Conjugated Polymer Nanoparticles as Efficient Fluorescence Bioimaging Nanoplatforms by Means of Fluorination Substitution-Induced Polymer Chains Planarity Reduction

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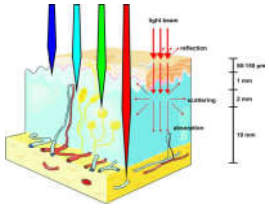
Introduction



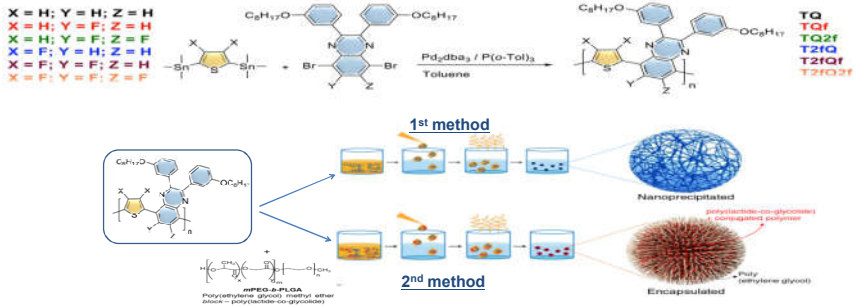
Light propagation through the tissues [6]

Fluorescence imaging techniques with high sensitivity, low cost and temporal resolution have become indispensable for biomedical imaging applications.[1] Fluorescent probes emitting in the far-red and near-infrared (NIR) spectral regions (650 - 1000 nm) is currently one of the main focuses for successful fluorescence-based bioimaging applications. the development of well-designed NIR fluorophores with highbrightness, good photostability, and large Stokes shifts is a profound need in modern bioimaging and clinical diagnosis.

Conjugated polymer nanoparticles (CPNs) have recently emerged as a new class of fluorescent nanoparticles because their fluorescent properties can be fine-tuned by an appropriate molecular design of conjugated polymers (CPs).[2] Given their superior fluorescent brightness and photostability as well as low cytotoxicity, the applicability of CPNs as fluorescent tags and sensors has been expanding significantly during the last few years.[3] Moreover, the absorbance in the UV-Vis and the NIR region, which is called the ***biological window***, allows deep tissue penetration, critical for medical applications such as imaging.

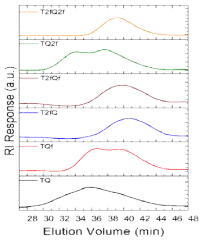
In this study, we developed a new family of aqueous CPNs as potential far-red and NIR fluorescence polymer probes with high photoluminescence quantum yields by utilizing simultaneously unfused, less rigid - polycyclic monomer building blocks and reducing the planarity of the polymer chains by anchoring fluorine atom(s) as substituents. Cellular toxicity and the ability of the obtained probes to be used as bioimaging agents and/or as potential cancer theranostics were investigated on breast cancer cell lines.

Synthesis and Preparation Method of Nanoparticles



Results

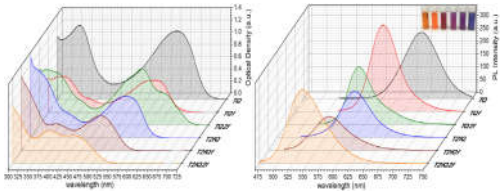
A series of donor-acceptor (D-A) conjugated polymers based on the thiophene as the electron donating and the quinoxaline as the electron deficient were synthesized based on the Stille cross-coupling polymerization procedure. The D-A polymers (TQ, TQf, TQ2f, T2fQ, T2fQ, T2fQ2f) were varying as regards the number of the fluoro atoms anchored onto the polymer backbone



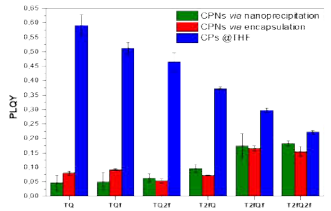
GPC chromatographs

Molecular weight characteristics and optical properties of the TQ based

Sample	M_n (g/mol)	M_w (g/mol)	PDI	λ_{max}^{sol} (nm)	PL_{max}^{sol} (nm)	PLQY
TQ	19900	64200	3.2	354, 600	668	0.59±0.04
TQf	11400	25800	2.3	338, 574	618	0.51±0.02
TQ2f	22600	52500	2.3	319, 557	592	0.47±0.03
T2fQ	6000	11300	1.9	538	600	0.37±0.01
T2fQf	8200	15600	1.9	387, 498	571	0.30±0.01
T2fQ2f	11200	16200	1.5	392, 456	544	0.22±0.01

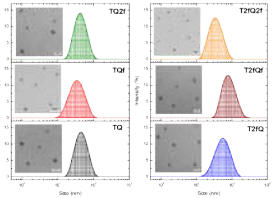


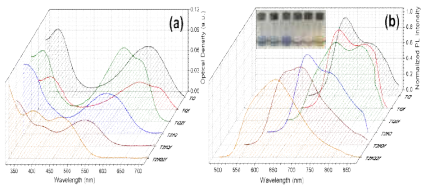
Absorption and photoluminescence spectra of TQs in THF solutions

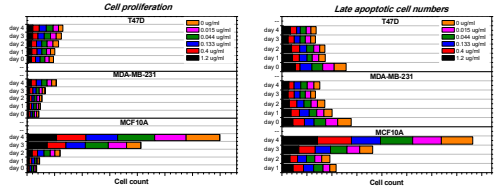


Comparison of the photoluminescence quantum yields for the TQ-based polymers in THF and their corresponding aqueous CPNs

CPNs via Nanoprecipitation	Z-Ave (nm)	PDI	PK1 mean (nm)	Z potential (mV)	TEM
TQ	40.98±0.12	0.13±0.01	47.26±0.56	-20.3	30.85±2.12
TQf	31.67±0.09	0.21±0.01	37.80±1.07	-19.6	24.04±1.01
TQ2f	40.55±0.12	0.18±0.01	46.51±0.48	-17.7	33.74±2.61
T2fQ	48.13±0.31	0.17±0.02	53.87±3.58	-21.0	33.50±1.45
T2fQf	75.03±1.22	0.16±0.02	84.65±4.94	-18.2	39.77±3.16
T2fQ2f	33.34±0.15	0.21±0.01	37.97±0.60	-15.5	27.45±3.11

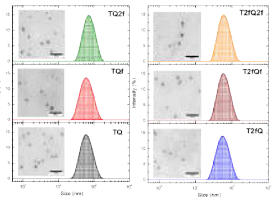


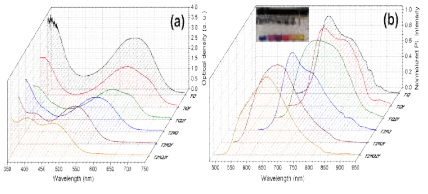


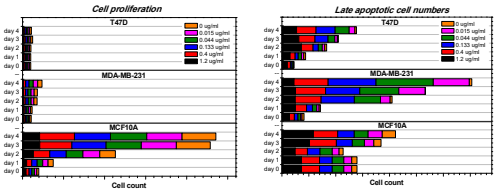


Cell proliferation and late apoptotic cell numbers of breast cancer cell lines treated with ***T2fQ2f encapsulated nanoparticles***

CPNs via Encapsulation	Z-Ave (nm)	PDI	PK1 mean (nm)	Z potential (mV)	TEM
TQ	57.71±0.20	0.11±0.01	65.64±0.46	-10.5	17.41±1.82
TQf	57.86±0.20	0.12±0.01	66.78±0.40	-11.0	19.01±2.06
TQ2f	69.40±0.42	0.11±0.01	75.54±9.46	-11.2	19.28±1.06
T2fQ	52.46±0.15	0.12±0.01	60.13± 0.41	-10.7	17.40±0.74
T2fQf	52.26±0.16	0.11±0.01	63.93±0.96	-10.7	19.55±0.91
T2fQ2f	58.32±0.13	0.13±0.01	69.13±7.22	-10.3	18.65±1.90







Size distribution of TQs nanoparticles in aqueous suspension determined by Dynamic Light Scattering measurements and their morphology by Transmission Electron Microscopy

UV-Vis absorption spectra and normalized emission spectra of TQ-based CPNs
In the inset of figures the CPNs in water are shown (from left to right; TQ, TQf, TQ2f, T2fQ, T2fQf, T2fQ2f).

Cell proliferation and late apoptotic cell numbers of breast cancer cell lines treated with ***T2fQf nanoprecipitated nanoparticles***

Conclusions

- Water-soluble nanoparticles comprising of the synthesized TQ derivatives were prepared by two different methods, enabling their potential use as low-bandgap fluorescent probes for bioimaging.
- All prepared CPNs indicate unimodal size distribution in aqueous suspension and the average particle size was determined less than 80nm, which is a compatible size for biological applications.
- The optical properties of the CPNs show light absorption in the UV-Vis region, while they demonstrated fluorescence emission in the far-red/NIR region.
- The far-red emitting TQ-based polymer nanoparticles with the three and four fluorine atoms on the repeat unit of the polymers demonstrate higher quantum yield values regardless of the preparation method.
- Generally, the CPNs produced through encapsulation were found to be less cytotoxic than the CPNs produced through nanoprecipitation.
- Encapsulated T2fQ2f CPNs seemed to be the least toxic to either the tumour cell lines T47D & MDA-MB-231 or the immortalized epithelial cell line MCF10A, not affecting cell proliferation and late apoptotic cell numbers in any tested concentration, making them suitable for bioimaging.
- Nanoprecipitated T2fQf CPNs seemed to have toxic effects possibly in a selective manner to the tumour cell lines, while the MCF10A cell proliferation rates and apoptotic cell numbers were not affected in any concentration, indicating their potential use as cancer theranostics.

References

1. R. Weissleder and M. J.Pittet, Imaging in the Era of Molecular Oncology, Nature 2008,425, 580
2. K. Li and B. Liu, Polymer Encapsulated Conjugated Polymer Nanoparticles for Fluorescence Bioimaging, J. Mater. Chem., 2012, 22, 1257.
3. Q. Zhao, X. Zhou, T. Cao, K. Y. Zhang, L. Yang, S. Liu, H. Liang, H. Yang, F. Li and W. Huang, Fluorescent/phosphorescent dual-emissive conjugated polymer dots for hypoxia bioimaging, Chem. Sci., 2015,6, 1825.
4. A. Patrizia, B. Kristian, C. K. A., F. T. H., G. A. W., G. S. O., H. S. M., H. M. R., J. Asta, K. David, K. Mladen, M. Johan, M. Pawel, N. Dominika, P. Jacques, W. B. C., G. Jakub, CA-Cancer. J. Clin. 2011, 61, 250-281.000

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