Electronic Supplementary Material (ESI) for Nanoscale Advances.

- Supporting Information -

Flavins Complex Gold Nanoparticles: Chemical Modeling Design, Physico-Chemical Assessment and Perspectives in Nanomedicine.

Celia Arib[a], Nadia Bouchemal[a], Maria Barile[b], Didier Paleni[c], Nadia Djaker[a], Nathalie Dupont[a], Jolanda Spadavecchia[a]*

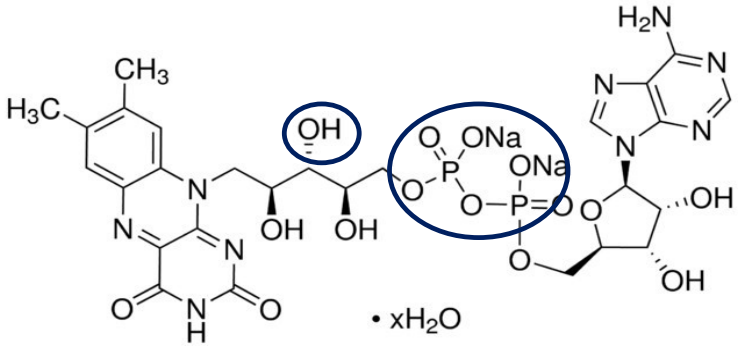
[a] *CNRS, UMR 7244, CSPBAT, Laboratoire de Chimie, Structures et Propriétés de Biomatériaux et d'Agents Thérapeutiques, Université Paris 13, Sorbonne Paris Nord,1 rue Chablis 93000 Bobigny, France.*

[b] *Dept. of Biosciences, Biotechnology and Biopharmaceutics, University of Bari "Aldo Moro" Via Orabona 470126 - Bari - ITALY*

[c] *BioEVEN start-up, 75 rue de Lourmel 75015 Paris*

* Corresponding author: jolanda.spadavecchia@univ-paris13.fr

ADDITIONAL SCHEMES



Scheme S1. Chemical structure of Flavin Adenin Dinucleotide (FAD). Blue rings are associated to site of complexation with gold ions.

ADDITIONAL TABLES-EXPERIMENTAL PART

Table S 1 : Chemical shifts $^{1}H/^{13}C$ and T1 (s) values

FAD

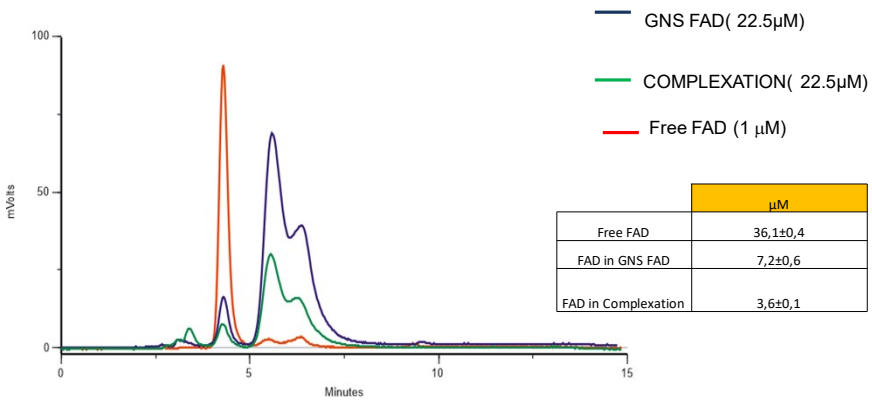
position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
^{1}H	5,85	4,53	4,49	4,35	4,29	4,26	4,03	3,91	4,34	4,09	7,52	2,36	2,29	7,52	8,28	7,85
^{13}C	86,98	74,69	69,89	83,43	67,52	64,9	71,08	72,3	69,05	67,55	116,29	20,53	18,46	130,14	139,14	139
T1 (^{1}H)	1,62										2,07	0,86	0,86	2,07	1,47	2,88

FAD-Au-PEG

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15/ 16
6,02	4,62	4,49	4,36	4,24	4,26	4,09	3,96	4,40	5,05	7,77	2,49	2,40	7,77	8,32 / 8,54
87,65									47,42	116,63			130,27	141,84 /145,15
1,97										1,96	0,90	0,91	1,96	1,33/1,43

ADDITIONAL FIGURES-EXPERIMENTAL PART

A)



	µM
Free FAD	36,1±0,4
FAD in GNS FAD	7,2±0,6
FAD in Complexation	3,6±0,1

B)

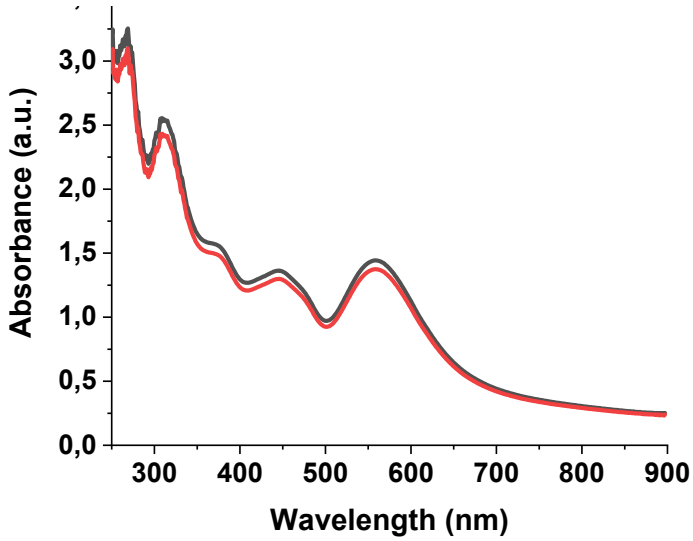


Figure S 1 A-B : Typical chromatograms with neutralyzed perchloric extract of free FAD (red line), FAD-$AuCl_2$-PEG (green line) ;FAD IN PEG AuNPs (GNS FAD) (blue line) **A)** ; Stability of FAD IN PEG-AuNPs before (black line) and after (red line) incubation in Dulbecco modified Eagle's Medium (DMEM) and stored for 72h **B)**.

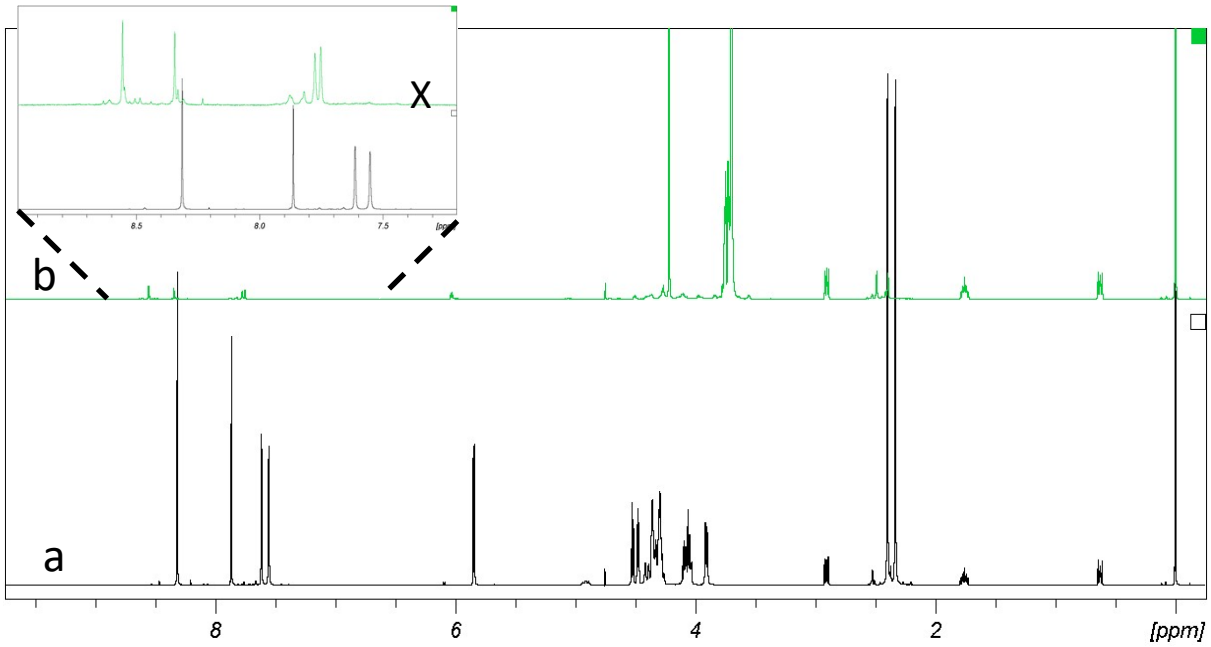
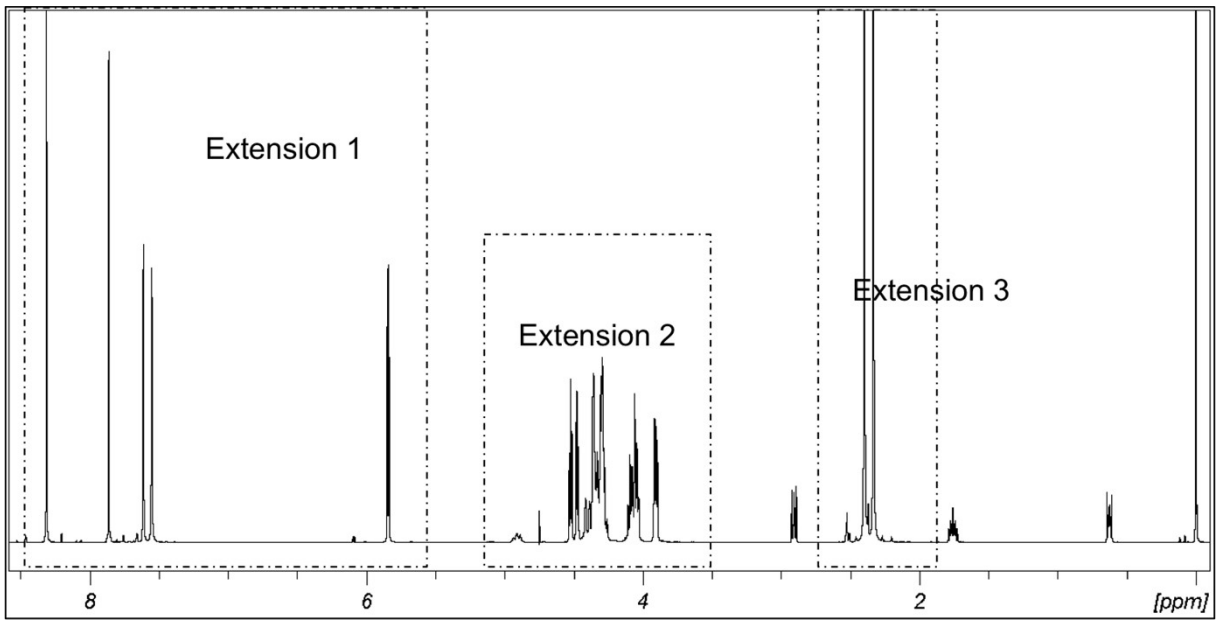


Figure S 2 :^{1}H NMR spectra of the FAD (a) and the complexed PEG/FAD-Au (b). T=300K – Internal reference : DSS (0/0.63/1.76/ 2.90 ppm)

As shown in extension (X) , the major change between the FAD spectra (a) and the PEG/FAD-Au spectra (b) is located in the aromatic area peaks (7.55 to 8.3 ppm), suggesting that FAD was complexed to the Au by forming bonds between aromatic protons of the FAD.



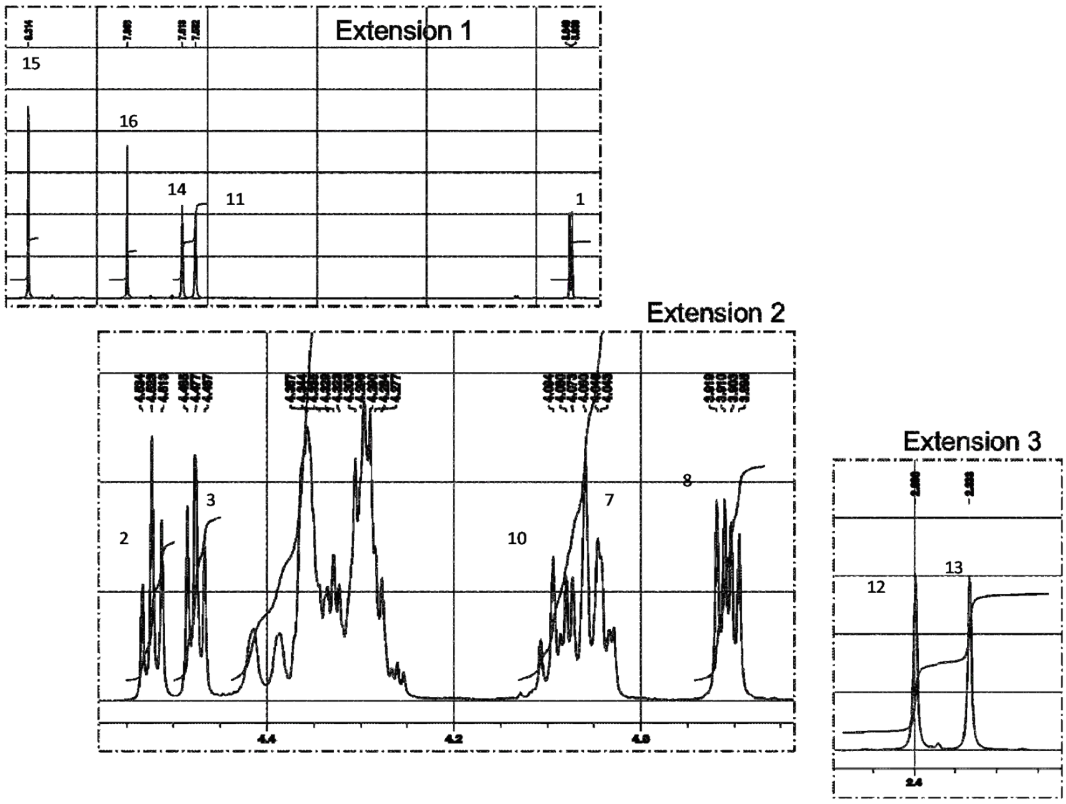


Figure S 2 bis : ^{1}H NMR spectrawith spectral assigments of FAD molecule

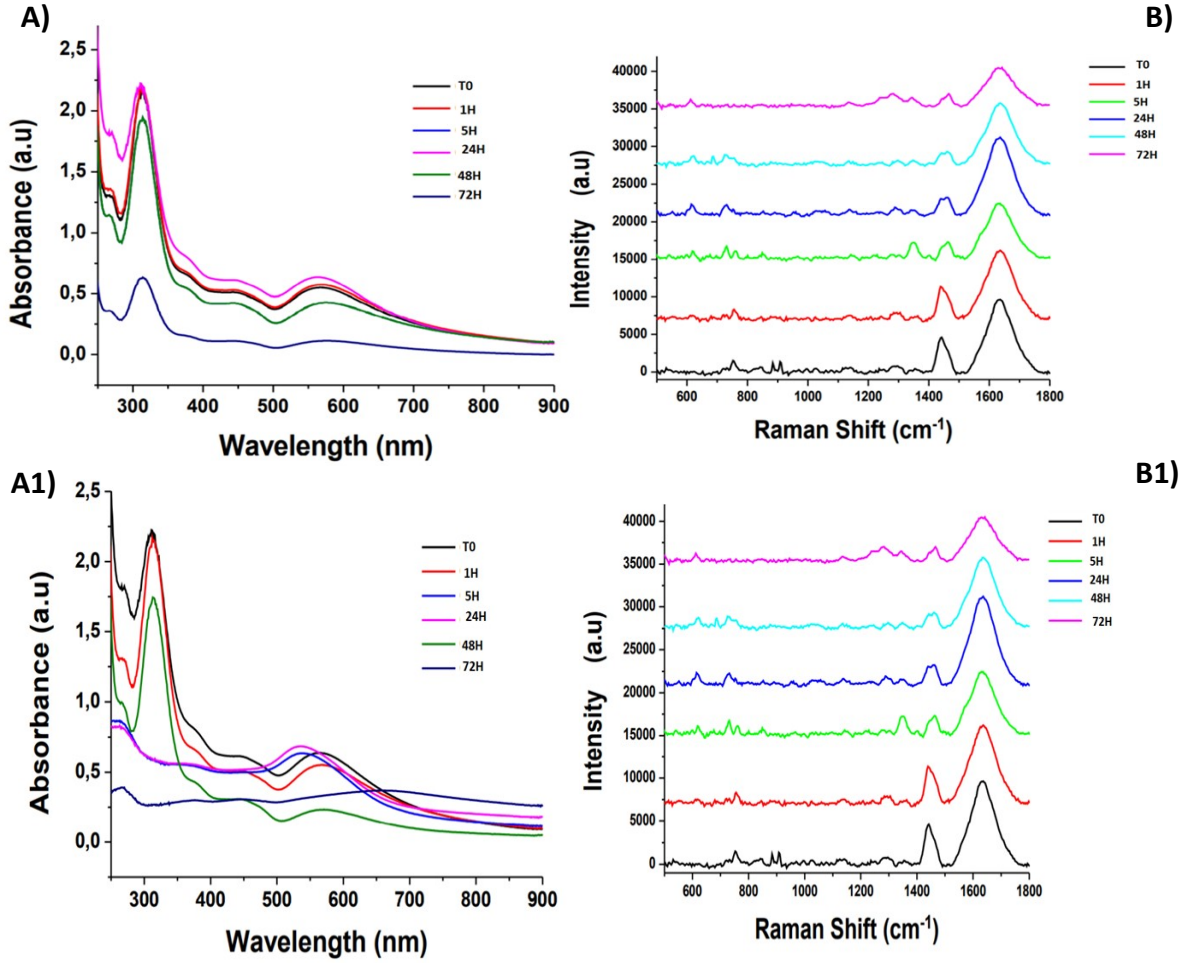


Figure S 3 : Monitoring FAD release from 0 h to 72 h at 37°C at pH 7 by UV-VIS (A) and pH 4 (A1); B) monitoring release of FAD IN PEG-AuNPs at pH 7 and pH 4 by Raman spectoscopy (B1).

Experimental DLS and Z potential measurements

Zeta Potential Report

v2.3

Malvern

Sample Details

Sample Name: FAD-PEG-Aunps_05052021_zeta 1
SOP Name: SOP (protocole) Au-NPS.sop
General Notes:

File Name: FAD-PEG-Aunps_0505202...
Record Number: 1
Date and Time: lundi 10 mai 2021 12:52:53
Dispersant Name: Water
Dispersant RI: 1,330
Viscosity (cP): 0,8872
Dispersant Dielectric Constant: 78,5

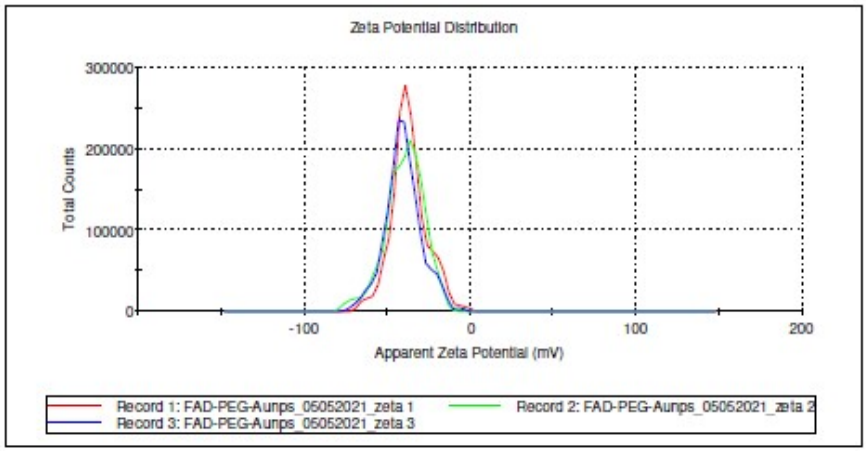
System

Temperature (°C): 25,0
Count Rate (kcps): 236,8
Cell Description: Clear disposable zeta c...
Zeta Runs: 12
Measurement Position (mm): 2,00
Attenuator: 8

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -36,7	**Peak 1:**	-36,7	100,0	10,9
Zeta Deviation (mV): 10,9	**Peak 2:**	0,00	0,0	0,00
Conductivity (mS/cm): 1,26	**Peak 3:**	0,00	0,0	0,00

Result quality See result quality report



Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: FAD-PEG-AuNPs_05052021_SIZE 1

SOP Name: SOP (protocole) Au-NPS-SIZE.sop

General Notes:

File Name:	FAD-PEG-Aunps_0505...	**Dispersant Name:**	Water
Record Number:	1	**Dispersant RI:**	1,330
Material RI:	0,20	**Viscosity (cP):**	0,8872
Material Absorbtion:	0,000	**Measurement Date and Time:**	lundi 10 mai 2021 12:45:59

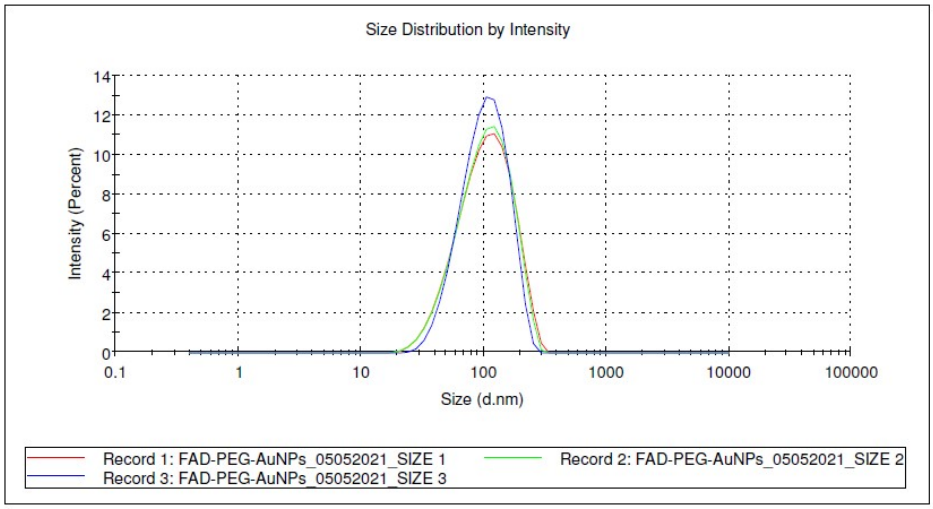
System

Temperature (°C):	25,0	**Duration Used (s):**	60
Count Rate (kcps):	296,1	**Measurement Position (mm):**	5,50
Cell Description:	Clear disposable zeta cell	**Attenuator:**	4

Results

			Size (d.nm...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	91,75	**Peak 1:**	114,4	100,0	54,05
PdI:	0,143	**Peak 2:**	0,000	0,0	0,000
Intercept:	0,907	**Peak 3:**	0,000	0,0	0,000

Result quality : Good



TABLES AND FIGURES: Theoretical part

Part B : Theoretical results

Table S-B1 :Main geometrical parameters and chemical descriptors for FAD (gazeous and solvated in water) ; FAD-PEG1 and FAD-PEG2 (solvated in water)

Chemical descriptors	FAD-PEG2	FAD-PEG1	FAD	FAD
Solvation	implicit water solvation, iefpcm			Gas phase
atoms	107	100	84	84
electrons	494	470	408	408
Charge	-2	-2	-2	-2
Spin	singlet	singlet	singlet	singlet
Dipolar moment μ (in Debye)	14,0152	17,6172	31,4391	16,1277
HOMO n°	247	235	204	204
LUMO n°	248	236	205	205
HOMOEnergy (in H)	-0,24105	-0,24099	-0,23037	-0,16120
LUMOEnergy (in H)	-0,11559	-0,11555	-0,11544	0,15387
HOMO Energy (in eV)	-6,56	-6,56	-6,27	-4,39
LUMO Energy (in eV)	-3,15	-3,14	-3,14	4,19
Gap (in eV)	3,41	3,41	3,13	8,57
Average linear polarizability **α total (in Bohr^3)**	749,17	715,83	623,38	483,44
Electron affinity A =-E(LUMO) (in H)	0,11559	0,11555	0,11544	0,15387

Ionization Potential I = -E(HOMO)(in H)	0,24105	0,24099	0,23037	0,16120
Global Hardness $\eta = (I-A)/2$	0,06273	0,06272	0,05746	0,15753
Softness S = $1/(2*\eta)$	7,97066	7,97194	8,70095	3,17390
Electronegativity $\chi = (I+A)/2$	0,17832	0,17827	0,17290	0,00367
Electrophilicity index **$\omega = \chi^2/2\eta$**	3,98938 E-03	3,98651 E-03	3,43596 E-03	4,23209E-06
Distance N(9A) - N(10) (in A)	14,164	14,009	13,588	12,418
dihedral angle N(9A) - P(A)-P-N10 (in °)	177,26	173,36	146,99	135,51
Mulliken charge on N(9A) atom (in adenine moiety)	-0,587	-0,587	-0,586	-0,578
Mulliken charge on P(A) atom (next to ribose moiety)	1,650	1,650	1,636	1,674
Mulliken charge on O (between P(A) and P)	-0,922	-0,922	-0,914	-0,902
Mulliken charge on P atom (next to ribityl moiety)	1,686	1,692	1,642	1,685
Mulliken charge on N(10) atom (in flavin moiety)	-0,668	-0,668	-0,668	-0,647

Table S-B2 :Main geometrical parameters and chemical descriptors for FAD; FAD-AuCl2; FAD-Au4and FAD-Au8 (solvated in implicit iefpcmwater)

Chemical descriptors	FAD	FADAuCl2	FAD-Au4	FAD-Au8
Solvation	implicit water solvation, iefpcm			
atoms	84	87	88	92
electrons	408	520	724	1040
Charge	-2	-1	-2	-2
Spin	singlet	singlet	singlet	singlet
Dipolar moment μ (in Debye)	31,4391	22,2791	41,7459	25,7008
HOMO n°	204	220	242	280
LUMO n°	205	221	243	281
HOMO Energy (in H)	-0,23037	-0,23095	-0,15575	-0,17453
LUMO Energy (in H)	-0,11544	-0,18224	-0,11395	-0,11129
HOMO Energy (in eV)	-6,27	-6,28	-4,24	-4,75
LUMO Energy (in eV)	-3,14	-4,96	-3,10	-3,03
Gap (in eV)	3,13	1,32	1,14	1,72
Average linear polarizability **α total (in Bohr^3)**	623,38	731,07	1210,38	1569,88
Electron affinity A =-E(LUMO) (in H)	0,11544	0,18224	0,11395	0,11129
Ionization Potential I = -E(HOMO)(in H)	0,23037	0,23095	0,15575	0,17453
Global Hardness η = (I-A)/2	0,05746	0,02435	0,02090	0,03162

Softness S = 1/(2*η)	8,70095	20,52967	23,92344	15,81278
Electronegativity χ= (I+A) /2	0,17290	0,20659	0,13485	0,14291
Electrophilicity index **$\omega = \chi^2/2\eta$**	3,436 E-03	2,079 E-03	7,601 E-04	1,292 E-03
Distance N_{9A}- N_{10}) (in Å)	13,588	14,210	12,897	14,431
dihedral angle N_{9A} - P_AP-N_{10} (in °)	146,99	167,53	129,56	173,65
Mulliken charge on N_{9A} atom (in adenine moiety)	-0,586	-0,581	-0,569	-0,573
Mulliken charge on P_A atom (next to ribose moiety)	1,636	1,714	1,695	1,703
Mulliken charge on O_c (between P(A) and P)	-0,914	-0,871	-0,883	-0,951
Mulliken charge on P atom (next to ribityl moiety)	1,642	1,738	1,684	1,726
Mulliken charge on N_{10} atom (in flavin moiety)	-0,668	-0,667	-0,675	-0,670

Table S-B3 : Calculated UV-Visible electronic transitions and main assignments for FAD optimized structure at the B3LYP/6-311G(d,p) TD-DFT level of theory in implicit iefpcm water.

No.	Wavelength (nm)	Osc. Strength	Major contribs
1	421	0.0	HOMO->LUMO (100%)
2	**418**	**0.1969**	**H-2->LUMO (94%)**
3	408	0.0	H-1->LUMO (98%)
4	389	0.0	H-3->LUMO (98%)
5	364	0.0	H-4->LUMO (98%)
6	358	0.0032	H-17->LUMO (22%), H-12->LUMO (52%), H-11->LUMO (14%)

Table S-B4 : Calculated UV-Visible electronic transitions and main assignments for FAD-PEG1 optimized structure at the B3LYP/6-311G(d,p) TD-DFT level of theory in implicit iefpcm water.

No.	Wavelength (nm)	Osc. Strength	Major contribs
1	**418**	**0,197**	**HOMO->LUMO (94%)**
2	392	0	H-1->LUMO (99%)
3	372	0	H-3->LUMO (67%), H-2->LUMO (25%)
4	362	0	H-3->LUMO (23%), H-2->LUMO (73%)
5	358	0,0037	H-18->LUMO (19%), H-14->LUMO (19%), H-13->LUMO (39%)
6	352	0,0001	H-5->LUMO (44%), H-4->LUMO (52%)

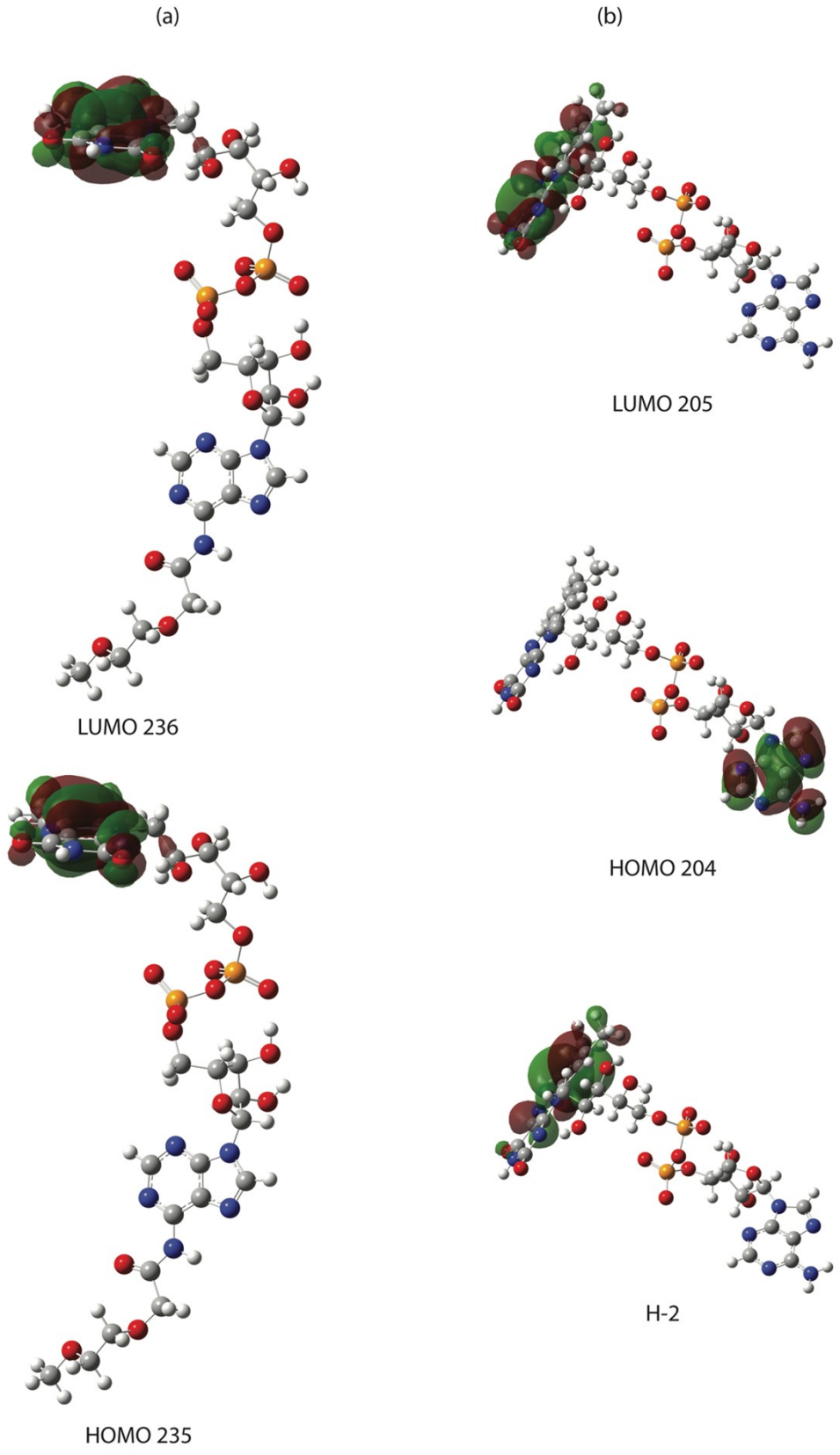


Figure SB-1: Graphical representation of HOMO, LUMO and eventually molecular orbitals involved in the more intensive UV-Vis electronic transitions for (a) FAD-PEG1 and (b) FAD computed using HF/6-311G(d,p). For the two systems, the more intensive transitions are relative to the flavin moiety.

Table SB-5 : Calculated electronic transitions and main assignments for FADAuCl2 optimized structure at the B3LYP/6-311G(d,p)/LANL2DZ TD-DFT level of theory in implicit iefpcm water.

No.	Wavelength (nm)	Osc. Strength	Major contribs
1	3169	0	H-1->LUMO (100%)
2	1873	0	H-2->LUMO (99%)
3	1306	0,0003	H-4->LUMO (99%)
4	1172	0	H-3->LUMO (100%)
5	1043	0,0007	H-6->LUMO (91%)

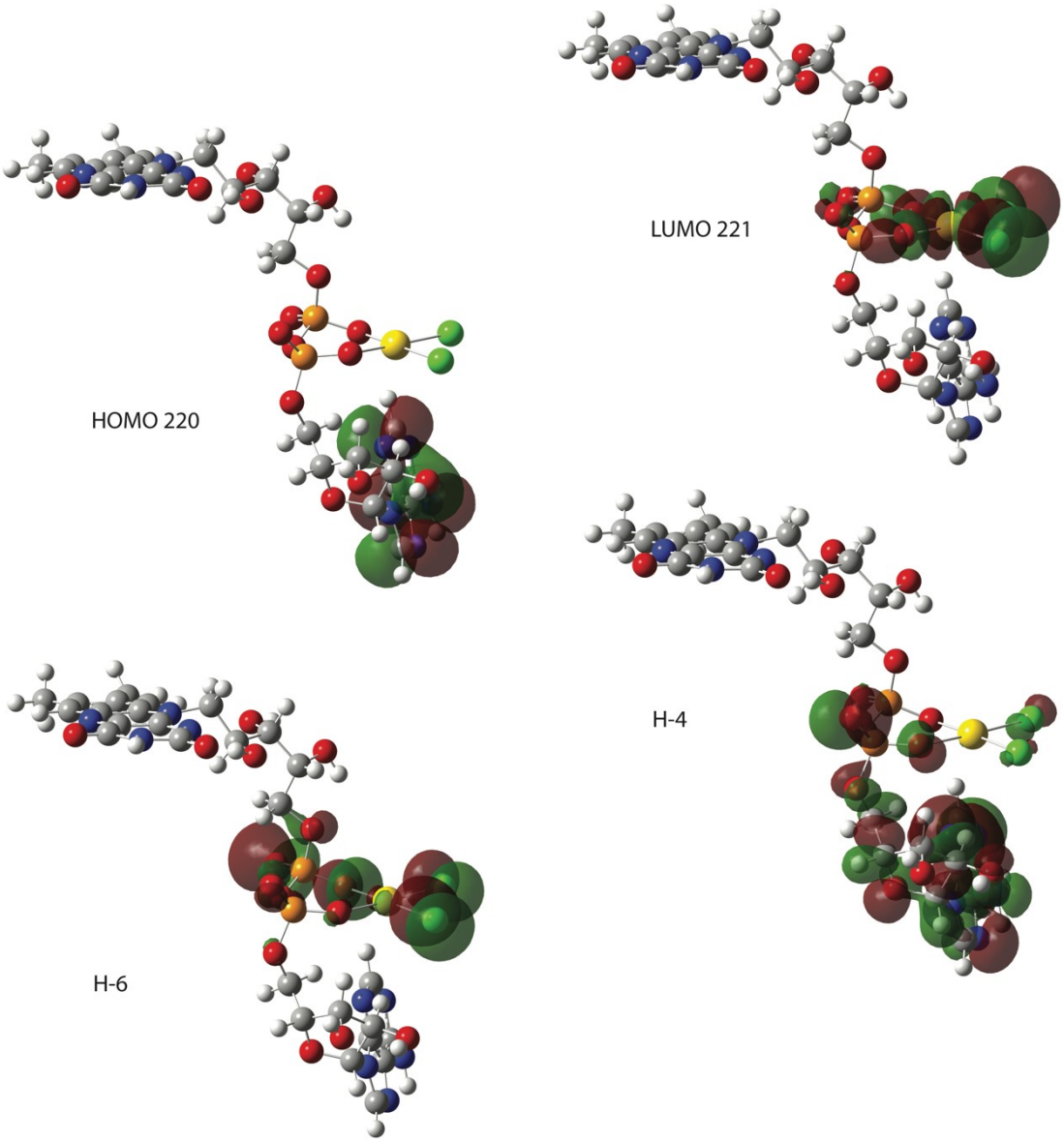


Figure S-B2: Graphical representation of HOMO, LUMO and eventually molecular orbitals involved in the more intensive UV-Vis electronic transitions for FAD-AuCl2 computed using HF/6-311G(d,p)/LANL2DZ. Surprisingly, flavin moiety is not involved in the transitions. Cl atoms modify the repartition of the energy scheme.

Table S-B6 : Calculated UV-Visible electronic transitions and main assignments for FAD-Au4 optimized structure at the B3LYP/6-311G(d,p)/LANL2DZ TD-DFT level of theory in implicit iefpcm water.

No.	Wavelength (nm)	Osc. Strength	Major contribs
1	3011	0,0026	HOMO->LUMO (105%)
2	1367	0,0009	HOMO->L+1 (100%)
3	1082	0,0089	HOMO->L+2 (99%)
4	520	0,0005	H-4->LUMO (91%)
5	**514**	**0,012**	**H-1->LUMO (85%)**
6	**486**	**0,0027**	**H-5->LUMO (90%)**

.

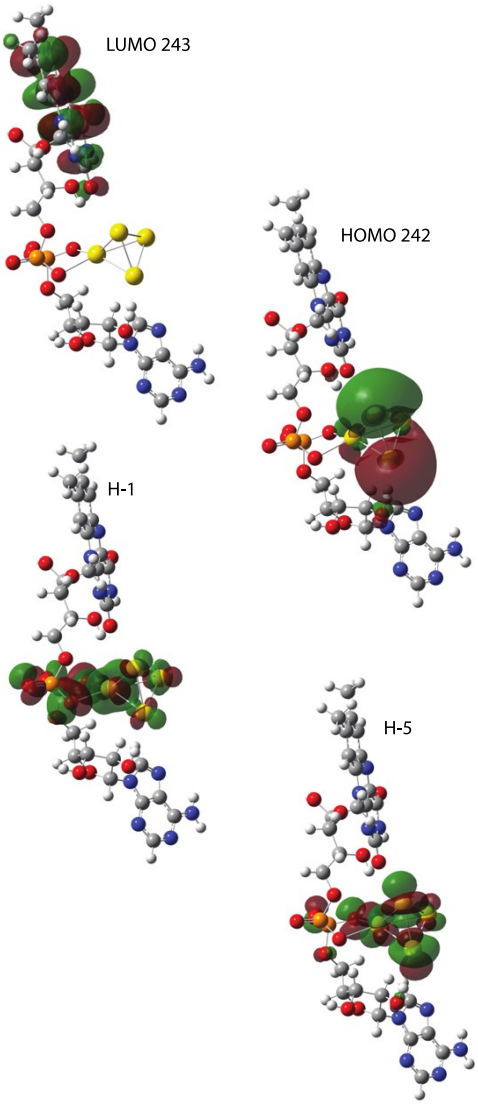


Figure SB-3: Graphical representation of HOMO, LUMO and eventually molecular orbitals involved in the more intensive UV-Vis electronic transitions for FAD-Au4 computed using HF/6-311G(d,p))/LANL2DZ. LUMO is located on flavin moiety, HOMO is centered on gold cluster whereas H-1 and H-5 include gold atoms and phosphate moieties

Table S-B7 : Calculated UV-Visible electronic transitions and main assignments for FAD-Au8 optimized structure at the B3LYP/6-311G(d,p)/LANL2DZ TD-DFT level of theory in implicit iefpcm water.

No.	Wavelength (nm)	Osc. Strength	Major contribs
1	857	0,0003	HOMO->LUMO (99%)
2	796	0,002	HOMO->L+1 (95%)
3	766	0,0012	HOMO->L+2 (97%)
4	**610**	**0,021**	**H-1->L+1 (10%), HOMO->L+3 (81%)**
5	**597**	**0,0285**	**HOMO->L+4 (81%)**
6	558	0,0004	H-1->LUMO (100%)

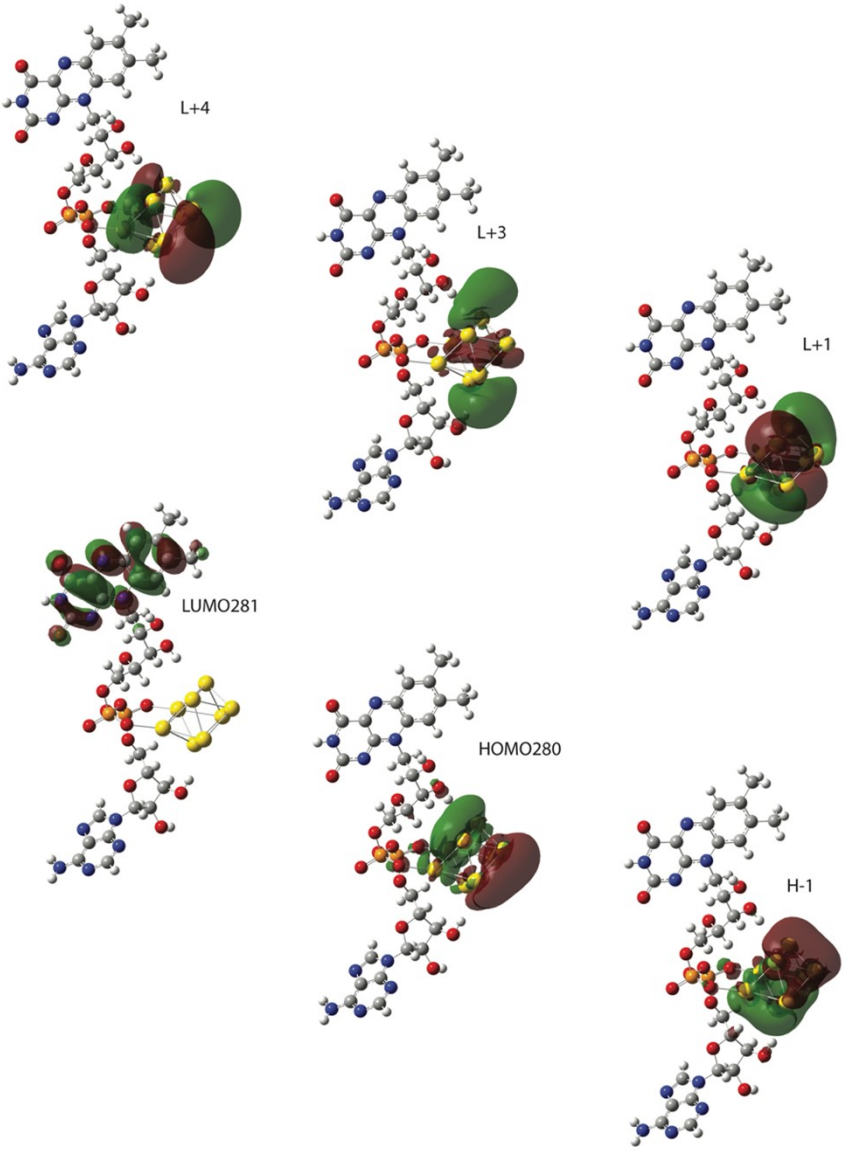


Figure S-B4: Graphical representation of HOMO, LUMO and eventually molecular orbitals involved in the more intensive UV-Vis electronic transition for FAD-Au8 computed using HF/6-311G(d,p))/LANL2DZ. Most of them; excluding the LUMO that is located on the flavin moiety, are located on the gold cluster.