RNA Crystal Improvement with Definitive Screening Designs

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When one or more crystallization leads have been obtained from prior knowledge or sparse matrix screening, the next step is determining which experimental factors are essential for crystal growth. This task is often done by varying one or two factors with evenly spaced factor levels, often at great expense in time and material. The Design of Experiments (DOE) approach offers experimental designs that can simultaneously vary from three to many factors with a relatively small number of samples. However, the interpretation of the results requires the fitting of linear models. Traditional DOE screening designs include two-level fractional factorials (introduced to protein crystallography by Carter and Carter in 1979) and optimal experimental designs where three or more factors are varied (introduced to protein crystallography by Carter and Yin in 1994). Most factors in vapor diffusion experiments cause a non-linear response in crystal quality and size. The non-linear response requires three factor levels to be detected. The newer Definitive Screening Designs (DSDs) have three factor levels (Jones and Nachtsheim 2011). We were attracted to DSDs because they require roughly half the samples the corresponding optimal designs require.

Here, we apply these designs to identify essential factors in the crystallization of several RNAs to optimize crystal size for single-crystal diffraction studies with synchrotron radiation. We used the hanging drop method for crystallization by vapor diffusion. We used the longest dimension of the largest crystal in a drop as our response variable. We used Response Surface Methodology (RSM) to identify the active factors in DSD experiments with 3 to 8 factors. The DSD experiments enabled us to eliminate the unimportant factors from downstream crystal size optimization experiments, saving us time and material. We envision an efficient workflow in which we screen experimental factors by using a DSD after sparse matrix screening and before optimizing the factors' levels with an optimal design or grid screens. After optimization, we replicate the lead many times to provide an abundant supply of large crystals for cryoscreening and diffraction studies.

To facilitate the adoption of our workflow by others, we implemented the designs in Excel spreadsheets that accommodate different combinations of chemical and physical factors. It takes a worker less than five minutes to customize the design for their project by entering the names of the factors, the concentrations of the stock solutions, the desired mean factor level, and the range of the factor levels. The spreadsheet computes the volumes of the stock solutions for each well. We also provide scripts to do the response surface analyses of the results (https://github.com/MooersLab/dsd4xtals). The README.md file on this site offers detailed explanations of the deployment of the designs and their subsequent analysis.

{1} Carter Jr, C.W. and Carter, C.W. (1979) Protein crystallization using incomplete factorial experiments. J. Biol. Chem, 254, 12219–12223.

{2} Carter Jr, C.W. and Yin, Y. (1994) Quantitative analysis in the characterization and optimization of protein crystal growth. Acta Crystallographica Section D: Biological Crystallography, 50, 572–590.

{3} Jones, B. and Nachtsheim, C.J. (2011) A class of three-level designs for definitive screening in the presence of second-order effects. Journal of Quality Technology, 43, 1-15.

Runs	Random	Treatment	A Coding	B Coding	C Coding	A Level	B Level	C Level
1	0.9615	1	0	1	-1	50	200	2
2	0.495	2	0	-1	1	50	20	12
3	0.0582	3	-1	0	-1	20	110	2
4	0.3303	4	1	0	1	80	110	12
5	0.5286	5	-1	-1	0	20	20	7
6	0.4419	6	1	1	0	80	200	7
7	0.4249	7	-1	1	1	20	200	12
8	0.2504	8	1	-1	-1	80	20	2
9	0.5961	9	0	0	0	50	110	7
10	0.1896	10	0	0	0	50	110	7
11	0.6254	11	0	0	0	50	110	7
12	0.497	12	0	0	0	50	110	7

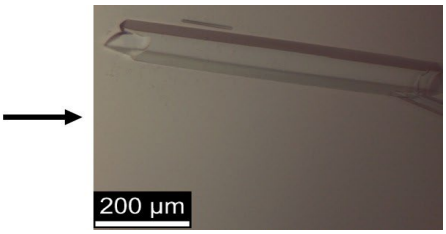

Figure 1