Purification of Fc-fusion proteins without: Chromatography, polymers, membranes or specific-ligands

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We introduce a new concept and a potentially general platform for purification of Fc- fusion proteins that does not rely on any resins, chromatographic media, membranes or specific-ligands, rather, makes use of aromatic [metal:chelator] complexes. We modified and significantly expanded our existing technology which was used to purify antibodies in our previous studies. Our fully aromatic complex is composed with the chelator: bathophenanthroline (batho) and metal ions: zinc or copper ions to form [(batho)3:Zn2+] or [(batho)3:Cu2+] complexes. Our technology captures the target proteins quantitatively *via* [cation:pi] and [pi:pi] interactions and allows their recovery at high yields (>80%, by densitometry) and purity (≥90%, by SDS-PAGE), while preserving their secondary structure, enzymatic activity and monomeric state. The entire process is performed at pH 7 there by avoiding complications that derive from exposure to harsh acidic conditions (*e.g.*, aggregation, partial denaturation). The monomeric dispersity was preserved after the elution and evaluated by the DLS (Dynamic light Scattering) and the native page gel electrophoresis. The secondary structure of the target protein was not affected during the purification process and confirmed by the technique of CD (Circular Dichroism). The biological activity of the purified protein was assed and was preserved. The cost-effectiveness and simple integration into future, industrial- scale downstream processing of therapeutic-grade biopharmaceuticals, will be discussed. In future, we are planning to apply the same purification technology on different type of therapeutic proteins as an efficient purification plat form while applying the necessary modification.

Method- Purification strategy:

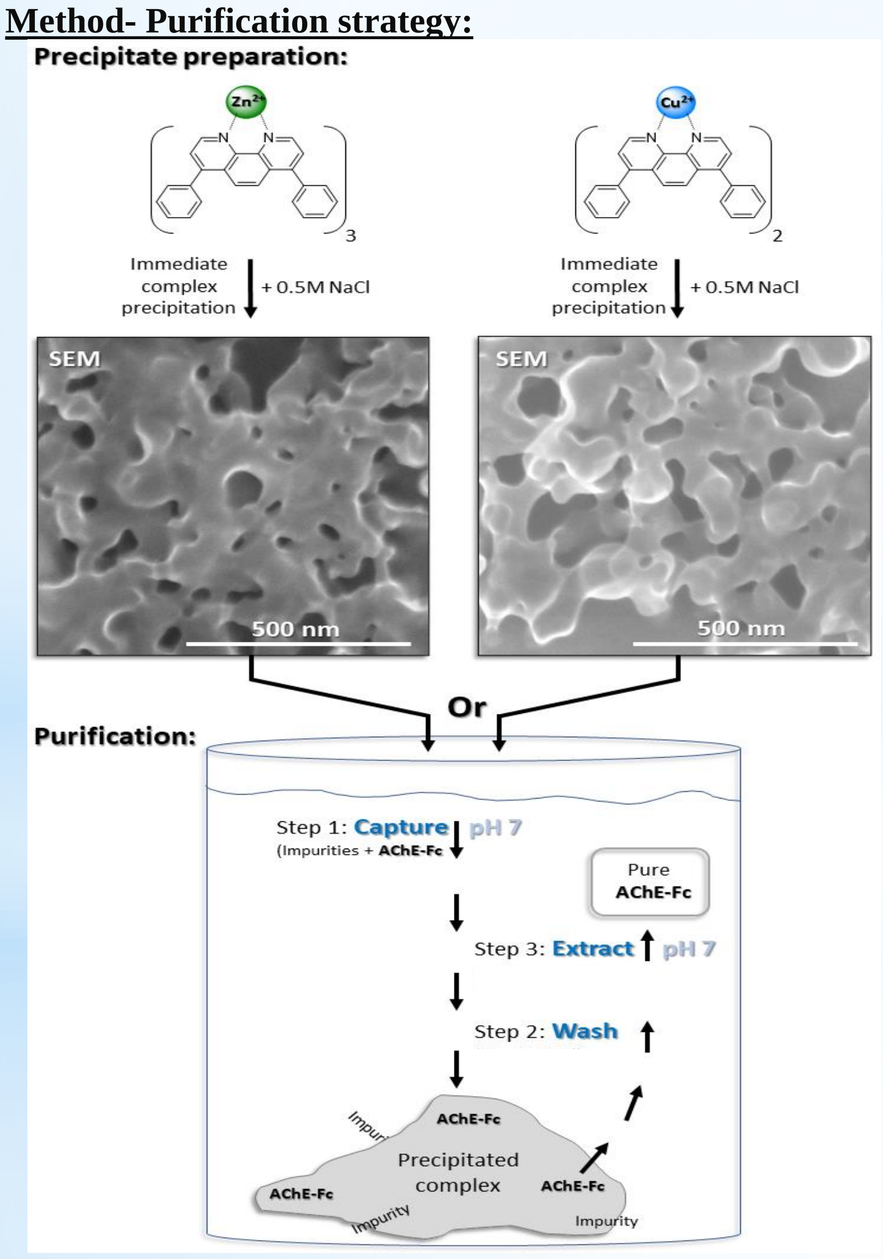

Figure 1. Illustration of a three-step purification protocol for AChE-Fc using [(Batho)3:cation] complexes.

Results:

Purity and yield

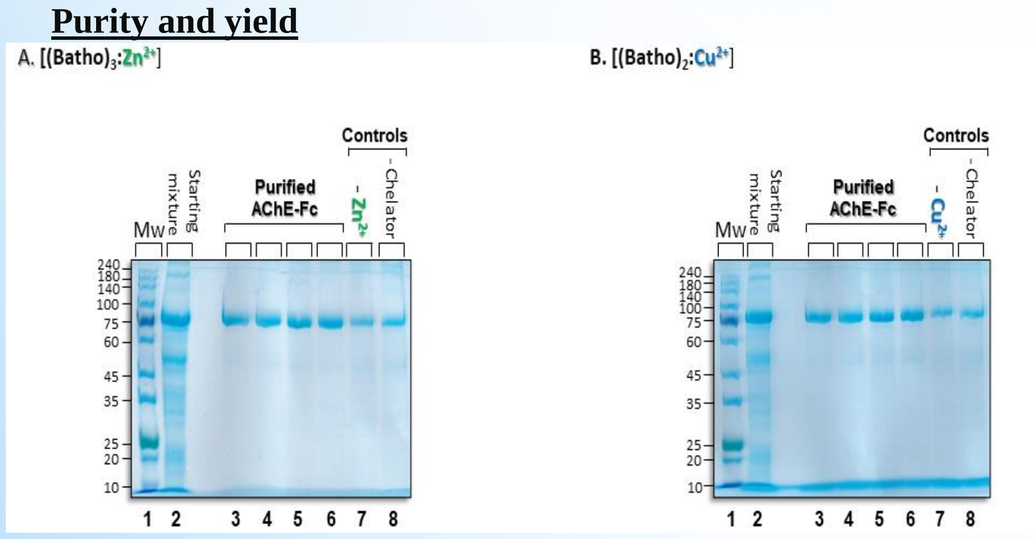

Figure 2. Purification of AChE-Fc

Lane 1: Molecular weight markers; lane 2: total amount of impure recombinant human AChE-Fc expressed in HEK293 cells added; lanes 3-6: Recovered AChE-Fc the capture and Washing-steps and followed by extraction; lanes 7-8: Controls: as in lanes 3-6 devoid of only the Zn2+ or only the chelator, respectively.

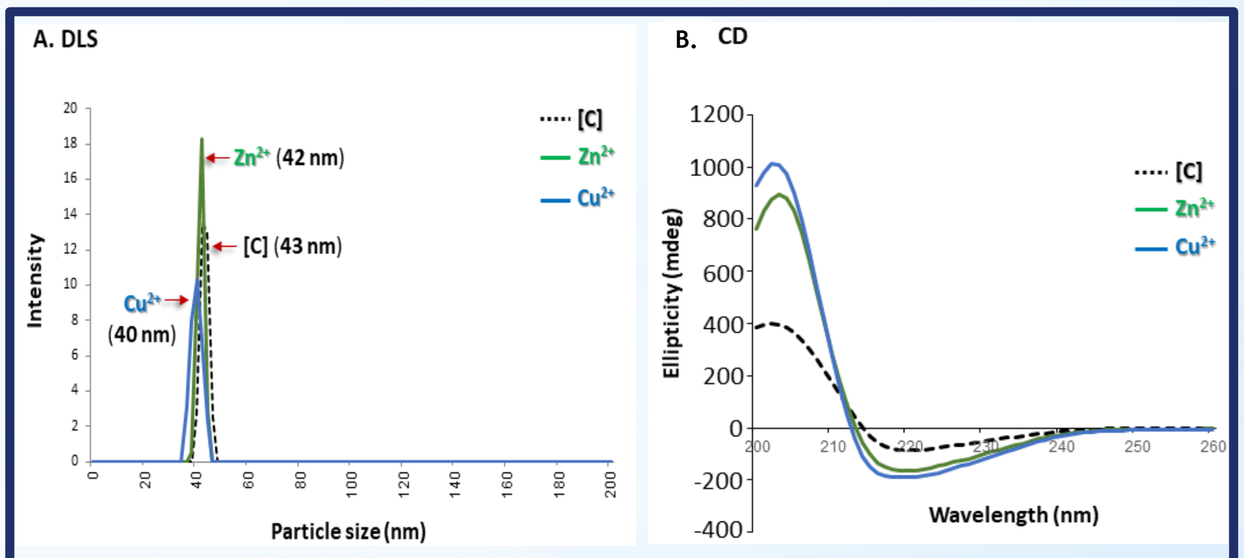

Figure 3: A.Dynamic light scattering (DLS) analysis. Particle size distribution of human AChE-Fc purified with either aggregates or with Protein A chromatography [C].

B. The far UV CD spectra of the AChE-Fc purified by Protein A chromatography [C] (black dotted line) is compared to AChE-Fc isolated with either the [(Batho)3:Zn2+] complex (green line) or [(Batho)3:Cu2+] complex (blue line)

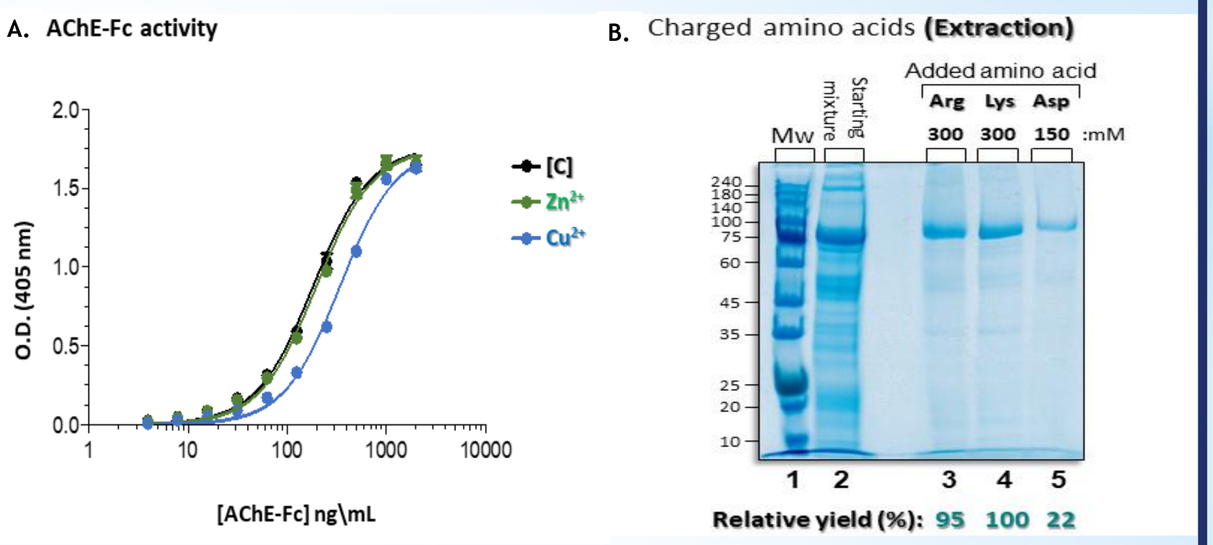

Figure 4: A. Ellman's assay applied on control and purified AChE-Fc. Acetylcholinesterase activity obtained with AChE-Fc purified via Protein A chromatography [C] (dotted black line), [(Batho)3:Zn2+] (green line) or [(Batho)3:Cu2+] (blue line).

B. Addition of the hydrophilic amino acids in the extraction step. Lane 1: Molecular weight markers; Lane 2: Total amount of impure recombinant human AChE-Fc added to each purification trial; Lanes 3-9: Recovered AChE-Fc in the absence (lane 3) or presence of indicated hydrophobic amino acids concentration added during the extraction step.

Conclusions:

1. **A Fc-fusion protein was isolated efficiently under mild conditions without any chromatographic step, specific ligand or any resin, rather with a precipitated aromatic [(batho)3:Zn2+] complex.**
2. **Preservation of the native-state and enzymatic activity combined with the observed high purity and yield should justify process upscaling.**
3. **Major interactions between the target Fc-fusion protein and the aromatic complex likely rely on [cation:π] and [π:π] interactions.**