Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

A survey of protein–protein complex crystallizations

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Received 21 November 2005
Accepted 31 March 2006

A survey of crystallization conditions was carried out for 650 published protein–protein complexes in the Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics (RCSB). This resulted in the establishment of a Protein Complex Crystallization Database (PCCD) and a set of configuration-space boundaries for protein-complex crystallizations. Overall, polyethylene glycol (PEG) based conditions accounted for 70–80% of all crystallizations, with PEG 3000–4000, 5000–6000 and 8000 being the most frequently used. The median values of PEG concentrations were between 10 and 20% and were inversely correlated with their molecular weights. Ammonium sulfate remained the most favorable salt precipitant, with a median concentration of 1.6 *M*. The crystallization pH for the vast majority of protein complexes was between 5.0 and 8.0. Overall, the boundaries for the crystallization configuration space of protein complexes appear to be more restricted than those of soluble proteins. This may reflect the limited stability and solubility of protein–protein complexes. Based on statistical analysis of the database, a sparse-matrix and a systematic buffer and pH screen were formulated to best represent the crystallization of protein complexes.

1. Introduction

It is well established that the first step in protein crystallization entails a search in a multidimensional configuration space for regions of successful crystal formation (McPherson, 1999). The combination of a practically unlimited number of possible trial conditions with an often very limited supply of protein samples makes protein crystallization a hit-or-miss process that lacks predictability. The first rational approach for macromolecular crystallization was an incomplete factorial design (Carter & Carter, 1979). Later, a sparse-matrix screening based on a limited number of successful crystallization conditions was introduced by Jancarik & Kim (1991). Since then, numerous kits have been developed by various research and commercial groups to popularize the sparse-matrix method (see, for example, Shieh *et al.*, 1995; Cudney *et al.*, 1994). Recently, attempts were made to analyze the crystallizations from structural genomics projects in an effort to better design or even predict protein crystallization conditions (Hui & Edwards, 2003; Tran *et al.*, 2004; Audic *et al.*, 1997; Rupp & Wang, 2004). However, a random (stochastic) sampling also appeared to be quite efficient in some cases (Segelke, 2001; Rupp *et al.*, 2002).

Over the years, it has become apparent that different classes of macromolecules show systematic bias in their pattern of crystallizations, suggesting that the assumption of equal probability in the incomplete factorial method is inaccurate (Hennessy *et al.*, 2000). This led to the development of crys-

tallization screens specific for certain classes of proteins, such as membrane and DNA-binding proteins. Likewise, the crystallization conditions for many protein complexes appear to be clustered somewhat differently from non-complexed soluble proteins. Previously, our survey of 200 published protein–protein complex crystallizations showed that polyethylene glycols (PEGs) were more successful than ammonium sulfate and that the pH of crystallizations was close to neutral (Radaev & Sun, 2002). With the rapid growth of structural entries in the Protein Data Bank (PDB; Baker & Dauter, 2005), the number of protein complexes in the data bank has also increased dramatically. To date, there are more than 650 protein–protein complex structures in the PDB, more than triple the number in our previous survey. Here, we report the results of a new survey on the crystallizations of these complexes. Based on the survey, we defined the crystallization configuration space for these proteins and formulated two 96-well format kits for initial crystallization screening of protein–protein complexes.

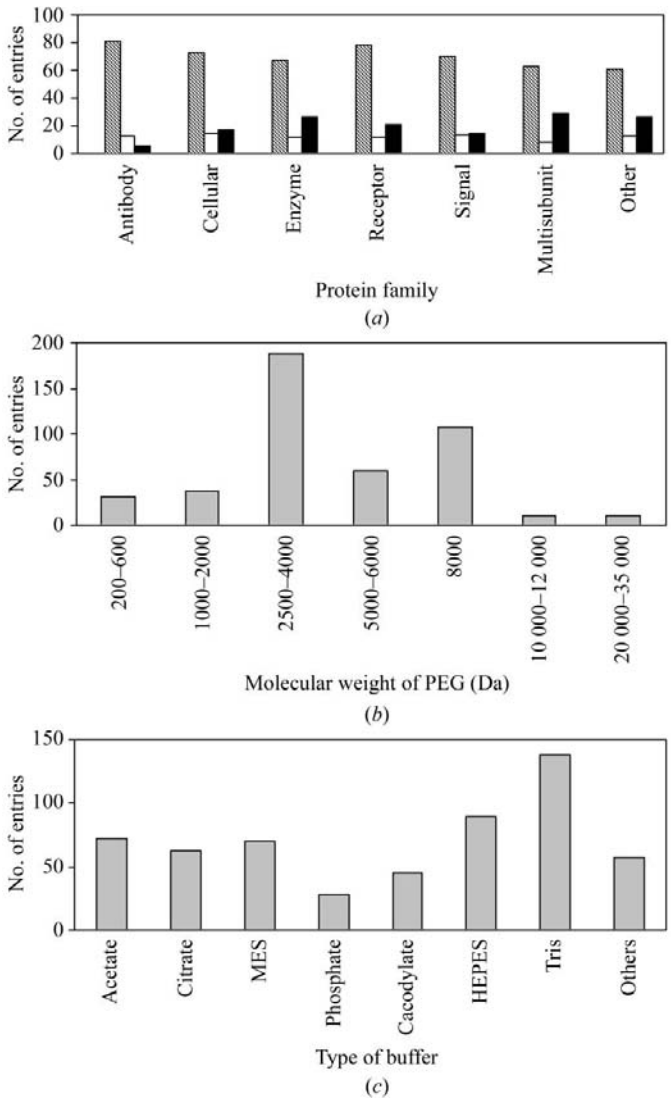

Figure 1
Distributions of (*a*) percentage of different types of precipitants used in the crystallization of various types of protein–protein complexes (hatched, PEG; white, ammonium sulfate; black, other salts and organic solvents, including 2-propanol, MPD and ethanol), (*b*) different types of PEG in protein-complex crystallizations (PEG MME and MPEG are combined with PEG according to their molecular weights) and (*c*) different types of buffers in protein-complex crystallizations.

Table 1
Boundaries in crystallization configuration space and median values for major crystallization parameters.

		Median value	
	Boundary conditions†	Protein complexes	Commercial kits‡
PEG 200–600 (%)	9–30	23	30
PEG 1000–2000 (%)	5–32	18	20
PEG 3000–4000 (%)	5–29	18	25
PEG 5000–6000 (%)	5–24	13	20
PEG 8000 (%)	3–24	13	20
PEG 10 000–12 000 (%)	5.5–18	14	17
PEG 20 000 (%)	3–18	10	10
Sulfate (*M*)	0.6–2.2	1.6	2
Phosphate (*M*)	0.8–1.9	1.0	1.4
Other salts (*M*)	0.6–3.6	1.4	1.5
MPD (%)	18–50	24	45
Other organics (%)	6–30	11	10
Protein concentration (mg ml^{-1})	4.5–37.5	11	
pH value	4.6–8.5	6.75	

† The boundaries for each parameter are defined to include 90% of all crystallization conditions. ‡ Commercial kits included here are Crystal Screen, Crystal Screen 2 and Index (Hampton Research Inc.), Wizard I and II (Emerald Biostructure) and JCSG (Qiagen Inc.).

2. Results and discussion

2.1. Establishing the Protein Complex Crystallization Database (PCCD)

To establish the Protein Complex Crystallization Database (PCCD), we first retrieved all published protein–protein complex structures from the PDB of the Research Collaboratory for Structural Bioinformatics (RCSB). After excluding multisubunit proteins such as free antibodies and major histocompatibility antigen–peptide complexes, the search resulted in 659 unique dissociable protein–protein complexes. They included 74 antibody–antigen complexes, 117 cellular protein complexes, 155 enzyme–inhibitor complexes, 121 receptor–ligand complexes, 71 signal transduction complexes, 52 large multicomponent protein complexes such as ribosomes and 69 other types of protein–protein complexes. Each entry contains the following information: the PDB code and description of the complexes, the method and temperature of crystallization, the protein concentration, precipitant, salt and additive concentrations, buffer concentration and its pH, space group and crystal lattice parameters and related reference. Owing to incomplete crystallization information in the Biological Macromolecule Crystallization Database (BMCD; Gilliland *et al.*, 1996) and the PDB, all crystallization parameters were derived from their respective publications.

2.2. Overall characteristics of protein–protein complex crystallizations

Analysis of the PCCD entries showed that vapor diffusion remains the method of choice in 96% of the cases surveyed. Microbatch and microdialysis constituted the remaining 3% and 1% of the crystallizations, respectively. Over half of the complexes (58%) were crystallized at room temperatures between 293 and 298 K, 24% were crystallized at 277 K and 14% at ambient temperatures between 289 and 292 K. Similar to our previous survey (Radaev & Sun, 2002), polyethelene glycols (PEGs) were the most successful precipitants for the complexes in all categories, constituting 70–80% of all crystallization conditions (Fig. 1*a*). Ammonium sulfate accounted for 10–15% and organic solvents and other salts for 5–25% of the surveyed crystallization conditions. Interestingly, the predominance of polyethylene glycols as precipitants was also found in the crystallization of non-protein complexes (Peat *et al.*, 2005; Kimber *et al.*, 2003; Rupp & Wang, 2004), suggesting that the success of PEG in crystallization is not limited to protein complexes.

2.3. Analysis of crystallization parameters in the PCCD

To define the boundaries of protein complex crystallization configuration space, we analyzed the statistical distributions of all crystallization parameters in the PCCD, such as pH, PEG and salt concentration (Fig. 2). Overall, most of them followed Gaussian distributions, suggesting the presence of an adequate number of entries in the PCCD. For crystals obtained using PEG, the usage of medium molecular-weight PEGs 3000–4000 was most frequent, representing over 40% of all PEG conditions, followed by PEG 8000 (25%) and PEG 5000–6000 (15%) (Fig. 1*b*). The overrepresentation of PEG 3000–4000 and PEG 8000 may in part be a consequence of their higher frequency in commercial kits. In general, lower molecular-weight PEGs displayed broader distributions than higher molecular-weight PEGs. The vast majority of complexes were crystallized between 10 and 20% PEG concentration, significantly lower than the 20–30% range present in most commercial crystallization kits (Table 1). This may be a consequence of the limited solubility of complexed proteins compared with their soluble counterparts. Moreover, the median PEG concentration decreased with the increase in molecular weight of PEGs. Many conditions with PEGs as major precipitants also included 200–300 m*M* salts as co-precipitants.

Ammonium sulfate was still the most frequently used salt, occurring in ∼50% of the cases with major salt precipitants. Other frequently used salt precipitants included lithium sulfate, sodium formate and sodium/potassium phosphate, each constituting ∼10% of the conditions. The concentrations of most salts varied between 1.0 and 2.0 *M*, except for sodium formate, whose concentration was up to 4.0 *M*. The median values for sulfates, phosphates and other salts were 1.6, 1.0 and 1.4 *M*, respectively (Fig. 2; Table 1). Organic solvents, such as methyl-2,4-pentanediol (MPD), 2-propanol and ethanol, constituted only 6% of the total entries in the PCCD. Of these, MPD appeared

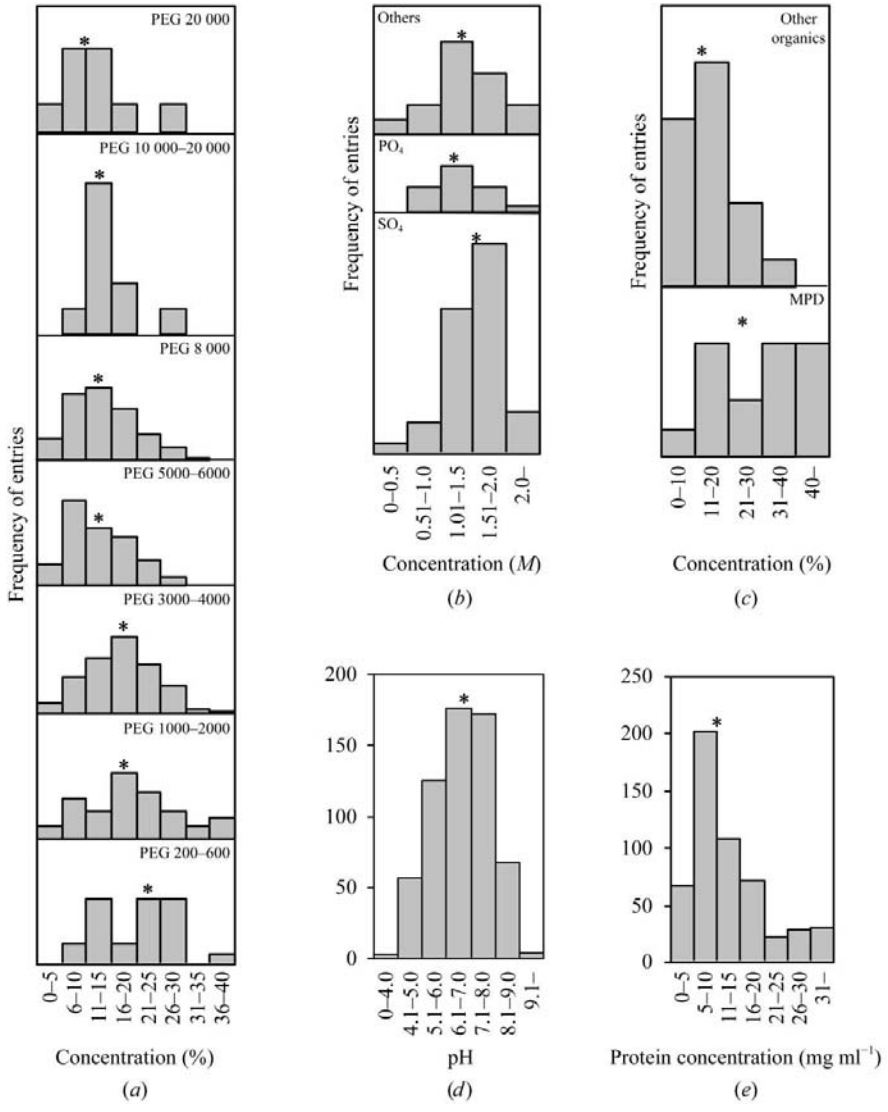

Figure 2
Concentration distributions for (*a*) different molecular-weight PEGs; (*b*) major salts and (*c*) MPD and other organic solvents. SO_4 includes ammonium, lithium, sodium and magnesium sulfates. PO_4 includes sodium/potassium phosphates. Others include sodium formate, NaCl and various acetates and citrates. (*d*) Distribution of pH and (*e*) protein concentrations observed in the crystallizations. An asterisk marks the median values.

to be more successful, with an even distribution between 10 and 70%.

The pH distribution showed a maximum between pH 6.0 and 8.0 (Fig. 2*d*), as extreme pH values often discourage protein complex formation. Only one complex was crystallized at a pH below 4.0 and four at a pH above 9.0. The five commonly used buffers were acetate, citrate, MES, HEPES and Tris (Fig. 1*c*). As regards protein concentration, most complexes were crystallized between 5 and 20 mg ml^{-1}, with a median concentration of 11 mg ml^{-1} (Fig. 2*e*).

2.4. Formulation of protein–protein complex crystallization screening kits

The design of crystallization conditions for certain classes of proteins should begin with defining the boundaries of their respective configuration space. For protein complexes, the crystallization configuration space is shown as two-dimensional projections of PEG *versus* pH and salt *versus* pH (Figs. 3*a* and 3*b*). Once the boundaries have been determined, the set of screening conditions can be obtained using at least two different approaches. The simplest method is to divide the configuration space evenly within the boundaries. However, owing to the multi-dimensional nature of a crystallization configuration space, a reasonable estimate of the crystallization grid sampling (*e.g.* 0.5 pH units, 5% in PEG concentration and 0.5 *M* in various salt concentrations) taking into account different types of buffers and additives would require at least 40 000 conditions to achieve uniform coverage. Furthermore, analysis of protein complex crystallizations showed that the distributions of the crystallization parameters are neither uniform nor random (Figs. 2 and 3). Alternatively, a probability-based approach can be used to divide the configuration space into clusters based on the frequency of crystallization hits and then take the centroids of each cluster as individual conditions. Specifically, a cluster analysis was carried out for each major precipitant including seven different PEGs, MPD, salts and organics (2-propanol and ethanol). The number of screening conditions assigned to each category was proportional to the number of observed crystallizations in that category. In all, the screening kit consisted of 66 PEG-based, 24 salt-based and six other precipitation conditions (Table 2; Figs. 3*c* and 3*d*).

The probability-based screen would in principle increase the chance of crystallization of protein complexes since it is based on the known successful crystallization conditions. However, its non-uniform sampling of the configuration space also creates underrepresented regions in the kit formulation. This increases the potential of failure for certain classes of proteins with unique precipitation profiles. A combination of probability and systematic sampling would allow us to reduce the number of dimensions in the configuration space and yet

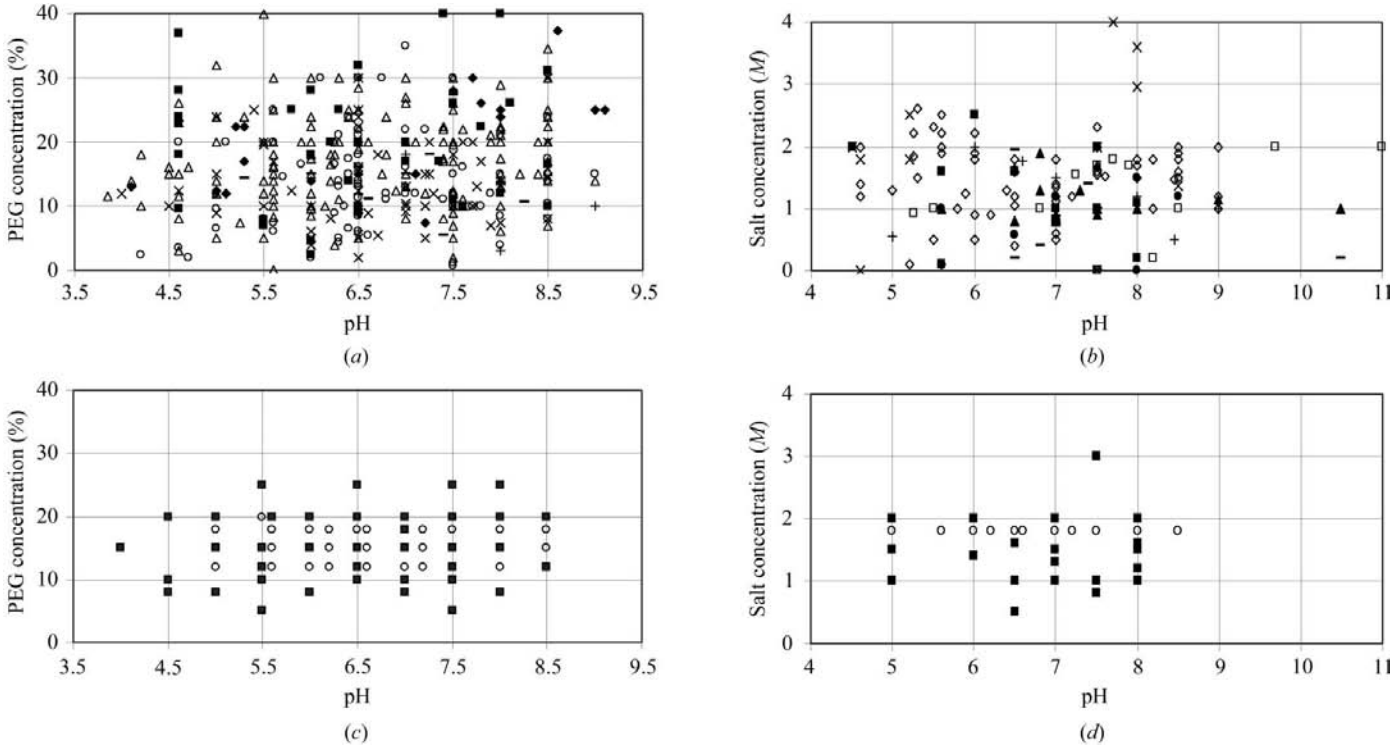

Figure 3
Precipitant concentration and pH distribution for protein complexes and their representation in the new protein-complex screening kits. (*a*) PEG concentrations *versus* pH distribution for PEG 200–600 (filled diamonds), PEG 1000–2000 (filled squares), PEG 2500–4000 (empty triangles), PEG 5000–6000 (crosses), PEG 8000 (empty circles), PEG 10 000–12 000 (dashes) and PEG 20 000 (plus signs). (*b*) Salt concentrations *versus* pH distribution for ammonium (diamonds) and lithium (empty squares) sulfates, sodium/potassium phosphate (filled triangles), sodium formate (crosses), acetate (dashes), citrate (filled circles), sodium/potassium chloride (filled squares) and others (plus signs). (*c*) PEG concentration *versus* pH and (*d*) salt concentration *versus* pH distributions for the protein complex screen (filled squares) and the buffer and pH screen (empty circles).

Table 2
List of conditions in the Protein Complex Screen.

	Precipitant	Salt	Additive	Buffer
1	25% PEG 350 MME			0.1 *M* Tris pH 8.0
2	15% PEG 400	0.1 *M* calcium acetate		0.1 *M* MES pH 6.0
3	20% PEG 400	0.1 *M* LiCl		0.1 *M* HEPES pH 7.5
4	25% PEG 400			0.1 *M* Tris pH 8.0
5	15% PEG 550 MME			0.1 *M* MES pH 6.5
6	25% PEG 1000	0.2 *M* NaCl		0.1 *M* Na/K phosphate pH 6.5
7	20% PEG 1500	0.1 *M* $(NH_4)_2SO_4$		0.1 *M* Tris pH 7.5
8	10% PEG 2000 MME	0.2 *M* $(NH_4)_2SO_4$		0.1 *M* Na acetate pH 5.5
9	20% PEG 2000 MME	0.2 *M* NaCl		0.1 *M* MES pH 6.0
10	15% PEG 2000 MME	0.1 *M* KCl		0.1 *M* Tris pH 8.0
11	25% PEG 2000 MME			0.1 *M* HEPES pH 7.5
12	5% PEG 4000	0.2 *M* Na acetate		0.1 *M* Na citrate pH 5.5
13	5% PEG 4000	0.2 *M* Li_2SO_4		0.1 *M* Tris pH 7.5
14	10% PEG 4000	0.1 *M* Ca acetate		0.1 *M* Na acetate pH 4.5
15	10% PEG 4000	0.2 *M* Na acetate		0.1 *M* Na citrate pH 5.5
16	10% PEG 4000	0.2 *M* NaCl		0.1 *M* MES pH 6.5
17	10% PEG 4000	0.1 *M* $MgCl_2$		0.1 *M* HEPES pH 7.5
18	10% PEG 4000		10% 2-propanol	0.1 *M* HEPES pH 7.0
19	15% PEG 4000	0.2 *M* ammonium acetate		0.1 *M* Na acetate pH 4.0
20	15% PEG 4000	0.1 *M* $MgCl_2$		0.1 *M* Na citrate pH 5.0
21	15% PEG 4000			0.1 *M* Na cacodylate pH 6.0
22	15% PEG 4000	0.15 *M* $(NH_4)_2SO_4$		0.1 *M* MES pH 6.0
23	15% PEG 4000			0.1 *M* HEPES pH 7.0
24	15% PEG 4000	0.1 *M* $MgCl_2$		0.1 *M* HEPES pH 7.0
25	15% PEG 4000	0.15 *M* $(NH_4)_2SO_4$		0.1 *M* Tris pH 8.0
26	20% PEG 4000			0.1 *M* Na citrate pH 4.5
27	20% PEG 4000	0.2 *M* ammonium acetate		0.1 *M* Na acetate pH 5.0
28	20% PEG 4000	0.2 *M* Li_2SO_4		0.1 *M* MES pH 6.0
29	20% PEG 4000			0.1 *M* Tris pH 8.0
30	20% PEG 4000	0.15 *M* $(NH_4)_2SO_4$		0.1 *M* HEPES pH 7.0
31	20% PEG 4000		20% 2-propanol	0.1 *M* Na citrate pH 5.6
32	20% PEG 4000	0.2 *M* NaCl		0.1 *M* Tris pH 8.0
33	25% PEG 4000			0.1 *M* Na cacodylate pH 5.5
34	25% PEG 4000	0.15 *M* $(NH_4)_2SO_4$		0.1 *M* MES pH 5.5
35	25% PEG 4000			0.1 *M* Na cacodylate pH 6.5
36	25% PEG 4000	0.2 *M* KI		0.1 *M* MES pH 6.5
37	25% PEG 4000	0.2 *M* NaCl		0.1 *M* HEPES pH 7.5
38	10% PEG 5000 MME		12% 1-propanol	0.1 *M* MES pH 6.5
39	15% PEG 5000 MME	0.1 *M* KCl		0.1 *M* HEPES 7.0
40	20% PEG 5000 MME	0.2 *M* $(NH_4)_2SO_4$		0.1 *M* Tris pH 7.5
41	8% PEG 6000	0.1 *M* $MgCl_2$		0.1 *M* MES pH 6.0
42	8% PEG 6000	0.15 *M* NaCl		0.1 *M* Tris pH 8.0
43	15% PEG 6000			0.1 *M* Na citrate pH 5.5
44	15% PEG 6000	0.1 *M* Mg acetate		0.1 *M* Na cacodylate pH 6.5
45	15% PEG 6000		5% MPD	0.1 *M* MES pH 6.5
46	15% PEG 6000	0.1 *M* KCl		0.1 *M* HEPES pH 7.5
47	15% PEG 6000			0.1 *M* Tris pH 85
48	20% PEG 6000			0.1 *M* Tris pH 8.5
49	8% PEG 8000	0.1 *M* Mg acetate		0.1 *M* Na acetate pH 4.5
50	8% PEG 8000			0.1 *M* Na citrate pH 5.0

retain uniform sampling on limited parameters, thus reducing the underrepresented regions. For example, if the concentrations of major precipitants were chosen close to their median values, then a systematic grid screening could be performed against buffers and pH. Specifically, a buffer and pH crystallization kit was designed to sample between the pH boundary of 4.5–8.5, while fixing the concentrations for PEG 400, PEG 2000, PEG 4000, MPEG 5000, PEG 6000, PEG 8000 and PEG 20 000 at 20, 18, 18, 18, 15, 15 and 12%, respectively, as well as for ammonium sulfate at 1.8 *M* (Table 3; Figs. 3*c* and 3*d*). The lower concentration for higher molecular-weight PEG reflects the reciprocal correlation between the PEG concentrations and their molecular weights. It is worth noting that this buffer and pH screen shares some of the characteristics, such as lower PEG and salt concentrations, with the Index Screen from Hampton Research Inc.

2.5. Testing of the new screens

We chose two protein complexes as test cases: the complex between NKG2D receptor and its ligand ULBP3 and that between the type III Fcγ receptor (FcγRIII) and the Fc portion of antibody IgG1. Both complexes failed to crystallize from commercial screening kits available in 2001, but were subsequently crystallized by systematic screening of various PEGs against pH in our laboratory. NKG2D is a 14 kDa C-type lectin-like activating receptor expressed on natural killer (NK) cells and certain types of T cells. ULBP3 is a

Table 2 (continued)

	Precipitant	Salt	Additive	Buffer
51	8% PEG 8000	0.2 *M* NaCl		0.1 *M* Na cacodylate pH 6.0
52	8% PEG 8000			0.1 *M* HEPES pH 7.0
53	8% PEG 8000			0.1 *M* Tris pH 8.0
54	12% PEG 8000	0.1 *M* Ca acetate		0.1 *M* Na cacodylate pH 5.5
55	12% PEG 8000			0.1 *M* Na phosphate pH 6.5
56	12% PEG 8000	0.1 *M* Mg acetate		0.1 *M* MOPS 7.5
57	12% PEG 8000	0.2 *M* NaCl		0.1 *M* HEPES 7.5
58	12% PEG 8000	0.2 *M* $(NH_4)_2SO_4$		0.1 *M* Tris 8.5
59	20% PEG 8000			0.1 *M* Na citrate pH 5.0
60	20% PEG 8000	0.2 *M* $(NH_4)_2SO_4$		0.1 *M* MES pH 6.5
61	20% PEG 8000			0.1 *M* HEPES 7.0
62	20% PEG 8000	0.2 *M* LiCl		0.1 *M* Tris 8.0
63	10% PEG 10 000	0.1 *M* Mg acetate		0.1 *M* MES pH 6.5
64	18% PEG 12 000			0.1 *M* HEPES pH 7.0
65	8% PEG 20 000	0.1 *M* NaCl		0.1 *M* Tris pH 8.0
66	15% PEG 20 000			0.1 *M* HEPES pH 7.0
67	0.5 *M* $(NH_4)_2SO_4$			0.1 *M* MES pH 6.5
68	1 *M* $(NH_4)_2SO_4$			0.1 *M* Na acetate pH 5.0
69	1 *M* $(NH_4)_2SO_4$			0.1 *M* MES pH 6.5
70	1 *M* $(NH_4)_2SO_4$			0.1 *M* Tris pH 8.0
71	1.5 *M* $(NH_4)_2SO_4$			0.1 *M* Na acetate pH 5.0
72	1.5 *M* $(NH_4)_2SO_4$			0.1 *M* HEPES pH 7.0
73	1.5 *M* $(NH_4)_2SO_4$			0.1 *M* Tris pH 8.0
74	2 *M* $(NH_4)_2SO_4$			0.1 *M* Na acetate pH 5.0
75	2 *M* $(NH_4)_2SO_4$			0.1 *M* HEPES pH 7.0
76	2 *M* $(NH_4)_2SO_4$			0.1 *M* Tris pH 8.0
77	1 *M* $(NH_4)_2SO_4$	1 *M* KCl		0.1 *M* HEPES pH 7.0
78	2 *M* Na formate			0.1 *M* Na acetate pH 5.0
79	3 *M* Na formate			0.1 *M* Tris pH 7.5
80	0.8 *M* $NaKHPO_4$			pH 7.5
81	1.3 *M* $NaKHPO_4$			pH 7.0
82	1.6 *M* $NaKHPO_4$			pH 6.5
83	1 *M* Na acetate			0.1 *M* HEPES pH 7.5
84	1 *M* Na citrate			0.1 *M* HEPES pH 7.0
85	2 *M* NaCl			0.1 *M* Na citrate pH 6.0
86	1 *M* Li_2SO_4			0.1 *M* MES pH 6.5
87	1.6 *M* Li_2SO_4			0.1 M Tris pH 8.0
88	1.4 *M* Na malonate			pH 6.0
89	1.2 *M* Na/K tartrate			0.1 *M* Tris pH 8.0
90	1.6 *M* $MgSO_4$			0.1 *M* MES pH 6.5
91	15% MPD		2% PEG 4000	0.1 *M* Na acetate pH5.0
92	25% MPD	50 m*M* Ca acetate		0.1 *M* Na cacodylate pH 6.0
93	50% MPD			0.1 *M* imidazole pH 7.0
94	10% 2-propanol	50 m*M* $MgCl_2$	5% PEG 4000	0.1 *M* MES pH 6.5
95	25% 2-propanol	0.2 *M* ammonium acetate		0.1 *M* HEPES pH 7.5
96	15% ethanol	0.1 *M* NaCl	5% MPD	0.1 *M* Tris pH 8.0

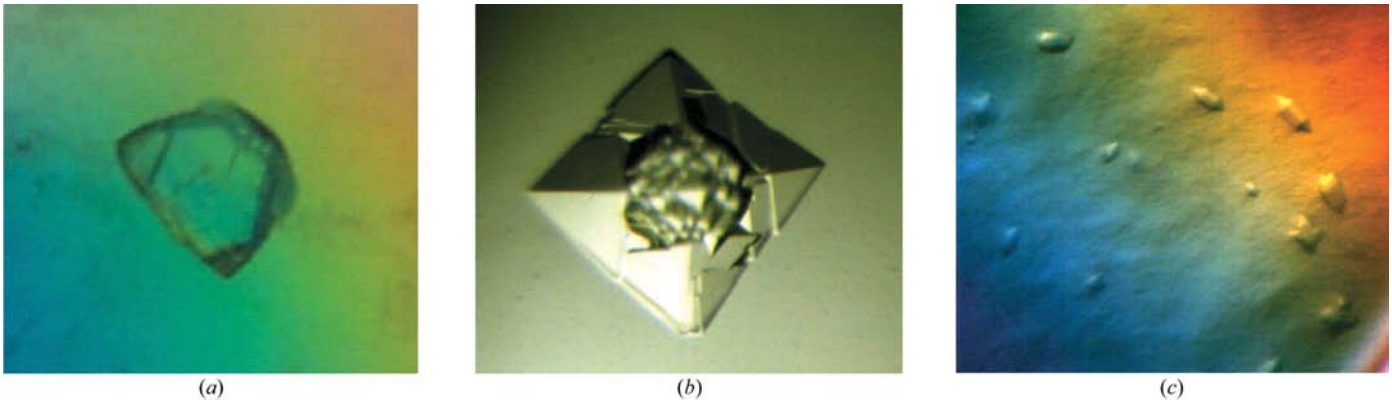

Figure 4
Crystals of NKG2D–ULBP3 and CD16–Fc complexes grown from the new protein–protein complex screen kits. NKG2D–ULBP3 crystals obtained from (*a*) microbatch (condition No. 33 of the Protein Complex Screen) and (*b*) vapor diffusion (condition No. 50 of the Buffer and pH Screen). (*c*) Crystals of CD16–Fc obtained from microbatch (condition No. 34 of the Protein Complex Screen).

Table 3
Buffer and pH Screen.

	Precipitant	Buffer/pH
1	20% PEG 400	0.1 *M* Na acetate pH 4.5
2	20% PEG 400	0.1 *M* Na citrate pH 5.5
3	20% PEG 400	0.1 *M* Na cacodylate pH 6.5
4	20% PEG 400	0.1 *M* Na HEPES pH 7.5
5	20% PEG 400	0.1 *M* Tris pH 8.5
6	18% PEG 2000	0.1 *M* Na acetate pH 5.0
7	18% PEG 2000	0.1 *M* Na citrate pH 5.6
8	18% PEG 2000	0.1 *M* Bis-Tris pH 6.2
9	18% PEG 2000	0.1 *M* $NaKHPO_4$ pH 6.5
10	18% PEG 2000	0.1 *M* Na cacodylate pH 6.0
11	18% PEG 2000	0.1 *M* MES pH 6.0
12.	18% PEG 2000	0.1 *M* MES pH 6.5
13.	18% PEG 2000	0.1 *M* ADA pH 6.6
14.	18% PEG 2000	0.1 *M* HEPES pH 7.0
15.	18% PEG 2000	0.1 *M* HEPES pH 7.5
16.	18% PEG 2000	0.1 *M* MOPS pH 7.2
17	18% PEG 2000	0.1 *M* Tris pH 8.0
18	18% PEG 2000	0.1 *M* Tris pH 8.5
19	18% PEG 4000	0.1 *M* Na acetate pH 5.0
20	18% PEG 4000	0.1 *M* Na citrate pH 5.6
21	18% PEG 4000	0.1 *M* Bis-Tris pH 6.2
22	18% PEG 4000	0.1 *M* $NaKHPO_4$ pH 6.5
23	18% PEG 4000	0.1 *M* Na cacodylate pH 6.0
24	18% PEG 4000	0.1 *M* MES pH 6.0
25	18% PEG 4000	0.1 *M* MES pH 6.5
26	18% PEG 4000	0.1 *M* ADA pH 6.6
27	18% PEG 4000	0.1 *M* HEPES pH 7.0
28	18% PEG 4000	0.1 *M* HEPES pH 7.5
29	18% PEG 4000	0.1 *M* MOPS pH 7.2
30	18% PEG 4000	0.1 *M* Tris pH 8.0
31	18% PEG 4000	0.1 *M* Tris pH 8.5
32	18% MPEG 5000	0.1 *M* Na acetate pH 5.0
33	18% MPEG 5000	0.1 *M* Na citrate pH 5.6
34	18% MPEG 5000	0.1 *M* Bis-Tris pH 6.2
35	18% MPEG 5000	0.1 *M* $NaKHPO_4$ pH 6.5
36	18% MPEG 5000	0.1 *M* Na cacodylate pH 6.0
37	18% MPEG 5000	0.1 *M* MES pH 6.0
38	18% MPEG 5000	0.1 *M* MES pH 6.5
39	18% MPEG 5000	0.1 *M* ADA pH 6.6
40	18% MPEG 5000	0.1 *M* HEPES pH 7.0
41	18% MPEG 5000	0.1 *M* HEPES pH 7.5
42	18% MPEG 5000	0.1 *M* MOPS pH 7.2
43	18% MPEG 5000	0.1 *M* Tris pH 8.0
44	18% MPEG 5000	0.1 *M* Tris pH 8.5
45	15% PEG 6000	0.1 *M* Na acetate pH 5.0
46	15% PEG 6000	0.1 *M* Na citrate pH 5.6
47	15% PEG 6000	0.1 *M* Bis-Tris pH 6.2
48	15% PEG 6000	0.1 *M* $NaKHPO_4$ pH 6.5
49	15% PEG 6000	0.1 *M* Na cacodylate pH 6.0
50	15% PEG 6000	0.1 *M* MES pH 6.0
51	15% PEG 6000	0.1 *M* MES pH 6.5
52	15% PEG 6000	0.1 *M* ADA pH 6.6
53	15% PEG 6000	0.1 *M* HEPES pH 7.0
54	15% PEG 6000	0.1 *M* HEPES pH 7.5
55	15% PEG 6000	0.1 *M* MOPS pH 7.2
56	15% PEG 6000	0.1 *M* Tris pH 8.0
57	15% PEG 6000	0.1 *M* Tris pH 8.5
58	15% PEG 8000	0.1 *M* Na acetate pH 5.0
59	15% PEG 8000	0.1 *M* Na citrate pH 5.6
60	15% PEG 8000	0.1 *M* Bis-Tris pH 6.2
61	15% PEG 8000	0.1 *M* $NaKHPO_4$ pH 6.5
62	15% PEG 8000	0.1 *M* Na cacodylate pH 6.0
63	15% PEG 8000	0.1 *M* MES pH 6.0
64	15% PEG 8000	0.1 *M* MES pH 6.5
65	15% PEG 8000	0.1 *M* ADA pH 6.6
66	15% PEG 8000	0.1 *M* HEPES pH 7.0
67	15% PEG 8000	0.1 *M* HEPES pH 7.5
68	15% PEG 8000	0.1 *M* MOPS pH 7.2
69.	15% PEG 8000	0.1 *M* Tris pH 8.0
70	15% PEG 8000	0.1 *M* Tris pH 8.5
71	12% PEG 20 000	0.1 *M* Na acetate pH 5.0
72	12% PEG 20 000	0.1 *M* Na citrate pH 5.6
73	12% PEG 20 000	0.1 *M* Bis-Tris pH 6.2
74	12% PEG 20 000	0.1 *M* $NaKHPO_4$ pH 6.5
75	12% PEG 20 000	0.1 *M* Na cacodylate pH 6.0
76	12% PEG 20 000	0.1 *M* MES pH 6.0
77	12% PEG 20 000	0.1 *M* MES pH 6.5
78	12% PEG 20 000	0.1 *M* ADA pH 6.6
79	12% PEG 20 000	0.1 *M* HEPES pH 7.0
80	12% PEG 20 000	0.1 *M* HEPES pH 7.5
81	12% PEG 20 000	0.1 *M* MOPS pH 7.2
82	12% PEG 20 000	0.1 *M* Tris pH 8.0
83	12% PEG 20 000	0.1 *M* Tris pH 8.5
84	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* Na acetate pH 5.0
85	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* Na citrate pH 5.6
86	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* Bis-Tris pH 6.2
87	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* $NaKHPO_4$ pH 6.5
88	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* Na cacodylate pH 6.0
89	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* MES pH 6.0
90	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* MES pH 6.5
91	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* ADA pH 6.6
92	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* HEPES pH 7.0
93	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* HEPES pH 7.5
94	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* MOPS pH 7.2
95	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* Tris pH 8.0
96	1.8 *M* $(NH_4)_2SO_4$	0.1 *M* Tris pH 8.5

24 kDa protein with a fold resembling the $\alpha1/\alpha2$ domain of class I major histocompatibility complex (MHC) antigen. The crystallization test trials for the NKG2D–ULBP3 complex were carried out using both the Protein Complex Screen (Table 2; Qiagen Inc.) and Buffer and pH Screen kits by microbatch as well as by vapor-diffusion methods. From microbatch experiments with an Oryx 6 robot crystallization station (Douglas Instruments), twinned crystals of tetragonal bipyramidal shape appeared in condition No. 33 of the Protein Complex Screen kit (25% PEG 4000, 100 m*M* sodium cacodylate pH 5.5) after 2 d (Fig. 4*a*). SDS–PAGE confirmed the presence of both NKG2D and ULBP3 in the crystals. The diffraction quality of the crystals was rather poor and was complicated by severe twinning. However, two major axes, ~60 and ~240 Å, were identified similar to the published unit-cell parameters $a = b = 62.05$, $c = 237.3$ Å (Radaev, Rostro *et al.*, 2001). Under vapor-diffusion conditions, crystals appeared in condition No. 50 of the Buffer and pH Screen (15% PEG 6000, 0.1 *M* MES pH 6.0; Fig. 4*b*). They grew as tetragonal bipyramids approximately 100 µm in size and diffracted to ~3.0 Å on a Rigaku 200 rotating-anode X-ray source with unit-cell parameters $a = b = 62.46$, $c = 238.5$ Å, which matched the previously published data.

The structure of the complex between the extracellular portion of FcγRIII (20 kDa) and Fc portion of IgG1 (50 kDa) was solved in two space groups: $P6_522$ and $P2_12_12_1$ (Radaev, Motyka *et al.*, 2001). Small crystals of the CD16–Fc complex with the characteristic bipyramidal shape reported previously (Radaev, Motyka *et al.*, 2001) appeared in a number of conditions: Nos. 19, 20, 26 and 34 of the Protein Complex Screen and Nos. 54 and 56 of the Buffer and pH Screen in both microbatch and vapor-diffusion settings (Fig. 4*c*). The condi-

tions spanned between 15 and 25% PEG 4000–6000 with pH ranging from 4.5 to 7.5.

It is interesting to note that additional crystallization conditions were obtained in both test cases compared with those previously reported. Although it is difficult to generalize from two specific test cases, the results suggest that the screening kits provide an adequate sampling of a sufficiently large area of crystallization configuration space and offer a good starting point for protein-complex crystallizations. In addition, tests of these screening kits on non-complexed proteins with limited solubility, such as cell-surface receptors, has also been conducted and appeared to be quite successful for these proteins with marginal solubility.

The authors would like to thank Mr Khanh Nguyen for his help in preparation and testing of the new screening kits. The PCCD can be accessed at http://red.niaid.nih.gov/programs.htm. This work was funded by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

References

Audic, S., Lopez, F., Claverie, J. M., Poirot, O. & Abergel, C. (1997). *Proteins*, **29**, 252–257.
Baker, T. & Dauter, Z. (2005). *Acta Cryst.* D**61**, 1.
Carter, C. W. Jr & Carter, C. W. (1979). *J. Biol. Chem.* **254**, 12219–12223.
Cudney, R., Patel, S., Weisgraber, K., Newhouse, Y. & McPherson, A. (1994). *Acta Cryst.* D**50**, 414–423.
Gilliland, G. L., Tung, M. & Ladner, J. (1996). *J. Res. Natl Inst. Stand. Technol.* **101**, 309–320.
Hennessy, D., Buchanan, B., Subramanian, D., Wilkosz, P. A. & Rosenberg, J. M. (2000). *Acta Cryst.* D**56**, 817−827.
Hui, R. & Edwards, A. (2003). *J. Struct. Biol.* **142**, 154–161.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kimber, M. S., Vallee, F., Houston, S., Necakov, A., Skarina, T., Evdokimova, E., Beasley, S., Christendat, D., Savchenko, A., Arrowsmith, C. H., Vedadi, M., Gerstein, M. & Edwards, A. M. (2003). *Proteins*, **51**, 562–568.
McPherson, A. (1999). *Crystallization of Biological Macromolecules.* Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.
Peat, T. S., Christopher, J. A. & Newman, J. (2005). *Acta Cryst.* D**61**, 1662–1669.
Radaev, S., Motyka, S., Fridman, W.-H., Sautes-Fridman, C. & Sun, P. D. (2001). *J. Biol. Chem.* **276**, 16469–16477.
Radaev, S., Rostro, B., Brooks, A., Colonna, M. & Sun, P. (2001). *Immunity*, **15**, 1039–1049.
Radaev, S. & Sun, P. D. (2002). *J. Appl. Cryst.* **35**, 674–676.
Rupp, B., Segelke, B. W., Krupka, H. I., Lekin, T., Schäfer, J., Zemla, A., Toppani, D., Snell, G. & Earnest, T. (2002). *Acta Cryst.* D**58**, 1514–1518.
Rupp, B. & Wang, J. (2004). *Methods*, **34**, 390–407.
Segelke, B. W. (2001). *J. Cryst. Growth*, **232**, 553–562.
Shieh, H. S., Stallings, W. C., Stevens, A. M. & Stegeman, R. A. (1995). *Acta Cryst.* D**51**, 305–310.