**2D Pascal’s Blot**

For larger proteins with many cysteine residues, it may be necessary to implement a 2D Pascal’s Blot method. This would involve:

1. Preparing 2PB and labelling the samples as set out in the [protocol file](https://github.com/JamesCobley/Pascals_Blot/blob/main/Protocol%20file%20Pascal%20Blot.docx).
2. Resolving the sample in the first pH dimension using an isoelectric focusing (IEF) gel.
3. Resolving the sample in the second molecular mass dimension on a suitable, as in pore-size optimised for the target, polyacrylamide gel.
4. Implementing the Pascal’s Blot method as set out in the protocol file.

The premise is that the dual pH and mass separation should enable the detection of a broader range of bands, in this case spots, that would otherwise be possible in conventional Pascal’s Blot. In this way, difficult to measure proteins can be better addressed.

Potential adaptations/variations on this theme include:

1. Tuning the size of the PEG-payload in 2PB to better resolve the target.
2. Using IP or gel electrophoresis to enrich the target or band of interest in an alkylated sample before 2PB labelling and subsequent 2D Pascal’s Blot.
3. A variation of 2, where 2PB is substituted for a cleavable, cysteine-reactive, FLAG payload to resolve cysteine redox proteoforms by mass and charge.