The [(bathophenanthroline)$_3$: Fe^{2+} complex as an aromatic non-polymeric medium for purification of human lactoferrin

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We describe a non-chromatographic, ligand-free platform for the efficient purification of recombinant human lactoferrin. The platform consists of a [metal: chelator] complex precipitate in the presence of osmotically active polyethylene glycol 6000 . Purification is achieved in three stages. Following formation of the complex, LF is captured under neutral conditions by the aggregated complexes (Step I), a washing step follows (Step II) and then, (Step III) LF is extracted in pure form with 100 mM tribasic Na citrate buffer (pH 7). Of the four complexes investigated, [bathophenanthroline (batho)3:Fe2+] was determined to be the most efficient. LF is recovered with high yield (~90%) and purity (≥97%, by SDS polyacrylamide gel electrophoresis) from an artificial contamination background comprising E. coli lysate proteins. Purified LF is demonstrated to be monomeric by dynamic light scattering ; to preserve its native secondary structure by circular dichroism spectroscopy; and, as apo-LF, to efficiently inhibit bacterial growth. Process yield is not affected by a 45-fold increase in LF concentration from 0.2 to 9 mg/mL. We provide evidence that protein capture relies on [cation:π] interactions between the lysine and arginine residues of LF with the fully aromatic [(batho)3:Fe2+] complexes. The use of [metal:chelator] complex aggregates is demonstrated to provide an economical and efficient avenue for LF purification.

Method- Purification strategy:

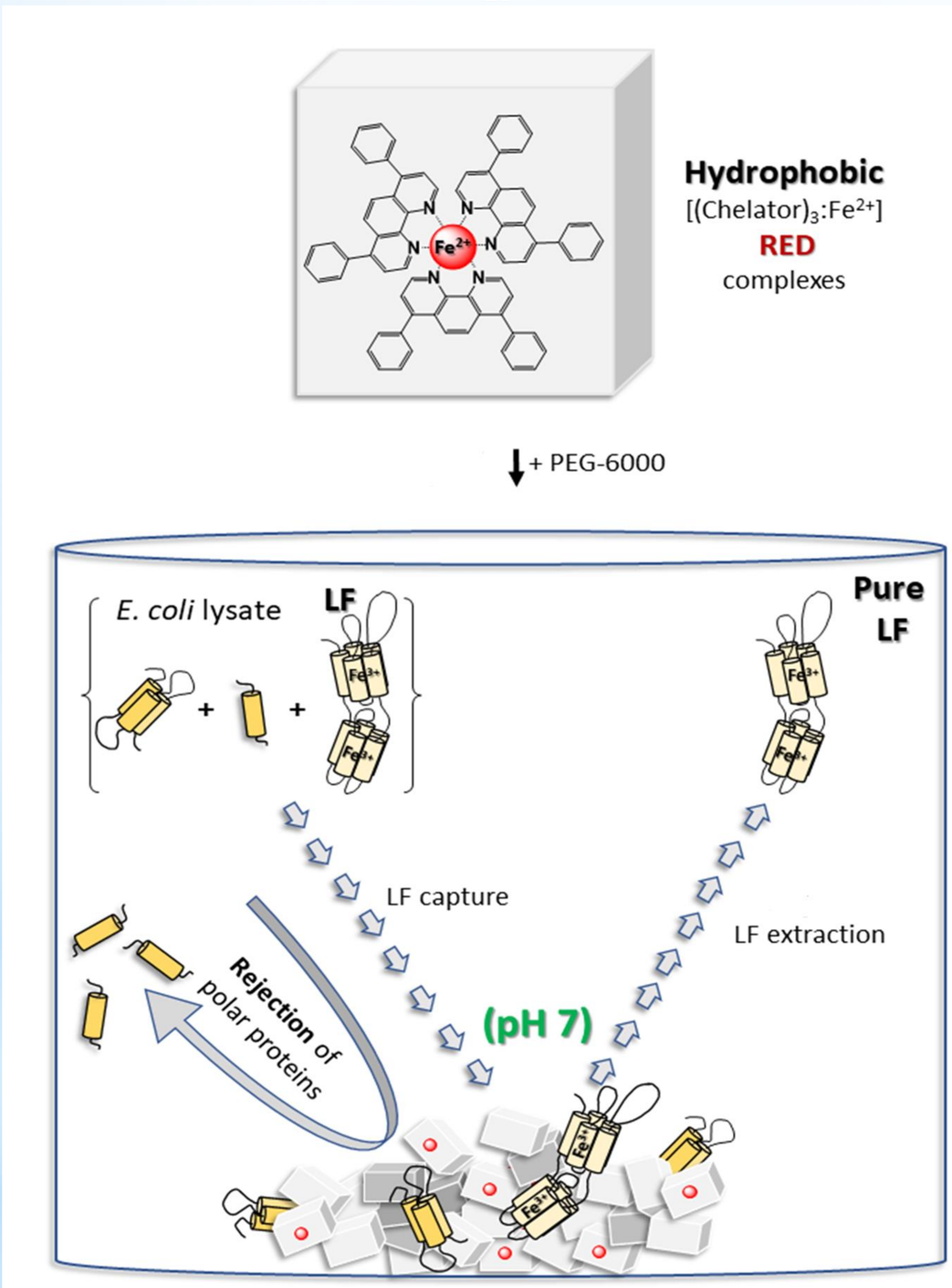

Figure 1. Illustration of lactoferrin purification using [bathophenanthroline (batho)3:Fe^{2+}] .

Results:

Purity and yield

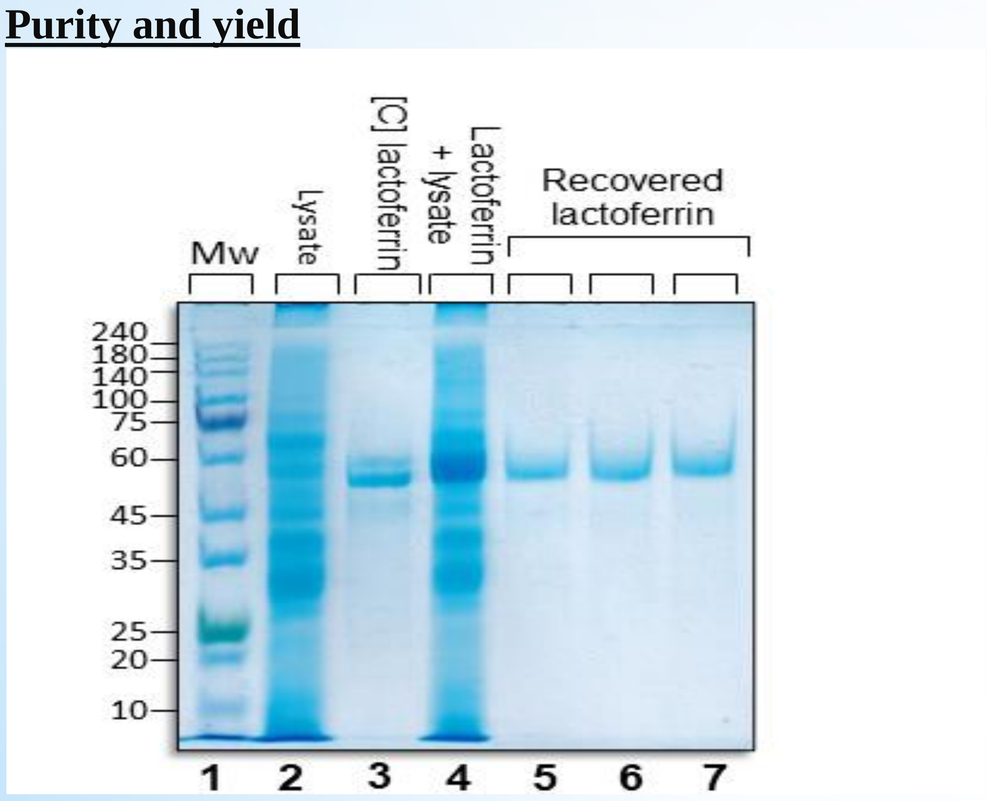

Figure 2.Purification of LF ,1: Molecular weight markers; lane 2; total amount of E. coli. lysate added; lane 3: Total amount of LF (≥90% purity); lane 4: Mixture of lanes 2 and 3; lanes 5-7: LF recovered by extraction.

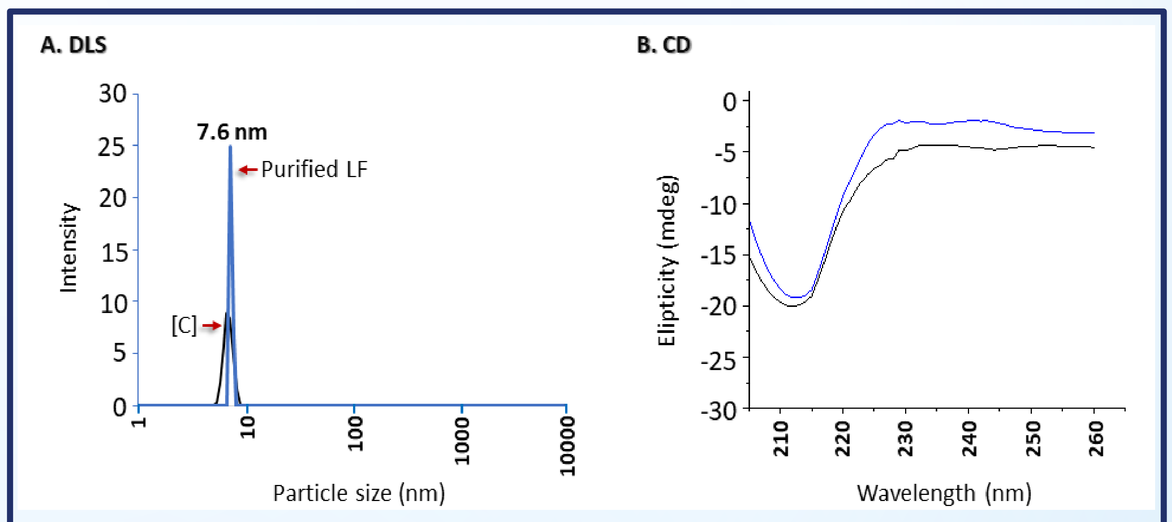

Figure 3. A. Particle size distribution of control [C] pure (≥90% purity) LF and LF recovered by the presented purification approach.

B. Circular dichroism (CD) analysis. Control - Black line, purified LF - blue line.

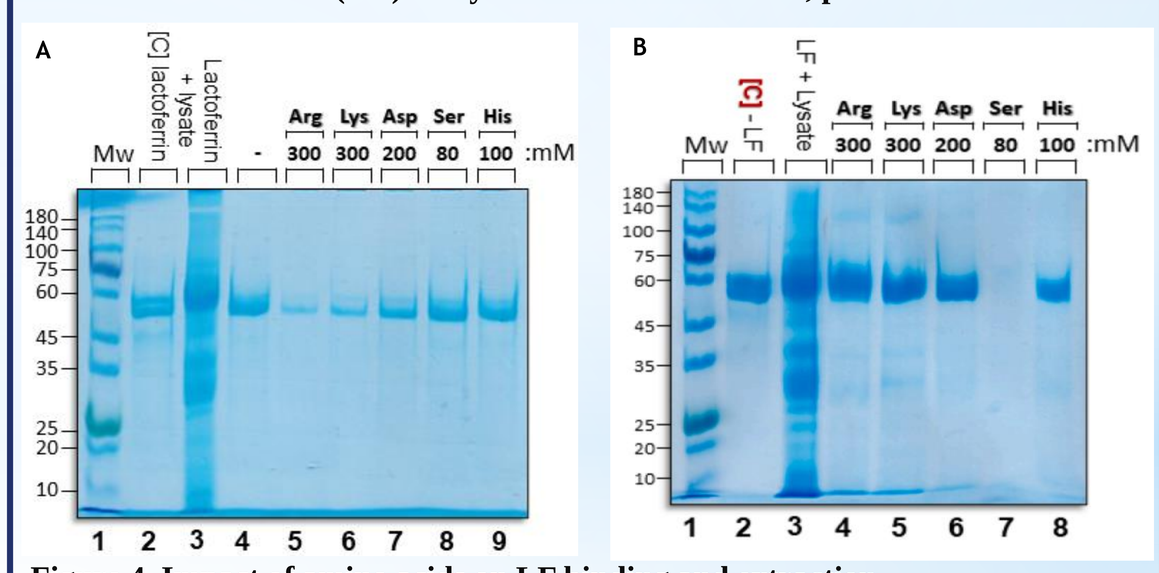

Figure 4. Impact of amino acids on LF binding and extraction.

A. lanes 1: Molecular weight markers; lanes 2: [C] Total amount of LF (≥90% purity) that was added; lanes 3: Mixture of the total amount of LF and E. coli. lysate; lanes 4: LF recovered by extraction; lanes 5-10: Indicated hydrophobic amino acids (all at pH 7) added in the binding step.

B. B. As in A, but when amino acid monomers are added at the extraction step.

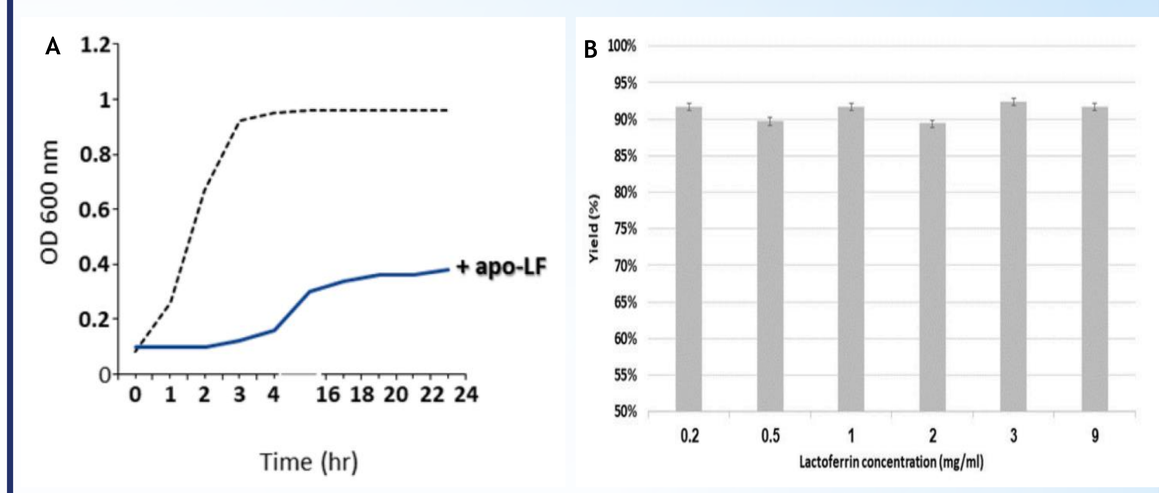

Figure 5. A. Inhibiting effect of purified apo-LF at 1.25 mg/mL on E. coli cell proliferation (blue line) and the dashed line shows growth in the absence of LF.

B. Process efficiency as a function of LF concentration

Conclusions:

1. **The positively charged side chains of human LF (i.e., Lys and Arg) appear to form multiple cation-π interactions with the fully aromatic [(batho)3:Fe2+] complex.**
2. **Preservation of LF native-state and biological activity combined with the observed high purity and yield should justify process upscaling.**

References:

Withanage T. J., Lal M., Salem H., Krichevski O., Wachtel E., and Patchornik G. The [(bathophenanthroline)3:Fe2+] complex as an aromatic non-polymeric medium for purification of human lactoferrin *J. Chromatography A* (2024) 1732, 465218.

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