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A crystallization screen based on alternative polymeric precipitants

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Most commercially available crystallization screens are sparse-matrix screens with a predominance of inorganic salts and polyethylene glycols (PEGs) as precipitants. It was noted that commercially available screens are largely unsatisfactory for the purpose of the crystallization of multimeric protein and protein–nucleic acid complexes. This was reasoned to be a consequence of the redundancy in screening crystallization parameter space by the predominance of PEG as a precipitant in standard screens and it was suggested that this limitation could be overcome by introducing a variety of other organic polymers. Here, a set of 288 crystallization conditions was devised based on alternative polymeric precipitants and tested against a set of 20 different proteins/complexes; finally, a screen comprising the 96 most promising conditions designed to complement PEG- and salt-based commercial screens was proposed.

1. Introduction

The precipitants currently utilized for protein crystallization can, by and large, be divided into four groups based on their physico-chemical characteristics: (i) inorganic salts, (ii) organic solvents, (iii) alcohols and (iv) organic water-soluble polymers (Patel *et al.*, 1995). Recent surveys of crystallization conditions published within the Protein Data Bank (PDB) clearly show that PEGs of various molecular mass are by far the most widespread polymeric compounds in use. Peat and coworkers scanned 8289 entries in the PDB and found around 4000 crystallization conditions containing a PEG component (Peat *et al.*, 2005). A similar study performed on 650 protein–protein complexes deposited within the PDB shows an even more pronounced dominance, with 70–80% of all conditions containing PEGs (Radaev *et al.*, 2006). Therefore, PEGs can be considered to be the most successful protein-crystallization agents (Gilliland *et al.*, 1994; McPherson, 1999; Kimber *et al.*, 2003).

Historically, the first structure of a protein crystallized in the presence of PEG was reported in 1975 (Wishner *et al.*, 1975). In 1976, the general usefulness of PEGs in protein crystallization was proposed (McPherson, 1976). The sparse-matrix screen devised by Jancarik and Kim in 1991 (Jancarik & Kim, 1991) contains 24 PEG-based conditions out of a total of 50, while 14 conditions are based on highly concentrated salts as precipitants. With some slight modifications, this screen is still commercially available and in use widely. Accordingly, current commercial crystallization screens are either dominated by PEGs or their monomethyl ethers (PEG MMEs), which can often directly replace a PEG precipitant component (Brzozowski & Tolley, 1994), or do not focus on polymers as

Table 1
Proteins used in this study.

Protein/complex	Abbreviation	Source organism	Supplier order No./ protein production	(Complex) molecular mass (kDa)	Known oligomerization state(s)
Commercially available 'benchmark' proteins					
Alcohol dehydrogenase	Adh	*Saccharomyces cerevisiae*	Sigma A3263	∼140.0	Homotetrameric
α-Amylase	Amy	*Bacillus subtilis*	Fluka 10069	∼47.0	Monomeric
DNAse I from bovine pancreas	DNAse	*Bos taurus*	Sigma D4263	∼62.0	Homodimeric
Ferritin type I from horse spleen	Fe	*Equus ferus*	Sigma F4503	∼440.0	24-mer complex of H and L subunits
Hen egg-white lysozyme	HEWL	*Gallus gallus*	Fluka 62971	14.3	Monomeric
Human insulin	I	*Homo sapiens*	Sigma I9278	5.8 (monomer)	Monomeric/homodimeric/ homohexameric
Core streptavidin	Sa	*Streptomyces avidinii*	Bacterial expression, refolding	∼269.0	Homotetrameric
Xylanase	Xy	*Trichoderma viride*	Fluka 95595	∼22	Monomeric
Proteins and complexes from past and ongoing research projects					
The four MBT repeats of Sfmbt in complex with peptide RHR^{me}KVLR	dSfmbt-4MBT	*Drosophila melanogaster*	Bacterial expression as cleavable hexahistidine-fusion protein	51.3	Monomeric, in complex with 7-amino-acid peptide ligand
The two MBT repeats of *sex-comb on midleg*	Scm-2MBT	*D. melanogaster*	Bacterial expression as cleavable hexahistidine-fusion protein	29.4	Monomeric
2-Methylisocitrate lyase	PrpB	*Escherichia coli*	Bacterial expression	128.0	Homotetrameric
3α-Hydroxysteroid dehydrogenase/ carbonyl reductase	HSDH	*Comamonas testosteroni*	Bacterial expression	52.8	Homodimeric
S-Adenosylmethionine:tRNA ribosyl transferase/isomerase (QueA)–tRNA stem loop complex	BsQueA	*Bacillus subtilis*	Bacterial expression as cleavable GST-fusion protein	38.5 + 4.5 (RNA)	Monomeric with an additional RNA component
S-Adenosylmethionine:tRNA ribosyl transferase/isomerase (QueA)–tRNA stem loop complex	EcQueA	*E. coli*	Bacterial expression as cleavable GST-fusion protein	39.4 + 4.5 (RNA)	Monomeric with an additional RNA component
S-Adenosylmethionine:tRNA ribosyl transferase/isomerase (QueA)–tRNA stem loop complex	HiQueA	*Haemophilus influenzae*	Bacterial expression as cleavable GST-fusion protein	40.6 + 4.5 (RNA)	Monomeric with an additional RNA component
Spliceosomal assembly complex 7	SAC7	*Homo sapiens*	Bacterial expression, *in vitro* reconstitution	∼70.0	Oligohexameric
Spliceosomal assembly complex 9	SAC9	*H. sapiens*	Bacterial expression, *in vitro* reconstitution	∼137.0	Oligooctameric
Spliceosomal assembly complex 10	SAC10	*H. sapiens*	Bacterial expression, *in vitro* reconstitution	∼135.0	Oligooctameric
Spliceosomal assembly complex including an RNA component	SAC-RNA	*H. sapiens*	Bacterial expression, *in vitro* reconstitution	∼115.0 + 4.0 (RNA)	Oligoheptameric with an additional RNA component
Cytokine receptor–ligand complex	CRLC	*H. sapiens*	Bacterial expression, refolding	∼66.0	Heterotetrameric (including the polypeptide hormone ligand)

precipitants. For example, 20 of the 48 conditions in the Hampton Research Natrix screen contain PEG or PEG MME, as do 28 of the 48 conditions in the Molecular Dimensions Ltd 3D Structure Screen, 66 of the 96 conditions in Molecular Dimensions ProPlex and 81 of the 96 conditions in Qiagen JCSG1. Moreover, many screens exclusively contain PEGs as precipitants (Hampton Research PEG/Ion, Molecular Dimensions Ltd PACT, Jena Bioscience JBScreen Classic 1–5 *etc*.). While this seems to make sense in the light of the obvious success of PEGs, the success rate might be biased by their widespread dominance within initial screens. In fact, a variety of alternative precipitants have recently been described as being useful for macromolecular crystallogenesis. In 1995, Patel and coworkers successfully evaluated six polymer classes with chemistry disparate from that of PEGs for their potential as crystallization precipitants (Patel *et al.*, 1995). However, apart from the PEG MMEs, which were introduced in 1994 (Brzozowski & Tolley, 1994), alternative polymers such as the Jeffamine polyetheramines (Guillet *et al.*, 2004; Liu *et al.*, 2006; Lloyd *et al.*, 1994; Cudney *et al.*, 1994), pentaerythritol propoxylate and pentareythritol ethoxylate (Gulick *et al.*, 2002), polyvinyl pyrrolidone, polypropylene glycol, polyvinyl alcohol and polyacrylate (Patel *et al.*, 1995) have so far only sporadically been introduced into standard crystallization screens. Some biopolymers or modified biopolymers, such as carboxymethyl cellulose (Patel *et al.*, 1995) or poly-γ-glutamate (Hu *et al.*, 2008), with a rather high molecular mass have also been reported to induce protein crystallization. Furthermore, di[poly(ethylene glycol)] adipate, a compound partly based on PEG chemistry, has recently been described to be useful for crystallogenesis (Kolenko *et al.*, 2009). However, the latter polymer types have not yet been included in commercial screens.

To close this gap, we set out to devise a screen that systematically searches for crystallization conditions with alternative polymeric precipitants. To expand the precipitant diversity even more, we also scanned the current water-soluble polymer market for chemical variants or alternatives to the

precipitants mentioned above. Owing to their particular properties as surface-active substances, ion exchangers and/or viscosity modifiers, many polymer variants have recently been developed. As an example, the anticipation of possible restrictions on phosphates for dishwasher products in the European Union has triggered the development of Sokalan CP 42, a modified polycarboxylate with particular antifilming and scale-inhibition properties (McCoy, 2005). However, the use of such polymers is often avoided owing to their prohibitive viscosity values within the useful concentration range as a precipitant.

Our screen entails a relatively narrow range of pH and salt concentrations centred on physiological values to increase its suitability for sensitive macromolecular complexes (Radaev *et al.*, 2006), while every condition contains at least one alternative polymeric precipitant.

2. Materials and methods

2.1. Polymeric precipitants and screen preparation

The Sokalan polymers CP 42, CP 5, CP 12 S, HP 56 and HP 66 K were a gift from BASF AG, Ludwigshafen, Germany. Walocel CRT 10 G and Walocel HM 100 were a gift from Dow Wolff Cellulosics GmbH, Walsrode, Germany. The Jeffamine polyether amines ED2003, SD2001, D2000, M2005, M2070 and T403 were a gift from Huntsman GmbH, Germany. Glascol W13 was a gift from Ciba AG, Basel, Switzerland. All other polymers were bought from Sigma–Aldrich GmbH, Germany. The Jeffamines were prepared as a 50%(*v*/*v*) solution in distilled water and titrated to pH 7.0 with HCl. The Sokalan polymers, polyacrylate and its copolymers, di[poly (ethylene glycol)] adipate and poly(ethylene imine) compounds were adjusted to pH 7.0 with either concentrated HCl or concentrated NaOH depending on the initial pH. Likewise, stock solutions of malonate, citrate, acetate and formate were adjusted to pH 7.0.

2.2. Proteins used in this study

Commercially available proteins were purchased from Sigma–Aldrich in lyophilized form [hen egg-white lysozyme (HEWL), xylanase from *Trichoderma viride* (Xy), alcohol dehydrogenase from yeast (Adh), α-amylase from *Bacillus subtilis* (Amy), DNAse I from bovine pancreas (DNAse)] and prepared as a 20 g l^{-1} solution in distilled water, except for HEWL, which was dissolved at 50 g l^{-1}. Ferritin type I from horse spleen (Fe) and human insulin (I) were obtained as

Table 2
The 288-condition polymer screen, split into three parts (*A*–*C*) to conveniently address the issue of 96 wells per plate.

The three parts do not feature particular differences. Conditions selected for the final 96-condition screen are marked with an asterisk (*). All percentages are given as weight per volume (*w*/*v*).

(*a*) Part *A*.

No.	Precipitant†	Salt/additive	Buffer‡	Hits§	Viscosity¶
1	18% polyvinyl alcohol type II	—	H 7.5	Xy	+++
2	38% acrylic acid/maleic acid copolymer (50:50), sodium salt	—	T 8.0		++
3*	50% polypropylene glycol 400	—	M 5.5	Adh, Amy, DNAse, Sa, Xy	0
4*	12% polyvinyl pyrrolidone K15	5% dimethyl sulfoxide	H 6.0	Adh, SAC9, Xy	0
5*	45% polyacrylate 2100, sodium salt	—	H 6.5	Adh, BsQueA, EcQueA, HEWL, HiQueA	+
6	5% Jeffamine ED2003	0.2 *M* sodium chloride	H 7.5	HEWL	0
7	6% Walocel CRT 10 G	0.2 *M* lithium chloride	M 6.0	Adh, HEWL	+++
8	24% polyvinyl pyrrolidone K15	0.2 *M* K/Na phosphate pH 6.25	—	Adh, Xy	+
9	15% Jeffamine T403	0.2 *M* lithium chloride	H 6.5	HEWL	0
10	5% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* magnesium sulfate	H 7.0	Xy	0
11	3% Walocel HM 100	0.3 *M* lithium sulfate	P 7.0		+++
12	14% polyvinyl alcohol type II	0.08 *M* magnesium chloride	—	I, Xy	++
13*	14% acrylic acid/maleic acid copolymer (50:50), sodium salt	—	—	Adh, Sa	0
14	10% Jeffamine T403	20% ethanol	—		0
15	15% polypropylene glycol 400	0.05 *M* magnesium formiate	—	Xy	0
16	12% polyvinyl pyrrolidone K15	0.2 *M* potassium acetate	H 6.5		0
17*	12.5% polyacrylate 2100, sodium salt	0.5 *M* ammonium phosphate pH 8.5		Adh, HEWL, Sa	0
18	10% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* sodium thiocyanate	H 7.0		0
19*	19% acrylic acid/maleic acid copolymer (50:50), sodium salt	—	T 8.5	Adh, BsQueA, I	0
20*	10% polypropylene glycol 400	—	—	Xy	0
21	15% polyacrylate 2100, sodium salt, 5% pentaerythritol ethoxylate (15/4 EO/OH)	—	M 6.0		0
22*	5% polyacrylate 2100, sodium salt	—	—		0
23	7% Walocel CRT 10 G	0.3 *M* sodium chloride	M 6.0	Adh, HEWL	+++
24	4% acrylic acid/maleic acid copolymer (50:50), sodium salt, 2.5% Jeffamine M2070	0.05 *M* imidazole pH 7.0	—	I	0
25*	24% polyvinyl pyrrolidone K15	0.16 *M* sodium citrate pH 7.0	T 8.0	Adh, HEWL	+
26	25% Jeffamine T403	0.2 *M* lithium sulfate	T 8.0		0
27*	25% pentaerythritol propoxylate (5/4 PO/OH)	—	M 6.0	DNAse, I, Xy	0
28*	24% polyvinyl pyrrolidone K15	0.1 *M* sodium sulfate	—	Adh, SAC9	+

Table 2 (continued)

No.	Precipitant†	Salt/additive	Buffer‡	Hits§	Viscosity¶
29	10% Jeffamine SD2001	0.2 *M* lithium sulfate	H 6.5		0
30	18% polyvinyl pyrrolidone K15, 10% PEG 400	0.35 *M* potassium chloride	H 7.0	Adh, HEWL	+
31	18% polyvinyl alcohol type II	0.2 *M* lithium sulfate	—	Adh	+++
32*	35% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* calcium chloride	H 6.5	Adh, BsQueA, EcQueA, I, Xy	0
33	50% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* potassium chloride	P 7.0	BsQueA, Sa	+
34	40% pentaerythritol ethoxylate (15/4 EO/OH)	10% ethanol	—	BsQueA, Sa	+
35*	35% polypropylene glycol 400	—	P 7.0	Adh, Xy	0
36	30% polyvinyl pyrrolidone K15	0.2 *M* sodium chloride	M 5.5		0
37	20% Jeffamine T403	0.3 *M* imidazole pH 7.0	—		0
38*	20% Jeffamine D2000, 10% Jeffamine M2005	0.2 *M* sodium chloride	M 5.5	Adh, HEWL, I, Sa, Xy	0
39	30% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* potassium chloride	T 8.0	Adh, Xy	0
40	10% Jeffamine ED2003	—	T 8.5	HEWL	0
41*	15% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* sodium thiocyanate	H 7.0	Adh, I, Xy	0
42*	5% polyvinyl alcohol type II, 10% Jeffamine T403	0.2 *M* potassium acetate	H 7.0	HEWL, Xy	+
43	24% acrylic acid/maleic acid copolymer (50:50), sodium salt	0.2 *M* potassium acetate	—	BsQueA	0
44*	45% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* sodium chloride	M 6.0	CRLC, EcQueA, HiQueA, Sa, Xy	0
45	20% polyvinyl alcohol type II	—	—	Adh	+++
46	9% acrylic acid/maleic acid copolymer (50:50), sodium salt	—	—	I, Sa	0
47*	8% polyvinyl alcohol type II	10% 1-propanol	H 7.0	Adh, Xy	+
48	8% Walocel CRT 10 G	0.2 *M* potassium chloride	P 7.0	Adh, Fe, HEWL, I	+++
49	30% Jeffamine T403	0.2 *M* magnesium sulfate	T 8.0		0
50	5% Walocel HM 100	—	—		+++
51	10% Walocel CRT 10 G	—	—	Adh, Sa	+++
52	50% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* sodium chloride	T 8.5		0
53	25% polypropylene glycol 400	10% 1-butanol	—	Xy	0
54	54% polyvinyl pyrrolidone K15	0.1 *M* lithium sulfate	—	CRLC, Xy	++
55	25% pentaerythritol ethoxylate (15/4 EO/OH)	0.3 *M* sodium chloride	T 8.5	Adh, HEWL	0
56*	30% polyvinyl pyrrolidone K15	0.1 *M* lithium sulfate	H 7.0	Adh, HEWL, Xy	+
57*	40% polypropylene glycol 400	0.2 *M* imidazole pH 7	—	Amy, Sa, Xy	0
58*	8% acrylic acid/maleic acid copolymer (50:50), sodium salt, 3% pentaerythritol ethoxylate (3/4 EO/OH)	0.06 *M* lithium sulfate	H 7.5	Adh, HEWL, I	0
59	19% acrylic acid/maleic acid copolymer (50:50), sodium salt	—	M 6.0	I, Sa	0
60	16% polyvinyl alcohol type II	0.2 *M* potassium acetate	—	Adh, HEWL	+++
61	55% polypropylene glycol 400	—	—	Adh, Sa, Xy	0
62*	35% Jeffamine SD2001	0.1 *M* sodium chloride	T 8.0	Adh, HEWL, Xy	0
63*	30% Jeffamine M600	10% dimethyl sulfoxide	—	CRLC, HEWL, Xy	0
64	5% Walocel CRT 10 G	0.2 *M* sodium chloride	T 8.5	HEWL, I	+++
65*	20% polypropylene glycol 400	10% 1-propanol	—	DNAse, Xy	0
66	15% polyacrylate 2100, sodium salt	0.3 *M* imidazole pH 7	—	Adh, I	0
67	4% Walocel CRT 10 G	0.3 *M* sodium chloride	H 6.8	Adh, HEWL	+++
68*	28% acrylic acid/maleic acid copolymer (50:50), sodium salt	—	H 6.5	BsQueA, EcQueA, HiQueA	+
69	15% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* ammonium sulfate	—		0
70	35% pentaerythritol ethoxylate (15/4 EO/OH), 5% Jeffamine SD2001	—	I 7.0	Sa, Xy	0
71	25% pentaerythritol ethoxylate (3/4 EO/OH)	0.2 *M* sodium citrate pH 7.0	T 8.5	I	0
72	20% pentaerythritol ethoxylate (15/4 EO/OH), 10% *n*-propanol	0.2 *M* sodium thiocyanate	H 7.0	I, Xy	0
73	5% Walocel CRT 10 G	—	—		++
74	5% pentaerythritol ethoxylate (15/4 EO/OH)	0.3 *M* ammonium sulfate	T 8.0		0
75*	15% Jeffamine ED2003	10% ethanol	—	HEWL, Sa, SCM-2MBT, Xy	0
76*	30% Jeffamine ED2003	0.2 *M* sodium chloride	M 6.0	Adh, HEWL, Sa, SCM-2MBT, Xy	0
77	40% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* lithium sulfate	M 5.5	Xy	+
78	20% pentaerythritol ethoxylate (3/4 EO/OH)	—	—	Xy	0
79*	25% Jeffamine SD2001	0.1 *M* sodium malonate	M 5.5	Adh, HEWL	0
80	3% Walocel CRT 10 G, 4% PEG 8000	0.2 *M* sodium chloride	P 7.0	HEWL	+
81*	15% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* sodium chloride	M 6.0	Xy	0
82	70% pentaerythritol ethoxylate (15/4 EO/OH)	—	0.15 *M* HEPES pH 7.0		+
83	17% Jeffamine M600	0.2 *M* sodium citrate pH 7.0	T 8.5		0
84	25% pentaerythritol ethoxylate (15/4 EO/OH)	0.16 *M* sodium citrate pH 7.0	M 6.0	Adh, HEWL	0
85*	25% di[poly(ethylene glycol)] adipate 900	0.2 *M* sodium chloride	T 8.0	Adh, HEWL, I	0
86	10% di[poly(ethylene glycol)] adipate 900	0.3 *M* sodium chloride	—	HEWL	0
87*	40% pentaerythritol propoxylate (5/4 PO/OH)	15% ethanol	—	Adh, Sa, Xy	0
88	42% polyvinyl pyrrolidone K15	0.1 *M* sodium malonate	T 8.0		+
89*	50% pentaerythritol propoxylate (5/4 PO/OH)	—	T 8.0	Adh, Amy, CRLC, EcQueA, Scm-2MBT, Xy	0
90	35% pentaerythritol ethoxylate (3/4 EO/OH)	0.2 *M* magnesium chloride	H 7.0	Xy	0
91*	12.5% polyvinyl pyrrolidone K15, 10% PEG 4000	0.2 *M* sodium chloride	T 8.0	Adh, Fe	+

Table 2 (continued)

No.	Precipitant†	Salt/additive	Buffer‡	Hits§	Viscosity¶
92	60% pentaerythritol ethoxylate (15/4 EO/OH)	—	H 7.5	EcQueA, Xy	0
93*	25% pentaerythritol propoxylate (5/4 PO/OH)	10% dimethyl sulfoxide, 0.1 *M* sodium chloride	—	Adh, HEWL, Xy	0
94	6% Walocel CRT 10 G	10% ethanol, 0.1 *M* sodium chloride	—	Adh, HEWL, I	++
95	2% polyvinyl alcohol type II, 2% PEG 8000	—	H 7.0	HEWL	0
96*	35% polyacrylate 2100, sodium salt	0.2 *M* ammonium sulfate	H 7.5	Adh, BsQueA, dSfmbt-4MBT, EcQueA, HEWL, HiQueA	0

(*b*) Part *B*.

No.	Precipitant†	Salt/additive	Buffer‡	Hits§	Viscosity¶
1	10% pentaerythritol propoxylate (5/4 PO/OH)	0.1 *M* sodium chloride	M 6.0	Xy	0
2	40% polyacrylate 2100, sodium salt	0.2 *M* potassium acetate	T 8.5	BsQueA, EcQueA, HiQueA	+
3*	30% pentaerythritol ethoxylate (15/4 EO/OH)	0.1 *M* magnesium formate	T 8.5	Adh, BsQueA, EcQueA, I	+
4	10% Jeffamine M2005	—	0.2 *M* HEPES pH 6.5		+
5*	35% di[poly(ethylene glycol)] adipate 900	0.2 *M* sodium chloride	T 8.0	Adh, BsQueA, HEWL, I	+
6	2% Walocel HM 100	0.1 *M* lithium chloride	0.1 *M* sodium-glycine pH 9.5		+++
7*	60% polypropylene glycol 400	—	T 8.0	Adh, BsQueA, EcQueA, Sa, Scm-2MBT	+
8*	30% pentaerythritol ethoxylate (15/4 EO/OH), 6% polyvinyl pyrrolidone K15	—	H 7.5	Adh, BsQueA, Xy	+
9	10% polyvinyl alcohol type I, 5% Jeffamine ED2003	0.2 *M* sodium chloride	H 6.5	Adh, HEWL, Xy	++
10	8% polyvinyl alcohol type II	0.3 *M* imidazole pH 7.0	—	Xy	++
11	6% polyvinyl pyrrolidone K15	0.1 *M* magnesium formate	H 7.0		0
12	36% polyvinyl pyrrolidone K15	—	M 6.0	Adh, BsQueA	++
13*	45% polypropylene glycol 400	10% ethanol	—	Adh, HEWL, Sa, Xy	+
14	30% Jeffamine M2005	0.1 *M* sodium chloride	M 6.0	Adh, HEWL	++
15*	10% pentaerythritol ethoxylate (3/4 EO/OH)	10% 1-butanol	—	DNAse, I, Xy	0
16	5% polyvinyl alcohol type II	0.1 *M* lithium sulfate	0.05 *M* sodium citrate pH 6.0		+
17	15% Jeffamine D2000	0.3 *M* sodium chloride	—	HEWL	0
18	12% polyvinyl alcohol type II	0.16 *M* sodium citrate pH 7	—	Adh	+++
19*	12.5% polyacrylate 2100, sodium salt, 6% Jeffamine SD2001		H 7.0	Adh, HEWL	+
20	4% Walocel CRT 10 G	0.3 *M* K/Na phosphate pH 6.25	—		+++
21*	6% polyvinyl pyrrolidone K15	—	H 6.5	Adh, SAC7, Xy	0
22*	20% Jeffamine ED2003	—	H 6.5	Adh, HEWL, Sa, Xy	+
23	18% polyvinyl pyrrolidone K15	0.2 *M* potassium acetate	T 8.5	Adh	+
24	45% pentaerythritol ethoxylate (3/4 EO/OH)	0.2 *M* sodium chloride	T 8.0	Xy	+
25	15% di[poly(ethylene glycol)] adipate 900	10% ethanol	—		+
26	25% di[poly(ethylene glycol)] adipate 900	0.3 *M* K/Na phosphate pH 6.25	—	Adh	+
27	20% pentaerythritol propoxylate (5/4 PO/OH)	0.3 *M* sodium chloride	T 8.0	HEWL	0
28	2% polyvinyl alcohol type II	0.3 *M* ammonium sulfate	—		0
29	4% polyvinyl alcohol type II	—	H 6.5		+
30*	25% Jeffamine D2000	0.2 *M* imidazole pH 7.0	—	HEWL, Xy	+
31	16% poly(ethylene imine) branched	0.2 *M* ammonium formate	T 8.0	HEWL	+
32	20% pentaerythritol ethoxylate (15/4 EO/OH), 8% poly(ethylene imine) branched	0.2 *M* ammonium formate	T 8.5	HEWL	+
33	40% pentaerythritol ethoxylate (3/4 EO/OH)	0.2 *M* sodium thiocyanate	H 6.5	Xy	+
34*	30% Jeffamine SD2001	0.2 *M* potassium chloride	H 6.5	Adh, HEWL	+
35	60% polyvinyl pyrrolidone K15	—	—	Xy	+++
36*	30% polypropylene glycol 400	0.1 *M* sodium chloride	—	Adh, Xy	0
37	35% Jeffamine T403	0.1 *M* sodium chloride	M 5.5	Xy	0
38	5% Walocel CRT 10 G, 10% polyacrylate 2100, sodium salt	0.1 *M* lithium chloride	H 6.8	Adh, HEWL, I	+++
39	8% poly(ethylene imine) branched		BT 6.0	HEWL	0
40	20% Jeffamine SD2001	15% *n*-propanol	—	Xy	0
41	10% polyvinyl alcohol type II, 10% Jeffamine D2000	0.2 *M* ammonium sulfate	T 8.0	Adh, HEWL	+++
42	15% pentaerythritol ethoxylate (3/4 EO/OH)	0.2 *M* potassium chloride	B 8.5		0
43	16% polyvinyl alcohol type II	0.2 *M* ammonium phosphate pH 8.5	—	Adh, HEWL, Xy	+++
44	3% Walocel HM 100	0.2 *M* magnesium sulfate	T 8.0		+++
45	20% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* sodium sulfate	H 6.5		0
46	10% pentaerythritol ethoxylate (3/4 EO/OH), 15% polyacrylate 5100, sodium salt	—	M 6.5	Adh	0

Table 2 (continued)

No.	Precipitant†	Salt/additive	Buffer‡	Hits§	Viscosity¶
47	9% Walocel CRT 10 G	0.2 *M* sodium chloride	T 8.5	I	+++
48	40% Jeffamine SD2001	—	H 7.0	HEWL, Sa, Xy	++
49*	30% di[poly(ethylene glycol)] adipate 900	0.2 *M* magnesium chloride	H 6.5	Adh, BsQueA, HEWL, HiQueA	0
50*	20% di[poly(ethylene glycol)] adipate 900	0.2 *M* magnesium sulfate	H 6.5	Adh, I	0
51	6% polyvinyl alcohol type II	0.08 *M* magnesium chloride	H 7.5	I, Xy	+
52*	35% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* potassium acetate	—	Adh, Xy	0
53	35% pentaerythritol ethoxylate (3/4 EO/OH)	10% 1-propanol	M 5.5	I	0
54*	20% pentaerythritol ethoxylate (15/4 EO/OH)	0.2 *M* potassium chloride	G 9.5	Adh, HEWL, I	+
55	5% Jeffamine T403	0.4 *M* sodium thiocyanate	—	HEWL	0
56*	40% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* sodium thiocyanate	H 7.0	Adh, BsQueA, EcQueA, I, Xy	+
57*	15% Jeffamine T403, 15% Jeffamine ED2003	0.2 *M* potassium chloride	H 6.5	Adh, HEWL, Xy	0
58	12% polyvinyl alcohol type II	0.2 *M* calcium chloride	B 8.5	Adh, HEWL, I	++
59	50% pentaerythritol ethoxylate (15/4 EO/OH)	0.1 *M* zinc acetate	0.15 *M* Tris pH 8.0		+
60*	15% pentaerythritol ethoxylate (15/4 EO/OH), 3% Jeffamine T403	0.2 *M* potassium acetate	M 6.0	Adh, HEWL, Xy	0
61	10% polyvinyl alcohol type II, 10% Jeffamine M2005	0.2 *M* sodium chloride	M 6.0	Adh, HEWL	++
62	30% pentaerythritol propoxylate (5/4 PO/OH)	0.2 *M* magnesium sulfate	H 6.5	Adh, EcQueA, I	0
63	24% poly(ethylene imine) branched	0.2 *M* potassium acetate	—	HEWL	++
64	50% polyacrylate 2100, sodium salt	—	—		++
65	10% polyacrylate 2100, sodium salt, 5% Jeffamine T403	—	—	HEWL, I	0
66*	30% polyacrylate 2100, sodium salt	0.1 *M* sodium malonate	H 7.0	Adh, HEWL, HiQueA, I	0
67*	10% Jeffamine D2000, 10% Jeffamine M2005	10% ethanol	—	HEWL, Xy	0
68	5% polyacrylate 2100, sodium salt, 6% polyvinyl pyrrolidone K15	0.2 *M* lithium acetate	BT 6.5	HEWL	0
69	10% pentaerythritol ethoxylate (15/4 EO/OH), 10% poly(ethylene imine) branched	10% ethanol	—	HEWL	0
70*	25% Jeffamine ED2003	0.1 *M* lithium sulfate	T 8.0	Adh, HEWL	+
71	20% polyacrylate 2100, sodium salt	10% ethanol	—	HEWL	0
72	12% poly(ethylene imine) branched	—	—	HEWL	0
73	2% Walocel CRT 10 G	0.2 *M* sodium citrate pH 7.0, 0.2 *M* imidazole pH 7.0		HEWL	+
74*	10% Jeffamine T403, 10% Jeffamine ED2003	—	T 8.0	Adh, HEWL, Xy	0
75	35% Jeffamine D2000	—	—	HEWL	+
76	9% Walocel CRT 10 G	—	H 6.5	Adh	+++
77*	25% polyacrylate 2100, sodium salt	0.1 *M* lithium sulfate	H 6.5	Adh, HEWL	0
78	10% pentaerythritol ethoxylate (15/4 EO/OH), 7% Jeffamine M2005	—	0.15 *M* Tris pH 8.5		0
79*	15% polyacrylate 2100, sodium salt	0.2 *M* magnesium chloride	H 7.5	Adh, HEWL, I	0
80	4% polyvinyl alcohol type II	0.2 *M* potassium acetate	H 7.0	HEWL	0
81	25% Jeffamine D2000	0.1 *M* lithium sulfate	H 7.5	HEWL	0
82*	40% Jeffamine D2000	—	H 6.5	HEWL, Xy	+
83*	10% polyacrylate 2100, sodium salt	0.5 *M* sodium chloride	T 8.0	Adh, HEWL	0
84	7% Walocel CRT 10 G	0.2 *M* magnesium sulfate	T 8.0	Adh, HEWL	+++
85	5% Jeffamine D2000, 5% Jeffamine M2005	0.2 *M* sodium chloride	—		0
86*	14% Jeffamine ED900, 11% Jeffamine SD2001	—	P 7.0	HEWL, Xy	0
87*	20% polyacrylate 2100, sodium salt	0.2 *M* sodium chloride	B 9.0	Adh, HEWL, I	0
88*	20% Jeffamine D2000	0.2 *M* sodium malonate	M 5.5	Adh, HEWL	0
89	35% Jeffamine M2005	0.2 *M* potassium chloride, 10% ethanol	—	HEWL, Xy	++
90	8% Walocel CRT 10 G	0.1 *M* zinc acetate	B 9.0		+++
91	30% Jeffamine D2000	—	0.2 *M* Tris pH 8.0	HEWL	0
92*	30% Jeffamine M2070	0.2 *M* potassium chloride	T 8.0	Adh, BsQueA, HEWL	0
93*	20% Jeffamine M2070	20% dimethyl sulfoxide	—	Adh, Xy	0
94*	40% pentaerythritol propoxylate (17/8 PO/OH)	0.2 *M* magnesium chloride	M 5.5	Adh, BsQueA, DNAse, EcQueA, HEWL, HiQueA, I, Xy	0
95	36% poly(ethylene imine) branched	—	M 6.5	HEWL	+++
96*	20% polyacrylate 5100, sodium salt	—	T 8.0	Adh, HEWL	0

(*c*) Part *C*. Some conditions were adjusted with NaOH or HCl to a final pH after mixing; this pH value is given in the column 'pH adj.' if applicable.

No.	Precipitant†	Salt/additive	Buffer‡	pH adj.	Hits§	Viscosity¶
1	55% glycerol ethoxylate	0.2 *M* potassium chloride	H 7.5			+
2	25% Sokalan HP 66 K	5% dimethylformamide	T 8.0		Adh	++
3	15% Sokalan HP 56, 5% PEG MME 5000	0.2 *M* lithium acetate	—			+
4	5% Sokalan HP 66 K, 5% Sokalan CP 42	—	—		HEWL	0
5	90% Glascol W13	—	M 6.0			+++
6	80% Glascol W13	10% dimethyl sulfoxide	H 7.5		Adh, HEWL, Xy	+++
7*	28% poly(ethylene imine) branched	—	H 7.0		HEWL, Sa	+
8	30% Sokalan CP 12 S	—	—			++

Table 2 (continued)

No.	Precipitant†	Salt/additive	Buffer‡	pH adj.	Hits§	Viscosity¶
9	20% Sokalan CP 12 S	0.2 *M* potassium citrate	T 8.0			+
10	4% poly(ethylene imine) branched	0.2 *M* ammonium acetate	H 7.0		HEWL	0
11*	20% Sokalan CP 7	0.1 *M* ammonium formate	H 7.0		Adh, HEWL, Sa	0
12	30% Glascol W13	—	M 6.0		HEWL, I	0
13	30% Sokalan CP 42	0.2 *M* potassium acetate			HEWL, HiQueA	++
14	20% Sokalan HP 66 K, 10% Glascol W13	—	—			++
15	40% Sokalan HP 56	0.2 *M* potassium acetate	—		Adh	+++
16*	20% Sokalan HP 56	0.2 *M* sodium sulfate	T 8.0		HEWL, I	+
17	30% Sokalan HP 56	0.1 *M* potassium chloride, 5% ethanol	—			++
18	30% Sokalan CP 7	—	M 6.0	5.8	HEWL	0
19*	25% Sokalan CP 7	0.1 *M* potassium chloride	H 7.0		Adh, HEWL, I	0
20	70% Glascol W13	0.2 *M* ammonium chloride	—		HEWL	++
21	50% glycerol ethoxylate	0.2 *M* ammonium acetate	BT 5.5			+
22	30% Sokalan HP 56	—	T 8.5			++
23	35% Sokalan HP 66 K	—	—		Adh, HEWL	+++
24	5% Sokalan HP 56	—	H 7.5			0
25*	20% Sokalan CP 5	0.3 *M* ammonium formate	H 7.0		Adh, HEWL	0
26	15% Sokalan HP 66 K	0.1 *M* potassium citrate	BT 6.0		HEWL	+
27	15% Sokalan CP 42	—	BT 6.5	6.5	Adh, HEWL	0
28*	40% glycerol ethoxylate	—	—		EcQueA, Sa, Xy	0
29	20% poly(ethylene imine) branched	10% 1-propanol	M 6.0	6.1	HEWL	+
30*	30% glycerol ethoxylate	—	T 8.5		Adh, Xy	0
31	20% Sokalan CP 12 S	0.1 *M* ammonium formate	M 6.0		BsQueA	+
32*	15% Sokalan HP 66 K, 3% poly(ethylene imine)	—	H 7.0	7.0	Adh, BsQueA, HEWL	+
33	10% PEG 3350, 6% poly(ethylene imine)	—	T 8.0	8.0	HEWL	0
34	5% PEG 3350, 3% poly(ethylene imine)	0.2 *M* sodium sulfate	BT 5.5		HEWL	0
35*	35% glycerol ethoxylate	0.2 *M* lithium citrate	—		Adh, HEWL, Xy	0
36*	30% glycerol ethoxylate	0.2 *M* ammonium acetate	M 6.5		BsQueA, I	0
37*	20% Sokalan CP 42	5% methanol	T 8.0		Adh, BsQueA, HEWL, HiQueA, Sa	+
38	35% Sokalan HP 56	—	BT 5.5	6.0	HEWL	++
39	10% Sokalan CP 5	0.2 *M* lithium citrate	—			0
40	10% Sokalan CP 42	0.2 *M* lithium citrate	H 7.5	7.5	Adh, HEWL	0
41*	25% Sokalan CP 42	10% tetrahydrofuran	T 7.0	7.0	BsQueA, HEWL, HiQueA, Sa, SAC9	+
42*	20% Sokalan CP 42	0.1 *M* lithium acetate	BT 6.0	6.0	Adh, HEWL, HiQueA	+
43	25% Sokalan CP 12 S	—	0.2 *M* BT 5.5	6.0		+
44	20% PEG 3350, 12% poly(ethylene imine)	0.2 *M* potassium chloride	M 6.5	6.5	HEWL	0
45	40% Sokalan CP 5	—	—		HEWL	++
46	25% Sokalan CP 5	—	T 8.0	8.4	Adh, HEWL	+
47*	15% Sokalan CP 12 S	0.1 *M* lithium citrate	BT 5.5	6.5	BsQueA, HEWL, HiQueA, Sa	+
48	45% glycerol ethoxylate	10% ethanol, 0.2 *M* potassium acetate	—		Adh	0
49	Sokalan CP 12 S	—	0.2 *M* BT 5.5			0
50	10% glycerol ethoxylate	0.2 *M* sodium sulfate	BT 6.0			0
51	5% Sokalan CP 42	0.2 *M* sodium sulfate	M 6.5		HEWL	0
52*	15% Sokalan CP 5	—	BT 6.0		Adh, HEWL	0
53*	25% Sokalan CP 42	—	BT 6.0		HEWL, HiQueA, Sa, SAC10, SAC9	+
54	35% Sokalan CP 5		H 7.0		HEWL	++
55	23% PEG 3350, 14% poly(ethylene imine)	—	T 8.5		HEWL	+
56*	25% Sokalan HP 66 K	0.2 *M* ammonium acetate	H 7.0		Adh, HEWL	++
57*	20% glycerol ethoxylate, 3% poly(ethylene imine)	—	T 8.5		Adh, BsQueA, HEWL	0
58	35% Sokalan CP 7	—	T 8.5		Adh, HEWL	++
59	5% Sokalan CP 7	—	M 6.0	6.0	HEWL	0
60	15% Sokalan CP 7	—	T 8.0			0
61	5% glycerol ethoxylate	0.2 *M* lithium acetate	BT 6.0			0
62	30% Sokalan HP 66 K	10% tetrahydrofuran	H 7.5		Adh	++
63	20% Sokalan HP 66 K	—	BT 6.5	6.3		+
64	15% glycerol ethoxylate	5% 2-propanol	—		Adh, Xy	0
65*	25% glycerol ethoxylate	0.2 *M* ammonium chloride	H 7.5		Adh, HEWL, I	0
66	10% Sokalan CP 7	0.2 *M* lithium acetate	H 7.5		HEWL	0
67	60% Glascol W13	0.2 *M* ammonium acetate	T 8.0			++
68	20% Sokalan HP 56		M 6.0	6.0	HEWL	+
69	50% Glascol W13	0.1 *M* potassium chloride	BT 5.5		HEWL	+
70*	40% Glascol W13	0.2 *M* potassium citrate			HEWL	+
71	20% Glascol W13	0.2 *M* sodium sulfate	H 7.5		HEWL	0
72*	30% polyacrylate 5100, sodium salt	10% ethanol	M 6.0		Adh, BsQueA, EcQueA, HEWL, HSDH, Sa, SAC-RNA, Scm-2MBT	+
73*	15% Sokalan CP 42	0.2 *M* potassium citrate	—		Adh, HEWL	0
74*	30% Sokalan CP 42	—	T 8.5		Adh, HEWL, HiQueA, Sa	++
75	20% glycerol ethoxylate	10% tetrahydrofuran	T 8.0		HEWL	0

Table 2 (continued)

No.	Precipitant†	Salt/additive	Buffer‡	pH adj.	Hits§	Viscosity¶
76	10% Sokalan HP 66 K	10% ethanol	T 8.0			0
77	25% glycerol ethoxylate	5% pyridine	H 7.0		Adh, Sa	0
78	15% PEG 3350, 9% poly(ethylene imine)	10% dimethyl sulfoxide	H 7.0		HEWL	0
79	10% Glascol W13	—	M 6.0			0
80*	25% Sokalan HP 56	0.2 *M* ammonium acetate	H 7.0		Adh, HEWL, Sa	+
81	15% Sokalan HP 56, 10% PEG MME 5000	0.1 *M* potassium citrate	M 6.0		Adh, HEWL	+
82	40% Sokalan CP 7	—	BT 6.5		Adh, HEWL	++
83	25% Sokalan CP 7	—	BT 5.5		Adh, HEWL	+
84	20% Sokalan CP 7	0.2 *M* ammonium sulfate	BT 6.5		Adh, HEWL	0
85	10% Sokalan CP 42	—	T 8.0	8.6	HEWL	0
86*	25% Sokalan CP 5	—	T 8.5	8.0	Adh, HEWL, HSDH	+
87	20% Sokalan CP 5	—	T 8.5	8.5	Adh, HEWL, I	0
88	5% Sokalan HP 66 K, 10% PEG 1000	—	T 8.5	8.4		0
89	10% Sokalan HP 66 K, 20% PEG 1000	—	BT 6.5	6.9	Adh	0
90	10% poly(vinyl pyrrolidone) K15, 10% PEG 4000	—	H 7.5		Adh	0
91*	10% poly(vinyl pyrrolidone) K15, 20% PEG 4000	0.2 *M* ammonium formate	—		Adh, BsQueA, Fe, HEWL, I	0
92	10% Sokalan HP 56, 10% PEG 1000	0.2 *M* potassium citrate	—		HEWL	0
93	20% poly(vinyl pyrrolidone) K15, 10% PEG 1000	10% dimethyl sulfoxide	—		Adh	0
94*	15% poly(vinyl pyrrolidone) K15, 25% PEG MME 5000	—	T 8.0		Adh, BsQueA, DNAse	+
95	15% Sokalan HP 56, 15% PEG 1000	—	M 6.0		Adh	+
96	15% poly(vinyl pyrrolidone) K15, 15% PEG 4000	0.2 *M* potassium acetate	BT 5.5	6.1	Adh	0

† Polymers: glycerol ethoxylate, Aldrich 441864; poly(ethylene imine), Aldrich 482595; poly(ethylene imine) branched, Aldrich 408727; Glascol W13, CIBA; Sokalan products, BASF; Jeffamines, Huntsman; Walocel, Dow Wolff Cellulosics; pentaerythritol ethoxylate (15/4 EO/OH), Aldrich 418730; pentaerythritol ethoxylate (3/4 EO/OH), Aldrich 416150; pentaerythritol propoxylate (17/8 PO/OH), Aldrich 418757; pentaerythritol propoxylate (5/4 PO/OH), Aldrich 418749; di[poly(ethylene glycol)] adipate 900, Aldrich 494852; polyvinyl alcohol type II, Sigma P8136; polyvinylpyrrolidone K15, Fluka 81390; maleic acid/acrylic acid copolymer (50/50), sodium salt, Aldrich 416061. For poly(ethylene imine), poly(ethylene imine) branched, Sokalan products, Jeffamines, di[poly(ethylene glycol)] adipate 900 and polyvinylpyrrolidone K15 the pH was adjusted to 7.0. ‡ Buffer abbreviations (0.1 *M*, unless stated otherwise, followed by pH value): T, Tris–HCl; M, MES–NaOH; BT, bis-tris–NaOH; H, HEPES–NaOH; P, potassium/sodium phosphate. § Protein abbreviations: HEWL, hen egg-white lysozyme; Xy, xylanase from *Trichoderma viride*; Adh, alcohol dehydrogenase from yeast; Amy, α-amylase from *Bacillus subtilis*; DNAse, DNAse I from bovine pancreas; Fe, ferritin type I from horse spleen; I, human insulin; Sa, strepavidin core; dSfmbt-4MBT, the four MBT repeats of Sfmbt from *Drosophila melanogaster* in complex with peptide RHR^{me}KVLR; Scm-2MBT, the two MBT repeats of *sex-comb on midleg* from *D. melanogaster*; HSDH, 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*; EcQueA, *S*-adenosylmethionine:tRNA ribosyl transferase/isomerase from *Escherichia coli*; HiQueA, *S*-adenosylmethionine:tRNA ribosyl transferase/isomerase from *Haemophilus influenzae*; BsQueA, *S*-adenosylmethionine:tRNA ribosyl transferase/isomerase from *B. subtilis* (these three proteins were in the presence of a cognate tRNA oligoribonucleotide); SAC7, SAC9, SAC10, SAC-RNA, CRLC, see §2. ¶ Symbols in the 'Viscosity' column: 0, low viscosity; +, elevated viscosity within the range commonly encountered in standard screens; ++, viscosity above the range commonly encountered in standard screens; +++, very high viscosity: problems with common crystallization robots are almost certain. However, with particular care manual handling using standard pipettes is possible. Reproducible pipetting of Walocel-containing solutions will necessitate a positive displacement pipette.

solutions and used without further treatment at concentrations of 4 and 10 g l^{-1}, respectively. Core streptavidin (SA) was expressed as inclusion bodies in *Escherichia coli*, refolded and purified to homogeneity as described by Chari *et al.* (2008) and used for crystallization experiments at 20 g l^{-1}. The four MBT repeats of Sfmbt from *Drosophila melanogaster* (dSfmbt-4MBT) were expressed and purified as described in Grimm *et al.* (2009) and used at 15 g l^{-1} in a 1:4 molar ratio complex with the modified peptide RHR^{me}KVLR, where ^{me}K represents a monomethyl lysine residue. The two MBT repeats of *sex-comb on midleg* (Scm-2MBT) were expressed and purified as described in Grimm *et al.* (2007); the protein solution was used at 20 g l^{-1}. 2-Methylisocitrate lyase (PrpB) from *E. coli* was prepared as described in Grimm *et al.* (2003) and 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni* (HSDH) was prepared as described in Grimm, Maser *et al.* (2000); both proteins were used at 8 g l^{-1}. The *S*-adenosyl-methionine:tRNA ribosyl transferase/isomerases (QueA) from *E. coli* (EcQueA), *Haemophilus influenzae* (HiQueA) and *Bacillus subtilis* (BsQueA) were expressed and purified as described in Grimm, Klebe *et al.* (2000) and mixed with a twofold molar ratio of substrate tRNA stem-loop fragment CUGCCUGU-CACGCAG in the presence of 2 m*M* magnesium chloride. The three different proteins were used at a concentration of 10 g l^{-1}. In addition, three spliceosomal assembly complexes, SAC7, SAC9 and SAC10, including up to eight protein subunits and a cytokine receptor/ligand complex (CRLC), as well as a spliceosomal assembly complex including a ribonucleic acid component (SAC-RNA) were included in the experiments. These complexes were used at a concentration of 8 g l^{-1}. See Table 1 for a detailed list of the different proteins.

2.3. Crystallization methods and X-ray diffraction experiments

HEWL and Adh were screened by the hanging-drop vapour-diffusion method in 24-well Linbro plates by mixing 1 µl reservoir screening solution with 1 µl protein solution. All other proteins were screened in 96-well crystallization plates using a 96-needle nanodrop crystallization robot (Cartesian Honeybee, Hamilton Bonaduz AG, Switzerland) by mixing 200 nl protein solution with 200 nl reservoir solution. Plates were inspected manually after 20 d incubation at 293 K and crystallization was assessed by visual evaluation. Representative crystal samples were measured under cryoconditions on a rotating-anode generator to verify the crystal parameters and to sort out possible salt or other small-molecule crystals. Crystals of SAC9 and Sfmbt were measured on beamline ID23-1 of the ESRF.

3. Results and discussion

We have developed a set of 288 crystallization conditions (Table 2), each containing at least one non-PEG polymer or a polymer mixture as precipitant. From polymers previously described as crystallization precipitants, we selected Jeffamine M-600 (Fig. 1*a*), pentaerythritol ethoxylate (Fig. 1*b*) and pentareythritol propoxylate (Fig. 1*c*), each with two different ethoxy/propoxy (EO/PO) ratios, polyvinylpyrrolidone K15 (Fig. 1*d*), polypropylene glycol 400 (Fig. 1*e*), polyvinyl alcohol type II (Fig. 1*f*), sodium polyacrylate 2100 and 5100 (Fig. 1*g*), a very low molecular-weight carboxymethyl cellulose (Walocel CRT 10 G; Fig. 1*h*), poly(ethylene imine) (Fig.1*i*) and di[poly (ethylene glycol)] adipate (Fig. 1*j*). To complement this set, we chose the following polymers or variants that are novel in their application to protein crystallization: the monoamines Jeffamine M2005 and M2070 (Fig. 1*a*), the primary diamines Jeffamine ED2003 (predominantly PEG backbone; Fig. 1*k*) and Jeffamine D2000 (polypropylene backbone; Fig. 1*l*), the secondary diamine Jeffamine SD2001 (Fig. 1*m*), the triamine Jeffamine T403 (Fig. 1*n*), a polyacryl amide (Glascol W13; Fig. 1*o*), glycerol ethoxylate (Fig. 1*p*), different acrylic acid/

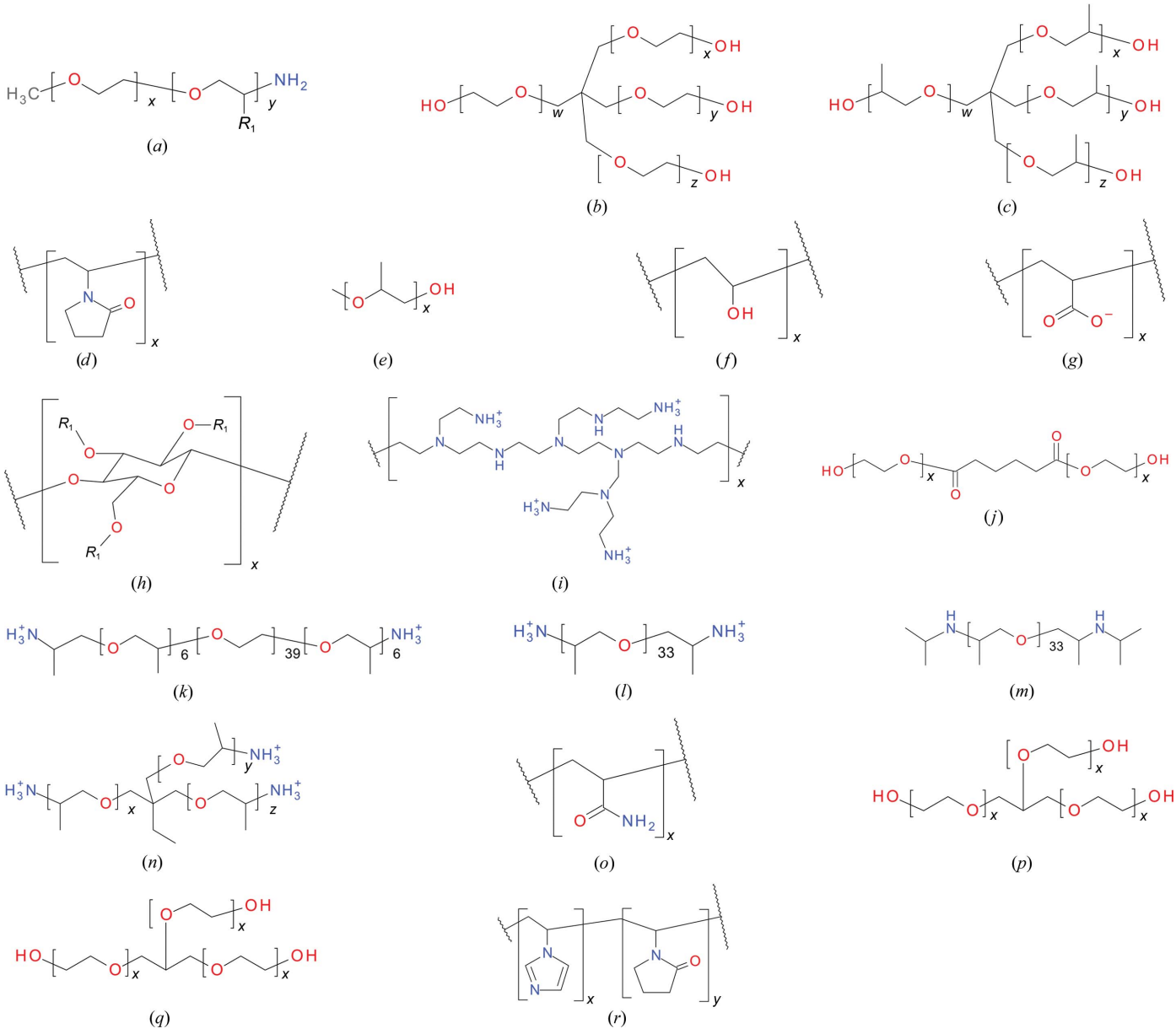

Figure 1
Structures of various polymeric precipitants. (*a*) M-type Jeffamines. R_1 = —H for EO or —CH_3 for PO. The PO/EO molar ratio is 29/6 for Jeffamine M2005, 10/31 for Jeffamine M2070 and 9/1 for Jeffamine M600. (*b*) Pentaerythritol ethoxylate. (*c*) Pentaerythritol propoxylate. (*d*) Polyvinyl pyrrolidone. (*e*) Polypropylene glycol. (*f*) Polyvinyl alcohol. (*g*) Polyacrylate. (*h*) Cellulose-based polymers. R_1 = —H, —CH_3 or —$CH_2CHOHCH_3$ (hydroxypropyl methylcellulose), —H or CH_2CO_2H (carboxymethyl cellulose). (*i*) Poly(ethylene imine). (*j*) Di[poly(ethyleneglycol)] adipate. (*k*) Jeffamine ED2003. (*l*) Jeffamine D2000. (*m*) Jeffamine SD2001. (*n*) T-type Jeffamines. (*o*) Polyacryl amide. (*p*) Glycerol ethoxylate. (*q*) Acrylic acid/maleic acid copolymer. (*r*) Vinylpyrrolidone/vinylimidazole copolymer.

Table 3
Crystallization statistics and properties of the various polymer species.

Precipitant	Chemical nature	Manufacturer	Approximate (average) molecular mass (kDa)	*K* value†	No. of conditions (out of 288) containing this precipitant	Hits obtained	Crystallization propensity‡	No. of different proteins crystallized	Proteins crystallized
Sokalan CP 42	Modified polycarboxylate, sodium salt	BASF	—	30	11	32	2.9	6	Adh, HEWL, HiQueA, Sa, SAC10, SAC9
Sokalan CP 12 S	Modified polyacrylate, sodium salt	BASF	3	20	6	5	0.8	4	BSQ, HEWL, HiQueA, Sa
Polyacrylate 2100 or 5100, sodium salt	Polyacrylate, sodium salt	Various	2.1 or 5.1	—	16	47	2.9	11	Adh, BsQueA, dSfmbt-4MBT, EcQueA, HEWL, HiQueA, HSDH, I, Sa, SAC-RNA, Scm-2MBT
Acrylic acid/maleic acid copolymer (50:50), sodium salt	Maleic acid/acrylic acid copolymer, sodium salt	Various	50	—	7	13	1.9	6	Adh, I, Sa, BsQueA, HiQueA, EcQueA
Sokalan CP 5	Maleic acid/acrylic acid copolymer, sodium salt	BASF	70	60	8	14	1.8	4	Adh, HSDH, HEWL, I
Sokalan CP 7	Maleic acid/acrylic acid copolymer, sodium salt	BASF	50	50	10	17	1.7	4	Adh, HEWL, I, Sa
Glascol W13	Polyacryl amide	CIBA		—	9	9	1.0	4	Adh, HEWL, I, Xy
PVA type II	Polyvinyl alcohol	Various	30–70	—	15	20	1.3	4	Adh, HEWL, I, Xy
Polypropylene glycol 400	Polypropylene glycol	Various	0.4	—	11	29	2.6	9	Adh, Amy, BsQueA, DNAse, EcQueA, HEWL, Sa, Scm-2MBT, Xy
Pentaerythritol ethoxylate (2 species)	Pentaerythritol ethoxylate	Various	0.27/0.80 depending on species	—	25	35	1.4	8	Adh, BsQueA, DNAse, EcQueA, HEWL, I, Sa, Xy
Pentaerythritol propoxylate (2 species)	Pentaerythritol propoxylate	Various	0.21	—	15	46	3.1	12	Adh, Amy, BsQueA, CRLC, DNAse, EcQueA, HEWL, HiQueA, I, Sa, SCM-2MBT, Xy
Glycerol ethoxylate	Glycerol ethoxylate	Various	1.0	—	13	20	1.5	7	Adh, BsQueA, EcQueA, HEWL, I, Sa, Xy

maleic acid copolymers (Sokalan CP 5, Sokalan CP 7 and a generic product; Fig. 1*q*), chemically modified polycarboxylates (Sokalan CP 42 and Sokalan CP 12 S), vinylpyrrolidone/vinylimidazole copolymers (Sokalan HP 66 K and Sokalan HP 56; Fig. 1*r*) and a hydroxypropyl methylcellulose (Walocel HM 100; Fig. 1*h*).

First of all, we intended to determine the general applicability of our screen for the production of diffraction-quality crystals. To this end, we obtained eight commercially available 'benchmark' proteins that exhibit high solubility and that have been crystallized before. All eight proteins could be crystallized within the 288 conditions of the screen. Xylanase (Fig. 2*a*), alcohol dehydrogenase, insulin, streptavidin core (Fig. 2*b*) and particularly HEWL (Figs. 2*c* and 2*d*) crystallized under a broad range of conditions (see Table 2 for detailed conditions and results and Table 3 for a statistical summary). All HEWL crystals belonged to the tetragonal crystal form known to appear in the presence of PEG as a precipitant, with unit-cell parameters equalling the published ones. During tests on a rotating-anode generator, diffraction exceeded 2.0 Å resolution with all HEWL crystal samples tested. Crystallization of amylase was limited to conditions containing polypropylene glycol 400 or pentaerythritol propoxylate, while DNAse I only crystallized in conditions containing polypropylene glycol 400, pentaerythritol propoxylate or pentaerythritol ethoxylate. Horse-spleen ferritin could be crystallized in the presence of the carboxymethyl cellulose Walocel CRT 10 G or a mixture of polyvinyl pyrrolidone K15 and PEG. The case of ferritin is particularly interesting since this protein has so far only been crystallized in the presence of cadmium ions (Laufberger, 1937; Granier *et al.*, 1997; Arosio *et al.*, 1983) and no cadmium was added externally. However, residual cadmium in the protein preparation (Hegenauer *et al.*, 1979) might still have played a role in crystallogenesis.

Encouraged by these results, we turned to 12 other proteins or protein complexes from ongoing or successfully completed structure-determination projects, including four protein complexes (SAC7, SAC9, SAC10 and SAC-RNA) that had failed within commercially available screens, including those targeted specifically to protein complexes. Of these 12 proteins, only the PrpB protein failed to yield crystals within our screen. This protein had previously been found to crystallize exclusively under high-salt conditions, which are excluded from this screen. In contrast, dSfmbt-4MBT, which

Table 3 (continued)

Precipitant	Chemical nature	Manufacturer	Approximate (average) molecular mass (kDa)	*K* value†	No. of conditions (out of 288) containing this precipitant	Hits obtained	Crystallization propensity‡	No. of different proteins crystallized	Proteins crystallized
Jeffamine M600	Poly(oxyalkylene) (monoamine)	Various	0.60	—	2	3	1.5	3	CRLC, HEWL, Xy
Jeffamine M2005, Jeffamine M2070	Poly(oxyalkylene) monoamines	Huntsman International	2.0	—	5	10	2.0	4	Adh, BsQueA, HEWL, Xy
Jeffamine ED2003	Poly(oxyalkylene) diamine, predominantly PEG backbone	Huntsman International	2.0	—	6	17	2.8	5	Adh, HEWL, Xy, Sa, SCM-2MBT
Jeffamine D2000	Poly(oxyalkylene) diamine, polypropylene backbone	Huntsman International	2.0	—	7	10	1.4	3	Adh, HEWL, Xy
Jeffamine SD2001	Secondary poly(oxyalkylene) diamine	Huntsman International	2.1	—	6	11	1.8	4	Adh, HEWL, Sa, Xy
Jeffamine T403	Poly(oxyalkylene) triamine	Huntsman International	0.57	—	7	3	0.4	2	HEWL, Xy
Jeffamine mixtures	Poly(oxyalkylene) amines	—	—	—	13	29	2.2	5	Adh, HEWL, I, Sa, Xy
Polyvinyl pyrrolidone K15	Polyvinyl pyrrolidone	Various	10	15	14	21	1.5	7	Adh, BsQueA, CRLC, HEWL, SAC7, SAC9, Xy
Poly(ethylene imine) and poly(ethylene imine) branched	Poly(ethylene imine)	Various	1.3 and 25 (branched species)	—	8	9	1.1	1	HEWL
Sokalan HP 66 K	Modified vinyl pyrrolidone/vinyl imidazole copolymer	BASF	—	38	7	7	1.0	2	Adh, HEWL
Sokalan HP 56	Vinyl pyrrolidone/vinyl imidazole copolymer	BASF	70	32	8	8	1.0	4	Adh, HEWL, I, SA
Di[poly(ethylene glycol)] adipate 900	Di[poly(ethylene glycol)] adipate	Various	0.9	—	7	14	2.0	5	Adh, BsQueA, HEWL, HiQueA, I
Walocel CRT 10 G	Carboxymethyl cellulose, very low molecular mass	Dow Wolff Cellulosics	—	—	14	22	1.6	5	Adh, Fe, HEWL, I, SA
Walocel HM 100	Hydroxypropyl methylcellulose, low molecular mass	Dow Wolff Cellulosics	—	—	4	0	0	0	
Other mixtures	—	—	—	—	32	44	1.4	7	Adh, BsQueA, DNAse, Fe, HEWL, I, Xy

† The *K* value of a polymer solution is defined as $K = \log(N_S/N_0)/c$, where N_S is the viscosity of the solution, N_0 is the viscosity of the solvent and c is the concentration in g ml^{-1}. Conditions: 1% active substance in water at pH 7. Values as given in the manufacturer's specifications. ‡ The crystallization propensity was defined as the number of hits with a certain polymer divided by the number of conditions containing that polymer. Some closely related polymer species have been grouped together in the table.

crystallized solely in a high-salt crystallization condition during extended screening experiments with conventional grid and sparse-matrix screens, could be crystallized in a different crystal form in the presence of polyacrylate (Fig. 2*e*). An X-ray diffraction data set was collected from one of these crystals on an ESRF synchrotron beamline and showed a significant improvement in resolution over the previous high-salt crystal form. Scm-2MBT was known to form crystals in the presence of higher molecular-weight PEGs. Here, from amongst the conditions of our screen it crystallized with comparable morphology in conditions containing polypropylene glycol 400 and pentarythritol propoxylate. Several crystal forms that differed from those obtained during previous screening experiments could be produced with the QueA protein in presence of a tRNA substrate. The diffraction limit of these crystals ranged between 7 and 5 Å. At the present stage it cannot be determined whether the crystals contain a complex between the protein and nucleic acid or only one of the two compounds. Two spliceosomal assembly complexes (SAC9 and SAC10) comprising up to eight subunits crystallized in the presence of Sokalan CP 42 (Fig. 2*f*) and two further crystal forms (from SAC9 and SAC7; Fig. 2*g*) were obtained, each in the presence of polyvinyl pyrrolidone K15. The presence of all the expected protein components of these complexes within the crystals has been confirmed by gel electrophoresis followed by silver staining. A spliceosomal assembly complex containing an RNA component (SAC-RNA) yielded crystals in the presence of polyacrylate. Despite an extensive effort with a variety of commercial screens, no crystals of these complexes could previously be obtained. Finally, the CRLC cytokine receptor–ligand complex formed diffraction-quality crystals (Fig. 2*h*) in the presence of polyvinyl pyrrolidone K15 and pentaerythritol propoxylate.

To score the specific propensity of a polymeric precipitant to induce protein crystallization, the ratio of all hits obtained with a particular polymer to the number of conditions containing that polymer was calculated (Table 3). A crystal was counted as a 'hit' if it was longer than 15 µm in at least one

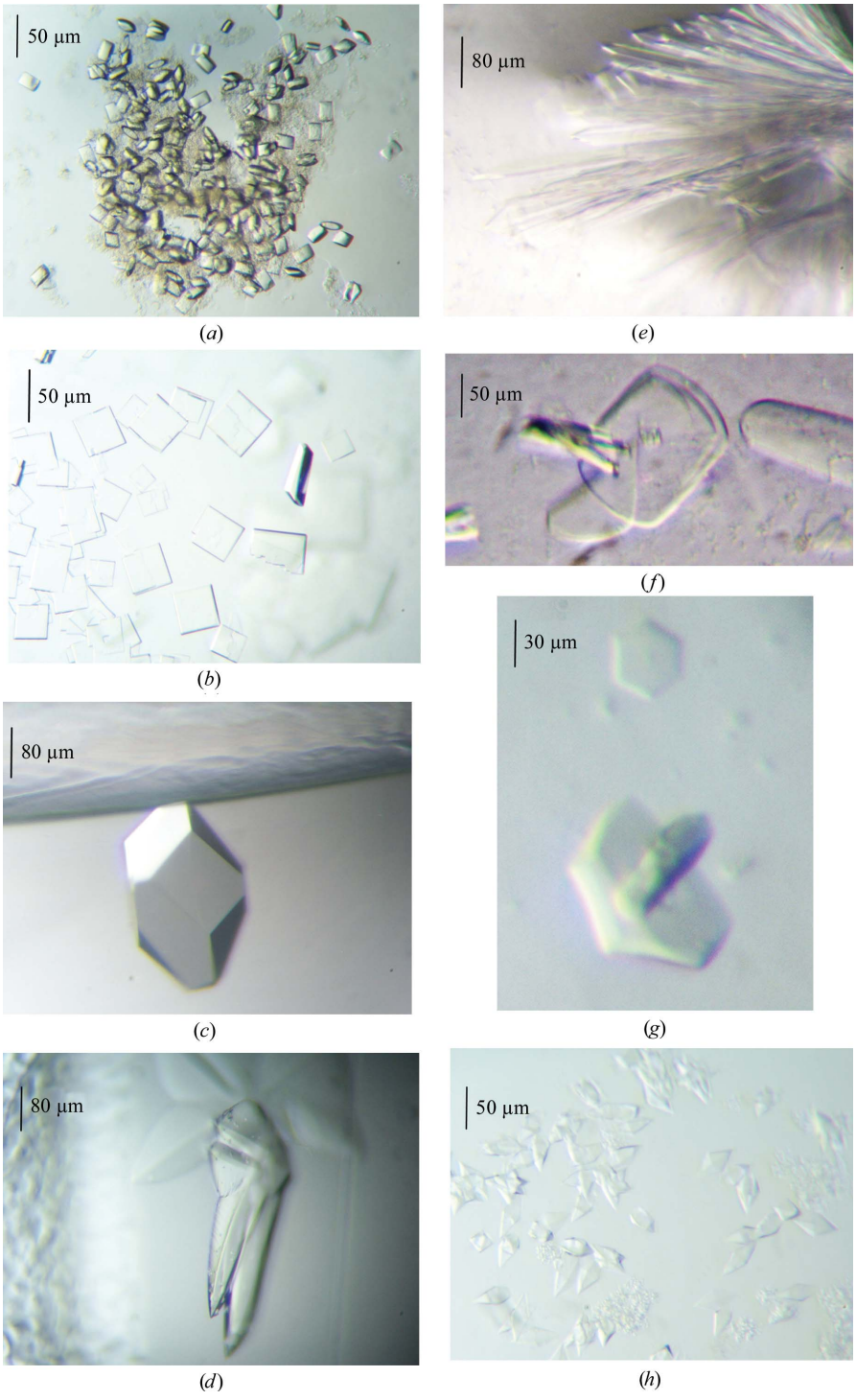

Figure 2
(*a*) Crystals of xylanase obtained in condition 93 of part *B* (20% Jeffamine M2070, 20% dimethyl sulfoxide). (*b*) Crystals of streptavidin core obtained in condition 22 of part *B* (5% polyacrylate 2100, sodium salt). (*c*) Lysozyme crystals obtained in condition 38 of part *C* (35% Sokalan HP 56, 0.1 *M* bis-tris pH 5.5). (*d*) Lysozyme crystals obtained in condition 13 of part *C* (30% Sokalan CP 42, 0.2 *M* potassium acetate). (*e*) Crystals of the four MBT repeats of Sfmbt from *D. melanogaster* obtained in condition 96 of part *A* (35% polyacrylate 2100, sodium salt, 0.2 *M* ammonium sulfate, 0.1 *M* HEPES pH 7.5). (*f*) Crystals of spliceosomal assembly complex (SAC) 9 obtained in condition 41 of part *C* (25% Sokalan CP 42, 10% tetrahydrofuran, 0.1 *M* Tris final pH 7.0). (*g*) Crystals of spliceosomal assembly complex (SAC) 7 obtained in condition 21 of part *B* (6% polyvinyl pyrrolidone K15, 0.1 *M* HEPES pH 6.5). (*h*) Crystals of the cytokine receptor–ligand complex obtained in condition 44 of part *A* [45% pentaerythritol propoxylate (5/4 PO/OH), 0.2 *M* sodium chloride, 0.1 *M* MES pH 6.0].

dimension. To detect possible salt crystals or those of small molecules, X-ray images were taken of around 30 samples. However, after an incubation period of 20 d no salt crystals were identified. Many hits of a certain protein appeared under different conditions with comparable morphology and could thus be identified as protein crystals with reasonable certainty.

The most successful polymers in this study in terms of crystallization propensity were pentaerythritol propoxylate, Jeffamine ED2003, the modified polycarboxylate Sokalan CP 42, polyacrylate and polypropylene glycol 400, which had crystallization propensities in the range 3.2–2.8. Looking at the number of different protein (complex) samples crystallized, 12 out of 20 species formed crystals in presence of pentaerythritol propoxylate, while 11 crystallized in the presence of polyacrylate and nine in the presence of polypropylene glycol (see Table 3 for the complete statistics). Particularly interesting is the case of the two spliceosomal assembly complexes SAC9 and SAC10. Both turned out to be resistant to crystallization in 22 different commercially available screens, including those targeted specifically to complexes. However, they formed diffraction-quality crystals in the presence of Sokalan CP 42, a polycarboxylate whose properties have been optimized towards its intended use in dishwasher tablets. As other polyacrylates within the screen do not induce the crystallization of SAC9 and SAC10, it is probable that the chemistry that makes Sokalan CP 42 suitable for household applications also makes it a suitable precipitant for the crystallization of these two particular complexes. It should be mentioned here that the manufacturer, BASF, does not disclose the exact chemistry of its Sokalan products. Likewise, dSfmbt-4MBT could previously only be crystallized under high-salt conditions within standard screens, while using sodium polyacrylate as a precipitant in our screen yielded a new crystal form that diffracted significantly better than the high-salt forms. On the other hand, in many conditions crystals appeared that were obviously identical to those previously observed with PEG precipitants. Particularly, in the case of HEWL

crystals were observed with all polymer classes (except Walocel HM 100, which did not yield any hits at all). All these crystals could be attributed to the tetragonal crystal form which is known to appear with PEG precipitants. This illustrates that to a certain degree many polymers are exchangeable as a precipitant. However, the chemical variety of polymers available today might provide the exact kind of chemical environment for success with more difficult proteins and complexes. The rationale for the popular crystallization additive screens is very similar: a library of salts, organic solvents, sugars and other small molecules is tested to select those chemicals that provide physico-chemical interactions that foster crystal growth or control nucleation. This study shows that exploiting the chemical diversity of polymeric precipitants can be equally or in some cases even more beneficial; the precipitant is, after water, almost always the predominant component of a crystallization experiment.

Based on the outcome of our experiments, we reduced the 288 tested conditions to a set of 96 by selecting those that were most effective with regard to the number of crystallization hits. To preserve chemical diversity, around 15 conditions were also chosen from polymers with lower crystallization propensity. Conditions that turned out to cause viscosity-related problems during our tests using a Cartesian Honeybee crystallization robot were eliminated. For this reason and owing to their glue-like properties, the cellulose-based Walocel polymers were completely excluded. The resulting crystallization screen is composed of the conditions marked with an asterisk in Table 2. To date, no systematic testing of cryocompatibility has been performed. However, all diffraction tests were performed under cryoconditions that were created by simply supplementing the mother liquor with 35%(*w*/*v*) glycerol. Only conditions containing carboxymethyl cellulose or hydroxypropyl methylcellulose necessitated the addition of glycerol to more than 35%(*w*/*v*) to achieve vitrification.

In conclusion, we also have to mention some of the difficulties associated with some of the alternative polymer classes. First of all, the chemical compatibility of several polymers is lower than that of PEGs. For example, charged polymers might effectively and quickly precipitate some oppositely charged molecular species. Some polymers exhibit only limited solubility in or miscibility with water, a feature that may strongly depend on the pH. Finally, several polymers are pH-active and special care must be taken in this direction. However, most crystallographers will happily accept those complications if a certain polymer turns out to be the 'silver bullet' for the crystallization of a difficult protein or macromolecular complex that has proven to be resistant to crystallization using standard screens.

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