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Fluorescent imaging agents for multiple applications: Conjugated Polymer Nanoparticles

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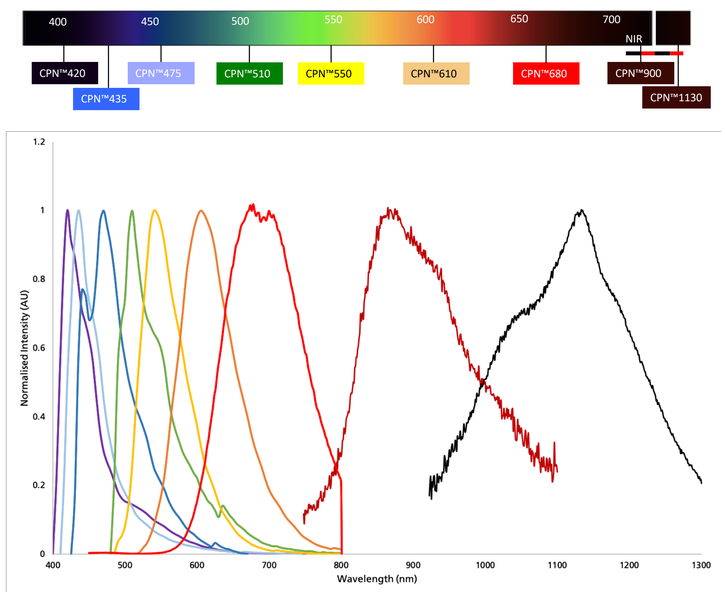
(Contributions from: CytoSmart, ONI, MDC, EM Analytical, Horiba, University of York, CPI, Aptamer Group)

Introduction

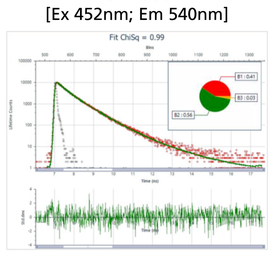
Conjugated Polymer Nanoparticles (CPNs™) are highly fluorescent, non-toxic, molecular bioimaging probes that can be used for a diverse range of existing cellular imaging applications. Derived from OLED television and visual display technology, CPNs offer immense brightness, making them useful for highly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

Wavelengths

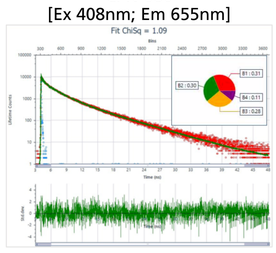
CPN wavelengths span the visible spectrum and extend into the near infrared region. Until now, near-IR imaging in clinical settings has been limited. However, CPNs such as CPN1150 (Ab 750nm / Em 1150nm) promise higher contrast, sensitivity, and tissue penetration depths. Moreover, an additional 8 wavelengths are under development.



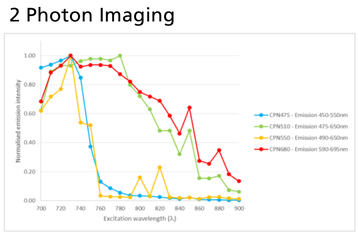
CPN-550
[Ex 452nm; Em 540nm]



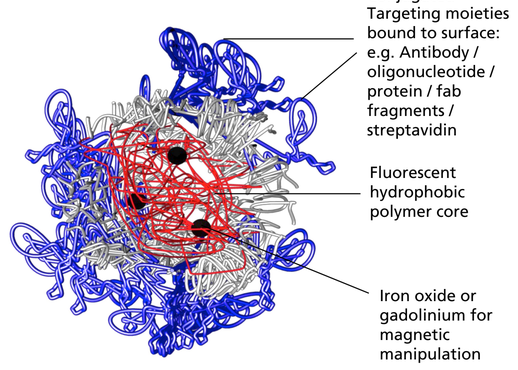
CPN-680
[Ex 408nm; Em 655nm]



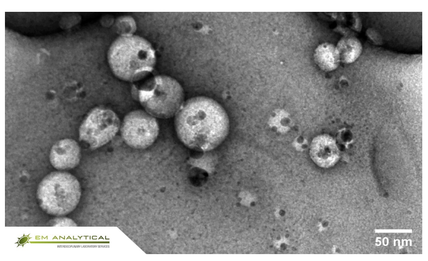
2 Photon Imaging



Excitation 700-900nm
Emission CPN475, CPN510, CPN550, CPN680



TEM of CPNs:



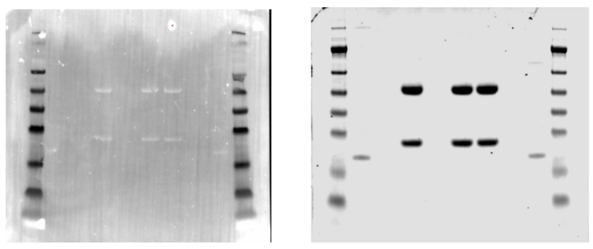
Biological Properties

The intense brightness of CPNs dramatically increases the sensitivity of applications such as flow cytometry, ELISA, IHC and microscopy. Using CPNs, single nanoparticles are detectible with flow cytometry and immunocytochemistry, enabling the study of individual proteins in samples and cells. Streptavidin and antibodies can be covalently conjugated to CPNs via the surfactant's carboxylic acid groups using *N*-ethyl-*N*'- dimethylaminopropyl-carbodiimide (EDC) chemistry. These targeted CPNs can be readily used in existing assays, with the increased brightness improving performance and increasing sensitivity. When conjugated to an oligonucleotide, the CPN-oligonucleotide complex is thermally stable and requires no cold storage. Other surface chemistries are also available, such as thiol and azide for click chemistry.

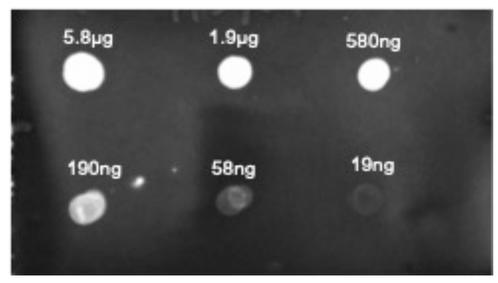
Applications

Microscopy		Flow Cytometry
1°/2° Antibodies	IHC	FRET
FISH	Genetics	Fluorescent ELISA
DNA Extraction	Molecular Imaging	Western Blotting
Genotyping		Lateral Flow Assays
qPCR		

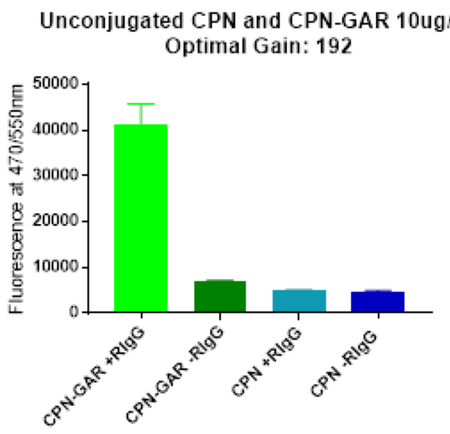
Western Blot

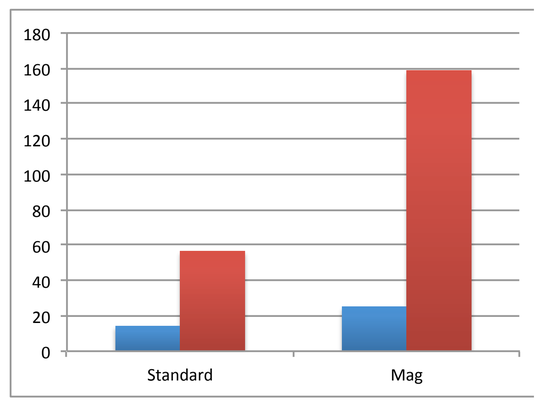


Western Blot with CPN 550 — SDS-PAGE gel with Comassie Stain



Dot blot for 1 µl dots of IgG at indicated quantities probed with CPN550+anti-Mouse IgG.





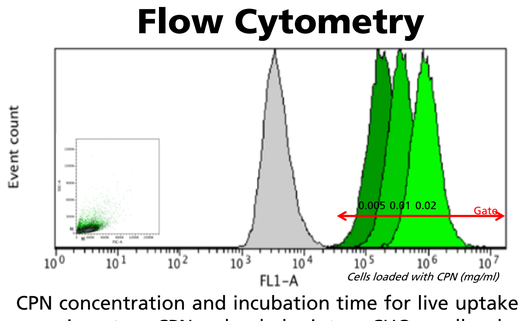
Antibody Conjugation

CPNs can be linked to a wide range of antibodies such as CD-4, CD-8, CD-34, INF-g, MOC-31, and mouse/rabbit IgG. Utilisation of the magnetic properties of CPNs increases binding by simple, careful placing of a magnetic field during conjugation. Sensitivity can be further increased when CPNs are applied to a range of applications, such as ELISA.

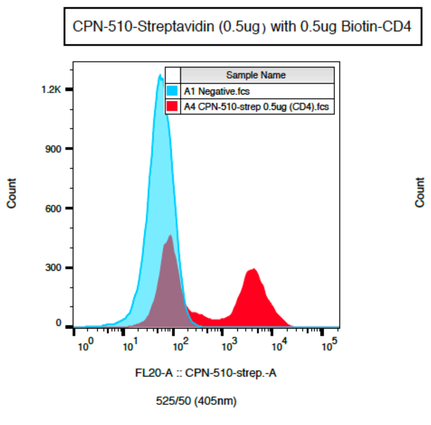
Use of CPNs in protein labelling and cell imaging

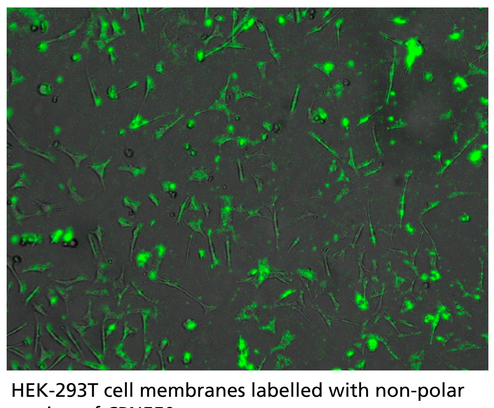
The intense fluorescent signal produce by CPNs make them ideal for labelling protein of interest, particularly those expressed at low levels, in cells and tissue samples. Reliable low level protein detection is an advantage for both flow cytometry and cell imaging studies. The stability of CPNs allows prolonged incubation times in cell media and labelled tissue samples can be fixed using formaldehyde without diminishing the CPN signal.

Flow Cytometry

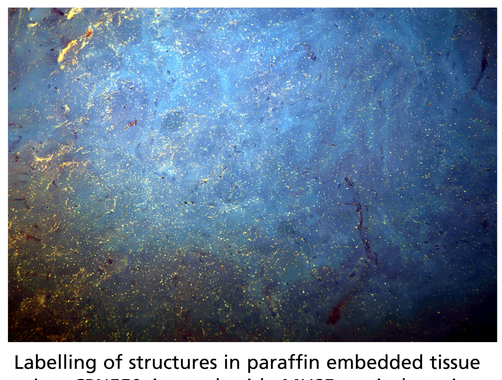


CPN concentration and incubation time for live uptake experiments. CPNs loaded into CHO cells by endocytosis at 0.02, 0.01 and 0.005mg/ml. The CPNs were clearly distinct from unloaded blank cells.





HEK-293T cell membranes labelled with non-polar version of CPN550



Labelling of structures in paraffin embedded tissue using CPN550 imaged with MUSE optical section imaging system

Conclusion

CPNs offer exciting potential for fluorescently labelling molecules and cells. Their immense brightness allow low-level proteins to be detected and rare cell types to be highlighted. This brightness allows lower levels of excitation to be used sparingly in delicate cells and tissues. The stability of CPNs also allows their use under high intensity illumination and 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 used on standard analysis platforms such as flow cytometry, immunocytochemistry and fluorescent microscopy.