Folding pathway of interleukin-1β

Sir — We recently reported the characterization of the folding pathway of the all-beta protein interleukin-1β, using a number of techniques including hydrogen-deuterium exchange and mass spectrometric analysis[1]. This latter combination of techniques is extremely powerful in that it allows for a population analysis of the native, unfolded and intermediate species as a function of time during refolding. We offered two mechanisms involving a transiently populated intermediate to account for the observed data:

U ⇌ I ⇌ N scheme 1

I ⇌ U ⇌ N scheme 2

We selected scheme 1 based on the apparent lag in native protein production which coincided with a build-up in the population of the intermediate species. The data are well fit by the mechanism we offered. But although our detection limit of protein is in picomole quantities and well above the calculated percentage of N in the early time points for an off-pathway mechanism (scheme 2)[2,3], deconvolution of the mass spectral data of closely separated peaks combined with the potential for wash-out of the deuterium label during sample workup may present potential problems in detecting small percentages of native protein in the early time points. At this time we can not definitively rule out the alternative mechanism. We are presently pursuing studies of an interleukin-1β species for which the rates of formation of I and N are closer in magnitude and the absence of N in the early time points is more easily resolved. This is a difficult and intriguing problem that we will continue to address.

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No crystals, no grant, revisited

Sir — For more than a decade study groups of the United States National Institutes of Health (NIH) routinely have denied funding for applications dealing with crystallization of membrane proteins, claiming that the outcome of such projects is too unpredictable to justify support. The practice is widely known as the 'no crystals, no grant rule', applied by the U.S. crystallographic community despite the fact that the NIH does not accept it as an official review criterion. This rule is irrational as it denies funding for the production of crystals, which is the key bottleneck in high resolution structural studies. The harmful effect of the mindless application of this rule on structural studies of membrane proteins in the United States was the subject of several communications[1–4].

According to Dr. P. C. Preusch of National Institute of General Medical Sciences (NIGMS), "European and Japanese groups are making much more progress on these structures than are their U. S. counterparts. This difference is due in large part to their ability to obtain funding for types of work that have been rendered unfundable in the U. S. by the 'no crystals, no grant' dictum" (ref. 2).

At a meeting convened by the NIH to discuss these problems there was general agreement about the damaging consequences of the 'no crystals, no grant' rule, and a report of this meeting ended with the promise: "Something useful will happen. Stay tuned" (ref. 5). Despite considerable effort by the NIGMS program administration to increase funding in this area, the rule is still in effect and continues to block efforts on the crystallization of membrane proteins.

The 'no crystals, no grant' rule was never announced or accepted by the NIH and reflects the arbitrariness of the U. S. crystallographic community. Its justification, that crystallization of membrane proteins is too risky to deserve financial support, was suspect from the outset, and became entirely untenable by the successful crystallization of several complex membrane proteins by relatively conventional methods in Europe and Japan, that are recognized by recent Nobel prizes. The lack of crystallographic data on most membrane proteins of great med-ical and scientific importance slowed research on them to a crawl and encouraged expensive, stop-gap efforts with low-yield mutagenesis techniques to fill the void in structural information. As a result the 'no crystals, no grant' rule proved penny-wise and pound-foolish even in a financial sense.

The ruthless application of this rule disrupted and in some cases effectively ended the research programs of membrane biochemists who devoted years of their life to develop the biochemical foundation for promising crystallization efforts, andmadesolidcon-tributions to the crystallography of membrane proteins. While this was going on, the richly-funded U. S. crystallographic community, with few exceptions, ignored the fundamental need for structural information on membrane proteins.

Based on the demonstrated success of membrane protein crystallization in Europe and Japan, no one can honestly claim any longer that such efforts are too unpredictable to deserve support. It is high time for the NIH to assure that scientific projects are judged only by legitimate scientific criteria and that censorship based on irrational rules is not permitted to enter into the evaluation process.

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