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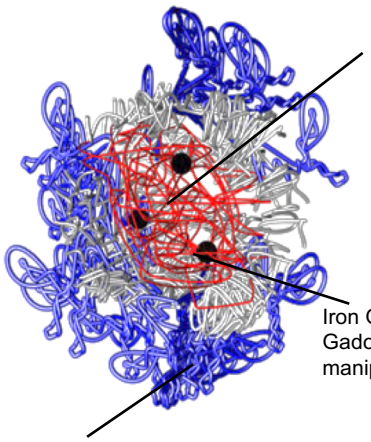
Conjugated Polymer Nanoparticles (CPNs™)
Highly Fluorescent Imaging Agents for Multiple Cellular Applications

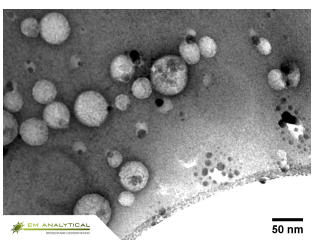
Conjugated Polymer Nanoparticles:
CPNs™ are exceptionally fluorescent, nanoparticulate, labelling probes that can be utilised in cellular imaging and diagnostics applications. They are highly stable in a wide range of pHs and temperatures, and produce very intense light emissions that are immensely brighter than conventional technologies. CPNs™ do not photo-bleach, (with over 12 months of stability), allowing significantly more sensitive detection of analytes than other fluorophores in techniques such as immunocytochemistry, ELISA and flow cytometry. The greater stability also makes the results highly reproducible. CPNs™ can be taken up by cells through endocytosis or targeted to specific regions by linking them to antibodies, proteins, nucleotides or other targeting moieties. CPNs™ match standard fluorescent filters and laser lines, and are available in a range of wavelengths covering the visible spectrum – including near-IR.

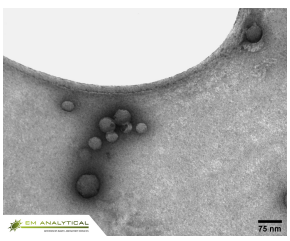


Why are they so much Brighter, Sensitive and Stable?
Derived from TV display OLED technology, the polymer core was originally designed to cope with years of electrical excitation, making it extremely stable. Quantum Yield (QY) is frequently and mistakenly assumed as a measure for brightness while the Extinction Coefficient's (ExCoeff) are often ignored. However, it is the extremely large ExCoeffs that give CPNs™ their incredible brightness and sensitivity.

Brightness = QY x ExCoeff

Structural Properties:
CPNs™ are water-soluble micelles with a light-emitting polymer core and are manufactured at an approximate size of 70-80 nm, although particles can be as small as 15-50 nm. Encapsulation of the core within a biocompatible surfactant also increases its hydrophilicity and this 'core-shell' structure provides a ready base for the covalent bonding of functionalising molecules for targeting proteins or nucleic acids. CPNs™ also incorporate iron oxide, allowing CPNs™ and the molecules or cells to which they are attached to be manipulated using magnets to direct movement and facilitate purification. The iron oxide can also be visualised by utilising Magnetic Resonance Imaging (MRI) as a contrast reagent.

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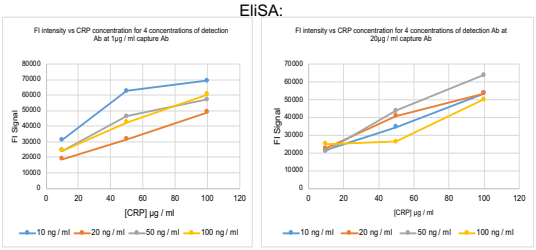
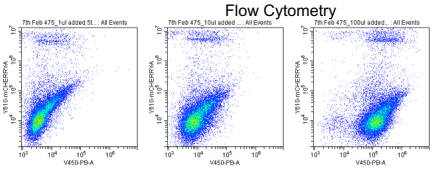
Biological Properties:

The intense brightness of CPNs™ dramatically increases the sensitivity of applications such as flow cytometry, ELISA, IHC and microscopy, with single nanoparticles being detectable in flow cytometry and immunocytochemistry. This enables 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 combination is thermally stable, requiring no cold storage. Other surface chemistries are also available, such as Thiol and Azide for click chemistry.

ElISA:

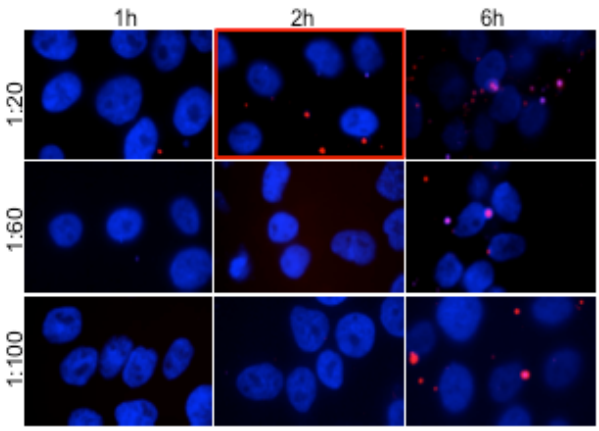


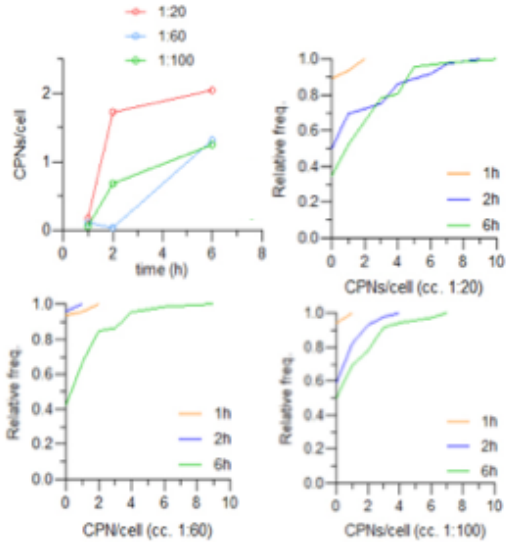
Flow Cytometry



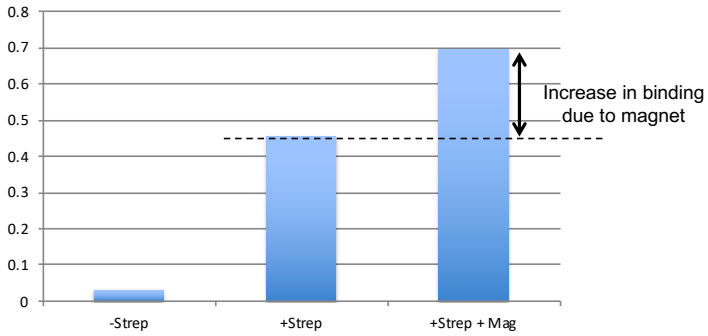
Cells 293T adherent: 24 well plate – 1ml volume with added 1µl 10µl or 100µl 475 CPN. Culture time 48 hr. Analysis by flow cytometry & confocal microscopy.

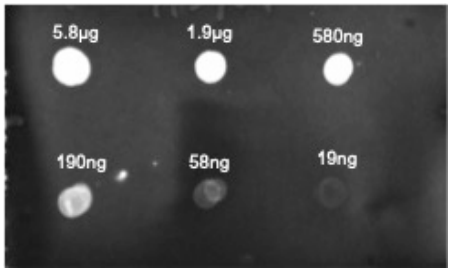
CPN concentration and incubation time for live uptake experiments





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™ during conjugation, by simple, careful placing of a magnetic, significantly increases binding. When applied to a range of applications, such as ELISA, this can further increase sensitivity.





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

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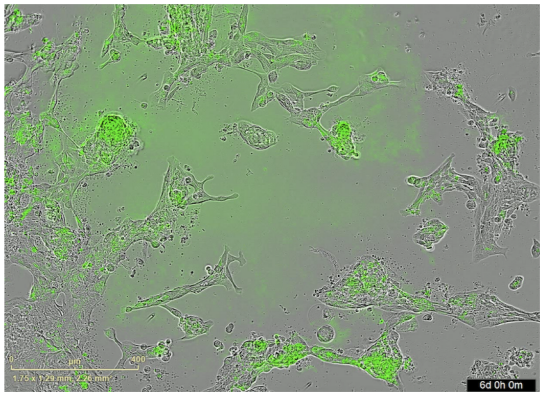
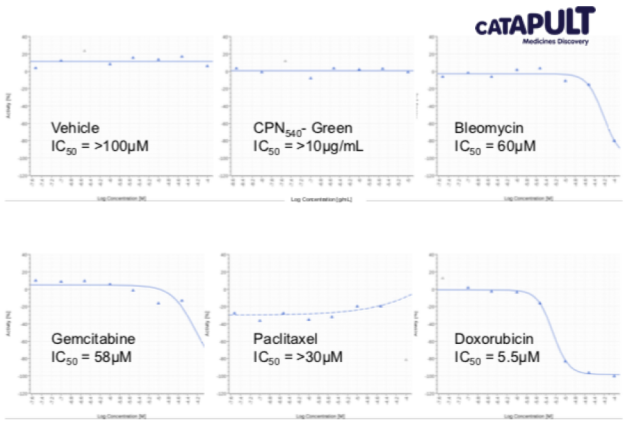
CPN 510 taken up by HCC70 over a 6 day period within an IncuCyte®, with no loss of fluorescence. Using the HCC70 cell line plated in 96 well ultra low adhesion plates, and incubated for ~24 hours allowing for spheroids to form. Spheroids were then treated with compound/ drug/ CPN over 3 days. Cell viability was correlated by ATP determination using Cell Titer Glo. Positive controls: Cytotoxic drugs paclitaxel; doxorubicin; bleomycin and gemcitabine. CPN540 (Green) does not appear to be cytotoxic to HCC70 cells at concentrations up to 10µg/mL.

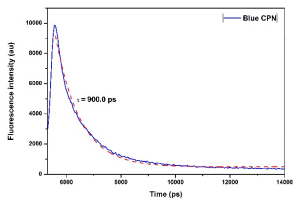




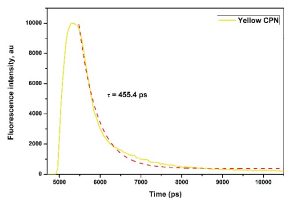
Excited and Fluorescence Lifetime:

The excited lifetime of CPNs™ is measured in nanoseconds, potentially allowing the use of a high repetition rate laser for time resolved measurements. Fluorophores with a shorter lifetime have generally been taken to mean a low quantum yield and therefore a weaker, dimer signal – this is not the case for CPNs™.

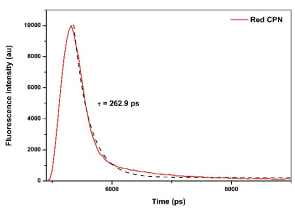
CPN 475



CPN 550



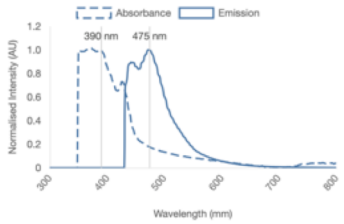
CPN 680



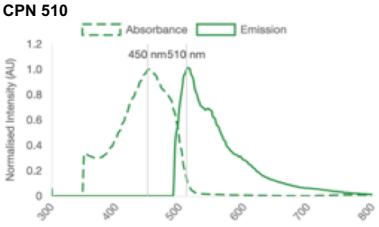
Wavelengths:

CPN™ wavelengths span the visible spectrum and extend into the near infrared. An additional 8 wavelengths are under development and will be brought to market by mid 2020. Near-IR imaging in clinical settings has been limited to date, and CPNs™, such as CPN1150, (ab 750nm / em 1150nm) promise higher contrast, sensitivity, and tissue penetration depths.

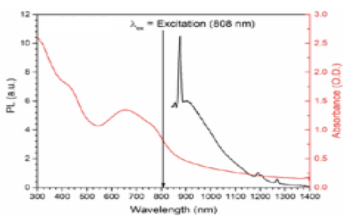
CPN 475



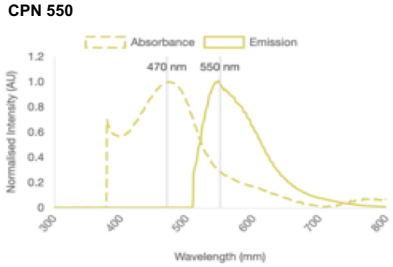
CPN 510



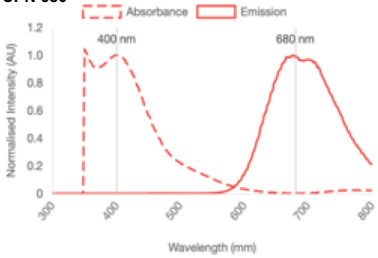
CPN 900



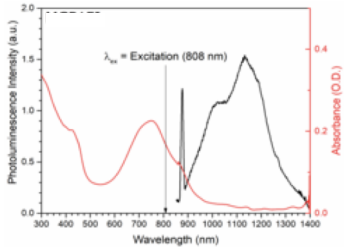
CPN 550



CPN 680



CPN 1150



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Protocol for Conjugation of CPN™ to Targeting Molecules, e.g. Streptavidin

CPNs™ are readily conjugated to proteins using EDC (N-ethyl-N'-dimethylaminopropyl-carbodiimide) to link amine groups (-NH2) on the protein to the carboxyl groups on the surface of the CPN™ (-COOH). Attachment of proteins such as antibodies or streptavidin will generate highly selective CPNs™ for the detection of target molecules. The brightness of the CPNs™ ensures the detection is highly sensitive, with single molecules being detected in flow cytometry and immunocyto / histochemistry. The affinity of targeting molecules varies greatly and an initial titration of the CPN™: targeting molecule ratio will need to be undertaken. Similarly, final usage dilution of the CPN™ - targeting molecule conjugate will need to be determined empirically.

Protocol

1. Add 50µl CPN™ (0.1mg/ml) to 1.5ml tube
2. Add 1µl of HEPES 1M
3. Add 1µl PEG 8000 5% w/v
4. Add 3µl streptavidin (1mg/ml) and vortex (several ratios of CPN™: targeting molecule should be tested to identify the optimum conditions, e.g. 10-100µl targeting molecule (1mg/ml))
5. Add 1µl freshly prepared EDC solution 5mg/ml
6. Shake mixture for 4 hours at room temperature
7. The CPN™:Streptavidin conjugates can then be purified from the reaction components by precipitating them using a magnet.
8. Re-suspend in 20µl sterile Resuspension Buffer (HEPES (20mM), BSA 0.1%, Tween-20 0.02%)

Reagents

- HEPES (1M), pH 7.4
- PEG 8000 (5% w/v)
- Streptavidin (1mg/ml)
- EDC [N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride] 5mg/ml
  (Freshly made and used immediately. Discard any unused)
- Bovine Serum Albumin (10% w/v)
- Tween-20
- Neodymium magnet