

Electronic Supplementary Information

Probing Conformational Dynamics of Photosystem I in Unconfined and Confined Space

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1. Experimental Section

1.1 Methods:

Preparation of physiological relevant buffers: Liposome has two types of solution namely Internal Buffer and the External Buffer. Inside the liposome is the Internal Buffer (IB) prepared by mixing Phosphate buffer saline (PBS 1X: 140 mM of NaCl, 2.7 mM of KCl, 8.1 mM Na₂HPO₄·2H₂O and 1.8 mM of KH₂PO₄ at pH 7.2), 2 M sucrose solution, dextran solution (400 mg/ mL) in Milli Q water while Outside the liposome it is External Buffer (EB) prepared by mixing PBS (1X), 20% glucose in water and BSA solution (100 mg/mL) in PBS. In order to prepare a liposome containing PSI solution, a solution is prepared by mixing the isolated PSI with internal buffer. Osmometer was used to measure the osmolarity of both the buffers and

solution of Internal buffer containing PSI. In order to maintain the shape of the liposome, osmolarity of EB was kept approximately 5-10 mOsm below than that of the IB. Iso-osmotic condition leads to changes in the shape of the liposome.¹

Preparation of Lipid-oil mixture:² In a 10 mL round bottom flask, chloroform solution of 2.5 mg of Egg PC, 0.5 mg of DOPS, 0.5 mg of POPG and 1% of biotin-DHPE was taken. Through nitrogen flush a thin film of lipid mixture was prepared, thereafter the flask was kept in high vacuum pump for 40 min. After that, the vacuum was released under nitrogen atmosphere. In the round bottom flask with the lipid film, 5 mL of mineral oil was added and was sonicated for 30 min in cold. It was thereafter incubation in hot air oven at 50 °C for 3 h. Then the flask was cooled down at room temperature and stored at 4 °C. Biotin-DHPE lipid was used for the immobilization of liposome on biotin surface,

Preparation of biotin functionalized Surface: Glass coverslips (50 X 50 mm) were sonicated with 3 M NaOH for 30 min and cleaned of any grease. After that they were rinsed with plenty of water and treated with piranha (2:3 mixtures of hydrogen peroxide and sulphuric acid) and sonicated for 45 min under fume hood. Thereafter the piranha solution was discarded and glass slides were thoroughly cleaned with water and dried under a stream of nitrogen gas. Surface functionalization was done through the following steps. Firstly, GOPTS was treated at 75 °C for 90 min for the silanization of glass surfaces. Next, silanised glass surfaces were treated with diamino-polyethylene glycol and heated at 75 °C for overnight. Afterwards, the polyethylene glycol functionalized surfaces were rinsed with plenty of water for complete removal of excess and unreacted diamino-polyethylene glycol from surfaces. Finally, polyethylene glycol surface was treated with Biotin-NHS for 1 h at 75 °C. Biotin functionalized surfaces were washed with DMF and plenty of water followed by drying under stream of air.¹

Construction of the flow chamber and Immobilization of liposomes:^{2,3} A flow chamber of 50 µL was constructed onto a microscopic glass slide using double sticky tape (Tesa, Hamburg, Germany) and biotin functionalized glass surface. Flow chamber was washed with EB before loading liposomes. Then, neutravidin solution (300 nM) in EB was loaded into the flow chamber and incubated for 10 min for the attachment of liposome to the biotin glass surface through biotin-avidin interaction. After 10 min, flow chamber was washed with EB, liposomes loaded

with PSI and without PSI were loaded into the flow chamber and it was sealed and observed under inverted fluorescence microscope.

References:

1. A. Biswas, A. Saha, B. Jana, P. Kurkute, G. Mondal, and S. Ghosh, *ChemBioChem*, **2013**, **14**, 689.
2. A. Saha, G. Mondal, A. Biswas, I. Chakraborty, B. Jana and S. Ghosh, *Chem. Commun.*, **2013**, 49, 6119-6121.
3. S. Nandi, P. Mondal, R. Chowdhury, A. Saha, S. Ghosh and K. Bhattacharyya, *Phys Chem Chem Phys.*, **2016**, **18**, 30444-30451.

Supplementary Figure

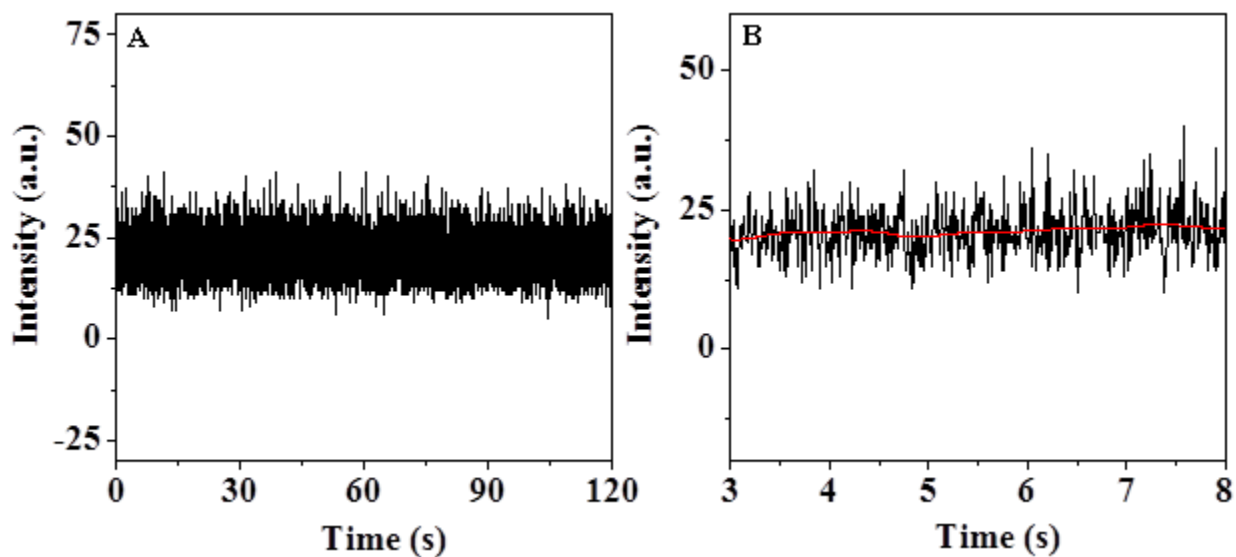


Figure S1: Fluctuation in fluorescence intensity of a control liposome recorded under a confocal microscope: (A) Full time scale (0-120 s); (B) expanded time scale (3-8 s).