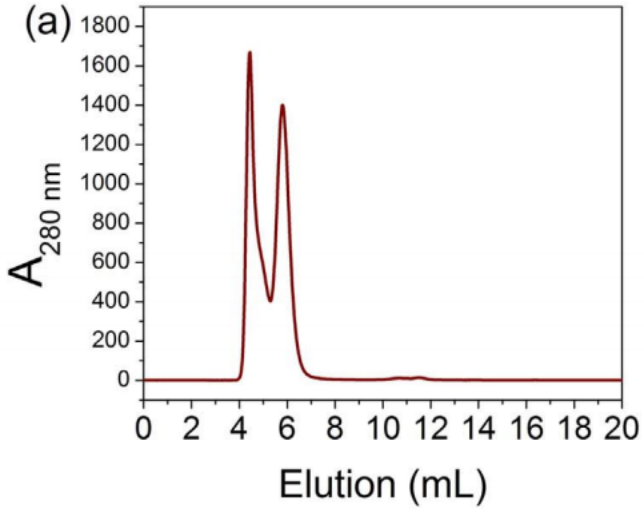
Your Name: Jess Lovescryo
Your PI's Name: Rea
Your collaborator Name(s): Chelsea Doescience
Collaborator PI's Name(s): Parker
Project Name (3-5 words): Apoferritin for benchmarking

Describe specimen, biological relevance, and brief background (maximum 500 words):

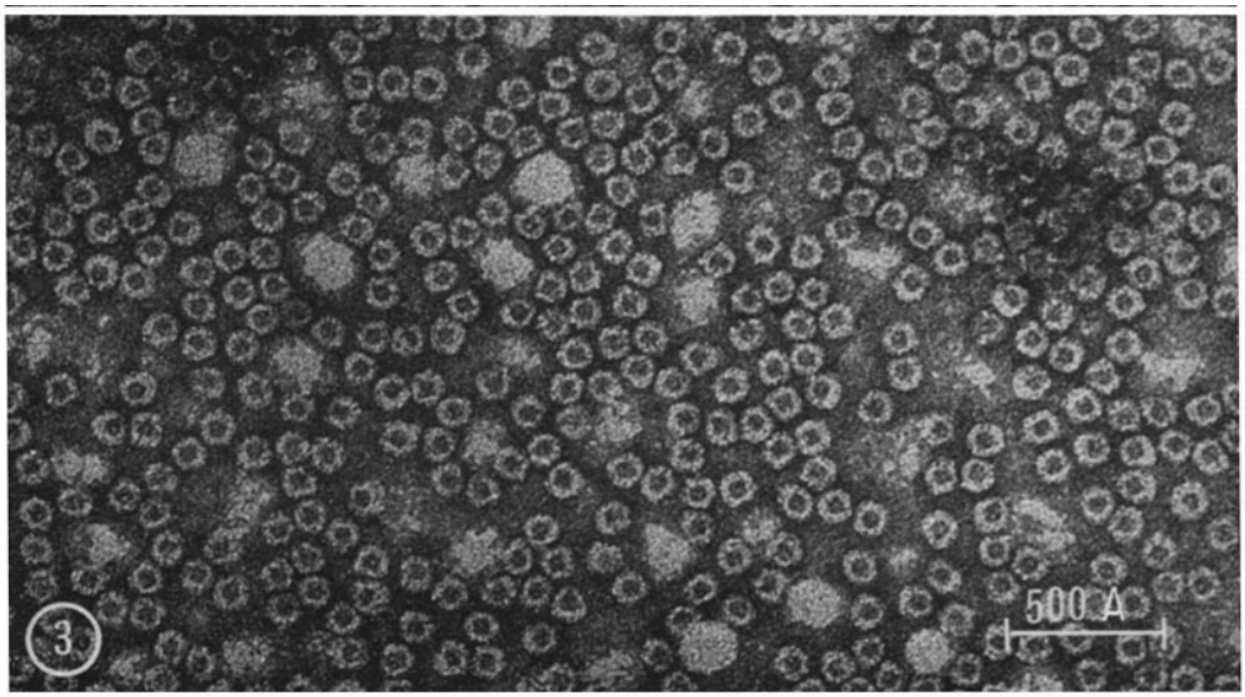
Specimen: Equine spleen apoferritin has a molecular weight of about 444,000 and is composed of 24 subunits (MW 18,500). These are arranged in 432 symmetry to form a nearly spherical hollow shell with outside and inside diameters approximately 130 Å and 75 Å respectively. The large cavity inside the molecule can store up to 4,500 Fe atoms.
Background: Until recent years, apoferritin had remained a difficult structure to determine by cryo-EM, even with the advent of direct electron detectors, because the contrast in individual particle images was insufficient to resolve the characteristic structural motif of a four α-helix bundle. Since then, multiple groups have reached resolutions better than 3 angstroms, thus establishing apoferritin as an appropriate benchmarking protein for TEMs. The goal of this project is to assure that our current instrumentation can achieve comparable resolutions to other TEM setups.
Biological significance: The ferritins are a class of iron-storage proteins in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called apoferritin. Apoferritin catalyzes the oxidation of Fe(II) which it retains inside the molecule as the ferric hydrolysate.

Specimen molecular weight: 440kD
Specimen dimensions: 13 nm sphere

Specimen screening on the Talos Arctica requires biochemistry-related files (evidence of biochemical homogeneity such SDS page, SEC traces etc.) and negative stain images or 2D classes:



Size exclusion chromatogram of commercial equine spleen ferritin at pH 4.5 using a SupelCo column on an Akta Purifier 900. Absorbance measured at 280 nm.



Apoferritin negatively stained with uranyl acetate. Image collected on a Phillips EM 200 operating at 80 kV. This is the sample I am using to plunge and prepare cryo grids for screening.

Phase plate (yes or no): no
Requested number of sessions (up to 4) on the Talos Arctica: 4