that the exclusion would not be consistent with a hypothesis of "pharmaceutical" proteins interacting with ice surface.

As evidence to the contrary, i.e., of interaction of non-antifreeze-proteins with ice interface, studies reported in [38, 39] are commonly invoked, and discussed below in some detail. Conformational changes of globular proteins were studied by employing the phosphorescence emission of tryptophan (Trp) residues as a monitor of the conformational changes of six proteins in response to variations in conditions of the medium [38]. Changes in well-structured compact cores of the macromolecules were monitored by the direct correlation between the phosphorescence lifetime τ and the rigidity of the protein matrix surrounding the chromophoric probe. The solidification of water markedly decreased τ and indicated unfolding related changes in conformation of the proteins, which was related primarily to the protein–ice interaction. Additionally, tryptophan florescence was employed to monitor unfolding of azurin mutant C112S from *Pseudomonas aeruginosa*. The thermodynamic stability (ΔG^0) of the macromolecule in frozen aqueous solutions was studied by introducing guanidinium chloride and monitoring tryptophan fluorescence for native and denatured states. The evaluation of the guanidinium chloride-induced unfolding in the frozen state allowed assessment of the thermodynamic stability of proteins in frozen solutions. The results obtained with C112S azurin demonstrated that the stability of the native fold may be significantly perturbed in the frozen solutions depending mainly on the size of the liquid solution pool in equilibrium with the solid phase. It was proposed that the effectiveness of stabilizing additives in preventing protein unfolding in the frozen state will be a combination of two influences: the ability to stabilize the N-state at low temperature and high-solute concentration (a preferential hydration mechanism), plus a specific action of the additive to contrast perturbations deriving from protein–ice interactions. We should note that, while these studies provided convincing evidence that destabilization of protein can be induced by formation of ice, it is not obvious if such destabilization is the results of the direct protein/ice interaction, as other mechanisms can be invoked.

In another study, concentration of the freeze-dried human interferon-γ on the surface of freeze-dried and spray-lyophilized materials was measured by electron spectroscopy for chemical analysis [18]. A higher concentration of the protein was observed on the surface of the dried materials, whereas the use of a surfactant (polysorbate 20) minimized the surface excess. The observations of the high surface concentration of a protein on the surface of freeze-dried cakes were taken as an evidence of protein sorption on solution/ice interface during the freezing step. This would certainly be a logical conclusion, but these studies did not provide a direct proof of protein sorption on the surface of ice crystals.

To reconcile the studies which showed rejection of non-antifreeze proteins from ice [37] with reports of both protein destabilization due to ice formation and the higher concentration of proteins on the surface of dried materials, we note that the formation of ice crystals during freezing usually results in formation of air bubbles [40], thus greatly increasing solution/air interface and potential for protein destabilization. Protein sorption on solution/air interface is well-documented, e.g., [41], and will not be discussed here. Therefore, protein sorption on the air bubbles created as