# The Use of Min Waves to Study Intrinsically Disordered Regions Involved in Phase Separation

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## Introduction and Theory

### 1 What is Phase Separation and Intrinsically Disordered Regions (IDRs)?

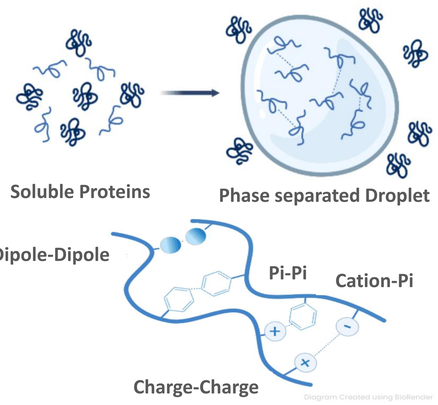
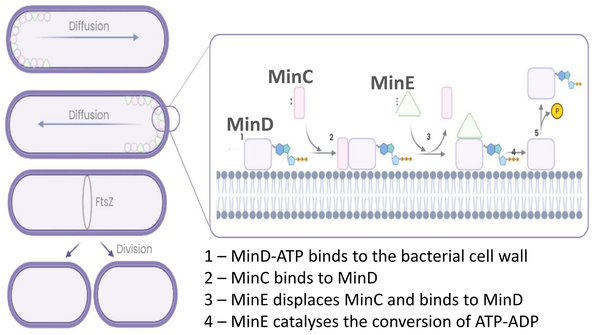



**Figure 1.** Figure 1a represents the phase separation of soluble proteins into their own "droplet". Figure 1b shows the type of interactions that causes a protein to phase separate, and these are the type of interactions that occur between intrinsically disordered regions.

### 2 What are Min Waves?




1 – MinD-ATP binds to the bacterial cell wall
2 – MinC binds to MinD
3 – MinE displaces MinC and binds to MinD
4 – MinE catalyses the conversion of ATP-ADP
5 – MinD-ADP then unbinds from the cell membrane and leaves

**Figure 2.** Diagrams representing the formation of Min waves.

### 3 Reproducing Min Waves + The Use of IDRs

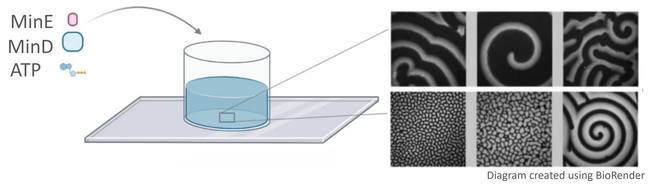



**Figure 3.** Diagram representing the reproduction of Min waves outside of bacterial cells. Min waves should give a consistent pattern. Therefor, this project aims to add IDR sequences to MinD and assess the pattern changes using mathematical models. These changes in the patterns should reflect the differing interaction strengths from the IDRs. Diagram adapted from *Loose et al.*

## Aims

- New method to look at IDR interactions
- Create a dynamic system that incorporates concentration gradients
- Create a system whereby the interactions can be clearly mapped

## Methods and Results

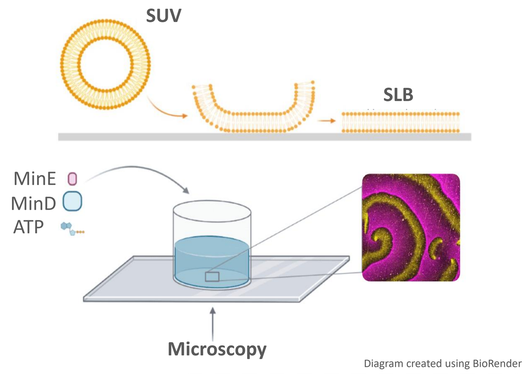
### 1 Min Wave Formation - Theory

**Step 1**

- Create small unilamellar vesicles (SUVs) with DOPC and DOPG lipids
- Using the sonication method.
- SUVs will collapse onto a hydrophilic glass surface to create supported lipid bilayers (SLBs).

**Step 2**

- Purify MinE and MinD
- Add them to a reaction chamber with lipid bilayers on the bottom
- Add adenosine triphosphase (ATP)
- Observe with confocal or total internal reflection (TIRF) microscopy




### 2 Supported Lipid Bilayer (SLB) Optimisation

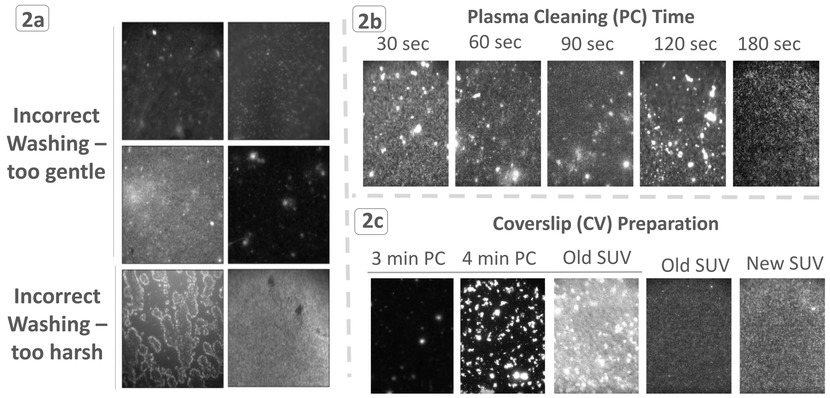



**Figure 2a to 2c.** Diagrams showing the optimisation of SLBs. Figure 2a depicts issues such as incorrect washing steps. Figure 2b shows the importance of plasma cleaning times to create the correct hydrophilic surface. Figure 2c shows the importance of using freshly prepared coverslips.

### 3 Protein Addition to Bilayers

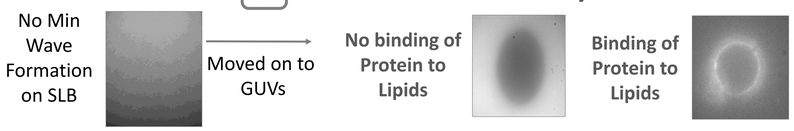
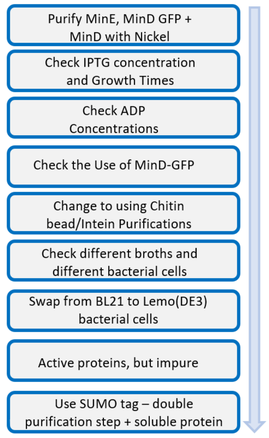



**Figure 3.** Addition of proteins to my lipid bilayers led to no change. Giant unilamellar vesicles (GUVs) were used as a diagnostic for lipid binding.

### 4 Protein Purification Optimisation

**4a Protein Purification Optimisation Steps**




**4b Bacterial Expression Plasmids Tried**

| Plasmid | Incubation Time | Incubation Temp. | Worked | Issue |
|---|---|---|---|---|
| **MinE-His** | 3 hrs | 37 | Yes | No issues |
| **MinD-His** | 18 hrs | 20 | No | Present in pellet, not active |
| **MinD-His/MinE** | 18 hrs | 20 | No | Present in pellet |
| **eGFP-MinD-His** | 18 hrs | 20 | Yes | Only use 20% |
| **MinE-Intein** | 3 hrs | 37 | Yes | No issues |
| **MinD-Intein** | 18 hrs | 20 | Yes | Active, but low yield |
| **SUMO-MinE** | 3 hrs | 37 | ? | ? |
| **SUMO-MinD** | 18 hrs | 20 | ? | ? |

**Figure 4a to 4b.** Figure 4a represents some of the optimisation steps followed or being followed to get pure, high yield and active protein. Figure 4b represents the plasmids that have been tired. His represents the use of a nickel tag, intein represents the use of intein tag and chitin bead column and SUMO tag represents the use of a SUMO tag to keep proteins soluble.

### 5 Current Wave Patterns

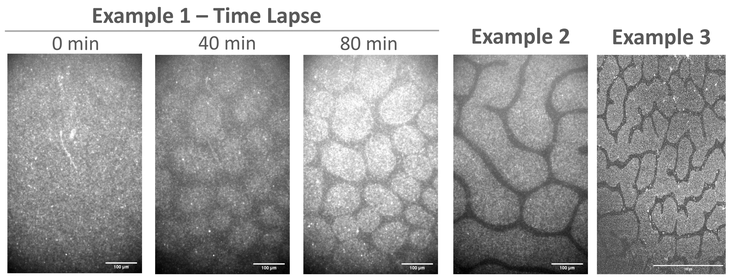



**Figure 5.** Figure representing the different wave patterns obtained so far. Example 1 shows a time lapse of how the waves form. Example 2 and 3 show the end of the wave formation for two other examples – at this point the waves remain static. Min waves are expected to regenerate in the presence of an excess amount of ATP (which was added in excess). However, these patterns remained static.

## Future Prospects and Ongoing Projects

- Purify MinD/MinE SUMO
- Purify MinD with an IDR attached
- Code for the different interactions to asses the IDR parameters of importance

## Conclusion

- Min waves are specific, concentration dependent reactions
- Optimisation of purifications needs to be performed with a SUMO tag
- Code for the different interactions will asses the IDR parameters of importance

### References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