Getting the most from a 50 µl stock of sample using the µSFM for refolding experiments

I – Introduction

Reducing sample consumption in stopped-flow experiments has always been a high priority as the cost of sample (proteins, peptides…) can be hundreds or thousands of dollars per millilitre. Typically for a refolding experiment (doing a 10 fold dilution) using a classical stopped-flow design the user would use 20-30 µl of sample per a single shot. So, considering you need to have about 9-10 shots for a good statistical analysis, and also considering the priming volume of the system, 500 µl is often considered as a minimum volume for your stock solution to schedule a series of experiments.

This application note demonstrates how the µSFM significantly reduces sample usage. Only 50 µl of precious sample would be required to plan a series of experiments!

II–Experimental setup

In this application note we use hen egg lysozyme to simulate an expensive protein. Only 50 µl of lysozyme at 0.5 mg/ml prepared in 6M Guanidine hydrochloride is used for the series of experiments. The refolding is initiated by diluting 10 times with water by applying a 1:9 mixing ratio with the µSFM. A MOS-200 is used to follow the intrinsic Tryptophan fluorescence.

MOS-200 equipped with a 150W xenon mercury lamp.

- Mode: fluorescence.
- Excitation wavelength: 280 nm
- Emission: 320 nm low pass band filter

µSFM equipped with 500 µl syringes

- Syringe 1: 500 µl of water
- Syringe 2: 50 µl of 0.5 mg/ml lysozyme in 6M GnD-HCl.
- Cuvette: µFC-08 (0.8 mm x 0.8mm)
- Mixer: Berger Ball
- Total flow rate: 1 ml/s.

The user can directly transfer the solutions to the µSFM syringe using the provided loading adaptor directly from Eppendorf™ tubes (see figure 1).

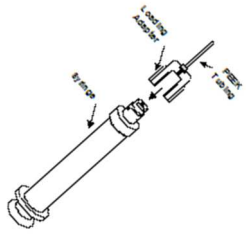

Figure 1: loading adaptor

Not a single drop of precious liquid is lost at this stage.

The mixing sequence used is shown in Figure 2. The user sets the total volume per shot and the mixing ratio. In this note the user pushes a total volume of 32 µl at each shot (3.2 µl of protein and 28.8 µl of water considering the 1:9 mixing ratio). This level of accuracy results from the nanolitre precision of the stepping motors used in the µSFM.

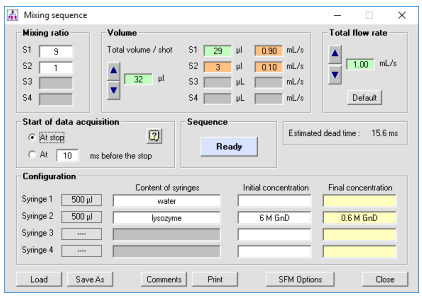

Figure 2: mixing sequence

As such, with this experimental set-up, 15 shots are obtained from the 50 µls loaded in syringe2.

III–Data acquisition

In less than 1 minute, the fluorescence signal for the 15 automatic replicates, each 2 seconds long is recorded.

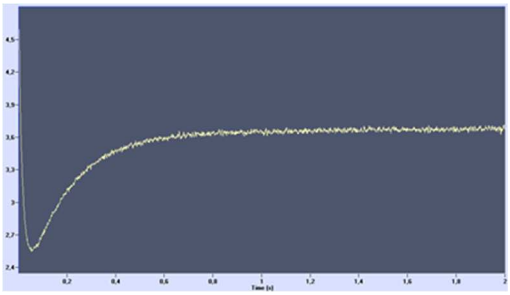

Figure 3: single shot of 3.2 µl of lysozyme

The traces are fitted independently using a two exponentials model and results of the fits are reported in table 1.

Shot Number	k1	k2
1	*trace distorted*	
2	*trace distorted*	
3	*trace distorted*	
4	*trace distorted*	
5	*60,9*	*5,8*
6	*67*	*5,4*
7	58,2	5,6
8	55,6	5,2
9	54	5,1
10	59,5	5,6
11	56	5,7
12	57,3	5
13	59,2	5,1
14	58,3	5,2
15	53,3	5,1
Average (7-15)	**56,8**	**5,3**
Deviation (7-15)	**2,2**	**0,3**

Table 1: Exponential fit results.

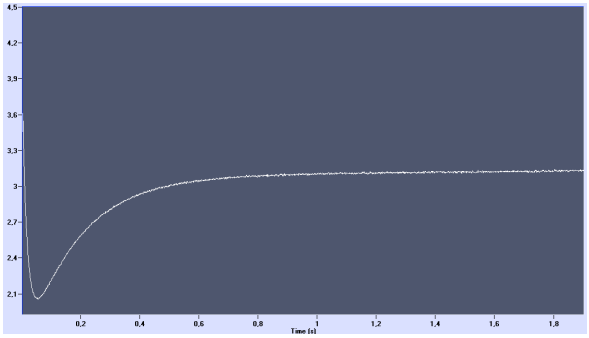

Figure 4: average trace (shot 7 to 15)

In this experiment, the first four traces are clearly distorted, while shots 5 and 6 show some deviations with respect to the remaining replicates and are discarded from the final analysis.

IV–Conclusion

With a stock of 50µl, we have obtained statistically valid kinetics. It has been possible to run 9 valuable shots.

With a stock of 500µl of sample, the user would have been able to do 150 experiments!