

Aggregation-Induced Stimulated Emission of 100% Dye Microspheres

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Dyes with aggregation-induced emission (AIE) properties have gained interest due to their bright luminescence in solid-state aggregates. While fluorescence from AIE dyes is widely exploited, relatively little is known about aggregation-induced stimulated emission. Here, stimulated emission of tetraphenylethene (TPE)-based organoboron AIE dyes, TPEQBN, in thin films and in microcavity lasers is investigated. Using femtosecond pump-probe spectroscopy, gain coefficients up to 230 cm⁻¹ at 500 nm are measured. Using rate equations, concentration- and pump-dependent gain dynamics as well as laser build-up dynamics are analyzed. During laser oscillation, radiative stimulated emission allows high instantaneous quantum yield greater than 90% to be achieved. Solid-state microspheres made of 100% AIE dyes are fabricated via microfluidic emulsion and solvent evaporation method. Coupled with high gain and high refractive index of 1.76, microspheres as small as 2 µm in diameter show lasing by nanosecond pumping with a threshold of \approx 10 pJ μ m⁻². Polymer coated, but not bare, microspheres are internalized by live cells and generate narrowband cavity-mode emission from within the cytoplasm. This work shows the potential of AIE dyes as laser materials.

1. Introduction

Unlike conventional organic dyes that lose their luminescence at high concentration, known as concentration quenching, aggregation-induced emission (AIE) molecules are the opposite: their quantum yield is extremely low when they are free in dilute solution, but they become highly emissive at solid-state aggregates.^[1–7] Such luminogens have been exploited for their applications in chemical sensing and bioimaging as well as optoelectronic devices.^[1–7] AIE molecules are typically made of fluorophores melded with a motif capable of generating intramolecular rotation or vibration. In dilute solution, the free rotation and vibration of the AIE motif cause non-radiative transition. However, the motions are restricted in aggregates or within viscous media, yet their structure prevents quenching-inducing π – π stacking, and this results in high quantum yield of emission.

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Recently, there have been interests in developing microspheres capable of generating cavity modified spectra or laser emission.^[8–11] These output spectra, which are distinctly different from conventional fluorescence, have potential to be useful for intracellular sensing, cell tracking, and multiplexed assays.^[8,9,11,12] Generating such emission requires materials with high gain and high refractive index. Dye-doped microspheres have shown lasing under nanosecond pumping,^[8,9] but their limited optical gain required relatively large diameters greater than 8 µm.^[8,13,14] AIE dyes have been utilized for microlasers,[15-20] and recently Liu et al. has demonstrated microbead lasers of 8.9 µm using 1 wt% AIE dye in epoxy resin.^[18] Considering the lack of aggregation-caused quenching, AIE dyes are promising laser materials. However, there have been very limited studies on aggregation-induced stimulated emission, and its full potential for miniaturizing microbead lasers remains to be explored.

Here, we show that while AIE aggregates offer high quantum yield for fluorescence or spontaneous emission, they are not free from significant non-radiative loss at a high concentration of excitons. Yet, we find that the quenching is substantially suppressed during laser oscillation by strong stimulated emission. As a result, we demonstrate laser generation from microbeads made entirely of AIE dyes with diameters as small as 2 µm. We describe detailed characterizations of aggregation-induced stimulated emission using femtosecond pump–probe absorption and provide insights into various relaxation and quenching kinetics at high excitonic concentrations. With biocompatible polymer coating, we realize lasing and cavity-enhanced spectral emission from AIE microspheres inside living cells.

2. Results and Discussion

2.1. Photophysical Properties of TPEQBN Thin Film Depends on Concentration

We chose and studied tetraphenylethene (TPE)-based organoboron TPEQBN among different AIE dyes. Its high extinction coefficient, high PL quantum yield, and low molecular weight are pre-requisite for high optical gain at high concentration in the solid state.^[21] Its molecular structure is shown in **Figure 1a**. TPEQBN includes N,C-chelate organoboron melded





Figure 1. Photophysical properties of TPEQBN film depends on concentration. a) Molecular structure of TPEQBN and photos of TPEQBN film samples under room light and UV lamp. The films are 50 nm thick and spin coated on quartz substrates. b) The molar extinction coefficient of TPEQBN dyes in toluene, and normalized PL spectra of TPEQBN-in-PMMA films with different dye weight concentrations. c) Transient PL decay curves of 10%, 50%, and 100% concentration samples. d) PL quantum yields for different TPEQBN concentrations. Circles, experimental data; curve, best fit with $78.7 - 7.6x^2 - 9.49x^3$ (%). e) Radiative and non-radiative transition rate constants. Circles, experiments. Curves, best fit with $k_r = 0.26 (1 - 0.1x^2 - 0.133x^3)$ and $k_{nr} = 0.074 (1 + 0.35x^2 + 0.41x^3)$. f) Refractive index, *n*, and extinction coefficient, *k*, of a 100% dye film.

with AIE motif of TPE. The TPE group has highly twisted phenyl rings on central alkene. This propeller-like conformation of the TPE molecule prevents quenching-inducing $\pi - \pi$ stacking and multiple interactions between C-H groups and phenyl rings allow rigid molecular conformation suppressing the non-radiative transition.^[22] TPEQBN-doped PMMA films 10% and 50% (by weight) concentrations and 100% TPEQBN film were prepared on quartz substrates by spin coating with 50 nm thickness. Figure 1a shows photographs of 10% and 100% TPEQBN films under room light and UV, respectively. 100% TPEQBN film under room light showed more yellowish color due to larger absorption from higher concentration. However, unlike conventional organic dyes, 100% TPEQBN film under UV showed much brighter fluorescence compared to 10% TPEQBN film. Figure 1b shows the PL spectra, showing a red shift of the peak from 497 to 529 nm due to the solvatochromic effect.^[23] The peak molar extinction coefficient (ϵ) of TPEOBN ($\approx 10^{-5}$ M) in toluene was measured to be 47 400 L mol⁻¹ cm⁻¹ at 395 nm. This value is similar to the extinction coefficients of conventional organoboron laser dyes in dilute solution; for example, $\varepsilon = 56\,000$ L mol⁻¹ cm⁻¹ of disodium-1,3,5,7,8-pentamethylpyrromethene-2,6-disulfonate-difluoroborate (pyrromethene 556).^[24] Figure 1c shows transient PL decay curves for 10%, 50%, and 100% dye samples, which were fit with a single exponential decay with a lifetime of 3 ns. The PL quantum yield measured with an integrating sphere was 0.78, 0.75, and 0.61 for the 10%, 50%, and 100% dye samples, respectively (Figure 1d). PL quantum yields of most organic dyes significantly drop as concentration increases by aggregation-induced quenching. In contrast, TPEQBN is less susceptible to aggregation-induced quenching. From the PL lifetime and quantum yield, the radiative (k_r) and non-radiative (k_{nr}) transition rates were calculated to be 0.26 and 0.074 ns⁻¹ for 10%, 0.25 and 0.084 ns⁻¹ for 50%, and 0.2 and 0.13 ns⁻¹ for 100% dye samples (Figure 1e). The refractive index of 100% TPEQBN films was 1.76 at the PL peak wavelength (λ) of 530 nm (c.f., n = 1.59 of polystyrene) (Figure 1f). The high refractive index is desirable to miniaturize lasers since the Q-factor of the microresonator increases nonlinearly with the refractive index difference against the surrounding.^[8,9]

2.2. Optical Gain Dynamics of TPEQBN

Femtosecond pump–probe absorption spectroscopy was performed to characterize optical gain for films with different concentrations at different pump intensities. **Figure 2**a,b show the transient absorbance spectra of 10% and 100% films at different pump–probe delay times (see Figure S1, Supporting Information for more data). Positive absorption change (ΔA) above 550 nm represents excited state absorption (ESA), and negative ΔA around 500 nm represents optical gain since TPE-QBN ground state absorption peak is well separated at 395 nm. Figure 2c,d plot the peak ESA and gain coefficients obtained at a pump fluence of 150 µJ cm⁻². Figure 2e,f plot gain coefficients as a function of the pump–probe delay time for different

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Figure 2. Optical pump-probe absorption and gain dynamics of TPEQBN films. a,b) Femtosecond pump-probe absorption spectra at a pump fluence of 150 μ J cm⁻² for 10% (340 nm thick) and 100% dye (120 nm thick) films. c) Peak ESA coefficients at a wavelength of \approx 610 nm. Lines are guides to the eye. d) Peak gain coefficients at a wavelength of \approx 490 nm. Lines are guides to the eye. e) The gain coefficients of three samples with different concentrations pumped at 150 μ J cm⁻². Circles, experimental data; lines, curve fits. f) Gain dynamics of 100% dye samples at different pump intensities. Circles, experiments; lines, curve fits.

concentration and pump levels. The maximum gain coefficient of 230 cm^{-1} was obtained with the 100% sample at 150 μJ $cm^{-2}.$

To explain the concentration-dependent quantum yield (Figure 1d), we considered nonlinear quenching described by $PL \propto An$ $-Bn^2 - Cn^3 - Dn^4$ where n is the number concentration of AIE molecules in unit volume. Accordingly, we can write: QY $= a - bx - cx^2 - dx^3$, where x = [0, 1] is the weight concentration of dye, where coefficients a to d are related to A to D, respectively. Curve fitting of the experimental data with the fourthorder polynomials yielded a = 78.7, b = 0, c = 7.60, d = 9.49. The lack of linear dependence indicates small quadratic dependence in quenching (B = 0) and is interesting. This may be a unique characteristic of AIE. Concentration quenching of typical dyes in solution or mobile carriers in semiconductors typically show a quadratic dependence as the collision probability increases with n^2 . This conventional situation is illustrated in Figure 3a. By comparison, excitons of solid-state AIE dyes are highly localized in the molecules due to strong exciton binding energy, as illustrated in Figure 3b. In this case, quenching can occur not only by collisions but also by Förster resonant energy transfer (FRET), depending on the concentration and pump power. The probability of FRET is inversely proportional to the intermolecular distance to the power six, $\approx l^{-6}$, where $l \propto x^{-\frac{1}{3}}$, taking spatial averaging of various local factors, such as dipole orientation, the spectral overlap of the donor emission and acceptor absorption, and the donor emission lifetime and assuming they are constant independent of TPEQBN concentration. So, the rate of FRET-type quenching events is proportional to $n^{3,[25]}$ Both collisional and FRET-mediated exciton annihilations have been previously studied.^[25–28] A commonly adopted model for pulsed excitation uses a collisional quenching rate $\propto Bn^2$ (*B* = time-independent constant) and a quenching rate of Bn^3 for FRET-induced annihilation.^[25] Alternatively, a quenching rate in the form of $\frac{B}{\sqrt{t}}n^2$ has been used, taking into account linear exciton diffusion in organic crystals.^[25,26,29]

To understand the time-dependent gain data (Figure 2e,f), we modeled the dye as a standard quasi-3 level system as depicted in Figure 3c, where molecules are pumped from the S0 to initial S1' states and then relaxed to the S1 level with a vibrational relaxation rate k_s . The excited molecules are then decayed to the S0 state with a transition rate of k_1 , which combines k_r and k_{nr} , or transitioned to excited states by ESA. The rate equations are

$$\frac{dn_2}{dt} = -k_s n_2 \tag{1}$$

$$\frac{dn_1}{dt} = k_s \, n_2 - k_1 n_1 - k_q n_1^q \tag{2}$$

where n_2 and n_1 are excitonic concentrations in the S1' and S1 levels, respectively, and for simplicity we have neglected ESA (k_{ESA}) and relaxation from the S2 level back to the S1 level (k'_1). The

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Figure 3. Illustration of quenching dynamics. Models for concentration quenching for a) mobile and b) immobile molecules in low and high concentration states. Circles represent molecules. Blue color indicates excited molecules. c) The energy level diagram and transition paths in optically pumped AIE molecules.

*q*th-order polynomial term with a decay rate of k_q has been included to describe Auger-type exciton–exciton quenching. For weak nonlinearity, q = 2 is appropriate, but for high n_1 , higher-order nonlinearity could be dominant. When the excitonic concentration is low, we can neglect the nonlinear term and find a solution ($k_s \gg k_1$).

$$n_1(t) = n_0 \left(e^{-k_1 t} - e^{-k_s t} \right)$$
(3)

where the initial conditions are n_2 (0) = n_0 and n_1 (0) = 0, where *t* is time delay between the femtosecond pump and probe pulses. For high concentrations where the nonlinear term is non-negligible, we find a solution: $n_1(t) = an_0e^{-k_1t}\left\{1 - \frac{k_q}{k_1}(an_0e^{-k_1t})^{q-1}\right\}^{-\frac{1}{q-1}}$, which is valid only when $k_s t \gg 1$ after all the molecules have settled in the S1 level, where $n_2(0) = 0$, $n_1(0) = n_0$, and $a \equiv \left(1 + \frac{k_q n_0^{q-1}}{k_1}\right)^{-\frac{1}{q-1}}$. Combining the two asymptotic solutions, we write an approximate ansatz.

$$n_{1}(t) \approx \frac{n_{0}a\left(e^{-k_{1}t} - e^{-k_{s}t}\right)}{\left(1 - \frac{k_{q}}{k_{1}}\left(n_{0}ae^{-k_{1}t}\right)^{q-1}\right)^{1/(q-1)}}$$
(4)

The measured gain, g(t), is proportional to $k_r n_1(t)$. Using Equation (4), we fitted the data in Figure 2e. The nonlinear term was necessary to fit the 50% and 100% dye data (but less essential for the 10% data), and q = 3 produced better fitting than q = 2. $k_1 = 0.33 \text{ ns}^{-1}$ was well within the best fit range of 95% confidence, which is consistent with the PL lifetime of 3 ns (Figure 1c). The overall results using three fitting parameters, n_0 , k_s , and $k_{q=3}$, were remarkably good. For x = 10% concentration, we determined $k_s \approx 0.6 \text{ ps}^{-1}$ and $\frac{k_3 n_0^2 a^2}{k_s} \approx 0.33$; from x = 50%, we ob-

tained $k_s \approx 0.85 \text{ ps}^{-1}$ and $\frac{k_3 n_0^2 a^2}{k_1} \approx 0.92$; and from x = 100%, we found $k_s \approx 1.49 \text{ ps}^{-1}$ and $\frac{k_3 n_0^2 a^2}{k_1} \approx 0.975$. The slight increase of k_s with increasing concentration may be attributed to increased exciton scattering due to heating. The instantaneous QY was estimated from $\frac{k_s n_1}{(k_1 n_1 + k_3 n_1^2)}$. It reaches the minimum of $(1 - \frac{k_3 n_0^2 a^2}{k_1}) \frac{k_r}{k_1}$ when $n_1(t) = n_0$. At 100% concentration we find the instantaneous QY is only 1.5% at ≈ 3 ps after the femtosecond pumping. At later times, as n_1 decreases, it gradually improved to the 60% level, that is, $\frac{k_r}{k_1 + k_{mr}}$. The drop in QY is due to the extreme density n_1 resulting from the pumping with a very high peak intensity of $\approx 1.1 \text{ GW cm}^{-2}$ (150 µJ cm⁻² and 100 fs). The minimum instantaneous QY of the 10% sample was 54%, comparable to its measured value of 78% by low-intensity continuous-wave excitation.

We also measured the pump–probe gain with lower pump fluences of 50 and 100 μ J cm⁻² up to 64 ps delay (Figure S1, Supporting Information) and fitted the data using Equation (4). We found that the data at 100 μ J cm⁻² were fit equally well with a quadratic term (Figure 2f). In lasing experiments described below, we used nanosecond (5 ns) pump pulses with greatly lower peak intensities of \approx 10 MW cm⁻². Under nanosecond pumping, nonlinear quenching is expected to be reduced, and the maximum gain will be attained with 100% dye concentration. Since the gain relaxation time is comparable to the pump duration t_p , the total available laser gain is $G(t_p) \sim \int_0^{t_p} g(t') dt'$, where g(t) is impulse gain at the pump intensity.

2.3. Numerical Modeling of AIE Microlasers

To gain insights into laser buildup, we extended the model to a microcavity laser with volume V_c filled with 100% dye AIE gain medium. A Gaussian pump pulse generates excitons at a rate of





Figure 4. Numerical simulations of AIE gain medium and microlaser. a) Simulation of the 120 nm thick 100% TPEQBN film for femtosecond pump at 150 μ J cm⁻². Circles were obtained from the experimental data in Figure 2e using a conversion factor of $n_1 = \text{Gain} \times 10^{17} \text{ cm}^{-2}$. b) Same sample as in (a) but for 5 ns pump pulse. Simulation for a 2 μ m 100% TPEQBN bead under nanosecond pump: c) Output laser emission versus pump fluence. d) Generated exciton density for several different cases. e) Number of photons in the microcavity. Inset shows the 110–150 ps period of relaxation oscillation. f) Instantaneous quantum yields well above (red) and near (yellow) threshold. The calculated quantum yields for the thin film in (a) and (b) are also shown.

 $n_{\rm p}$ per volume: $n_{\rm p} = \frac{n_0}{t_{\rm p}} * (4ln2/\pi)^{0.5} e^{-4ln2 t^2/t_{\rm p}^2}$, where n_0 is the total number of excitons created by the absorption of a single pump pulse. We can write $n_0 = \frac{E_{\rm p}}{h_{\rm v}} (1 - 10^{-\epsilon l})l$, which is the total number of absorbed photons by a pump pulse, where $E_{\rm p}$ is the pump pulse energy fluence per area, hv is the photon energy, ϵ (51 000 cm⁻¹; Figure S2, Supporting Information) is the extinction coefficient, and l is the thickness of the AIE gain medium. The expanded rate equations are^[30,31]

$$\frac{dn_2}{dt} = n_{\rm p} - k_{\rm s} n_2 \tag{5}$$

$$\frac{dn_1}{dt} = k_s n_2 - k_r n_1 \left(1 + \beta q\right) - k_{nr} n_1 - k_2 n_1^2 - k_3 n_1^3 \tag{6}$$

$$\frac{dq}{dt} = k_{\rm r} n_1 \left(1 + \beta q\right) V_{\rm c} - k_{\rm c} q \tag{7}$$

Equation (7) describes the photon building up within the microcavity, where *q* represents the number of photons in the cavity mode to be lased, and k_c is the cavity output coupling rate. β is a reciprocal of the total number of optical modes, $\sim V_c/(\lambda/n)^3$, in the microcavity (known as spontaneous emission factor). We have separate k_1 to k_r and k_{nr} and added a stimulated emission

term $k_r n_1(1 + \beta q)$. Physically, this term describes the interaction of each exciton with the entire cavity modes, which are similar to free-space modes for large $1/\beta$, and also with the particular cavity mode with q photons. This single-mode lasing model can be extended to multiple-mode lasing by including additional cavity modes.

To verify our earlier analytical results, we first numerically solved the equations simulating the pump probe measurement in the open geometry ($\beta = 0$). Figure 4a plots $n_p(t)$ and $n_1(t)$ for $t_p = 100$ fs and $E_p = 150 \,\mu$ J cm⁻² in excellent agreement to the experimental data (circles, Figure 2e). We have used $k_1 = 0.33 \,\mathrm{ns}^{-1}$, $k_r = 0.20 \,\mathrm{ns}^{-1}$, $k_{\rm nr} = 0.13 \,\mathrm{ns}^{-1}$, $k_2 = 1.25 \times 10^{-19} \,\mathrm{cm}^3 \,\mathrm{ns}^{-1}$, and $k_3 = 0.94 \times 10^{-38} \,\mathrm{cm}^6 \,\mathrm{ns}^{-1}$. A critical exciton density at which the nonlinear decay becomes greater than the linear decay is given by $k_1 \, n_c = k_2 \, n_c^2 + k_3 n_c^3$. Using the coefficients for 100% AIE, we find $n_c = 2.3 \times 10^{18} \,\mathrm{cm}^{-3}$. So, $n_1 > n_c$ is affected by the nonlinear quenching. For the same parameters, nanosecond pumping with $t_p = 5$ ns results in lower and broader profile of $n_1(t)$ (Figure 4b). At the peak we obtained $n_1 = 4.21 \times 10^{18} \,\mathrm{cm}^{-3}$.

Next, we simulated a microbead made of 100% TPEQBN with a radius, *r*, of 1 µm. We used $V_c = \frac{4\pi}{3} r^3$, $\beta = 0.001$, $l = \frac{4}{3} r$, and $k_c = 1.9 \text{ ps}^{-1}$ assuming a cavity Q-factor, Q_c , of 2000 ($k_c = 2\pi v/Q_c$). Figure 4c plots the total output photons, $\int_{-\infty}^{\infty} q(t')dt'$, for different pump fluence. The light-in–light-out curve shows a clear threshold at 0.45 mJ cm⁻². Above threshold, as the intracavity photon





Figure 5. Self-assembled 100% AIE dye microbead lasers. a) Schematic and workflow for the fabrication of 100% dye microspheres. b) SEM images of representative microspheres with different diameters controlled by the flow rates. Scale bar: 2 μ m. c) Representative output spectra from a single microsphere below and above lasing threshold, respectively. WGM modes are designated by polar indices and polarization states. d) The peak intensity of the TE³⁰ mode at different single-pulse pump fluences. Circles, experiments; line, curve fit. e) Representative time-averaged above-threshold spectra of four microspheres with different diameters.

density increases, stimulated emission occurs increasingly more, and the number of excitons, or population inversion, is saturated and clamped to its threshold level. This is evident in the $n_1(t)$ profile in Figure 4d. For comparison, it shows n_1 for the cases of $\beta = 0$ (no cavity) and $k_1 = k_2 = 0$, respectively, as well as the pumping profile n_p . Figure 4e shows the laser output profiles, q(t), for two different pump levels E_p of 0.5 and 4 mJ cm⁻², respectively. The oscillations in the beginning of output profiles occur when $t_p < 2\pi/k_r$, known as relaxation oscillation. We defined instantaneous quantum yield, QY, to be the ratio of radiative over non-radiative transitions.

$$QY = \frac{k_r n_1 (1 + \beta q) V_c}{k_r n_1 \beta q + k_1 n_1 + k_2 n_1^2 + k_3 n_1^3}$$
(8)

Figure 4f shows QY(*t*) for different cases. Interestingly, during laser oscillation well above threshold at $E_p = 4$ mJ cm⁻², QY is >90% because the stimulated emission by intracavity light increases the radiative decay rate over both linear and nonlinear spontaneous non-radiative decay rates combined. Near the threshold, QY is ≈30%. For comparison, we also calculated QY for the free-space TPEQBN thin film used in the pump–probe experiment for femtosecond and nanosecond pump pulses. The significant nonlinear loss of QY was clearly seen (Figure 4f). The >90% quantum yield during laser operation demonstrates that 100% AIE dyes are an excellent gain material for lasers.

2.4. Self-Assembled 100% AIE Dye Microbead Lasers

We synthesized 100% dye microbeads using the oil-in-water microemulsion and solvent evaporation technique in a microfluidic setup (Figure 5a). Individual microbeads are composed entirely of AIE dyes at the maximum achievable dye concentration. The mass density of the 100% dye aggregates is expected to be ≈ 1.14 g cm⁻³.^[21] Droplets of 1 wt% TPEQBN solution in dichloromethane (DCM) were generated in polyvinyl alcohol (PVA)-containing water. The collected microdroplets in PVA water were left at room temperature until the DCM solvent was fully evaporated, leaving solid-state microspheres with 100% dye concentration. The size of the droplets was determined by flow rates, which we controlled by pressure pumps. Figure 5b shows scanning electron microscopy (SEM) images of representative microspheres fabricated with a range of dye-solution pressure from 0.75 psi (yielding 2.0 µm diameter) to 0.9 psi (2.8 µm diameter) at a constant water channel pressure of 1 psi. Excellent spherical shape with surface smoothness was shown in Figure 5b. Each batch generated at specific pressure levels had a monodispersed size with a diameter variation <1% (Figure S3, Supporting Information).

We used a hyperspectral microscope and an optical parametric oscillator (OPA, 5 ns, 20 Hz) pump laser tuned at 440 nm to characterize the 100% dye microbeads. Figure 5c shows the output spectra of a 2.6 μ m TPEQBN microbead at two pump fluences below and above threshold, averaged over 100 pulses.





Figure 6. Cell affinitive AIE microbead lasers for in vitro imaging. a) Zeta potential of uncoated microspheres. b) Bright-field image of HeLa cells 24 h after incubation with uncoated microspheres. c) Zeta potential of PEI-coated microspheres. d) HeLa cells incubated with PEI-coated microspheres. e) Confocal microscopy images of TPEQBN microbeads (green) in RFP-expressing HeLa cells (red). Center, z-projection; right and bottom, cross-sections. f) Viability of cells 24 and 48 h after incubation with PEI-coated microbeads. g) Time-lapse imaging of microspheres in cells. h) Emission spectrum of an intracellular TPEQBN microsphere (arrow in (g)). Scale bars in (e) and (g): 20 μm.

Above threshold, the typical multi-peak spectra revealed whispering gallery modes (WGM) characterized by polar indices and polarization states. Figure 5d shows a single-shot peak intensity of the dominant cavity mode at 511 nm with a full-width-at-half-maxima linewidth of 0.44 nm. It shows a threshold at approximately 0.7–0.8 mJ cm⁻². The pulse-to-pulse variation is largely due to multimode lasing and mode competition, as well as pump beam instability. Figure 5e shows lasing of four different microspheres. To the best of our knowledge, this result represents the first demonstration of all-AIE-dye microsphere lasers.

2.5. Cell Affinitive AIE Microbead Lasers for In Vitro Imaging

Conjugated molecules tend to have negative surface charges due to their electron-rich aromatic groups.[32] The zeta potential of 100%-TPEQBN microspheres in water was measured to be -16 mV (Figure 6a). When incubated with HeLa cells in vitro, bare microspheres remain separated from the cells (Figure 6b) because of the negatively charged surface of the cell membrane. To create positive surface charges, we used polyethylenimine (PEI).^[33] We added fully dried TPEQBN microspheres into 5 wt% PEI solution and agitated for 2 h. The resulting PEI-coated AIE microspheres had a zeta potential of +26 mV (Figure 6c). PEI coating showed no apparent effects on the shape and lasing threshold of TPEQBN microbeads (Figure S4, Supporting Information). The PEI-coated particles showed affinity with HeLa cells and resulted in cellular uptake after overnight incubation in the culture condition (Figure 6d). TPEQBN microbeads are internalized into HeLa cells through macropinocytosis.[34] Confocal fluorescence microscopy confirmed internalization of 4 µm microspheres (Figure 6e). There were no differences in cell viability for cells with microspheres over 48 h (Figure 6f). We pumped the intracellular microspheres and obtained above-threshold WGM spectra from individual microspheres (Figure 6g,h). The modal peak wavelengths were stable within <0.2 nm over 2.5 h as measured every 30 min (Figure S5, Supporting Information). The 4 μ m intracellular TPEQBN microbead laser is two times smaller in diameter or eight times smaller in volume, compared to conventional dye-doped polystyrene microbeads.^[8,9,14]

3. Conclusions

The high gain and high refractive index of AIE aggregates are desirable characteristics for their applications as the gain medium and cavity material. Although higher gain coefficients and refractive indices are available with perovskites and inorganic semiconductors, the organic small-molecular nature of AIE dyes is a distinct advantage over the solid-state crystals. It may be possible to combine AIE dyes with high-index dielectrics to further miniaturize laser devices. With high brightness and barcode-like cavity-mode features, AIE microspheres may be useful in multiplexed imaging and assays. Our work paves the way for many applications of aggregated-induced stimulated emission.

4. Experimental Section

Materials: TPEQBN was purchased from Lumtec. Poly(vinyl alcohol) (PVA; M_w 13 000–23 000, 87–89%) and polyethyleneimine (PEI; $M_n \approx 1800$, $M_w \approx 2000$) were purchased from Sigma-Aldrich.

Microsphere Fabrication: The microdroplet generation was done with glass microfluidic chips (14 μ m channel size, Dolomite) connected to pressure pumps (Flow EZ, Fluigent), using 1 wt% TPEQBN in methylene



chloride as oil phase and 2 wt% PVA in water as continuous phase, forming an oil-in-water emulsion. The emulsion was left at room temperature for 12 h without agitation until the methylene chloride in oil phase fully evaporated. Solidified TPEQBN microbeads were washed by three rounds of centrifugation (1000 rcf, 2 min) and resuspended in water to remove residual surfactants. Microsphere size was controlled by adjusting the flow rates of the oil phase and continuous phase. All processes were performed in glass vials.

Optical Characterizations of Thin Films: TPEQBN thin films were fabricated on quartz substrates by spin coating at 1000 rpm for 1 min, followed by 70 °C baking for 5 min. A frequency-doubled 382 nm picosecond pulsed laser (VisIR-765, PicoQuant), a photon counting avalanche photodiode (PDM series, Micro Photonics Devices), and spectrograph (shamrock 303i, Andor) were used for transient PL measurements. A variable-angle spectroscopic ellipsometer (J.A. Woollam M-2000FI) was used to measure the optical constants as well as the thickness of TPEQBN thin films. The incident angle was varied from 55° to 75° in steps of 5°. The collected data were analyzed using Complete EASE software (J.A. Woollam Co. Inc.). A stylus profilometer was used in the thickness measurement. Femtosecond pump-probe absorption spectroscopy was performed with a femtosecond pulsed laser and transient absorption spectrometer (Helios, Ultrafast Systems). A Ti:sapphire regenerative amplifier (Spitfire, Spectra-Physics) and optical parametric amplifier (TOPAS, Spectra-Physics) were used. The pump beam wavelength was 365 nm. Repetition rates of the probe and pump beams were 1000 and 500 Hz, respectively.

Optical Characterizations of Microbeads: A custom-built hyperspectral microscope was used for PL measurements.^[11] The setup used a 50×, 0.6-NA air objective lens (Nikon), a motorized XY translational sample stage (MLS-203, Thorlabs), a nanosecond pump laser (Opolette HE 355 LD, Optotek), an EMCCD camera for wide-field imaging, and an EMCCD-coupled spectrometer (Newton, Andor) with a spectral resolution of 0.1 nm.

Electron Microscopy: SEM images were obtained using a Phenom Pharos (Nanoscience) at 5 kV.

Cell Culture Experiments: Red fluorescence protein (RFP)-expressing HeLa human cervical cancer cell-line cells (from GenTarget InC) and normal HeLa cells (from ATCC) were cultured in Dulbecco's modified Eagle medium supplemented with 10% v/v fetal bovine serum and 1% v/v penicillin–streptomycin at 37 °C in a humidified CO₂ incubator. For cytotoxicity evaluation, HeLa cells were seeded in a 96-well plate at \approx 10 000 cells per well and incubated for 24 h for settlement. Then, the cells were immersed with fresh whole media containing microspheres in different particle-to-cell ratios and incubated for 24 or 48 h. Their viability was quantified using CCK-8 assay (ApexBio). Cells without incubation with microspheres were used as a control normalization group. Fluorescence microscope images were taken by confocal laser scanning microscopy (Olympus FV3000).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

S.-H.Y. has financial interests in LASE Innovation Inc., a company focused on commercializing technologies based on laser particles that were re-

viewed and are managed by Massachusetts General Brigham in accordance with their conflict of interest policies. All other authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

aggregation-induced emission, cell tagging, microlasers, optical gain, stimulated emission

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