High-level expression and purification of recombinant human Apolipoprotein A-I in *Pichia pastoris*

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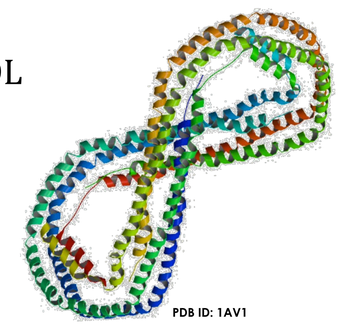
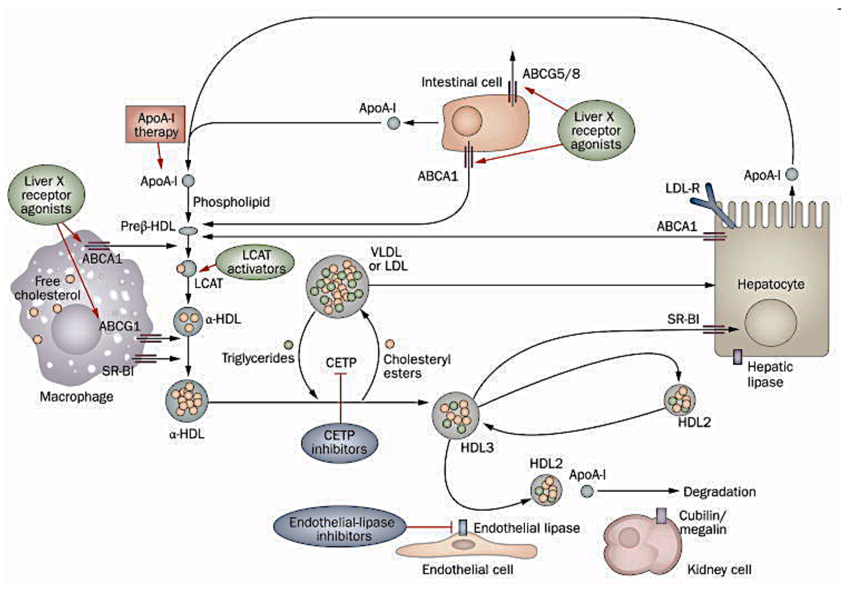
ABSTRACT. The High Density Lipoprotein (HDL) complex, commonly addressed as the "good cholesterol" helps reduce the risk of cardiovascular disorders mainly due to its ability to remove accumulated cholesterol from arteries (via reverse cholesterol transport), in addition to posessing anti-inflammatory and anti-thrombotic properties. These protective effects of HDL are mediated mainly by Apolipoprotein A-I (ApoA1) which is the major protein component of HDL. ApoA1 is a lipid binding protein and promotes cholesterol efflux from peripheral tissues to the liver for excretion. An increase in the plasma levels of ApoA1 is generally accpted to be cardioprotective, making it a potential therapeutic. However, recombinant ApoA1 is rapidly cleared from the plasma and is also prone to oxidation by myeloperoxidase.

Two naturally occuring variants of ApoA1, namely the Milano & Paris mutants, are characterised by a single point mutation resulting in the introduction of a Cysteine residue. Populations with ApoA1-Milano have been reported to have a healthier cardiovascular system even with low plasma levels of ApoA1/HDL. Functional comparison of native and mutant ApoA1 variants would enhance our understanding of their mechanisms of action. In this study, we present a method that has been streamlined to facilitate high-level secreted expression of recombinant human ApoA1 in its native form (without the use of any affinity tag) by the methylotropic yeast *Pichia pastoris* in a bioreactor, followed by a comparison of several purification methods (two-phase extraction, cold acetone precipitation, ion-exchange chromatography and mixed-mode chromatography) to efficiently recover the protein with highest efficiency. This process would not only yield the protein with maximum functionality, but also promises immense industrial implications.

Apolipoprotein A-I (ApoA1)

- Major protein component of HDL
- Key effector functions:
  - Uptake of cholesterol and phospholipids from tissues
  - Activation of LCAT
- Plethora of variants



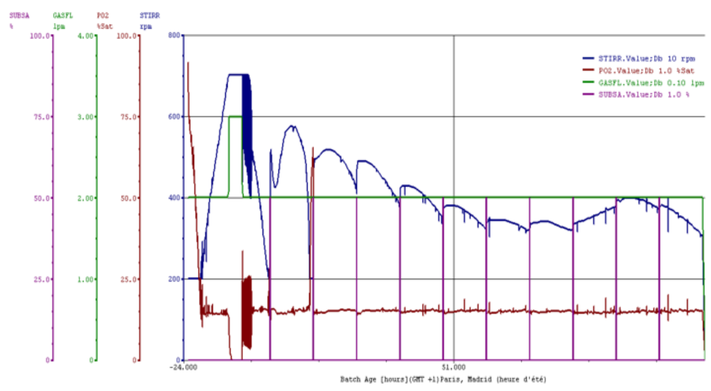


Source: Lipids Online Slide Library

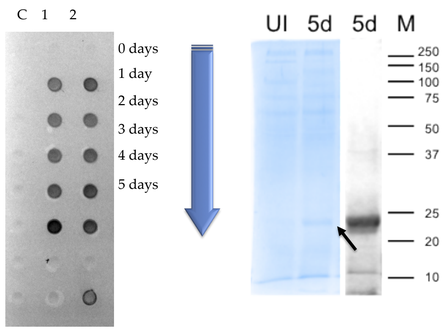
rhApoA1 expression

Host Organism *Pichia pastoris* | **Induction of Expression** Methanol, 0.5% | **Duration of Induction** 5 days (120hrs)

Expression profile (2l bioreactor):

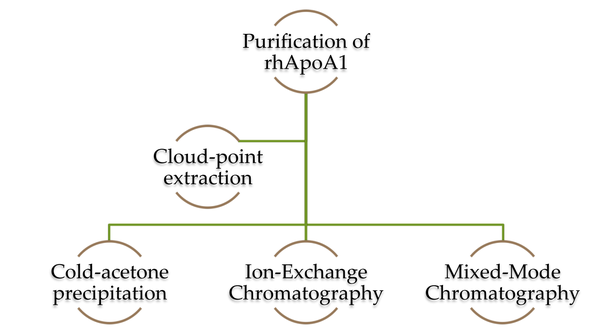


Protein expression:

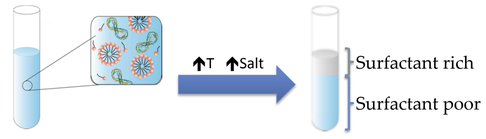


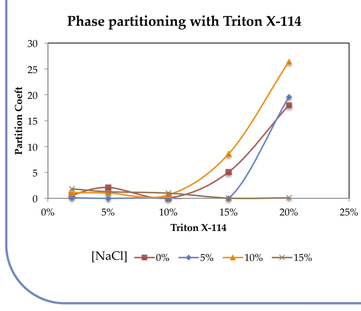
- Secreted expression at the expected Mol. Wt. of ~25kDa
- Verified by western blotting
- Reproducible high expression levels

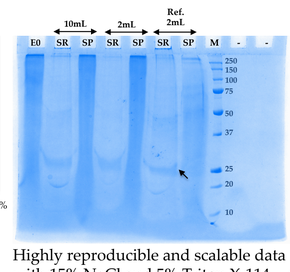
rhApoA1 purification



Cloud point extraction:







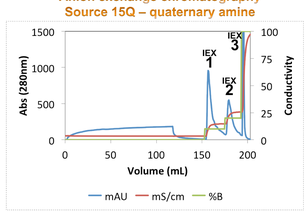
Highly reproducible and scalable data with 15% NaCl and 5% Triton X-114

Ion-Exchange Chromatography:

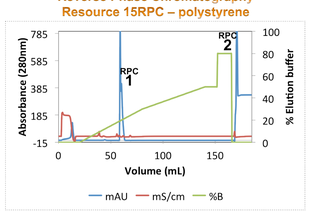
Strategy:

Desalt *Pichia* expression broth → Anion-Exchange (Q-amine) → Reverse-Phase Chromatography → Purified ApoA1

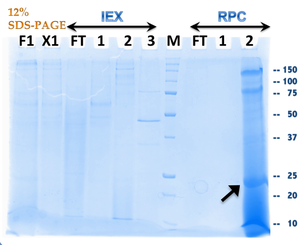
Anion exchange chromatography
Source 15Q – quaternary amine



Reverse Phase Chromatography
Resource 15RPC – polystyrene



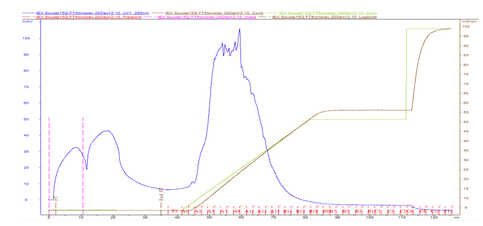
12% SDS-PAGE

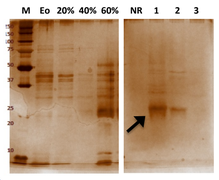


- Protein recovered in high concentration
- Aggregates observed
- Lengthy sample pretreatment required
- Not suitable for scaling up

Cold-acetone precipitation:

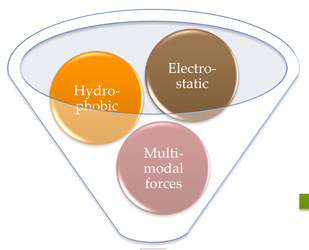
Precipitation with 20% cold acetone (impurities) → Increase acetone content to 40% → Reduce pH to 5.8 and increase acetone content to 60% → Polishing with Q-sepharose IEX chr.





- Large quantities of cold acetone required
- Long incubation times at sub-zero temperatures
- Not suitable for scale up

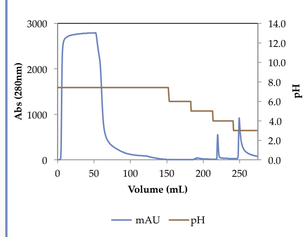
Mixed-mode chromatography:

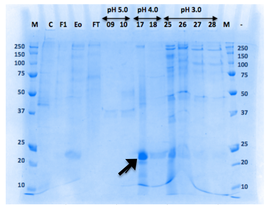


HEA Hypercel™ – $CH_2 – (CH_2)_4 – CH_3$

Mixed-mode chromatography

Binding	Elution
• *hydrophobic attraction*	• *electrostatic repulsion*





Conclusion

High-level expression of rhApoA1 with *Pichia pastoris* without the use of affinity tags.

Single-step purification of rhApoA1 from the broth using Mixed-Mode chromatography without any pretreatment, while maintaining the integrity of the protein for future functional applications.

Direct industrial application in cost-effective production of therapeutic rhApoA1.

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