Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTARY INFORMATION

Amino Acid-Stabilized Luminescent Gold Clusters for Sensing Pterin and its Analogues

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Calculation formula for relative QY: $\varphi = \varphi_S \frac{FA_S n^2}{F_S A n_S^2}$, where φ – QY of the sample, φ_S – QY of the reference, F – integral of the sample emission, F_S – integral of the reference emission, A – absorption of the sample, A_S – absorption of the reference, n – refractive index of the sample, n_S – refractive index of the reference.

LOD calculation procedure

The values of limit of detection (LOD) can be obtained using the following formula (1):

$$LOD = {}^{3.3\sigma}/_{S} \qquad (1)$$

, where σ is the residual standard deviation (also known as "root MSE" – root mean square of the error), and S is the slope of the calibration curve. In our case, we used the slope of the calibration curve in the linear range. We calculated σ as the root-mean-square approximation error obtained using the formula (2):

$$\sigma = \sqrt{\chi^2_{red.}} \qquad (2)$$

In the formula (2), $\chi^2_{red.}$ means the reduced "chi-squared":

$$\chi^2_{red.} = \frac{\chi^2}{\nu} = \frac{\chi^2}{N - p} \qquad (3)$$

In the formula (3), χ^2 is the residual sum of squares (RSS), ν are degrees of freedom (which is equal to $N - p$), N is the number of data points, p is the number of fit function parameters β. The value of RSS used above can be calculated using the formula (4):

$$\chi^2 = \sum_{i=1}^{N} w_i \left(y_i - f\left(x_i, \beta_1, \dots, \beta_p\right)\right)^2 \qquad (4)$$

This value is minimized during the fit to find the optimal fit function parameters β.

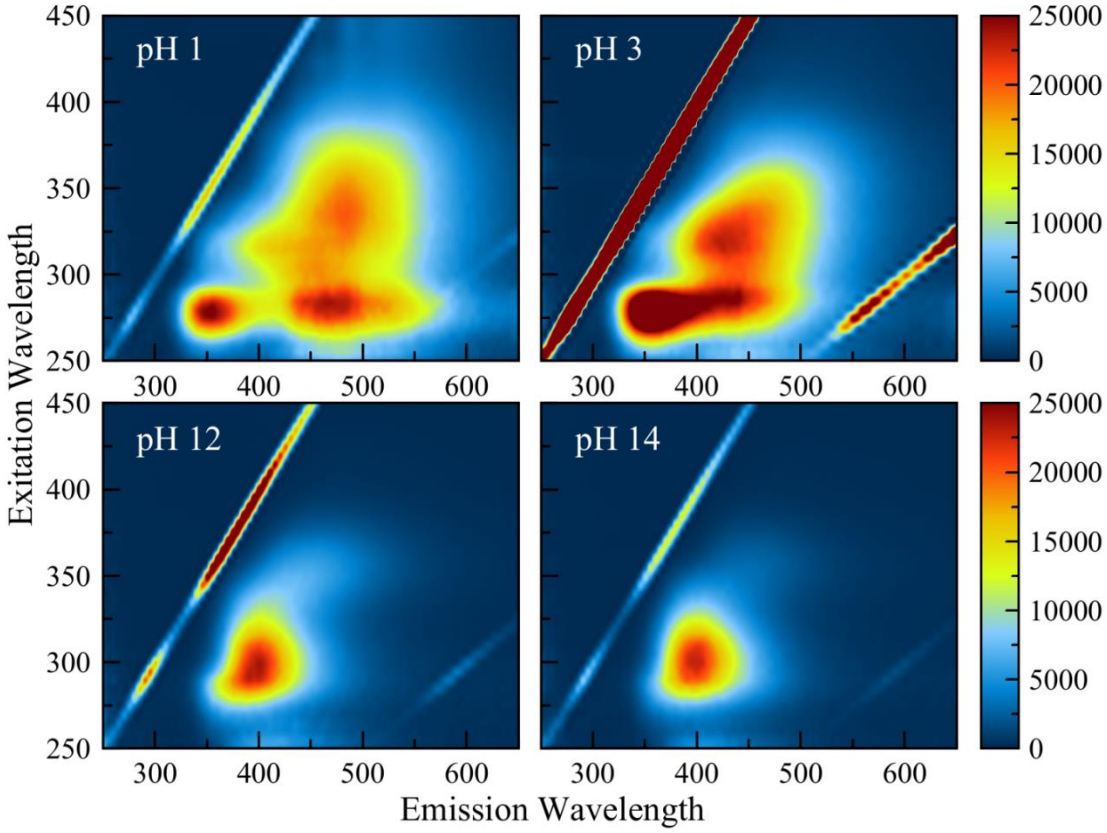

Figure S1. *2D contour plots for Trp-Au NCs in acidic (top) and alkali (bottom) conditions. In present work, Trp-Au NCs were used at pH 12 (bottom left).*

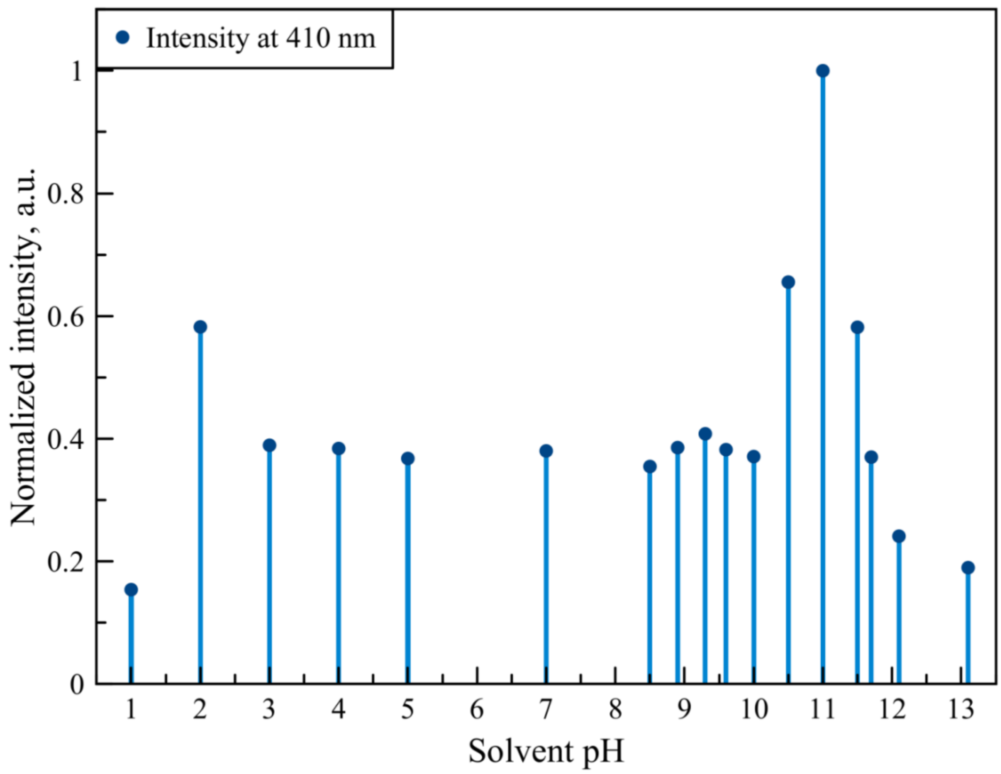

Figure S2. *Relative emission intensity at 410 nm of DOPA-Au NCs versus solvent pH. The [Au]:[DOPA] ratio is 5.*

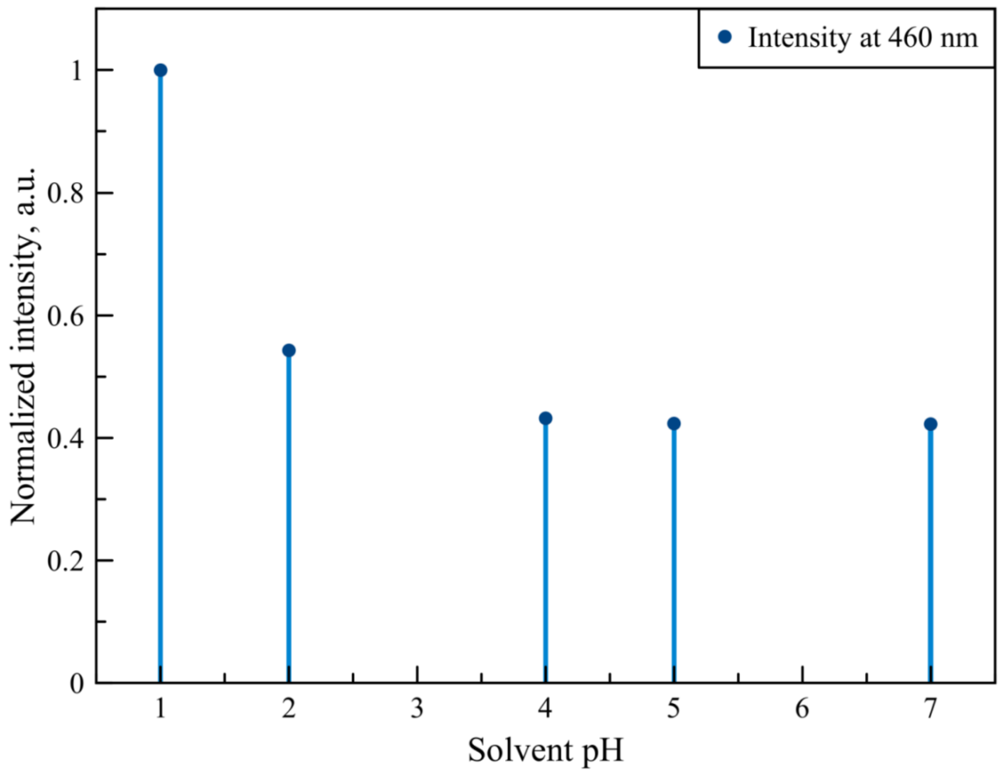

Figure S3. *Relative emission intensity at 460 nm of Trp-Au NCs versus solvent pH. The [Au]:[DOPA] ratio is 2.*

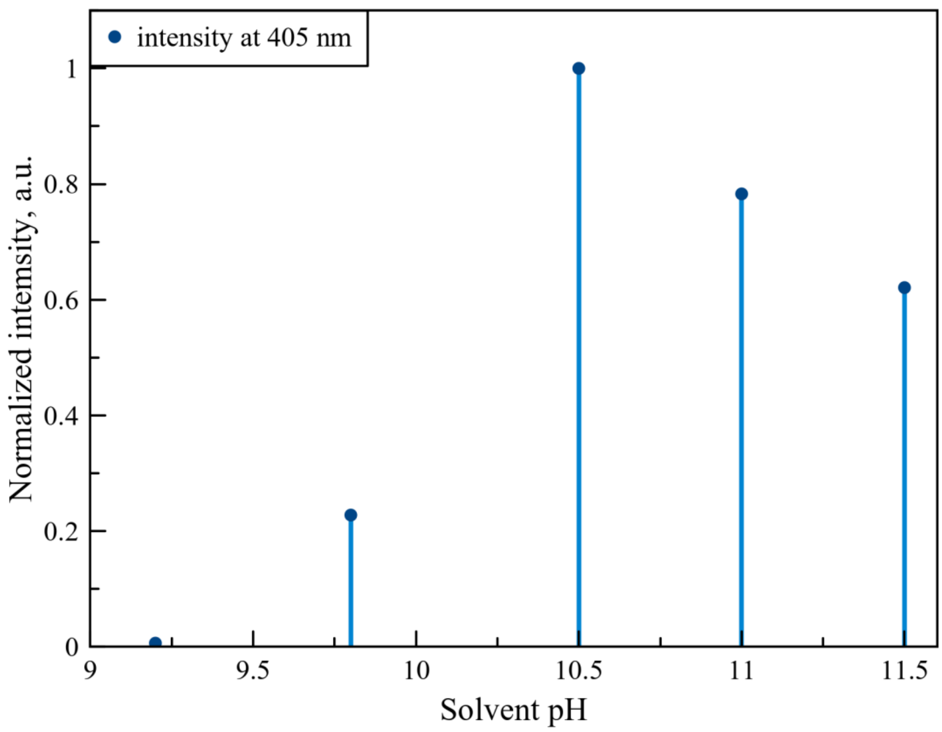

Figure S4. *Relative emission intensity at 405 nm of Tyr-Au NCs (a) versus solvent pH. The [Au]:[Tyr] ratio is 0.33.*

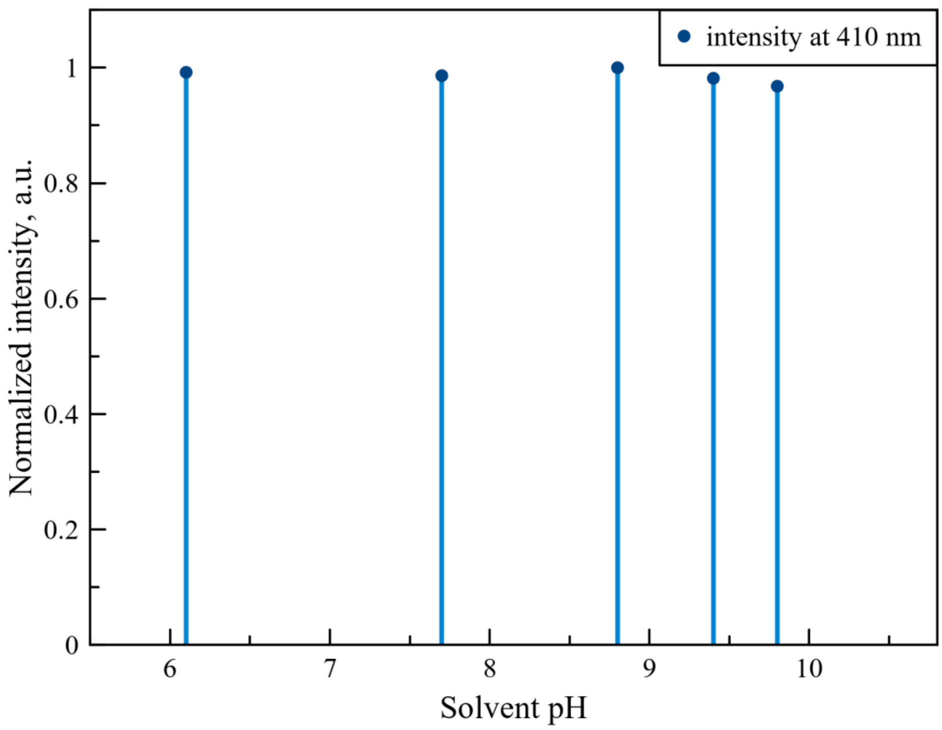

Figure S5. *Relative emission intensity at 410 nm of Tyr-Au NCs (b) versus solvent pH. The [Au]:[Tyr] ratio is 0.5.*

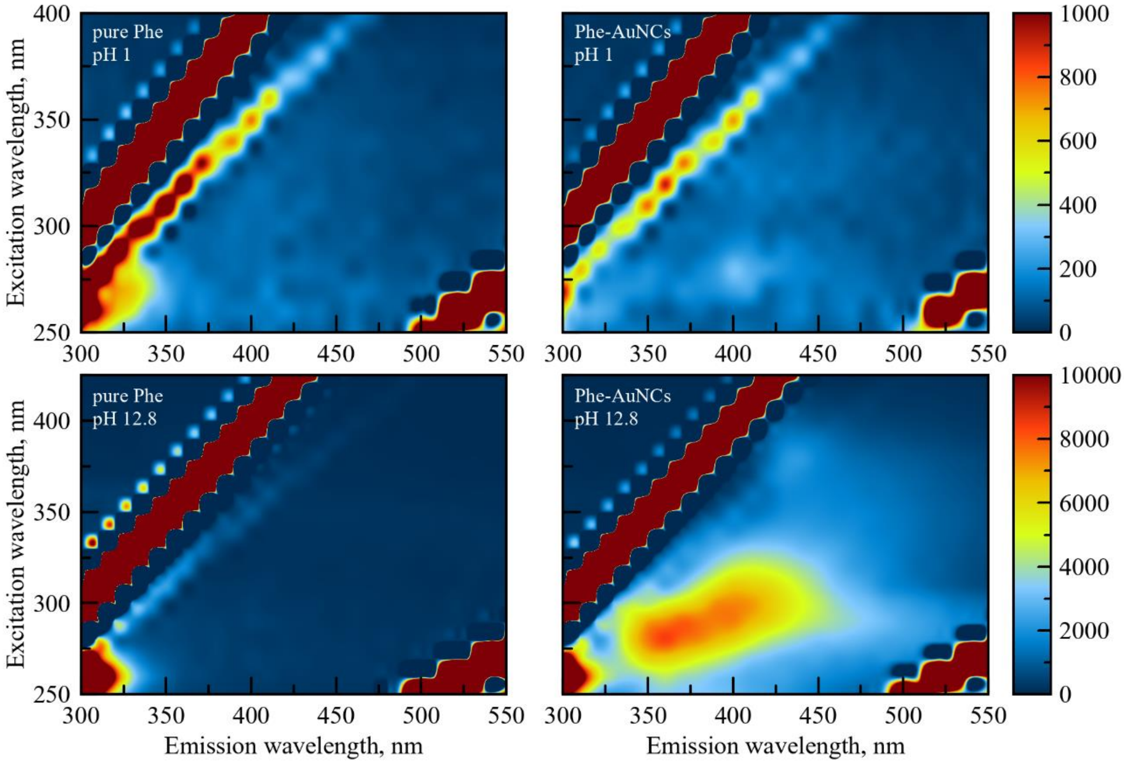

Figure S6. *2D contour plots for Phe-Au NCs in acidic (top) and alkali (bottom) conditions*

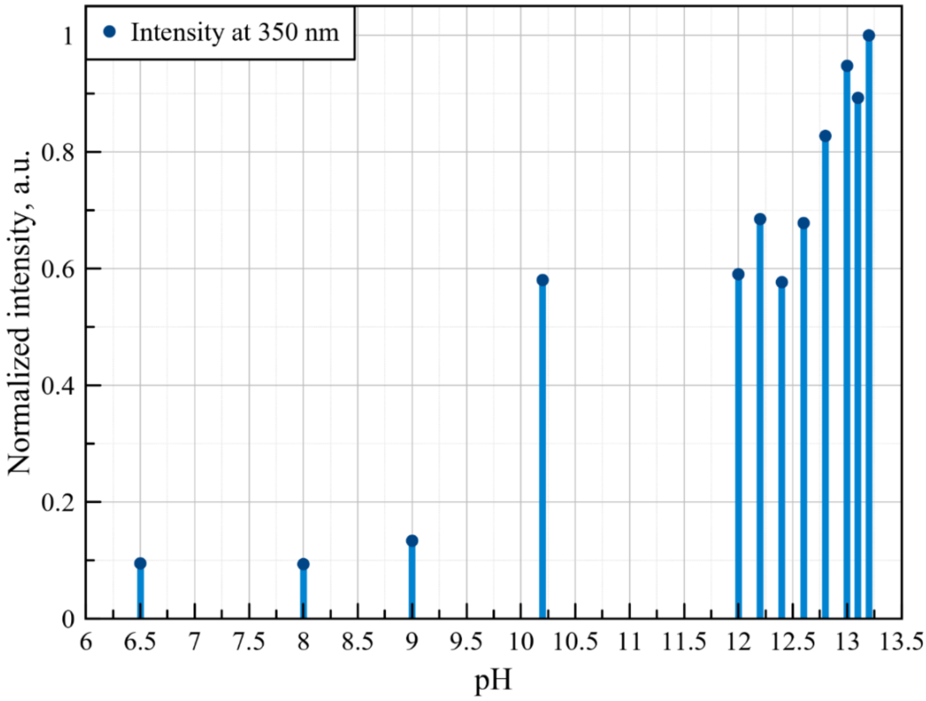

Figure S7. *Relative emission intensity at 350 nm of Phe-Au NCs under different pH values.*

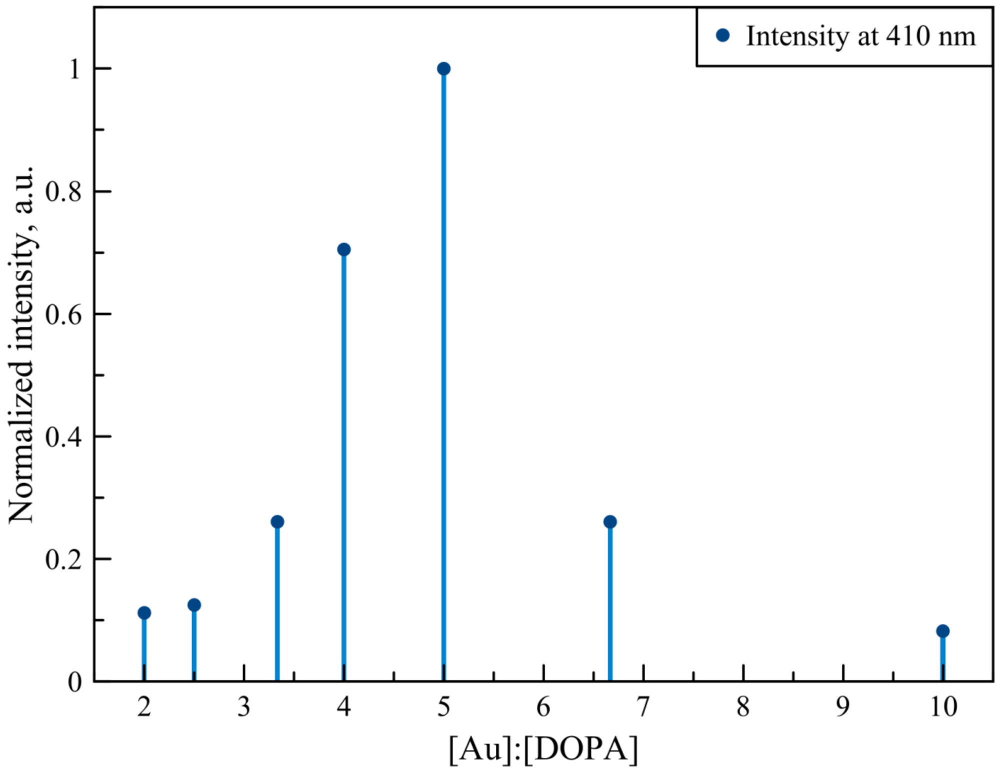

Figure S8. *Relative emission intensity at 410 nm of DOPA-Au NCs versus [Au]:[DOPA] ratio. The solvent pH is 11.*

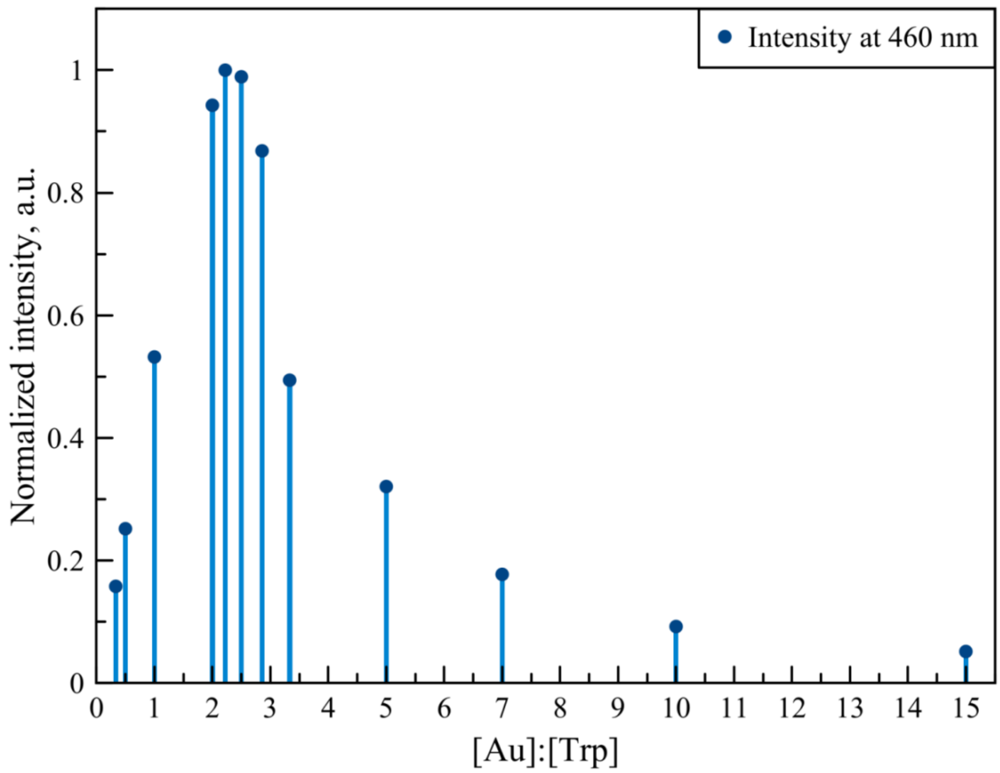

Figure S9. *Relative emission intensity at 460 nm of Trp-Au NCs versus [Au]:[Trp] ratio. The solvent pH is 1.*

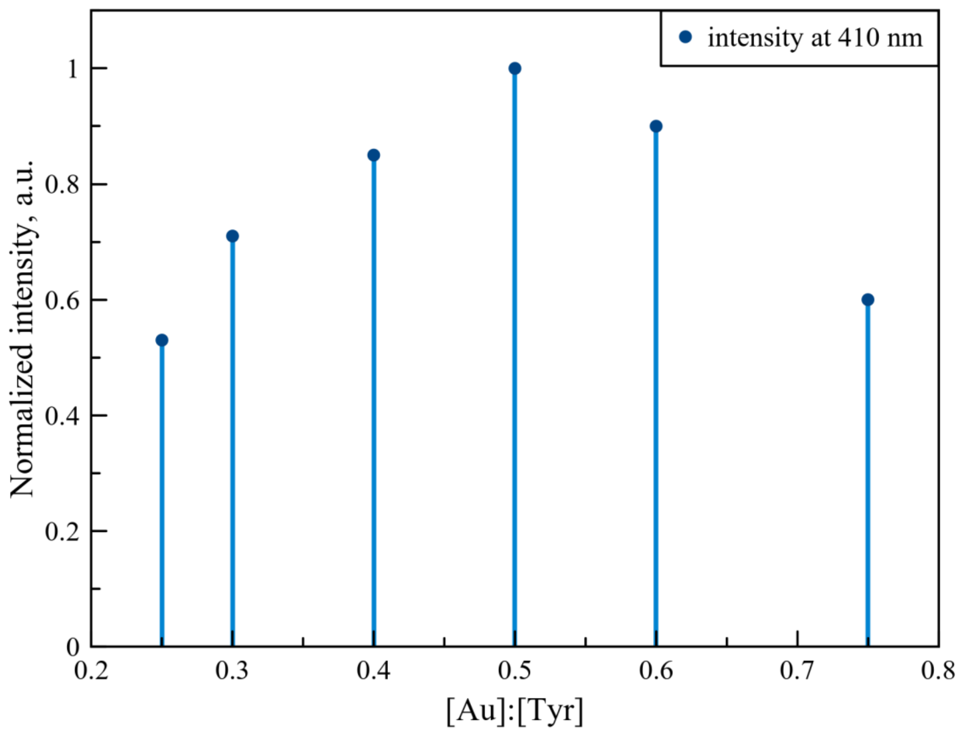

Figure S10. *Relative emission intensity at 410 nm of Tyr-Au NCs (b) versus [Au]:[Tyr] ratio. The solvent pH is 7.*

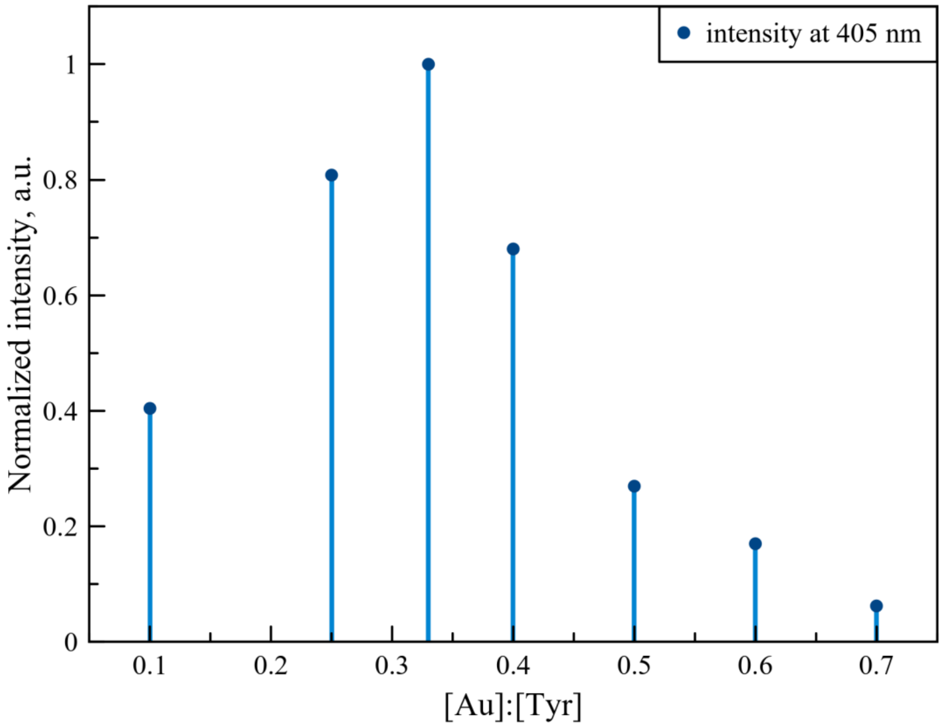

Figure S11. *Relative emission intensity at 405 nm of Tyr-Au NCs (a) versus [Au]:[Tyr] ratio. The solvent pH is 10.5.*

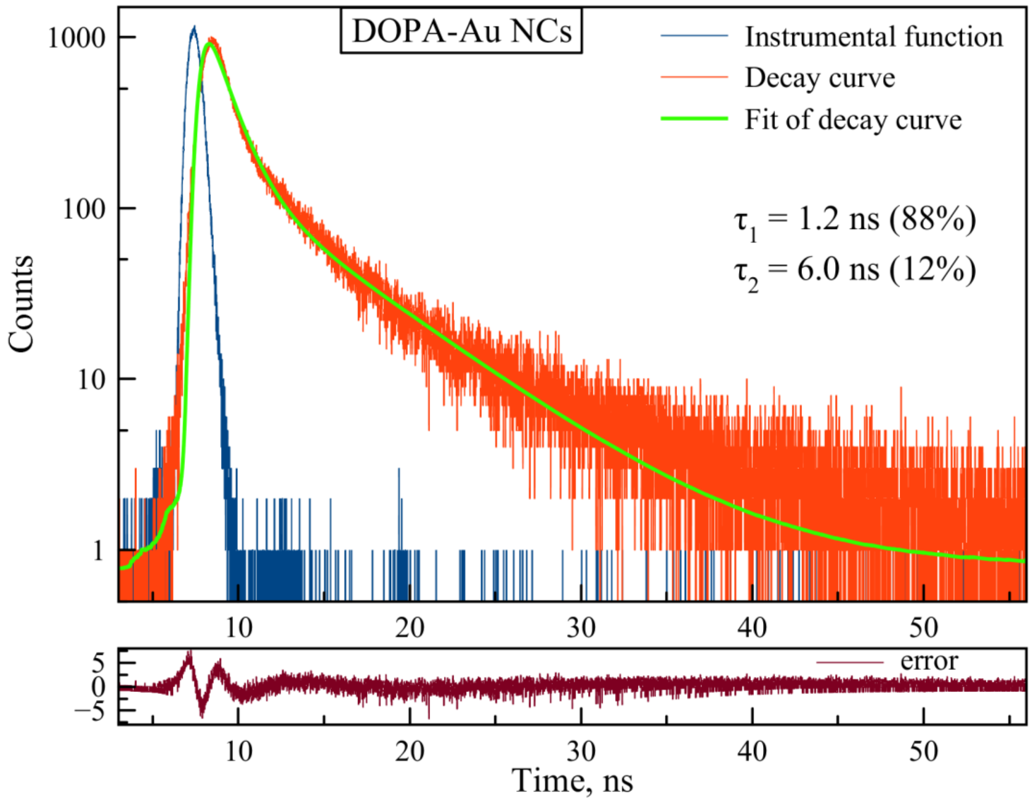

Figure S12. *Fluorescence decay of DOPA-Au NCs (λ_{ex} 340 nm, λ_{em} 415 nm). Fit: 0.88exp(-t/1.2) + 0.12exp(-t/6.0).*

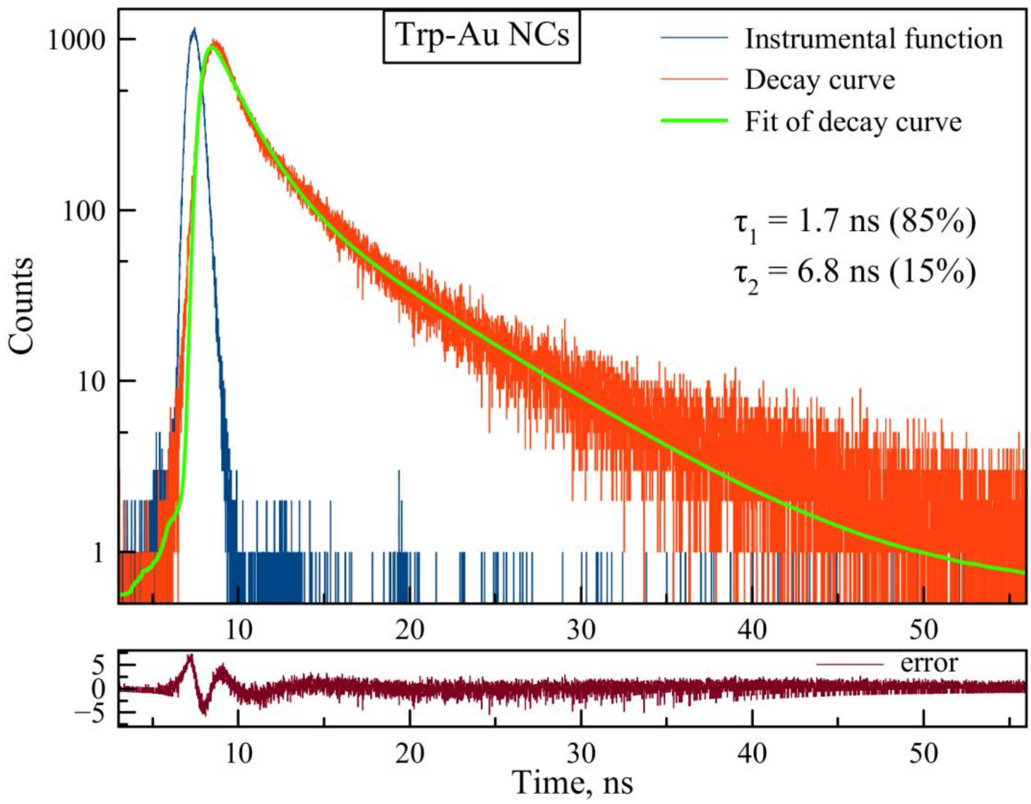

Figure S13. *Fluorescence decay of Trp-Au NCs (λ_{ex} 340 nm, λ_{em} 460 nm).*
Fit: 0.85exp(-t/1.7) + 0.15exp(-t/6.8).

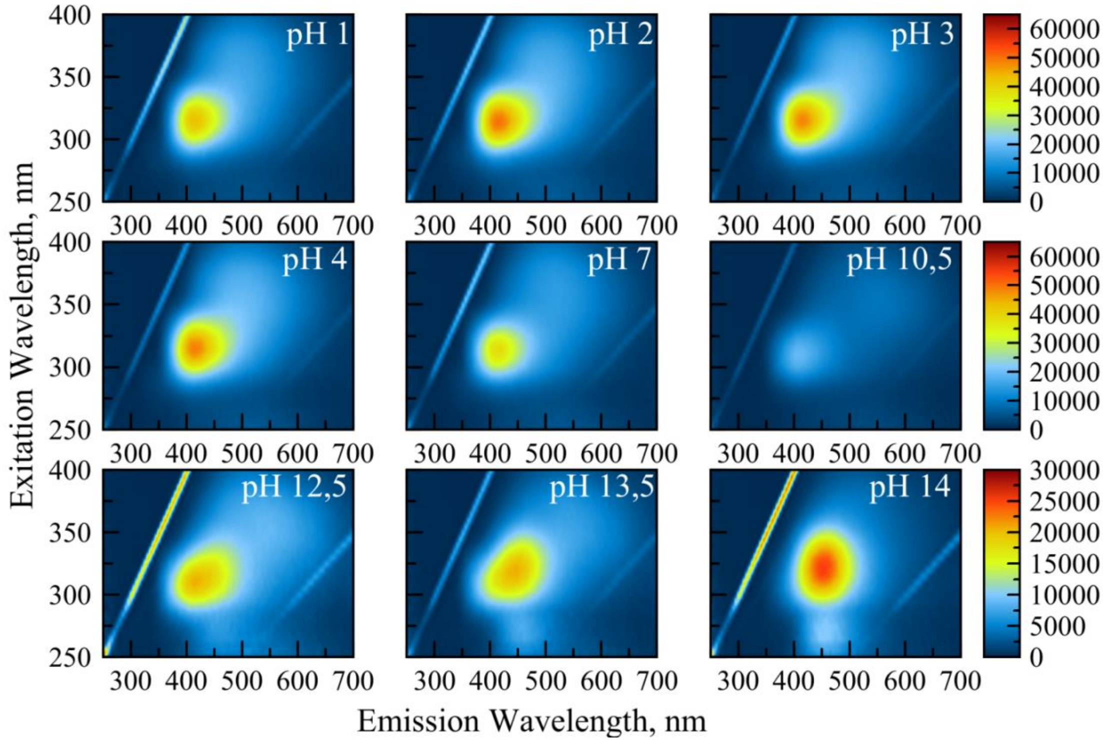

Figure S14. *2D contour plots for DOPA-Au NCs in various pH conditions.*

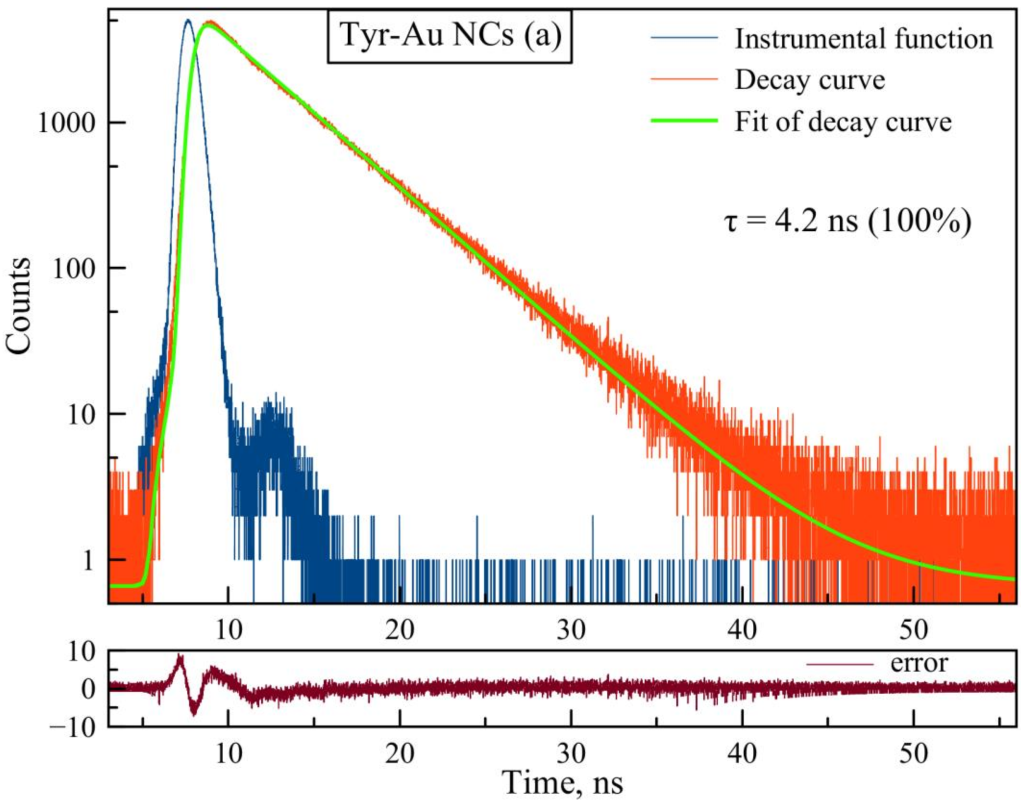

Figure S15. *Fluorescence decay of Tyr-Au NCs (a) (λ_{ex} 340 nm, λ_{em} 405 nm).*

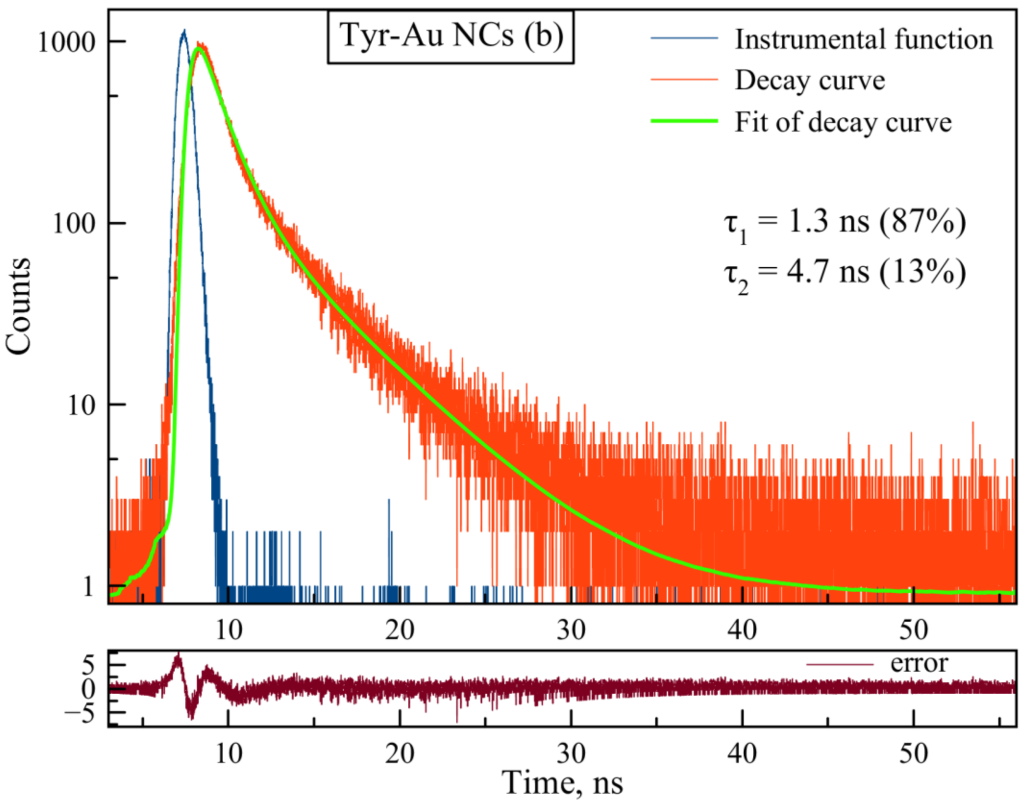

Figure S16. *Fluorescence decay of Tyr-Au NCs (b) (λ_{ex} 266 nm, λ_{em} 410 nm).*
Fit: 0.87exp(-t/1.3) + 0.13exp(-t/4.7).

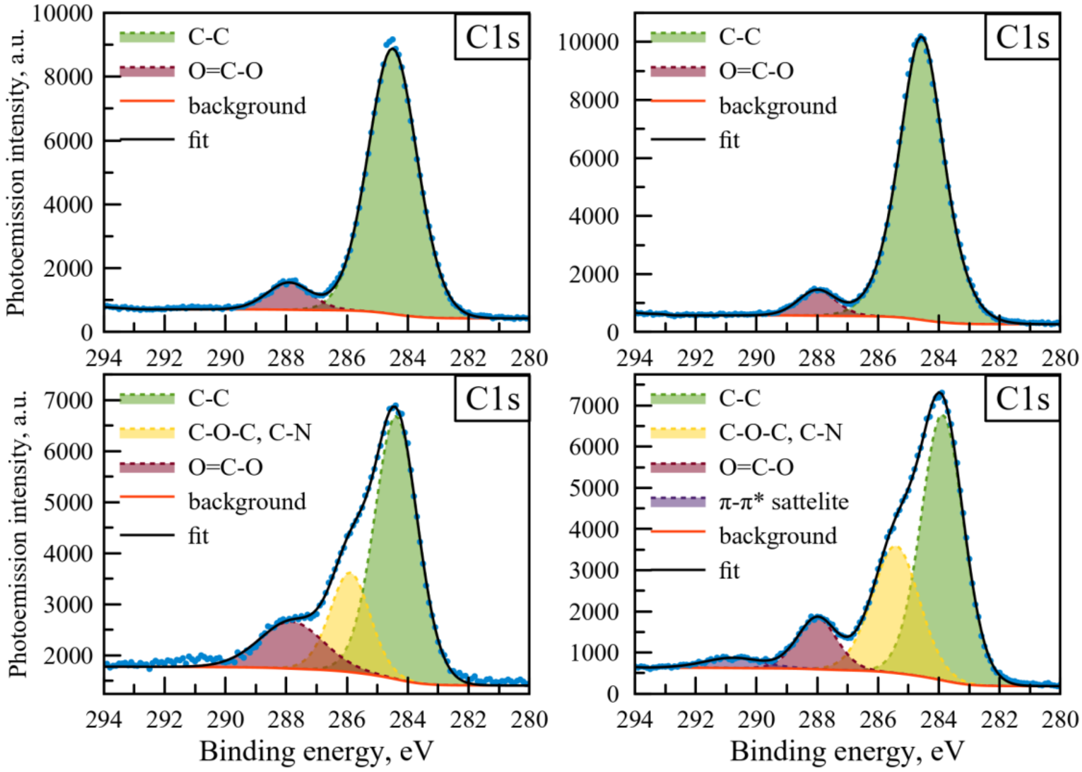

Figure S17. *Left: C(1s) XPS spectra of Tyr (top) and Tyr-Au NCs (b) (bottom). Right: C(1s) XPS spectra of Tyr (top) and Tyr-Au NCs (a) (bottom).*

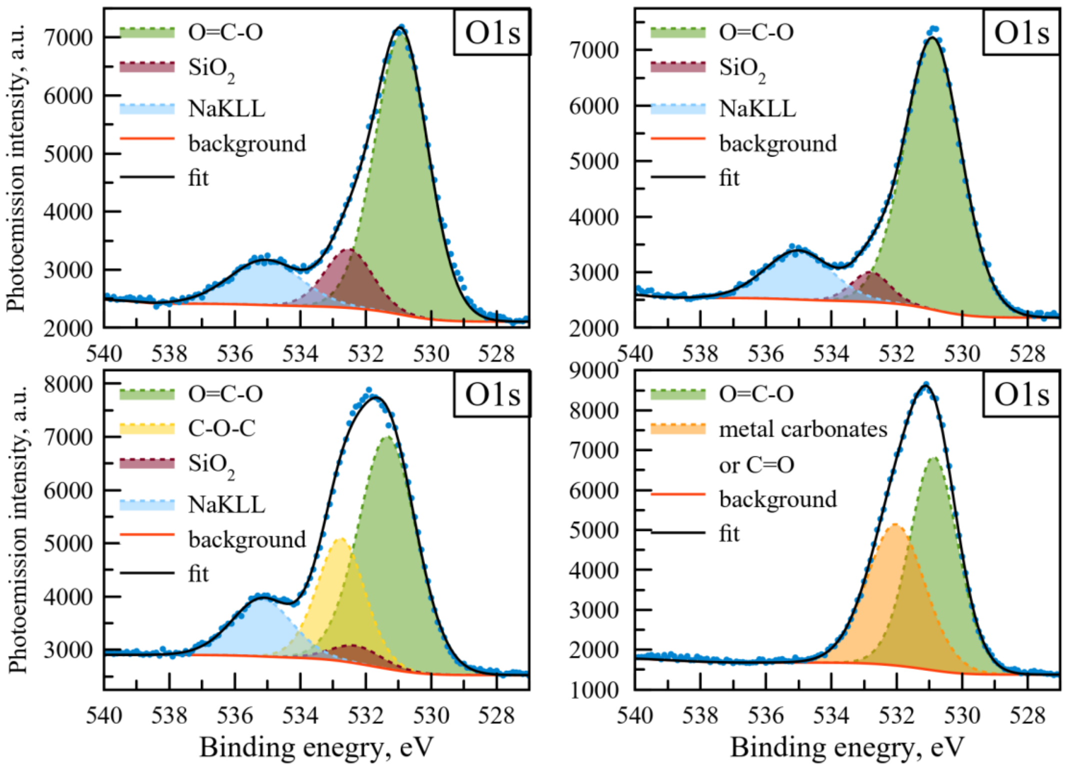

Figure S18. *Left: O(1s) XPS spectra of Tyr (top) and Tyr-Au NCs (b) (bottom). Right: O(1s) XPS spectra of Tyr (top) and Tyr-Au NCs (a) (bottom).*

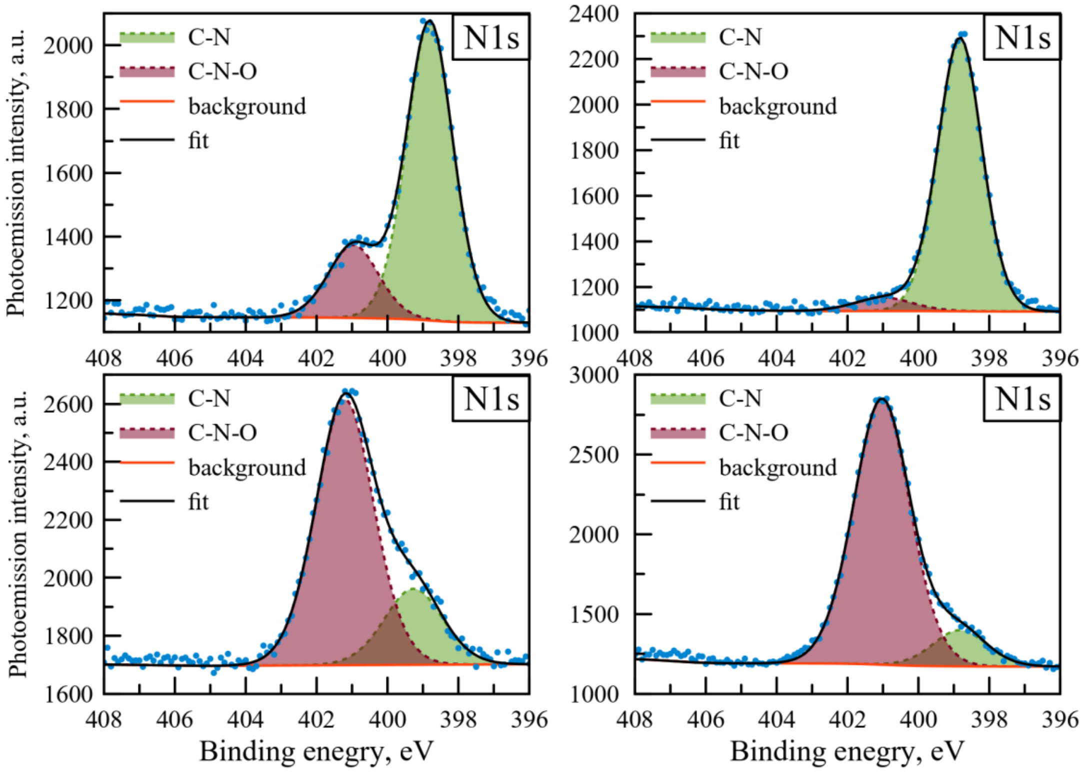

Figure S19. *Left: N(1s) XPS spectra of Tyr (top) and Tyr-Au NCs (b) (bottom). Right: N(1s) XPS spectra of Tyr (top) and Tyr-Au NCs (a) (bottom).*

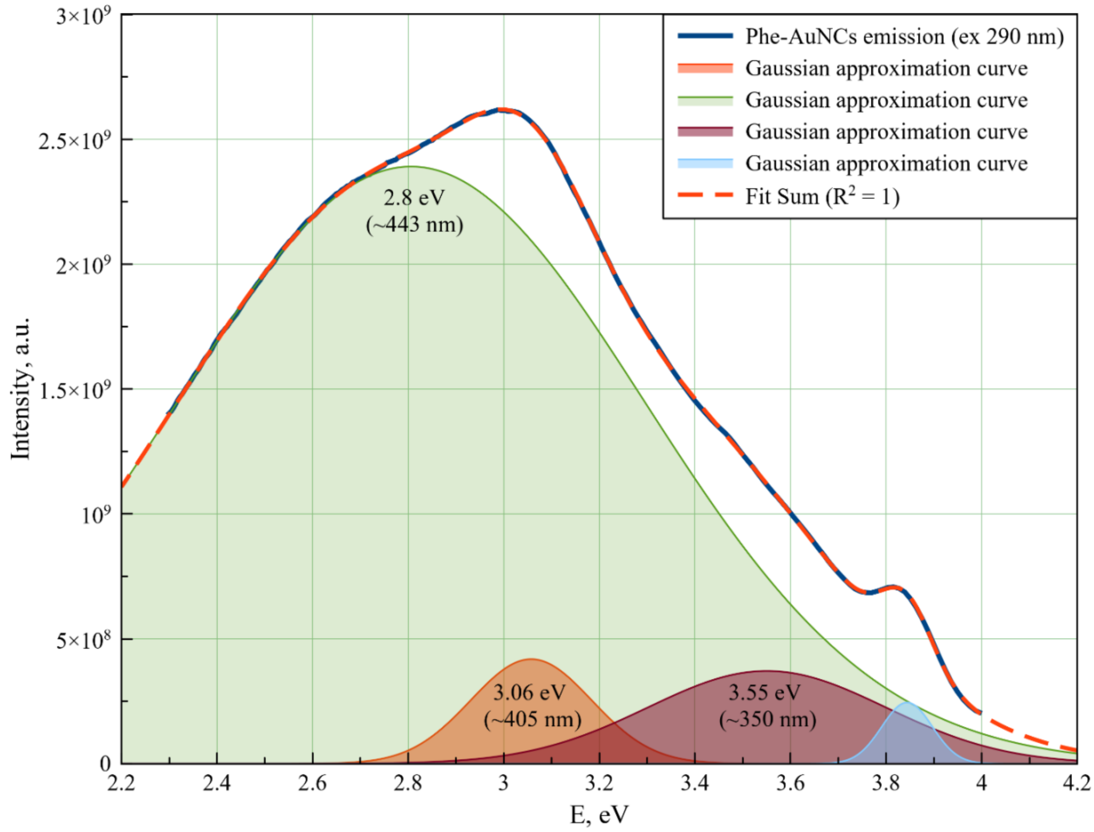

Figure S20. *Decomposition of the emission spectrum of Phe-Au NCs into three Gauss peaks.*

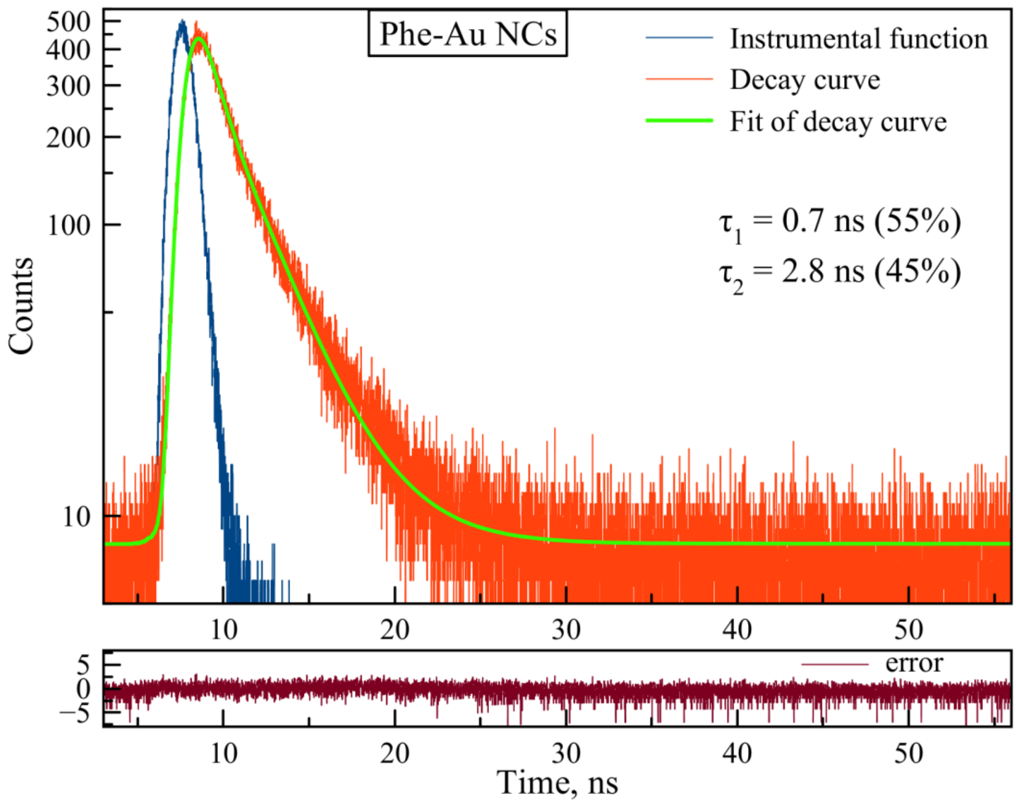

Figure S21. *Fluorescence decay of Phe-Au NCs (λ_{ex} 266 nm, λ_{em} 350 nm). Fit: 0.55exp(-t/0.7) + 0.45exp(-t/2.8).*

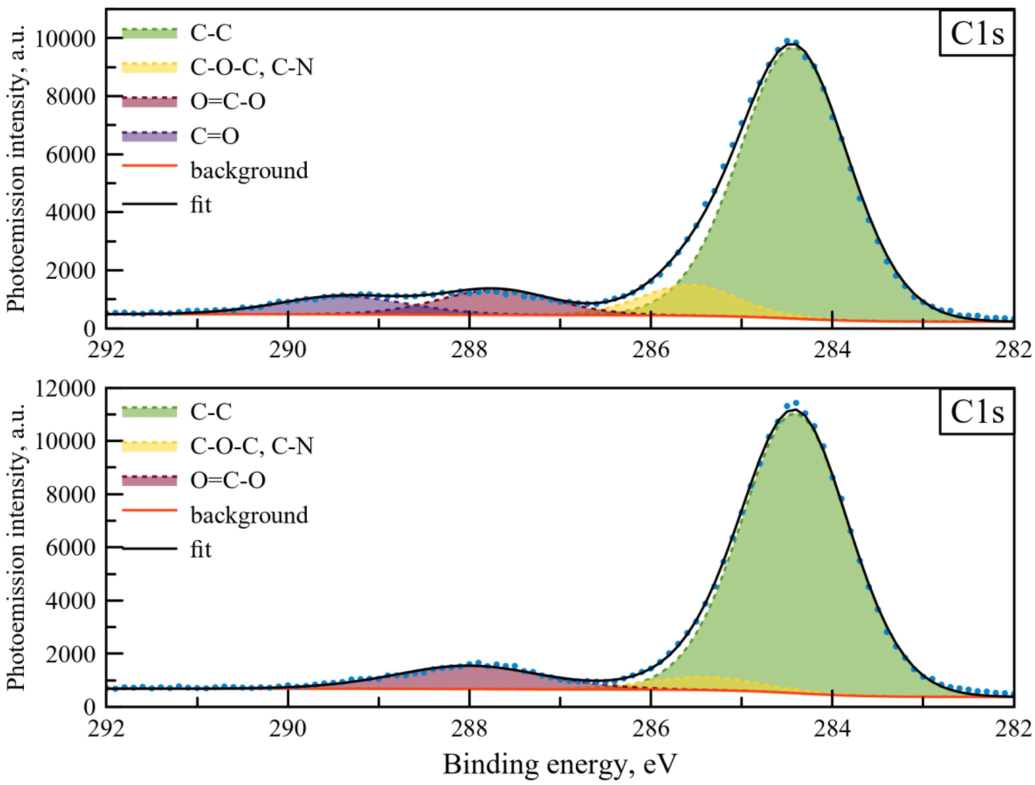

Figure S22. *C(1s) XPS spectra of Phe (top) and Phe-Au NCs (bottom).*

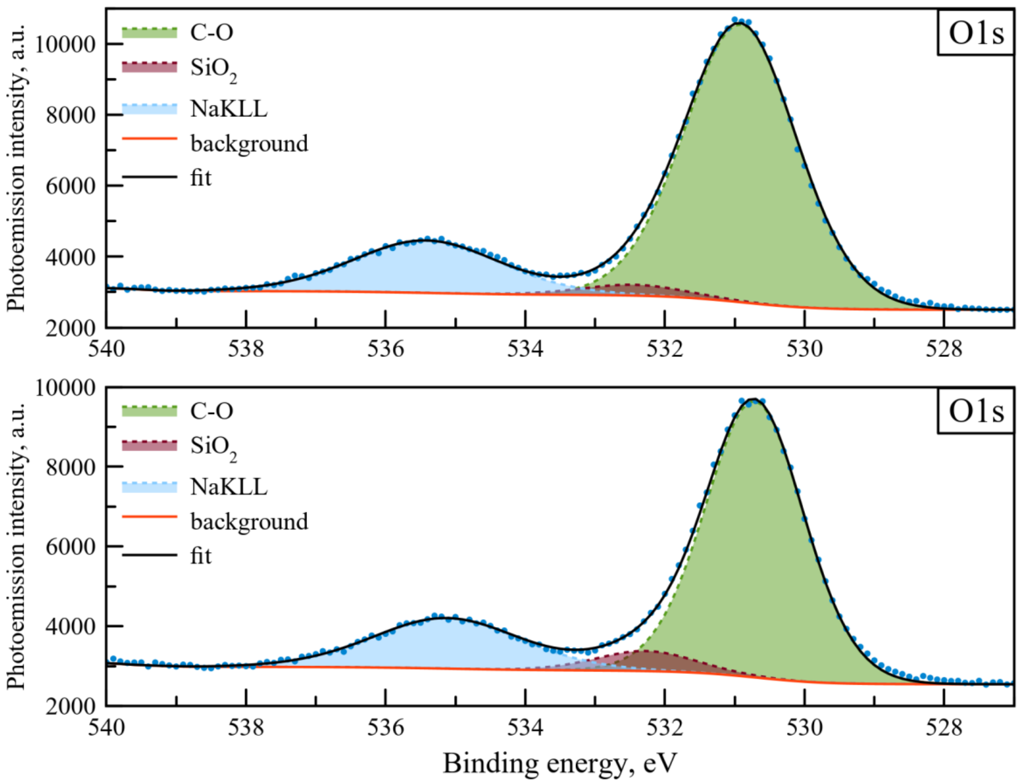

Figure S23. *O(1s) XPS spectra of Phe (top) and Phe-Au NCs (bottom).*

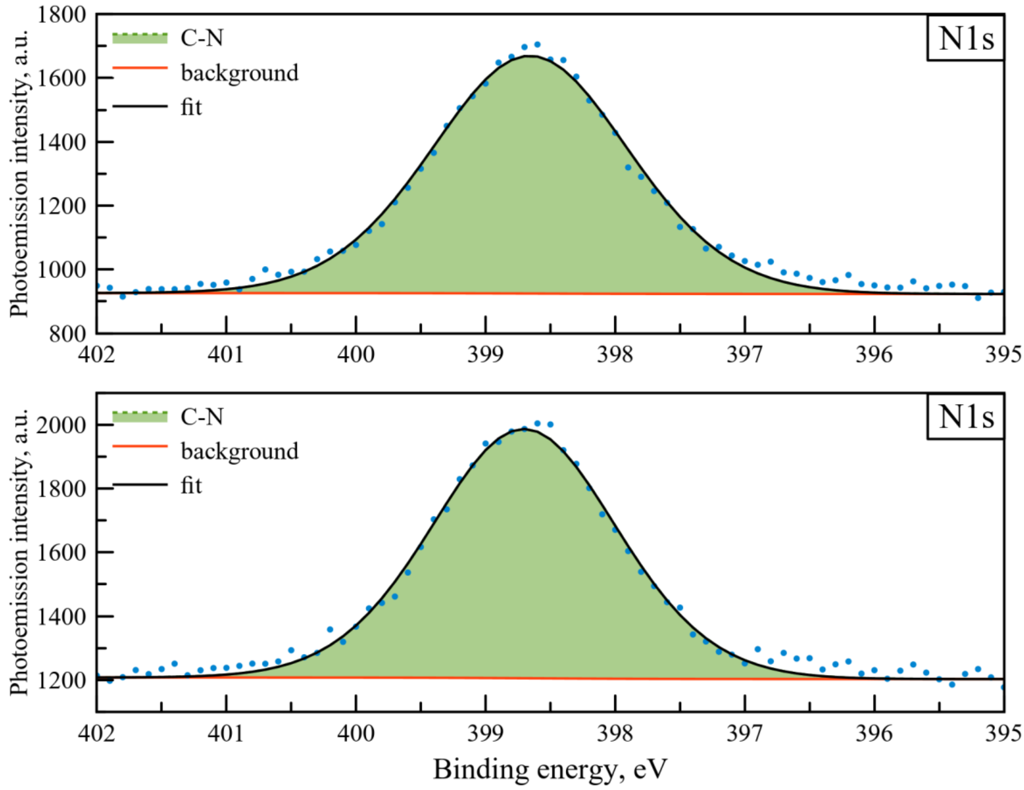

Figure S24. *N(1s) XPS spectra of Phe (top) and Phe-Au NCs (bottom).*

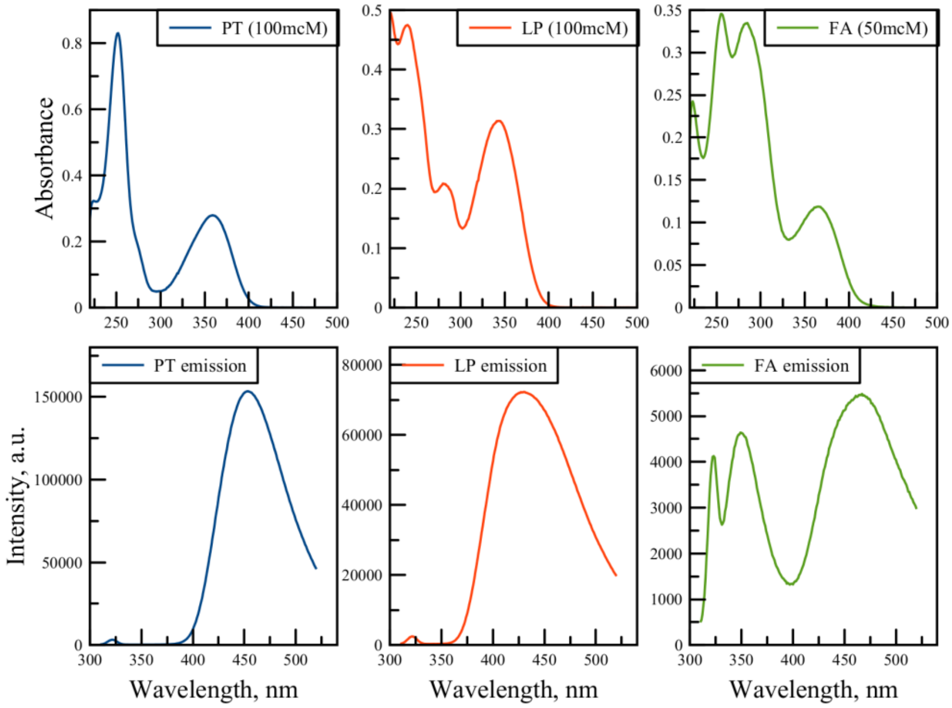

Figure S25. *Absorption (top) and emission (bottom) spectra of Ptr (left), Lep (in center) and FA (right).*

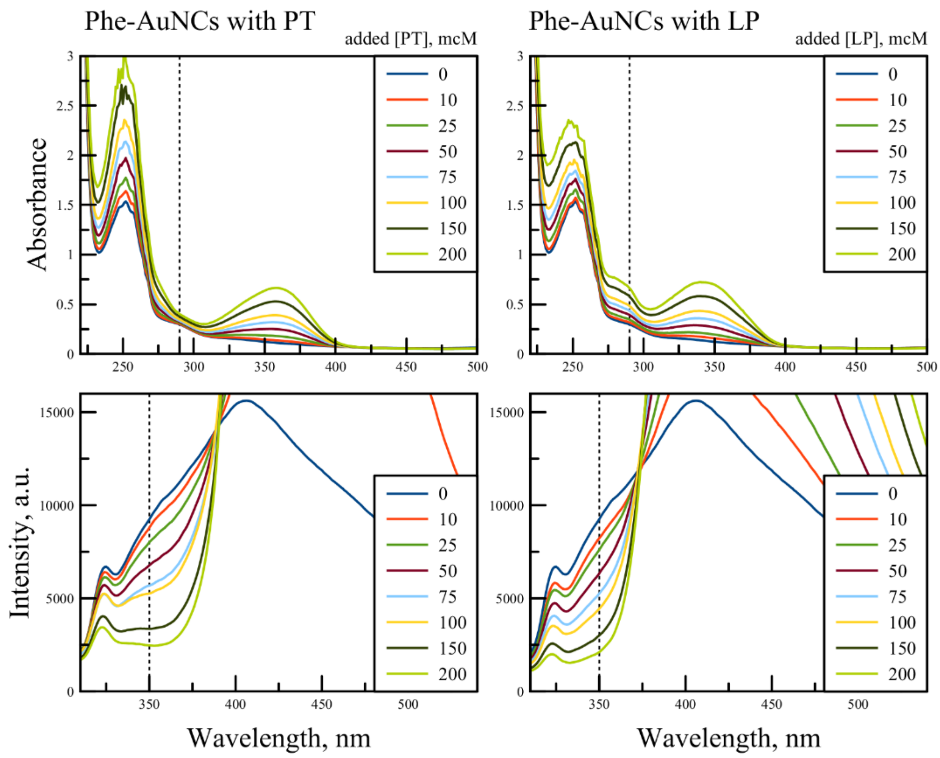

Figure S26. *Absorption (top) and emission (bottom) spectra of Phe-Au NCs in the presence of Ptr (left) and Lep (right).*

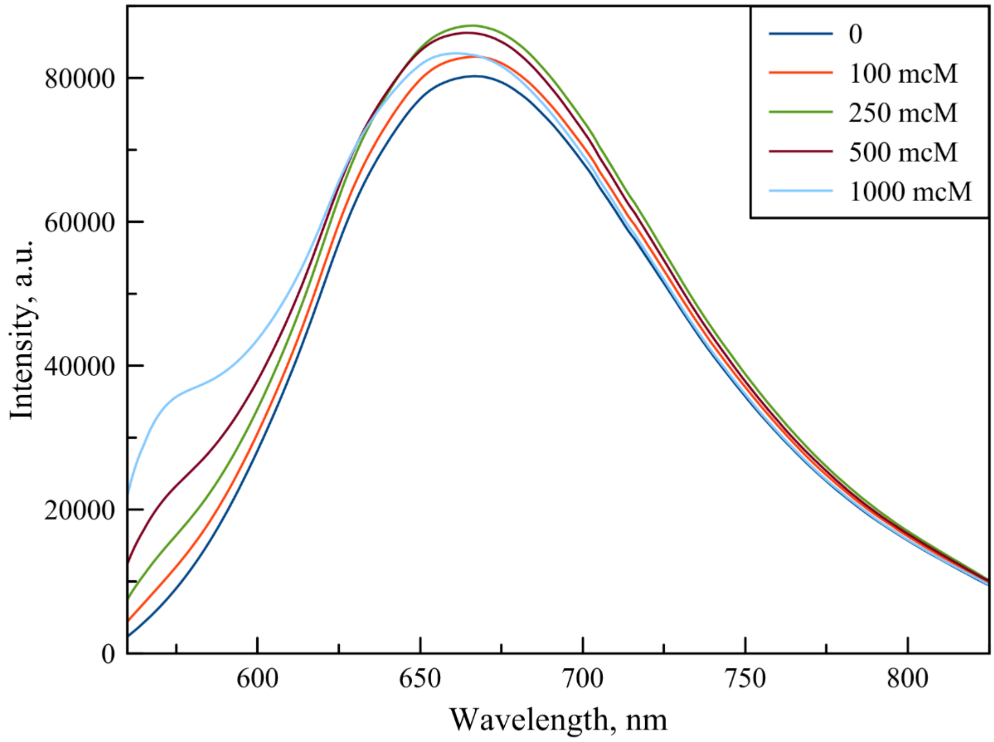

Figure S27. *Emission spectra of BSA-Au NCs in the presence of different Ptr concentrations. BSA-Au NCs synthesis protocol: [BSA] = 1 mg/ml; initial pH 12.5; [Au]:[BSA] = 140:1.*

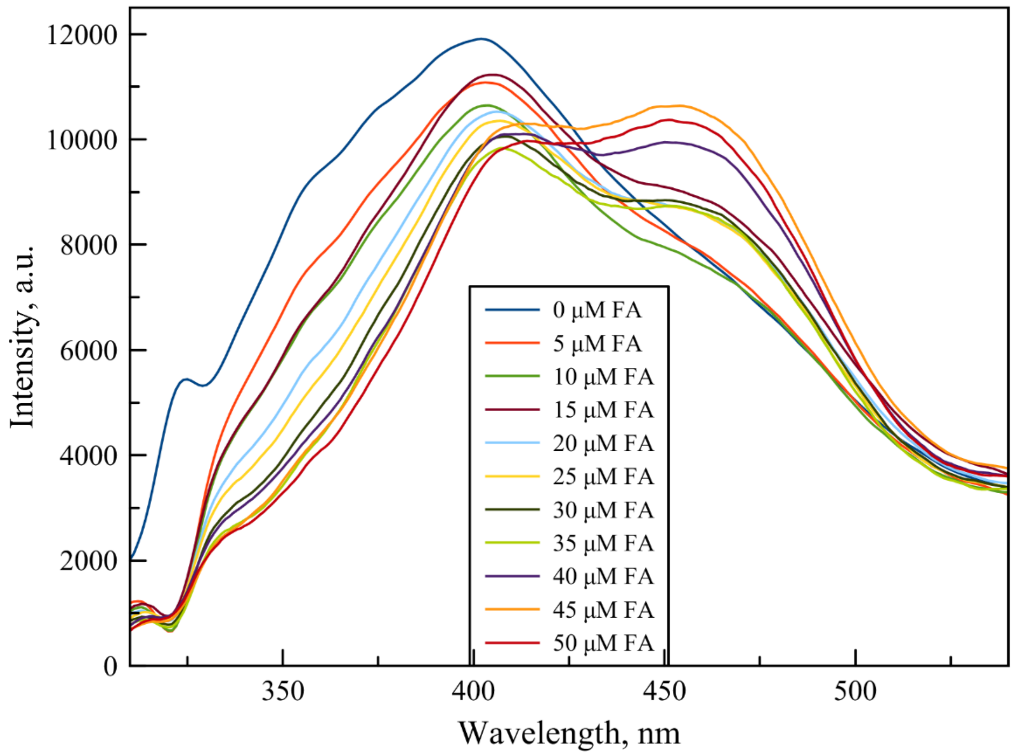

Figure S28. *Emission spectra of Phe-Au NCs (excitation at 290 nm) in the presence of FA.*

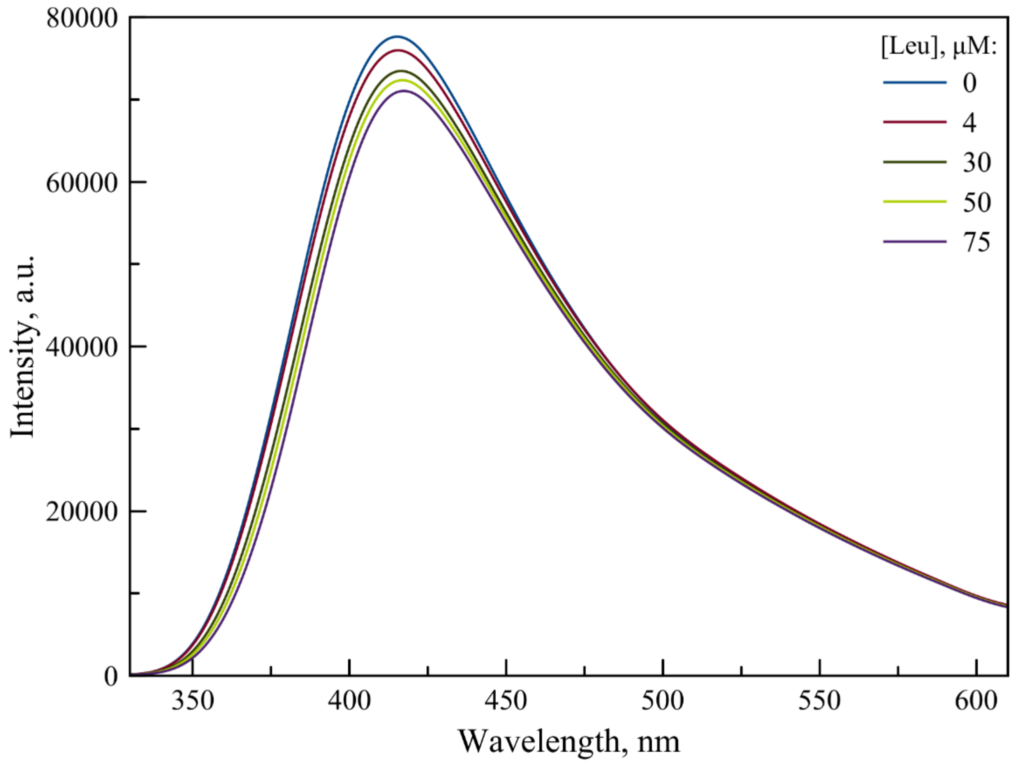

Figure S29. *Emission spectra of DOPA-Au NCs (excitation at 315 nm) in the presence of Lep.*

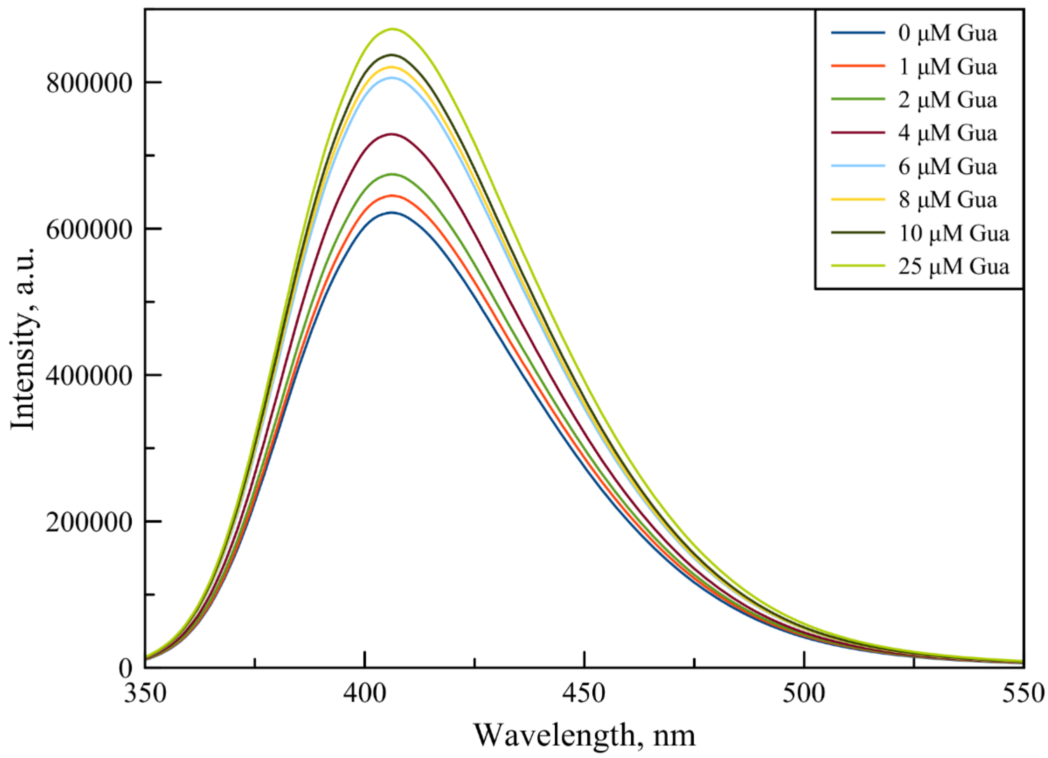

Figure S30. *Emission spectra of Tyr-Au NCs (a) (excitation at 315 nm) in the presence of guanine.*

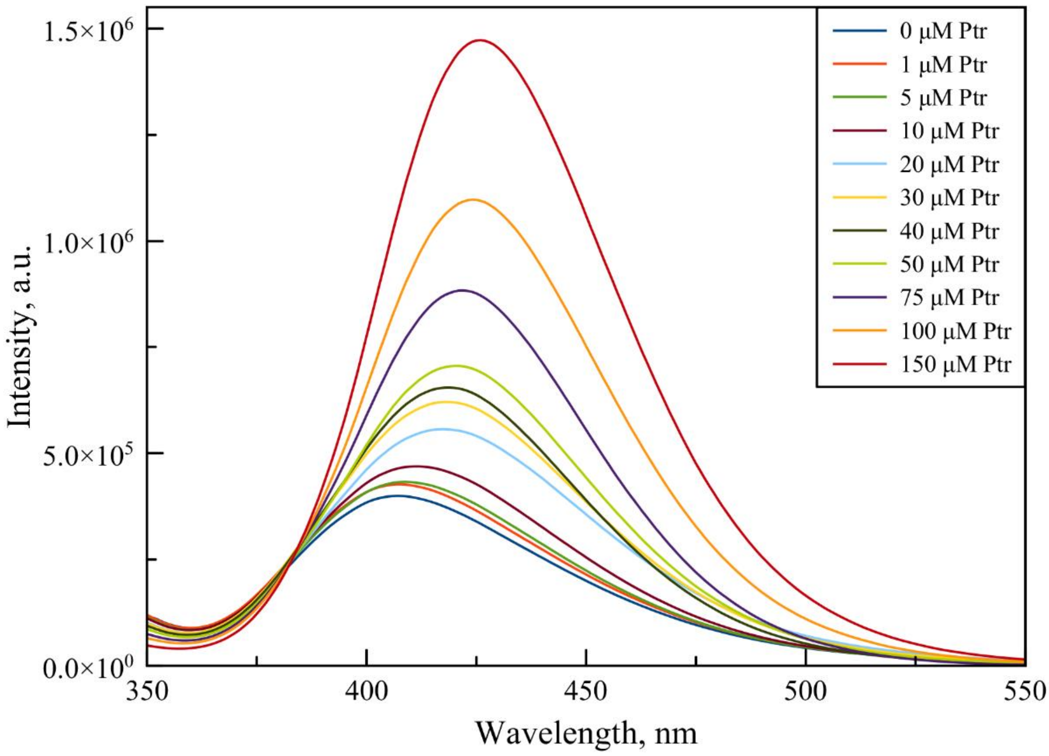

Figure S31. *Emission spectra of Tyr-Au NCs (b) (excitation at 285 nm) in the presence of Ptr.*

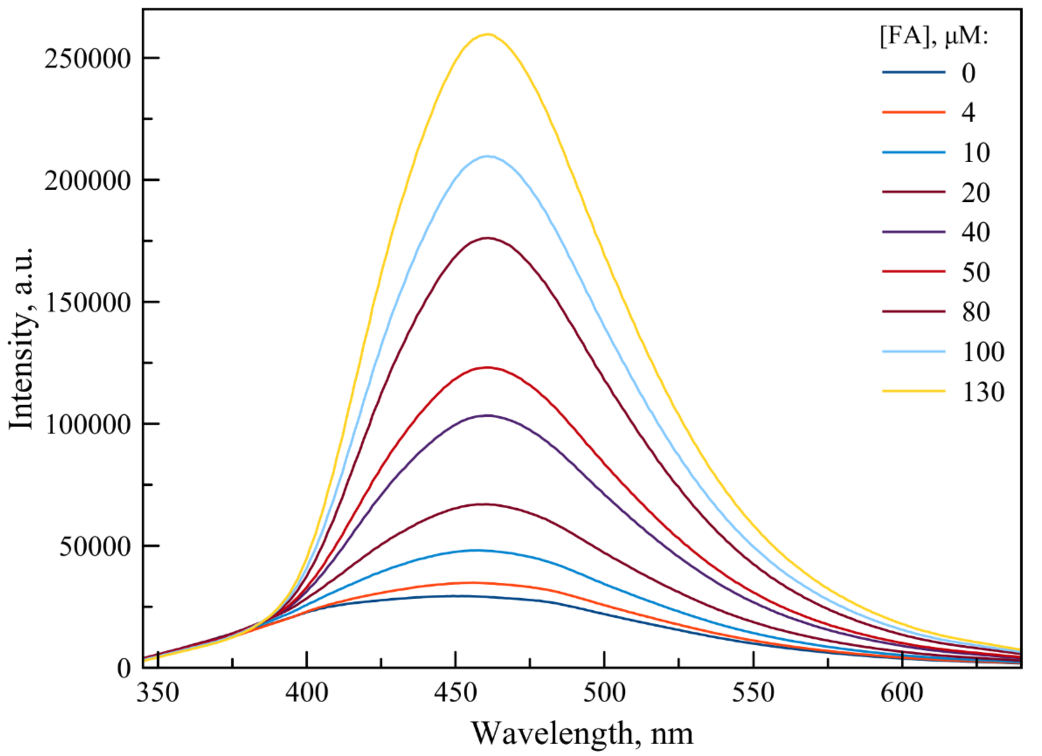

Figure S32. *Emission spectra of Trp-Au NCs (excitation at 330 nm) in the presence of FA.*

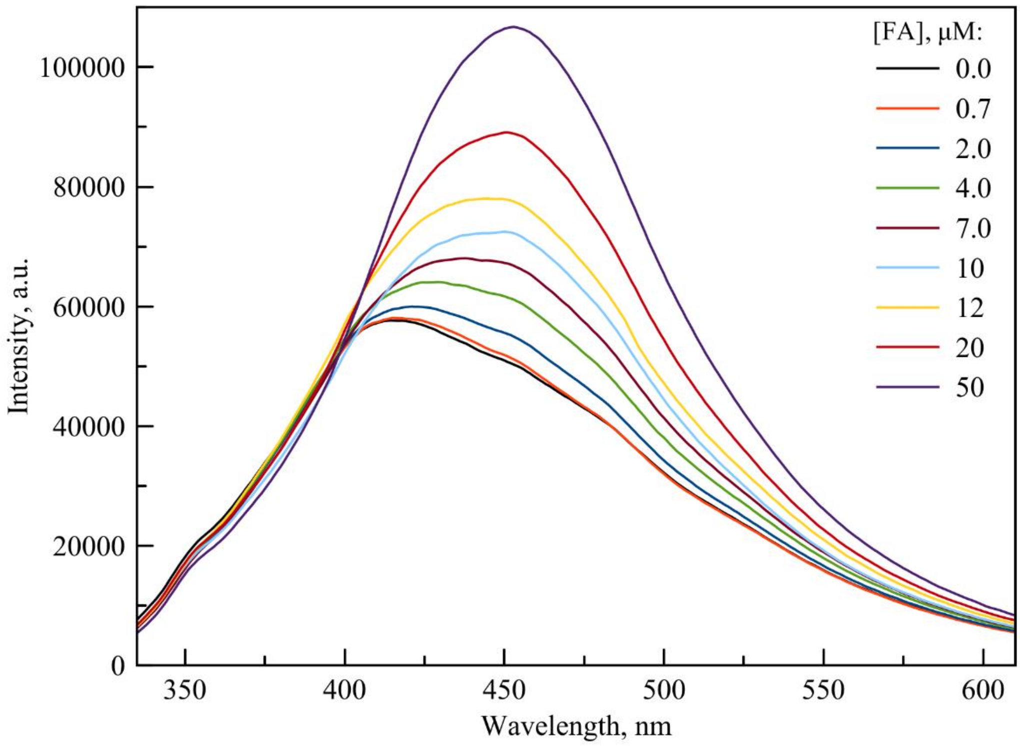

Figure S33. *Emission spectra of Trp-Au NCs (excitation at 330 nm) in serum in the presence of FA.*

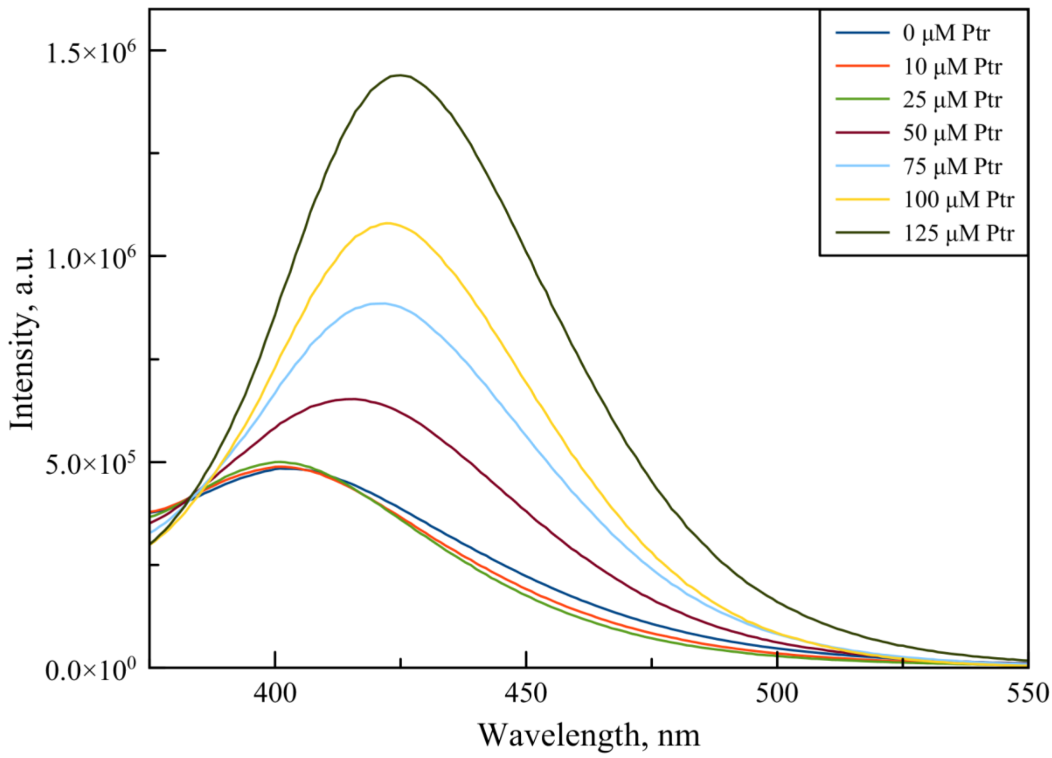

Figure S34. *Emission spectra of Tyr-Au NCs (b) (excitation at 285 nm) in serum in the presence of Ptr.*

Table S1. *Table of dependence of normalized emission intensity at 410 nm of DOPA-Au NCs on the synthesis parameters: [Au]:[DOPA] ratio and solvent pH.*

DOPA-AuNCs Intensity at 410 nm		[Au]:[DOPA]								
		10.0	6.7	5.0	4.0	3.3	2.5	2.0	1.0	0.5
Solvent pH	13.1	--	--	0.19	--	--	--	--	--	--
	12.0	--	--	0.24	0.21	0.10	--	--	--	--
	11.5	--	0.19	0.58	0.67	0.27	--	--	--	--
	11.0	0.08	0.26	1.00	0.70	0.26	0.12	0.11	--	--
	10.5	--	0.33	0.66	0.37	0.23	--	--	--	--
	10.0	--	0.26	0.37	0.25	--	--	--	--	--
	9.6	--	0.26	0.38	0.25	--	--	--	--	--
	8.9	--	0.26	0.39	0.25	--	--	--	--	--
	8.5	0.16	0.27	0.35	0.34	0.20	--	--	--	--
	7.0	0.17	--	0.38	--	0.21	0.14	--	--	--
	5.0	0.16	--	0.37	--	0.19	0.14	0.14	0.06	0.04
	4.0	0.13	--	0.38	--	0.19	0.15	0.13	0.06	0.04
	3.0	0.08	--	0.39	--	0.22	0.15	--	--	--
	2.0	0.07	--	0.58	--	0.25	0.13	0.06	0.05	0.03
	1.0	0.03	--	0.15	--	0.05	0.05	0.06	0.05	0.04

Table S2. *Table of dependence of normalized emission intensity at 460 nm of Trp-Au NCs on the synthesis parameters: [Au]:[Trp] ratio and solvent pH.*

Trp-Au NCs Intensity at 460 nm		[Au]:[Trp]									
		15	5	3.3	2.9	2.5	2.2	2.0	1.0	0.50	0.33
Solvent pH	7.0	--	--	--	--	--	--	0.40	0.22	0.12	0.07
	5.0	--	--	--	--	--	--	0.40	0.21	0.11	0.06
	4.0	--	--	--	--	--	--	0.41	0.23	0.13	0.07
	2.0	0.12	0.34	0.32	0.40	0.45	0.46	0.51	0.42	0.38	0.25
	1.0	0.05	0.32	0.49	0.87	0.99	1.00	0.94	0.53	0.25	0.16

Table S3. *Normalized emission intensity at 350 nm of Phe-Au NCs in dependence of [Phe]:[Au] ratio and dilution rate of obtained complexes after synthesis.*

Phe-AuNCs *Intensity at 350 nm*		[Phe]:[Au] ratio			
		0	2	5	10
dilution rate	-	0,061	-	-	0,404
	5	-	-	1,000	0,948
	50	-	0,281	0,274	0,165

Table S4. *Synthesis conditions and photopysical properties of different AA-Au NCs.*

AA	[Au]/[AA]	Solvent pH*	Sensor for	$\lambda_{ex}/\lambda_{em}$, nm	QY, %	Reference
Tyr	1.30	5.0	Al^{+3}, Fe ions	383/498	1.7	https://doi.org/10.1039/C4AY01137F
	0.55	2.0	tyrosinase	385/470	2.5	https://doi.org/10.1016/j.aca.2014.05.050
	0.50	7.0	guanine	285/405	7	This work
	0.33	10.5	pterin	315/405	26	This work
DOPA	5.0	4.0-10.3	ascorbic acid (through Fe ions)	360/545	--	https://doi.org/10.1016/j.snb.2017.02.151
	2.6	4.5	Fe ions	360/525	1.7	https://doi.org/10.1021/ac203362g
	1.8	4.0	hROS	360/464	--	https://doi.org/10.1021/acs.analchem.0c01147
	5.0	11.0	leucopterin	315/415	1.0	This work
Trp	0.37	5.0	Fe ions	305/450	2.9	https://doi.org/10.1016/j.snb.2016.11.052
	2.20	1.0	Folic acid	330/460	0.7	This work
Phe	0.50	6.0	I^-	320/390	--	https://doi.org/10.1038/s41598-022-05155-5
	0.10	13.0	pterin leucopterin folic acid	290/350	4.3	This work

Table S5. *LOD values for analytes under different detection methods.*

Analyte	Method/Fluorescent sensor	LOD, μM	Reference	Year
Guanine	electrochemistry	0.06	https://doi.org/10.1007/s00604-021-04926-7	2021
	carbon dots	0.0064	https://doi.org/10.1016/j.aca.2021.338977	2021
	NFR-Ag NCs on nanosheets	1.85	https://doi.org/10.1016/j.talanta.2018.09.097	2018
	Tyr-Au NCs	0.7	This work	
Leucopterin	liquid chromatography with mass spectrometry	0.0072	https://doi.org/10.1016/j.jchromb.2013.05.004	2013
	capillary electrophoresis	0.56	https://doi.org/10.1016/j.chroma.2014.02.019	2014
	DOPA-Au NCs	2.8	This work	
	Phe-Au NCs	9	This work	
Pterin	liquid chromatography with mass spectrometry	0.0006	https://doi.org/10.4155/bio.12.131	2012
	liquid chromatography	0.018	https://doi.org/10.1016/j.jpba.2013.12.012	2013
	capillary electrophoresis	0.1	https://doi.org/10.3390/molecules24061166	2019
	Tyr-Au NCs	4.4	This work	
	Phe-Au NCs	11	This work	

Folic Acid	flow injection analysis	0.068	https://doi.org/10.1002/pca.704	2003
	capillary electrophoresis	0.02	https://doi.org/10.1016/j.chroma.2005.11.052	2006
	SERS	0.009	https://doi.org/10.1021/nn201606r	2011
	absorbance (reaction inhibition)	0.82	https://doi.org/10.1016/j.saa.2011.06.015	2011
	BSA-Au NCs	0.042	https://doi.org/10.1016/j.snb.2014.03.075	2014
	BSA-Au NCs with Au NPs	0.065	http://doi.org/10.1007/s00604-014-1442-z	2015
	liquid chromatography with mass spectrometry	0.0006	https://doi.org/10.1016/j.jpha.2015.05.004	2015
	electrochemistry	0.01	https://doi.org/10.1016/j.bios.2016.05.095	2016
	carbon dots	0.04	https://doi.org/10.1016/j.snb.2018.01.227	2018
	carbon dots	0.38	https://doi.org/10.1016/j.saa.2019.117931	2019
	carbon dots	0.28	https://doi.org/10.1007/s00216-020-02507-w	2020
	ovalbumine-CuNCs	0.18	https://doi.org/10.1016/j.talanta.2018.11.067	2018
	D-Trp-Au NCs	5.8	https://doi.org/10.1186/s40543-021-00266-6	2021
	L-Trp-Au NCs	0.2	This work	
	Phe-Au NCs	6	This work	