# The role of iron binding on bovine lactoferrin structure and dynamics

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**Introduction:** Bovine lactoferrin (bLF) is a 76 kDa glycoprotein that belongs to the transferrin family. It is an iron binding protein widely known by its multiple functions, such as antiviral activity. bLF can be found in many body fluids, like milk, blood, tears and saliva. The three-dimensional structure is composed by two lobes, N and C, and the iron binding sites are located into the two domains. Despite the importance of bovine lactoferrin, few studies demonstrate the differences in structural stability between the iron-saturated (holo) and iron free lactoferrin (apo). Since these changes can affect the functionality of lactoferrin, it is important to investigate the differences in the stability of apo and holo forms. **Objectives:** With this goal, we used urea and high hydrostatic pressure to promote disturbances in bLf structure and to evaluate the protective effect of this ion. **Materials and Methods:** Changes in secondary and tertiary structures of lactoferrin were monitored by spectroscopic techniques such as fluorescence spectroscopy and circular dichroism. Fluorescence Spectroscopy was applied using an intrinsic probe, tryptophan, and an extrinsic probe, bis-ANS (4,4′-Dianilino-1,1′-binaphthyl-5,5′-disulfonic acid dipotassium salt). **Results and Discussion:** Our results clearly show that iron binding strongly stabilizes the tertiary structure and preserves the secondary structure of the protein under pressure or high concentration of urea. Moreover, it was possible to correlate the bLf iron binding and the interaction of this protein with Vero cells. We observed by two-photon microscopy that the kinetics of internalization of holo-bLf is faster, when compared to the apo form. It demonstrates that the presence of iron ions improves the bLf-cells interaction. We also verified if high hydrostatic pressure effect could cause changes in the kinetics of intracellular traffic in apo and holo lactoferrin. **Conclusions:** These studies are important to understand the bLF structure/stability and its functions due to its growing applications as an antiviral agent.

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