thermoscientific

Cryo-EM: High speed epitope mapping

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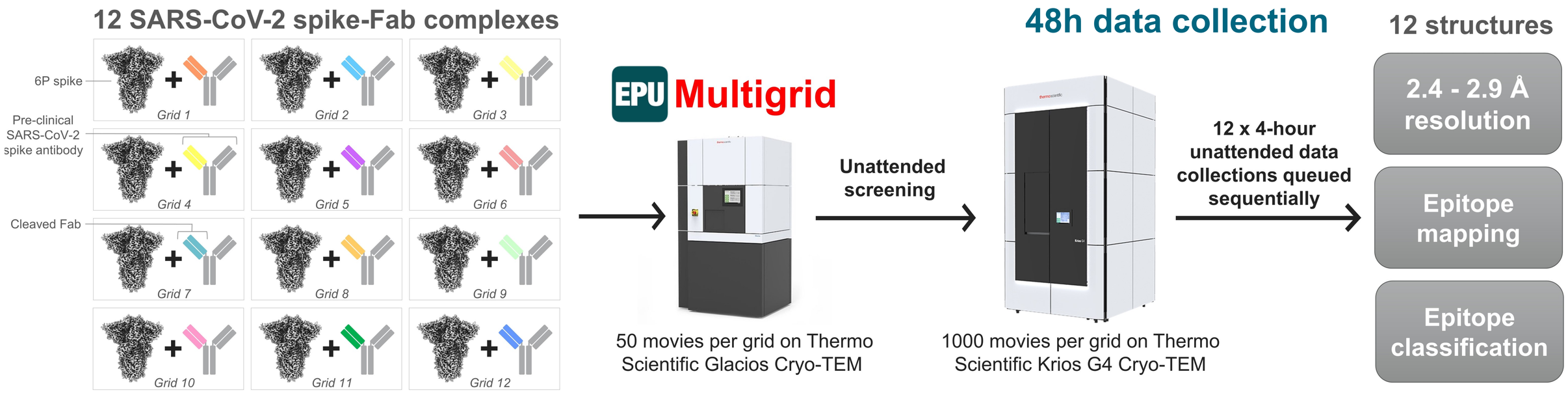
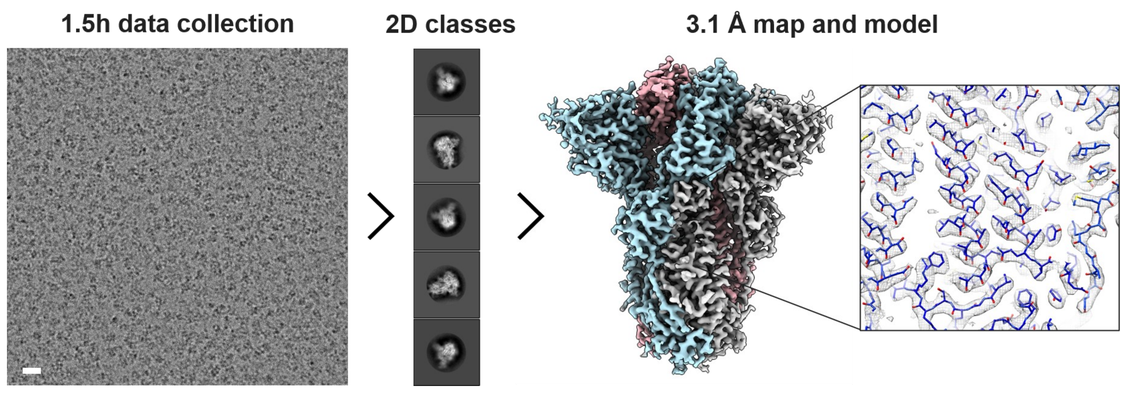
12 structures in 48h



Cryo-electron microscopy (cryo-EM) is a powerful tool for epitope mapping of antibodies that block SARS-CoV-2 virus entry. Defining the epitopes of neutralizing antibodies allows us to understand how antibodies can confer protective immunity against SARS-CoV-2. Here we show how 12 sub-3-angstrom reconstructions of spike protein in complex with 12 distinct Fabs can be obtained from a 2-day unattended microscopy session using EPU Multigrid.

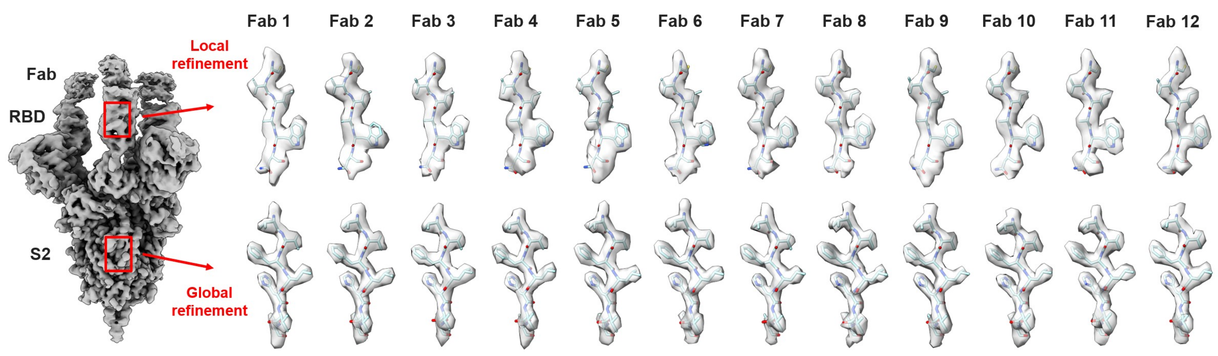
METHODS



Grid	Quantifoil R1.2/1.3
Camera	Selectris X Energy Filter and Falcon 4 Detector
Slit width (eV)	10
Nominal magnification	130,000x
Pixel size (Å)	0.929
Dose rate (e-/pix/sec)	5.3
Exposure time (sec)	8.32
Total dose (e-/Å^2)	48
Fractionation	EER
Autofocus	After centering
Hole centering	AFIS (6 µm image shift)
Number of images	12 x 1000
Throughput	~250/hour

Table 1. Cryo-EM data acquisition parameters.

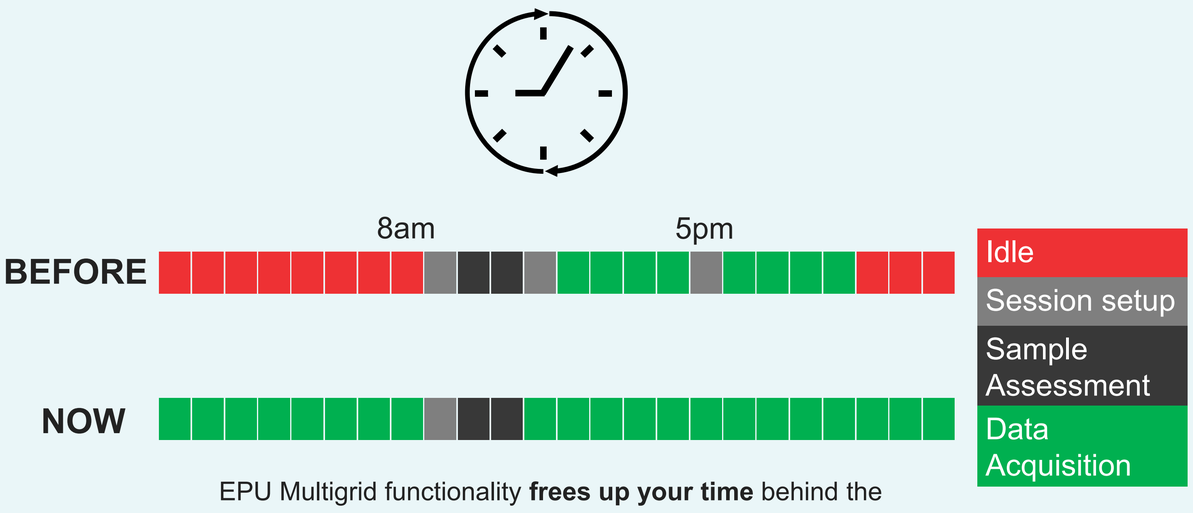
RESULTS



Spike-Fab complex	Global resolution (Å)	Local resolution (Å)
Fab 1	2.8	3.7
Fab 2	2.7	4.0
Fab 3	2.9	3.7
Fab 4	2.9	3.7
Fab 5	2.8	4.2
Fab 6	2.5	3.7
Fab 7	2.9	3.4
Fab 8	2.4	3.1
Fab 9	2.8	3.9
Fab 10	2.6	3.4
Fab 11	2.5	3.4.
Fab 12	2.9	3.4

Table 2. Global and local resolutions achieved for each spike-FAB complex

Round-the-clock productivity and sample throughput



EPU Multigrid functionality **frees up your time** behind the microscope, while **maximizing productivity**

1. Acquire an Atlas on every grid to inspect samples
2. For each grid that you want to acquire:
   - Load and select Grid squares
   - Set foil hole settings such as ice filter and define an acquisition template. EPU will select the holes and prepare the grid squares automatically
   - Reload preferences or re-use previous session settings to set-up each grid in less than 15 minutes
3. Start a queued session

Advantage of EPU Multigrid

- Reduce dedicated operator time from 8 to 3 hours for Glacios and Krios
- Fit sample assessment and high-resolution data acquisition in one session
- Tools can be used 24/7 due to unattended operation

Methods

Spike-Fab-complex grids were vitrified in duplicate and screened using Glacios cryo-TEM. Twelve best grids, one grid per complex, were taken forward for a 48-hour unattended EPU Multigrid session using Krios G4 cryo-TEM.

Results

We have obtained 12 sub-3Å structures of the SARS-CoV-2 spike protein in complex with RBD-binding Fabs. Our reconstructions are well resolved at the epitope-paratope interface despite the relatively small size of each dataset. Sample optimization and screening, as well as the use of both the Selectris X Energy Filter and the E-CFEG resulted in high quality datasets.

Conclusion

Significant advances have been made in the speed, quality, and automation of cryo-EM data collection. The cryo-EM's resolution revolution will be followed by a throughput revolution, which increases efficiency and reduces cost per dataset, and enable fragment-based drug discovery and epitope mapping of large antibody panels.

In summary, cryo-EM is now poised to emulate the success of macromolecular crystallography thanks to faster data collection and EPU Multigrid.

Acknowledgements

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Antibody discovery was performed at Takeda Pharmaceuticals

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