

Fluorescent imaging agents for multiple applications

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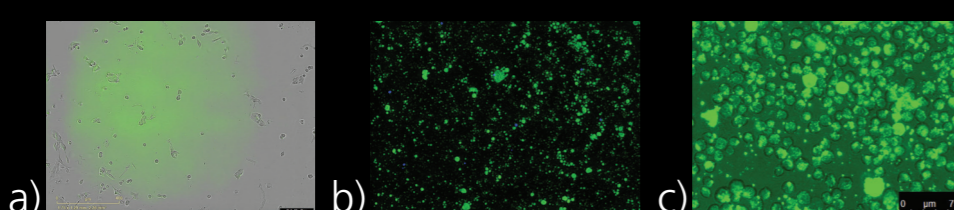
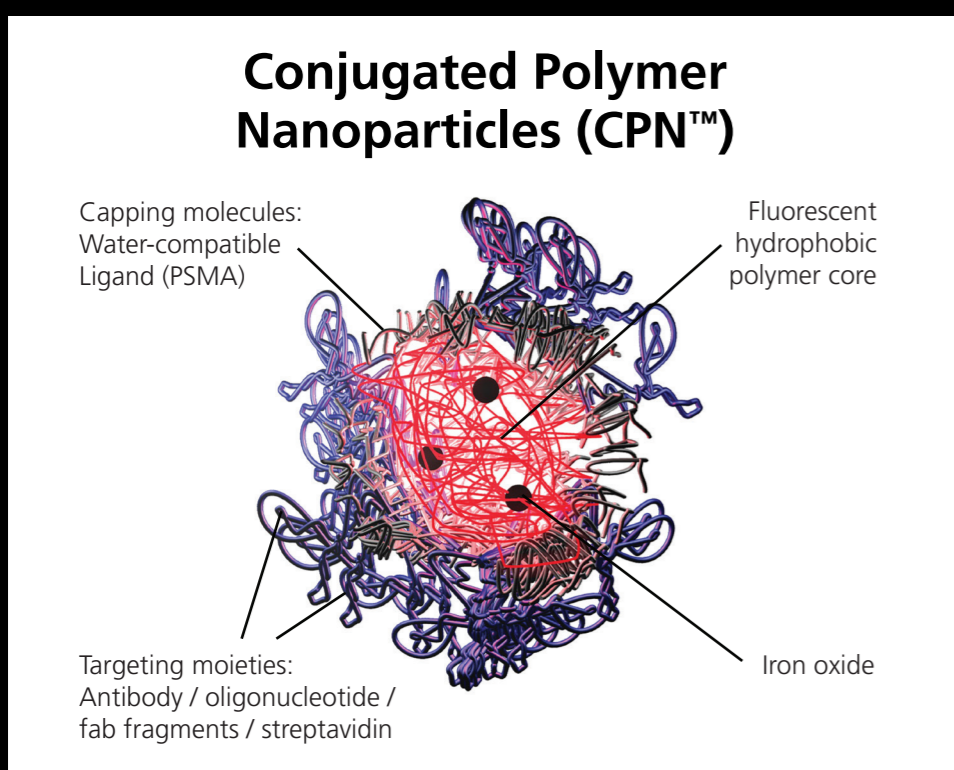
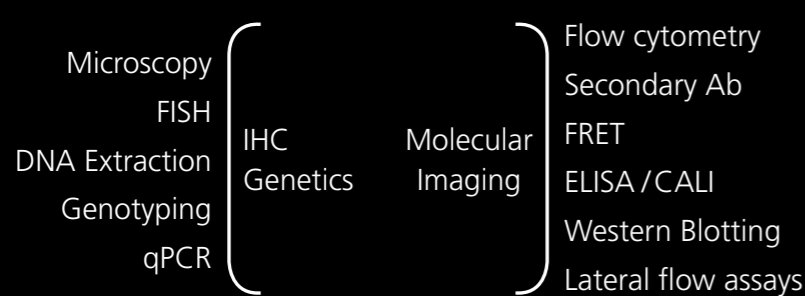
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Introduction

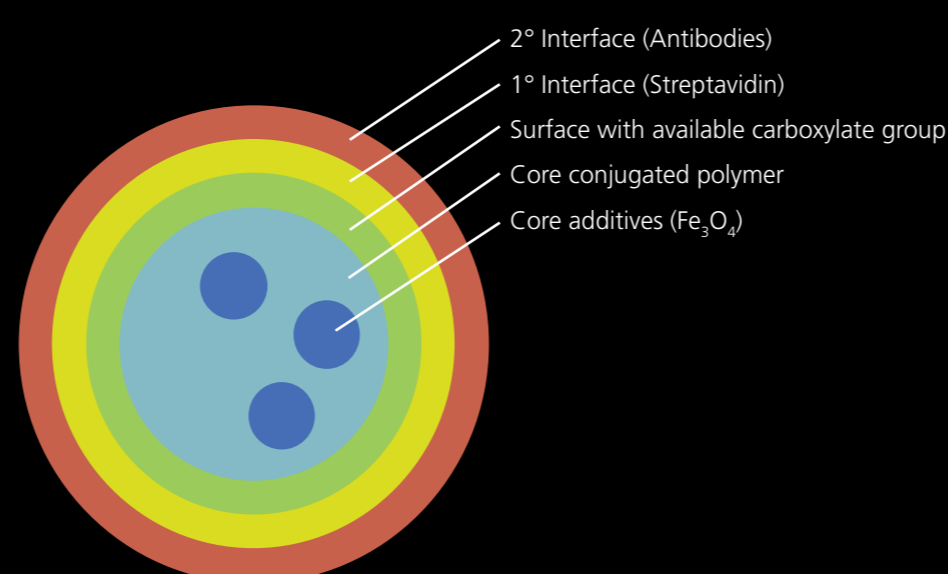
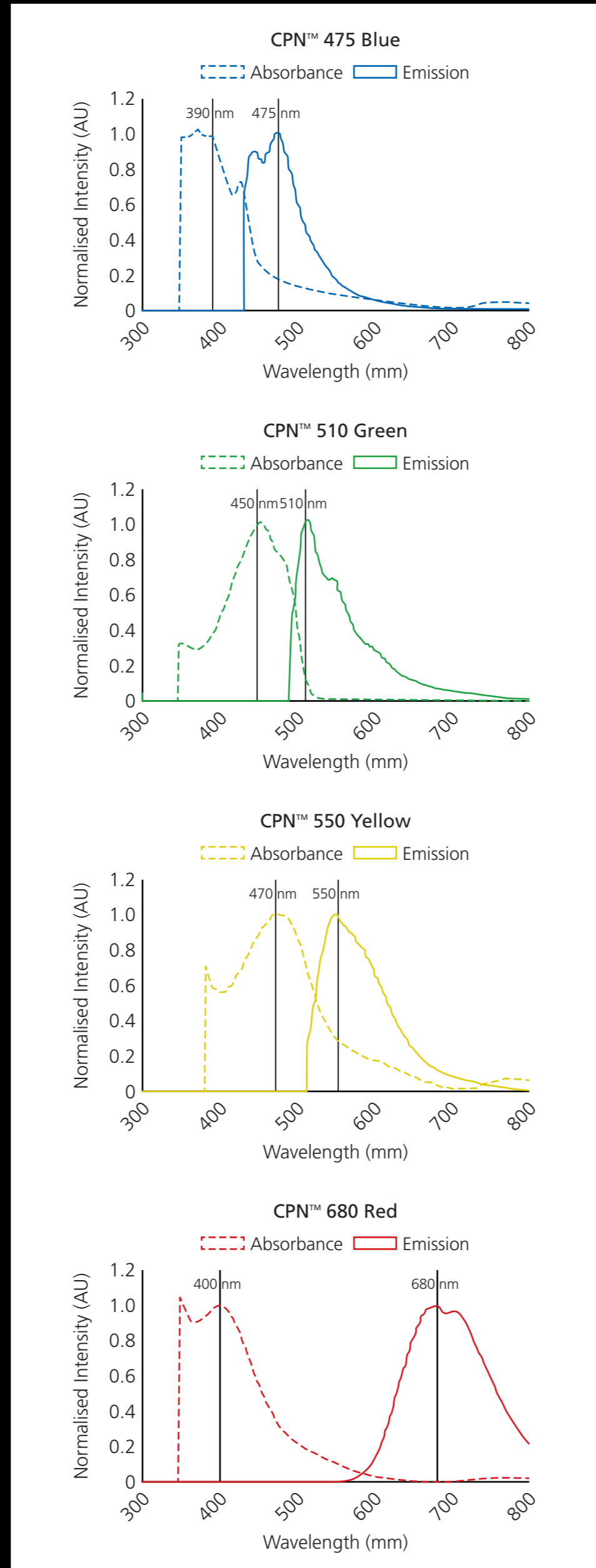
Conjugated Polymer Nanoparticles (CPN™) are highly fluorescent, non-toxic, molecular bioimaging probes that can be used for a diverse range of existing cellular imaging applications. Derived from OLED television and visual display technology, CPNs offer immense brightness. They have exceptional stability across a wide range of pH and temperatures and are not prone to photo-bleaching. CPNs have a range of emission spectra covering the visible spectrum and are compatible with standard fluorescent filters and laser lines. CPNs can label targeted cells through endocytosis or linkage to specific targeting moieties, such as antibodies or binding proteins. The emission intensity of CPNs makes them useful for highly sensitive imaging techniques such as immunocytochemistry and flow cytometry. Furthermore, the stability of CPNs means results obtained are highly reproducible. Here, we will explore the applicability of CPNs in various cell biology systems.

Applications

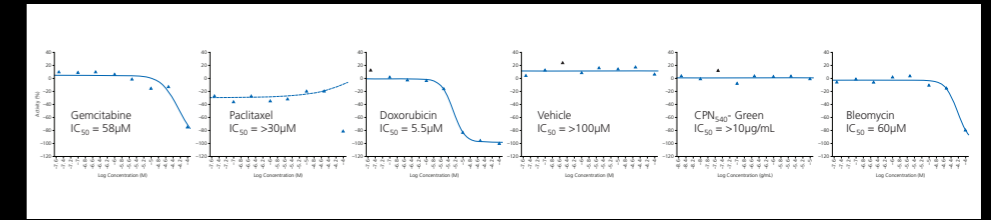


a) The fluorescent uptake of CPN 550 Yellow by HCC70 cells over 6 days imaged on an IncuCyte; b) CPNs can be directly visualised on a standard microscope - Image of CPN550 nanoparticles in suspension; c) Fluorescent microscope image of CPN loaded into CHO cells by endocytosis.

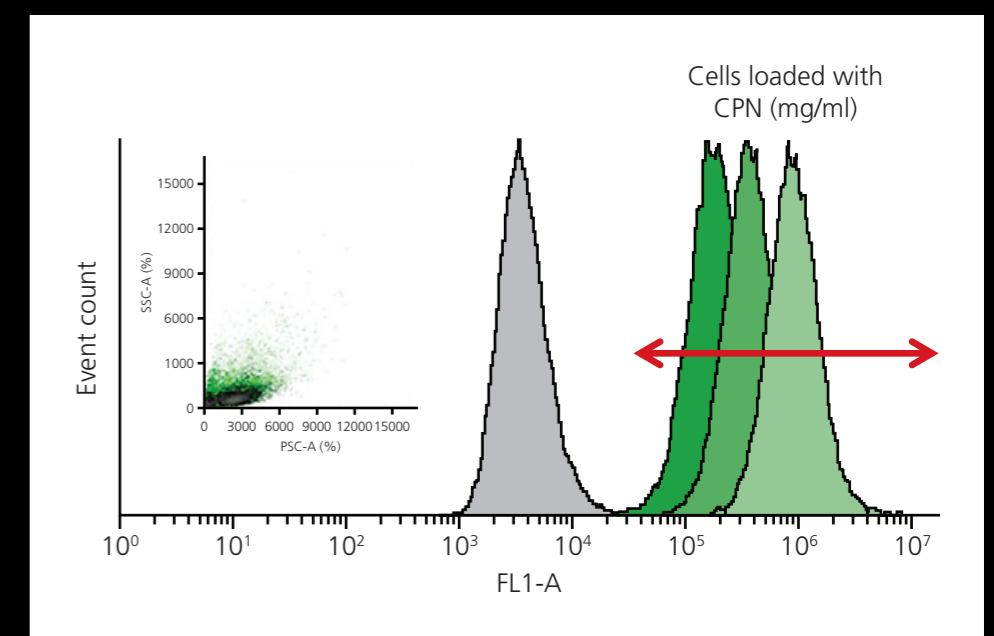
CPNs have emission wavelengths spanning the visible spectrum. Excitation and emissions spectra for the 4 available colours of CPN.



CPNs Have A Multi-Layer Structure



The CPN 550 Yellow from the culture media were readily taken into the mammalian epithelial cell line HCC70 by endocytosis. The CPNs were trafficked through the endosomal/lysosomal pathway and displayed a punctuate, perinuclear distribution. Each punctum is understood to be a single CPN contained in a vesicle. The cell culture was continuously imaged over 6 days to identify any indication of cytotoxicity from the CPNs. The graphs in the lower panel show that for up to 10mg/ml, corresponding to the highest concentration tested, the CPNs were non-toxic. Other cytotoxins were used as a reference, which induced cell death.



CPNs loaded into CHO cells by endocytosis at 0.02, 0.01 and 0.005mg/ml. The CPNs were clearly distinct from unloaded blank cells.

CHO cells, grown in suspension, were also loaded with CPN550 from the culture media by endocytosis. These cells were then readily sorted using flow cytometry. The CPNs had an intense punctate perinuclear distribution, which was readily visualised using FITC filters on a standard fluorescent microscope. These cells were then sorted using BD Accuri™ C6 Plus flow cytometer. The fluorescent signal was directly proportional to the concentration of the CPN in the culture media and correlated to the number of CPNs acquired per cell.

Conclusion

CPNs offer exciting potential for fluorescently labelling molecules and cells. The immense emissions allow for the detection of low-level proteins and highlight rare cell types. The signal strength also means that lower levels of excitation can be used in sparingly delicate cells and tissues. The robustness of the CPNs also allows them to be used under high intensity illumination if required and in extreme experimental conditions, with temperatures up to 120°C and a pH ranging from 4 to 10.

The CPNs can be linked to targeting proteins and can be utilised on standard analysis platforms such as flow cytometry, immunocytochemistry and fluorescent microscopy.