Electronic Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Dynamics of the Intrinsically Disordered Protein CP12 in its association with GAPDH in the green alga *Chlamydomonas reinhardtii*: a fuzzy complex*

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CP12 sequences

CP12 sequences alignment with CLUSTAL 2.1.

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[Arabidopsis thaliana]          KAAPEGGISDVVEKSIKEAQET-CAGDPVSGECVAAWDEVEELSAAAS 99
[Chlamydomonas reinhardtii]   --SGQPAVDLNKKVQDAVKEAEDA-CAKG-TSADCAVAWDTVEELSAAVS 46
[Synechococcus elongatus]     ------MSDIQEKIEQARQEAHAISEEKGATSPDAAAAWDAVEELQAEAA 44
                                     .:.. ::.: :**.      . .* :...*** ****.* .:

[Arabidopsis thaliana]        HARDKKKADG--SDPLEEYCKDNPETNECRTYDN 131
[Chlamydomonas reinhardtii]   HKKDAVKADVTLTDPLEAFCKDAPDADECRVYED 80
[Synechococcus elongatus]     HQRQQ-KSET--EPFFGDYCSENPDAAECLIYDD 75
                              * ::  *::       :  :*.: *:: **  *::
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"*****" means that the residues in that column are identical in all sequences in the alignment.

"**:**" means that conserved substitutions have been observed.

"**.**" means that semi-conserved substitutions are observed.

MALDI-ToF mass spectrometry analyses

All mass analyses were performed on the MALDI-ToF mass spectrometer Microflex II from Bruker Daltonics (Deutschland).

CP12 Tyr78^{nox}

Determination of global mass of labeled and unlabeled CP12.

Samples of 20 to 30 pmoles of CP12 (unlabeled) and labeled CP12 Tyr78^{Nox} were prepared by dilution in 10µL of 0.1% trifluoroacetic acid (TFA) in water (v/v) before being spotted onto a MALDI Target plate (1 µL) and added of a saturated solution of matrix α-cyano-4-hydroxycinnamic acid (1 µL) of 70% acetonitrile in water, 0.1% TFA (v/v). The global mass was measured on the MALDI-ToF mass spectrometer Microflex II from Bruker Daltonics (Deutschland) in the range from 2000 to 20000 Da and in a linear and positive mode. External mass calibration was performed on the averaged $[M+H]^+$ from the Protein Calibrant I (Bruker Daltonics).

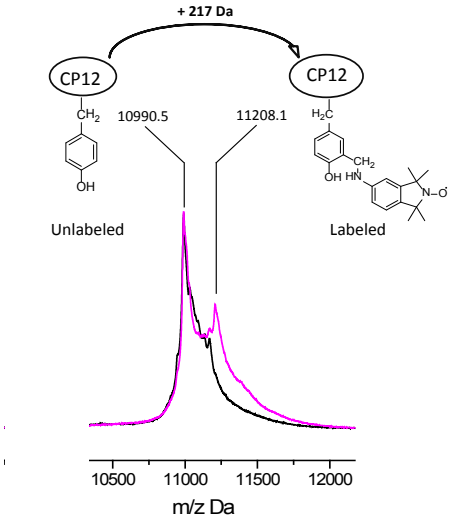

Figure SI.1: MALDI-ToF analyses: superimposed spectra of unlabeled (10990.5 Da, black) and labeled CP12 Tyr78^{Nox} (11208.1 Da, magenta).

MALDI-ToF peptide mass fingerprint of the wild type CP12 (without previous reduction and alkylation)

Samples of 60 to 100 pmoles were digested by Trypsin at a ratio enzyme to substrate of 1/50 (w/w) (Sigma, St Louis, MO, USA) for 4 hours at 37°C. The digested solutions were then acidified by 1 µL of 12.5% trifluoroacetic acid (TFA) in water (v/v), vacuum dried and then dissolved in 0.1% TFA in water (v/v) before being spotted onto a MALDI Target plate (1 µL) and added of a saturated solution of matrix α-cyano-4-hydroxycinnamic acid (1 µL) of 70% acetonitrile in water, 0.1% TFA (v/v). Tryptic peptides were analyzed on the MALDI-ToF mass spectrometer Microflex II from Bruker Daltonics in the range from 600 to 5000 Da. Data acquisition was operated in positive and reflectron mode. External mass calibration was performed on the mono-isotopic $[M+H]^+$ from the peptide calibration standard (Bruker Daltonics). A peak list was generated by a PMF (Peptide Mass Fingerprint) method from the FlexAnalysis software and manually checked. The experimentally measured peptide masses were compared with the theoretical tryptic peptides calculated from the sequence of CP12, with variable modifications of the cysteine residues in reduced (S- or -SH) or oxidized (-S-S-, -2 Da) states, of tryptophane and tyrosine by the spin label **1** [Mannich bridge structure increased mass by 217 Da (nitroxide –NO•) or 218 (reduced state of nitroxide –NOH) or benzoxazine structure increased mass by 229 Da (oxidated state of nitroxide –NO•) or 230 (reduced state of nitroxide –NOH)].

GHHHHHHHHHHSSGHIEGRHMSGQPAVDLNKKVQDAVKEAEDACAKGTSADCAVAWDTVEEL SAAVSHKKDAVKADVTLTDPLEAFCKDAPDADECRVYED (sequence coverage 100%)

Fragments detected after Trypsin digestion	Measured MH+	Calculated MH+	Modification
HMSGQPAVDLN**K**	1296.804	1296.629	
HMSGQPAVDLN**K**	1313.861	1312.624	1*M oxidized
HMSGQPAVDLN**KK**	1424.944	1424.724	
KDAV**K**ADVTLTDPLEAFC**K**	2064.280	2064.061	
GHHHHHHHHHHSSGHIEG**R**	2270.228	2270.015	
GTSADCAVAWDTVEELSAAVSH**K**	2346.598	2347.08	
DAV**K**ADVTLTDPLEAFC**K**DAPDADEC**R**	2904.923	2906.311	2*C oxidized
DAV**K**ADVTLTDPLEAFC**K**DAPDADEC**R**VYED	2998.773	2999.285	2*C oxidized
EAEDACA**K**GTSADCAVAWDTVEELSAAVSH**K**	3289.651	3290.487	2*C oxidized
K	3801.259	3802.746	2*C oxidized
VQDAV**K**EAEDACA**K**GTSADCAVAWDTVEELS AAVSH**K**	3929.173	3930.841	2*C oxidized
VQDAV**K**EAEDACA**K**GTSADCAVAWDTVEELS AAVSH**KK**			

Table SI.1: Peptide Mass Fingerprint of the unlabeled CP12. Identified peptides are described by their measured MH+, calculated MH+ and the corresponding sequence. The last column indicates the modification of the residues. Sequence of the unlabeled CP12 has been covered by the identified peptides at 100% (amino acids are indicated in bold and underlined in the sequence).

GHHHHHHHHHHSSGHIEGRHMSGQPAVDLNKKVQDAVKEAEDACAKGTSADCAVAWDTVEEL SAAVSHKKDAVKADVTLTDPLEAFCKDAPDADECRVYED (sequence coverage 100%)

Fragments detected after Trypsin digestion	Measured MH+	Calculated MH+	Modification
VYED	755.09	755.212	Y+218[a]+12[b]
HMSGQPAVDLN**K**	1296.804	1296.629	
HMSGQPAVDLN**KK**	1424.980	1424.724	
KDAV**K**ADVTLTDPLEAFC**K**	2076.083	2076.061	K+12[b]
GHHHHHHHHHHSSGHIEG**R**	2270.272	2270.015	
GTSADCAVAWDTVEELSAAVSH**K**	2347.234	2347.08	
DAV**K**ADVTLTDPLEAFC**K**DAPDADEC**R**VYED	2998.927	2999.285	
DAV**K**ADVTLTDPLEAFC**K**DAPDADEC**R**VYED	3225.848	3228.285	Y+217+12[b]
EAEDACA**K**GTSADCAVAWDTVEELSAAVSH**KK**DAV**K**	3702.288	3703.714	2*C oxidized
VQDAV**K**EAEDACA**K**GTSADCAVAWDTVEELSAAVSH**K**	3801.201	3802.746	2*C oxidized
VQDAV**K**EAEDACA**K**GTSADCAVAWDTVEELSAAVSH**KK**	3929.055	3930.841	2*C oxidized

Table SI.2: Peptide Mass Fingerprint of the labeled CP12. Identified peptides are described by their measured MH+, calculated MH+ and the corresponding sequence. The last column indicates the modification of the residues. Sequence of the unlabeled CP12 has been covered by the identified peptides at 100% (amino acids are indicated in bold and underlined in the sequence).

[a] 218 = mass increment corresponding to reduced state of nitroxide –NOH

[b] 12 = mass increment corresponding to the imine formation between the Lysine (K) amino group and formaldehyde present in the reaction mixture.

CP12 Cys23^{proxyl} and Cys31^{proxyl}

For global mass analyses, the protein solutions of unlabeled and labeled CP12 variants Cys23^{proxyl} and Cys31^{proxyl}, at 30µM each in 50mM Tris pH 8, 0.1M NaCl, were directly spotted onto a MALDI Target plate at 1µL each one, and immediately acidified by addition of a saturated solution of matrix α-Cyano-4-hydroxycinnamic acid (1 µL of 70% acetonitrile in water at 0.1% TFA (v/v)). The spots were desalted by adding 2x 2µL of 0.1% TFA in water (v/v) then quickly removed and air-dried before analysis. Global mass spectra were acquired in linear and positive mode, using an external calibration on the Protein calibration Standard I (Bruker Daltonics).

For peptide mass fingerprint analyses, the protein solutions (2µL, 60pmol) were diluted ten times in 100mM ammonium bicarbonate pH 8, and submitted to reduction by 10mM DTT for 45 min at 56°C in the dark and to subsequently alkylation by 55mM Iodoacetamide for 30 min at room temperature in the dark. Digestion by Trypsin was performed at a ratio enzyme to substrate of 1/50 (w/w) (Sigma, USA) for 4 hours at 37°C. The digested solutions were then acidified by adding 1µL of 12.5% TFA in water (v/v), before being spotted onto a MALDI Target plate in the same conditions as described above. Spectra were acquired in positive and reflectron mode and external mass calibration was applied using the Peptide calibration Standard (Bruker Daltonics). A peak list was generated by Peptide Mass Fingerprint method in the FlexAnalysis software and processed in the Biotools software (Bruker Daltonics) including the following parameters: variable modifications of the methionine residues by oxidation (increased mass by 16 Da) and of the cysteine residues by i) the proxyl (**-O**$^{\cdot}$ form (increased mass of 237 Da) or **-OH** form in reductive conditions (increased mass of 238 Da) or ii) the carbamidomethyl group (increased mass of 57 Da) or iii) the reduced (-SH) or oxidized (-S-S-) states.

MALDI-ToF global mass analyses of unlabeled CP12 Cys31 and labeled CP12 Cys31^{proxyl}

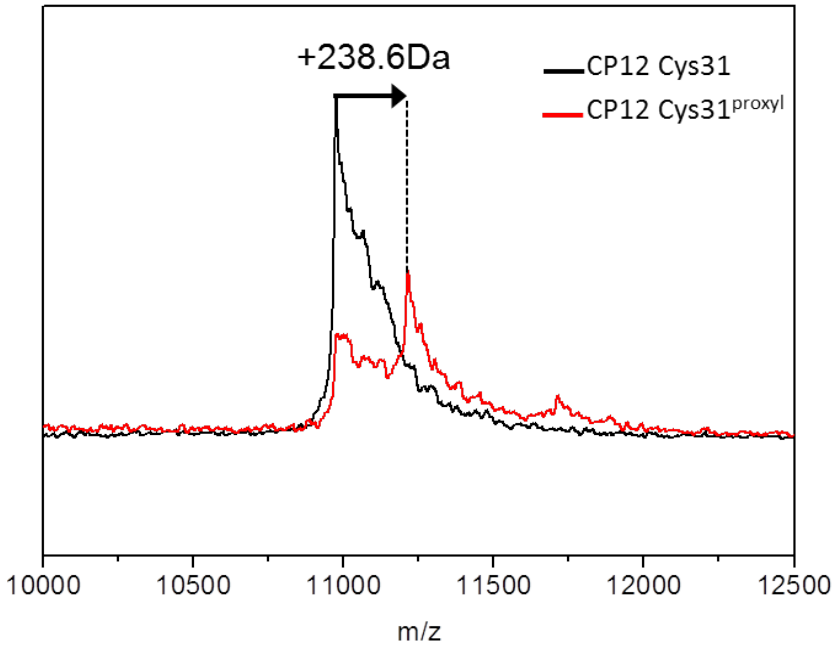

Figure SI.2: MALDI-ToF global mass analyses of unlabeled CP12 Cys31 (black line) and labeled Cys31^{proxyl} (red line). The mass increment of 238.6Da corresponds to the grafting of a single label to CP12.

MALDI-ToF Peptide Mass Fingerprint of the reduced-alkylated unlabeled CP12 Cys31 after Trypsin digestion

No	*Theo. Mo*	*Dev(Da)*	*Range*	*PC*	*Sequence*	*Modification*
1	1295.629	0.017	[20-31]	0	HMSGQPAVDLNK	
2	1423.724	-0.001	[20-32]	1	HMSGQPAVDLNKK	
3	1496.573	-0.009	[89-101]	1	DAPDADE**C**RVYED	(1-SH)
4	2269.015	-0.021	[1-19]	0	GHHHHHHHHHHSSGHIEGR	
5	2346.080	-0.053	[47-69]	0	GTSAD**C**AVAWDTVEELSAAVSHK	(1-SH)
6	2403.101	-0.052	[47-69]	0	GTSAD**C**AVAWDTVEELSAAVSHK	(1 Carbamidomethyl C)
7	2474.175	-0.035	[47-70]	1	GTSAD**C**AVAWDTVEELSAAVSHKK	(1-SH)
8	2492.084	-0.026	[75-97]	1	ADVTLTDPLEAF**C**KDAPDADE**C**R	(C Oxidized S-S)
9	2531.196	-0.027	[47-70]	1	GTSAD**C**AVAWDTVEELSAAVSHKK	(1 Carbamidomethyl C)
10	2998.285	-0.008	[75-101]	2	ADVTLTDPLEAF**C**KDAPDADE**C**RVYE	(C Oxidized S-S)
11	3411.512	0.03	[71-101]	3	DAVKADVTLTDPLEAF**C**KDAPDADE**C**RVYED	(Oxidized S-S)

GHHHHHHHHHHSSGHIEGRHMSGQPAVDLNKKVQDAVKEAEDASAK**GTSADCAVAWDTVEELS AAVSHKKDAVKADVTLTDPLEAFCKDAPDADECRVYED**
Coverage: 86% in bold highlighted in grey

MALDI-ToF Peptide Mass Fingerprint of the reduced-alkylated unlabeled CP12 Cys31^{proxyl} after Trypsin digestion

No	*Theo. Mo*	*Dev(Da)*	*Range*	*PC*	*Sequence*	*Modification*
1	819.361	0.030	[39-46]	0	EAEDASAK	
2	1295.629	0.004	[20-31]	0	HMSGQPAVDLNK	
3	1311.624	-0.43	[20-31]	0	HMSGQPAVDLNK	(1Oxidation M)
4	1423.724	-0.006	[20-32]	1	HMSGQPAVDLNKK	
5	1439.719	-0.061	[20-32]	1	HMSGQPAVDLNKK	(1Oxidation M)
6	2269.015	-0.015	[1-19]	0	GHHHHHHHHHHSSGHIEGR	
7	2346.080	0.096	[47-69]	0	GTSAD**C**AVAWDTVEELSAAVSHK	(1-SH)
8	2403.101	-0.001	[47-69]	0	GTSAD**C**AVAWDTVEELSAAVSHK	(1Carbamidomethyl C)
9	2474.175	-0.017	[47-70]	1	GTSAD**C**AVAWDTVEELSAAVSHKK	(1-SH)
10	2492.084	-0.002	[75-97]	1	ADVTLTDPLEAF**C**KDAPDADE**C**R	(C Oxidized S-S)
11	2531.196	-0.022	[47-70]	1	GTSAD**C**AVAWDTVEELSAAVSHKK	(1Carbamidomethyl C)
12	2584.212	-0.004	[47-69]	0	GTSAD**C**AVAWDTVEELSAAVSHK	(1Proxyl – **OH**, 238 C)
13	2712.203	0.077	[47- 70]	1	GTSAD**C**AVAWDTVEELSAAVSHKK	(1Proxyl – **OH**, 238 C)
14	2998.285	0.171	[75-101]	2	ADVTLTDPLEAF**C**KDAPDADE**C**RVYED	(C Oxidized (S-S)
13	3124.473	-0.114	[47-74]	2	GTSAD**C**AVAWDTVEELSAAVSHKKDAVK	(1Proxyl - **O**$^{\cdot}$, 237 C)

GHHHHHHHHHHSSGHIEGRHMSGQPAVDLNKKVQDAVK**EAEDASAKGTSADCAVAWDTVEELSAAVSHKKDAVKADVTLTDPLEAFCKDAPDADECRVYED**
Coverage 94% in bold highlighted in grey

Table SI-3: MALDI-ToF Peptide Mass Fingerprint of the labeled "C23S" by Proxyl, after reduction-alkylation and Trypsin digestion. Identified peptides are described by their theoretical Monoisotopic mass (*Theo. Mo*), standard deviation (*Dev.*) in Da, the range in the sequence of CP12 (numbered with the Tag of CP12), partial cleavage (*PC*) by Trypsin, the corresponding sequence and the modification.

MALDI-ToF global mass analyses of unlabeled CP12 Cys23 and labeled CP12 Cys23^{proxyl}

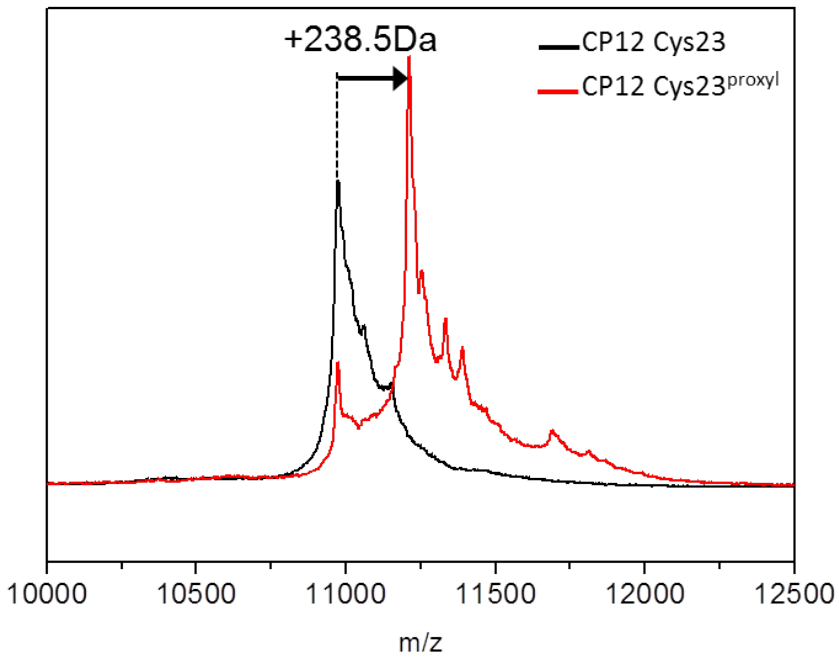

Figure SI.3: MALDI-ToF global mass analyses of unlabeled CP12 Cys23 (black line) and labeled Cys23^{proxyl} (red line). The mass increment of 238.5Da corresponds to the grafting of a single label to CP12.

EPR analyses

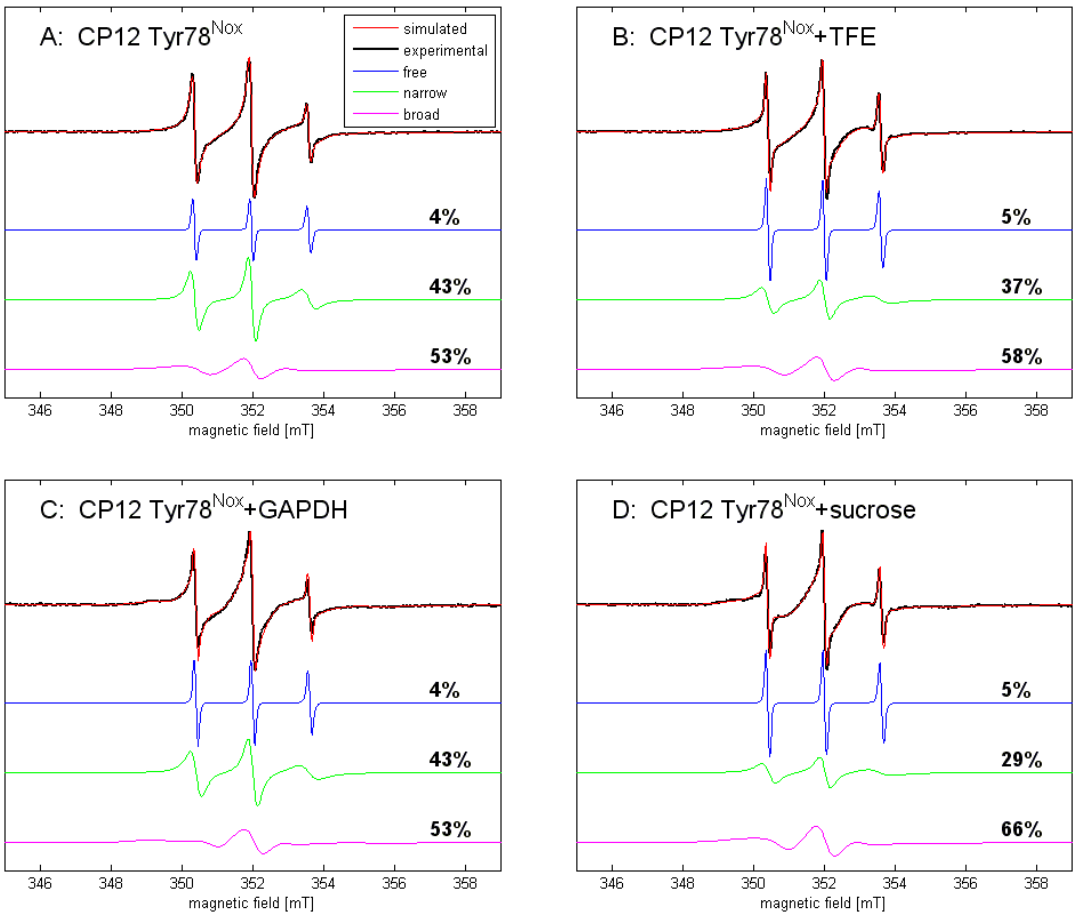

Figure SI.4: Experimental EPR spectra of CP12 Tyr78^{Nox} (black line) alone (A), in TFE 30% v/v (B), in presence of equimolar tetrameric GAPDH (C) and in 30% w/v sucrose (D). The simulated spectra using EasySpin software (red line) are superimposed on the experimental ones. The three components required to obtain the best fits are: free label in solution (blue line), narrow component (green line) corresponding to the fast-motion component and broad component (pink line) corresponding to the slow-motion component.