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Protein dissociation under pressure using fluctuation correlation spectroscopy

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**Protein dissociation under pressure using fluctuation correlation spectroscopy.**

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**Abstract**

The dissociation of multimeric proteins under pressure has provided important information about protein-protein interactions. The volume of protein subunits is generally smaller than the volume of the native protein and an increase in pressure causes subunit dissociation. However, most of the present methods to detect dissociation are based on spectroscopic signals that are related to the native and the dissociated state. These signals do not directly report the dissociation, but rather changes in protein conformation that could depend on the actual separation of the subunits. These spectroscopic changes frequently occur over a relatively large range of pressure and part of the signal change may simply be due to the formation of dissociation intermediates. Our new approach is based on the direct measurement of the number of fluorescent elements in a given volume by fluctuation correlation spectroscopy (FCS). We designed a pressure cell that fits our FCS instrument. The cell is based on a quartz capillary of about 50  $\mu\text{m}$  internal diameter and 350  $\mu\text{m}$  external diameter. The cell is coupled to a pressure pump that can produce about 3.5kbar. We have studied the dissociation of several multimeric proteins to directly assess the degree of protein dissociation at each pressure and compared the results with the changes in spectroscopic signals. Support: NIH, PHS P41 5 RR03155.