DEVELOPMENT OF A VERSATILE MONITORING TECHNIQUE FOR REAL-TIME PROTEIN ACTIVITY TRACKING WITHIN CELLULAR ENVIRONMENTS AND BIOMIMETIC TISSUE ENGINEERING SCAFFOLD SYSTEMS

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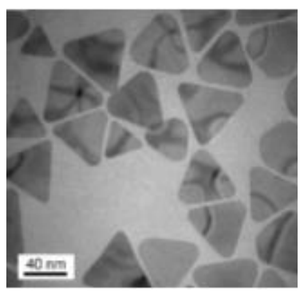
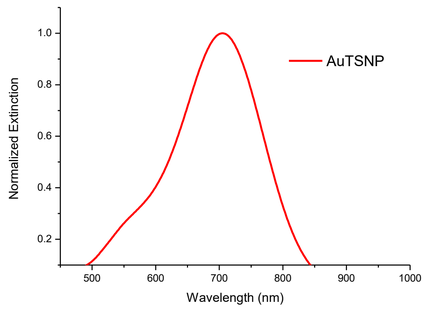
Introduction

Crowded cellular environments with complex and intricate molecular interactions underpin biological processes. High biological noise is intrinsic within these biological systems, and this poses critical challenges to the in situ detection and measurement of biomolecular and protein activities important to advancing approaches to disease and injury treatment. Currently the techniques available to characterize protein behaviours in living biological systems are highly elaborate and are generally greatly hindered by the high background noise of the cellular environments. Here we present a versatile and straight forward technique for monitoring proteins and protein interactions within cells, based on a novel nano-bio-technology method based on nanoparticles Local Surface Plasmon Resonance (LSPR). High sensitive gold edge coated triangular silver nanostructures (AuTSNP), which exhibit a highly sensitive spectral response to the molecular interactions on their surfaces, are used to probe protein behaviours within complex cellular and tissue regeneration environments. In this work, monitoring of the dynamic behaviour of a critical extracellular protein, Fibronectin (Fn) in its active form, within the presence of bone tissue regeneration scaffolds and living cells (C2C12 myoblasts, MC3T3-E1 pre-osteoblasts) is presented. The excellent sensitivity and straight forward application within cellular environments, demonstrates AuTSNP as powerful new tools to signature protein conformational transitions and monitor essential protein activity.



TEM image of AuTSNP

LSPR



Nanoplate UV-Vis spectrum

Materials & Methods

Synthesis of AuTSNP

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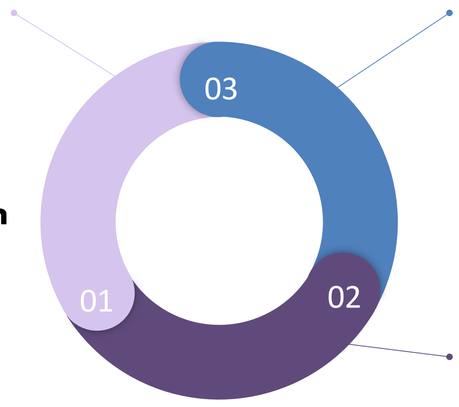
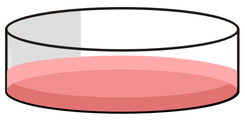
Bone tissue regeneration scaffold preparation



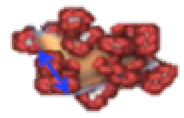
Plasmonic spectral monitoring over 32 hours

C2C12 and MC3T3 cell culture

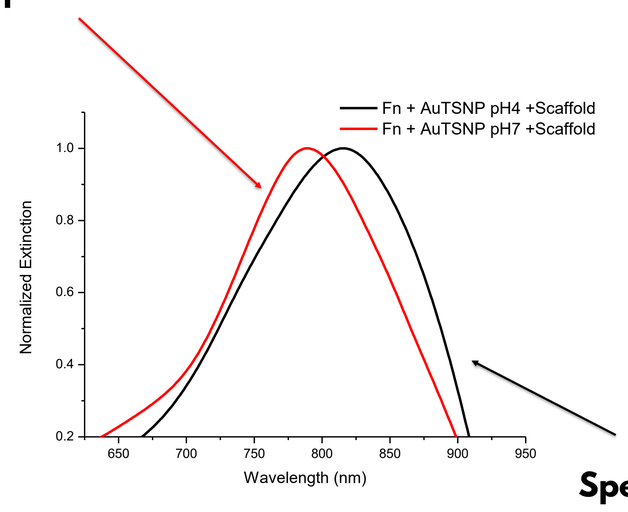


Results & discussion

Spectral signature of compact conformation

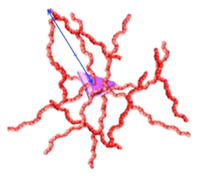


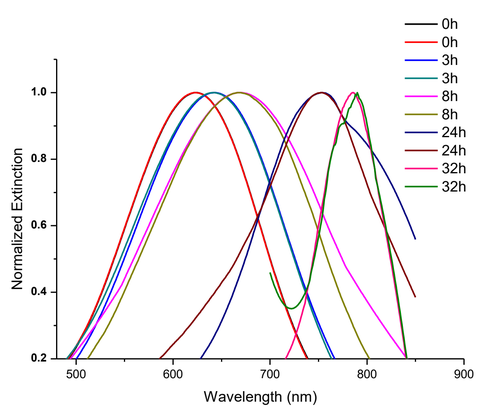
Compact Fn loaded AuTSNP (pH 7)



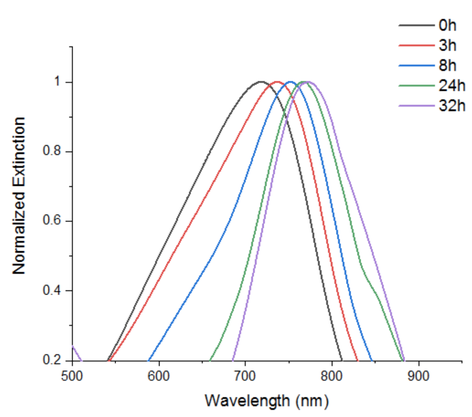
Spectral signature of extended conformation

Extended Fn loaded AuTSNP (pH 4)





Active Fn PEG-Np incubated with C2C12 myoblasts and tissue scaffold



Active Fn PEG-Np incubated with MC3T3-E1 pre-osteoblasts

High sensitive LSPR Fn functionalised AuTSNP were used to monitor the conformation transitions of Fn in the presence of C2C12 myoblast cells and bone tissue regeneration scaffolds over time from 0 to 32h. As Fn unfolds from a compact conformation to form fibrils in which Fn displays a highly extended conformation, LSPR spectra exhibit large red shifts. A similar behaviour is observed when fibronectin functionalised AuTSNP are incubated with MC3T3-E1 pre-osteoblasts and monitored over the same time. These results correlate with the most recent models on Fn conformational activity within the cellular environment, demonstrating the potential of the AuTSNP to provide critical detailed information on dynamic protein conformational response and behaviour.

References

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3. Devine, D. M., Hoctor, E., Hayes, J. S., Sheehan, E. & Evans, C. H. Extended release of proteins following encapsulation in hydroxyapatite/chitosan composite scaffolds for bone tissue engineering applications. *Mater. Sci. Eng. C* 84, 281–289 (2018).
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