Acoustic Liquid Handling Applied to Protein Crystallography—Miniaturization, Formulating, Transferring, Monitoring, & LCP Formation

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Abstract
Acoustic liquid handling—using sound to move fluids and suspensions—has been widely accepted in the pharmaceutical field. It is scalable to laboratory needs and now has been applied to protein crystallography processes. The technology uses low-energy pulses of sound to eject fluids from an open source eliminating any place of fluid contact. We believe that acoustic liquid handling offers a new tool of multiple dimensions that can be used in many different crystallographic applications.

INTRODUCTION
The Labcyte Echo 550 series requires no liquid handling by using acoustic coupling to create a sound field that couples with a protein crystallization fluid. The acoustic field creates a central droplet in a microwell, which is used with a new tool of multiple dimensions that can be used in many different crystallographic applications.

EXPERIMENT 1A: Transfer of Glycerol Solutions: Measurement of Accuracy and Reproducibility

Transfer of glycerol solutions (5–60% by volume) was carried out on an Echo 555 liquid handler. The Echo liquid handler moves fluids without any physical contact with the sample. A transducer moves towards the plate holding the fluids and emits acoustic energy, which creates a central droplet in the microwells. Two microwells (6 x 3) were set up with 10 µL of 5%, 10%, 20%, 30%, 40%, 50%, and 60% glycerol solution. The test was performed in triplicate to ensure accuracy and precision.

EXPERIMENT 1B: Transfer of Glycerol Solutions: Measurement of Reproducibility

Transfer of glycerol solutions (5–60% by volume) was carried out on a Labcyte Echo 555 liquid handler. Two microwells (6 x 3) were set up with 10 µL of 5%, 10%, 20%, 30%, 40%, 50%, and 60% glycerol solution. The test was performed in triplicate to ensure accuracy and precision.

EXPERIMENT 2: Formulation of Microintermediate Solutions through Spot-on-Contact

Methods
Formulate microintermediate solutions from transfer-on-contact.

EXPERIMENT 3: Transfer of Microcrystals

Methods
Microcrystals were transferred from one mesh to another mesh. The acoustic droplet-ejection system was calibrated using a 384-well plate. The microcrystals were transferred from one mesh to another mesh. The transfer was performed in triplicate to ensure accuracy and precision.

EXPERIMENT 4: Non-invasive monitoring of protein crystalization fluids

Methods
Pulses of acoustic energy were directed into microwells containing protein crystallization fluids. At any interface between materials where the acoustic impedance is not identical, some of the energy will be reflected back towards the transducer (i.e., emitted). The reflected energy will be transferred to the near-field region, where it is detected by a transducer. The amplitude of the energy reflected back to the transducer (R2) is a function of the acoustic impedance of the material through which the acoustic pulse has passed and the acoustic impedance of the substance to which the pulse is entering. The acoustic impact is affected by the temperature of the materials through which the acoustic energy passes.

EXPERIMENT 5: Transfer of Acoustic phase cubic components to facilitate the self-assembly of nanoliter-scale environments for membrane protein crystallization

Methods
50 nL of anisotropic (MDM) water was transferred to a destination plate followed by immediate (225 nL) molecule in methanol (21 by weight) transferred “spot-on-contact.” After 2 minutes the cell plate was placed in a 4°C nitrogen bath. The microwells were observed at 15 min intervals until the samples had repeated four times with a 3 minute delay time between cycles. After the 15th minute of transfer, the plate was transferred to an incubator at 35°C.

SUMMARY

These experiments show that acoustic droplet ejection is a viable tool for transfer in protein crystallography. The acoustic droplet-ejection system was calibrated using a 384-well plate. The microcrystals were transferred from one mesh to another mesh. The transfer was performed in triplicate to ensure accuracy and precision. It is possible to transfer the protein solution crystallization fluids non-invasively with the analytical functions that are included with an Echo liquid handler. It is also possible to transfer all the components needed to form a lipidic cubic phase acoustically. The LCP appears to be more advantageous for handling large volumes than other liquid handlers.