Selective and sensitive detection of Nitric Oxide in aqueous and biological settings using a novel fluorescent Cobalt-MOF

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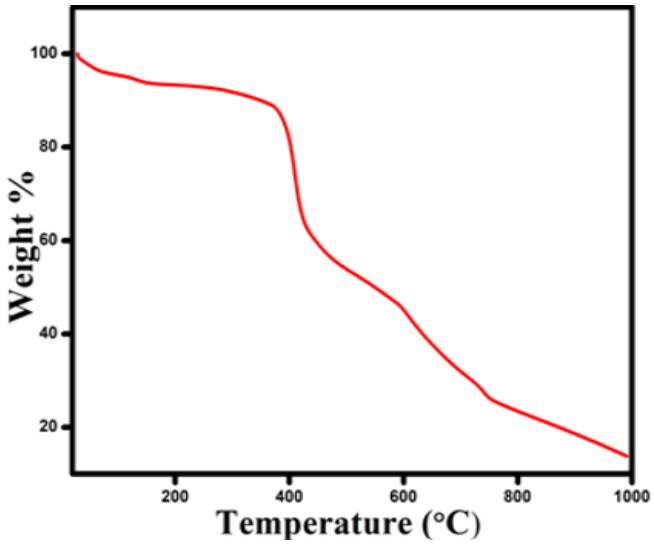

Figure S1. TGA curve of PUC-9 NPs

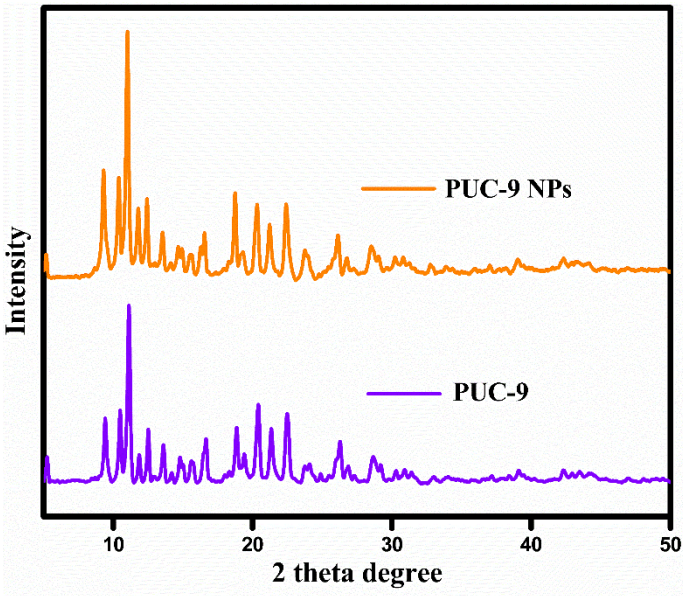

Figure S2. PXRD spectra of PUC-9 and PUC-9 NPs.

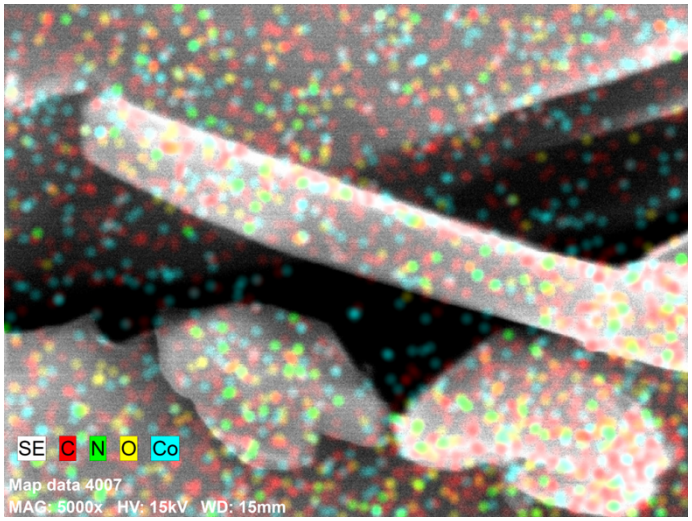

Figure S3. Overall elemental mapping of PUC-9.

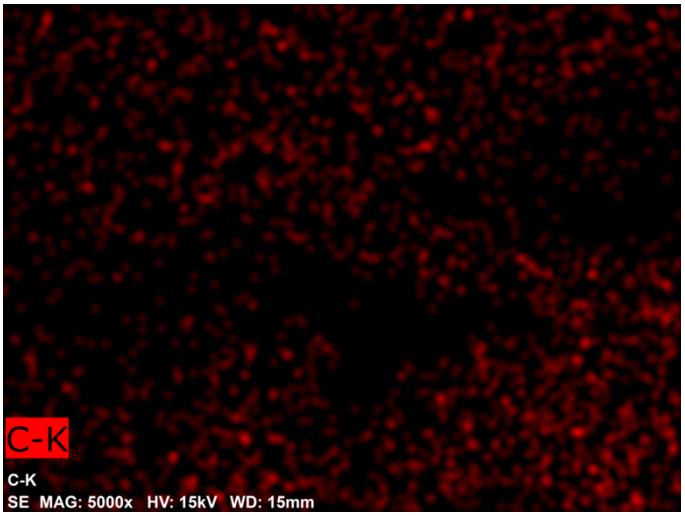

Figure S4. Elemental mapping of PUC-9 to confirm the presence of Carbon.

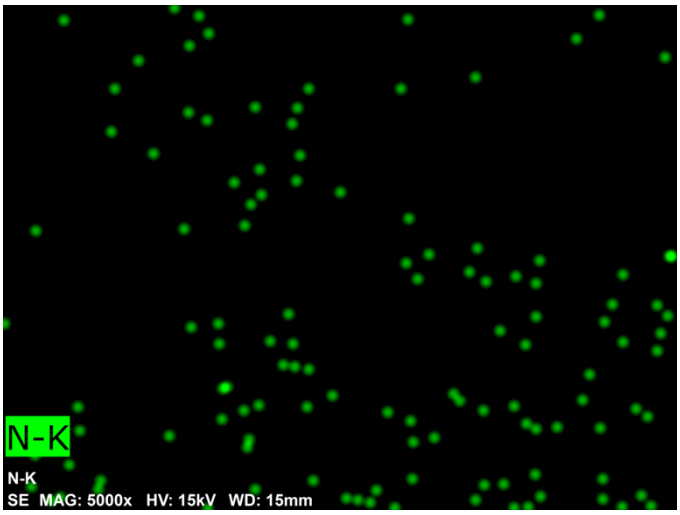

Figure S5. Elemental mapping of PUC-9 to confirm the presence of Nitrogen.

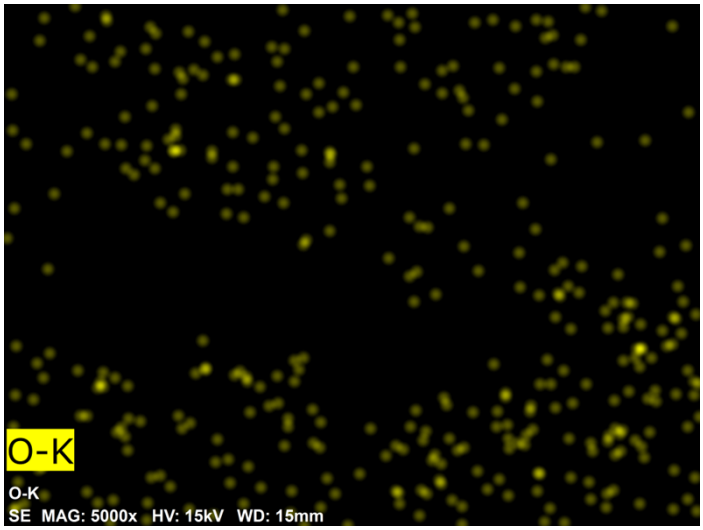

Figure S6. Elemental mapping of PUC-9 to confirm the presence of Oxygen.

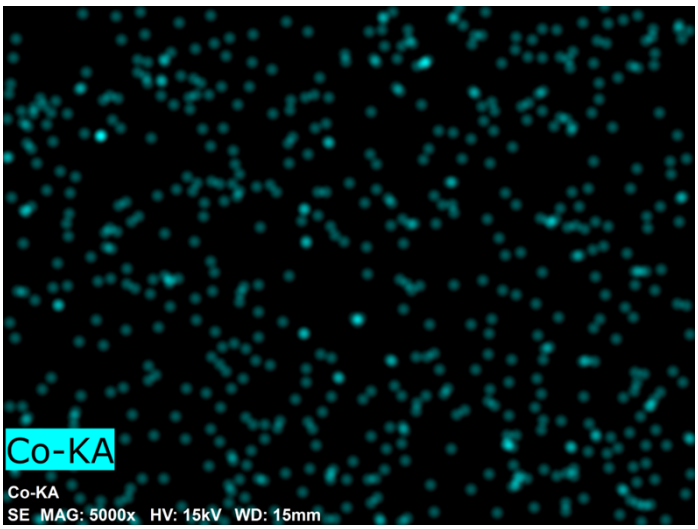

Figure S7. Elemental mapping of PUC-9 to confirm the presence of Cobalt.

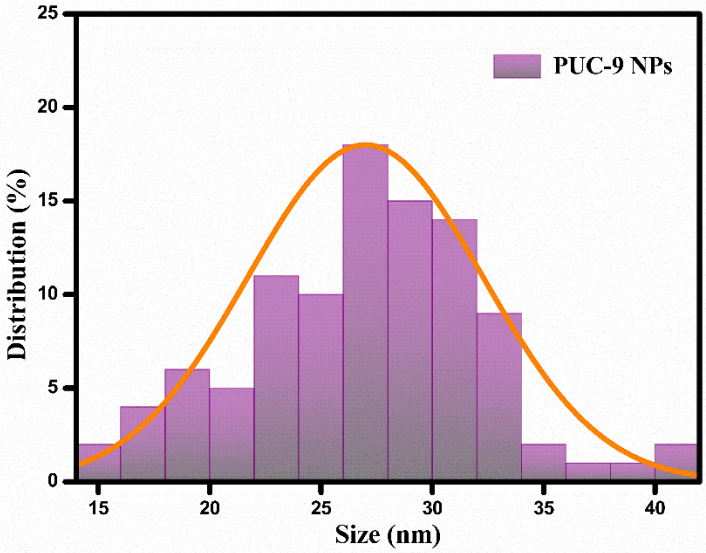

Figure S8. Size distribution histogram plot of PUC-9 NPs.

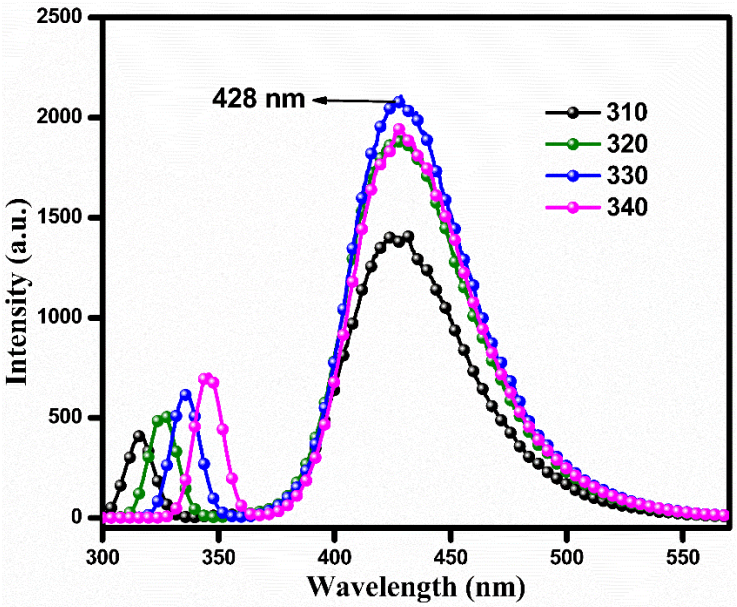

Figure S9. Fluorescence spectra of PUC-9 at different excitation wavelength (310-340 nm).

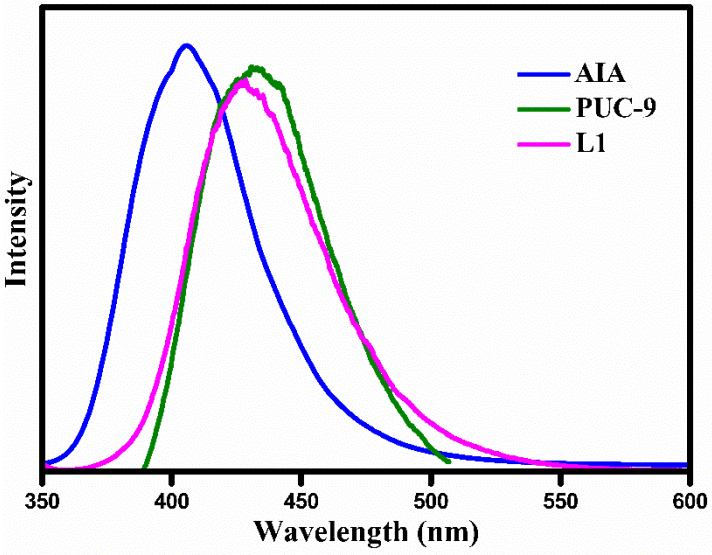

Figure S10. Comparison of emission spectra of PUC-9 with its ligands.

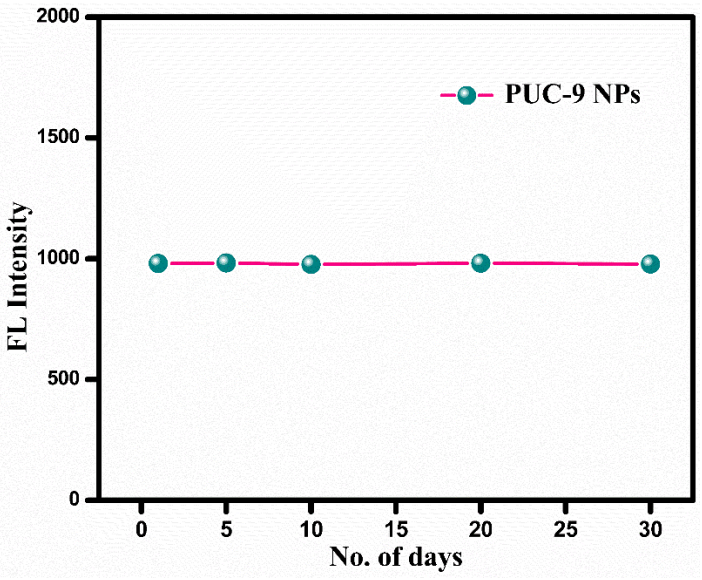

Figure S11. The fluorescence intensity of PUC-9 NPs remained unchanged over a 30-day incubation period, indicating their stable fluorescence properties. This consistent intensity suggests that the NPs maintain their structural integrity and fluorescent characteristics without degradation or significant alteration in aqueous media.

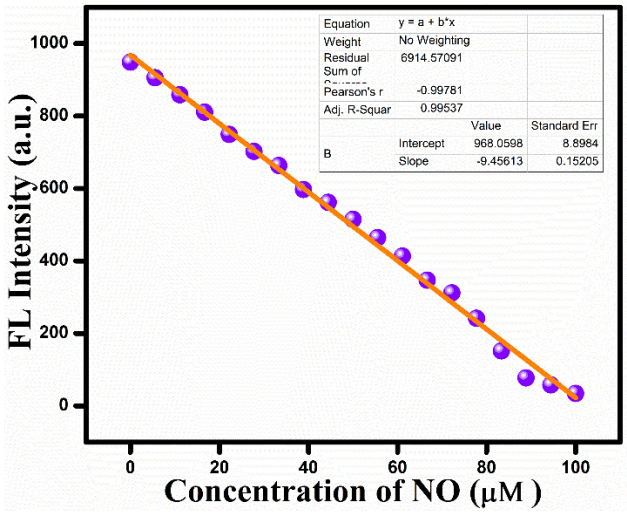

Figure S12. Calibration curve of PUC-9 in the presence of different▪NO concentration.

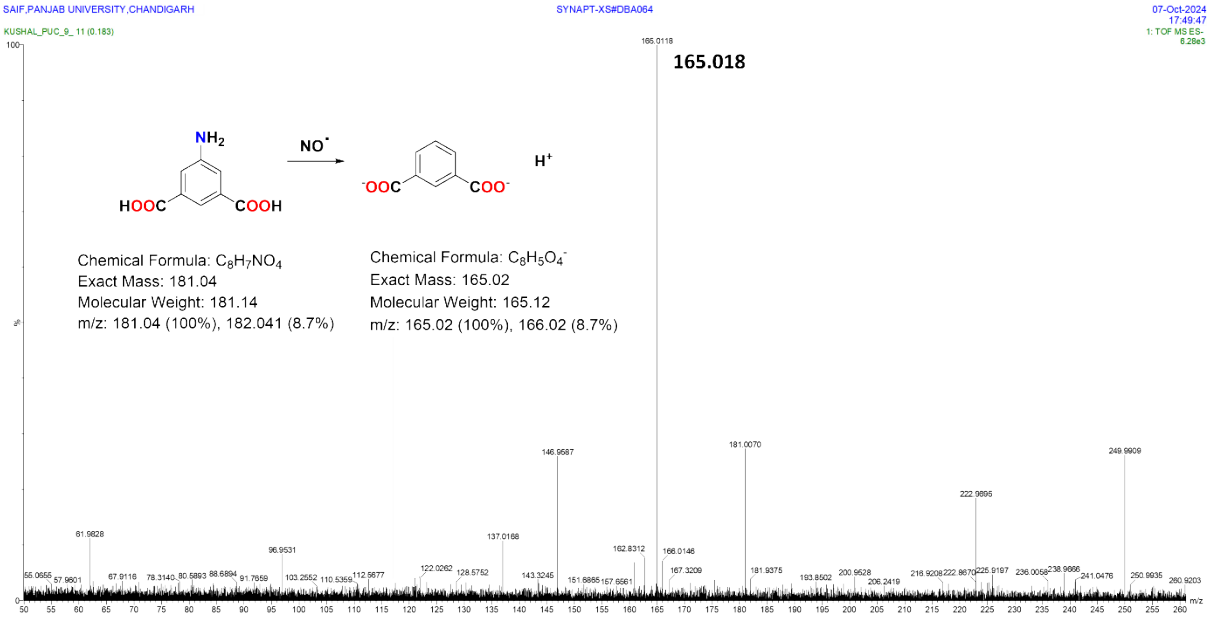

Figure S13. Mass spectra of 5-aminoisophthalic acid following the addition of ▪NO with a perfectly matching peak at 165.018 (100% intensity).

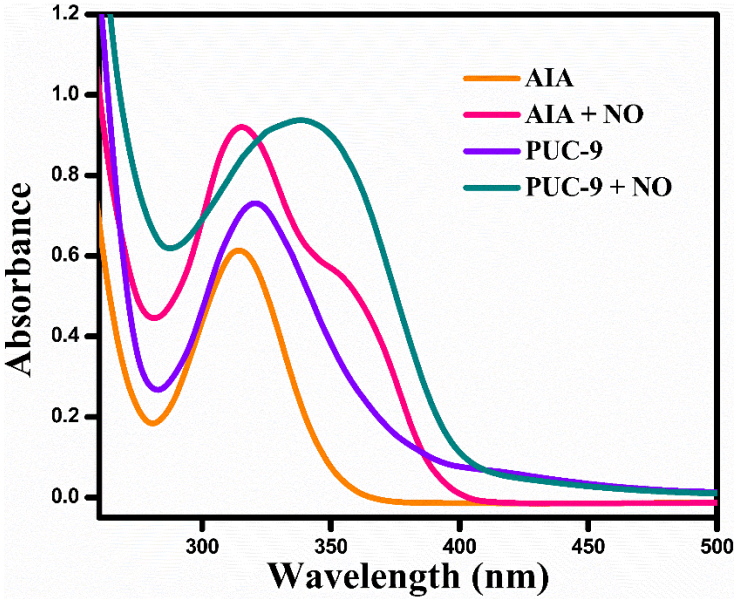

Figure S14. Absorbance spectra of PUC-9 and 5-amino isophthalic acid before and after addition of ▪NO.

Cell toxicity Assay:

Using the MTT assay, which is used widely for the estimation of cell toxicity, we observed that there is non-significant impact on the survival of THP-1 macrophages following exposure to PUC-9 up to 0.5 and survival remains >80% even after prolonged exposure (>6hr) **(Figure S15).**

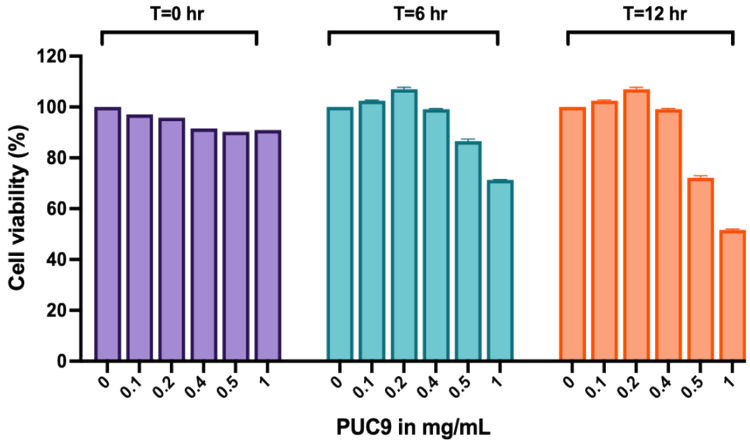

Figure S15: MTT cell viability assay on treatment with different PUC-9 concentrations: Data shows that no toxicity in THP-1 cells was observed up to 0.5 mg/mL even at >6hr of exposure. The experiments were performed at least twice in triplicates. Data represent Mean±SEM of the experiments performed at least twice in triplicates. Statistical significance was determined using One-Way ANOVA with Tukey's post-hoc correction (*p≤0.05, **p ≤0.01, ***p≤0.001).

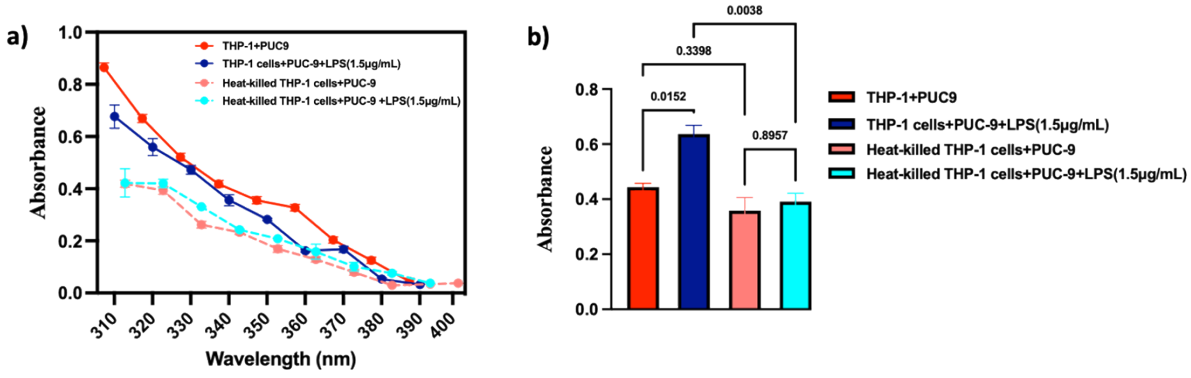

Figure S16: Absorbance-based detection of ▪NO production in THP-1 cells using PUC-9: **a)** Absorbance spectra of PUC-9 following low LPS stimulation in live and heat-killed THP-1 cells (dead cells). Live cells showed increased absorbance due to ▪NO production following exposure to LPS; dead cells alone or in presence of LPS showed no significant change in absorbance. **b)** Histogram summarizing absorbance values of PUC-9 spectra. The bar represents absorbance averaged over **310–340 nm,** measured in triplicates, and plotted as mean

± SEM. Data represent Mean±SEM from N=2 independent experiments, each in triplicate n= 3. Statistical significance was determined using One-Way ANOVA with Tukey's post-hoc correction (*p≤0.05, **p ≤0.01, ***p≤0.001).

Table S1. Crystal data and Structure Refinement for **PUC-9.**

CCDC	2402336
Identification code	PUC-9
Empirical formula	$C_{34}H_{19}CoN_7O_{10}$
Formula weight	744.49
Temperature/K	293(2)
Crystal system	triclinic
Space group	P-1
a/Å	10.0211(2)
b/Å	10.1174(4)
c/Å	20.8976(3)
α/°	93.905(2)
β/°	101.679(2)
γ/°	104.872(2)
Volume/Å³	1989.19(10)
Z	2
ρcalc g/cm³	1.243
μ/mm⁻¹	0.490
F(000)	758.0
Crystal size/mm³	0.009 × 0.007 × 0.006
Radiation	Mo Kα (λ = 0.71073)
2Θ range for data collection/°	6.534 to 54.718
Index ranges	-11 ≤ h ≤ 12, -13 ≤ k ≤ 12, -26 ≤ l ≤ 25
Reflections collected	27506
Independent reflections	8425 [Rint = 0.0504, Rsigma = 0.0633]
Data/restraints/parameters	8425/6/470
Goodness-of-fit on F²	1.031
Final R indexes [I>=2σ (I)]	R1 = 0.0521, wR2 = 0.1391
Final R indexes [all data]	R1 = 0.0766, wR2 = 0.1508
Largest diff. peak/hole / e Å⁻³	0.51/-0.44

Table S2. Comparison of properties of PUC-9 with other fluorescent probes used for the detection of ▪NO.

Name of the sensor	**Detection method**	**LOD value**	**Detection in Living Cells**	**Cytotoxicity**	**Ref.**
Sensor-**L**^{3} based on Rhodamine-B-en **(2)** and 2-(pyridin-2-ylmethoxy)benzaldehyde **(1)**	Fluorescence	83.4 nM	Yes, by imaging in HepG2 cell lines	less than 30% cytotoxicity after 24 hours	[1]
UiO66@NH_2	Fluorescence	0.575 μM	No	unknown	[2]
CuCo-PTC MOF	Fluorescence	0.15 μM	No	unknown	[3]
FP-NO	Fluorescence	47.6 nM	Yes, imaging and flow cytometry analysis of exogenous ▪NO in MCF-7 cells	Low cytotoxicity	[4]
Bodipy dye	Fluorescence	30 nM	Yes, by imaging in HL-7702 cells	unknown	[5]
PABA@MOF-808.	Fluorescence	0.715 μM	No	Unknown	[6]
PUC-2	Fluorescence	0.08 μM	Yes, in THP-1 cells	Minimal toxicity (90-95% survival even when exposed for >12 h)	[7]
PUC-9	Fluorescence	19 nM	Yes, by using fluorescence imaging, absorbance and fluorescence spectra in THP-	Minimal toxicity, survival remains >80% even after	This study

			1 cells	prolonged exposure (>6hr)	

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