









# How is the TEM image formed?





















ASCEM	https://cryoem.ibc.sinica.edu.tw/							
	Open to everyone							
	CEM1	CEM2	Vitrobot	CEM3	CEM4			
	JEM-1400	Tecnai F20	Type IV	Talos Arctica	Titan Krios G3			
	120 kV	200 kV		200 kV	300 kV			
Negative staining			#2 #3	Autoloader VPP	Autoloader VPP			
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Gatan CCD	Gatan CCD DE-20		Falcon 3EC	Gatan BioQuantum (K3) Falcon 3EC			
thin super cooled vitreous ice layer	Yu-,	Hua Huiju	Kondo	Irene	Hsiung			
Cryo-EM	Screening			Data Collection				

# Successful sample preparation



## The 1.51 Å cryo-EM structure of apoferritin at ASCEM











Ice embedded virus particles (cryoEM micrographs)









#### Key improvements: Beam-induced motion can be corrected





Ice embedded virus particles (cryoEM micrographs)



## Orientation determination



# 3D reconstruction need many views









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### Molecular weight vs difficulty for cryo-EM structural targets

# **CryoEM SPA – Success Factors**

#### Key concepts:

- Protein size (dimension/molecular weight) is large enough to be "seen" in micrographs.
- High purity and good homogeneity of sample.
- Symmetric proteins are easier cases.
- A specimen with thin ice layer made carefully
- >1-million particles are suggested to collect for data process.
- Well clean-up for junk particles for 3D reconstructions
- Diverse orientations are needed
- Good computation resources accessible.

# The smallest resolvable protein?

#### 2800 movies, 560萬顆

# ~48kDa















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上機費用	上班時間	<sup>、</sup> 班時間 週六、週日)						
CEM1 (JEOL JEM-1400)	400 / 時,學術界費率         200 / 時           2,000 / 時,業界費率         1,000 /	· 學術界費率 時,業界費率						
CEM2 (FEI Tecnai G2 F20 TWIN)	500 / 時, 學術界費率 300 / 開 4,000 / 時, 業界費率 2,400 /	, 學術界費率 時, 業界費率						
	13.000 / 天,學術界費率		Holey Grid	390				
CEM3 (FEI Talos Arctica 200 keV)	100 000 / 天,業界要素	UltrAuFoil Grid	950					
	10,000/天/天介貞平		C-clip plus ring	950				
CEM4 (FEI Titan Krios 300 keV)	18,0007人,学們芥賀率	Data Collection (4 hours)	5200					
	140,000 / 大,業界費率	Data Collection (Full Day)	13000					
為推廣冷凍電顯學術研究及應用,按學儀處建議並於2	Basic operation by Staff (41	2000						
01日起學術界費率不冉區分院、內外兩種經費費率,	Grid +2 nm C (Quantifoil, H	660						

https://reservation.iis.sinica.edu.tw/servlet/SignInHandler								
案件申請 - CEM 3, Talos Arctica								
		*申請蘋別:	ASCEM-3 Academic user V					
		申請人:	張淵智 (kondo@gate.sinica.edu.tw)	電話	27872553			
		所屬老師單位類別:	政府機構 ・	所屬老師公司/單位:	: 中央研究院 🗸			
		所屬老師部門:	儀器服務中心	*所屬老師或主管	: 張淵智 🗸			
		*經費來源:	請選擇 く	*計畫名稱				
		*預約時段;	選擇時段					
您好・張淵智	現在時間是:2024/05/	*Overnight data collection is available only for afternoon applicants, if needed. :	O Yes, I understand					
▶ 案件 ( <u>新申請</u> , 待息	<u>載理,未結業,已結業,綜</u>	*A New User is recommended to take Talos EPU Training Course first. :	Cotit, I've also booked a training course this time. I've been trained and can operate Taios EPU independently. Unore of the above applies, I need to contact CEMS staff for possible assistance.					
ASCEM: Cryo-E	M Facility (108起) - 限 設	*All frozen grids (up to 6 grids per session) and the grid storage information form must be provided to ASCEM at least 2 business days prior to the scheduled time.	○ Yes, I understand.					
申請	ASCEM Training Cour	*Sample Name (Abbreviation) :						
申請	CEM 1, JEM1400 Cry	*Has the sample been checked with an electron microscope? :	○ Yes ○ No					
		*Molecular Weight (kDa) :						
中調	CEMIZ, FEI TEUTIALFZ							
申請	CEM 3, Talos Arctica	"Buffer Composition (salt, detergent, glycerol etc.) :						
申請	CEM 4, Titan Krios	*Number of Grids :						
		*Service Mode :	請選擇 ~					
申請	CryoEM software supp	Contact Staff :	請選擇 🖌					
申請	FEI Vitrobot-2	*Do "on-the fly motion correction"? :	O Yes (Default parameter) Yes (User defined parameter)					
申請	FEI Vitrobot-3	"Need to transfer motion corrected data to DiCOS? :	Vo Vo Ves, DiCOS group name: No					
申罪	FEI Vitrobot-3	Other Notes :	- 10					
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