Crystal structures of Kelch–DPP III-loop-peptide and of DPP III–Kelch

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Crystallization of the DPP III ETGE loop–Kelch domain complex

- crystallization by sitting drop vapour diffusion technique, Oryx 8 robot (Institute of Biochemistry, Graz University of Technology, Graz, Austria)

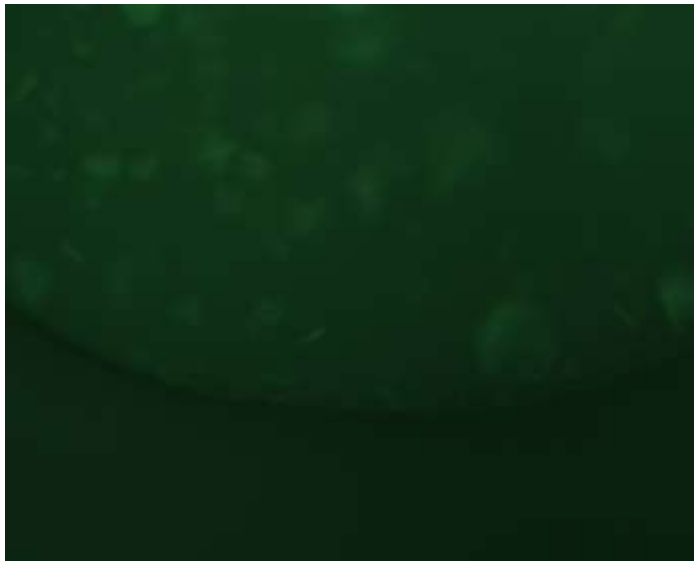
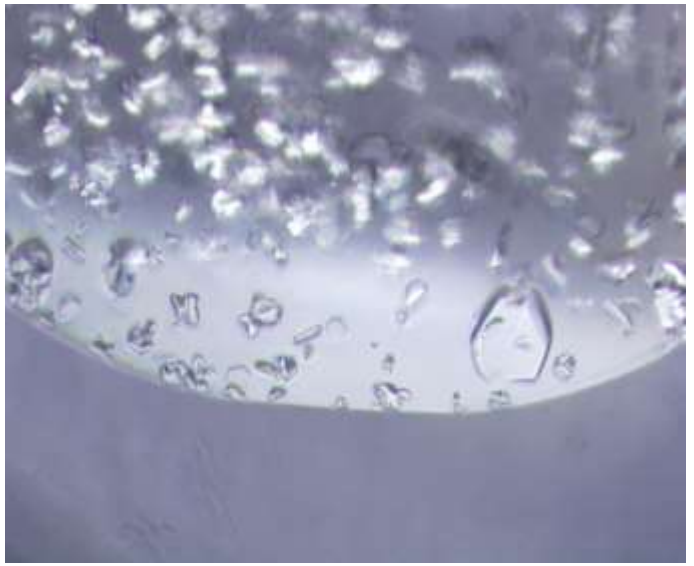
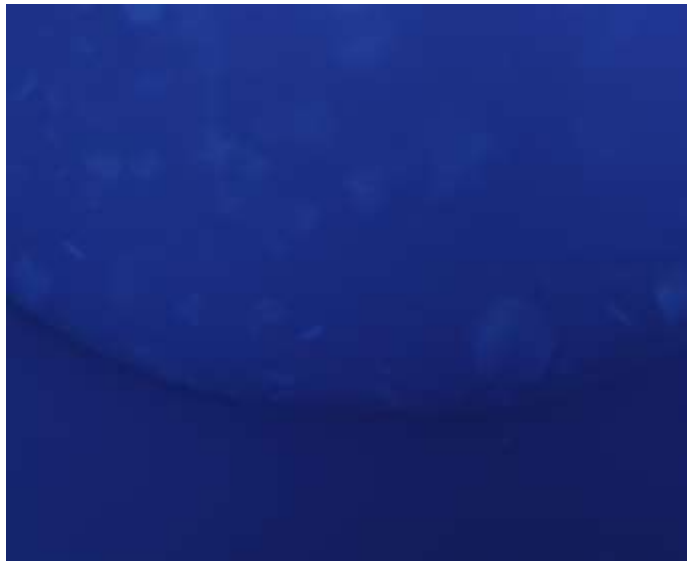
- 16 °C → SALTRX 1 & 2, STRUCTURE SCREEN 1 & 2, PACT PREMIER, JCSG SCREEN, PGA SCREEN

- 4 °C → SALTRX 1 & 2, MORPHEUS SCREEN

- protein samples after SEC or IEC mixed with 11 AA peptide (VINP**ETGE**QIQ) in different molar ratios (1:1 or 1:4)
- 2016 different crystallization conditions

- good diffracting crystals, Elettra - XRD1, XRD2



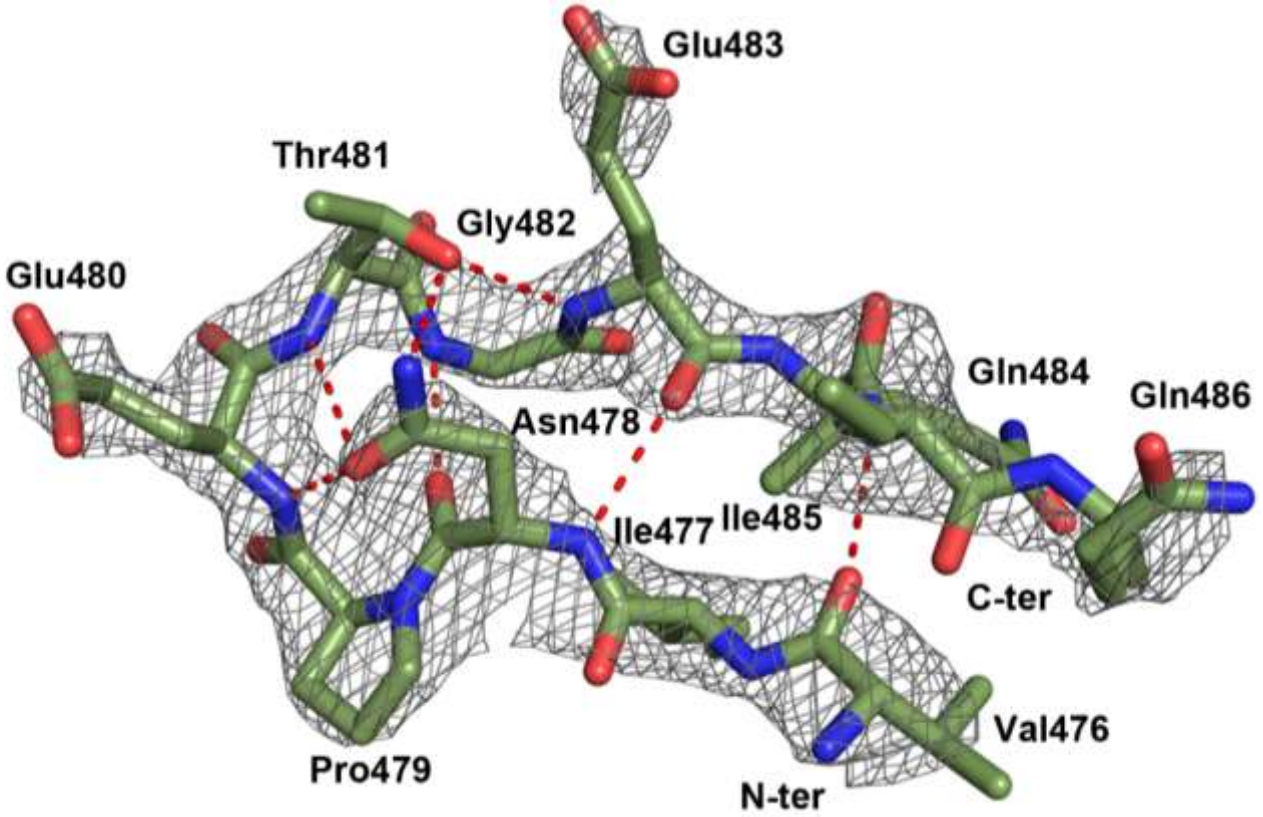


- 1.8 M sodium acetate pH 7, 0.1 M Bis-Tris propane buffer pH 7
- Kelch : 11 AA peptide = 1:1
- crystals appeared after 2 weeks of equilibration at 289 K

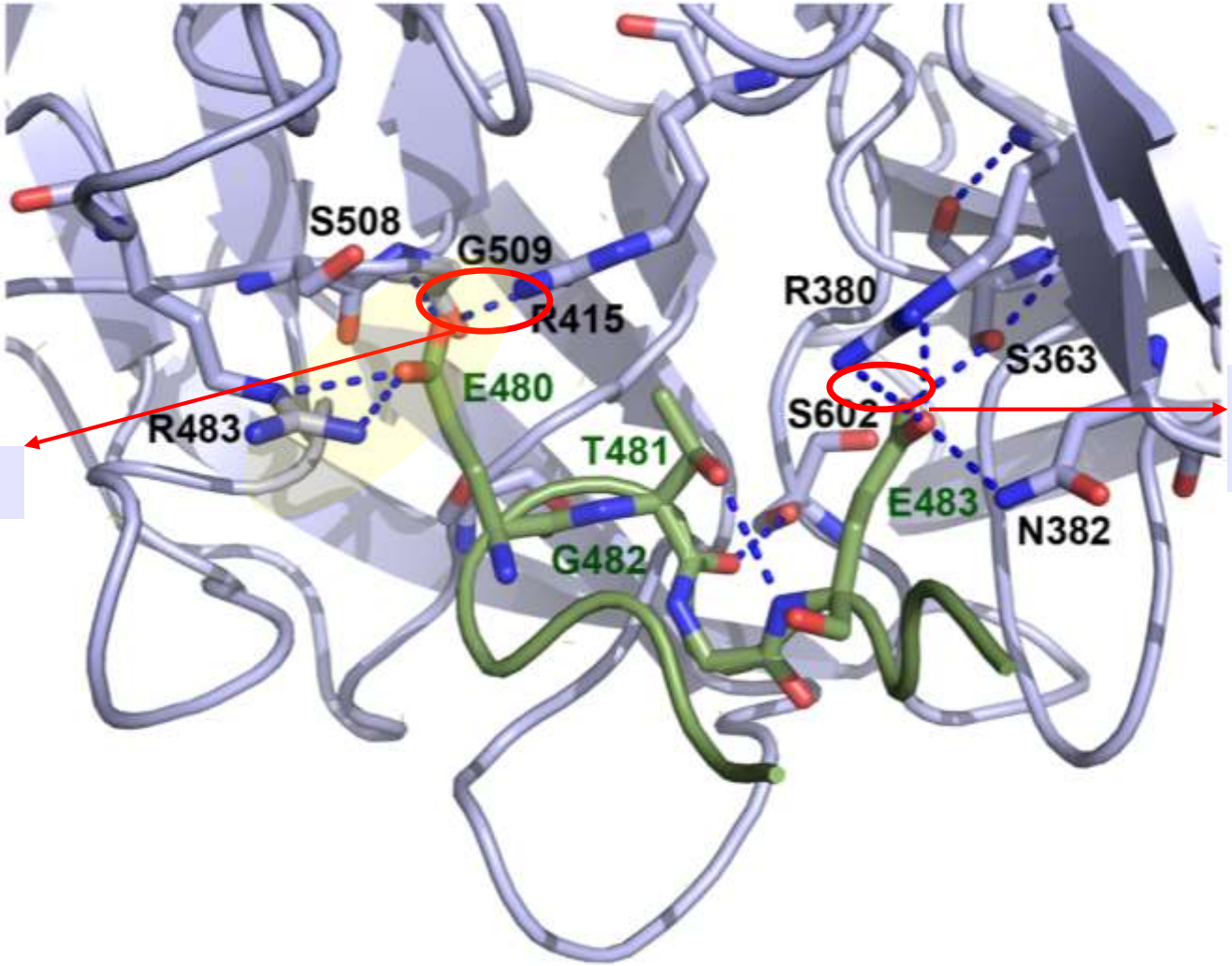
The X-ray structure of the DPP III ETGE loop–Kelch domain complex

		Kelch:11 AA
Crystallographic data		
Crystal system		Trigonal
Space group		$P3_121$
Wavelength / Å		1.000
Unit cell parameters / Å, °		$a = b = 75.296(1)$, $c = 127.539(2)$ $\alpha = \beta = 90$, $\gamma = 120$
Z	unit cell	6
	asymmetric unit	1
Resolution range / Å		65.21 – 2.70 (2.80 – 2.70)
R_{sigma}		0.020 (0.448)
$<I/\sigma(I)>$		40.66 (2.30)
Completeness / %		99.5 (100)
Redundancy		18.1 (19.1)

Refinement	
R_{work} / R_{free}	0.1809/ 0.2584
Protein atoms	2324
Water molecules	9
Sodium atoms	1
Mean B / Å^2	69.133
R.m.s.d. from ideal value	
Bond length / Å	0.007
Bond angles / °	1.499
Geometry	
Ramachandran favored / %	93.56
Ramachandran outliers / %	0.34
Rotamer outliers / %	6.88



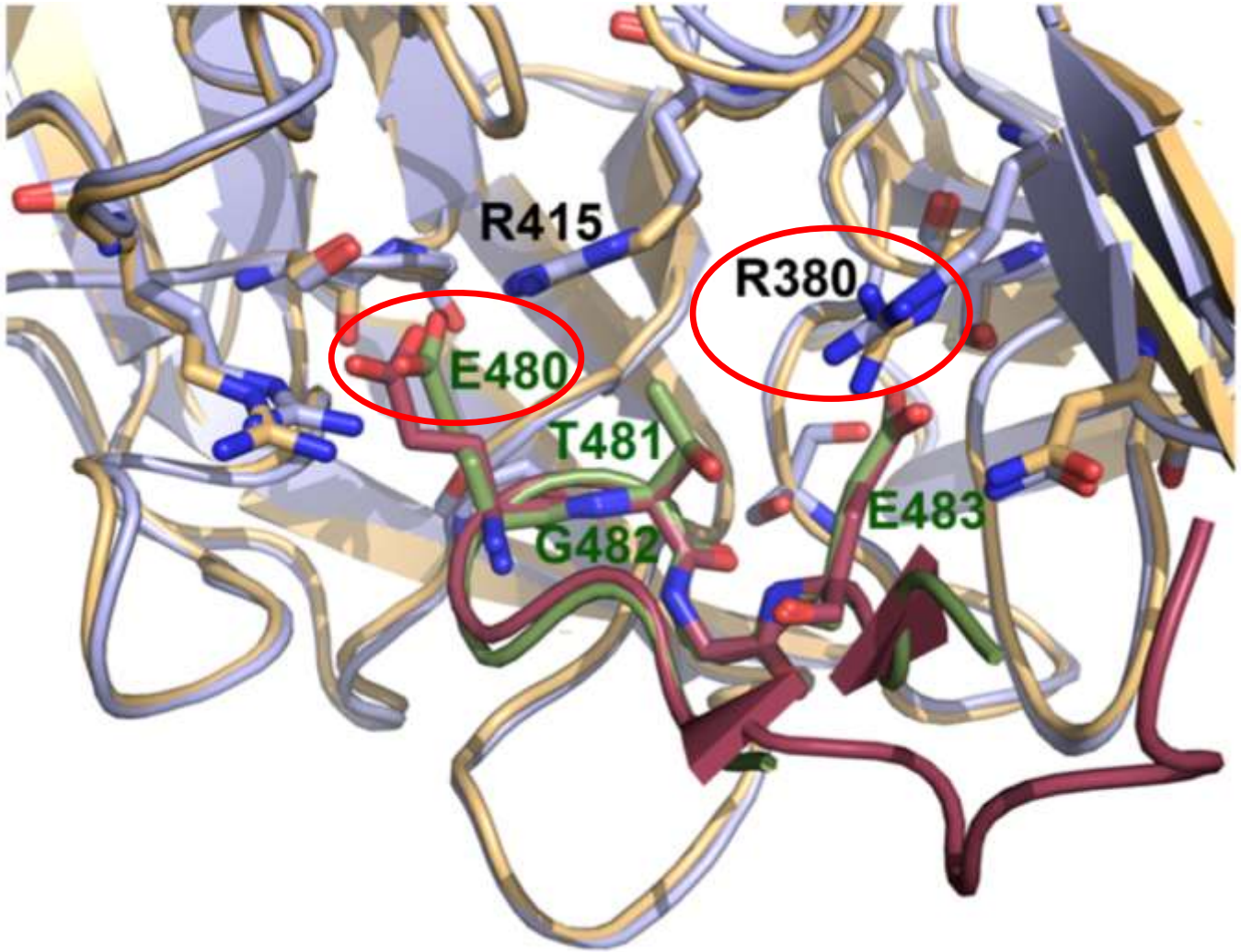
The 11 AA peptide (stick representation) with intramolecular hydrogen bonds shown as red dashed lines and the $2F_o$-F_c electron density map contoured at a 1.6 σ level is shown as grey mesh.



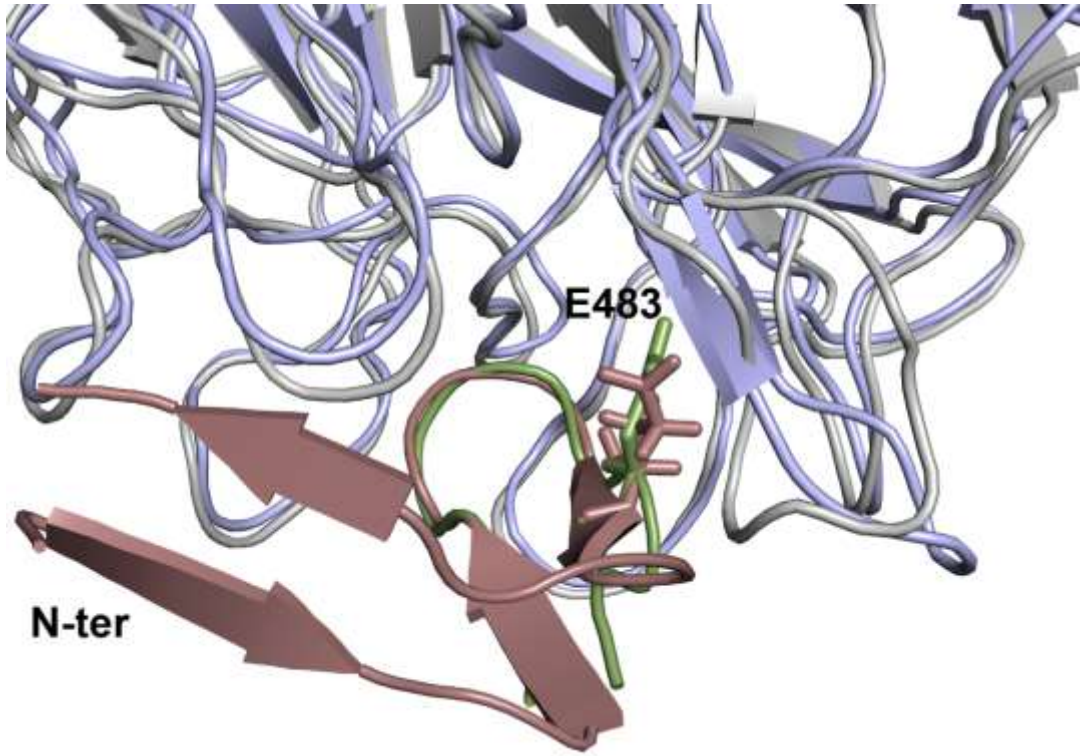
Glu480 OE2···Arg415 NH2

Glu483OE1···Arg380 NH1
→ 0.6 Å longer

The Kelch and the ETGE motif residues in the X-ray structure involved in direct interactions are shown as sticks (oxygen atoms in red, nitrogen atoms in blue) and are labeled. Polar interactions within 3.5 Å are shown as blue dashed lines.



Overlay of the NRF2 peptide (raspberry colour)–Kelch domain complex (light orange, PDB ID: 2FLU) and the DPP III peptide (green) – Kelch (light blue, PDB ID: 6TG8) complex structures.

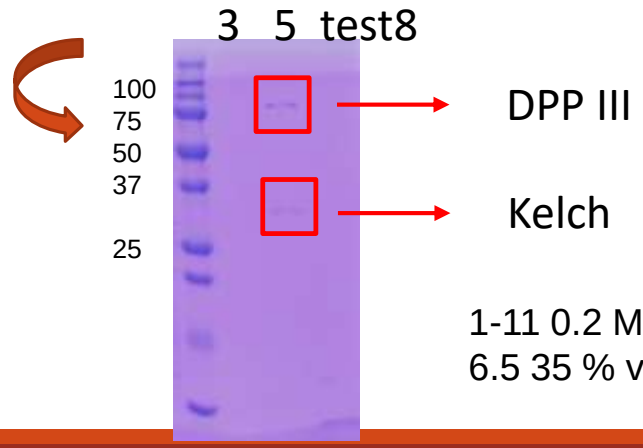


Overlay of the 24 AA peptide (brown) and the Kelch domain (gray) after ASMD followed by 150 ns of productive MD simulations on the 11 AA peptide (green) in its X-ray complex with Kelch (light blue).

Crystallization of the DPP III–Kelch complex

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

- non diffracting crystals, Elettra - XRD2



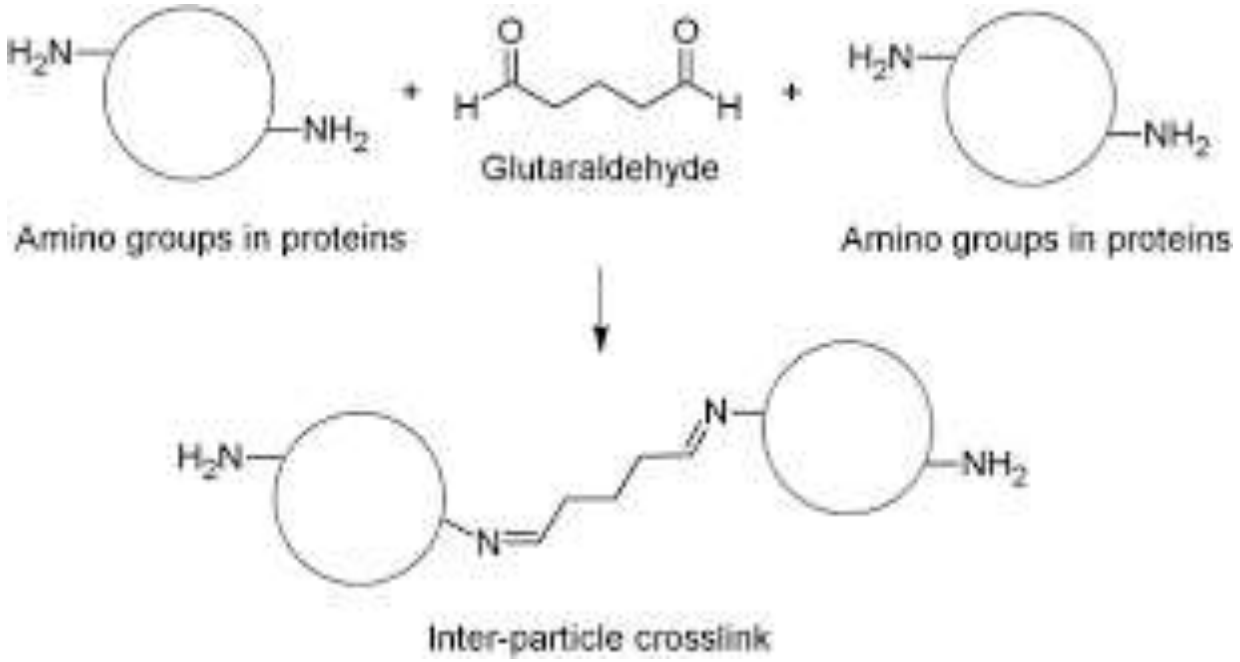
- protein samples after SEC in 1:1 molar ratio (DPP III variants: N6, R623W)
- 2304 different crystallization conditions

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)

FUTURE WORK

- purification of Kelch:DPP III complex by SEC
- seeding in new conditions
- addition of substrates (thermostability screening)
- crystallization with different mutants
- cross-linking the protein complex in a dilute solution using various concentrations of gluteraldehyde (or similar) and subsequently isolating the covalently cross-linked complex using SEC

THANK YOU FOR YOUR ATTENTION!


H2N
NH2
O
H
O
H
Glutaraldehyde
H2N
NH2
Amino groups in proteins
Amino groups in proteins
H2N
N
N
NH2
Inter-particle crosslink