

*Supporting Information*

**D-Penicillamine-Stabilized Gold Nanoclusters as a Selective  
Fluorescent Sensor for Tetracycline**

*Luis Marco-Sabater, Elena Zaballos-García, Jorge Escorihuela\* and Julia Pérez-Prieto\**

## **1. Experimental details.**

### **1.1. Materials.**

All the chemicals and solvents used in this thesis degree were of analytical grade and used without any additional purification. Gold(III) chloride ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ), sodium chloride ( $\text{NaCl}$ ), potassium chloride ( $\text{KCl}$ ), magnesium chloride ( $\text{MgCl}_2$ ), calcium chloride ( $\text{CaCl}_2$ ), aluminum chloride ( $\text{AlCl}_3$ ), sodium nitrate ( $\text{NaNO}_3$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), copper sulphate ( $\text{CuSO}_4$ ), L-phenylalanine (L-Phe), L-valine (L-Val), L-histidine (l-His), tetracycline and ampicillin were purchased from Sigma-Aldrich. D-penicillamine was purchased from BLD Pharmatech. For all aqueous solutions, high purity deionized water from a Millipore system was used.

### **1.2. Equipment.**

The following technical instruments were used for analysis and characterization. Centrifugation was performed on a Beckman Coulter's Microfuge 16 benchtop centrifuge. UV-vis absorption spectra were recorded on a PerkinElmer 1050+ UV/vis/NIR spectrometer. All the measurements were performed using 1cm×1cm path length quartz cuvettes. Fluorescence spectra were recorded on a FLS1000 photoluminescence spectrometer from Edinburgh Instruments. The quantum yield was measured with a Hamamatsu C9920-02 absolute PL Quantum Yield Measurement System. The pH measurements were carried out by using a Crison GLP 21 pH meter. Transmission electron microscopy (TEM) images were acquired using a HITACHI HT7800 microscope with a filament of LaB6 operating at 100 kV. Fourier transform infrared (FT-IR) spectra were recorded on a Thermo Scientific Nicolet iS10. X-ray photoelectron spectroscopy (XPS) spectra were acquired with VG-Microtech Multilab 3000 equipment. The  $^1\text{H}$  spectrum were registered at room temperature in a Bruker AvanceIII 300 spectrometer, with a 300 MHz Bruker magnet. The chemical shifts ( $\delta$ ) are reported in ppm using deuterium oxide, 99.9% atom ( $\text{D}_2\text{O}$ ) as solvent.

### **1.3. Procedures.**

#### **1.3.1. Preparation of AuNC@D-Pen.**

A freshly prepared aqueous solution of  $\text{HAuCl}_4$  (50  $\mu\text{L}$ , 50 mM) was diluted in water (1 mL), and an aqueous solution of D-penicillamine (53  $\mu\text{L}$ , 1 M) was added. The mixture was set for 5 days at room temperature, obtaining a white precipitated (fluorescent under UV-light) and a colourless solution. The precipitate was isolated by centrifugation at 10000 rev/min for 15 min. The supernatant was slowly removed without disturbing the precipitate, which had been washed

two times by dispersion in water and precipitation by centrifugation at 10000 rev/min for 15min. After purification, the **AuNC@D-Pen** were dispersed in water or the solutions were diluted with buffer solution, meanwhile, to avoid the effect of the pH change.

### **1.3.2. Fluorescence detection of tetracycline.**

Fluorescence experiments were conducted at room temperature using an excitation wavelength of 310 nm. Tetracycline solutions of varying concentrations were freshly prepared and sequentially added to 3 mL of **AuNC@D-Pen** solution (citrate buffer, pH 4.3, with an absorbance of 0.4 at 305 nm). Photoluminescence spectra were recorded at room temperature immediately after each addition of tetracycline. The concentration of tetracycline was plotted on the x-axis, while the corresponding PL intensity was plotted on the y-axis. Finally, a linear correlation curve was generated to determine the concentration of tetracycline.

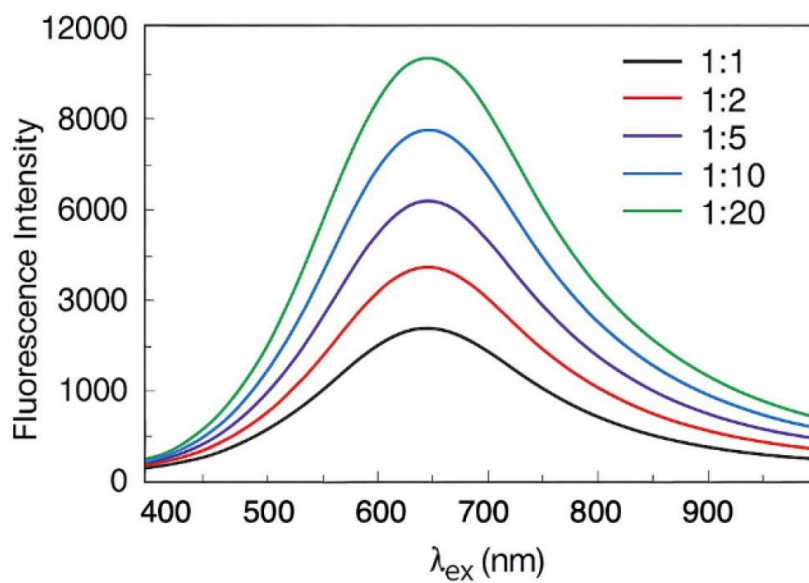
### **1.3.3. Selectivity studies of AuNC@D-Pen.**

To evaluate the selectivity of the prepared **AuNC@D-Pen**, a series of selective experiments were performed. Initially, 5  $\mu$ L of a 0.05 M TC solution was added to a solution of **AuNC@D-Pen** to establish the baseline photoluminescence (PL) intensity as a control. Subsequently, 5  $\mu$ L of 0.05 M solutions of different potential interfering analytes, such as common anions and cations (NaCl, KCl, MgCl<sub>2</sub>, AlCl<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>, MnCl<sub>2</sub>, CaCl<sub>2</sub>, and MgSO<sub>4</sub>), amino acids (L-Phe, L-Val, and L-His), and ampicillin, were added to the **AuNC@D-Pen** solution. Fluorescence intensity values were measured after an incubation time of 5 minutes at room temperature.

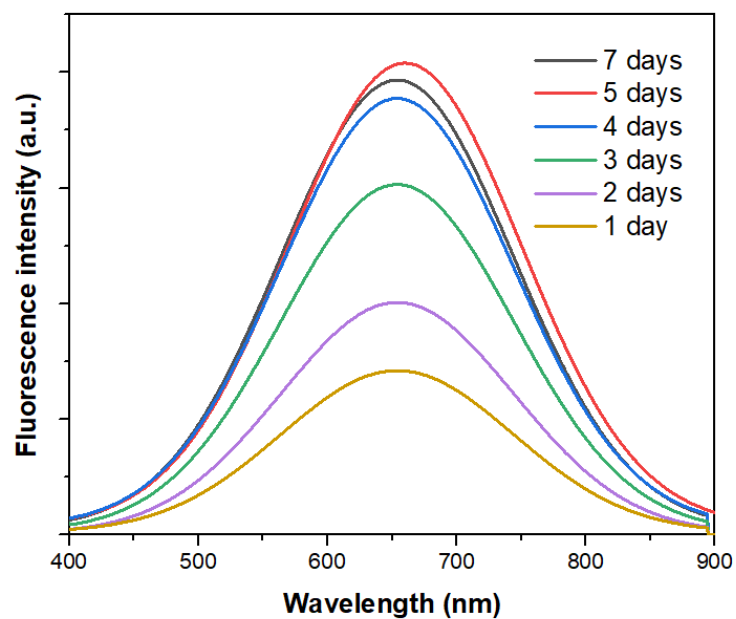
### **1.3.4. Selectivity studies of AuNC@D-Pen.**

We performed recovery experiments using both tap water and lake water. Tap water was tested for TC without pretreatment. For TC detection in lake water, the sample was initially centrifuged to remove suspended particles. Next, water samples were adjusted at pH around 4.0 by adding HCl solution. Known concentrations of tetracycline (0.8, 1.2, 2.5, and 5.0  $\mu$ M) were added to each water sample, and the resulting solutions were analysed under the same optimized conditions used for the calibration curve. For each concentration level, three independent measurements were carried out, allowing the calculation of both the recovery percentage and the corresponding relative standard deviation (RSD).

#### 1.4 Additional figures.

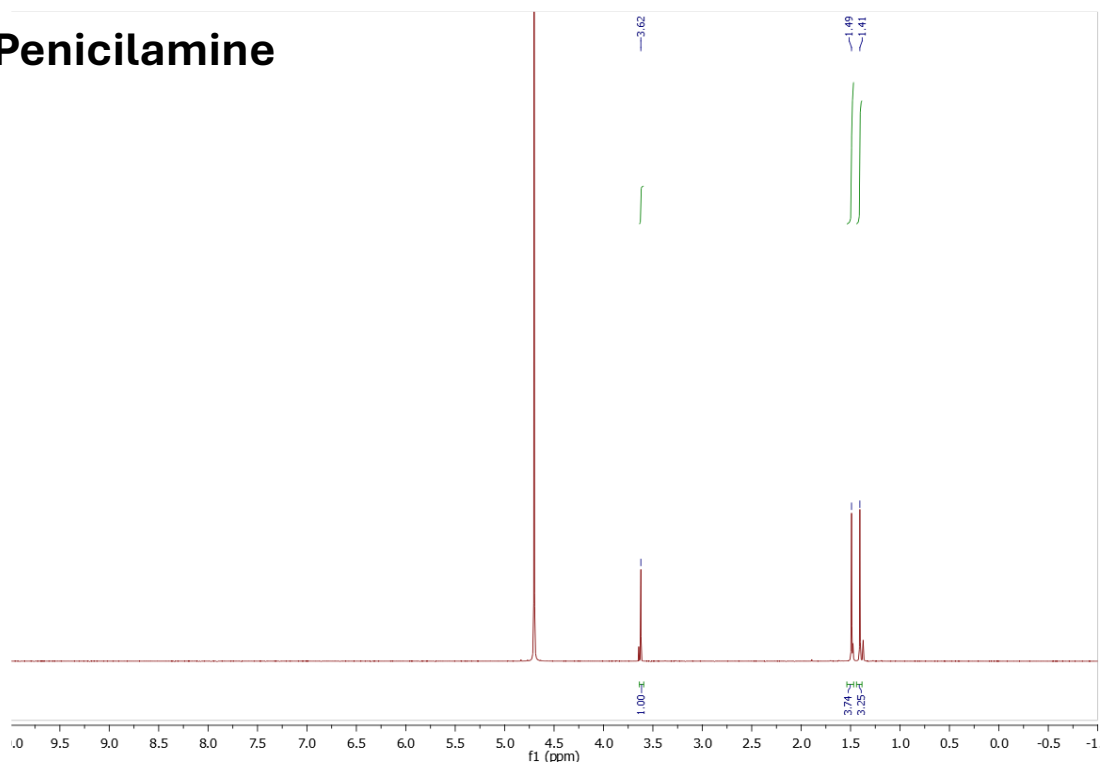


**Figure S1.** HAuCl<sub>4</sub>/D-Pen molar ratio effect on the fluorescence of AuNC@D-Pen.

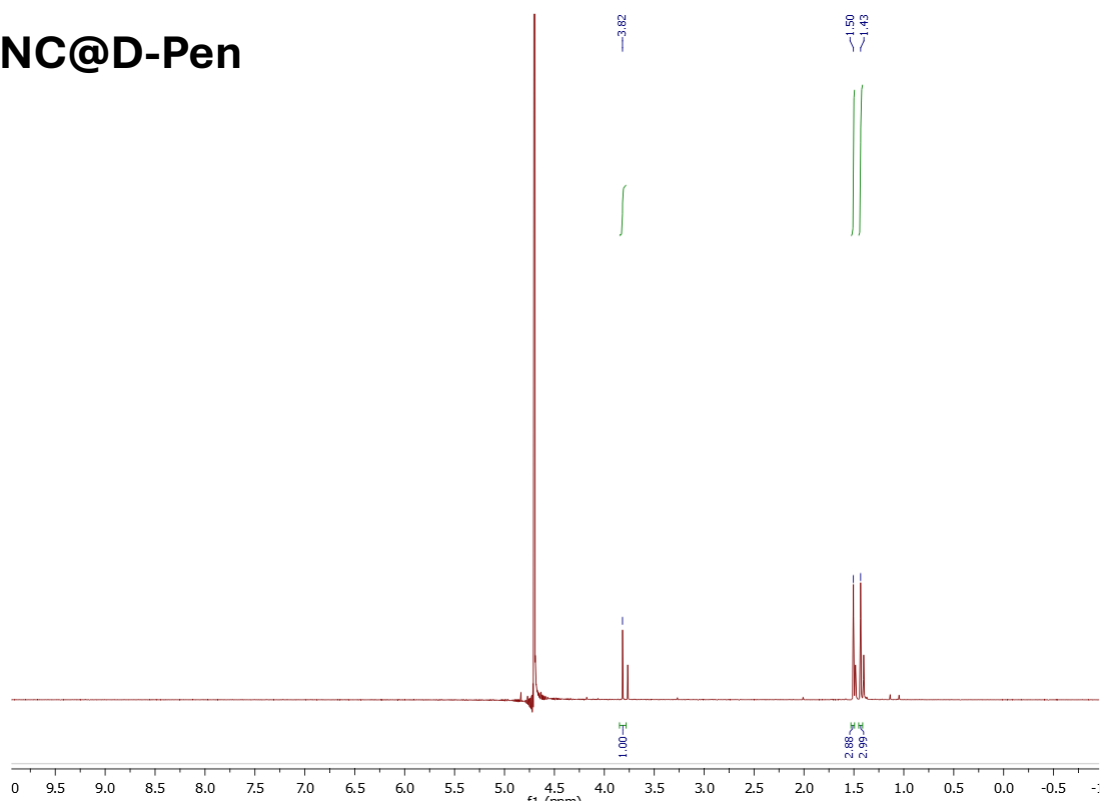


**Figure S2.** Reaction time effect on the fluorescence intensity of AuNC@D-Pen.

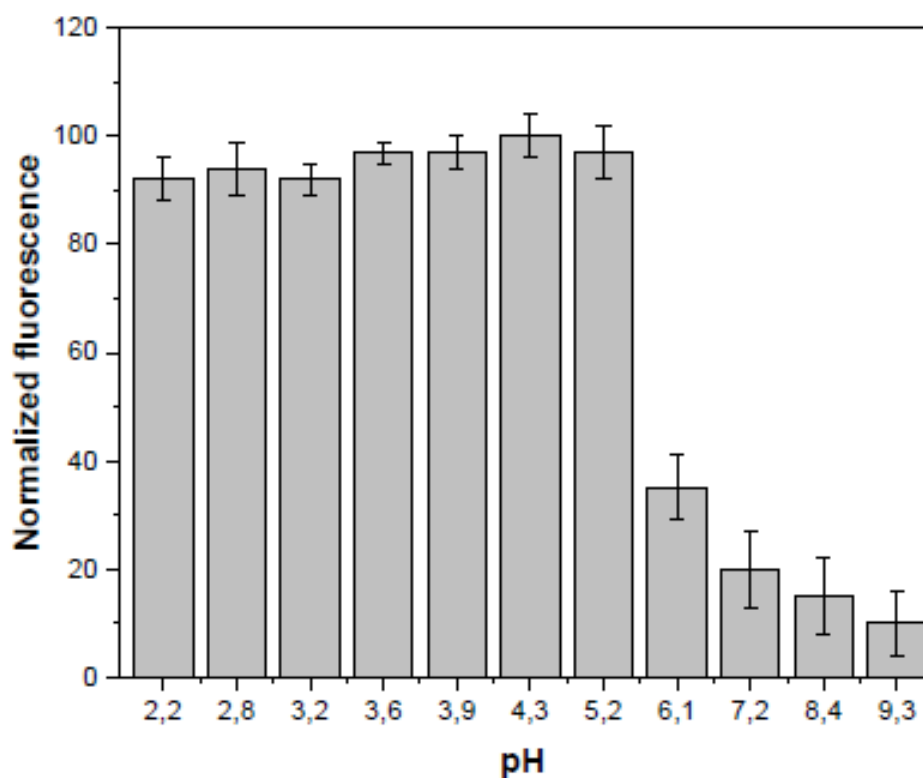
## D-Penicilamine



## AuNC@D-Pen



**Figure S3.**  $^1\text{H}$  NMR (300 Mz,  $\text{D}_2\text{O}$ ) of D-Penicilamine (top) and D-Penicilamine and  $\text{HAuCl}_4$  after 2 h (bottom).



**Figure S4.** pH effect on the fluorescence intensity of AuNC@D-Pen.

## 1.5. Computational details.

DFT calculations were performed at the B3LYP-D3/def2TZVP (SDD for Au) level of theory using Gaussian 16. To this end, a model consisting of 18 gold atoms and 14 D-penicillamine ligands was modeled.

### 1.5.1. Cartesian coordinates of optimized structure

79	0.241805000	-0.673956000	1.653161000	79	2.863827000	1.069737000	2.438293000
79	-2.257242000	0.928007000	0.383311000	79	-4.818733000	-1.916877000	-0.812376000
79	0.691109000	1.641077000	0.107229000	79	-4.471415000	1.002174000	-2.713708000
79	1.100526000	-1.004837000	-1.055552000	79	5.813920000	-0.902296000	0.962772000
79	-2.986748000	-1.780430000	2.528374000	16	2.263750000	-1.779664000	-3.205028000
79	-1.749724000	-1.647886000	-0.531926000	16	-1.122076000	-0.917789000	3.701225000
79	-1.198754000	0.578617000	-2.167480000	16	-1.213742000	-4.082869000	-0.727348000
79	-0.604419000	2.634307000	2.807478000	16	-2.885280000	2.051472000	2.493058000
79	0.601400000	-3.997344000	0.782606000	16	0.291046000	3.856484000	-0.957498000
79	-1.986206000	3.537804000	-1.542843000	16	2.478882000	-3.778151000	2.193837000
79	2.909327000	-1.607299000	1.075347000	16	-0.324223000	1.859147000	-4.138656000
79	3.413898000	0.671608000	-0.439024000	16	4.095705000	2.018573000	-2.424664000
79	1.896376000	1.912711000	-3.318935000	16	1.689572000	3.183118000	3.068675000
79	4.363176000	-1.869320000	-2.112081000	16	-4.698185000	-2.869983000	1.337053000

16	-4.282281000	3.254226000	-2.057391000	1	-6.121876000	-3.570477000	-4.289933000
16	6.445330000	-2.086301000	-1.003780000	1	-2.467445000	1.463233000	-6.063954000
16	5.012598000	-0.178732000	3.063970000	1	2.660749000	4.509875000	-2.925464000
16	-4.837024000	-1.311839000	-3.109878000	1	0.736046000	-3.088553000	-5.635215000
6	4.498627000	3.848486000	-2.015436000	6	5.841173000	-6.195584000	2.987617000
6	3.726661000	4.731188000	-3.048235000	8	5.723239000	-7.297782000	2.500973000
6	7.812480000	-1.175277000	-1.972229000	8	6.985708000	-5.760667000	3.523208000
6	7.738412000	-1.722185000	-3.432942000	6	7.298457000	3.316839000	4.052169000
6	3.689845000	-5.220662000	1.898634000	8	7.641127000	3.256407000	5.210637000
6	4.687706000	-5.148428000	3.102870000	8	7.691782000	4.310402000	3.243467000
6	6.309740000	0.899193000	3.962943000	6	8.808034000	-1.033925000	-4.319852000
6	6.307034000	2.332838000	3.359871000	8	8.672848000	0.081096000	-4.769536000
6	1.927793000	-3.539646000	-3.880831000	8	9.898988000	-1.781006000	-4.527224000
6	0.565662000	-3.511538000	-4.642350000	6	-4.880349000	-6.003838000	-0.851819000
6	-0.323719000	1.218586000	-5.937983000	8	-5.624609000	-5.833324000	-1.867644000
6	-1.750723000	0.649129000	-6.226426000	8	-5.173537000	-6.199109000	0.334828000
6	-6.591424000	-1.496248000	-3.878570000	6	1.566588000	1.755914000	6.250665000
6	-6.953814000	-3.016170000	-3.838412000	8	1.137257000	1.348937000	7.451324000
6	-4.912933000	4.451115000	-3.387378000	8	2.263274000	1.057570000	5.545192000
6	-4.953256000	5.872394000	-2.709138000	6	-6.165800000	4.423366000	1.299124000
6	-3.968064000	3.626201000	2.380915000	8	-5.975528000	5.300199000	0.478995000
6	-5.205396000	3.227075000	1.517728000	8	-7.249765000	4.391343000	2.077185000
6	-6.366430000	-2.877827000	2.247543000	6	-7.456007000	-4.047252000	4.284881000
6	-6.140768000	-3.836009000	3.462184000	8	-7.684446000	-5.327135000	4.554188000
6	0.323702000	5.175433000	0.340446000	8	-8.171857000	-3.141381000	4.657938000
1	1.382513000	5.356211000	0.545330000	6	-1.055974000	-2.749801000	7.505627000
1	-0.130568000	4.796604000	1.252970000	8	-2.073463000	-2.756263000	8.373719000
6	-0.376059000	6.470030000	-0.074558000	8	-0.060811000	-3.419163000	7.666279000
6	-0.562438000	-2.205998000	5.003506000	6	-0.388298000	7.433442000	1.145037000
6	-1.283979000	-1.758896000	6.320595000	8	-0.162923000	8.695757000	0.812810000
6	-2.503875000	-5.265047000	-0.095553000	8	-0.614510000	7.057171000	2.281128000
1	-3.162759000	-4.742163000	0.593080000	6	-3.617540000	6.659206000	-2.718652000
1	-1.969919000	-6.032103000	0.464046000	8	-3.790310000	7.973891000	-2.775823000
6	-3.338399000	-5.936217000	-1.209767000	8	-2.509907000	6.155904000	-2.638516000
6	1.842449000	3.940898000	4.810640000	6	-8.188833000	-3.301623000	-4.756323000
6	1.018384000	3.148187000	5.866056000	8	-8.138451000	-3.263804000	-5.964990000
1	4.130631000	-5.434966000	4.003768000	8	-9.290280000	-3.593766000	-4.066588000
1	5.314931000	2.759299000	3.545693000	6	-1.895397000	0.263787000	-7.713780000
1	6.765127000	-1.423696000	-3.841142000	8	-1.723120000	-1.045885000	-7.988042000
1	-2.977423000	-6.952713000	-1.386968000	8	-2.141949000	1.068780000	-8.577223000
1	0.040338000	2.939331000	5.409748000	6	3.882929000	6.236728000	-2.739768000
1	-4.836857000	2.960523000	0.524017000	8	3.210184000	6.824383000	-1.916164000
1	-5.454355000	-3.331240000	4.152900000	8	4.830732000	6.838488000	-3.460727000
1	-0.810425000	-0.823465000	6.644376000	6	-0.088818000	-4.954284000	-4.822258000
1	-1.429398000	6.251786000	-0.282014000	8	-1.293754000	-4.991260000	-4.366454000
1	-5.188187000	5.719685000	-1.647315000	8	0.579288000	-5.829739000	-5.354266000

7	4.216769000	4.517989000	-4.421471000	1	5.884942000	-0.156438000	5.809132000
1	5.131331000	6.153893000	-4.107942000	1	6.486873000	1.505044000	6.027482000
1	3.488844000	4.749022000	-5.093462000	6	7.679834000	0.221128000	3.837354000
7	-5.979700000	6.796426000	-3.223937000	1	7.635683000	-0.810093000	4.196296000
1	-6.883002000	6.637714000	-2.791325000	1	8.048651000	0.194861000	2.806422000
1	-6.091084000	6.734129000	-4.232416000	1	8.400068000	0.768443000	4.454321000
7	-5.991174000	2.148537000	2.147957000	6	4.070791000	4.173673000	-0.583355000
1	-7.189718000	3.523428000	2.550824000	1	2.999642000	4.033162000	-0.445592000
1	-5.394882000	1.521010000	2.681264000	1	4.597931000	3.524484000	0.117650000
7	-5.594492000	-5.163544000	3.143878000	1	4.318875000	5.209761000	-0.336253000
1	-6.918978000	-5.797114000	4.116272000	6	6.024037000	3.984868000	-2.155328000
1	-4.612609000	-5.233536000	3.385150000	1	6.383523000	3.718959000	-3.151329000
7	6.532023000	2.438846000	1.911085000	1	6.322401000	5.018684000	-1.947416000
1	7.314364000	4.094660000	2.359549000	1	6.526972000	3.340892000	-1.429497000
1	5.715079000	2.118360000	1.400928000	6	9.135571000	-1.541940000	-1.278948000
7	7.971722000	-3.175290000	-3.494675000	1	9.138038000	-1.180330000	-0.247003000
1	9.695041000	-2.656508000	-4.124022000	1	9.315275000	-2.619185000	-1.261446000
1	7.505516000	-3.575721000	-4.304534000	1	9.969612000	-1.065744000	-1.805252000
7	-3.308925000	-5.257754000	-2.549570000	6	7.581125000	0.336421000	-1.933330000
1	-3.292638000	-4.241693000	-2.437932000	1	8.376808000	0.852312000	-2.476041000
1	-4.282507000	-5.501458000	-2.882991000	1	6.627917000	0.612307000	-2.387612000
7	-7.286670000	-3.512927000	-2.497977000	1	7.582881000	0.681174000	-0.894843000
1	-6.970623000	-2.889754000	-1.761074000	6	2.857505000	-6.511022000	1.972121000
1	-6.855905000	-4.423433000	-2.302729000	1	2.150603000	-6.557906000	1.138512000
7	-2.055487000	-0.530078000	-5.390757000	1	3.532449000	-7.367977000	1.923626000
1	-1.998639000	-0.239269000	-4.411231000	1	2.289665000	-6.565960000	2.906273000
1	-1.587641000	-1.517181000	-7.147050000	6	4.399544000	-5.105424000	0.549633000
7	-0.518266000	-2.705866000	-3.991110000	1	5.047653000	-5.975886000	0.408765000
1	-0.256726000	-2.288374000	-3.093519000	1	3.670022000	-5.083089000	-0.262067000
1	-1.237169000	-3.500084000	-3.879561000	1	5.011123000	-4.201918000	0.475649000
7	5.305163000	-3.845743000	3.388828000	6	1.973415000	-4.581820000	-2.762510000
1	5.539680000	-3.336795000	2.539292000	1	1.204035000	-4.419426000	-2.007121000
1	6.789828000	-4.832627000	3.808543000	1	1.833643000	-5.577509000	-3.193191000
1	7.606013000	-3.652353000	-2.674594000	1	2.947589000	-4.545786000	-2.269902000
1	4.693792000	-3.240987000	3.928426000	6	3.022599000	-3.838611000	-4.918910000
1	7.313998000	1.857591000	1.617278000	1	3.995018000	-3.941982000	-4.429550000
1	-4.774627000	8.086390000	-2.886987000	1	2.765726000	-4.778751000	-5.417246000
1	4.460203000	3.542836000	-4.578474000	1	3.094645000	-3.047354000	-5.671372000
1	-2.530121000	-5.502440000	-3.196660000	6	-6.351741000	3.979352000	-3.671809000
1	-0.908188000	-1.966484000	-4.599123000	1	-6.812173000	4.588706000	-4.456066000
1	-3.033706000	-0.793192000	-5.511840000	1	-6.339348000	2.945519000	-4.026800000
1	-6.442878000	1.581328000	1.435237000	1	-6.981320000	4.027085000	-2.778348000
1	-5.670346000	-5.433362000	2.159997000	6	-4.076418000	4.404613000	-4.669207000
1	-8.969533000	-3.658609000	-3.125795000	1	-3.024617000	4.616251000	-4.475479000
6	5.836560000	0.867431000	5.426533000	1	-4.158717000	3.420736000	-5.139706000
1	4.801979000	1.202077000	5.528448000	1	-4.452858000	5.145300000	-5.388323000

6	-4.356894000	3.963151000	3.831538000	1	-6.816398000	-0.813549000	1.802963000
1	-4.869159000	3.140473000	4.335639000	1	-7.687676000	-1.472450000	3.217866000
1	-3.464672000	4.217391000	4.409585000	7	0.732260000	3.855908000	7.128566000
1	-5.020718000	4.835221000	3.839994000	1	1.561323000	4.315226000	7.499449000
6	-3.202397000	4.794345000	1.763595000	1	0.685673000	2.138965000	7.839090000
1	-2.325411000	5.043689000	2.363962000	1	0.018173000	4.565698000	7.004688000
1	-2.876611000	4.560746000	0.746724000	7	-2.730432000	-1.490697000	6.248426000
1	-3.841102000	5.680302000	1.716485000	1	-2.909134000	-0.587272000	5.821150000
6	0.788283000	0.197429000	-6.192377000	1	-2.750902000	-2.155623000	7.981075000
1	0.724620000	-0.666125000	-5.531312000	6	-0.945181000	-3.629372000	4.588095000
1	0.753900000	-0.147439000	-7.232517000	1	-0.507320000	-3.880284000	3.619133000
1	1.764901000	0.656524000	-6.021393000	1	-0.563269000	-4.332712000	5.334253000
6	-0.079147000	2.483057000	-6.780759000	1	-2.030028000	-3.760456000	4.506589000
1	0.880495000	2.928669000	-6.505696000	6	0.954140000	-2.058121000	5.162073000
1	-0.056118000	2.231846000	-7.844517000	1	1.241429000	-1.035772000	5.416237000
1	-0.862067000	3.229724000	-6.627066000	1	1.284736000	-2.720250000	5.965276000
6	-6.409830000	-0.985985000	-5.318170000	1	1.463522000	-2.339991000	4.240410000
1	-5.707341000	-1.605274000	-5.882386000	1	-3.204440000	-2.183030000	5.672471000
1	-7.360972000	-1.017644000	-5.851816000	6	3.326702000	4.047614000	5.174901000
1	-6.047115000	0.046118000	-5.302557000	1	3.444967000	4.557069000	6.139906000
6	-7.624443000	-0.637844000	-3.138861000	1	3.796148000	3.067743000	5.249408000
1	-8.611014000	-0.787555000	-3.591865000	1	3.859583000	4.649513000	4.433202000
1	-7.700562000	-0.883186000	-2.077105000	6	1.245815000	5.351336000	4.644626000
1	-7.366730000	0.421470000	-3.218441000	1	0.183045000	5.328150000	4.389042000
6	-7.415682000	-3.446889000	1.281225000	1	1.372637000	5.930388000	5.566258000
1	-7.584839000	-2.754875000	0.451696000	1	1.753496000	5.892180000	3.843893000
1	-7.128252000	-4.416689000	0.866551000	7	0.157920000	7.183189000	-1.235894000
1	-8.366058000	-3.555008000	1.814453000	1	0.017224000	8.649336000	-0.166426000
6	-6.735942000	-1.457491000	2.684156000	1	-0.409693000	7.002731000	-2.058944000
1	-5.976425000	-1.040227000	3.352937000	1	1.137624000	6.976731000	-1.427173000

