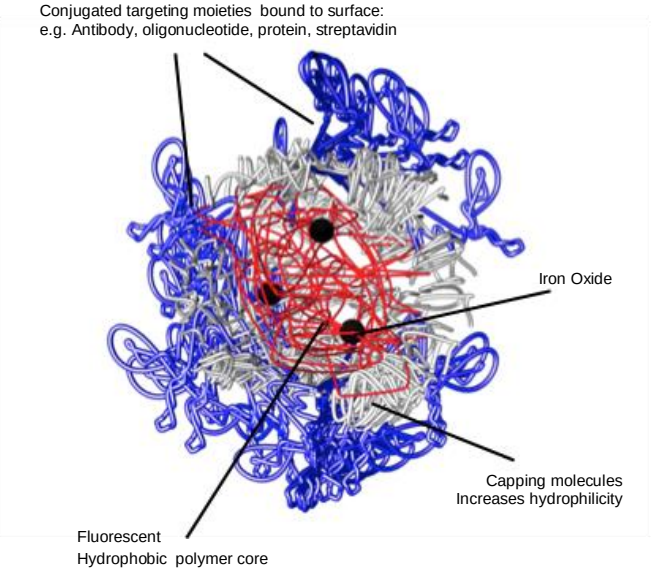
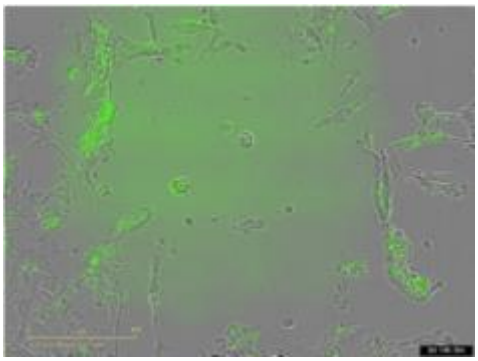
Conjugated Polymer Nanoparticles (CPNs™) CPN™ 510

CPNs™ are highly fluorescent nanoparticles containing semiconductor light emitting polymer cores encapsulated within a surfactant. CPNs™ have fluorescent properties significantly exceeding those of other in vitro labelling agents, intense brightness due to exceptional extinction coefficients and outstanding photo-, thermo- and chemical stability. CPNs™ have shown no toxicity and are ideal for use with live cell systems. CPNs™ are enabled with surface accessible carboxylic groups for the conjugation of a wide range of molecules including antibodies, streptavidin and nucleic acids. This allows CPNs™ to be used in a diverse array of binding and targeting applications, such as flow cytometry, immunohistochemistry, immunocytochemistry, and high content screening. CPNs™ also incorporate iron oxide, allowing their magnetic manipulation and the purification and quantification of bound molecules or cells. Further, the iron oxide allows CPNs™ to be used as contrast agents in Magnetic Resonance Imaging (MRI). CPNs™ range in fluorescence emission wavelengths from 450 nm to 680 nm.



Applications

- Flow cytometry - Cell surface, low abundance targets
- Cell imaging/tracking – CPNs loaded in to by endocytosis
- Immunohistochemistry – Readily linked to secondary Ab
- Fluorescent ELISA – Signal is time independent
- Fluorescent In Situ Hybridisation – Sensitive and stable signal
- Western blotting - Allows linear quantification of signal



HCC70 cells passively loaded with CPN510 over 3 days in culture

Structural Properties:

Conjugated Polymer Nanoparticles are water-soluble micelles compromising of a Light Emitting Polymer and are around 100 nm in size. CPNs™ exhibit luminescence or fluorescence with light emission wavelengths from 475 nm to 680 nm. They are encapsulated within a biocompatible surfactant, increasing the hydrophilicity and allowing them to form micelles. This ‘core-shell’ structure, consisting of the polymer forming the core and the surfactant the surrounding shell, provides a ready base on which to covalently bond functionalising molecules, such streptavidin, antibodies, targeting proteins or nucleic acids. CPNs™ also incorporate iron oxide into their core. This allows 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 be also be visualised using Magnetic Resonance Imaging (MRI), acting as a contrast reagent.

Biological Properties:

CPNs™ are readily conjugated to biomolecules such as antibodies or streptavidin allowing their use in a wide range of molecular biological applications. The intense brightness of the CPNs™ dramatically increases the sensitivity of these applications, with single nanoparticles being detectable in flow cytometry and immunocytochemistry, allowing the study of individual proteins in samples and cells. The streptavidin and antibodies are covalently conjugated to the CPNs™ via the surfactant's carboxylic acid groups using N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC) chemistry. The CPNs™ conjugates can be used in 'end user' assays at concentrations matching those of other conjugated fluorophores. Due to differences in assay systems working dilutions should be determined by titration assay. The CPNs™ are both thermal and photostable, however once conjugated to biological materials, they should be stored at 2-6°C.

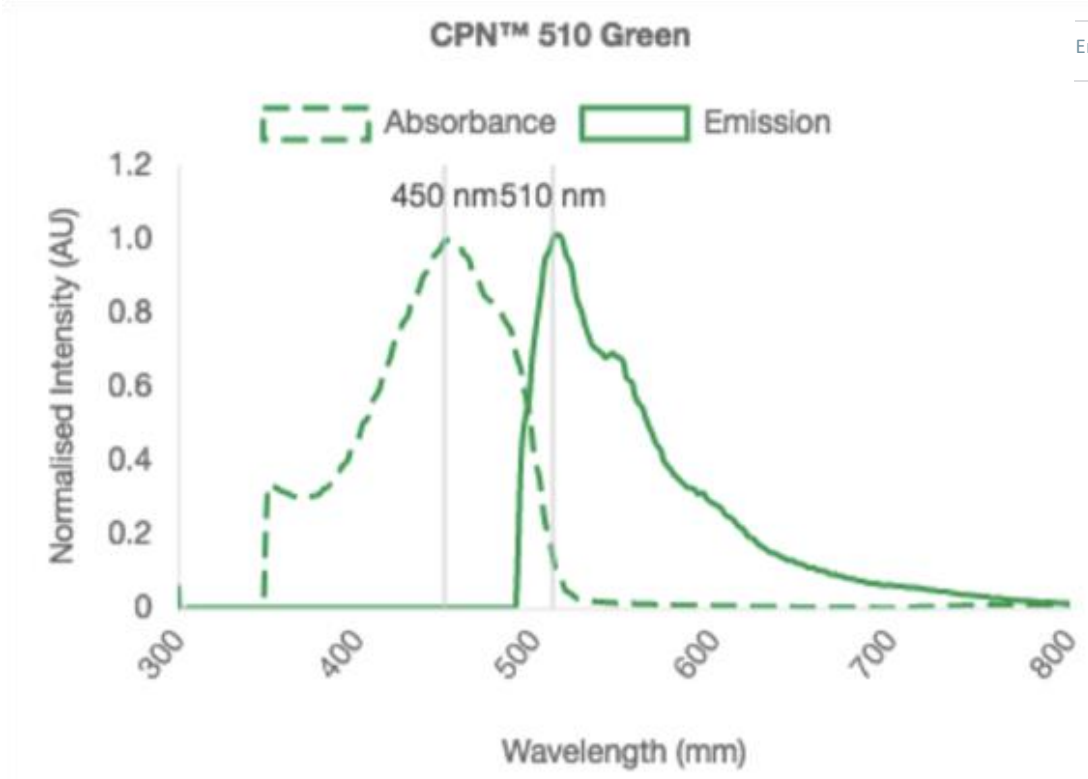
Optical Properties:

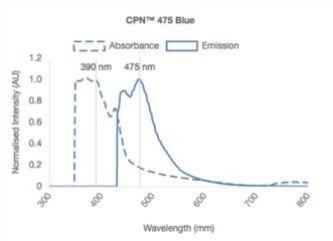
The color of light emitted by CPNs™ is dependent on the specific polymer core creating a common platform of labels across the visible spectrum. CPNs™ are extremely bright and photostable, with samples stored under ambient temperature and lighting conditions retaining their fluorescence for over two years.

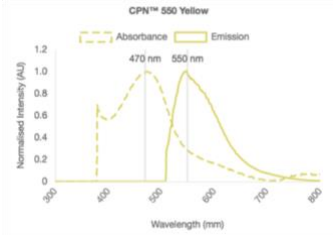
Material	Amount	Concentration	Storage	Stability
CPN™ 475	250 µl	0.1 mg/ml	Ambient	When stored as directed, product is stable for 12 months

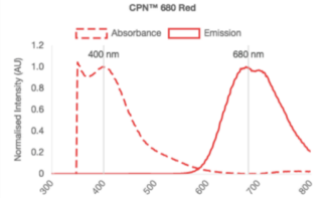
Emission Spectra:

Hydrodynamic diameter	80 nm
Excitation maximum	450 nm
Emission maximum	510 nm









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 CPN™ 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 870µl of water.
2. Add 20µl of HEPES 1M
3. Add 20µl PEG 8000 5% w/v
4. Add 40µl 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 20µl freshly prepared EDC solution 5mg/ml
6. Shake mixture for 4 hours at room temperature
7. Add 20µl BSA (10% w/v) and leave to shake for a further half an hour.
8. The CPN™: streptavidin conjugates can then be purified from the reaction components by precipitating them using a magnet.
9. Re-suspend in 40µl PBS: The linked protein may make the suspension susceptible to microbial contamination. It is recommended that 3.2mM (0.02%) sodium azide (or alternative antimicrobial) is added and stored at 4^{O}C.

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)

Phosphate buffered saline, pH 7.4

Neodymium magnet

Sodium azide, 0.1M

Samples of magnetic CPNs excited with 365 nm light