Experiment 6.2 – Gel Separation of Proteins Methods

Purpose:

The purpose of this experiment was to evaluate different ways of trying to separate out RFP in an agarose gel.

Setup:

This was actually a large set of experiments that all ran on a similar basis. Each one had lanes loaded with a pure RFP sample and then tested to evaluate how each approach worked at concentrating/separating the protein. All of these tests are qualitative and since RFP showed up just fine on our UV cameras used for gel imaging it was easy to see which approach would work and was worth further study.

Procedure:

Each of these experiments involved loading a pure RFP solution of about 30 ul in a well and running it on a gel at 120V and then checked at various times under UV.

Results:

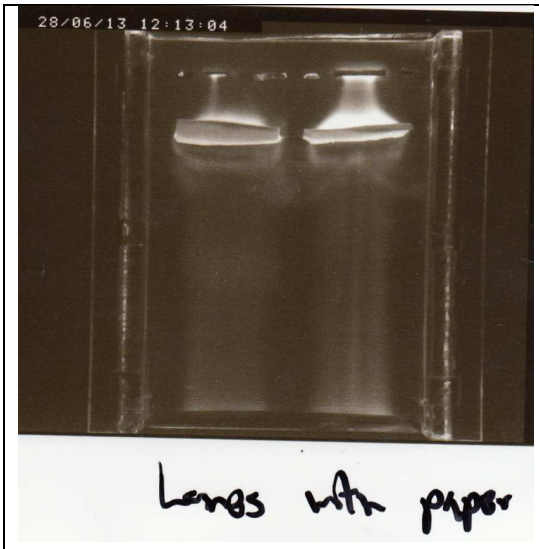

Figure 1 Folded paper to block RFP passage

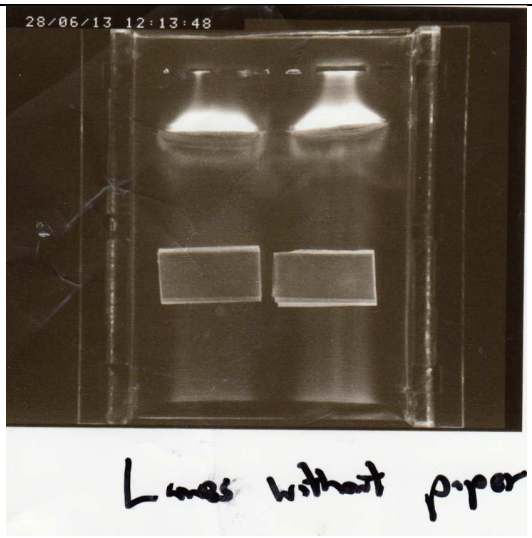

Figure 2 Folded paper removed to show little accumulation in paper

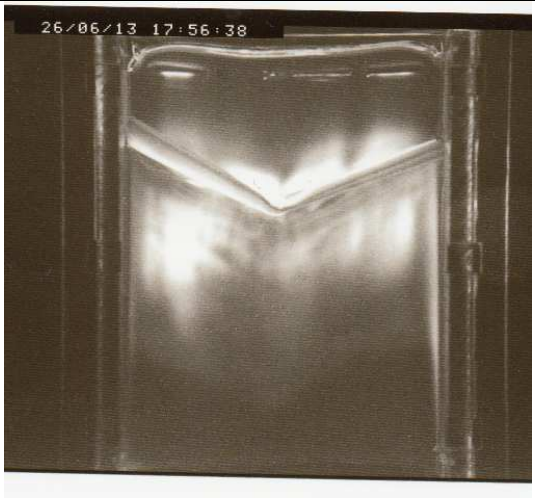

Figure 3 Folded V paper to test flow

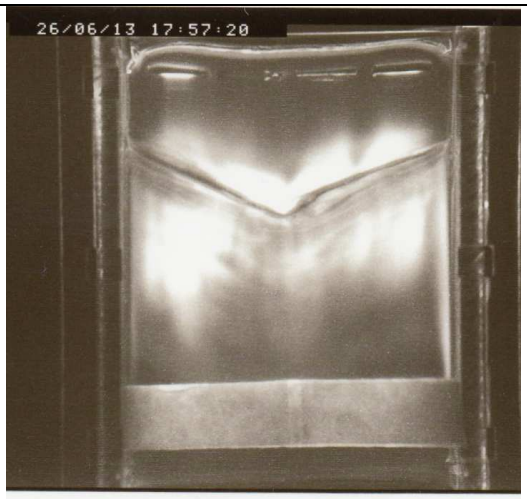

Figure 4 Folded V paper with paper removed

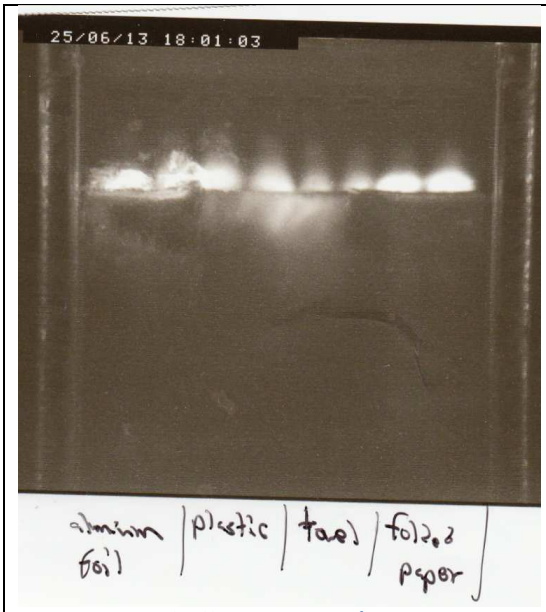

Figure 5 Tests of different blocking/accumulation methods

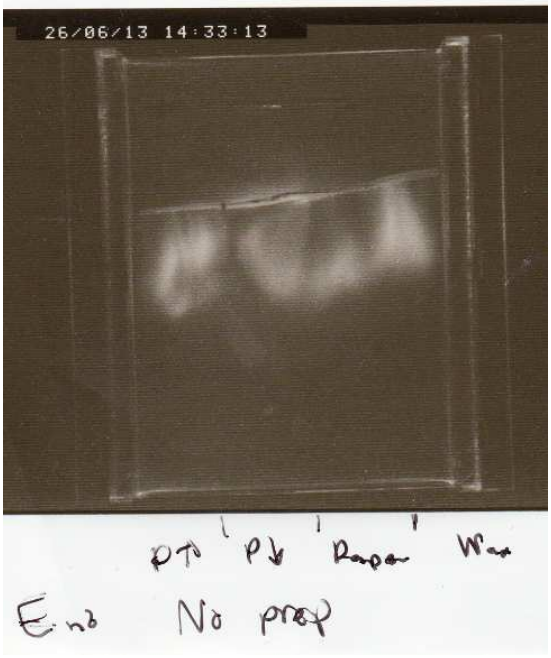

Figure 6 Tests of parafilm blocking

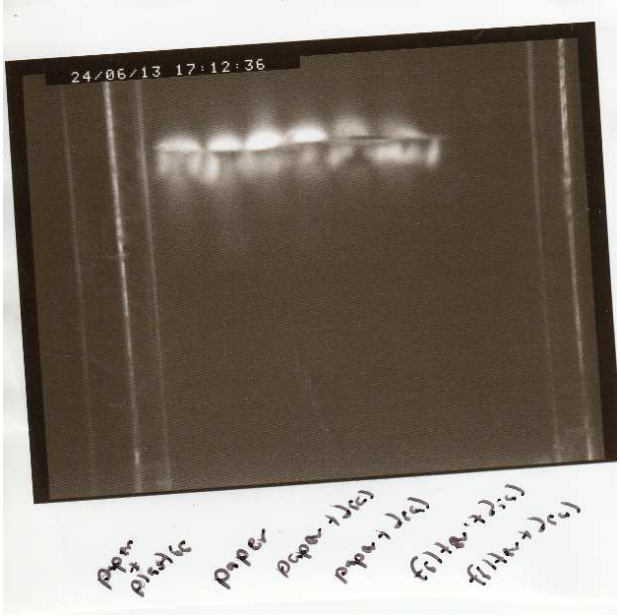

Figure 7 Tests for accumulation

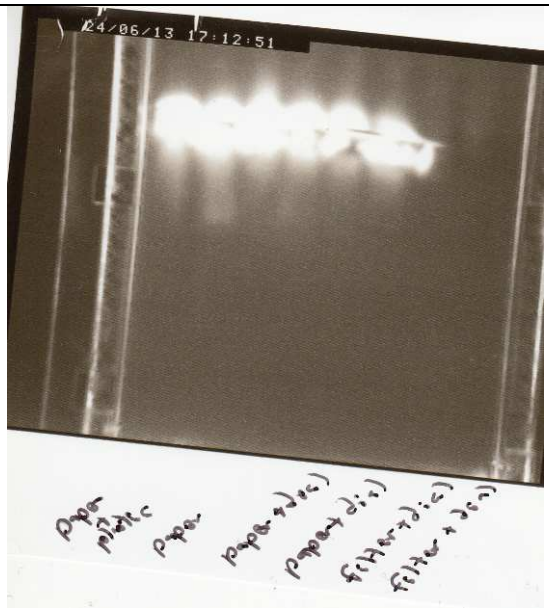

Figure 8 Tests if accumulation runs too long

Discussion:

Figure 1 shows the results of the gel running into folded paper and it is easy to see that the RFP has concentrated above the paper with little running past. In Figure 2 the paper is removed and it can be seen that very little RFP has accumulated in the paper.

Figure 3 represents an attempt to channel the RFP by making a V shaped piece of folded paper. The idea was that maybe the RFP would end up running down along the V and concentrating in the tip of it. This did not happen. Instead once RFP reaches a high enough concentration it just runs through the paper but does not seem to run along it at all. In Figure 4 it can be easily seen that the RFP did not really accumulate in the paper. It just seems to run through.

Figure 5 actually represents one of our earliest attempts at protein separation where we tried to use aluminum foil, a piece of transparent plastic, a piece of paper towel and folded paper. Out of all these approaches the folded paper worked the best by far. The aluminum foil actually started to react chemically and destroyed most of the RFP in that lane.

In Figure 6 we used some parafilm were we tried to use paper side facing up, paper side facing down, just the paper and just the wax. It is pretty easy to see that none of these shows an promise for filtering RFP.

Figure 7 represents an attempt to combine paper with plastic, paper with dialysis tubing and filter paper with dialysis tubing. All of these approaches leaked quite a lot. In Figure 8 the system was for about another 10 minutes and RFP just runs through all of these. The contrast had to be turned up on this one to better image the RFP which is why it looks so intense.

Based on all of these we decided on the folder paper in a simple straight cut as the most appropriate method. So long as RFP does not run into the paper for more than about 10 minutes the concentration gradient won't drive it through the paper. This proved to be ideal for our usage case and generally results in 25%-45% recovery of RFP in various experiments. While being cheap and easy to do and still getting rid of the detergents, RNA and DNA in the source.