**Class I MHC Refold Protocol**

1) Prepare 250ml refold buffer (see below) and leave to stir in the cold room (4$^{o}$C) for several hours.

For a 250 ml refold:

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| 100 mM | Tris-HCl pH8 (25 ml of 1M stock) |
| 0.4 M | L-Arginine Hydrochloride (21.06 g) |
| 0.5 mM | Oxidised Glutathione (0.0775 g) |
| 1.5 mM | reduced Glutathione (0.385 g) |
| 2 mM | EDTA (1ml of 0.5 M stock) |

Check the pH it should be around 7.8

Then chill to 4$^{o}$C

2) Add 0.2 mM PMSF (500µl of 0.1M stock) to the 250ml refold buffer. This is added just before pulsing with β2M inclusion body (see stage 3).
3) Add DTT (final concentration 2mM) to the β2M inclusion body and split into 3 aliquots. In this example we are refolding 5 mg of β2M (which is in 300 µl of solubilisation denaturant buffer)
4) Place one of these aliquots (100µl in this example) into 30 ml universal tube and dilute with 20 ml of refold buffer and this mixture is added slowly drop by drop into the vortex of the refold mix (this is called pulsing).
5) Repeat stage 4 for remaining 2 aliquots of β2M
6) After the last pulse the refolding mix is left stirring gently at 4$^{o}$C for 30mins
7) Next add peptide (2-3 mg dissolved in 1 ml of DMSO) into the refolding buffer making sure that the tip does not enter the buffer itself as the DMSO will solidify if cold.
8) Then add DTT (final concentration 2mM) to the HLA-A2 inclusion body (comprised of 5mg in 600 µl solubilisation buffer in this example) and split into 3 aliquots. Place first aliquot (200µl in this example) into 30 ml universal tube and dilute with 20 ml of refold buffer and this mixture is added slowly drop by drop into the vortex of the refold mix (this is the first pulse). Repeat with remaining two aliquots.
9) Stage 8 is repeated 4 more times (e.g. pulse refold buffer with 4 x 5 mg of HLA-A2 heavy chain) over the course of 3 days (usually pulse once in the morning and once at night). At the end 25 mg of heavy chain will have been added into the 250ml refold.
10) After final HLA-A2 heavy chain pulse leave refold stirring overnight at 4$^{o}$C.
11) The next day concentrate down the 250ml refold buffer mix to 10ml using a 10k cut off filter in the Amicon stirred cell at 4$^{o}$C. **It is important that you keep the flow through as this contains the peptide, L-arginine and glutathione which can be used a further 2 times to refold HLA-A2.**
12) The 10ml sample is filtered through a 0.45µm filter and then loaded onto the Superdex S200 gel filtration column that has been pre-equilibrated with 20mM Tris pH 8, 50mM NaCl.
13) Note that the MHC-I complex elutes at ~210 ml and unbound β2M elutes at ~260ml.