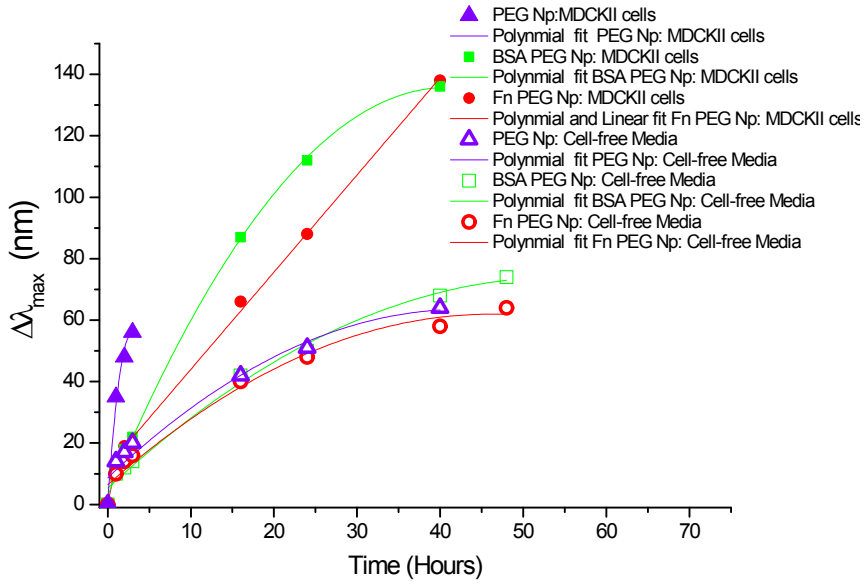
Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

PEG coated AuTSNPs (PEG-Np) and BSA PEG-Np are used to provide control measurements and examine the spectral response of the nanoplates in cell-free media and in the presence of the MDCKII cells. As shown in SI Figure 1, after 40 hours incubation in cell free media, the peak spectral maxima are typically located at 772 nm for PEG-Np, 772 nm for BSA PEG-Np and 766 nm for Fn PEG-Np. Non-specific binding by cell media proteins and molecules are believed to be generally responsible for these spectral red shifts. The spectral red shift due to this non-specific binding follows a nonlinear profile, which can be readily fitted to a second order polynomial as shown in SI Figure 1. Note that the presence of the Fn, which is a relatively large protein (440kDa) compared with BSA (66.5), acts to reduce non-specific binding as indicated by the lower shifts for the Fn PEG-Np compared to PEG-Np and BSA PEG-Np.

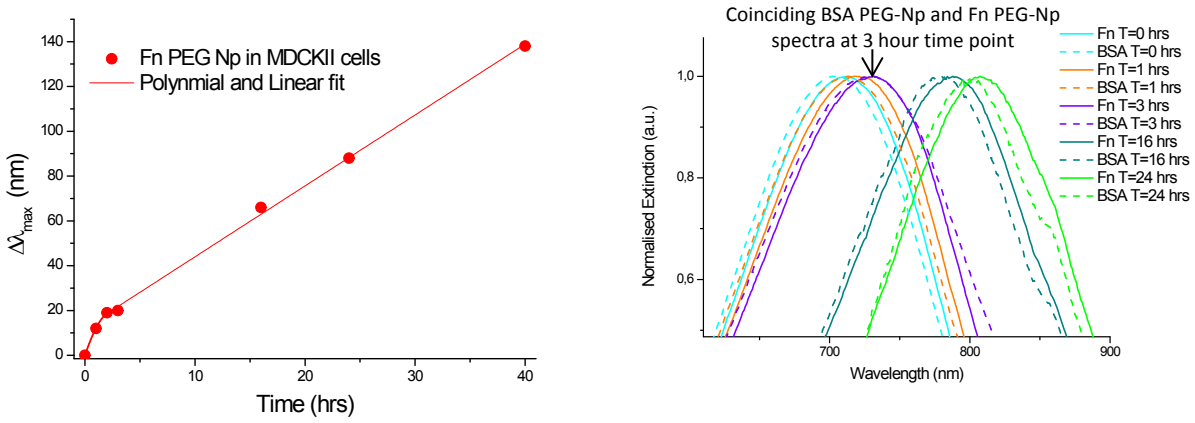


SI Figure 1 LSPR spectral shift of PEG-Np (violet), BSA PEG-Np (green), Fn PEG-Np (red) in the presence of MDCKII cells (solid symbols) and cell-free media (open symbols) with nonlinear polynomial curve fits and a linear fit in the case of Fn PEG-Np in the presence of MDCKII cells.

As observed in SI Figure 1, in the presence of MDCKII cells, both PEG-NP and BSA PEG-Np follow a similar nonlinear spectral shift profile, associated with non-specific binding. The rate of spectral shift is significantly increased compared to that for cell media without the presence of the MDCKII cells, particularly for the PEG-Np, which lack the presence of surface protein protection. This increased rate is believed to be associated with cellular activity including, cellular degradation uptake and digestion of the PEG-Nps. A significant difference is noted for the Fn PEG-Np which exhibits a linear red shift response in contrast to the nonlinear red shift profile of PEG-NP, BSA PEG-NP in both cell free media and in the presence of MDCKII cells and Fn in cell free media.

On closer inspection the Fn PEG Np spectral shift response appears to follow a nonlinear trend up to the 3 hour time point as shown in SI Figure 2a) after which a distinct transition to a linear progression

is observed. The work of Vogel et al has shown that Fn added to cells in the manner carried out during this work takes up to 4 hours for the fabrication of an Fn matrix to occur.[34,40] This time scale correlates strongly with the transition in behaviour of Fn PEG-Np incubated with MDCKII observed in SI Figure 2a) and b). The 3 hour time point coincides with a slowing down of the rate of the Fn PEG-Np spectral shift compared to BSA PEG-Np, observed in Si Figure 2b) where the BSA PEG-NP spectra shift catches up with the Fn PEG-NP spectral shift at the 3 hour time point. These results provide evidence that from the 3 hour time point the Fn PEG-Np have been actively integrated into the ECM and the Fn associated with the Fn PEG-Np is undergoing incorporation, via the reported route of cellular integrin binding induced Fn uncoiling, extension and assimilation into Fn fibril networks. Further evidence that the Fn PEG-Np spectral response in the presence of MDCKII cells is due to Fn conformational activity, is given by the fact that the overall Fn PEG-Np spectral shift is larger than that of BSA PEG-Np despite the fact that Fn PEG-Np having demonstrated strongly reduced non-specific binding compared to BSA PEG-Np in cell free media as seen in SI Figure 1 and the absence of observed non-specific binding in the serum assay.

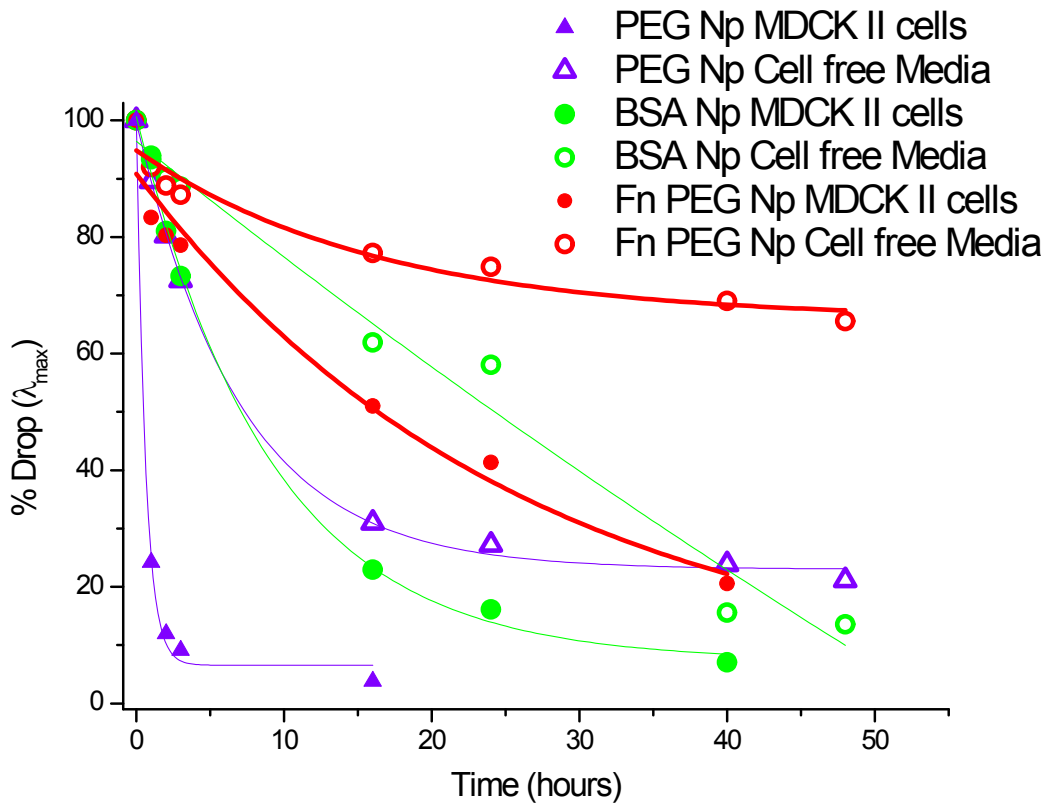


SI Figure 2 a) Spectral shift of Fn PEG-Np (red) in the presence of MDCKII cells with polynomial curve fits for up to 3 hours incubation followed by a linear fit for the remaining incubation duration. b) UV-Vis LSPR spectra of Fn PEG-Np and BSA PEG-Np incubated with MDCKII cells from 0 to 24 hours.

Note that in the case of the serum assay shown in Figure 3c) overnight incubation of Fn PEG-Np in serum, under the conditions for CTSB cleavage at pH 4, the spectral max location is measured to be 747 nm, which is10 nm blue shifted from the pH 4 buffer conditions previously observed. This indicates that the presence of the serum protein or EDTA may inhibit the full extension of the Fn indicating the high sensitivity of the PEG-Nps to subtle changes in the Fn conformation. The blue shifted peak spectral maximum is significant in indicating the absence of any non-specific binding due to the serum proteins. This is evidence that Fn provides strong inhibition of non-specific binding acting as a functional molecule providing good specificity and selectivity enabling the successful operation of the CTSB cleavage assay. A blue shift will not be recorded if the Fn molecules are not sufficiently close to the PEG-Np surface upon CTSB cleavage. The 6 nm blue shift recorded on the addition of CTSB indicates the continued presence of the Fn close to the PEG-NP surface and the capacity to provide sensitive recording within such a complex medium.

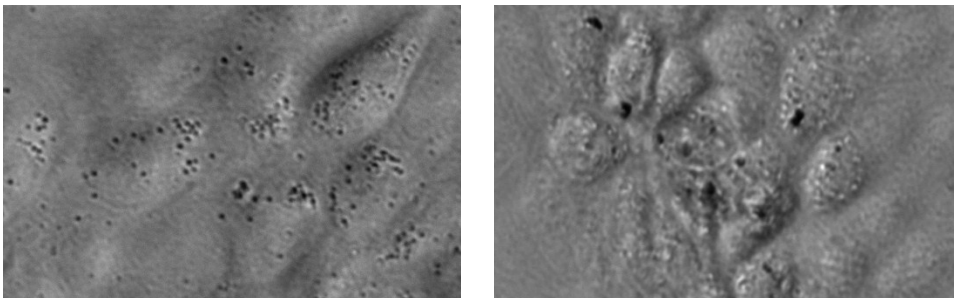
Cellular degradation is distinguished as a reduction in the amplitude of the PEG-Np, BSA PEG-Np and Fn PEG-Np rather than spectral shifting. A spectral narrowing with a reduction in the spectral fullwidth half maximum (fwhm) is also associated with cellular digestion which results in the degradation of the Nps. SI figure 3 shows the progression of the amplitude of the peak spectral

maxima over time in cell free media and in the presence of MDCKII cells for the PEG-Np, BSA PEG-Np and Fn PEG-Np. The PEG-Np show the most rapid degradation under both conditions with a particularly fast degradation in the presence of the MDCKII cells. This decrease in the PEG-Np spectral amplitude in the presence of MDCKII cells, is accompanied by a sharp decrease in the fwhm from 174 at 3 hours to 112 at 16 hours. The stability of the BSA PEG-Np is improved over the PEG-Np which is attributed to the presence of the protecting influence of the BSA. A gradual reduction is observed in the BSA PEG-Np spectral fwhm in the presence of MDCKII cells from 166 nm up to 3 hours, reducing to 150 nm at 16 hours, 142 nm at 24 hours and 132 nm at 40 hours. The Fn PEG-Np show good stability in cell free media. In the presence of MDCKII cells the Fn PEG-Np fwhm is a constant 163 nm up to 40 hours reducing to 106 nm at 48 hours demonstrating their longevity which is expected due to their increasing incorporated within Fn fibril in the ECM.



SI Figure 3. Percentage drop in spectral peak maxima with time of PEG-Np (violet), BSA PEG-Np (green), Fn PEG-N-p (red) in the presence of MDCKII cells (solid symbols) and cell-free media (open symbols) with exponential decay fits as a guide to the eye.

Dark spots visible in certain bright field images of MDCKII cells exposed to nanoplates such as those shown in SI figure 4, are indicative of bulk silver deposits associated with degraded nanoplates. These observations indicate the active role of cellular digestion mechanisms in the degradation of the nanoplates and a rate of degradation, which is strongly dependent on the functionality of the surface molecules.



SI Figure 4. Bright field images of MDCK II cells incubated for 72 hours with i) BSA PEG-Np and ii) Fn PEG-NP.

The strong protective nature of Fn against non-specific binding significantly delays the cellular digestion process enabling sufficient time to effectively probe the Fn incorporation process within the ECM, prior to cellular degradation. Cellular degradation is a natural and continuous process within cellular life cycles. ECM evolution is an example, wherein older Fn molecules are degraded by cathespsins such as CTSB and new Fn is generated. It would be contrary to the nature of cellular processes if the nanoplates were not degraded and disposed of over time by cellular digestion processes and maintained long term stability within such cellular environments.