Synthesis and photophysics of red emitting RNA templated PbSe nanostructures†

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Electronic Supporting information†

Experimental Section:

Materials. Lead acetate (Qualigens), sodium hydroxide and sodium borohydride (Merck), Se powder (99.99%, 100 mesh), ribonucleic acid derived from Torula yeast type VI (RNA) (Sigma); nitrogen gas (Grade 1, purity >99.99%) (Sigma, India) and all other chemicals were of analytical grade and used as received.

In this work, the used RNA sample was a heterogeneous mixture of RNA molecules of varied molecular weight(s) and length(s), and no specific sequence was employed.

Equipment. Electronic spectra were recorded on Shimadzu UV2100 and Carry 5000 spectrophotometers in 1 cm quartz cell. PLQY was measused by absolute method with the help of integrating sphere accessory attached with Horiba Jobin Yvon Nanolog spectrofluorophotometer by calibrating it with fluorescein dye. Emission measurements were made on Shimadzu RF-5301PC and Horiba Jobin Yvon Nanolog spectrofluorophotometers in 1 cm quartz cell. 2D and 3D images of fresh and aged particles the colloidal solutions were recorded using tapping mode on a NTEGRE (NTMDT) atomic force microscope (AFM) with

scaning frequency of 1.5 Hz at room temperature (20 ± 2°C). Surface morphologies and elemental analysis of synthesized nanosystems were performed on QUANTA 200-FEG Digital Scanning Electron Microscope with EDAX facility equipped with CCD camera. Electron micrographs and selected area electron diffraction were measured on a FEI-TECNAI 200 kV Digital TEM with an image analysis system having variable magnifications up to 2800000×, respectively. Time resolved emission spectroscopy (TRES) and fluorescence lifetime in nanosecond time domain were recorded in 1 cm quartz cell on a Horiba Jobin Yvon "FluoroCube Fluorescence Lifetime System" equipped with NanoLEDs and LDs. Hamamatsu (R3809 U) photomultiplier and a thermoelectrically cooled TBX-04-D detector was used to detect the emitted photons. Anisotropy measurements, were made using an automated polarisation accessory (Model 5000U-02). The decay curves were analyzed using multi-exponential iterative reconvolution technique, the software, which was provided by IBH. Data analysis was carried out by DAS 6.3 software. X-Ray diffraction patterns were recorded on a Brukers D8 advanced X-Ray diffractometer using Cu Kα line (1.5418 Å) of the X-Ray source at 40 kV and 30 mA. The diffraction patterns were recorded in 2θ range of 10° to 100°.IR spectra were recorded on a Thermo Nicolet Nexus FTIR spectrophotometer in mid IR range (4000 - 400 cm^{-1}) in KBr medium and in far IR range (600 - 50 cm^{-1}) in polystyrene. The proton NMR spectra were recorded on Bruker Avance 500 (500 MHz) spectrometer in H_2O and D_2O media. Optical image was recorded using Nikon eclipse LV100 digital microscope.

Synthesis of RNA-templated colloidal PbSe solution:

Colloidal PbSe was prepared by adding 0.15 ml of 0.1 mol dm^{-3} lead acetate to the 100 ml deaerated aqueous solution containing 0.022g RNA at 4 °C followed by the slow injection of 390

μL of freshly prepared NaHSe (38×10^{-3} mol dm^{-3}).[5] It results in the formation of reddish brown PbSe colloidal solution. To this reaction mixture excess of lead acetate (1.5×10^{-4} mol dm^{-3}) was added drop wise to makeup the effective concentrations of PbSe and Pb^{2+} at 1.5×10^{-4} mol dm^{-3} each and Pb/Se = 2. The pH of solutions was maintained 8.5 at each step.

$$x\ Pb^{2+} + x\ HSe^{-} \rightleftharpoons (PbSe)_x + H^{+} \quad (1)$$

Characterization:

Sample for TEM were prepared by applying a drop of the colloidal sample on a carbon coated copper grid G-200 (size 3.05 mm). The excess of colloidal solution was removed with the help of a tissue paper. The coated grid was dried in dark at room temperature for about 30 min to evaporate the remaining moisture. Electron micrographs of these samples were recorded at different magnifications by scanning the dried grid under the electron microscope at an accelerating voltage of 200 kV. Selected area electron diffraction (SAED) patterns of these samples were recorded to find out the structure of different phases in the colloids samples. Sample for atomic force microscope (AFM) and field emission scanning electron microscope. Indexing of electron diffraction pattern was carried out using a ratio method and Miller indices were then assigned corresponding to different rings. Solid sample(s) of as prepared PbSe for XRD were obtained from their colloidal solution(s) by removing excess of water on a Buchi R-114 Rotavapor at 35°C.

Fig.S1A presents the optical absorption and emission spectra of the optimized sample of colloidal PbSe QDs.

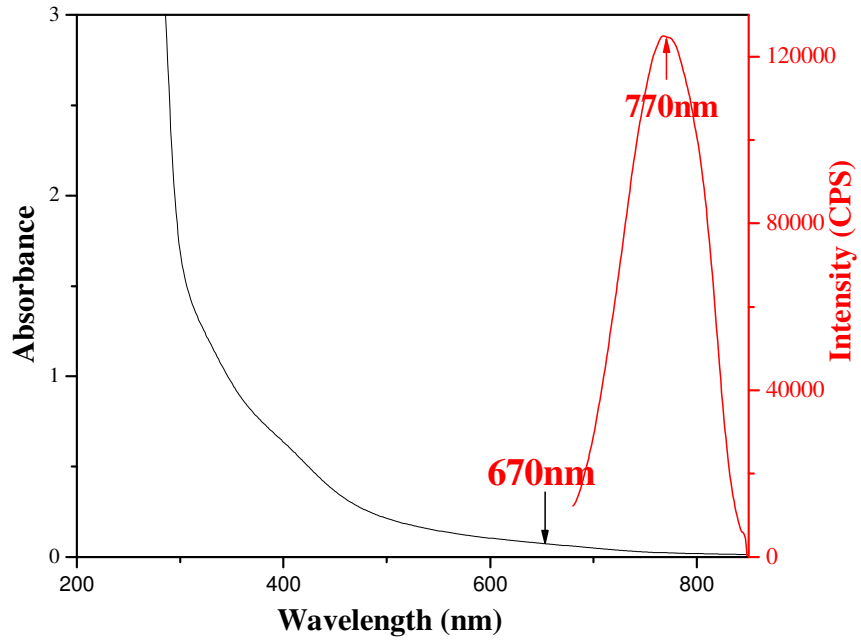


Fig.S1A Absorption and emission spectra of the optimized sample **SP1.**

Optimized sample of colloidal PbSe QDs containing [RNA] = 0.022g/100ml, molar ratio of [Pb/Se] = 2 at pH 8.5. **(SP1)**. [Optimization was carried out by varying [RNA] = 0.013 to 0.025g/100ml; molar ratio of [Pb/Se] = 1 – 3; excitation energy (4.1 eV/ 300 nm - 1.8 eV/ 700 nm); pH from 8.5-10.5.]

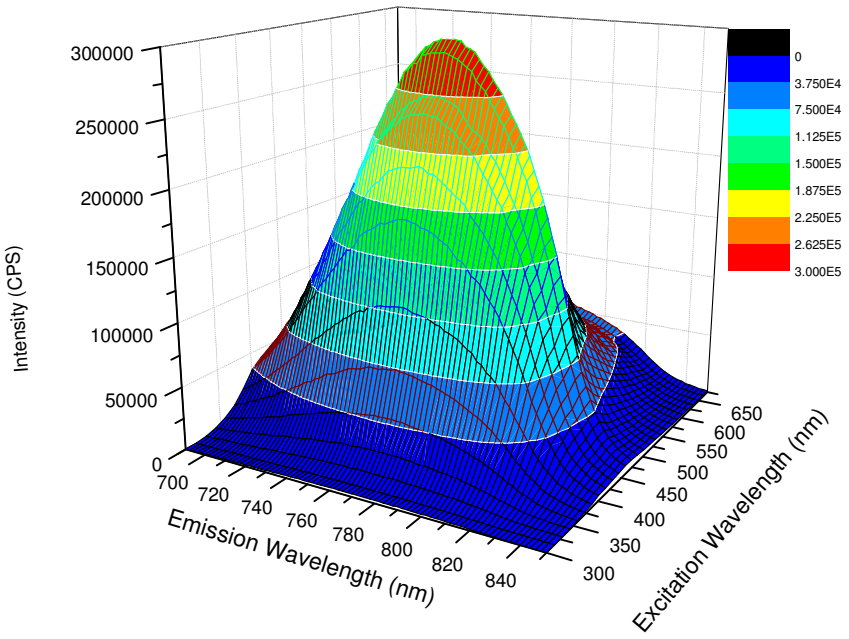


Fig.S1B 3D excitation – emission spectra of **SP1.**

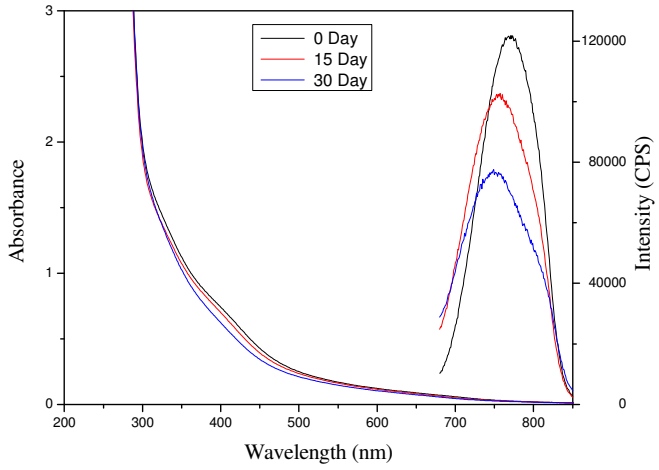


Fig.S1C Absorption and emission spectra of fresh (a) and aged (b) **SP1.**

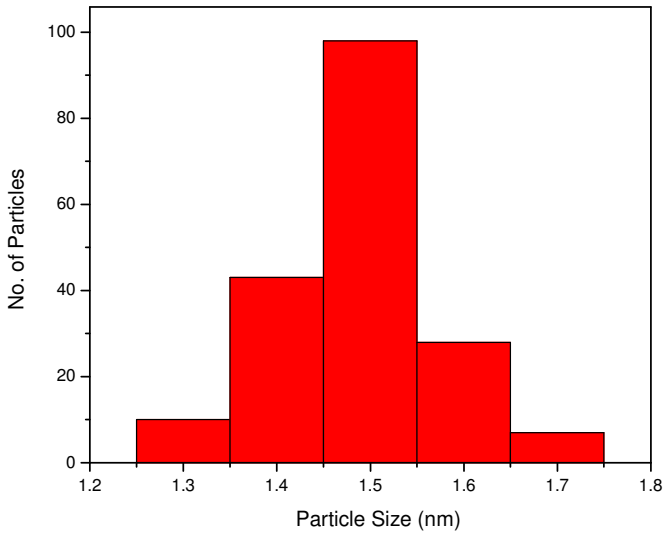


Fig.S2A Size histogram of QDs observed in the TEM image of fresh **SP1 given in Fig. 2a**.

L **R**

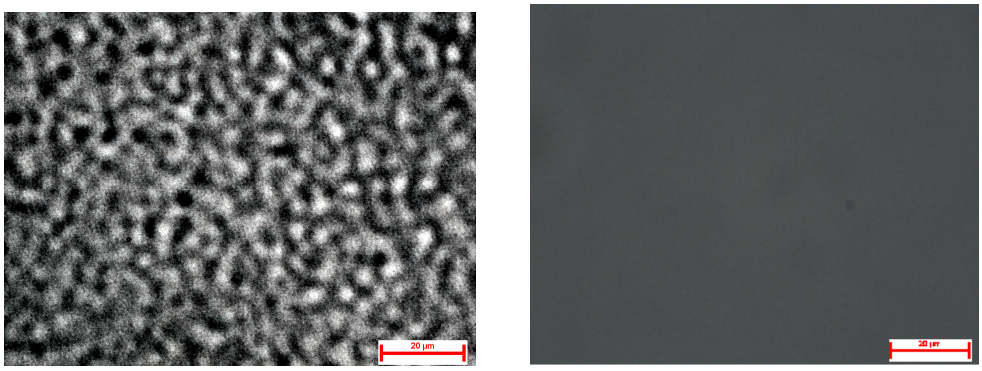


Fig.S2B Optical image (100 ×) of aged **SP1** on glass substrate **(L)** and bare glass substrate **(R).**

Scale bar in the above figure corresponds to 20 µm.

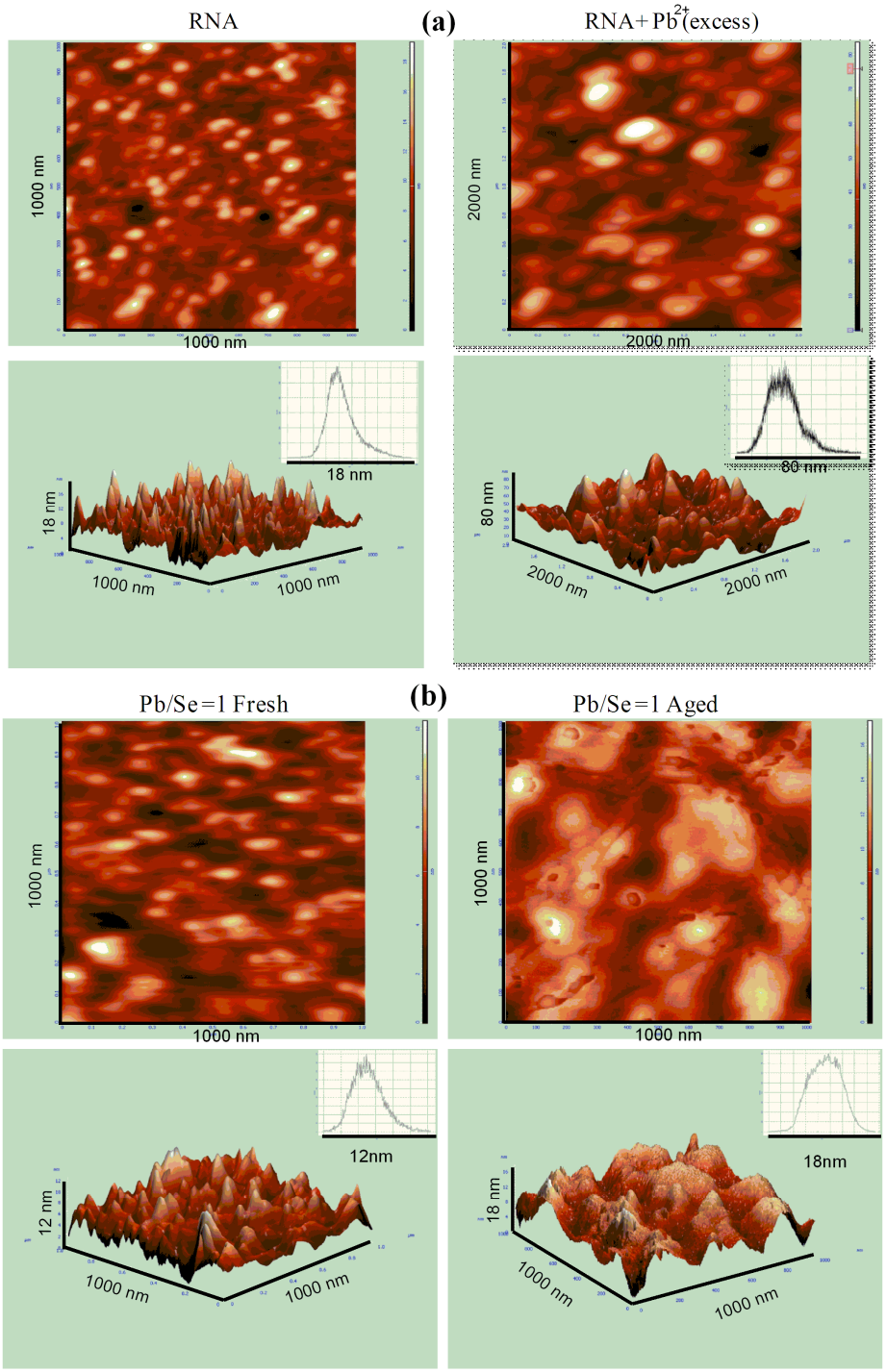


Fig.S2C 2D and 3D AFM images of RNA; RNA+Pb^{2+}(a).

RNA capped PbSe with [Pb]/[Se]=1: fresh; aged (b).

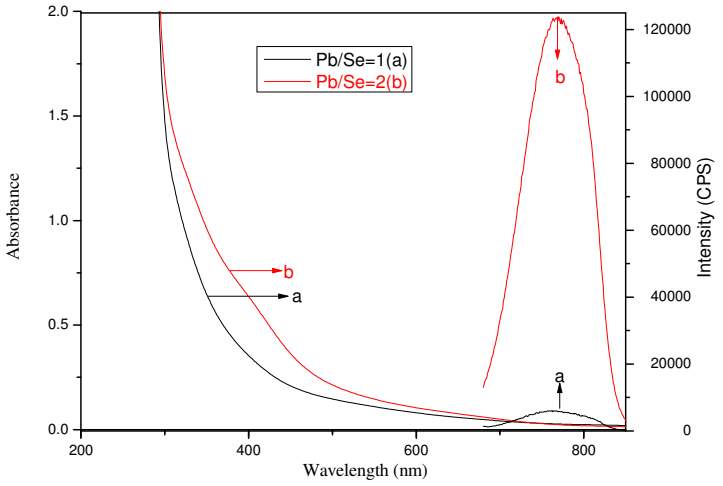


Fig.S2D Effect of Pb/Se molar ratio: 1.0 (a) and 2.0 (b) on the electronic and emission spectra of PbSe QDs at pH 8.5.

IR study:

The IR spectra of SP1 in both mid-ir and far-ir ranges were observed to be fairly different to each of RNA, and Pb^{2+}- RNA as regard to the shape, intensity and energy of various bands (Figs.S3X and S3Y). The addition of Pb^{2+} to RNA results in the disappearance and / or marked shift in the vibration peak (cm^{-1}) corresponding to moieties: 1693 (G&U), 1642 (A&C), 1544 (G), 1469 (U), 1281(C), 1421 (in plane C2´-OH), 1385 & 1355 (purine in anti and syn conformation, respectively); 968 (RNA backbone).

An examination of the IR spectra of these samples in far IR region (Fig. S3Y) exhibit the presence of broad dip masked with several sharp peaks in 250-80 cm^{-1} range. The broad dip is known to arise due to PbSe stretching and the sharp peaks are contributed due to Pb^{2+}-RNA, thus indicating an interaction of PbSe with the matrix.

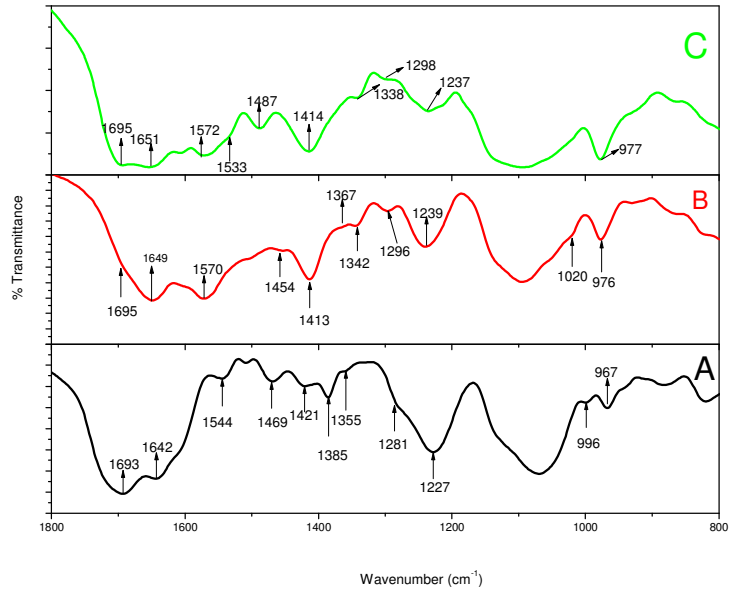


Fig.S3X

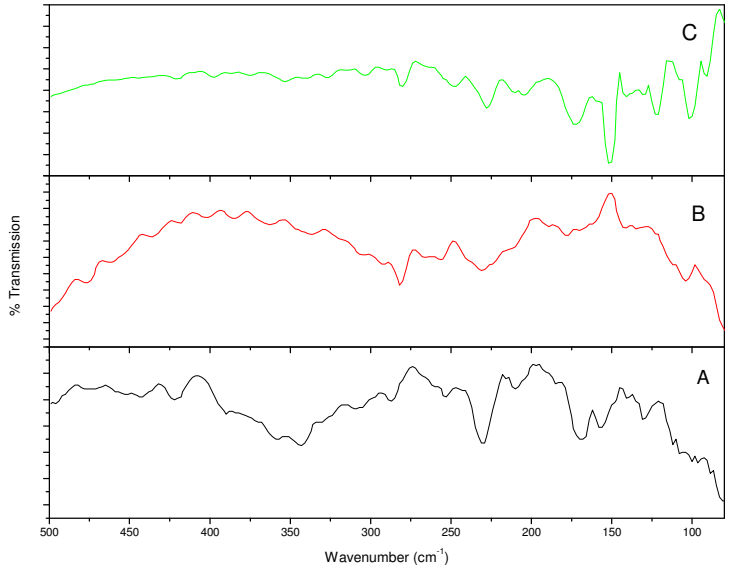


Fig.S3Y

Fig.S3 Mid IR (X) and Far IR spectra (Y) of - RNA (A); RNA+Pb^{2+} (B); PbSe on RNA matrix (C). (H. Arakawa, J. F. Neault, H. A. Tajimir-Riahi, Biophys. J. 2001, **81**, 1580.)

NMR study:

The sites of the interaction of RNA matrix were further probed by recording the ^{1}H NMR of RNA, Pb^{2+} - RNA and PbSe – RNA (Fig.S4). The ^{1}H NMR of RNA exhibits all the characteristic peaks due to aromatic protons of pyrimidine and purine (H_2, H_8, H_6) bases, sugar base (H_1', H_5) and sugar protons (H_2', H_3', H_4', H_5') resonating between 7.5 to 8.4, 5.5 to 6.2 and 3.6 to 4.5 ppm, respectively.

^{1}H NMR **^{31}P NMR**

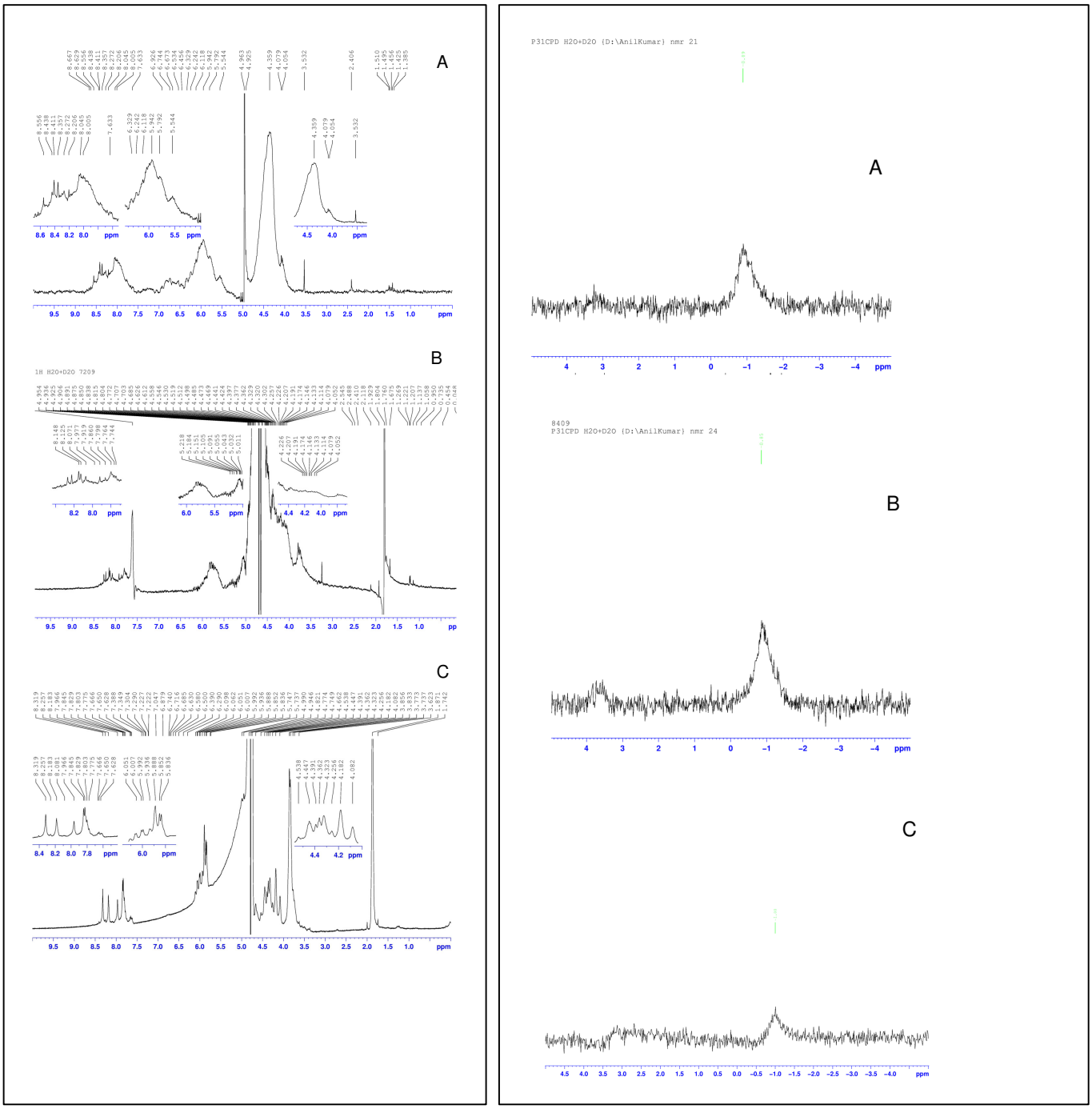


Fig.S4 Proton NMR (Left) and ^{31}P NMR spectra (Right) of - RNA (A); RNA+Pb^{2+} (B); PbSe on RNA matrix (C).

Fluorescence Lifetime measurements:

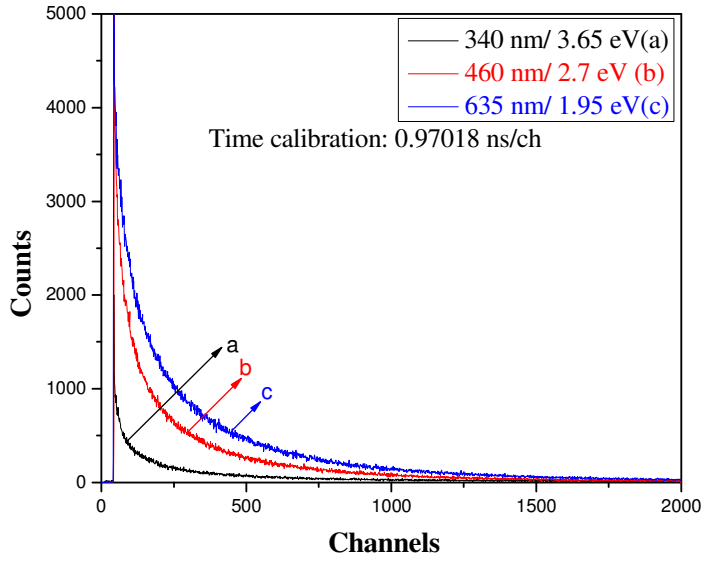


Fig.S5A Effect of excitation energy on emission lifetime of **SP1.**

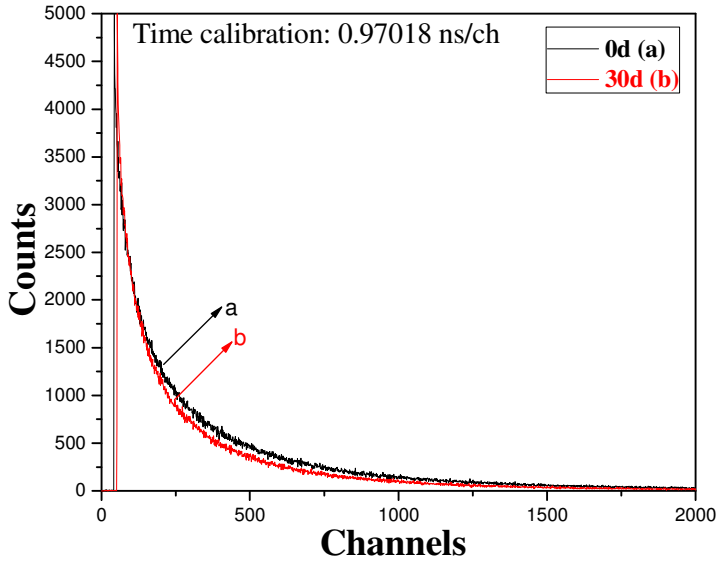


Fig.S5B Decay curves of fresh (red) and aged (green) **SP1**

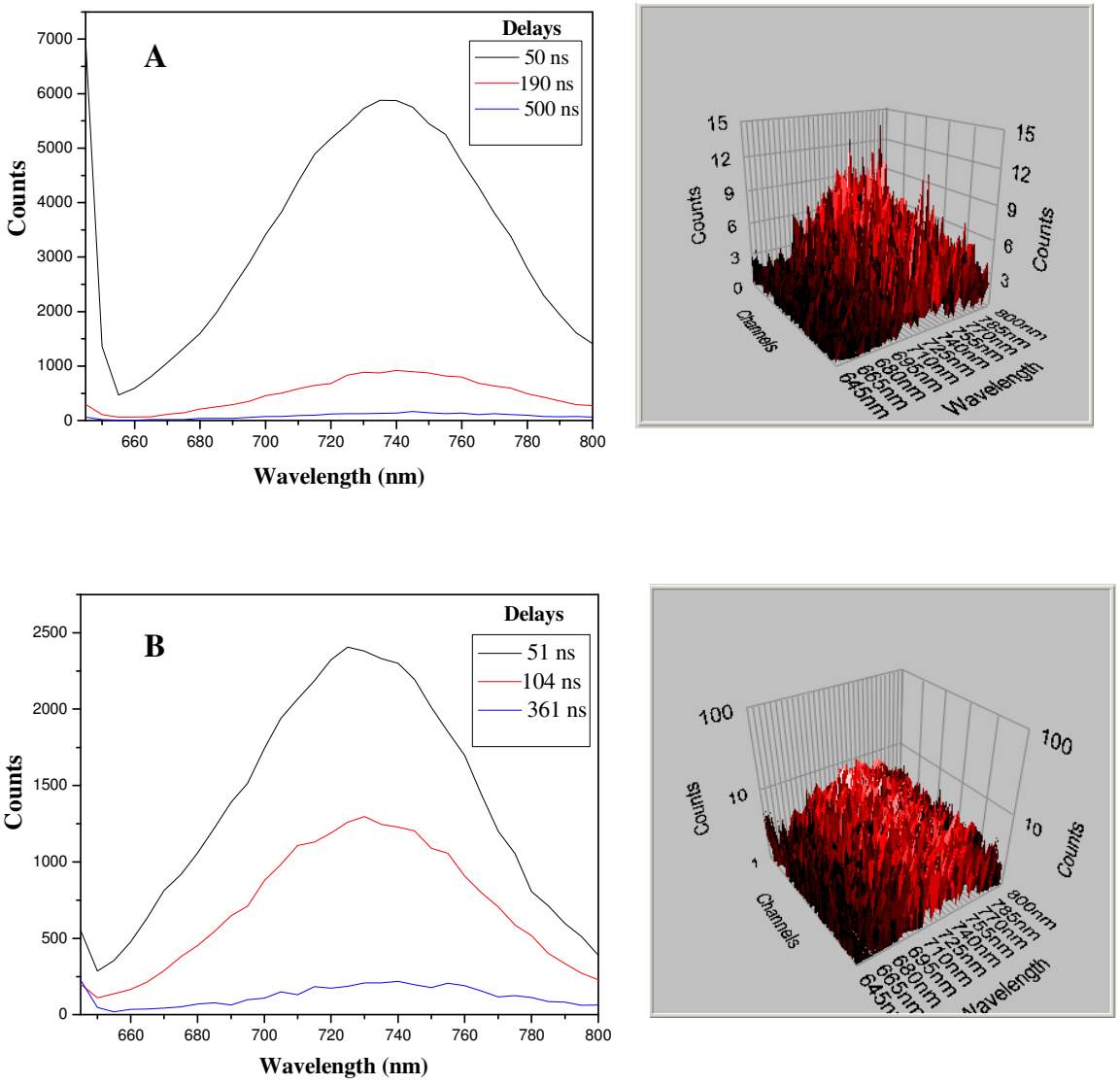


Fig.S6 Time- resolved emission spectra of fresh (A) and aged for 30 days (B) **SP1** alongwith their 3D images.

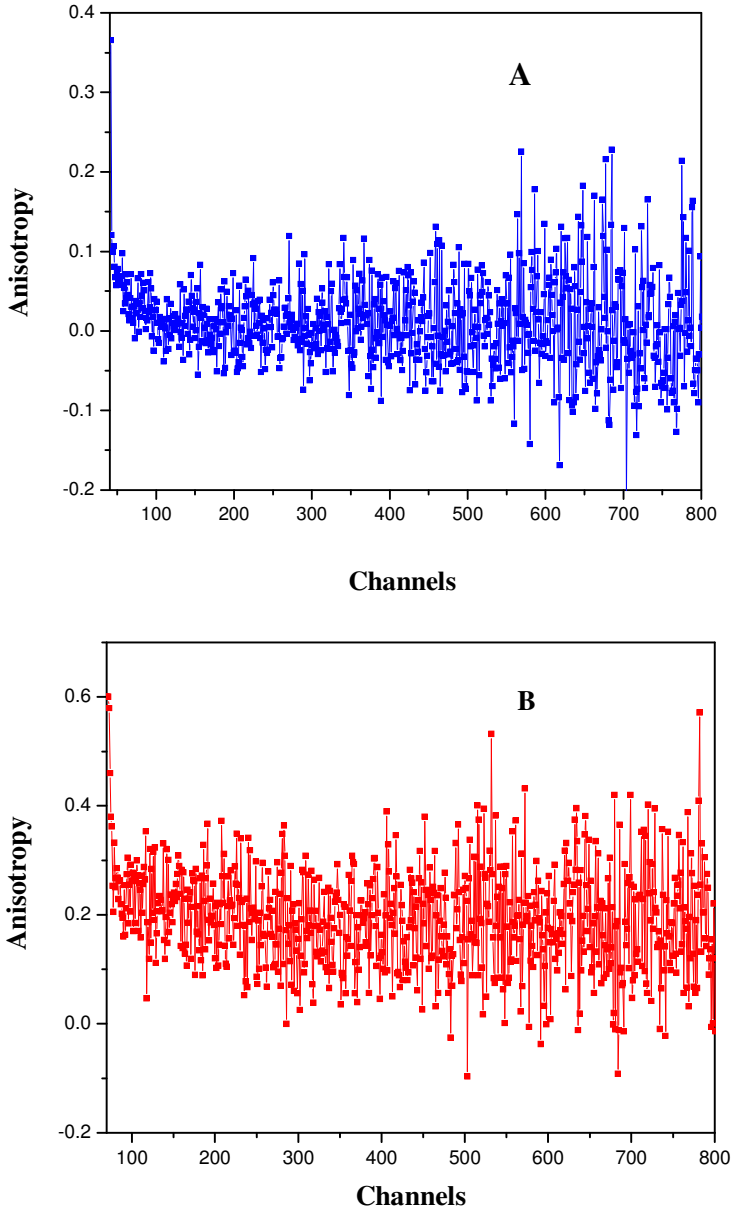


Fig.S7 Time- resolved anisotropy measurements of fresh (A) and aged (B) **SP1**.

Table S1 Mid IR spectra of RNA; RNA+Pb^{2+} and PbSe on RNA matrix.

Moiety/Functional group	**RNA (cm^{-1})**	**Pb^{2+} - RNA (cm^{-1})**	**PbSe on RNA matrix (cm^{-1})**
In plane vibrations: G&U	1693 (br)	1695 (br)	1695 (br) (Change in shape)
A&C	1642 (m)	1649(s)	1651(br)
G	1544(m)	1570(br)	1572(br)
U (medium)	1469(m)	1454(w)	1487(s)
In plane C2´-OH	1421(br)	1413(s)	1414(s)
Purine in anti confm.	1385(s)	1367(sh)	disappeared
Purine in syn confm.	1355 (sh)	1342 (m)	1338 (sh)
C	1281 (sh)	1296 (m)	1298 (br)
Assym. Stretch PO_2^{2-}	1227 (br)	1239 (br)	1237 (br)
In Ribose vib. due to 2´-OH	996 (br)	996(sh)	disappeared
RNA backbone	968 (m)	976 (s)	977(s)

Table S2A Effect of excitation energy on emission lifetime of **SP1** (λ_{ex}=635 nm; λ_{em}=770 nm).

Excitation Wave-length (λ_{ex} in nm)	**Lifetime (ns)**						**<τ> (ns)**	**χ^2**
	Component 1		**Component 2**		**Component 3**			
	τ_1	**Emission %**	**τ_2**	**Emission %**	**τ_3**	**Emission %**		
340	2.31 (0.89)	3.70	50.78 (0.31)	28.39	312.3 (0.12)	67.91	226.6	1.12
460	8.18 (0.52)	4.29	75.68 (0.40)	30.16	356.0 (0.18)	65.56	256.6	1.18
635	29.23 (0.26)	7.68	140.75 (0.23)	32.84	456.5 (0.13)	59.48	320.0	1.21

(Value in bracket is pre-exponential factor corresponding to respective τ)

TABLE S2B Effect of aging on the emission lifetime of **SP1** (λ_{ex} = 635 nm; λ_{em} = 770 nm).

Aged (in days)	**Lifetime (ns)**						**<τ> (ns)**	**χ^2**
	Component 1		**Component 2**		**Component 3**			
	τ_1	**Emission %**	**τ_2**	**Emission %**	**τ_3**	**Emission %**		
0	29.23 (0.26)	7.68	140.75 (0.23)	32.84	456.5 (0.13)	59.48	320.0	1.22
30	5.33 (0.32)	1.92	81.40 (0.30)	27.43	368.63 (0.17)	70.65	282.9	1.26

(Value in bracket is pre-exponential factor corresponding to respective τ)

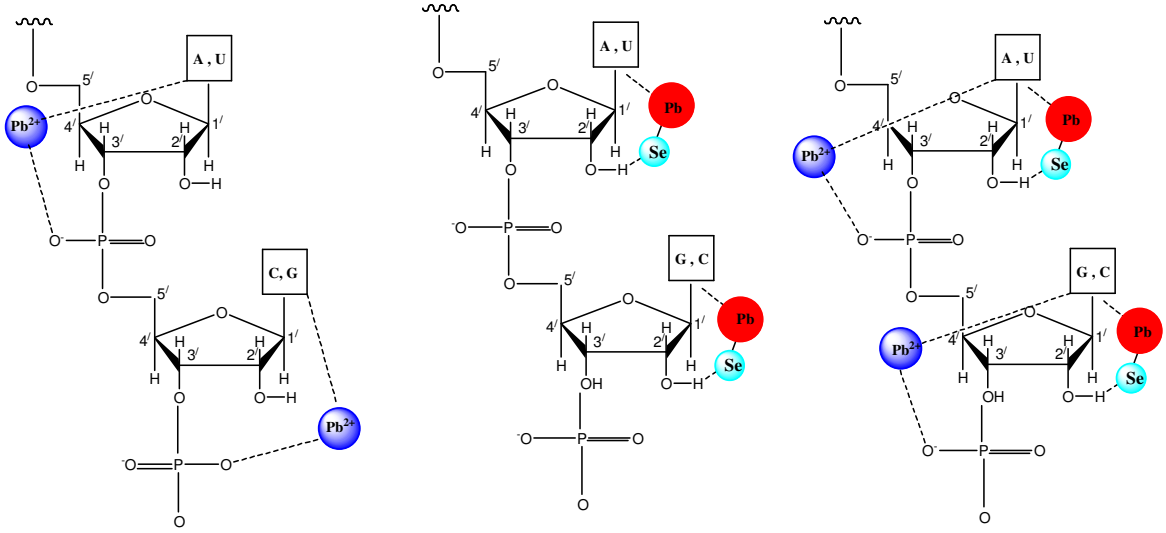
TABLE S3A Steady State Anisotropy and polarization of fresh and aged SP1 (λ_{ex}=670nm; λem=770nm).

SP1	**Anisotropy**	**Polarization**
Fresh	0.020	0.030
Aged	0.035	0.052

TABLE S3B Time resolved anisotropy data of fresh and aged **SP1** (λ_{ex}=635nm; λ_{em}=770nm).

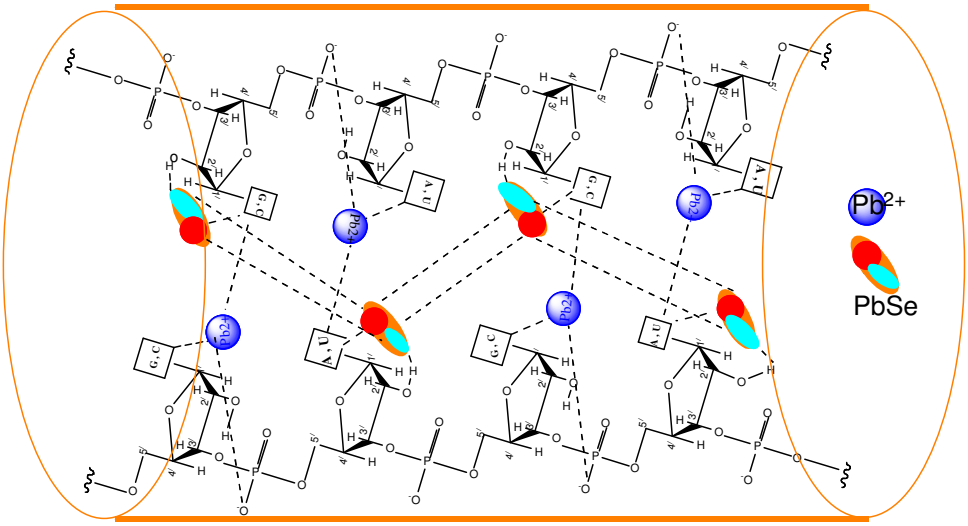
Aged (days)	**Component 1**		**Component 2**		χ^2
	Θ_1 (ns)	**Emission %**	**θ_2 (ns)**	**Emission %**	
0	0.41 (0.26)	4.54	24.6 (0.09)	95.46	0.97
30	0.76 (0.73)	11.5	54.0 (0.08)	88.53	1.08

Schemes:



a b c

Scheme S1A: Pb^{2+}- RNA (a); Stoichiometric PbSe - RNA (b); PbSe - RNA in the presence of excess Pb^{2+} (**SP1**) Fresh (c).



Scheme S1B: **SP1** (Aged).