Supporting Information

Non-invasively visualizing cell-matrix interactions in two-photon excited supramolecular hydrogels

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1. General Information

Chemical reagents and solvents were purchased from Aladdin and used without further purification. ^{1}H NMR and ^{13}C NMR were obtained on a Bruker Advance III 400 Instrument operating at 400 MHz. HRMS were recorded on a Water Q-Tof Mass Instrument. Fluorescence images were taken on a Olympus IX73 microscope and a two photon microscope (A1RMP, Nikon, Tokyo, Japan).

2. Experimental Procedures

SEM: Samples were prepared by depositing dilute solutions (approximately 0.5 mg/ml) of gelators on silicon wafer, freeze dried overnight, and sprayed with a thin gold layer. SEM images were taken on a FEI QUANTA 250 microscope.

Single-photon and two-photon fluorescence microscope: Samples were prepared by placing 200 μL of flocculent hydrogels on a glass slide, washed with deionized water for three times, and then freeze dried

overnight. Fluorescence images were taken on a Olympus IX73 microscope and a two photon microscope (A1RMP, Nikon, Tokyo, Japan).

UV-Vis absorption: The solution (2×10^{-5} mol/L) of G_1-G_3 were tested using instrument of Lambda 20 from Perkin Elmer, Inc., USA, respectively.

Fluorescence spectra: The solution (2×10^{-5} mol/L) of G_1-G_3 were tested using LS 50B from Perkin Elmer, Inc., USA, respectively.

Preparation of single crystals G_2-G_3: Crystals suitable for X-ray diffraction was obtained by slow evaporation in n-hexane/tetrahydrofuran/dichloromethane (v/v/v 1:1:1) and n-hexane/tetra-hydrofuran (v/v 1:1) solution at room temperature for G_2 and G_3, respectively.

Single crystal X-ray diffraction: Single-crystal data were collected on a Bruker SMART Apex II CCD-based X-ray diffractometer with Mo-Kαradiation (λ = 0.71073 Å) at 293 K. The empirical absorption correction was applied by SADABS program (G. M. Sheldrick, SADABS, program for empirical absorption correction of area detector data; University of Göttingen, Göttingen, Germany, 1996). The structure was solved using direct method, and refined by full-matrix least-squares on F2 (G. M. Sheldrick, SHELXTL97, program for crystal structure refinement, University of Göttingen, Germany, 1997).

X-ray powder diffraction (PXRD): Xerogels and crystals of G_1-G_2 were tested using a D8 Advance instrument from Bruker-AXS Company.

Rheology measurements: The rheological properties of hydrogels G_1-G_3 were measured with a Rotary Rheometer (Gemini HRnano). The dynamic frequency sweep measurements were performed using a sinusoidal shear strain of constant peak amplitude (0.01%) over a range of frequencies (0.1-100 $rads^{-1}$) at 25℃.

3. Additional Experimental Data and Figures

3.1 Gelation properties of **G_1 –G_{14}**.

For 7-substituted coumarin-derived gelators by pyridine (G_4-G_5, G_7-G_8, G_{10}-G_{11}, G_{13}-G_{14}), they could not form hydrogels in water/DMSO (99.5%w/0.5%w) solution at room temperature (RT) by "solvent-mediated"method. While, they can form gels in water/DMSO (50%w/50%w) under heating (over 50℃) and cooling by "temperature-mediated" method (Figure 1 and S1). The result suggested that gelation property has no direct relationship with 4-methyl coumarin or 4-hydrogen coumarin in those chemical structures. However, if substitue pyridine group was replaced by benzene (G_1, G_2), the gelation property was closely related with 4-methyl coumarin or 4-hydrogen coumarin. G_1 could form hydrogels in water/DMSO (99.5%w/0.5%w) at room temperature (RT) by "solvent-mediated"method (Figure 1 and S1), while, G_2 only self-assembled into hydrogel in water/DMSO (50%w/50%w) under heating and cooling by "temperature-mediated" method.

For 6-substituted coumarin-derived gelators by pyridine (G_6, G_9, G_{12}), all of them could self-assemble into hydrogels in water/DMSO (95.5%w/0.5%w) at room temperature (RT) by "solvent-mediated"method. While, substitute benzene group based gelator (G_3) only formed gel in water/DMSO (50%w/50%w) under heating and cooling by "temperature-mediated" method. Clearly, pyrindine or benzene has influence on the self-assembly ability of gelators. If pyridine or benzene was replaced by other analogous groups (NG_1, NG_2), they could not form hydrogels either in water/DMSO (99.5%w/0.5%w) or in water/DMSO (50%w/50%w) solution, demonstrating the important role of pyridine or benzene groups for this type of gelators. Thus, G_1, G_6, G_9, and G_{12} should have stronger self-assembly ability in aqueous solution compared with other gelators, which may be ascribed to the different chemical structures of gelators.

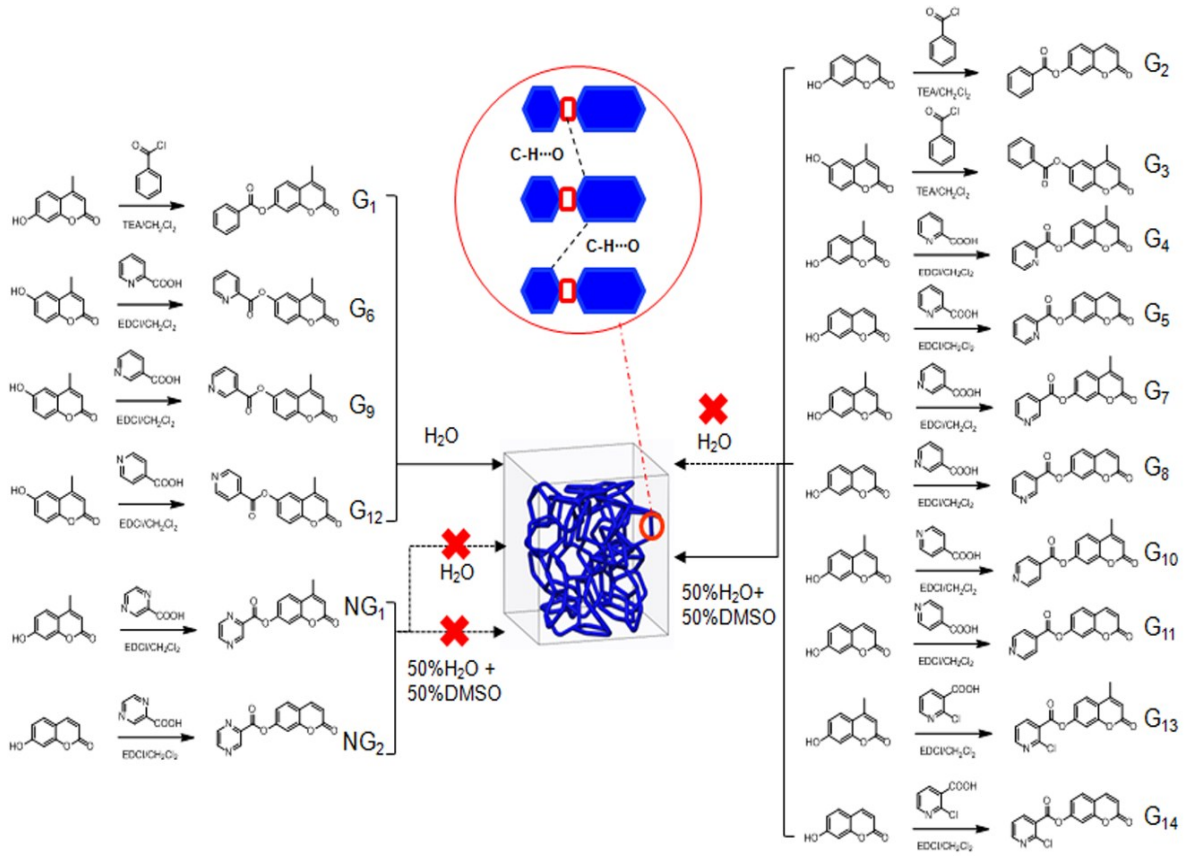


Figure S1 Schematic demonstration of gelators self-assembly in water/DMSO (99.5%w/0.5%w or 50%w/50%w) through C-H···O bonds. G_1, G_6, G_9, and G_{12} could self-assemble in aqueous solution under room temperature (final DMSO concentration of 0.5%). G_2-G_5, G_7-G_8, G_{10}-G_{11}, G_{13}-G_{14} only self-assemble into gels in water/DMSO (50%w/50%w) by heating and cooling. NG_1 and NG_2 could not form hydrogels in water/DMSO (99.5%w/0.5%w or 50%w/50%w) solution.

3.2 Photographs of the hydrogels self-assembled from $\mathbf{G_1}$ –$\mathbf{G_{14}}$.

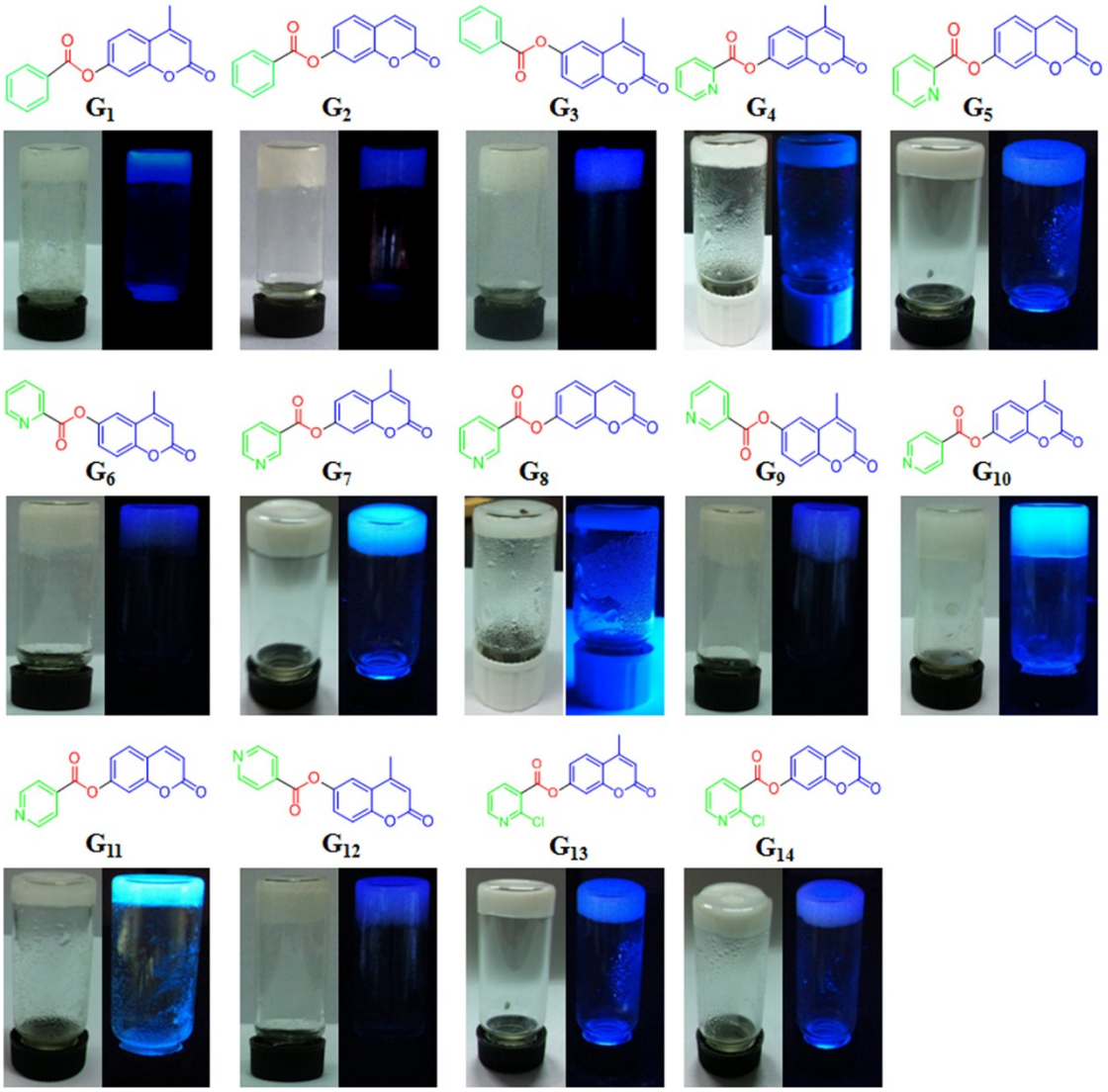


Figure S2 Photographs of the hydrogels from G_1 –G_{14} before and after UV light irradiation (365 nm).

3.3 Rheological properties of hydrogels $\mathbf{G_1}$-$\mathbf{G_3}$.

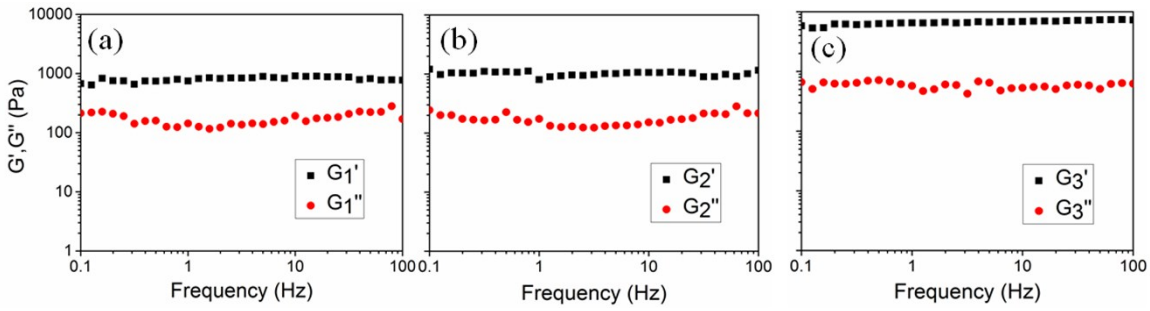


Figure S3 Rheological measurement of frequency sweep for hydrogels (a):G_1; (b):G_2; (c):G_3 at a strain of 0.01% over a range of 0.1-100 $rads^{-1}$.

3.4 The assembly ability of **G_1-G_3** in water.

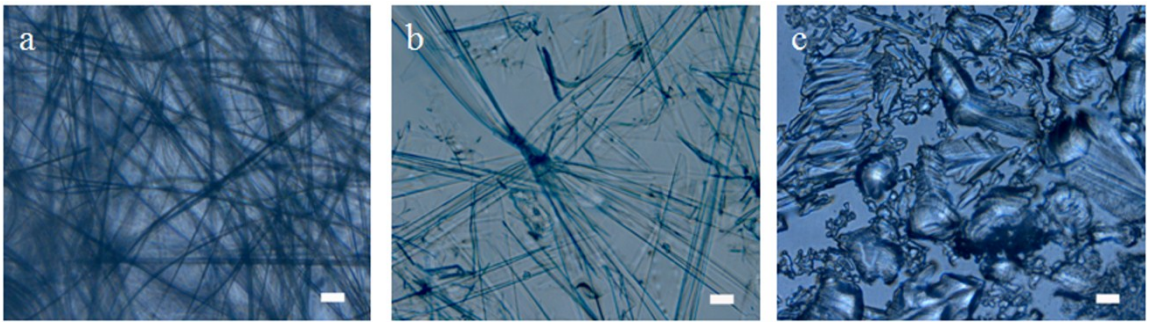


Figure S4 Optical microscope images of G_1-G_3 assembly in water. The result suggested that G_1 have the strongest capability of self-assembling into fibers but **G_2** and **G_3** formed crystals. Scale bar = 20 μm.

3.5 Crystal and experimental data of **G_2**.

Table S1A Crystal data and structure refinement for **G_2**.

Empirical formula	$C_{16}H_{10}O_4$
Formula weight	266.24
Temperature	298(2) K
Wavelength	1.54178 Å
Crystal system, space group	Monoclinic, P 21/c
Unit cell dimensions	a = 6.6490(5) Å α = 90°
	b = 28.779(2) Å β = 115.909(3)°
	c = 7.2200(6) Å γ = 90°
Volume	1242.70(16) $Å^3$
Z, Calculated density	4, 1.423 Mg/m^3
Absorption coefficient	0.857 mm^{-1}
F(000)	552
Crystal size	0.20 x 0.10 x 0.08 mm
Theta range for data collection	3.07 to 68.41°
Limiting indices	-7<=h<=8, -34<=k<=34, -8<=l<=8
Reflections collected / unique	11930 / 2266 [R(int) = 0.0235]
Completeness to theta = 25.242	99.4 %
Max. and min. transmission	0.9346 and 0.8474
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2266 / 0 / 182
Goodness-of-fit on F^2	1.050
Final R indices [I>2sigma(I)]	R1 = 0.0355, wR2 = 0.1046
R indices (all data)	R1 = 0.0423, wR2 = 0.1110
Extinction coefficient	0.0060(9)
Largest diff. peak and hole	0.187 and -0.133 e. $Å^{-3}$

Table S1B Bond lengths [Å] and angles [deg] for **G_2**.

O(1)-C(7)	1.1978(17)
O(2)-C(7)	1.3589(16)
O(2)-C(8)	1.3938(15)
O(3)-C(10)	1.3788(14)
O(3)-C(14)	1.3827(16)
O(4)-C(14)	1.2064(16)

C(1)-C(2)	1.382(2)
C(1)-C(6)	1.386(2)
C(1)-C(7)	1.4816(19)
C(2)-C(3)	1.381(2)
C(2)-H(2A)	0.9300
C(3)-C(4)	1.366(3)
C(3)-H(3A)	0.9300
C(4)-C(5)	1.365(3)
C(4)-H(4A)	0.9300
C(5)-C(6)	1.393(2)
C(5)-H(5A)	0.9300
C(6)-H(6A)	0.9300
C(8)-C(9)	1.3772(18)
C(8)-C(13)	1.3927(18)
C(9)-C(10)	1.3805(18)
C(9)-H(9A)	0.9300
C(10)-C(11)	1.3920(16)
C(11)-C(12)	1.4001(18)
C(11)-C(16)	1.4332(17)
C(12)-C(13)	1.3739(19)
C(12)-H(12A)	0.9300
C(13)-H(13A)	0.9300
C(14)-C(15)	1.4428(19)
C(15)-C(16)	1.3329(19)
C(15)-H(15A)	0.9300
C(16)-H(16A)	0.9300
C(7)-O(2)-C(8)	122.67(11)
C(10)-O(3)-C(14)	121.97(10)
C(2)-C(1)-C(6)	119.46(14)
C(2)-C(1)-C(7)	122.54(13)
C(6)-C(1)-C(7)	118.00(14)
C(3)-C(2)-C(1)	120.13(16)
C(3)-C(2)-H(2A)	119.9
C(1)-C(2)-H(2A)	119.9
C(4)-C(3)-C(2)	120.49(19)
C(4)-C(3)-H(3A)	119.8
C(2)-C(3)-H(3A)	119.8
C(5)-C(4)-C(3)	119.96(16)
C(5)-C(4)-H(4A)	120.0
C(3)-C(4)-H(4A)	120.0
C(4)-C(5)-C(6)	120.61(17)
C(4)-C(5)-H(5A)	119.7
C(6)-C(5)-H(5A)	119.7
C(1)-C(6)-C(5)	119.35(17)
C(1)-C(6)-H(6A)	120.3
C(5)-C(6)-H(6A)	120.3
O(1)-C(7)-O(2)	123.57(13)
O(1)-C(7)-C(1)	125.74(13)
O(2)-C(7)-C(1)	110.69(12)
C(9)-C(8)-C(13)	121.76(12)
C(9)-C(8)-O(2)	124.24(11)
C(13)-C(8)-O(2)	113.90(11)
C(8)-C(9)-C(10)	117.49(11)
C(8)-C(9)-H(9A)	121.3
C(10)-C(9)-H(9A)	121.3
O(3)-C(10)-C(9)	116.32(10)
O(3)-C(10)-C(11)	120.61(11)

C(9)-C(10)-C(11)	123.07(12)
C(10)-C(11)-C(12)	117.36(12)
C(10)-C(11)-C(16)	118.11(11)
C(12)-C(11)-C(16)	124.53(11)
C(13)-C(12)-C(11)	121.00(11)
C(13)-C(12)-H(12A)	119.5
C(11)-C(12)-H(12A)	119.5
C(12)-C(13)-C(8)	119.29(12)
C(12)-C(13)-H(13A)	120.4
C(8)-C(13)-H(13A)	120.4
O(4)-C(14)-O(3)	116.23(13)
O(4)-C(14)-C(15)	126.75(13)
O(3)-C(14)-C(15)	117.02(11)
C(16)-C(15)-C(14)	121.56(12)
C(16)-C(15)-H(15A)	119.2
C(14)-C(15)-H(15A)	119.2
C(15)-C(16)-C(11)	120.69(12)
C(15)-C(16)-H(16A)	119.7
C(11)-C(16)-H(16A)	119.7

Table S1C Geometrical parameters of hydrogen bonds in crystal $\mathbf{G_2}$.

D-H···A	D-H(Å)	H···A(Å)	D···A(Å)	D-H···A(deg)	Symmetry-for-A
Intra C2-H2A...O2	0.93	2.40	2.7136	100	---
C4-H4A…O4	0.93	2.49	3.2719	142	1-x,1/2+y,3/2-z
Intra C9-H9A...O1	0.93	2.39	2.8244	108	---
C12-H12A…O3	0.93	2.53	3.4378	166	-1+x,y,z
C16-H16A…O4	0.93	2.47	3.3583	160	-1+x,y,z

:: No Classic Hydrogen Bonds Found

Table S1D Short Ring-Interactions with Cg-Cg Distances in crystal $\mathbf{G_2}$.

Analysis of Short Ring-Interactions with Cg-Cg Distances < 6.0 Angstrom	
Cg(I) = Plane number I	
Cg-Cg = Distance between ring Centroids (Ang.)	
Cg(I)	Cg-Cg
Cg(1) [1] -> Cg(1)	3.8101
Cg(1) [1] -> Cg(3)	3.6290
Cg(2) [1] -> Cg(2)	3.9568
Cg(3) [1] -> Cg(1)	3.6290
Cg(3) [1] -> Cg(3)	4.8155
Min or Max	3.629

3.6 Crystal and experimental data of $\mathbf{G_3}$.

Self-assembly mechanism: Compound $\mathbf{G_3}$ also gave crystals suitable for X-ray diffraction analysis upon crystallization from n-hexane/tetrahydrofuran solution with the same method to $\mathbf{G_2}$. Colorless single crystals of $\mathbf{G_3}$ belonged to a monoclinic space group P c and no classic hydrogen bonds or solvent molecules were found in self-assembly (Table S2A, S2B, S2C, for crystallographic details). It was found that the main driving forces in the self-assembly process are two types of C-H···O intermolecular hydrogen bonds. Firstly, head-tail fashion of one-dimension (1D) supramolecular polymer was formed through the intermolecular hydrogen bonds between the C=O of lactone and a hydrogen of benzene (C5-H5A···O4), which have the a bond length of 2.59 Å (H5A···O4). Then 1D supramolecular polymeric strands entangled with each other to form 3D crystal networks through intermolecular hydrogen bonds (C10-H10A···O1)

between the C=O of benzene and the 8-hydrogen atom of coumarin with the bond length of 2.51 Å (H10A···O1) (Table S2C). In addition, structure analysis of **G_3** shows that there were no π-π stacking occurred in the molecular self-assemby (the minimum Cg-Cg distances are > 4.0 Å, Table S2D).

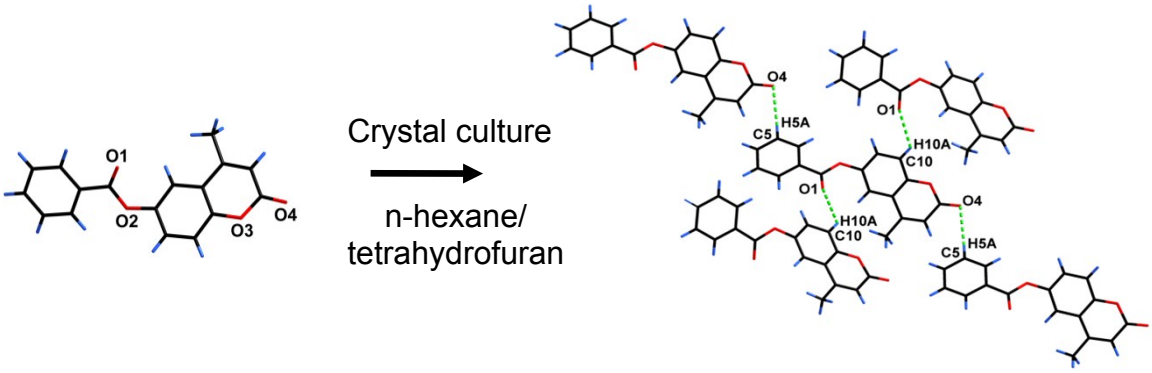


Table S2A Crystal data and structure refinement for **G_3**.

Empirical formula	$C_{17}H_{12}O_4$
Formula weight	280.27
Temperature	100(2) K
Wavelength	1.54178 Å
Crystal system, space group	Monoclinic, P c
Unit cell dimensions	a = 13.1267(4) Å α = 90°
	b = 3.86890(10) Å β = 93.9450(10)°
	c = 12.7810(4) Å γ = 90°
Volume	647.56(3) $Å^3$
Z, Calculated density	2, 1.437 Mg/m^3
Absorption coefficient	0.850 mm^{-1}
F(000)	292
Crystal size	0.20 x 0.10 x 0.05 mm
Theta range for data collection	3.37 to 68.13°
Limiting indices	-15<=h<=15, -4<=k<=4, -15<=l<=15
Reflections collected / unique	7022 / 2370 [R(int) = 0.0240]
Completeness to theta = 25.242	99.3 %
Max. and min. transmission	0.933 and 0.847
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2272 / 2 / 190
Goodness-of-fit on F^2	1.070
Final R indices [I>2sigma(I)]	R1 = 0.0371, wR2 = 0.1058
R indices (all data)	R1 = 0.0372, wR2 = 0.1060
Absolute structure parameter	0.02(18)
Largest diff. peak and hole	0.350 and -0.429 e. $Å^{-3}$

Table S2B Bond lengths [Å] and angles [deg] for **G_3**.

O(1)-C(7)	1.203(3)
O(2)-C(7)	1.369(3)
O(2)-C(8)	1.404(2)
O(3)-C(11)	1.381(2)
O(3)-C(16)	1.381(2)
O(4)-C(16)	1.208(2)
C(1)-C(6)	1.394(3)
C(1)-C(2)	1.397(3)
C(1)-C(7)	1.484(3)

C(2)-C(3)	1.389(3)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.382(3)
C(3)-H(3A)	0.9500
C(4)-C(5)	1.395(3)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.389(3)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(8)-C(13)	1.374(3)
C(8)-C(9)	1.391(3)
C(9)-C(10)	1.387(3)
C(9)-H(9A)	0.9500
C(10)-C(11)	1.388(3)
C(10)-H(10A)	0.9500
C(11)-C(12)	1.396(3)
C(12)-C(13)	1.406(3)
C(12)-C(14)	1.455(3)
C(13)-H(13A)	0.9500
C(14)-C(15)	1.347(3)
C(14)-C(17)	1.498(3)
C(15)-C(16)	1.447(3)
C(15)-H(15A)	0.9500
C(17)-H(17A)	0.9800
C(17)-H(17B)	0.9800
C(17)-H(17C)	0.9800
C(7)-O(2)-C(8)	119.02(15)
C(11)-O(3)-C(16)	121.71(15)
C(6)-C(1)-C(2)	119.93(18)
C(6)-C(1)-C(7)	117.98(17)
C(2)-C(1)-C(7)	122.08(17)
C(3)-C(2)-C(1)	119.9(2)
C(3)-C(2)-H(2A)	120.1
C(1)-C(2)-H(2A)	120.1
C(4)-C(3)-C(2)	120.0(2)
C(4)-C(3)-H(3A)	120.0
C(2)-C(3)-H(3A)	120.0
C(3)-C(4)-C(5)	120.56(19)
C(3)-C(4)-H(4A)	119.7
C(5)-C(4)-H(4A)	119.7
C(6)-C(5)-C(4)	119.6(2)
C(6)-C(5)-H(5A)	120.2
C(4)-C(5)-H(5A)	120.2
C(5)-C(6)-C(1)	120.0(2)
C(5)-C(6)-H(6A)	120.0
C(1)-C(6)-H(6A)	120.0
O(1)-C(7)-O(2)	123.21(18)
O(1)-C(7)-C(1)	125.50(19)
O(2)-C(7)-C(1)	111.29(16)
C(13)-C(8)-C(9)	122.07(18)
C(13)-C(8)-O(2)	122.09(18)
C(9)-C(8)-O(2)	115.64(17)
C(10)-C(9)-C(8)	119.40(18)
C(10)-C(9)-H(9A)	120.3
C(8)-C(9)-H(9A)	120.3
C(9)-C(10)-C(11)	118.70(18)
C(9)-C(10)-H(10A)	120.6

C(11)-C(10)-H(10A)	120.6
O(3)-C(11)-C(10)	116.02(17)
O(3)-C(11)-C(12)	121.56(16)
C(10)-C(11)-C(12)	122.42(18)
C(11)-C(12)-C(13)	118.03(17)
C(11)-C(12)-C(14)	118.04(18)
C(13)-C(12)-C(14)	123.93(18)
C(8)-C(13)-C(12)	119.38(18)
C(8)-C(13)-H(13A)	120.3
C(12)-C(13)-H(13A)	120.3
C(15)-C(14)-C(12)	118.76(18)
C(15)-C(14)-C(17)	121.90(17)
C(12)-C(14)-C(17)	119.34(18)
C(14)-C(15)-C(16)	123.09(18)
C(14)-C(15)-H(15A)	118.5
C(16)-C(15)-H(15A)	118.5
O(4)-C(16)-O(3)	116.97(18)
O(4)-C(16)-C(15)	126.34(19)
O(3)-C(16)-C(15)	116.69(17)
C(14)-C(17)-H(17A)	109.5
C(14)-C(17)-H(17B)	109.5
H(17A)-C(17)-H(17B)	109.5
C(14)-C(17)-H(17C)	109.5
H(17A)-C(17)-H(17C)	109.5
H(17B)-C(17)-H(17C)	109.5

Table S2C Geometrical parameters of hydrogen bonds in crystal $\mathbf{G_3}$.

D-H···A	D-H(Å)	H···A(Å)	D···A(Å)	D-H···A(deg)	Symmetry-for-A
C5-H5A...O4	0.95	2.59	3.5170	164	-1+x,-1+y,z
C10-H10A…O1	0.95	2.51	3.1452	124	x,2-y,-1/2+z

:: No Classic Hydrogen Bonds Found

Table S2D Short Ring-Interactions with Cg-Cg Distances in crystal $\mathbf{G_3}$.

Analysis of Short Ring-Interactions with Cg-Cg Distances < 6.0 Angstrom	
Cg(I) = Plane number I	
Cg-Cg = Distance between ring Centroids (Ang.)	
Cg(I)	Cg-Cg
Cg(1) [1] -> Cg(3)	4.6556
Cg(3) [1] -> Cg(1)	4.4618
Min or Max	4.462

3.7 Power XRD patterns of crystal and xerogel from hydrogelators $\mathbf{G_2}$ and $\mathbf{G_3}$.

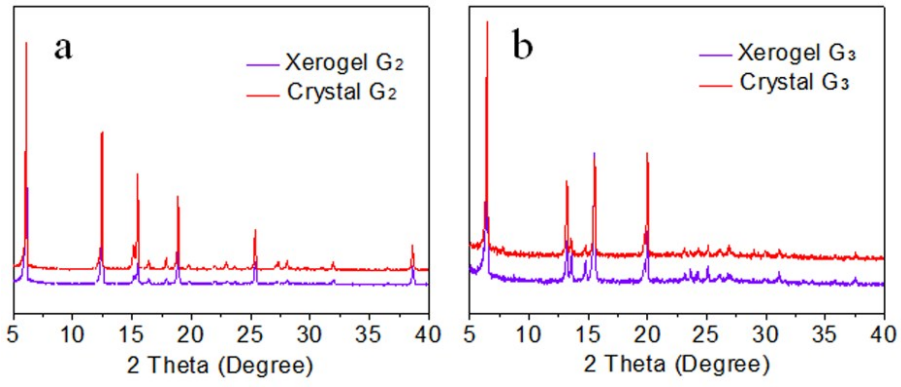


Figure S5 Power XRD patterns of crystals and xerogels self-assembled from hydrogelators a) G_2 and b) G_3. Here, PXRD patterns of two states could be essentially same with each other, implying the same packing modes in both single crystal and xerogel state.

3.8 Fluorescence spectra of hydrogelators **G_1-G_3**.

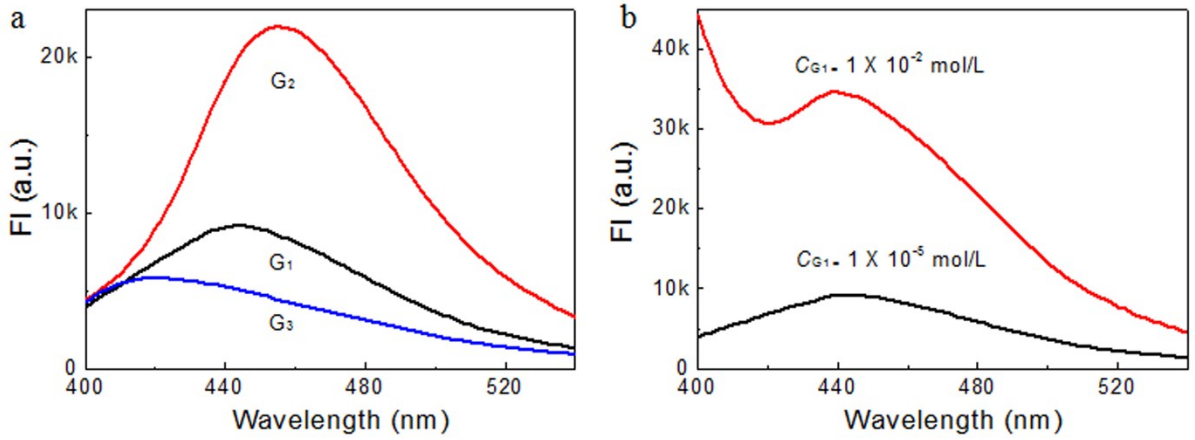


Figure S6 a) The fluorescent emission spectra of G_1-G_3 nanofibrous solution, respectively. b) Fluorescence spectra of nanofibrous solution with increasing G_1 concentration.

3.9 Fluorescent images of the nanofibers self-assembled from **G_2**, **G_3**.

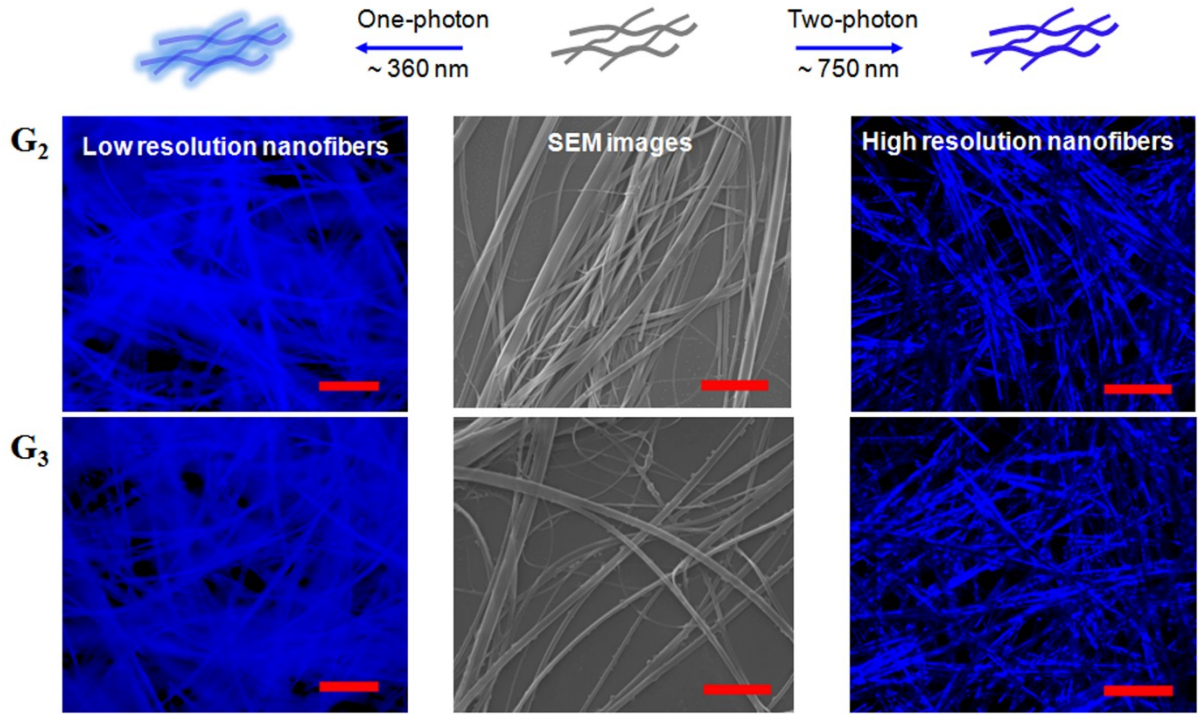


Figure S7. SEM image of G_2 and G_3 nanofibers. Scale bar = 5 μm. Single-photon fluorescent images of G_2 and G_3 nanofibers under UV excitation. Scale bar = 300 μm. Two-photon fluorescent images of G_2 and G_3 nanofibers under NIR excitation. Scale bar = 50 μm.

3.10 Fluorescent images of A549 cells cultured on G_1 hydrogels films.

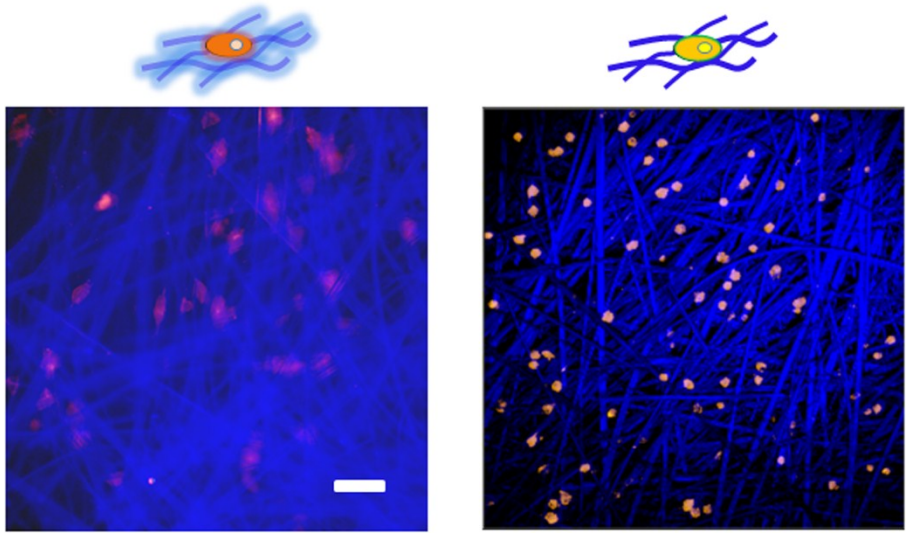


Figure S8. 2D cell-substrate imaging blue nanofibers under the excitation of a) UV light at the wavelength of 360-370 nm and b) NIR light at the wavelength of 750 nm. Red or yellow A549 cells are excited at the wavelenght of 530-550 nm or 544 nm. The images are overlay of the nanofibers and cells at the same sample site. Scale bar = 50 μm.

3.11 Cell viability of A549 cells incubated with hydrogelators G_1-G_3.

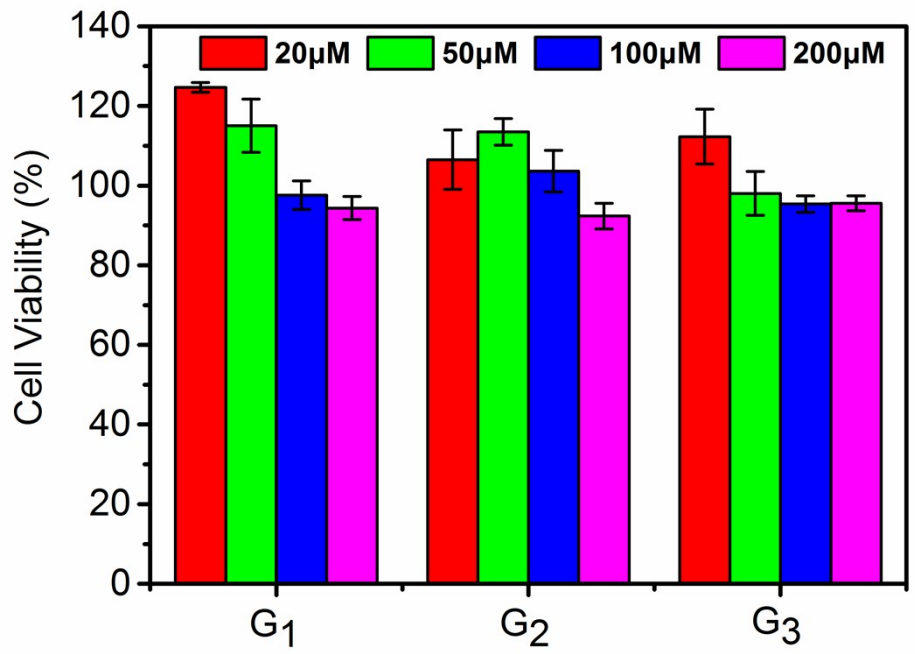


Figure S9. After culturing human lung carcinoma (A549) cells in the presence of hydrogelators G_1, G_2, or G_3 (the concentration: 20-200 μM) for 24 hours, over 90% (by CCK-8 assays) of survival cells suggested the good biocompatibility for all of them.

3.12 Two-photon excited fluorescent images of A549 cells cultured in 3D G_1 hydrogels.

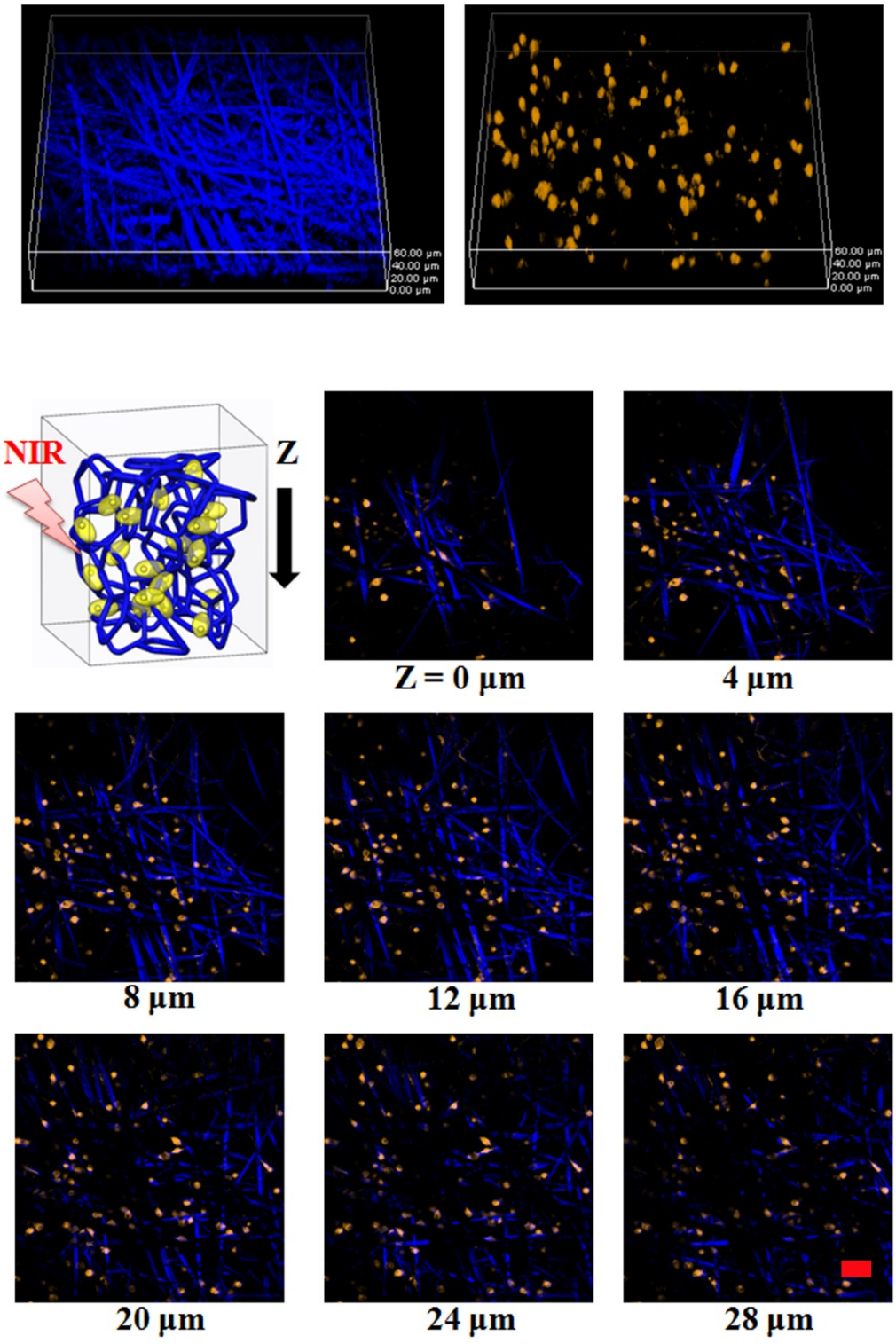


Figure S10. above) Volume view of 3D cell-substrate imaging. below) Slices view of the overlaid images of both nanofibers and A549 cells at the same depths. The blue nanofibers are excited by NIR laser at the wavelength of 750 nm. Yellow cells are excited at the wavelenght of 544 nm. Scale bar = 20 µm.

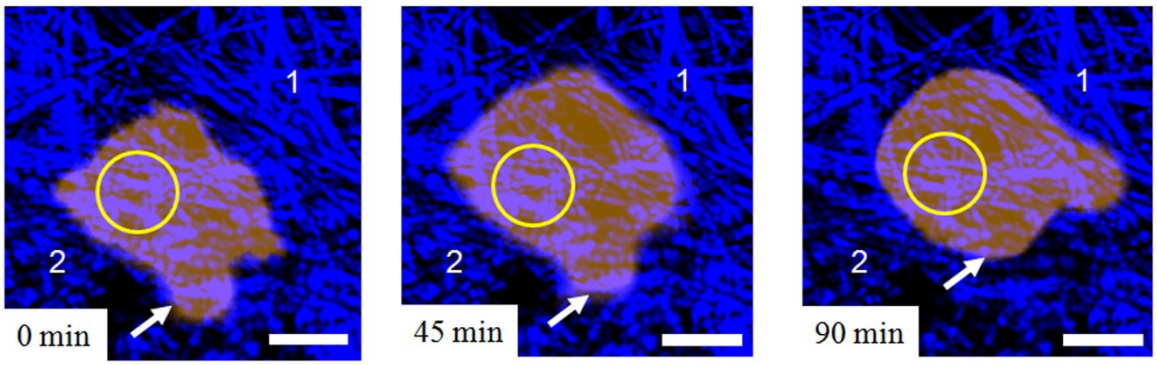
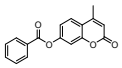
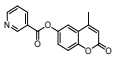
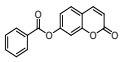
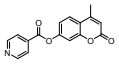
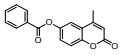
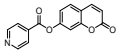
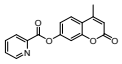
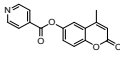
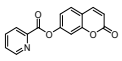
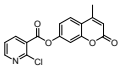
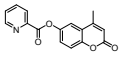
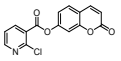
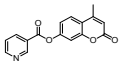
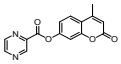
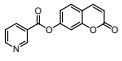
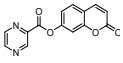


Figure S11. The simultaneous confocal fluorescent images of cells and nanofibers in the same scanning transverse section after 8 h culture and the dynamic imaging of cells migrating through 3D nanofibrous structures from 0 to 90 min. Scale bar = 10 μm.

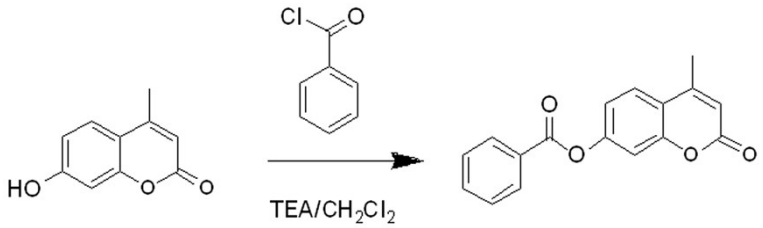
Table S3. Coumarin-based supramolecular platform for cell-matrix imaging.

Entry	Molecular Structure	Gelation solvent	Temperature (℃)	Cell imaging	Entry	Molecular Structure	Gelation solvent	Temperature (℃)	Cell imaging
G_1		PBS(0.5%DMSO)	RT[a]	2D/3D	G_9		PBS(0.5%DMSO)	RT[a]	2D/3D
G_2		PBS(50%DMSO)	88[b]	2D	G_{10}		PBS(50%DMSO)	93[b]	2D
G_3		PBS(50%DMSO)	78[b]	2D	G_{11}		PBS(50%DMSO)	95[b]	2D
G_4		PBS(50%DMSO)	85[b]	2D	G_{12}		PBS(0.5%DMSO)	RT[a]	2D/3D
G_5		PBS(50%DMSO)	82[b]	2D	G_{13}		Methanol	45[b]	2D
G_6		PBS(0.5%DMSO)	RT[a]	2D/3D	G_{14}		Methanol	53[b]	2D
G_7		PBS(50%DMSO)	87[b]	2D	NG_1		Non-gelator	---	---
G_8		PBS(50%DMSO)	92[b]	2D	NG_2		Non-gelator	---	---

Gel preparation methods: (a) “solvent-mediated” method; (b) “temperature-mediated” method.

4. Synthesis and Characterizations

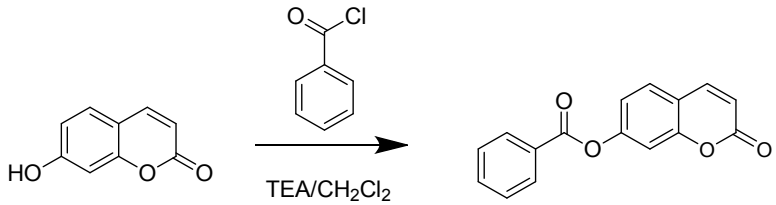
Synthesis of G_1:



A solution of 7-hydroxy-4-methylcoumarin (1.76 g, 10 mmol) and TEA(1.50 g, 15 mmol) in CH_2Cl_2 (20 ml) was stirred at 0°C for 30 min, then a solution of benzoyl chloride (2.10 g, 15 mmol) in CH_2Cl_2(5 ml) was added dropwise. The mixture was stirred for overnight at room temperature. The resulting solution was washed three times with water (5 ml), saturated $NaHCO_3$ solution (5 ml). Then the organic layer was collected and dried over anhydrous Na_2SO_4. Purification with column chromatography (CH_2Cl_2:EA=5:1) gave the product (2.32 g, 83%)as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.19(dd, J_1=1.6Hz J2=0.8Hz, 2H), 7.65(d, J=8.4Hz, 2H), 7.53(t, J_1=8.0Hz, 2H), 7.22(m, 2H), 5.28(s, 1H), 2.45(s, 3H). ^{13}C NMR (400MHz, $CDCl_3$) δ = 164.7, 160.7, 154.5, 153.6, 152.2, 134.3, 130.5, 129.1, 129.0, 128.9, 125.7, 118.5, 118.1, 114.8, 110.8, 18.9. HRMS (ESI) calcd for $C_{17}H_{12}O_4$ $[M+H]^+$ 280.0736; found 281.0828.

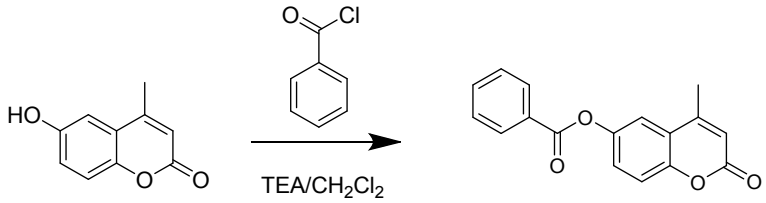
G_2-G_3 were prepared using the same procedure for **G_1**.

Synthesis of G_2:



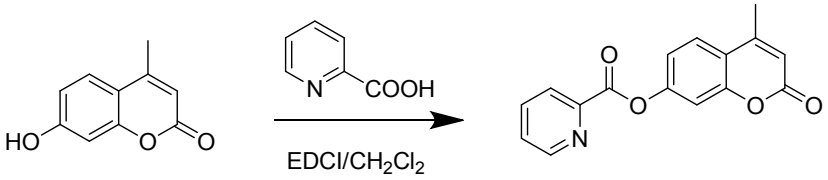
7-hydroxycoumarin (1.62 g, 10 mmol), benzoyl chloride (2.10 g,15 mmol) and TEA (1.50 g, 15 mmol) in CH_2Cl_2 (25 ml) yielded **G_2** as white powder (2.12 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.20(t, J=3.2Hz, 2H), 7.65-7.71(m, 2H), 7.53(t, J=8.0Hz, 3H), 7.25(s, 1H), 7.20(m, 1H), 6.41(d, J=9.6Hz, 1H). ^{13}C NMR (400MHz, $CDCl_3$) δ = 164.7, 160.5, 154.9, 153.7, 143.1, 134.3, 130.5, 128.9, 128.8, 118.8, 116.9, 116.3, 110.8. HRMS (ESI) calcd for $C_{16}H_{10}O_4$ $[M+H]^+$ 266.0579; found 267.0671.

Synthesis of G_3:



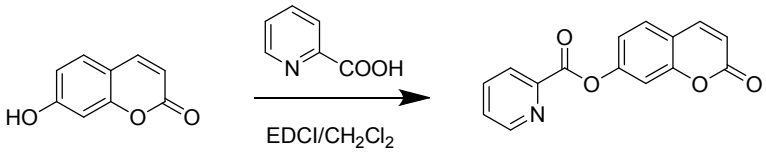
6-Hydroxy-4-methylcoumarin (1.76 g, 10 mmol), benzoyl chloride (2.10 g, 15 mmol) and TEA(1.50 g, 15 mmol) in CH_2Cl_2 (25 ml) yielded **G_3** as white powder (2.46 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.19(s, 2H), 7.65(d, J=7.6Hz, 1H), 7.53(t, J=8.0Hz, 2H), 7.46(t, J_1=0.8Hz, 1H), 7.38(d, J=0.8Hz, 1H),7.25(s, 1H), 6.33(s, 1H), 2.42(s, 3H). ^{13}C NMR (400MHz, $CDCl_3$) δ = 165.1, 160.3, 151.6, 150.9, 146.8, 133.9, 130.1, 130.0, 128.8, 128.6, 128.3, 125.3, 120.5, 118.0, 117.3, 115.6, 18.6. HRMS (ESI) calcd for $C_{17}H_{12}O_4$ $[M+H]^+$ 280.0736; found 281.0812.

Synthesis of G_4:



4-Methylumbelliferone (1.76 g, 10 mmol), 2-Picolinic Acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_4** as white powder (2.44 g, 85.01%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.87(d, J=4.0Hz, 1H), 8.30(d, J=8.0Hz, 1H), 7.95(t, J=2.0Hz, 1H), 7.68(d, J=8.0Hz, 1H), 7.60(t, J=4.0Hz, 1H), 7.30(d, J=2.0Hz, 1H), 7.25(s, 1H), 6.30(s, 1H), 2.46(d, J=1.2Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.4, 160.7, 154.3, 153.4, 152.2, 150.4, 146.9, 137.6, 128.0, 126.3, 125.7, 118.4, 114.8, 110.9, 18.9. HRMS (ESI) calcd for $C_{16}H_{12}NO_4$ 282.0766 $[M+H]^+$; found 282.0771.

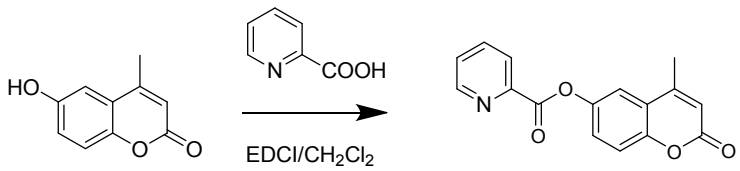
Synthesis of G_5:



To a solution of 7-hydroxycoumarin (1.62 g, 10 mmol), 2-Picolinic Acid (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) was added EDCI (2.10 g, 11 mmol). The mixture was stirred for 2 hours at room temperature. The resulting solution was washed three times with water (5 ml), saturated $NaHCO_3$ solution (5 ml). Then the organic layer was collected and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give the product **G_5** as white powder (2.45 g, 91.76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.87(d, J=4.0Hz, 1H), 8.29(d, J=7.6Hz, 1H), 7.95(t, J=8.0Hz, 1H), 7.72(d, J=9.6Hz, 1H), 7.60(t, J=4.0Hz, 1H), 7.55(d, J=8.4Hz, 1H), 7.3(s, 1H), 7.23(d, J=8.0Hz, 1H), 6.43(d, J=9.6Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.4, 160.4, 154.8, 153.5, 150.4, 146.9, 143.0, 137.6, 128.9, 128.0, 126.3, 118.7, 117.1, 116.4, 110.8. HRMS (ESI) calcd for $C_{15}H_{10}NO_4$ 268.0610 $[M+H]^+$; found 268.0609.

Other gelators were prepared by using the similar above procedure.

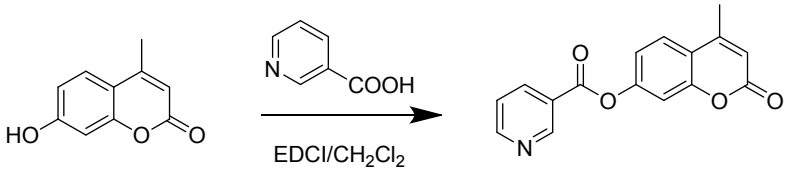
Synthesis of G_6:



To a solution of 6-hydroxy-4-methylcoumarin (1.76 g, 10 mmol), 2-Picolinic Acid (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) was added EDCI (2.10 g, 11 mmol). The mixture was stirred for 2 hours at room temperature. The resulting solution was washed three times with water (5 ml), saturated $NaHCO_3$ solution (5 ml). Then the organic layer was collected and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give the product **G_6** as white powder (2.50 g, 88.96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.84(dd, J_1=4.8Hz J_2=0.8Hz, 1H), 8.27(dd, J_1=8.0Hz J_2=0.8Hz, 1H), 7.93(td, J_1=7.6Hz J_2=1.2Hz, 1H), 7.58(m, 1H), 7.50(d, J=2.4Hz, 1H), 7.43(dd, J_1=8.8Hz J_2=2.0Hz, 1H), 7.38(d, J=8.8Hz, 1H), 6.32(s, 1H), 2.40(s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.9, 160.4, 151.8, 151.3, 150.2,

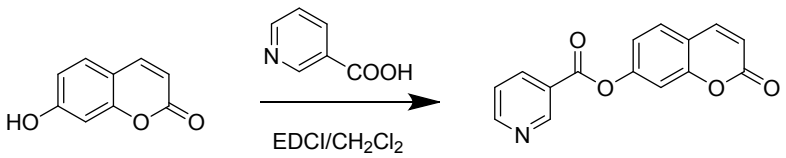
147.0, 146.9, 137.4, 127.8, 126.1, 125.4, 120.8, 118.2, 117.4, 115.9, 18.7. HRMS (ESI) calcd for $C_{16}H_{11}NO_4$ [M+H]+ 282.0766; found 282.0739.

Synthesis of G_7:



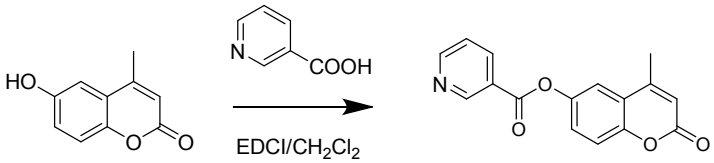
4-Methylumbelliferone (1.76 g, 10 mmol), Nicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_7** as white powder (2.51 g, 89.32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=9.4(s, 1H), 8.89(dd, J_1=4.8Hz J_2=1.6Hz, 1H), 8.46(d, J=8.0Hz, 1H), 7.68(d, J=8.8Hz, 1H), 7.48-7.51(m, 1H), 7.27(d, J=2.0Hz, 1H), 7.22(dd, J_1=8.8Hz J_2=2.4Hz, 1H), 6.31(d, J=1.2Hz, 1H), 2.47(d, J=1.2Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.5, 160.5, 154.6, 154.4, 153.0, 152.0, 151.6, 137.9, 125.8, 125.1, 123.7, 118.4, 118.2, 115.0, 110.7, 18.9. HRMS (ESI) calcd for $C_{16}H_{12}NO_4$ 282.0766 $[M+H]^+$; found 282.0755.

Synthesis of G_8:



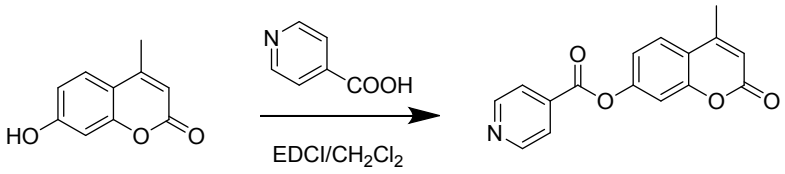
7-hydroxycoumarin (1.62 g, 10 mmol), Nicotinic acid(1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_8** as white powder (2.35 g, 88.01%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.40 (d, J = 1.6 Hz, 1H), 8.89 (dd, J_1 = 4.8 Hz J_2 = 1.6Hz, 1H), 8.49 (dt, J_1 = 8.0 Hz J_2 = 1.6 Hz, 1H), 7.73 (d, J = 9.6Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.51-7.54 (m, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.20 (dd, J_1 = 8.4 Hz J_2 = 2.4 Hz, 1H), 6.44(d, J = 9.6 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.5, 160.4, 154.9, 154.6, 153.1, 151.6, 143.0, 137.9, 129.0, 125.1, 123.8, 118.5, 117.2, 116.5, 110.7. HRMS (ESI) calcd for $C_{15}H_{10}NO_4$ 268.0610 $[M+H]^+$; found 268.0610.

Synthesis of G_9:



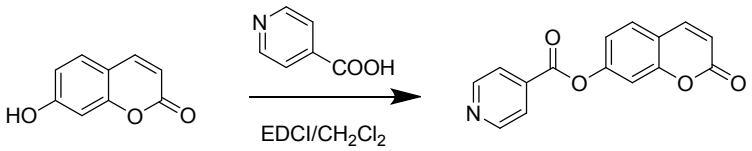
6-Hydroxy-4-methylcoumarin (1.76 g, 10 mmol), Nicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) yielded **G_9** as white powder (2.43 g, 86.47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.40 (d, J = 2.0 Hz, 1H), 8.88 (dd, J_1 = 4.8 Hz J_2 = 1.6 Hz, 1H), 8.45 (dt, J_1 = 8.0 Hz J_2 = 1.6 Hz, 1H), 7.50 (m, 1H), 7.47 (t, J = 1.6 Hz, 1H), 7.40 (d, J = 2.4 Hz, 2H), 6.35 (d, J = 1.2 Hz, 1H), 2.43 (d, J = 1.2 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 164.0, 160.3, 154.3, 151.6, 151.4, 151.3, 146.5, 137.8, 125.3, 125.2, 123.7, 120.8, 118.3, 117.3, 116.0, 18.7. HRMS (ESI) calcd for $C_{16}H_{11}NO_4$ [M+H]+ 282.0766; found 282.0757.

Synthesis of G_{10}:



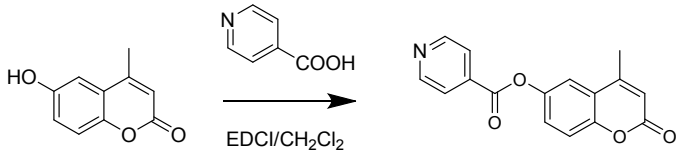
4-Methylumbelliferone (1.76 g, 10 mmol), Isonicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_{10}** as white powder (2.60 g, 90.59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.89 (d, J = 6.0 Hz, 2H), 8.01 (d, J = 6.0 Hz, 2H), 7.68 (d, J = 8.8 Hz, 1H), 7.26 (d, J = 2.4 Hz, 1H), 7.20 (dd, J_1 = 8.8 Hz J_2 = 2.4 Hz, 1H), 6.31 (d, J = 1.2 Hz, 1H), 2.47 (d, J = 1.2 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.4, 160.5, 154.4, 152.9, 152.0, 151.2, 136.2, 125.9, 123.4, 118.5, 118.0, 115.0, 110.6, 18.9. HRMS (ESI) calcd for $C_{16}H_{12}NO_4$ 282.0766 $[M+H]^+$; found 282.0764.

Synthesis of G_{11}:



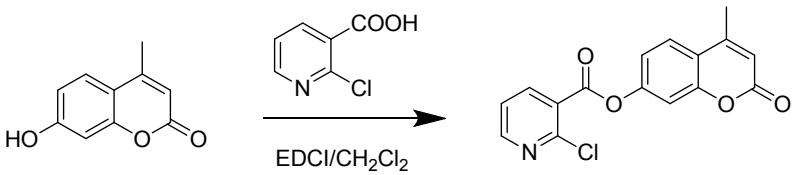
7-hydroxycoumarin (1.62 g, 10 mmol), Isonicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10 mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20 ml) yielded **G_{11}** as white powder (2.28 g, 85.39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.89 (d, J = 6.0 Hz, 2H), 8.01 (d, J = 6.0 Hz, 2H), 7.73 (d, J = 9.6 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.20 (dd, J_1 = 8.4 Hz J_2 = 2.4 Hz, 1H), 6.44 (d, J = 9.6 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ=163.4, 160.4, 154.9, 153.0, 151.2, 143.0, 136.2, 129.0, 123.4, 118.4, 117.3, 116.7, 110.7. HRMS (ESI) calcd for $C_{15}H_{10}NO_4$ 268.0610 $[M+H]^+$; found 268.0607.

Synthesis of G_{12}:



6-Hydroxy-4-methylcoumarin (1.76 g, 10 mmol), Isonicotinic acid (1.23 g, 10 mmol), EDCI (1.23 g, 10mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) yielded **G_{12}** as white powder (2.35 g, 83.62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.88 (dd, J_1 = 4.4 Hz J_2 = 1.6 Hz, 2H), 8.01 (dd, J_1 = 4.4 Hz J_2 = 1.6 Hz, 2H), 7.47 (d, J = 2.4 Hz, 1H), 7.40 (m, 2H), 6.36 (d, J = 1.2 Hz, 1H), 2.44 (d, J = 1.2 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 163.9, 160.3, 151.6, 151.4, 151.0, 146.4, 136.3, 125.1, 123.2, 120.8, 118.2, 117.2, 116.1, 18.7. HRMS (ESI) calcd for $C_{16}H_{11}NO_4$ [M+H]+ 282.0766; found 282.0758.

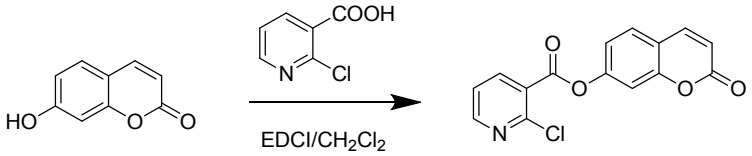
Synthesis of G_{13}:



7-Hydroxy-4-methylcoumarin (1.76 g, 10 mmol), 2-Chloronicotinic acid (1.57 g, 10 mmol), EDCI (1.23 g, 10mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) yielded **G_{13}** as white powder (2.67 g, 84.76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.62 (dd, J_1 = 4.8 Hz J_2 = 2.0 Hz, 1H), 8.38 (dd, J_1 = 8.0 Hz J_2 = 2.0 Hz,

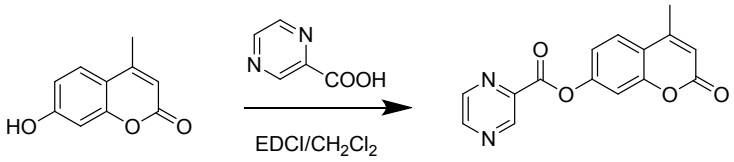
1H), 7.68 (d, J = 8.4 Hz, 1H), 7.43 (m, 1H), 7.22 (m, 2H), 6.30 (s, 1H), 2.46 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 182.0, 158.8, 153.5, 153.2, 152.9, 152.4, 148.9, 141.6, 126.7, 125.3, 123.3, 118.3, 118.1, 114.0, 110.2, 18.2. HRMS (ESI) calcd for $C_{16}H_{10}ClNO_4$ $[M+H]^+$ 316.0386; found 316.0773.

Synthesis of G_{14}:



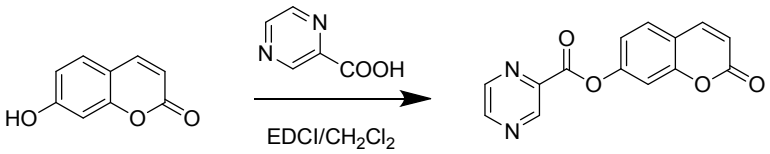
7-hydroxycoumarin (1.62 g, 10 mmol), (1. g, 10 mmol), EDCI (1.23 g, , 2-Chloronicotinic acid (1.57 g, 10 mmol), EDCI (1.23 g, 10mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) yielded **G_{14}** as white powder (2.13 g, 70.76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.62 (dd, J_1 = 4.8 Hz J_2 = 2.0 Hz, 1H), 8.38(dd, J_1 = 8.0 Hz J_2 = 2.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.43 (m, 1H), 7.22 (m, 2H), 6.43 (d, J = 9.6 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 162.5, 160.1, 154.8, 153.8, 152.9, 149.3, 144.2, 141.9, 129.8, 125.6, 123.9, 119.1, 117.4, 116.5, 110.7. HRMS (ESI) calcd for $C_{15}H_8ClNO_4$ $[M+H]^+$ 302.0241; found 301.0220.

Synthesis of NG_1:



7-Hydroxy-4-methylcoumarin (1.76 g, 10 mmol), 2-Pyrazinecarboxylic acid (1.24 g, 10 mmol), EDCI (1.23 g, 10mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) yielded **NG_1** as white powder (2.05 g, 72.69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.48 (d, J = 1.2 Hz, 1H), 8.88 (d, J = 2.4 Hz, 1H), 8.83 (m, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.24-7.31 (m, 2H), 6.31 (s, 1H), 3.74 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 162.0, 156.2, 153.7, 152.7, 150.9, 146.8, 145.2, 144.9, 143.6, 125.2, 118.3, 116.4, 114.9, 112.5, 18.9. HRMS (ESI) calcd for $C_{15}H_{10}N_2O_4$ $[M+H]^+$ 282.0641; found 282.0635.

Synthesis of NG_2:



7-hydroxycoumarin (1.62 g, 10 mmol), 2-Pyrazinecarboxylic acid (1.24 g, 10 mmol), EDCI (1.23 g, 10mmol) and DMAP (60 mg, 0.5 mmol) in CH_2Cl_2 (20ml) yielded **NG_2** as white powder (1.88 g, 70.15%). ^{1}H NMR (400 MHz, d-DMSO) δ = 9.44 (d, J = 1.2 Hz, 1H), 8.85 (d, J = 2.4 Hz, 1H), 8.81 (m, 1H), 7.73 (d, J = 9.6 Hz, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.24-7.30 (m, 2H), 6.42 (d, J = 9.6 Hz, 1H). ^{13}C NMR (400 MHz, d-DMSO) δ = 161.8, 159.6, 154.0, 152.6, 148.7, 146.3, 145.0, 143.7, 142.2, 129.7, 118.6, 117.2, 115.9, 110.3. HRMS (ESI) calcd for $C_{14}H_8N_2O_4$ $[M+H]^+$ 269.0580; found 269.0562.