Supporting Information

1. Calculation concerning the oligomerization:

This calculation has been applied to the equilibrium between dimer and tetramer, whatever the nature of the other oligomeric form, which does not interfere in the calculation. Here we have considered an hexamer.

The expression of the equilibrium constant for the dissociation of the tetramer, K_{tet}, as a function of the concentrations at equilibrium is:

$$K_{tet} = [D']^2 / ([T']*C) \quad \text{where } C = 1 \text{ mol.L}^{-1} \tag{1}$$

[D'] and [T'] represent the concentration of dimer and tetramer respectively.

$$C_0 = [D] + [T] + [H] \tag{2}$$

[D], [T], [H] and C_0 represent the concentration of dimer, tetramer and hexamer and the total protein concentration, respectively, all expressed in monomer unit, and linked to the concentrations of dimer and tetramer by:

$$[D'] = [D] /2 \tag{3}$$

$$[T'] = [T] /4 \tag{4}$$

Using equations (3) and (4), the expression of K_{tet} is now:

$$K_{tet} = ([D]/2)^2 / ([T]/4) \tag{5}$$

further simplified to:

$$K_{tet} = [D]^2 / [T] \tag{6}$$

The percentage of protein in each oligomeric form is:

$$\%T = [T]*100 / C_0 \tag{7}$$

$$\%H = [H]*100 / C_0 \tag{8}$$

K_{tet} is expressed as a function of C_0, [T] and [H], by replacing [D] in (6) using equation (2):

$$K_{tet} = (C_0 - [T] - [H])^2 / [T] \quad (9)$$

Using the equations (7) and (8), [T] and [H] are replaced in equation (9) by expressing them as a function of C_0, %T and %H:

$$K_{tet} = 0.01*C_0*(100 - \%T - \%H)^2 / \%T \quad (10)$$

By applying the Log function to equation (10) we obtain equation (11):

$$\text{Log} (\%T / (0.01*(100 - \%T - \%H)^2) = \text{Log } C_0 - \text{Log } K_{tet} \quad (11)$$

Finally plotting of Log ($\%T / (0.01*(100 - \%T - \%H)^2)$) as a function of Log C_0 yields a straight line with a slope equal to 1, intersecting the x-axis at Log C_0 = Log K_{tet}.

The values of % T and % H were determined by measuring the areas under each peak:

$\%T = A2*100 / (A1 + A2 + A3)$ and

$\%H = A3*100 / (A1 + A2 + A3)$

A1, A2 and A3 represent the areas under the peak of the dimer, tetramer and hexamer, respectively.

2. Supplementary tables and figure concerning the mass analysis

Table 1S: Cysteine alkylation pattern of Fur dimer and monomer.

Fur dimer (50 µM) and monomer (50 µM) in Tris/HCl 0.1 M (pH = 8), 0.1 M KCl, 50 mM EDTA, without and with DTT (200 µM), were treated with iodoacetamide (6 mM) during 24h and analyzed by electrospray mass spectrometry. In the designation Fur*+n, the star is for the N-terminal methionine excised form of Fur and n for the number of alkyl groups covalently linked.

	Dimer		Monomer	
	Mass (± 3 Da)	Designation	Mass (± 3 Da)	Designation
+ EDTA	16891 17022	Fur*+4 Fur+4	16660 16791	Fur* Fur
+ EDTA + DTT	16891 17022	Fur*+4 Fur+4	16891 17022	Fur*+4 Fur+4

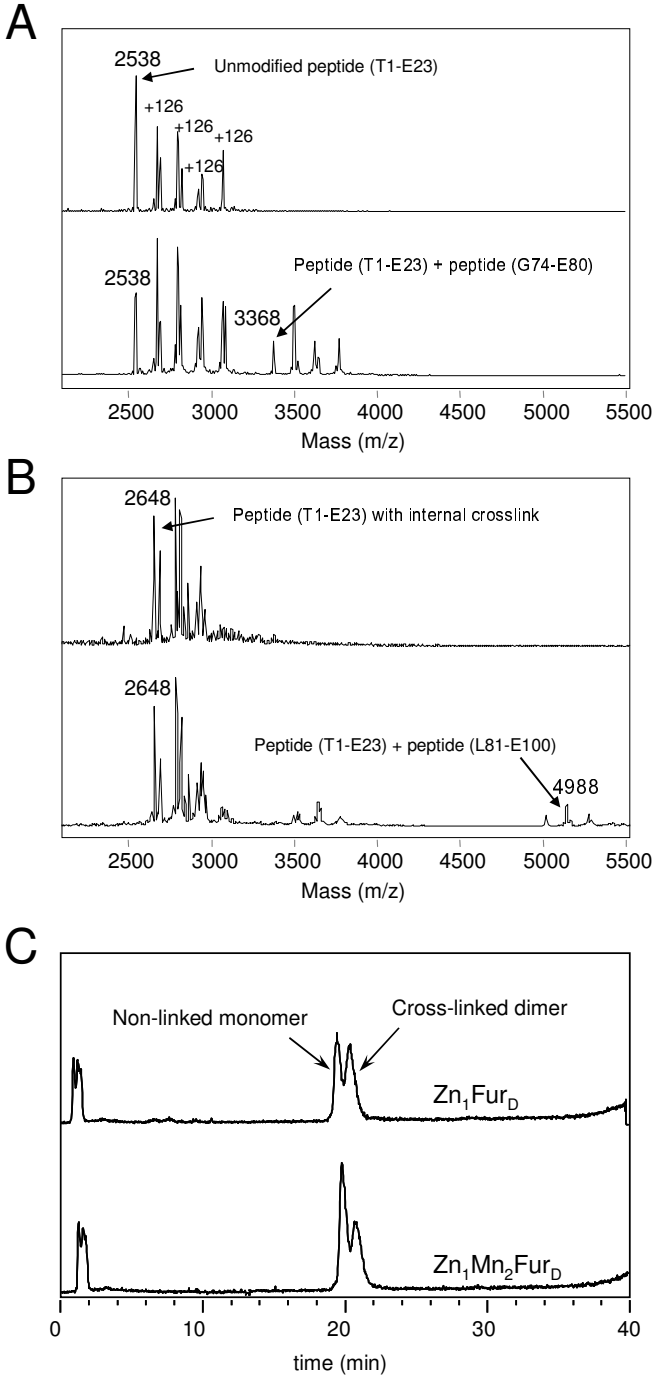
Table 2S: Peptide map of endoproteinase Lys-C digested monomer.

Fur monomer (25 µM) in 100 mM ammonium bicarbonate (pH = 7.9), without and with DTT (200 µM), was digested with endoproteinase Lys-C and the crude peptides were analyzed by MALDI-TOF mass spectrometry.

number	Fragments	Expected	Measured - DTT	Measured + DTT
1	99 – 117	2177.1	2177.1	2177.1
2	22 – 41	2349.2	2349.3	2349.2
3	78 – 98	2460.2	**2458.3**	**2460.2**
4	118 – 148	3547.6	**3545.7**	**3547.7**
5	42 - 77	4034.1	4034.0	4034.1

Figure S1. DMA crosslink experiments

(**A, B**) The dimer was treated with DMA and the non-linked monomer (**upper spectrum**) and covalently cross-linked dimer (**lower spectrum**) were purified by HPLC and digested with endoproteinase Glu-C. The resulting peptides were separated by HPLC and each fraction was analyzed by MALDI-TOF mass spectrometry. The figures shows the spectrum of the two HPLC fractions in which peptide (T1-E23) was eluted (**A, B**). The spectrum of the fractions containing peptide T1-E23, gives a singly charged ion at m/z 2538 (**A**) corresponding to the intact peptide or m/z 2648 (**B**) that is due to internal DMA crosslink. The 126 Da mass adducts correspond to fixation of one DMA molecule on one or several of the four lysines of the peptide or on the N-terminal amino group. In the case of the covalent dimer (**lower spectra**), new species are detected with m/z 3368 (**A**) and m/z 4988 (**B**). (**C**) HPLC profil of DMA treated Zn_1Fur_D (**upper profil**) and $Zn_1Mn_2Fur_D$ (**lower profil**).


A
2538
Unmodified peptide (T1-E23)
+126
+126
+126
+126
2538
Peptide (T1-E23) + peptide (G74-E80)
3368
2500
3000
3500
4000
4500
5000
5500
Mass (m/z)
B
2648
Peptide (T1-E23) with internal crosslink
2648
Peptide (T1-E23) + peptide (L81-E100)
4988
2500
3000
3500
4000
4500
5000
5500
Mass (m/z)
C
Non-linked monomer
Cross-linked dimer
Zn1FurD
Zn1Mn2FurD
0
10
20
30
40
time (min)