DETECTING MULTIPLE SUBSTATES AND PATHWAYS IN PROTEINS: THE CASE OF THE GFP

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Abstract:

The Green Fluorescent Protein, GFP, a biopolymer that has found many relevant applications,[1] appears as an ideal candidate for detailed conformational investigations, since it is naturally fluorescent and its chromophore, buried inside the protein, is particularly sensitive to its environment. In particular, the fluorescence of GFPmut2, a GFP mutant engineered to enhance emission and stability, carries information on the chromophore intrinsic switching rate between the anionic and the neutral chemical states, K_{AN}, a parameter that has been exploited to unveil the existence of protein substates and hints on the folding/unfolding dynamics.[2, 3]

Summarizing, by means of advanced spectroscopic investigations on native and unfolding GFPmut2 single molecules and by processing the resulting K_{AN} signal we show clear evidence of:

i) the existence of different substates in the folded/native protein;
ii) the occurrence of discrete unfolding pathways;
iii) regular oscillations between substates near protein unfolding;[3]
iiii) strong resonance effects under applied electric or acoustic fields;[3]
iiiii) correlation among switching rates of the folded protein substates, pre-unfolding oscillation frequencies and unfolding pathways properties.

These results prove that the GFP molecules, prepared under the same protocol, can be found in different substates and that they evolve along specific and reproducible unfolding paths in agreement with predictions.[4] Each substate accessible to a molecule may correspond to a (slightly) different biological function as indirectly suggested, e.g., by non-exponential kinetics for some proteins.[5, 6]

It is likely that most proteins behave as seen here but this will be probably unveiled in the near future by single molecule experiments if suitable probes, as sensitive as the GFP chromophore, become available.

We conclude by stating that the evidence of conformational and dynamical multiplicity of the GFP proteins has been revealed by single molecule investigations accompanied by advanced microspectroscopic techniques whereas the more traditional measures on ensembles (solutions, e.g.) would have yielded only average values and certainly missed the points described here.

Selected references

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