Supporting information

Detection and Quantification of ATP in Human Blood Serum

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General

Synthesis of S1 and S2 was performed according to the literature procedures. Starting materials are used without further purification. Standard laboratory techniques was performed in all the synthesis. Spectrometer at 25 °C. Mass spectrometry studies were performed using Shimadzu LCMS-8030 liquid chromatograph mass spectrometer (ESI) or Shimadzu Axima Performance MALDI-TOF mass spectrometer. All the optical measurements were performed in acetonitrile. Acetonitrile is dried over 4Å molecular sieves. Optically dilute solutions (0.1 A) were used for all photophysical experiments. Fluorescence emission spectra were acquired using an Edinburgh single photon counting spectrofluorimeter (FLSP 920).

Isotherms were constructed using emission maximum of each titration. Absorption spectra were recorded using a Hitachi U-3010 spectrophotometer. All the optical measurements were performed using a quartz cuvette with a path length of 1 cm at room temperature. The absolute quantum yields were measured using a Hamamatsu Quantaurus absolute quantum yield spectrometer QY-C11347.

Preparation of Polymer chips[1]

The multi-well 10 x 21 (sub-microliter) glass slides were fabricated by ultrasonic drilling of microscope slides (well diameter: 1000 ± 10 μm, depth: 250 ± 10 μm). Sensor solutions in polymer solution (4% poly (ether-urethane) in THF w/w) were prepared. In a typical array, 200 nL of sensor-polymer solutions were pipetted into each well of the multi-well glass slides and dried. Then, water (400 nL) was pipetted into each well and dried to form hydrated gel matrix. Finally, analytes were added as aqueous solutions into each well and the chip was dried at room temperature for 1 hr. Images from the sensor array were recorded using a Kodak Image Station 440CF (for preliminary experiments) and a Kodak Image Station 4000MM PRO (for qualitative and quantitative experiments). After acquiring the images, the integrated (nonzero) gray pixel

value (n) is calculated for each well in each channel. Images of the sensor chip were recorded before (b) and after (a) the addition of an analyte. The final responses (R) were evaluated as indicated in the following equation:

$$R = \sum_{n} \frac{a_n}{b_n} - 1$$

Thus obtained data for qualitative analysis were then analyzed using Linear Discriminant Analysis (LDA). Qualitative analysis was performed using Support Vector Machine (SVM) algorithm.

Synthesis of S1 and S2

Synthesis of S1 and S2 were reported previously.[2]

S1

(3S,7S,11S)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7,11-triyl)tris(3-(4-dimethylamino)naphthalen-1-yl)thiourea

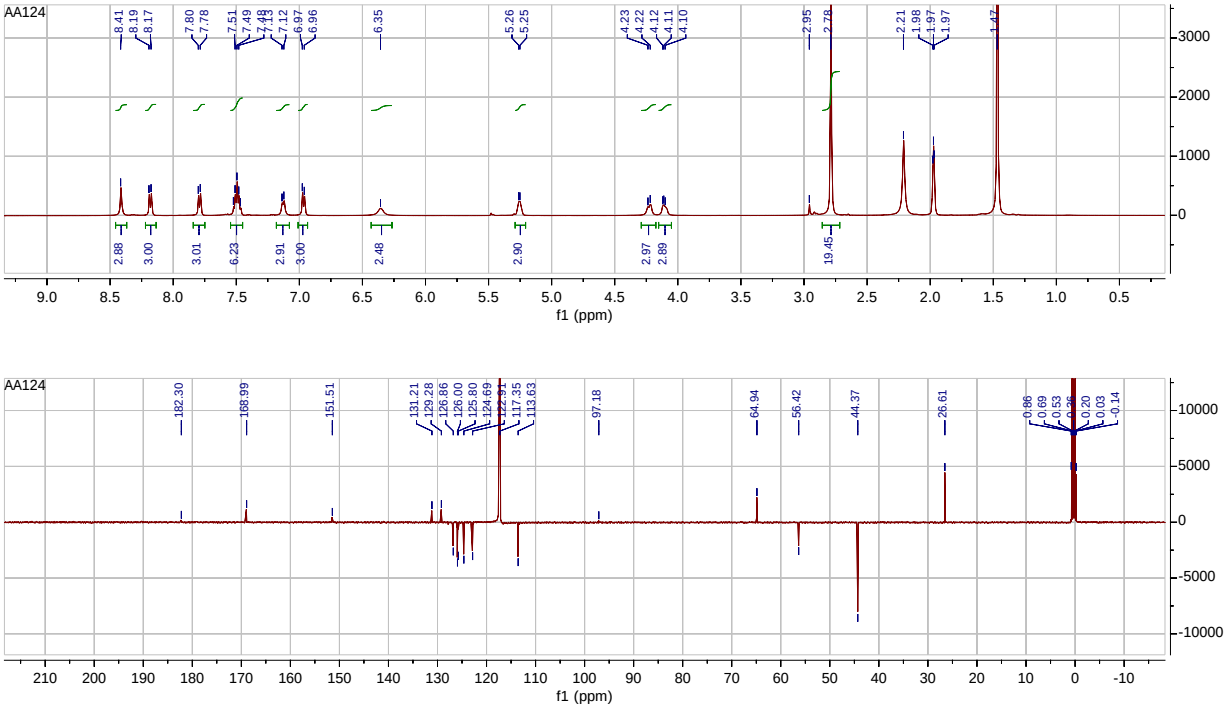
^{1}H NMR (500 MHz, CD_3CN) δ 8.41 (s, 1H), 8.18 (d, J = 8.3 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.54 – 7.45 (m, 2H), 7.13 (d, J = 7.3 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 6.35 (s, 1H), 5.25 (d, J = 4.8 Hz, 1H), 4.22 (d, J = 9.0 Hz, 1H), 4.15 – 4.05 (m, 1H), 2.78 (s, 7H). ^{13}C NMR (126 MHz, CD_3CN) δ 168.99, 151.51, 131.21, 129.28, 126.86, 126.00, 125.80, 124.69, 122.91, 117.35, 113.63, 64.94, 56.42, 44.37, 26.61. MS (ESI) m/z: 946.38 $[M]^+$.

S2

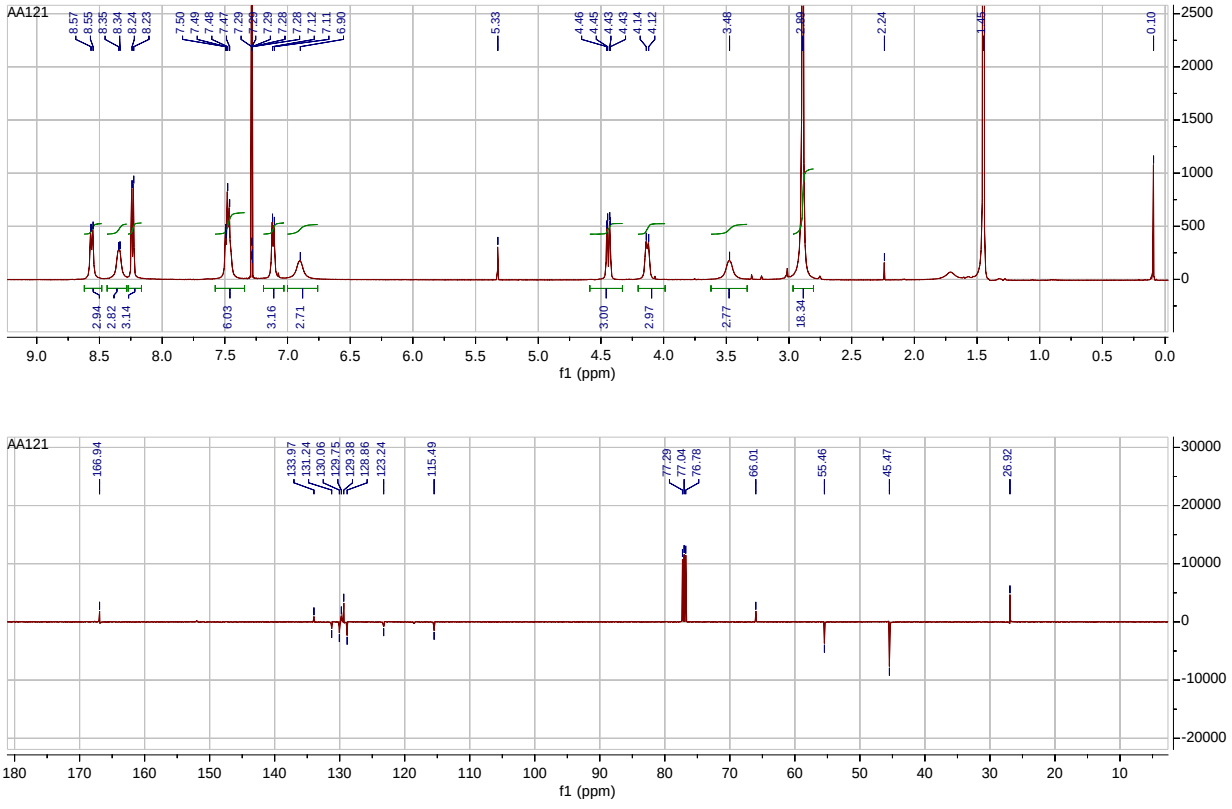
(3S,7S,11S)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7,11-triyl)tris(5-(dimethylamino)naphthalene-1-sulfonamide

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (d, J = 8.3 Hz, 1H), 8.35 (d, J = 5.8 Hz, 1H), 8.24 (d, J = 6.9 Hz, 1H), 7.48 (dd, J = 10.8, 4.7 Hz, 2H), 7.12 (d, J = 7.3 Hz, 1H), 6.90 (s, 1H), 4.44 (dd, J = 11.3, 3.0 Hz, 1H), 4.13 (d, J = 9.6 Hz, 1H), 3.48 (s, 1H), 2.89 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.94, 133.97, 131.24, 130.06, 129.75, 129.38, 128.86, 123.24, 115.49, 77.29, 77.04, 76.78, 66.01, 55.46, 45.47, 26.92. MS (ESI) m/z: 961.31 $[M]^+$.

^{1}H and ^{13}C-APT NMR Spectra of S1



^{1}H and ^{13}C-APT NMR Spectra of S2



MS Spectra of S1 and S2

EI DIP mass spectra were recorded using a Shimadzu QP5050A.

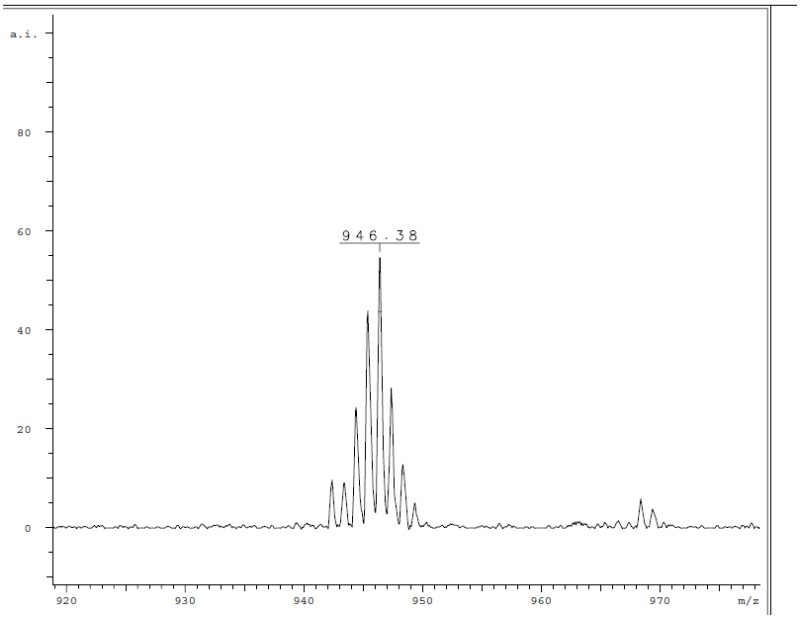


Figure: MS Spectrum of **S1**.

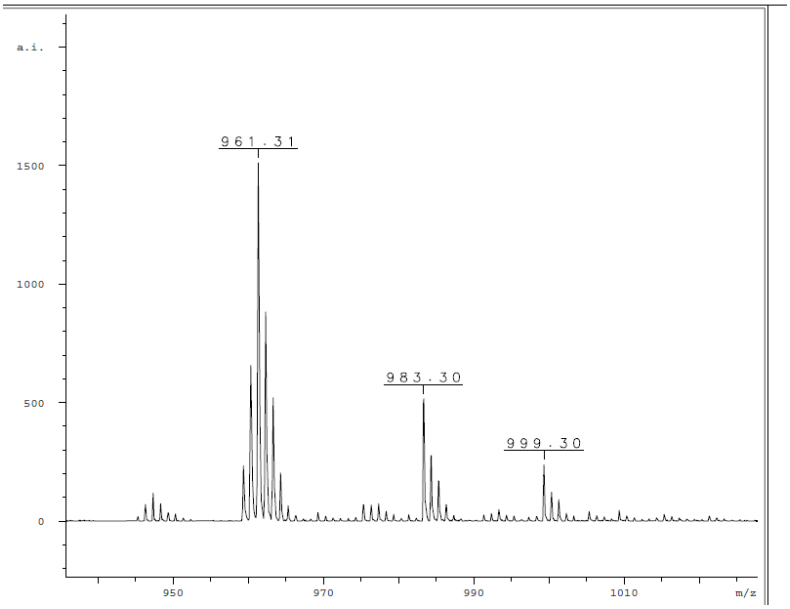
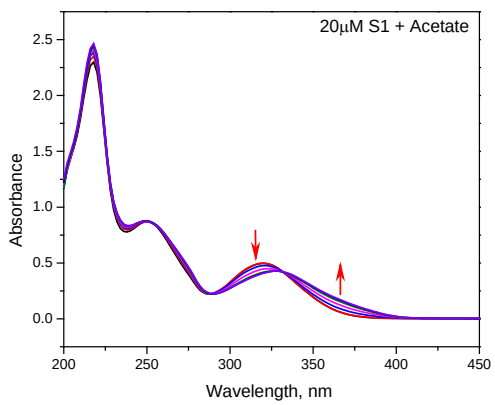
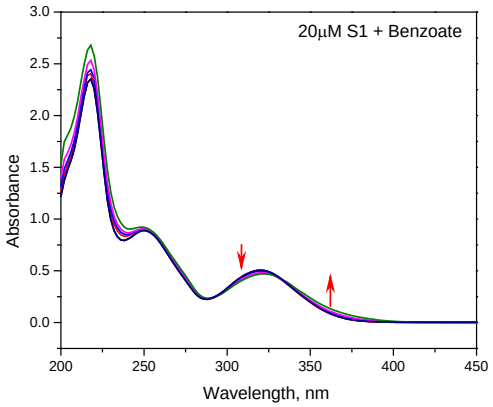
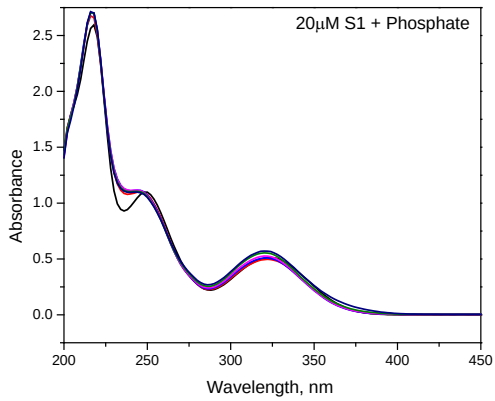


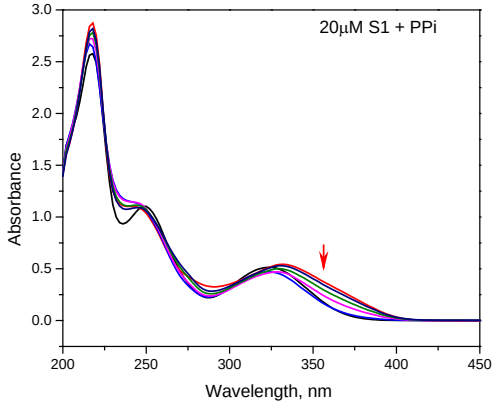
Figure: MS Spectrum of **S2**.

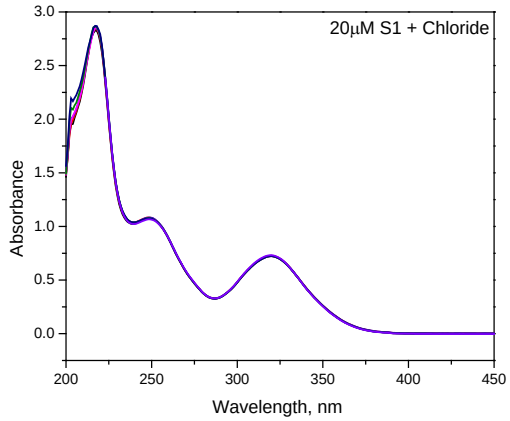
UV-Vis absorption titrations of S1 and S2

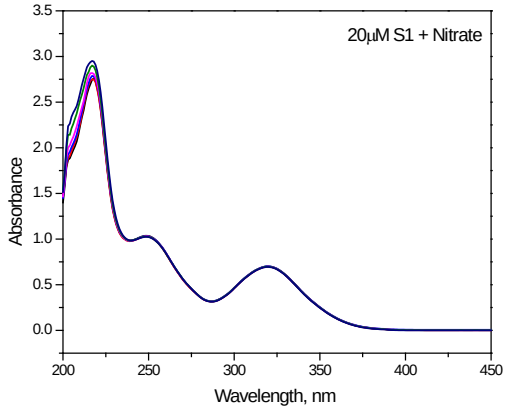


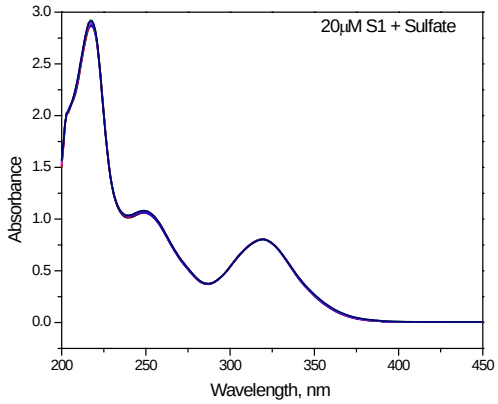


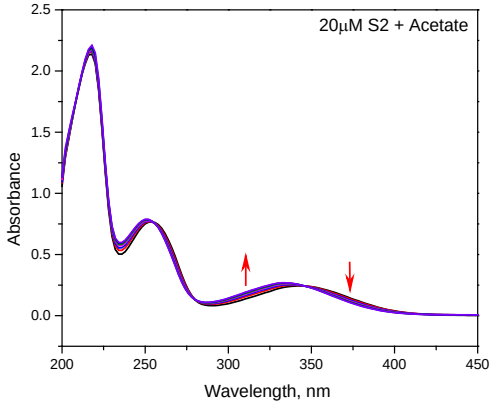


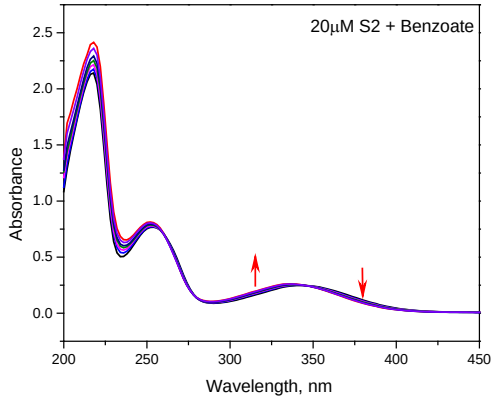


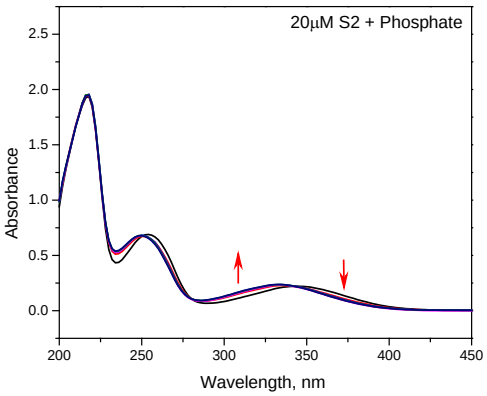


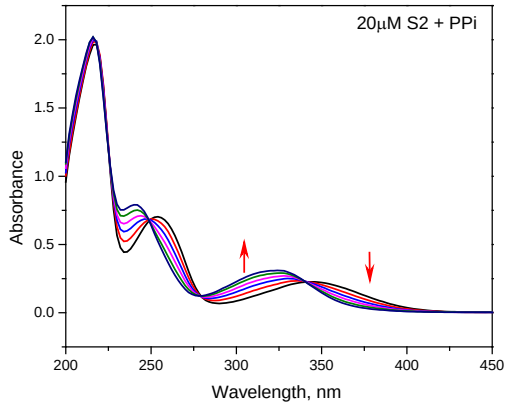


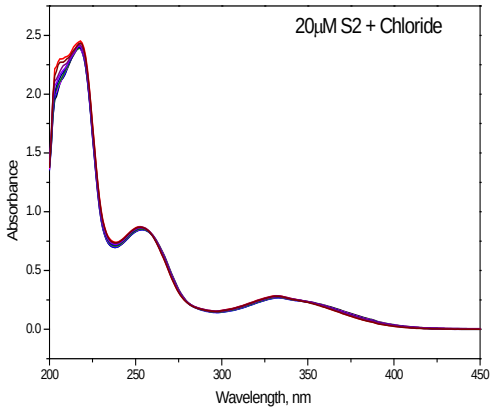

20μM S1 + Sulfate
Absorbance
Wavelength, nm

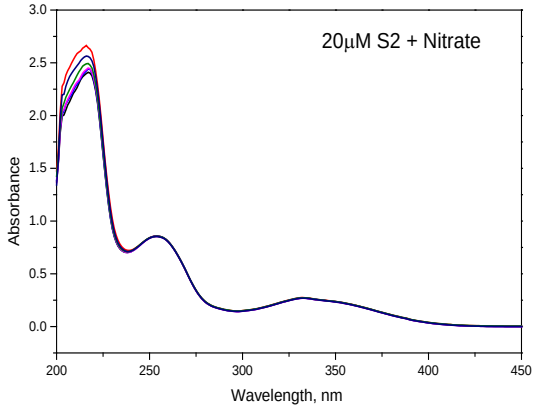

20μM S2 + Acetate
Absorbance
Wavelength, nm

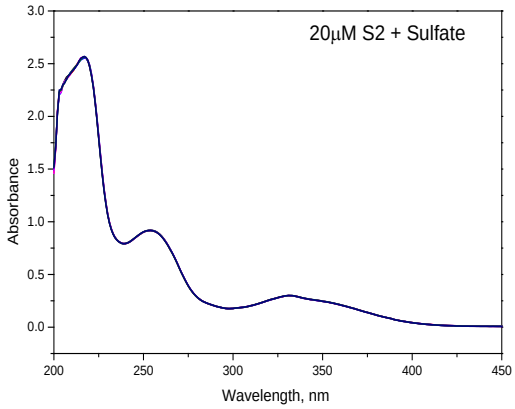

20μM S2 + Benzoate
Absorbance
Wavelength, nm


20μM S2 + Phosphate
Absorbance
Wavelength, nm

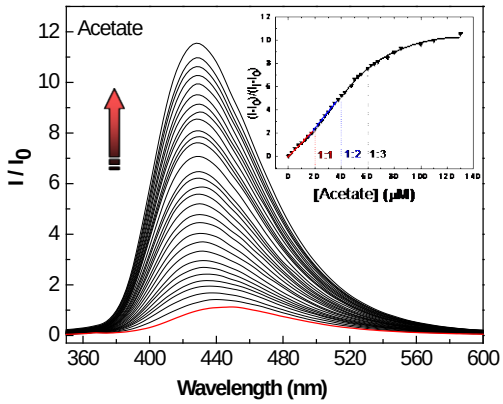

20μM S2 + PPi
Absorbance
Wavelength, nm

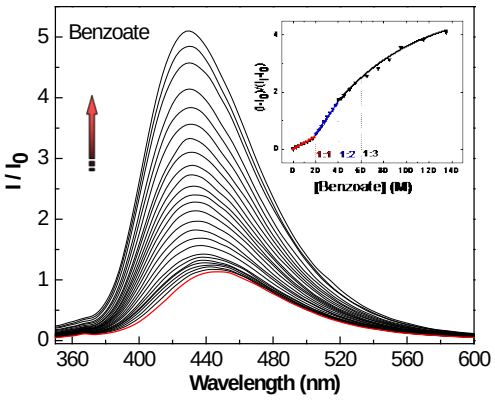

20μM S2 + Chloride
Absorbance
Wavelength, nm

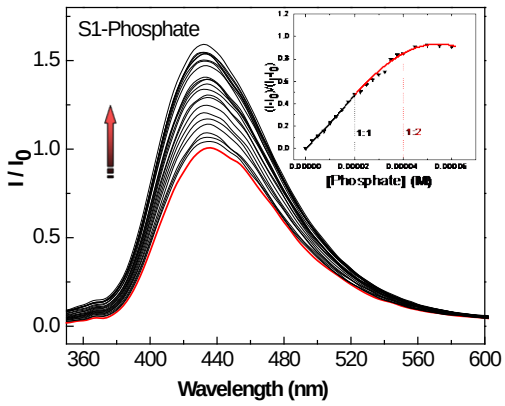


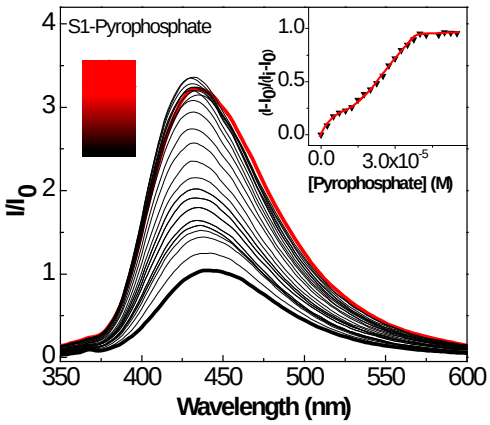


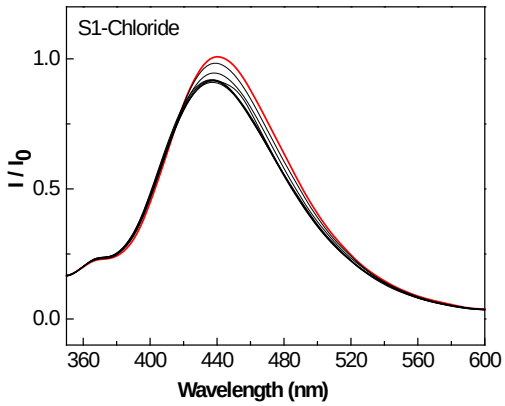
Fluorescence titrations of S1 and S2

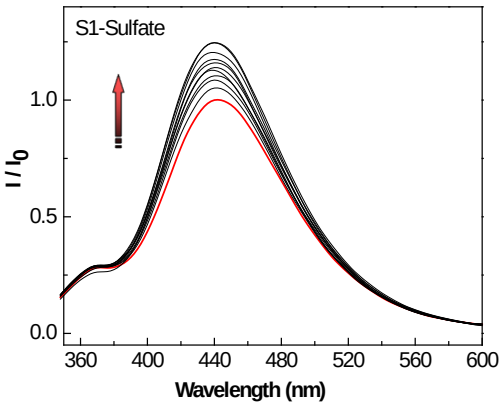


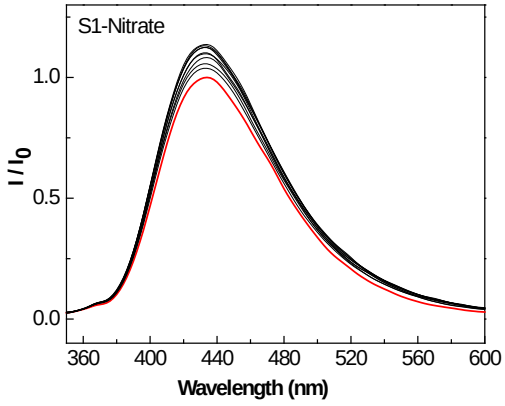


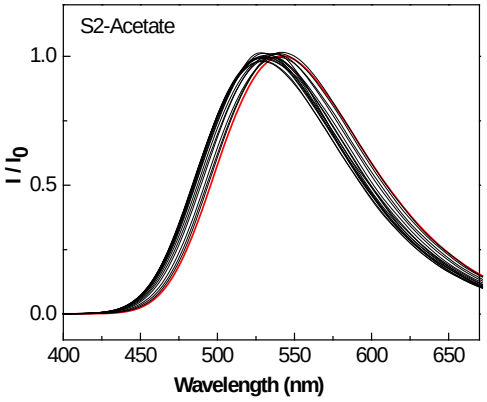


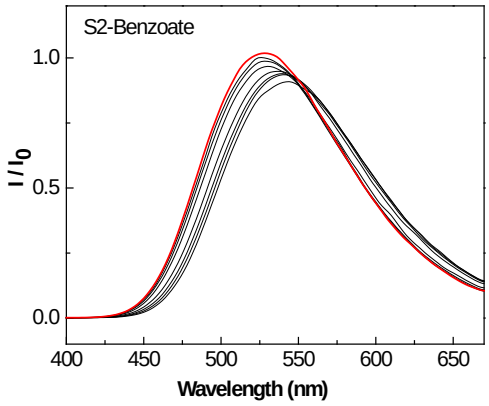


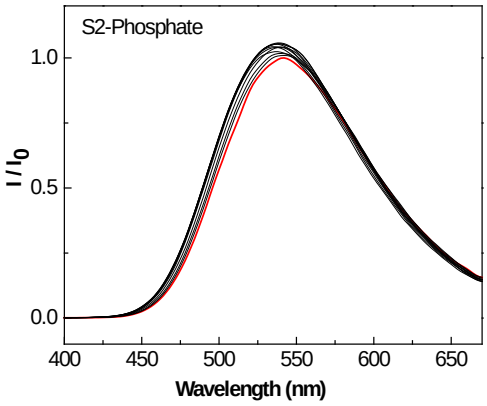

S1-Chloride
I / I0
Wavelength (nm)

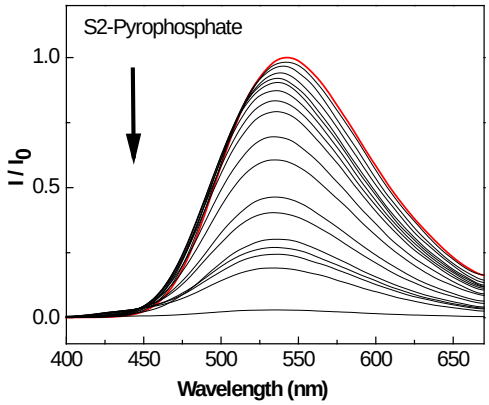

S1-Sulfate
I / I0
Wavelength (nm)

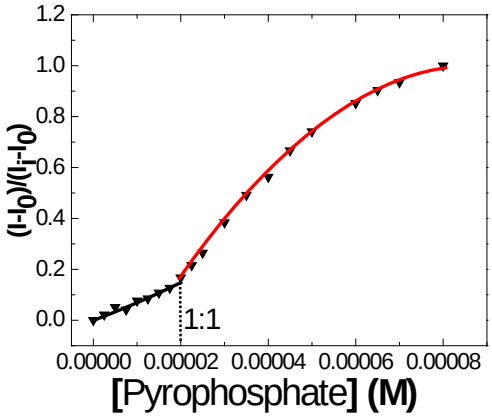

S1-Nitrate
I / I0
Wavelength (nm)

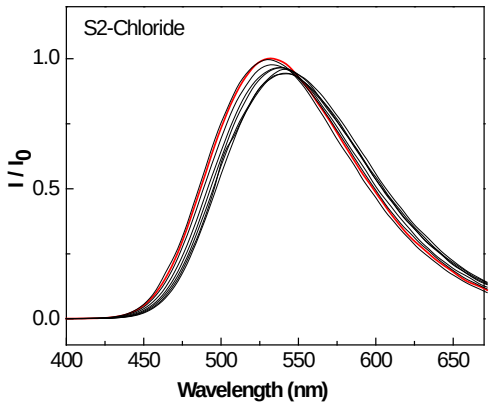

S2-Acetate
I / I0
Wavelength (nm)

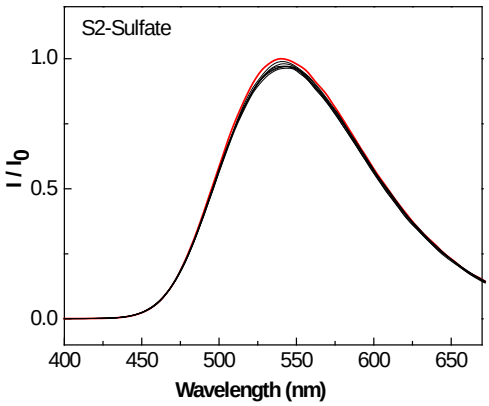

S2-Benzoate
I / I0
Wavelength (nm)

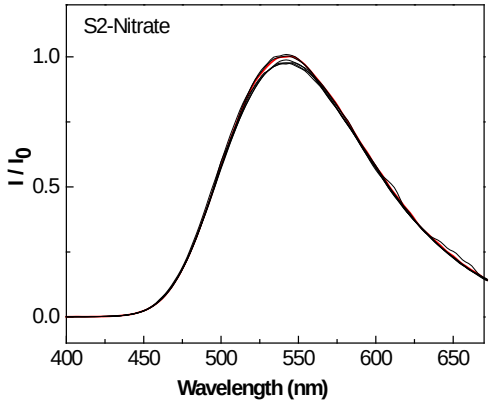

S2-Phosphate
I / I0
Wavelength (nm)


S2-Pyrophosphate
I / I0
Wavelength (nm)


(I-I0)/(Ii-I0)
1:1
[Pyrophosphate] (M)


S2-Chloride
I / I0
Wavelength (nm)

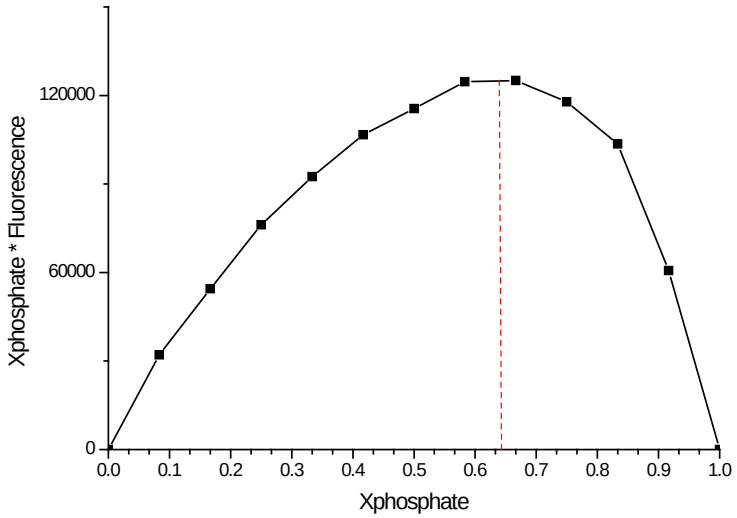

S2-Sulfate
I / I0
Wavelength (nm)


S2-Nitrate
I / I0
Wavelength (nm)

Job`s plot experiments

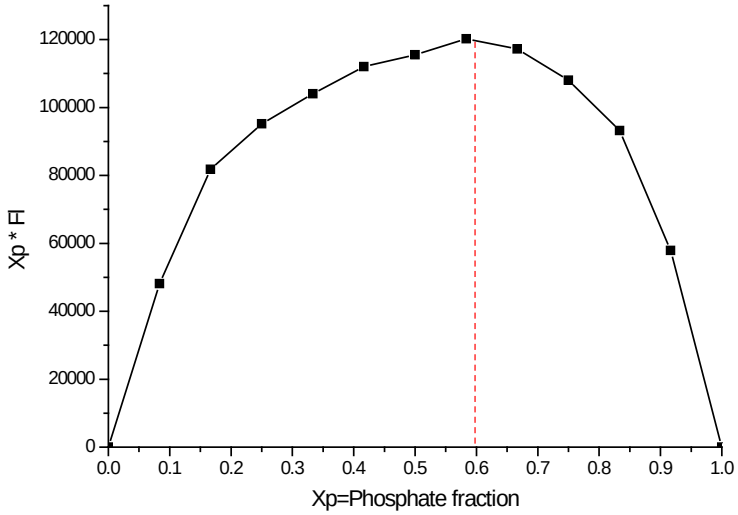
S1– Phosphate Job`s plot in Acetonitrile

Result: **1:2 Binding**



S2– Phosphate Job`s plot in Acetonitrile

Result = **1:2 Binding**



Qualitative analysis

Linear discriminant analysis (LDA)

Table. The jackknifed classification matrix of qualitative analysis of 10 analytes and a control using S1 and S2 in hydrogel matrix.

Jackknifed Classification Matrix	ADP	AMP	ATP	Acetate	Benzoate	Chloride	Nitrate	Phosphate	Pyrophosphate	Serum	Sulfate	%correct
ADP	10	0	0	0	0	0	0	0	0	0	0	100
AMP	0	10	0	0	0	0	0	0	0	0	0	100
ATP	0	0	10	0	0	0	0	0	0	0	0	100
Acetate	0	0	0	10	0	0	0	0	0	0	0	100
Benzoate	0	0	0	0	10	0	0	0	0	0	0	100
Chloride	0	0	0	0	0	10	0	0	0	0	0	100
Nitrate	0	0	0	0	0	0	10	0	0	0	0	100
Phosphate	0	0	0	0	0	0	0	10	0	0	0	100
Pyrophosphate	0	0	0	0	0	0	0	0	10	0	0	100
Serum	0	0	0	0	0	0	0	0	0	10	0	100
Sulfate	0	0	0	0	0	0	0	0	0	0	10	100
Total	10	10	10	10	10	10	10	10	10	10	10	100

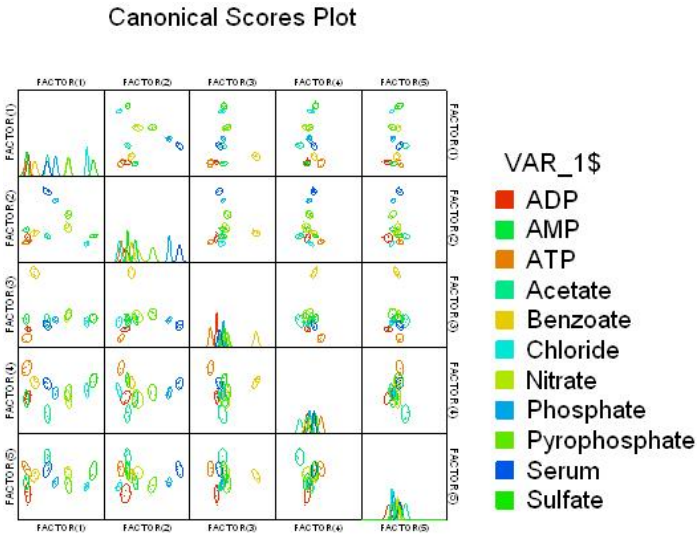


Figure. The canonical scores plot of qualitative analysis of 10 analytes and a control using S1 and S2 in hydrogel matrix.

Semi-quantitative Analysis

Semi-quantitative assay for ATP-Na in water

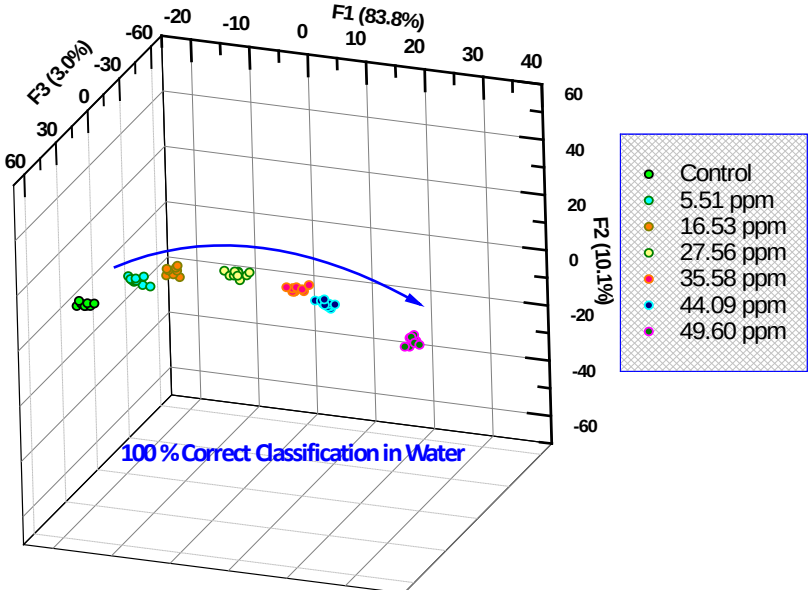


Figure. Linear discriminant analysis (LDA) of ATP-Na in hydrogel matrix. LDA shows the trend depending on increasing concentration of ATP.

Table. The jackknifed classification matrix of Semi-quantitative analysis of ATP-sodium salt using S1 and S2 in hydrogel matrix.

Jackknifed Classification Matrix								
	10	**30**	**50**	**70**	**80**	**90**	**water**	**%correct**
10	10	0	0	0	0	0	0	100
30	0	10	0	0	0	0	0	100
50	0	0	10	0	0	0	0	100
70	0	0	0	10	0	0	0	100
80	0	0	0	0	10	0	0	100
90	0	0	0	0	0	10	0	100
water	0	0	0	0	0	0	10	100
Total	10	10	10	10	10	10	10	100

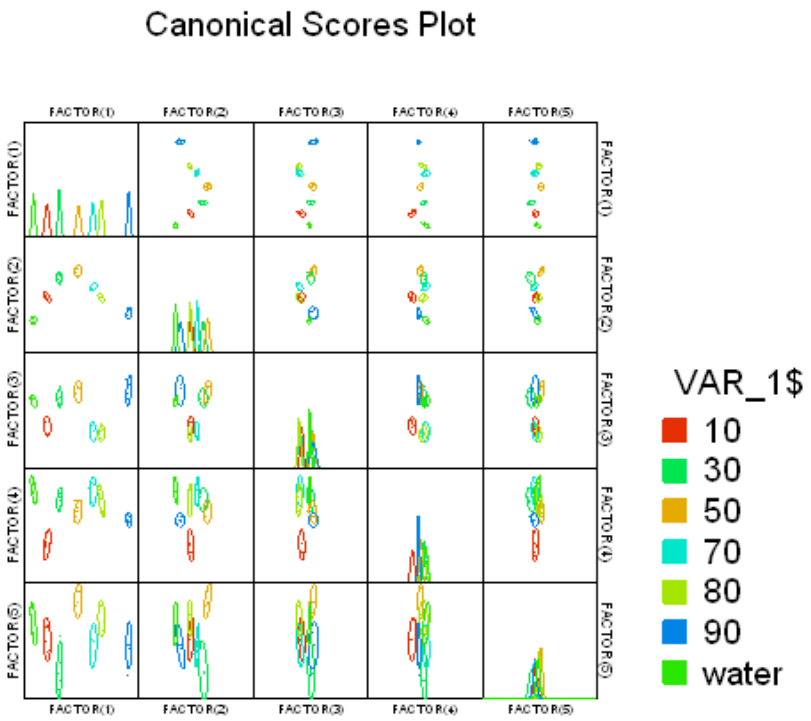


Figure. The canonical scores plot of Semi-quantitative analysis of ATP tri-sodium salt using S1 and S2 in hydrogel matrix.

Semi-quantitative assay for ATP-Na in buffer

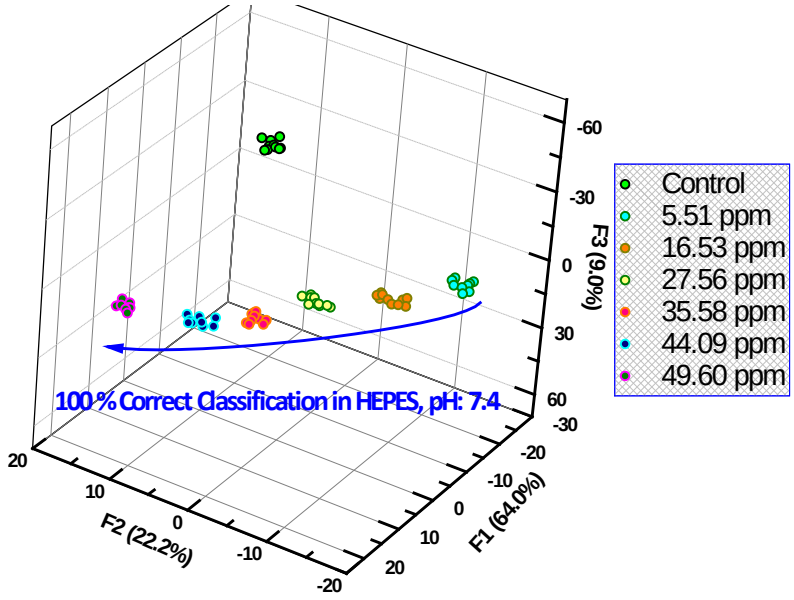


Figure. Linear discriminant analysis (LDA) of ATP-Na in hydrogel matrix. LDA shows the trend depending on increasing concentration of ATP in a buffer solution (25 mM HEPES, pH 7.4).

Table. The jackknifed classification matrix of Semi-quantitative analysis of ATP-sodium salt using S1 and S2 in hydrogel matrix.

Jackknifed Classification Matrix								
	10	30	50	70	80	90	water	%correct
10	10	0	0	0	0	0	0	100
30	0	10	0	0	0	0	0	100
50	0	0	10	0	0	0	0	100
70	0	0	0	10	0	0	0	100
80	0	0	0	0	10	0	0	100
90	0	0	0	0	0	10	0	100
water	0	0	0	0	0	0	10	100
Total	10	10	10	10	10	10	10	100

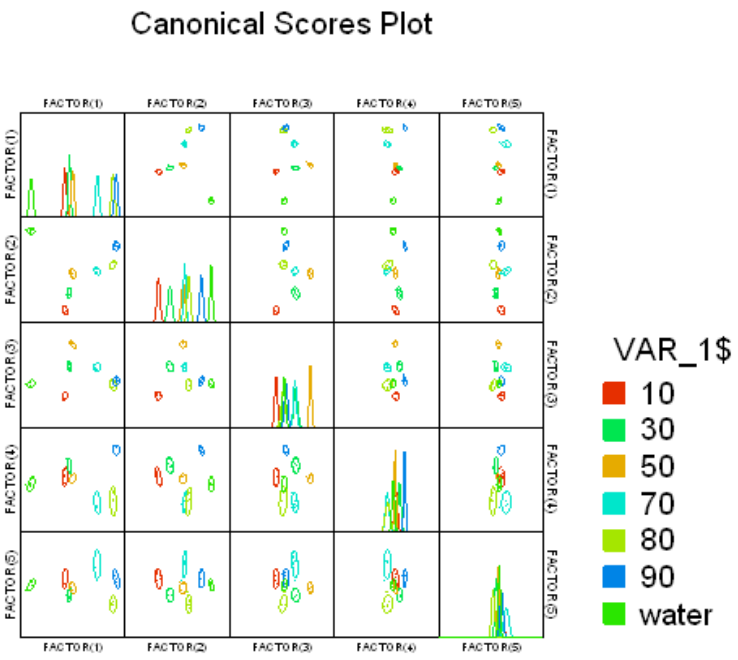


Figure. The canonical scores plot of Semi-quantitative analysis of ATP tri-sodium salt by using S1 and S2 in hydrogel matrix.

Semi-quantitative assay for ATP-Na in human blood serum

Table. The jackknifed classification matrix of Semi-quantitative analysis of ATP-sodium salt using S1 and S2 in hydrogel matrix in human blood serum.

Jackknifed Classification Matrix							
	10 uM ATP	30 uM ATP	50 uM ATP	70 uM ATP	80 uM ATP	90 uM ATP	%correct
10 uM ATP	9	0	0	0	0	0	100
30 uM ATP	0	9	0	0	0	0	100
50 uM ATP	0	0	10	0	0	0	100
70 uM ATP	0	0	0	9	0	0	100
80 uM ATP	0	0	0	0	9	0	100
90 uM ATP	0	0	0	0	0	8	100
Total	9	9	10	9	9	8	100

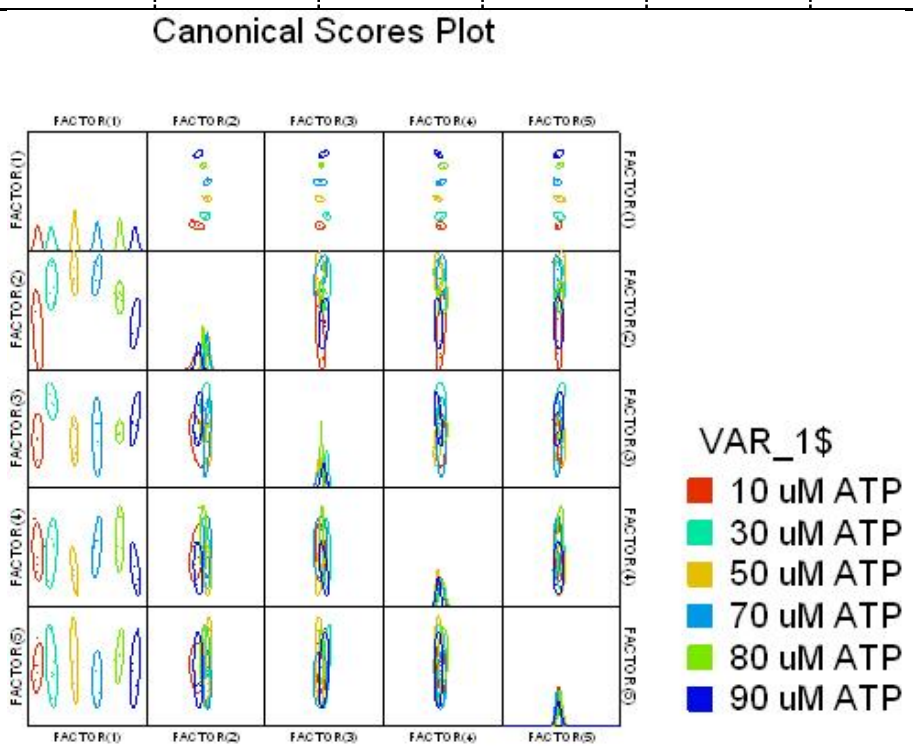


Figure. The canonical scores plot of Semi-quantitative analysis of ATP tri-sodium salt using S1 and S2 in hydrogel matrix in human blood serum.

References

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