

Supporting Information

Nanomechanical insight of pancreatic cancer cell membrane during Receptor Mediated Endocytosis of targeted Gold Nanoparticles

Tanmay Kulkarni¹, Debabrata Mukhopadhyay^{1,2} Santanu Bhattacharya^{1,2}*

¹Department of Biochemistry and Molecular Biology, Mayo College of Medicine and Science, Jacksonville, FL, USA.

²Department of Physiology and Biomedical Engineering, Mayo College of Medicine and Science, Jacksonville, FL, USA.

Corresponding Author: *Santanu Bhattacharya, PhD

Email: Bhattacharya.santanu@mayo.edu

Method:

PEGylation of PTP-GNP

For PEGylating we have used ten-fold diluted PTP-GNP and mixed with 2.5 mg/ mL mPEG₃-SH for 30 minutes at room temperature. PEGylated PTP-GNP (PTP-GNP-PEG) was further purified using ultracentrifugation at 38 000 rpm. We monitored no effective change in hydrodynamic size change of GNPs.

Result:

Effect of PTP-GNP protein corona formation on receptor dependent endocytosis process

From both nanomechanical studies with AFM and fluorescence studies with streptavidin-biotin complex reaction, we observed a sudden recovery in membrane stiffness as well as the plectin-1 receptor expression at the 30th minute. This abnormal behavior during the dynamic receptor mediated endocytosis process could be due to the protein corona formation around the PTP-GNP due to prolonged exposure to various components in complete DMEM media. We speculate that due to the corona formation, there fails to exist plectin-1 targeted peptide available to interact with the free plectin-1 receptors on the Panc1 cell membrane. This would explain the sudden recovery of plectin-1 receptors thereby, recovering the membrane stiffness. To test our hypothesis, we pretreated Panc1 cells with PTP-GNP for 30 minutes followed by replacing the media containing PTP-GNP with fresh complete medium containing PTP-GNP for upto 2 hours and corresponding plectin-1 expression was monitored by streptavidin-biotin reaction (Figure S3A). We observed a similar trend in the average fluorescence intensity compared to the Figure 5A wherein, we saw a sudden increase in average fluorescence intensity at the 30th minute thereby recovering complete plectin-1 expression at the 2-hour time point. Interestingly, the complete recovery of the plectin-1 fluorescence expression was observed to be delayed by ~90 minutes compared to Figure 5A. To ascertain our hypothesis of protein corona formation, we treated the Panc1 cells with PEGylated PTP-GNP consisting of very minimal free reactive species available on its surface to form a protein corona and observed the average fluorescence intensity corresponding to the plectin-1 receptor upto 24 hours. We observed a substantial increase in time duration until which, the receptor mediated endocytosis process continues to occur due to delayed protein corona formation. We saw the receptor mediated endocytosis process extend from 30 minutes for PTP-GNP treatment to over 8 hours for PEGylated PTP-GNP after which, the receptor expression was observed to begin recovery process as observed from Figure 5A and S3B. A complete recovery of plectin-1 receptor expression was observed at 16-hour time point and maintained it until 24 hours' time point (Figure S3B). Above results confirm our hypothesis of protein corona formation around PTP-GNP prohibiting from undergoing receptor mediated endocytosis process due to unavailability of free peptide on GNP surface to react with plectin-1 receptors on Panc1 cell membrane surface.

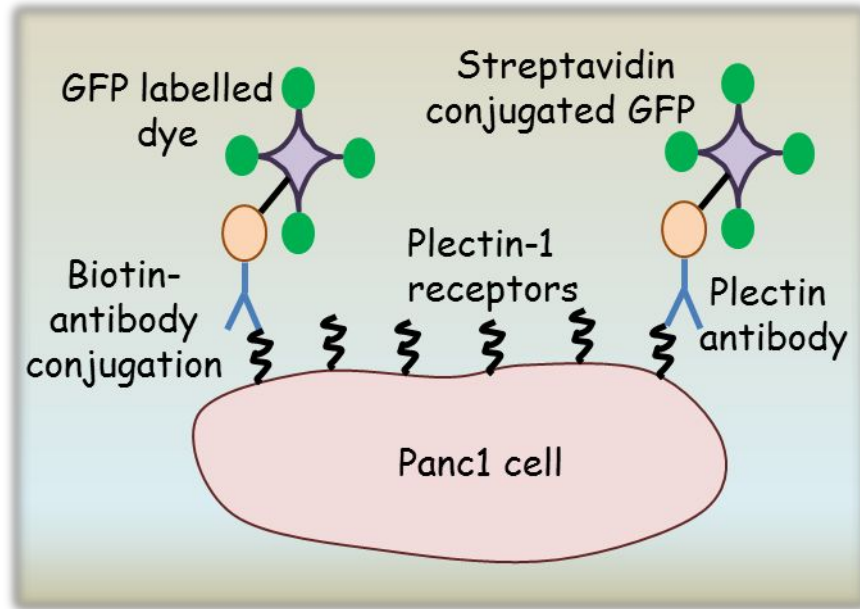


Figure S1. A schematic representing classical streptavidin-biotin reaction to evaluate plectin-1 surface receptor expressions.

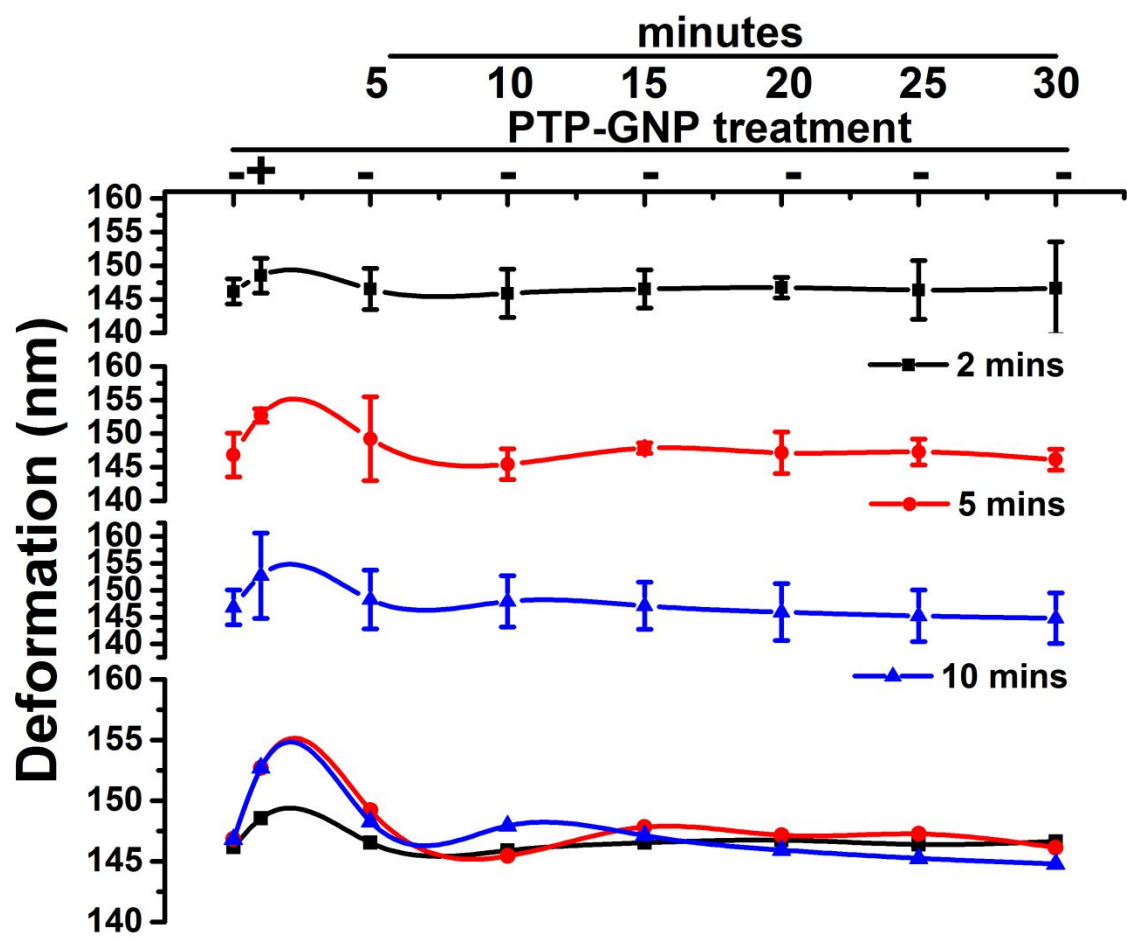


Figure S2. Dynamic recovery of Panc1 cell membrane's deformation upon exposure to PTP-GNP for various time durations.

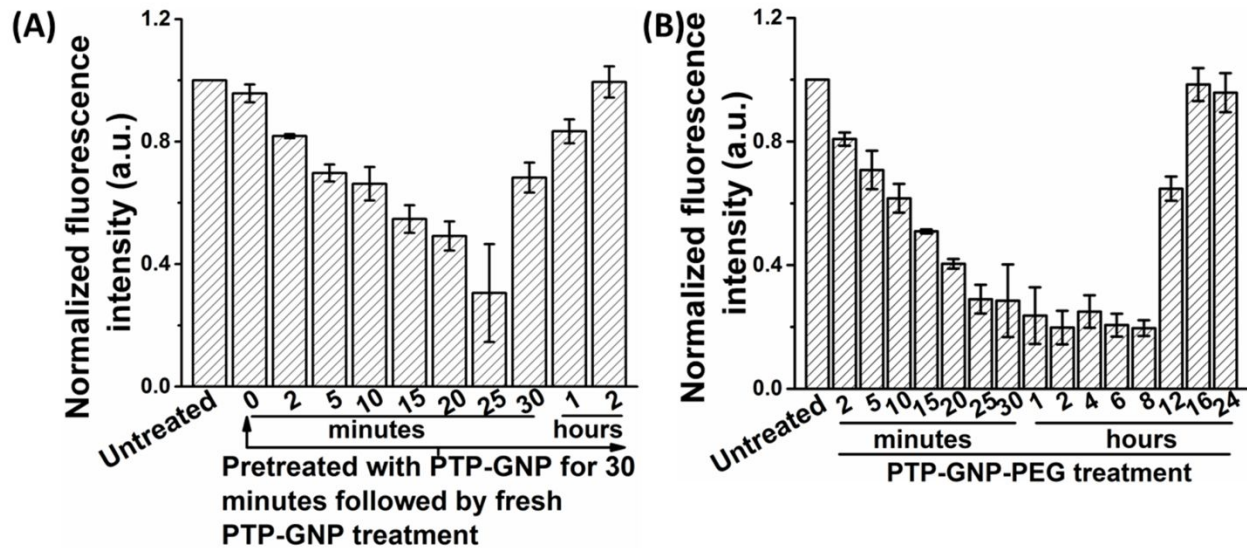


Figure S3. Effect of PTP-GNP protein corona formation on receptor mediated endocytosis process. (A) Complete recovery of Plectin-1 receptor expression on the Panc1 cell surface implying protein corona formation upon treatment with PTP-GNP. (B) Prolonged plectin-1 receptor mediated endocytosis process in the presence of PEGylated PTP-GNP.