**Lab Book**

|  |  |
| --- | --- |
| **Date** | **Tasks done** |
| 02.08.2012 | Open and closed guided structures w/o catchers, anchors and locks were prepared |
| 03.08.2012 | A gel shift assay was run with the aforementioned structures.  |
| 10.08.2012 | TEM Session |
| 21.08.2012 | TEM Session |
| 24.08.2012 | Open, closed guided and closed not guided structures, all of them with catchers (no anchors, no locks), were prepared. TEM Session |
| 27.08.2012 | Open, closed not guided and guided structures were put for PCR. |
| 28.08.2012 | TEM Session |
| 30.08.2012 | A new set of Core Oligos was pipetted |
| 31.08.2012 | TEM Session |
| 03.09.2012 | Open and closed guided structures were prepared in 2 different versions: With a 5’ Biotinylated catcher strand and conventional locks and with conventional catchers and conventional locks. In addition Two other closed guided structures were prepared, this time containing the aptamer locks. One with the 5’biotinylated catcher (69) and the other with conventional catchers. TEM Session |
| 06.09.2012 | The concentration of the constructs assembled on 03.09 was measured in Nanodrop. The constucts were Dialyzed for two hours for TEM and Gel shift assay purposes. |
| 07.09.2012 | TEM Session |
| 10.09.2012 | The constructs from 03.09 containing the conventional catcher strands were corrected and a new solution of biotinylated catcher complementary strands was prepared and mixed with Quantum Dots.  |
| 11.09.2012 | A gel shift Assay with all the previously mentioned structures was run. Results negative. There were 2 mistakes in the gel: No scaffold in the structures and the amount of construct was varied from lane to lane leading to not comparable results. |
| 12.09.2012 | New assemblies were prepared and left for PCR, concentrations and ratios of QDs, Catcher complementary oligos and origamis were recalculated.  |
| 13.09.2012 | Newly PCR constructs were dialyzed and a new gel to test the effect of dialysis was planned. Not enough material; gel was delayed. |
| 14.09.2012 | A gel comparing structures with 5’ biotinylated catcher (+QDs) and conventional catcher ones (+QDs+Catcher complementary) with and without dialysis was run. Result, the bands were to dim to resolve something, still not quantum dot binding.  |
| 17.09.2012 | Two gels were run. The first one comparing constructs prepared on 27.08 with the ones done on 12.09. The second one comprised the same gel run on 14.09 but doubling the amount of construct used. Results: Gel 1-> Constructs from 12.09 are smeared down in a weird fashion, possible problem with construct. Gel2->Bands are very dim again and there’s still not Quantum Dot binding to the structures.  |
| 19.09.2012 | A new set of tubes was prepared to check if the MgCl2 concentration affects the assemblies. The concentrations used were 8mM, 10mM, 12mM, and 14mM. The aforementioned gel was run. Results: A lower concentration of MgCl2 gives clearer bands; higher concentrations give more diffuse bands. Concentration picked> 8mM.  |
| 21.09.2012 | A gel shift assay was run with open and closed structures in the 5’ Biotinylated and catcher complementary 3’ Biotinylated versions, adding QDs and QDs+Catcher complements respectively. Results: Very weird. No Quantum Dot binding, structures look very similar to the scaffold, no smear at all.  |
| 24.09.2012 | New gel comparing constructs from 27.08 (FB14) with constructs of 20.09, and aiming for Quantum dot binding (FB8). Results: The constructs with FB14 have wider and more diffuse bands compared with the ones with FB8, which continue to display strong bands near the scaffold and very diffuse dimer-like bands. Note: the electrodes of the chamber were probably dirty because the gel ran uneven.  |
| 28.09.2012 | A PAGE gel was run to discard the fact that the catchers are not working properly. The two aforementioned kinds of catchers were used (5’ Biot. and conventional for 3’Catcher complementary) and loaded with Quantum Dots. Results: Positive for 5’ Biotinylated version but inconclusive for some lanes of the 3’ version containing middle hybridization products as a control. This might be because the amount used was too low. Test again.  |
| 02.10.2012 | TEM Session |
| 08.10.2012 | To test convincingly the catcher’s in 3’ version functionality, an additional gel was run isolating all of the components of hybridization and binding and comparing them with their hybridized version. Results: The hybridization and quantum dot binding works flawlessly.  |
| 09.10.2012 | A whole new set of constructs for the GUV and Aptamer group was assembled. The constructs made comprise: Open , closed and closed guided versions with catchers and anchors; open and closed labeled with Alexa 647 at the edge strand; and two closed not guided versions with fluorescent aptamer locks, one with BHQ (Black Hole Quencher) and the other without it.  |
| 10.10.2012 | TEM Session |
| 11.10.2012 | A gel for testing again open and closed structures in the catcher complementary 3’ version was done. Quantum Dots and catcher complements were added as well as some alexa 647 labeled catcher complements, to test if they can also bind to the structure, which they should. The main change done here is that the structure was left incubating for 1h with the QDs+catcher complements mix and the alexa oligos for half an hour. Results: There was a quantum dot binding to the structure however it occurred both in the open and the closed versions. In the Alexa lane there wasn’t any observed fluorescence. Update: The Alexa result was expectable, as Alexa 647 is not excitable with UV light.  |