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SUPPLEMENTARY INFORMATION FOR MICROFILM EDITION

According to our experimental results, we privileged model b from scheme I, described by the following differential equations:

$$\frac{dI_R}{dt} = -k_2 I_R \quad (1)$$

$$\frac{dI_M}{dt} = -k_3 I_M \quad (2)$$

$$\frac{dI_{VR}}{dt} = -k_1 I_{VR} \quad (3)$$

$$\frac{dI_S}{dt} = k_2 I_S + k_3 I_M - k_4 I_S \quad (4)$$

$$\frac{dN}{dt} = k_1 I_{VR} + k_4 I_S \quad (5)$$

The analytical solution of the system used for fitting the experimental kinetic traces of thioredoxin refolding process can be expressed as a sum of exponentials:

$$S(t) = I_{VR,0}(S_{VR} - S_N)e^{(-k_1 t)} + I_{R,0}(S_R + \frac{S_S k_2 - S_N k_4}{k_4 - k_2})e^{(-k_2 t)} + I_{M,0}(S_M + \frac{S_S k_3 - S_N k_4}{k_4 - k_3})e^{(-k_3 t)} +$$
$$+(S_N - S_S)(\frac{k_2 I_{R,0}}{k_4 - k_2} + \frac{k_2 I_{M,0}}{k_4 - k_3})e^{(-k_4 t)} + (I_{VR,0} + I_{M,0} + I_{R,0})S_N \quad (6)$$

$S(t)$ represents the observed signal, $I_{VR,0}$, $I_{R,0}$, $I_{M,0}$ represent the fractions of the intermediates accumulated after the burst phase. S_i represents the specific signals (signal corresponding to the population of each intermediate) and k_i are the experimental rate constants determined in the refolding process.

The next step was to fit the kinetic traces. Three refolding traces at final 0.315 M GuHCl, after 15 s, 1800 s and overnight unfolding time, were selected. The selection was done because double-jump reactivation experiments had been performed under the same conditions, which allowed us to estimate the value of I_{VR}, at the above mentioned unfolding times (5-10 % for the

1800 s or overnight unfolding time and between 55-60 %, in the case of 15 s unfolding time). All

the fittings gave similar values for the specific signals (only the fittings for 2 traces are represented

in fig.1 for clarity).

In order to integrate the scheme Ib with the schemes II, and finally to verify the integral model

(given in the scheme III), we proceed to describe the equilibria established in the denatured state,

written in a general form:

$$X \underset{}{\overset{k_1}{\rightleftharpoons}} Y \underset{}{\overset{k_2}{\rightleftharpoons}} Z \qquad (7)$$
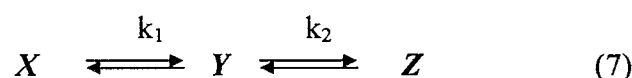
where:

$$\frac{dX}{dt} = -k_1 \times x + k_{-1} \times y, \quad (8)$$

$$\frac{dY}{dt} = k_1 \times x + k_{-2} \times z - (k_{-1} + k_2) \times y, \quad (9)$$

$$\frac{dZ}{dt} = k_2 \times y - k_{-2} \times z, \qquad (10)$$

$$Co = x + y + z, \qquad (11)$$

x, y and z are the concentrations of the species X, Y, and Z, and C_0 is the total protein

concentration. After replacing the y and z variables and arrangement of the terms, we obtain:

$$\frac{d^2X}{dt^2} + (k_1 + k_{-1} + k_2 + k_{-2}) \times \frac{dX}{dt} + \left[(k_1 + k_{-1})(k_2 + k_{-2}) \right] \times x = k_{-1} \times k_{-2} \times Co, \qquad (12), \text{ with the}$$

characteristic equation: $r^2 + \left(\sum_{i=\pm 1} k_i \right) \times r + \left[(k_1 + k_{-1})(k_2 + k_{-2}) - k_{-1} k_2 \right] = 0 \qquad (13)$

The discriminant of the characteristic equation (13) is always positive and the free term is also positive, so there are two positive solutions and the general solution of equation (12) will have the form:

$$X(t) = A_{a,x}e^{r1 \times t} + A_{b,x}e^{r2 \times t} + R_x \quad (14),$$

where $r_{1,2} = -k_{obs1, obs2}$:

$$k_{obs1,2} = -\frac{1}{2} \times \left[(k_1 + k_{-1} + k_2 + k_{-2}) \pm \sqrt{[(k_1 + k_{-1}) - (k_2 + k_{-2})]^2 + 4 k_{-1} \times k_2} \right] \quad (15)$$

Symmetrically for Y and Z:

$$Y(t) = A_{a,y}e^{r1 \times t} + A_{b,y}e^{r2 \times t} + R_y \quad (16)$$

$$Z(t) = A_{a,z}e^{r1 \times t} + A_{b,z}e^{r2 \times t} + R_z \quad (17)$$

Inspection of equations 14, 16 and 17 indicates that the variation of the amplitude of each species should follow a bi-phasic relaxation process. We calculate the k_1, k_{-1}, k_2, k_{-2}, (0.01 s^{-1} to 3.5 10^{-4} s^{-1}) corresponding to equilibrium 7 using equations 14 - 17 and assuming that the relative amplitudes of the very rapid, rapid and medium refolding phases (at infinite unfolding time) are equivalent to the fraction of the corresponding unfolded species.

Note that the above given assumption is already incorporated in the general kinetic model (scheme III) and reflects the dramatic differences between the rates of the reactions occurring in the denatured state (0.01 − 3x10^{-4} s^{-1}) and the rate of accumulation of the intermediates (k > 250 s^{-1}). Using the specific signals and the fractions derived from fitting the refolding kinetic data, we simulate the variation of the amplitude of the three rapid refolding phases with the unfolding time according to scheme IIa (fig. 2), and thus to verify indirectly the proposed model from scheme III.
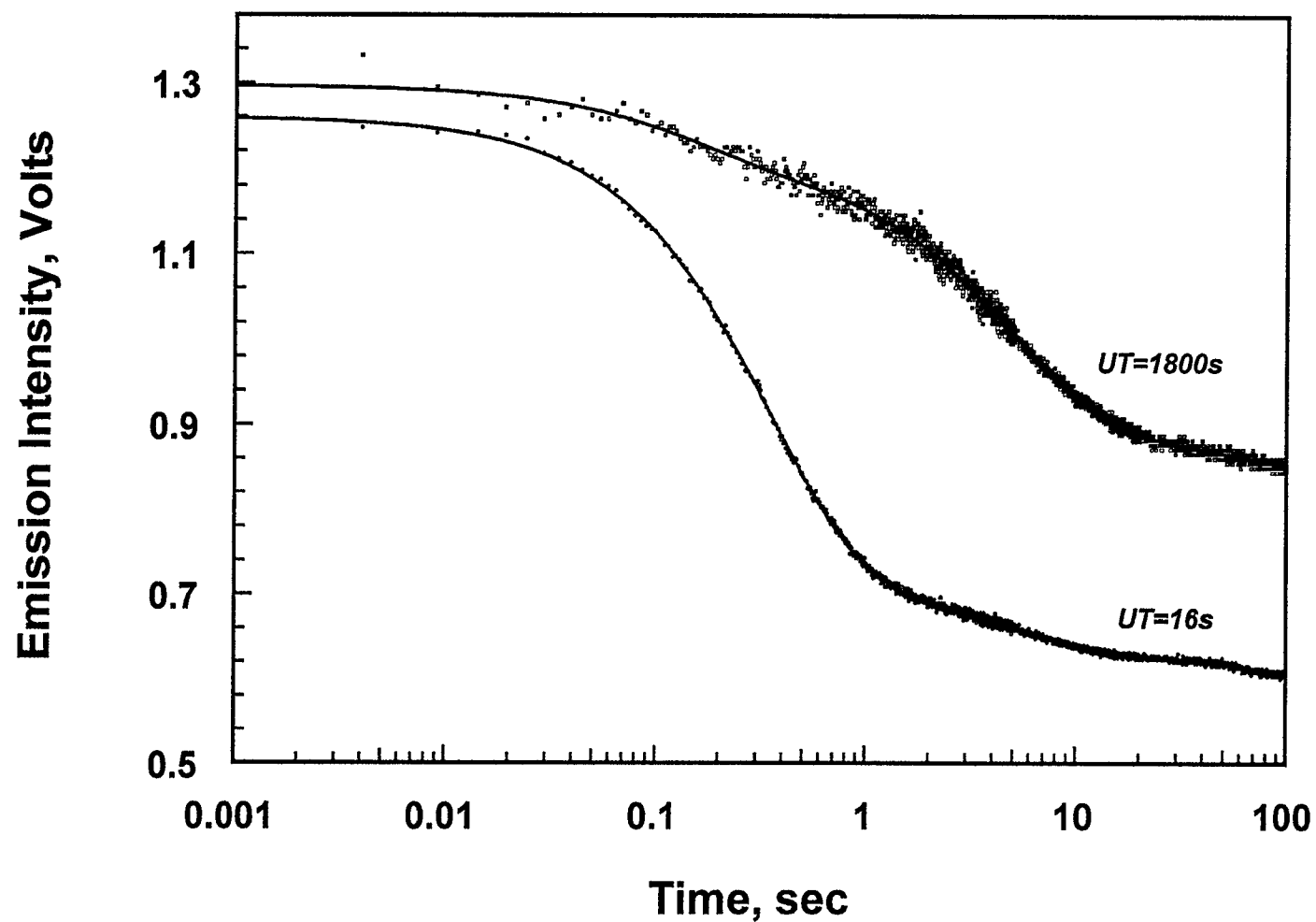
Fig.1   The refolding kinetic profiles of 6.3 M GuHCl-denatured protein at 16 s and 1800 s times of unfolding, obtained by stopped-flow multimixing experiments. The solid lines represent the fits according to the model from scheme Ib and described by the eq. 6
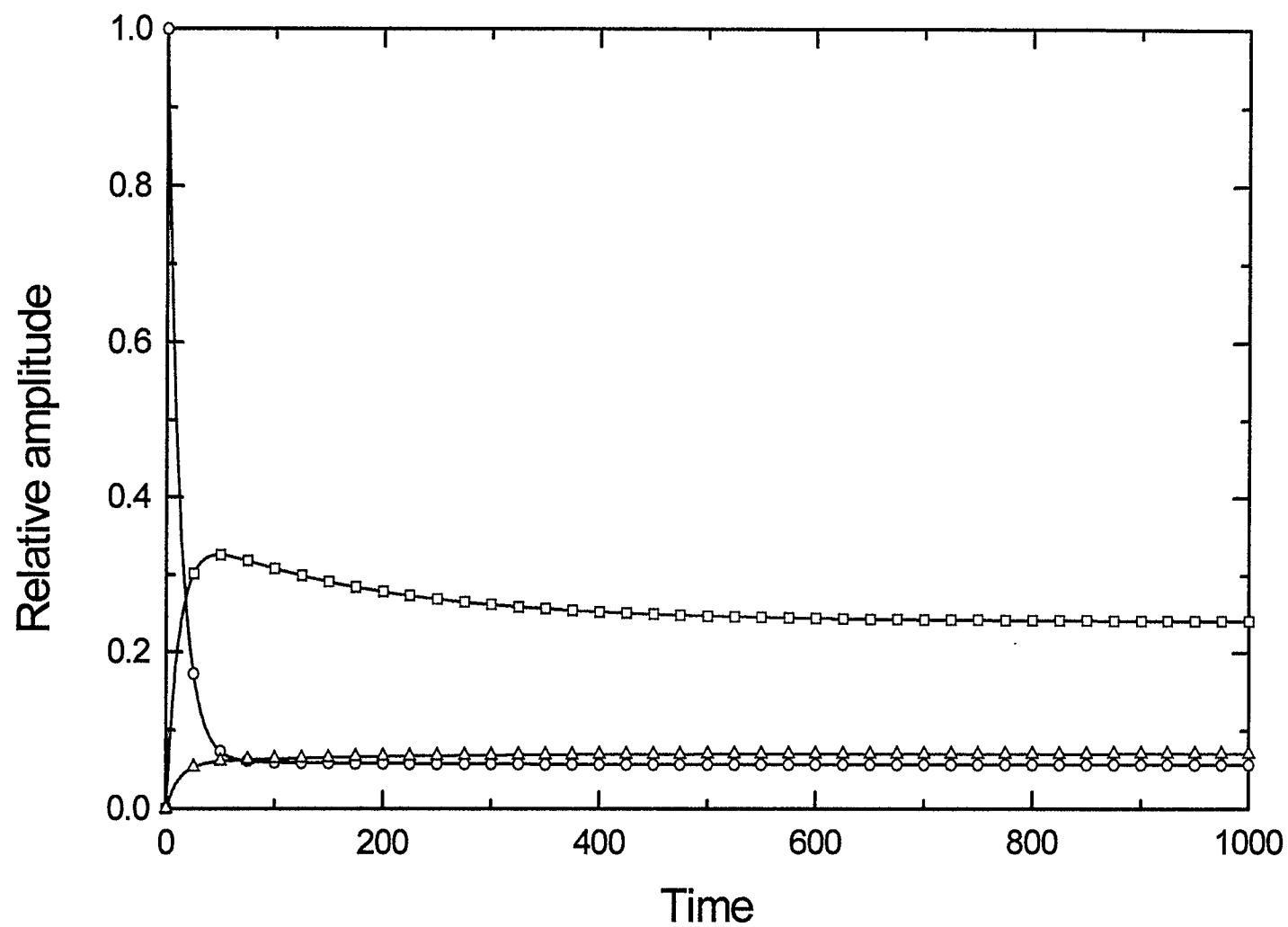
Fig. 2 Simulation of he amplitude variation of the very rapid (circles), rapid (squares) and medium phase (triangles) with the unfolding time, according to the equilibrium described in the scheme II a.