Which quench flow for my lab?

I. What is a quench flow?

The chemical quench flow instrument, commonly called quench flow, is a member of the rapid kinetics instruments family. It is used to follow reactions on the milliseconds to seconds timescale. Depending on the application it can be used as an alternative to stopped-flow (for example when no optical detection is possible). Alternatively it can be used as a complement to stopped-flow (when you wish to trap an intermediate of reaction for structural analysis).

In a quench flow instrument, the reaction is initiated by mixing two reactants. The solution is allowed to age in a delay line (also called the ageing line or ageing loop) for a user-defined time before being stopped by a third sample (the quencher) in a second mixer. The solution is then collected for external analysis. By collecting samples at various ageing times the user can build the kinetics of the reaction.

Other quenching methods are available. The reaction can be quenched by cold, eg. by ejecting the aged solution to a cryo-bath for further EPR, NMR or Mossbaeuer analysis, this is known as the freeze quench technique. Light can also be used, a laser or xenon flash lamp stops the reaction, this are optical quench methods. These two techniques are not described in this application note, but BioLogic SFM can address these two applications and the SFM can be converted from quench flow to freeze quench or optical quench in minutes.

At a glance
In this document you will learn:

- The basics of quench flow
- How to choose the right instrument for your application, simply by asking the right questions
- About the different ageing methods to age your reaction before quenching.
- The benefits of each quench flow model.

II. Three ageing methods

1. Using continuous flow (the traditional method)

In continuous flow mode, the solutions are injected at a constant flow rate in the mixers. The user selects the reactant volumes and how fast he/she wants to inject. The ageing time is the time spent by the reactants to move from mixer 1 (where the reaction is initiated) to mixer 2 (where the reaction is stopped). The ageing time can thus be easily calculated by dividing the volume of the delay line by the flow rate of solutions going through the line. An example of driving sequence is given in Figure 1 using a 74µl delay line. The user injects 50µl of each solution (1:1:1 mixing ratio) in 25ms so flow rate in the delay line is 4ml/s: thus, ageing time is 74/4=18.5ms.

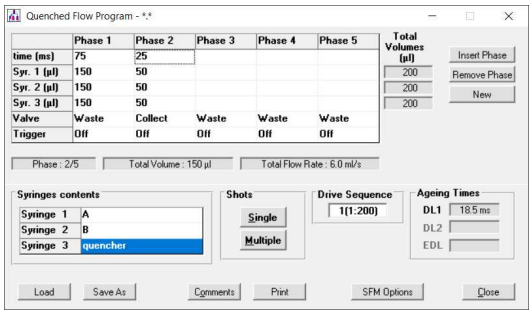

Figure 1 Example of sequence in continuous flow mode using a 74µl delay line.

The limitation of this mode is the ability to generate turbulent mixing as mixers require a minimum flow rate (1ml/s) to generate turbulent conditions.

Similarly, the flow rate of 12ml/s (maximum flow rate) is linked to the speed of the diverting valve and motor specifications. So, for a given delay line, only a range of ageing time is achievable. For example, using a 74µl delay line, ageing times from 6ms to 74ms can be obtained. To reach longer times the user sets a longer delay line. However, delay line volumes cannot be increased constantly, due to mechanical constraints. Also, the larger the delay line, the more samples needed for the washing step (to clean and equilibrate delay line between experiments) and the experiment itself. So, this ageing method may require larger sample volumes, which may create issues. For all these reasons the continuous flow method is generally preferred for ageing time below 300ms.

2. Using Interrupted flow

The interrupted flow mode is the traditional method used to reach ageing times higher than 300 ms. This pushing mode differs from the continuous flow mode as the experiment is carried out in several phases. First, the ageing line is filled with the reactants. The samples are then allowed to age in the delay line for a pre-defined time before being pushed to mixer 2 for quenching. In this mode there are the same limitations as before for minimum flow rate (1ml/s), but this is less of a problem, as most of the ageing is made from the incubation phase between the loading and emptying phase. An example of such a sequence is given in Figure 2.

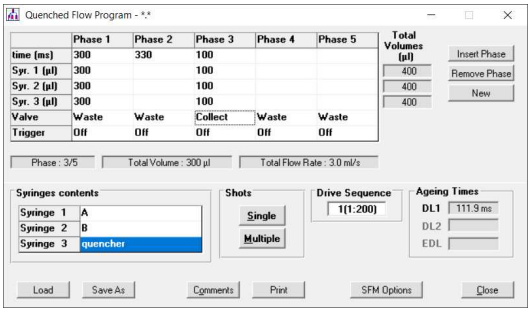

Figure 2 Example of sequence in interrupted flow mode using a 224µl delay line.

Phase 1 of the sequence is used to wash the delay line from previous experiments and the last µl will be used to load the delay line. This will be followed by a 330 ms incubation to the mixing. Phase 3 is the collect phase where the ageing line is emptied at exactly the same flow rate as has been loaded in phase 1. It is crucial to have the same mixing ratio and flow rate in phase 1 and 3 to collect a homogeneous aged solution. Because the flow rate is the same in both phases the loading/emptying time can be calculated easily: it is the volume of the delay line divided by flow rate. In the example in Figure 2, the volume of the ageing line is 224µl; and the flow rate in the ageing line is 2 ml/s, so the loading/emptying time is 224/2=112 ms. Adding the incubation time, the total ageing time for this experiment would thus be 442 ms. One can clearly see that the minimum flow rate is no longer a limitation in this mode, as users can keep the flow rate constant at each shot and just vary the incubation time.

This mode can only be used with long delay lines which would thus require more samples compared to experiments using short ageing times. This mode also has its own limitations as you cannot collect more sample than the volume you incubate in the ageing line, which could be limiting for some analysis techniques that require 300-400µl of sample. In such cases, accumulation of experiments is required.

3. Using pulse flow

As explained before, in instruments using continuous flow modes, the mixers cease to operate correctly at decreasing flow rates (increasing ageing times). This necessitates switching to interrupted mode which is not recommended if samples quantities need to be used sparingly.

This scenario is negated by the pulsed-flow mode since the instantaneous flow rate in the pulses ensure a correct mixing of the reacting solutions.

In fact, the pulse mode is a combination of the continuous flow and interrupted flow modes. Instead of filling the delay line continuously, the delay line is filled by micro-pulses separated by several micro incubation times (see Figure 3). The ageing time is then defined by the duration of the n pulses to flow solutions from mixer 1 to mixer 2 and the incubation times between pulses. Figure 3 a_n represents the ageing time of the solution when it is quenched and collected. By accumulating more than n pulses the user can collect as much solution as required for analysis.

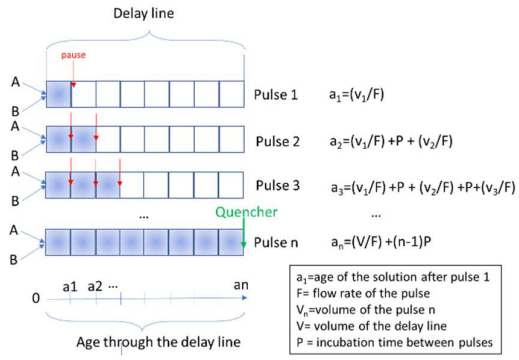

Figure 3 Principle of pulse flow mode

The individual pulses sizes vary from 0.3 to 2µl and are automatically set by the instrument to adapt to the flow line geometry and to the ageing conditions. The mean flow rate value is achieved by varying the frequency of the pulses. The pulse frequency does not modify the turbulent conditions of each pulse.
So, it means long ageing times can be reached without changing the delay line, but just changing the incubation time between pulses. Thanks to the sub-microliter precision of stepping motors, pulse mode can then be applied to small delay lines. This means:
- lower volumes of sample are required per experiment
- no need to change the delay line volume when increasing ageing time
- the same amount of sample is required, whatever the ageing time.

From a user's perspective, the pulse design (volume, numbers and frequency) is transparent as it is fully software controlled. The user only sets the volume of sample to mix and the ageing time he wants to reach. The Biokine software will automatically generate the right pulse parameters (see Figure 4)

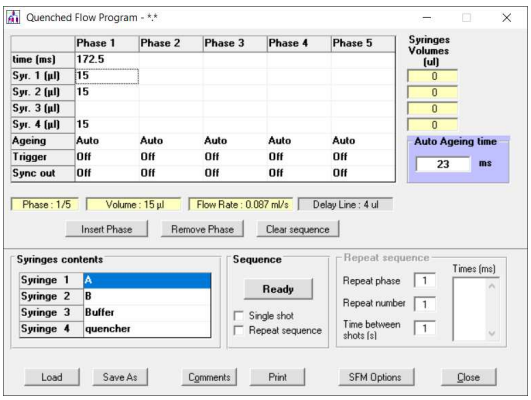

Figure 4 Example of mixing sequence in pulse flow mode using a 4µl delay line

Using this unique push mode developed at BioLogic, reaction times from a few milliseconds to seconds can be obtained with only one single ageing line, and the sample consumption is the same whatever the ageing time selected. This mode is thus ideal for applications requiring limited, small quantities of samples, as only 10µl of sample are required.

III. What choices exist?

Two types of quench flow are available from BioLogic. A modular unit, which is a SFM-3000/4000 stopped-flow accessory and a microvolume unit (QFM-4000) optimized for small volumes and single mixing.

1. SFM-3000/Q, SFM-4000/Q

The SFM-3000/Q and SFM-4000/Q quench-flow systems are based on the stopped-flow chassis of SFM-3000 and SFM-4000. A quench flow diverting valve is used to replace the optical observation head of the stopped-flow.

Figure 5 SFM-3000/Q or SFM-4000/Q

The switch from stopped-flow to quench flow is made in few minutes, so this approach is highly advantageous when the high levels of modularity are important to the user.

The SFM-3000/Q has 3 syringes and 2 mixers, it can thus only address single mixing applications (1 mixing step before quench).

SFM-4000/Q has 4 syringes and 3 mixers, it can thus address single mixing applications and double mixing applications (2 mixing steps before quench). It should be noted that the SFM-3000/Q can be upgraded to SFM-4000/Q

if double mixing becomes a user requirement at a later date.

The two systems use a set of delay lines so users need to adjust the delay line volume to the ageing times he/she wants to reach. All ageing modes are possible using these models: continuous flow mode is used for the smallest lines and thus shorter ageing times (<300ms). Interrupted mode or pulse flow mode are used for longer times. It should be noted that pulse mode is only available for single mixing application and that size pulses are limited to 12µl in this configuration.

The SFM-3000/Q and SFM-4000/Q are suitable for ageing times from 2 ms to several seconds/minutes. The sample consumption depends on the delay line volume and on the selected mixing ratio.

2. QFM-4000

The QFM-4000 has 4 syringes and 2 mixers. It is therefore only suitable for single mixing applications (one mixing step before quenching). The QFM-4000 is based on the pulse flow technique, only ageing points shorter than 5 ms would be used in continuous flow mode using this instrument.

Figure 6 QFM-4000

Reaction times from a few milliseconds to seconds can be obtained with only one single ageing line. The QFM-4000 achieves such a large time range with a single ageing line, by operating in pulsed flow mode.

The QFM-4000 uses a single delay line for all ageing times. The delay line volume is between 3 and 4 µl and is factory calibrated. As the total flow rate in the pulse does not need to be high, driving syringes are smaller (compare to the SFM-3000/4000 configuration) and injection of only 10-15µl of samples is possible at each shot. A schematic demonstrating the principles of the QFM 4000 is given in Figure 7. Three syringes are connected to mixer 1, two are used for reactants and one is used for the buffer to wash the delay line and the flow path between experiments.

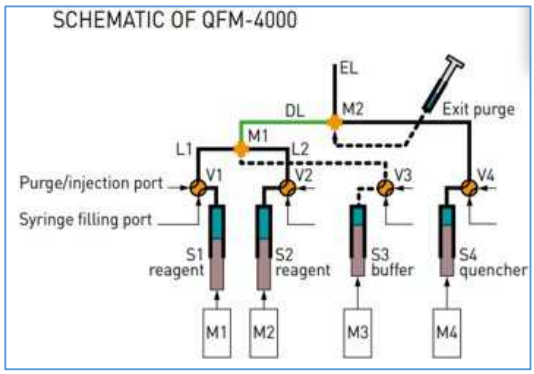

Figure 7 QFM-4000 Schematic

The quencher moves directly to mixer 2 and an air purge is available to recover all quenched solution and to ensure no precious solution is lost in the instrument.

A special zero dead volume mode is available when users do not wish to carry out a full kinetics, but just want to catch a reaction intermediate. In this mode, the sample is loaded manually in the a storage line (L1 and L2 in Figure 7) and buffer is used to propel the sample in the delay line.

As shown in Figure 4, the Biokine software interface is very user friendly. The user only needs to select the sample volume to mix and the ageing time. Shots at different ageing times can be accumulated very quickly (as there is no need to change delay lines or to calibrate), therefore a complete kinetics series is carried out in minutes, when this can take hours with rival instruments.

The QFM-4000 is now recognised as the benchmark instrument for microvolume handling and its user-friendly interface/operation.[1,]

IV. Identifying the right instrument. Ask the right questions.

1. Do I need single mixing or/and double mixing quench flow?

If you need double mixing quench flow, so two mixing steps before quench, then it means you need 3 mixers and 4 syringes. In such a scenario, SFM-4000/Q is the only real choice. Typical double mixing quench applications are H/De exchange experiments[3] during refolding of proteins or radioactive labelling[4,5].
If you only need to do single mixing then you required modularity level will be a key factor in your choice of instruments.

2. Do I only need quench flow?

If you want a dedicated quench flow unit to run single mixing with no real plans to upgrade to stopped-flow or freeze quench in the future, then QFM-4000 would be the choice. If you want to have some modularity (now or later) then you should consider the SFM-3000/Q or SFM-4000/Q, as you can swap the mixing head quickly and switch from one application to the other easily. Both SFM can carry out single mixing quench-flow. The SFM-4000/Q includes three mixers and 4 syringes, so would provide the greatest modularity. For example, such a setup would enable you to carry out concentration dependence studies while doing single mixing quench by using the buffer in syringe 1.

3. Are my samples extremely precious? Do I have to take great care to manage sample quantities?

If sample consumption is your main concern, then the QFM-4000 is your preferred instrument. Thanks to the pulse mode described above, and the single delay line, sample consumption is reduced to 10-15µl per shot and dead volume can also be reduced to zero. Sample consumption will also be the same for all ageing times. So with 200µl of solutions in a vial you will have enough to execute a full kinetics.

In comparison, in a SFM configuration the sample consumption will depend on the volume of the delay line used. Using a 1:1 mixing ratio, typically 100 to 200µl per shot would be required for an experiment. Sample consumption can be reduced by changing the mixing ratio but not at the volume level of QFM-4000.

4. Do I need to apply a high mixing ratio between my samples?

If your samples are precious but you need to apply high mixing ratios then the SFM-3000/4000 would be the right choice, as the microdelay line used in the QFM-4000 in its standard configuration is not adapted to asymmetric ratio. The volume of the delay can be customized to allow large mixing ratios, but in such a case, sample consumption will also increase.

V. Conclusion

This note describes the different solutions offered by BioLogic to address chemical quench flow applications and the questions one should ask to identify the right instrument. The choice depends on the number of mixing steps, the modularity required, and the cost of samples. Instrument price could also be a factor. A question tree is provided in Figure 8 to summarize the points discussed in this note.

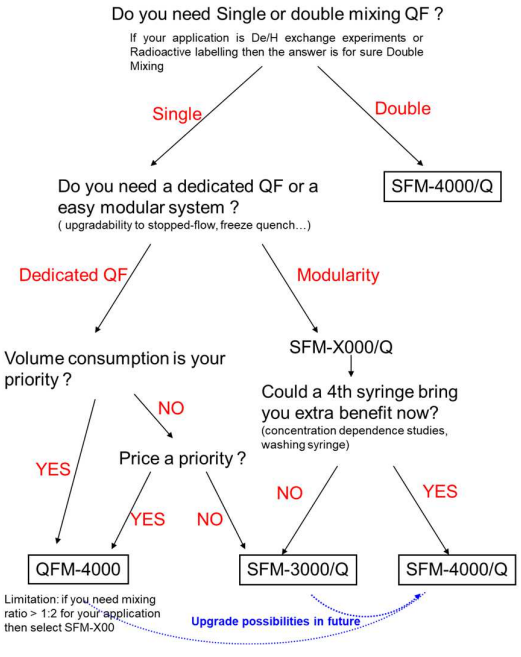

Figure 8 Schematics: how to choose the right quench flow instrument?

REFERENCES

1) **A. Philippe, C. Lionne, A. Sanchez , A.F. Pagano, R. Candau, Experimental Physiology (2019) ,vol 104, issue 8, Pages 1274-1285**
2) **S. Kaushik, S. Marques, P. Khirsariya, K. Paruch, L. Libichova, J. Brezovsky, Z. Prokop, R. Chaloupkova, J. Damborsky, FEBS journal, Volume 285, Issue 8, April 2018, Pages 1456-1476**
3) **C.Nishimura, H.J. Dyson, P.Wright , Journal of Molecular Biology, 27 May 2011, 411(1):248-263**
4) **A.Kaserer, B. Andi, P. Cook, A. West, *Methods Enzymol*. 2010 ; 471: 59–75**
5) **A.P. Einholm, B. Vilsen and J.P. Andersen, The Journal of Biological Chemistry (2004), 279, 15888-15896**

Revised in 09/2019

Scientific articles are regularly added to BioLogic's Learning Center:
https://www.biologic.net/topics/

To access all BioLogic's application notes, technical notes and white papers click on Documentation:
https://www.biologic.net/documents/