Progress in synthesis and characterization at the single particle level of aggregation induced emission nano-objects inside microfluidic devices

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Vincent G. Colin[1], **Pierre Frère**[1], **Matthieu Loumaigne**[1]
[1]*MOLTECH-Anjou (UMR6200), Univ. Angers, France*
Tel: (+33) 2 41 73 52 53, e-mail: matthieu.loumaigne@univ-angers.fr

ABSTRACT
Aggregation Induced Emission (AIE) is a relatively young field of research that is radically changing the way we use fluorophores for "real-life" applications. In this novel photophysical phenomenon, chromogens that are not fluorescent in solution become highly emissive in the aggregate or solid state. Consequently, aggregation is no more a threat for fluorophore based application and priors test that relied on 'turn-off' mechanisms can now be transferred to more sensitive "turn-on" detection scheme. Here we will present new results obtained on a series of small AIEgen push-pull molecules whose emission span the visible spectrum. The aggregates are created and characterized inside microfluidic chips. This short paper will mainly focus on technical aspects of such measurements.
Keywords: Aggregation Induced Emission, microfluidics, single particle spectroscopy, burst analysis

1. INTRODUCTION

Aggregation Induced Emission is a relatively young field of research that could radically change the way we use fluorophores for "real-life" applications. It was discovered in 2001 by Ben Zhong Tang et al [1]. In this novel photophysical phenomenon, chromogens that are not fluorescent in solution become highly emissive in the aggregate or solid state. Such luminogens with AIE attribute have been referred to as AIEgens. Consequently, aggregation is no more a threat for fluorophore based application and priors test that relied on 'turn-off' mechanisms can now be transferred to more sensitive "turn-on" detection scheme. Ben Zhong Tang and his team have studied a lot this phenomenon and regularly publish very comprehensive reviews[2], [3].

Most of the traditional organic luminophores have planar structures and are usually disc-like flat molecules and consequently, their aggregates tend to experience intense intermolecular $\pi - \pi$ stacking interactions that quench the luminophores. This process is known as Aggregation-Caused Quenching (ACQ).

During the past decade the AIE mechanisms have been deciphered and can now mostly be resume in one acronym : RIM, i.e. Restriction of Internal Motion. In order to illustrate this mechanisms let's consider one archetypal AIEgen : tetraphenylethene (TPE) In TPE, four phenyl rings are linked to a central ethene rod through single bonds, and can thus rotate against the ethene stator. Stated differently, the molecule is conformationally flexible. Hence, the isolated molecules of TPE in a dilute solution can undergo active intramolecular rotations, which serves as a relaxation channel from the excited state to the ground state through non-radiatively decay. In aggregate state, however, the multiple inter and intramolecular interactions constrain the TPE molecule and suppress the rotations of the phenyl rings which blocks the radiationless relaxation channel and opens the radiative decay pathway. The Restriction of Internal Rotation (RIR) and the Restriction of Internal Vibration (RIV), that can be merged into the notion of Restriction of Internal Motion (RIM), explain why AIEgen molecules can have weak quantum yield when diluted in solution, but display a bright fluorescence emission while aggregated.

Here we will present new results obtained on a series of small AIEgen push-pull molecules whose emission span the visible spectrum. The aggregates can be synthesized and characterized inside microfluidic chips. This short paper will mainly focus on the technical aspects of such measurements.

2. MATERIALS AND METHODS

2.1 Microfabrication

Microfluidic chips are created via soft-lithography without the use of a clean room. The microfabrication is based on a largely adapted process firstly described by [4]. A dry photoresist film (Elga FP450, $50\,\mu\mathrm{m}$ thick) is attached to a microscope glass slide with an office laminator (FGK 220, $0.56\,\mathrm{m\,min^{-1}}$, $110\,^{\circ}\mathrm{C}$) . The substrates are cleaned through sonication in an acetone solution during 5 minutes, then rinsed with deionized water. After respecting a hold time of 20 minutes, the photoresist is exposed to UV light through a photomask (JDphoto data) with the negative of the microfluidic design. Irradiation is performed by a LED (Chanzon $365\,\mathrm{nm}/400\,\mathrm{mA}$) during 7 minutes, corresponding to an energy of $300\,\mathrm{mJ\,cm^{-2}}$. Development is initiated after 15 minutes of hold time in an aqueous potassium carbonate solution (1% wt) at $35\,^{\circ}\mathrm{C}$ under medium magnetic stirring.

Afterwards, a PDMS (PolyDiMethylSiloxane) mixture (Eleco RTV615, elastomer and curing agent, 10:1) is poured onto the master in order to obtain the inverse replica. The sample is first degassed under low vaccuum (roughly $0.1\,\mathrm{bar}$) for 10 minutes, and then cured in an oven for 24 hours at $50\,^{\circ}\mathrm{C}$. The replica is then peeled

off the master and bond to a microscope cover slide through plasma treatment, and the microfluidic circuit thus obtained can be used after 10 hours rest.

The control of the flows in the microchannels is achieved through a piezoelectric controller (Elveflow OB1 MK3) allowing a regulation either in pressure or flow rate on the different outlets.

2.2 AIE Molecules

The AIEgen molecules used in this study are synthesized at Moltech-Anjou and will be thoroughly described in a future publication. They are small push-pull molecules with a carbazole donor group and a variety of different acceptor groups (fluoro, cyano and nitro). The change of acceptor significantly shifts the emission spectra of the AIE aggregates and the 3 compounds appear respectively as blue, green and orange. Moreover, these 3 molecules only differ by a few atoms, which would induce a very limited steric hindrance upon a supposed co-aggregation or co-crystallization. Another advantage of the similarity between these molecules is the common absorption band they share around $405\,\mathrm{nm}$.

2.3 Preparation of the sample

The molecules were dissolved in DMSO because of its compatibility with PDMS and aggregation was triggered by water. Two way of preparing the co-aggregates were studied. In the first one called "mix of aggregates", we created two solutions containing an AIE dye in DMSO. The aggregation is triggered by a large addition of water. Then, the two solutions containing AIE aggregates are mixed. In the second one, called "mix of dyes", the two AIEgen are firstly mixed in DMSO and the aggregation is triggered by the addition of a large amount of water.

Fluorescence spectra have been measured in solution with a Jasco FP-8500 spectrofluorometer, for solutions prepared with a concentration of $2.5 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$.

2.4 Optical setup

The epifluorescence setup is built around an Olympus IX73 inverted microscope. The excitation source is a Fianium supercontinuum pulsed white laser (displaying a spectrum ranging from $400\,\mathrm{nm}$ to $2500\,\mathrm{nm}$). The infrared part of the spectrum is separated by a SuperK Split spectral splitter (NKT Photonics), mostly for safety issues. A narrow excitation band (about $10\,\mathrm{nm}$ wide, centered around $405\,\mathrm{nm}$) is then selected out of the visible spectrum obtained by a bandpass filter (Thorlabs FB405-10). The unpolarized laser beam is expanded in order to fit the back focal aperture of a water-immersion high numerical aperture objective (NA = 1.2, 60x, Olympus). The laser is focalised up to the diffraction limit (irradiance of $3.5\,\mathrm{MW\,cm^{-2}}$). The light emitted by the excited AIE particle is collected by the same microscope objective and sent through a dichroic beamsplitter (Semrock Di03-R405), an emission filter (Semrock BLP01-405R-25), and the lens tube onto a $25\,\mu\mathrm{m}$ core optical fiber (Thorlabs FG025LJA) that acts as a confocal pinhole. The $25\,\mu\mathrm{m}$ core matches the size of one Airy spot for the confocal filtering. The photons exiting the optical fiber are directed to an avalanche photodiode (PDM Micro Photon Devices, $100\,\mu\mathrm{m}$ active surface diameter). The corresponding electronic pulses are sent to a single photon counting module (SPC-130EM Becker&Hickl). Alternatively, the sample can be illuminated with a white LED lamp (SOLIS-3C) or a UV LED (same make as mentioned in section 2.1) with the corresponding image being formed onto a Canon EOS 70D camera.

2.5 Burst analysis

The raw data, consisting of arrival times measured by the photon counting card, are processed via a homemade software (pySPC[1]) implemented in the Python programming language (Python 3.6), with the numpy (version 1.15.4) and scipy (1.1.0) modules.

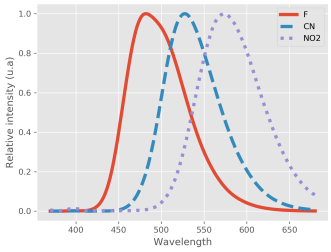
Automatic selection of the individual bursts emitted by the AIE aggregates is based on a "binning and thresholding" method.

Binning time is set at half of the diffusion time of the AIE aggregates that is obtained via Fluctuation Correlation Spectroscopy (FCS) of the raw data. This relatively long binning time permits to average and smooth out possible short emission fluctuations of the aggregate like triplet state blinking. This short modulation would lead to an artificial separation of one burst into a subset of several short bursts.

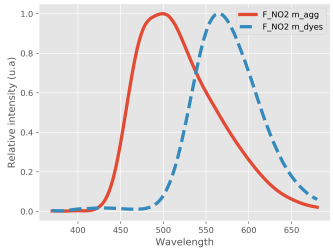
Two thresholds are then drawn, the first one, T_{burst}, in order to define a minimum number of photons per bin needed to identify a burst and the second one, T_{flank}, for detecting the end of the burst. The algorithm scans consecutively all the time bins of the signal, and each occurrence of the signal of a bin being higher than T_{burst} marks the start of a new burst. Then, the left and right boundaries of this new burst will be extended, until the number of photons per bin drops below T_{flank}.

The values of T_{burst} and T_{flank} are based on the poissonian statistics that govern the experimental signal [5].

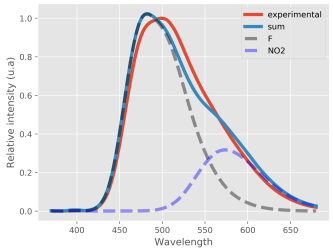
[1] https://github.com/MLoum/pySPC



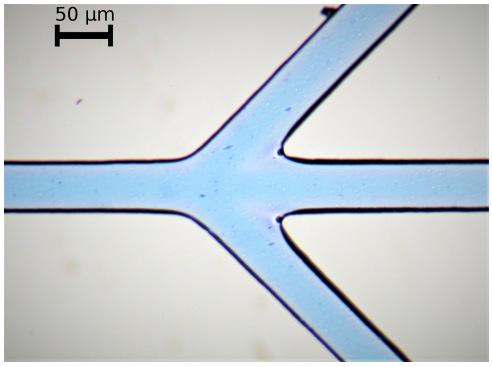
(1a) Emission spectra of AIE aggregates in water for different acceptor groups.



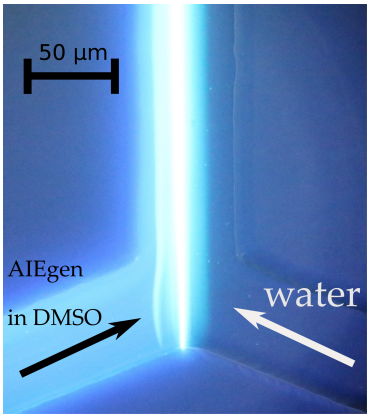
(1b) Emission spectra of the Fluoro-Nitro combination for the "mix of aggregates" and the "mix of dyes" cases



(1c) Graph showing that the emission spectra of the Fluoro-Nitro mix of aggregates can be seen as the weigthed sum of the emission spectra of Fluoro and Nitro pure compounds.



(2a) Image of a master made of (blue) dry film photoresist.



(2b) Color image of AIEgen molecules in DMSO aggregating at the boundaries with water inside a Y-shaped microfluidic junction.

3. Results

3.1 Emission spectra of AIE aggregates

Figure 1a shows the emission spectra of the 3 different kinds of AIEgen aggregates formed in water (almost 100% in mass compared to the remaining DMSO). The effects of the change of acceptor group is clearly visible and the color of emission can be tuned from blue to orange.

Figure 1b shows the difference of emission spectra between a mix of aggregates and a mix of dyes when using the fluoro and nitro compound. Whereas the spectra of the mix aggregates can be seen as a weighted sum of the spectra of the pure Fluoro and the pure Nitro compounds (cf fig. 1c), the spectra emanating from the mix of dyes is totally different. The main striking fact is the disappearance of the blue part of the spectra.

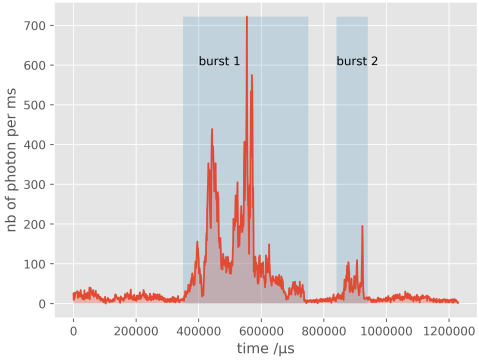
3.2 Aggregation triggered inside microfluidics chips

Figure 2a shows an image under the microscope of a master microfabricated by the process described in section 2.1. According to the constructor specifications for the dry film photoresist, the resolution of $50\,\mu\mathrm{m}$ is attained. The edges of the micro-structure are slightly smoothed out.

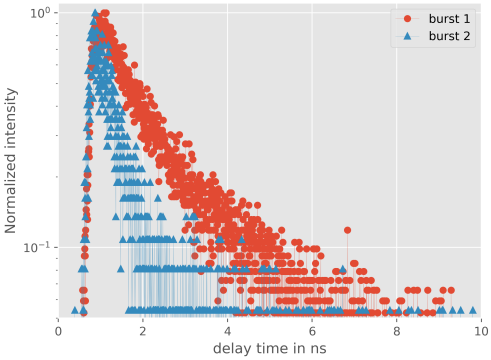
Figure 2b shows a Y-junction where the AIEgen molecules in DMSO come in contact with water. This triggers the aggregation of the molecule as attested by the large increase of luminescence of the aggregate along the diffusion boundaries.

3.3 Lifetime measurements of single AIE aggregates in solution

Figure 3a shows a time trace of the intensity of fluoro AIEgen aggregates where two bursts have been identified by the algorithm described in section 2.5. Figure 3b shows that the fluorescence decay is quite different for these two bursts.



(3a) Photon intensity time trace with the identification of two bursts.



(3b) Example of the polydispersity of the AIE aggregates based on the difference of fluorescence decay of the two bursts of figure 3a

4. DISCUSSION

The drastic change in emission (cf. fig 1b) upon the aggregation of two similar yet different AIE molecules is a good first evidence of the co-aggregation of these two molecules. Nonetheless, this is a proof at the macroscopic scale, and single particle measurements still need to be carried out to ascertain this hypothesis.

The measurements made on AIE aggregates made of only one type of molecule (cf fig 3b) tends to show that the aggregates are already quite polydisperse. A burst per burst approach seems very promising but robust statistical analysis will be needed in order to get a good overview of the sample characterization.

Finally, the study in microfluidic channels offers some undeniable advantages, such as monitoring of the aggregation process along the channel length, or real time switching of some chosen parameters (such as solvent ratio, or temperature). Different flow geometries are also considered in order to modify the aggregate shapes.

5. CONCLUSIONS

In this short communication, we have shown that AIE aggregates can be formed and studied at the single particle level inside microfluidic channels. We have also given experimental proofs of the formation of AIE co-aggregates. We are currently working on the identification of these co-aggregates at the single particle based on their emission spectra by using photon time of flight spectroscopy[6].

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