Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Styryl dye formation promoted by catalytic centers of piperazine bound to silica surface traced by single molecule fluorescence microscopy

*Aline M. Lino and Marcelo H. Gehlen**

Instituto de Química de São Carlos – Universidade de São Paulo – Brazil

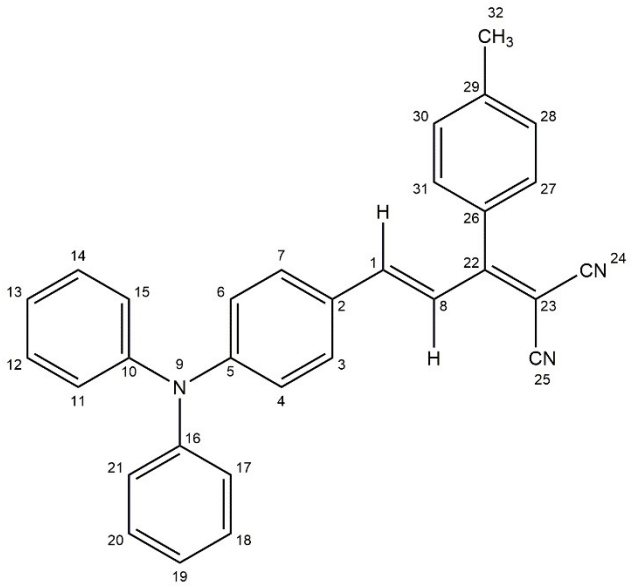
*corresponding author: marcelog@iqsc.usp.br

Supplementary Information

Reagents, solvents and surfactants were purchased from Sigma-Aldrich, Fischer or PanReach Co. and were used as received unless otherwise specified.

Synthesis of (E) -2- [3- [4- (diphenylamine) phenyl] -1- (p-tolyl) - allylidene] -malononitrile (DFTAM). 126 mg of 4-diphenilamino benzaldehyde and 78 mg of 2- [1- (4-methylphenyl) ethylidene]-malononitrile (Aldrich Co.) were mixed in 5 mL of absolute ethanol and 15 μL of the catalyst piperidine was added. The reacting system was refluxed during 6 h. The product DFTAM was obtained in a net yield of 57 % and it was initially purified in a chromatographic silica column using toluene as eluent.

HPLC coupled to Mass spectra determination of (E) -2- [3- [4- (diphenylamine) phenyl] -1- (p-tolyl) - allylidene] -malononitrile (DFTAM) were performed with a Water® equipment (separation module 2695 with a column C_{18-4} Inertsil and methanol/water operating gradient; UV detector 2996; hexapole Micromass analizer model ZQ). Agilent spectrometer model 500/54 was used to adquire the ^{1}H and ^{13}C NMR spectra of dye in $CDCl_3$. FTIR characterization was achieved on a Shimadzu model IRAffinity 1 spectrometer with the dye dispersed on KBr pellet.



DFTAM molecular structure

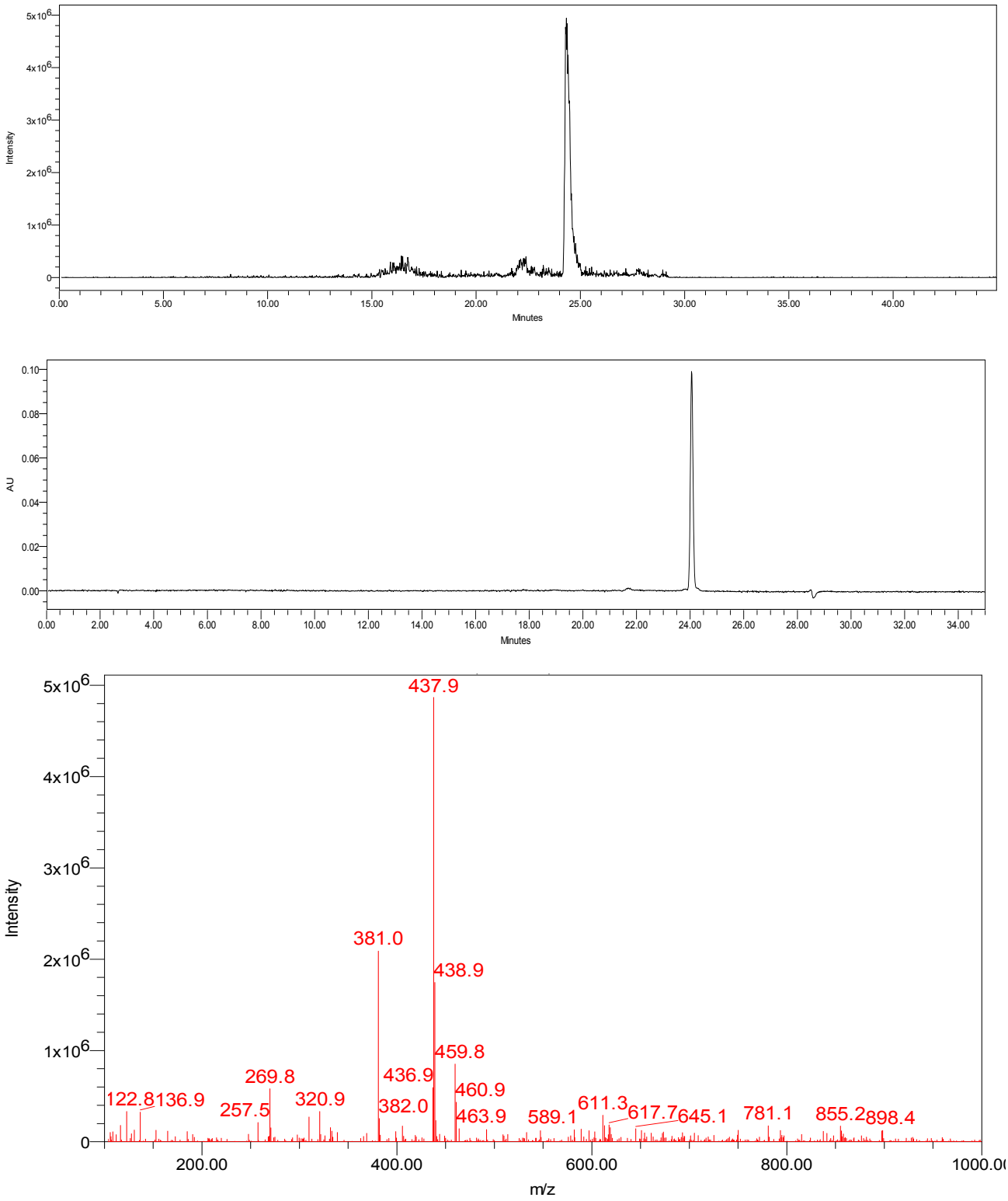


Figure S1. *HPLC data of DFTAM and the corresponding mass spectrum showing $[M+H]^+$ m/z = 437.9 of the DFTAM ($C_{31}H_{23}N_3$) molecular ion.*

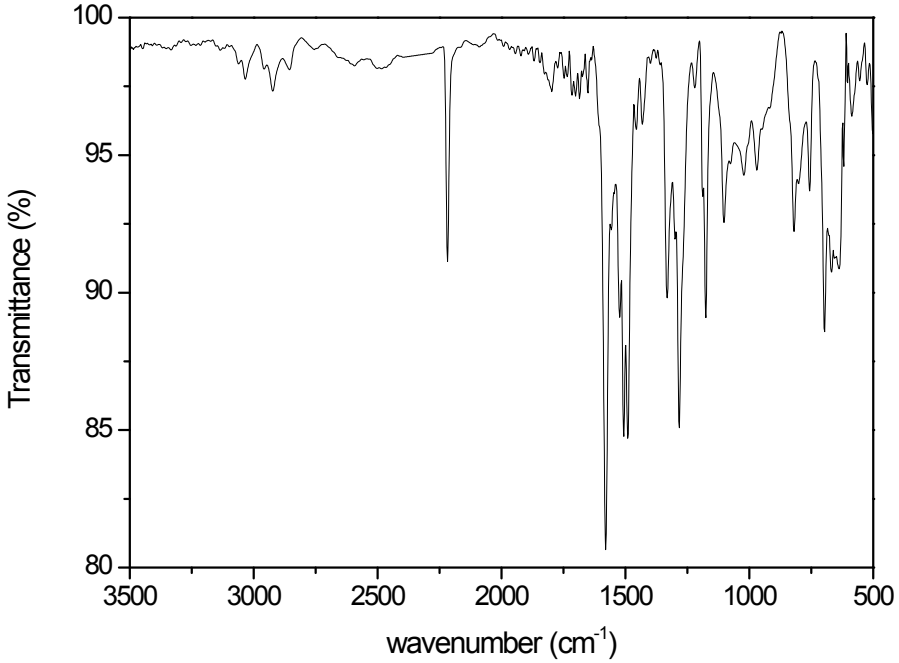
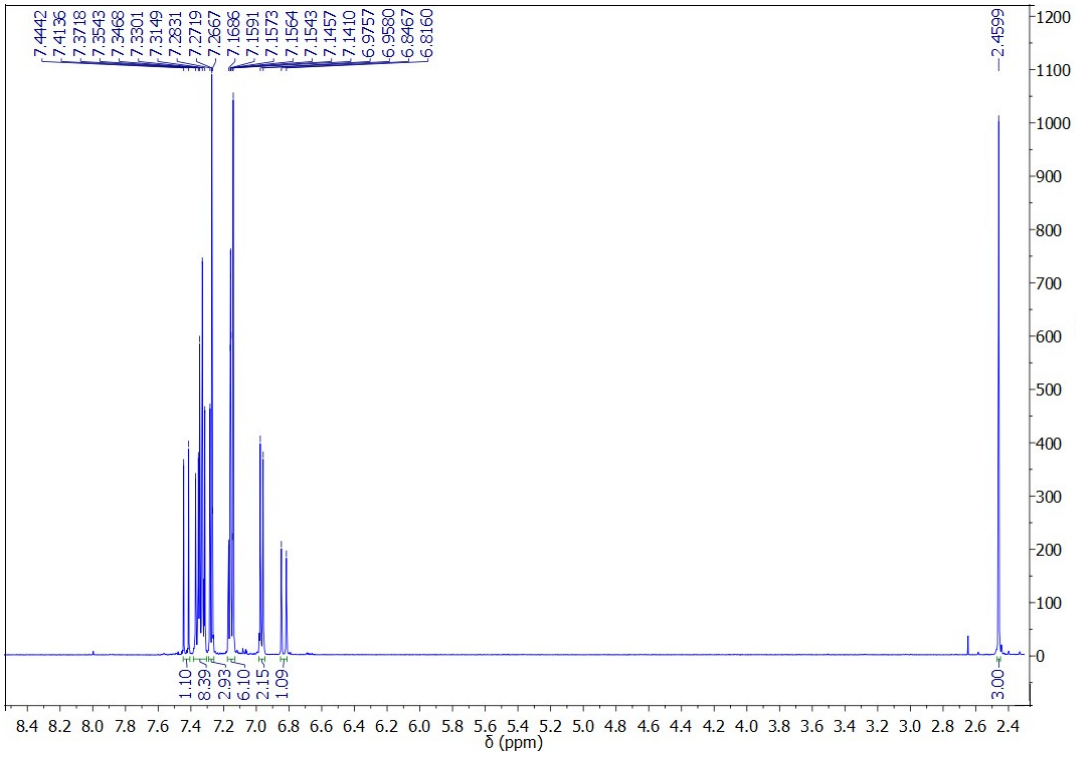


Figure S2. *FTIR spectrum of DFTAM*



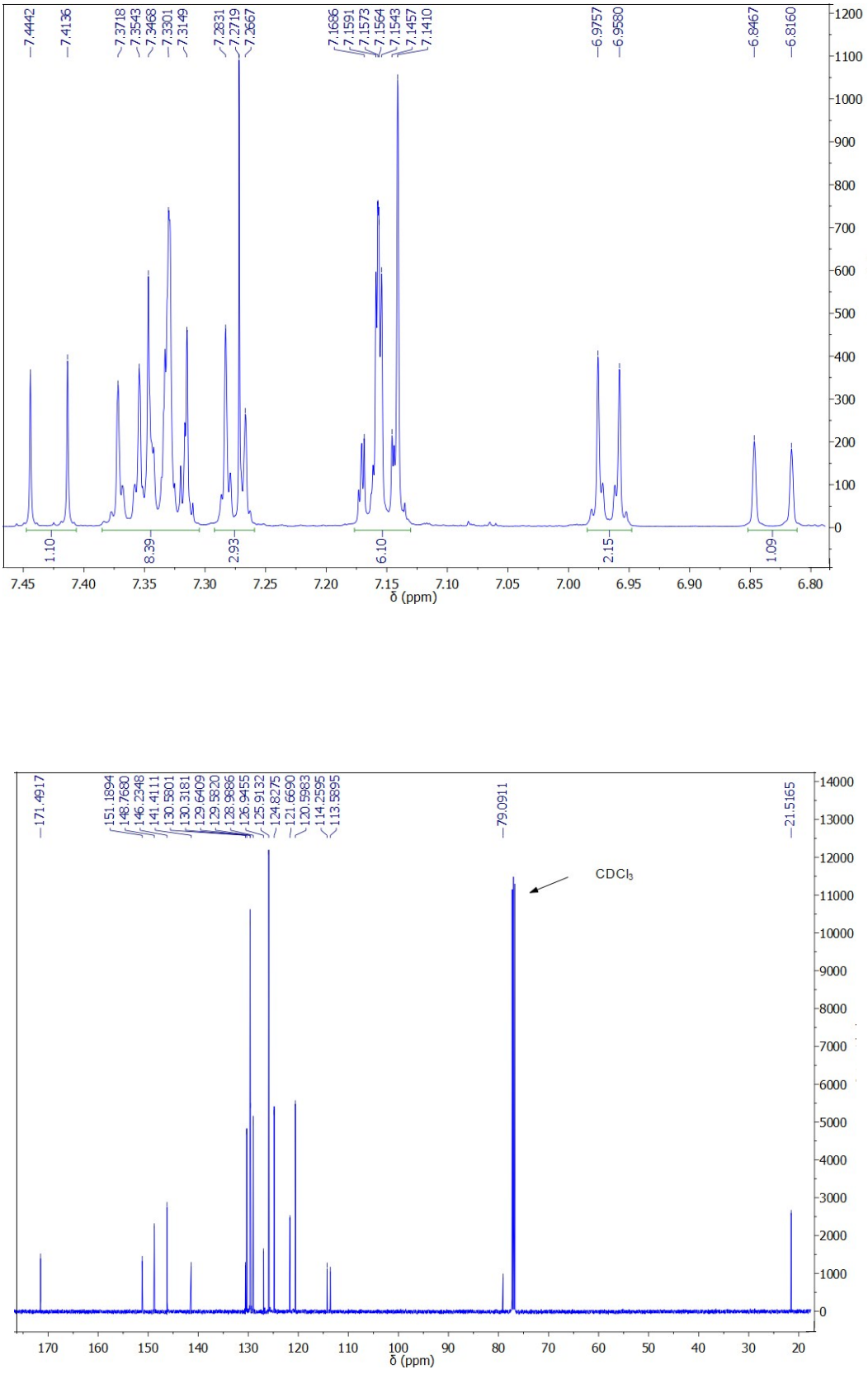
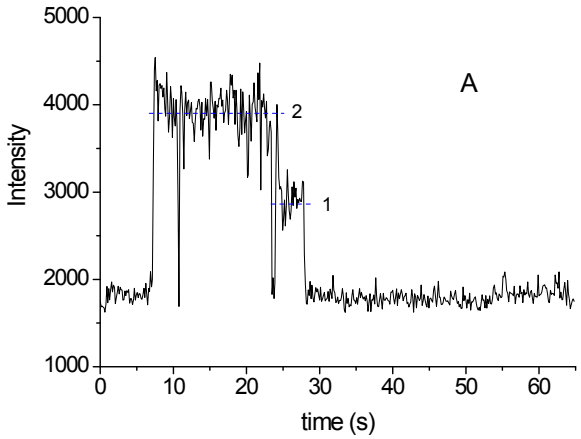
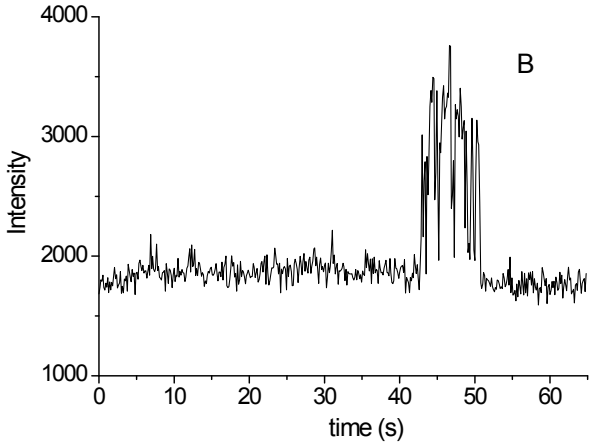


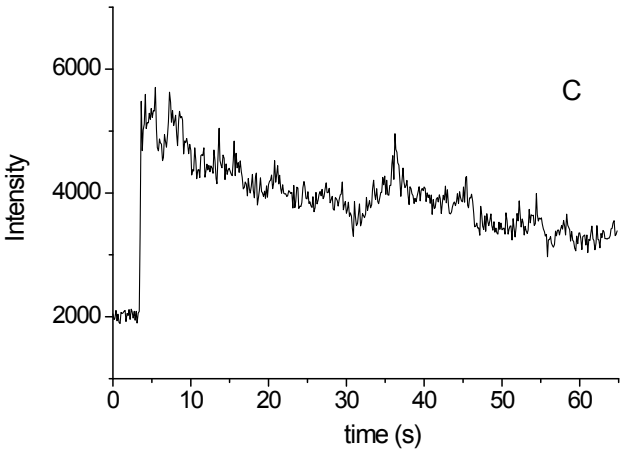
Figure S3. *NMR ^{1}H and ^{13}C spectra of DFTAM in $CDCl_3$*

Table S1. *NMR ^{13}C and ^{1}H peaks of DFTAM in $CDCl_3$*

Position	^{13}C (ppm)	^{1}H (ppm)
1	126.9	6.83 (d, *J* = 15.4 Hz)
2	130.6	-
3	130.3	7,27 (d, *J* = 8.1 Hz)
4	120.6	6,96 (d, *J* = 7.9 Hz)
5	151.2	-
6	120.6	6,96 (d, *J* = 7.9 Hz)
7	130.3	7,27 (d, *J* = 8.1 Hz)
8	121.7	7,42 (d, *J* = 15.4 Hz)
9	-	-
10	148.8	-
11	126.0	7.15 (m)
12	129.6	7.34 (m)
13	129.5	7.15 (m)
14	129.6	7.34 (m)
15	126.0	7.15 (m)
16	146.2	-
17	126.0	7.15 (m)
18	129.6	7.34 (m)
19	129.5	7.15 (m)
20	129.6	7.34 (m)
21	126.0	7.15 (m)
22	171.4	-
23	79.1	-
24	113.6	-
25	114.2	-
26	126.9	-
27	129.0	7.34 (m)
28	124.8	7.34 (m)
29	141.4	-
30	124.8	7.34 (m)
31	129.0	7.34 (m)
32	21.5	2.46 (s)


A
Intensity
time (s)
1
2
5000
4000
3000
2000
1000
0
10
20
30
40
50
60


B
Intensity
time (s)
4000
3000
2000
1000
0
10
20
30
40
50
60


C
Intensity
time (s)
6000
4000
2000
0
10
20
30
40
50
60

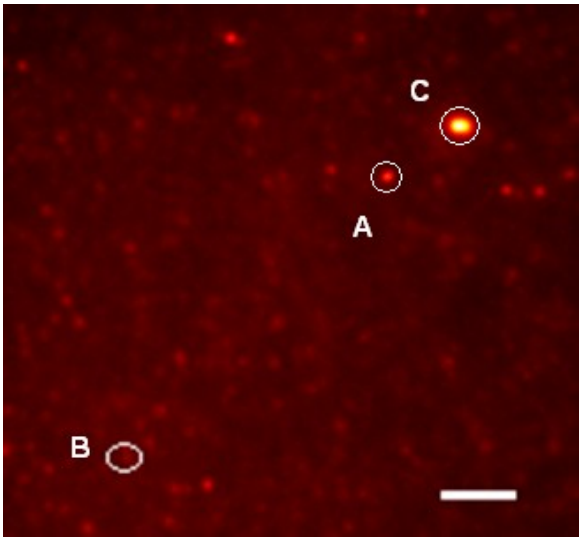


Figure S4. *Intensity-time trajectories of catalytic regions showing different behavior during Knoevenagel condensation reaction forming the fluorescent DFTAM observed in TIRFM in the absence of added water traps. (A) Discrete transitions to lower dye number; (B)SM bursting events after a long inert period; (C) trajectory of a high emissive catalytic region with possible photo bleaching of the dye. Integrated intensity image with indication of the three spots analyzed. 2μm scale bar.*