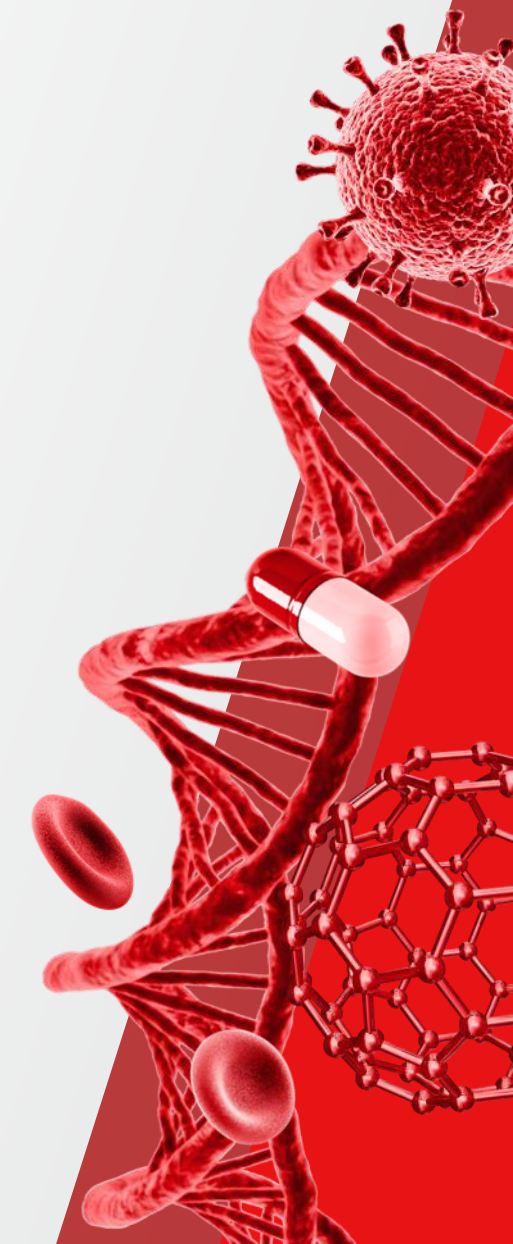


SLAC – Krios G2 – Workflow Validation Report

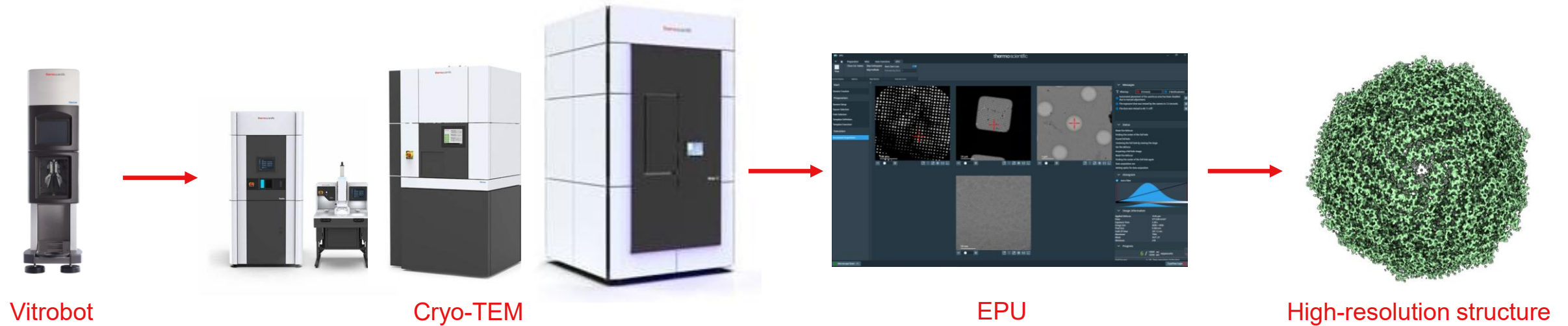
Francis E. Reyes, Ph.D

Jul 13 2026

 The world leader in serving science



Scope of SPA workflow validation



Aim: Execute the Single Particle Analysis (SPA) workflow validation (WV) to ensure functionality across all components of the pipeline.

Description: Vitrified protein (Apo ferritin) is prepared using a Vitrobot Mark IV. Movies are collected using EPU software. Movies are processed in CryoSPARC to generate a high-resolution density map. The density map is used to fit a deposited PDB model in UCSF ChimeraX. The workflow is accomplished using Thermo Fisher hardware and software, unless otherwise noted.

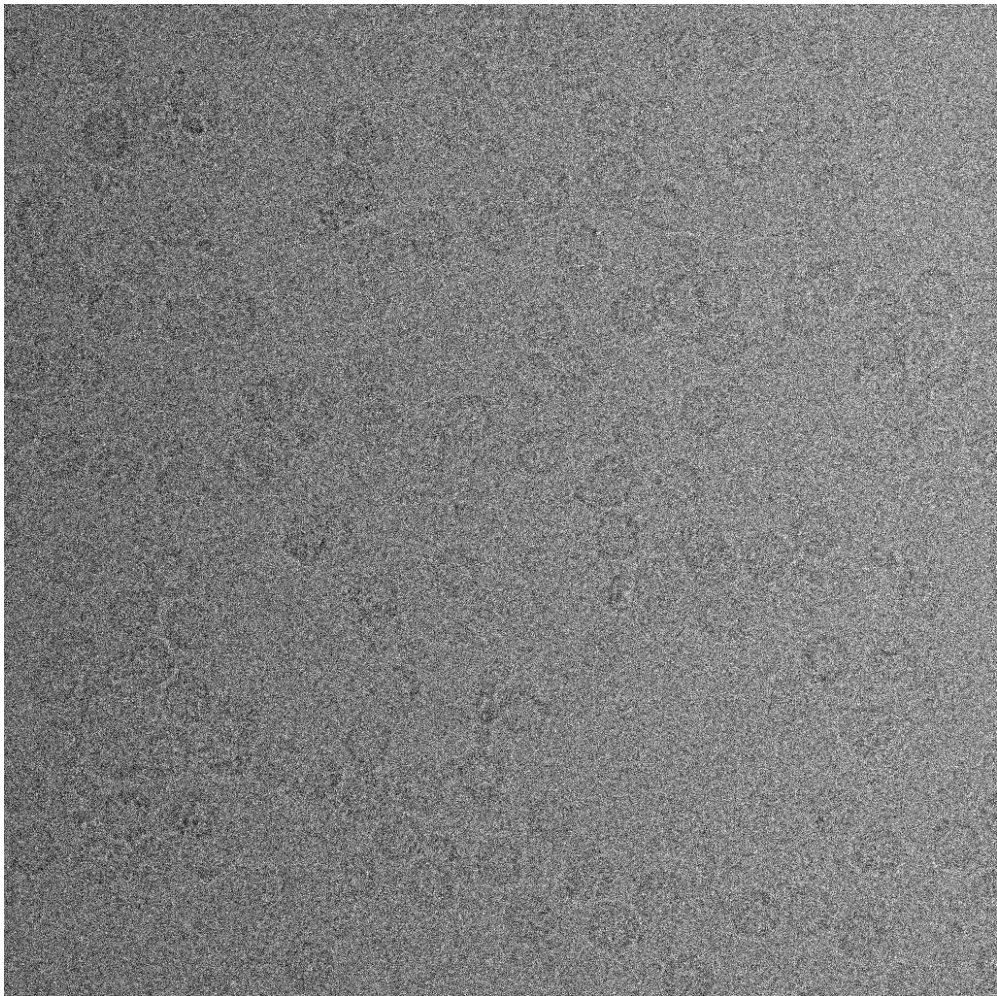
System configuration and workflow details



System serial number	9924174
System type	Krios G2
Detector	Selectris Falcon 4i
Spherical aberration	2.7 mm
Phase plate	No
Sample	Apoferritin
Data acquisition software	EPU 3.10.0.8733
SPA processing software	CryoSPARC 4.7.1
Sample preparation	Jonathan Herrmann and Helen Donelick (June, 2026)
Data transfer and CryoSPARC setup	Francis Reyes (Jul 9 2026)
Data acquisition	Francis Reyes (Jul 10 2026)
Data processing	Francis Reyes (Jul 13 2026)

Experimental conditions

Representative micrograph (215 kx mag),



Dose rate (camera)	8.9 e ⁻ pix ⁻¹ s ⁻¹
Total dose (specimen)	30 e ⁻ Å ⁻²
Exposure time	1.2 s
Nominal pixel size (190kx)	0.60 Å pix ⁻¹
Refined pixel size (190kx)	0.57 Å pix ⁻¹
Sample	Apoferritin (Vitroease)
Number of frames	41
Compressed tiff fractions	21 (~1.4 e ⁻ Å ⁻² frame ⁻¹)
Images per hole	5
Beam-image shift radius	12 µm
Delay after image shift	5 s
Defocus range	[-0.5, -1.2] µm
C2 aperture	50 µm
Objective aperture	No
Grid type	QF R2/1 200 mesh (C on Cu)

Experimental conditions



Data acquisition settings in EPU

Presets

Data Acquisition

Import

Export

Camera

Falcon 4i

Fractions

Manual

Compression

Yes

Align

No

Dose (e/Å²)

30.00

Exp. Time (s)

1.20

Fractions (Nr)

21

Frames (Nr)

41

Measure

8.91

e/px/s

24.69

e/Å²/s

Get

Set

NanoProbe

215000x

(μm)

-3.00

Spot Size

5

Ill. Area (μm)

0.55

C2 Aperture

50

Insert Slit

Link Settings

Preview

Preset Selection

Camera Settings

Exposure Settings

Dose Rate

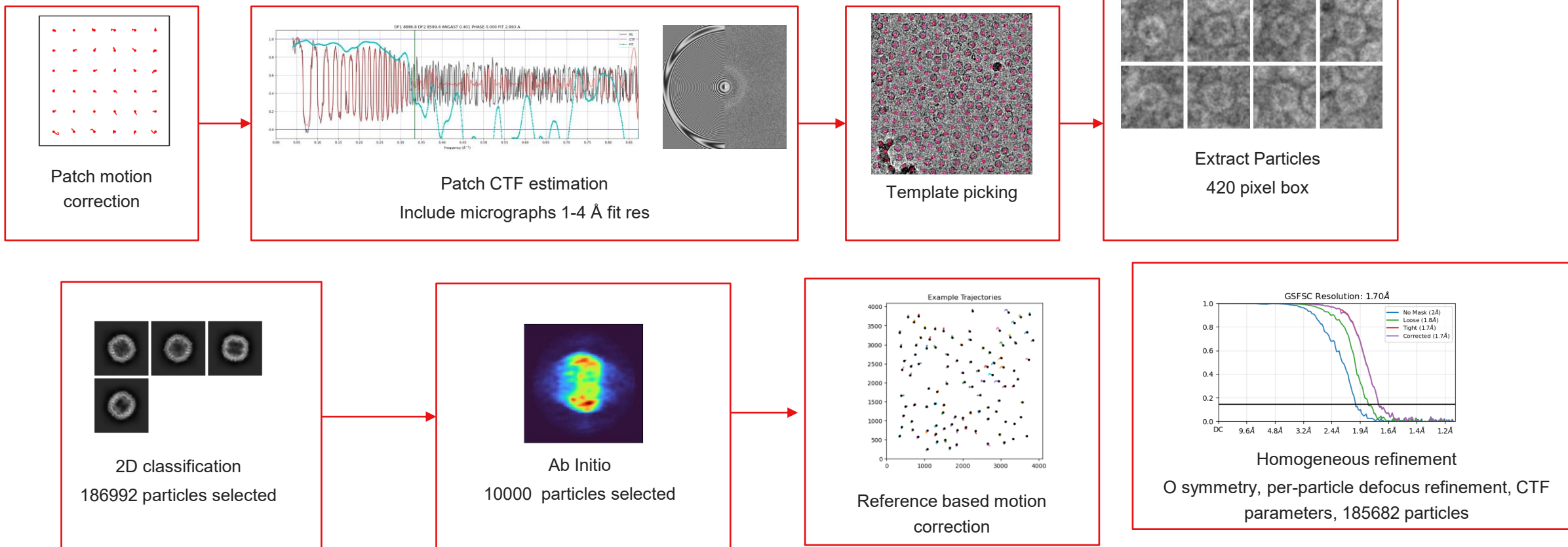
Optics Settings

Acquisition

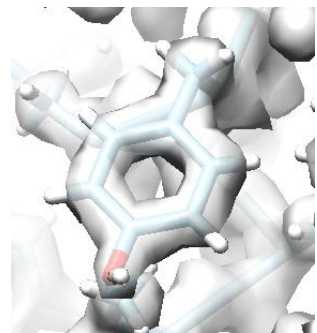
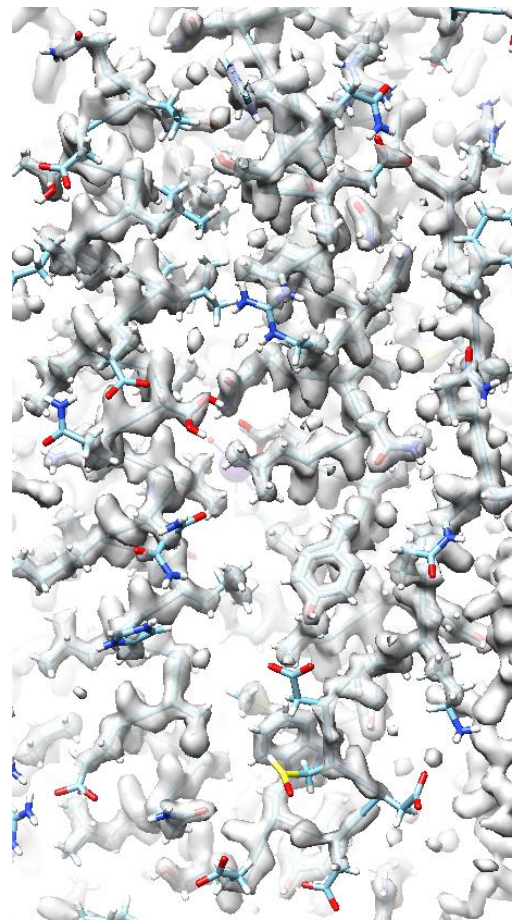
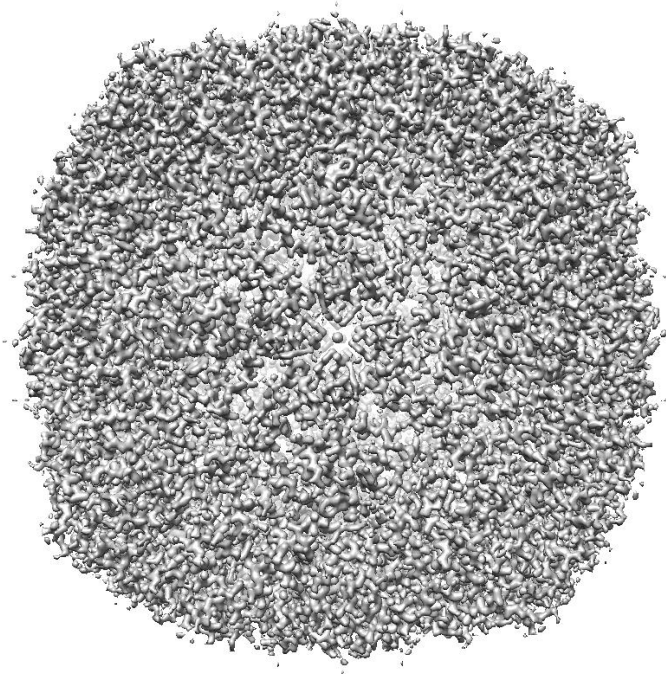
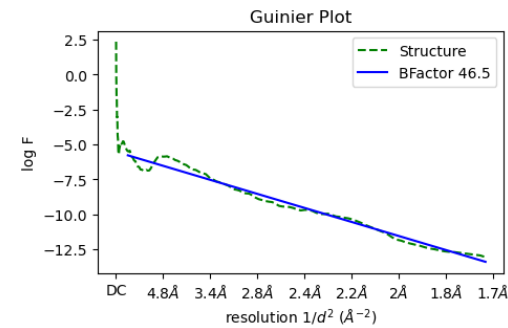
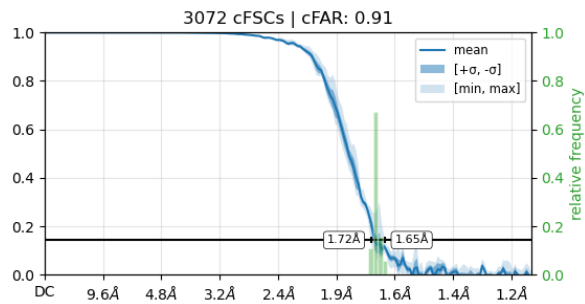
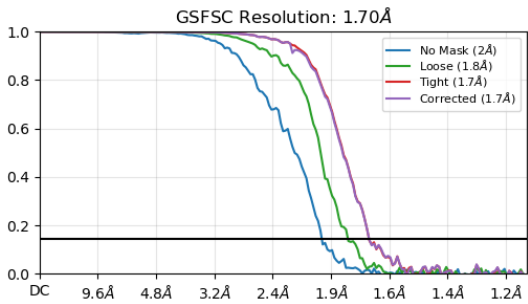
Representative data processing – 215 kx

No. of movies acquired	1825
No. of movies accepted	1692
Acceptance criterion	CTF 1-4 Å
Motion correction algorithm	Patch motion correction (CryoSPARC Live)
CTF estimation algorithm	Patch CTF (CryoSPARC Live)
Particle picking	Template (CryoSPARC Live)
Particle box size	420 pix
No. of particles picked	428,680
No. of particles post-2D	186,992
Symmetry imposed	0

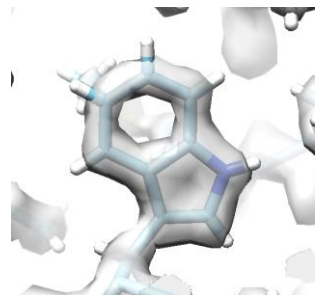
Representative data processing – 215 kx AFIS



Representative result – 215 kx AFIS



Y29



W93

Fit with 6Z6U

Table of datasets and processing results



Magnification (EFTEM)	Collection method	Nominal pixel size	Refined pixel size	Accepted micrographs	Particles in final reconstruction	Final resolution	B-factor	Residual beam tilt
215kx	Fast (AFIS)	0.60 Å pix ⁻¹	0.572 Å pix ⁻¹	340	185682	1.70 Å	46.5 Å ²	[0.06, -0.05] mrad

Validation

- ✓ Standard protein grid has collectable squares: **PASS**
- ✓ Acquisition of movies via EPU: **PASS**
- ✓ Successful 3D reconstruction to specification: **PASS**
- ✓ Calibrate pixel size for collected magnifications: **PASS**

Box link with final reconstructions:

<https://thermofisher.box.com/s/3e9i78wnavunm1jkf1j2jk45xiz637mn>