

intensity when doing DLS measurement. After the protein has been placed in the new buffer, concentrate it and set up crystallization trials.

[0051] If conditions are not optimal after performing the initial buffer screening, proceed to use additives. At final concentration, common additives used are 5% glycerol, 10% glycerol, 25, 50, and 100 mM NaCl, 0.1%, 1% octylglucoside, 10 mM betamercaptoethanol, 5 mM dithiothreitol, 2 mM CHAPS. The procedure is as follows to perform additive screening: (A) Use only enough protein to test for the additives. Calculate how much protein needed: # of additives \times protein (2 mg/ml) \times 20 μ l. Estimate some loss during concentration and use this amount to exchange the protein into the best buffer condition found above (Step 12) at a concentration of 50 mM. Use a concentrator (Apollo, Ultrafree, etc.) to concentrate the protein: (a) add the selected buffer to the protein (1:5 dilution, invert gently), concentrate down; (b) add more buffer (1:5 dilution) concentrate again; (c) repeat step (b) one more time. Alternatively the protein can be dialysed against the selected buffer and then concentrated to 2 mg/ml. (B) Based on the protein's properties, decide which additives are going to be tested with the selected buffer (e.g., if it has cysteines it may be preferable to use betamercaptoethanol or dithiothreitol). (C) The total volume of the sample to be 15-20 μ l and the protein concentration to be 1-2 mg/ml. Place 15-20 μ l of protein (1-2 mg/ml) in 0.5 ml eppendorf tubes and add stock additives to each of the tubes to desired concentration. Mix gently by pipetting up and down. (D) Let these samples incubate for 30-60 minutes at room temperature, but preferably overnight. (E) Spin the samples in a microfuge for 5 min and perform DLS on each sample, compare to the results from Step 12, and determine the optimal condition. (F) The optimal buffer with the best additive is then used to exchange the protein using a concentrator as indicated in Step A. Crystallization trials are then performed.

TABLE 6

List of Additives (final concentration):
10 mM BME
2 mM CHAPS
5 mM DTT
5% glycerol
10% glycerol
25 mM NaCl
50 mM NaCl
100 mM NaCl
0.1% Octylglucoside
1.0% Octylglucoside

[0052] The present embodiments, examples, methods, and procedures are meant to exemplify and illustrate the invention and should in no way be seen as limiting the scope of the invention. Variations in the compositions, buffers, additives and their concentrations and various modifications of the methods and applications thereof of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention.

[0053] Any patents or publications mentioned in this specification are indicative of levels of those skilled in the art to which the invention pertains and are hereby incorporated by reference to the same extent as if each was specifically and individually incorporated by reference.

REFERENCES

- [0054] D'Arcy, A. (1994). *Acta Cryst.* D50, 469-471.
- [0055] Ferre-D'Amare, A. R. & Burley, S. K. (1997). *Methods Enzymol.* 276, 157-166.
- [0056] Habel, J. E., Ohren, J. F. & Borgstahl, G. E. O. (2001). *Acta Cryst.* D57, 254-259.
- [0057] Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* 24, 409-411.
- [0058] Laemmli, U. K. (1971). *Nature* (London), 22, 680-685.
- [0059] LePre, C. A. & Moore, J. M. (1999). *Journal of Biomolecular NMR*, 12, 493-499.
- [0060] Zulauf, M. & D'Arcy, A. (1992). *J. Cryst. Growth*, 122, 102-106.

What is claimed is:

1. A method for optimum solubility screening to crystallize proteins comprising the following steps:
 - (a) providing a reservoir of buffer in a container, wherein the container has a cover slip;
 - (b) depositing an aliquot of said buffer either on the cover slip or on a pedestal in the container above the buffer reservoir in each container;
 - (c) placing a protein in said aliquot of said buffer to form a protein solution drop;
 - (d) inverting the cover slip onto (over) the container;
 - (e) incubating the container at a given temperature for a sufficient time; and
 - (f) evaluating said protein solution drop for solubility and monodispersity.
2. The method of claim 1, wherein the steps are repeated or performed in parallel with a set of at least 4 buffers for each protein provided in order to screen for the optimal solubilization conditions.
3. The method of claim 2, wherein the set is comprised of 24 buffers.
4. The method of claim 2, wherein the set of 24 buffers is Table 1.
5. The method of claim 1, further comprising step (g) exchanging the protein into the buffer wherein the protein solution drop is soluble and monodispersed to conduct crystallization trials.
6. The method of claim 1, wherein the evaluating step is carried out by dynamic light scattering.
7. The method of claim 1, wherein a protein solution drop is monodisperse if its radius is <5 nm and polydispersity is <30%.
8. The method of claim 1, wherein if after step (f) the protein solution drops are not soluble or monodispersed, steps a-f are repeated with the buffer which showed the lowest monodispersity, further comprising step (c.1) adding an aliquot of an additive to said protein solution drop to find the best condition.