Supplementary Information for:-

THE INTERFACE BETWEEN CATALYTIC AND HEMOPEXIN DOMAINS IN MATRIX METALLOPROTEINASE-1 CONCEALS A COLLAGEN BINDING EXOSITE

Laurence H. Arnold, Louise Butt, Stephen H. Prior, Chris Read, Gregg B. Fields, Hideaki Nagase, Rob Visse and Andrew R. Pickford

Suppl. Table S1. Oligonucleotides for Site-Directed Mutagenesis of MMP-1.

Mutant	Direction	Sequence
D299A	Forward	GTGATGTTCTTTAAAGCGAGATTCTACATGCGCAC
	Reverse	GTGCGCATGTAGAATCTCGCTTTAAAGAACATCAC
R300A	Forward	GAAGTGATGTTCTTTAAAGACGCGTTCTACATGCGCACAAATCC
	Reverse	GGATTTGTGCGCATGTAGAACGCGTCTTTAAAGAACATCACTTC
F301A	Forward	GATGTTCTTTAAAGACAGAGCGTACATGCGCACAAATCCC
	Reverse	GGGATTTGTGCGCATGTACGCTCTGTCTTTAAAGAACATC
F308A	Forward	CATGCGCACAAATCCCGCCTACCCGGAAGTTGAG
	Reverse	CTCAACTTCCGGGTAGGCGGGATTTGTGCGCATG
Y309A	Forward	GCACAAATCCCTTCGCCCCGGAAGTTGAGCTC
	Reverse	GAGCTCAACTTCCGGGGCGAAGGGATTTGTGC
F316A	Forward	CGGAAGTTGAGCTCAATGCGATTTCTGTTTTCTGGCC
	Reverse	GGCCAGAAAACAGAAATCGCATTGAGCTCAACTTCCG
S318A	Forward	GTTGAGCTCAATTTCATTGCGGTTTTCTGGCCACAACTG
	Reverse	CAGTTGTGGCCAGAAAACCGCAATGAAATTGAGCTCAAC
V319A	Forward	CTCAATTTCATTTCTGCGTTCTGGCCACAACTGCC
	Reverse	GGCAGTTGTGGCCAGAACGCAGAAATGAAATTGAG
P325A	Forward	GTTTTCTGGCCACAACTGGCGAATGGGCTTGAAGCTGC
	Reverse	GCAGCTTCAAGCCCATTCGCCAGTTGTGGCCAGAAAAC
R337A	Forward	CTTACGAATTTGCCGACGCGGATGAAGTCCGGTTTTTC
	Reverse	GAAAAACCGGACTTCATCCGCGTCGGCAAATTCGTAAG
D338A	Forward	GAATTTGCCGACAGAGCGGAAGTCCGGTTTTTC
	Reverse	GAAAAACCGGACTTCCGCTCTGTCGGCAAATTC
Q352A	Forward	GTACTGGGCTGTTGCGGGACAGAATGTGC
	Reverse	GCACATTCTGTCCCGCAACAGCCCAGTAC
Q354A	Forward	GGCTGTTCAGGGAGCGAATGTGCTACACG
	Reverse	CGTGTAGCACATTCGCTCCCTGAACAGCC
H358A	Forward	GGACAGAATGTGCTAGCGGGATACCCCAAGGAC
	Reverse	GTCCTTGGGGTATCCCGCTAGCACATTCTGTCC
P361A	Forward	GAATGTGCTACACGGATACGCGAAGGACATCTACAGCTCC
	Reverse	GGAGCTGTAGATGTCCTTCGCGTATCCGTGTAGCACATTC

Suppl. Table S2. Chemical Shift Assignments for HPX-1 at pH 4.8 and 25°C.

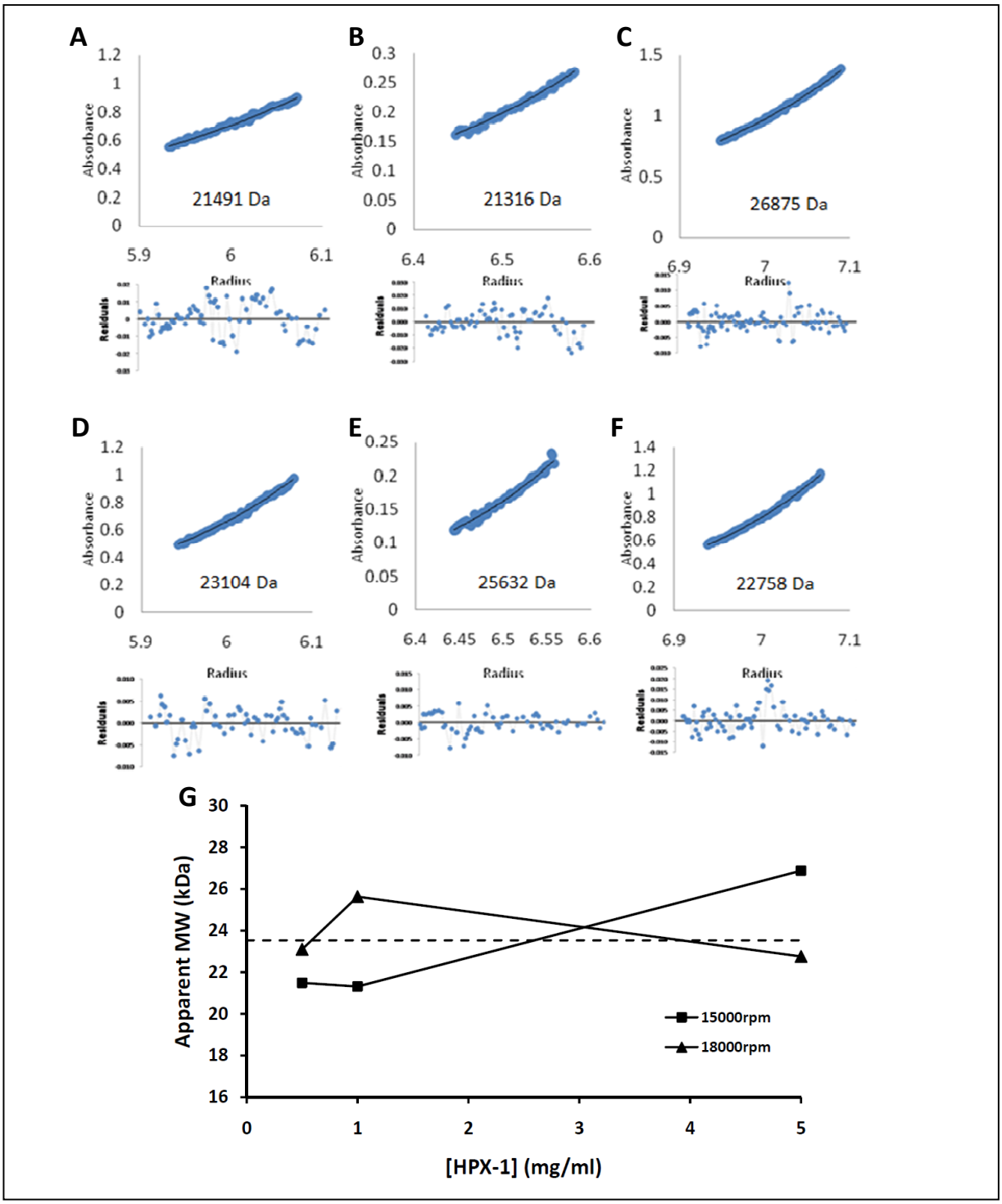
Residue	H	N	C	Cα	Cβ	Residue	H	N	C	Cα	Cβ
Pro272	-	n.d.	177.03	61.61	31.48	Gln323	8.24	115.42	174.53	54.84	27.50
Gln273	8.45	121.45	174.41	53.95	27.84	Leu324	7.37	122.79	172.84	50.39	40.07
Thr274	8.12	121.11	170.80	58.65	67.75	Pro325	-	n.d.	171.86	60.16	30.33
Pro275	-	n.d.	173.41	61.21	30.57	Asn326	7.47	112.27	174.50	50.23	38.47
Lys276	8.09	122.06	174.83	53.51	31.99	Gly327	7.86	111.29	173.75	46.66	-
Ala277	8.18	126.01	175.62	53.12	16.89	Leu328	8.37	123.10	174.79	53.52	41.19
Cys278	7.84	110.64	173.52	52.79	38.03	Glu329	8.60	118.45	174.32	55.29	30.75
Asp279	7.45	124.37	175.95	52.71	40.84	Ala330	7.76	118.89	174.30	50.22	20.75
Ser280	8.72	124.88	173.92	59.09	61.71	Ala331	8.90	125.70	172.75	50.18	21.94
Lys281	8.52	122.80	174.62	54.20	30.15	Tyr332	8.99	117.42	169.33	55.63	38.88
Leu282	7.19	125.66	172.48	55.31	41.30	Glu333	7.80	123.90	174.02	52.41	31.43
Thr283	6.74	114.55	170.90	57.35	69.44	Phe334	9.33	130.70	173.92	52.43	36.78
Phe284	9.17	116.24	174.98	56.35	41.13	Ala335	9.34	132.70	178.91	54.45	16.65
Asp285	9.21	124.23	174.60	54.15	40.65	Asp336	8.06	118.52	175.22	55.11	38.96
Ala286	7.79	121.26	173.43	50.39	19.27	Arg337	6.30	114.80	172.98	52.48	30.12
Ile287	8.72	123.88	172.03	59.11	43.09	Asp338	7.99	122.54	172.24	52.94	38.28
Thr288	8.59	118.79	171.25	58.75	68.09	Glu339	7.04	116.02	173.52	52.74	34.35
Thr289	6.91	117.14	174.18	59.81	68.40	Val340	8.75	125.71	172.40	59.08	31.70
Ile290	8.50	120.35	174.37	59.98	38.25	Arg341	8.82	128.07	172.12	52.90	31.36
Arg291	10.41	133.19	175.09	55.70	27.20	Phe342	8.72	121.59	173.49	55.23	42.52
Gly292	9.33	108.38	172.98	44.07	-	Phe343	8.81	121.53	173.67	56.13	40.55
Glu293	7.84	121.98	173.98	53.28	30.34	Lys344	8.75	123.54	174.85	56.81	34.59
Val294	8.33	122.96	172.40	61.24	30.27	Gly345	10.20	124.11	172.47	45.48	-
Met295	8.51	128.27	170.48	52.83	34.47	Asn346	8.09	123.46	172.75	49.86	35.31
Phe296	8.97	125.31	173.61	54.20	39.66	Lys347	7.83	120.97	172.07	52.83	35.97
Phe297	8.80	119.94	174.28	56.50	39.41	Tyr348	8.51	118.02	170.56	53.41	40.42
Lys298	9.17	125.91	172.48	54.33	33.94	Trp349	9.13	122.67	171.63	56.79	33.77
Asp299	10.17	130.93	174.61	55.68	38.31	Ala350	n.d.	n.d.	173.55	48.13	19.73
Arg300	7.42	123.49	174.22	54.80	29.67	Val351	9.08	120.12	173.03	57.55	33.84
Phe301	9.07	123.97	172.66	56.67	41.30	Gln352	8.44	125.64	175.15	53.63	30.84
Tyr302	9.00	118.19	171.87	53.73	41.25	Gly353	9.50	120.53	173.21	45.49	-
Met303	9.06	120.35	173.08	52.45	35.58	Gln354	9.00	126.96	173.34	55.11	28.47
Arg304	9.06	125.11	173.31	53.24	32.97	Asn355	8.03	119.50	172.75	51.50	38.96
Thr305	9.03	120.21	170.89	59.12	68.89	Val356	8.68	127.20	175.05	61.30	30.08
Asn306	8.94	125.39	172.57	49.34	39.78	Leu357	8.31	132.13	175.67	54.29	40.35
Pro307	-	n.d.	175.32	62.68	30.48	His358	8.71	121.26	174.57	55.78	27.39
Phe308	8.44	118.62	173.71	56.74	37.79	Gly359	8.62	113.57	171.01	43.31	-
Tyr309	7.93	121.48	173.10	53.47	37.97	Tyr360	7.30	118.93	171.81	55.45	36.49
Pro310	-	n.d.	175.27	62.49	30.14	Pro361	-	n.d.	173.85	59.98	n.d.
Glu311	7.85	121.33	174.38	54.88	29.38	Lys362	7.86	120.86	172.99	52.92	35.64
Val312	8.42	125.82	173.30	60.42	31.43	Asp363	8.20	119.75	176.53	51.68	42.31
Glu313	8.33	130.87	172.15	53.78	30.03	Ile364	7.98	114.71	174.86	63.12	-
Leu314	8.25	131.05	173.91	52.31	43.07	Tyr365	7.61	127.10	177.64	59.10	34.91
Asn315	7.93	124.99	171.60	49.46	40.57	Ser366	9.55	118.77	175.48	59.23	60.63
Phe316	8.95	120.95	176.17	56.09	37.99	Ser367	6.96	116.53	172.40	59.48	62.13
Ile317	8.94	127.76	175.75	65.63	n.d.	Phe368	7.03	116.41	173.44	56.56	39.51
Ser318	8.64	113.04	173.76	58.67	61.51	Gly369	7.51	105.19	174.34	44.21	-
Val319	7.38	121.84	174.73	63.13	30.27	Phe370	6.36	117.55	171.48	56.36	37.35
Phe320	7.14	123.28	173.91	57.93	38.85	Pro371	-	n.d.	176.06	60.66	30.89
Trp321	8.27	116.35	171.77	53.57	28.05	Arg372	8.58	119.90	174.13	56.19	27.25
Pro322	-	n.d.	176.35	63.18	30.55	Thr373	6.98	107.22	174.25	60.65	67.08

Suppl. Table S2. Chemical Shift Assignments for HPX-1 at pH 4.8 and 25°C (continued).

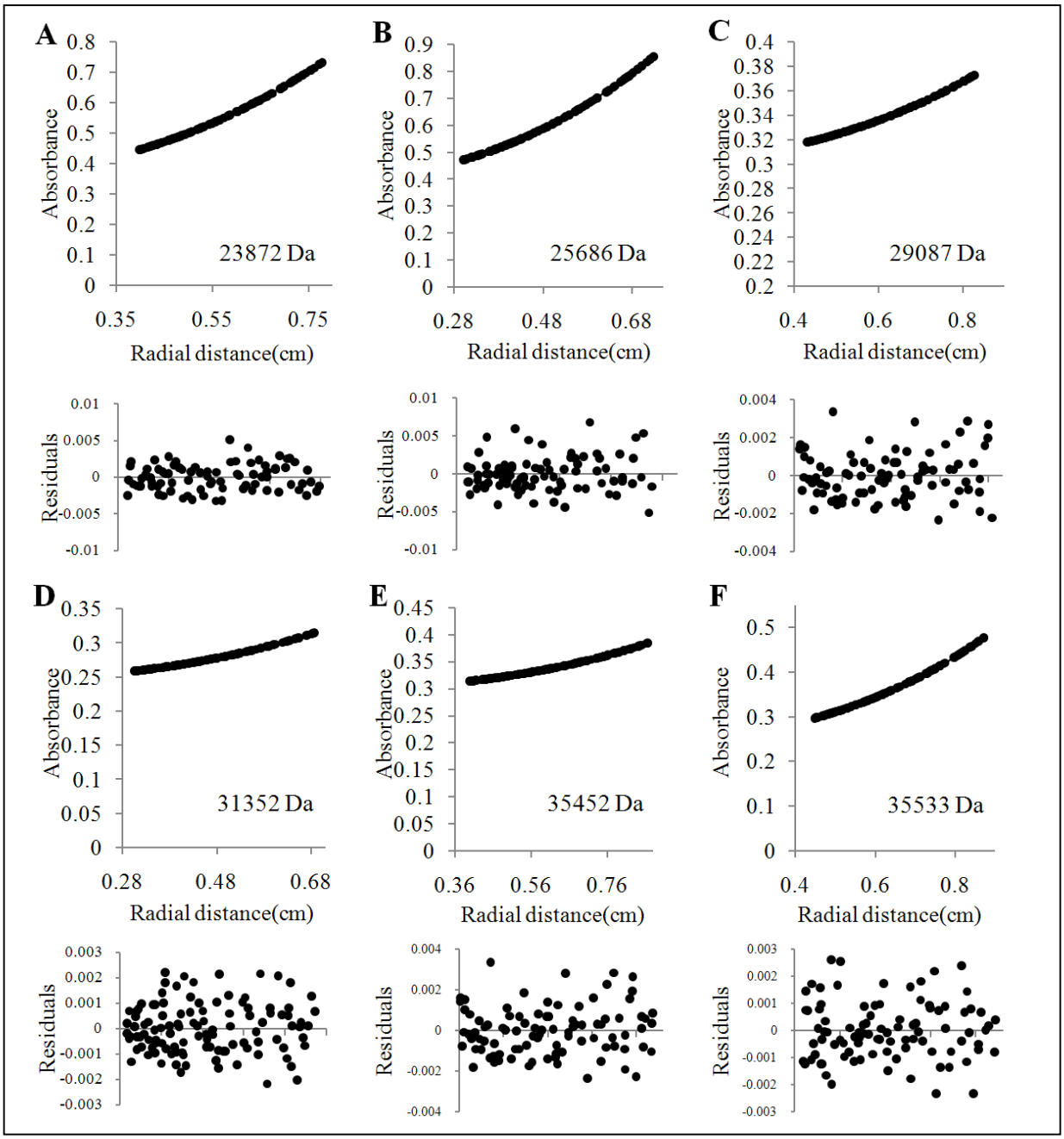
Residue	H	N	C	Cα	Cβ	Residue	H	N	C	Cα	Cβ
Val374	7.76	126.03	173.49	62.18	30.00	Ile422	7.46	110.33	171.60	42.70	n.d.
Lys375	8.88	129.27	173.46	54.23	32.31	Gly423	7.98	116.66	171.52	53.69	29.69
His376	7.22	114.07	170.14	52.77	29.86	His424	7.28	120.19	172.14	53.64	33.79
Ile377	10.10	125.53	173.19	57.45	n.d.	Lys425	9.97	128.78	173.73	60.16	32.91
Asp378	8.52	124.99	173.99	55.04	n.d.	Val426	9.14	127.14	174.77	54.06	39.86
Ala379	7.93	120.33	173.42	50.58	21.17	Asp427	7.94	121.84	172.72	49.89	20.68
Ala380	8.91	126.33	172.15	50.14	22.56	Ala428	8.45	121.21	169.21	57.77	32.20
Leu381	8.08	117.99	171.24	53.51	43.80	Val429	7.81	120.48	169.35	55.02	39.34
Ser382	8.69	120.32	172.65	55.37	63.71	Phe430	8.83	122.86	173.10	52.36	34.09
Glu383	9.17	132.87	175.14	54.54	29.82	Met431	8.85	126.97	174.11	55.69	35.37
Glu384	8.23	131.5	175.72	57.2	28.45	Lys432	9.94	130.05	173.35	54.27	38.61
Asn385	9.15	117.66	174.63	54.39	36.68	Asp433	8.65	104.21	171.81	43.57	n.d.
Thr386	7.79	109.77	174.78	60.47	69.79	Gly434	7.81	119.29	173.13	55.59	39.97
Gly387	8.52	112.88	170.17	44.82	-	Phe435	8.82	121.14	172.37	52.12	38.12
Lys388	7.73	117.59	173.49	51.30	32.04	Phe436	8.71	124.56	172.68	56.02	36.88
Thr389	9.31	124.54	170.84	58.66	68.85	Tyr437	8.73	122.34	174.30	56.20	41.89
Tyr390	8.93	127.90	172.02	54.61	39.31	Phe438	8.95	121.01	174.44	55.33	39.30
Phe391	8.67	121.11	173.05	54.54	n.d.	Phe439	9.19	124.05	173.30	54.06	29.40
Phe392	8.99	125.00	173.31	57.01	n.d.	His440	9.93	123.62	173.22	45.51	n.d.
Val393	9.52	127.54	172.94	60.83	33.73	Gly441	8.52	118.00	173.01	60.55	67.19
Ala394	9.53	134.36	172.96	52.78	15.28	Thr442	8.12	125.00	171.55	54.50	30.98
Asn395	8.37	118.24	173.90	52.08	36.35	Arg443	8.01	123.40	172.07	50.91	n.d.
Lys396	8.56	124.01	172.00	54.12	n.d.	Gln444	9.64	128.68	173.05	54.40	31.57
Tyr397	9.10	116.62	170.54	55.01	41.23	Tyr445	8.29	127.37	172.09	54.07	32.80
Trp398	9.41	124.29	172.91	56.87	n.d.	Lys446	9.83	133.75	171.76	55.32	42.21
Arg399	7.61	116.59	169.70	52.56	n.d.	Phe447	8.40	130.05	172.97	48.60	41.50
Tyr400	9.56	132.31	170.97	55.48	41.91	Asp448	7.46	110.33	171.60	42.70	n.d.
Asp401	7.71	129.13	174.28	51.29	42.00	Pro449	-	n.d.	176.01	62.09	30.83
Glu402	8.92	125.41	176.84	55.99	n.d.	Lys450	8.02	119.82	176.59	57.45	30.43
Tyr403	8.06	119.34	177.06	57.95	35.79	Thr451	6.82	107.76	172.92	59.72	68.54
Lys404	8.81	122.53	175.00	55.44	31.29	Lys452	7.94	120.25	172.41	55.68	27.55
Arg405	7.91	118.16	173.67	55.14	24.70	Arg453	6.53	116.55	173.58	52.66	32.30
Ser406	6.96	112.04	172.39	55.26	64.13	Ile454	8.47	123.26	174.85	59.64	37.08
Met407	9.04	127.42	175.46	54.04	n.d.	Leu455	9.23	130.72	175.92	54.49	41.83
Asp408	8.68	129.52	n.d.	52.05	38.37	Thr456	7.14	111.20	169.43	59.20	70.23
Pro409	-	n.d.	176.30	61.47	30.60	Leu457	8.22	128.32	174.29	52.74	41.97
Gly410	8.64	110.15	171.52	43.89	-	Gln458	9.06	125.91	174.03	52.06	31.79
Tyr411	7.60	119.94	175.28	55.73	37.06	Lys459	8.63	122.62	177.79	54.62	31.73
Pro412	-	n.d.	n.d.	59.67	n.d.	Ala460	8.32	126.88	175.27	53.56	16.35
Lys413	7.96	120.99	173.40	53.10	35.44	Asn461	7.63	114.00	177.04	50.80	36.45
Met414	8.73	119.34	176.85	53.35	29.56	Ser462	7.35	117.19	173.93	61.02	n.d.
Ile415	8.57	128.09	175.92	65.26	37.82	Trp463	7.99	123.87	174.39	57.00	25.93
Ala416	9.19	119.61	177.08	52.45	16.85	Phe464	7.24	118.45	173.36	52.43	36.72
His417	6.96	113.50	174.14	56.15	28.53	Asn465	8.11	118.94	173.18	52.33	35.71
Asp418	6.85	117.70	172.51	53.37	40.36	Cys466	8.48	121.25	173.92	54.44	39.68
Phe419	8.42	120.81	169.66	52.77	36.15	Arg467	8.57	125.46	174.59	54.79	29.32
Pro420	-	n.d.	176.06	62.33	30.14	Lys468	8.40	125.25	173.80	54.80	31.55
Gly421	8.17	111.18	173.54	43.89	-	Asn469	8.00	127.70	177.86	53.13	38.83

n.d. : not determined

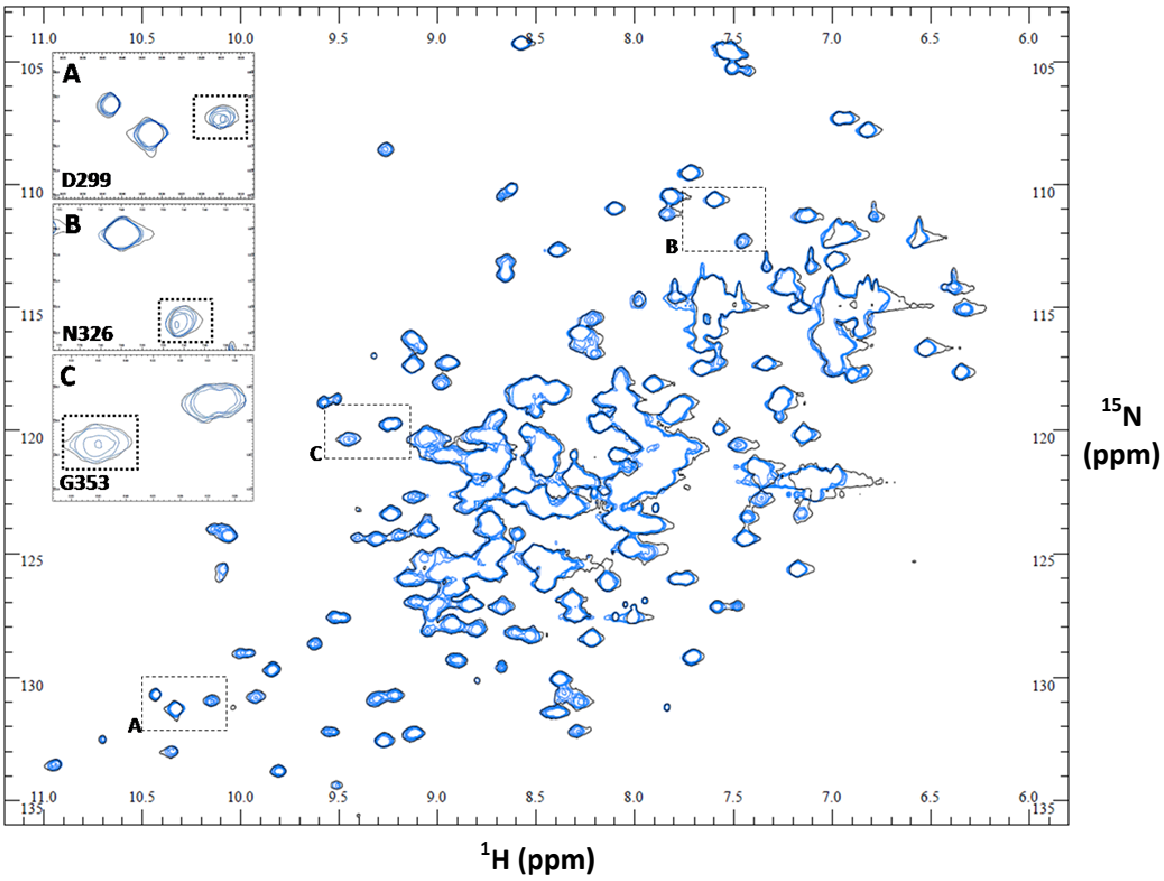
Suppl. Figure S1: **SE AUC analysis of MMP-1 HPX domain.** Data recorded at **(A–C)** 15000rpm and **(D–F)** 18000rpm are shown for HPX concentrations of **(A, D)** 0.5mg/ml, **(B, E)** 1.0mg/ml and **(C, F)** 5.0mg/ml. For each, the calculated MW are shown and the residuals are displayed below. **(G)** Plot of apparent MW *versus* protein concentration for rotor speeds of 15000 and 18000 rpm. The horizontal dashed line indicates the theoretical MW of HPX-1 (23.6 kDa).



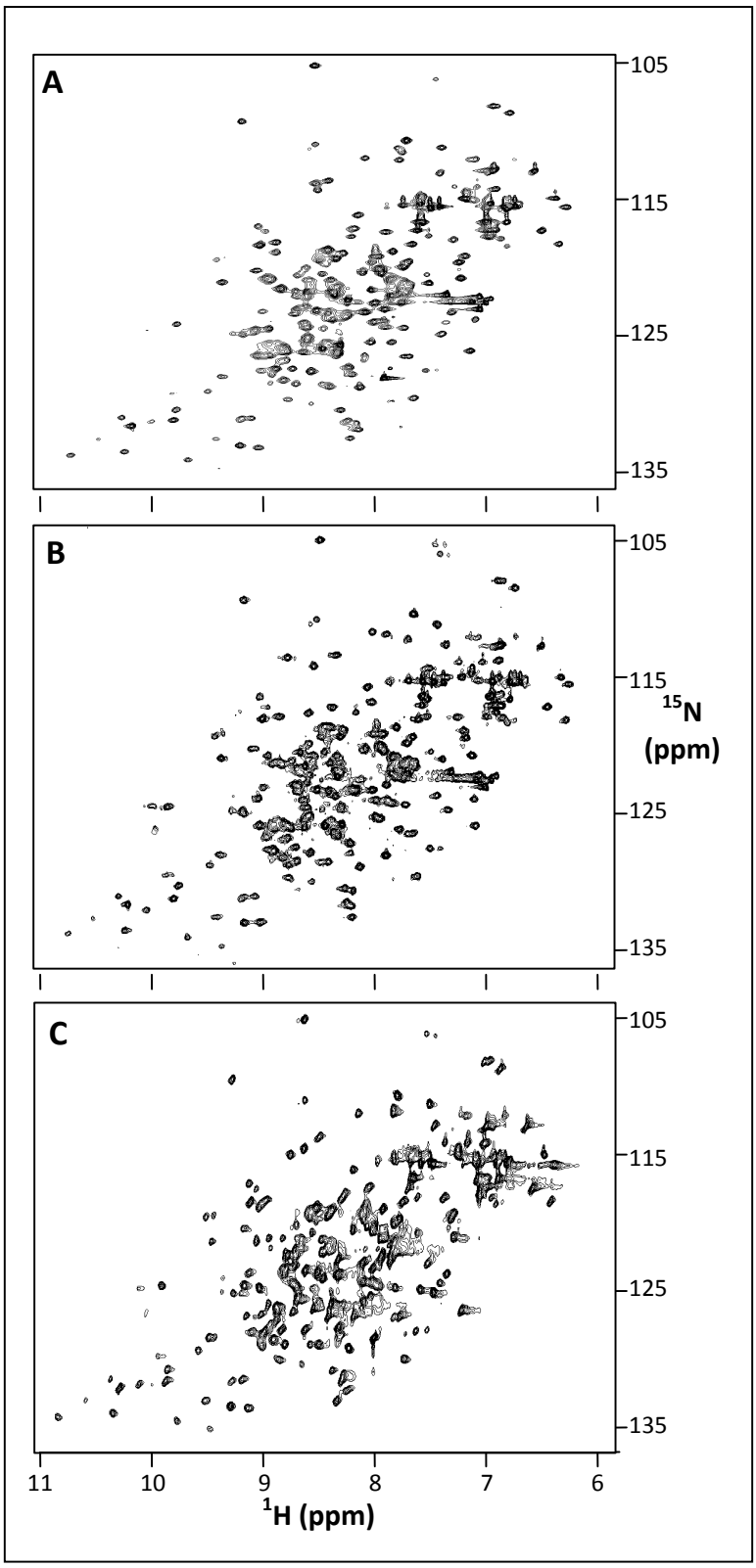
Suppl. Figure S2: SE AUC analysis of HPX:THP complex. Data recorded at 15000 rpm are shown for α1(I)772–787 THP:HPX molar ratios of **(A)** 0:1 (i.e. free HPX), **(B)** 1:1, **(C)** 2:1, **(D)** 5:1, **(E)** 10:1 and **(F)** 20:1. The apparent MW is displayed for each molar ratio. The residuals displayed beneath each plot are randomly dispersed showing a good fit of the model to the data.



Suppl. Figure S3. NMR-Monitored Titration of ^{15}N-labelled HPX-1 with α1(I)772–787 THP. Overlay of the ^{1}H,^{15}N-HSQC spectra of free HPX-1 (in black) with the first six points from the α1(I)772–787 THP titration (i.e. HPX-1:THP molar ratios of 1:0 to 1:1.2). Each spectrum has only one contour to highlight the loss of intensity upon addition of the THP. The inset portions show example losses of spectral intensity due to line broadening of the backbone $^{1}H_{N}$-$^{15}N_{H}$ cross-peaks for **(A)** D299, **(B)** N326 and **(C)** G353.



Suppl. Figure S4: NMR analysis of WT and mutant HPX-1 proteins. Extracts of the $^{1}H^{15}N$-HSQC spectra of **(A)** WT HPX-1 **(B)** mutant HPX-1(F301A), and **(C)** HPX-1(V319A) are shown. Each spectrum was recorded at pH 4.8 and 25°C.



Suppl. Figure S5: SAXS analysis of proMMP-1*, MMP-1* and MMP-1*(F301A). (A-C) Experimental scattering data for **(A)** proMMP-1*, **(B)** MMP-1* and **(C)** MMP-1*(F301A). The logarithm of the scattering intensity is plotted against the momentum transfer. The overlayed red lines in (A) and (B) are scattering patterns simulated from the crystal structure coordinates of proMMP-1* and MMP-1*, respectively, using the program *CRYSOL*. **(D, E)** Orthogonal views of the average low resolution solution state models of (D) proMMP-1*, and (E) MMP-1* generated by *ab initio* modeling from the simulated scattering data (the red lines in parts (A) and (B), respectively). Each has been automatically overlaid over their crystal structures using the program *SUPCOMB*. In the crystal structures, the PRO domain is coloured red, the CAT domain green and the HPX domain blue. The good agreement between the crystal and simulated solution models validates the *ab initio* modeling technique.