SUPPORTING INFORMATION

Easy and rapid chemosensing method for identification of accumulated Tin in algae: A solemnstrives to protect marine eco-system

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1. NMR Studies:

^{1}H NMR of AFLin DMSO-d_6 :

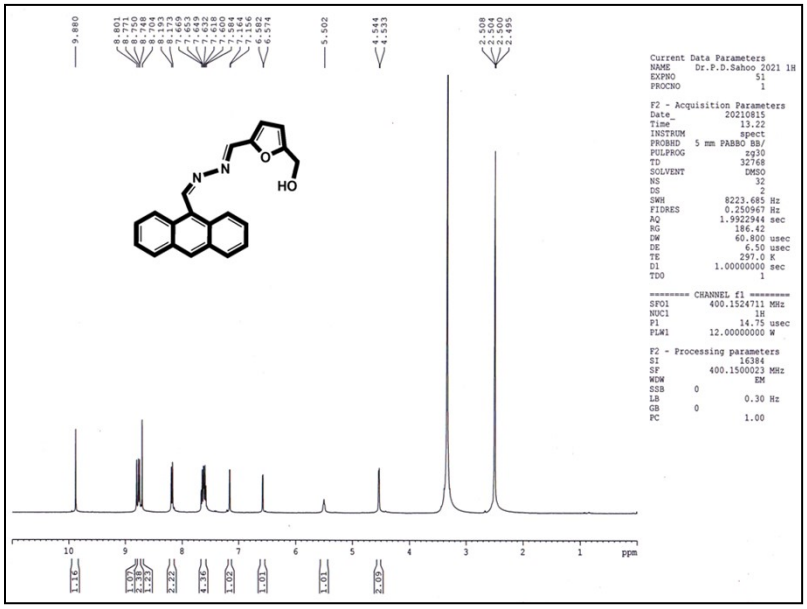


Fig. S1. ^{1}H NMR of **AFL** in DMSO-d_6 (400 MHz).

^{13}C NMR of AFL in DMSO-d_6:

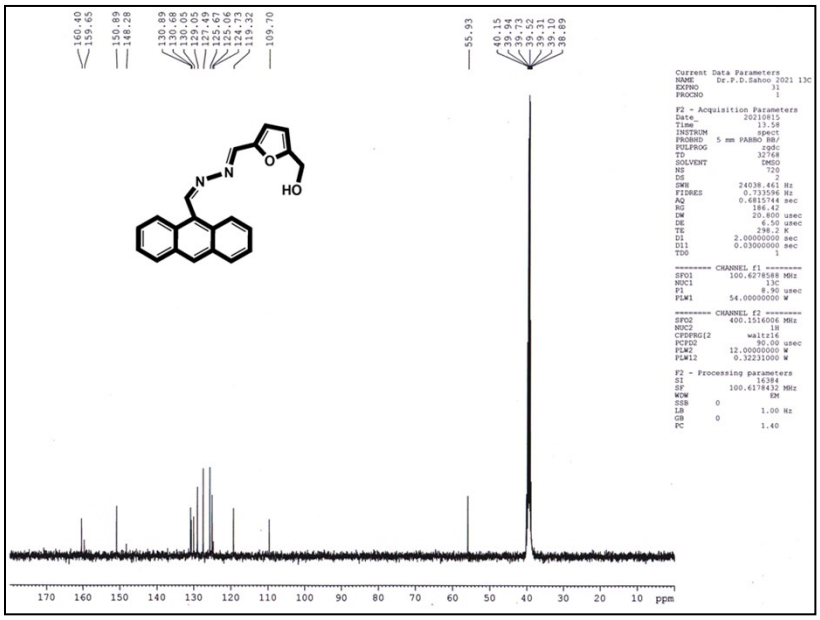


Fig. S2. ^{13}C NMR of **AFL** in DMSO-d_6 (400 MHz).

Mass spectrum of AFL:

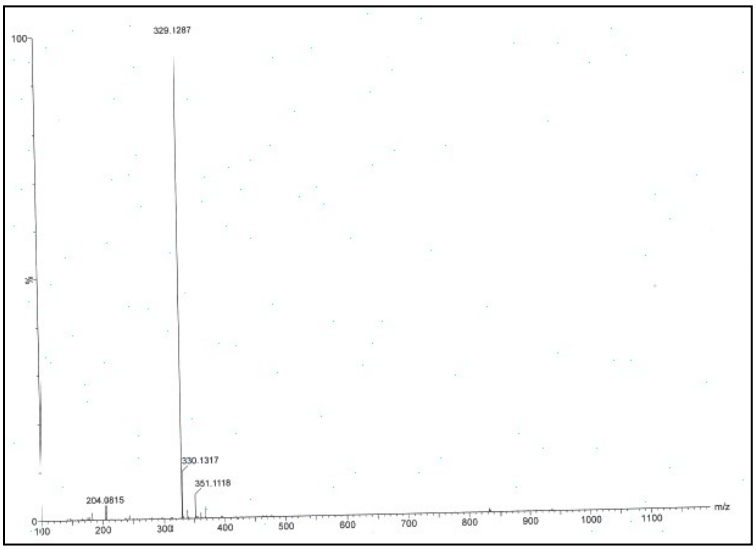


Fig. S3. HRMS of **AFL**

2. Materials and Instruments

9-anthracenecarboxaldehyde, 5-(hydroxymethyl) furfural, methanol, acetonitrile and all the reagents were purchased from Sigma-Aldrich Pvt.Ltd. Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Solvents were dried according to standard procedures. Elix Millipore water was used throughout all experiments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra, $CDCl_3$, DMSO-d_6 and for NMR titration DMSO-d_6 and D_2O were used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. The following abbreviations are used to describe spinmultiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.The mass spectrum (HRMS) was carried out using a micromass Q-TOF Micro™ instrument by using Acetonitrile as a solvent. Fluorescence spectra were recorded on a Perkin Elmer Model LS 55 spectrophotometer. UV spectra were recorded on a SHIMADZU UV-3101PC spectrophotometer.

Leica TCS SP8 laser scanning confocal microscope systemwas used for confocal microscopy. Images obtained through section scanning were analyzed by the LasX software with excitation at 400 nm monochromatic laser beam, and emission spectra were integrated over the range 483 nm (single channel) with 10X magnification.

3. UV-vis and fluorescence titration. A stock solution of **AFL** (1 μM) was prepared in water-acetonitrile (1:1, v/v). Sn^{2+} solution of concentration 10 μM was prepared in Millipore water. All experiments were carried out in aqueous medium at neutral pH. During the titration, each time a 1 μM solution of **AFL** was filled in a quartz optical cell of 1 cm optical path length

and Sn^{2+} stock solution was added into the quartz optical cell gradually by using a micropipette. Spectral data were recorded at 1 min after the addition of Sn^{2+}.

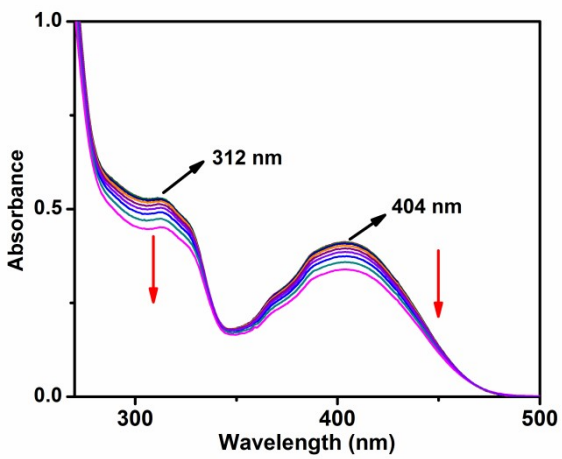


Fig. S4. UV−vis absorption spectra of **AFL** (1 μM) upon incremental addition of Sn^{2+} up to 1 μM in H_2O:CH_3CN (1:1, v/v) at pH 7.2 (10 mM Tris-Cl buffer)

4. Determination of fluorescence quantum yield

The quantum yield can be calculated as follows:

$\Phi D = \{F_D \times A_S \times (n_D)^2 / F_S \times A_D \times (n_S)^2\} \times \Phi S$

Where Φs is the fluorescence quantum yield of the standard (Coumarin 1 in ethanol, 0.73, 25°C), F_D and F_S are the integral areas of fluorescence intensity of the chromophore and the standard at the same excitation wavelength, respectively, A_D and A_S are the absorbances of the chromophore and the standard at the defined excitation wavelength, respectively, and n_S and n_D are the refractive indices at 25°C of the solvents of the standard (ethanol) and of the chromophore, respectively.

5. Evaluation of the association constants for the formation of AFL-Sn^{2+} complex:

By Fluorescence Method:

Binding constant of the chemosensor **AFL** was calculated through emission method by using the following equation:

$$1/ (I - I_0) = 1/K(I_{max} - I_0) [G] + 1/(I_{max} - I_0) \quad \text{……..(ii)}$$

Where I_0, I_{max}, and I represent the emission intensity of free **AFL**, the maximum emission intensity observed in the presence of added Sn^{2+}at 483 nm (λ_{ex}= 400 nm),the emission intensity at a certain concentration of the Sn^{2+} respectively and [G] is the concentration of the guest Sn^{2+}.

Binding constant calculation graph (Fluorescence method):

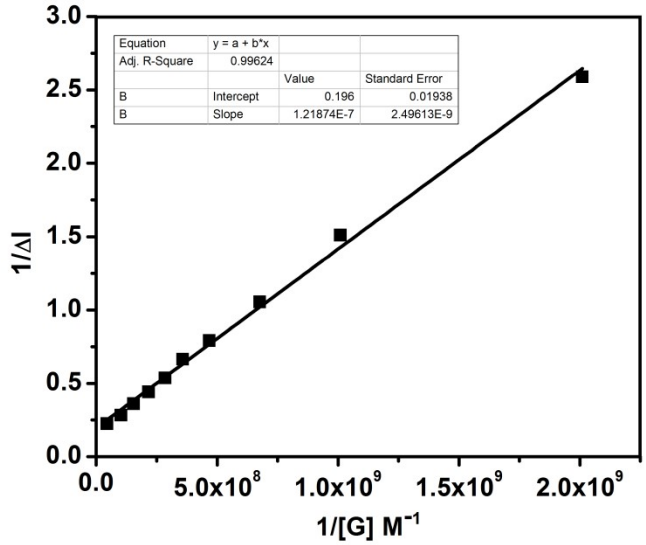


Fig. S5. Linear regression analysis for the calculation of association constant value by fluorescence titration method

The association const. (K_a) of **AFL** for sensing Sn^{2+} was determined from the equation:

K_a = intercept/slope.From the linear fit graph we get intercept= 0.196, slope = 1.21874×10^{-7}. Thus we get, $\mathbf{K_a}$= (0.196) / (1.21874×10^{-7}) = $\mathbf{1.60 \times 10^6\ M^{-1}}$.

6. Calculation of limit of detection (LOD) of AFLwith Sn^{2+}:

The detection limit of the chemosensor **AFL** for Sn^{2+} was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without $Sn^{2+}$was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of **AFL** for sensing $Sn^{2+}$was determined from the following equation.

$$LOD = K \times SD/S$$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

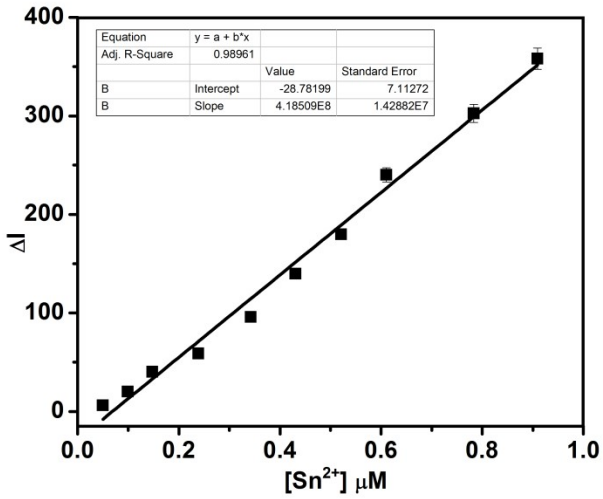


Fig. S6. Linear fit curve of **AFL** at 483 nm with respect to Sn^{2+} concentration. Standard deviations are represented by error bar (n=3).

For **AFL** with Sn^{2+}**:**
From the linear fit graph we get slope = 4.18509×10^{8}, and SD value is 12.54253
Thus using the above formula we get the Limit of Detection = 8.990×10^{-8} M, i.e 89.9 nM or 90 nM. Therefore **AFL** can detect Sn^{2+} up to this very lower concentration by fluorescence technique.

7. Job's plot for determining the stoichiometry of binding by fluorescence method:

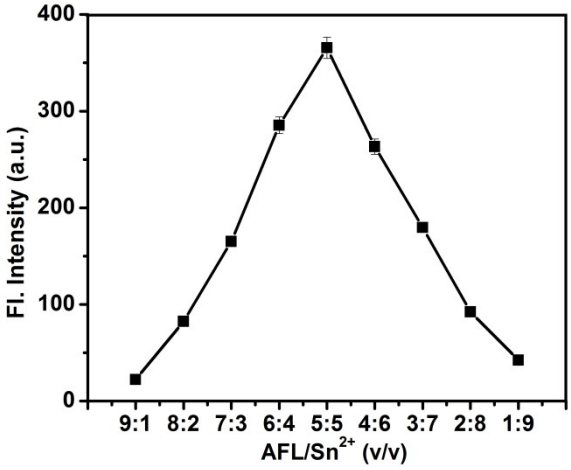


Fig. S7. Job's plot of **AFL** (1μM) with Sn^{2+} (1μM) in acetonitrile-water (1:1, v/v), at neutral pH =7.2 (10 mM Tris-Cl buffer), by fluorescence method ($\lambda_{ex.}$= 400 nm), which indicate 1:1 stoichiometry for **AFL** with Sn^{2+} ion. Standard deviations are represented by error bar (n=3).

8. Reversible cycle analysis of chemosensing probe AFL

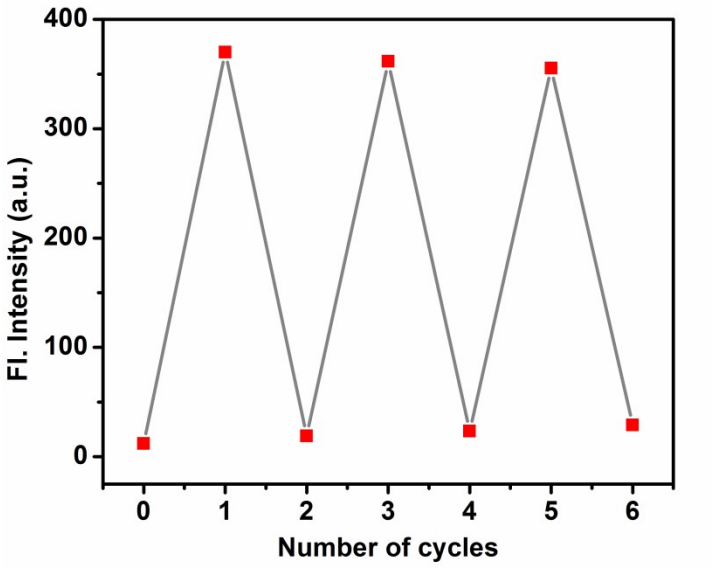


Fig. S8. Reversibility cycle analysis of **AFL** (1× 10^{-6} M) with Sn^{2+} (1× 10^{-6}) in the presence of EDTA (1× 10^{-5} M) in acetonitrile-water (1:1, v/v), buffered with 10 M Tris-Cl buffer (pH 7.2), $\lambda_{ex.}$= 400 nm.

9. Selectivity in presence of other analytes

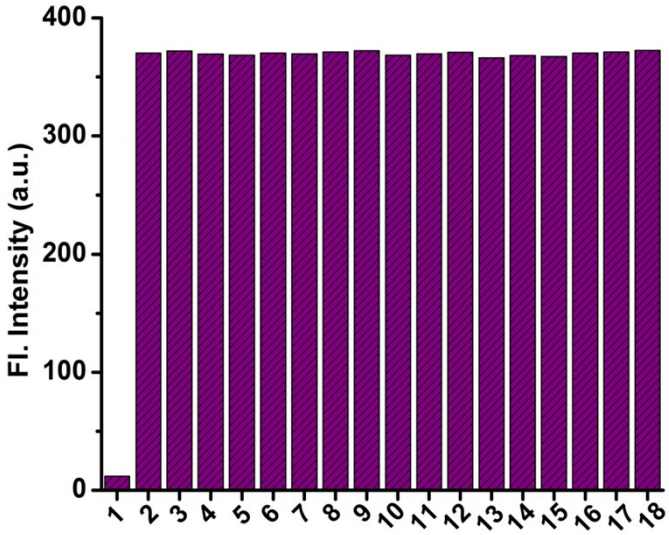


Fig. S9. Histogram representing competitive fluorescence spectra of **AFL** with different bio relevant cations at 483 nm (λ_{ex}= 400 nm) in CH_3CN-H_2O (1:1, v/v), at neutral pH.[From left to right: 1) Only **AFL**, **AFL** with 2) Sn^{2+}, 3) Sn^{2+}+ Sn^{4+} 4) Sn^{2+}+ Zn^{2+}, 5) Sn^{2+}+ Cu^{2+}, 6) Sn^{2+}+ Hg^{2+}, 7) Sn^{2+}+ Cd^{2+}, 8) Sn^{2+}+ Mg^{2+}, 9) Sn^{2+}+ Pb^{2+}, 10) Sn^{2+}+ Fe^{2+}, 11) Sn^{2+}+ Cr^{2+}, 12) Sn^{2+}+ Ni^{2+}, 13) Sn^{2+}+Co^{2+},14) Sn^{2+}+ Mn^{2+}, 15) Sn^{2+}+ Ca^{2+}, 16) Sn^{2+}+ Al^{3+}, 17) Sn^{2+}+ Na^{+} and 18) Sn^{2+}+K^{+}].

10. pH Titration

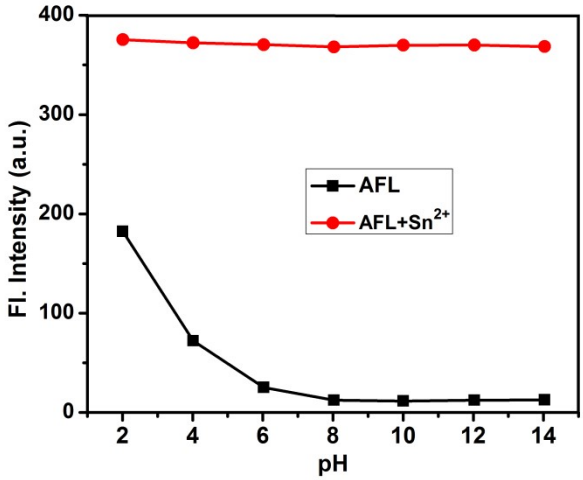


Fig. S10. Fluorescence responses of probe **AFL** (black) and **AFL-Sn^{2+} complex** (red) in different pH conditions in water-acetonitrile (1:1, v/v) (λ_{ex}= 400 nm).

11. ^{1}H NMR titration studies

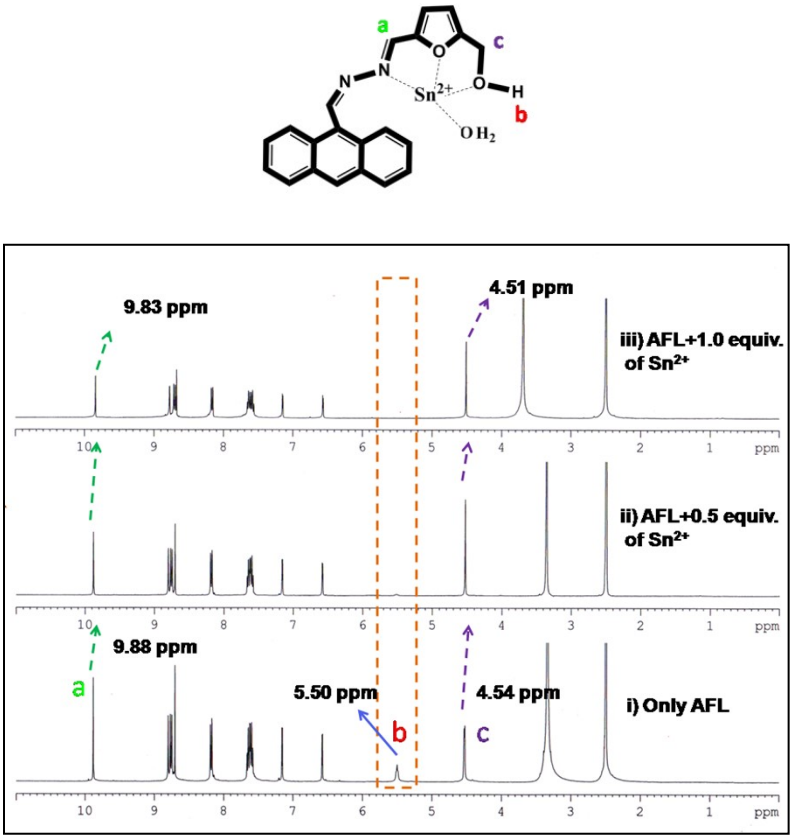
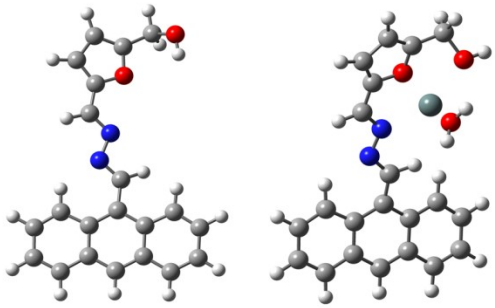


Fig. S11. ^{1}H NMR titration [400MHz] of **AFL** in DMSO-d_6 at 25^{0}C and the corresponding changes after the addition of 1 equiv. of Sn^{2+} from (i) only **AFL**, (ii) **AFL**+ 0.5 equiv. of Sn^{2+} (iii) **AFL**+ 1.0 equiv. of Sn^{2+}

12. Energy minimized structures of AFL and AFL-Sn^{2+} complex



AFL
E= - 1070.0319 a.u.

AFL-Sn^{2+} complex
E= - 1149.5865 a.u.

Fig. S12. Energy minimized structures of **AFL** and **AFL-Sn^{2+}complex** from B3LYP level

13. Mass spectrum of AFL-Sn^{2+} complex:

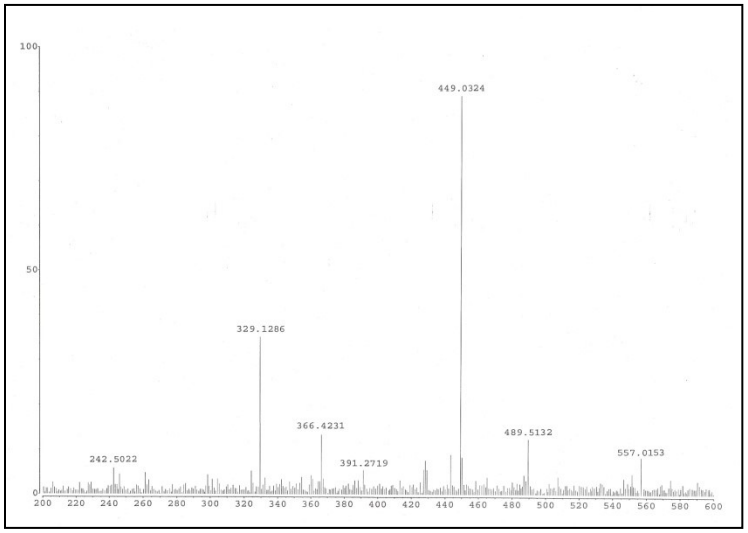


Fig. S13. HRMS of **AFL-Sn^{2+} complex**

14. Table S1 Details of the geometry optimization in Gaussian 09 program.

Details	**AFL**	**AFL-Sn^{2+}complex**
Calculation method	B3LYP	B3LYP
Basis set	6-311G(d, p)	6-311G (d, p) /LANL2DZ
E(CAM-B3LYP) (a.u.)	-1070.0319	-1149.5865
Charge, Multiplicity	0, 1	+2, 1
Solvent (CPCM)	Water	Water

15.Table S2. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of all the molecules and complexes. The data were calculated by TDDFT//B3LYP/6-311G(d,p)/LANL2DZ based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c](%)
AFL	$S_0 \rightarrow S_1$	2.6039eV476.15 nm	0.6611	H → L (70%)
	$S_0 \rightarrow S_4$	3.5528eV348.98 nm	0.3413	H → L+1 (55%)
AFL-Sn^{2+}complex	$S_0 \rightarrow S_4$	3.1579eV 392.61 nm	0.0754	H-1 → L (67%)
	$S_0 \rightarrow S_7$	3.4399eV 360.43 nm	0.2616	H-2 → L (48%)

[a]Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength.[b]Oscillator strength. [c]H stands for HOMO and L stands for LUMO.

16. Table S3. Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

Species	**E_{HOMO} (a.u)**	**E_{LUMO} (a.u)**	**ΔE(a.u)**	**ΔE(eV)**	**ΔE(kcal/mol)**
AFL	-0.20594	-0.09813	0.10781	2.93	67.65
AFL-Sn^{2+}complex	-0.21964	-0.13535	0.08429	2.29	52.89