Reply to Marchenko et al.: Flux analysis of GroEL-assisted protein folding/unfolding

Using NMR-based relaxation experiments, we showed that exchange between the folded state (F) of a metastable SH3 domain and a folding intermediate (I) is an order of magnitude faster when the SH3 domain is bound to apo GroEL than in free solution (1). We did not consider fluxes through the apo GroEL-assisted and unassisted pathways.

Marchenko et al. (2) note that the approximate rate constants for the GroEL-assisted interconversion between the F and I states ($k_{F\leftrightarrow F\text{-}G\leftrightarrow I\text{-}G\leftrightarrow I}$ and $k_{I\leftrightarrow I\text{-}G\leftrightarrow F\text{-}G\leftrightarrow F}$) are slower than the corresponding rate constants (k_{FI} and k_{IF}) for direct interconversion, as the binding of I to GroEL is slower than the interconversion between the GroEL-bound F and I states under the conditions of the NMR experiments [i.e., $(k_{on}^{app}+k_{off}^{IG})<(k_{FI}^{G}+k_{IF}^{G})$, where k_{on}^{app} is a pseudo-first-order association rate constant given by $k_{on}[G]$; see scheme in Fig. 1]. On this basis, Marchenko et al. (2) conclude that our data provide "strict experimental evidence that apo GroEL does not accelerate protein folding, although it does accelerate one of its steps," and therefore corroborates their earlier hypothesis that the interaction of GroEL with folding intermediates hinders the formation of native structure (3).

However, Marchenko et al. (2) fail to take into account that binding of F and I to GroEL are second-order processes dependent upon the concentration of apo GroEL. The relative contributions of GroEL-assisted and unassisted pathways can be assessed by steady-state flux analysis (4).

The flux through parallel and serial reaction paths is given by $F_{parallel}=\Sigma F_i$ and $F_{serial}=[\Sigma(1/F_i)]^{-1}$, respectively, where F_i is the flux of the ith reaction step. For the kinetic scheme in Fig. 1, the fluxes between states F and I through the GroEL-assisted and unassisted pathways are given by

$$Flux^{F\leftrightarrow I}_{GroEL\text{-}assisted}=\left\{(k_{on}[F][G])^{-1}+\left(k_{FI}^{G}[F\text{-}G]\right)^{-1}+\left(k_{off}^{IG}[I\text{-}G]\right)^{-1}\right\}^{-1}$$

and

$$Flux^{F\leftrightarrow I}_{GroEL\text{-}unassisted}=k_{FI}[F],$$

respectively, where [G] is the concentration of free SH3 binding sites on GroEL (assumed to be one per GroEL cavity). $Flux^{F\leftrightarrow I}_{GroEL\text{-}assisted}$ and $Flux^{F\leftrightarrow I}_{GroEL\text{-}unassisted}$ are plotted as a function of total GroEL concentration in Fig. 1*A*, and the corresponding ratio of fluxes is shown in Fig. 1*B*. In the NMR experiments, the total concentration of GroEL 14 mer is 8.6 μM (corresponding to 17.1 μM in cavities and 120 μM in subunits), and, under these conditions, $Flux^{F\leftrightarrow I}_{GroEL\text{-}assisted}$ is indeed slower than $Flux^{F\leftrightarrow I}_{GroEL\text{-}unassisted}$. However, when the total concentration of GroEL is increased about sixfold (~51 μM), the GroEL-assisted pathway predominates. Moreover, the total flux between the F and I states is always increased in the presence of GroEL. Exactly the same conclusions are reached using the formalism of Marchenko et al. (2) when the dependence of the apparent rate constants ($k_{F\leftrightarrow F\text{-}G\leftrightarrow I\text{-}G\leftrightarrow I}$ and $k_{I\leftrightarrow I\text{-}G\leftrightarrow F\text{-}G\leftrightarrow F}$) on GroEL concentration are taken into account.

Thus, for any given protein substrate, the relative importance of the GroEL-assisted pathway will depend upon the concentration of GroEL and the balance of the various rate constants depicted in the kinetic scheme shown in Fig. 1. Indeed, even a GroEL minichaperone can facilitate protein folding in vivo (5). The SH3 domain used in our study (1) is a model substrate that folds rapidly on its own. The unassisted folding of obligate GroEL substrates, however, may be slow, and, therefore, in such instances, acceleration of folding/unfolding on the surface of GroEL is likely to be functionally important.

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1 Libich DS, Tugarinov V, Clore GM (2015) Intrinsic unfoldase/foldase activity of the chaperonin GroEL directly demonstrated using multinuclear relaxation-based NMR. *Proc Natl Acad Sci USA* 112(29):8817–8823.
2 Marchenko NY, Marchenkov VV, Semisotnov GV, Finkelstein AV (2015) Strict experimental evidence that apo-chaperonin GroEL does not accelerate protein folding, although it does accelerate one of its steps. *Proc Natl Acad Sci USA* 112:E6831–E6832.
3 Marchenkov VV, et al. (2004) [The interaction of the GroEL chaperone with early kinetic intermediates of renaturing proteins inhibits the formation of their native structure]. *Biofizika* 49(6): 987–994. Russian.
4 Hammes GG, Chang Y-C, Oas TG (2009) Conformational selection or induced fit: a flux description of reaction mechanism. *Proc Natl Acad Sci USA* 106(33):13737–13741.
5 Chatellier J, Hill F, Lund PA, Fersht AR (1998) In vivo activities of GroEL minichaperones. *Proc Natl Acad Sci USA* 95(17):9861–9866.
6 Clore GM (1983) Computer analysis of transient kinetic data. *Computing in Biological Science*, eds Geisow MG, Barrett AN (Elsevier North-Holland, Amsterdam), pp 313–348.

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The authors declare no conflict of interest.

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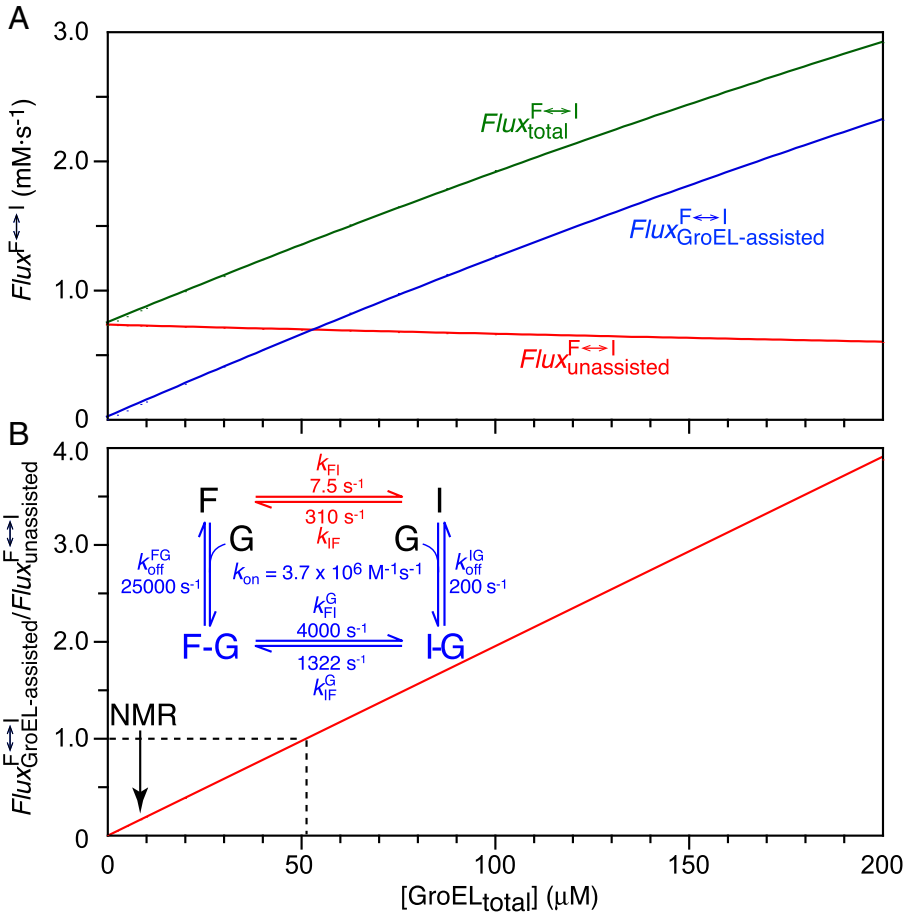

Fig. 1. Fluxes at equilibrium for the F to I interconversion through the GroEL-assisted and unassisted pathways. (*A*) Total (green), GroEL-assisted (blue), and GroEL-unassisted (red) fluxes as a function of total GroEL concentration. $Flux^{F\leftrightarrow I}_{total}$ is given by the sum of the fluxes through the GroEL-assisted and unassisted pathways. Note that the decrease in the GroEL-unassisted flux with increasing GroEL concentration is due to the concomitant decrease in the concentration of the free folded state F. (*B*) Ratio of GroEL-assisted to unassisted fluxes. *Inset* in *B* depicts the reaction scheme and rate constants (1) for the direct, unassisted (red) and GroEL-assisted (blue) interconversion between the F and I states of the metastable SH3 domain. Concentrations of all species at equilibrium were calculated by integrating the differential equations describing the reaction scheme shown in *Inset* until the steady state is reached (6). The total concentration of SH3 domain is 100 μM. The total concentration of GroEL is expressed in terms of the GroEL 14mer.