Supporting Information to

Stability of an aggregation-prone partially folded state of human profilin-1 correlates with aggregation propensity

Edoardo Del Poggetto[1], Angelo Toto[2], Chiara Aloise[1], Francesco Di Piro[1], Ludovica Gori[1], Francesco Malatesta[2], Stefano Gianni[2, 3], Fabrizio Chiti[1], Francesco Bemporad[1, *]

From the [1]Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Viale G. B. Morgagni 50, 50134, Firenze (Italy); [2]Dipartimento di Scienze Biochimiche 'A. Rossi Fanelli', Sapienza University of Rome, 00185 Rome, Italy; [3]Istituto Pasteur - Fondazione Cenci Bolognetti and Istituto di Biologia e Patologia Molecolari del CNR;

[*]To whom correspondence should be addressed: Francesco Bemporad, [1]Department of Biomedical, Experimental and Clinical Sciences "Mario Serio", University of Florence, Viale G. B. Morgagni 50, 50134, Firenze (Italy); francesco.bemporad@unifi.it; tel.: 0039-055-275-1211

Supporting Table 1

denaturant	Urea	GndHCl	GndSCN
Equilibrium $\Delta G_{U-F}^{H_2O}$ (KJ/mol)	23.5 ± 2.0§	23.5 ± 2.0§	23.5 ± 2.0§
Equilibrium m (KJ/(mol · M))	7.7 ± 1.5	22.8 ± 4.0	48.8 ± 8.0
Equilibrium C_m (M)	3.0 ± 0.3	1.0 ± 0.2	0.48 ± 0.05
Kinetic $\Delta G_{U-F}^{H_2O}$ (KJ/mol)	25.8 ± 2.0§§	25.8 ± 2.0§§	25.8 ± 2.0§§
Kinetic $\Delta G_{U-PF}^{H_2O}$ (KJ/mol)	3.4 ± 2.0§§§	3.4 ± 2.0§§§	3.4 ± 2.0§§§
Kinetic m (KJ/(mol · M))	8.2 ± 1.5	20.5 ± 4.0	44.0 ± 8.0
Kinetic C_m (M)	3.1 ± 0.3	1.3 ± 0.2	0.59 ± 0.06
Kinetic $k_{F1}^{H_2O}$ (s^{-1})	1490 ± 70*	1490 ± 70*	1490 ± 70*
Kinetic $k_{U1}^{H_2O}$ (s^{-1})	400 ± 40**	400 ± 40**	400 ± 40**
Kinetic $k_{F2}^{H_2O}$ (s^{-1})	2.34 ± 0.1***	2.34 ± 0.1***	2.34 ± 0.1***
Kinetic $k_{U2}^{H_2O}$ (s^{-1})	0.0004 ± 0.00004+	0.0004 ± 0.00004+	0.0004 ± 0.00004+
Kinetic m_{F1} (KJ/(mol · M))	-1.31 ± 0.13++	-1.31 ± 0.13++	-1.31 ± 0.13++
Kinetic m_{U1} (KJ/(mol · M))	0.26 ± 0.03+++	0.26 ± 0.03+++	0.26 ± 0.03+++
Kinetic m_{F2} (KJ/(mol · M))	-4.30 ± 0.23	-10.80 ± 0.36	-23.80 ± 0.29
Kinetic m_{U2} (KJ/(mol · M))	2.29 ± 0.05	8.10 ± 0.05	18.59 ± 0.18

§, *, **, ***, +, ++, +++ These sets of parameters were shared in the fitting procedure performed as described in the Data analysis section in the main text. Experimental conditions were 37 °C in a 50 mM phosphate buffer with 150 mM NaCl at pH 7.4; final profilin-1 concentration was 2.65 μM in the stopped-flow experiments and 29.1 μM in T-jump relaxation kinetics.

§§ $\Delta G_{U-F}^{H_2O}$ was calculated as $\Delta G_{U-F}^{H_2O} = -R \cdot T \cdot \ln\left(\frac{k_{F1}^{H_2O} \cdot k_{F2}^{H_2O}}{k_{U1}^{H_2O} \cdot k_{U2}^{H_2O}}\right)$

§§§ $\Delta G_{U-PF}^{H_2O}$ was calculated as $\Delta G_{U-PF}^{H_2O} = -R \cdot T \cdot \ln\left(\frac{k_{F1}^{H_2O}}{k_{U1}^{H_2O}}\right)$

Supporting Table 2

parameter		pH = 7.4	pH = 6.5	pH = 5.5	pH = 4.5	pH = 4.0	pH = 3.5
Equilibrium $\Delta G_{U-F}^{H_2O}$ (KJ/mol)	WT	26.5 ± 2.5	26.5 ± 2.5	23.7 ± 2.5	21.1 ± 2.5	11.7 ± 2.5	4.2 ± 2.5
	G118V	17.1 ± 2.5	17.6 ± 2.5	12.9 ± 2.5	10.1 ± 2.5	2.5 ± 2.5	-
Equilibrium m_{eq} (KJ/(mol · M))	WT	7.59 ± 0.50	8.33 ± 0.50	9.16 ± 0.50	9.99 ± 0.50	10.4 ± 0.50	10.9 ± 0.50
	G118V	7.59 ± 0.50	8.33 ± 0.50	9.16 ± 0.50	9.99 ± 0.50	10.4 ± 0.50	10.9 ± 0.50
Equilibrium C_m (M)	WT	3.49 ± 0.20	3.18 ± 0.20	2.59 ± 0.20	2.11 ± 0.20	1.12 ± 0.20	0.38 ± 0.20
	G118V	2.25 ± 0.20	2.11 ± 0.20	1.41 ± 0.20	1.01 ± 0.20	0.24 ± 0.20	-
Kinetic $k_{F2}^{H_2O}$ (s^{-1})	WT	1.73 ± 0.17	3.55 ± 0.36	3.80 ± 0.38	2.27 ± 0.23	1.11 ± 0.11	0.94 ± 0.09
	G118V	1.29 ± 0.13	2.60 ± 0.26	2.81 ± 0.28	2.60 ± 0.26	1.93 ± 0.19	-
Kinetic $k_{U2}^{H_2O}$ (s^{-1})	WT	$(3.94 \pm 0.39) \cdot 10^{-5}$	$(8.08 \pm 0.81) \cdot 10^{-5}$	$(2.68 \pm 0.27) \cdot 10^{-4}$	$(4.57 \pm 0.46) \cdot 10^{-4}$	$(9.99 \pm 1.00) \cdot 10^{-3}$	$(1.76 \pm 0.17) \cdot 10^{-1}$
	G118V	$(1.31 \pm 0.13) \cdot 10^{-3}$	$(2.15 \pm 0.22) \cdot 10^{-3}$	$(1.55 \pm 0.16) \cdot 10^{-2}$	$(4.35 \pm 0.44) \cdot 10^{-2}$	$(6.95 \pm 0.70) \cdot 10^{-1}$	-
Extrapolated $k_{U2}^{H_2O}$ (s^{-1})	WT	$(4.54 \pm 0.45) \cdot 10^{-5}$	$(1.76 \pm 0.18) \cdot 10^{-4}$	$(9.91 \pm 0.99) \cdot 10^{-4}$	$(1.25 \pm 0.12) \cdot 10^{-3}$	$(2.03 \pm 0.20) \cdot 10^{-3}$	$(1.60 \pm 0.16) \cdot 10^{-1}$
	G118V	$(1.70 \pm 0.17) \cdot 10^{-2}$	$(1.59 \pm 0.16) \cdot 10^{-1}$	$(7.59 \pm 0.76) \cdot 10^{-1}$	$(4.88 \pm 0.49) \cdot 10^{-2}$	$(8.36 \pm 0.84) \cdot 10^{-1}$	-
Calculated $k_{F2}^{H_2O}$ (s^{-1})	WT	1.99 ± 0.20	7.73 ± 0.77	14.1 ± 0.14	6.19 ± 0.62	0.23 ± 0.02	0.85 ± 0.08
	G118V	16.8 ± 0.17	193 ± 19	138 ± 14	2.92 ± 0.29	2.32 ± 0.23	-
Calculated $\Delta G_{U-PF}^{H_2O}$ (KJ/mol)	WT	0.35 ± 2.50	1.93 ± 2.50	3.24 ± 2.50	2.48 ± 2.50	-3.92 ± 2.50	-2.40 ± 2.50
	G118V	6.36 ± 2.50	10.7 ± 2.50	9.65 ± 2.50	0.29 ± 2.50	0.458 ± 2.50	-
Kinetic m_{F2} (KJ/(mol · M))	WT	5.22 ± 0.50	5.01 ± 0.50	5.52 ± 0.50	5.47 ± 0.50	6.85 ± 0.50	9.92 ± 0.50
	G118V	3.72 ± 0.50	3.40 ± 0.50	3.87 ± 0.50	7.43 ± 0.50	8.71 ± 0.50	-
Kinetic m_{U2} (KJ/(mol · M))	WT	2.37 ± 0.50	3.32 ± 0.50	3.64 ± 0.50	4.52 ± 0.50	3.55 ± 0.50	0.98 ± 0.50
	G118V	4.22 ± 0.50	4.93 ± 0.50	5.29 ± 0.50	2.56 ± 0.50	1.69 ± 0.50	-
b	WT	0.14 ± 0.01	0.003±0.003	-0.11 ± 0.01	-0.49 ± 0.05	-0.74 ± 0.07	1.32 ± 0.13
	G118V	1.14 ± 0.10	1.25 ± 0.12	1.43 ±0.14	5.94 ± 0.60	5.79 ± 0.058	-
c	WT	0.04 ± 0.05	-0.07 ± 0.05	-0.09 ± 0.05	-0.09 ± 0.05	0.00 ± 0.05	-0.04 ± 0.05
	G118V	-0.12 ± 0.05	-0.24 ± 0.05	-0.35 ± 0.05	-0.02 ± 0.05	0.00 ± 0.05	-

Experimental conditions were 2.65 μM profilin-1 in 10 mM buffers with an ionic strength of 30 mM achieved using NaCl and 25 °C. Buffers employed were TRIS (pH 7.4), MES (pH 6.5), acetate (pH 5.5, 4.5 and 4.0), formate (pH 3.5).

Supporting Table 3

variant	WT	A20T	C71G	M114T	E117G	G118V	Q139L
Equilibrium $\Delta G_{U-F}^{H_2O}$ (KJ/mol)	26.2 ± 0.6[1]	19.8 ± 0.4[2]	23.8 ± 0.2[3]	15.7 ± 0.2[3]	26.9 ± 0.1[3]	18.0 ± 0.2[3]	33.9 ± 0.3[2]
Equilibrium m_{eq} $(\mathrm{KJ/(mol \cdot M)})$	7.3 ± 0.2[1]	10.6 ± 0.2[2]	6.7 ± 0.1[3]	8.4 ± 0.1[3]	8.2 ± 0.1[3]	8.0 ± 0.1[1]	9.4 ± 0.1[2]
Equilibrium C_m (M)	3.57 ± 0.01[1]	1.87 ± 0.01[2]	3.57 ± 0.01[3]	1.86 ± 0.01[3]	3.26 ± 0.01[3]	2.25 ± 0.01[1]	3.59 ± 0.01[2]
Kinetic $k_{F2}^{H_2O}$ $(\mathrm{s^{-1}})$	1.02	3.86	1.10	0.48	2.36	1.13	4.85
Kinetic $k_{U2}^{H_2O}$ $(\mathrm{s^{-1}})$	$(1.48 \pm 0.15) \cdot 10^{-5}$	$(4.52 \pm 0.45) \cdot 10^{-4}$	$(4.74 \pm 0.47) \cdot 10^{-3}$	$(9.52 \pm 0.95) \cdot 10^{-4}$	$(5.88 \pm 0.59) \cdot 10^{-5}$	$(1.68 \pm 0.17) \cdot 10^{-3}$	$(2.00 \pm 0.20) \cdot 10^{-6}$
Extrapolated $k_{U2}^{H_2O'}$ $(\mathrm{s^{-1}})$	$(1.70 \pm 0.17) \cdot 10^{-5}$	$(6.90 \pm 0.69) \cdot 10^{-3}$	$(9.20 \pm 0.92) \cdot 10^{-2}$	$(4.10 \pm 0.41) \cdot 10^{-3}$	$(1.41 \pm 0.14) \cdot 10^{-4}$	$(2.19 \pm 0.22) \cdot 10^{-2}$	$(6.11 \pm 0.41) \cdot 10^{-6}$
Calculated $\Delta G_{U-PF}^{H_2O}$ (KJ/mol)	0.34 ± 0.50	6.76 ± 0.50	7.35 ± 0.50	3.62 ± 0.50	2.17 ± 0.50	6.36 ± 0.50	2.77 ± 0.50
β_T^1	0.59 ± 0.02[4]	0.59 ± 0.02[4]	0.59 ± 0.02[4]	0.59 ± 0.02[4]	0.59 ± 0.02[4]	0.59 ± 0.02[4]	0.59 ± 0.02[4]
β_T^2	0.79 ± 0.03[5]	0.79 ± 0.03[5]	0.79 ± 0.03[5]	0.79 ± 0.03[5]	0.79 ± 0.03[5]	0.79 ± 0.03[5]	0.79 ± 0.03[5]
ThT fluorescence (a.u.)	14.77 ± 1.48[3]	68.49 ± 6.85[2]	72.11 ±7.00[3]	30.48 ± 3.00[3]	18.09 ± 2.00[3]	53.71 ± 5.00[3]	21.76 ± 2.18[2]

[1] Data from (1);

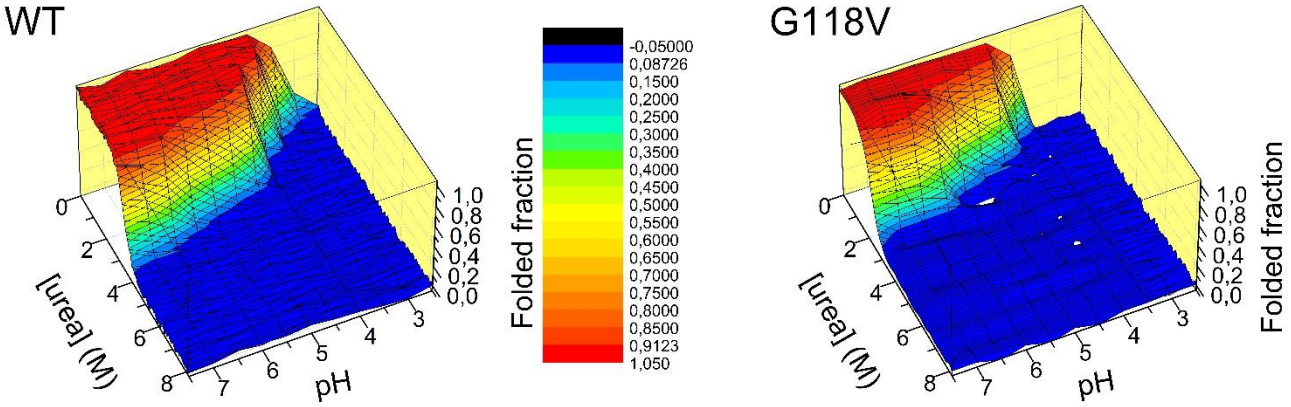
[2] Data from (2);

[3] Data from (3);

[4, 5] These parameters were shared in the fitting procedure;

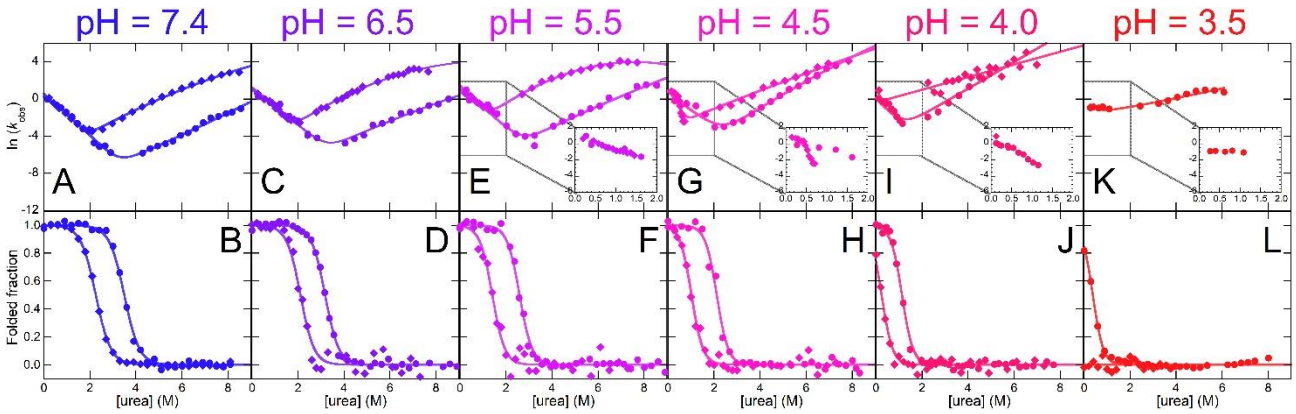
Experimental conditions were 2.65 μM profilin-1 in 20 mM TRIS, 2 mM DTT, 0.1 mM $CaCl_2$, 0.2 mM NaN_3, pH 7.3, 25 °C.

Supporting Figure 1



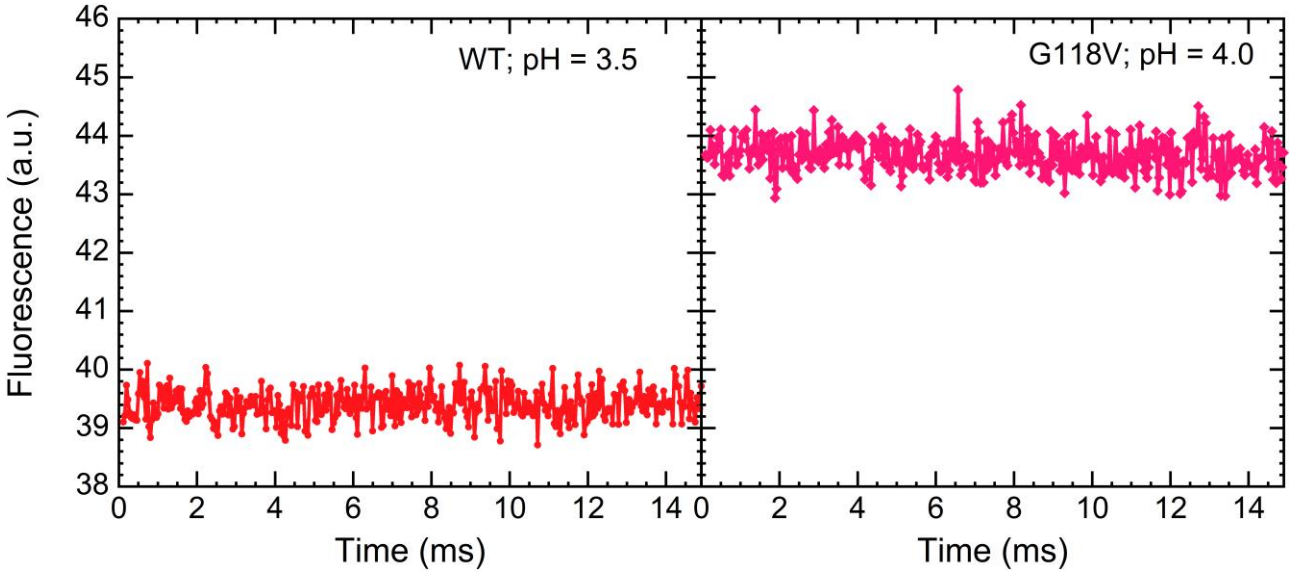
Supporting Figure 1: Fraction of folded profilin-1 as a function of urea concentration and pH. Data are shown for the WT (left) and G118V (right) variants.

Supporting Figure 2



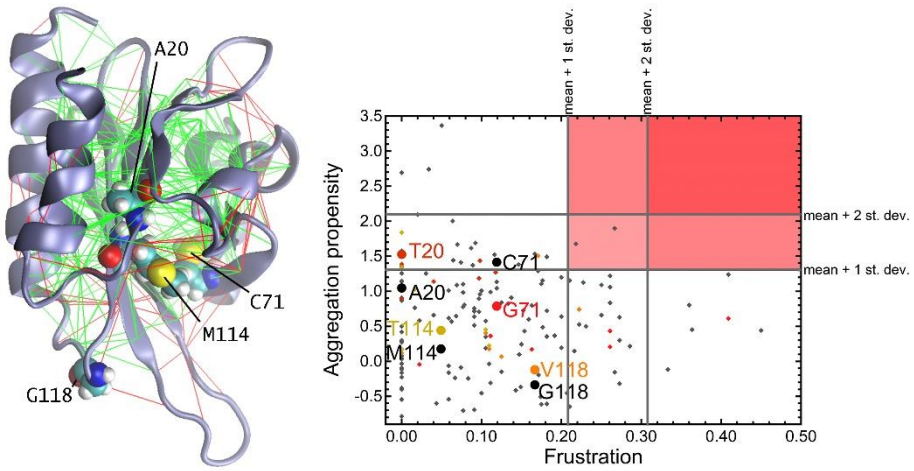
Supporting Figure 2: Chevron plots (A, C, E, G, I, K) and equilibrium unfolding curves (B, D, F, H, J, L) for WT (circles) and G118V (diamonds) profilin-1, carried out at pH of 7.4 (A, B), 6.5 (C, D), 5.5 (E, F), 4.5 (G, H), 4.0 (I, J) and 3.4 (K, L). Colour varies from blue to red as pH decreases from 7.0 to 3.5, following the same colour scale shown in Figure 4 (main text). Panels E, G, I, K report an inset with zooms at urea concentrations ranging from 0 to 2 M urea, in order to show down-ward curvatures in the refolding limbs of the chevron plots. Continuous lines in panels A, C, E, G, I, K represent best fits to equation (1) (see Data analysis section in main text). Continuous lines in panels B, D, F, H, J, L represent best fits to the equation edited by Santoro & Bolen (4).

Supporting Figure 3



Supporting Figure 3: T-jump experiment of WT (left) and G118V (G118V) profilin-1, carried out in the absence of denaturant at pH 3.5 (10 mM formate buffer with 100 mM NaCl) for WT and at pH 4.0 (10 mM acetate buffer with 100 mM NaCl) for G118V. Each trace is the average of 20 measurements.

Supporting Figure 4



Supporting Figure 4: Comparison between frustration and aggregation propensity of profilin-1. We calculated frustration with Protein frustratomoter 2 (5) and aggregation propensity with the Zyggregator method (6). Frustrated and non-frustrated contacts are represented as red and green lines in the profilin-1 structure (PDB code 1PFL) on the left-hand side. The right-hand panel shows the correlation between frustration and aggregation propensity. Each position of the sequence is represented as a grey diamond. The residues that involve the most amyloidogenic mutations studied here are represented as black circles. The effect of the mutations C71G, A20T, G118V and M114T are depicted in red, dark orange, orange and yellow respectively (circles for the mutated residues, diamonds for nearby residues). The continuous lines correspond to the values of the average + 1 and 2 standard deviations. These boundaries illustrate that no residues exceedingly frustrated and aggregation-prone (pink regions) there exist in the profilin-1 sequence.

Supporting References

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