

# Integrated Michaelis-Menten equation in DynaFit.

## 1.

### Application to EGFR protein kinase

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#### Abstract

This brief Technical Note contains detailed step-by-step instructions for how to determine EGFR protein kinase substrate kinetic parameters using a newly proposed mathematical method (Reytor Gonz'alez *et al.*, 2016) recently published in the journal *Analytical Biochemistry*, <http://dx.doi.org/10.1016/j.ab.2016.11.001>. All kinetic determinations are fully automatic, using a combination of the software package DynaFit (Kuzmič, 1996) and a Perl language interpreter. Detailed instructions and download links are also provided showing how to equip a MS Windows-based computer for the fully automated kinetic analysis.

#### *Key words:*

EGFR protein kinase; enzyme progress curves; global fit; integrated Michaelis-Menten equation; Lambert omega function; substrate kinetics;

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## 1. Introduction

The main purpose of this Technical Note is to provide additional supporting information for an currently submitted for publication in the journal *Analytical Biochemistry*, see ref. [1]. The Note contains step-by-step instructions on how to download, install, and execute the software package DynaFit [2, 3] supplemented by (1) Perl [4] automation scripts; and (2) all requisite experimental data; and (3) DynaFit input files.

## 2. Instructions

This section contains step-by-step instructions on how to perform fully automated determination of substrate kinetic parameters for EGFR protein kinase, as described in ref. [1].

### 2.1. Prerequisites

The software automation package described in this document will execute on any computer running the MS Windows operating system (version XP/SP3, Vista, 7, 8, 8.1, or 10). It is required that a suitable Perl language interpreter [4] is pre-installed to achieve full automation and integration of the Windows operating system with the DynaFit software package [2, 3].

#### 2.1.1. Testing Perl version currently installed

To test whether or not the Perl interpreter is currently installed, and if so which version is installed, please follow these steps:

1. Open the command window: Start ... Programs ... Accessories ... Command Prompt
2. Type the following command, followed by the Enter key: `perl -v`
3. Check whether or not the Perl interpreter is installed on the Windows system, according to the screen captures below.

Screen capture when Perl is already installed on the system:

```
C:\>perl -v
This is perl 5, version 20, subversion 1 (v5.20.1) built for MSWin ...
(with 1 registered patch, see perl -V for more detail)

Copyright 1987-2014, Larry Wall

Binary build 2000 [298557] provided by ActiveState ...
Built Oct 15 2014 22:10:49
C:\>
```

Screen capture when Perl not yet installed on the system:

```
C:\>perl -v
'perl' is not recognized as an internal or external command,
operable program or batch file.

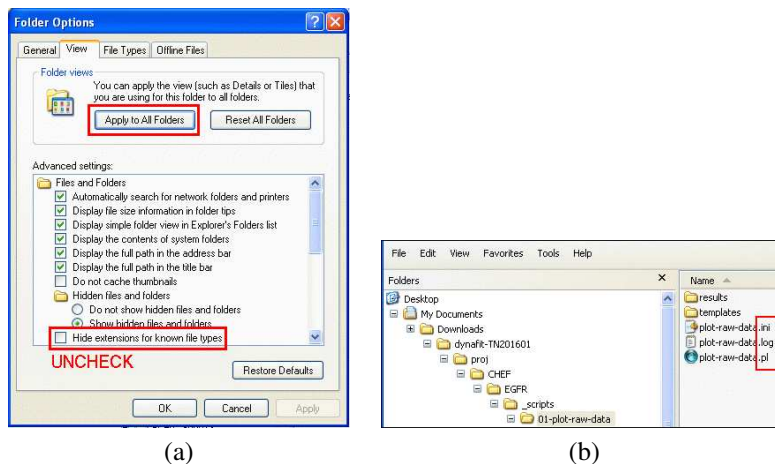
C:\>
```

If the Perl interpreter is already installed, skip the immediately following installation step and proceed directly to the download step, section 2.2 below. Otherwise download and install Perl as shown in section 2.1.2

### 2.1.2. Installing Perl for Windows

The Perl interpreter package is available free of charge. Downloading and installing the Perl software package does not require the end user to provide any information to the software publisher, [www.activestate.com](http://www.activestate.com). To download and install the Perl interpreter for Windows please follow these steps:

1. Point the web browser to <http://bit.ly/2flKnDY><sup>1</sup>
2. Select either the 32 bit version or the 64 bit version, depending which operating system is installed on the computer:
  - 32 bit: “Download ActivePerl [version] for Windows (x86)”
  - 64 bit: “Download ActivePerl [version] for Windows (64-bit, x64)”
3. Download the installer and follow the usual installation steps.
4. Check whether the Perl interpreter is correctly installed, but following the steps described in in section 2.1.1



**Figure 1:** Un-hide file extensions of known file types in MS Windows.

### 2.1.3. Configure Windows to “un-hide” file extensions

By default, the Windows operating system “hides file extensions of known file types”. For example, a given directory / folder might contain three distinct files with unique file names, as follows:

1. File determine-Km.pl (a Perl script)
2. File determine-Km.ini (a configuration information for the above Perl script)
3. File determine-Km.log (an output file generated by the Perl script)

<sup>1</sup><http://www.activestate.com/activeperl/downloads>

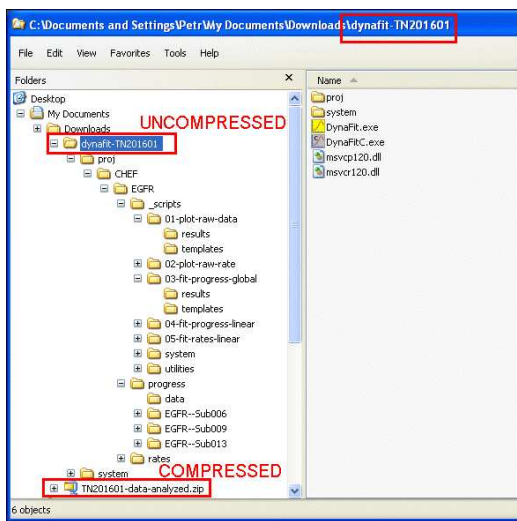
Note the file names are distinct, in that the file name *extensions* (in this case .pl, .ini, .log) are different. However, because all three file name extensions are “known” to the Windows operating system, the operating system will “hide” them from the user in Windows Navigator and will display all three files as if they had the file name, *determine-Km*. This can be very confusing to the researcher attempting to utilize the software automation package described in this Technical Note. It is therefore important to reveal full file names, including file name extensions, as they actually exist under Windows.

To reveal (or “un-hide”) file extensions of known file types under the MS Windows operating system, please follow the following steps, see also *Figure 1*.

1. Start Windows Navigator: Start ... Programs ... Accessories ... Windows Explorer
2. Select menu Tools ... Folder Options
3. Select tab View
4. Locate item Hide extensions for known file types
5. Un-check the associated check box
6. Click the [Apply] button
7. Click the [Apply to All Folders] button
8. Click the [OK] button

## 2.2. Download the DynaFit / EGFR demonstration package

To download and setup the demonstration package, please follow these steps:



**Figure 2:** File and directory layout after extracting the download ZIP archive file.

1. Point a web browser to <http://bit.ly/2flPzIf<sup>2</sup>>
2. Download the ZIP file to any location on the target computer system.

<sup>2</sup><http://www.biokin.com/publications/technotes/data/TN201601-data-analyzed.zip>

3. IMPORTANT: Unzip the compressed archive directory. This will create a new directory dynafit-TN201601. See *Figure 2*.

The *extraction* (“un-archiving”) of the downloaded ZIP compressed file is very important in particular under the Windows 10 operating system. Depending on the settings of the Windows Explorer operating system component, the operating system might make it appear as if the ZIP archive were an “actual” directory or folder. However, the DynaFit software package will not operate correctly unless and until the ZIP archive is fully extracted into a *separate uncompressed directory*.

### 2.3. Determine substrate kinetic constants for EGFR

To analyze the EGFR substrate kinetic data included in this report, please follow these steps:

1. Navigate into the directory dynafit-TN201601/proj/CHEF/EGFR/\_scripts, see *Figure 2*
2. Navigate in turn to the following subdirectories and double click on the corresponding Perl scripts (file extension .pl):
  - Script 01-plot-raw-data/plot-raw-data.pl:  
Plot raw experimental data.
  - Script 02-plot-raw-rate/plot-raw-data.pl:  
Plot raw instantaneous rates.
  - Script 03-fit-progress-global/fit-progress-global-all.pl:  
Determine kinetic constants by global fit of combined progress curves.
  - Script 04-fit-progress-linear/fit-progress-linear-all.pl:  
Determine initial rates using presumably “initial linear” regions of each progress curve.
  - Script 05-fit-rates-linear/fit-rates-linear.pl:  
Determine Michaelis constants from the “initial linear” rates obtained in the immediately preceding step.

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### Bibliographic Information

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## References

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