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SCAN ME

Fluorescent imaging agents for multiple applications: Conjugated Polymer Nanoparticles

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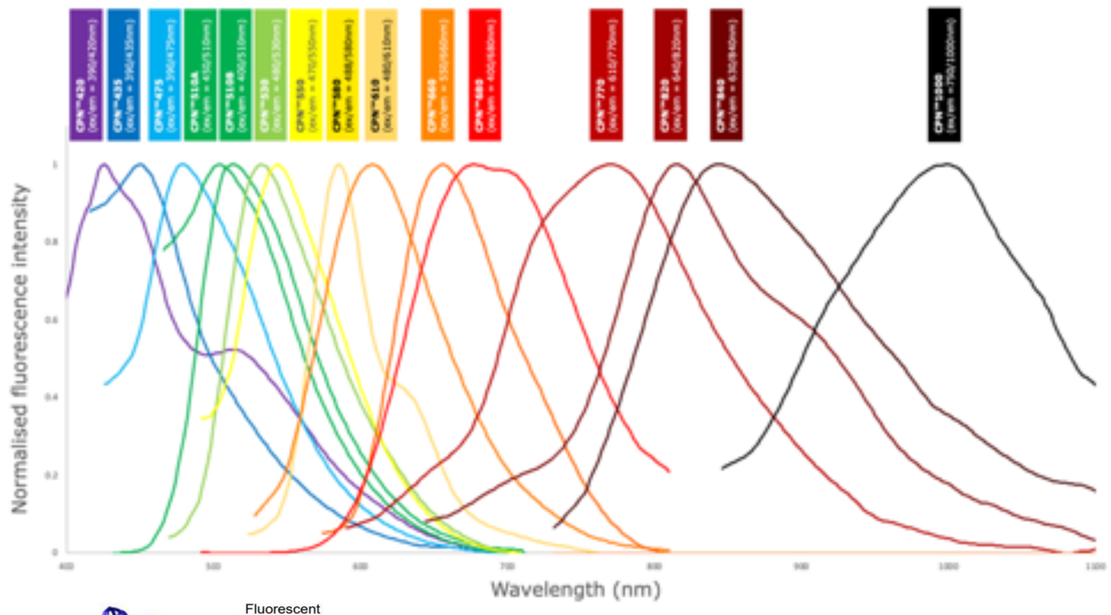
(Contributions from: CytoSmart, Medicines Discovery Catapult, EM Analytical, Horiba, University of York, CPI)

Introduction

Conjugated Polymer Nanoparticles (CPNs™) are highly fluorescent, non-toxic, molecular bioimaging probes that can be used for a diverse range of applications. CPNs™ offer immense brightness, making them useful for highly sensitive imaging techniques, including immunocytochemistry, microscopy and flow cytometry. CPNs™ have a range of excitation and emission spectra covering the visible and near infrared 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. Furthermore, CPNs have application in therapeutics with the potential for use in photothermal/photodynamic therapy. Here, we will explore CPNs in a variety of applications.

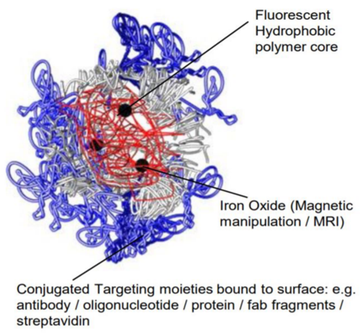


Imaging
Microscopy
1°/2° Antibodies
Flow Cytometry
FISH
FRET

Quantification
Lateral Flow assays
Fluorescent ELISA
Western Blotting
DNA Extraction
qPCR

Therapeutics
In-vivo imaging
Photothermal Therapy
Drug Development

Reagents **Diagnostics** **Therapeutics**

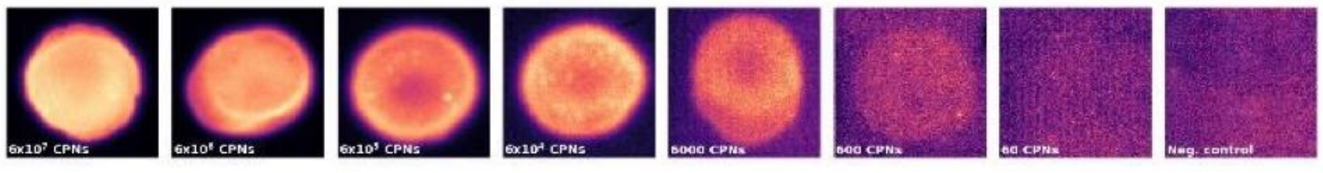


Biological Properties:

The intense brightness of CPNs dramatically increases the sensitivity of applications such as lateral flow tests, ELISA, immunohistochemisty, microscopy and flow cytometry. 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.

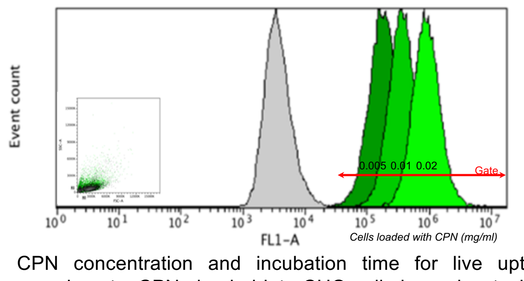
Use of CPNs in protein labelling and cell imaging

The intense fluorescent signal produced by CPNs make them ideal for labelling proteins 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. CPN's stability allows for prolonged incubation times in cell media and labelled tissue samples can be fixed using formaldehyde without diminishing the CPN signal. CPNs are highly sensitive with quantitative detection of just a few hundred individual CPNs.



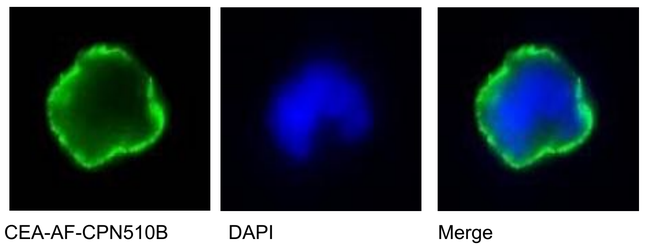
CPN 610 dilution series with images taken using the Brightline (Chelsea Tech LTD and Stream Bio LTD) fluorometer.

Flow Cytometry



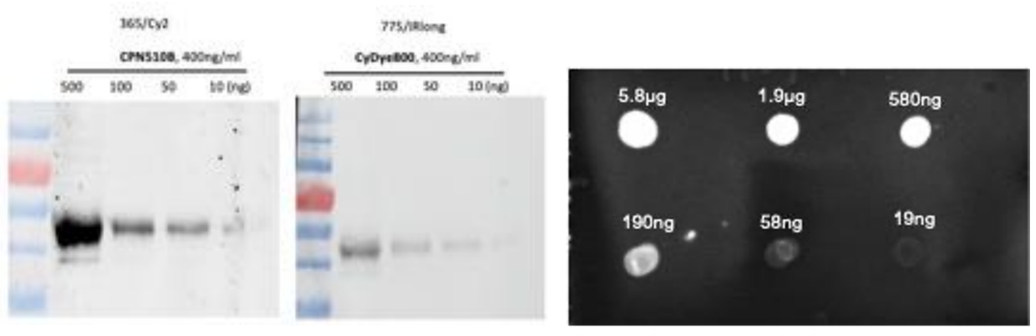
CPN concentration and incubation time for live uptake experiments. CPNs loaded into CHO cells by endocytosis at 0.02, 0.01 and 0.005mg/ml.

Cell Imaging



CEA-AF-CPN510B DAPI Merge

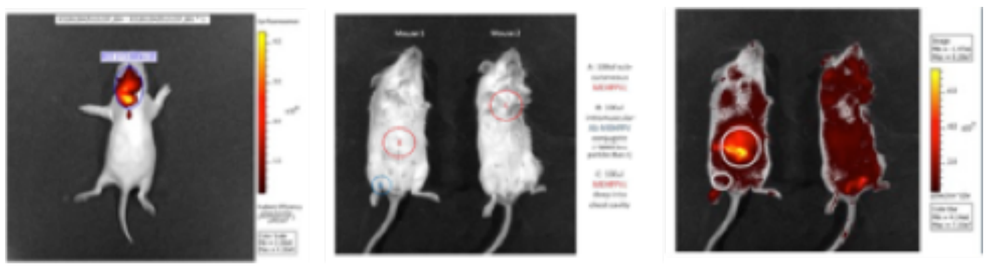
LoVo cells were fixed in 100% methanol at -20°C and followed by incubation with CPN510B conjugated CEA-Affimers (1:20).in PBS.



Western blots produced by CPN510B, and CyDye 800 at matching concentrations.

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

In Vivo Imaging and Photothermal therapy



CPN550 injected into three areas by dstl, visible though deep tissue

The brightness and non-toxic nature of CPNs makes them ideal *in vivo* imaging probes. The fluorescent properties enable a good tissue depth penetration, this has been applied in tumour margin demarcation with CPN680 showing advantages over more commonly used fluorophores. CPNs can be used in photothermal therapy with PT-CPNs designed with specific cores that when excited give rise to significant amounts of photothermal energy, superior to gold nanorods and carbon nanotubes.

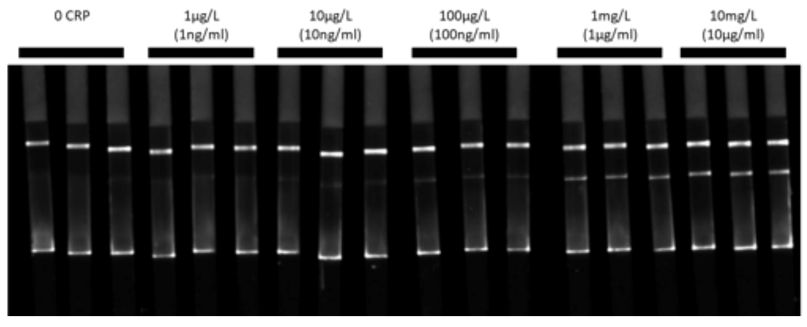
Lateral Flow Tests

CPNs are outstanding tools for use in lateral flow diagnostic tests. CPNs high fluorescent intensity enables low levels of accurate target detection enabling the potential for early disease diagnosis.

The wide CPN color range facilitates an ultrasensitive multiplexed, mass screening capability. Furthermore, IR CPNs have the potential to be used as visual signals with a greater absorbance than gold nanoparticles. The magnetic properties of CPN also allows for magnetic enhancement of lateral flow devices.

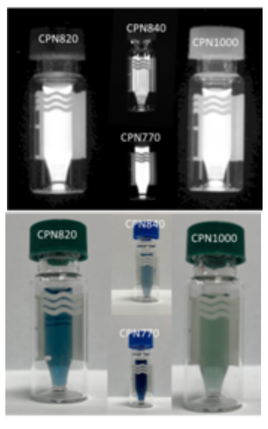
Several CPN based lateral flow devices are currently in development for heart attacks, brain injury and viral pathogens.

Limit of detection 800 times better than the standard tests and 30 times better than the high sensitivity tests according to FDA regulatory information



CPN based lateral flow tests showing signal from 10mg/L (10ug/ml) to 10ug/L (10 ng/ml)) with clear and consistent controls lines visible.

Near Infrared (NIR) CPNs



The unique properties of our CPNs offer advantages over conventional IR dyes and can enhance IR imaging. The Near infrared CPNs enable deep tissue imaging in biological tissues as blood and skin appear transparent in these spectral regions.

NIR CPNs can be utilised for fluorescence guided surgery with emission profiles matching the emission of the only clinically approved NIR dye ICG, whilst also providing a higher degree of stability and greater fluorescence.

CPN770, CPN820, CPN840, CPN1000: Greyscale images of infrared fluorescence and colour images under ambient lighting conditions.

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 2 to 10. The CPNs can be linked to targeting proteins and can be used on standard analysis platforms such as fluorescent microscopy, immunocytochemistry and flow cytometry. CPNs also have potential for diagnostic and therapeutic applications and *in vivo* imaging platforms.

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