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Results in Chemistry 3 (2021) 100127

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.elsevier.com/locate/rechem

Fluorogenic probes for thioredoxin reductase activity

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ARTICLE INFO

Keywords:
Thioredoxin reductase
Thioredoxin
Selenocysteine
Fluorescent probes
Biosensors
Enzyme activity
Cellular redox status
Cancer detection

ABSTRACT

The thioredoxin system is vital in maintaining the cellular redox state and is implicated in various cancers and other diseases. It comprises of thioredoxin reductase, thioredoxin and NADPH. Various methods have been developed for the detection and quantification of the thioredoxin reductase enzyme. Most of these methods suffer the shortfall of poor specificity, hence there is need to develop more robust and specific techniques. Recently, there has been an increased interest in fluorescent probes that can target thioredoxin reductase. This review highlights the progress in the synthesis and application of the different fluorescent probes that have been employed for this purpose.

1. Introduction

Thioredoxin reductase enzymes (EC 1.8.1.9) in mammals are a group of selenoproteins that belong to a family of homodimeric pyridine-nucleotide oxidoreductases. They control the cell redox state by functioning as reducing equivalents for many proteins and cellular processes. These enzymes exist in three main isoforms; in the cytosol as TrxR1, in mitochondria as TrxR2 and as a third form, thioredoxin-glutathione reductase (TGR) which is mainly expressed in male germ cells [1]. TrxR2 differs from TrxR1 in possessing an *N*-terminal mitochondrial import sequence [2].

Each monomer of TrxR (Fig. 1) has a molecular weight of 55–60 kDa depending on its cellular location, and includes an FAD prosthetic group and an NADPH binding site, both of which are required for the enzyme's catalytic process [3,4]. TrxR has 64% sequence homology to glutathione reductase, which includes a -Cys-Val-Asn-Val-Gly-Cys- redox-active site [5]. However, TrxR also has a second redox active site on the C-terminal that incorporates the unique selenocysteine (Sec) residue (Fig. 1), affording this enzyme significantly greater catalytic efficiency and broader substrate specificity compared to other oxidoreductase enzymes that utilize cysteine (Cys) [5–8]. Engineered TrxR, with Cys replacing the Sec residue, displays reduced catalytic efficiency of ~ 2 orders of magnitude. Sec has a pK_a ~ 5.2, whereas Cys residues typically have a pK_a closer to ~ 8.5. Therefore, the selenol residue in mammalian TrxR is efficiently deprotonated at physiological pH, enhancing its nucleophilicity in comparison to Cys. The insertion of Sec into the TrxR sequence occurs through the UGA codon, which is normally a stop codon in protein translation [9].

The thioredoxin reductase system is comprised of TrxR, NADPH and thioredoxin (Trx). NADPH transfers electrons to the redox active site of TrxR via an NADPH-FAD charge transfer complex which reduces -Cys-Sec- on the C-terminal active site (Fig. 2). Sec becomes exposed on the surface of the enzyme to reduce the disulfide (-Cys-Cys-) of Trx [1,10].

Trx, the major substrate for TrxR, is a protein disulfide oxidoreductase which can reduce disulfides at a rate five orders of magnitude faster than dithiothreitol (DTT) [11]. In its reduced form, Trx controls cellular redox potential and plays a protective role by scavenging ROS-derived radicals [6,12]. As a reducing equivalent for other proteins, it protects cells from tumor necrosis factor, anti-Fas antibody and ischemic reperfusion injury [13,14]. Other known functions of Trx include inhibiting the pro-apoptotic protein, ASK-1 and PTEN, a tumor suppressor protein [15,16]. Besides its cytoprotective properties, Trx also activates redox factor-1 (Ref-1) and promotes DNA repair through the reduction of ribonucleotide reductase, amongst other proteins responsible for cell growth and survival.

Overall, the thioredoxin system can promote growth and prevent apoptosis of cancer cells [19]. High levels of TrxR have been found in colorectal carcinomas, breast cancer, prostate cancer and human lung carcinoma, at levels that are ~ ten-fold greater than in normal tissues [20–23]. In melanoma cells TrxR levels can correlate with metastases where it aids growth and promotes DNA synthesis while inhibiting apoptosis-inducing proteins and tumor suppressing proteins

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https://doi.org/10.1016/j.rechem.2021.100127
Received 22 November 2020; Accepted 12 March 2021

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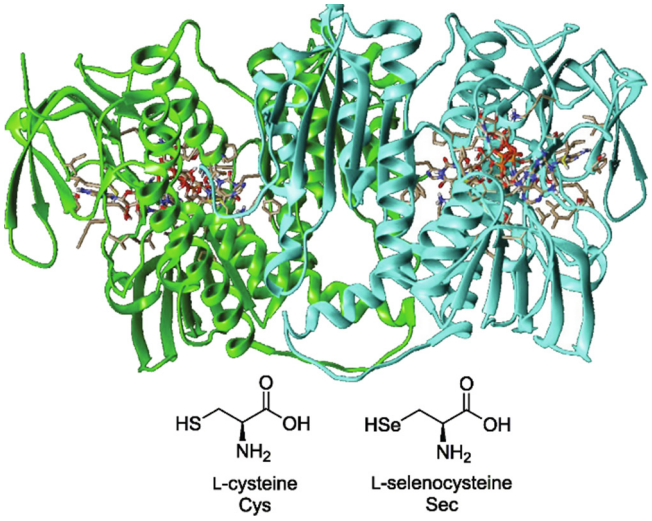

Fig. 1. (top) A subunit of TrxR showing the A chain (cyan) and the B chain (green). Structures were obtained from the protein data bank and visualized using UCSF Chimera [17,18] **(bottom)** Sec, the selenium analog of Cys, is a unique residue found on the TrxR active site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

such as p53 [20,21,24–29]. A good example of the requirement of this enzyme in cancerous cells is the reverting of their morphology to normal cells when TrxR is knocked down via siRNA. This finding points to the role of TrxR in cancer progression [29]. Additional investigations have also shown inhibited TrxR expression in Parkinson's disease and stroke [30,31].

The importance of TrxR in disease necessitates useful and practical methods for monitoring this enzyme. A common commercial method is the DTNB assay which employs Ellman's reagent in the colorimetric quantification of TrxR in the presence of NADPH. DTNB is reduced to yellow colored TNB^{2-}, and the absorption shifts from 305 nm to 412 nm. A modified version of this assay is the insulin end-point assay in which TrxR reduces Trx which goes on to reduce the insulin disulfide bonds. The free insulin thiols will then reduce DTNB to TNB^{2-}. This is called an end-point or discontinuous assay since it can be quenched by a chaotrope such as guanidium hydrochloride before quantifying the free thiols. DTNB is however a substrate for other biological reducing species, and in cell extracts, background activity can range from 15 to 40 %. In addition, the assay can only be used in cells after they have been lysed [32].

The SC-TR assay is a method involving selenocystine (SC) reduction by TrxR that is readily monitored via the consumption of NADPH (λ_{abs} 340 nm). This method not only allows quantification of TrxR but gives the relative abundance of TrxR to Trx. It was developed for use with spectrophotometry and is conducive for high throughput screening in multi-well plates. The SC-TR assay is highly specific since selenocystine cannot be reduced by biothiols including glutathione reductase. A limitation is the use of UV–Vis absorption which is subject to interference from other biomolecules absorbing in the same region as NADPH [32].

Immunological methods have been used to monitor TrxR activity not only in melanoma cells but also in blood plasma. However, these techniques are generally regarded to be not cost effective and prone to error and false negatives [25,26,33].

Fluorescent probes can have enhanced utility in the detection of TrxR due to their sensitivity and capability to give high temporal and spatial resolution. In comparison to the methods described to date, fluorescent probes are also a potential non-invasive way to detect biological analytes [34,35]. Ideal fluorescent probes should be highly sensitive such that they can be used in low concentrations, down to the nanomolar range. Fluorophores with a high signal to background ratio are therefore of interest, with near-infrared fluorophores being desirable [36].

In this focused review, we highlight fluorescent probes that have been synthesized and used for the detection of thioredoxin reductase activity. Additional related probes have also been used to target selenols and thioredoxin, but these will not be covered [37–39].

2. Probes for monitoring TrxR activity

The Fang group designed, synthesized, and evaluated the first off–on fluorescent probe selective for TrxR, TRFS-green (Fig. 3), which provides a convenient and direct method to detect and assay the TrxR enzyme [40]. It consists of a five-membered dithiolane ring conjugated to a fluorescent naphthalimide via a carbamate linkage.

TRFS-green has a λ_{em} = 480 nm at λ_{exc} = 373 nm which shifts to λ_{em} = 538 nm at λ_{exc} = 438 nm when the probe is reduced by TrxR in the presence of NADPH under physiological conditions. A 10 μM solution of TRFS-green in the presence of TrxR (75 nM) and NADPH (200 μM) leads to a gradual increase in fluorescence over 180 min. The proposed turn-on mechanism for TRFS-green was validated via HPLC monitoring.

When the disulfide bond is reduced, a nucleophilic thiolate is formed which promotes intramolecular cyclization to give a five membered cyclic oxathiolanone and the fluorescent naphthalimide. TRFS-green is selective against small reducing thiols and structurally-related glutathione reductase and lipoamide dehydrogenase. Hep G2

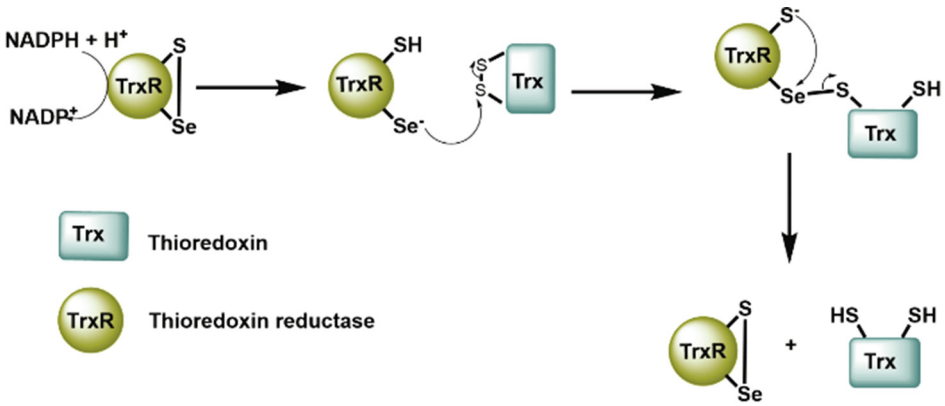

Fig. 2. The thioredoxin reductase system catalytic mechanism.

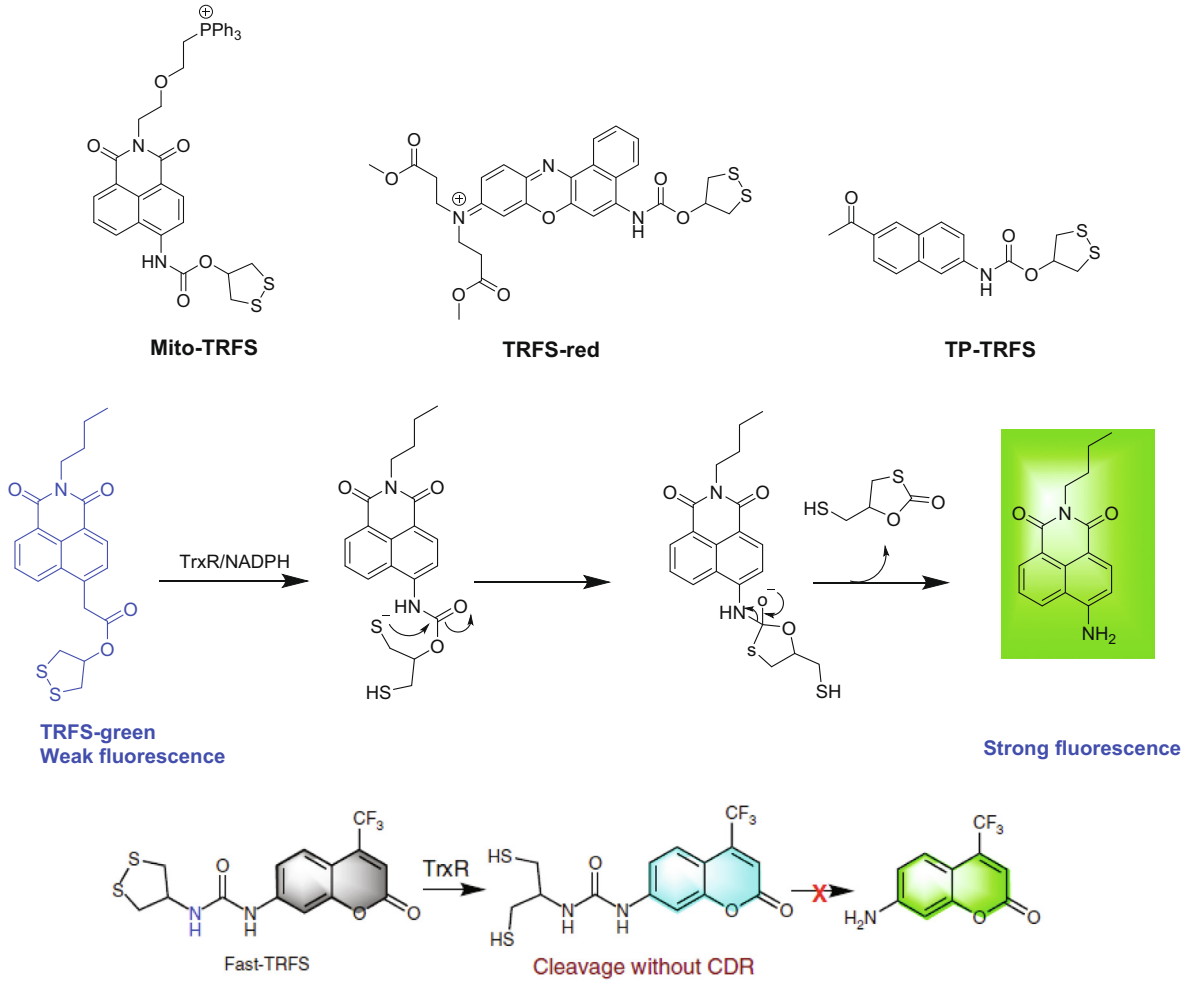

Fig. 3. The TRFS series of probes synthesized by Fang and co-workers. Turn-on mechanisms for TRFS-green and Fast-TRFS are also shown (adapted with permission from [40,41], Copyright 2013, 2019, American Chemical Society). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cells and cell lysates containing TrxR also show reduction of TRFS-green, but not in the presence of 2,4-dinitrochlorobenzene (DNCB), a known inhibitor of TrxR. Despite the selectivity of this probe, it still has relatively low binding efficiency with a K_m of 189 μM, and the fluorescence increase does not plateau after 3 h [40]. The lengthy time for the signal to plateau is due to the slow cyclization-driven step to uncage the fluorophore [41]. TRFS-green has also been used to image the TrxR activity in HeLa cells in different studies [42,43].

A red-shifted probe, TRFS-red (Fig. 3), was synthesized by conjugating a Nile blue fluorophore with the dithiolane quencher. TRFS-red has $\lambda_{max,abs}$ at 530 nm that is shifted to 615 nm in the presence of TrxR and NADPH. [44] When excited at 615 nm, the reduced probe fluoresces at 661 nm and the increase in fluorescence plateaus at 120 min. TRFS-red also has an improved 90-fold fluorescence increase compared to TRFS-green's ~ 35-fold increase. This is a result of the positively charged iminium cation on TRFS-red that enhances affinity between the probe and the negatively charged C-terminal of TrxR. TRFS-red is also selectively reduced by isolated TrxR and with HeLa cells. The turn-on response of 10 μM TRFS-red in cells is faster (1 h) in comparison to TRFS-green (4 h). This probe predominantly localizes in the cytosol, showing higher selectivity for TrxR1 [44].

A third probe in the TRFS series, Mito-TRFS, was synthesized to selectively target mitochondrial TrxR, TrxR2 [31]. Mitochondria are known to produce ROS which can cause oxidative stress in disease. Since TrxR is involved in controlling ROS levels in cells, its activity can be used to monitor disease states.

In a manner similar to TRFS-green, Mito-TRFS has a naphthalimide fluorophore conjugated to the 1,2-dithiolan-4-ol moiety, as the TrxR recognizing motif. A triphenylphosphonium group is present in Mito-TRFS for mitochondrial targeting. This probe has $\lambda_{max,abs}$ = 375 nm and emits at 480 nm. In the presence of TrxR, the disulfide bonds are reduced, uncaging the fluorophore and the probe emits at 540 nm at λ_{exc} = 438 nm. The turn-on response of Mito-TRFS is relatively rapid, with a 30-fold increase that plateaus in 1 h. As in the case of TRFS-red, the improved kinetics is attributed to the positive charge on Mito-TRFS that facilitates binding to the negatively charged enzyme active site while enhancing the hydrophilic nature of the probe. Fluorescence colocalization experiments confirm Mito-TRFS distribution in mitochondria. This is the first probe to directly detect TrxR2 activity in living cells. The study also showed a decline in TrxR2 activity in Parkinson's disease [31]. Mito-TRFS was additionally employed in a separate study to image TrxR2 in HeLa cells [45].

In a related study, the Fang group altered the linker units and TrxR recognition moieties to evaluate properties enabling faster reaction times and heightened selectivity [41]. Findings include the fact that 6-membered ring dithianes are not responsive to the enzyme, in contrast to 5-membered ring dithiolanes. This results from heightened ring strain and reactivity associated with smaller rings. Cyclic diselenides, in contrast, are more inclined towards reduction by GSH.

Among all the synthesized probes, Fast-TRFS (Fig. 3) stands out due to its quick turn-on response. HPLC-MS data confirms the turn-on mechanism of Fast-TRFS being due to cleavage of the disulfide bond with no cyclization-driven release (CDR) of the fluorophore. However, with prolonged incubation (240 min), there is a decrease in the fluorescence due to re-oxidation of the thiols to the disulfide.

Fast-TRFS shows an 80-fold increase in fluorescence that peaks at ~ 5 min (λ_{exc} = 345 nm, λ_{em} = 460 nm). Other potential reducing/interfering agents such as Trx, glutathione reductase, diaphorase, amidehydrolase, bovine serum albumin, GSH, NADPH, Cys and Sec have minimal interference to TrxR. This probe displays a higher selectivity for TrxR over GSH (50 fold) compared to TRFS-red (12.8) and TRFS-green (15.2).Treatment of HeLa cell lysates with Fast-TRFS also gives a more rapid and intense (70-fold) fluorescence increase in comparison to previously reported probes, enabling dose-dependent visualization of TrxR. The researchers have used Fast-TRFS in a potential TrxR assay technique and also in the identification of TrxR inhibitors which may be useful for cancer treatment [41].

The first two-photon fluorescent probe for TrxR, TP-TRFS (Fig. 3), was recently synthesized through the conjugation of 2-acetyl-6-aminonaphthalene to a 1,2-dithiolane quenching moiety [30]. In two-photon fluorescence, a fluorophore is excited by the absorption of two photons which have half the energy and double the wavelength of a single photon required to achieve that excitation. It is advantageous in allowing non-damaging and deeper imaging or tissue penetration, making the probe ideal for use in imaging living organisms [46,47].

A 10 μM solution of TP-TRFS shows an ~ 15-fold fluorescence increase at 490 nm in the presence of 50 nM TrxR within 3 h. The probe also responds in Hep G2 cell lysates and shows diminished signaling in the presence of auranofin or DNCB, the TrxR inhibitors. The fluorescence also increases in HeLa cells but is weaker when TrxR is knocked out. TP-TRFS has a turn-on response in zebrafish, and is thus the first probe to be used in detecting TrxR in living organisms. Two-photon fluorescence imaging with TP-TRFS is also used to show declined TrxR in the brain of mice with cerebral ischemic reperfusion injury. The data supports previously reported studies on declining TrxR mRNA in the ischemic cerebral cortex, and may help in understanding and in developing therapy for stroke [48].

Bu and co-workers synthesized the probe TR-green (Fig. 4) based on the Michael addition of TrxR to the probe's α,β-unsaturated ketone [49]. This strong covalent binding allows localized imaging of the enzyme and detection at nanomolar levels. The mechanism of covalent binding via conjugate addition is the same as that of the known TrxR inhibitor curcumin whereby the Sec498 and Cys497 residues are alkylated [50]. TR-green is synthesized by conjugating a coumarin fluorophore to a substituted furan ring through an α,β-unsaturated ketone linker. This quenches the fluorescence through intramolecular charge transfer (ICT) from the electron-rich fluorophore to the electron poor aromatic ring through the extended conjugation. Conjugate addition to the Sec residue of TrxR thus results in a fluorescence signal at 500 nm when TR-green is excited at 440 nm. From a series of related probes synthesized in this study, TR-green is the most selective and inhibitory for TrxR due to its 5-methylfuran moiety. Other probes can be attacked by thiols through a similar Michael addition reaction.

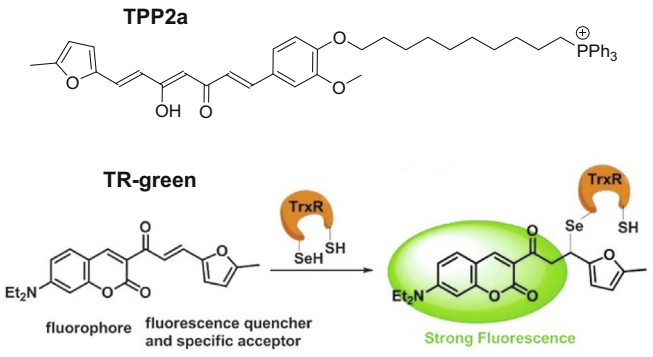

Fig. 4. Mitochondria-targeting **TPP2a** and **TR-green**, synthesized by Bu and co-workers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The Bu group went on to create an improved inhibitor of TrxR2 that also functions as an effective probe. TPP2a (Fig. 4) is synthesized by conjugating a TrxR2 inhibitor, a furan analog of curcumin, to PPh_3 for mitochondria targeting [51]. Since TrxR is essential in combating the effects of ROS in cancerous cells, its inhibition by TPP2a leads to mitochondrial apoptosis in HeLa cancer cells. Interestingly, TPP2a is more active (IC_{50} = 1.44 mM) against the cancer cells compared to the furan-curcumin inhibitor scaffold (IC_{50} = 24.22 mM).

TPP2a shows environmentally sensitive fluorescence, (λ_{ex} = 440 - nm, λ_{em} = 520 nm). In the presence of thiols, the fluorescence is relatively low but increases in the presence of BSA, TrxR, and other proteins such as Trx, glutathione reductase, tubulin, horseradish peroxidase and superoxide dismutase.

To circumvent the non-specific signaling, the researchers cleverly coordinated the β-diketone of TPP2a to a cupric ion, resulting in fluorescence quenching through the disruption of conjugation. This results in selectivity for TrxR because the coordination of cupric ion is weaker than preferential coordination of the β-diketone moiety with the K29 residue on the *N*-terminal of the TrxR active site. This binding is rationalized through molecular docking of the probe onto the TrxR active site. The simulated data shows that TPP2a attains a folded conformation in the hydrophobic active site and its β-diketone forms two hydrogen bonds with the K29 residue of TrxR.

The Singh group designed carbon-dot based probes, Biotin-CD-Naph, that were conjugated to a naphthalimide moiety through a disulfide linkage (Fig. 5) [52]. This makes a FRET pair that can be used in the selective targeting of thioredoxin reductase. Carbon dots offer advantages such as good water solubility, inertness in biological environments, photostability and easy surface functionalization [53–56].

FRET pairs are advantageous in monitoring cell activities since the fluorescence intensity of both the donor and the acceptor are considered, limiting the influence of external factors, and allowing reproducibility of results. The carbon dots are the donors whereas the 3-aminonaphthalimide fluorophore is the acceptor. The cystamine linkage allows the donor and acceptor to be in a favorable distance for FRET transfer. Biotin is also attached onto the CD surface, promoting endocytosis into cancer cells which overexpress biotin receptors on their surfaces. The emission wavelength of CDs is known to shift depending on the excitation therefore the emission energy can be adjusted to suit that of the acceptor. This makes CDs ideal donors in a FRET pair. The same property can be used to minimize background noise by shifting the excitation to a higher wavelength.

In the presence of TrxR, the emission wavelength of the nanosensor at 565 nm decreases whilst that at 450 nm (CD fluorescence) increases and plateaus in 80 min. The reduction of the disulfide disrupts the FRET pair, and this results in fluorescence. The probe is highly specific to TrxR and the fluorescence ratio I_{450}/I_{565} increases steadily over time with a limit of detection of 7.2×10^{-8} M. The nanosensor can image TrxR in human breast cancer MCF-7 and cervical cancer HeLa cell lines. Cancer cells have about 70% reduction in viability when treated with 100 μg/mL of the nanosensor. This is likely due to the release of naphthalimide which resembles amonafide, a chemotherapeutic drug.

A second generation carbon dot-based probe, fCD-Cu^{2+}, was synthesized and evaluated for thioredoxin reductase screening by the Singh group via conjugation of 3-3′-dithiodipropanoic acid (DTPA) moieties to the carbon dots (Fig. 5) [57]. The blue fluorescence of the CDs is quenched in the presence of Cu^{2+} ions via the chelating effect of DTPA. The quenching mechanism is both static and dynamic

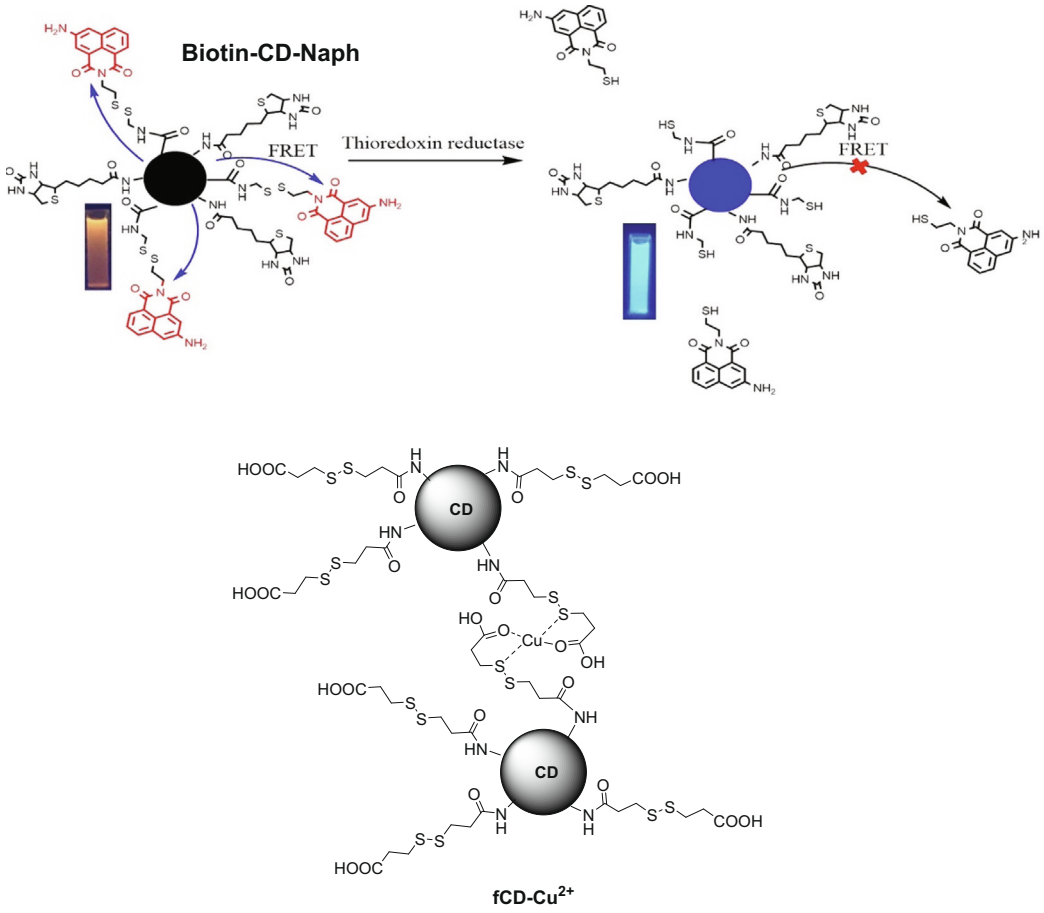

Fig. 5. Carbon dot-based TrxR probes synthesized by the Singh group ((Adapted with permission from [52]. Copyright 2017, American Chemical Society).

and was based on the transfer of electrons to the empty d orbitals of Cu^{2+}. In the presence of TrxR, the disulfide bonds are reduced and 3-mercaptopropinoic acid is released as a bidentate Cu^{2+} ion chelate, in a cation displacement assay. When exposed to TrxR, a linear increase in the fluorescence of the functionalized CDs is observed that plateaus after 100 min (λ_{ex} = 340 nm, λ_{em} = 446 nm). The probe exhibits minimal toxicity to mammalian MCF-7 cells, but shows promising cytoxicity to cancer cells, and is thus a promising theragnostic tool.

Holmgren and co-workers evaluated two probes for their selectivity to mammalian Trx and TrxR. The probes consisted of fluorescein isothiocyanate-labeled insulin (FiTC-insulin) and di-eosin-glutathione disulfide (Di-E-GSSG). They were prepared by modifying the amino groups of GSSG with either FiTC or EITC [58]. Di-E-GSSG is reduced in the presence of Trx, TrxR and NADPH with a K_m of 4.3 μM (λ_{ex} = 520 nm, λ_{em} = 545 nm). This probe is shown to be a better substrate for Trx in comparison to TrxR due to the large size and hydrophobicity of Di-E-GSSG that renders unfavorable binding to TrxR. Di-E-GSSG is also a poor substrate for glutathione reductase (GR), even at high enzyme concentrations. The K_m for this GR reaction is 6.3 μM, with a low turnover.

FiTC-insulin has an average of two fluorophores per insulin molecule. When the disulfide between the A and B chain of insulin is reduced, a fluorescence increase at 520 nm occurs (λ_{ex} = 480 nm). When incubated with Trx, TrxR and NADPH, an increase in fluorescence is observed and the K_m is 9.7 μM. FiTC-insulin can detect low levels of Trx (4–20 nM) and TrxR (0.4–2 nM). Trx activity can be determined in U937, human Burkitt's lymphoma and K562 cell lines, and in homogenized skin biopsies, with quantification of low levels of 0.35 ng Trx per μg of protein. FiTC-insulin can also be used in determining Trx in serum and plasma. However, this is a relatively laborious method involving initially reducing Trx with DTT and gel filtration to remove the excess DTT. These studies led to the development of a commercial TrxR assay kit [59].

Recently, a unique off–on probe (MPSE) containing a benzoselazole conjugated to a naphthalimide fluorophore (Fig. 6) was synthesized to detect TrxR2 [60].

In the presence of TrxR, the fluorescent MPSE (λ_{em} = 550 nm) reacts to form a non-emissive molecule, MP, due to PET quenching. MPSE serves as a theragnostic via its ability to detect mitochondrial TrxR and to trigger apoptosis in cancer cells. In apoptotic cells, damaged mitochondria are phagocytosed by lysozymes and the acidic envi-

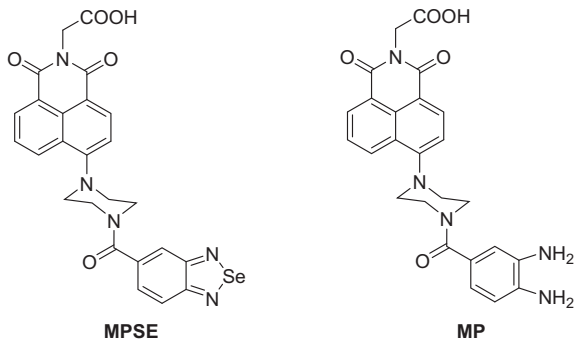

Fig. 6. MPSE and the non-emissive analog, MP.

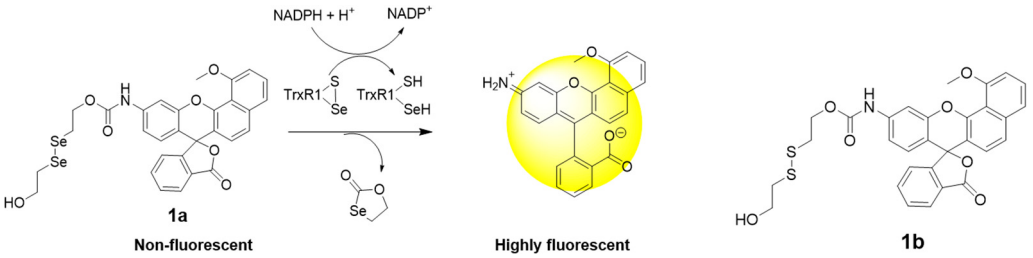

Fig. 7. The first diselenide probe for TrxR **(1a)** monitoring and its disulfide analog **1b**.

ronment of the lysozomes promotes a turn-on response of the probe (MP-H^+ λ_{em} = 470 nm) such that the apoptotic cycle can be monitored. The greater ICT in MPSE is responsible for the larger Stokes shift and more red-shifted emission in comparison to MP-H^+. The theragnostic properties are attributed to the probe promoting the expression of caspases and apoptotic proteins while diminishing antiapoptotic protein levels. This is accompanied by an increase in the release of ROS and inhibition of the MAPK signal transduction pathway that is essential for cancer cell proliferation.

Recently, the synthesis and evaluation of the first diselenide-based probe for the selective detection of thioredoxin reductase was reported (Fig. 7) [61]. This involves conjugation of a linear diselenide moiety, a fluorescence quencher, to a fluorescent seminaphthorhodafluor. The design rationale was assisted by molecular docking studies onto the TrxR active site. The diselenide probe **(1a)** has a more favorable binding energy of −11.1 kcal/mol compared to its disulfide analogue **(1b)** which has a value of −9.6 kcal/mol.

Nucleophilic attack of the diselenide moiety by the Sec residue of TrxR is envisioned to be faster in comparison to the corresponding disulfide-functionalized probe due to the greater electrophilicity of Se. The selenoate formed is also a better leaving group in comparison to thiolates. Additionally, the cylization-driven release of the fluoreophore is faster for the diselende probe compared to the disulfide due to greater nucleophilicity of the selenolate, which attacks the carbamate group.

Probe **1a** exhibits a relatively quick turn-on response to TrxR, reaching maximum fluorescence within 30 min. **1a** also has a low K_m value of 15.89 μM, which shows high affinity to the enzyme. In HCC827 lung adenocarcinoma cells, the diselenide probe exhibits fluorescence that is diminished in the presence of the TrxR inhibitor DNCB. As anticipated, the disulfide analog also fluoresces in HCC827 cells but the emission is lower (~1.6-fold) compared to **1a**. Probe **1a** shows potential as a scaffold for the development of theragnostics, since it affords relatively low IC_{50} values of 3.5 μM and 4.8 μM with SK-MEL-5 and A375 melanoma cells lines respectively.

3. Conclusion

The development of probes for monitoring TrxR activity is of significant current interest due its role in cancer, oxidative stress and other significant disease states such as ischemic repurfusion injury and Parkinsons disease [29,30]. The Sec residue on the TrxR active site gives this enzyme significantly greater catalytic efficiency and broader substrate specificity compared to other oxidoreductase enzymes that utilize cysteine (Cys) [5–8]. This unique residue allows targeted detection of TrxR using different fluorescent probes. Many of the probes synthesized and evaluated to date target TrxR in cancer cell lines, and some also exhibit selective cytotoxicity and show promise as theragnostics. There is still a need to develop more probes with greater kinetic and theragnostic properties. One such way would be to design probes with a turn-on response that is not dependent on the cyclization-driven step, as was done for Fast-TRFS. The TrxR-recognition moiety can be selenium-based, as designed by the Strongin group, to allow rapid nucleophilic attack by TrxR which leads to a faster response [60,61]. Molecular docking simulation-guided design of fluorescent probes is an interesting avenue that can lead to more rapid discovery of the best probes targeting TrxR. This has been utilized in the design of ethaselen, an orally administered antineoplastic drug which is under phase I clinical trials and covalently binds to overexpressed TrxR in cancers [62]. Docking simulations have also been used to compare the binding of diselenide and disulfide probes and related compounds onto the TrxR active site [61,63]. The progress summarized herein should aid researchers in initiating additional studies of enhanced TrxR probes and theragnostics, and in broadening applications of TrxR study to additional disease states.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding: This work was supported by Portland State University.

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