Electronic Supplementary Information for the paper:

Nickel(II)-promoted specific hydrolysis of zinc finger proteins

Agnieszka Belczyk-Ciesielska[a], Brigitta Csipak[b], Bálint Hajdu[b], Aleksandra Sparavier[a], Masamitsu N. Asaka[c], Kyosuke Nagata[c], Béla Gyurcsik[b,*] and Wojciech Bal[a,*]

[a]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a; 02-106, Warsaw, Poland*

[b]*Department of Inorganic and Analytical Chemistry, MTA-SZTE Bioinorganic Chemistry Research Group, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary*

[c]*Nagata Special Laboratory, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, 305-8575 Tsukuba, Japan*

Table S1. Expected products of P-1MEY and P-1MEY# hydrolysis. Ni(II)-cleavage sites are underlined, (L) and (S) denote long and short hydrolytic products. Masses were calculated by ProtParam, Expasy. Mass spectra of the cleaved products are shown in Fig. S8.

Cleavage site	Protein sequence	M_W calc. (Da)
-	*P-1MEY*	13012.3
SRH (L)	GHHHHHHHHHHSSGHIEGRHMLEPGEKPYKCPECGKSFSQSSNLQKHQRTHTGEKPYKCPECGKSFSQSSDLQKHQRTHTGEKPYKCPECGKSFSRSDHL	11506.6
SGH (L)	SGHIEGRHMLEPGEKPYKCPECGKSFSQSSNLQKHQRTHTGEKPYKCPECGKSFSQSSDLQKHQRTHTGEKPYKCPECGKSFSRSDHLSRHQRTHTGKKTS	11496.7
SGH + SRH (L)	SGHIEGRHMLEPGEKPYKCPECGKSFSQSSNLQKHQRTHTGEKPYKCPECGKSFSQSSDLQKHQRTHTGEKPYKCPECGKSFSRSDHL	9991.1
SGH+SDH (L)	SGHIEGRHMLEPGEKPYKCPECGKSFSQSSNLQKHQRTHTGEKPYKCPECGKSFSQSSDLQKHQRTHTGEKPYKCPECGKSFSR	9538.6
SRH (S)	SRHQRTHTGKKTS	1523.6
SGH (S)	GHHHHHHHHHHS	1533.5
SDH (S)	SDHLSRHQRTHTGKKTS	1976.1
-	*P-1MEY#*	13001.3
SGH (L)	SGHIEGRHMLEPGEKPYKCPECGKSFSQSSNLQKHQRTHTGEKPYKCPECGKSFSQSSDLQKHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	11485.8

accggcgaaaaaccgtataaatgtcccgaatgcgg**gaagtcattttcccgcagcgataac**

T G E K P Y K C P E C G K S F S R S D N

ctggt*t*cgccatcagcgtacccatactggtaaaaaaacctcttaa*ggatcc*

L V R H Q R T H T G K K T S - G S

Scheme S1. Oligonucleotide primer design: The segments of the DNA sequence encoding for P-1MEY# protein, including primer hybridization sites (1MEY#-R in bold/underlined and 1MEY#-F in bold/italic). The introduction of the desired point mutations resulted in base pair mismatch at the sites highlighted by red background. In parallel the protein sequence is also shown to indicate the resulting mutations (also highlighted by red background). By these changes Asn (N) and Val (V) were introduced instead of His (H) and Ser (S), respectively.

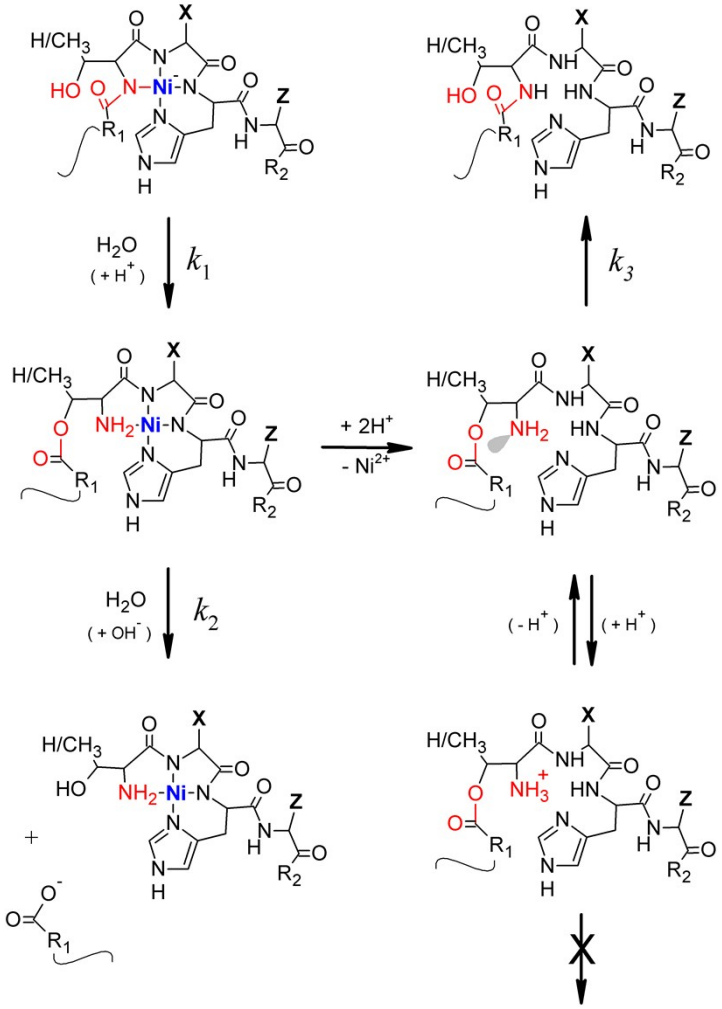
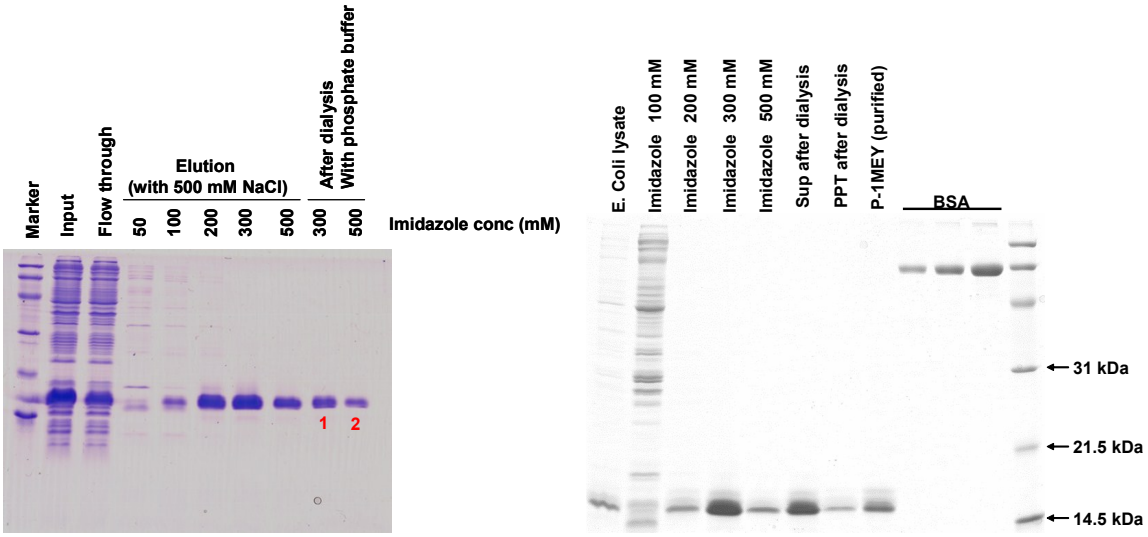


Fig. S1. Molecular mechanism of Ni(II)-dependent hydrolysis of the X-(Ser/Thr)-X-His-X sequence, including the reactivity of ester intermediate.[31,35] The charge displayed at the Ni(II) center takes into account the negative charges of the deprotonated peptide-nitrogens.

(a)



(b)

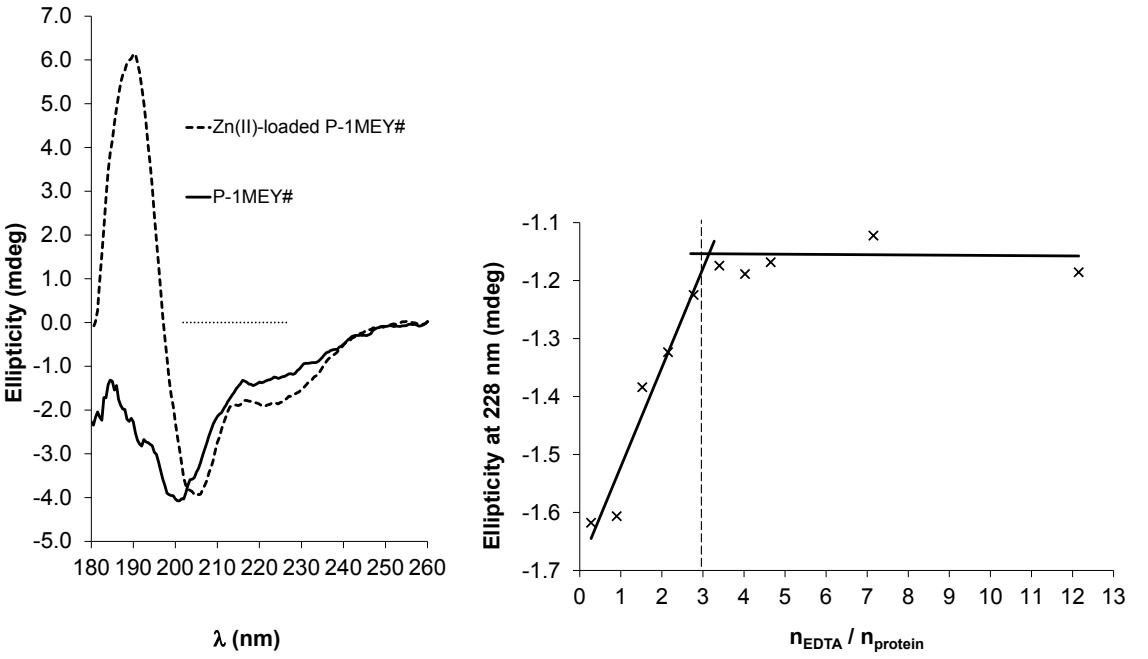


Fig. S2. (a) Monitoring of the protein purification by SDS-PAGE. The largest amounts of both the P-1MEY (left panel) and the P-1MEY# (right panel) proteins were eluted at 300 mM imidazole from the Ni(II)-charged IMAC resin. (b) Circular dichrosim spectra of the Zn(II)-loaded P-1MEY# protein and its apo form (left panel). The titration of the P-1MEY# protein by EDTA monitored through the change in the CD intensity (right panel).

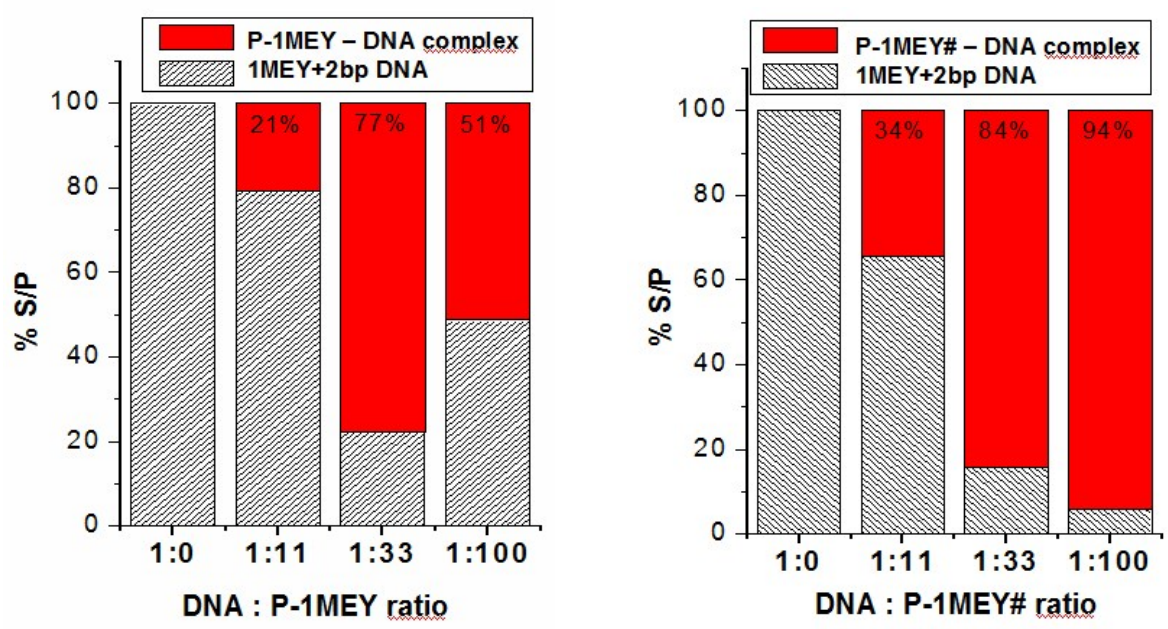


Fig. S3. Quantification of the EMSA experiment shown in Fig. 1 by densitometric analysis (Gel QuantNET). The comparison of the DNA-protein interaction in case of the 1:33 samples shows similarity, but the P-1MEY# binds DNA slightly more efficiently (with ~10% higher affinity) than P-1MEY, what is also represented by the unbound DNA fraction present in the latter case. The figure at 100× protein excess is not as clear because of the supplementary non-specific binding with the 1MEY+2bp DNA probe (similarly to that seen with the non-specific DNA probe, Fig. 1. lane 8.) The smaller intensity of the bands here may arise partially from the band splitting and eventually from the less material loaded on the gel. Nevertheless, at high protein excess almost all the DNA is bound by both P-1MEY and P-1MEY#.

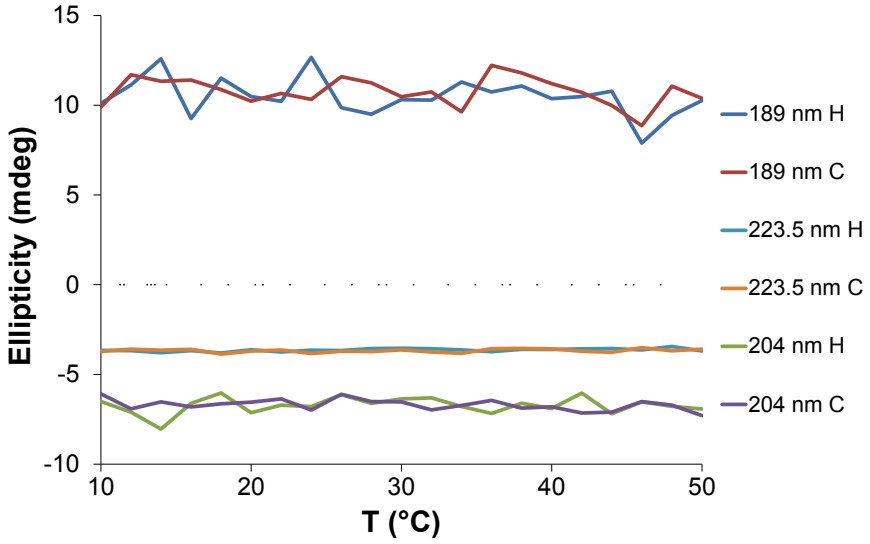


Fig. S4. The protein stability check by CD spectroscopy. Temperature dependence of CD signals of P-1MEY at selected wavelegths. H and C stand for the heating and cooling procedures, respectively.

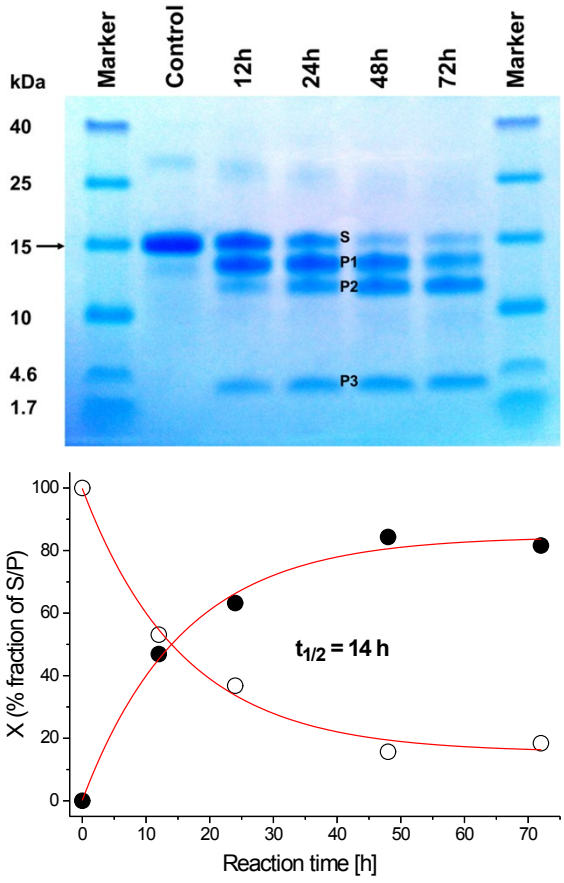
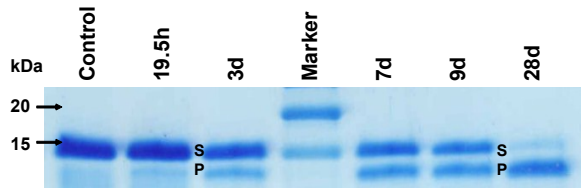
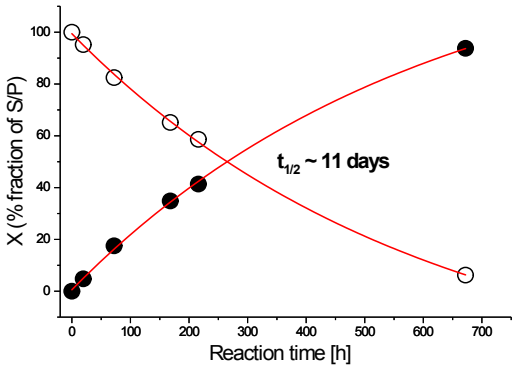


Fig. S5. Top: Tricine-SDS-PAGE monitoring of the P-1MEY protein hydrolysis (pH 8.2, 37°C in 100 mM HEPES buffer). A control experiment without Ni(II) ions was also set up. $c_{protein}$ = 40 μM, $c_{Zn(II)}$ = 100 μM, $c_{Ni(II)}$ = 400 μM, S – substrate, P – products: P1 – mixture of the cleaved protein fragments lacking either the N- or the C-terminal peptide, P2 – central part of the protein, P3 – N or C-terminal short peptide products.

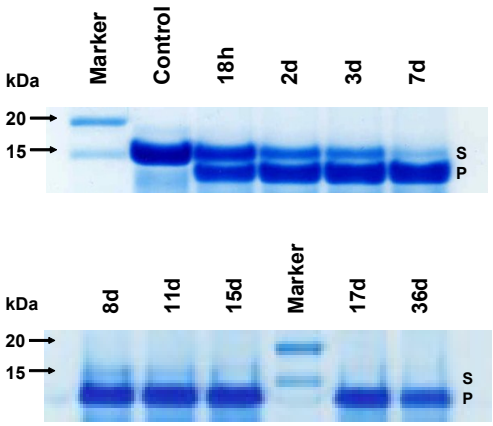
Bottom: Densitometric analysis of the P-1MEY hydrolysis products in Tricine-SDS-PAGE. The image was processed by ImageJ program. The percentage of the original protein and the sum of the products are plotted as a function of time. The half-life of P-1MEY at 37°C and pH 8.2 was estimated as ca. 14 h. In the control experiment without Ni(II) no changes were observed after 72 hours of incubation, showing that the protein itself was stable.

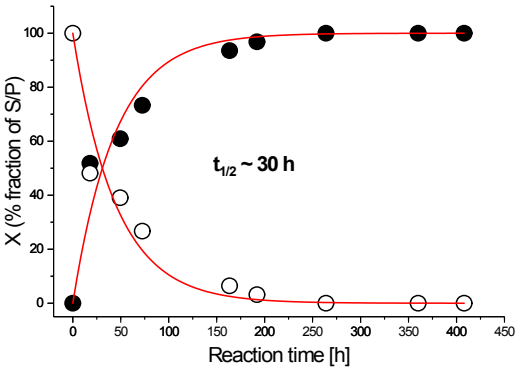
(a)


kDa
Control
19.5h
3d
Marker
7d
9d
28d
20
15
S
P
S
P

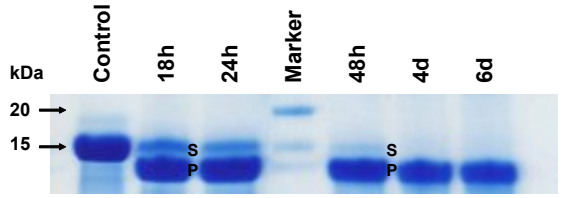

X (% fraction of S/P)
Reaction time [h]
t1/2 ~ 11 days

(b)


kDa
Marker
Control
18h
2d
3d
7d
20
15
S
P
kDa
8d
11d
15d
Marker
17d
36d
20
15
S
P


X (% fraction of S/P)
Reaction time [h]
t1/2 ~ 30 h

(c)


kDa
Control
18h
24h
Marker
48h
4d
6d
20
15
S
P
S
P

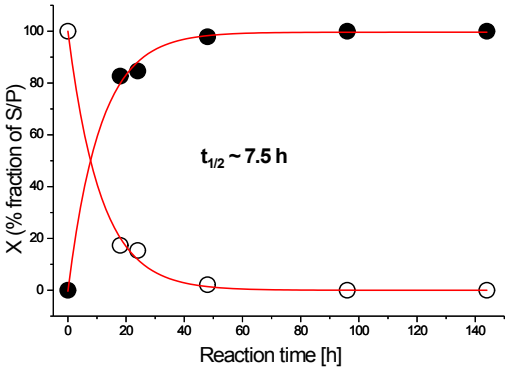

X (% fraction of S/P)
Reaction time [h]
t1/2 ~ 7.5 h

Fig. S6. The Ni(II)-dependent hydrolysis of P-1MEY# protein at (a) 37°C, pH 7.4; (b) 37°C, pH 8.2 and (c) 50°C, pH 8.2. **Left:** electrophoretic analysis. (S – substrate, P – product). Incubation times are given in hours (h) and days (d), M – protein ladder Precision Plus Protein™ Dual Xtra Standards BIO-RAD (molecular masses in kDa) in all Tricine-SDS-PAGE images. **Right:** Kinetic plots derived from the densitometric analysis of the tricine-SDS-PAGE images by ImageJ program.

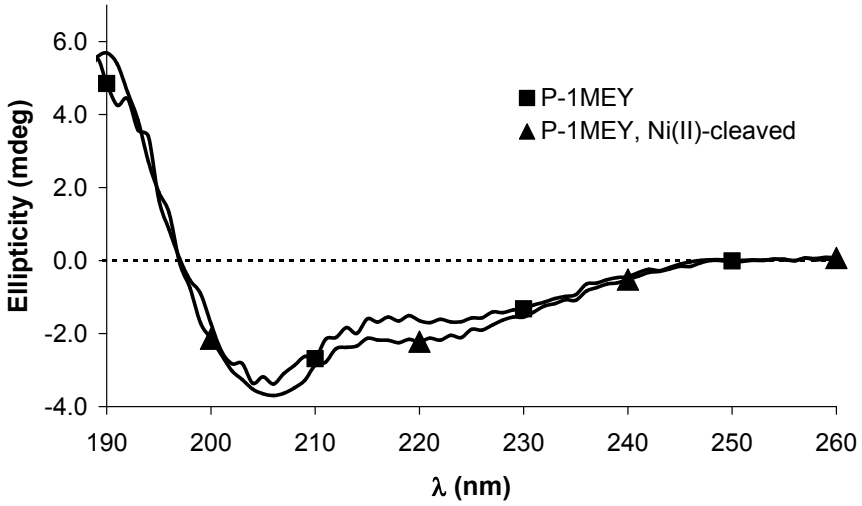
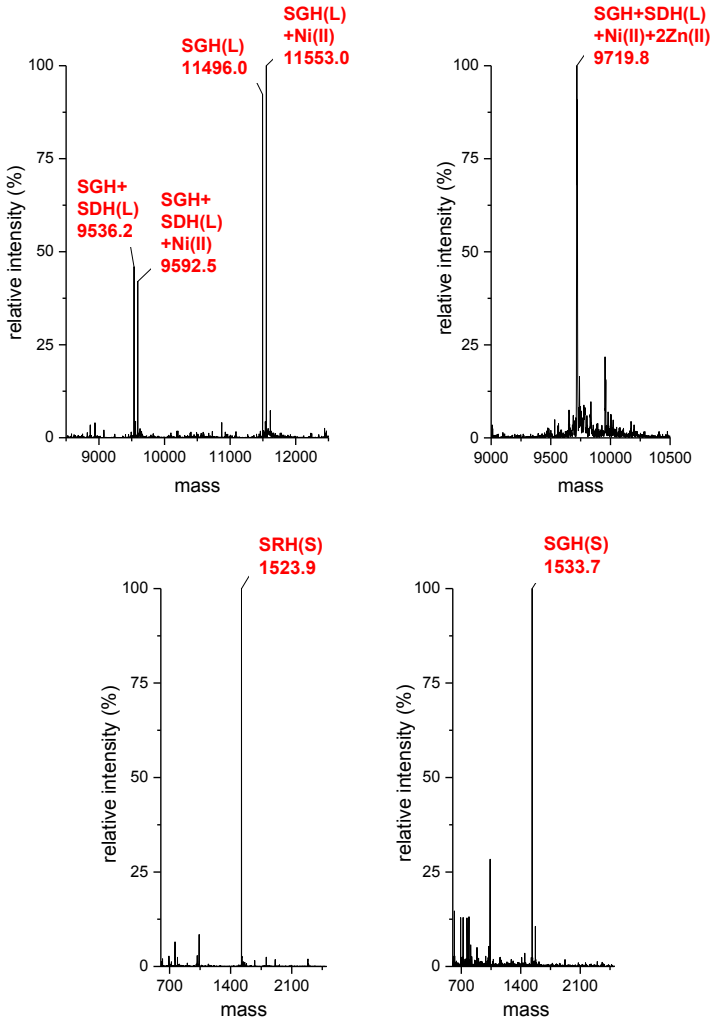


Fig. S7. Circular dichroism spectra of the P-1MEY protein and its Ni(II)-cleaved product (5 mM phosphate buffer, pH 7.4) at 3.1 μM protein concentration in a 2 mm quartz cell. The cleavage reaction was carried out at 50°C and pH 8.2 as described above, and the sample buffer was exchanged before the CD measurement.

(a)


SGH(L)
+Ni(II)
11553.0
SGH(L)
11496.0
SGH+
SDH(L)
9536.2
SGH+
SDH(L)
+Ni(II)
9592.5
SGH+SDH(L)
+Ni(II)+2Zn(II)
9719.8
SRH(S)
1523.9
SGH(S)
1533.7
relative intensity (%)
mass

(b)

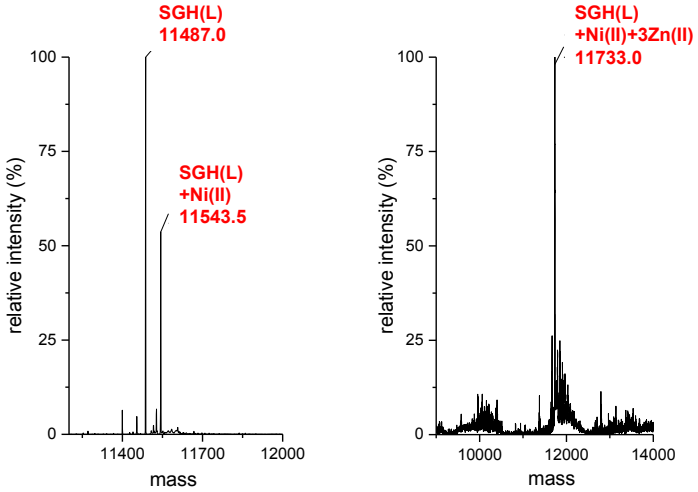

SGH(L)
11487.0
SGH(L)
+Ni(II)
11543.5
SGH(L)
+Ni(II)+3Zn(II)
11733.0
relative intensity (%)
mass

Fig. S8. Mass spectra of the cleaved protein products detected in acidified (left panels) and neutral (right panels) solutions. (a) SGH(L), SGH+SDH(L), SRH(S) and SGH(S) cleavage products of P-1MEY protein (b) SGH(L) cleavage products of P-1MEY# protein. (For the explanation of the nomenclature see Table S1.) It is worth mentioning that the Ni(II) ions could remain partially bound to the proteins even under acidic conditions, due to the inertness of the ATCUN-like complex.