

GPS-YNO2 Manual

Prediction of Tyrosine Nitration Sites

Version 1.0.1 14/08/2014

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Statement

1. **Implementation**. The softwares of the CUCKOO Workgroup are implemented in JAVA (J2SE). Usually, both of online service and local stand-alone packages will be provided.

2. **Availability**. Our softwares are freely available for academic researches. For non-profit users, you can copy, distribute and use the softwares for your scientific studies. Our softwares are not free for commercial usage.

3. **GPS**. Previously, we used the GPS to denote our Group-based Phosphorylation Scoring algorithm. Currently, we are developing an integrated computational platform for post-translational modifications (PTMs) of proteins. We re-denote the GPS as Group-based Prediction Systems. This software is an indispensable part of GPS.

4. **Usage**. Our softwares are designed in an easy-to-use manner. Also, we invite you to read the manual before using the softwares.

5. **Updation**. Our softwares will be updated routinely based on users' suggestions and advices. Thus, your feedback is greatly important for our future updation. Please do not hesitate to contact with us if you have any concerns.

6. **Citation**. Usually, the latest published articles will be shown on the software websites. We wish you could cite the article if the software has been helpful for your work.

7. Acknowledgements. The work of CUCKOO Workgroup is supported by grants from the National Basic Research Program (973 project) (2006CB933300, 2007CB947401, 2007CB914503, and 2010CB912103), Natural Science Foundation of China (90919001, 30700138, 30900835, 30830036, 30721002, 30871236, and 90913016), Chinese Academy of Sciences (KSCX1-YW-R65, KSCX2-YW-R-139, INFO-115-C01-SDB4-36), and National Science Foundation for Post-doctoral Scientists (20080430100).

Introduction

The 1998 Nobel Prize in Physiology or Medicine was awarded for discoveries concerning nitric oxide (NO) as a signaling molecule in the cardiovascular system. NO acts as a freely-diffusible signaling molecule and second messenger which can regulates the production of cyclic GMP (cGMP). The following studies showed that an interplay involving excess NO, transition metal centers and oxidants may generate protein cysteine S-nitrosylation as well as protein tyrosine nitration (PTN) (1-5). Though lots of work contributed to dissect the mechanisms of PTN previously (6-8), our understanding of PTN is still fragmentary. However, it is noticed that when oxidants, such as superoxide radicals (O_2^{\bullet}) and hydrogen peroxide (H_2O_2) , are presented with transition metal centers in NO metabolism, reactive nitrogen species such as peroxynitrite anion (ONOO⁻) and nitrogen dioxide (NO₂) will format, which resulted in nitration of protein tyrosines (Figure 1). Although this process was questioned by the complexity of the cellular environment, for instance, the transition metal could be replace by heme peroxidase, it was assured that PTN could be triggered in vivo. Though originally addressed in early in vitro protein chemistry studies, recent studies led to the discovery that cellular PTN has important implications on histone modification (9), protein activity regulation (10), epitope recognition (11) and so on. Furthermore, PTN showed its significance in biochemical processes critical for physiology and pathology including signal transduction, immune response, cell death, aging, neurodegeneration and so on (1-5). However, as a covalent modification linked to NO signaling pathway, our understanding of PTN and its relationship with S-nitrosylation is still fragmentary.

Combined with the conventional experimental identification of substrates for PTN (3,10), large-scale detection of cellular nitrated proteins was introduced for a global view with the development of the biotechnology including the antibodies to recognize the nitrotyrosine, the method for selective enrichment of nitrotyrosine-containing peptides and the mass spectrometer (12-14). In light of the advance of biotechnologies, lots of studies touched on systematic analysis of nitrated proteins to provide insights into the biological roles of PTN (13,15-20). Previously, Souza et al. and Elfering et al. investigated the consensus sequence around the nitration sites, which resulted in opposed conclusions (21-22). However, a recent proteomic work with 335 PTN sites in 267 proteins from human Jurkat cells of which lysate was treated by peroxynitrite resulted in no conclusive sequence preference for PTN (16). Nowadays, the desire to map the PTM sites without the time-consuming and expensive experimental methods has motivated the development of computational approaches, which was shown to be able to rapidly generate helpful information for further experimental verification.

Although there have been ~170 databases and computational tools developed for PTM analyses especially phosphorylation (http://www.biocuckoo.org/link.php) (23), *in silico* prediction of tyrosine nitration sites in proteins is still a great challenge.

In this work, 1.066 experimentally verified PTN sites within 554 unique proteins were collected from the scientific literature and public databases. In our previous study, the GPS 2.0 (Group-based Prediction System) algorithm was developed for the prediction of kinase-specific phosphorylation sites (24), and we just improved the method substantially and released the GPS 3.0 algorithm for prediction of S-nitrosylation sites (25). Here we developed a novel computational software of GPS-YNO2 1.0 for prediction of tyrosine nitration sites. The leave-one-out validation and 4-, 6-, 8- and 10-fold cross-validations were adopted to evaluate the prediction performance and system robustness. The performance of the GPS-YNO2 1.0 was satisfying, with an accuracy of 76.51%, a sensitivity of 50.09% and a specificity of 80.18% under the low threshold condition. As applications of GPS-YNO2 1.0, we predicted 325 (~88%) of these the collected substrates without bona fide sites with at least one potential nitration site. Obviously, these prediction results might be of great help for further experimental verification. Finally, the online service and local packages of GPS-YNO2 1.0 were implemented in JAVA 1.4.2 and are freely available at: http://yno2.biocuckoo.org/.

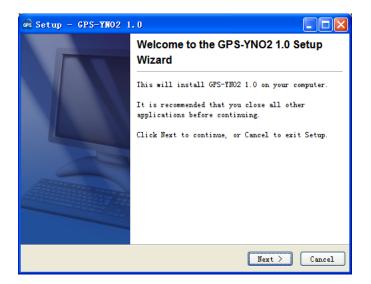
💼 GPS-YNO2	1.0	,				
File Tools Help						
Predicted Site						
Positio	n	Peptide	Sco	ore	Cutoff	Cluster
(L						
Enter sequer	nce(s) in FASTA 1	format				
]
Threshold				Console		
🔾 High	Medium	Low		Example	Clear	Submit

GPS-YNO2 1.0 User Interface

Download & Installation

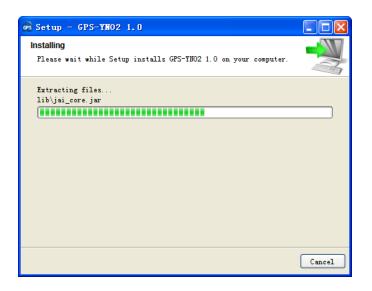
The GPS-YNO2 1.0 was implemented in JAVA (J2SE), and could support three major Operating Systems (OS), including Windows, Linux/Unix or Mac OS X systems. Both of online web service and local stand-alone packages are available from: <u>http://yno2.biocuckoo.org/</u>. We recommend that users could download the latest release.

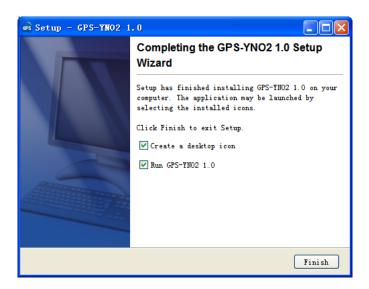
Please choose the proper package to download. After downloading, please double-click on the software package to begin installation, following the user prompts through the installation. And snapshots of the setup program for windows are shown below:



📾 Setup - GPS-YN02 1.0
Select Destination Directory Where should GPS-TMO2 1.0 be installed?
Select the folder where you would like GPS-YNO2 1.0 to be installed, then click Next.
C:\Program Files\GPS-TN02
Required disk space: 77.5 MB
Free disk space: 4,401 MB
<pre></pre>

😪 Setup - GPS-YN02 1.0	×
Select Start Menu Folder Where should Setup place the program's shortcuts?	3
Select the Start Menu folder in which you would like Setup to create the program's shortcuts, then click Next.	
Group-based Prediction System	
Tools For Entertainment Tools For Network	
Tools For Scientific Research Tools For Study	
Tools For System 管理工具	
✔ Create shortcuts for all users	
🗌 Don't create a Start Menu folder	
< Back Next > Cancel	





Finally, please click on the **Finish** button to complete the setup program.

Prediction of Tyrosine Nitration Sites

1. A single protein sequence in FASTA format

The following steps show you how to use the GPS-YNO2 1.0 to predict tyrosine nitration sites for a single protein sequence in FASTA format.

(1) Firstly, please use "Ctrl+C & Ctrl+V" (Windows & Linux/Unix) or "Command+C & Command+V" (Mac) to copy and paste your sequence into the text form of GPS-YNO2 1.0

ile Tools Help					
Predicted Sites			1		
Position	Peptide	Score		Cutoff	Cluster
nter sequence(s) i	n FASTA format				
	3 protein epsilon, P62259)				
	ERYDEMVESMKKVAGMDVELT				
	FYYKMKGDYHRYLAEFATGN				
	ALLRDNLTLWTSDMQGDGE		1	IREGEALINFOVETTE	EILINGEDRAGREARARD
	IQEERDINE IEW ISDMQGDGE		102		
hreshold		Co	nsole		
	ledium O Low		nsole Example	Clear	Submit

Note: for a single protein, the sequence without a name in raw format is also OK. However, for multiple sequences, the name of each protein should be presented.

📾 GPS-YN02 1.0							
File Tools Help							
Predicted Sites							
Position	Peptide	Sco	ore		Cutoff	Cluster	
Enter sequence(s) in F	ASTA format						
>Example (MOUSE 14-3-3 pr							
MDDREDLVYQAKLAEQAERY		EERNLLSVAY	KNVIGARRAS	WRIISSIEQI	KEENKGGEDKLK	MIREYRQMVETELKLICCDI	
LDVLDKHLIPAANTGESKVFY				ELPPTHPIF	RLGLALNFSVFYY	EILNSPDRACRLAKAAFDD	
AIAELDTLSEESYKDSTLIMQL	LRDNLTLWTSDMQGDGEE	QNKEALQDVE	DENQ				
Threshold			Console				
🔾 High 🛛 🖲 Med	ium 🔾 Low	o Ali	Exam	nple	Clear	Submit	

(2) Choose a **Threshold** that you need, the default cut-off is **Medium**.

(3) Click on the **Submit** button, then the predicted tyrosine nitration sites will be shown.

LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNI	Score 2.925	Cutoff	Cluster					
Predicted Sites Position Peptide 9 DDREDLVYQAKLAEQ 20 LAEQAERYDEMVESM 49 RNLLSVAYKNVIGAR 85 KLKMIREYRQMVETE 131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNU			Cluster					
Position Peptide 9 DDREDLVYQAKLAEQ 20 LAEQAERYDEMVESM 49 RNLLSVAYKNVIGAR 85 KLKMIREYRQMVETE 131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNU			Cluster					
9 DDREDLVYQAKLAEQ 20 LAEQAERYDEMVESM 49 RNLLSVAYKNVIGAR 85 KLKMIREYRQMVETE 131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNU			Cluster					
20 LAEQAERYDEMVESM 49 RNLLSVAYKNVIGAR 85 KLKMIREYRQMVETE 131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNU	2.925							
49 RNLLSVAYKNVIGAR 85 KLKMIREYROMVETE 131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNU		1.16	Cluster D					
85 KLKMIREYROMVETE 131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNU	1.286	0.554	Cluster A					
131 MKGDYHRYLAEFATG 152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNI	1.642	1.16	Cluster D					
152 AENSLVAYKAASDIA 214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNE	2.466	0.828	Cluster C					
214 DTLSEESYKDSTLIM Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNE	2.679	1.065	Cluster B					
Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNE	1.294	1.16	Cluster D					
>Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELT LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNI	1.749	0.554	Cluster A					
Threshold	Console							
◯ High	Console	mple Clear	Submit					

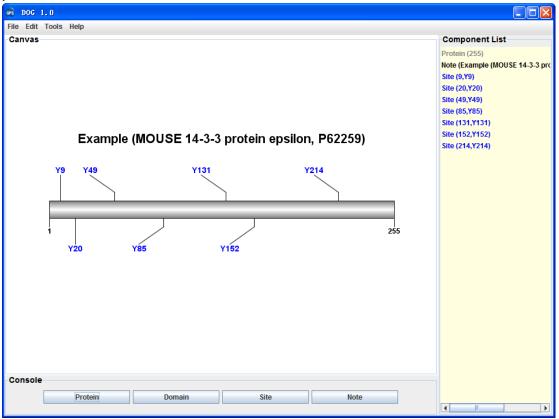
(4) Then please click on the **RIGHT** button in the prediction form. You can use the "**Select All**" and "**Copy Selected**" to copy the selected results into Clipboard. Then please copy the results into a file, eg., an EXCEL file for further consideration. Also, you can choose "**Export Prediction**" to export the prediction results into a tab-delimited text file.

GPS-YNO2 1.0								
ile Tools Help								
Predicted Sites								
Position	Peptide	Score		Cutoff	Cluster			
9	DDREDLV <mark>Y</mark> QAKLAEQ	2.925		1.16	Cluster D			
20	LAEQAERYDEMVESM	1.286		0.554	Cluster A			
49	RNLLSVAYKNVIGAR	1.642	Select All		Cluster D			
85	KLKMIREYROMVETE	2.466	Copy Select		Cluster C			
131	MKGDYHRYLAEFATG	2.679	Export Resi Visualize		Cluster B			
152	AENSLVA <mark>Y</mark> KAASDIA	1.294		1.16	Cluster D			
214	DTLSEESYKDSTLIM	1.749		0.554	Cluster A			
Enter sequence(s) in FASTA format >Example (MOUSE 14-3-3 protein epsilon, P62259) MDDREDLVYQAKLAEQAERYDEMVESMKKVAGMDVELTVEERNLLSVAYKNVIGARRASWRIISSIEQKEENKGGEDKLKMIREYRQMVETELKLICCDI LDVLDKHLIPAANTGESKVFYYKMKGDYHRYLAEFATGNDRKEAAENSLVAYKAASDIAMTELPPTHPIRLGLALNFSVFYYEILNSPDRACRLAKAAFDD AIAELDTLSEESYKDSTLIMQLLRDNLTLWTSDMQGDGEEQNKEALQDVEDENQ								
Threshold Console								

Again, you can also click the "Export Prediction" in File menu to export the results.



If you choose the Visualize function, the given protein and its predicted sites will be visualized with DOG (Domain Graph, Version 1.0), an illustrator of protein domain structures.



2. Multiple protein sequences in FASTA format

For multiple protein sequences, there are two ways to use the GPS-YNO2 1.0.

A. Input the sequences into text form directly. (Num. of Seq \leq 2,000)

If the number of total protein sequences is not greater than 2,000, you can just use "Ctrl+C & Ctrl+V" (Windows & Linux/Unix) or "Command+C & Command+V" (Mac) to copy and paste your sequences into the text form of GPS-YNO2 1.0 for prediction.

e Tools Help								
redicted Sites								
Position	Peptide	Score	Cutoff		Cluster			
50	LHFEGRNYEASVDSL	0.116	0		Cluster C			
115	ANAPIGLYRLSLEAS	0.28	0		Cluster B			
125	SLEASTGYQGSSFVL	0.558	0		Cluster A			
149	WCPADAVYLDSEEER	0.139	0		Cluster B			
159	SEEERQEYVLTQQGF	0.052	0		Cluster C			
168	LTQQGFIYQGSAKFI	0.483	0		Cluster B			
225	HGCQRVKYGQCWVFA	0.251	0		Cluster B			
252	PTRVVTNYNSAHDQN	0	0		Cluster C			
266	NSNLLIEYFRNEFGE	0.239	0		Cluster B			
302	RPDLOPGYEGWOALD	0.061	0		Cluster B			
320	QEKSEGTYCCGPVPV	0.171	0		Cluster B			
339	EGDLSTKYDAPFVFA	0	0		Cluster C			
394	REDITHTYEXAMPLE	0.062	0		Cluster C			
430	TNNTAEEYVCRLLLC	0.041	0		Cluster C			
443	LCARTVSYNGILGPE	0.951	0		Cluster A			
455	GPECGTKYLLNLNLE	0.719	0		Cluster A			
Enter sequence(s) in FASTA format Example1 IAEELVLERCDLELETNGRDHHTADLCREKLVVRRGQPFWLTLHFEGRNYEASVDSLTFSVVTGPAPSQEAGTKARFPLRDAVEEGDWTATVVDQ DDCTLSLQLTTPANAPIGLYRLSLEASTGYQGSSFVLGHFILLFNAWCPADAVYLDSEEERQEYVLTQQGFIYQGSAKFIKNIPWNFGQFEDGILDICLI Example2 ISWIGSVDILRRWKNHGCQRVKYGQCWVFAAVACTVLRCLGIPTRVVTNYNSAHDQNSNLLIEYFRNEFGEIQGDKSEMIWNFHCWVESWMTRPD QPGYEGWQALDPTPQEKSEGTYCCGPVPVRAIKEGDLSTKYDAPFVFAEVNADVVDWIQQDDGSVHKSINRSLIVGLKISTKSVGRDEREDITHTY Example3 IRIRVGQSMNMGSDFDVFAHITNNTAEEYVCRLLLCARTVSYNGILGPECGTKYLLNLNLEPFSEKSVPLCILYEKYRDCLTESNLIKVRALLVEPVIN								
reshold		Consol	e					
reshold	Medium O Low	-	e xample	Clear	Submit			

B. Use Batch Predictor tool.

If the number of protein sequences is very large, eg., yeast or human proteome, please use the **Batch Predictor**. Please click on the **"Batch Predictor**" button in the **Tools** menu.

Tools	Help		
Batch	Predictor	Ctrl-B	F

The following steps show you how to use it:

(1) Put protein sequences into one or several files (eg., SC.fas, CE.fas, and etc)

with FATSA format as below:

Most importantly, the name of each protein should be presented.

(2) Click on the **Batch Predictor** button and then click on the **Add File** button and add one or more protein sequence files in your hard disk.

💼 Batch Predictor					×
Sequence File List					
		-			
	Remove All	Remove	Add File		
Result File List					
Result Export Fold	ar				>>
Threshold			Console		
🔾 High 🔹 Medium	◯ Low	o Ali	Clea	r	Submit

		Batch Pred Sequence File					
ee 打开 查看: 百 CE.fas DM.fas HS.fas SC.fas	FASTA Seq				Remove	Add File	
文件名:	"CE.fas" "DM.fas"	"HS.fas" "SC.fas"					
文件类型:	所有文件			-			
			打开	取消			
			Result Export Fol	der			>>
		Threshold				Console	
		🔾 High	Medium	◯ Low	O All	Clear	Submit

Then the names of added files will be shown in the Sequence File List.

📾 Batch Predictor					
Sequence File List					
CE.fas					
DM.fas					
HS.fas					
SC.fas					
	Remove All	Remove	Add File		
Result File List					
Result Export Fold	ler			>>	
Threshold			Console		
🔾 High 💿 Medium	◯ Low		Clear		Submit

(3) The output directory of prediction results should also be defined. Please click on the >> button to specify the export fold.

😰 Batch Pre						×
Sequence Fil	e List					
CE.fas DM.fas	🖻 保存					
HS.fas						
SC.fas	保存: 📑	Predict Results		- A A A		
Result File Li						
	文件名:	C:\Predict Results				
	文件类型:	Folder			-	
				保存	取消	
	Result Expo	ort Folder			>>	
Threshold				Console		
🔾 High	Medium	○ Low) Ali	Clear	Sub	mit

(4) Please choose a proper threshold before prediction. Then please click on the **Submit** button, then the **Batch Predictor** begin to process all of the sequence files that have been added to the list. The result of prediction will be export to the **Prediction Export Fold**, and the name of result files will be shown in the **Prediction File List**.

🗪 Batch Predictor					
Sequence File List					
CE.fas					
DM.fas					
HS.fas					
SC.fas					
	Remove All	Remove	Add File		
Result File List					
C:\Predict Results\CE.ccd.fas					
C:\Predict Results\DM.ccd.fas					
C:\Predict Results\HS.ccd.fas					
C:\Predict Results\SC.ccd.fas					
Result Export Folde	er C:\Predict Resu	lts		>>	
Threshold			Console		
🔾 High 💿 Medium	O Low		Clear		Submit

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Release Note

- 1. Dec. 20th, 2009, the online service and the local stand-alone packages of GPS-YNO2 1.0 were released.
- 2. Aug. 14th, 2010, GPS-YNO2 was updated for the new sites and retraining.
- 3. Aug. 14th, 2014, GPS-YNO2 was updated for the curation of nitration sites.