

June 21, 2012: Molecular Shuttles

- **Goal:** To use streptavidin as the bridge in the molecular shuttles experiment as opposed to using biotin, as in the previous experiments. This would allow for us to use two different filters to view the beads and microtubules, thereby making it possible to distinguish between the two microtubule-layers of the dendrimer-like structure.
- **Materials:**

Name	Location	Abbreviation
BRB80	Lab bench	
MT100 (50/50 Rhodamine/biotin)	Lab bench	
1.00 μ m Biotinylated Beads	-4 C fridge	
Unlabelled streptavidin	-4 C fridge	
Alexa 488 labeled streptavidin	-4 C fridge	
Rhodamine labeled tubulin	-20 C freezer	
Biotinylated tubulin	-20 C freezer	
Rhodamine labeled MT100	Lab bench (from Ofer)	
MgCl ₂	-20 C freezer	Mg
GTP	-20 C freezer	
DMSO	-20 C freezer	
Taxol	-20 C freezer	Tx
Casein	-20 C freezer	Cs
DG	-20 C freezer	
GO	-20 C freezer	
Catalase	-20 C freezer	Cat
DTT	-20 C freezer	
ATP	-20 C freezer	

Make 50/50 MT100

1. Prepare microtubule growth buffer in a microcentrifuge tube.

Volume	Reagent	[Stock]	[Final]
21.8 μ L	BRB80		
1 μ L	Mg	100 mM	4 mM
1 μ L	GTP	4 mg/mL	0.16 mg/mL
1.2 μ L	DMSO	5%	0.0024%
25 μ L	Final Volume		

2. Pipet 6.25 μ L microtubule growth buffer into rhodamine labelled tubulin aliquot. Wrap in foil and do not expose this to light.
3. Pipet 6.25 μ L microtubule growth buffer into biotinylated tubulin aliquot.

4. Add all contents of biotinylated tubulin aliquot to rhodamine tubulin labelled aliquot.
5. Let aliquot sit on ice for 5 minutes.
6. Incubate at 37 C for 30 minutes.

Make MT100:

Prepare MT100 Solution:

Volume	Reagent
980 μL	BRB80
10 μL	Taxol
10 μL	Tubulin from aliquot
1000 μL	Final Volume

This is light sensitive--wrap in foil.

Create Bead solution

Volume	Reagent
249 μL	BRB80
1 μL	Biotinylated beads
250 μL	Final Volume

Prepare standard solutions.

BRB80CT

Volume	Reagent	[Stock]	[Final]
196 μL	BRB80		
2 μL	Taxol	1mM	10 μM
2 μL	Casein	20mg/mL	0.2mg/mL
200μL	Final volume		

Unlabeled Streptavidin Solution

Volume	Reagent	[Stock]	[Final]
59 μL	BRB80 CT		
1 μL	Unlabeled STV	10 μM	0.15 μM
60 μL	Final Volume		

Motility Solution (keep in dark/wrap in foil):

Volume	Reagent	[Stock]	[Final]
75 μL	BRB80CT		
1 μL	DG	2M	200mM
1 μL	GO	20mg/mL	0.2mg/mL
1 μL	ATP	100mM	1mM
1 μL	Cat	0.8mg/mL	0.08mg/mL

1 μ L	DTT	1M	100mM
20 μ L	MT100		
100μL	Final volume		

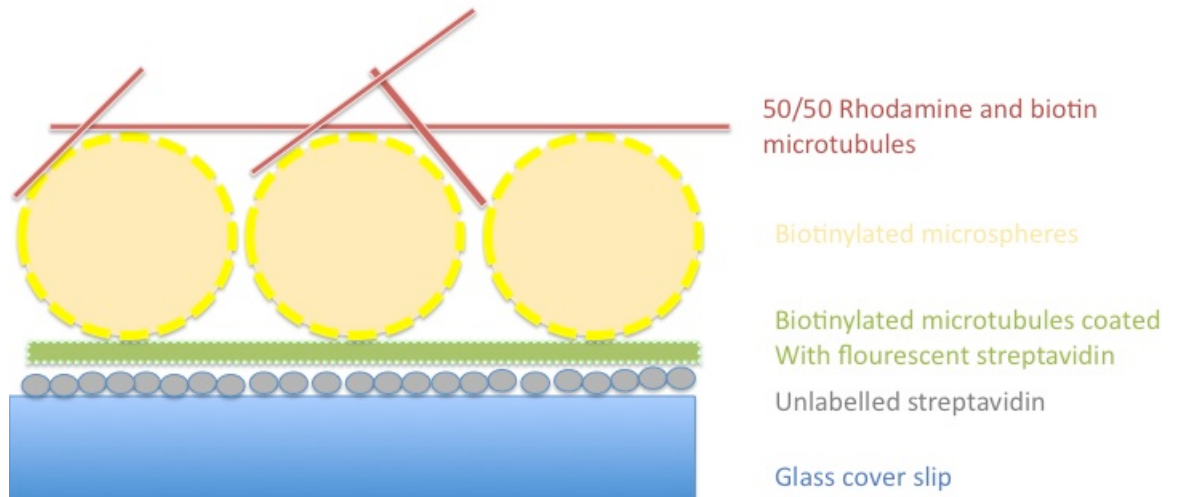
Antifade solution:

Volume	Reagent	[Stock]	[Final]
95 μ L	BRB80CT		
1 μ L	DG	2M	200mM
1 μ L	GO	20mg/mL	0.2mg/mL
1 μ L	Cat	0.8mg/mL	0.08mg/mL
1 μ L	DTT	1M	100mM
100μL	Final volume		

Using STV as a bridge instead of MTs

1. Flow through 20 μ L unlabeled, undiluted streptavidin.
2. Wait 15 minutes.
3. Flow through 20 μ L antifade solution.
4. Flow 20 μ L biotinylated microtubules.
5. Wait 5 minutes.
6. Flow 20 μ L antifade.
7. Flow 20 μ L labeled streptavidin solution.
8. Wait 10 minutes.
9. Flow through 20 μ L antifade.
10. Image
11. Flow through 1:250 dilution of 1 μ M unlabeled biotinylated beads.
12. Wait 10 minutes.
13. Flush with 20 μ L antifade.
14. Flow through 20 μ L unlabeled streptavidin solution.
15. Wait 10 minutes.
16. Flush with 20 μ L antifade.
17. Flow 20 μ L 50/50 microtubules.
18. Wait 10 minutes
19. Flush with 20 μ L antifade.
20. Resume imaging.

Experiment design: Molecular Shuttles



Results:

- Trial 1
 - Saw images of dried up areas in the slide. The beads were tethered by microtubules but not to the surface.
 - Images of the flow cell after step 10 of **using STV as a bridge instead of MTs**, showed a lot of haze and fluorescence. Antifade was therefore used twice to flush the flow cell as opposed to once.
- Trial 2
 - Saw images of interesting aggregates of microtubules. Again—these were not in solution.
 - In this trial, the waiting time for step 5 of **using STV as a bridge instead of MTs** was increased to 15 minutes.
- Trial 3
 - After step 8, the flow cell was imaged (between flushing with labeled streptavidin and flushing with antifade). Microtubules appeared to be present in the sample. They again, formed aggregates that we eventually determined were situated in dried up portions of the flow cell.

Conclusions:

- In the next set of experiments, it will be important to get rid of the haze that is caused by the fluorescently labeled streptavidin. There are multiple avenues that can be taken to do so:
 - Use a smaller concentration of labeled streptavidin (instead of using the stock, dilute it 1:60 (1 μ L streptavidin, 59 μ L BRB80CT). This should reduce the concentration to 0.16 μ M.
 - Wash through 3 or 4 times with antifade.