P450 Site of Metabolism

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Document Conventions

In addition to the use of italics for names of documents, the font conventions that are used in this document are summarized in the table below.

Font	Example	Use
Sans serif	Project Table	Names of GUI features, such as panels, menus, menu items, buttons, and labels
Monospace	`$SCHRODINGER/maestro`	File names, directory names, commands, environment variables, command input and output
Italic	*filename*	Text that the user must replace with a value
Sans serif uppercase	CTRL+H	Keyboard keys

Links to other locations in the current document or to other PDF documents are colored like this: Document Conventions.

In descriptions of command syntax, the following UNIX conventions are used: braces { } enclose a choice of required items, square brackets [ ] enclose optional items, and the bar symbol | separates items in a list from which one item must be chosen. Lines of command syntax that wrap should be interpreted as a single command.

File name, path, and environment variable syntax is generally given with the UNIX conventions. To obtain the Windows conventions, replace the forward slash / with the backslash \ in path or directory names, and replace the $ at the beginning of an environment variable with a % at each end. For example, `$SCHRODINGER/maestro` becomes `%SCHRODINGER%\maestro`.

Keyboard references are given in the Windows convention by default, with Mac equivalents in parentheses, for example CTRL+H (⌘H). Where Mac equivalents are not given, COMMAND should be read in place of CTRL. The convention CTRL-H is not used.

In this document, to *type* text means to type the required text in the specified location, and to *enter* text means to type the required text, then press the ENTER key.

References to literature sources are given in square brackets, like this: [10].

P450 Site of Metabolism

Cytochrome P450 enzymes play an integral role in the metabolism pathway of drugs. These heme-containing enzymes catalyze a variety of reactions, such as hydroxylation, dealkylation, and double-bond oxidation, that result in the degradation of small molecules. Predicting the sites of metabolism of drug-like molecules would give medicinal chemists better control of the metabolic stability of molecules they design.

For the 3A4 isoform of cytochrome P450, ligand-based reactivity models have been shown to be highly predictive. The success of these predictions is thought to be due to the lack of orientational preference of ligands in the 3A4 binding site, which is highly flexible. For other isoforms with regioselective preferences, it is more difficult to predict the reactivity with ligand-based models. This is particularly true for differentiating ligands with very subtle differences, such as inversions of stereo centers and small functional group modifications away from the sites of metabolism. In such cases, a model that includes information about the binding mode to the isoform of interest is necessary.

For a given atom of a molecule to be a significant site of metabolism by a P450 enzyme, it must have some degree of reactivity in the absence of the enzyme and also be accessible to the reactive heme iron center. To address both of these requirements, the P450 Site of Metabolism workflow combines induced-fit docking (IFD) for the determination of accessibility to the reactive center with a rule-based approach to intrinsic reactivity.

1 Methodology

The reactivity rules have been parameterized to predict atomic reactivity profiles for promiscuous P450 enzymes that are thought to be mostly independent of structural restrictions on the binding poses. The reactivity is predicted with a linear free energy approach based on the Hammett and Taft scheme, where the reactivity of a given atom is the sum of a baseline reactivity rate and a series of perturbations determined by the connectivity.

The induced-fit docking approach is a variation on the current protocol (see the *Induced Fit Docking* manual). The initial sampling is enhanced by generating multiple starting conformations, so that a wider range of poses is found in the initial docking stage. The initial docking includes van der Waals scaling of the receptor and alanine mutation of the most flexible residues. In the Prime refinement stage, any residue with an atom within 5 Å of any ligand pose is selected for side-chain prediction. The subsequent minimization includes the ligand, side chains, and backbones of the flexible residues. The ligand is then redocked into each of the

low-energy protein conformations, determined by a 40 kcal/mol cutoff. There is no final scoring stage, since all poses are considered in determining which atoms are sufficiently accessible to the reactive heme iron. Any atom within the cutoff distance of 5 Å from the heme iron is considered as a potential site of metabolism.

2 Running the Calculation

The calculation can be set up from the P450 Site of Metabolism - Perform Calculation panel, which you open by choosing Workflows → P450 Site of Metabolism → Perform Calculation or Tasks → P450 Site of Metabolism → Perform Calculation.

The ligands can be taken from the Workspace (the included entries), the selected entries in the Project Table, or from a file. You can make this selection from the Take ligands from option menu. If you choose File, click Browse to navigate to the file, which must be in Maestro format. The ligands must be properly prepared before you run the calculation: 3D structures with all hydrogens. If you have 2D structures, or SMILES strings for the structures, you can prepare them with LigPrep (Applications > LigPrep)—see the *LigPrep User Manual* for details.

Next, choose the isoform that you are interested in from the CYP Isoform option menu. Three isoforms are available: 2C9, 2D6, and 3A4. For 3A4, only the intrinsic reactivity is calculated. For the others, an induced-fit docking calculation is also performed.

When you have made your choices, click Start to start the calculation. The Start P450 Job dialog box opens, in which you can name the job, choose a host, and set the number of processors used for the Prime and the Glide stages of the induced-fit docking calculation. The induced-fit docking run can take some time, so you should consider running it on multiple processors. When you click Start in this dialog box, the job is started, and the Monitor panel opens to show the status of the job.

If you want to adjust the induced-fit protocol, you can click Write to write the input file for modification. The Write P450 Job dialog box opens. It is identical in function to the Start P450 Job dialog box, because all the job information is included in the input file. See Chapter 5 of the *Induced Fit Docking* manual for information on the input file.

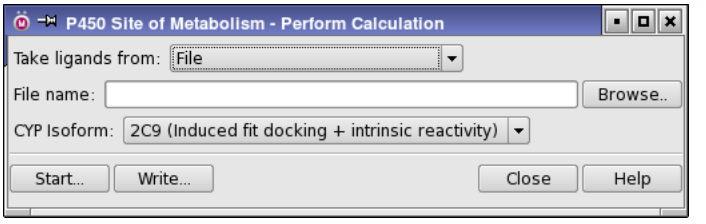

Figure 1. The P450 Site of Metabolism - Perform Calculation panel

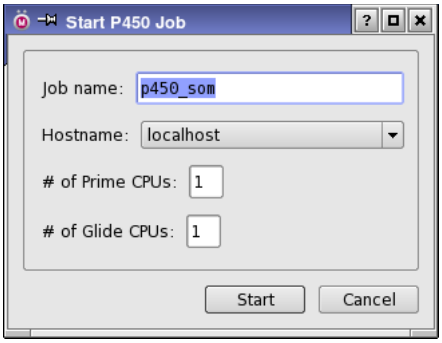

Figure 2. The Start P450 Job dialog box

When you have modified the input file, you can run the calculation with the command

`$SCHRODINGER/ifd` *jobname*`.inp`

3 Examining the Results

When the job finishes, you can examine the results in the P450 Site of Metabolism - Examine Results panel, which you open by choosing Workflows > P450 Site of Metabolism > Examine Results or Tasks > P450 Site of Metabolism > Examine Results.

First, the results must be imported. To do so, click Import Results, and navigate to the file *jobname*`-CYPligs-out.maegz`. When you import the file, the first ligand in the file is displayed in the display area as a 2D structure, annotated with reactivity information. The ligand name and the CYP isoform are shown above the display area.

The type of annotation can be set by choosing a Show reactivity option:

- Fe-accessibility from IFD—Label atoms with the accessibility of the atoms to the iron. This is defined as the natural logarithm of the number of poses for the atom in which the atom was within 5 Å of Fe. Larger values indicate greater accessibility.
- Intrinsic reactivity—Label atoms with the intrinsic reactivity for 3A4, calculated with Hammett and Taft methodology. Positive values are more reactive, negative values are less reactive.
- Overall SOM score—Linear combination of the accessibility and the intrinsic reactivity. The results are displayed as green circles, in which the radius is proportional to the score. Larger scores mean higher reactivity. When you display this score, you can click on one of the green circles to view a representative pose for that site in the Workspace.

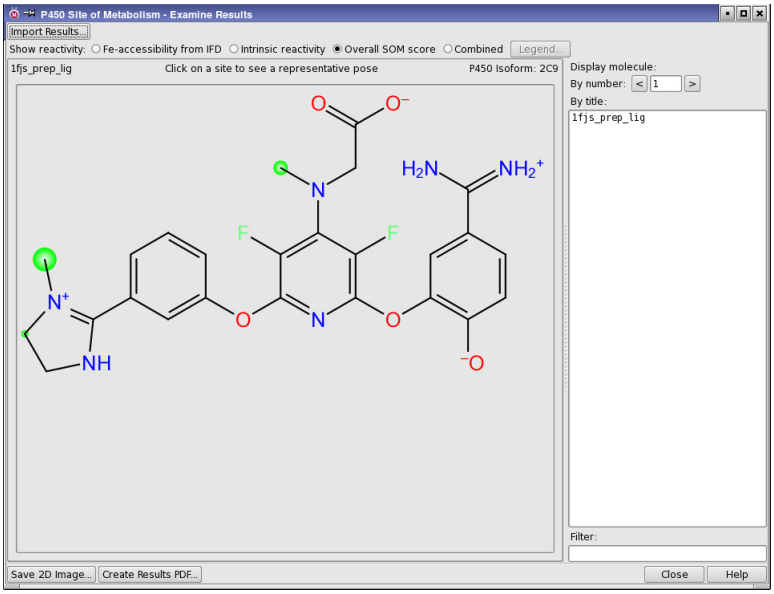

Figure 3. The Examine Results panel, showing the Overall SOM score.

- Combined—Show all three annotations at the same time, in graphical form. An explanation of the annotations is given in a panel when you click Legend. The description of the annotations is summarized below.

  The Fe-accessibility is indicated by a set of green rays drawn out from the atom to a given maximum length. Each full-length ray represents one unit of accessibility, so the number of full-length rays is the integer part of the score. The remaining partial ray represents the decimal part of the score.

  The circles represent the overall score and the intrinsic reactivity. The radius of the circle is proportional to this score. The intensity of the red color of the circle is proportional to the intrinsic reactivity. The blue perimeter indicates whether the atom passed the filtering stage, which has cutoffs for the intrinsic reactivity and the number of poses.

If you ran predictions on more than one molecule, you can step through the molecules with the controls to the left of the display area. Click the arrow buttons to display the next or previous molecule, enter the molecule number (index in the input file) in the text box, or select the molecule by its title in the By title list. You can filter the list by entering text in the Filter text box.

You can save a copy of the annotated 2D structure as an image, in TIFF, JPEG, or PNG format. Click Save 2D Image, navigate to the desired location, and name the file. The format is determined automatically by the file extension. The images are illustrated in Figure 4.

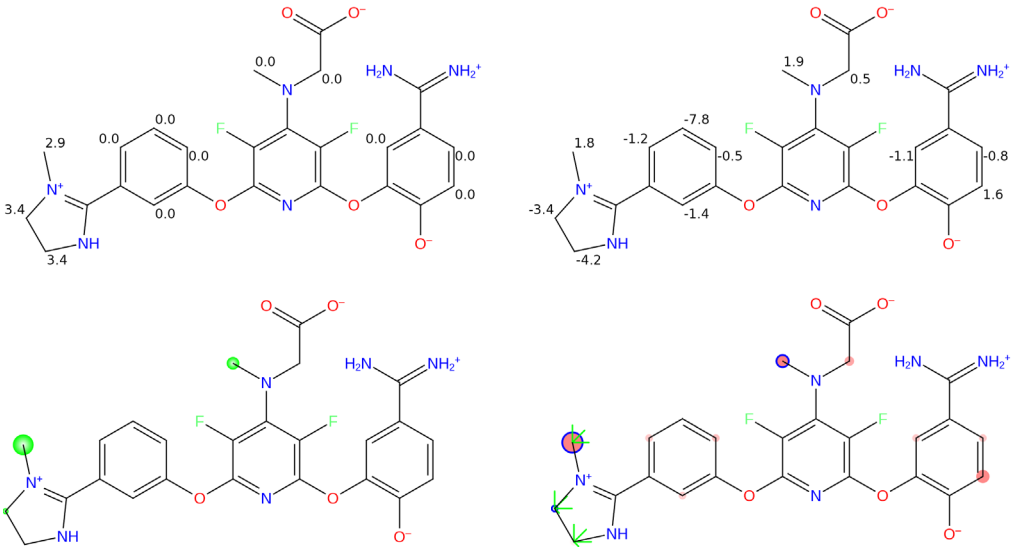

Figure 4. 2D images of scoring displays. Top to bottom, left to right: Fe-accessibility, Intrinsic reactivity, Overall SOM score, Combined.

You can also export the results for all molecules to a PDF file. The results are arranged in three columns, one for each property.

Getting Help

Information about Schrödinger software is available in two main places:

- The `docs` folder (directory) of your software installation, which contains HTML and PDF documentation. Index pages are available in this folder.
- The Schrödinger web site, http://www.schrodinger.com/, particularly the Support Center, http://www.schrodinger.com/supportcenter, and the Knowledge Base, http://www.schrodinger.com/kb.

Finding Information in Maestro

Maestro provides access to nearly all the information available on Schrödinger software.

To get information:

- Pause the pointer over a GUI feature (button, menu item, menu, ...). In the main window, information is displayed in the Auto-Help text box, which is located at the foot of the main window, or in a tooltip. In other panels, information is displayed in a tooltip.

  If the tooltip does not appear within a second, check that Show tooltips is selected under General → Appearance in the Preferences panel, which you can open with CTRL+, (⌘,). Not all features have tooltips.
- Click the Help button in a panel or press F1 for information about a panel or the tab that is displayed in a panel. The help topic is displayed in your browser.
- Choose Help → Online Help or press CTRL+H (⌘H) to open the default help topic in your browser.
- When help is displayed in your browser, use the navigation links or search the help in the side bar.
- Choose Help → Manuals Index, to open a PDF file that has links to all the PDF documents. Click a link to open the document.
- Choose Help → Search Manuals to search the manuals. The search tab in Adobe Reader opens, and you can search across all the PDF documents. You must have Adobe Reader installed to use this feature.

For information on:

- Problems and solutions: choose Help → Knowledge Base or Help → Known Issues → *product*.
- Software updates: choose Maestro → Check for Updates.
- New software features: choose Help → New Features.
- Scripts available for download: choose Scripts → Update.
- Python scripting: choose Help → Python Module Overview.
- Utility programs: choose Help → About Utilities.
- Keyboard shortcuts: choose Help → Keyboard Shortcuts.
- Installation and licensing: see the *Installation Guide*.
- Running and managing jobs: see the *Job Control Guide*.
- Using Maestro: see the *Maestro User Manual*.
- Maestro commands: see the *Maestro Command Reference Manual*.

Contacting Technical Support

If you have questions that are not answered from any of the above sources, contact Schrödinger using the information below.

E-mail: help@schrodinger.com
USPS: Schrödinger, 101 SW Main Street, Suite 1300, Portland, OR 97204
Phone: (503) 299-1150
Fax: (503) 299-4532
WWW: http://www.schrodinger.com
FTP: ftp://ftp.schrodinger.com

Generally, e-mail correspondence is best because you can send machine output, if necessary. When sending e-mail messages, please include the following information:

- All relevant user input and machine output
- P450 Site of Metabolism purchaser (company, research institution, or individual)
- Primary P450 Site of Metabolism user
- Installation, licensing, and machine information as described below.

Gathering Information for Technical Support

This section describes how to gather the required machine, licensing, and installation information, and any other job-related or failure-related information, to send to technical support.

For general enquiries or problems:

1. Open the Diagnostics panel.
   - **Maestro:** Help → Diagnostics
   - **Windows:** Start → All Programs → Schrodinger-2012 → Diagnostics
   - **Mac:** Applications → Schrodinger2012 → Diagnostics
   - **Command line:** `$SCHRODINGER/diagnostics`
2. When the diagnostics have run, click Technical Support.

   A dialog box opens, with instructions. You can highlight and copy the name of the file.
3. Attach the file specified in the dialog box to your e-mail message.

If your job failed:

1. Open the Monitor panel in Maestro.

   Use Applications → Monitor Jobs or Tasks → Monitor Jobs.
2. Select the failed job in the table, and click Postmortem.

   The Postmortem panel opens.
3. If your data is not sensitive and you can send it, select Include structures and deselect Automatically obfuscate path names.
4. Click Create.

   An archive file is created in your working directory, and an information dialog box with the name of the file opens. You can highlight and copy the name of the file.
5. Attach the file specified in the dialog box to your e-mail message.
6. Copy and paste any log messages from the window used to start Maestro (or the job) into the email message,or attach them as a file.
   - **Windows:** Right-click in the window and choose Select All, then press ENTER to copy the text.
   - **Mac:** Start the Console application (Applications → Utilities), filter on the application that you used to start the job (Maestro, BioLuminate, Elements), copy the text.

If Maestro failed:

1. Open the Diagnostics panel.

   - **Windows:** Start → All Programs → Schrodinger-2012 → Diagnostics
   - **Mac:** Applications → Schrodinger2012 → Diagnostics
   - **Linux/command line:** `$SCHRODINGER/diagnostics`

2. When the diagnostics have run, click Technical Support.

   A dialog box opens, with instructions. You can highlight and copy the name of the file.

3. Attach the file specified in the dialog box to your e-mail message.

4. Attach the file `maestro_error.txt` to your e-mail message.

   This file should be in the following location:

   - **Windows:** `%LOCALAPPDATA%\Schrodinger\appcrash`
     (Choose Start → Run and paste this location into the Open text box.)
   - **Mac:** `Documents/Schrodinger`
   - **Linux:** Maestro's working directory specified in the dialog box (the location is given in the terminal window).

5. On Windows, also attach the file `maestro.EXE.dmp`, which is in the same location as `maestro_error.txt`.

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