

## Rapid Kinetic and Spectroscopy instruments

### SFM20, SFM300&400 - high dilutions and variable ratio mixing – part II (updated August 13, 2009)

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The possibility to obtain variable mixing ratio by a simple programming of the instrument (i.e. without changing the syringes) is one of the major advantages of the Bio-Logic stopped-flow instruments. The microprocessor control of the stepping motors giving 6400 steps per revolution of the motor gives a smooth and quasi continuous movement of the syringe over a very large range of flow rate.

A few examples of significant experiments to test and apply this property are described below.

#### INSTRUMENTS USED

Stopped-flow : SFM-300 equipped with FC-08 cuvette. Syringes 1 and 2 were the standard 10 mL syringes while the syringe N°3 was the small 1.9 mL syringe.

Spectrometer : MOS-250 in fluorescence mode.

- Excitation : 297 nm
- Emission 340 nm
- Band pass 10 nm
- 150 W Xe(Hg) lamp

#### TEST REACTION

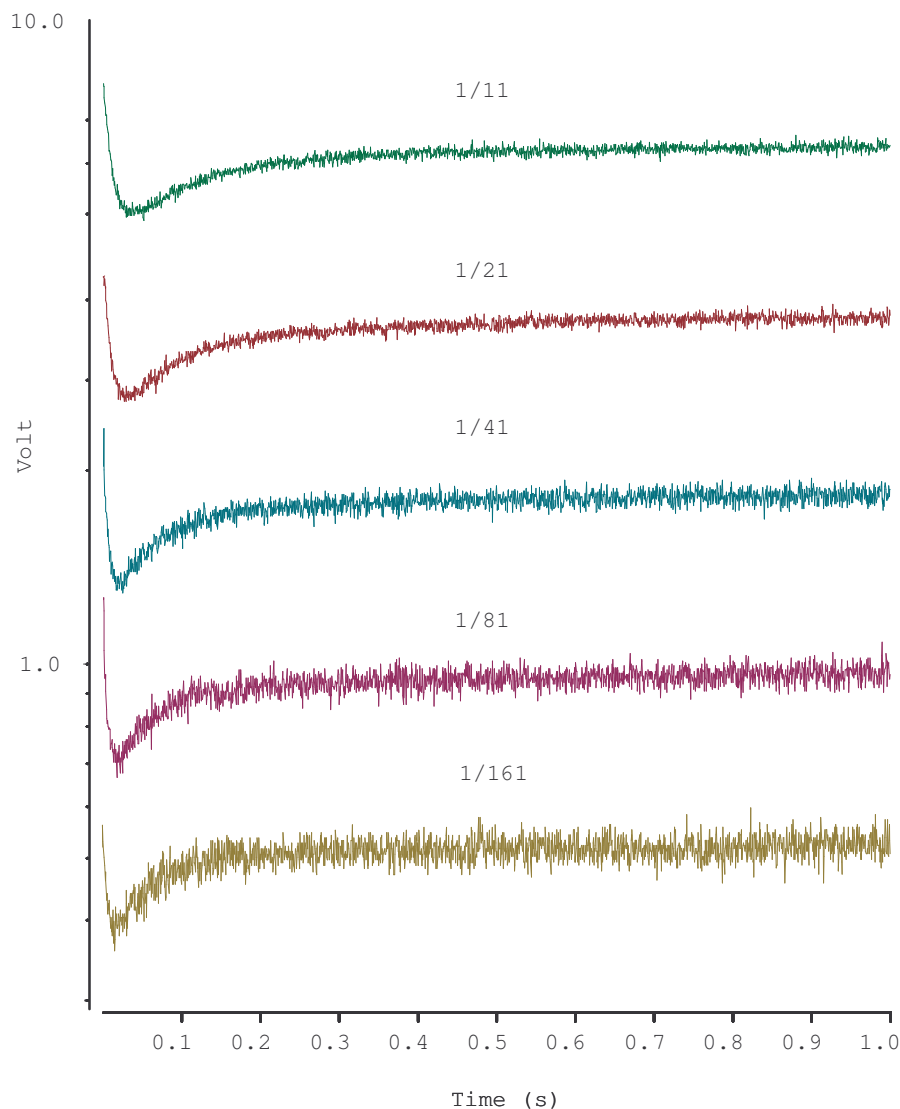
Refolding of lysozyme. Hen egg lysozyme, (1 mg/mL) denatured in 6 M guanidine chloride was set in the instrument syringe N°3. Refolding reaction was initiated by diluting this solution in 50 mM Pi buffer pH 7.2.

## HIGH DILUTION TEST

High dilution factors were obtained by mixing at each shot : **15  $\mu\text{L}$**  of denatured enzyme against variable amount of the dilution buffer. It is important to note that these experiments were obtained with a unique load of the instrument and that the dilution ratio were obtained by simple reprogramming of the syringe movements.

Dilutions from **1/11** to **1/161** were then obtained by mixing the 15  $\mu\text{L}$  enzyme solution with 150 to 2400  $\mu\text{L}$  of buffer. The two syringes N°1 and N°2 were used to deliver the dilution buffer keeping the total flow rate high enough (10 mL/s) to ensure that the dead time of the experiment remained nearly constant and equal to **1.5 ms**.

Results are shown below.



Note the logarithmic scale on the ordinate. Kinetics of refolding are well observed in spite of the progressive reduction of the signal due to increasing dilution of the enzyme. At 1/161 dilution, the final enzyme concentration was **6.2  $\mu\text{g/mL}$** .

Note also the change in rate constants due to the decrease of final guanidine concentration (from 540 mM to 3 mM)

## CHANGE OF CONCENTRATION OF ONE REACTANT

This test was executed to demonstrate how to modify the concentration of one reactant without changing the concentration of the other.

To demonstrate this we used the same reaction as above. In this case, however, the final concentration of enzyme was kept constant, while many final guanidine concentrations were assayed.

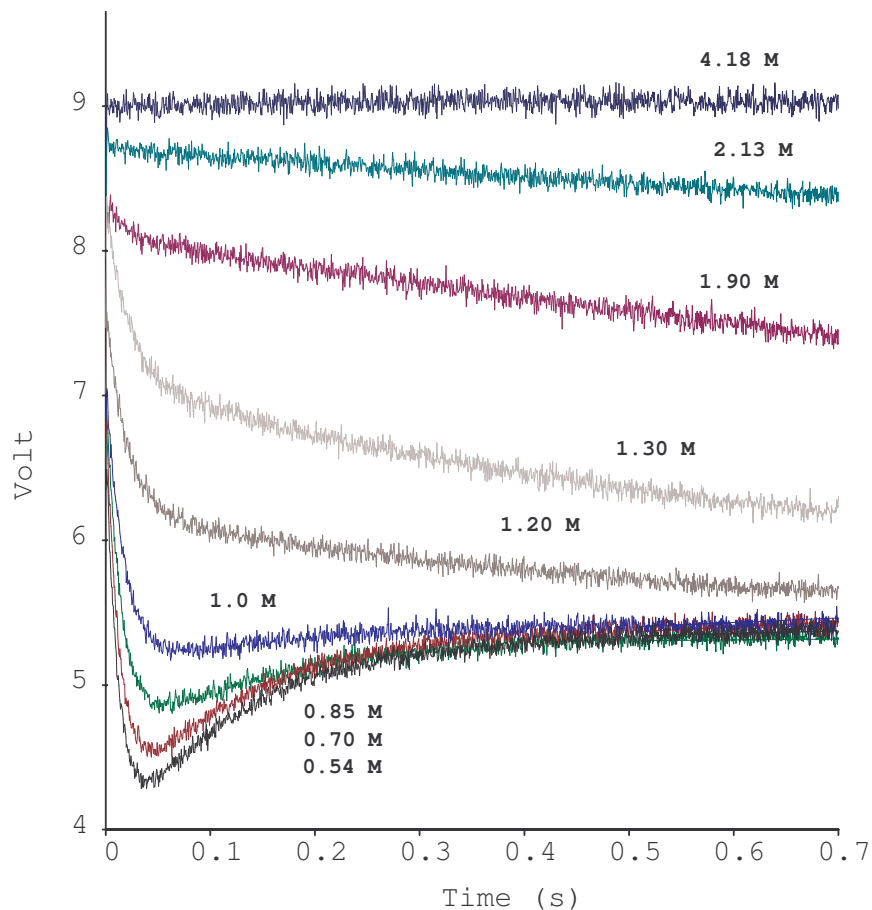
The sequence of shot was as followed :

Flow duration (ms)-----	40
S1 (buffer) (μL) -----	240 - V
S2 (4 M Guanidine) (μL) -----	V
S3 (Enzyme + 6 M Guanidine) (μL) -----	24

“V” value was varied from one shot to the other from 0 μL to 240 μL therefore giving a final guanidine concentration varying from 0.54 M to 4.18 M.

At the same time final lysozyme concentration was constant and equal to 90 μg/mL.

The plots in the figure below shows how the refolding kinetics and final lysozyme fluorescence is dependent on the final guanidine concentration. The plot below shows kinetics obtained for the whole set of final guanidine concentrations. (as indicated on the graph).



Here also it is important to point out that this series of shots were obtained with a single filling of the syringes and with simple reprogramming of the instrument from the software