Purification of Human H chain Ferritin

Cloning performed by the Protex facility at the University of Leicester, protein purification by Dr. Louise Fairall, Leicester Institute of Structural and Chemical Biology and electron microscopy by Dr. Christos Savva, Midlands Regional Cryo-Electron Microscopy Facility.

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We kindly request that any work published using this plasmid acknowledges the Protex facility at the University of Leicester, Louise Fairall and Christos Savva. **This plasmid is only for academic use**.

The plasmid LF2422 contains ferritin human H chain cloned into pGEX2T with a TEV site instead of a thrombin site.

Ferritin Nucleic Acid sequence:

ATGACGACCGCGTCCACCTCGCAGGTGCGCCAGAACTACCACCAGGACTCAGAGGCCGCCATCAAC
CGCCAGATCAACCTGGAGCTCTACGCCTCCTACGTTTACCTGTCCATGTCTTACTACTTTGACCGCGA
TGATGTGGCTTTGAAGAACTTTGCCAAATACTTTCTTCACCAATCTCATGAGGAGAGGGAACATGCT
GAGAAACTGATGAAGCTGCAGAACCAACGAGGTGGCCGAATCTTCCTTCAGGATATCAAGAAACCA
GACTGTGATGACTGGGAGAGCGGGCTGAATGCAATGGAGTGTGCATTACATTTGGAAAAAAATGT
GAATCAGTCACTACTGGAACTGCACAAACTGGCCACTGACAAAAATGACCCCCATTTGTGTGACTTC
ATTGAGACACATTACCTGAATGAGCAGGTGAAAGCCATCAAAGAATTGGGTGACCACGTGACCAAC
TTGCGCAAGATGGGAGCGCCCGAATCTGGCTTGGCGGAATATCTCTTTGACAAGCACACCCTGGGA
GACAGTGATAATGAAAGCTAA

Sequencing primers:

pGEX5’ GGG CTG GCA AGC CAC GTT TGG TG

pGEX3’ CCG GGA GCT GCA TGT GTC AGA GG

Protein sequence after TEV cleavage:

G A M T T A S T S Q V R Q N Y H Q D S E A A I N R Q I N L E L Y A S Y V Y L
S M S Y Y F D R D D V A L K N F A K Y F L H Q S H E E R E H A E K L M K L Q
N Q R G G R I F L Q D I K K P D C D D W E S G L N A M E C A L H L E K N V
N Q S L L E L H K L A T D K N D P H L C D F I E T H Y L N E Q V K A I K E L G
D H V T N L R K M G A P E S G L A E Y L F D K H T L G D S D N E S

Plasmid Recovery

- Using gloves and a new razor blade cut around the marked circle on the filter paper and place the circle into a 1.5ml Eppendorf tube.
- Add 50ul of autoclaved MQ water and vortex briefly.
- Leave on bench for 20 minutes.
- Vortex briefly.
- Spin on a desktop centrifuge at max for 1 minute.
- Take out 10ul and use to transform XL1 or DH5α cells and plate on Ampicillin plates.
- Use transformants to propagate more plasmid.
- We recommend to re-sequence the plasmid prior to overexpression and purification.

Overexpression

Day 1.

- Transform 1ul of plasmid into chemically competent *E.coli* (E.g. BL21(DE3) or Rosetta (DE3) and plate on plates containing Ampicillin (and Chloramphenicol for Rosetta). Leave at 37°C overnight.

Day 2.

- Use a few colonies to begin a starter culture (e.g. 60ml) in LB Amp media (and Chloramphenicol for Rosetta). Grow at 37°C until A_{600nm} =0.3
- Inoculate 10 ml of starter culture into each 1 liter LB Amp (and Chloramphenicol for Rosetta) media. Grow at 37°C until an A_{600nm}=0.1
- Induce with 40 micromolar IPTG and grow overnight at 20°C.
- **Note:** You may also try inducing at A_{600nm}=0.8 with 1 mM IPTG and grow overnight at 20°C. Please see https://doi.org/10.1080/10826068.2016.1141300

Day 3.

- Spin down cells (e.g. 4K for 10 min at 4°C)

Protein Purification

Conditions described below were for 1.5 liters of *E. coli*, 14 grams wet cell paste total.

Buffers:
Lysis Buffer (50 ml):
1X PBS
Roche Complete Tablet
0.5% Triton X-100
0.5 mM TCEP

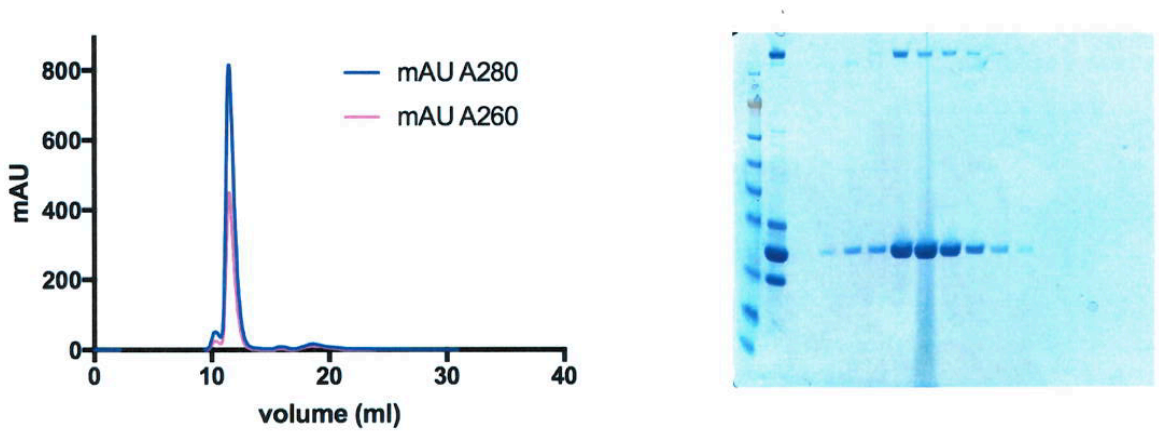
Wash Buffer (100 ml)
1X PBS
0.5% Triton X-100
0.5 mM TCEP

Cleavage Buffer for TEV and Gel Filtration buffer (500 ml, 0.2μm filtered)
50 mM Tris pH 7.5
100 mM NaCl
0.5 mM TCEP

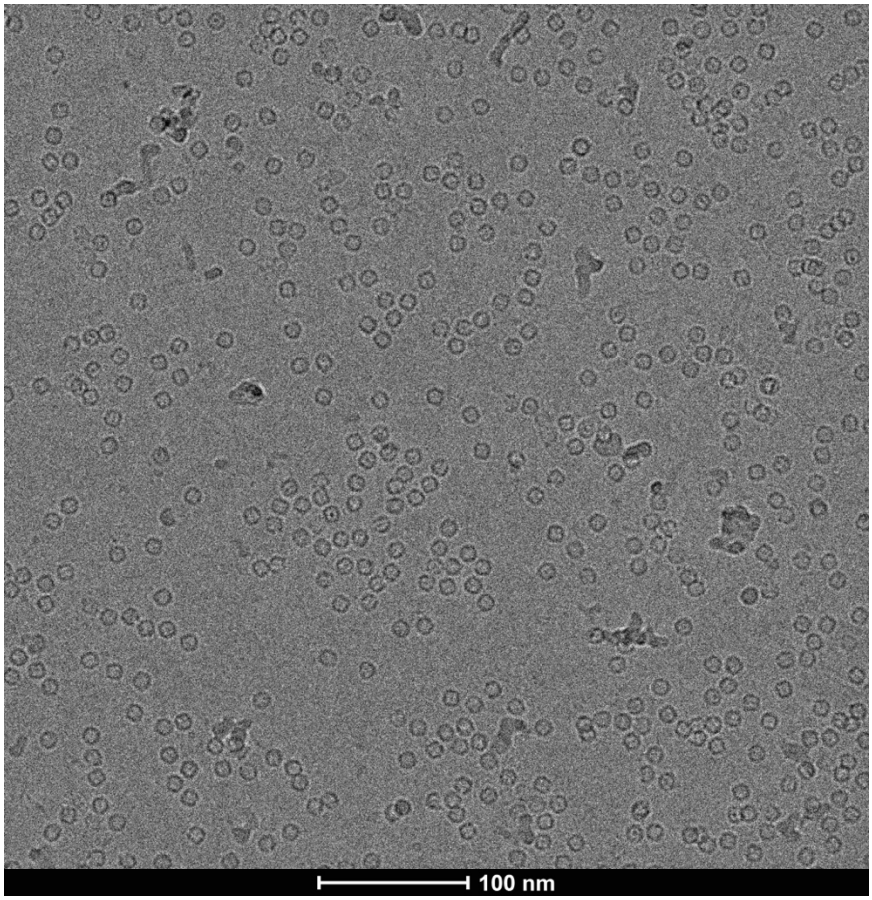
Day 1
- Resuspend cell pellets in 50 ml lysis buffer on ice with pipetting and some vortexing
- Lyse cells either by sonication, cell disruptor, or French Press (depending on equipment available conditions will vary). We used sonication.
- Spin at 30,000 rpm for 20 min (Beckman JA-30.50 Ti, 8 x 50 ml rotor or equivalent).
- Add supernatant to 1ml of GST resin (1.25 ml 80% slurry) and leave at 4°C on roller for 30 min to bind
- Spin for 2 min at 4K to pellet resin, Remove supernatant
- Add some wash buffer and transfer to 15ml Falcon tube
- Wash 4 x 15ml with wash buffer, spinning and discarding supernatant in between
- Spin for 2 min at 4K
- Discard supernatant
- Wash with 3 x 15ml cleavage buffer
- Discard supernatant
- Add 10 ml cleavage buffer
- Measure protein concentration using Biorad assay or A_{280nm} (EC=17570 M^{-1} cm^{-1})
- Add TEV to cleave from resin (1:100 TEV:GST-ferritin. E.g. for 10 mg ferritin use 100ug TEV). **Note:** If each 24mer has even one GST un-cleaved it will remain bound so cleavage must proceed as much as possible.
- Leave on roller at room temperature overnight.

Day 2

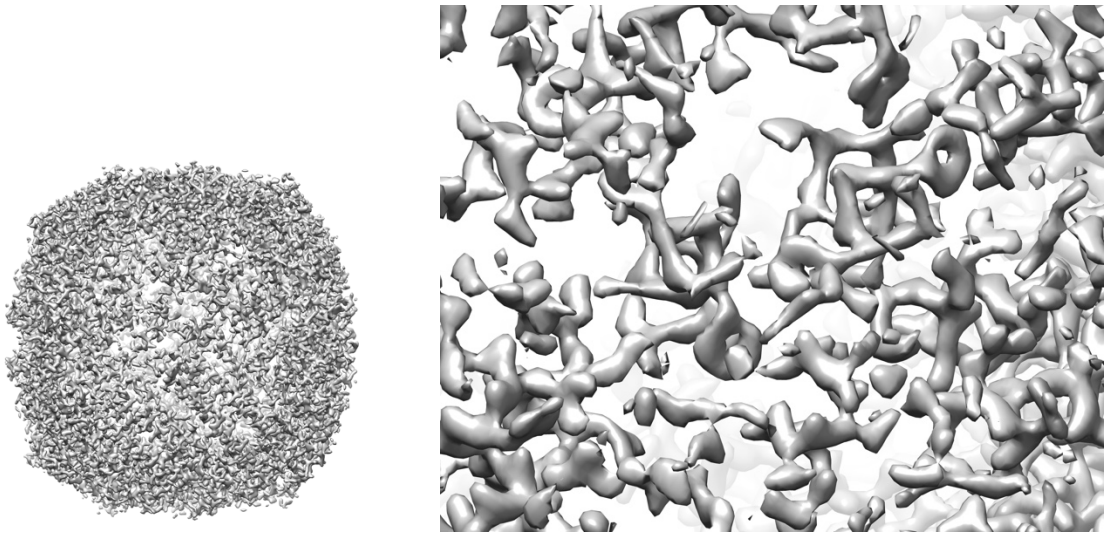
- Concentrate to 0.5ml (e.g. using an Amicon Ultra 15 with a 10,000 MWCO)
- Load onto a 25ml Superdex S200 size exclusion column
- Run fractions on SDS PAGE and pool central peak
- Measure protein concentration and if required concentrate to >2mg/ml. At this concentration there is a good number of particles per hole but some may prefer higher especially if recording images at high magnification
- Alternatively use graphene oxide and dilute ferritin to ~0.2 mg/ml for good coverage



Chromatogram from S200 size exclusion column and SDS PAGE of the central peak



Micrograph of ferritin at 2mg/ml on Quantifoil R1.2/1.3 Au grids. The image is 4Kx4K at a pixel size of 1.34Å. Grids were glow discharged for 30 sec at 30 mA on a GloQube and frozen on a Vitrobot MKIV with 4 sec blot, blot force 10 at 4°C.



Reconstruction at 2.2Å on graphene oxide using the Falcon 3 on the Leicester Titan Krios at a pixel of 0.54Å from ~175,000 particles.