

# CRYSTALLIZATION OF THE KEAP1-DPP III COMPLEX

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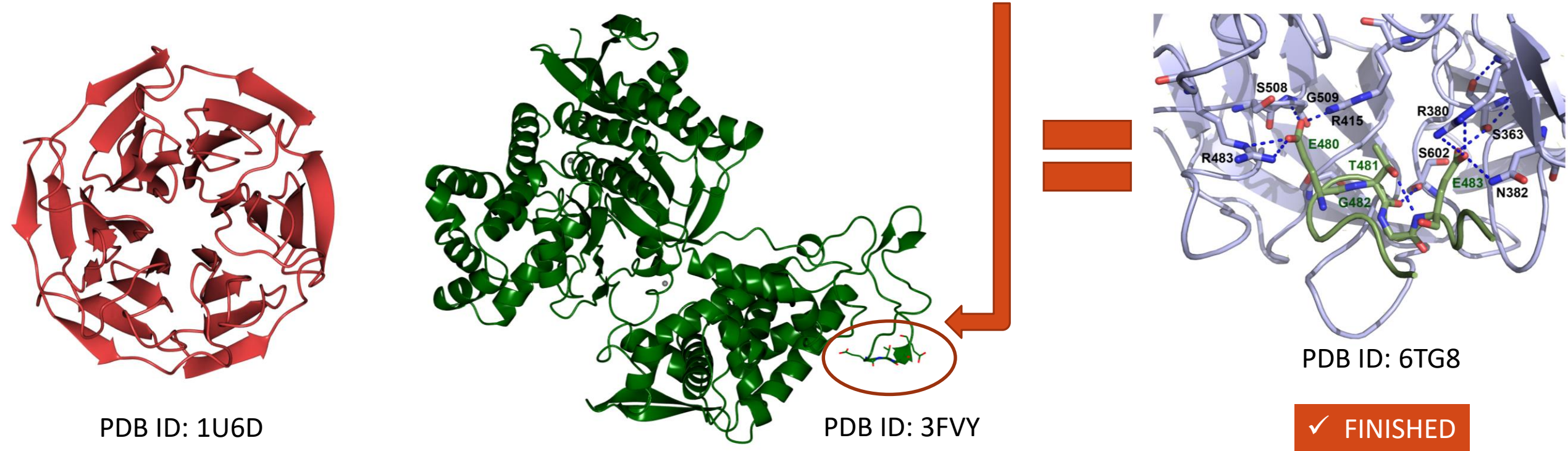
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# Overview

- Aims of structural characterization
- Crystallization of the Kelch domain:DPPIII and Keap1:DPP3 complexes
- Future work

✓ Crystal structure determination:

1. Kelch domain of Keap1 + VINP **ETGE** QIQ

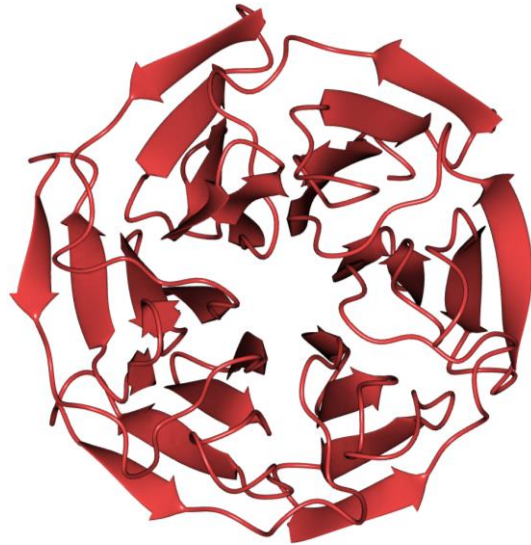


✓ Crystal structure determination:

**2. Kelch domain of Keap1**



**DPPIII**



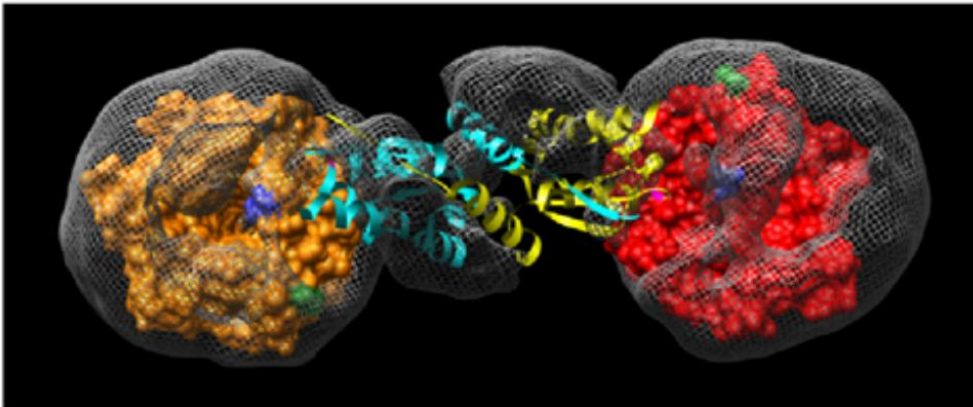
PDB ID: 1U6D



PDB ID: 3FVY

✓ Crystal structure determination:

## 3. full length Keap1



Top view of the Keap1 homodimer model

Ogura *et al.* *PNAS*, **107** (2010) 2842-2847.



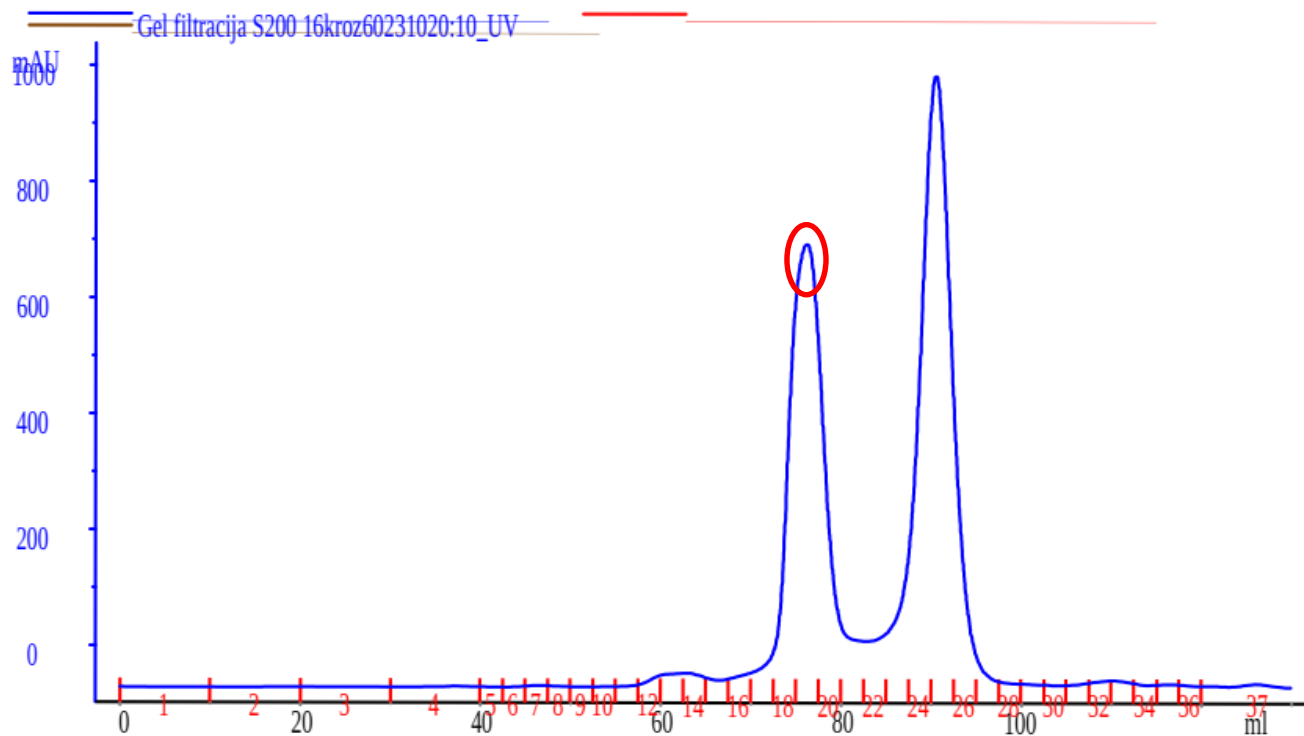
## DPPIII



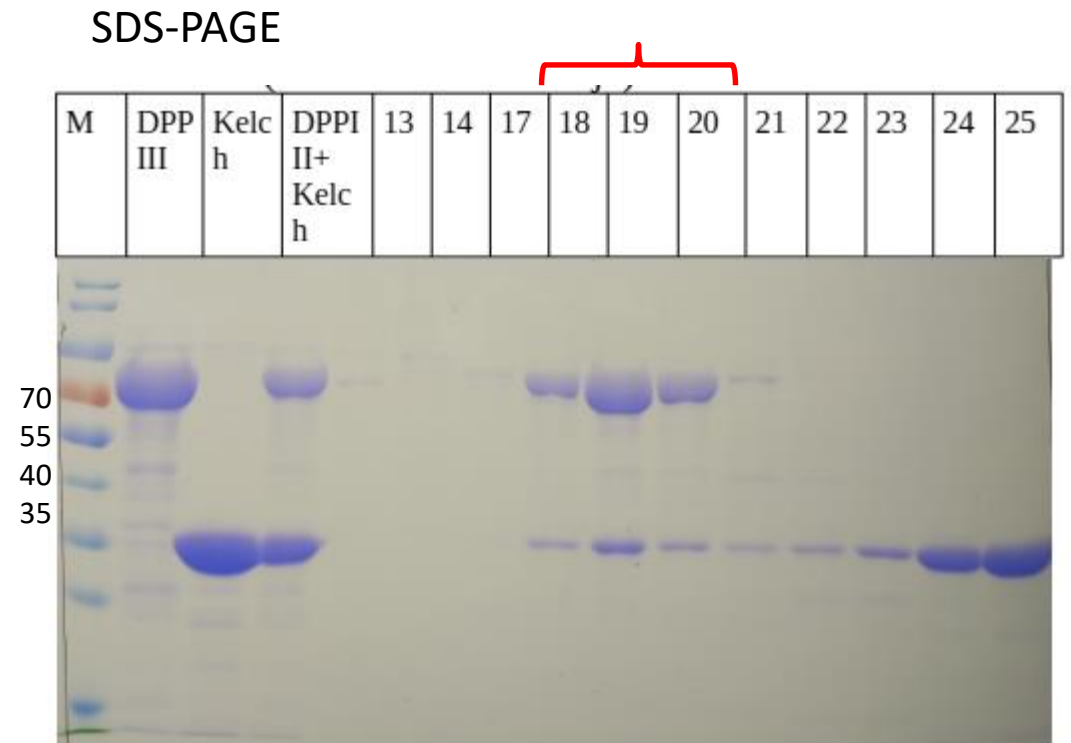
PDB ID: 3FVY

# Crystallization of the DPP III–Kelch complex

- 2 approaches:
  - ✓ purification by the SEC and then incubation prior the crystallization
  - ✓ purification of the Kelch:DPPIII complex by the SEC

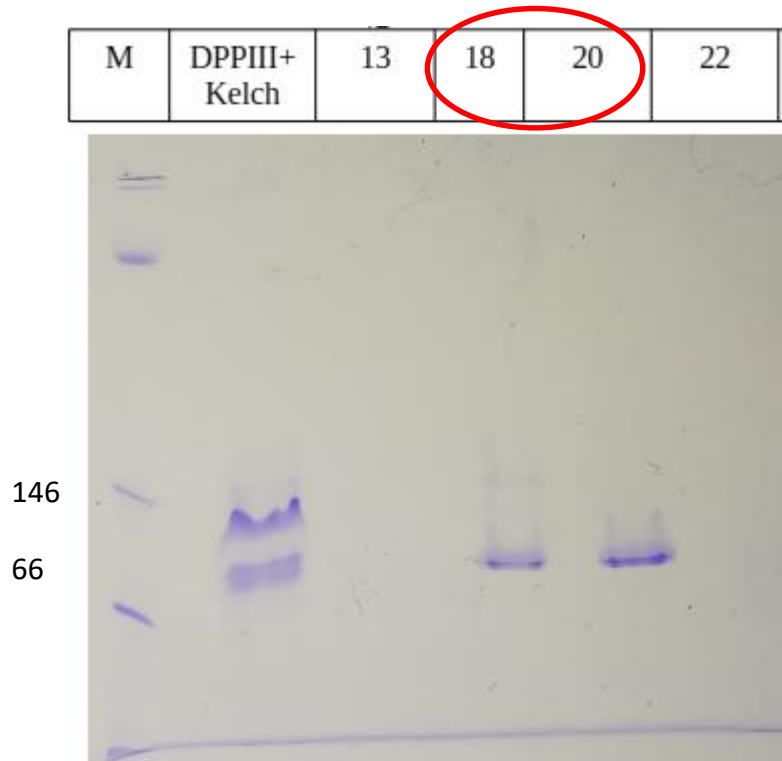


SDS-PAGE (loadano 5uL frakcija)



## NATIVE-PAGE

crystallization !



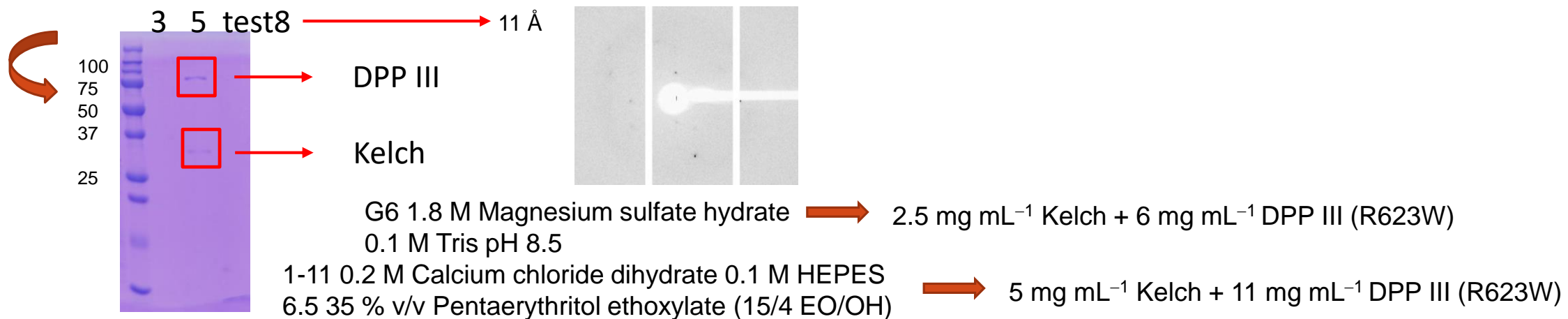
$M_r(\text{Kelch}) = 37\ 000$   
 $M_r(\text{DPPIII}) = 80\ 000$

- crystallization by sitting drop vapour diffusion technique, Oryx 8 robot (Institute of Biochemistry, Graz University of Technology, Graz, Austria)
- 16 °C → MIDAS +, SALTRX 1 & 2, PEG'S II SCREEN, STRUCTURE SCREEN 1 & 2, PACT PREMIER, INDEX SCREEN, STURA/MACRO SOL



- protein samples after SEC in 1:1 molar ratio (DPP III variants: N6, R623W)
- 2304 different crystallization conditions

- non or poor diffracting crystals, Elettra - XRD2









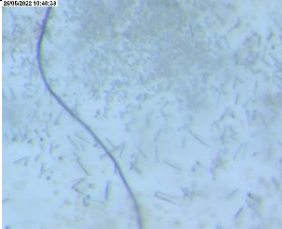
# Crystallization of the DPP III–Keap1 complex

- crystallization by sitting drop vapour diffusion technique, Oryx 8 robot (University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia)
- 18 °C → LMB JCSG, STRUCTURE SCREEN 1 & 2, PACT PREMIER



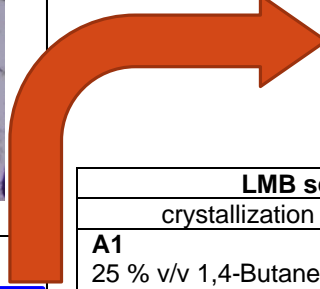
- protein samples after SEC in 1:1 and 1:2 molar ratio (Keap1:DPP III R623W)
- 768 different crystallization conditions

- 5 days after setting up the experiment

LMB screen crystallization condition	Drop Keap1:DPP3= <b>1:1</b> Keap1:DPP3= <b>1:2</b>	
<b>F2</b> 16 % w/v PEG 4000/ 0.1 M Sodium citrate 5.8 0.1 M Ammonium sulfate 20 % v/v Glycerol	multiple crystals	
<b>H5</b> 3 M Sodium chloride 0.1 M Tris 7.5	✗	multiple crystals
<b>F8</b> 18 % w/v PEG 4000 0.1 M Tris 9.0 0.3 M Sodium acetate trihydrate	✗	
<b>H11</b> 1.6 M Sodium/potassium phosphate 6.0		
<b>G11</b> 20 % w/v PEG 8000 0.1 M CAPS 9.0 0.2 M Magnesium chloride hexahydrate	multiple crystals	✗
<b>E11</b> 28 % w/v PEG 4000 0.1 M Sodium citrate 5.2 0.2 M Ammonium acetate	✗	multiple crystals
<b>G12</b> 20 % w/v PEG 8000 0.1 M CHES 9.5	✗	






SEEDING



1 day after setting up the experiment with seeds of the condition F8 (S)

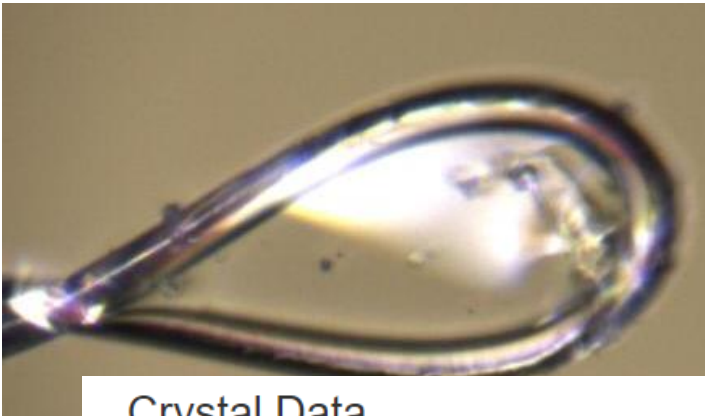


LMB screen crystallization condition (C)	Keap1:DPP3= <b>1:2 (P)</b>	
	<b>P:S:C=3:1:2</b>	<b>P:S:C=2:1:3</b>
<b>A1</b> 25 % v/v 1,4-Butanediol 0.1 M Tris 8.0	multiple crystals	
<b>G2</b> 3.5 % w/v PEG 6000 0.1 M Bis-Tris propane 7.1 0.1 M Potassium chloride	bigger needle shaped crystals <b>DISAPPEARED AFTER 2 WEEKS</b>	bigger needle shaped crystals <b>DISAPPEARED AFTER 2 WEEKS</b>
<b>E9</b> 40 % v/v PEG 400 0.1 M Tris 8.4 0.2 M Lithium sulfate	many small needle shaped crystals	
<b>F9</b> 18 % w/v PEG 5000 MME 0.1 M MES 6.5 0.2 M Ammonium sulfate	many small needle shaped crystals	many small needle shaped crystals
<b>F10</b> 20 % v/v PEG 600 0.1 M Sodium cacodylate 5.6 0.15 M Potassium thiocyanate/ 0.2 M Sodium chloride	many small needle shaped crystals	many small needle shaped crystals
<b>G10</b> 10 % w/v PEG 8000 0.1 M HEPES 7.5 9 % v/v Ethylene glycol	many small needle shaped crystals	many small needle shaped crystals
<b>H11</b> 1.6 M Sodium/potassium phosphate 6.0		many small crystals

- 60 crystals tested at the Elettra - XRD2

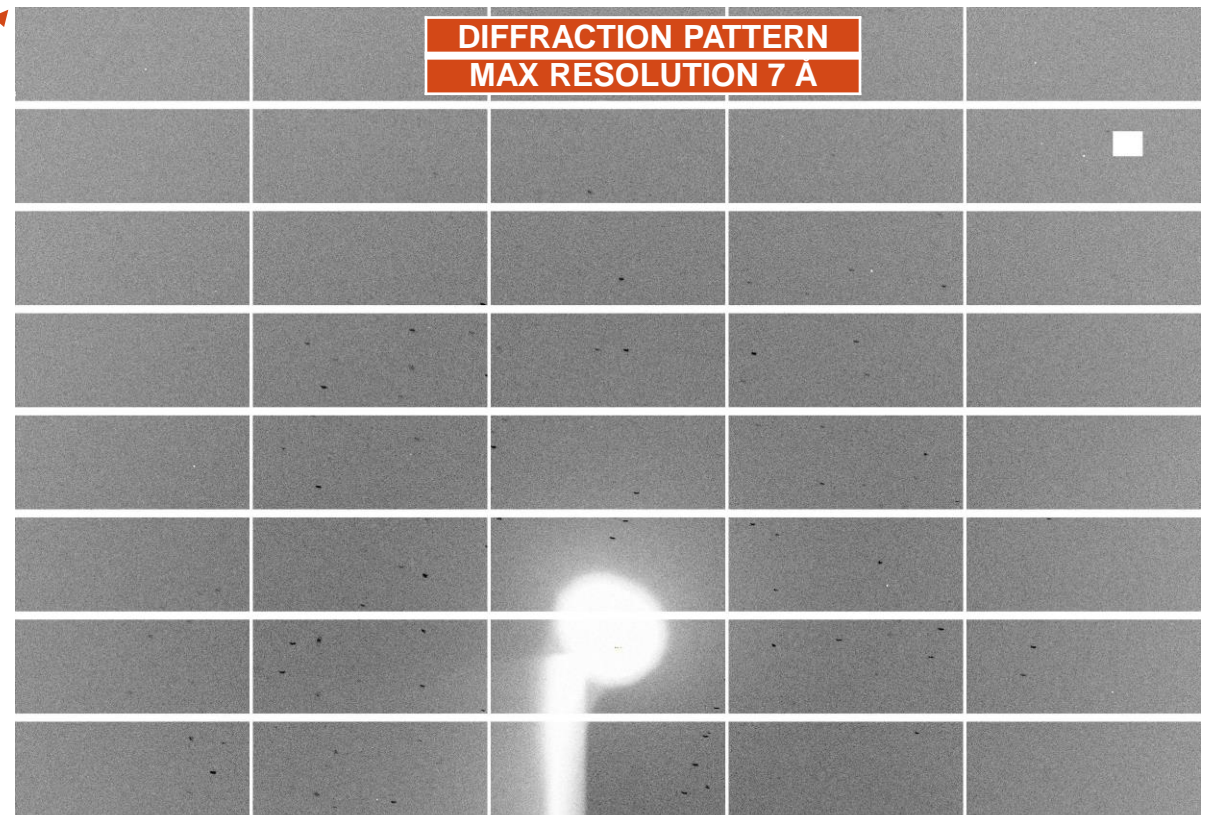


**CRYSTALLIZATION CONDITION**  
**LMB F2 + SEEDS**



Crystal Data

Unit Cell	
Length ( Å )	Angle ( ° )
a = 49.812	$\alpha = 90$
b = 151.378	$\beta = 100.04$
c = 53.721	$\gamma = 90$



**DIFFRACTION PATTERN**  
**MAX RESOLUTION 7 Å**

Symmetry	
Space Group	P 1 2 1 1

**DPPIII (PDB ID: 3FVY)**

**Unit Cell: 151.378 Å 151.378 Å 221.702 Å**  
**90.000° 90.000° 120.000°**  
**Space Group: R3**

## FUTURE WORK

- optimization of crystallization of the Keap1:DPPIII complex  
(seeding in new conditions)
- cryo- EM microscopy (size of the complex 220 kDa)

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**THANK YOU FOR YOUR ATTENTION!**