Supporting Information for

Competing and simultaneous click reactions at the interface and in solution

Doungporn Yiamsawas, Manfred Wagner, Grit Baier, Katharina Landfester*, and Frederik R. Wurm*

Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

landfester@mpip-mainz.mpg.de, wurm@mpip-mainz.mpg.de

1. Experimental Part

1.1 Materials

For the kinetic study, bucillamine was obtained from Biozol diagnostic Vertrieb GmbH. 1,4-bis(3-(2-pyridyldithio)propionamido) butane (BPB) and 1,1′-(Methylenedi-4,1-phenylene) bismaleimide, triethylene amine. (TEA) THF-d_8, D_2O, and chloroform-*d* were obtained from Sigma-Aldrich. The oil-soluble surfactant polyglycerol polyricinoleate (PGPR) was used for the preparation of the inverse miniemulsion systems. Hexamethylcyclo trisiloxane was used as internal standard. All chemicals were used without further purification.

1.2 Solution reaction of thiol-maleimide and thiol-disulfide interchange reaction

Appoximately 2.2 mg (10 µmol) of bucillamine and ca. 2.2 mg (10 µmol of hexamethylcyclo trisiloxane was dissolved in 500 ml of THF-d_8. 50 µl (10 µmol) of 0.3 M TEA solution in THF-d8 was also dropped in this solution. And, separately, a second solution of 3.6 mg (1 µmol of bismaleimide in 250 ml of THF-d_8 was also prepared. These two solution was mixed in NMR tube and immediately introduced in the NMR spectrometer with the temperature of the experiment previously equilibrated (298 K or 283 K). In case of thiol-disulfide interchange reaction, a solution of 4.8 mg (10 µmol) of BPB in 150 ml of THF-d_8 was prepared instead of bismaleimide solution. For dual reaction, 1.8 mg (0.5 µmol) of bismeimide and 2.4 mg (0.5 µmol) of BPB in 150 ml of THF-d_8 was prepared and mixed with bucillamie solution.

1.3 Interfacial reaction of thiol-maleimide and thiol-disulfide interchange reaction in inverseminiemulsion

For kinetics study of thiol-maleimide reaction, ca. 2.2 mg (10 µmol) of bucillamine and 0.5 mg of NaCl were dissolved in 100 mg of D_2O. 500 µl of a stock solution of

chloroform-d/PGPR (0.8 wt%) was added and the mixture was stirred at 1000 rpm for 1 h. Meanwhile, a second solution composed of 3.6 mg (10 μmol) of bismaleimide and ca. 2.2 mg (10 μmol) of hexamethylcyclo trisiloxane was dissolved in 150 μl of chloroform-d. After 1 h, the pre-emulsificated mixture was submitted to a pulsed ultrasonication process in an ice bath for 60 s (2.5 s sonication, 5 s paused) at 60% amplitude using a 1/8" tip Branson 450W sonifier. The obtained miniemulsion was immediately transferred into a conventional NMR tube. A NMR spectrometer (Bruker Avance III 700 MHz) was previously equilibrated at the temperature of the experiment (283 K). The second solution containing the bismaleimide and hexamethylcyclo trisiloxane used as reference for the spectrum, were added to the NMR tube, homogenized and the tube was immediately introduced in the spectrometer. In case of thiol-disulfide interchange reaction, a solution of 4.8 mg (10 μmol) of BPB in 150 ml of chloroform-d was prepared instead of bismaleimide solution. For dual reaction, 1.8 mg (0.5 μmol) of bismaleimide and 2.4 mg (0.5 μmol) of BPB in 150 ml of chloroform-d was prepared. For each experiment, ^{1}H-NMR spectra were collected consecutively, using 32 transients with an 11 ms long 90° pulse. A 12600 Hz spectral width together with a recycling delay time of 3 s, resulting in a total time of 5 min for the collection of each spectrum and a total time of 600 min, was used for each experiment.

1.4 Determination of fluorescent dye sulforhodamine (SR101) after degradation of capsules by glutathione

The capsules were prepare by following the procedure in 1.3 for two "click" reactions thiol-maleimide addition and thiol-disulfide exchange. 10 μl of SR101 dye (5.5 mg/ml) were added into water phase.

The capsules were re-dispersed in phosphate buffer solution at pH7 after the formation of the capsules in inverse miniemulsion. The capsules dispersion in cyclohexane was added into 4 ml of the buffer solution. The mixture was stirred for 24 h with open cap in order to guarantee evaporation of the solvent.

To 1 ml of the aqueous capsule dispersion (in buffer solution), 500 μL of glutathione in phosphate buffer solution (10 mg/ml) was added. The mixture was kept stirring over 7 hours and then centrifuge at 10000 rpm for 30 min. The supernatant was investigated by fluorescence spectroscopy in order to determine the amount of released SR101. As control, a capsule dispersion that fluorescent dye was encapsulated was incubated with phosphate buffer at pH7, room temperature for 7 hour. The fluorescence signal of SR101 at difference concentrations was used to set up a calibration curve (Note: SR101 has an absorption

maximum at 550 nm and the emission was measured at 605 nm). The total release of SR101 from the degraded capsule by enzyme was calculated from the calibration curve.

1.5 Gel permeation chromatography (GPC) analysis

The apparent molecular weight was determined by gel permeation chromatography (Agilent Technologies 1260 Infinity). After 10 hour of reaction from NMR spectrometer, the samples directly analyzed by GPC. Solutions of the final materials with concentrations of 1 mg mL^{-1} were prepared in DMF, injected at a flow of 1 ml min-1 with DMF as eluent phase. The signal was detected with a UV-Vis detector S-3702 (Soma) and the molecular weight was obtained with the software PSS-WinGPC UniChrom (PSS) against PS standards.

1.6 Kinetics

The curves of the kinetics measurements were fitted using the software Origin 9.0.

1.7 ^{1}H NMR and 2D ^{1}H,^{1}H TOCSY

The ^{1}H NMR experiments were recorded with a 5 mm BBI ^{1}H/Xz- gradient on a 700 MHz spectrometer with a Bruker Avance III system. For the ^{1}H NMR spectrum 64 transients were used with an 11µ s long 90° pulse and a 12600 Hz spectral width together with a recycling delay of 5 s. The assignment was accomplished by ^{1}H,^{1}H TOCSY (total correlated spectroscopy). The spectroscopic widths of the homo-nuclear 2D TOCSY, COSY and NOESY experiments were typically 13600 Hz in both dimension (f1 and f2) and the relaxation delay 2s. The “tocsy” mixing time used in the 2D TOSCY was kept at 100 ms.

1.7 2D ^{1}H, ^{13}C-HSQC

The experiments were done with a 5 mm triple resonance TXI ^{1}H/^{13}C/^{15}N probe with z-gradient on the 850 MHz spectrometer on a Bruker Avance III system. The data were analysed with Topspin 3.1 software from the Bruker Company.

For a ^{1}H NMR spectrum 128 transients were used with an 9,4µs long 90° pulse and a 17600 Hz spectral width together with a recycling delay of 5s.

The proton (850 MHz), carbon (213 MHz) were measured in a mixture of DMSO-d_6 at 298,3K and the spectra were referenced with the residual DMSO-d_5H at $\delta(^1H)$ = 2,49 ppm, DMSO-d_6 $\delta(^{13}C$ heptett) = 40,3 ppm.

The 2D $^1H,^{13}C$-HSQC (heteronuclear single quantum correlations via double inept transfer and phase sensitive using Echo/Antiecho-TPPI gradient selection with decoupling during acquisition) and HSQC-edited (with multiplicity editing during selection step) experiments were run with 2048 points in f2 and 512 points in f1 dimension. The following parameters were used to obtain optimal results: $^1J_{CH}$=145Hz for optimizing observable intensities of cross peaks from one bond ^1H-^{13}C correlation.

The spectral widths for ^1H and ^{13}C were 12000 Hz (14 ppm) for ^{13}C and 43000 Hz (199 ppm).

Before Fourier transformation, the data were zero filled to 1024 points in f1 and multiplied by a window function (q-sine bell or sine bell) in both dimension.

The carbon signals of the product from miniemulsion was extracted from the cross peaks $^1H,^{13}C$-HSQC due to the poor signal intensities in a 1D ^{13}C NMR measurement.

2. Additional Figures

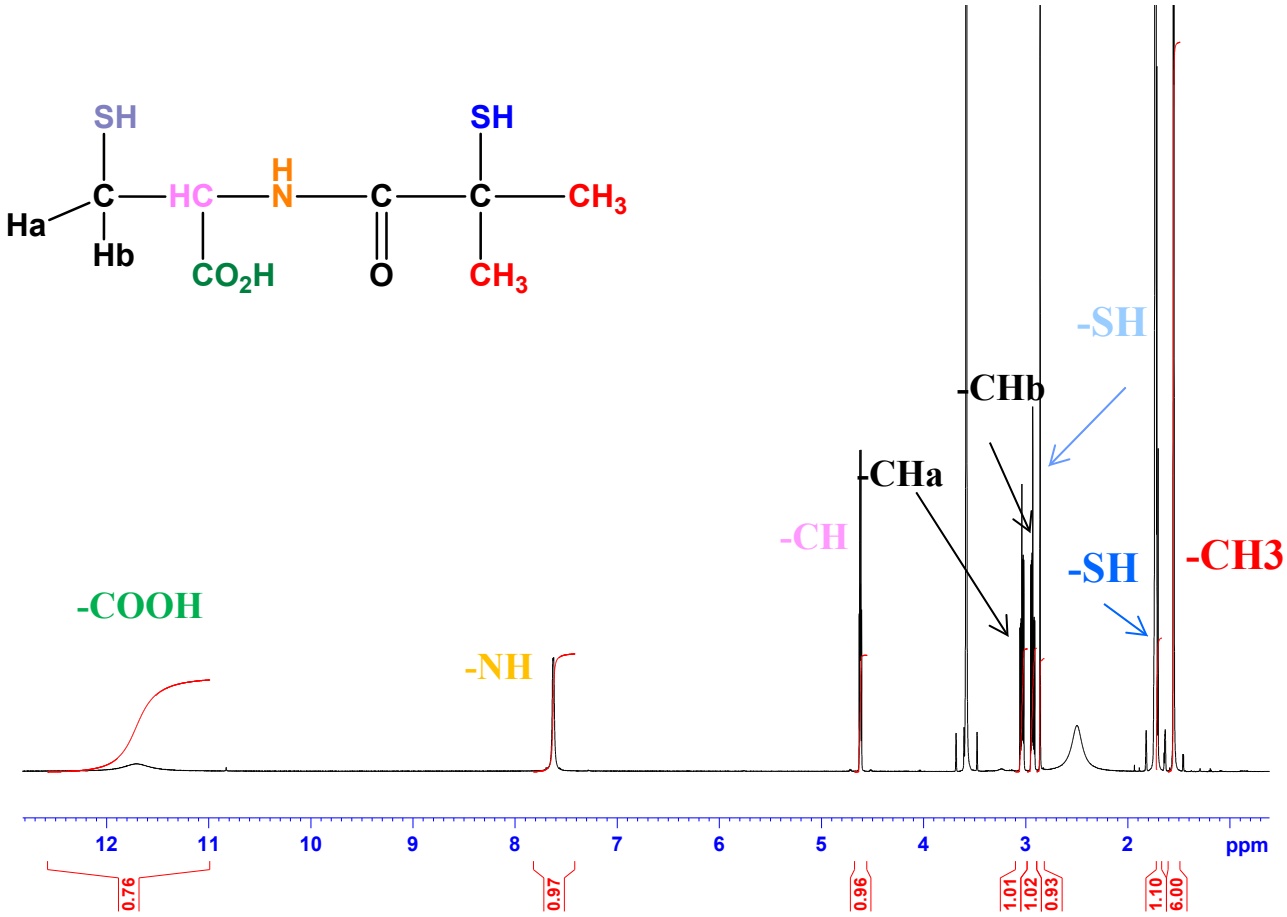
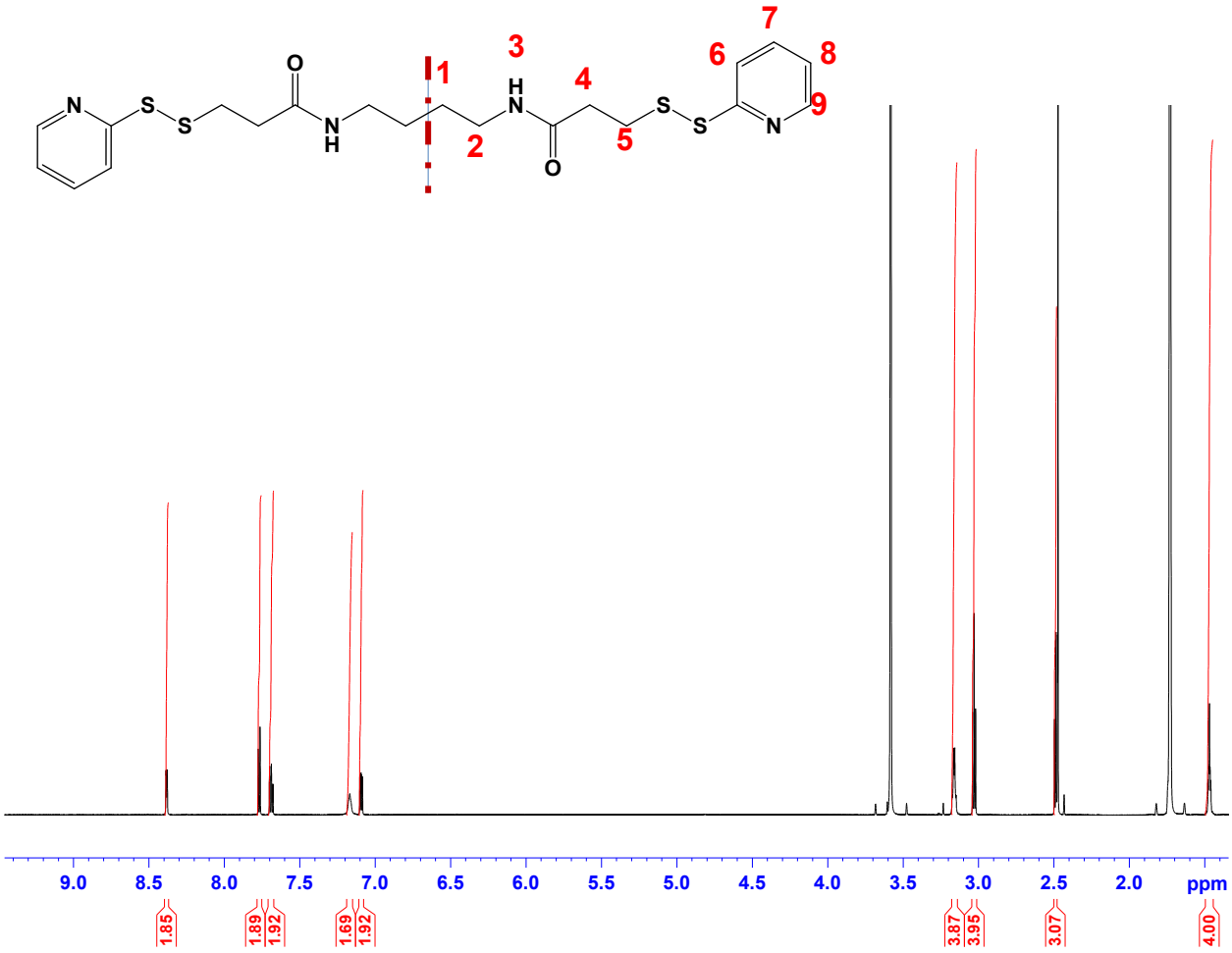


Fig. S1 ^{1}H NMR spectrum of bucillamine in THF-d_8 (700 MHz, 298 K).

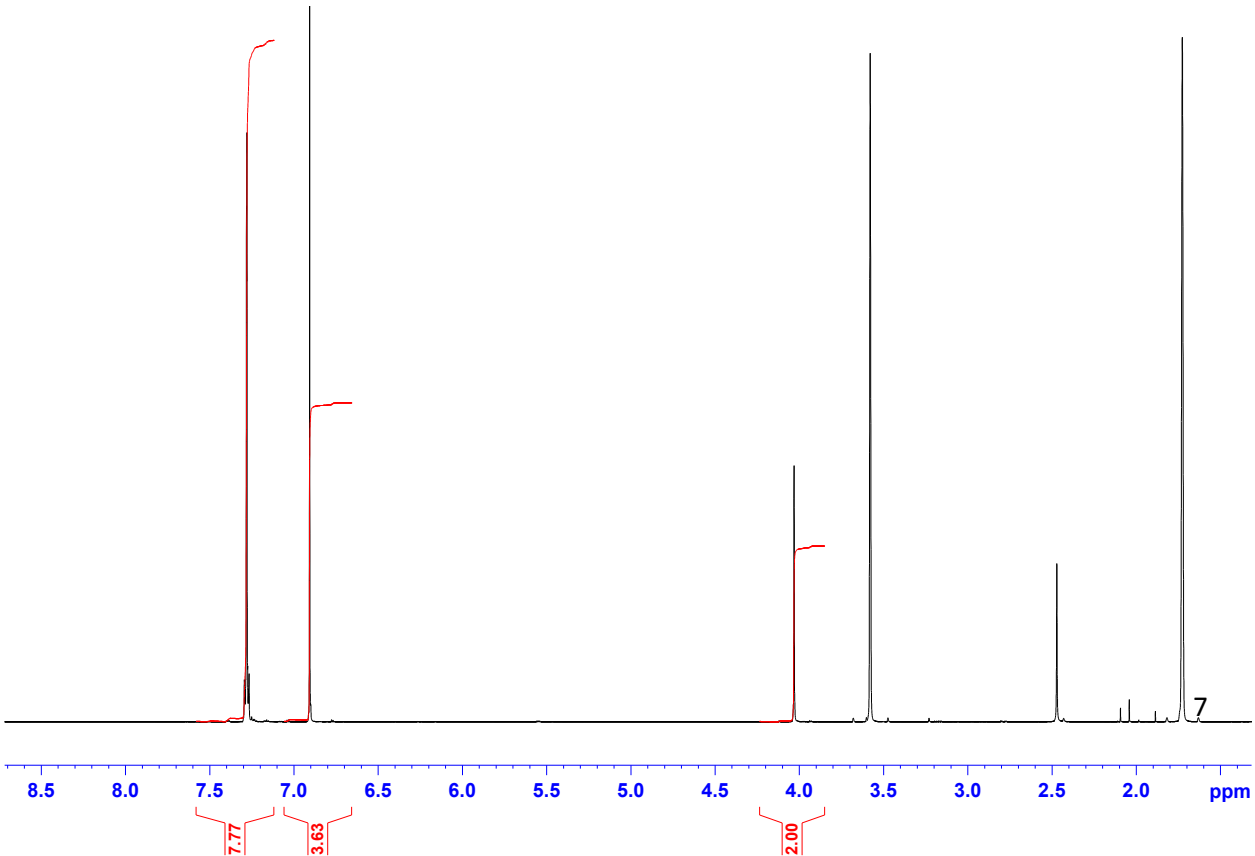

7
6
8
3
1
4
9
2
5
N
S
S
O
N
H
H
N
O
S
S
N
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
1.85
1.89
1.92
1.69
1.92
3.87
3.95
3.07
4.00

(BPB) disulfide

Folow –CH signal pyridine ring

9 6 7 3 8 2 5 4 1

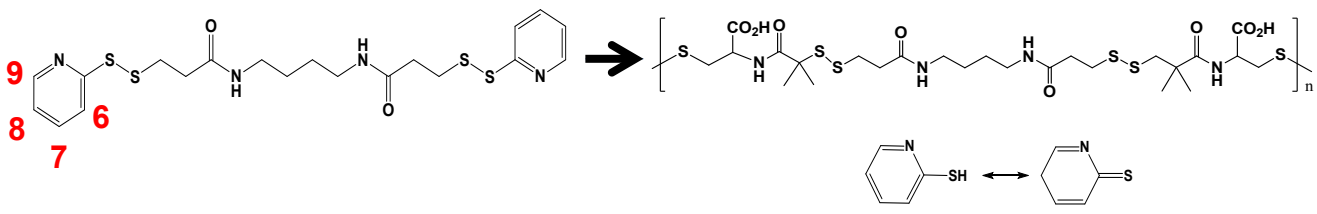
Fig. S2 ^{1}H NMR spectrum of 1,4 bis (3-(2-pyridyldithio)propionamido) butane (BPB) in THF-d_8 (700 MHz, 298 K).



Folow –CH signal maleimide

3 2 1

Fig. S3 ^{1}H NMR spectrum of 1,1′-(Methylenedi-4,1-phenylene) bismaleimide in THF-d_8 (700 MHz, 298 K).



8

-CH signal in pyridyl ring decreased

9 6 8

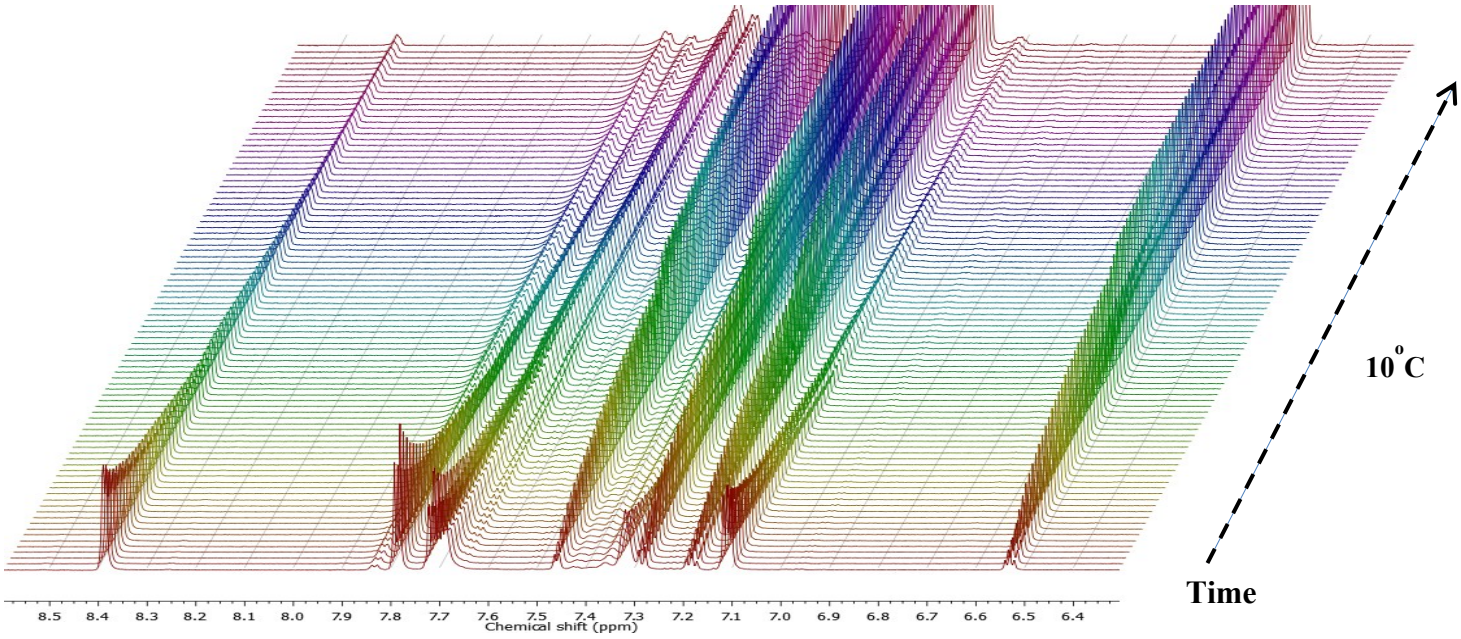


Fig. S4 Overlay of ^{1}H NMR spectra of dithiol-disulfide interchange reaction in THF-d_8 (700 MHz at 283 K).

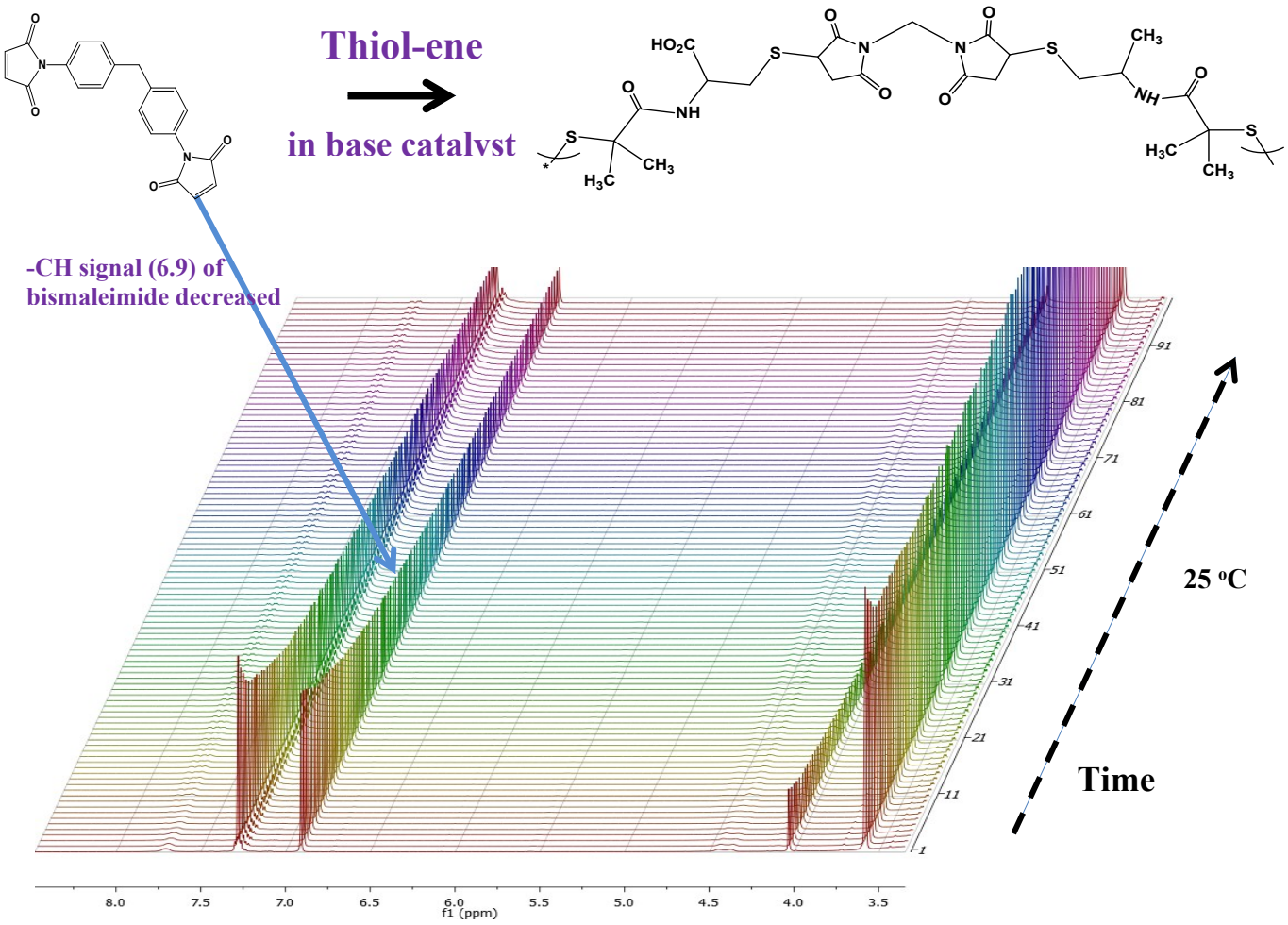


Fig. S5 Overlay of ^{1}H NMR spectra of thiol-maleimide click reaction in THF-d_8 (700 MHz at 298 K).

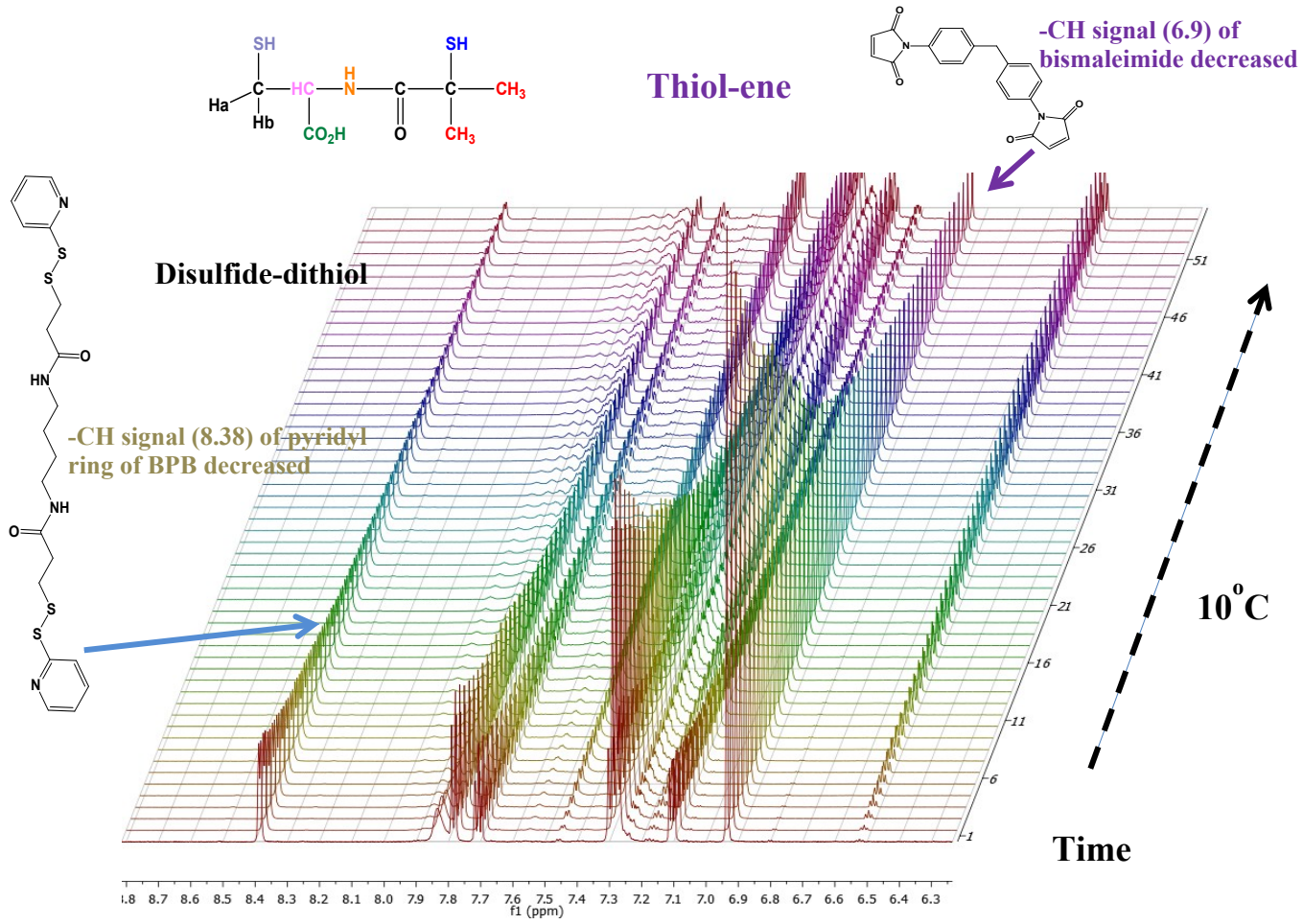


Fig. S6 Overlay of ^{1}H NMR spectra of dual reaction thiol-disulfide interchange and thiol-maleimide click reaction in THF-d_8 (700 MHz at 283 K).

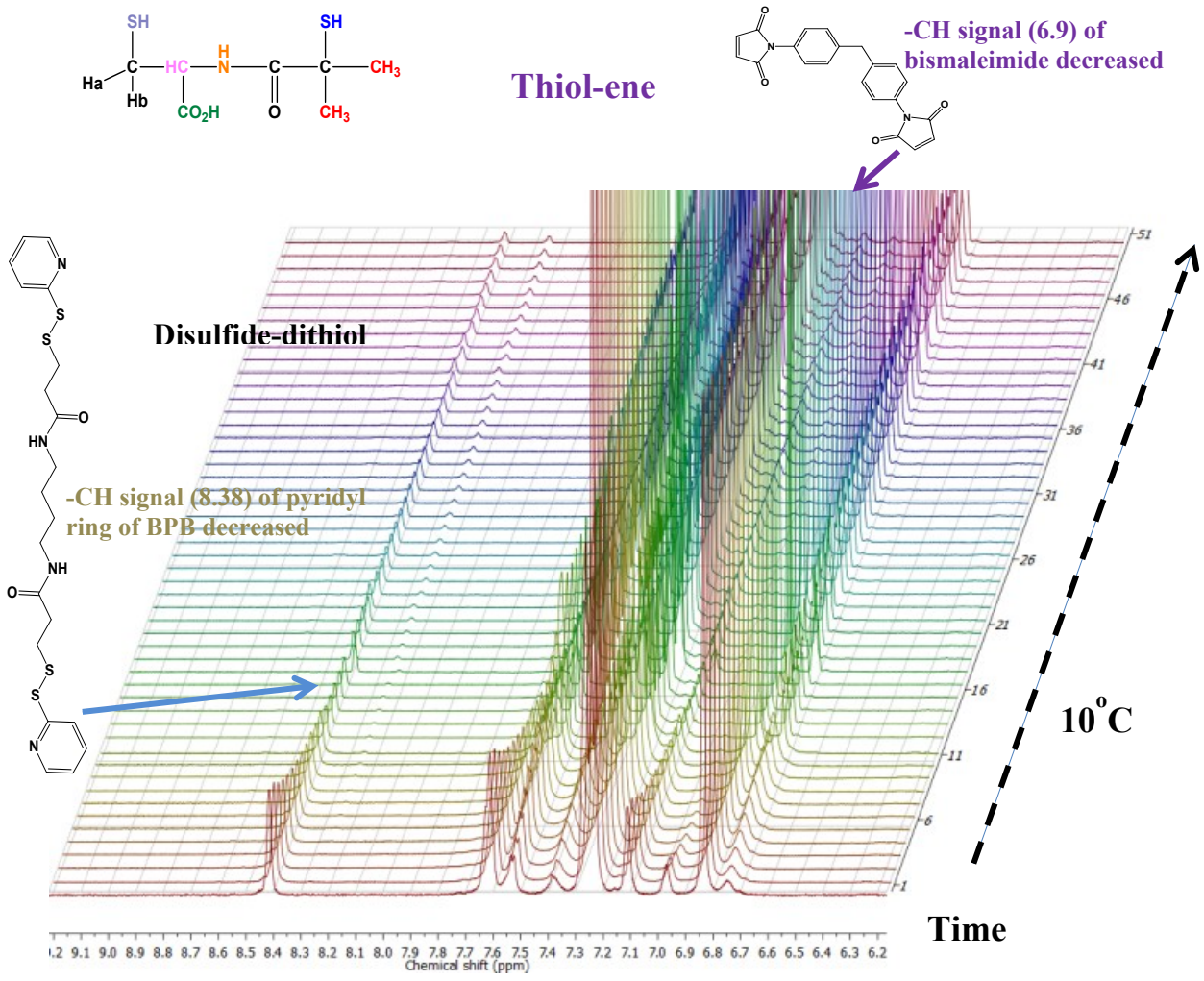


Fig. S7 Overlay of ^{1}H NMR spectra of dual reaction thiol-disulfide interchange and thiol-maleimide click reaction in miniemulsion (700 MHz at 283 K).

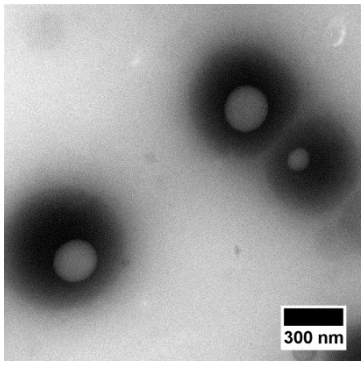


Fig. S8 TEM images of the nanocapsules obtained in miniemulsion process by the thiol-maleimide reaction (images taken directly from the dispersion in chloroform).

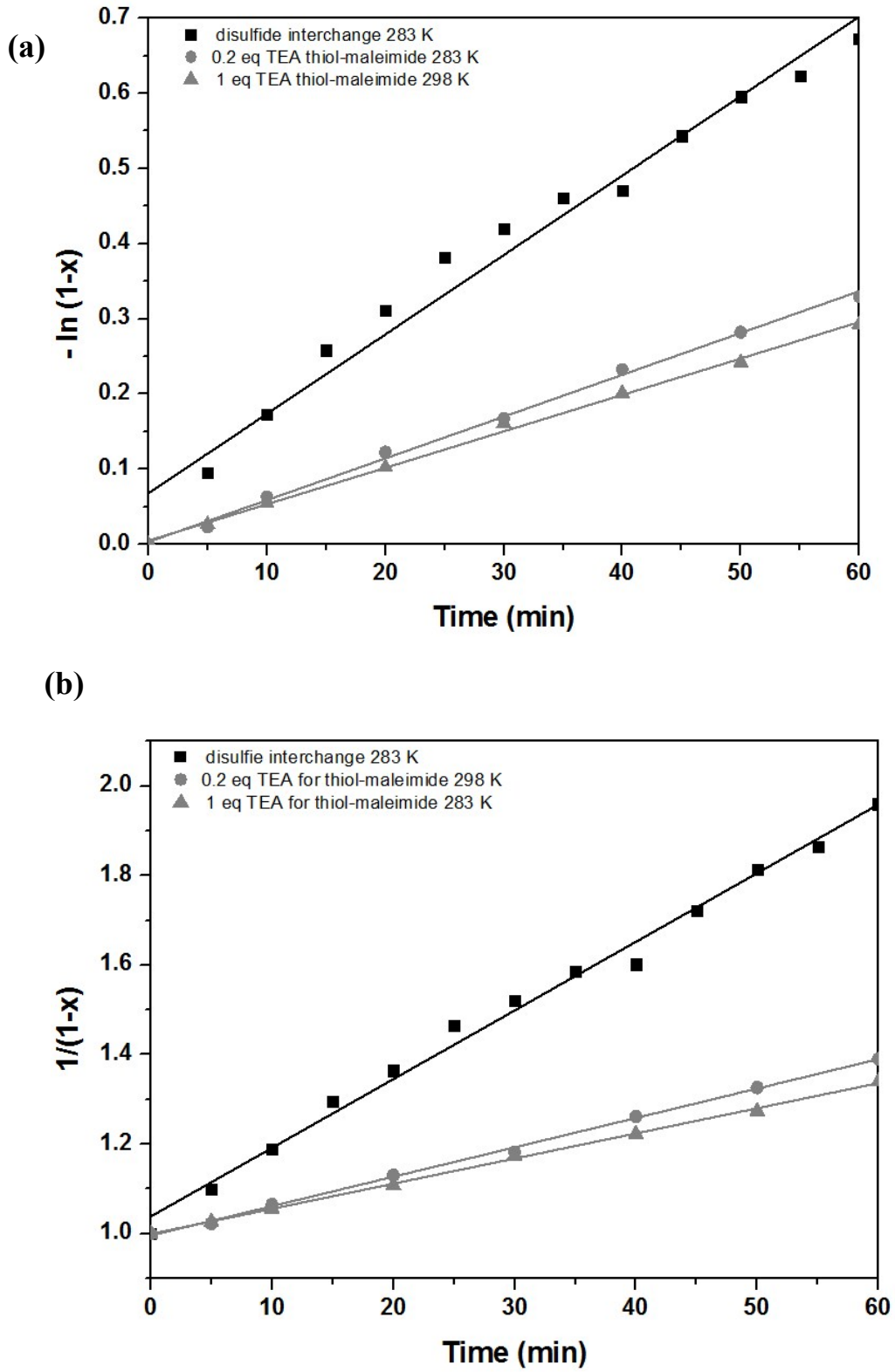


Fig. S9 Pseudo-first (a), second (b) order reaction kinetics for the first hour of thiol-disulfide interchage and thiol-maleimide click reaction in THF-d_8 by ^{1}H NMR.

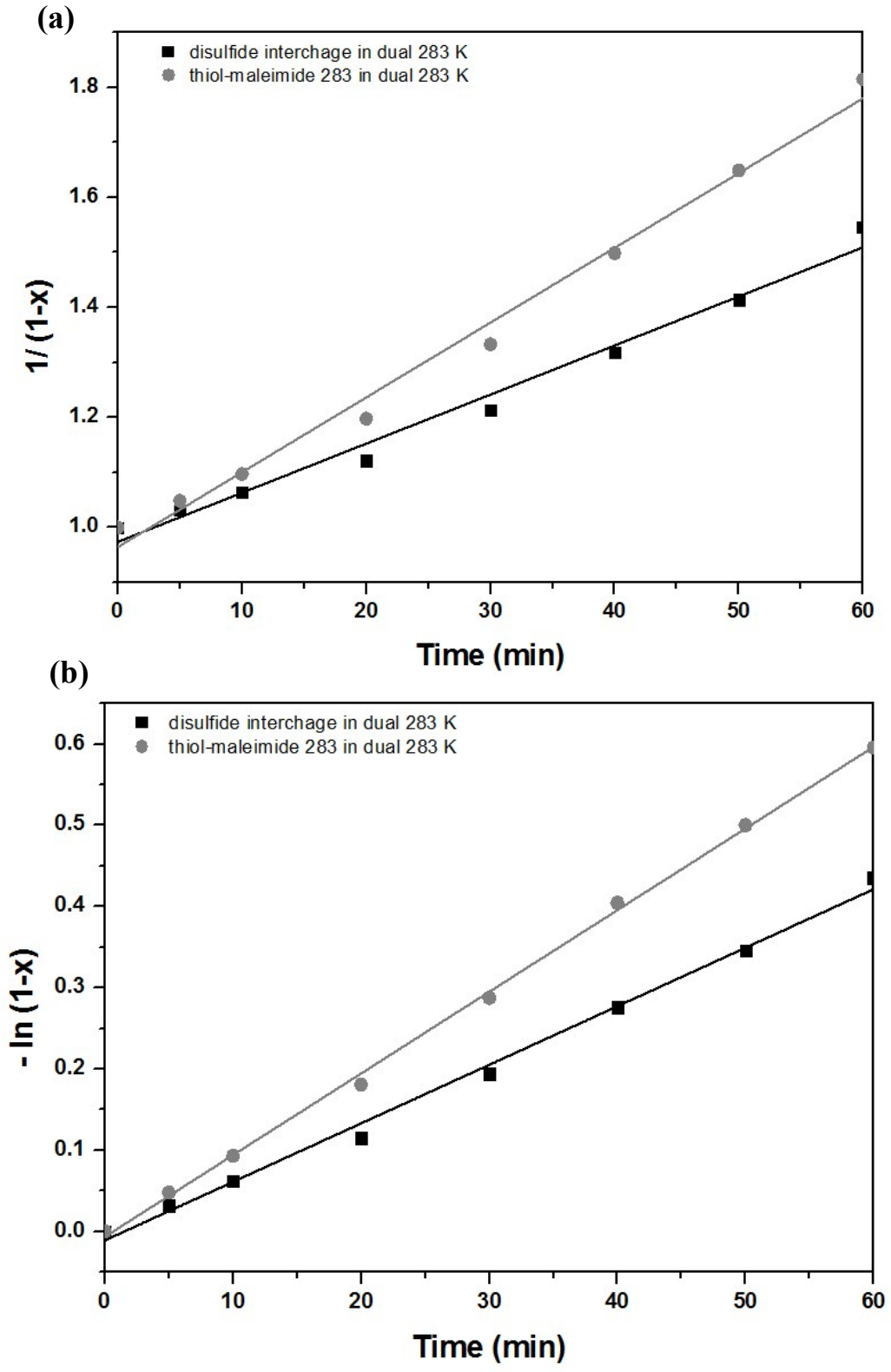


Fig. S9B Pseudo-first (a), second (b) order reaction kinetics for the first hour of dual reactions thiol-disulfide interchange and thiol-maleimide click reaction at 1 eq. of TEA, 283 K in THF-d_8 by ^{1}H NMR.

Table S1 Kinetic rate constants (k) and linear fitting values (R) describing the kinetics of thiol-disulfide interchange and thiol-maleimide click reaction

Kinetic order	Rate constant	Thiol-disulfide @283 K	Thiol-maleimide		Dual	
			0.2 eq TEA @ 298 K	1 eq TEA @283 K	Disulfide @283 K	Maleimide @283 K
			solution			
1	**k_1 (min^{-1})**	0.0105	0.00556	0.00485	**0.00721**	**0.01006**
	R	0.972	0.997	0.997	**0.99445**	**0.99861**
2	**k_2 (min^{-1} mol^{-1} L)**	**0.01534**	**0.00657**	**0.00561**	0.00892	0.01359
	R	**0.9917**	**0.99841**	**0.99869**	0.98238	0.98912
			miniemulsion			
1	**k_1 (min^{-1})**	0.02402	-	0.01939	0.08092	0.03623
	R	0.9959	-	0.77874	0.63287	0.42648
2	**k_2 (min^{-1} mol^{-1} L)**	**0.03127**	-	**0.02278**	0.07048	0.04974
	R	**0.9969**	-	**0.81509**	0.62503	0.4939

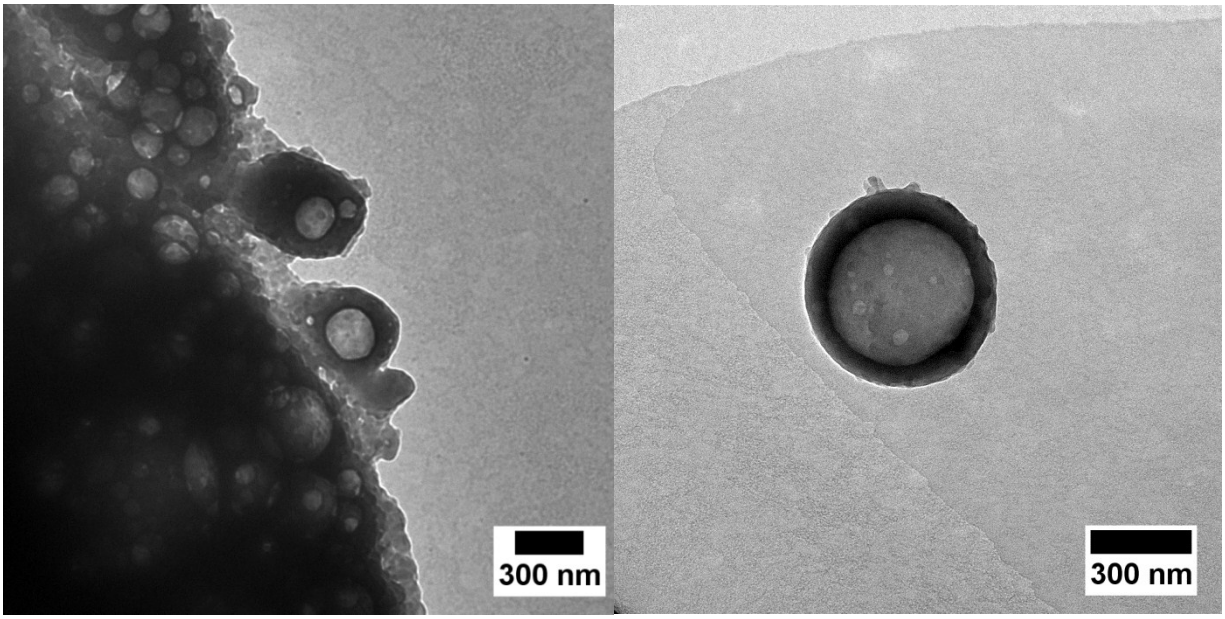
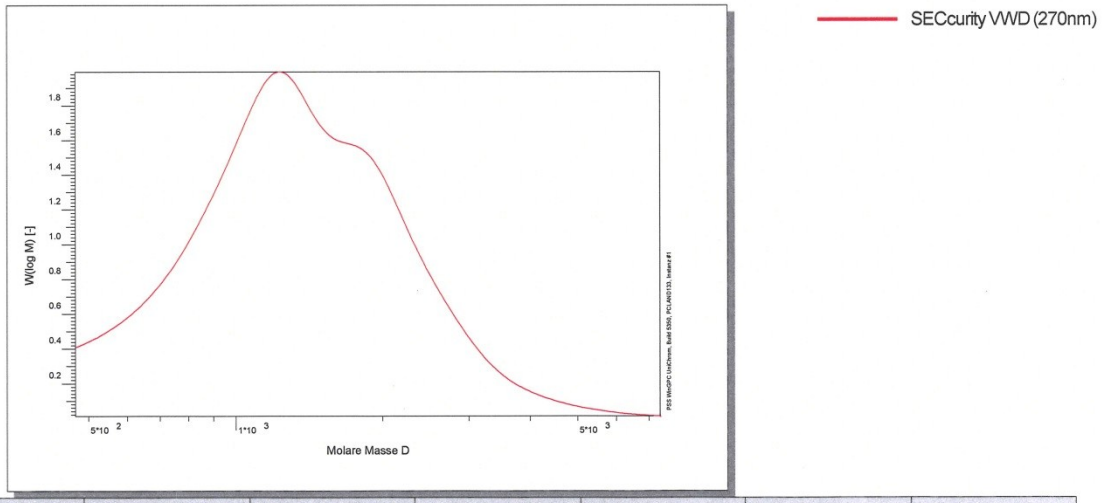


Fig. S10 TEM images of the dual reaction nanocapsules obtained in miniemulsion process (after redispersion in water).

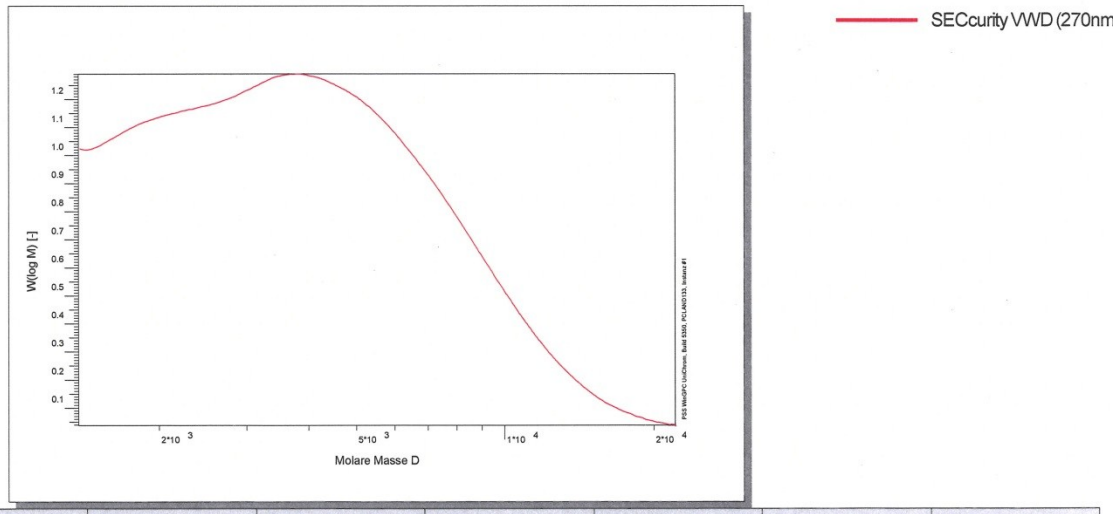
(a)

Molecular weight distribution:



Name	Mn	Mw	D	Vp	Mp	Area
SECcurity VW	1200,67	1516,24	1,26	33,51	1208,56	0,11

(b) **Molecular weight distribution:**



Name	Mn	Mw	D	Vp	Mp	Area
SECcurity VW	3225,08	4638,39	1,44	31,77	3474,19	0,02

Fig. 11 GPC analysis of the product of the dual reaction thiol-disulfide interchange and thiol-maleimide click reaction at 283K in solution (a) and miniemulsion (b).

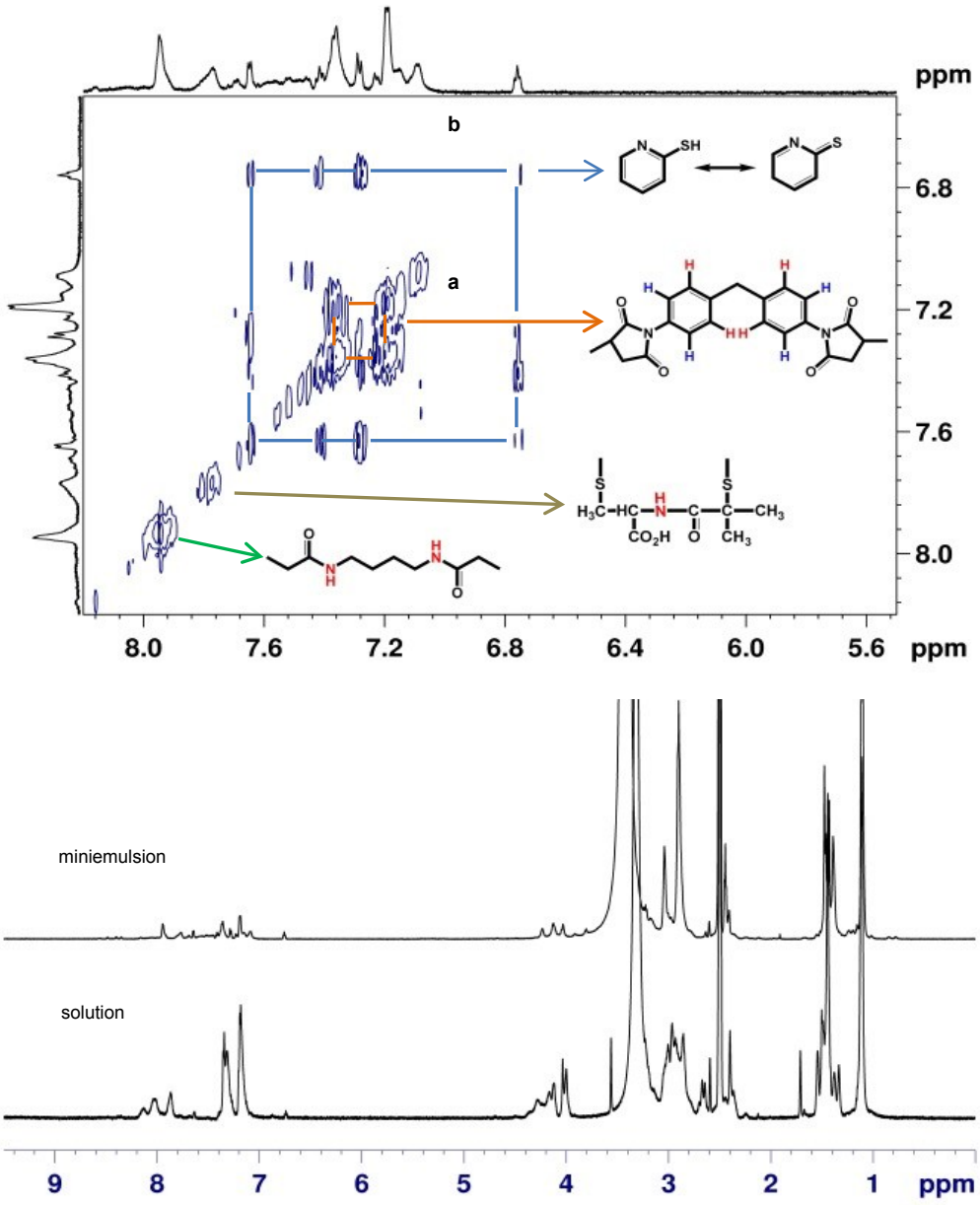


Fig. S12 (Upper) TOSCY NMR (700 MHz) measurement (^{1}H-^{1}H correlation) of the product from dual reaction in miniemulsion at 283 K in DMSO-d_6 and (lower) ^{1}H NMR spectra of the product from dual reaction in solution and miniemulsion process in DMSO-d_6 .

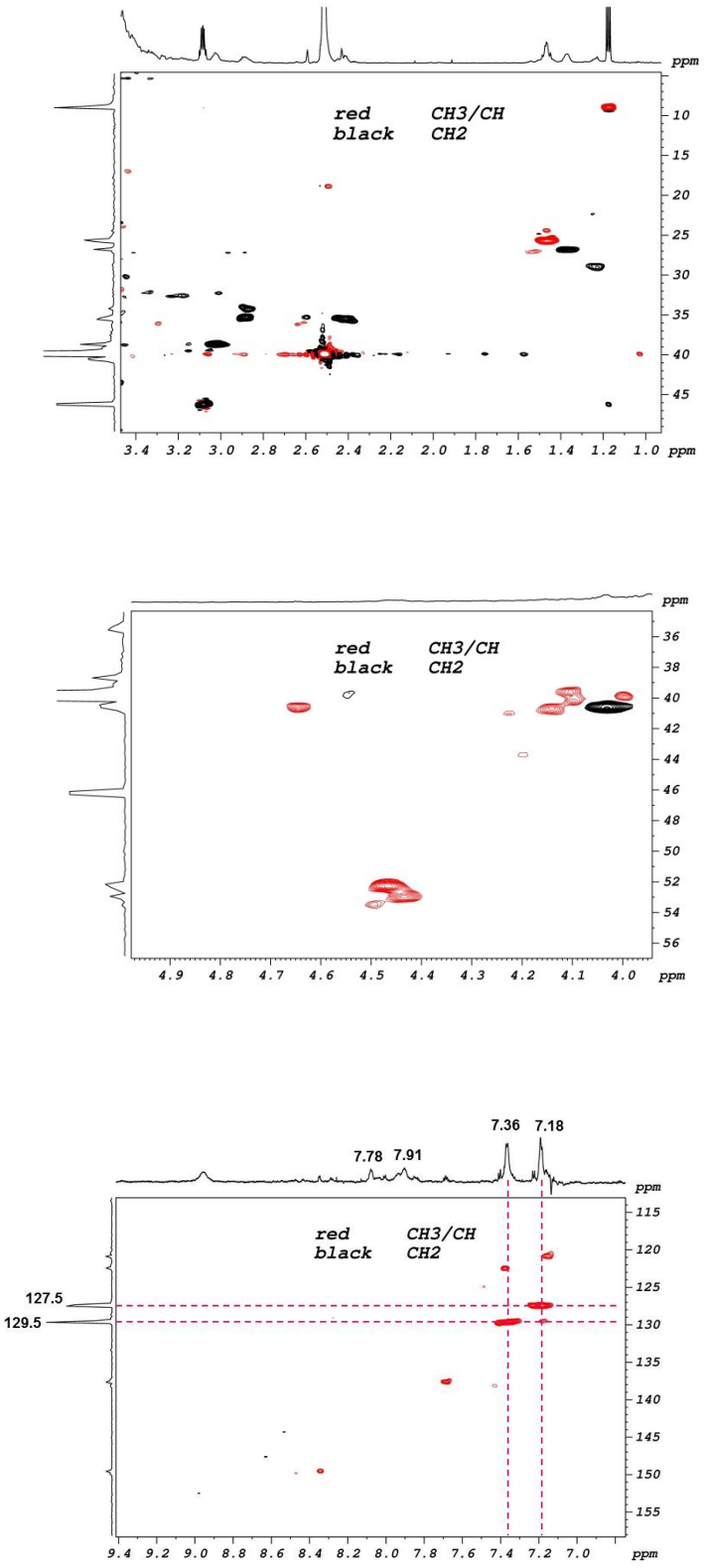


Fig. S13 2D $^{1}H,^{13}C$-HSQC of the product from dual reaction in miniemulsion in (700 MHz, DMSO-d_6, 298 K)

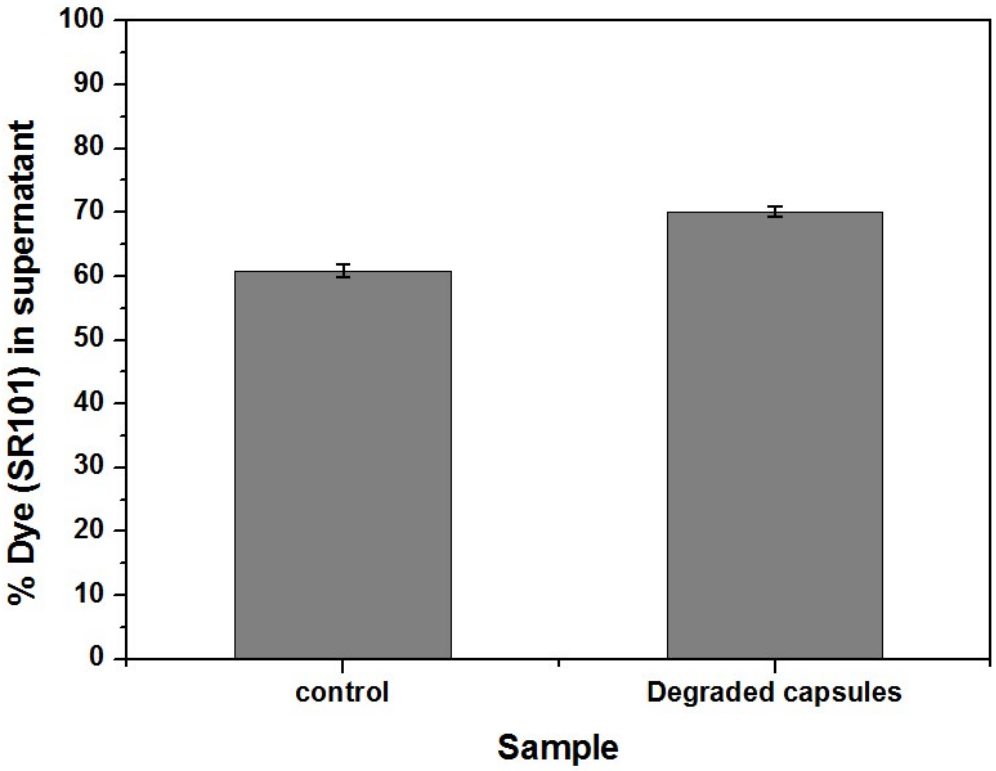


Fig. S14 Percentage of SR101 dyes in supernatant from glutathione responsive capsules after cleavage by glutathione for 7 hours, room temperature at pH7.

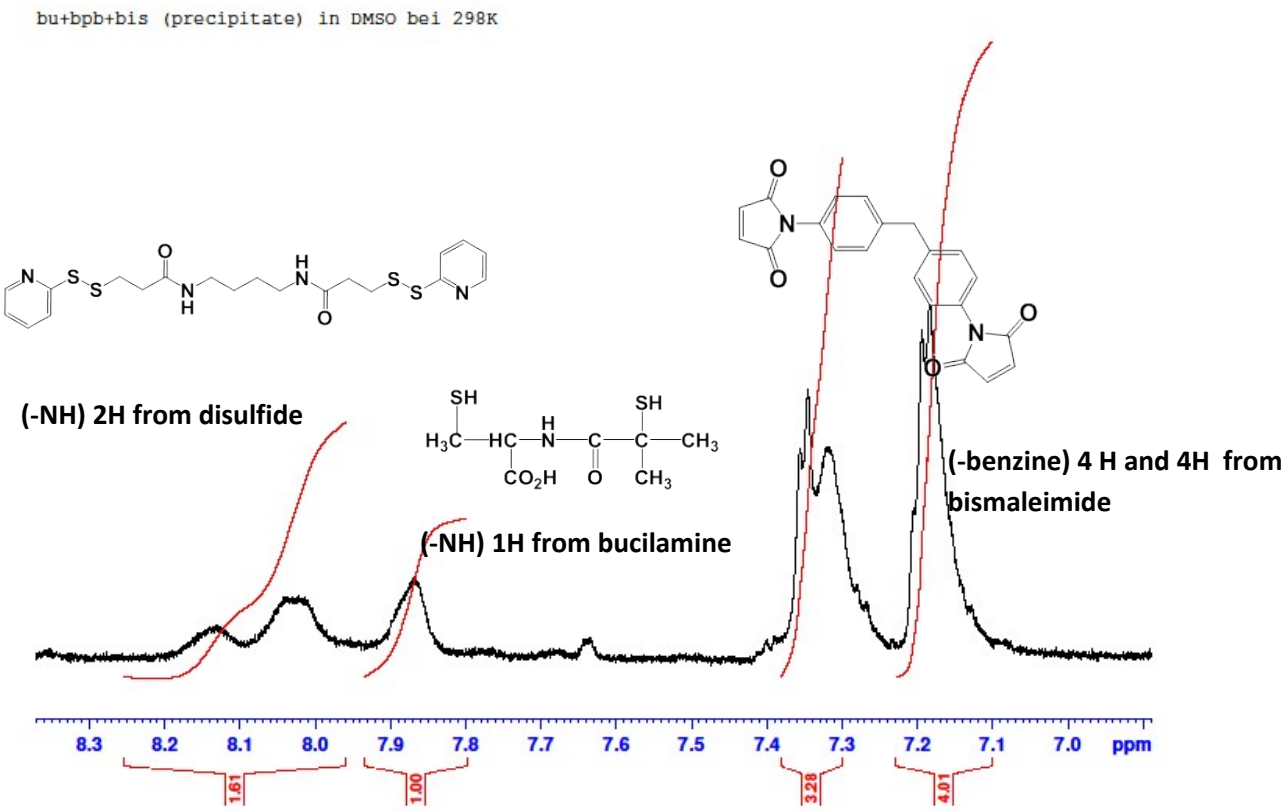


Fig. S15 Integration from ^{1}H NMR of the from dual reaction in miniemulsion at 298 K..