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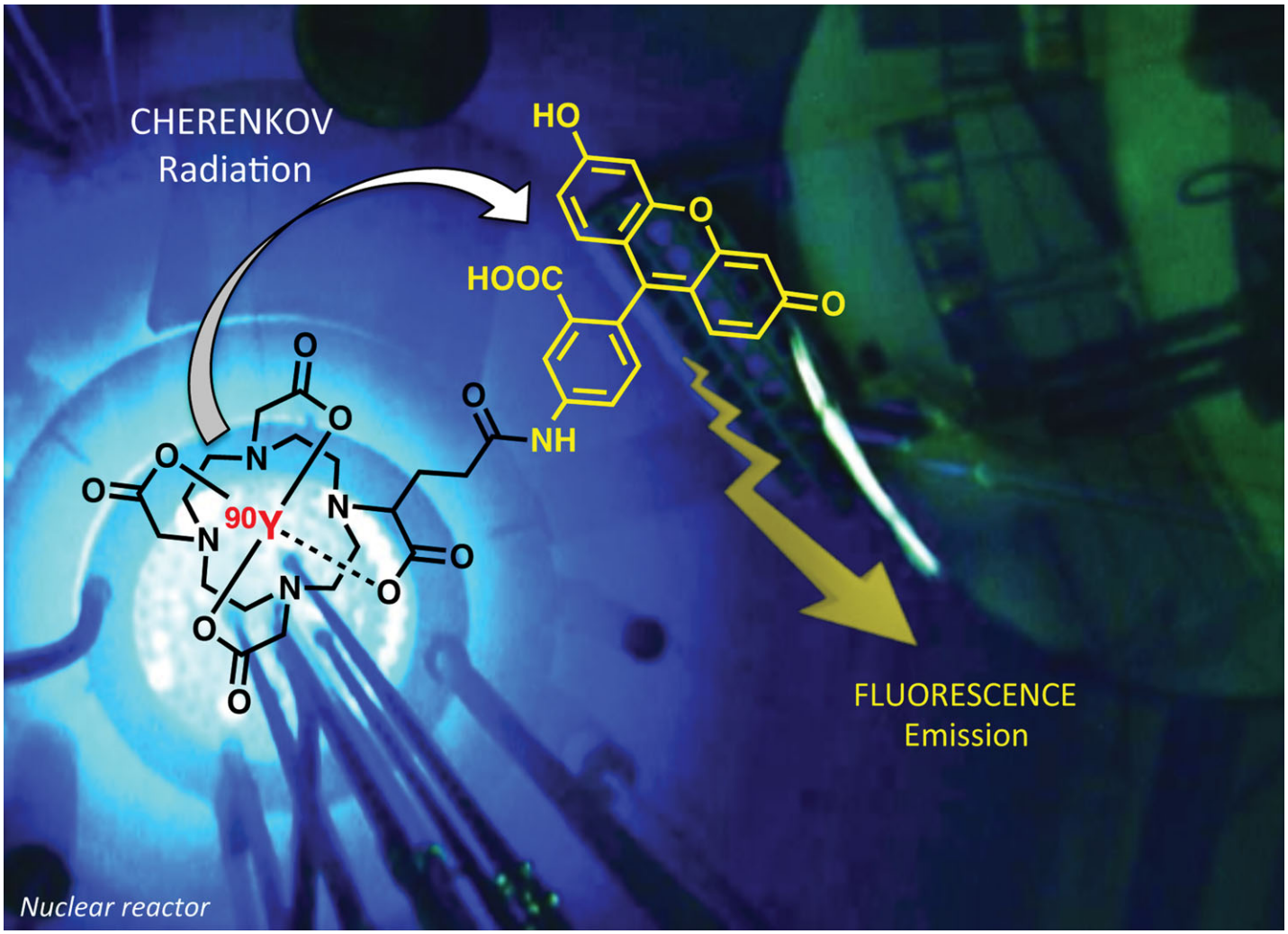
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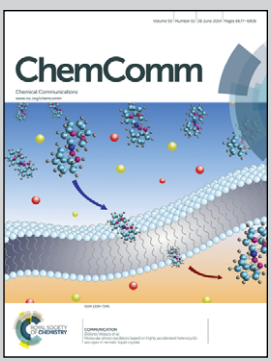


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Inter/intramolecular Cherenkov radiation energy transfer (CRET) from a fluorophore with a built-in radionuclide

Some radionuclides emit optical light, the Cerenkov Radiation (CR, *i.e.* the blue glow in nuclear reactors), which can activate fluorophores. Key parameters were addressed to optimize such processes (energy of the emitted particle, concentrations, λ_{max}, $\Phi_F \times \varepsilon$, η).

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See Richard A Decréau *et al.*, *Chem. Commun.*, 2014, **50**, 6711.

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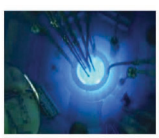
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Inter/intramolecular Cherenkov radiation energy transfer (CRET) from a fluorophore with a built-in radionuclide†

Yann Bernhard,[a] Bertrand Collin[b] and Richard A. Decréau*[a]

The Cherenkov radiation (CR) from [^{18}F]-FDG, [^{177}Lu]-$LuCl_3$ and [^{90}Y]-YCl_3 was detected and CR energy transfer (CRET) to several fluorophores was examined. Subsequent fluorescence emission was found to be a function of the position of absorption bands with respect to the CR peak, energy of emitted particles, radionuclide/fluorophore loading, and fluorophore brightness. A variant of the best fluorophore with a built-in radionuclide was synthesized to achieve inter- and intra-molecular CRET.

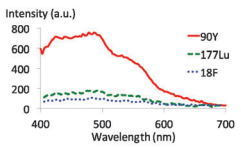
Cherenkov radiation (CR) is the light emitted when a particle exceeds the speed of light in an insulating medium.[1] Such particles that may be β^-, β^+ (and eventually α) are emitted during radioactive decay.[1] CR is typically the blue glow observed in nuclear reactors (Fig. 1A). It is a variant of chemiluminescence, and is one of the many ways of light production besides bioluminescence, fluorescence, and phosphorescence, *etc.* CR has a continuous spectrum in the 250–1000 nm window, but is mostly blue-weighted (300–600 nm).[1,2] CR is beginning to be used in biomedical applications: β^-/β^+ emitting radiopharmaceuticals (for PET and RIT) are now to be considered bimodal by essence.[3–8] Hence, the potential of Cherenkov luminescence imaging (CLI) may be significant because it does not require external irradiation, nor does it suffer from auto-fluorescence. It is only since 2009 that CLI became possible, with the design of ultrasensitive photon imagers.[3–8] Chemists have been poorly involved in this area so far, but there is a need to design probes that could achieve an efficient Cherenkov radiation energy transfer (CRET) process, *i.e.* CR absorption by fluorophores,[9] lanthanides,[10] Quantum Dots (QDs)[11–14] and subsequent luminescence emission. In these seminal studies, the radionuclides were exogenous, and the QDs are potentially toxic.



A

*M	KeV	hv
^{90}Y	2280	70
^{18}F	600	3
^{177}Lu	400	-

B



C

Fig. 1 (A) Cherenkov radiation (CR) observed in a nuclear reactor. (B) Correlation between the intensity of the emitted CR, the energy of emitted β^-/β^+ particles, the reported yield in photons per decay,[8,15] and the luminescence intensity. (C) Spectroscopic detection of CR emitted by [^{90}Y]-YCl_3 (16.2 MBq), [^{18}F]-FDG (46.5 MBq), and [^{177}Lu]-$LuCl_3$ (318.3 MBq) in saline buffer (zoom in the 400–700 nm region).

Herein, several parameters have been carefully examined from a chemistry standpoint to optimize an intermolecular CRET: the energy of an exogenous radioactive source (*i.e.* energy donor), and the optical properties of the fluorophore (*i.e.* energy acceptor), such as the maximum absorption band compared to the CR spectrum, and both constitutive parameters of brightness ($\Phi_F \times \varepsilon$), such as the fluorescence quantum yield (Φ_F), and the molar extinction coefficient (ε). With the best leads, subsequent inter-/intramolecular CRET was achieved using the best fluorophore lead with a built-in/endogenous radionuclide.

CR was detected from a solution of radionuclides: under the apparatus detection limit, CR stretches from 300 to 600 nm (Fig. S1, ESI†). The intensity of the Cherenkov radiation was examined as a function of the energy of the emitted particle (from 400 to 2280 keV) that affects its velocity, but not as a function of its charge (β^- *vs.* β^+) (Fig. 1). Hence, at different radioactivity levels (9–350 MBq), it was found that the intensity of the CR increases from ^{177}Lu (400 keV, β^+) to ^{18}F (600 keV, β^+) to ^{90}Y (2280 keV, β^-) (Fig. 1B and table). This correlates with Mitchell's simulation,[15] which in light of Frank–Tamm formula,[16] suggests *ca.* 3 and 70 photons emitted per decay of ^{18}F and ^{90}Y nuclei, respectively. Note that the CR intensity is also known to be a function of the refractive index η of the medium.[3]

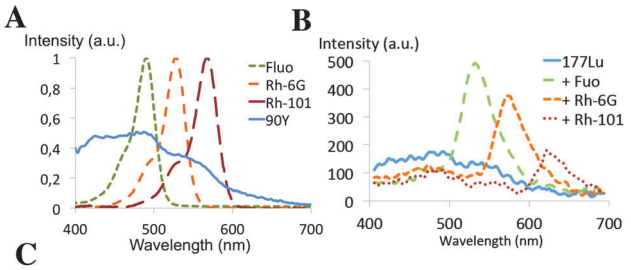
Subsequent intermolecular CRET was examined for a constant level of radioactivity, with a series of fluorophores having comparable brightness (*i.e.* comparable Φ_F, and comparable ε) but with

[a] *Institut de Chimie Moléculaire de l'Université de Bourgogne (ICMUB), UMR 6302 CNRS-Université de Bourgogne, BP 47870, F-21078, Dijon Cedex, France. E-mail: Richard.Decreau@u-bourgogne.fr*

[b] *Comprehensive Cancer Center George-François Leclerc (CGFL), Nuclear Medicine Department, Preclinical Imaging Platform, 21079 Dijon, France*

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C

	Fluorescein	Rhodamine 6G	Rhodamine 101
λabs/λem (nm)	490 / 514	530 / 552	576 / 600
$\Phi_F \times \varepsilon$	73,000	109,000	95,000

Fig. 2 (A) Position of the absorbtion λ_{max} of fluorophores with respect to the CR emission spectrum. (B) Fluorescence emission of fluorophores (0.4 mM) upon CRET (and CR radiation remaining at the bottom of the spectrum) (^{177}Lu 180–190 MBq). Working solution: 0.15 M (0.9%) NaCl solution/MeOH (or 0.1 M NaOH solution) 6 : 4 vol., at RT (see ESI†).

absorption spectra that differ in the position of their maximum absorption bands. Our first results show the following trend, *i.e.* fluorophores absorbing at the CR peak emission wavelength (*i.e.* 495 nm) undergo intense fluorescence emission. On the other hand, the fluorophore emission intensity decreases when the fluorophore λ_{max} shifts away from the CR peak (*i.e.* the overlap is less). Hence, the intensity of fluorescence goes down from fluorescein (λ_{max} 495 nm, *i.e.* a perfect match with the CR peak emission wavelength) to rhodamine 6G (λ_{max} 528 nm, *ca.* 30 nm shift from the CR peak) to rhodamine 101 (λ_{max} 560 nm, *ca.* 60 nm shift) (Fig. 2). Other fluorophores, the λ_{max} of which are on the edge of the CR (in the Agilent Cary-Eclipse fluorimeter detection limit), undergo either a limited emission or no emission at all (Table S5, ESI†). Such a fluorophore is porphyrin TPPS (λ_{ex} 420 nm), which emits at *ca.* 660 nm (Fig. S1, ESI†). The CRET ratios were calculated (see ESI†) by using the Piwnica-Worms method[11] that was adapted from methods developed using FRET/BRET (Tables S3, S4 and S7, ESI†).[17–19]

As shown for the CR, the resulting CRET is proportional to the flux of photons, *i.e.* the energy of the radionuclide, which for a given radioactivity goes up from ^{177}Lu (β^+, 400 keV) to ^{18}F (β^+, 600 keV) to ^{90}Y (β^-, 2280 keV) (Fig. 1B). Under comparable levels of radioactivity, the amount of photons delivered to the fluorophore vary considerably from one radionuclide to another: so does the resulting CRET emission (Fig. 3), and hence the CRET ratios (Tables S3, S4 and S7, ESI†).

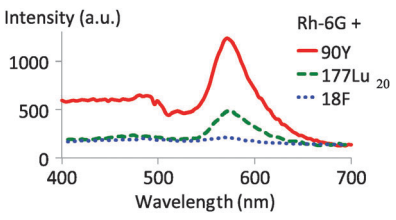


Fig. 3 The intensity of the rhodamine 6G (0.4 mM) fluorescence emission is a function of the energy of the particle emitted during the radionuclide decay: 400 keV (^{177}Lu, 180 MBq), 600 keV (^{18}F, 61.5 MBq), and 2280 keV (^{90}Y, 9.5 MBq), *i.e.* the amount of emitted optical CR photons.

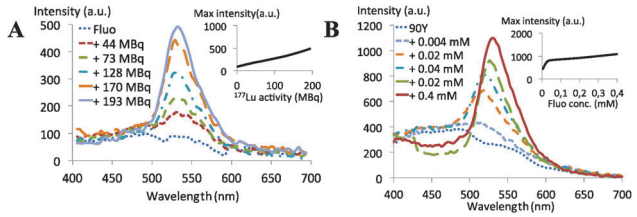


Fig. 4 (A) Increase of the intensity of the fluorescence emission signal of fluorescein (0.4 mM) is a function of the quantity of [^{177}Lu]-$LuCl_3$ (*i.e.* radioactivity). Inset: monitoring of the increase at λ_{em} of fluoreseine (526–529 nm). (B) Increase of fluorescence upon addition of fluoreseine at constant activity (6.9–9.5 MBq). Inset: fluorescence monitored at maximum intensity (508–526 nm) upon addition of [^{90}Y]-YCl3.

When the radioactivity of a given radionuclide, such as ^{177}Lu, drops from 350 MBq to 50 MBq, the intensity of fluorescence emission drops accordingly (Fig. 4A). A linear relationship was observed between the number of MBq and the intensity of luminescence (AU) (inset Fig. 4A). The detection limit was 50 MBq for ^{177}Lu (and 1 MBq for ^{90}Y). When no radioactivity was used, no fluorescence was observed. Similarly, the intensity of fluorescence was linearly dependent upon the concentration of the fluorophore added (detection limit 10 μM) (Fig. 4B and inset).

Among the fluorophores mentioned above, fluorescein was found to be the best lead because its λ_{max} matches the most intense peak of the CR spectrum, and hence the overlap between the two spectra is maximized. Hence, it was appended with a radionuclide to afford **1** (Fig. 5A). Target **1** was synthesized upon reaction of 5-aminofluoresceine with DOTAGA-anhydride to afford the fluorescein–DOTA conjugate **1** (Fig. S5, ESI†). Metallation of **1** with non-radioactive $^{89}YCl_3$ was achieved to optimize the metallation conditions and to fully characterize the ^{89}Y-DOTA–fluorescein complex ^{89}Y-**1** (^{1}H-NMR, HRMS: Fig. S15 and S16, ESI†). The same conditions were applied for the reaction with [^{90}Y]-YCl_3 to afford the radiolabeled complex ^{90}Y-**1**, which was monitored by radio-TLC (Fig. S8, ESI†). Subsequent fluorescein fluorescence emission was observed at pH 8, which was the result of both inter- and intramolecular CRET processes (a pure intramolecular CRET process would require a single-molecule fluorescence spectroscopy study).

In conclusion, this study presented a use of the optical light emitted by radionuclides, the Cherenkov radiation (CR), which is blue-weighted (300–600 nm). The yield in photons is a function of the energy of the radionuclide, ^{90}Y is more energetic

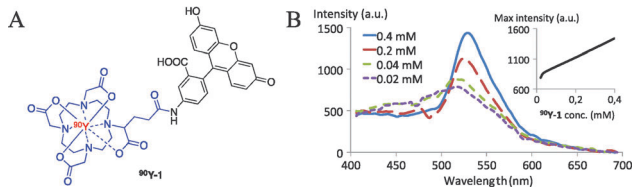


Fig. 5 (A) Structure of ^{90}Y-**1**: a fluorescein–DOTA conjugate with a built-in radionuclide (synthesis in Fig. S5, ESI†). (B) ^{90}Y-**1** fluorescence emission upon inter- and intramolecular CRET processes at various concentrations (and constant activity, 9 MBq). Inset: monitoring of the increase at λ_{em} of fluoreseine (520 nm).

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(2280 keV) hence more luminescent than ^{177}Lu (400 keV). CR energy transfer (CRET) onto fluorophores was achieved with subsequent fluorescence emission: these processes were found to be a function of: (a) the drug/radionuclide loadings, (b) the energy of the emitted particle (*i.e.* that addresses its velocity and hence its yield in photons), and (c) a good match between the maximum absorption band of the fluorophore and the CR peak emission wavelength. Such CRET studies rely on substantial concentrations of fluorophores, with radionuclide/fluorophore molar ratios up to $1:10^6$ (whether the radionuclide is exogenous or endogenous), which corresponds to the standards of radiolabelling.[20] CR is a promising radiation that may be used for radioactive optical imaging in medicine, especially as optical sensors become even more sensitive. Hence, upon careful design of the (chemiluminescent) donor/(fluorescent) acceptor couple, CRET may appear as a convenient approach to transfer a portion of the blue-centered CR light towards the NIR region of the spectrum where tissues are more transparent. Hence, it appears necessary to better comprehend CR and develop CRET molecular probes for CLI.

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