Protein crystallization with alternative polymer precipitants

T. Skálová,[1] J. Dušková,[1] J. Hašek, [1] P. Kolenko,[1] T. Kovaľ,[2] A. Štěpánková,[1] J. Dohnálek[1]

[1] *Institute of Macromolecular Chemistry, AS CR, v.v.i., Heyrovského nám. 2, 162 06 Praha 6, Czech Republic,* [2] *Institute of Physics AS CR, v.v.i., Na Slovance 2, 182 21 Praha 8, Czech Republic*

t.skalova@gmail.com

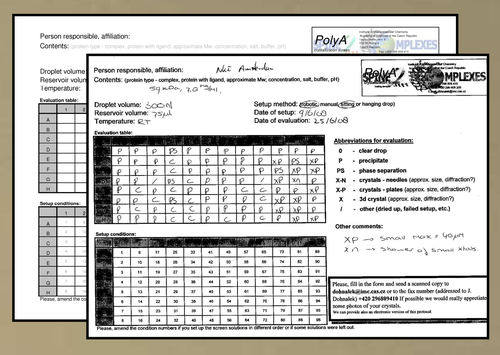
Introduction

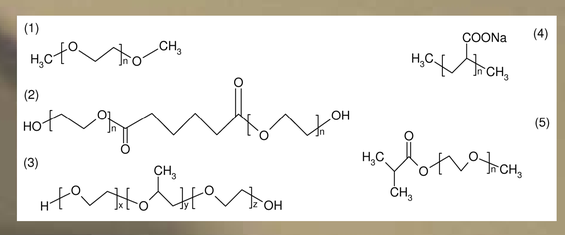
Use of polymer-based precipitants is widely spread in the current protein crystallization screens. In spite of that, the variety of the used polymeric compounds is limited. Our effort was focused on widening the spectrum of available polymer precipitants with potential applications in crystallization of complexes.

PolyA and Poly B crystallization screens

As a result of polymer testing, eight polymers were selected on the basis of their solubility and other properties at varying pH and salt concentrations. After preliminary testing on proteins, new crystallization screens PolyA and PolyB based on these eight polymers were formulated. Each screen uses 4 polymers and contains 96 conditions, altogether 2 screens x 4 polymers x 24 salt & pH = 192 crystallization conditions in PolyA and PolyB. The screens were tested in the frame of SPINE2-Complexes project and some good quality protein crystals have been obtained. The work was published (Skálová *et al*., J. Appl. Cryst., 2010, 43, 737-742).

Testing of the screens





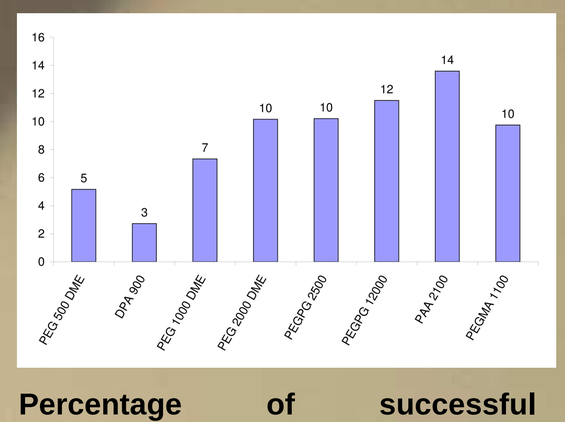
Chemical structure of polymers used in PolyA and PolyB. (1) Polyethylene glycol dimethyl ether, (2) di[poly(ethylene glycol)] adipate, (3) poly(ethylene glycol-*ran*-propylene glycol), (4) poly(acrylic acid), sodium salt, (5) poly(ethylene glycol) methyl ether methacrylate.

Polymers included in the screens PolyA and PolyB	**Polymer no.**	**Acronym**	**Average molecular weight**	**State**	**Stock solution**	**Producer, cat. no.**
Polyethylene glycol 500 dimethyl ether	5	PEG 500 DME	500	liquid	100 % (v/v)	Fluka 81313
Di(poly(ethylene glycol))adipate	6	DPA 900	900	liquid	100 % (v/v)	Aldrich 494852
Polyethylene glycol 1000 dimethyl ether	7	PEG 1000 DME	1 000	solid	65% (w/v)	Fluka 81312
Polyethylene glycol 2000 dimethyl ether	8	PEG 2000 DME	2 000	solid	65 % (w/v)	Fluka 81314
Poly(ethylene glycol-*ran*-propylene glycol)	10	PEGPG 2500	2 500	liquid	50 % (v/v)	Aldrich 438197
Poly(ethylene glycol-*ran*-propylene glycol)	11	PEGPG 12000	12 000	liquid	40 % (v/v)	Aldrich 438200
Poly(acrylic acid), sodium salt;	12	PAA 2100	2 100	solid	40 % (w/v)	Polysciences 06568
Poly(ethylene glycol) methyl ether methacrylate	13	PEGMA 1100	1 100	solid	50 % (w/v)	Aldrich 447951
Polymers considered unsuitable	**Polymer no.**	**Reason for rejection**	**Average molecular weight**	**State**	**Stock solution**	**Producer, cat. no.**
Polypropylene glycol P 1200	1	cloudy solution with salts	1 200	liquid	100 % (v/v)	Fluka 81370
Polypropylene glycol P 2000	2	phase separation with salts	2 000	liquid	100 % (v/v)	Fluka 81380
Polypropylene glycol triol 4800	3	cloudy solution with salts	4 800	liquid	100 % (v/v)	Fluka 17285
Polypropylene glycol diol 3000	4	phase separation with salts	3 000	liquid	100 % (v/v)	Fluka 17284
Polyvinyl alcohol 4-98 (Mowiol 4-98)	9	low solubility	22 000	solid	-	Fluka 81382
Poly(ethylene glycol) (200) adipate	14	low surface tension	530	liquid	50 % (v/v)	Polysciences 21509
Poly(ethylene glycol) phenyl ether acrylate	15	non miscible with water	324	liquid	-	Aldrich 407348
Poly(ethylene glycol) distearate	16	insoluble in water	930	solid	-	Aldrich 205413

Survey of the tested 16 polymers. Precipitants chosen for the protein crystallization screens PolyA and PolyB are in the first part of the table. Stock solution concentration is shown as used in the reported trials and for PolyA and PolyB preparation.

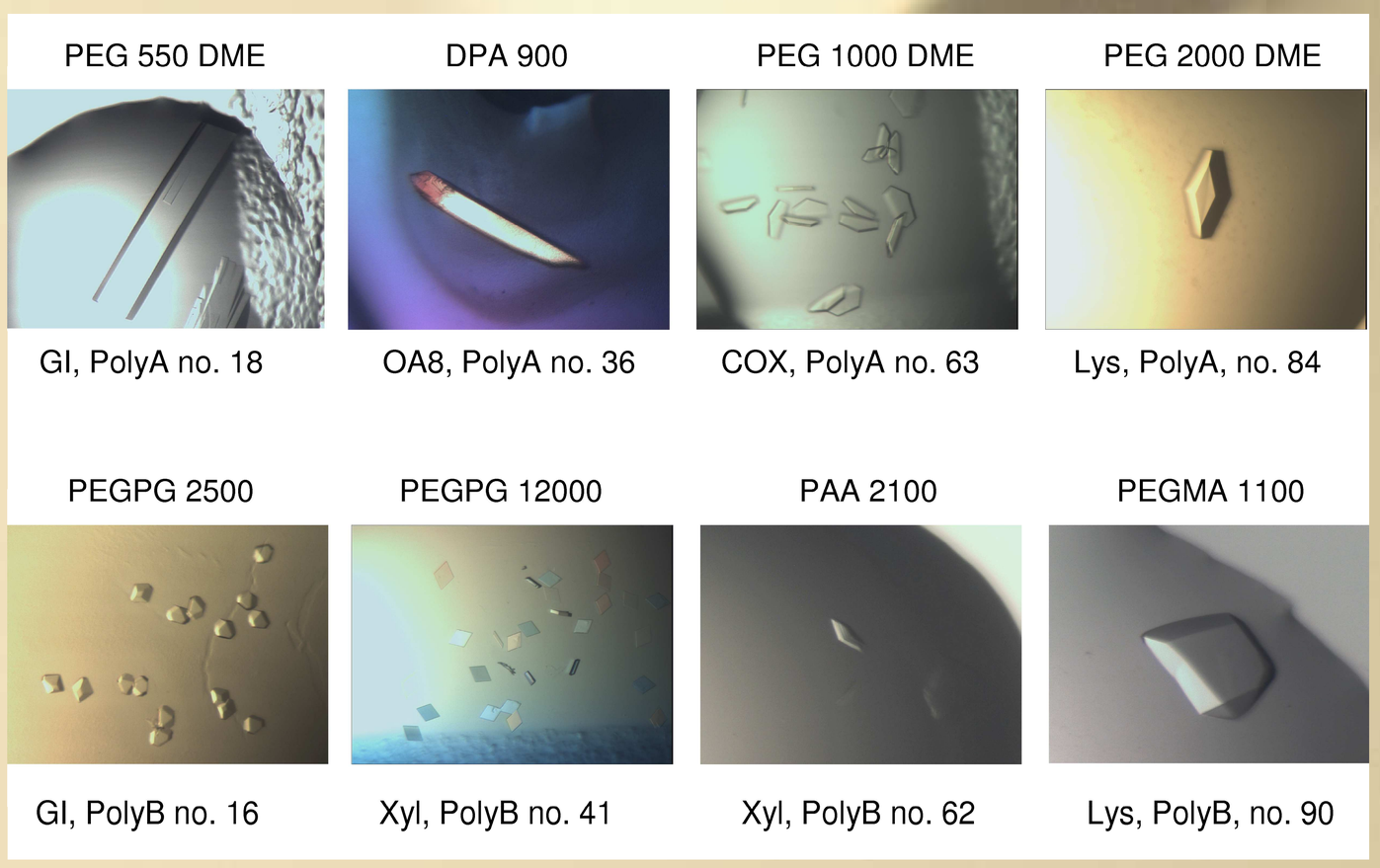
Salt	**Buffer** (0.1 M)	PolyA				PolyB			
		30% (v/v) PEG 500 DME	40% (v/v) DPA 900	25% (w/v) PEG 1000 DME	35% (w/v) PEG 2000 DME	30% (v/v) PEGPG 2500	20% (v/v) PEGPG 12000	30% (w/v) PAA 2100	40% (w/v) PEGMA 1100
None	none	**1**	**25**	**49**	**73**	**1**	**25**	**49**	**73**
None	sodium acetate/HCl, pH 4.5	**2**	**26**	**50**	**74**	**2**	**26**	**50**	**74**
0.2 M ammonium acetate	sodium acetate/HCl, pH 4.5	**3**	**27**	**51**	**75**	**3**	**27**	**51**	**75**
None	BIS-TRIS/HCl, pH 5.5	**4**	**28**	**52**	**76**	**4**	**28**	**52**	**76**
0.2 M ammonium sulfate	BIS-TRIS/HCl, pH 5.5	**5**	**29**	**53**	**77**	**5**	**29**	**53**	**77**
0.2 M sodium chloride	BIS-TRIS/HCl, pH 5.5	**6**	**30**	**54**	**78**	**6**	**30**	**54**	**78**
0.2 M ammonium acetate	BIS-TRIS/HCl, pH 5.5	**7**	**31**	**55**	**79**	**7**	**31**	**55**	**79**
0.2 M magnesium chloride	BIS-TRIS/HCl, pH 5.5	**8**	**32**	**56**	**80**	**8**	**32**	**56**	**80**
None	BIS-TRIS/HCl, pH 6.5	**9**	**33**	**57**	**81**	**9**	**33**	**57**	**81**
0.2 M ammonium sulfate	BIS-TRIS/HCl, pH 6.5	**10**	**34**	**58**	**82**	**10**	**34**	**58**	**82**
0.2 M sodium chloride	BIS-TRIS/HCl, pH 6.5	**11**	**35**	**59**	**83**	**11**	**35**	**59**	**83**
0.2 M calcium chloride	MES/NaOH, pH 6.5	**12**	**36**	**60**	**84**	**12**	**36**	**60**	**84**
0.01M zinc sulfate	MES/NaOH, pH 6.5	**13**	**37**	**61**	**85**	**13**	**37**	**61**	**85**
0.2 M ammonium acetate	HEPES/NaOH, pH 7.0	**14**	**38**	**62**	**86**	**14**	**38**	**62**	**86**
0.2 M magnesium chloride	HEPES/NaOH, pH 7.0	**15**	**39**	**63**	**87**	**15**	**39**	**63**	**87**
None	HEPES/NaOH, pH 7.5	**16**	**40**	**64**	**88**	**16**	**40**	**64**	**88**
0.2 M ammonium sulfate	HEPES/NaOH, pH 7.5	**17**	**41**	**65**	**89**	**17**	**41**	**65**	**89**
0.2 M sodium chloride	HEPES/NaOH, pH 7.5	**18**	**42**	**66**	**90**	**18**	**42**	**66**	**90**
None	Tris/HCl, pH 8.5	**19**	**43**	**67**	**91**	**19**	**43**	**67**	**91**
0.2 M ammonium sulfate	Tris/HCl, pH 8.5	**20**	**44**	**68**	**92**	**20**	**44**	**68**	**92**
0.2 M sodium chloride	Tris/HCl, pH 8.5	**21**	**45**	**69**	**93**	**21**	**45**	**69**	**93**
0.2 M ammonium acetate	Tris/HCl, pH 8.5	**22**	**46**	**70**	**94**	**22**	**46**	**70**	**94**
0.2 M magnesium chloride	Tris/HCl, pH 8.5	**23**	**47**	**71**	**95**	**23**	**47**	**71**	**95**
0.1 M sodium chloride	glycine/NaOH, pH 9.0	**24**	**48**	**72**	**96**	**24**	**48**	**72**	**96**

Formulation of the crystallization screens PolyA (solutions 1-96) and PolyB (solutions 1-96). Solution with a given number (shown in the table) contains salt and buffer indicated in the corresponding row and polymer at the top of the corresponding column.

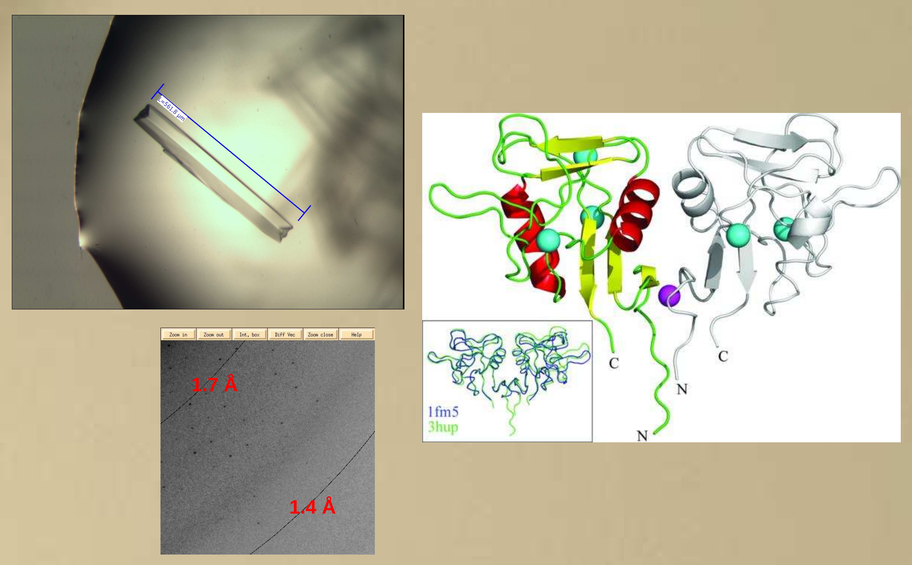


Percentage of successful crystallization hits for each polymer in the screens PolyA and PolyB (ca. 500 experiments performed per polymer).

Crystals obtained with PolyA and PolyB



Structure of CD69 obtained using a novel polymer



Beautiful crystals of human CD69 with lengths up to 561.8 μm were obtained using hanging drop vapor diffusion method with 1 μl of protein and 1 μl of precipitant solution: 30 % (*v*/*v*) di[poly(ethylene glycol)] adipate and 0.1M imidazole, pH 6.6. The diffraction data were measured at ID14-1 BESSYII (Berlin) and processed up to 1.37 Å resolution. The structure was deposited under PDB code 3HUP and published (Kolenko *et al*., Acta Cryst. (2009), F65, 1258–1260). The high resolution structure of the protein yielded details about asymmetry of the dimer interface and binding of a sodium ion at the dimer interface.

Conclusions

Eight of the sixteen selected polymers were subjected to complex experimental testing which led to the formulation of two novel protein crystallization screens PolyA and PolyB. This work extends the pool of available polymeric protein precipitants and confirms applicability of polyethylene glycol dimethyl ethers, di(poly(ethylene glycol)) adipate, poly(ethylene glycol-ran-propylene glycol), poly(acrylic acid) sodium salt, and polyethylene glycol methyl ether methacrylate. The tests showed significance of polymer type for results of a crystallization trial and confirmed the importance of variation of the same crystallization condition by exchanging polymeric precipitants. PAA 2100 and PEPG 12000 proved to be the most successful candidates for protein crystallization screening. Polymer DPA 900 led to new structural results for human CD69.

This research was supported by the European Commission (integrated project SPINE2-Complexes no. LSHG-CT-2006-031220), by GA AV CR (project IAA500500701), and by GA CR (project 305/07/1073).