JCSG Top96: A Rationally Designed Crystallization Screen Based on over 1000 Deposited Protein Structures

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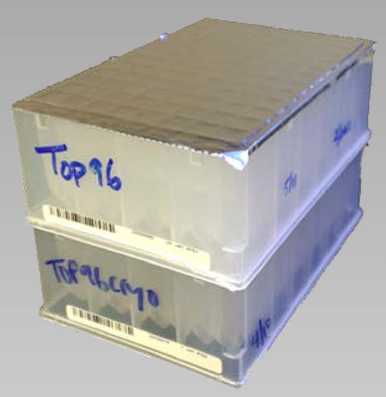

Large scale high-throughput structural biology and structural genomics biology (HT-SG) centers, such as the Joint Center for Structural Genomics (JCSG), present a unique opportunity for the analysis of large systematic datasets of crystallization conditions for a diverse set of protein targets from both bacterial and mammalian origin. The crystallization pipeline developed at the JCSG has resulted in over 165,000 screened crystals and close to 1400 unique protein structures to date. Analysis of these data (07/1/2000 - 9/23/2010) reveals a high level of redundancy in crystallization conditions with 55% (631/1144 deposited JCSG structures) of the structures coming from 25% of the conditions routinely used.

Previous rationally designed crystallization screens, such as our own JCSG+, have focused mainly on the propensity to grow crystals, but not on the analysis of the diffraction properties of the resulting crystals. Using data from 1144 deposited JCSG protein structures, a new minimal 96-well structure-based crystallization screen has been designed - *JCSG Top96* - incorporating the 96 most successful crystallization conditions with respect to PDB deposition. Several trends within this new screening set are identified including the above average performance of PEG in combination with other precipitants and the below average performance of single precipitant conditions. To further build on this screening strategy, the most successful cryo-protectant for each crystallization condition was identified and the *JCSG Top 96 Cryo* screen was formulated. The *JCSG Top96* and *JCSG Top96 Cryo* screens are particularly suitable for proteins available in limited quantities where conservation of material is of high importance. These screens can be used by both large-scale HT-SG platforms and smaller-scale studies in individual laboratories. This high efficiency crystallization screen will help reduce costs and will also enable evermore challenging problems in biological and biomedical research to be addressed using X-ray crystallography.

The ***JCSG Top96 screen*** **is a novel single plate crystallization screen** collated from **10 years of PDB depositions by the JCSG**. The screen is a follow up to previous JCSG screens such as the JCSG Core Suite and JCSG+:

- ***JCSG Top96* conditions** available at: http://www.jcsg.org/top96
- **Large scale HT-SG** structure based screen.
- **Minimal screen, increased efficiency**, saves money and protein.
- **Data mined from:**
  - **5.4 million** crystallization experiments.
  - **145,000** crystals.
- **1144 JCSG protein structures** deposited in PDB → ***Fig. 1***.
- **55%** of JCSG structures crystallize in 96 conditions → ***Fig. 1***.
- **~4-fold better performance** than JCSG+, only 16 conditions in common, JCSG+ → 168 PDB, *JCSG Top96* → 631 PDB.
- **Conditions organized in plate** according to precipitant and concentration → ***Fig. 2***.
- **7 precipitant classes** → ***Fig. 3 and Fig. 4***.
- **Above average** performance of PEG combinations → ***Fig. 4***.
- **Enrichment of PEG** combination precipitants → ***Fig. 5***.
- **Design of *JCSG Top96 Cryo*** from diffraction/PDB data → ***Fig. 6***.

1) *JCSG Top96* Design

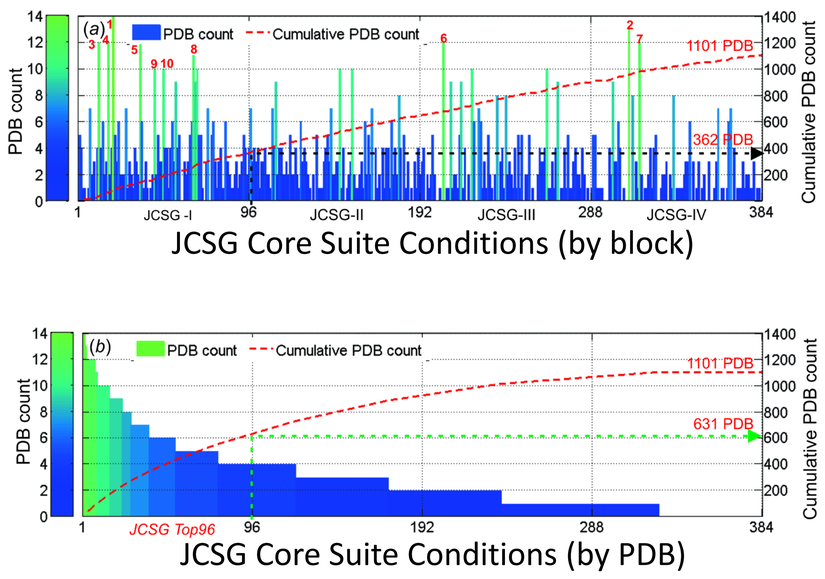

Figure 1: **Selection of *JCSG Top96* conditions**. (a) 1144 JCSG PDBs were ranked by screen (1101 from JCSG Core Suite conditions) and (b) sorted to select the best 96.

2) *JCSG Top96* Plate Layout

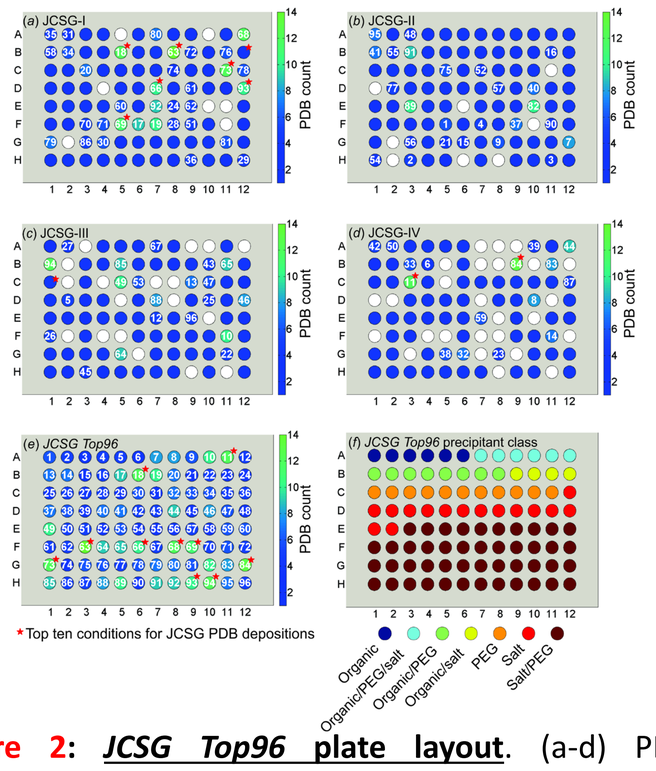

Figure 2: ***JCSG Top96* plate layout**. (a-d) PDB depositions from each of the JCSG Core Suite blocks and (e) *JCSG Top96*, well colored according to PDB counts, (f) *JCSG Top96*, wells colored by precipitant class.

3) *JCSG Top96* Precipitant Class

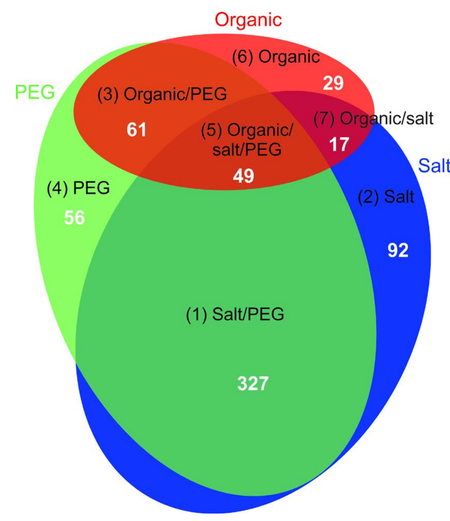

Figure 3: **Seven precipitant classes of the *JCSG Top96* screen**. Proportional Venn diagram showing the overlap of PEG (green), salt (blue) and organic (red) precipitants. Numbers in white represent PDB depositions.

4) *JCSG Top96* Performance

Figure 4: **Performance of the seven precipitant classes of *JCSG Top96***. (a-c) PDB and *JCSG Top96* counts, (d) difference from mean. Note above average performance for all PEG combination precipitant classes shown in (d).

5) *JCSG Top96* Enrichment

Figure 5: **Enrichment of PEG combination precipitant classes**. (a) JCSG Core Suite, (b) *JCSG Top96* and (c) *JCSG Top96 Cryo*. PEG combination precipitants (green) are enriched at the expense of the single precipitant classes (blue).

6) *JCSG Top96 Cryo*

Figure 6: **Selection of optimal cryo-protectant for each of the *JCSG Top96* conditions**. PDB deposition data used to determine cryo-protectant that most frequently gives clear freeze (visually and diffraction-based) for each condition. (a-f) cryo-protectants in use at JCSG.

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