

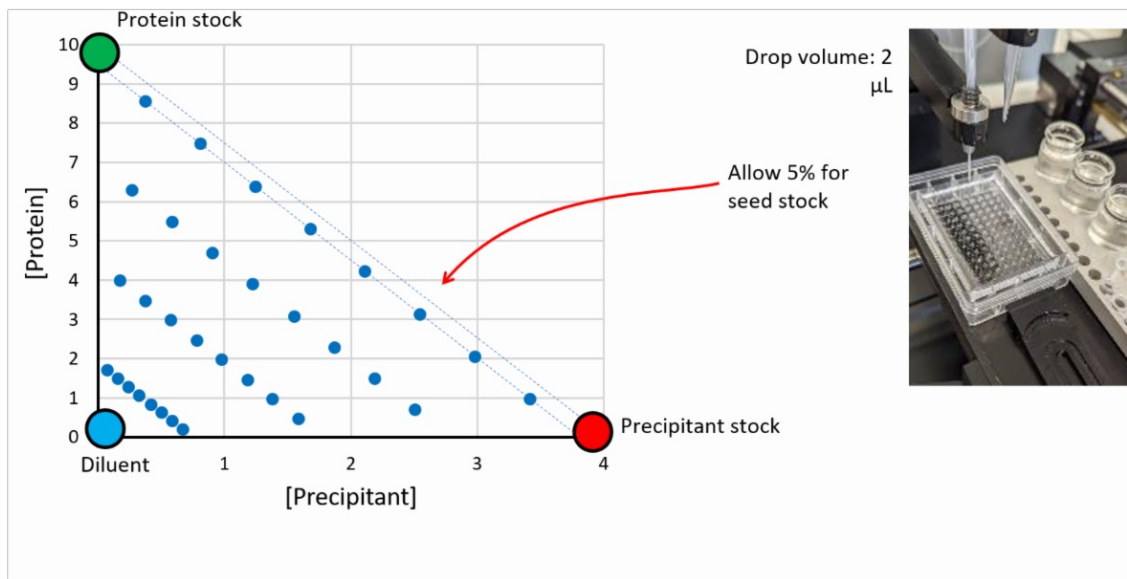
Dear Stefan

Explore your protein's phase diagram with our innovative and powerful new experiment script.

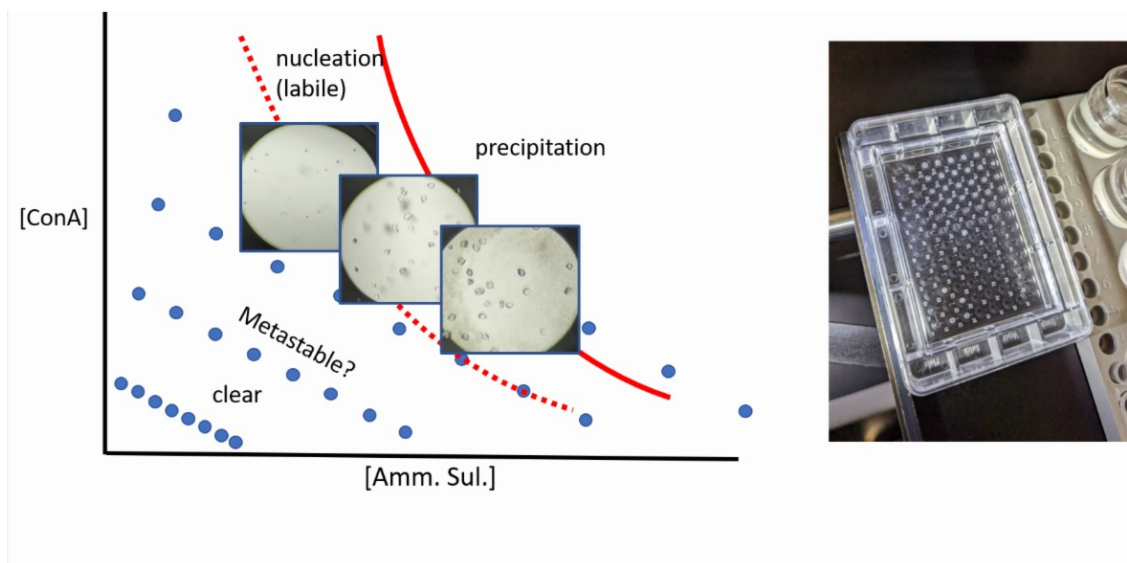
Douglas Instruments recommends a new approach to explore crystallization space systematically. The new experiment design samples a diagonal path across the phase diagram that is parallel to the expected solubility curve. This can give greater coverage of crystallization space than a typical protein-precipitant 2D concentration gradient, without requiring additional protein.

**Case study using concanavalin A:**

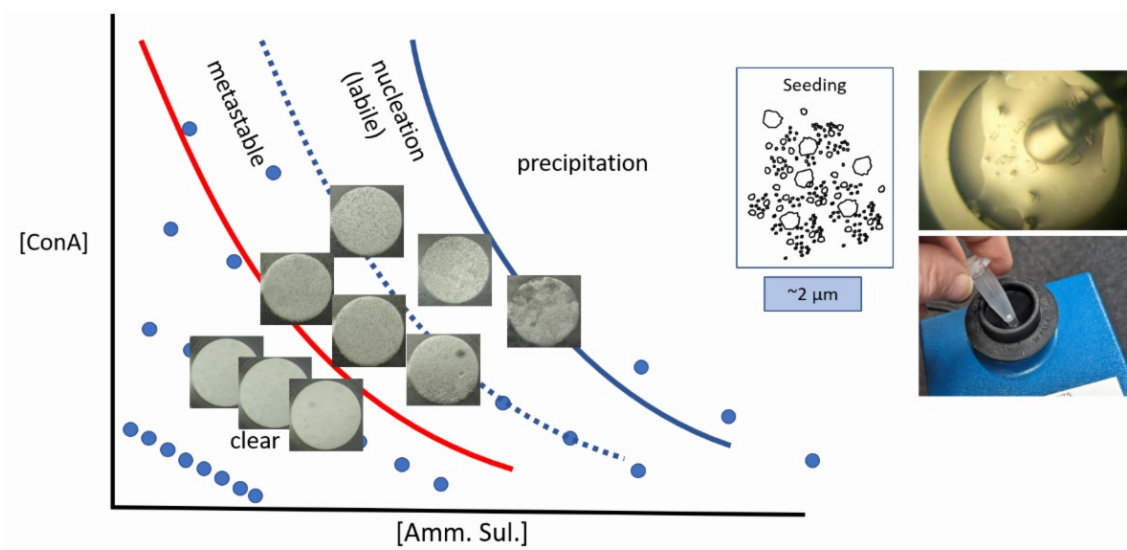
**Experiment design:** phase diagram experiment using concentrated protein (green), precipitant cocktail (red) and diluent (blue)



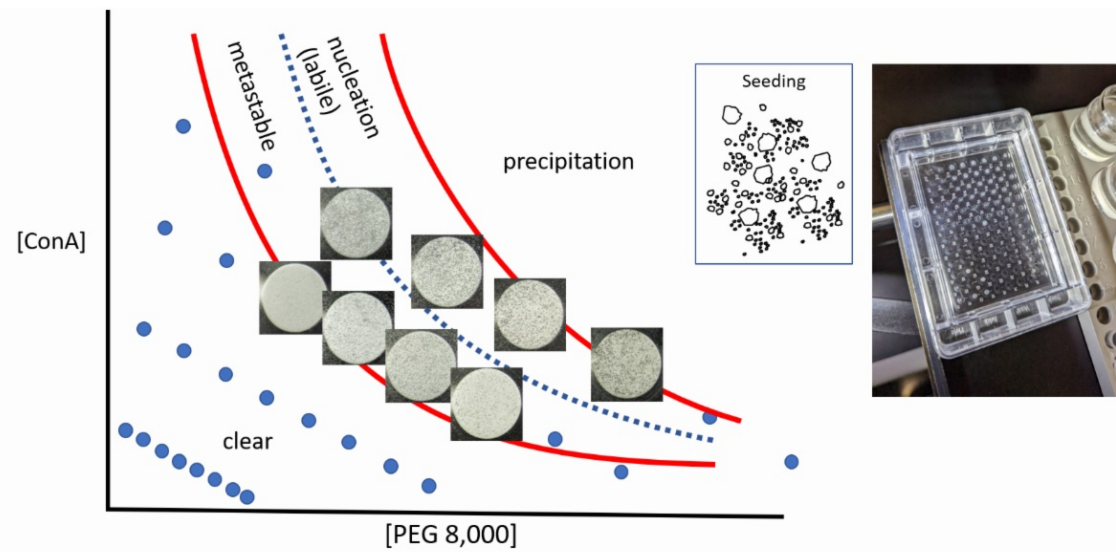
**Experiment 1:** three drops had crystals without seeding. This showed us the rough upper and lower boundaries of the nucleation zone



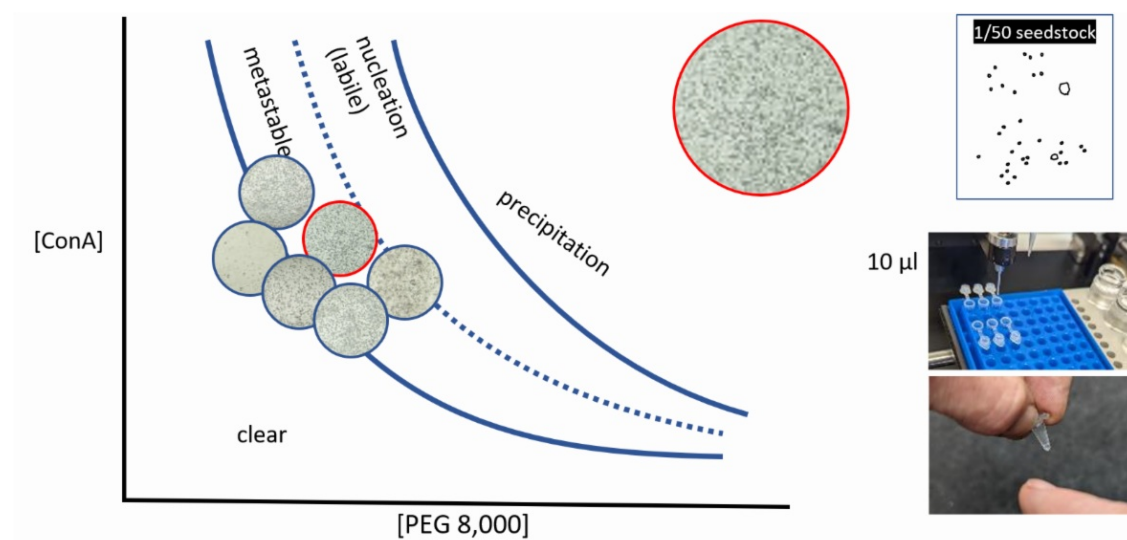
**Experiment 2:** we used one of the drops from experiment 1 to make a seedstock, and repeated the experiment with microseeding. This gave us the lower boundary of the metastable zone



**Experiment 3:** we repeated the same experiment with 50% PEG 8,000 replacing ammonium sulfate



**Scaling up:** using an Oryx8 we dispensed larger volumes to six PCR tubes with a 1/50 dilution of the seed-stock, at slightly lower PEG concentration. The PCR tubes were flicked, not vortexed.



#### Key benefits of new experiment:

1. **Expanded crystallization space:** Achieve broader testing with a systematic approach that can help identify optimal conditions for serial data collection, MicroED, or neutron diffraction.
2. **Simple and versatile:** this method is highly adaptable and often requires no changes between projects. Simply provide the protein and a pre-mixed precipitant cocktail at the desired maximum concentration, when requested by the software.
3. **Seeding:** run experiments both with and without seeds to identify the metastable regions of the phase diagram. Seeding can also be utilized to control crystal size and density.

#### Conferences:

Douglas Instruments is attending the following meetings:

#### ECM 34, Padova

26 August - 30 August 2024

#### Rhine-Knee Regional Meeting, Konstanz

30 September - 2 October 2024

#### Visit our booth for:

Demonstrations

rMMS microseeding toolkits

Updates about our latest projects including crystallization methods for serial crystallography.

#### Recently published research using Oryx protein crystallization robots:

#### Rationally seeded computational protein design of $\alpha$ -helical barrels

Albanese, K.I., Petrenas, R., Pirro, F., Naudin, E.A., Borucu, U., Dawson, W.M., Scott, D.A., Leggett, G.J., Weiner, O.D., Oliver, T.A. and Woolfson, D.N., 2024.

Nature Chemical Biology, pp.1-9.

#### Protein Thermodynamic Properties, Crystallisation, and the Hofmeister Series

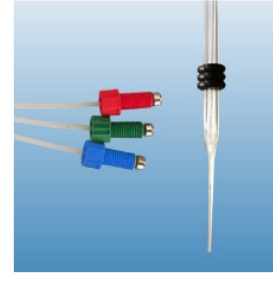
## Products available from Douglas Instruments



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