

Perspective

A Short Review on Cryoprotectants for 3D Protein Structure Analysis

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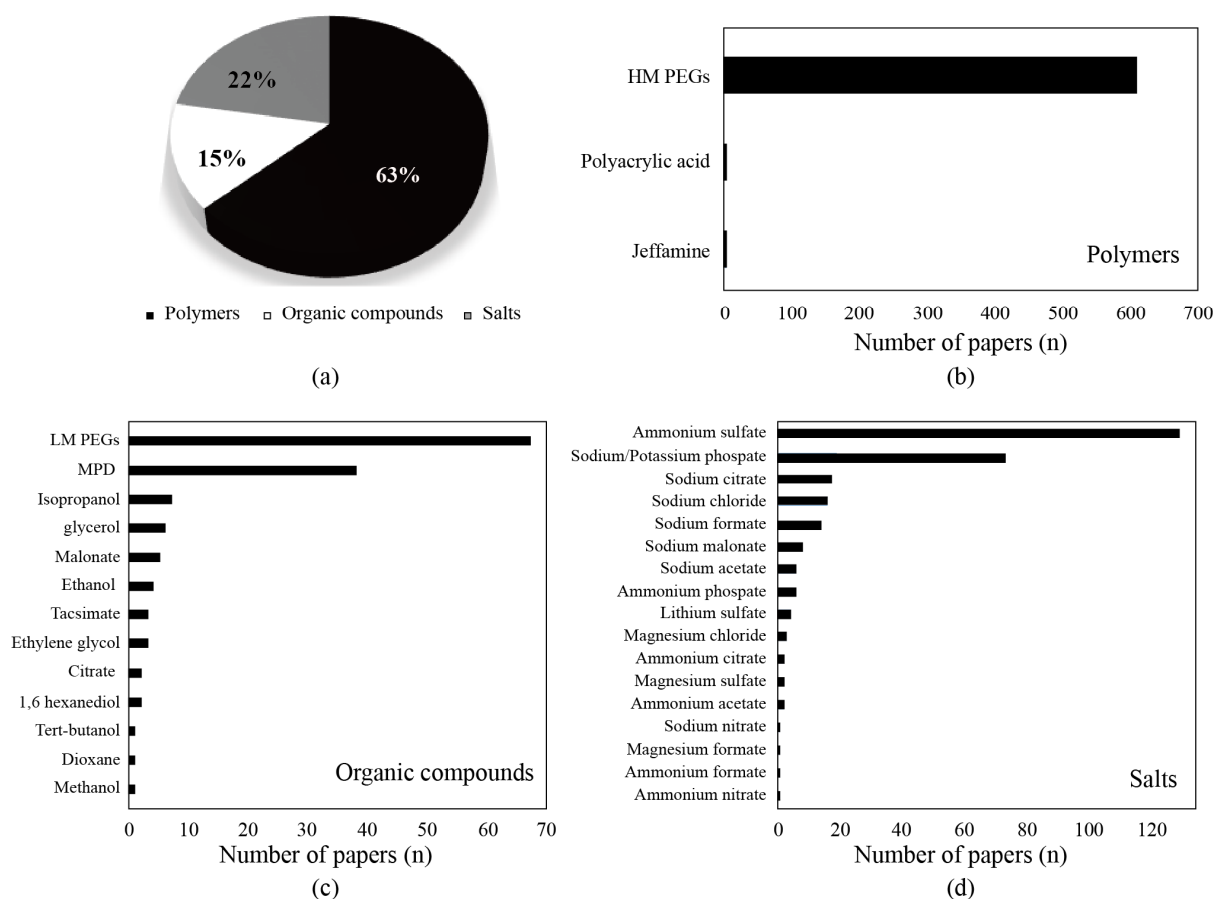


Figure S1. The statistic analysis of precipitants for crystallization in the structures reported to the PDB in the period of 2015 to 2021(996 publications are selected randomly). The types of precipitant: (a).Polymers are shown as black , organic compounds as white and salts as gray. (b).The most frequently applied polymers are HM(high molecular weight) PEGs. (c). The most frequently and widely used organic compounds are LM(low molecular weight) PEGs. MPD (2-Methyl-2,4-pentanediol), isopropanol glycerol, malonate, ethanol, tascimate, ethylene glycol, citrate, 1,6 hexanediol, tert-butanol, dioxane, methanol are used frequency orders of applied precipitating agents by crystallization. (d). The most frequently used salt as precipitating agents is ammonium sulfate. Sodium/potassium phosphate, sodium citrate, sodium chloride, sodium formate, sodium malonate, sodium acetate, ammonium phosphate, lithium sulfate, magnesium chloride, ammonium citrate, magnesium sulfate, ammonium acetate, sodium nitrate, magnesium formate, ammonium formate, ammonium nitrate are used frequency orders of applied precipitants for crystallization.

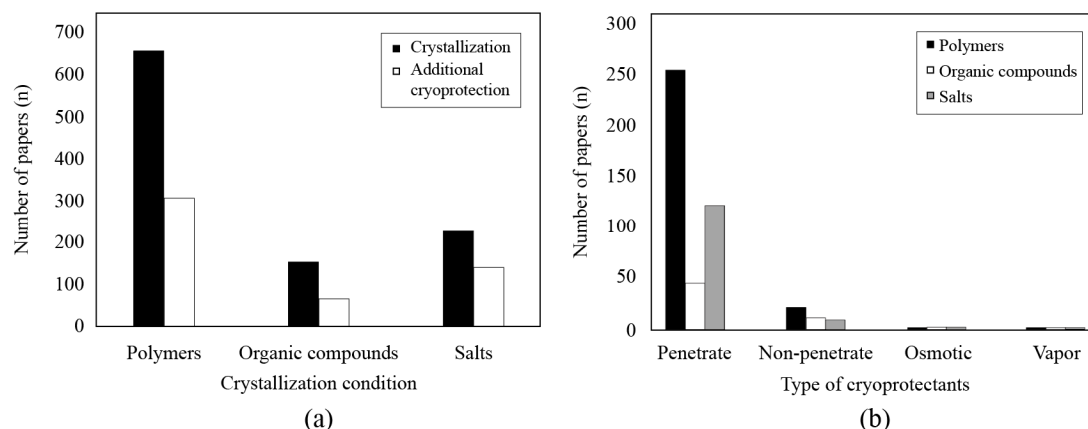


Figure S2. The use of additional cryoprotection. (a) The bar graph represents the publication numbers (Y-axis) versus crystallization conditions (X-axis). The publication numbers used direct crystallization conditions as cryoprotectants are shown as black bar and additional cryoprotections have been used are shown as white bar. (b) The used cryoprotectants after crystallization which are classified according to penetrate, non-penetrate, osmotic and vapor, are represented against the publication numbers. Polymers are represented as black bar, organic compound as white bar and salts as gray bar. The analyzed protein structures which have been published in the period of 2015 – 2021, are selected randomly (n = 996).

Table S1. Lists of commercially available cryoprotectants from two representative companies.

Hampton research			Molecular Dimensions		
No.	Conc.	Cryoprotectant	No.	Conc.	Cryoprotectant
Same products*					
Penetrate					
1	100%	(+/-)-2-Methyl-2,4-pentanediol (MPD)	1	100%	MPD
2	100%	1,2-Propanediol	2	100%	1,2-Propanediol
3	100%	2,3-Butanediol	3	100%	2,3-Butanediol
4	100%	Glycerol	4	100%	Glycerol
5	100%	Ethylene glycol	5	100%	Ethylene glycol
6	50%	Diethylene glycol	6	50%	Diethylene glycol
7	100%	Dimethyl sulfoxide (DMSO)	7	100%	DMSO
8	100%	Polyethylene glycol (PEG) 400	8	100%	PEG 400
9	30%	D-(+)-Maltose monohydrate	9	30%	D-(+)-Maltose monohydrate
Non-penetrate					
10	50%	PEG 1,000	10	50%	PEG 1,000
11	50%	PEG 3,350	11	50%	PEG 3,350
12	50%	PEG monomethyl ether (MME) 5,000	12	50%	PEG MME 5,000
13	50%	PEG 8,000	13	50%	PEG 8,000
14	50%	PEG 10,000	14	50%	PEG 10,000
Other products					
Penetrate					
15	50%	Pentaerythritol propoxylate (5/4 PO/OH)	15	30%	D-Glucose
16	100%	Polypropylene glycol P 400	16	50%	Polypropylene glycol MME 500
17	70%	D-(+)-Sucrose	17	12.5%, 12.5%, 37.5%, 12.5%	Diethylene glycol, MPD, 1,2-Propanediol, DMSO
18	70%	D-Sorbitol	18	25%, 25%, 25%	Diethylene glycol, Glycerol, 1,2-Propanediol
19	35%	meso-Erythritol	19	25.0%, 12.5%, 12.5%, 12.5%, 12.5%	Diethylene glycol, Ethylene glycol, MPD, 1,2-Propanediol, Glycerol
20	70%	Xylitol	20	12.5%, 25%, 12.5%, 12.5%, 12.5%	Ethylene glycol, MPD, 1,2-Propanediol, DMSO, Glycerol
21	15%	myo-Inositol	21	12.5%, 12.5%, 25%, 12.5%, 12.5%	Diethylene glycol, Ethylene glycol, 1,2-Propanediol, DMSO, Glycerol
22	20%	D-(+)-Raffinose pentahydrate	22	12.5%, 12.5%, 12.5%, 12.5%, 25%	Diethylene glycol, MPD, 1,2-Propanediol, DMSO, Glycerol

23	50%	D-(+)-Trehalose dihydrate	23	25%, 12.5%, 12.5%, 12.5%, 12.5%	MPD, 1,2-Propanediol, DMSO, Glycerol, Ethylene glycol
24	70%	D-(+)-Glucose monohydrate	24	12.5%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5%	Diethylene glycol, MPD, 1,2-Propanediol, DMSO, Glycerol, Ethylene glycol
25	100%	PEG MME 550	25	25%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5%	MPD, 1,2-Propanediol, DMSO, Glycerol, Ethylene glycol, 1,4-Dioxane
26	100%	PEG 200	26	12.5%, 25%, 12.5%, 12.5%, 12.5%	Ethylene glycol, MPD, Glycerol, 2,3-Butanediol, DMSO
27	6.0 M	1,6-Hexanediol	27	25%, 12.5%, 37.5%, 12.5%	Diethylene glycol, MPD, 2,3-Butanediol, 1,4-Dioxane
28	6.0 M	L-Proline	28	25%, 12.5%, 25%, 12.5%	Diethylene glycol, Glycerol, 2,3-Butanediol, 1,4-Dioxane
29	4.0 M	Trimethylamine N-oxide dihydrate	29	12.5%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5%	Diethylene glycol, Ethylene glycol, Glycerol, 2,3-Butanediol, DMSO, 1,4-Dioxane
30	50%, 25%	Ethylene glycol, 3-(1-Pyridinio)-1-propanesulfonate (NDSB-201)	30	12.5%, 12.5%, 12.5%, 25%, 25%	Diethylene glycol, Glycerol, 1,2-Propanediol, DMSO, 1,4-Dioxane
31	100%	Tacsimate™ pH 7.0	31	12.5%, 12.5%, 12.5%, 25%, 37.5%	Ethylene glycol, Glycerol, 1,2-Propanediol, DMSO, 1,4-Dioxane
32	50%	NDSB-201	32	25%, 25%, 25%, 25%	Diethylene glycol, Ethylene glycol, Glycerol, 1,4-Dioxane
			33	12.5%, 25%,	Diethylene glycol, Ethylene glycol,

	12.5%, 25%, 25%	1,2-Propanediol, DMSO, 1,4-Dioxane
34	12.5%, 12.5%, 12.5%, 12.5%, 25%, 25%	Ethylene glycol, Glycerol, 1,2-Propanediol, 2,3-Butanediol, DMSO, 1,4-Dioxane
35	0.3 M, 0.3 M	Xylitol, D-Glucose
36	30%	xylitol
37	30%	D-Trehalose
38	30%	Sucrose
39	12.5%, 12.5%, 12.5%, 12.5%, 20%, 30%	Diethylene glycol, Glycerol, 1,2-Propanediol, 2,3-Butanediol, DMSO, 1,4-Dioxane
40	0.3 M, 0.3 M, 0.3 M	D-Trehalose, Sucrose, D-Maltose
41	100%	1,4-Dioxane
42	12.5%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5 mM	Diethylene glycol, Ethylene glycol, MPD, 1,2-Propanediol, DMSO, NDSB 201
43	12.5%, 25%, 12.5%, 12.5%, 12.5%, 12.5 mM	Diethylene glycol, Ethylene glycol, MPD, 1,2-Propanediol, Glycerol, NDSB 201
44	12.5%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5 mM	Diethylene glycol, MPD, 1,2-Propanediol, DMSO, Glycerol, NDSB 201
45	12.5%, 12.5%, 12.5%, 12.5%, 12.5%, 12.5 mM, 12.5%	Diethylene glycol, MPD, 1,2-Propanediol, DMSO, Glycerol, NDSB 201, Ethylene glycol
46	12.5%, 12.5%, 12.5%,	Diethylene glycol, MPD, 1,2-Propanediol,

	25%,	DMSO,
	12.5%,	Glycerol,
	12.5 mM	NDSB 201
47	12.5%,	Diethylene glycol,
	12.5%,	MPD,
	12.5%,	1,2-Propanediol,
	12.5%,	DMSO,
	12.5%,	Glycerol,
	12.5 mM,	NDSB 201,
	12.5%	1,4-Dioxane
48	12.5%,	Diethylene glycol,
	12.5%,	MPD,
	12.5%,	1,2-Propanediol,
	12.5%,	Glycerol,
	12.5 mM,	NDSB 201,
	25%,	Ethylene glycol,
	12.5%	1,4-Dioxane
49	12.5%,	Diethylene glycol,
	12.5%,	1,2-Propanediol,
	12.5%,	DMSO,
	12.5%,	Glycerol,
	12.5 mM,	NDSB 201,
	12.5%	1,4-Dioxane
50	12.5%,	Diethylene glycol,
	12.5%,	Ethylene glycol,
	12.5%,	MPD,
	12.5%,	Glycerol,
	12.5%,	2,3-Butanediol,
	12.5%,	DMSO,
	12.5 mM	NDSB 201
51	12.5%,	Diethylene glycol,
	25%,	Ethylene glycol,
	12.5%,	MPD,
	12.5%,	Glycerol,
	12.5%,	2,3-Butanediol,
	12.5 mM	NDSB 201
52	12.5%,	Diethylene glycol,
	12.5%,	MPD,
	12.5%,	Glycerol,
	12.5%,	2,3-Butanediol,
	25%,	DMSO,
	12.5 mM	NDSB 201
53	12.5%,	Diethylene glycol,
	12.5%,	MPD,
	12.5%,	Glycerol,
	12.5%,	2,3-Butanediol,
	12.5%,	DMSO,
	12.5%,	1,4-Dioxane,
	12.5 mM	NDSB 201
54	12.5%,	Diethylene glycol,
	12.5%,	Ethylene glycol,
	12.5%,	MPD,

	12.5%,	Glycerol,
	12.5%,	2,3-Butanediol,
	12.5%,	1,4-Dioxane,
	12.5 mM	NDSB 201
55	12.5%,	Diethylene glycol,
	12.5%,	MPD,
	12.5%,	1,2-Propanediol,
	12.5%,	DMSO,
	12.5%,	Glycerol,
	12.5 mM,	NDSB 201,
	12.5%	Ethylene glycol
56	100 mM	NDSB-201

Non-penetrate

33	50%	Polyvinylpyrrolidone K 15
34	80%	PEG 1600
35	50%	PEG 4,000

Others (include the mixture)

36	5.0 M	Lithium acetate dihydrate	57	2.5 M	Lithium sulfate
37	10.0 M	Lithium chloride	58	2.5 M	Lithium formate monohydrate
38	4.0 M	Lithium formate monohydrate	59	2.5 M	Sodium malonate dibasic monohydrate
39	8.0 M	Lithium nitrate	60	1.0 M	Sodium sulfate
40	2.0 M	Lithium sulfate monohydrate	61	2.5 M	Sodium formate
41	3.4 M	Sodium malonate pH 7.0	62	0.3 M,	Sodium malonate dibasic
				0.3 M,	monohydrate, Sodium sulfate,
				0.3 M	Sodium formate
42	3.4 M	Magnesium acetate tetrahydrate			
43	5.0 M	Sodium chloride			
44	7.0 M	Sodium formate			
45	7.0 M	Sodium nitrate			
46	1.0 M	Sodium sulfate decahydrate			
47	30%,	PEG 3,350,			
	20%,	Glycerol			
48	30%,	PEG 400,			
	10%,	PEG 20,000,			
	5%,	Glycerol,			
	5%	NDSB-201			

* Blue colored written products names from No. 1 to 14 are the same selling items by two different companies.