HrZZ
Hrvatska zaklada za znanost
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CRYSTALLIZATION OF THE KEAP1-DPP III COMPLEX

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Overview

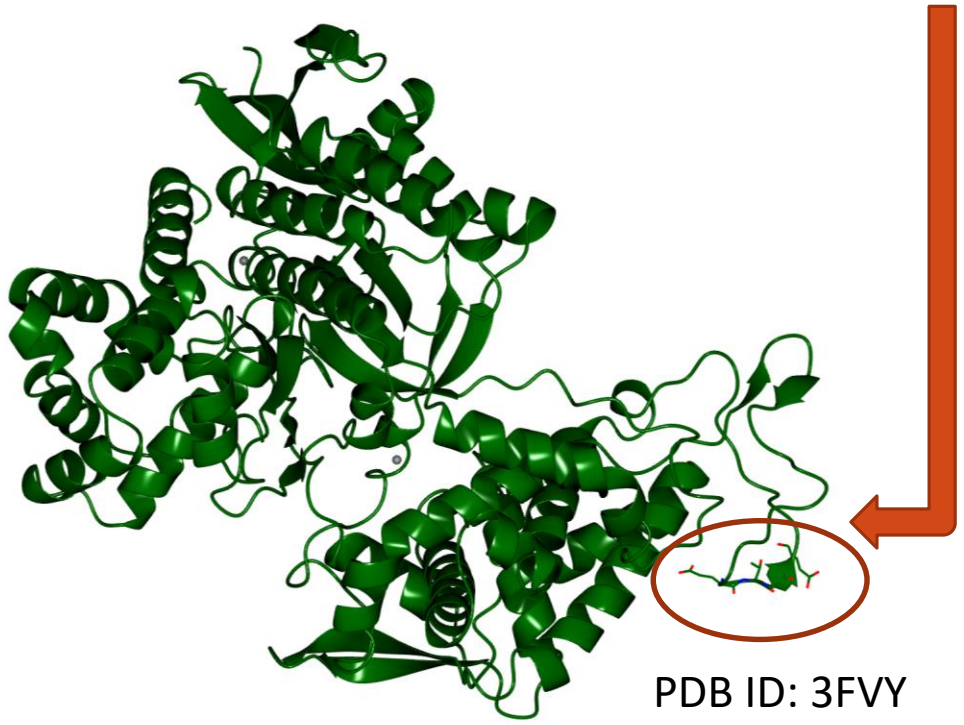
- Aims of structural characterization
- Crystallization of the Kelch domain:DPPIII and Keap1:DPP3 complexes
- Future work

AIMS:

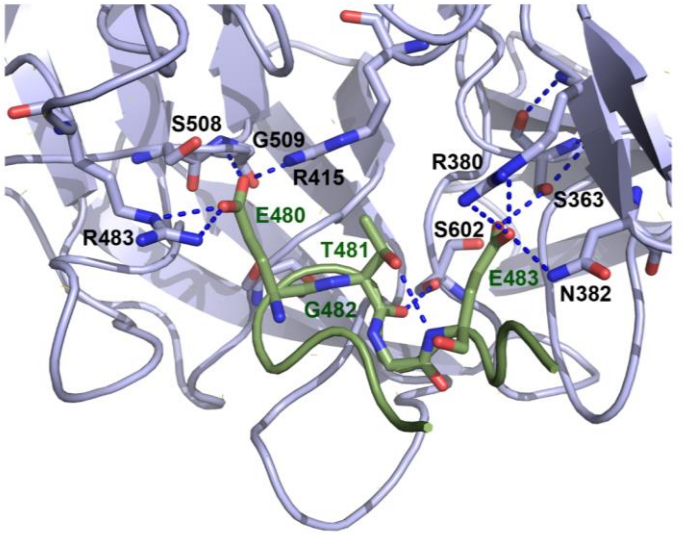
✓ Crystal structure determination:

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

PDB ID: 1U6D



PDB ID: 3FVY



PDB ID: 6TG8

✓ FINISHED

AIMS:

✓ Crystal structure determination:

2. Kelch domain of Keap1

PDB ID: 1U6D

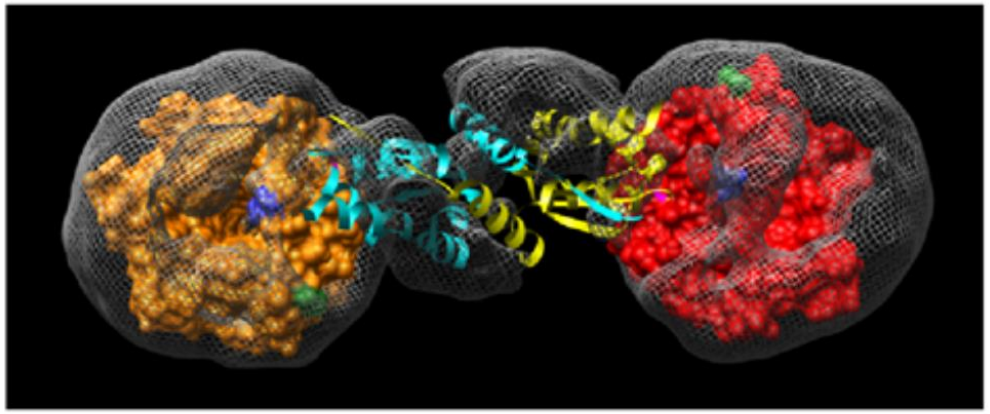
DPPIII

PDB ID: 3FVY

AIMS:

✓ Crystal structure determination:

3. full length Keap1



Top view of the Keap1 homodimer model

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

DPPIII

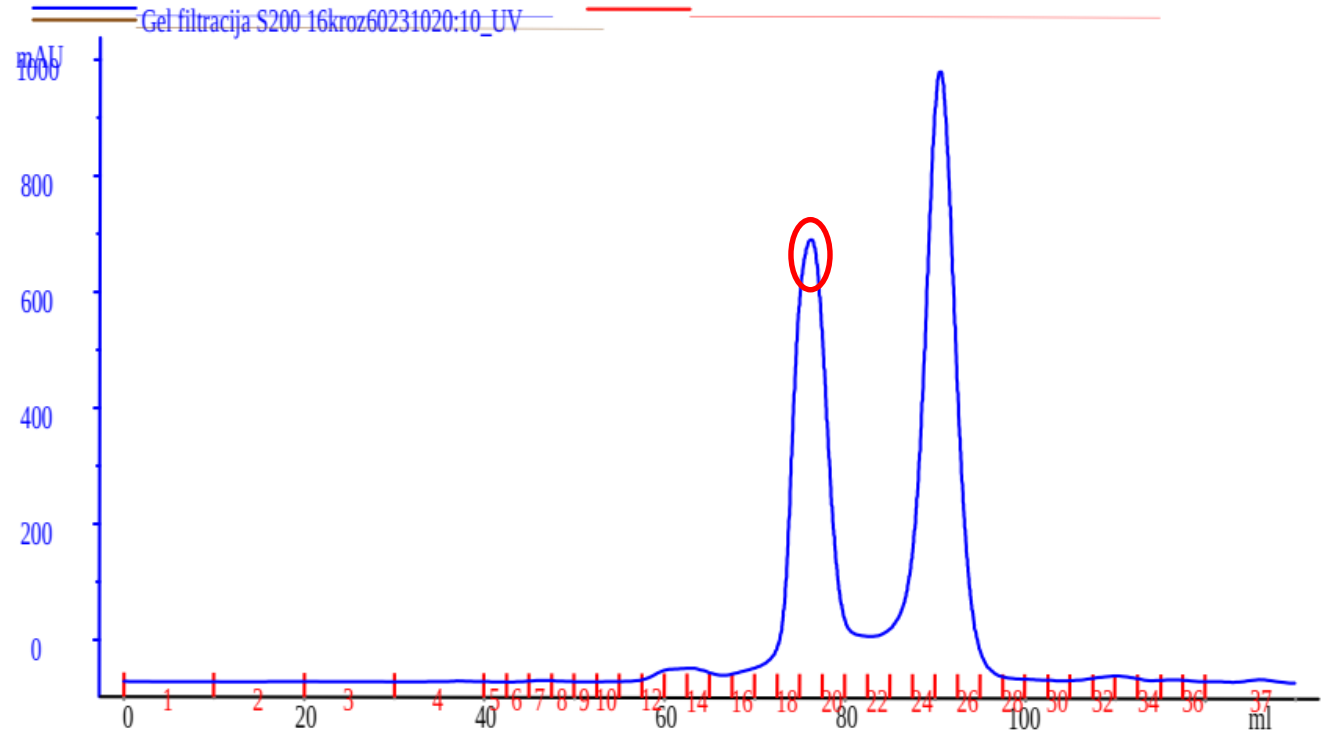
PDB ID: 3FVY

Crystallization of the DPP III–Kelch complex

- 2 approaches:

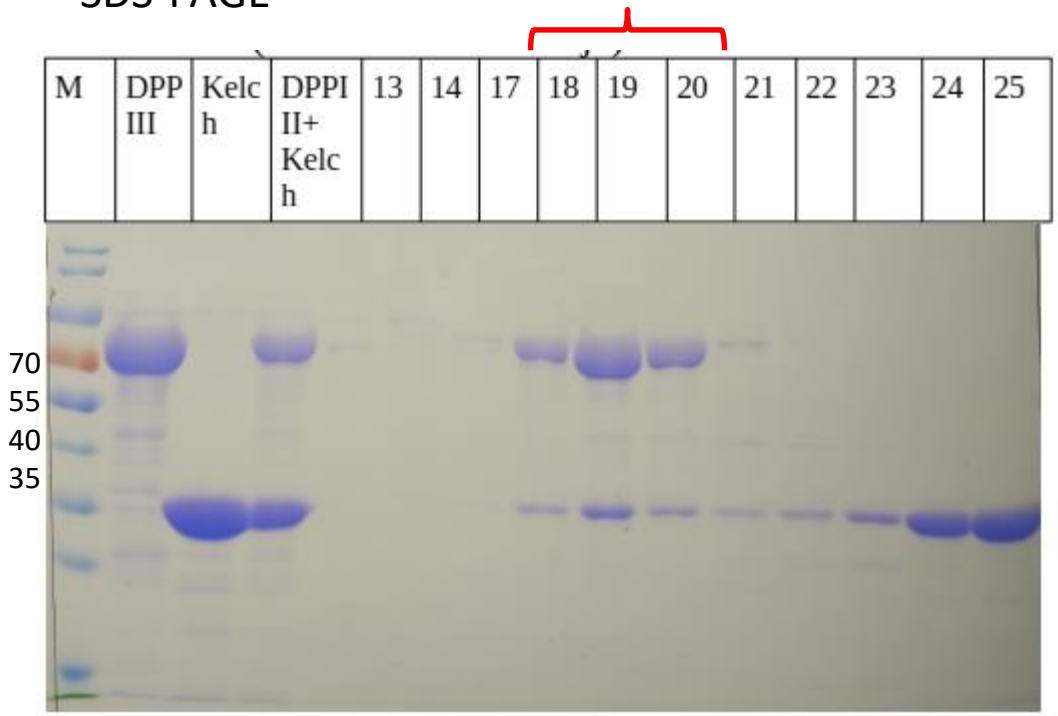
✓ purification by the SEC and then incubation prior the crystallization

✓ purification of the Kelch:DPPIII complex by the SEC



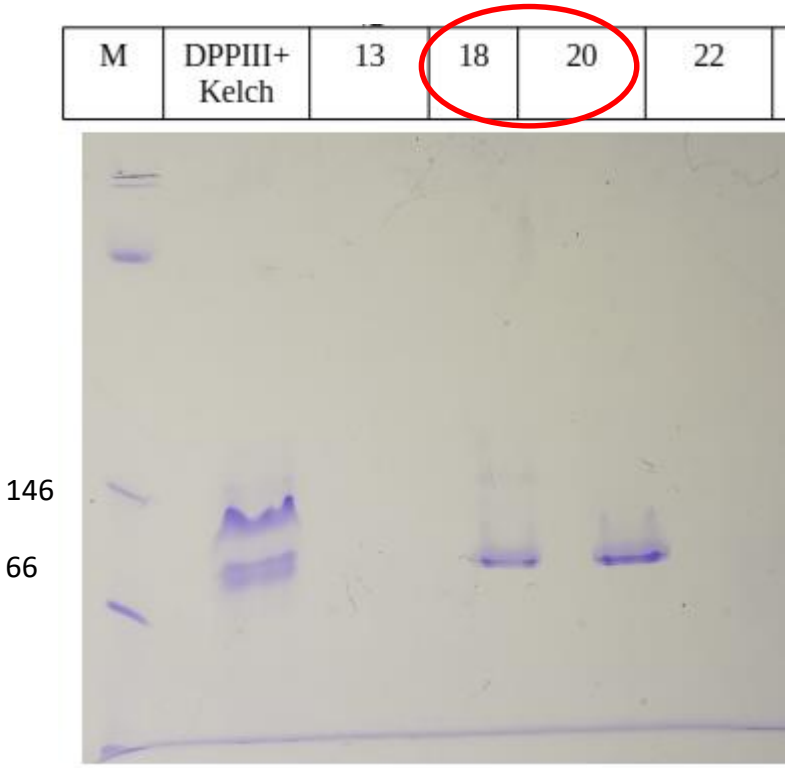
SDS-PAGE (loadano 5uL frakcija)

SDS-PAGE



NATIVE-PAGE

crystallization !

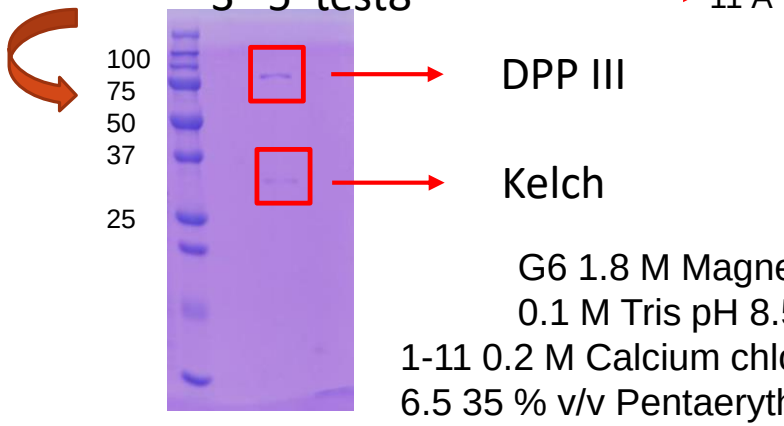


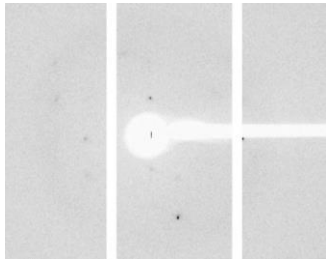
M_r(Kelch) = 37 000
M_r(DPPIII) = 80 000

- 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





G6 1.8 M Magnesium sulfate hydrate
0.1 M Tris pH 8.5
→ 2.5 mg mL^{-1} Kelch + 6 mg mL^{-1} DPP III (R623W)

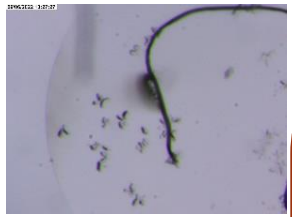
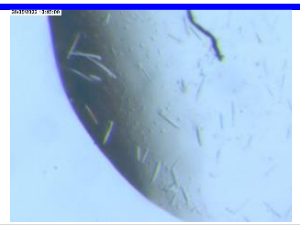
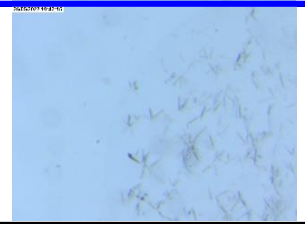
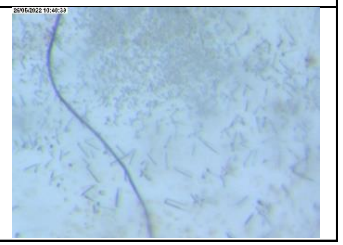
1-11 0.2 M Calcium chloride dihydrate 0.1 M HEPES
6.5 35 % v/v Pentaerythritol ethoxylate (15/4 EO/OH)
→ 5 mg mL^{-1} Kelch + 11 mg mL^{-1} DPP III (R623W)

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

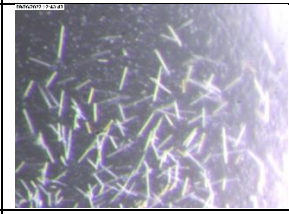
- protein samples after SEC in 1:1 and 1:2 molar ratio (Keap1:DPP III R623W)
- 768 different crystallization conditions

- 5 days after setting up the experiment

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

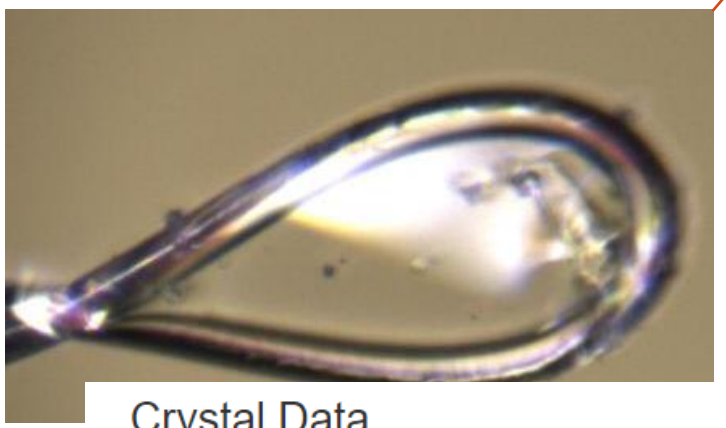
SEEDING

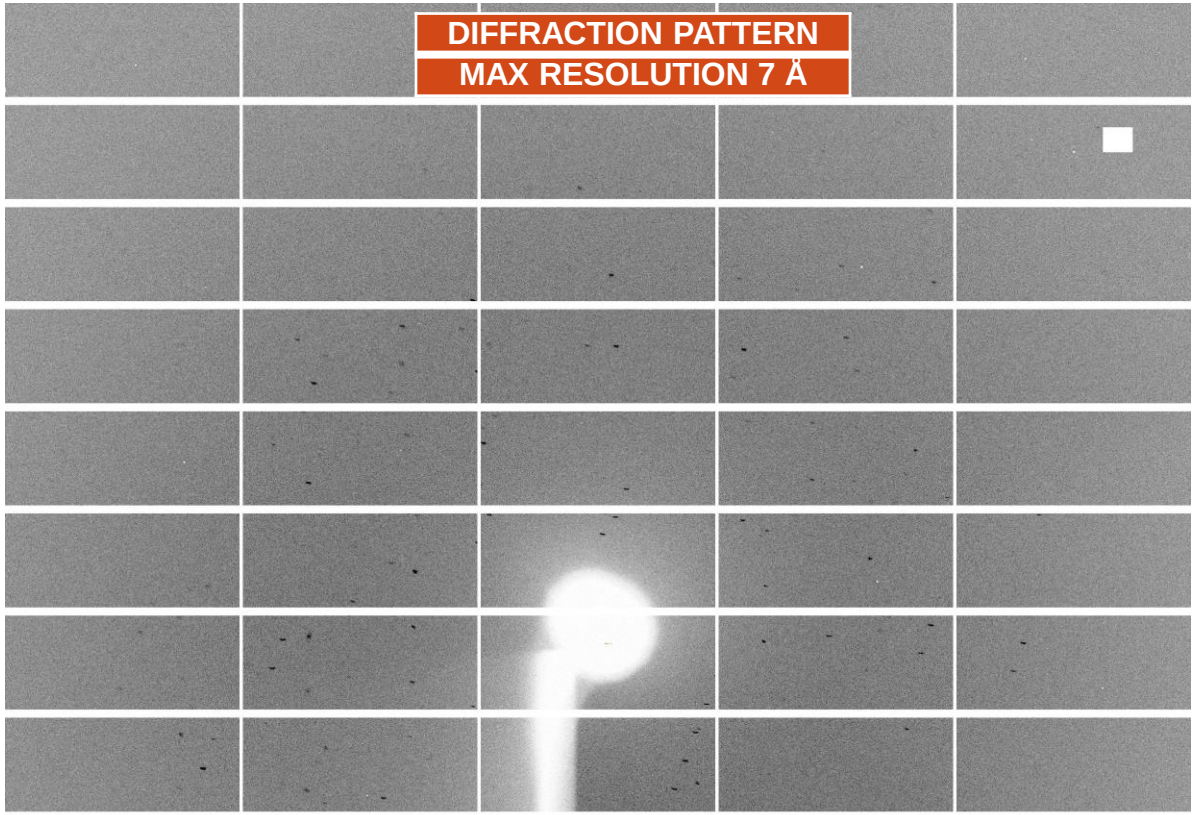
1 day after setting up the experiment with **seeds** of the condition F8 (**S**)

LMB screen crystallization condition (C)	Keap1:DPP3=1:2 (P) P:S:C=3:1:2	 P:S:C=2:1:3
A1 25 % v/v 1,4-Butanediol 0.1 M Tris 8.0	multiple crystals	
G2 3.5 % w/v PEG 6000 0.1 M Bis-Tris propane 7.1 0.1 M Potassium chloride	bigger needle shaped crystals DISSAPEARED AFTER 2 WEEKS	bigger needle shaped crystals DISSAPEARED AFTER 2 WEEKS
E9 40 % v/v PEG 400 0.1 M Tris 8.4 0.2 M Lithium sulfate	many small needle shaped crystals	
F9 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
F10 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
G10 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
H11 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	α = 90
b = 151.378	β = 100.04
c = 53.721	γ = 90

Symmetry	
Space Group	P 1 21 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)

THANK YOU FOR YOUR ATTENTION!