Non-chromatographic, Ligand-free Platform for Immunoglobulin Purification

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Antibodies are extensively used in medicine for therapeutic and diagnostic purposes. They are traditionally purified by 2-3 chromatographic steps, where the first exploits a protein–ligand called Protein A. The latter is used by most, if not all, pharmaceutical companies since it binds to diverse antibody types with high affinity and specificity and leads to high process yields (>90%) and purity (>95%) within a single chromatographic step. However, as the antibody concentration increases, antibody purification becomes challenging, since a single industrial-scale Protein A column cannot capture the entire antibody population. Therefore, we developed an alternative, non-chromatographic antibody purification platform that quantitatively captures antibodies regardless of their concentration. The studied strategy relied on aromatic [metal/chelator] complexes rather than on resins conjugated to Protein A. Two aromatic complexes were evaluated and were composed of a commercially available chelator called bathophenanthroline (batho) bound to either Fe2+ or Zn2+. Such water-insoluble complexes (i.e., [(batho)3:Fe2+] or [(batho)3:Zn2+]) allow for (a) an efficient separation of IgG antibodies from their mixture with IgM antibodies or (b) the purification of an Fc-fusion protein composed of the Fc-domain of an IgG1 bound to the enzyme acetylcholinesterase. The process yield (>85%, by densitometry) and purity (>95%, by SDS-PAGE) values are encouraging. The recovered targets were monomeric (by dynamic light scattering and Native-PAGE) and preserved their secondary structure (by circular dichroism) and catalytic activity. Purification was achieved at pH~7, thereby circumventing the exposure of antibodies to harsh acidic conditions and, hence, antibody aggregation.

Method- Purification strategy:

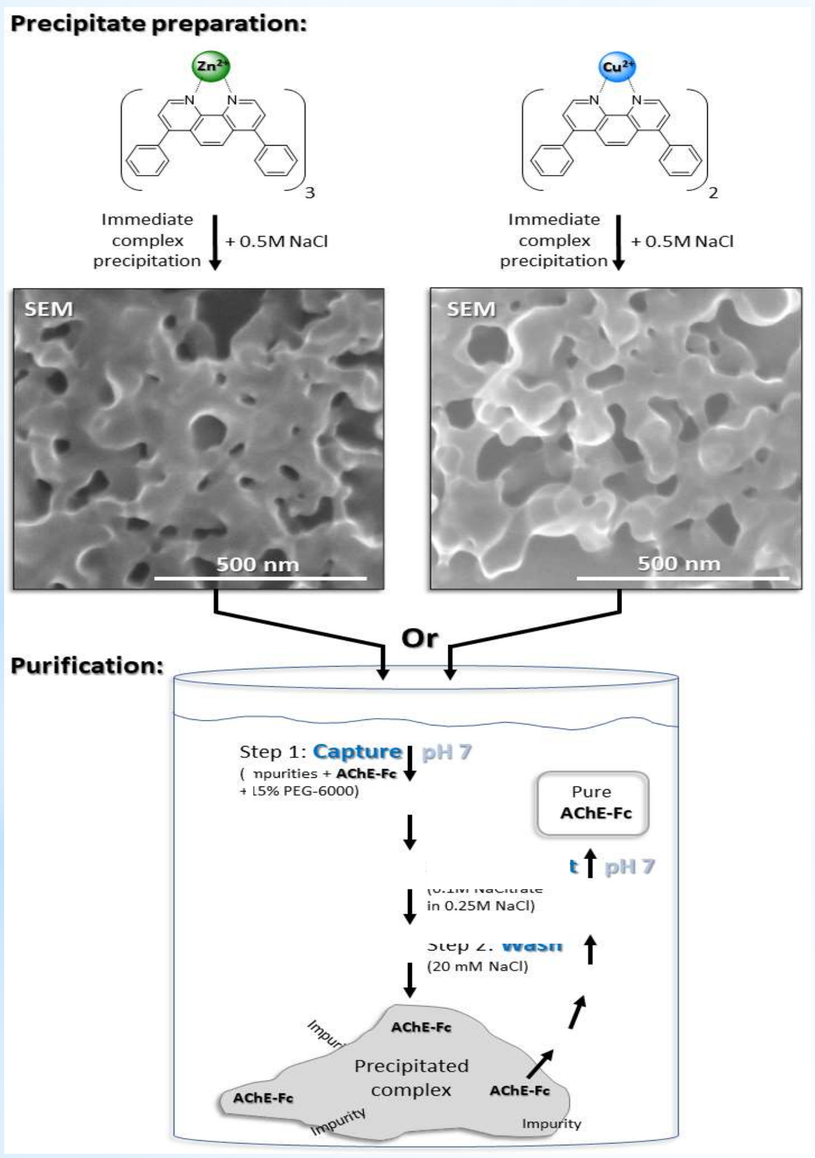

Figure 1. Illustration of a three-step purification protocol for AChE-Fc using [(Batho)3:cation] complexes.

Results:

Purity and yield

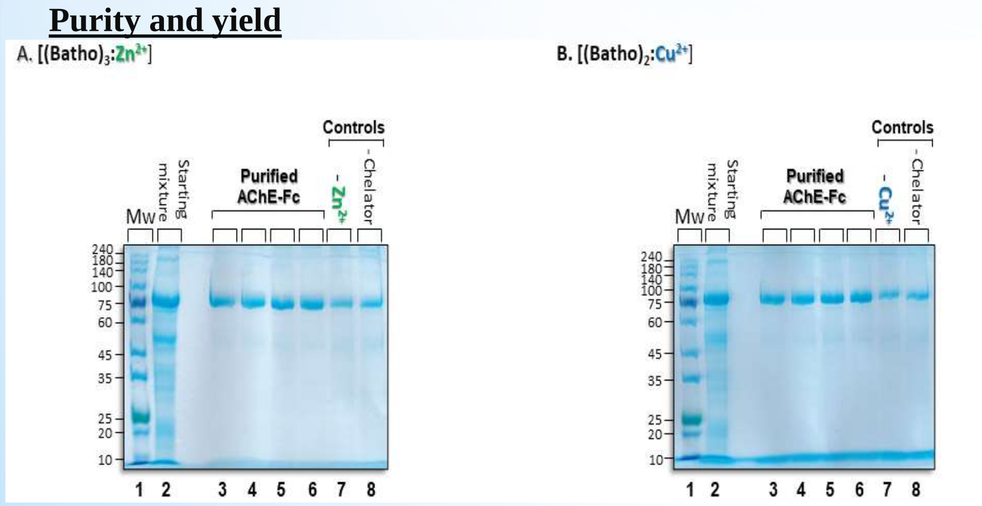

Figure 2. Purification of AChE-Fc

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.

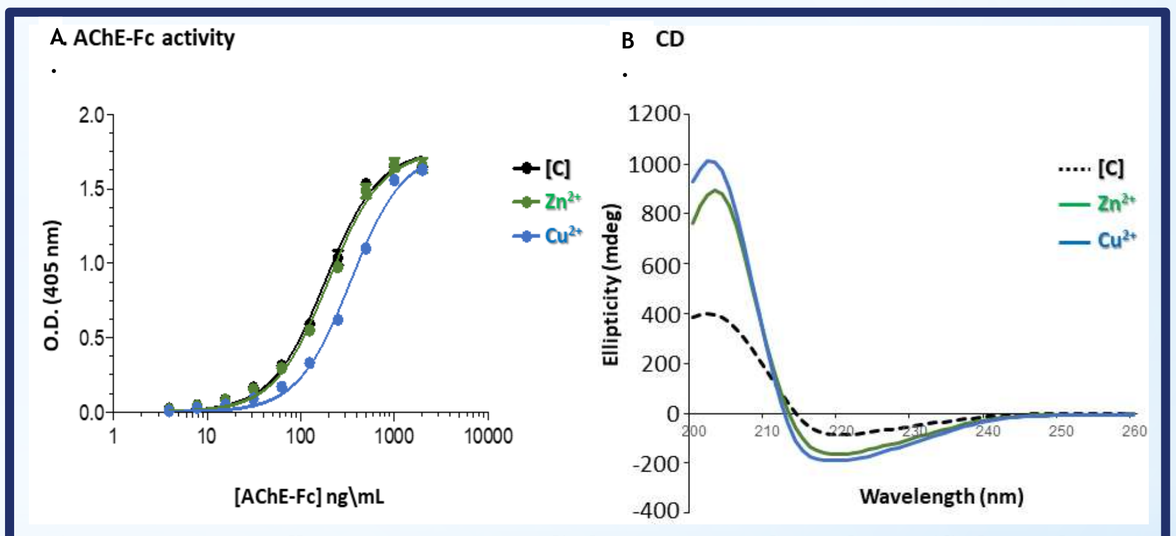

Figure 3: A. Ellman's assay applied on control and purified AChE-Fc. Acetylcholinesterase activity obtained with AChE-Fc purified via Protein A chromatography [C] (dotted black line), [(Batho)3:Zn2+] (green line) or [(Batho)3:Cu2+] (blue line).

B. 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) .

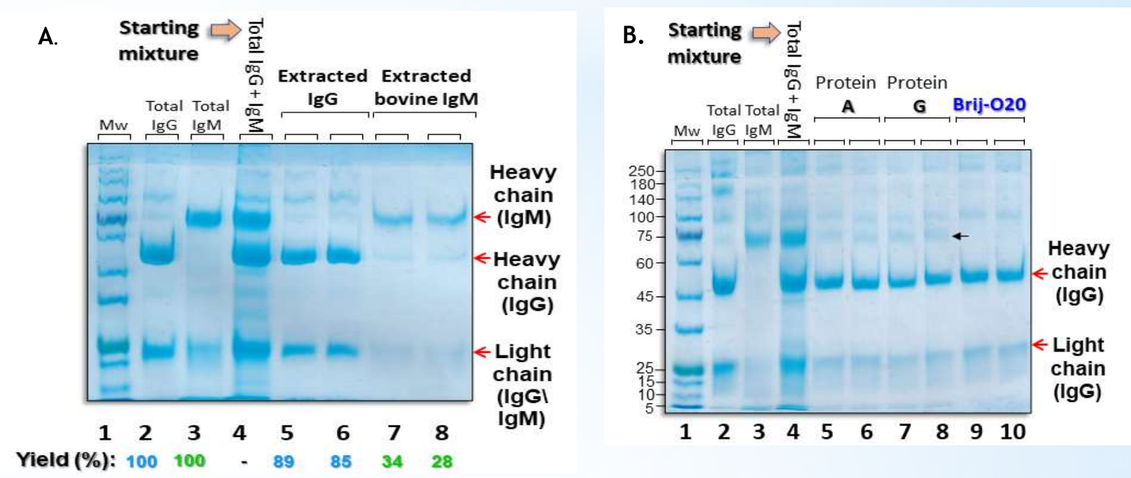

Figure 4: A. Purification of IgG and IgM. Lane 1: molecular weight markers; Lanes 2-3: control - total amounts of IgG and IgM; Lane 4 - starting mixture; Lanes 5-6: IgG extracted; Lanes 7-8: extracted IgM . % Yield of IgG or IgM is indicated below the relevant lanes.

B. Comparison of the conjugated micelle purification platform with Protein A or Protein G chromatography for IgG:IgM mixtures (molar ratio 9:1). lane 4: mixture of lanes 2 and 3; lanes 5-6 and 7-8: recovered IgG via Protein A or Protein G spin columns, respectively; lanes 9-10: recovered IgG using Brij-O20\DDM\Tyr\bathophenanthroline\Fe2+].

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 were observed.**

1. **Human polyclonal IgG can be purified with good yield (85-90%) and purity (≥95%) from either bovine or human polyclonal IgM at close to neutral pH and at 10°C with the use of Tween-20 or Brij-O20 micelles supplemented with DDM, and Tyr, and conjugated by the amphiphilic [(bathophenanthroline)3:Fe2+] complex.**