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# **Assembly of archaeal, salty and fatty viruses: when a bit of grease helps**

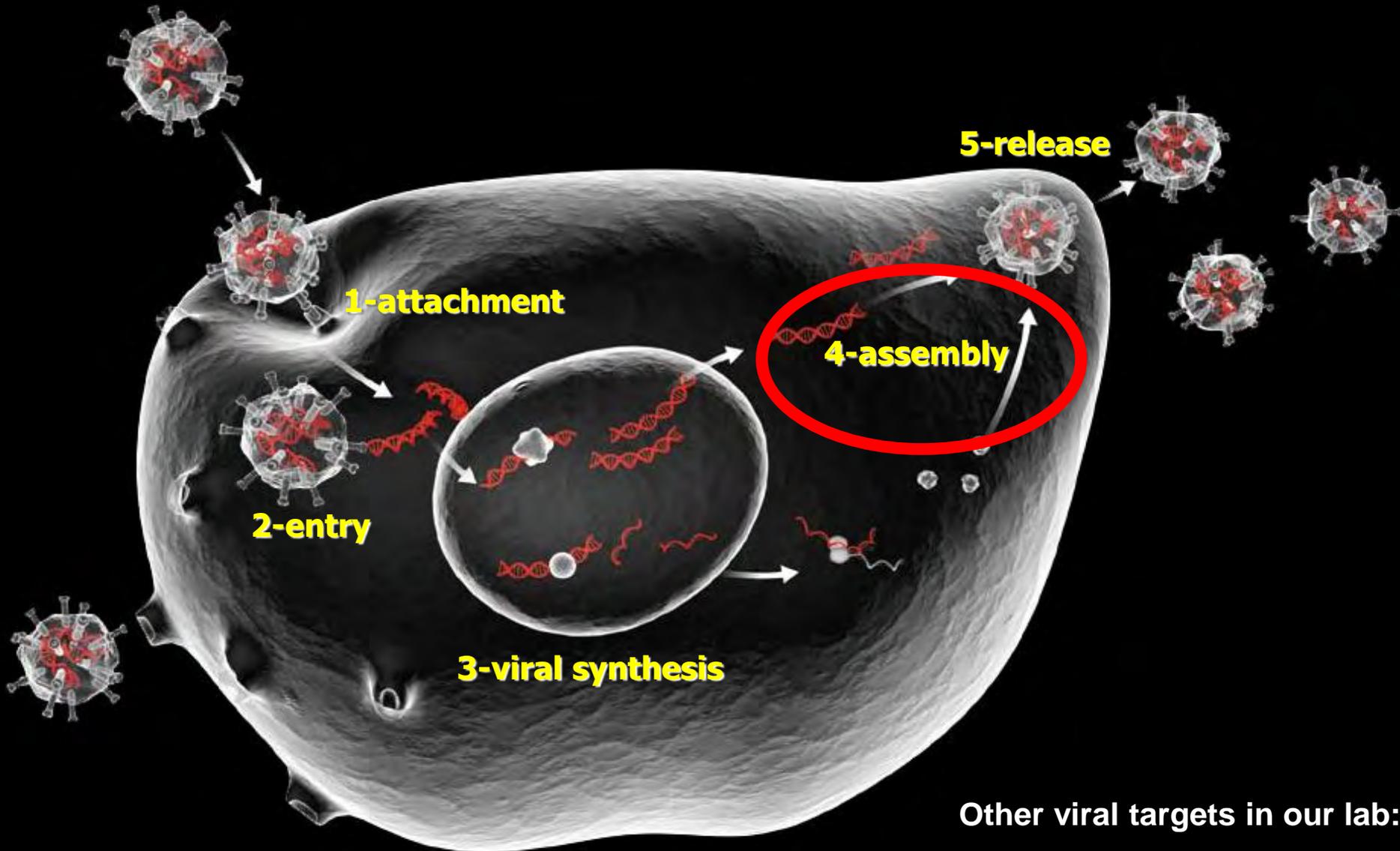
***RES Users' Meeting  
20 September 2016 - León***

# Talk Overview

- **brief introduction**
- **archaeal virus HHIV-2**  
*(what we know so far....)*
- **'overboost' of info from HR cryo-EM**
- **unfolding 'story' on viruses with vertical single  $\beta$ -barrel MCPs**

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# Virus Lifecycle



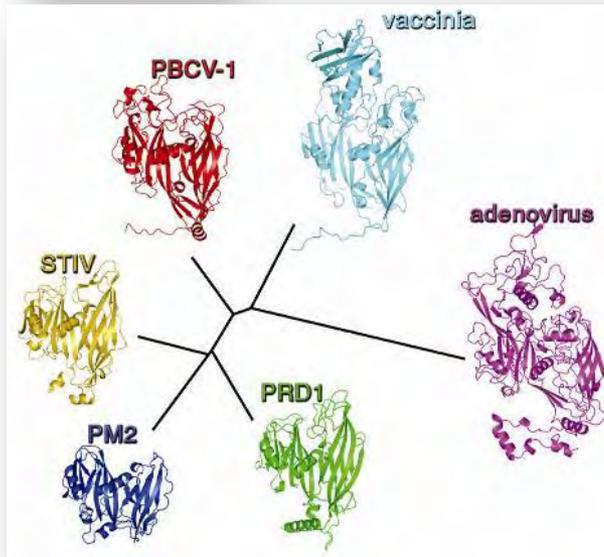
Other viral targets in our lab:

- *Flavivirus*
- *Bunyavirus*

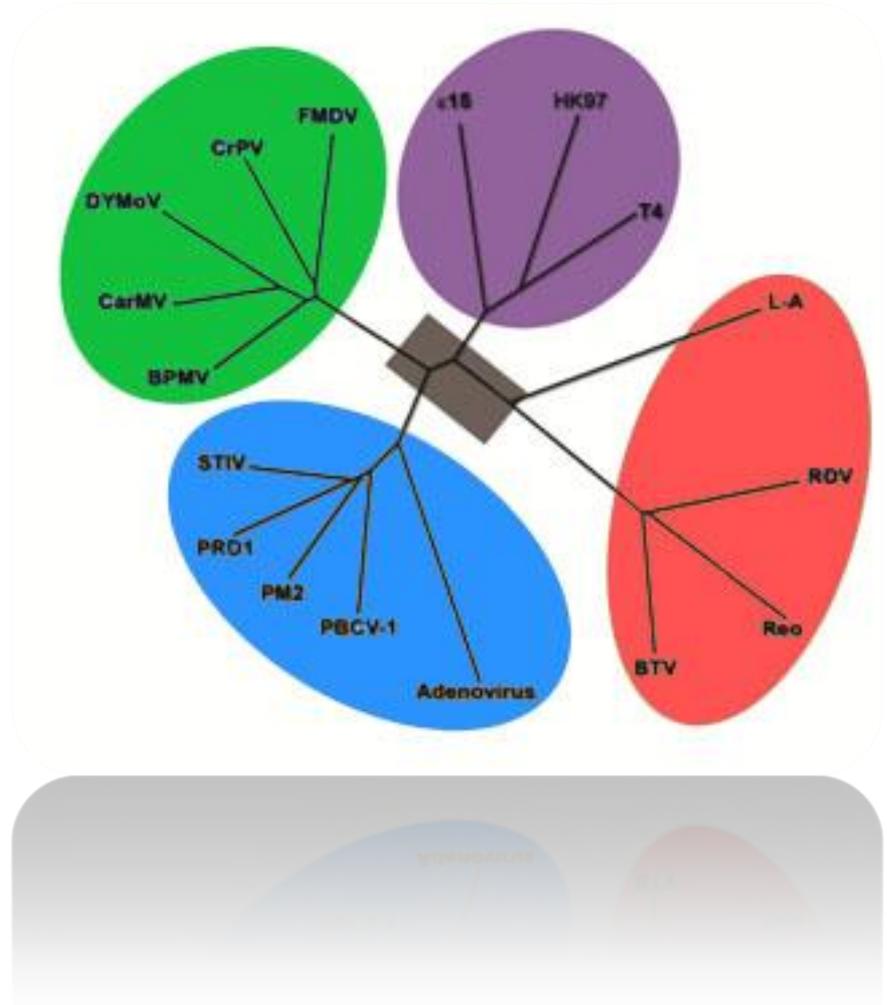
# Structure Unifies the Viral Universe



double  $\beta$ -barrel



Comparison of coat protein structures and virion architecture provides a basis for grouping viruses previously considered unrelated.



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- HHIV-2 isolated from the largest salt-pan in Europe (45000 km<sup>2</sup>) located in Margherita di Savoia (FG-Italy);
- Most of haloarchaeal virus (<50) look like tailed-phages;
- The viral genome is a linear double-stranded DNA molecule of ~30 kb.



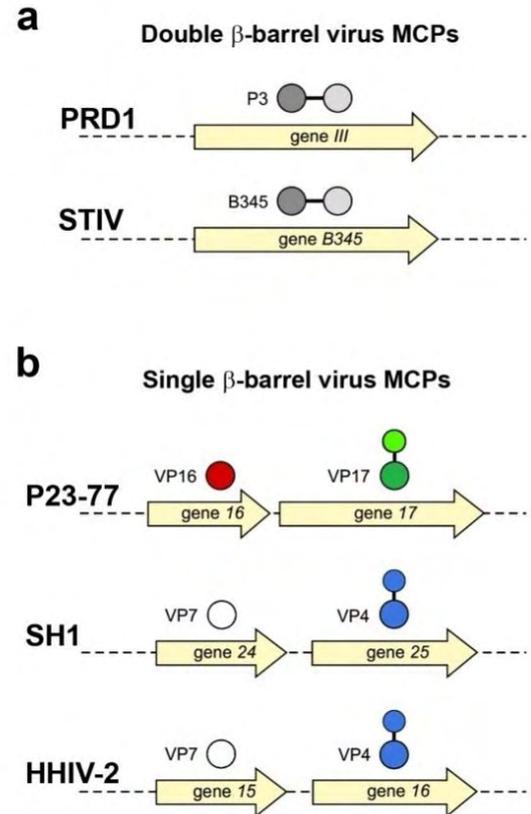
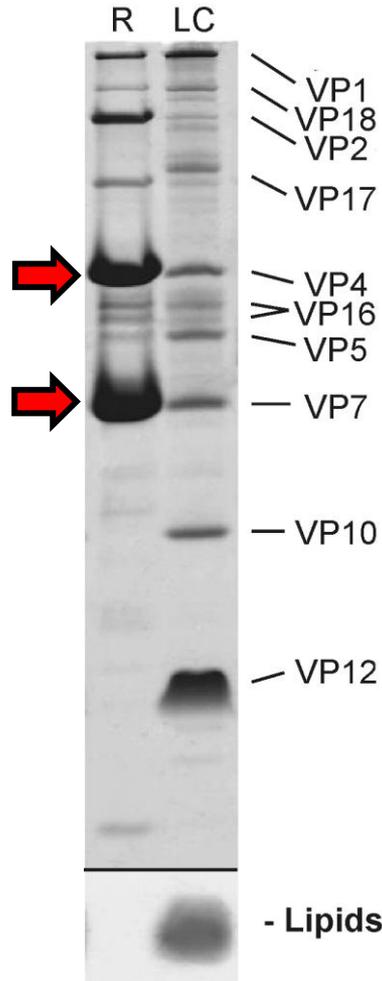
...only few archaeal icosahedral virus structures are known: STIV\*\* SH1, STIV2, HSTV-1

\*\*Veesler et al., PNAS, 2013 (3.9 Å res)

***What solutions have been provided by Evolution for the assembly of archaeal viruses living in such harsh environments?***

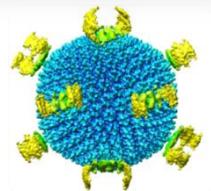
# HHIV-2 virion contains ~10 structural proteins and lipid components acquired from the host membrane

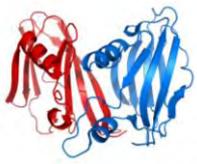
possess 2 Major Capsid Proteins (MCPs)



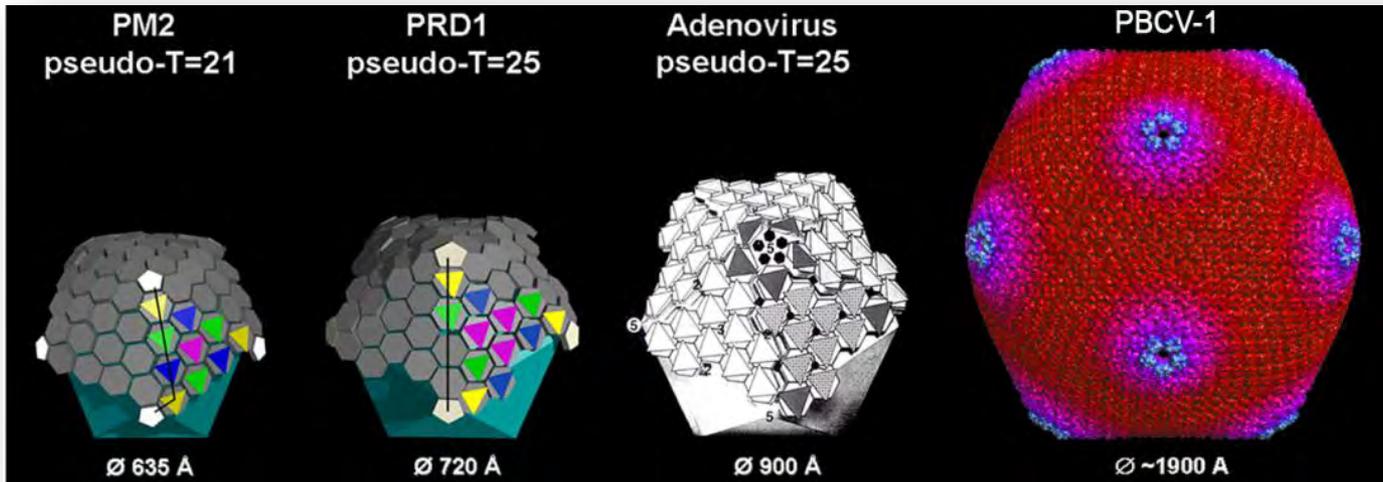
VP4 and VP7 and the major membrane associated proteins share > 70% sequence homology with the corresponding proteins in archaeal SH1 virus

Jääliñoja *et al.* PNAS 2008 (~10 Å resolution):





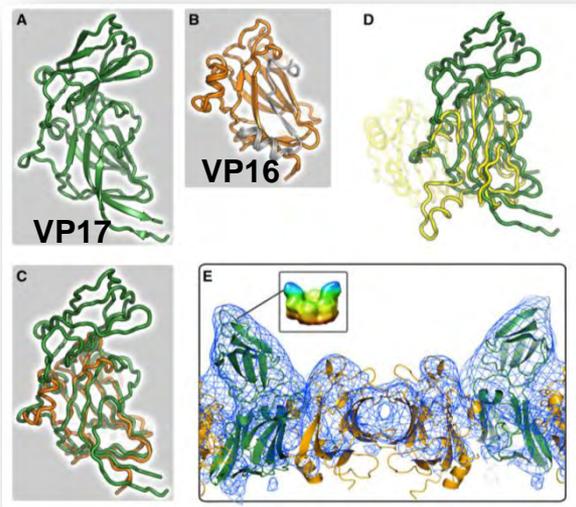
# Double $\beta$ -barrel MCP: PRD1-adenovirus lineage



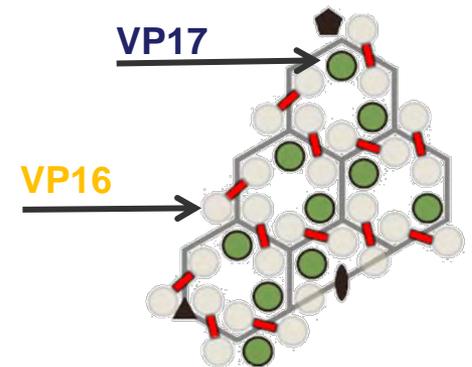
Abrescia\*, Cockburn\* et al., *Nature* 2004

## Recently... two single $\beta$ -barrel MCPs in *Thermus* phage P23-77

Rissanen et al. *Structure* (2013) from Jaana Bamford and Dave Stuart groups

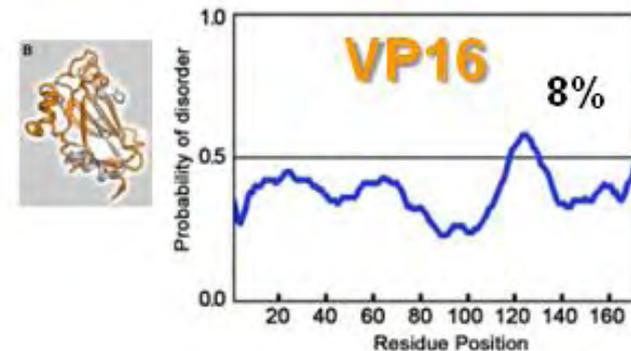
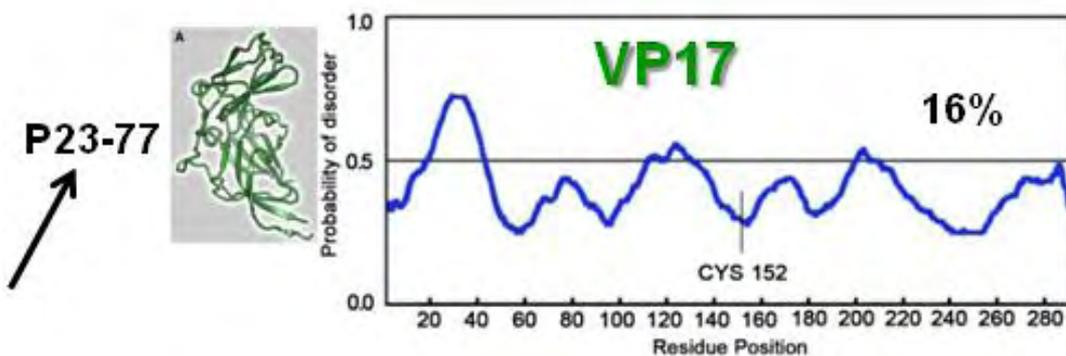


Happonen et al., *J. Virol.* 2010  
 P23-77 map at 14 Å resolution



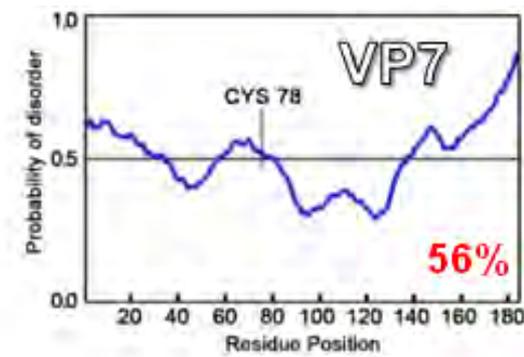
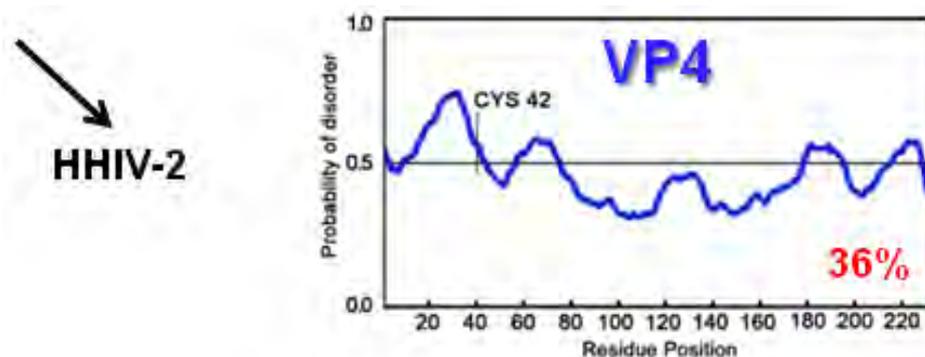
**VP16-VP16 building block for assembly**

# HHIV-2 VP7/VP4 differs from P23-77 MCPs *in silico* and in solution

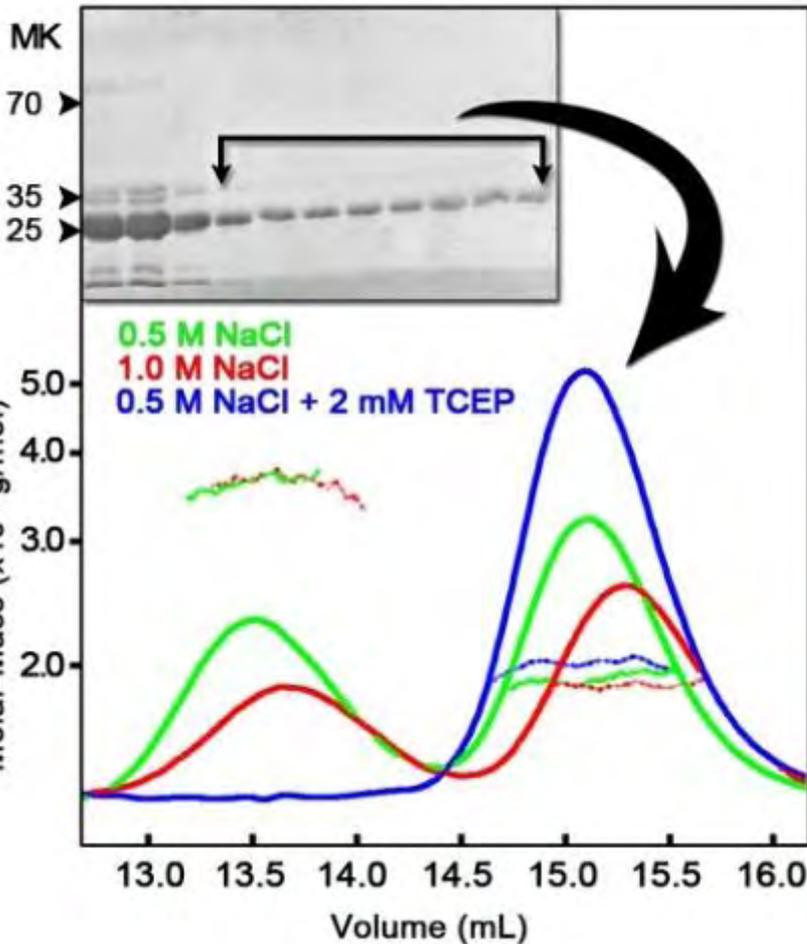
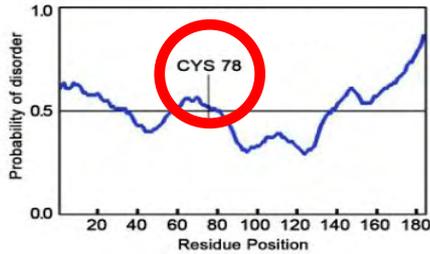


Disorder  
Prediction  
(RONN)

sequence similarity < 20%

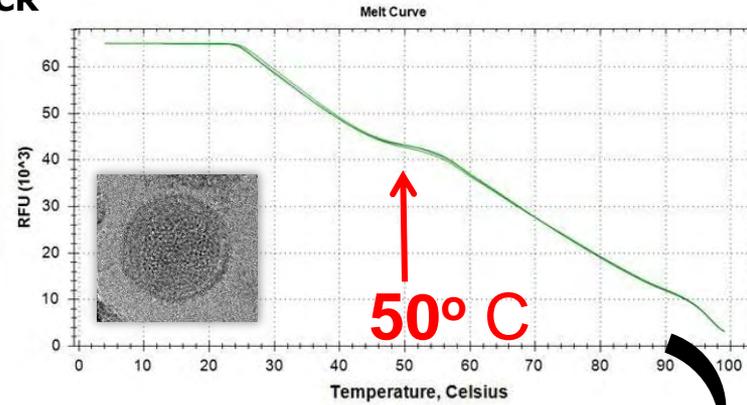


# VP7 monomer-dimer switching (recombinant protein)

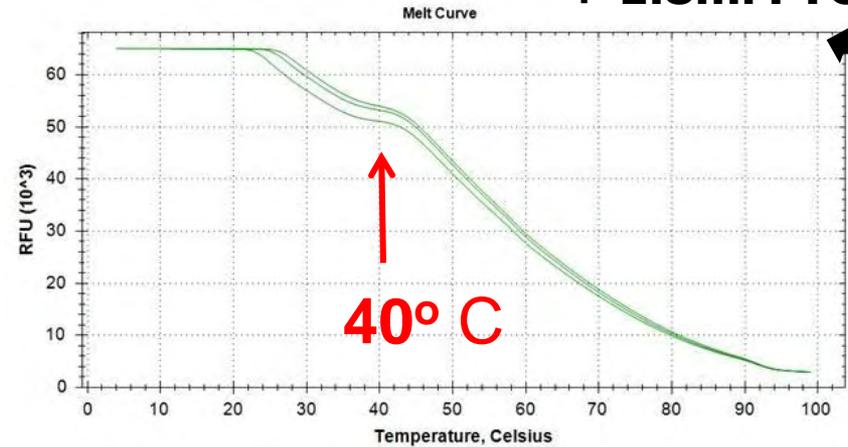


# HHIV-2 thermo-stability

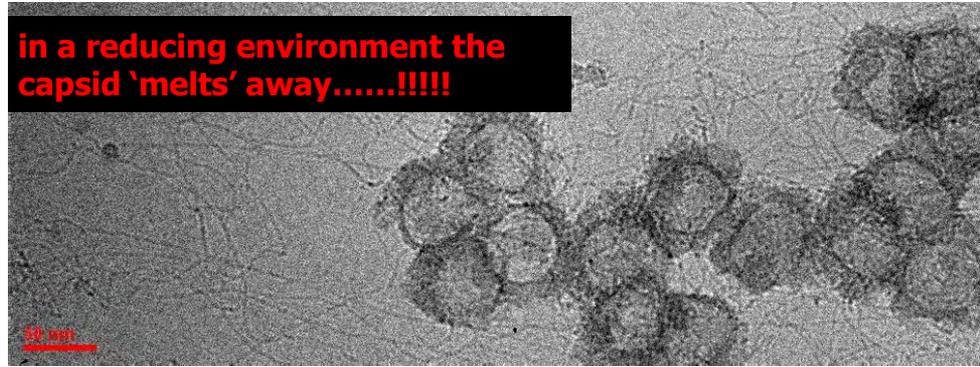
SYBR-Green –  
CFX96 RT-PCR



+ 1.8mM TCEP

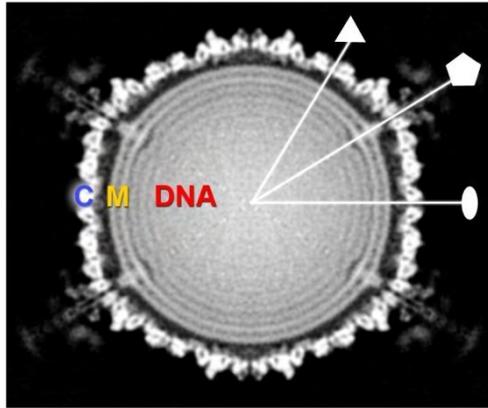


in a reducing environment the  
capsid 'melts' away.....!!!!

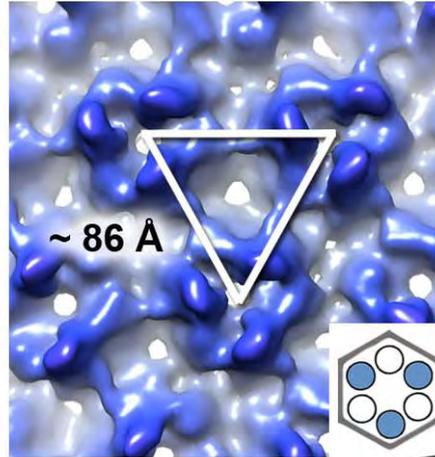


# Virus and overall capsid organization at 11 Å resolution

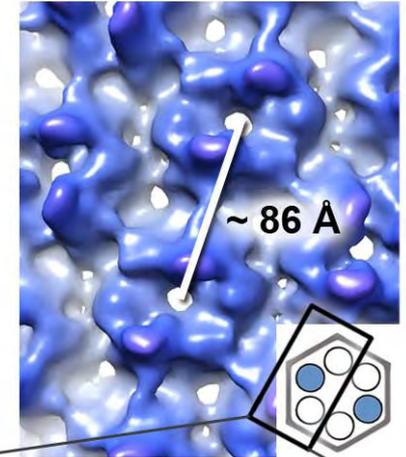
*data collected back in 2011 using in-house 200kV FEG microscope*



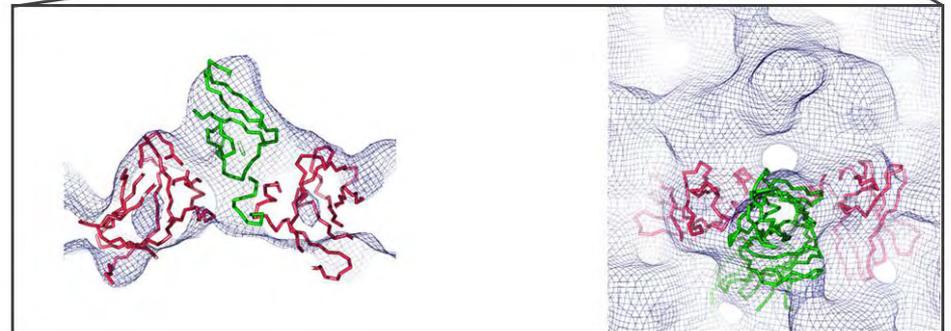
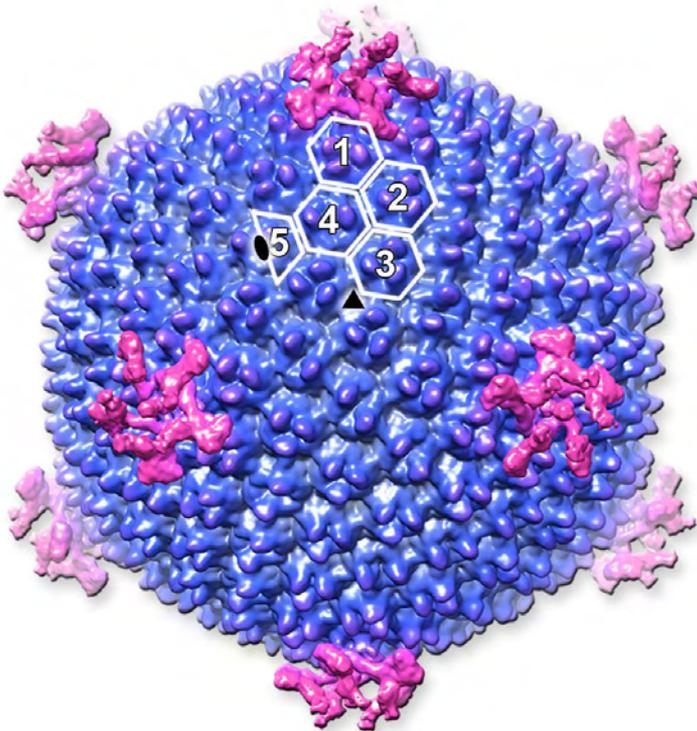
three- tower



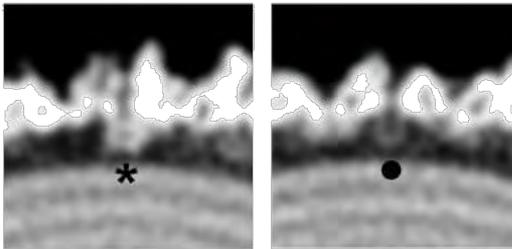
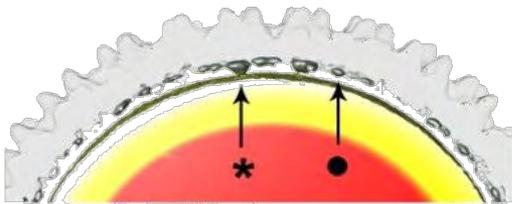
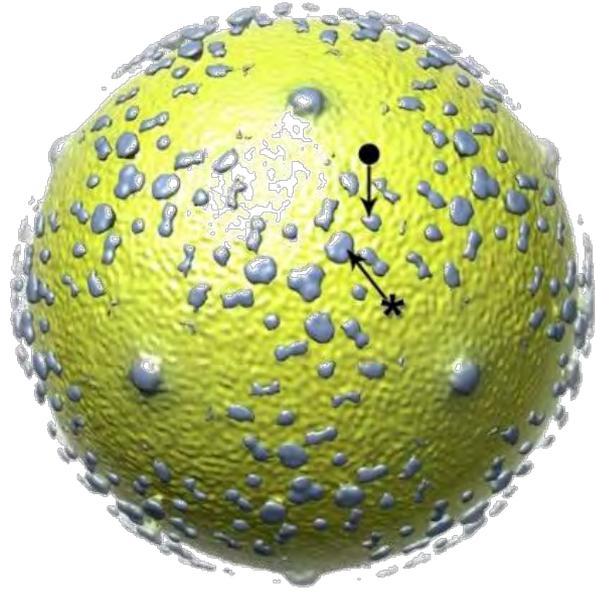
two- tower



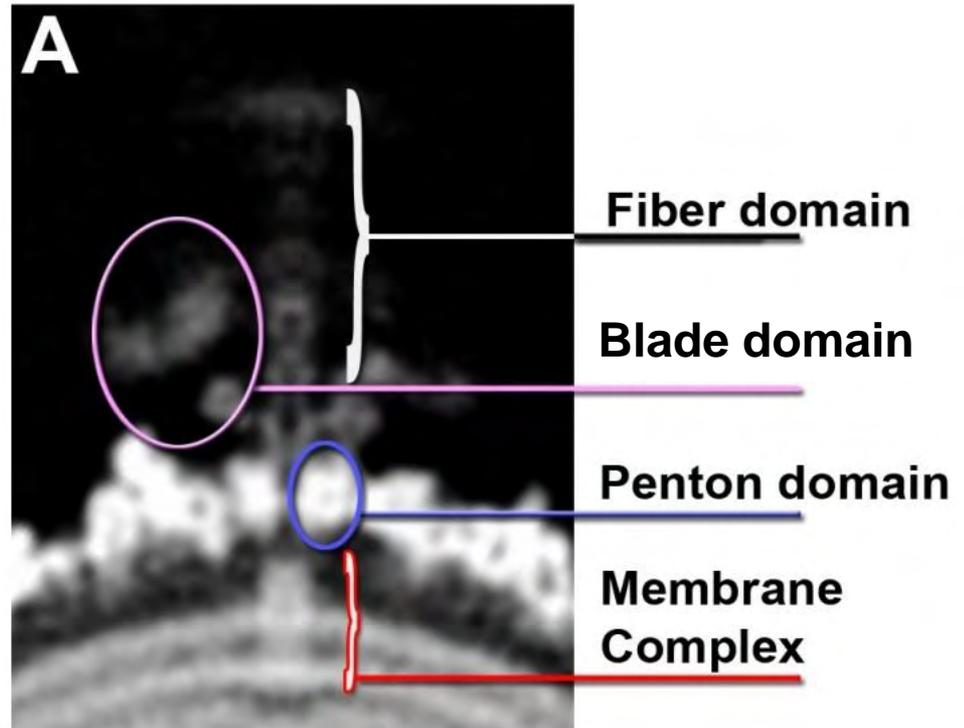
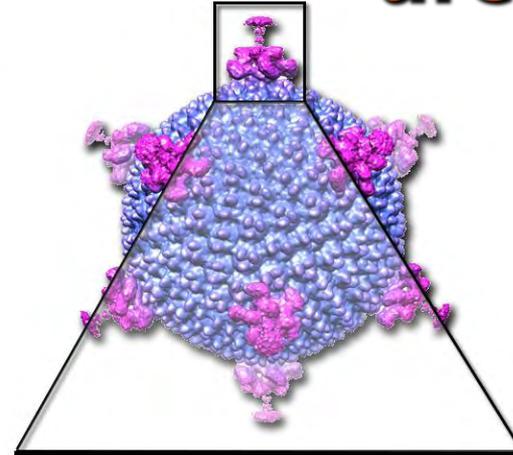
VP4  
VP7



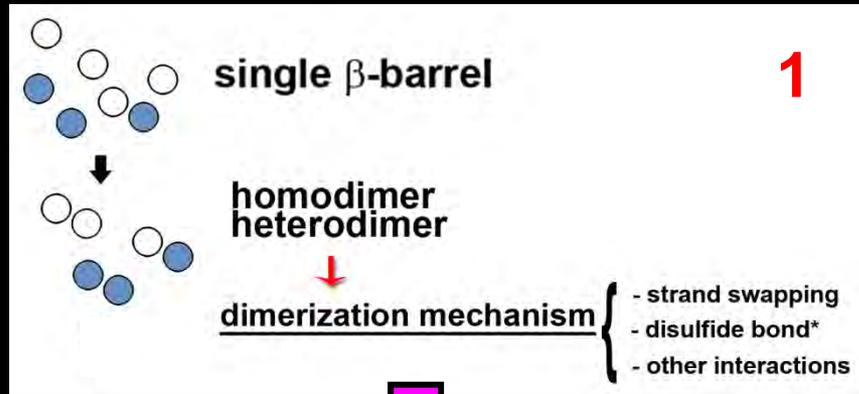
# Capsid cross-talks with vesicle (??)



# Vertex architecture

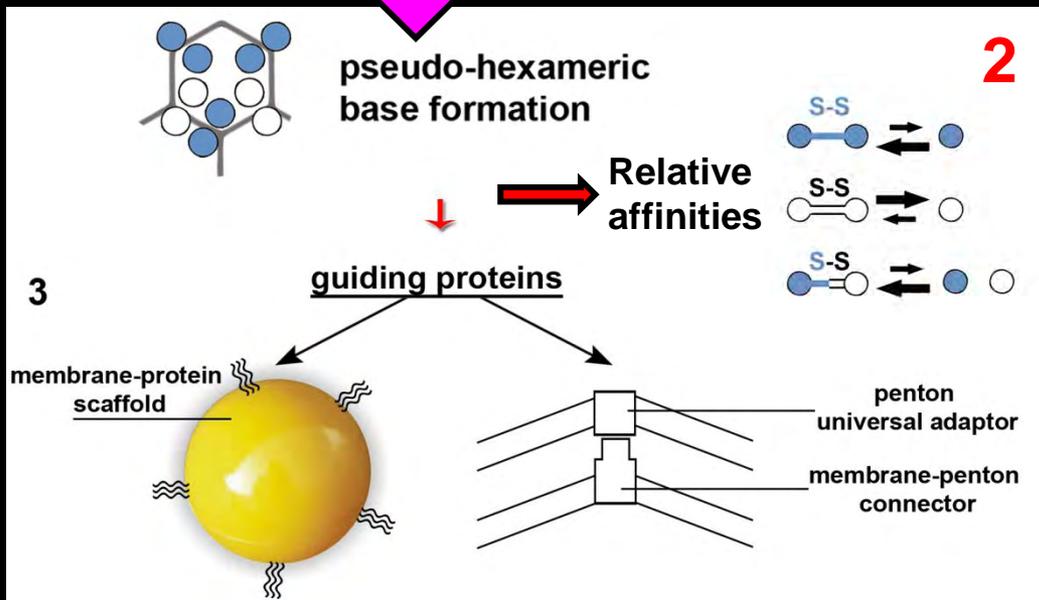


# Modularity of assembly: the *Meccano* model

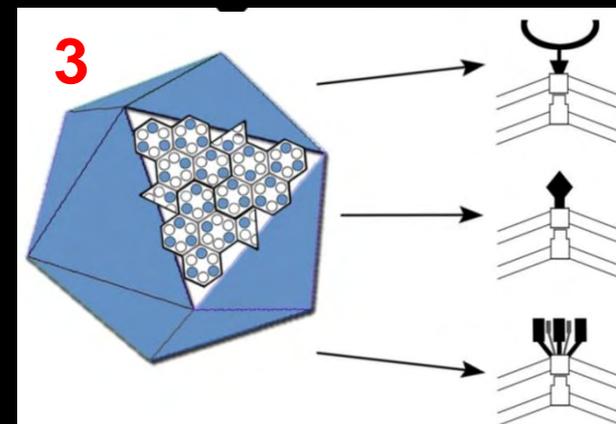


..... the dimerization mechanism might be virion specific and influence the assembly events

**2** There must be a 'GPS' system guiding the interactions of dimeric/monomeric species onto vesicle  $\rightarrow$  relative affinities vs. capsid-membrane interactions. VP7 behaving as IDP?



**3** HHIV-2 illustrates the deconvolution of the virus architecture from the virus-host interacting apparatus



and yet very unsatisfying.....

# IN-HOUSE

Key question:  
What are the interactions that drive the assembly of single  $\beta$ -barrel viruses?  
**Electron Microscope and computing**

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# 2014-2015

## The Electron Microscopy Revolution

Revolution in detection technology & software algorithm

(direct detection cameras and bayesian processing as in Relion; Scheres et al. JMB 2012 )

# 2015-2016

## The democratization of cryo-EM



CORRESPONDENCE

### The democratization of cryo-EM

**To the Editor:** The cryo-electron microscopy (cryo-EM) community is in the throes of a 'resolution revolution'<sup>1</sup>. Years of technical development have led to hardware and software that can provide refined atomic structures with resolutions adequate for obtaining novel biological insights and for structure-based drug design<sup>2,3</sup>. However, these advances come at the cost of a multi-million-dollar investment that is required to establish a cutting-edge microscope, a detector and computational support. We believe that there is now the opportunity, and need, to provide democratic access to maximize the impact of cryo-EM on basic and applied science.

Possible models for such access are already emerging, following lessons learned from the experience accumulated over the

# High-end EM infrastructure across Europe

Several Titan Krios (300 kV) are present or being commissioned across mainly northern Europe:

**Germany:** 10-15 Titan Krios

**UK :** LMB 2, Pharma 1, Crick 2, Leeds 2, eBIC 2

**Netherlands:** 2 Titan Krios (NeCEN)

**Switzerland:** 5

**France:** 3 Titan Krios, in process - Paris area and 1 Strasbourg and 1 to be at ESRF-Grenoble

**Czech Republic:** 1 Titan Krios

**Sweden:** 2 Titan Krios

**Spain:** 0

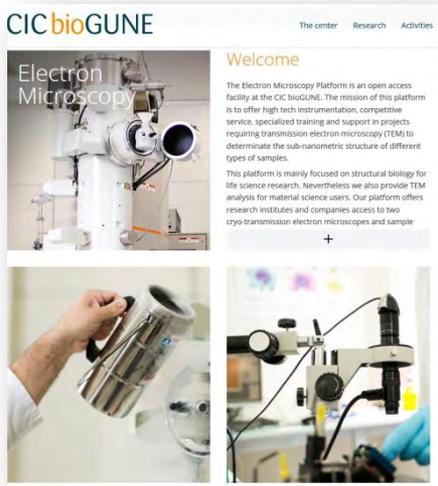
**MAYBE ONE DAY IN BILBAO ?**



# High-Resolution cryo-EM work-flow

*(right now in Spain)*

feeders



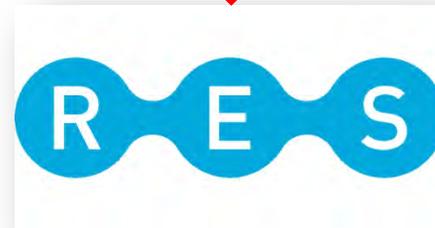
hub-1

hub-2

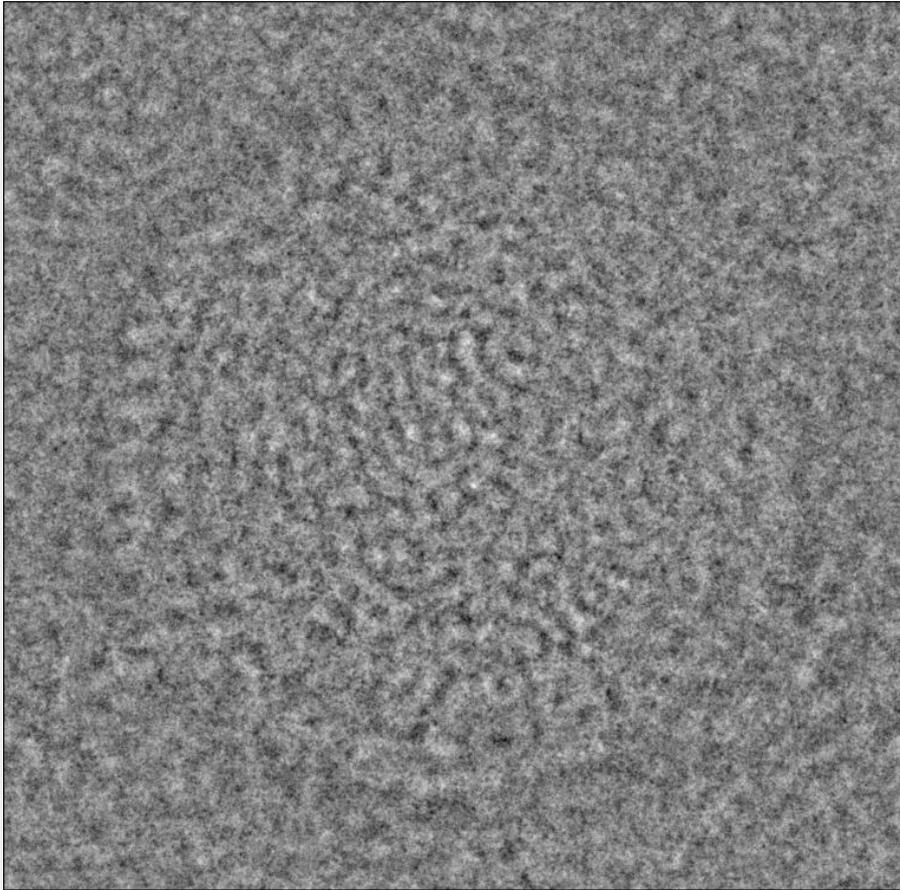
200kV JEOL JEM-2200FS

for past 9 yrs as national/international  
hub for external services/collaborations

Image Processing



# Our reasons for Picasso-RES:

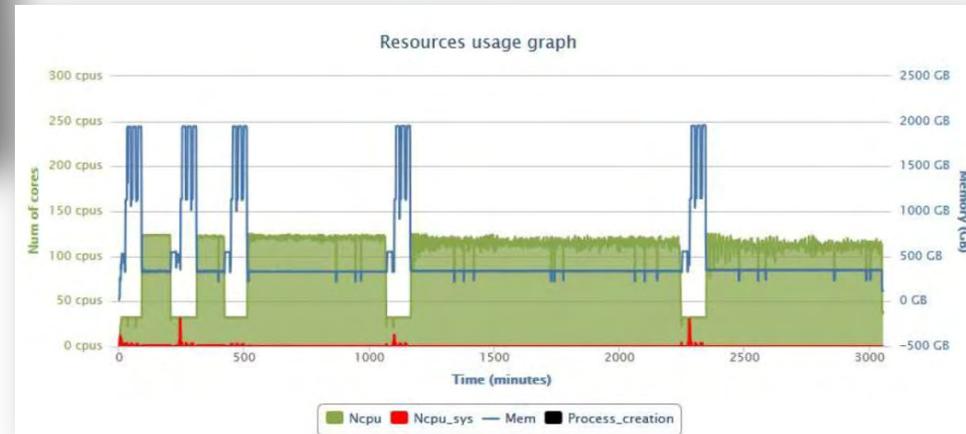


**Huge box size  
760x760**

**Relatively large  
number of particles**



**Huge RAM requirement**



**so right now....**

***(work in progress)***

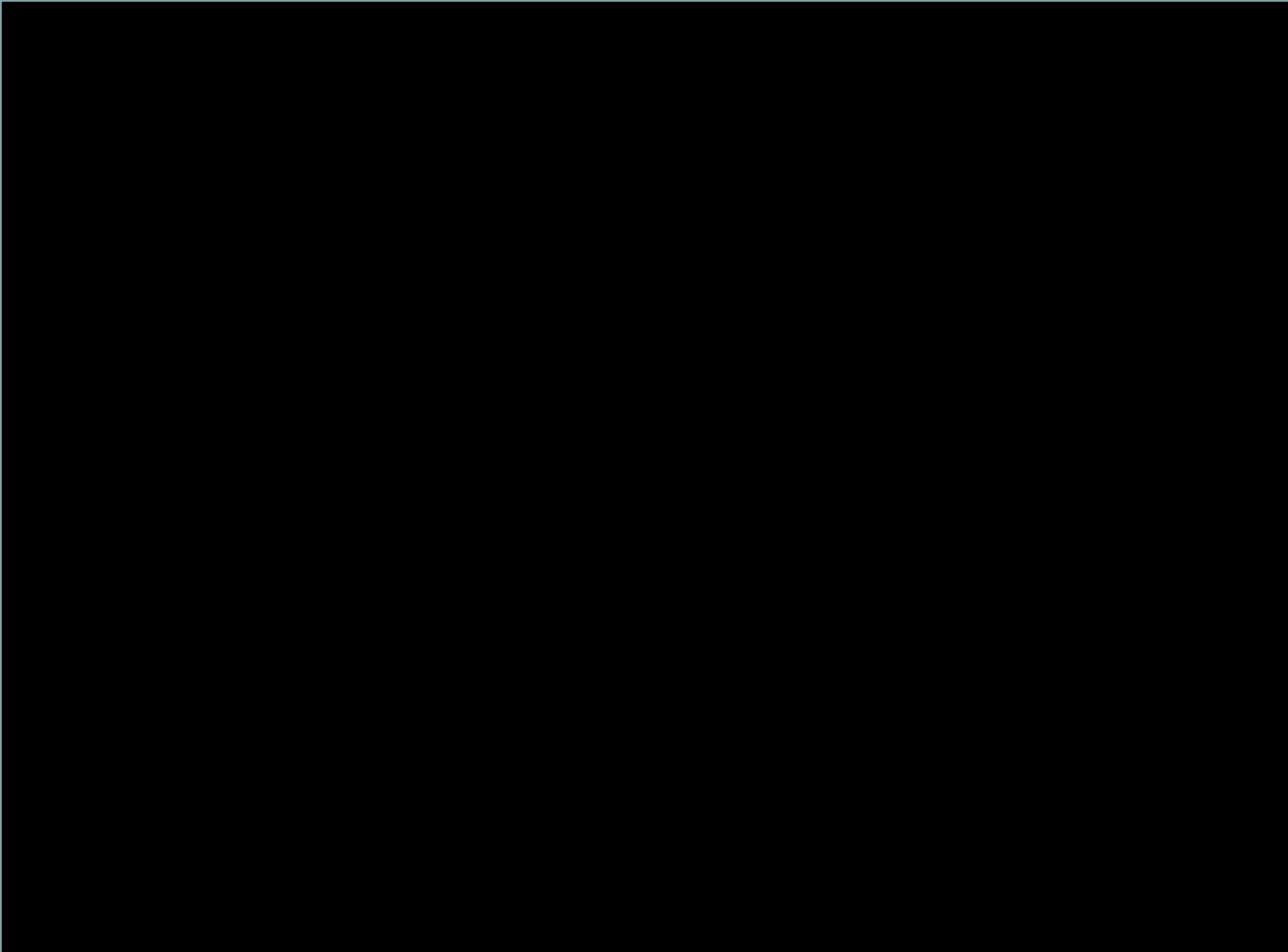
# HHIV-2

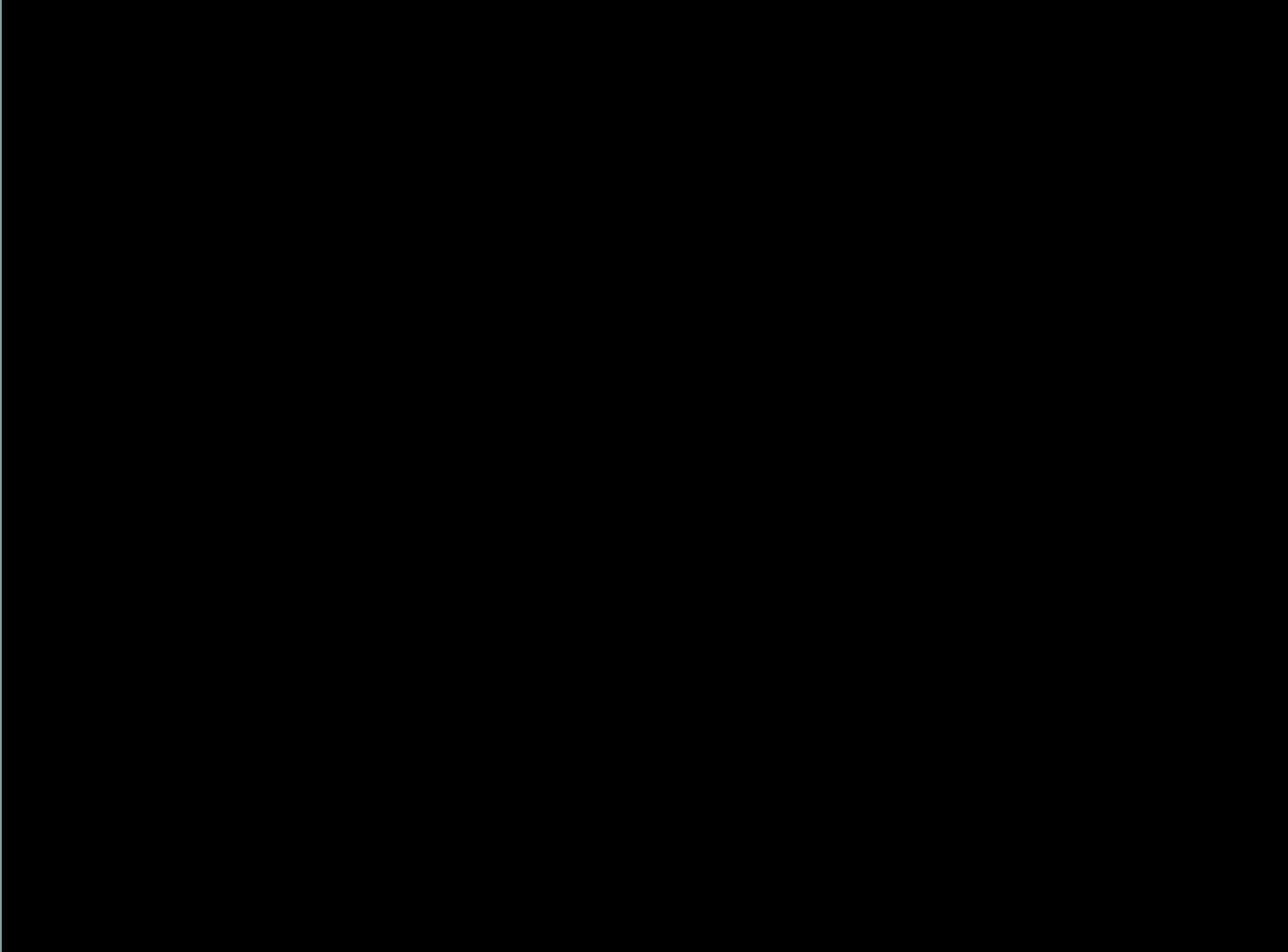
4.4 Å

~800 Å

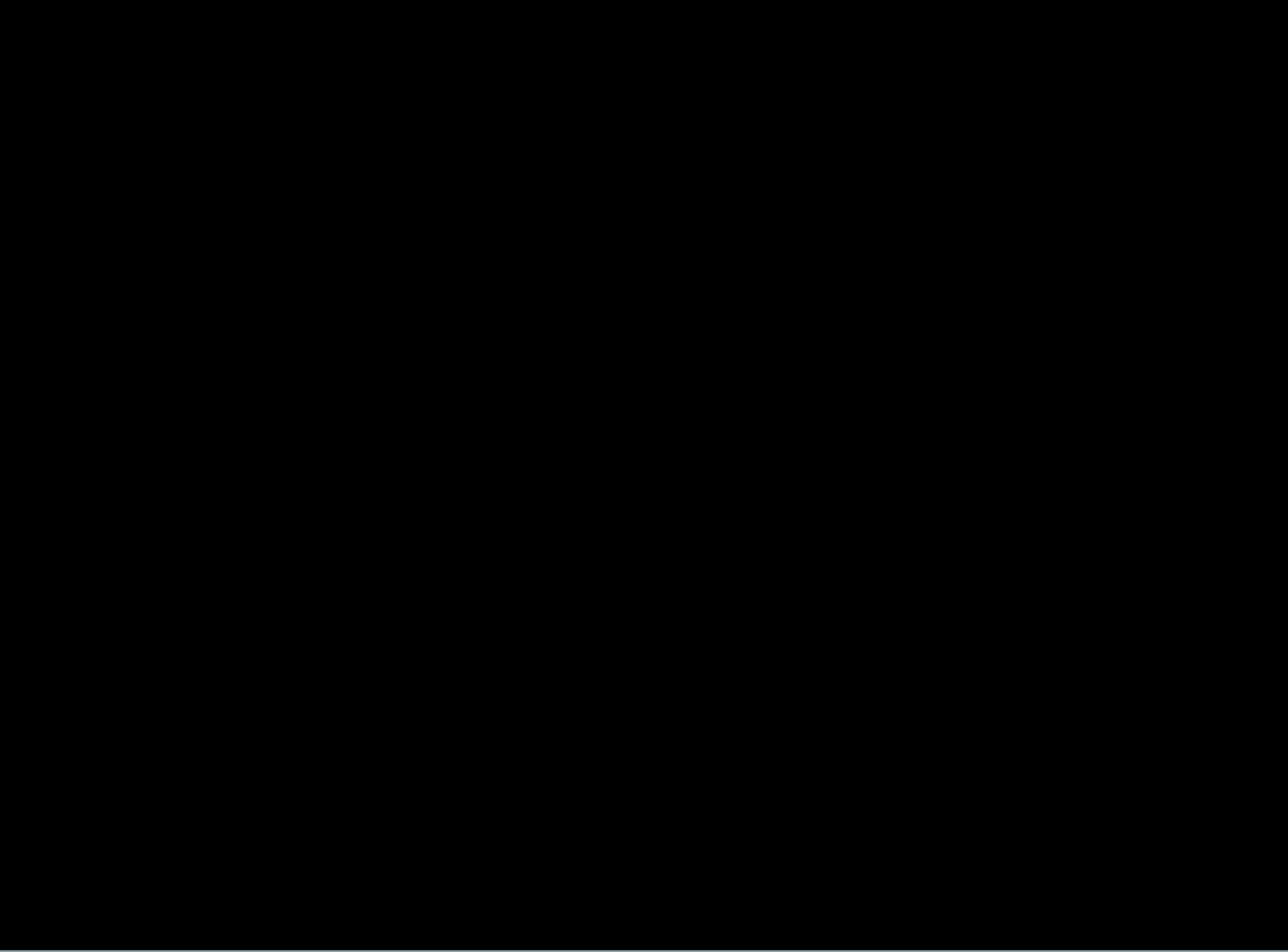
mapsize  
1.7 Gb









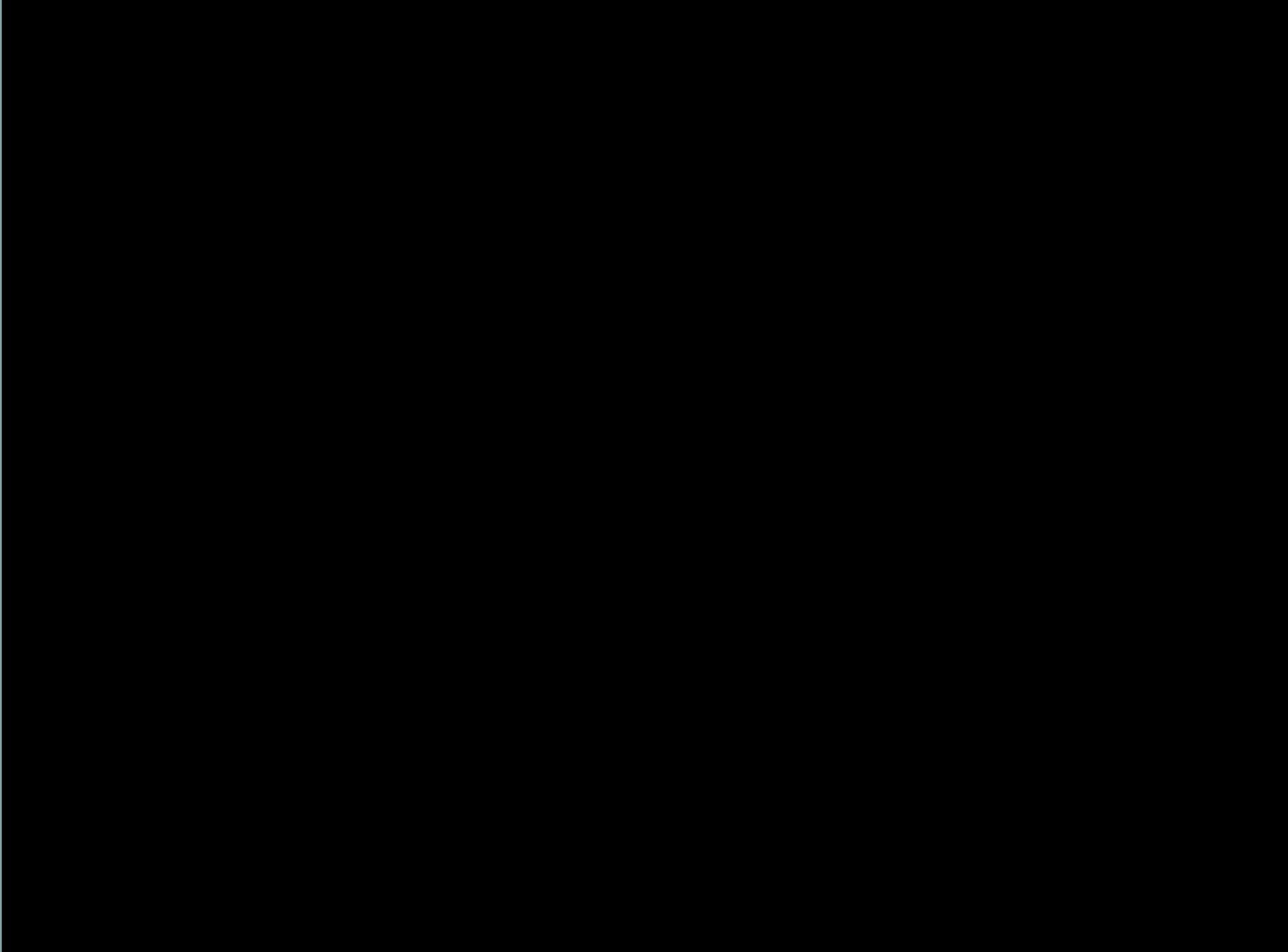








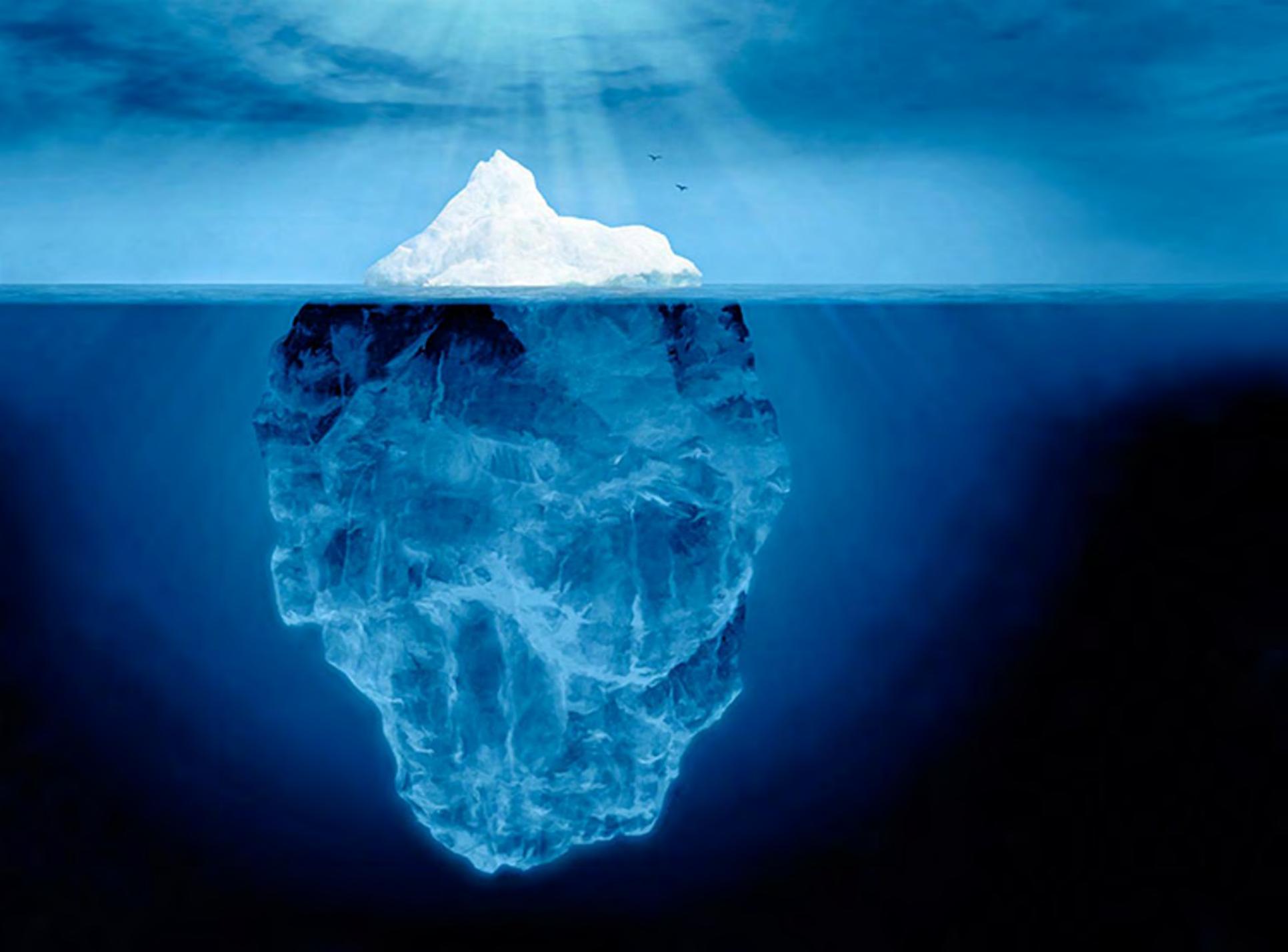








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# Acknowledgments

D. Bamford, H. Oksanen, S. Jaakkola  
*Helsinki University*



Daniel Castaño-Diez  
*C-CINA*



## Current Lab. Members:

Hani Y Boshra  
Diego Charro  
Stavros Azinas  
Isaac Santos  
Beatriz Gonzalez



CIC bioGUNE EM Platform  
David Gil-Carton



**ikerbasque**  
Basque Foundation for Science



**CIC bioGUNE**  
Biozientzietako Ikerkuntza Kooperatiboko Zentroa  
Centro de Investigación Cooperativa en Biociencias

