Supplementary information for

A Simple Approach for Discrimination of Nucleotides Based on A Water-Soluble Polythiophene Derivative

Zhiyi Yao, Xueling Feng, Wenjing Hong, Chun Li* and Gaoquan Shi*

Department of Chemistry and the Key Laboratory of Bio-organic Phosphorous Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China. Fax: +86-10-6277-1149; Tel:+86-10-6279-8909; E-mail:gshi@tsinghua.edu.cn; chunli@mail.tsinghua.edu.cn.

Experimental Section

Materials. All chemicals were purchased from Aldrich and Beijing Chem. Reagents Co. (Beijing, China) and were used as received. Water-soluble polythiophene derivative, poly(3-(4-Methyl-3'-thienyloxy)propyltrimethylammonium) (PMTPA), was synthesized and purified as reported previously.[S1] Aqueous stock solutions of PMTPA and nucleotide phosphates (XNPs) were prepared in pure water. The concentrations of the nucleotides were determined by using $\varepsilon_{259\ (ATP,\ ADP,\ AMP)} = 1.54 \times 10^4\ M^{-1}\ cm^{-1}$, $\varepsilon_{262\ (UTP,\ UDP,\ UMP)} = 1.00 \times 10^4\ M^{-1}\ cm^{-1}$, $\varepsilon_{268\ (TTP,\ TDP,\ TMP)} = 9.6 \times 10^3\ M^{-1}\ cm^{-1}$, $\varepsilon_{253\ (GTP,\ GDP,\ GMP)} = 1.37 \times 10^4\ M^{-1}\ cm^{-1}$, and $\varepsilon_{272\ (CTP,\ CDP,\ CMP)} = 9.10 \times 10^3\ M^{-1}\ cm^{-1}$, respectively, in phosphate buffer (100 mM, pH 7.0).

Measurements. Absorption spectra were collected by using a Hitachi 3010 UV-visible spectrometer.

Sample Preparation, Data Acquisition and Analysis. To keep all tests at a consistent condition, all measurements were performed at 25 ^{o}C. Aqueous PMTPA stock solution was diluted to the desired concentration in 10 mM HEPES buffer and then mixed with the certain XNP. The concentration of PMTPA was kept at a constant concentration of 0.1 mM and the analyte was added with three different concentrations. There were three measurements at each concentration and total nine measurements for one sample.

All data for LDA were collected from absorption spectra of PMTPA/XNP. The absorbance at 700, 581, 560, 536, 500, 474, 464, 454, 444, and 410 nm were submitted to perform variable as the following formula: variable 1 = $(A_{581}-A_{700})/A_{410}$, variable 2 = $(A_{560}-A_{700})/A_{410}$, variable 3 = $(A_{536}-A_{700})/A_{410}$, variable 4 = $(A_{500}-A_{700})/A_{410}$, variable 5 = A_{474}/A_{410}, variable 6 = $A_{464}/A_{410,}$ variable 7 = $A_{454}/A_{410,}$ variable 8 = $A_{444}/A_{410.}$

In the process of data analysis, linear discrimination analysis (LDA) was used and Jackknifed classification matrices were taken to evaluate discrimination results. The main results are listed as follow:

Table S1. Average values of eight variables for fifteen XNPs from absorption spectra

VAR	ADP	AMP	ATP	CDP	CMP	CTP	GDP	GMP	GTP	UDP	UMP	UTP	TDP	TMP	TTP
1	0.468	0.073	0.787	0.169	0.068	0.436	0.982	0.430	0.971	0.313	0.069	0.615	0.321	0.100	0.589
2	0.535	0.107	0.802	0.242	0.107	0.531	0.896	0.540	0.917	0.450	0.108	0.731	0.457	0.145	0.813
3	0.728	0.199	0.948	0.409	0.196	0.700	1.161	0.704	1.126	0.686	0.191	0.896	0.680	0.256	0.933
4	0.896	0.455	0.937	0.733	0.411	0.909	1.114	0.815	1.086	0.955	0.399	0.976	0.951	0.507	1.041
5	1.062	0.734	1.147	0.988	0.650	1.185	1.191	0.915	1.217	1.084	0.643	1.186	1.123	0.762	1.229
6	1.076	0.819	1.128	1.033	0.730	1.179	1.188	0.943	1.203	1.094	0.727	1.163	1.133	0.837	1.202
7	1.077	0.896	1.101	1.062	0.811	1.159	1.167	0.969	1.174	1.092	0.812	1.132	1.129	0.906	1.165
8	1.072	0.958	1.074	1.072	0.884	1.128	1.137	0.991	1.136	1.083	0.890	1.099	1.113	0.962	1.125

Table S2. Summary of the Jackknifed classification matrix for fifteen XNPs.

	ADP	AMP	ATP	CDP	CMP	CTP	GDP	GMP	GTP	UDP	UMP	UTP	TDP	TMP	TTP	%correct
ADP	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
AMP	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	100
ATP	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	100
CDP	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	100
CMP	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	100
CTP	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	100
GDP	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	100
GMP	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	100
GTP	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	100
UDP	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	100
UMP	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	100
UTP	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	100
TDP	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	100
TMP	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	100
TTP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	100
Total	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	100

Table S3. Summary of the Jackknifed classification matrix for the pink-purple group.

	ADP	ATP	GTP	GDP	GMP	UTP	TTP	%correct
ADP	9	0	0	0	0	0	0	100
ATP	0	9	0	0	0	0	0	100
GTP	0	0	9	0	0	0	0	100
GDP	0	0	0	9	0	0	0	100
GMP	0	0	0	0	9	0	0	100
UTP	0	0	0	0	0	9	0	100
TTP	0	0	0	0	0	0	9	100
Total	9	9	9	9	9	9	9	100

Table S4. Summary of the Jackknifed classification matrix for the yellow-orange group.

	AMP	CMP	CDP	CTP	UDP	UMP	TDP	TMP	%correct
AMP	9	0	0	0	0	0	0	0	100
CMP	0	9	0	0	0	0	0	0	100
CDP	0	0	9	0	0	0	0	0	100
CTP	0	0	0	9	0	0	0	0	100
UDP	0	0	0	0	9	0	0	0	100
UMP	0	0	0	0	0	9	0	0	100
TDP	0	0	0	0	0	0	9	0	100
TMP	0	0	0	0	0	0	0	9	100
Total	9	9	9	9	9	9	9	9	100

Table S5. Average values of eight variables for fifteen XNPs along with monophosphate and pyrophosphate from absorption spectra

VAR	ADP	AMP	ATP	CDP	CMP	CTP	GDP	GMP	GTP	UDP	UMP	UTP	TTP	TDP	TMP	Pi	PPi
1	0.468	0.073	0.787	0.169	0.068	0.436	0.982	0.430	0.971	0.313	0.069	0.615	0.321	0.100	0.589	0.035	0.073
2	0.535	0.107	0.802	0.242	0.107	0.531	0.896	0.540	0.917	0.450	0.108	0.731	0.457	0.145	0.813	0.067	0.119
3	0.728	0.199	0.948	0.409	0.196	0.700	1.161	0.704	1.126	0.686	0.191	0.896	0.680	0.256	0.933	0.139	0.224
4	0.896	0.455	0.937	0.733	0.411	0.909	1.114	0.815	1.086	0.955	0.399	0.976	0.951	0.507	1.041	0.353	0.547
5	1.062	0.734	1.147	0.988	0.650	1.185	1.191	0.915	1.217	1.084	0.643	1.186	1.123	0.762	1.229	0.610	0.908
6	1.076	0.819	1.128	1.033	0.730	1.179	1.188	0.943	1.203	1.094	0.727	1.163	1.133	0.837	1.202	0.697	0.991
7	1.077	0.896	1.101	1.062	0.811	1.159	1.167	0.969	1.174	1.092	0.812	1.132	1.129	0.906	1.165	0.787	1.053
8	1.072	0.958	1.074	1.072	0.884	1.128	1.137	0.991	1.136	1.083	0.890	1.099	1.113	0.962	1.125	0.868	1.084

Table S6. Summary of the Jackknifed classification matrix for fifteen XNPs along with monophosphate and pyrophosphate.

	ADP	AMP	ATP	CDP	CMP	CTP	GDP	GMP	GTP	UDP	UMP	UTP	TDP	TMP	TTP	Pi	PPi	%correct
ADP	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
AMP	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
ATP	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
CDP	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	100
CMP	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	100
CTP	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	100
GDP	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	100
GMP	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	100
GTP	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	100
UDP	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	100
UMP	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	100
UTP	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	100
TDP	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	100
TMP	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	100
TTP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	100
Pi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	100
PPi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	100
Total	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	100

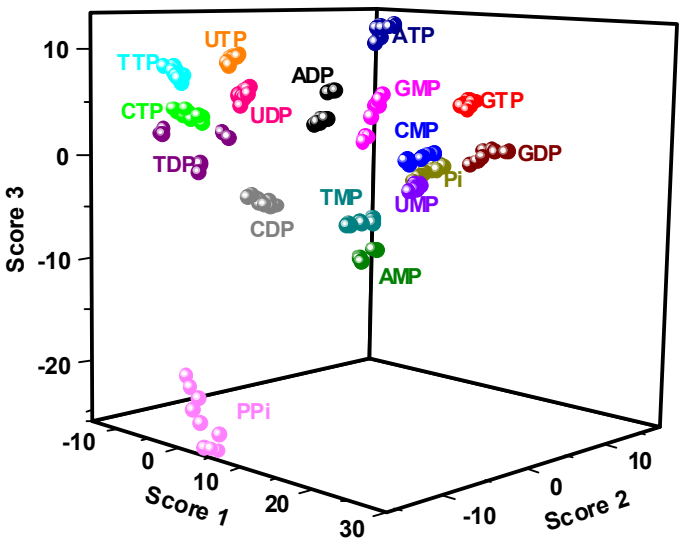

Figure S1. Three-dimensional LDA score plot for discrimination of fifteen XNPs along with monophosphate and pyrophosphate.

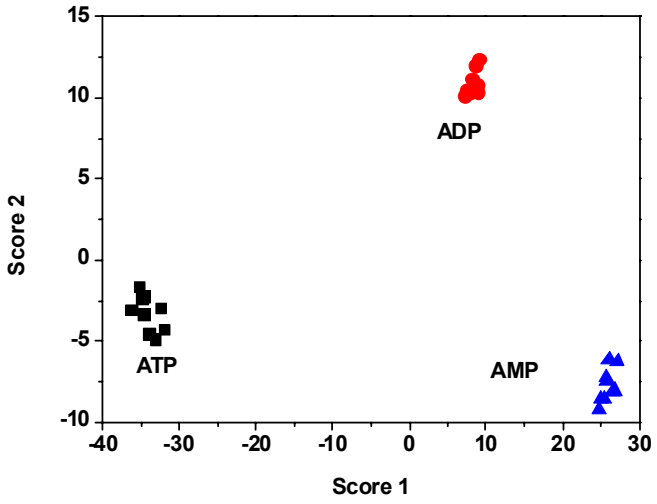

Figure S2. Two-dimensional LDA score plot for discrimination of ANPs.

Reference

(S1) C. Li, M. Numata, M. Takeuchi, S. Shinkai, *Angew. Chem. Int. Ed.* **2005**, *44*, 6371–6374.