Hanna lab- Expression and purification of human Vitronectin

Human Vitronectin (VN) starting from residue 62 (without the Somatomedin B-like domain) was nicely expresses in bacteria as inclusion bodies. The VN inclusion bodies were solubilized by denaturation with Urea and refolded. The refolded protein is difficult to handle (it tends to form a gel which is an intrinsic property of the protein). Therefore, we were unable to use standard chromatographic procedures because the protein tends to clog filtration devices and columns. The protocol which we finally used at the ISPC is as follows:

Human Vitronectin (non-tagged) cloned in the expression vector pET3c was expressed in *E. coli* Rosetta2(DE3)pLysS (Addgene vector 30223 – by James Thomson). Expression was performed in LB media supplemented with chloramphenicol (17μg/ml) and ampicillin (100 μg/ml). Protein induction was done by addition of IPTG to a final concentration of 200 μM, followed by over-night growth at 37 °C. The bacteria were harvested by centrifugation at 5000*g* for 15min at 4 °C. The supernatant was discarded and the pellet was frozen at -20 °C. To lyse the bacteria, the pellet was thawed on ice and resuspended in 100 ml cold PBS containing 0.5M NaCl, lysozyme (1mg/ml), 24U/ml DNase, 1mM PMSF, and complete protease inhibitor (Roche, Indianapolis, IN). The lysate was sonicated on ice and centrifuged at 20,000*g* for 30min at 4 °C. The supernatant was removed and the pellet containing inclusion bodies of VN was washed with PBS containing 0.5M NaCl followed by an additional wash with 40ml cold BPS containing 2M urea. The suspension was centrifuged at 20,000*g* for 30 min at 4°C. The pellet was resuspended in 100ml buffer (20mM Tris 7.5, 150mM NaCl, 8M Urea, 3mM DTT). The suspension was stirred for 1h and the insoluble material was removed by centrifugation at 20,000*g* for 30 min at 4 °C and discarded.
To refold and purify denatured VN, the supernatant was first diluted x4 with 20mM Tris 7.5, 150mM NaCl, 3mM DTT (to yield 2M urea solution). This solution was allowed to stir at 4°C for 1h. The filtered soup was rocked with 10ml Q–Sepharose beads (GE Healthcare, pre-equilibrated with 20mM Tris 7.5, 150mM NaCl, 3mM DTT and 2M urea) for 2 h at 4 °C. The resin was collected by centrifugation at 1500*g* for 5min at 4 °C and the supernatant was removed. The beads were washed with the binding buffer and the protein was finally eluted from the beads with 20mM Tris 7.5,1M NaCl, 2M Urea, 3mM DTT. The fractions containing VN were pooled and dialyzed extensively against PBS at 4 °C followed by centrifuged at 18,000*g* for 5min at 4 °C. The pure protein was divided into aliquots, flash frozen with liquid nitrogen and stored at -80 °C. We obtained over 100ml of protein at 4 mg/ml.