Isolation of the Inner Membrane Fraction:

- After harvesting the cells, wash in PBS buffer to remove residual media
- Lyse the cells using a homogenizer with 3x30s, incubate on ice between each round
- Centrifuge the cells and transfer supernatant to a new tube
- Add appropriate amounts of protease inhibitors
- Centrifuge the supernatant at 4°C for 1.5 h at the highest possible rpm (if an ultracentrifuge is available, centrifuge at 100,000 x g)
- After the centrifugation step, a dark brown residue should be visible at the bottom of the tube
- Carefully remove the entire supernatant
- Add PBS buffer and let the tubes incubate at 4°C for 1h
- Carefully resuspend the pellet without introducing any bubbles
- Add appropriate detergent for the solubilization of the desired membrane protein. In our case, 0.624 M SDS was used
- Pursue with further analysis