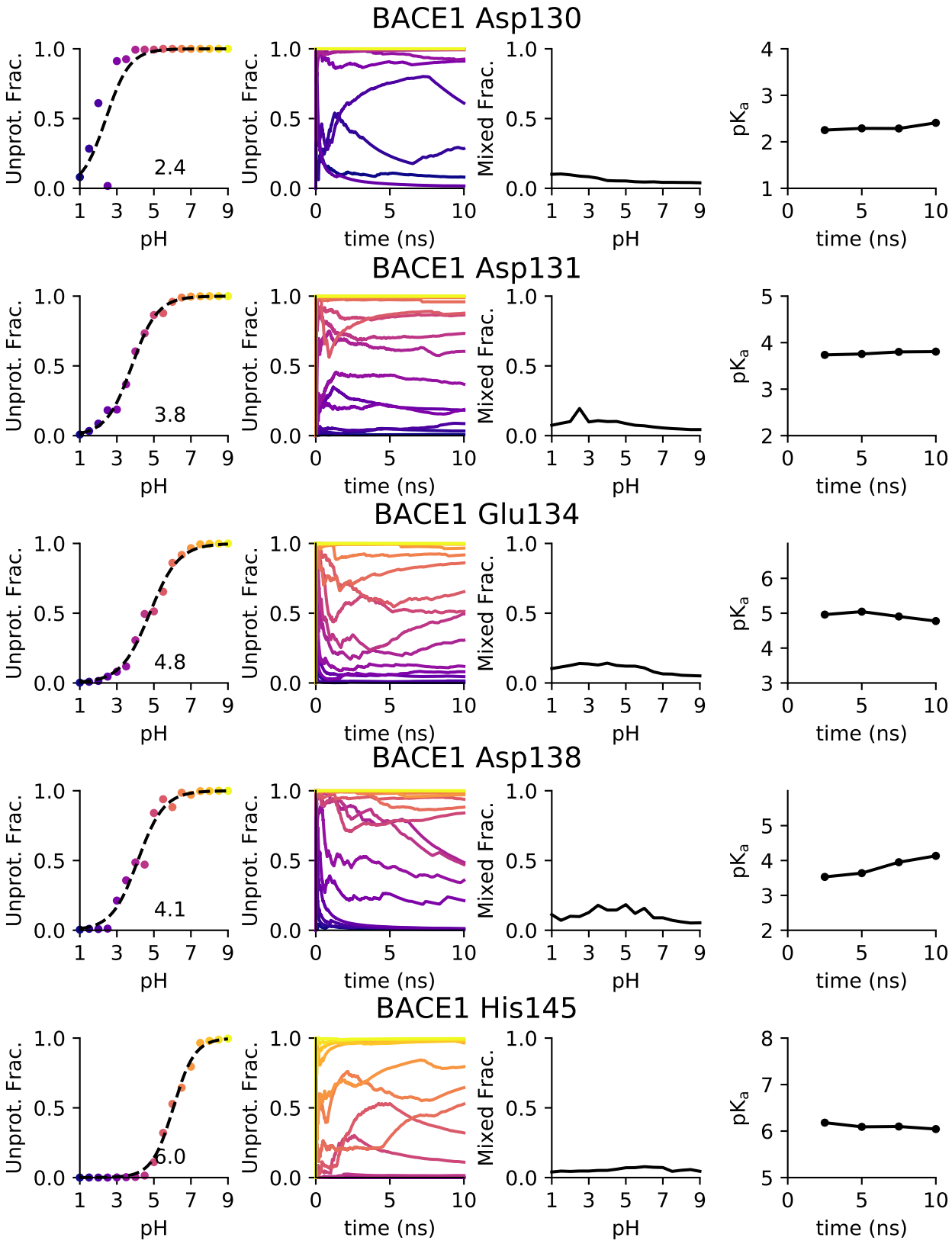

SNase Glu73
SNase Glu75
SNase Asp77
SNase Asp83
SNase Asp95
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
3.8
3.4
2.0
2.6
4.3

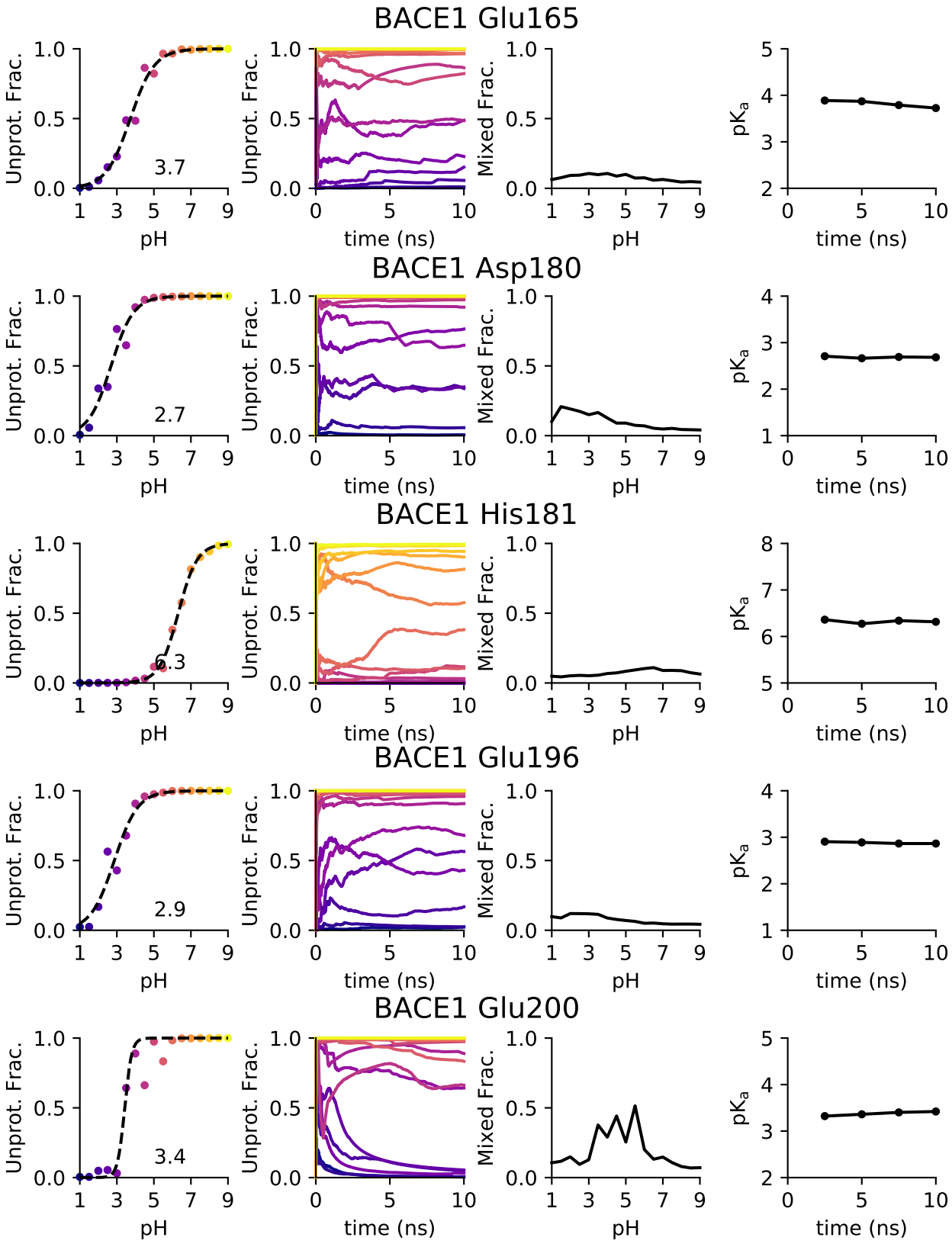

SNase Glu101
SNase His121
SNase Glu122
SNase Glu129
SNase Glu135
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)
3.9
6.7
3.3
4.2
3.9

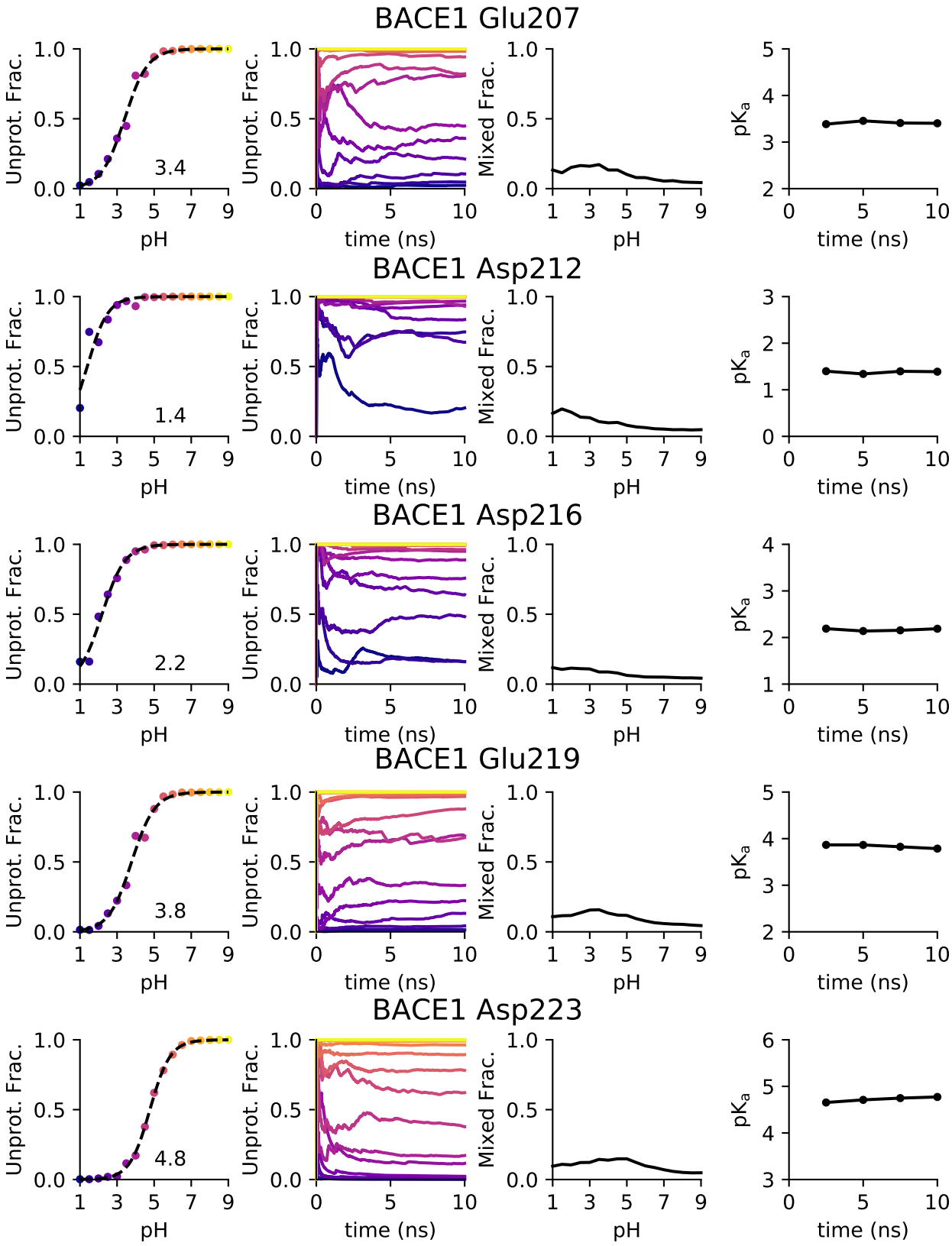

BACE1 Glu1
Unprot. Frac.
pH
4.2
time (ns)
Mixed Frac.
pK$_a$
BACE1 Asp4
5.1
BACE1 Glu17
4.5
BACE1 Asp32
6.8
BACE1 His45
5.5

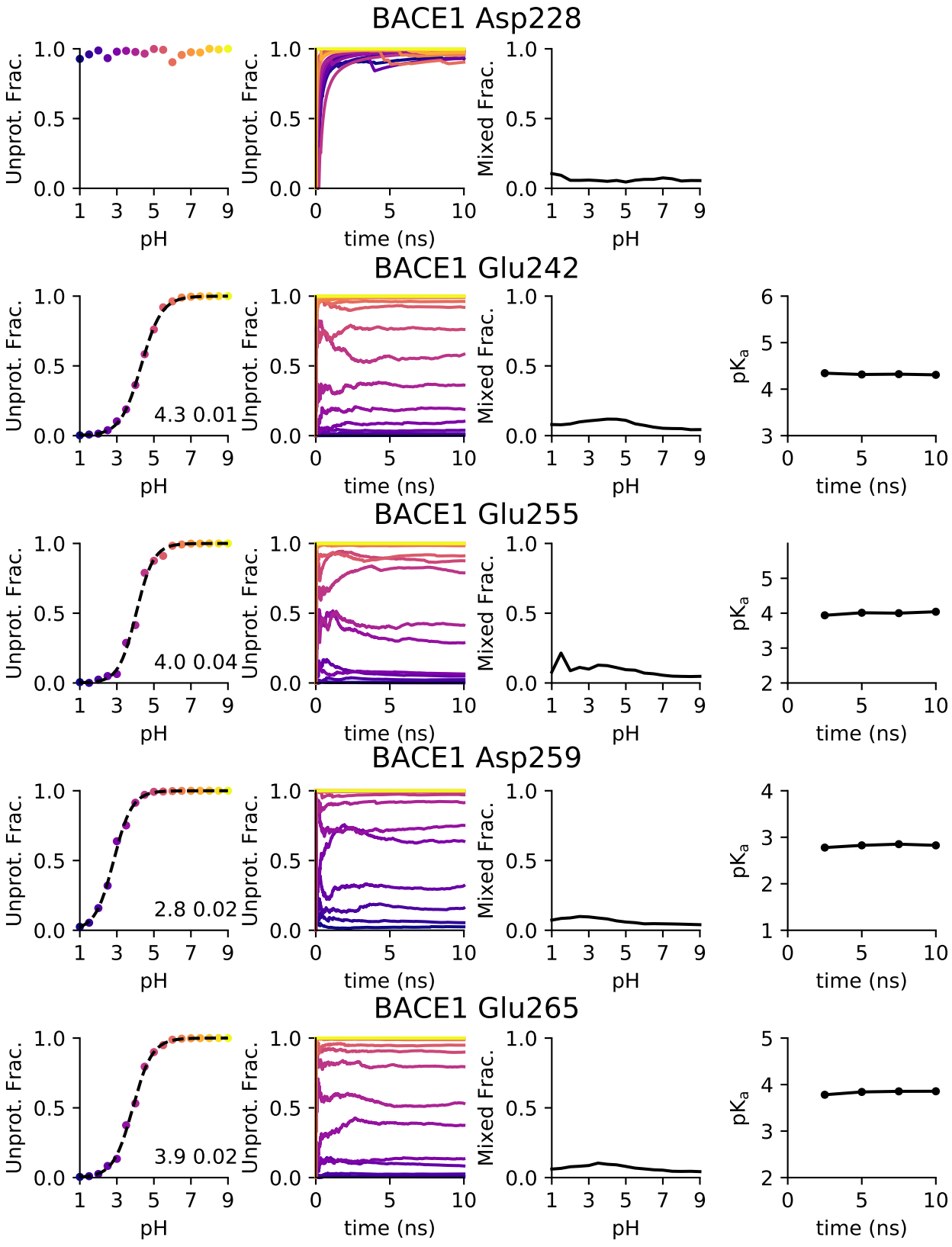

BACE1 His49
Unprot. Frac.
pH
6.1
time (ns)
Mixed Frac.
pK$_a$
BACE1 Asp62
2.3
BACE1 Glu77
4.4
BACE1 Glu79
4.4
BACE1 Asp83
3.4

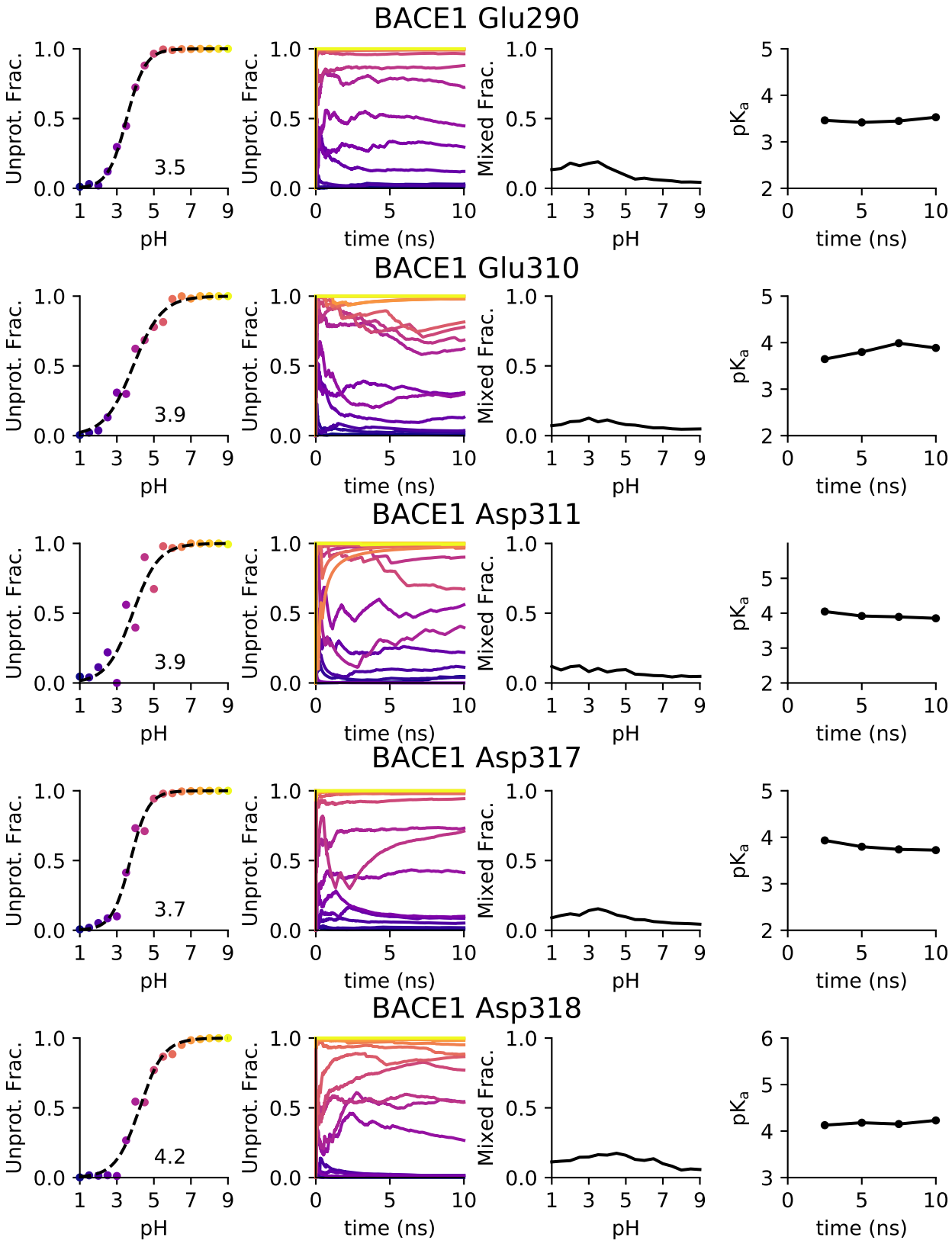

BACE1 His89
Unprot. Frac.
pH
7.1
time (ns)
Mixed Frac.
pK$_a$
BACE1 Glu104
3.7
BACE1 Asp106
2.8
BACE1 Glu116
5.7
BACE1 Glu125
4.1

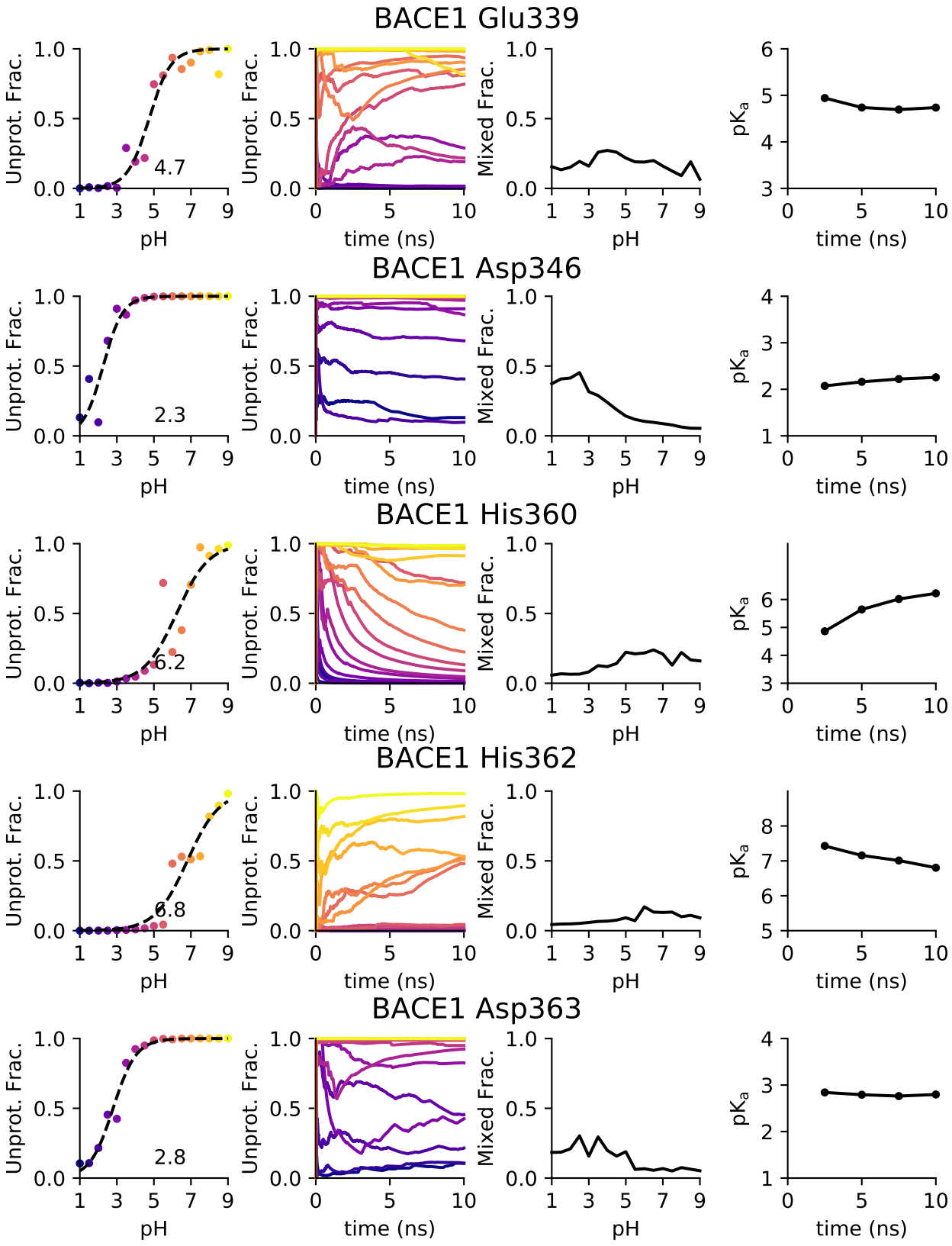

BACE1 Asp130
Unprot. Frac.
pH
2.4
time (ns)
Mixed Frac.
pK$_a$
BACE1 Asp131
3.8
BACE1 Glu134
4.8
BACE1 Asp138
4.1
BACE1 His145
6.0


BACE1 Glu165
Unprot. Frac.
pH
3.7
time (ns)
Mixed Frac.
pK$_a$
BACE1 Asp180
2.7
BACE1 His181
6.3
BACE1 Glu196
2.9
BACE1 Glu200
3.4


BACE1 Glu207
Unprot. Frac.
pH
3.4
time (ns)
Mixed Frac.
pK$_a$
BACE1 Asp212
1.4
BACE1 Asp216
2.2
BACE1 Glu219
3.8
BACE1 Asp223
4.8


BACE1 Asp228
BACE1 Glu242
4.3 0.01
BACE1 Glu255
4.0 0.04
BACE1 Asp259
2.8 0.02
BACE1 Glu265
3.9 0.02
Unprot. Frac.
Mixed Frac.
pK$_a$
pH
time (ns)


BACE1 Glu290
Unprot. Frac.
1.0
0.5
0.0
3.5
1 3 5 7 9
pH
Unprot. Frac.
0 5 10
time (ns)
Mixed Frac.
pH
pK$_a$
5 4 3 2
time (ns)
BACE1 Glu310
3.9
BACE1 Asp311
3.9
BACE1 Asp317
3.7
BACE1 Asp318
4.2
6 5 4 3


BACE1 Glu339
Unprot. Frac.
pH
4.7
time (ns)
Mixed Frac.
pK$_a$
BACE1 Asp346
2.3
BACE1 His360
6.2
BACE1 His362
6.8
BACE1 Asp363
2.8

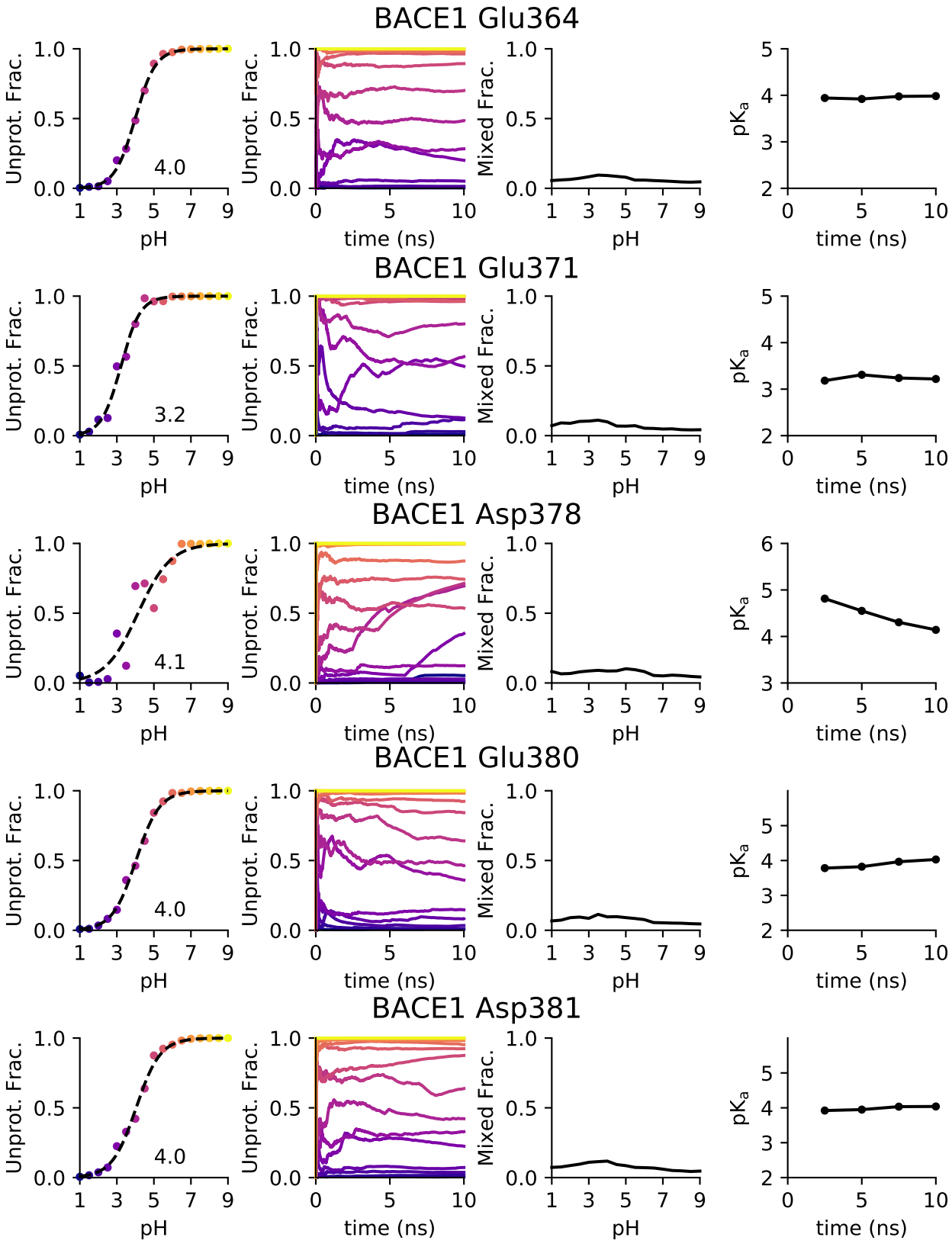

BACE1 Glu364
Unprot. Frac.
pH
4.0
time (ns)
Mixed Frac.
pK$_a$
BACE1 Glu371
3.2
BACE1 Asp378
4.1
BACE1 Glu380
4.0
BACE1 Asp381
4.0

Figure S6: **Titration data, convergence of protonation-state sampling, mixed fractions, and convergence of pK_{a} estimates for all titratable residues in 11 proteins discussed in the main text.** The plots are arranged from left to right. **First column.** Titration data showing the unprotonated fraction at each pH and the best fit to the generalized Henderson-Hasselbalch equation (dashed black line). **Second column.** Cumulative running estimate of the unprotonated fraction as a function of time for each pH (the color of each curve corresponds to the pH in the titration data plot). **Third column.** Fraction of the time that λ is between the value of 0.2 and 0.8 as a function of pH. **Fourth column.** Running estimate of the pK_{a} as a function of time evaluated every 0.5 ns. For BACE1, results from 10-ns single pH simulation are presented.