# Supporting Information
# Coupling high throughput microfluidics and Small-Angle X-ray scattering to study protein crystallization from solution

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## Fabrication of microfluidics chips

Table S1. **Parameters for fabrication of a dry film mold**

| Steps | Details |
|---|---|
| Lamination 1st layer | 50 µm |
| Exposure | 10 seconds at 25mW/m2 |
| Lamination 2ndlayer | 100 µm |
| Exposure | 10 seconds at 25mW/m2 |
| Lamination 3rd layer | 200µm |
| Exposure | 15 seconds at 25mW/m2 |
| Post Exposure Bake | 55 seconds by hotplate at 100oC |
| Development | 12 minutes in 1% w/w Na2CO3 |
| Rinse and dry | 5 minutes in 1% w/w CaCO3 and water |
| Post bake | 55 seconds at 100oC |

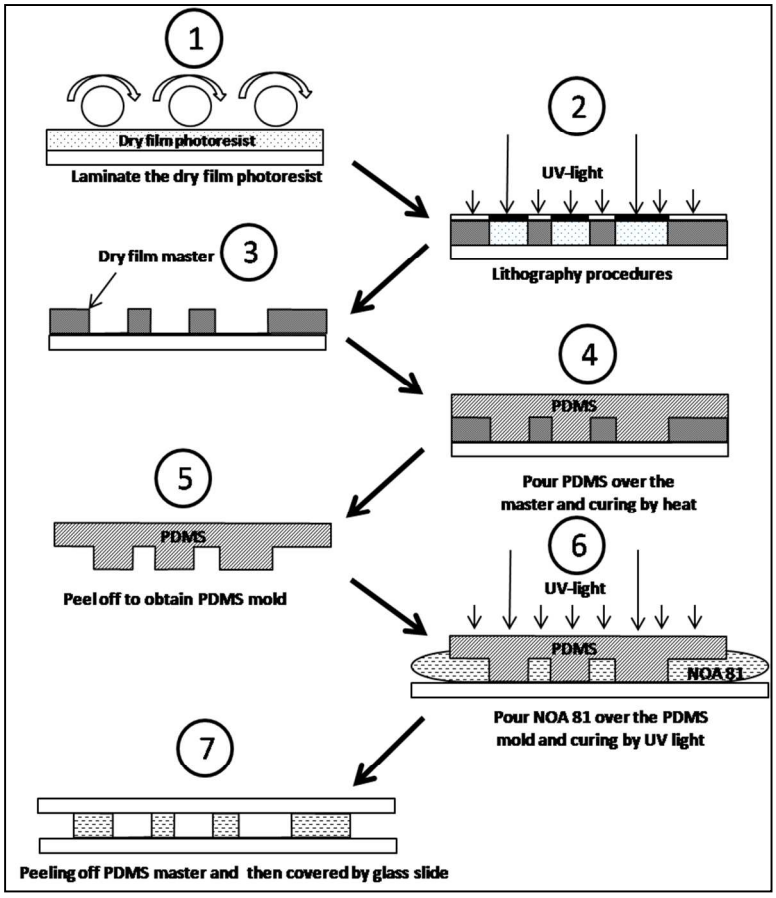


Figure S1. Schematic overview of device construction: 1-3) Dryfilm master was fabricated after laminating and processing by soft lithography procedure 4-5) A PDMS master was formed from dryfilm mold after curing at 65°C for 2 hours. 6)-7) NOA closed – channel protocol.

## Purification of protein solutions

Dynamic light scattering experiments on lysozyme diluted in acetate buffer showed that commercial lysozyme is not only composed by monomers. Indeed, the hydrodynamic radius of a single lysozyme molecule is supposed to be around 2 nm and the DLS graph presented above highlight unusual bumps around 100 nm due to larger aggregates. In crystallization/nucleation experiments, these aggregates could lead to non-expected results and a misunderstanding of the nucleation phenomenon and must therefore be removed from protein solution.

In order to purify the protein solutions, samples are centrifuged through membrane centrifugal filters with molecular weight cut-off of 50 kDa (Amicon, Merck Millipore, Germany) for 20 minutes. The molecular weight of lysozyme being 14 kDa, monomers are consequently passed through the membrane and can be obtained in the lower part of the filter while aggregates are retained in the upper part. DLS graphs of the lysozyme solution before and after purification are presented above.

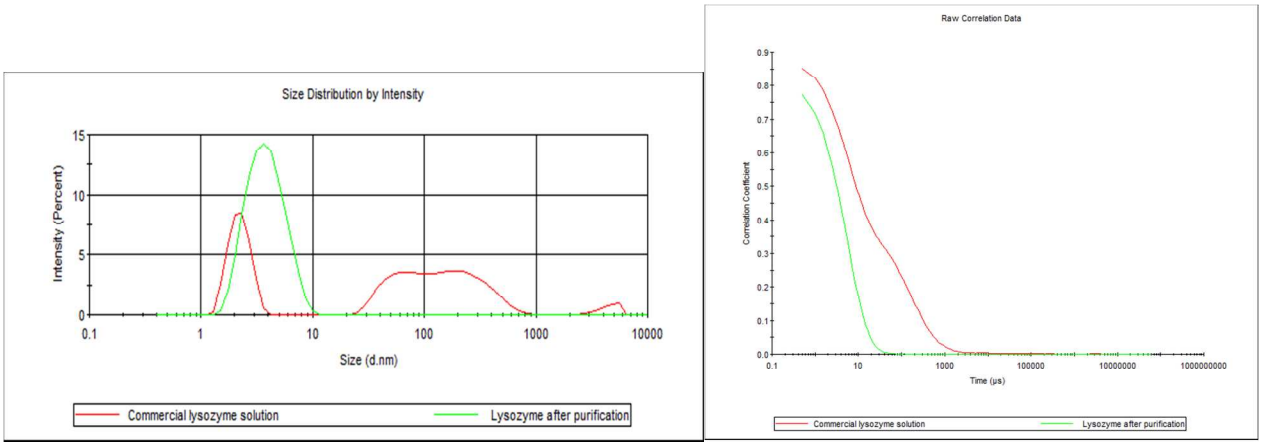


Figure S2: DLS experiments on commercial lysozyme solution before (red) and after (green) purification. Intensity vs size (left) and correlogram (right).

During the process, due to removal of aggregates, total protein concentration is approximately divided by 3. To obtain a final concentration of pure monomer lysozyme of 150 mg/mL, one could start with a 450 mg/mL initial protein solution. Nevertheless, such high concentration solution tends to form a gel and is impossible to filter through membrane. To overcome this problem, it is better to start with a lower concentration of protein solution and to concentrate it after. To concentrate the pure monomeric solutions, samples are centrifuged through membrane centrifugal filters with molecular weight cut-off of 3kDa (Amicon, Merck Millipore, Germany) for 30 min. Lysozyme monomers are retained and collected in the upper part of the filter while solvent is passed through the membrane.

With this two-step process, starting with a 150 mg/mL commercial lysozyme solution, one can obtain an approximately 50 mg/mL pure monomeric solution, and then concentrate it to more than 100 mg/mL as presented in the figure above.

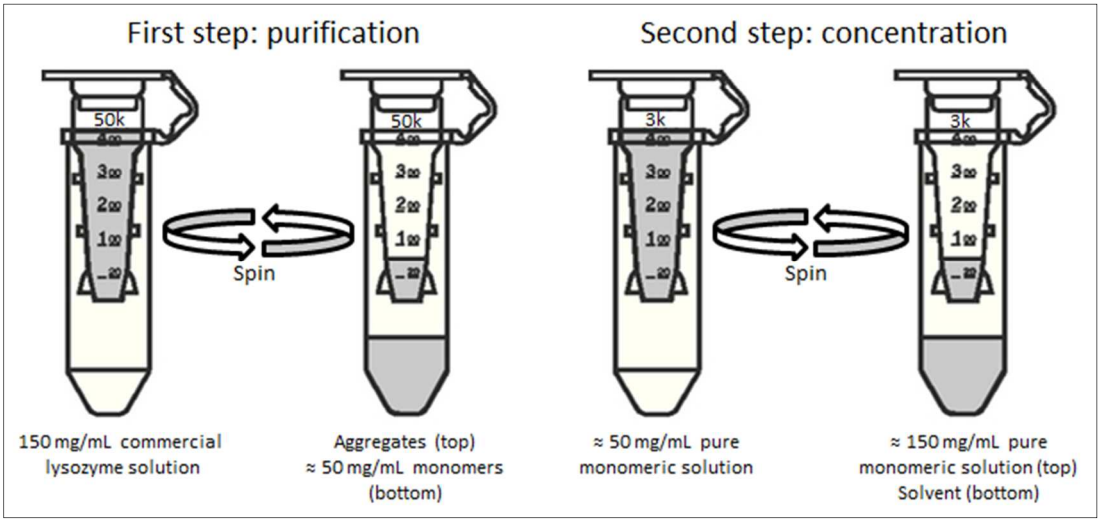

Figure S3: Process of purification and concentration of lysozyme.

## Experiments on determination of proteins interactions in solution

Table S2. **Operating conditions: Flowrates of incoming stock solutions and concentrations of different compounds in the droplets.**

| Flow Rates (μL/min) | | | | Concentrations | |
|---|---|---|---|---|---|
| Oil | Lysozyme | NaCl | Buffer NaAc | Lysozyme (mg/mL) | NaCl (mM) |
| 4 | 0.5 | 0 | 2.5 | 21.7 | 0 |
| 4 | 0.5 | 0.15 | 2.35 | 21.7 | 100 |
| 4 | 0.5 | 0.30 | 2.20 | 21.7 | 200 |
| 4 | 0.5 | 0.45 | 2.05 | 21.7 | 300 |
| 4 | 0.5 | 0.60 | 1.90 | 21.7 | 400 |
| 4 | 1.2 | 0 | 1.8 | 52 | 0 |
| 4 | 1.2 | 0.15 | 1.65 | 52 | 100 |
| 4 | 1.2 | 0.30 | 1.50 | 52 | 200 |
| 4 | 1.2 | 0.45 | 1.35 | 52 | 300 |
| 4 | 1.2 | 0.60 | 1.20 | 52 | 400 |
| 4 | 2.0 | 0 | 1.0 | 86.7 | 0 |
| 4 | 2.0 | 0.15 | 0.85 | 86.7 | 100 |
| 4 | 2.0 | 0.30 | 0.70 | 86.7 | 200 |
| 4 | 2.0 | 0.45 | 0.55 | 86.7 | 300 |
| 4 | 2.0 | 0.60 | 0.40 | 86.7 | 400 |